POSTER ABSTRACTS

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Investigating the effect of diet on life history characteristics of Anopheles arabiensis infected with Microsporidia MB, a Plasmodium falciparum blocking symbiont

Boanyah G[1], Koekemoer L[2], Bukhari T[1], Herren J[1]
[1]University of the Witwatersrand, [2]International Centre of Insect Physiology and Ecology

Background
The symbiont Microsporidia MB (MB) found in Anopheles arabiensis tested in Kenya has shown malaria blocking potency against the transmission of the Plasmodium parasite. MB density is high in mosquito gonads, which is linked to horizontal (sexual) and vertically (transovarial) transmission from one mosquito to another. Maximizing MB density and transmission is important for maintaining heavily infected mosquito colonies for experiments and ultimately trial releases. We have investigated how environmental factors such as diet affect the MB An. arabiensis symbiosis phenotype.
Methods
F1 larvae of G0 females confirmed to be An. arabiensis and infected with MB were either combined (Isogroup lines (IGLs)) or reared separately (Isofemale lines (IMLs)) depending on the experiments. Four diet regimes, Tetramin 0.07, Tetramin 0.3, Gocat 0.3 and Cerelac 0.3 milligram per larva (mg/larva) were tested on F1 IGLs for larva diet. IGLs reared on Tetramin 0.3 mg/larva were fed on either 1% or 6% glucose diet to determine adult survival. Larva of IMLs were fed on Tetramin 0.07mg and Tetramin 0.3mg for larva experiment. The adult experiment on IMLs were reared on 1% and 6% respectively.
Results
We found that amongst the four larval diet regimes tested for An. arabiensis development in the presence of Microsporidia MB, Tetramin 0.3 mg/larva gave the fastest larva development, highest adult emergence, largest male size mosquitoes, greatest prevalence, and density of MB. Also, adult MB mosquitoes fed on 6% glucose survived longer than negatives whilst with 1% glucose diet there was no significant difference between MB+ & -. However, development time and wing size, and the survival of adult were not significantly different between MB infected and uninfected An. arabiensis under the Tetramin 0.07 and 1% glucose diet respectively, suggesting that the MB conferred fitness advantage was diet dependent.
Conclusion
Microsporidia MB has no negative effect on the development of Anopheles arabiensis even under low diet conditions. Identified best larval and adult diet regimes can be used for mass rearing of MB colonised An. arabiensis for future trial release. Knowledge on the effect of diet is important for understanding MB spread in An. arabiensis in the field.

Transcriptomics and metabolomics-based investigation of the chemical communication between two Plant-growth-promoting Rhizobacteria (PGPR) strains

Mmotla K[1], Mhlongo M[2], Mashabela D[3], Mafuna T[4], Allie F[5]
[1]Mr K Mmotla, [2]Dr MI Mhlongo, [3]Mr MD Mashabela, [4]Dr T Mafuna, [5]Dr F Allie

Background
Plant growth-promoting rhizobacteria (PGPR) refer to advantageous microorganisms that inhabit the rhizosphere. PGPR plays a role in stimulating plant growth and enhancing plant resistance to both biotic and abiotic stresses. For PGPR to effectively fulfill their roles, they engage in intricate interactions with one another, a phenomenon that occurs within the rhizosphere. This collaborative synergy among PGPR species within the rhizosphere is essential for them to perform their functions optimally. Nevertheless, the precise mechanisms and dynamics of PGPR-PGPR interactions remains the subject of ongoing research. This study aimed to Investigate the impact of metabolite-mediated cross-feeding mechanisms on the metabolome of bacterial strains, unraveling the dynamic interplay between metabolite exchange.

Methods
Two PGPR strains, Bacillus megaterium and Bacillus licheniformis were grown in 50 ml flask M9 containing LB media and incubated at 30 °C 160 rpm for 24 h in a shaking incubator until optical density of 1 M was reached. They were centrifuged, pellet and supernatant were harvested. For cross-feeding, 0.1% of the inoculum (receiver) was transferred into 50ml flask that contained 15 ml extracts from late-log phase (donor), this was carried out in triplicates. The strains that were not cross-fed were used as a control. The Optical density (OD) was measured and recorded at every 6 h interval until 36 h using laboratory spectrophotometer at 600nm. The pellet and supernatant were stored in -80 °C. The extracts were analysed using ultra-high performance liquid chromatography-mass spectrometry (UHPLC-MS), and machine-learning techniques were applied to mine the data to discover significant biomarkers.

Results
The presence of metabolites derived from B. licheniformis resulted in a reduction in the growth of B. megaterium. Conversely, the metabolites produced by B. megaterium had a stimulating effect on the growth of B. licheniformis.

Conclusion
This reciprocal relationship suggests a dynamic interplay between the two bacterial species, wherein the metabolites from each strain exert contrasting influences on the growth patterns of the other. This collaborative approach among PGPR not only enhances plant growth but also exemplifies the intricate and mutually beneficial interactions that occur in the rhizosphere, contributing to more sustainable and productive agriculture.

Structural and biochemical characterization of Aspergillus niger RING finger domain toward new anti-cancer drug discovery

George M[1], Masamba P[1], Kappo A[1]
[1]University Of Johannesburg

Background: Cancer has been increasing in incidence and prevalence in recent years and has been the most common and widely diagnosed disease globally. The Global Cancer Observatory 2020 revealed an estimated 19 million new cancer cases, which is set to increase to 28 million by 2040. In South Africa, prostate, breast, and cervix-uteri cancer are the leading cancer types. Pro-cancer proteins have been widely studied. One of these proteins is the Retinoblastoma-binding protein 6 (RBBP6), a multi-domain splicing-associated protein comprising a RING (Really Interesting New Gene) finger domain. A previous bioinformatics study showed the existence of homologues of the human RING finger domain in Aspergillus niger and Saccharomyces cerevisiae. Therefore, this study aimed to characterize the A. niger RING finger domain, shown to have a substitution of a cysteine residue for aspartic acid, and subsequently used this protein for the discovery of new anti-cancer compounds.
Methods: Bioinformatic analyses were conducted to predict the protein's 3D structure and identify possible anti-cancer compounds. In vitro studies were performed by heterologous expression in bacterial cells and followed by purification. Various biochemical characterizations were then used to determine the protein's overall chemical structure and molecular interactions to validate the results of the computational studies.
Results: The A. niger RING finger domain consists of two α-helices and two anti-parallel β-sheets. Four possible anti-cancer ligands with high docking scores and favourable ADMET properties were identified. The A. niger RING protein was recombinantly expressed in various bacterial cell lines and purified. The protein was subjected to various characterizations such as Ultraviolet-visible spectroscopy, Fourier Transform-Infrared spectroscopy, and Raman spectroscopy to elucidate the functional groups within the protein. The secondary structure elements were found to be the same as those from the in silico studies.
Conclusions: This study provides a foundational basis to ascertain the hypothesized structural difference between the A. niger RING finger domain and its human form. More so, this study further provides a biochemical basis for the structural determination of the protein prior to in vitro testing and evaluation of the new anti-cancer compounds and the subsequent design of their derivatives.

Exploring Glycolytic Proteins as Potential Drug Targets for Developing Anti-schistosomal Treatments

Motlhatlhedi K[1], Pilusa N[1], Masamba P[1], Kappo A[1]
[1]University Of Johannesburg

Background: Schistosomiasis is a devastating parasitic disease caused by the Schistosoma species. It affects over 260 million people worldwide, with the highest morbidity and mortality rates in sub-Saharan Africa. Despite its significant impact on public health, Schistosomiasis remains one of the neglected tropical diseases. Praziquantel (PZQ) is currently the only drug that treats all schistosomiasis infections due to its availability, cost-effectiveness, and minimal side effects. However, recent studies showed the emergence of PZQ-resistant strains due to drug pressure. Additionally, PZQ does not kill the parasite during the crucial reproduction stage, as the disease results directly from eggs' entrapment in host tissue, increasing vulnerability to opportunistic infections. Therefore, discovering new druggable targets or vaccine candidates for schistosomiasis is critical. Glycolytic proteins have emerged as possible drug targets and vaccine candidates for treating schistosomiasis. Since proteins do not function alone, disrupting essential protein-protein interactions using small molecule inhibitors has become a more promising approach to resolving drug resistance in schistosomiasis. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and Triosephosphate isomerase (TPI) are glycolytic proteins that play a crucial cooperative role in generating energy for the worm's motility and survival. Therefore, disrupting this critical GAPDH~TPI protein complex should decrease the worms' energy levels, thus creating an unfavourable environment for the parasite to thrive. This study, therefore, aimed to characterize the GAPDH~TPI protein complex to discover, design, and develop new anti-schistosomal drugs. Methods: The study commenced by conducting in silico characterization of the two proteins to obtain their 3D homology models. These models were then utilized for molecular docking using HDOCK to confirm their interaction. Results: The homology models of the proteins revealed their respective secondary structure elements, while the molecular docking results validated their putative interaction and further revealed the identity of the amino acids involved in the interaction. Conclusion: Overall biochemical characterization of the GAPDH~TPI protein complex showed the need for wet-lab studies to ascertain this interaction and its potential use as a drug target for schistosomiasis.

the effect of curcumin derivatives on SARS-CoV-2 spike s1 protein induced oxidative stress on macrophage cells

Nkwana M[1], Makola R[1], Matsebatlela T[1]
[1]Department Of Biochemistry, University Of Limpopo

The ongoing COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has emerged as a global health crisis. The disease is characterized by an exaggerated immune response leading to oxidative stress, particularly in macrophage cells that play a crucial role in the innate immune defense against viral infections. The overproduction of reactive oxygen species (ROS) and the dysregulation of inflammatory responses exacerbate the severity of COVID-19. Curcumin derivatives have shown potent antioxidant and anti-inflammatory properties in various pathological conditions. This study was aimed at investigating the potential of curcumin derivatives in ameliorating COVID-19 induced oxidative stress in macrophage cells. Cell viability was assessed by using two assays, absorbance-based (MTT) and fluorescence-based (PI) and curcumin derivatives shown to exert no cytotoxic effects on non-infected Raw 264.7 cells. The Annexin V/ PI assay was employed to demonstrate that curcumin derivatives induce apoptosis as mode of cell death on Raw 264.7 cells. An ELISA assay was used to determine the stimulatory effects of curcumin derivatives on IL-6 expression in this cell line. The results obtained showed that these derivatives suppress the production of IL-6 as SARS-CoV-2 infected curcumin derivative cells produced less IL-6 compared to SARS-CoV-2 infected control cells. The reduction in the production of ROS and NO was observed in SARS-CoV-2 infected curcumin derivative treated cells as opposed to untreated SARS-CoV-2 infected controls, using muse oxidative stress kit and DAF-2 DA, respectively. our study demonstrates that curcumin derivatives possess remarkable antioxidative and anti-inflammatory properties and can mitigate COVID-19 induced oxidative stress in macrophage cells.

Biochemical and spectroscopic characterization of S. mansoni NAD-binding domain of Glyceraldehyde–3–phosphate dehydrogenase towards new anti-schistosomal discovery

Pilusa N[1], Motlhatlhedi K[1], Masamba P[1], Kappo A[1]
[1]University Of Johannesburg

Background: Schistosomiasis is a water-borne parasitic disease of poverty that causes significant morbidity and mortality in disadvantaged communities. It has infected over 261 million individuals, and millions more are at risk. Its infection may seem mild; however, it is attributed to chronic clinical complications inclusive of cancer and HIV/AIDs. Although great efforts were made toward the control and elimination of schistosomiasis, it is still difficult to contain due to shortcomings of the sole treatment drug, Praziquantel, signifying the need for alternative treatment. There is renewed interest in metabolic enzymes as druggable targets, such as GAPDH, which was previously characterized as a possible vaccine target for schistosomiasis. Schistosomes depend on glycolysis for survival, and GAPDH is a key glycolytic enzyme and has been shown to correlate to drug resistance in schistosomiasis. Therefore, targeting GAPDH should deprive schistosomes of the energy needed for survival. Thus, this study was designed to characterize the S. mansoni NAD-binding domain of GAPDH towards developing an alternative treatment for the disease. Methods: To achieve this aim, in silico studies were used to predict and confirm the protein structure, ascertain its physiochemical properties, and evaluate the antigenicity of the protein. Thereafter, in vitro studies were done whereby the protein was expressed in inclusion bodies, solubilized, purified, and subjected to other various characterizations. Results: In silico analysis predicted the NAD-binding domain of S. mansoni GAPDH has two α-helices and six β-sheets. Protein-protein interactions were identified and used to predict antigenic sites, while three ligands with excellent ADMET properties were predicted. In vitro studies showed successful heterologous expression in bacteria, solubilization, purification, and various spectroscopic characterizations validating results from in silico studies regarding secondary structural elements of the protein. Conclusion: Overall, this study revealed the NAD-binding domain of S. mansoni is stable and a probable antigen that can be employed as a druggable target against schistosomiasis. Additionally, the study provides the basis for structure-based drug discovery for the development of an alternative anti-schistosomal treatment.

Keywords: ADMET, GAPDH, Glycolysis, NAD-binding domain, Praziquantel, Schistosomiasis

Biochemical and computational characterization of Hsp70.14 protein towards the discovery and development of new anti-cancer compounds

Ndaba T[1], Masamba P[2], Kappo A[3]
[1]Student Member, [2]Co-supervisor, [3]Supervisor

Background: Cancer remains one of the leading causes of death, with over 10 million cases worldwide. Several therapeutic approaches have been developed over the years in the treatment of cancer such as surgery, chemotherapy, and hormone therapy, all of which display deficiencies in terms of specificity. New therapeutic approaches are, therefore, under constant development, aimed at eliminating specific pathways exploited by cancerous cells. One of these mechanisms to be investigated is the molecular interaction between the cancer-associated heat shock protein 70.14 (Hsp70.14) and BAG2 protein to determine how this interaction contributes to the progression of cancer. Disruption of this interaction through the discovery and development of protein-protein interaction (PPI) modulators could serve as a potential therapeutic approach against the development of cancer. The present study was thus aimed at the heterologous recombinant expression, purification, and characterization of the Hsp70.14 protein.

Methodology: Hsp70.14 was expressed in competent Top10 E. coli cells, purified using Ni-NTA affinity chromatography, and biophysically characterized using FTIR and Raman spectrometry. Additionally, in silico methods were used to computationally characterize and predict the structure of the Hsp70.14 protein and that of its hypothesized interacting partner, BAG2.

Results: The results showed that Hsp70.14 protein predominantly contains hydrophilic residues, and its structure is made up of two α-helices and three anti-parallel β-strands, whilst BAG2 is comprised of three α-helices, with both structures successfully validated using a Ramachandran plot and a Q-MEAN Swiss model. In-vitro studies conducted on the Hsp70.14 protein displayed FTIR results revealing a high number of carbonyl and hydroxyl groups present in the protein, whilst the data from Raman spectroscopy displayed symmetric C-C stretching and CH2 twisting vibrations present in the fingerprint region of the proteins respectively.

Conclusion: The characterizations thus provide the basis for the structural determination of the protein and the subsequent identification of the residues important in the interaction with the BAG2 partner towards the discovery and design of new anti-cancer biopharmaceuticals

In-silico Analysis and Evaluation of Inhibitors for SARS-CoV-2 Spike Protein-HSPA8 Complex in COVID-19 Therapeutics Development.

Navhaya T[1], Matsebatlela T[2], Makhoba X[3]
[1]University Of Limpopo, [2]University of Limpopo, [3]University of South Africa

Background
The coronavirus disease 2019 (COVID-19) pandemic left the world economy in shambles and had a strenuous effect on the health systems of all countries worldwide, necessitating continuous efforts to identify novel therapeutic strategies. COVID-19 virus calls into action the chaperonin system that assists the virus in viral entry and replication. This research project focuses on the in-silico discovery of potential inhibitors targeting the SARS-CoV-2 spike protein-HSPA8 complex, aiming to unveil promising candidates for developing effective COVID-19 therapeutics. Using advanced computational techniques, this study delves into the sophisticated molecular interactions between the viral spike protein and a crucial cellular chaperone, HSPA8. Through comprehensive virtual screening, this study aims to identify small molecules that have the potential to disrupt the formation of the viral-chaperone protein complex, thereby impeding viral entry and replication.
Methods
This ongoing research project employs a multidisciplinary approach combining cheminformatics, structural bioinformatics (homology modelling and analysis), molecular docking (protein-protein docking and protein-ligand docking), and molecular dynamic simulations.
Results
Preliminary findings indicate that the selected candidates satisfy all bioavailability conditions, required medicinal chemistry properties, physiochemical properties, water solubility and drug-likeness. Preliminary findings from protein-protein docking showed multiple extensive bonds formed between the spike protein and the heat shock protein of interest. Preliminary findings from protein-ligand docking have highlighted promising small molecules that exhibit favourable binding affinities and stabilities against the SARS-CoV-2 spike protein-HSPA8 complex. Extensive computational modelling and simulations are underway to optimize and validate the potential candidates further.
Conclusion
Navigating through the complexities of this research, the end goal is to subsidize the development of targeted therapeutic interventions for COVID-19. The nature of this ongoing project underscores the commitment to refine and expand the knowledge and understanding of the molecular interactions involved, paving the way for identifying potent inhibitors and their translation into practical clinical applications. This abstract is a picture of the progress to date and offers insight into the ongoing study of potential drug candidates targeting the SARS-CoV-2 spike protein-HSPA8 complex.

Cytotoxic properties and high-resolution respirometry mitochondrial activities of Eriocephalus racemosa against MDA-MB 231 triple-negative breast cancer

Adu-Amankwaah F[1]
[1]Stellenbosch University

Triple-negative breast cancer (TNBC) represents a significant global health crisis due to its resistance to conventional therapies and lack of specific molecular targets. This study explored the potential of Eriocephalus racemosa (E. racemosa), a fynbos plant, as an alternative treatment for TNBC. The cytotoxic properties and high-resolution respirometry mitochondrial activities of E. racemosa against the MDA-MB 231 TNBC cell line were evaluated.
Methods: Hexane solvent extraction of E. racemosa was performed, while mass spectrometry-based metabolite profiling was used to identify the phytochemical constituents of the extracts. The crude extract was further tested against MDA-MB 231 cancer cells to determine its cytotoxicity. The mode of cell death was confirmed using flow cytometry, and we assessed the activities of caspases 3, 8, and 9 using a multiplex activity assay kit. High-resolution respirometry measurements of mitochondrial function in the MDA-MB 231 cell line were conducted using the Oroboros O2K.
Results: Metabolite profiling of E. racemosa extract identified the presence of quinolines and derivatives, stigmastanes and derivatives, triterpenoids, and four unknown compounds. The extract demonstrated promising cytotoxic activity, with a half maximal inhibitory concentration (IC50) of 12.84 µg/mL. Further, the extract induced apoptosis in the MDA-MB-231 cancer cell line, similar to the reference drug cisplatin (17.44% and 20.25%, respectively) when compared with untreated cells. Caspase 3 activities confirmed the induction of the apoptosis pathway in both cisplatin and the crude plant extracts. Additionally, caspase 8 and 9 activities confirmed the activation of both the intrinsic and extrinsic apoptosis pathways in the plant crude extracts. High-resolution respiratory measurements showed elevated mitochondrial activities in all components examined except for complex-IV activities.
Conclusion: It was concluded these findings support further exploration of E. racemosa as a potential therapeutic agent for TNBC, offering a promising avenue for the development of targeted treatments with minimal adverse effects.

Alpha glucosidase and α-amylase activities of Amaranthus spinosus

Snyman N[1], Nkobole N, Prinsloo G
[1]University Of South Africa

Diabetes mellitus is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. Insulin is a hormone that regulates blood glucose. Hyperglycemia, also called raised blood glucose, is a common effect of uncontrolled diabetes and, over time, leads to severe damage to many of the body's systems, especially the nerves and blood vessels. Amaranth is well recognised as a highly nutritious superfood with significant nutraceutical characteristics. It has been extensively utilised due to its well-rounded nutritional composition and functional attributes, which have demonstrated notable therapeutic advantages. Amaranth has extreme adaptability to adverse growing conditions, but it is also an edible crop and contains some important antidiabetic properties. The main aim of the study is to report on the antidiabetic properties of Amaranthus spinosus. A. spinosus showed lower activity than acarbose in the α-glucosidase enzyme (IC50= 237.06 µg/mL, acarbose=36.98 µg/ mL). With regard to α-amylase, the crude extract showed good activity (IC50=3.37 µg/ mL). In conclusion, A. spinosus leaves possess noticeable in vitro α-amylase and moderate α-glucosidase inhibitory activities.

Metabolite profiling of two Salvia species using molecular networking and application of molecular docking and network pharmacology to investigate potential inhibitors and therapeutic mechanisms of Salvia species against COVID-19 treatment

Msobo A[1], Piater L[2], Koorsen G[3], Tsotetsi T[3], Mhlongo M[1]
[1]University Of Johannesburg, [2]University of Johannesburg, [3]University of Johannesburg, [4]University of Johannesburg, [5]University of Johannesburg

Background: Salvia species are used traditionally to treat various ailments and have been reported to have potential inhibitory activity against SARS-CoV-2. However, not much is known about these species as their chemical composition remains poorly explored and there is a lack of direct evidence on the potential of these species as therapeutics. Hence, the need to investigate/identify their active compounds and determine their mechanisms of action. Methods: In this study, an LC-MS-based metabolomics combined with molecular networking was employed to decipher the chemical composition of two Salvia species (Africana lutea and dolomitica) and identify and annotate their active compounds. Furthermore, molecular docking and network pharmacology were used to investigate potential inhibitors and action mechanisms of these identified compounds against COVID-19 viral and human targets, respectively. Results: Statistical analysis (PCAs) revealed distinct groupings and clear separations between the two species, implying significant metabolite profile differences. A total of 83 compounds were identified from the two species belonging to flavonoids, hydroxycinnamic acids, and terpenes, the major classes of compounds found in Salvia species. These compounds demonstrated maximum inhibitory activity against the 5 important viral proteins and revealed therapeutic mechanisms against COVID-19 infections through anti-inflammatory effects. Conclusion: Overall, this study highlighted the use of metabolomics, molecular docking, and network pharmacology in identifying the active compounds and underlying therapeutic mechanisms of Salvia species in treating COVID-19. Through these findings, we lay the foundation for further studies of Salvia and Salvia-based drugs for treating COVID-19.

Temperature-dependent extraction and chromatographic recovery and characterisation of ellagitannins with potent antioxidant and glycaemic control properties from ‘Wonderful’ pomegranate peel

Chukwuma C[1], Mashile B[1], Makhafola T[1]
[1]Centre For Quality Of Health And Living (cqhl), Central University Of Technology, Bloemfontein

Background: Pomegranate peel contains bioactive ellagitannins including punicalagin, a major bioactive principle. However, studies have not optimally recovered and evaluated these ellagitannins for their antioxidant and glycaemic control potential. In the present study, a temperature-dependent extraction was employed to optimally recover bioactive ellagitannins and punicalagin from pomegranate (variety ‘Wonderful’) peel.
Methods: Pomegranate peel was extracted with distilled water at different temperatures (25, 37, 50, 65, 78 and 95 °C). The extracts were evaluated for phenol and flavonoid contents, and in vitro antioxidant and enzyme inhibitory activities. XAD16N resin was used to recover tannins from the extracts and punicalagin was purified from the tannin recovered from the 78 °C crude extract. LC–MS was used to characterise the ellagitannins profile of the tannin recovered from the 78 °C crude extract. The dose-dependent anti-lipid peroxidative and glucose uptake potentials of the punicalagin and its parent crude extract and tannin were measured.
Results: The extract obtained at 78 °C had the highest phenolic, flavonoid, tannin and punicalagin contents. The tannin recovered from the 78 °C extract had stronger 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging, oxygen radical scavenging (IC50 = 9.64 vs. 15.6 and 25.6 µg/mL, respectively), anti-linoleic acid peroxidative (IC50 = 9.97 vs. 19.9 and 27.3 µg/mL, respectively) and antiglycation (IC50 = 29.1 vs. 34.2 and 79.1 µg/mL, respectively) activities than the extract and purified punicalagin. Similarly, the cellular anti-lipid peroxidative and glucose modulatory effect of the tannin (IC50 = 3.67 µg/mL; EC50 = 14.8 µg/mL) was better than that of the extract (IC50 = 24.3 µg/mL; EC50 = 55.0 µg/mL) and punicalagin (IC50 = 59.0 µg/mL; EC50 = 112 µg/mL), which may be attributed to constituent compounds (punicalagin, ellagic acid, granatin A, corilagin, casuarinin, gallic acid and quercetin hexoside) in the tannin. The α-amylase inhibitory data suggested that punicalagin influences the enzyme inhibitory potential of pomegranate peel, since it outperformed the extract, the tannin and acarbose (IC50 = 31.9, 59.8 and 54.5 µg/mL, respectively).
Conclusions: Tannins/ellagitannins purified from pomegranate peel aqueous extract obtained at 78 °C may be promising dietary supplements for exerting glycaemic control and mitigating oxidative stress.

Isolation of bioactive compounds from Ximenia caffra var traditional medicinal plant to investigate antimalarial activity against plasmodium falciparum.

Maboya K[1]
[1]University Of Johannesburg

Background: Malaria is a life-threatening disease spread to humans through the bite of infected Anopheles mosquitoes and is prevalent in tropical regions where it poses a threat to humankind. Of the five Plasmodium parasites known to cause malaria in humans, P. vivax and P. falciparum are the only two species recognized to cause deadly manifestations. Further, due to the emergence of resistance that continues to challenge the existing antimalarials, there is a critical need for the development of new strategies, including the study of medicinal plants as they harbour a wide array of biological active compounds that have therapeutic potential and therefore, targeting proteins such as PfHsp70-1, which is vital for the development of the parasite and is a potential avenue worth exploring. Materials and methods: For extraction, the leaves Ximenia caffra var were crushed into a fine powder and then macerated using hexane, ethyl acetate, dichloromethane, and methanol for 3 days and the extracts were filtered and concentrated using a Buchi rotavapor. The crude extract was then subjected to isolation using TLC and silica gel chromatography and the compounds isolated were screened for their anti-plasmodial activity using SYBR green assay and cytotoxicity was confirmed using MTT assay. The PfHsp70-1 protein was targeted by expressing, purifying and characterizing the protein in the presence of the isolated compounds, the cells were made competent using CaCl2 then they were transformed with a pQe30 plasmid having the pfHsp70-1 gene. The expression was induced using 1Mm IPTG and the samples will be expressed on a 12% SDS-PAGE gel. Results: the isolated bioactive compounds have shown significant antimalarial activity in vitro with a decrease in parasite viability, indicating potential antimalarial activity and the compounds exhibited selective toxicity against malaria parasites while sparing normal cells. The PfHsp70-1 protein was successfully expressed, purified, and resolved at the expected theoretical size of 74 kDa. Conclusion: Ximenia caffra var plants have been found to have antimalarial activity that can be further be considered as potential antimalarial drugs that can be optimized and developed into prospective PfHsp70-1 inhibitors, which is crucial in the endeavour to fight and combat malaria.

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Lawrence S[1]
[1]Scatterlings
asdf asfasf esf ef

Carbon monoxide alleviates salt-induced oxidative damage in Sorghum bicolor by inducing the expression of proline biosynthesis and antioxidant genes

Ikebudu V[1], Nkuna M[1], Ndou N[1,2], Ajayi F[2], Chivasa S[3], Cornish K[4], Mulaudzi T[1]
[1]Life Sciences Building, Department of Biotechnology, University of the Western Cape, [2]SensorLab, Department of Chemical Sciences, University of the Western Cape, [3]Biosciences Department, Durham University, [4]Department of Horticulture and Crop Science; Department of Horticulture and Crop Science; Department of Food, Agriculture and Biological Engineering; The Ohio State University; Ohio Agricultural Research and development Center

Abstract: Crop growth and yield are affected by salinity, which causes oxidative damage to plant cells. Plants respond to salinity by maintaining cellular osmotic balance, regulating ion transport, and enhancing the expression of stress-responsive genes, thereby inducing tolerance. As a byproduct of heme oxygenase (HO)-mediated degradation of heme, carbon monoxide (CO) regulates plant responses to salinity. This study investigated a CO-mediated salt stress tolerance mechanism in sorghum seedlings during germination. Sorghum seeds were germinated in the presence of 250 mM NaCl, treated with CO donor (1 and 1.5 µM hematin), also a HO inhibitor; zinc protoporphyrin IX (5 and 10 µM ZnPPIX, a CO scavenger; and hemoglobin (0.1 g/L Hb). Salt stress decreased germination index (47.73 %) and root length (74.31 %), while hydrogen peroxide (H_2O_2) (193.5 %), and proline (475 %) contents increased. This increase correlated with induced HO (137.68 %) activity and transcripts of ion-exchanger and antioxidant genes. Salt stress increased the Na^+/K^+ ratio (2.06) and altered vascular bundle structure, metaxylem pit size (42.2 %), and primary and secondary metabolites. However, exogenous CO (1 µM hematin) increased the germination index (63.01 %) and root length (150.59 %) while H_2O_2 (21.94 %) contents decreased under salt stress. Carbon monoxide further increased proline (147.62 %) and total soluble sugars (60.40 %), restored vascular bundle structure, decreased Na^+/K^+ ratio (1.46), metaxylem pit size (31.2 %), and damaged primary and secondary metabolites under salt stress. Carbon monoxide increased HO (30.49 %) activity, protein content (13-fold), and antioxidant gene transcripts. The alleviatory role of CO was abolished by Hb and slightly inhibited by ZnPPIX under salt stress. These results suggest that CO elicited salt stress tolerance by reducing oxidative damage through osmotic adjustment and by modifying HO1, ion exchanger, and antioxidant transcripts.

Keywords: Antioxidant, carbon monoxide, epidermis, hematin, heme oxygenase salinity, Oxidative stress; ROS, vascular bundles

Plasma 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D Levels in Breast Cancer Risk in Mali: A Case–Control Study

BISSAN A[1,2,3], Ly M[4], Amegonou A[3], Sidibe F[3,5], Koné B[3], Barry N[6], Tall M[2], Timbiné L[2], Kouriba B[2,3], Reynier P[7], Ouzzif Z[1]
[1]Biochemistry, Metabolic and Molecular Unit, Faculty of Medicine and Pharmacy of Rabat, Mohammed V University of Rabat, [2]Charles-Merieux Center for Infectiology (CMIC) of Bamako, [3]Biology Teaching and Research Department, Faculty of Pharmacy, University of Sciences, Techniques and Technologies of Bamako, [4]University Hospital of Luxembourg, [5]University Hospital of Point G, [6]Pharmaceutical Biochemistry Laboratory, Cheikh Anta Diop University, [7]Department of Biochemistry and Molecular Biology, University Hospital of Angers

Background: Breast cancer is the most prevalent cancer found in women in Mali. The aim of the current study was to determine the association between metabolites circulating in the blood, 25(OH)D and 1,25(OH)2D, and vitamin D levels with the risk of breast cancer in Malian women.

Methods: We conducted a prospective case–control study from August 2021 to March 2022. Control subjects were matched to cases according to age (within 5 years). The patients' clinical stage was determined by the oncologist according to the tumour–nodes–metastasis (TNM) classification system.

Results: We observed no differences in the mean 25(OH)D ($p = 0.221$) and 1,25(OH)2D ($p = 0.285$) between cases and controls. However, our findings indicate a more pronounced inverse association in the first level of plasma 25(OH)D, while the risk function decreases at higher levels. This observation takes strength with 1,25(OH)2D by a significant association between the first quartile and breast cancer as a risk factor ($p = 0.03$; OR= 71.84; CI: 1.36–3785.34).

Conclusions: These outcomes showed a possible association between 25(OH)D and 1,25(OH)2D in decreasing the risk of breast cancer.

Evaluating the Therapeutic Potential of Curcumin and Synthetic Derivatives: A Computational Approach to Anti-Obesity Treatments

Moetlediwa M[1], Jack B[1], Mazibuko-Mbeje S[2], Pheiffer C[1], titinchi S[3], Salifu E[1], Ramharack P[1]
[1]South African Medical Research Council And North-west University, [2]North-West University, [3]University of the Western Cape

Natural compounds such as curcumin, a polyphenolic compound derived from the rhizome of turmeric, have gathered remarkable scientific interest due to their diverse metabolic benefits including anti-obesity potential. However, curcumin faces challenges stemming from its unfavorable pharmacokinetic profile. To address this issue, synthetic curcumin derivatives aimed at enhancing the biological efficacy of curcumin have previously been developed. In silico modelling techniques have gained significant recognition in screening synthetic compounds as drug candidates. Therefore, the primary objective of this study was to assess the pharmacokinetic and pharmacodynamic characteristics of three synthetic derivatives of curcumin. This evaluation was conducted in comparison to curcumin, with a specific emphasis on examining their impact on adipogenesis, inflammation, and lipid metabolism as potential therapeutic targets of obesity mechanisms. In this study, predictive toxicity screening confirmed the safety of curcumin, with the cur-cumin derivatives demonstrating a safe profile based on their LD50 values. The synthetic curcumin derivative 1A8 exhibited inactivity across all selected toxicity endpoints. Furthermore, these com-pounds were deemed viable candidate drugs as they adhered to Lipinski's rules and exhibited favorable metabolic profiles. Molecular docking studies revealed that both curcumin and its synthetic derivatives exhibited favorable binding scores, whilst molecular dynamic simulations showed stable binding with Peroxisome proliferator-activated receptor gamma (PPARγ), Cy-clooxygenase-2 (COX2), and fatty acid synthase (FAS) proteins. The binding free energy calculations indicated that curcumin displayed potential as a strong regulator of PPARγ (−60.2 ± 0.4 kcal/mol) and FAS (−37.9 ± 0.3 kcal/mol), whereas 1A8 demonstrated robust binding affinity with COX2 (−64.9 ± 0.2 kcal/mol). In conclusion, the results from this study suggest that the three synthetic curcumin derivatives have similar molecular interactions to curcumin with selected therapeutic targets. However, in vitro and in vivo experimental studies are recommended to validate these findings

Androstachys johnsonii Prain (Picrodendraceae) exerts its antioxidant and anti-inflammatory potentials by preventing reactive oxygen species production and regulating the expression of inflammatory mediators in LPS-stimulated RAW 264.7 macrophages.

Mfotie Njoya E[1], Tabakam G[1], Chukwuma C[1], Mashele S[1], Makhafola T[1]
[1]Central University Of Technology

Background: Androstachys johnsonii Prain (Picrodendraceae) is widely used because of the strength, durability, and hardness of its wood, but its medicinal use is kept secret by traditional healers, according to a survey. Considering that inflammation and oxidative stress are major risk factors for the progression of various chronic diseases and disorders, we resolved to investigate the antioxidant and anti-inflammatory potentials of A. johnsonii using in vitro and cell-based assays.
Methods: The antioxidant activity of Androstachys johnsonii hydroethanolic leaf extract (AJHLE) was evaluated using the ABTS, DPPH, and FRAP assays. Its cytotoxic effect was assessed on RAW 264.7 macrophages using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. Then, its anti-inflammatory effect was evaluated by measuring the nitric oxide (NO) production inhibitory activity in lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages using Griess reagent and the 15-lipoxygenase (15-LOX) inhibitory activity using the ferrous oxidation xylenol orange assay. Moreover, its preventive effect on reactive oxygen species (ROS) production was determined using 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA) staining, and its regulatory effect on the expression of pro-inflammatory mediators such as interleukins (IL)-1β, IL-10, tumour necrosis factor-α (TNF-α), as well as cyclooxygenase-2 (COX-2) was quantified using commercially available kits.
Results: It was found that AJHLE strongly inhibits radicals such as ABTS•+, DPPH•, and Fe3+-TPTZ with IC50 values of 9.07 µg/mL, 8.53 µg/mL, and 79.09 µg/mL, respectively. Besides, AJHLE induced a significant ($p<0.05$) cytotoxic effect at 100 µg/mL, and when tested at non-cytotoxic concentrations, it inhibited NO and ROS production in LPS-stimulated RAW 264.7 macrophages in a concentration-dependent manner with IC50 values of 24.06 µg/mL and 10.49 µg/mL, respectively. Furthermore, AJHLE showed that its anti-inflammatory action occurs via the enzymatic inhibition of 15-LOX activity, the downregulation of COX-2, TNF-α, and IL-1β expression, and the upregulation of IL-10 expression. A chemical investigation showed that AJHLE contains significant amounts of phenolics, flavonoids, lignan glycosides, hydrolysable tannins, and quinic acid derivatives, which support its antioxidant and anti-inflammatory activities.
Conclusion: These findings suggest that A. johnsonii is a potential source of therapeutic agents against oxidative stress and inflammatory-related diseases, and the major compounds identified may be responsible for its significant antioxidant and anti-inflammatory potentials.

Development of an Escherichia coli vaccine to vaccinate sheep pre-lambing

Seyama S[1,2], Kappo A[1], Masamba P[1], Ramagoma F[2]
[1]University Of Johannesburg, [2]Onderstepoort Biological Products

Background: Colibacillosis is a condition that causes prominent levels of mortality in lambs. There are two forms: a bacteremia form, caused by invasive strains of E. coli in 2-3 weeks old lambs, and a neonatal coliform diarrhoea form, predominantly affecting 2-8 days old lambs. In neonatal form, enterotoxigenic strains of E. coli (ETEC) colonize the small intestine and produce an enterotoxin that clinically manifests into diarrhoea and a watery mouth. Most ETEC strains possess a common surface antigen, K99. This K99 pili adhesins enables the organism to adhere to the intestinal epithelium, colonize the small intestine and secrete toxins; studies have shown that lambs vaccinated with K99 antigen are protected from colibacillosis. This study evaluates cross-protective efficacy between the five E. coli serotypes manufacturing the OBP colibacillosis vaccine. Methods: In the present study, the molecular detection of E. coli virulence was performed by PCR. Results: The presence of heat-stable toxins (ST), K99, and F41 fimbriae adhesins in E. coli types 1, 4, and 5 was confirmed. More so, E. coli type 5 has STa, while STb toxins are not present in types 1 and 4. It was also shown that E. coli type 3 had F17 fimbriae adhesion, while E. coli type 2 exhibited no bands. Additionally, E. coli types 1, 3, 4, and 5 were confirmed as enterotoxigenic E. coli strains (ETEC). Conclusion: It is hypothesized that E. coli type 5 could cross-protect against E. coli type 1 and 4 because of the presence of STa toxin, which is not present in types 1 and 4, and thus has been found to disrupt intestinal fluid homeostasis, causing hypersecretion of fluid and electrolytes.

Spectroscopic Characterization of the Interaction between Divalent Cations and S. mansoni Universal Stress G4LZI3 Protein

Mahamba L[1], Masamba P[1], Kappo A[1]
[1]University Of Johannesburg

Background: Schistosomiasis is a parasitic infection caused by blood flukes of the genus Schistosoma. It is a poverty-related neglected tropical disease that mostly affects people in tropical and subtropical countries, especially in places with poor sanitation and limited access to safe drinking water. It has been reported that about 30% of individuals in the sub-Saharan region have bladder cancer connected to schistosomiasis. Moreover, despite its consistent use for treating all schistosome infections for over five decades, the mechanism of action of praziquantel remains unclear and over the past few years, there have been increasing reports of schistosome resistance to this drug. This study aimed to recombinantly express, purify, and biophysically characterize the interaction between selected divalent cations and the S. mansoni Universal stress G4LZI3 protein towards the elucidation of the mechanism of action of this drug.
Method: The protein's 3D structure was modelled using MODELLER and validated using a Ramachandran plot. Thereafter, wet lab experiments were performed by transforming competent E. coli cells with the Universal stress G4LZI3 plasmid, followed by recombinant expression of the protein to obtain sufficient quantities before purification on a Nickel-NTA affinity column. Lastly, biophysical characterization was performed using Fourier Transform-Infrared Spectroscopy and Raman spectroscopy to determine the functional groups within the protein and provide preliminary data on the interaction between the protein and the divalent cations.
Results: The G4LZI3 protein consists of four α-helices and four β sheets and the Ramachandran plot showed that no stereochemical parameters were violated during model building, rendering the predicted structure acceptable. The protein was successfully over-expressed in M15 bacterial cells and purified to homogeneity using affinity chromatography. The characterization results agreed with the in silico characterization and Raman spectroscopy showed possible interaction between the protein and ligand.
Conclusion: The Raman spectroscopy results confirmed the hypothesized interaction between the G4LZI3 protein and $CaCl_2$, which have been proposed to play an important role in regulating certain biochemical processes within the worm.

Gene-specific DNA Methylation profiles in whole blood of South African women with Gestational Diabetes Mellitus

Masete M[1,2], Pheiffer C[1,2,3], Adam S[2,4], Dias S[1]
[1]Biomedical Research and Innovation Platform (BRIP), South African Medical Research Council, [2]Department of Obstetrics and Gynecology, School of Medicine, Faculty of Health Sciences, [3]Centre for Cardio-Metabolic Research in Africa (CARMA), Division of Medical Physiology, [4]Diabetes Research Centre, Faculty of Health Sciences

BACKGROUND
Gestational diabetes mellitus (GDM) is associated with adverse short- and long-term effects for mothers and their offspring. DNA methylation is an epigenetic mechanism that plays an important role in the pathophysiology of GDM. Recently, our laboratory demonstrated altered genome-wide DNA methylation of genes key to metabolic regulation in women with GDM. The current study aims to expand on this and validate altered DNA methylation in the complete sample set of South African women with/without GDM.

METHODS
Women with (n=63) and without (n=118) GDM were recruited at <28 weeks of pregnancy. DNA was isolated from whole blood and quantified using Qubit fluorometry. DNA methylation levels of three CpG sites corresponding to differentially methylated genes, peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A), protein tyrosine phosphatase receptor type N2 (PTPRN2) and Insulin gene enhancer protein (ISL1), were selected based on the relevance to GDM pathophysiology were validated using pyrosequencing.

RESULTS
GDM is an insulin resistant state, as expected fasting insulin was higher in women with GDM (p=0.03) when compared to women without GDM. Methylation levels of PPARGC1A were lower, PTPRN2 were higher and ISL1 was significantly higher in CpG3 (p=0.01) and CpG7 (p<0.001) when compared to non-GDM groups. ISL1 gene is transcription factor that binds to insulin enhancer region of the insulin gene. In silico analysis showed E2F-1, WT1 and GCF transcription factors predicted to bind within margin less or equal than 15% of ISL1 gene.

CONCLUSION
These findings associate high levels of ISL1 methylation with GDM and insulin resistance in South African women.

Investigation of the differences between Southern and Northern African scorpions of the Buthidae family based on their proteomic profiles.

ZINYEMBA N[1], Offor B[1], Piater L[1]
[1]Department of Biochemistry, University of Johannesburg, Auckland Park, 2006

Background: Snake envenomation was designated a neglected tropical disease by the World Health Organisation in 2018. However, other envenomation cases are just as overlooked, such as that by scorpions, which account for 1.4 million reported cases globally, of which 3,000 result in death. A growing concern is the decreased anti-venom production and the limited variety available to treat scorpion envenomations. This research aimed to produce proteomic profiles for three African scorpions (Androctonus crassicauda, Buthus occitanus and Parabuthus granulatus), thus aiding the knowledge that could contribute to more robust and efficient anti-venom production.
Methods: Proteomic profiles were generated for different scorpions using biochemical techniques such as protein quantitation, protein activity assays, and electrophoresis. The samples' proteins were quantified using the Amido Black and Bicinchoninic acid assays. The quantified samples were then used to conduct and generate protein profiles using one (1DE) and two-dimensional gel electrophoresis (2DE). The hyaluronidase and proteinase assays were then performed to determine the protein activity within each sample.
Results: The protein profiles produced using 1DE and 2DE showed that there may be common proteins present amongst all three scorpions and distinct protein spots amongst different gels. Large unresolved bands towards the bottom of the 1DE gel are shared amongst all species. The hyaluronidase activity assay continuously revealed that A. crassicauda has the highest protein activity. Furthermore, the proteinase assay indicated that the scorpion venoms contained enzymes with peptide-cleaving activities, which could be susceptible to snake anti-venom, as some neutralising activity was observed.
Conclusion: It was revealed that scorpions from different genera and different locations appeared to have similar protein profiles. In both 1DE and 2DE, there was a differential spot pattern amongst similar areas on the gels, which could be accounted for by the scorpions' differing geographical origins, diets, and sexes. The proteinase activity assay indicated that all the scorpions have such activity of some calibre, and that snake anti-venom could have some neutralising activity on scorpion venom. Profiling these scorpion venoms can aid in producing robust scorpion anti-venom, which can have neutralising activity against scorpions of varying genera.

Proteomic profiling of medically important African spitting and non-spitting cobra snake venoms

Offor B[1], Piater L[1]
[1]University Of Johannesburg

Background: Snakebite envenoming is a neglected tropical disease (NTD) that results from the injection of snake venom of a venomous snake into animals and humans. It affects mainly people from tropical and subtropical regions across the globe. In Africa, there are over 100,000 snakebite envenomings and over 20,000 deaths per annum. Lack of epidemiological data and underestimated morbidity and mortality figures contribute to difficulties in snakebite prevention and antivenom treatment. Snake venom contains a cocktail of compounds dominated by proteins and peptides which make up the toxins with various pathological implications including cytotoxicity, neurotoxicity and haematoxicity. These toxins have species and genus-specific variations which contribute massively to diverse differences in venom toxicity that can undermine the efficacy of antivenom. Profiling the proteomes of medically important African snake venoms will aid in the development of safer and more effective antivenoms within the continent. Additionally, it will upscale the therapeutic application of animal venom in the treatment of diseases including cancer. Methods: Here, the venom of 2 spitting cobras (Naja pallida and Naja woodi) and 3 non-spitting cobras (Naja anchietae, Naja annulifera and Naja nivea) were fractionated by reverse phase-high performance liquid chromatography (RP-HPLC). The peak fractions were analysed by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Using label-free LC-MS/MS the toxins were identified and grouped into families based on their relative abundance in the venom. Results: Proteomes of the spitting and non-spitting cobras were dominated by the three-finger toxins (3FTXs) family. While the non-spitting cobra venoms were dominated by the cytotoxins (CTX) (N. anchietae–83.62%, N. annulifera–81.10% and N. nivea–70.00%), that of the spitting cobras were dominated by CTX (N. pallida–50.55% and N. woodi–55.78%) and phospholipase A2 (PLA2) (N. pallida–39.34% and N. woodi–31.40%). Other notable toxin families identified in this study with varied abundance across these Naja species include snake venom metalloproteinase (SVMP), cysteine-rich secretory protein (CRISP), nerve growth factor (NGF), Kunitz-type serine protease inhibitor (KSPI). Conclusions: The data generated from this study will be useful for the development of effective antivenom against snakebite envenomation and possibly for therapeutic application.

The investigation of the metabolic reprogramming in barley leaf tissue treated with 3,5-dichloroanthranilic acid, an inducer of plant immunity

SAIDI T[1], Hamany Djande C[1], Pretorius C[1], Piater L[1], Dubery I[1]
[1]Departmen of Biochemistry, University of Johannesburg, Auckland Park, 2006

Background: Barley classifies as a cereal crop, a grass crop from the Poaceae family grown for their edible seeds. Within a South African context, barley is mainly cultivated for malting purposes. Despite the absence of an adaptive immune system similar to that observed in animals, plants like barley can be triggered to activate various immune mechanisms, resulting in biochemical and molecular reconfigurations therein. Such alterations can be explored using metabolomics defined as the complete systematic identification and quantification of the metabolic processes found in a biological system. Aim: The investigation and determination of the metabolic changes in barley after treatment with a chemical inducer 3,5-dichloroanthinilic acid (3,5-DCAA). Hypothesis: The treatment of barley with 3,5-DCAA will induce metabolic reprogramming depicted as markers of plant immunity. Methodology: Barley (cv 'Elim') was cultivated for 21 days and treated with 3,5-DCAA. Leaf samples were harvested in 12 h intervals post treatment (0, 12, 24, 36 h) and metabolites were extracted in 80% methanol. The samples were analysed using ultra-high performance liquid chromatography-high definition mass spectrometry (UHPLC-HDMS). Unsupervised (Principal component analysis, PCA) and supervised (orthogonal projection to latent structures-discriminant analysis, OPLS-DA) learning methods were used to mine the generated data. The annotation of treatment related metabolites was done using the mass spectral information obtained in comparison to those in the databases or published literature. Results: Differences between samples treated with 3,5-DCAA and the untreated control groups were observed. Features responsible for these differences were identified and 48 metabolites were annotated. The annotated metabolites fell into the class of amino acids and derivatives, organic acid compounds, fatty acid derivatives, phenolic acid derivatives, alkaloids and flavonoids. Conclusion: The compound 3,5-DCAA was found to elicit metabolic changes consistent with plant immune responses in the barley.

Proteomic exploration of the recently re-classified forest cobra Naja species and the potential cytotoxic activity of fractioned toxins in cancer cell lines.

Motsa P[1], Piater L[2], Offor B[3]
[1]University Of Johannesburg, [2]University of Johannesbug, [3]University of Johannesburg

Background: Snake envenomation is a devastating public health issue that affects millions of individuals globally, especially in rural areas of developing countries where the prevalence is high. In 2009, it was recognized by the World Health Organisation (WHO) as a neglected tropical disease (NTD) and in 2017 it was elevated to Category A on the Neglected Tropical Diseases list. Conventional antivenom remains the main therapy treatment for severe snake envenomation and is in critically short supply in many of these developing countries.
Methods: To determine and verify the distinct proteomic variations in the snake venom compositions 1D and 2D electrophoresis will be used. This will be followed by quantifying and comparing the enzymatic activities of the venom samples using biochemical assays. The venomics aspect of this study will be broken down into two main categories, the separation and isolation of proteins, and the acquisition of protein structural information for protein identification and characterization. For the cancer cell line investigation, the cytotoxicity of the venom samples will be monitored through the use of the Alamar blue assay and flow cytometry using annexin V-FITC.
Results: The proteomic profiles from 1D and 2D electrophoresis will offer a reasonable approximation of how many different types of protein molecules are present in each venom sample. The enzymatic activities of the venoms will be assayed to further highlight variations between the venoms. Venomic analysis will uncover the overall protein composition of each snake venom and identify the toxins that will be assigned to families and expressed as percentages of total protein content. The cytotoxic effects these toxins have on tumor cells will determine if they are credible candidates to be explored for their therapeutic potential.
Conclusion: The proteomic analysis of the different forest cobra snake venoms will provide important insights into the intra-genus variations and similarities in relation to species, diet, and geographic location. The venomic proteome information generated from the venoms will add to the growing knowledge of potential therapeutic leads in the development of antivenoms and other therapeutics used in the treatment of diseases such as cancer.
Keywords: Snake envenomation, antivenom, venomic, toxins

Proteomic exploration of the venomic differences of North and South African scorpion species.

Pillay S[1], Piater L[1], Offor B[1]
[1]Department of Biochemistry, University Of Johannesburg, Auckland Park, 2006

Background: Envenomation is the exposure to poison or toxins injectable by animals such as snakes, scorpions, spiders, insects, or marine life upon biting/stinging prey or a victim. Worldwide, in tropical and sub-tropical regions scorpion envenomation poses a serious threat to public health, however, it is highly neglected with many occurrences and deaths not being reported. The aim of this study was to assess the proteomic profiles of two North and one South African scorpion species according to their geographical distribution, where specific differences and similarities would aid in the development of more effective antivenoms. Methods: The proteomic profiles of Leiurus quinquestriatus, Androctonus amoreuxi and Parabuthus transvaalicus were determined by 1-dimensional gel electrophoresis where potential venomic proteins were separated by their molecular weight, and 2-dimensional gel electrophoresis where protein similarities between the species were identified from the spots that separated based on their charge and molecular weight. The enzyme activity of the venom was assayed by the hyaluronidase and proteinase assays. Results: For both 1D and 2D gel electrophoresis a similar banding pattern was observed for each species, however, notable variations in the band/spot intensities could be observed. For 1D a similar banding pattern was observed between 25-70 kDa, while variations were observed between 35-55 kDa for all species. For 2D, on a pH 4-7 strip, similar spots were observed for all species between 10-15 kDa and 50-250 kDa, and between 5-10 kDa, 20-30 kDa, and 50-100 kDa on the pH 3-10 strip. Through the hyaluronidase and proteinase assays, the venom of P. transvaalicus was found to have the highest hyaluronidase activity which aids in venom spread after injection, and the highest proteinase activity. In contrast A. amoreuxi was the opposite having the lowest proteinase and hyaluronidase activity. When treated with two distinct antivenoms it was seen that the proteinase activity of L. quinquestriatus was the most effected followed by A. amoreuxi and P. transvaalicus, which was notably the least affected. Conclusion: These findings contribute valuable information that can aid in further understanding the major toxins found in these species' venoms and for the development of effective antivenoms.

Phytochemical investigation and evaluation of Ziziphus mucronata towards the discovery and development of novel antimalarial pharmaceutical agents.

Buthelezi N[1]
[1]University Of Johannesburg

Background: The continued development of resistance by Plasmodium falciparum towards most of the currently available antimalarials is a burdening hazard that emphasizes the need for the development of alternative strategies to help alleviate malaria. The study of traditional medicinal plants in search of phytochemicals with antimalarial potential and targeting them against the parasite and proteins deemed essential in the development of the parasite is seen as an appealing avenue. As such, this study focused on the phytochemical evaluation and validation of Ziziphus mucronata's antimalarial potency. Methods: Inspired by its ethnobotanical reputation of being effective against malaria, Ziziphus mucronata was collected and sequentially extracted using hexane (HEX), ethyl acetate (ETA), dichloromethane (DCM), and methanol (MTL). The crude extracts were then screened for their antimalarial (parasite lactate dehydrogenase (pLDH) assay) and cytotoxic (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay) potential, followed by the isolation of the active compounds from the DCM extract using silica gel chromatography. Structural elucidation was performed using spectroscopic techniques (NMR: 1H, 12C, and DEPT), and the compounds were then targeted against the P. falciparum heat shock protein 70-1 (PfHsp70-1) using Autodock Vina, followed by in vitro validation assays using ultraviolet-visible (UV-VIS) spectroscopy and the malate dehydrogenase (MDH) chaperone activity assay. Results: All the extracts except the methanol extract displayed considerable antimalarial potential with varying IC50 values, HEX (11.69 ± 3.84 µg/ml), ETA (7.25 ± 1.41 µg/ml), and DCM (5.49 ± 0.03 µg/ml). The extracts also exhibited minimal cytotoxicity except for the ETA and DCM, with CC50 values of 10.96 and 10.01 µg/ml, respectively. Isolation and structural characterization of the active compounds from the DCM extracts revealed that betulinic acid (19.95 µg/ml) and lupeol (7.56 µg/ml) were responsible for the observed antimalarial activity. Molecular docking suggested a strong binding affinity between PfHsp70-1, betulinic acid (-6.8 kcal/mol), and lupeol (-6.9 kcal/mol). Meanwhile, the in vitro validation assays revealed the disruption of the protein structural elements and chaperone function. Conclusion: This study proves that Z. mucronata has considerable antimalarial potential and supports the notion of considering medicinal plants as a source of prospective antimalarial agents.

Exploration of iso-mukaadial acetate and ursolic acid acetate as potential inhibitors of Plasmodium falciparum Hsp70-1 (PfHsp70-1)

Tshililo G[1], Salomane N[1]
[1]University Of Johannesburg

Malaria is an infectious disease caused by the genus Plasmodium parasites, with Plasmodium falciparum being the most lethal agent. P. falciparum uses heat shock proteins (Hsps) to overcome the human immune response. One of the Hsps that P. falciparum expresses is the PfHsp70-1, an antimalarial drug target. This study focused on expressing and purifying the PfHsp70-1 and investigating the efficacy of the plant-derived bioactive compounds iso-mukaadial acetate (IMA) and ursolic acid acetate (UAA) as novel inhibitors of PfHsp70-1. Competent Escherichia coli (E. coli) BL21 (DE3) cells were transformed with pQE30/PfHsp70-1 plasmid using heat-shock transformation, with the expression of the protein induced using 1 mM Isopropyl β-d-1-thiogalactopyranoside (IPTG) and analyzed using 12% Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel and western blot analysis. The protein was purified using Nickel Affinity chromatography and the interaction between the compounds and PfHsp70-1 was evaluated using malate dehydrogenase (MDH) and luciferase assay. The PfHsp70-1 was successfully expressed and purified and was found to resolve at an expected theoretical size of 70 kDa through SDS-PAGE and western blot analysis. PfHsp70-1 prevented the heat-induced aggregation of MDH and luciferase. However, the study compounds IMA and UAA disrupted the chaperone activity of PfHsp70-1. This renders IMA and UAA as potential antimalarial drug candidates.

Anticancer potential of Cotyledon orbiculata crude extracts on cervical cancer cells

Marema T[1], Laka K[1], Mbita Z[1]
[1]University Of Limpopo

Background
In women, cervical cancer is the leading cause of mortality and rated the fourth most common cancer, worldwide. These soaring numbers can be attributed to lack of adequate healthcare facilities for both early diagnosis and effective treatment regimes, especially in both underdeveloped and developed countries. Thus, it is important to find both specific innovative biomarkers and novel, affordable and effective anticancer alternative strategies. Medicinal plants are gaining a lot of interest as alternative source of anticancer drugs. Therefore, this study was aimed at identifying potential anticancer molecular mechanisms of Cotyledon orbiculata crude extracts against cervical cancer cells using a proteomics approach to identify novel biomarkers and drug targets.
Methods
Cotyledon orbiculata (C. orbiculata) leaves were collected, dried, and extracted using water, methanol, acetone, and hexane. Both qualitative and quantitative techniques were used to determine the phytochemical constituents of the extracts. Cervical CaSki (ATCC®CRL-1550™) and HeLa (ATCC®CCL-2™) cancer cells were used as cervical cancer study models and non-cancer Hek-293 (ATCC®CRL-1573™) were utilized as non-cancer model cells. In order to assess the cytotoxicity of C. orbiculata crude extracts, the MTT assay was used. A TACSTM Annexin V-FITC Apoptosis Detection kit was utilized to determine the potential of the C. orbiculata crude extracts to induce apoptosis. Lastly, Proteome profilers were used to determine molecular targets of both the acetone and methanol crude extracts.
Results
The acetone, methanol, and hexane crude extracts significantly ($P<0.0001$) reduced the viability of both HeLa and CaSki cervical cells after 24 and 48 hours, respectively. The IC50s were obtained and exhibited minimal cytotoxic effect toward the non-cancerous, Hek-293 cells. The C. orbiculata crude extracts induced late apoptosis on CaSki and HeLa cancer cells. The Acetone and Methanol IC50s upregulated Cleaved-caspase-3, Catalase, Cytochrome c, TRAIL R2/DR5, Fas/TNFRSF6/CD95, HO-2/HMOX2, HSP27, PON2, P21/CIP1/CDKN1A, P27/Kip1, Phospho-Rad17(S635) and XIAP proteins.
Conclusion
This study showed that the C. orbiculata extracts contain potential anticancer compounds, which target both the intrinsic and extrinsic apoptotic pathways. Most importantly, the C. orbiculata crude extracts downregulated potential novel biomarkers for cervical cancer development. Therefore, this plant contains potential templates for future anticancer drug development.
Key words: Phytochemicals, Cytotoxicity, Apoptosis

Vitellaria paradoxa Mitigates Sodium Arsenite-Induced Locomotor Deficit and Oxidative Stress in Drosophila melanogaster

Oyibo A[1,2,3], Abolaji A[4], Omoboyowa D[5], Odunola O[2]
[1]Focus Area for Human Metabolomics, Faculty of Natural and Agricultural Sciences, North-West University (Potchefstroom C, [2]Cancer Research and Molecular Biology Laboratories, Department of Biochemistry, University of Ibadan, , [3]Department of Biochemistry, College of Natural and Applied Sciences, Chrisland University,, [4]Drosophila Laboratory. Molecular Drug Metabolism and Toxicology Unit, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, , [5]Department of Biochemistry, Adekunle Ajasin University, A

Arsenic is classified as a class 1 environmental carcinogen, with subsurface water ingestion being the main cause of exposure in Africa. Therefore, the focus of arsenic research is on mitigating exposure and/or eradicating its harmful effects. Consequently, the protective effect of the Ethyl Acetate Fraction (EACF) of the Ethanol Leaf Extract of Vitellaria paradoxa (ELVp) against Sodium Arsenite (SA)-induced toxicity in Drosophila melanogaster was evaluated. Gas Chromatography-Mass spectrometry (GC-MS) was used to analyze the EACF. The compounds determined by GC-MS were molecularly docked against D. melanogaster's glutathione-S-transferase-2 (GST-2). The treatment of D. melanogaster (Harwich strain) with EACF was conducted to ascertain its impact on longevity. Subsequently, EACF (1.0 and 3.0 mg/5 g food) and/or SA (0.0625 mM) were fed to D. melanogaster for a duration of five days. The beneficial effects of EACF in reducing SA-induced toxicity were then evaluated by analyzing the fly's emergence rate, locomotor activity, oxidative stress, and antioxidant biomarkers. In the in-silico study, the twelve active ingredients of the EACF demonstrated varying degrees of binding affinity against GST-2, which was like the co-crystallized ligand (glutathione). EACF increased the lifespan of D. melanogaster by 20.0% compared to the control group and decreased the SA-induced decline in emergence rate and locomotor performance by 178.2% and 20.5%, respectively. Additionally, EACF mitigated the effects of SA-induced reduction in total thiol and non-protein thiols, as well as the suppression of GST and catalase activities ($p < 0.05$). These results were corroborated by the histology of the fat body of D. melanogaster. Due to its potent antioxidant qualities, EACF prevented sodium arsenite-induced oxidative stress and improved the antioxidative status of D. melanogaster. Hence, our findings suggest that the ethyl acetate fraction of Vitellaria paradoxa leaves will serve as a good prophylactic against arsenic toxicity.

Impact of Microbial Cross-Feeding on Chemical Communication Among Two PGPR Strains (Bacillus megaterium and Pseudomonas fluorescens) and the Pathogenic Pseudomonas syringae DC300 Using Metabolomics.

Sibanyoni N[1], Piater L[1], Kerchev P[2,3], Mhlongo M[1]
[1]Department of Biochemistry, Faculty of Science, University of Johannesburg, [2]Department of Molecular Biology and Radiobiology, Faculty of Agri Sciences, Mendel University in Brno, [3]Phytophthora Research Centre, Faculty of Agri Sciences, Mendel University in Brno

Plant growth-promoting rhizobacteria (PGPR) have gained increasing attention due to their ability to enhance plant growth and protect against pathogens. Among these, Bacillus megaterium (BM) and Pseudomonas fluorescens (N04) have demonstrated promising plant growth-promoting properties. However, the influence of microbial interactions and chemical communication between these PGPR strains and the pathogenic P. syringae DC300 remains largely unexplored. The study aims to use an LC-MS/MS-based metabolomics approach in combination with multivariate statistics to investigate how microbial cross-feeding between the PGPR strains Bacillus megaterium (BM) and Pseudomonas fluorescens (NO4) influences chemical communication and metabolite production, and its impact on the pathogenic bacterium Pseudomonas syringae DC300. Cultures of B. megaterium, P. fluorescens and P. syringae DC300 were grown in a minimal liquid medium until they reached an optical density of 1. Subsequently, 0.1 (OD) of the respective microbes (receiver) were cross-fed into a filter-sterilised media (donor) and controls were grown in pure media. The cell turbidity was measured every six hours, and samples were collected at 6-hour intervals (0, 6, 12, 18, 24, and 36), followed by extraction of metabolites. An LC-MS-based metabolomics approach and statistical analysis were used to investigate the metabolic perturbation caused by cross-feeding. The growth curves indicated that PGPR-PGPR cross-feeding maintained growth similar to controls, while PGPR-Pathogen BMd → Pstr exhibited a significant decrease, and Pathogen-PGPR (Pstd → NO4r) significantly decreased NO4 growth. Metabolic analysis revealed a time-dependent metabolic perturbation in the cross-fed organism, indicating the metabolic re-adjustment of the receiver microorganism to donor media. Metabolite annotation revealed metabolites that belong to the following metabolite classes: amino acids, organic acids, sugars, lipids, organoheterocyclic compounds, and benzenoids. The results showed that PGPR-PGPR cross-feeding maintained growth akin to controls, contrasting with significant growth reductions observed in PGPR-pathogen and pathogen-PGPR interactions. Metabolite analysis emphasised the diverse metabolites classes involved in microbial cross-feeding interactions. This study contributes to understanding the complex interactions between PGPR strains and pathogenic bacteria in the rhizosphere. Expanding this experimental approach to diverse communities could aid in modeling and simulating bacterial interactions within intricate microbiomes.

Key words: Cross-feeding, PGPR, microbial interactions, chemical communication, pathogenic bacteria, metabolomics

The inhibitory effects of Momordica balsamina leaf methanol extract on the inflammatory-induced metastasis in triple-negative breast cancer cell line via the IL-6/JAK2/STAT3 pathway.

Mohale T[1], Poopedi K[1], Riedel-van Heerden S[2], Mbazima V[1]
[1]University Of Limpopo, [2]2Biomedical Research and Innovation Platform, South African Medical Research Council

The inhibitory effects of Momordica balsamina leaf methanol extract on the inflammatory-induced metastasis in triple-negative breast cancer cell line via the IL-6/JAK2/STAT3 pathway.

T.E Mohale1, K.W Poopedi1, S Riedel-Van Heerden2, V.G Mbazima1.

1Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo, Sovenga, 0727, South Africa.
2Biomedical Research and Innovation Platform, South African Medical Research Council, P O Box 19070, 7505 Tygerberg, Cape Town, South Africa.
Email: mohaletshwarelo98@gmail.com; vusi.mbazima@ul.ac.za

The overexpression of interlekin-6 (IL-6) in triple-negative breast cancer (TNBC) cells and the tumour microenvironment drives inflammatory-mediated tumour cell survival, cancer progression, metastasis, and immunosuppression through the activation of the IL-6/JAK2/STAT3 pathway. This study investigated the potential anti-metastatic effects of Momordica balsamina methanol extract (MBME) in IL-6-activated MDA-MB-231 breast cancer cell line. Triple-negative breast cancer MDA-MB-231 and kidney HEK-293 cells were exogenously activated with IL-6 (50 ng/ml) during treatment. The cytotoxicity of the MBME on MDA-MB-231 and HEK-293 cells was determined using the MTT and annexin-V assays, where MBME (≤100 µg/ml) exhibited minimal toxicity on the viability of IL-6-activated MDA-MB-231 and HEK-293 cells and further validated by non-significant induction of apoptotic cell death from Annexin-V results. The transwell cell invasion, wound healing, cell adhesion and extracellular matrix (ECM)-protein adhesion assays showed that the MBME inhibits IL-6-induced invasive, migratory, adhesive, and potential to attach to ECM-proteins in MDA-MB-231 cells. The mechanism of action of the MBME in IL-6-activated MDA-MB-231 cell was assessed using Real-time PCR and western blot where an upregulation in Bax and downregulation in Bcl-2, JAK2, STAT3, MMP-2 and MMP-9 mRNA expression was observed after treatment with MBME. Furthermore, MBME decreased the protein levels of MMP-2, MMP-9, vimentin, pSTAT3 while increasing the levels of TIMP-3 protein in IL-6-activated MDA-MB-231 cells. Moreover, a significant inhibition in the activity of MMP-2 and MMP-9 was observed in extract-treated IL-6-activated MDA-MB-231. In conclusion, the MBME exhibits the potential to inhibit the IL-6-induced metastatic effects of MDA-MB-231 cells by downregulating the IL-6/JAK2/STAT3 pathway.

Keywords: Triple-negative breast cancer, Metastasis, Interleukin-6, Momordica balsamina.

Physcion exhibits anti-bone resorbing effect in ovariectomized rats and in silico activity against RANK receptor

Mada S[1], Abaya P[2], James D[2], Tanko M[3]
[1]1, Department of Biochemistry, Federal University , [2]2, Department of Biochemistry, Ahmadu Bello University , [3]3 Veterinary Teaching Hospital, Faculty of Medicine Ahmadu Bello University
Postmenopausal osteoporosis is a subclinical disease and a global public health disorder associated with morbidity, mortality and deterioration of the quality of life. This study was design to investigate the effect of physcion (PHY) on selected bone resorbing cytokines in ovariectomized (OVX) rats and also carried out molecular docking studies between PHY and receptor-activator of nuclear factor kappa-β (RANK) receptor. Forty female Wistar rats weighing between 180-190 g were either ovariectomized (n=32) or sham-operated (n=6). The animals were divided into five groups (n = 6): Sham, OVX, OVX + PHY (50 mg/kg), OVX + PHY (100 mg/kg) and OVX + Alendronate (5 mg/kg). At the end of six weeks treatments, body weight gain, serum estrogen, RANK-ligand (RANKL), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) levels were measured. The result indicated that treatment of OVX rats with PHY significantly ($p<0.05$) reduced excessive bodyweight gain compared to untreated OVX rats. Similarly, treatment of OVX rats with different concentrations of PHY significantly ($p<0.05$) improved estrogen level and reduced RANKL level when compared to untreated OVX rats. Likewise, treatment of OVX rats with PHY significantly ($p<0.05$) suppressed IL-6 and TNF-α levels when compared to untreated OVX rats. Moreover, molecular docking studies between PHY and RANK receptor indicated a favourable inhibitory interaction with a binding energy of -7.10 kcal/mol and demonstrated a possible drug-like property based on Lipinski's rule. This study demonstrated that physcion exhibits anti-bone resorptive effects via reduction of bone resorbing cytokines levels in OVX rats and demonstrated a potential drug-like property in silico. Thus PHY could be a potential therapeutic agent against excessive bone loss.

An insight into the Mechanisms of Actions of Selected Bioactive Compounds against Epigenetic Targets of Prostate Cancer: Implications on histones modifications

Muhammad A[1], Sanusi Katsayal B[1], Ebiegberi Forcados G[2], Pharmacy Yusuf A[4], Aisha Lawal Y[1], Aisha Jibril S[1], Nuraddeen H[1], Mubarak Ibrahim M[1], Zubairu Sadiq I[1], Bello Abubakar M[3], Malami I[6], Babangida Abubakar I[5]

[1]Ahmadu Bello University, [2]Biochemistry Division, National Veterinary Research Institute, Vom. Nigeria, [3]Department of Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria, [4]Department of Biochemistry, School of Life Sciences, Federal University of Technology, Minna, Niger State, Nigeria, [5]Department of Biochemistry, Kebbi State University of Science and Technology Aliero, Nigeria , [6]Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria

Prostate cancer is a leading cause of morbidity and mortality among men globally. In this study, we employed an in silico approach to predict the possible mechanisms of action of selected novel compounds reported against prostate cancer epigenetic targets and their derivatives, exhausting through ADMET profiling, drug-likeness, and molecular docking analyses. The selected compounds: sulforaphane, silibinin, 3, 3`-diindolylmethane (DIM), and genistein largely conformed to ADMET and drug-likeness rules including Lipinski's. Docking studies revealed strong binding energy of sulforaphane with HDAC6 (− 4.2 kcal/ mol), DIM versus HDAC2 (−5.2 kcal/mol), genistein versus HDAC6 (− 4.1 kcal/mol), and silibinin against HDAC1 (− 7.0 kcal/mol) coupled with improved binding affinities and biochemical stabilities after derivatization. Findings from this study may provide insight into the potential epigenetic reprogramming mechanisms of these compounds against prostate cancer and could pave the way toward more success in prostate cancer phytotherapy.

DETOXIFICATION IN ISOVALERIC ACIDEMIA: A NEW PERSPECTIVE

van der Sluis R[1]
[1]North-West University

Background
Isovaleric acidemia (IVA), due to isovaleryl-CoA dehydrogenase (IVD) deficiency, results in the accumulation of isovaleryl-CoA, isovaleric acid and secondary metabolites. The increase in these metabolites decreases mitochondrial energy production and increases oxidative stress. This contributes to the neuropathological features of IVA. A general assumption in the literature exists that glycine N-acyltransferase (GLYAT) plays a role in alleviating the symptoms experienced by IVA patients through the formation of isovalerylglycine. GLYAT forms part of the phase II glycine conjugation pathway in the liver and detoxifies excess acyl-CoA's namely benzoyl-CoA. However, very few studies support GLYAT as the enzyme that conjugates isovaleryl-CoA to glycine.
Methods
In this study we evaluated the ability of GLYAT and GLYATL1, a paralogue of GLYAT, to form isovalerylglycine using both in silico modelling and in vitro enzyme vallidation.
Results
The relative enzyme activity studies showed that both GLYAT and GLYATL1 can form isovalerylglycine. Furthermore, contrary to established treatment methodologies, increasing the glycine concentration in vitro did not result in an increase in the formation of isovalerylglycine.
Conclusions
This study supports the recommendation that primary substrates of GLYAT (e.g. preservatives such as benzoate) and GLYATL1 be limited through dietary changes in IVA patients to promote the formation of isovalerylglycine. The complex interplay between genetic variations, metabolite concentrations, dietary supplementation, and the kinetics of GLYAT and GLYATL1 likely account for the observed interindividual variation in the amount of isovalerylglycine excreted by IVA patients.

MOLECULAR DOCKING AND STRUCTURE-ACTIVITY RELATIONSHIP ANALYSIS OF TARGET COMPOUNDS AGAINST GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE IN AZITHROMYCIN-RESISTANT NEISSERIA GONORRHOEAE

Yakobi S[1], Zuma L[1], Pooe O[1]
[1]University Of Kwazulu Natal

Background
The emergence of drug-resistant strains of Neisseria gonorrhoeae poses a significant global health challenge, necessitating the development of novel antimicrobial agents. This study focuses on exploring the potential of phenolic compounds to target glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a crucial enzyme involved in glycolysis and implicated in various pathological mechanisms in N. gonorrhoeae.

Methods
Among the phenolic compounds evaluated, quercetin was selected for its significant binding affinity to N. gonorrhoeae-derived GAPDH. Structural integrity assessments were conducted using Procheck software, and molecular docking simulations were performed to confirm the binding capacity of quercetin. Molecular dynamics simulations were employed to further explore the stability and flexibility of the quercetin-N. gonorrhoeae GAPDH complex.

Results
The results revealed that quercetin exhibited substantial binding affinity to N. gonorrhoeae-derived GAPDH. Structural integrity assessments and molecular docking simulations confirmed the binding capacity of quercetin to the target enzyme. Molecular dynamics simulations provided insights into the stability and flexibility of the quercetin-N. gonorrhoeae GAPDH complex, highlighting specific interactions between quercetin and amino acid residues crucial for antimicrobial action.

Conclusions
The findings of this study provide valuable insights into the potential of quercetin-based therapeutics targeting drug-resistant N. gonorrhoeae. The significant binding affinity of quercetin to N. gonorrhoeae-derived GAPDH, along with insights into the interactions between quercetin and specific amino acid residues, suggest its potential as a promising antimicrobial agent. These findings contribute to addressing the urgent need for novel antimicrobial strategies against drug-resistant N. gonorrhoeae strains.

A liquid chromatography-electrospray tandem mass spectrometry method to investigate the metabolites and co-factors of the methionine-homocysteine cycle in women using combined oral contraceptives and non-users

Jacobs T[1], van Breda S[1], Venter G[1], Erasmus E[1]
[1]Focus Area for Human Metabolomics, North-west University

The methionine-homocysteine cycle is a multi-step process involving analytes such as betaine, S-adenosyl-methionine, and 5-methyl-tetrahydrofolic acid. These analytes act as methyl-group donors during the methylation of catechol estrogens with the assistance of catechol-O-methyltransferase (COMT) to limit the production of carcinogenic products during biotransformation. Researchers have demonstrated that women with polymorphisms in the COMT-pathway, which affect the methylation rate negatively, are more prone to develop breast cancer. Furthermore, our research has shown that there is an increased formation of catechol estrogens which are not effectively converted into methoxy-estrogens in combined oral contraceptives (COC) users. Whether this is due to the inhibition of the COMT enzyme or to limited co-factor availability, is not known. Therefore, the aim of this study was to develop a serum-based liquid chromatography-electrospray tandem mass spectrometry (LC-ESI-MS/MS) method to quantify the metabolites and co-factors of the methionine-homocysteine cycle, to investigate the effect of COC's on this pathway.

We developed an in-house method capable of simultaneously detecting and quantifying 18 metabolites and co-factors of the homocysteine-methionine cycle in serum. Protein precipitation and dilution was done as sample preparation after which the samples were analysed using LC-ESI-MS/MS. Due to the analytes of interest having different concentrations, some analytes which were investigated at lower concentrations resulted in less-than-optimal precision and accuracy during partial method validation. We applied the method to serum samples of the eBOSS (estrogen biotransformation and oxidative stress status) study, including both COC-users and non-users, to compare the levels of the analytes of interest. The results showed significant difference in betaine, choline, dimethyl glycine, total cysteine, and related ratios between users and non-users. This suggests COC use may alter the methionine-homocysteine cycle by reducing the amount of available methyl group donors by increasing the COMT-enzyme activity due to synthetic hormone use.

Development and validation of an in-house hydrophilic interaction liquid chromatography- electrospray ionization- tandem mass spectrometry method to quantify N1,N12-diacetylspermine levels in urine samples.

Oosthuizen N[1], Venter G[1], Erasmus E[1]
[1]Focus Area - Human Metabolomics, Biochemistry Department, North-West University

Over the last decades a significant increase in breast cancer (BC) incidences has been observed worldwide and it is also the most commonly diagnosed cancer amongst women. A lower survival rate for patients has been observed in low-income countries when compared to high-income countries, partly due to early detection being less successful in low-income countries. Therefore, there is a high demand for the availability of more biomarkers that can detect BC in patients, even at the earliest stages of development. Increasing evidence suggests that oestrogen-DNA adducts (EDAs) can potentially be utilized as BC biomarkers. There is, however, still controversy about their use as biomarkers and at which stage of BC and at which concentration the EDAs would be able to indicate potential cancer development. N1,N12-Diacetylspermine (DiAcSpm) has however been reported as a biomarker for BC in various studies and is reportedly more sensitive than previously recognized biomarkers such as serum carcinoembryonic antigen (CEA). DiAcSpm levels can thus be compared with EDA levels to determine the efficacy of EDAs as potential biomarkers.

In this study we developed an in-house hydrophilic interaction liquid chromatography-electrospray ionization-tandem mass spectrometry (HILIC LC-ESI-MS/MS) method to quantify and compare DiAcSpm levels between different breast cancer stages, between patients and controls, and to determine any correlation between the urinary DiAcSpm levels and measured urinary EDA levels of breast cancer patients. The method was validated by investigating the following parameters: linearity, matrix effect, selectivity, intra- and inter-day accuracy and precision, repeatability, carry-over, and stability. A calibration curve was constructed using the relative response of 10 DiAcSpm concentrations and yielded a correlation coefficient of 0,9997. The precision and accuracy of the method was tested at 7 different concentrations ranging from 7 ng/mL to 2000 ng/mL and resulted in a relative standard deviation of less than 15% for precision and the percentage accuracy was calculated to be close to 100% except for the lower limit of quantification (LLOQ) and low quality control sample (LQC), which had values of 130% (for intra- and inter-day) and 120% (inter-day) respectively.

Prevalence and genetic diversity of selected enteric pathogens in a peri-urban and rural daycare center from Vhembe District; South Africa.

Munzhedzi L[1], Kabue J, Traore A, Potgieter N
[1]University Of Venda, [2]University of Venda, [3]University of Venda, [4]University of Venda

Child daycare centers (DCC's) host a variety of pathogenic enteric microorganisms which are the most common cause of diarrhoea worldwide leading to a high mortality rate. A cross-sectional study was carried out in Daycare centers in rural South Africa with a total of 84 samples including fomites (n=29), handwash (n=33) and clinical samples (22). E. coli, salmonella and shigella were determined using standard culture methods, multiplex PCR and sequencing. Human norovirus, sapovirus and rotavirus strains were detected using multiplex Real time RT-PCR, amplified by conventional R-T PCR and Sanger sequencing. The prevalence of bacteria: Salmonella 0% (0/166), Shigella 0% (0/166) E. coli 38% (63/166). The predominant E. coli pathotype was EAEC, which was successfully sequenced (12 from rural daycare center and 7 from peri-urban daycare center) and shared a common ancestor to those circulating globally. The prevalence of Viruses: RoT 13% (22/166), NoV was 7% (12/166; G1 (2/166) and G2 (10/166)), and SoV was 3% (5/166). Genotyping of RoT showed all positive were rotavirus A and NoV revealed norovirus GII.1. No statistical significance was observed between the two daycare centers (p=0.02). The high prevalence of E. coli pathotypes, NoV (G1 and G2), RoT A, and SoV circulating in the daycares is of high concern as these pathogens are associated with high deaths in children under the age of 5 years in these settings. Also, the phylogenetic analysis in both daycare centres indicated that the circulating strains in this study were closely related, showing a possible transmission route in the daycare centres, and shared common ancestor with those circulating globally, which were reported in outbreaks in the daycare environment as well as cases of diarrhoea globally. More stringent hygiene practices should be implemented by the DCC principals for their workers to ensure a reduction or eradication of any possible transmission of these pathogens from the workers to the children and the environment. The data from this study will aid in vaccine development of the circulating strains in the region and will add to existing data in daycare centers as the data is limited especially in Africa.

Using insilico approaches to identify allosteric inhibitors targeting Plasmodium falciparum prolyl-transferRNA synthetase for antimalarial drug development

Ramahala R[1], Olotu F[1], Tastan Bishop O[1]
[1]Rhodes University (Research Unit In Bioinformatics)

Background
Malaria, an infectious disease caused by Plasmodium falciparum (Pf), is a serious global health concern causing high mortality and morbidity rate. Eradication of malaria is hindered by the emergence of drug resistance in Plasmodium parasites and non-selective toxicity of potential hit candidates. This necessitates the development of novel selective antimalarial drugs that target housekeeping enzymes and arrest multiple stages of the parasitic life-cycle. Prolyl-tRNA synthetase (ProRS), a vital component of the protein translation machinery, accurately ligates L-proline to its cognate tRNA. This housekeeping enzyme is a chemical-genetically validated antimalarial drug target because it catalyses aminoacylation throughout the Pf life cycle and ensures translation fidelity.

Methods
In this study, we employed structural bioinformatics methodologies to predict binding sites outside the PfProRS' active sites and identify new selective inhibitors targeting these sites. To achieve this, the allosteric sites were predicted using SiteMap, DoGSiteScorer, FTMap and PyVOL. Subsequently, an active PfProRS inhibitor provided by MMV (Medicines for Malaria Venture) was optimised by searching chemical databases for 70% Tanimoto similar analogs. To identify potential hits, analogs were docked to PfProRS and the human homolog using AutoDock VINA. The stability of the identified hits and ligand-protein complexes was elucidated using Molecular dynamics (MD) simulations.

Results
Firstly, two allosteric sites were predicted: one near active site and one at the dimer interface. Furthermore, the two predicted allosteric pockets were highly conserved across the Plasmodium species, excluding the human. Secondly, 23 benzimidazole-derivatives were identified as promising hit candidates having strong binding affinity to the two allosteric sites on PfProRS.

Conclusion
In conclusion, this study presents an opportunity for scientists to develop efficacious antimalarial drugs with high selectivity and utilise these 23 hits as a starting point to combat drug resistance. Further validation techniques like hit-to-lead optimisation can be employed to validate these 23 promising hits.

Bioassay-guided evaluation of potential antimalarial activities from Ximenia caffra, Ziziphus mucronata and Ricinus communis.

Masisa S[1], Mhlongo N[2], Kappo A[1], Simelane M[1]
[1]University of Johannesburg, [2]University of KwaZulu-Natal

Background: The increasing resistance of Plasmodium parasites to currently available antimalarial therapy poses a great challenge in the treatment of malaria. Plants have been used as a primary source of novel pharmacologically active compounds to make drugs since ancient times, and more than 80% of the rural population relies on traditional medicine for their primary healthcare needs. Aim: To explore the in-vitro antimalarial potentials of Ximenia caffra, Ziziphus mucronata, and Ricinus communis by extracting, isolating, and characterizing bioactive compounds. Methods: Traditionally used medicinal plants, Ximenia caffra, Ziziphus mucronata, and Ricinus communis, were collected and sequentially extracted using several solvents. Plant crude extracts were screened for asexual antiplasmodial activity. Extracts with promising antimalarial activity were considered for isolation with column chromatography. Results: Crude dichloromethane and ethyl acetate Z. mucronata extracts exhibited high antimalarial activity with IC50 values of 5.494 µM and 7.227 µM, respectively. Pure compounds isolated from these extracts were later, with spectroscopic techniques characterized to be betulinic acid, methyl betulinate, and lupeol. Conclusion: The antimalarial activity of the crude extracts and the potential of the isolated compounds confirm that these medicinal plants could serve as effective agents for treating malaria.

Molecular mechanisms of atazanavir and Ritoataz tablets against human cervical cancer cells

Makgoo L[1], Mosebi S[2], Mbita Z[1]
[1]University Of Limpopo, Department of Biochemistry, Microbiology and Biotechnology, Private Bag X1106, Sovenga, 0727, [2]University of South Africa, Department of Life and Consumer Sciences, Private Bag X06, Florida 1710

Background: Cervical cancer is a Human Papilloma virus (HPV)-related disease, which is on the rise in a number of countries including South Africa. Human Immunodeficiency Virus Protease Inhibitors (HIV-PIs) have attracted a lot of attention for anticancer drug development. However, it remains unclear whether it worth the effort to repurpose HIV-PIs for the treatment of HPV-associated cervical cancer. This study was aimed at determining the anticancer effects of atazanavir HIV protease inhibitor and Ritoataz tablets on CaSki cervical cancer cells.
Methods: MTT viability assay was used to evaluate the effect of atazanavir and Ritoataz tablets on the viability of CaSki cervical cancer cell line and non-cancerous cells (HEK-293). Further confirmation of the MTT assay was performed by analysing the effect of atazanavir and Ritoataz tablets IC50s on CaSki and non-cancerous cells (HEK-293) using the Muse™ Count & Viability assay. To confirm mode of death induced by atazanavir and Ritoataz tablets in HPV-associated cervical cancer cells, apoptosis was performed using Annexin V Assay. In addition, Muse™ Cell Cycle assay was used to check whether atazanavir and Ritoataz tablets promote or halt cell cycle progression in cervical cancer cells.
Results: Atazanavir and Ritoataz tablets did not affect the viability of non-cancerous cells (HEK-293) but it decreased the viability of the CaSki cervical cancer cells in a dose-dependent manner. Atazanavir and Ritoataz tablets induced apoptosis in HPV related cervical cancer cells. Furthermore, it also induced cell cycle arrest thus halting cell cycle progression.
Conclusion: The use of HIV drugs as potential cancer therapeutics can be a promising strategy because based on this study, atazanavir and Ritoataz tablets have shown anticancer properties notably in HPV related cervical cancer cells.

HPLC-MS/MS and spectrophotometric analysis of oxidative stress markers in Black South African women with Luminal B breast cancer

du Plessis C, Venter G[1], Erasmus E[1]
[1]North-west University

Lifestyle, diet, oral contraceptive use, menopausal status, and body composition are known modifiable risk factors for breast cancer as they influence metabolic processes that lead to reactive oxygen species (ROS) production and eventually oxidative stress. However, the impact of oxidative stress on breast cancer in Black South African women is poorly understood. Hence, we hypothesized that understanding the relationship between oxidative stress status and breast cancer in Black South African (BSA) women could lead to improved screening and prevention strategies in low and middle-income countries. This study aims to investigate the role of oxidative stress in the etiology of breast cancer in Black South African women by evaluating the differences in oxidative stress status between Black South African women with Luminal B breast cancer and matched controls. The study involves measuring oxidative stress markers in serum and urine samples from a combined ± 800 women with Luminal B breast cancer and matched controls. Two spectrophotometric assays – the Derivatives-Reactive Oxygen Metabolites (d-ROMs) assay and the Ferric Reducing Ability of Plasma (FRAP) assay – were used. Results indicated no statistically significant difference in serum antioxidant capacity (p-value = 0.6) or serum peroxide levels (p-value = 0.4) between cases and controls. However, while direct comparison with oxidative stress between cases and controls showed no difference, comparison with cofactors like HIV status, menopausal status, and oral contraceptive use, did have a statistical difference. Therefore, further analysis using High Pressure Liquid Chromatography tandem Mass Spectrometry (HPLC-MS/MS) in this study will be used to evaluate F2-isoprostanes in urine samples to assess lipid peroxidation in vivo as another series of oxidative stress markers. This further investigation with liquid chromatography could clarify the differences in oxidative stress status between the two groups.

Role of AtNOGC1 “an Arabidopsis thaliana nitric oxide binding protein with guanylyl cyclase activity” in regulating nutrient starvation.

Gavhi P[1], Iwuoha E, Koo A, Mulaudzi T
[1]University Of The Western Cape

AtNOGC1 is an Arabidopsis thaliana protein, with a guanylyl cyclase (GC’s) activity and senses nitric oxide (NO) via its heme nitric oxide (HNOX) binding motif. Upon activation by NO in the presence of Reactive Oxygen Species (ROS) and abscisic acid (ABA), AtNOGC1 produces high levels of cyclic guanosine monophosphate (cGMP). Cyclic GMP further activates protein kinase, ion gated channels and phosphodiesterase’s, and mediate plants stress responses. In silico data indicated a high AtNOGC1 transcript under nitrogen starvation, suggesting a possible role in nitrogen fixation. Nitrogen is a major limiting factor in agriculture, hence its overuse as fertilizers. Moreover, nitrogen use efficiency of crops is low (33%), suggesting that more than 60% of the fertilizers get lost into the environment, resulting in soil toxicity. This study reports on the effects of nitrogen starvation in Arabidopsis and the possible role of AtNOGC1 to confer tolerance. Sterilised wild type A. thaliana col 0, seeds were sown in media supplemented with or without 2 mM KNO3 in addition to 100 µM sodium nitroprusside (NO) and 30 µM ABA. The seeds were germinated for 25 days under 16/8 hrs light/dark at 23 ℃. Results showed that nitrogen starvation affected growth, as observed by reduced plant biomass (300%), root (170%) and shoot (300%) lengths. There was also a reduction in chlorophyll a (87%), chlorophyll b (46%) and total chlorophyll (69%) under nitrogen starvation. Interestingly, treatment with NO, ABA and a combination (NO+ABA) improved growth and chlorophyll content in Arabidopsis seedlings under nitrogen starvation. Furthermore, nitrogen starvation induced oxidative damage, due to over-production of ROS [H2O2 (208%)] and malondialdehyde (207%), which correlated with increased proline (95%) and total soluble sugars (TSS; 300%). However, treatment with of NO and ABA, prevented oxidative damage and reduced TSS (~50%), thus showing a high degree of osmotic adjustment. Moreover, Real Time PCR, showed that AtNOGC1 transcript was expressed at high levels under nitrogen starvation, but treated with NO and ABA led to a gradual decrease in AtNOGC1 transcript. These findings suggest that AtNOGC1 conferred nitrogen stress tolerance through its interaction with NO and ABA.

Extracellular vesicles discharged by brain cells contribute to revealing insights into the elevated prevalence of dementia in individuals with schizophrenia

Fernández-Rhodes M[1,2], Gallart-Palau X[2], Serra A[1]
[1]Dept. Medical Basic Sciences - University of Lleida - +Pec Proteomics - IRBLLEIDA, [2]IRBLLEIDA - +Pec Proteomics - Neuroscience Area

Background
Schizophrenia (SCHZ) is commonly accompanied by a range of co-occurring conditions, including an increased likelihood of age-related dementia. On the other hand, extracellular vesicles (EVs) play a vital role in facilitating molecular communication between different cells and systems in the human body. Although EVs have been linked to brain-related disorders, neurodegeneration, and dementia, the influence of brain EVs on the development and progression of dementia in individuals with SCHZ remains unclear.

Methods
In this study, we employed state-of-the-art, discovery-driven proteomics techniques to examine the proteome compositions of brain EVs (bEVs) in post-mortem brain tissue samples, with a particular focus on the prefrontal cortex (BA9). Our cohort of study participants included individuals with schizophrenia (SCHZ) and those with progressive Alzheimer's disease (AD) (n=30).

Results and Discussion
Brain extracellular vesicles (bEVs) in both diseases exhibit consistent regulatory patterns in proteins that are associated with critical neurodegenerative processes related to aging. Some of these proteins, such as Glial Fibrillary Acidic Protein (GFAP), Immunoglobulins, Myelin Basic Protein (MBP), and Microtubule Protein TAU, consistently show strikingly similar regulatory patterns in bEVs in both schizophrenia (SCHZ) and Alzheimer's disease (AD). These findings highlight the potential shared mechanisms underlying neurodegeneration and suggest that there might be a convergence of pathways within brain EVs that contribute to the progression of dementia in individuals with SCHZ.

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Exploring the apoptosis-inducing potential of Cannabis sativa and cannabidiol (CBD) in pancreatic cancer in vitro

Tshabalala D[1], Sebutsoe X[1], Motadi L[1]
[1]Department Of Biochemistry, Faculty Of Science, University Of Johannesburg

Background: Pancreatic cancer remains one of the deadliest and most aggressive types of cancer. This is due to the limited treatment options and poor prognosis. Recently, there has been a surge of interest in exploring the therapeutic effects of Cannabis sativa, notably its derivative cannabidiol (CBD). CBD's anti-cancer, pro-apoptotic, and anti-inflammatory properties could potentially help with the aggressive nature of the disease. Additionally, studies have highlighted that CBD can alleviate pain caused by both the disease and the conventional treatment. It has also been shown to manage chemotherapy-induced symptoms and enhance the effectiveness of the current chemotherapeutic drugs underscoring its potential as an adjunctive therapy. Methods: To address the limited treatment options of the diseases, this study aimed to explore the apoptosis-inducing potential of crude Cannabis sativa and CBD in pancreatic cancer cells. This entailed treating the pancreatic cancer cells with crude Cannabis sativa and CBD to assess their viability using AlarmaBlue assay, XCELLigence, and adenosine triphosphate detection assay. Apoptosis induction was analysed by checking the morphology of the cells under the microscope, using Annexin V/ Propidium iodide staining followed by flow cytometric analysis for the precise quantification of apoptotic cells. Moreover, apoptosis was investigated by checking the intensity of deoxyribonucleic acid (DNA) fragmentation using agarose gel electrophoresis and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay. Lastly, the expression of apoptosis-related genes was evaluated using quantitative polymerase chain reaction (qPCR) and western blotting. Results: This study revealed a significant reduction in cell viability following treatment with crude Cannabis sativa and CBD. This was followed by a robust induction of apoptosis highlighted by cell shrinkage, DNA fragmentation, and the upregulation of pro-apoptotic genes. Conclusion: these findings shed light on the therapeutic potential of Cannabis sativa and CBD, offering new avenues for future research and clinical interventions.

Investigating the apoptotic effects of green synthesized silver and gold nanoparticles conjugated to neutralizing antibodies.

Tsotetsi N[1], Choene M[1], Motadi R[1]
[1]University Of Johannesburg

Background
Cancer is a global burden, exerting a significant strain on population and health systems. Cancer accounted for an estimated 18.1 million cases and 9.9 million deaths in 2020. Cervical cancer is the second most diagnosed malignancy, and the leading cause of cancer-related death in South African women. Although cervical cancer can be successfully treated if diagnosed early, associated side effects of current therapies necessitate new therapeutic approaches including the discovery of new drugs with little to no side effects. It is believed that nanomaterials have properties that can serve as an alternative approach to combating cancers and many other diseases. Silver and gold nanoparticles which are commonly used in optic imaging of tumours and to treat bacterial infection recent research has suggested they also possess anti-cancer properties however the mechanism they use to exert anticancer properties are not known. Therefore, this study will investigate the apoptotic potential of biologically synthesized NP in HeLa cells.
Methods
Metallic silver and gold nanoparticles were synthesized biologically using Cannabis sativa plant extract. To characterize them Uv-vis spectrum was used to measure the wavelength of the synthesized CBD-Ag and CBD-Au NP. To determine the functional groups responsible for reduction and capping of the nanoparticles, FT-IR spectrum was used. To determine the crystallographic nature of the synthesized NP X-ray diffraction was used. The particles were imaged using HR-TEM and Zeta potential was used to assess the surface charges. To assess cell viability Hela cells were treated with in serial dilutions ranging from (0 - 200µg/ml) for 24hr. The cell viability was assessed using alamar blue assay from which the inhibitory concentrations (IC50) of 24.17 µg/ml and 71.21µg/ml were obtained and will be used in all subsequent assays.

Key words: Cancer, nanoparticles, cannabis sativa

Evaluation of Leptospermum petersonii methanol and ethyl acetate crude extracts for anti-metastatic and apoptotic effects against pancreatic and prostate cancer cells

Matlou M[1], Sebutsoe X[1], Motadi L[1], Choene M[1]
[1]Department of Biochemistry, faculty of Science, University of Johannesburg, South Africa

Background: The pinnacle in cancer-related mortality is metastasis (approximately 90%), which is the migration of cancer cells from the primary tumour to nearby tissues and distant organs. Due to drawbacks of conventional cancer therapy, interest in medicinal plants for their potential as therapeutic agents has increased in the academic and pharmaceutical worlds. The aim of the project is to investigate natural sources (Leptospermum petersonii) for active compounds that could inhibit metastasis of cancer cells and activate apoptotic activity against metastatic pancreatic and prostate cancer cells. Methods: The research utilized different techniques (alamarblue assay, xcelligence system, ADP assay, wound healing assay, migration assay, invasion assay, caspase assay, fluorescence microscopy and RT-PCR) in the analysis and determination of anti-metastatic activity and stimulation of apoptotic genes against pancreatic (cell line MIA-PACA2) and prostate cancer (cell line PC3). AlamarBlue assay was used to test for cellular viability of normal and cancerous cell lines upon treatment with the crude extracts. Induction of apoptosis was determined by analyzing the morphology of cells using microscopy and by analysis of the activation of caspase 3/7 using caspase assay. Analysis of cancer metastasis was conducted using migration, invasion and wound healing assay of which RT-PCR was used to evaluate anti-metastatic genes. Results: The study illustrated that L. petersonii significantly reduced cellular viability of MIA PACA2 and PC3. Caspase assay confirmed activation of apoptosis due to upregulation of caspase 3/7. Upon further testing, the study found substantial evidence of upregulation and activation of anti-metastatic genes. Conclusion: In conclusion, we anticipate the development of the next generation of customized cancer therapies that target cancer cell migration for greater efficacy of treatment in which these therapies should benefit patients' chances of survival at any stage of the disease by limiting key driving features of metastasis.

Biochemical Properties of an Extracellular Thermo-acidophilic β-D-glucosidase from Fusarium oxysporum CPOA-2 for Application in Biomass Valorization

Ayodeji A[1,2], Bamidele O[2], Kolawole A[2], Olajuyigbe F[2], Ajele J[2]
[1]Enzymology and Molecular Biotechnology Laboratory, Department of Biochemistry, Joseph Ayo Babalola University, [2]Enzymology and Microbial Biotechnology Laboratory, Department of Biochemistry, Federal University of Technology

Background
The saccharification of cellulosic biomasses requires the activity of rate-limiting enzyme, β-D-glucosidase amongst other hydrolytic enzymes. Here, we describe some biochemical properties of a purified fungal β-glucosidase and its usability in the production of fermentable sugars from agro-waste cassava peel biomass.
Methods
Microorganisms identified by molecular gene sequence analysis were screened for cellulolytic activity. Optimally produced β-glucosidase was subjected to ammonium sulphate precipitation prior to apparent homogeneity purification on DEAE-Sephadex A-50 anion-exchanger and Sephadex G-100 gel filtration columns. Biochemical properties and inhibition of the purified enzyme were studied.
Results
The Fusarium oxysporum CPOA-2 β-D-glucosidase (foxBGL) was optimally produced at pH 5.5 and 37 °C on the 6th day of submerged fermentation. The homogenously purified β-D-glucosidase has a subunit molecular mass of 86.5 kDa determined by SDS-PAGE and native molecular weight of 136.2 kDa as determined by gel filtration. The purified foxBGL was acidic-thermostable, remarkably retaining over 50% of initial activity after 120 min of incubation at 70 °C. Activity of foxBGL was stimulated by Na+, K+, Cu2+, Mn2+, Mg2+, Co2+ but inhibited by Pb2+, Al3+, EDTA and SDS. This foxBGL was very active on pNPG and cellobiose; with moderate or low activity on salicin, maltose, methylcellulose, α-cellulose, carboxymethyl cellulose, Avicel® and trehalose. The Km and Vmax were 0.72 mM and 2.17 mM/min, respectively for pNPG and 0.85 mM and 1.25 mM/min for cellobiose. Glucose competitively inhibited foxBGL with Ki value of 7.4 mM.
Conclusion
This purified β-D-glucosidase possesses remarkable and desirable properties suitable for application in biomass valorization and production of fermentable sugars.

Coping with the ESKAPE pathogens: Evolving strategies, challenges and future prospects

Aloke C[1,2], Achilonu I[1]
[1]Protein Structure-Function and Research Unit, School of Molecular and Cell Biology, Faculty of Science, University of the Witwatersrand, Braamfontein, Johannesburg, 2050, [2]Department of Medical Biochemistry, Alex Ekwueme Federal University Ndufu-Alike

Background
Globally, the ESKAPE pathogens (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species) are the major cause of nosocomial infections. These pathogens are multidrug resistant, and their negative impacts have brought serious health challenges and economic burden on many countries worldwide.
Methods
We conducted a comprehensive scientific literature search employing major search engines including Scopus, Pubmed/Medline, Google Scholar, etc. The search involved medical subject captions including ESKAPE pathogens, antimicrobial peptides, bacteriophage, photodynamic therapy, antibiotics, etc. Thus, this narrative review exploits different emerging alternative therapeutic strategies including combination antibiotics, antimicrobial peptides ((AMPs), bacteriophage and photodynamic therapies used in the treatment of the ESKAPE pathogens, their merits, limitations, and future prospects.
Results
Our findings indicate that ESKAPE pathogens exhibit resistance to drug using different mechanisms including drug inactivation by irreversible enzyme cleavage, drug-binding site alteration, diminution in permeability of drug or drug efflux increment to reduce accumulation of drug as well as biofilms production. However, the scientific community has shown significant interest in using these novel strategies with numerous benefits although they have some limitations including but not limited to instability and toxicity of the therapeutic agents, or the host developing immune response against the therapeutic agents.
Conclusions
Thus, comprehension of resistance mechanisms of these pathogens is necessary to further develop or modify these approaches in order to overcome these health challenges including the barriers of bacterial resistance.

Cannabidiol and Cannabis Sativa as a potential treatment in vitro prostate cancer cells silenced with RBBp6 and PC3 xenograft

Jantjies Z[1], Moleya B[1], Sebutsoe X[1], Motadi L[1]
[1]University Of Johannesburg

Prostate cancer is the second most frequently occurring carcinoma in males worldwide and one of the leading causes of death in men around the world. Recent studies estimate that over 1.4 million males are diagnosed with prostate cancer on an annual basis, with approximately 375,000 succumbing to the disease annually. With current treatments continuing to show severe side effects, there is a need for new treatments. In this study, we looked at the effect of cannabis sativa extract, cannabidiol, and cisplatin on prostate cancer cells, PC3. In addressing the above questions, we employed the MTT assay to measure the antiproliferative effect on PC3 cells following treatment with varying concentrations of Cannabis sativa extract, cisplatin, and cannabidiol. xCELLigence was also used to confirm the IC50 activity in which cells were grown in a 16-well plate coated with gold and monitor cell attachment. Caspase 3/7 activity was also measured using 96 well plates following treatment. Western blot and qRT-PCR were also used to measure the gene expression of tumor suppressor genes, p53, Bax, and Bcl2. Animal studies were employed to measure the growth of PC3-mouse-derived cancer to evaluate the effect of compounds in vivo. From the treatment with varying concentrations of Cannabis sativa extract, cannabidiol, and cisplatin, we have observed that the three compounds induced antiproliferation of PC3 cancer cell lines through the activation of caspase 3/7 activity. We also observed induction of apoptosis in these cells following the silencing of retinoblastoma binding protein 6 (RBBP6), with upregulation of p53 and bax mRNA expression, and a reduction in Bcl2 gene expression. The growth of tumors in the mouse models was reduced following treatment with cisplatin and cannabidiol. We demonstrated that cannabidiol is a viable therapy to treat prostate cancer cells, in combination with silencing of RBBP6. This suggests that cannabidiol rather than Cannabis sativa extract may play an important role in reducing cancer progression.

Investigating the expression patterns of the novel TSPO splice variant, PBR-s , in cervical cancer cells

Maenetja K[1], Mbita Z, Laka K
[1]Department Of Biochemistry, Microbiology, And Biotechnology, University Of Limpopo.
Investigating the expression patterns of the novel TSPO splice variant, PBR-s , in cervical cancer cells

K. P Maenetja, Z. Mbita and K. Laka*

Department of Biochemistry, Microbiology, and Biotechnology, University of Limpopo, Private Bag X1106, Sovenga 0727, Polokwane, South Africa.
*Correspondence: Email: Zukile.Mbita@ul.ac.za

Cervical cancer remains one of the major contributors of cancer-related fatalities in women globally, despite advancements in diagnostic tools and treatment modalities. The incidence of cervical cancer continues to rise, underscoring the urgency to identify new biomarkers and therapeutic targets. One promising biomarker is the TSPO (Translocator protein) gene, involved in cancer development and progression, exhibiting altered expression and alternative splicing across various cancers. This study focused at investigating the expression pattern of the novel TSPO spliced variant PBR-s (Peripheral-type benzodiazepine receptor) in cervical cancer, CaSki cell line, under the influence of various anticancer agents. The impact of various anticancer agents Cobalt chloride (CoCl2), Curcumin, Arsenic trioxide (A2O3), and Sodium butyrate (NaBu) on the survival of CaSki cells was evaluated through the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay and count and viability assay. Cell death in CaSki cells was further evaluated using the Muse® Annexin V and Dead Cell Assay, along with the Muse® MitoPotential Assay. Additionally, the expression of the PBR-s gene was assessed using conventional polymerase chain reaction The results showed a notable decrease in the viability of CaSki cells, along with increased late apoptotic cells and disruption of mitochondrial membrane potential under the effect of Curcumin, A2O3, and NaBu with minimal effect observed on cells subjected to CoCl2. Additionally, the expression of PBR-s varied under the influence of different anticancer agents, showing both increasing and decreasing activity. The results suggested that reduced expression levels of the PBR-s variant may promote apoptosis induction, while heightened expression may have antiapoptotic effects in CaSki cells.
Keywords : Cervical cancer, Apoptosis, PBR-s, and anticancer agents.

Functional comparison of in vitro models for RYR1 and STAC3 gene knockdown.

Vorster A[1], Schoonen M[1], Smuts I[2], Van der Westhuizen F[1]
[1]1 Focus Area for Human Metabolomics, Mitochondria Research Group, North-West University,
[2]Department of Paediatrics and Child Health, Steve Biko Academic Hospital, University of Pretoria

Background

RyR1 (Ryanodine Receptor 1) and STAC3 (SH3 and cysteine-rich domain 3) are pivotal components of the excitation-contraction coupling (ECC) machinery in skeletal muscle cells and mutations in RyR1 and STAC3 can lead to impaired ECC and muscle weakness, emphasizing the importance of studying these proteins in the context of muscle health and disease.

In the ICGNMD (International Centre for Genomic Medicine in Neuromuscular Diseases) study, 58 congenital myopathy patients were recruited and assessed at Steve Biko Academic Hospital in Pretoria, South Africa. 28 patients were clinically diagnosed with King Denborough syndrome (KDS), which is usually associated with RYR1 mutations. However, 25 patients had a pathogenic STAC3 mutation (c.851G>C, p.Trp284Ser), and three had a compound heterozygous variant, including a novel STAC3 deletion (c.834_836del). This suggests that STAC3 mutations in our population contribute to a KDS phenotype. The disease mechanism of the interaction between STAC3 and RYR1 is still unclear and was investigated in this study using an in vitro model.

Methods

A C2C12 differentiated cell line was used and STAC3 and RYR1 protein expression was knocked down using siRNA. Expression levels were measured using RT-PCR and western blot. The knockdown cell lines were then analyzed using seahorse flux analyzer to monitor mitochondrial involvement, The cells were also subjected to staining for fluorescent microscopy. the cells were stained with mitotracker green, mitosox and Rhod-2am.

Results

Expression of these genes was reduced, and downstream functional analyses revealed a lower oxygen consumption rate, suggesting altered energy production which hinders the ECC in muscle cells – as seen in this cohort where patients present with extremely low muscle tone and severe muscle weakness. During the fluorescent microscopy it was also noted that the affected cells had a higher calcuim concentration in the sitoplasm then the normal control cells.

Conclusion

We present initial in vitro findings that STAC3 has an association with mitochondrial function and that this model may be useful to investigate and compare the pathophysiology of STAC3 and RYR1 dysfunction.

Identification of multi stage allosteric inhibitors against Plasmodium falciparum prolyl-transferRNA synthetase for antimalarial drug development

Ramahala R[1], Olotu F[1], Tastan Bishop O[1]
[1]Rhodes University (research Unit In Bioinformatics)

Background
Malaria is a serious global health concern causing high mortality and morbidity rate annually. Eradication of malaria is partly hindered by the emergence of drug resistance in Plasmodium parasites. This necessitates the development of new antimalarial drugs with novel strategies. One way is to identify drugs that can be effective in the multiple stages of the parasitic life cycle which would slow down the resistance process. Aminoacyl tRNA synthetase (aaRS) are crucial to the survival of the parasite and play a role in each of its stages. Recent studies indicated Prolyl-tRNA synthetase (ProRS) which ligates L-proline to its cognate tRNA, as a validated antimalarial drug target, hence the main interest in this study. As the active site of the protein is highly conserved between parasite and human homologs, we aimed to identify potential allosteric modulators.

Methods
4NCX and 4HVC (PDB ids) were retrieved from Protein Data Bank for Plasmodium and human ProRS protein (PfProRS and HsProRS) respectively; the missing residues were modelled using Modeller. The allosteric sites of the dimeric protein were predicted for both organisms using SiteMap, DoGSiteScorer, FTMap and PyVOL. A PfProRS inhibitor (unpublished data) was searched against chemical databases to identify analogs with 70% Tanimoto similarity. Identified analogs were docked to PfProRS and HsProRS via AutoDock VINA. The stability of the identified ligand-protein complexes was elucidated using Molecular dynamics (MD) simulations.

Results
Two allosteric sites were predicted: one near active site and one at the dimer interface. These predicted allosteric pockets were highly conserved across the Plasmodium species, but not in HsProRS. Overall, 23 potential hits with a common scaffold were identified having strong binding affinity to either of allosteric sites on PfProRS with high selectivity against HsProRS.

Conclusion
In conclusion, this study presents 23 potential hits as a starting point to further develop as allosteric inhibitors. It further provides a well established computational drug discovery pipeline that can be applicable to other aaRSs.

Harnessing solvent dynamics: optimizing loloatin antimicrobial activity

Bezuidenhout S[1], Rautenbach M[2]
[1]Stellenbosch University, [2]Stellenbosch University
Harnessing solvent dynamics: optimizing loloatin antimicrobial activity

Sherwin Bezuidenhout and Marina Rautenbach
From BIOPEP, Department of Biochemistry, Stellenbosch University, Stellenbosch 7600, South Africa
Email: mra@sun.ac.za

The rapid rise in antimicrobial resistance, and failure of conventional antibiotics to treat bacterial infections has caused a shift towards 'green' biocides to combat these infections, and one such 'green' biocide, and frontrunner in this regard, are antimicrobial peptides. The loloatins (Lols) are a family of antimicrobial cyclic decapeptides produced by Brevibacillus laterosporus. In the current study, the effect of aromatic amino acid supplementation of the growth media on loloatin production was analysed via mass spectrometry, where it was observed that amino acid supplementation had little to no significant effect on the loloatin production profile. Additionally, ion mobility mass spectrometry (IMMS) was used to investigate the loloatin oligomerisation states in a range of organic solvent (ethanol) concentrations, where it was observed that there is a loss of loloatin dimer formation at higher concentrations of organic solvent (70%-90% ethanol) when the peptide is kept in solution for 16 hours or longer. It is hypothesized that peptide dimers are responsible for the potent antimicrobial activity of the Lols, and as such, the Lols were tested in a solid surface activity assay with a fresh (1 hour) and overnight (16 hours) incubation of peptide (250 µg/mL) in 60%- and 70% ethanol, dried onto a surface, and challenged with Staphylococcus aureus. It was observed that at 60% ethanol, there was no significant difference in activity between the fresh and overnight incubations, however, at 70% ethanol, the overnight incubation had improved activity in comparison to the fresh peptide incubation at the same ethanol concentration. All these findings suggest that at 70% ethanol and higher, the hydrophobic environment leads to the formation of higher order oligomers which are deposited onto the surface after drying, resulting in improved activity.

Key words: antimicrobial peptides, loloatins, solid surface activity, antimicrobial activity, oligomers

Investigating the in vitro anti-cancerous activity of O. sanctum and O. kilimandscharicum against malignant melanoma

Moyo B[1], Mhlongo M[1], Sitole L[1]
[1]University Of Johannesburg

Background: The increased mortality rate among cancer patients over the last decade has prompted for the development of alternative treatment options for cancer. According to the World Health Organisation, over 10 million cancer-related deaths were recorded in 2020. However, naturally derived products from medicinal plants such as Ocimum sanctum and Ocimum kilimandscharicum have shown promise against numerous respiratory diseases. The aim of this study was to explore the potential anticancer effect of Ocimum sanctum and Ocimum kilimandscharicum on skin cancer.

Methodology: A sum of 8 extracts were obtained from the two plants via sequential extraction using Hexane, dichloromethane, Ethyl acetate and Methanol in increasing polarity. The 8 extracts were treated on A375 cells to determine cell cytotoxicity using the Almar blue assay, where etoposide and 1% DMSO were used as controls. The IC50 values obtained ranged from 29- 40µg/ml. The extracts were treated against the HEK293 cell line to determine their cytotoxic effects on non-cancerous cells. Two (2) extracts were found to be toxic against the HEK 293 cell line and were excluded from future experiments.

Results: In vitro assays were employed to determine the anticancer properties induced by the medicinal crude extracts starting with light microscopy which showed evidence of cell blebbing and reduction in cell size treated at IC50. The Caspase 3/7 assay and fluorescence microscopy were used to confirm apoptotic induction by the crude extracts. At IC50 the crude extracts exhibited high caspase activity, nuclear pyknosis and karyorrhexis were observed thus confirming apoptotic activity. Flow cytometry was used to confirm the mode of cell death. The results showed more than 50% of cell population were undergoing early and late apoptosis after 24-hour treatment.

Conclusion: O. sanctum and O. kilimandscharicum both show promising results as complementary medicine and potential alternative cancer treatment, although the latter has more requirements due to the drug discovery pipeline. Future research would require bio-guided fraction for the isolation of bioactive compounds to verify the results obtained in vitro.

Keywords: Apoptosis, Anticancer, Ocimum sanctum, Ocimum kilimandscharicum, Melanoma

Pharmacokinetics of cannabidiol after intrabuccal administration in Mice

Omotayo O[1], Bhengus S[1], Venter K[1], Lemmer Y[2,3], Mason S[1]
[1]Human metabolomics, North-west University, Potchefstroom, [2]Council for Scientific and Industrial Research (CSIR) Next Generation Health, [3]Preclinical Drug Development Platform at the Faculty of Health Sciences of the North-West University

Background: In recent years, there has been an increased interest in the pharmacological and therapeutic properties of cannabidiol. The use of this compound has seen an upsurge due to its alleged benefits in managing various illnesses, including neurological conditions and their symptoms. Despite the increase in the use of this drug, the pharmacokinetic profile still needs to be studied. There is a need to identify the optimum dose of cannabidiol that can penetrate the brain for safe therapeutic use. Hence, using male and female C3HeB/FeJ (Kramnik) mice, and the intrabuccal route for the administration of CBD, this study examined the optimum dose/concentration at which cannabidiol could penetrate the brain.

Methods: The concentration of CBD in the brains of male and female C3HeB/FeJ mice was examined 6 hours following a once-off administration of cannabidiol at three different concentrations (10, 20, and 30 mg/kg) to the mice. Liquid chromatography with tandem mass spectrometry (LC-MS/MS) was used to analyze and determine the concentration of cannabidiol in the brain samples.

Results and conclusions: All administered doses yielded a quantifiable level of cannabidiol in the brain. Mice administered with 30 mg/kg had a high concentration of cannabidiol in their brain samples with a great variation, while mice administered with the lowest dose of CBD (10 mg/kg) showed steady concentrations of cannabidiol in the brain. When compared across the sexes of the mice, no significant difference ($p>0.05$) was seen in the concentration of cannabidiol recorded in the brains of the male and female mice. Hence, based on the findings of this study, sex is not a major determinant of the bioavailability of CBD in the brain, and administration of 10 mg/kg cannabidiol would result in the bioavailability of this compound in the brain with minimal variation.

Keywords: Cannabidiol; Kramnik mice; optimum dose of cannabidiol; pharmacological properties; therapeutic properties

Metabolomics and Proteomics of FFPE Samples Unveils New Avenues for Precision Medicine

Isaiah A[1], Vlok M[2], Luies L[1], Williams A[1], Loots D[1], Chegou N[2], Tutu van Furth M[3], van der Kuip M[3], Mason S[1]
[1]North-west University. Potchefstroom campus, [2]Stellenbosch University, [3]Amsterdam University Medical Centre

Background: Formalin-fixed paraffin-embedding (FFPE) is a widely adopted preservation technique for tissue samples in medical facilities, exclusively utilized for immunohistochemical studies. New approaches for extracting useful information from FFPE tissue are making this type of sample (re)-emerge as being useful in biomedical research. In the realm of 'omics, specifically metabolomics and proteomics, we have made a breakthrough in detecting metabolites and proteins within these tissues. Historically, extracting materials of sufficient quantity and quality from FFPE samples posed significant challenges.
Method: Recent advances have led to the development of an optimized unified protocol that integrates both metabolomics and proteomics approaches into a single workflow.
Results: This innovative protocol allows for the measurement of metabolites and proteins with good yield, demonstrating high repeatability and reproducibility. Our findings illuminate the capability of the unified protocol to revitalize the utility of FFPE samples, traditionally overlooked in research, by providing a means to extract biologically relevant data.
Conclusion: This approach paves the way for groundbreaking discoveries in biology and medicine, as it enhances our understanding of biological processes and disease mechanisms. By applying this method in future metabolomics and proteomics studies, we could significantly contribute to the advancement of precision medicine.

The Metabolic Consequences of a High Dose of Cannabidiol Treatment Over Two Weeks in Mice

Bhengu S[1], Omotayo O[1], Mason S[1]
[1]North-West University, Focus Area for Human Metabolomics, Biochemistry Department

Background
Conventional medicine typically imposes adverse side-effects, and its cost cannot be overlooked, as well. Therefore, this has led to the increase in the search for alternative and complementary plant medicinal products, including those derived from cannabis. Cannabidiol (CBD), a non-psychoactive component of Cannabis sativa, has been evaluated for its anti-inflammatory and neuroprotective properties, among several other therapeutic properties. Animal models are needed to assess the biochemistry of CBD; however, there are insufficient studies in mice, if any, that have investigated metabolic alterations induced by CBD treatment and whether there is any toxicity thereof. Therefore, this study aims to investigate the metabolic effects of cannabidiol treatment over two weeks in mice.

Methods
Untargeted metabolomics using 1H-NMR was used for the analysis of urine, feces, serum, and cerebrospinal fluid, to determine the global metabolite profile of mice treated daily with 10 mg/kg of CBD via the intrabuccal route for two weeks.

Results
The results show moderate differentiation of the CBD-treated mice from the controls. Furthermore, some makers of hepatic stress were detected but overall, no deleterious metabolic effects or any hepatoxicity.

Conclusion
Therefore, the 10 mg/kg dose, route of administration, and duration of treatment have been proven to be safe and therefore can be used by the scientific community for any further research into CBD or assessing translatability to humans.

Assessment of HepG2 Cell Growth and Viability in Response to Hormone Incubation.

du Preez M[1], Venter G[1]
[1]Human Metabolomics, North-West University

Background:
The function of estrogen includes the regulation female reproductive organ development and function as well as the development of secondary sexual characteristics. Combined oral contraceptives (COCs) function by prevent ovulation and thus preventing pregnancy; these drugs contain a combination of natural and synthetic estrogens and induce a constantly huge hormone overload into the body and can influence estrogen metabolism. Both natural and synthetic estrogens have been found to regulate cellular pathways, importantly the biotransformation pathway, whereas both have been implicated in breast cancer development. Numerous studies have been conducted to explore the hormone-influenced cellular effects; however, these studies often utilize varying concentrations, which most often are supraphysiological. Thus, this study aimed to start the experimental procedure by assessing the effect of different concentrations on growth and viability of HepG2 cells.

Methods:
HepG2 cells were seeded in the presence of various hormones and at different concentrations. The hormones included estradiol (E2), ethinyl estradiol (EE), 2-hydroxyestradiol (2-OH-E2), 4-hydroxyestradiol (4-OH-E2), progestin drospirenone (DRSP), and a DRSP/EE combination. Cell count and viability was assessed using the Muse® Cell Analyser.

Results:
The hormone treatments, particularly E2 and EE, exhibited varying effects on the cell proliferation where the higher concentrations generally resulted in an increased cell number over time. However, the proliferation was not accompanied by a sustained cell viability, especially not at the supraphysiological concentrations. Interestingly, the highest concentrations of E2 and EE resulted in significant decreases in cell viability, highlighting potential cytotoxic effects associated with these (supraphysiological) doses.

Conclusion:
The highest concentration in all hormones tested presented with an overall reduced cell viability, while the lower concentrations of these hormones did not result in excessive fluctuations in the viability. It is not known what the effect of the lower concentrations would have been on cell viability beyond 72 hours. It may be important to investigate this, since medications such as COC are taken every day and constant exposure could yield dissimilar results. These findings show the importance of assessing the cytotoxic effect of cell treatments and taking this in consideration when designing downstream experiments to investigate other cellular effects.

Investigating the effects of chloroform and methanol extract of Dicerocaryum senecioides and Flaveria trinervia on metastatic breast cancer cells

Sebei T[1], Mbazima V, Chokoe P[1]
[1]Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo, Faculty of Science and Agriculture

Plants have been used for centuries in various traditional healing practices worldwide. Research has revealed that some of these plants contain bioactive compounds with potential anticancer properties, not only inhibiting the growth of tumours but also mitigating metastasis. Dicerocaryum senecioides and Flaveria trinervia have both been reported to have anti-inflammatory and antioxidative effects, which can contribute to inhibiting metastatic processes such as migration of metastatic cells from their primary site and their subsequent adhesion at a secondary organ. Therefore, this study aimed to Investigate the potential anti-metastatic effects of Dicerocaryum senecioides and Flaveria trinervia chloroform and methanol extracts on MDA-MB-231 breast cancer cells. The effect of the extracts on the viability of MDA-MB-231 and HEK 293 human embryonic kidney cells was assessed using the cell counting kit-8 (CCK-8) kit. To determine the mode of cell death induced by the extracts, annexin-V and dead cell assay was employed. The effect of the extracts on reactive oxygen species formation was assessed with the Muse® Oxidative Stress Kit. A transwell assay was used to microscopically assess the anti-migratory and adhesive effects of the extracts on MDA-MB-231 cells. The effects of the extracts on the enzymatic activity of the matrix metalloproteinases (MMPs) was assessed using gelatin-zymography. The findings revealed that there was no significant effect on cell viability at concentrations below 200 µg/ml ($p < 0.05$). The extracts induced apoptotic cell death in a concentration dependent manner. Reactive oxygen species formation were suppressed in the MDA-MB-231 cells treated with the extracts. Moreover, the extracts suppressed cell invasion, migration, and adhesion. The D. senecioides chloroform extract inhibited up to 75% adhesion of the cells while its methanol extract inhibited 60%. The F. trinervia chloroform extract mitigated cell adhesion up to 60% and methanol inhibited 65%. Furthermore, both extracts inhibited MMP-2 and -9 activity in MDA-MB-231 by up to 40%. Thus, this study highlighted Dicerocaryum senecioides and Flaveria trinervia as potential sources of compounds with anti-metastatic activity. Further studies should focus on isolating and characterizing these active compounds, followed by in vivo and clinical trials for efficacy evaluation.

Investigating tissue specific changes in the proteome of Ndufs4 deficient mouse model.

Khumalo S[1], Lindeque J[1], Venter M[1]
[1]Focus Area For Human Metabolomics
Investigating tissue-specific changes in the proteome of Ndufs4 deficient mouse model.

Sibonelo Glen Khumalo1, Jeremie Zander Lindeque1, Marianne Venter1

1 Human Metabolomics, Faculty of Natural and Agricultural Sciences, North-West University (Potchefstroom Campus), South Africa.

Background: Complex I (CI, OMIM 25010) deficiency is the most common cause of OXPHOS related defects in humans. Patients with CI deficiency exhibit similar functional characteristics to Leigh Syndrome (LS), an infantile neurodegenerative disorder characterised by asymmetric, focal lesions in specific brain regions. Additionally, tissue-specificity is a common feature in LS, where tissues with high demand for OXPHOS derived energy are more prone to CI deficiency. This poses a substantial challenge in accurate diagnoses and prognoses of diseases linked to CI deficiency. We herein aimed to address these complexities by utilising mass spectrometry-based proteomics as tool to generate hypotheses and elucidate the mechanistic processes underlying perturbations associated with CI deficiency using the well characterised Ndufs4 knockout (KO) mouse model.

Methods: We conducted comprehensive proteome wide-profiling utilising liquid chromatography with tandem mass spectrometry (LC-MS/MS) on six distinct tissues (brainstem, cerebellum, olfactory bulb, heart, kidney, and liver) collected from Nduf4 KO (n=9) and wild-type (n=9) mice. Subsequently, we performed bioinformatic analysis on differential abundant proteins (DAPs) specific to each tissue.

Results: Overall, our quantitative and comparative proteome profiling resulted in the identification of more than 1000 proteins in each tissue based on 1% FDR. Differential abundance analysis revealed that the Ndufs4 subunit was downregulated across the tissues, with varying level of abundance. Functional enrichment analysis, including gene ontology (GO) terms and biological pathways, of DAPs specific to each tissue revealed distinct enrichment of GO terms. Pathways enrichment analysis of DAPs demonstrated significant enrichment of pathways related to fatty acid metabolism, translation, biological oxidations, ketone body metabolism, and neurotransmitter release cycle.

Conclusion: In conclusion, this study revealed that various tissues employ different cellular responses to Ndufs4 deficiency. Understanding these tissue-specific responses can provide valuable insights into the molecular mechanisms underlying CI deficiency-related diseases.

Evaluating phytochemicals, anti-bacterial, anti-biofilm and anti-motility effects of four medicinal plants against nosocomial pathogens

Mnisi J[1], Matotoka M[1], Masoko P[1]
[1]Department of Biochemistry, Microbiology and Biotechnology,University Of Limpopo

Background
Nosocomial infections are continually gaining resistance to known antibiotics due to having variety of mechanisms working against the antibiotics. This necessitates the discovery of novel antibacterial drugs. The study aims to evaluate the antimicrobial properties and anti-virulence effects of Gardenia volkensii, Carissa Bispinosa, Peltophorum africanum and Senna Petersiana against Pseudomonas aeruginosa and Staphylococcus aureus.
Methods
The stem powders were extracted with hexane, acetone and methanol. Major phytochemicals were quantified using spectrophotometric methods. The ability of the plant extracts to inhibit the growth of P. aeruginosa and S. aureus was evaluated using the broth micro-dilution assays. Anti-biofilm activities were evaluated using the 96 well microtiter plate assay and the crystal violet assay. Furthermore, the anti-motility activities of the extracts were evaluated on a soft agar media.
Results
The acetone extracts of P. africanum and S. petersiana exhibited higher phenolic (294.17 ± 4.73 and 269.89 ± 3.05 mg GAE/g) and flavonoid contents (807.26 ± 2.79± 2.79 and 755.87 ± 5.59 mg QE/g). P. africanum had the lowest MIC (0.078 mg/mL and 0.31 mg/mL) against P. aeruginosa and S. aureus respectively. P. africanum extract had notable inhibition of the initial cell adhesion of P. aeruginosa, where over 60% inhibitions were determined at sub-MICs. At 4MIC G. volkensii and C. bispinosa had moderate activity against P. aeruginosa early cell adhesion. The preformed biofilms were challenging for the extracts to inhibit however the 4MIC of some extracts had inhibitions above 50% against P. aeruginosa. Furthermore, the 4MIC of the plant extracts exhibited the highest level of activity, effectively suppressing 100% of the motility of both P. aeruginosa.
Conclusion
The results indicated that the plant extracts are more effective at inhibiting the initial cell adherence stage of biofilm formation. This was observed more in Gram-negative bacterium. It was revealed that the P. africanum acetone stem bark extract could be a potential candidate for further investigations in drug discovery to treat infectious nosocomial diseases induced by biofilm formers.

Diversity and associative transcriptomics of the voltage-gated sodium channel of Rhipicephalus microplus

Tadmor C[1], Stutzer C[1], Olivier N[1], van der Merwe A[1], Maritz-Olivier C[1]
[1]University Of Pretoria

Background: The cattle tick, Rhipicephalus microplus is well known as vector for various tick-borne diseases (TBDs). Along with high levels of acaricide resistance and ability to adapt to changes in the environment, the TBDs transmitted by this species currently affect some 80% of the global cattle population. Globally, chemical acaricides such as synthetic pyrethroids (SPs) have been utilised as the main control strategy for tick populations. However, prolonged SP usage has resulted in increased resistant R. microplus populations. A total of five single nucleotide polymorphisms (SNPs) in the voltage-gated sodium (VGS) channel have been validated to be associated with SP resistance in R. microplus. These SNPs have previously been utilised for the rapid detection of the SP resistance genotype of R. microplus populations through PCR-based assays. However, these SNPs are confined to Domains II and III of the VGS channel and the presence of additional possible SP resistance-linked SNPs in the remainder of the R. microplus VGS channel remain unknown. Therefore, this study aimed to detect novel mutations within R. microplus in South Africa across the entire VGS channel coding sequence and describe the SNP diversity.
Method: RNA sequencing data from various R. microplus tissues and life stages were used for de novo R. microplus transcriptome assembly. The VGS channel was identified and validated using published fragments of the channel. A predicted structural model for the entire VGS channel was generated. Variant analysis was performed to obtain novel mutations. These novel mutations were validated using PCR to confirm their presence in an R. microplus field sample.
Results: A total of 19 novel mutations of high confidence were identified across the entire VGS channel. Three of these mutations were found to occur within and around Domain IV (T5043C, G5271A and C5734T). Two of these mutations were successfully validated within an R. microplus field sample.
Conclusion: This research emphasizes the importance of analysing the entire VGS channel for novel mutations potentially linked to pyrethroid resistance. Furthermore, this research provides a foundation for future pyrethroid docking studies and sequencing of the VGS channel in R. microplus.

Structural bioinformatics analysis of human NAT2 enzyme function and comparison of all variants that possess known phenotype

Veldman W[1], Tastan Bishop O[1]
[1]Rhodes University

Background
The Tuberculosis drug Isoniazid is primarily metabolized by acetylation via the hepatic enzyme arylamine N-acetyltransferase 2 (NAT2). Several single nucleotide polymorphisms in the coding exon region of the NAT2 gene result in NAT2 alleles that differ in NAT2 metabolic activity and therefore differ in the rate of elimination of Isoniazid. This often leads to either drug-underexposure or hepatoxicity.
In the current study we ran molecular dynamics (MD) simulations of enzymes with varying residue substitutions, represented by the different alleles, to find functional differences between them that are linked to the differences in metabolic activity.

Methods
700 nanosecond MD simulations were run using 17 variants plus the reference allele, representing all human NAT2 alleles with known phenotype. The variants were compared to reference using a combination of enzyme structure, residue root-mean square fluctuation (RMSF), hydrogen bonding, and network centrality to determine the allosteric effects of the residue substitutions on enzyme function. Network centrality can be defined as the measurement of inter-residue communication within an enzyme.

Results
In four variants classified with the decreased activity phenotype, a pattern of hydrogen bonding and network centrality changes from reference was discovered in the Inter-domain. This pattern is linked to separation of Domain I and the Inter-domain. Interestingly, the catalytic acetyl-cysteine residue of variant R64Q lost internal hydrogen bonds which may be linked to the large conformational changes seen in this variant enzyme. Remarkably, the addition of the R197Q and K268 residue substitutions to R64Q mitigated the large changes from reference seen in the R64Q variant. The enzyme may do this through allosteric propagation from the enzyme locations of the respective residue substitutions then across the Inter-domain which vertically links Domain I to the active-site loop and horizontally links the left and right flanks of the enzyme. The two variants with rapid phenotype, the same phenotype as reference, showed the least changes compared to reference.

Conclusion
In summary, the combined MD analyses of enzyme structure, residue RMSF, hydrogen bonding, and network centrality proved helpful in the determination of allosteric effects on enzyme function caused by the residue substitutions of each NAT2 variant.

Determining sub-lethal neurotoxic concentrations of quinolinic acid in a primary neuronal cell line.

Meyer M[1], Loots D, Williams M, Mason S
[1]North West University
Melize Meyer, Du Toit Loots, Monray Williams, Shayne Mason

North-West University, Focus Area for Human Metabolomics, Biochemistry Department, Potchefstroom, South Africa

Background: Neurons are arguably the most important cells in our bodies, responsible for controlling an array of daily physiological functions. Neuronal cell lines have been used in numerous in vitro studies in order to assess the mechanisms of action of neurons and evaluate the neurotoxicity of specific chemicals. Quinolinic acid (QA) forms part of the tryptophan metabolism, specifically a downstream metabolite of the kynurenine pathway (KP). QA is used as a substrate to produce NAD+ in normal physiological conditions. However, due to inflammation, QA may be produced in excess, via the KP via the activation of indoleamine 2,3-dioxygenase (IDO-1) which leads to an increased tryptophan catabolism. This is problematic since QA has been proven to have both excitotoxic and neurotoxic properties. However, there is no general consensus in the literature as to what concentrations of QA are lethal to human neurons.

Methods:
A primary human neuronal cell line was grown using neuronal media with added neuronal growth supplement and penicillin/streptomycin solution. Neuronal cell viability was determined using a MTT assay at various QA concentrations of between 50-150nM.

Results:
We were able to experimentally determine at which concentrations QA is lethal to a neuronal cell line. Our results are given both qualitatively and quantitatively.

Conclusion:
The results from this study, will enable us to apply sub-lethal concentrations of QA to activated and inactivated microglial (brain immune) cell lines, in order to determine its mechanisms of action or toxicity, and in so doing evaluate the biological role of QA in the immune response during neuroinflammation. Furthermore, this would additionally give clues as to why QA production is increased during inflammation, or whether it is simply an intermediary product of other pathways that are activated, e.g. NAD+ synthesis.

Mitochondrial morphology and function in lung cancer cells: an in vitro model system for metastasis

Lindeque R[1], Labuschagne S[1]
[1]North-West University

Background: Despite being the leading cause of cancer related deaths, lung cancer metastasis is not very well understood. Especially with regards to the highly dynamic metabolism that metastasising cancer cells require. Current models of metastasis are lacking in accessibility and practicality for metabolic studies. This study aims to establish an in vitro model for metastasis that can be used for metabolic studies and that reflects the in vivo microenvironments of cancer cells by making use of physiologically relevant media.
Methods: To establish this model, a lung adenocarcinoma cell line (A549), were used to model the extracellular matrix interaction of the three main cellular phases of metastasis namely: existence in the primary tumour, circulating tumour cells and colonised cells forming a secondary tumour. The extracellular matrix attachment of the primary tumour was modelled by culturing cells in a monolayer, while the circulating tumour cells were grown as suspension cells, forming spheroids. The secondary tumour cells were grown by transferring these spheroids to cell culturing plates to reestablish extracellular matrix attachment. Fluorescent probes were used to assess the mitochondrial mass, mitochondrial superoxide and mitochondrial membrane potential. In addition, a Seahorse XF96 mitochondrial stress test revealed the oxygen consumption rates (OCRs) of each respective cell group.
Results: Results showed that upon detachment the cells drastically reduced their mitochondrial OCR, experienced reduced mitochondrial membrane potential and highly elevated mitochondrial superoxide. Interestingly, upon reattachment the cells quickly reverted to a state similar to that of the attached cells.
Conclusions: This remarkable display of metabolic plasticity and a reversible switch of energy metabolism suggests that the survival of circulating tumour cells depends on metabolic adaptation. In addition, the recolonisation of these cells requires an intrinsic ability to switch from a low energy state back into a high energy state. This model was established in supraphysiological media, however, to reflect circulating nutrient conditions and to close the gap in translatability between in vitro results and in vivo applications a physiological relevant media will be used in future studies.

Lithium induces oxidative stress, apoptotic cell death and G2/M phase cell cycle arrest in A549 cells

Ramushu P[1], Matsebatlela T[1]
[1]University Of Limpopo

Background: Though lithium was first identified for treatment of mania/depression, in the recent years' attention has been focused on its anti-cancer potential due to its affordability, stability and safety. Lung cancer ranks first as the main cause of death in males and has high mortality rates with low survival rates. In this study, lung adenocarcinoma (A549) cells were treated with various concentrations of lithium chloride with the hopes of eliciting anti-inflammatory and anti-cancer effects.
Methods: The in vitro cytotoxic effects of Lithium chloride was assessed using the MTT [3-(4, 5-dimethythiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay, Muse® cell death and cell cycle analysis. Nitric oxide measurement assay and oxidative stress assay was done to check the inflammation parameters after LiCl administration.
Results: MTT assay was used to demonstrate that LiCl had minimal cytotoxic effects on Raw 264.7 macrophage cells below the concentration of 40 mM. Lithium showed cytotoxic effects on the A549 cells mainly at the concentration of 80 and 100mM. Cell death assay showed that the lithium chloride induced apoptosis in A549 cells. Cell cycle analysis showed that Lithium chloride induced G2/M cell cycle arrest in A549 thus regulating apoptosis.
Conclusion: This study shows that lithium chloride can be used as prooxidant and ant-lung cancer treatment with reduced side effects since its less toxic to normal cells at concentration of 20 mM.

Aqueous extracts of Dodonaea viscosa induce potent and selective cytotoxicity in Diffuse Large B Cell Lymphoma cells.

Yekelo B[1], Mowla S[1]
[1]Department of Pathology, Division of Haematology, Faculty of Health Sciences, University of Cape Town, Observatory

Introduction

Diffuse Large B cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma worldwide. Additionally, it occurs with high frequency among people infected with HIV, where it follows a highly aggressive course. Approximately 30-40% of DLBCL patients who receive therapy relapse or develop refractory disease, indicating the need for improved therapy. Plant-derived bioactive compounds have proven to be effective in treating cancer. Aqueous extract from Dodonaea viscosa (DVE), a traditional medicinal plant, was assessed for its cytotoxic activity against DLBCL cells.

Methods

The IC50 of DVE against two DLBCL cell lines (HBL-1 and SU-DHL-4) was determined, relative to a non-cancerous lymphoblastoid cell line (LCL) (PB-B95-8H) using viability assays. Effects on proliferation were investigated using proliferation-tracking, and colony formation in semi-solid medium. Effects on the cell cycle were assessed using staining with propidium iodide and flow cytometry. Effect on apoptosis was determined using microscopy, Annexin V incorporation assay, caspase activity assay, and western blotting to assess the expression of apoptotic markers.

Results

DVE selectively inhibited the proliferation of the DLBCL cell lines compared to a non-cancerous LCL as evidenced by a favourable selectivity index of 2.25 for SU-DHL-4 and 3 for HBL-1. Moreover, cellTrace assays showed a 2.3-fold and 1.3-fold reduction in the proliferation of the DVE-treated DLBCL daughter cell population (SU-DHL-4 and HBL-1, respectively), while the LCLs (PB-B95-8H) were less affected. Similarly, DVE more potently affected the colony formation of DLBCL cells as observed over a 7-day period. While cell cycle phases remained unaffected, DVE induced cell death as evidenced by a sub-G1 peak in DLBCL cell populations. Typical morphological features of apoptosis were identified in the DVE-treated DLBCL cells, which was corroborated with Annexin V assays, caspase-3/7 activity assays, as well as expressions of cleaved caspase-3 and cleaved PARP-1.

Conclusion

The study showed that DVE is selectively cytotoxic to DLBCL cells relative to a non-cancerous control cell line, demonstrating that it is a promising source of bioactive compounds with anti-cancer activity.

Investigating antimycobacterial activity and cytotoxicity effects of the crude and isolated compound from Senna petersiana.

Mothupi F[1], Masoko P[1]
[1]University Of Limpopo

Tuberculosis (TB), an infectious disease which led to more than 1.4 million deaths in 2018 remains a global health threat. The global health system is burdened by antibiotic resistance which contribute to the loss of efficacy of antitubercular drugs. To compensate for the urgent need for discovery of novel TB drugs, Senna petersiana was adopted for drug discovery studies because medicinal plants can serve as good templates from which novel drugs can emerge. Senna petersiana is traditionally used for treatment of TB and related infections. Solvents of varying polarity were used to exhaustively extract phytochemicals from the ground leaves of Senna petersiana, followed by evaluation of antimycobacterial activity of the extracts during bioassay guided fractionation. n-Hexane fractions showed promising bioactivity against Mycobacterium smegmatis, hence their subjection to column chromatography for isolation of bioactive compound. The compound isolated was elucidates as β-sitosterol which displayed remarkable antimycobacterial activity against Mycobacterium smegmatis on bioautographic assay with minimum inhibitory concentration (MIC) of 2.5 mg/mL. Although the qualitative bioactivity of this compound was remarkable, the quantitative bioactivity assay disputes the use of β-sitosterol as TB drug lead because high MIC values may be an indication that bioactive agents of this compound are at a very low concentration. Furthermore, it was not surprising to observe the cytotoxicity effects of the crude acetone extract of Senna petersiana on tryptophan hydroxylase-1 macrophage cells to be toxic at higher concentrations. As a result, the use of β-sitosterol as antitubercular drug candidate may not be recommended due to toxicity risk.

Key words: Tuberculosis, Antimycobacterial activity, β-sitosterol, cytotoxicity

Epigenomic analysis of HIV-associated B-cell lymphoma: An investigation of the impact of Activation Induced Cytidine Deaminase (AICDA/AID) overexpression

Makofane L[1], Ramorola B[1], Alves de Souza Rios L[1], Wilmer T[2], Mowla S[1]
[1]Department of Pathology, University of Cape Town, [2]Biomedical Research and Innovation Platform, South African Medical Research Council

Background: Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL) are highly aggressive subtypes of B-NHL that occur with high frequencies among HIV-infected individuals and thus pose a major clinical challenge in HIV endemic regions. DNA methylation is an epigenetic form of gene regulation, and the modification of methylation profiles is a prominent feature of cancer, including lymphoma. Activation-induced cytidine deaminase (AICDA/AID) is an enzyme that introduces mutations in specific motif regions of the DNA by deaminating cytosine into uracil, playing an essential role in antibody diversity. This enzyme is overexpressed in both BL and DLBCL. Due to its deaminating function, AID has also been recently recognized as a DNA demethylator. However, its impact on epigenetic modulation of gene expression in aggressive lymphomas has yet to be defined.

Methods: AID-overexpressing B cell line models, as well as controls, were generated using lentiviral transduction. A panel of lymphoma-driver genes was selected for analysis using information from the published literature on lymphoma biology. Changes in expression will be assessed using qPCR. Changes in DNA CpG methylation patterns within gene promoters will be analyzed via bisulfite conversion and pyrosequencing.

Results and Discussion: The human AICDA Open Reading Frame (ORF) was cloned into the lentiviral vector, pCDH-CMV-MCS-EF1-copGFP. This plasmid allows for the expression of AICDA and the green fluorescent protein (GFP), which is used for selection. Successful cloning was confirmed through western blotting using an AICDA-specific antibody via transfection into HEK293 cells. The pCDH-CMV-hAID plasmid, along with packaging and envelope plasmids were transfected into HEK293T cells to produce lentiviruses, which were used to transduce the DLBCL cell line HBL-1. Successfully transduced cells are currently being expanded in culture, following cell sorting. A panel of lymphoma-driver genes, including PIM1, FANCA, BCL2, BCL6, MYC, SIRT1, PVT1, FEN1, TERT, and others, which have altered expressions in lymphoma, potentially via methylation, have been identified through a comprehensive analysis of the published literature. Current and future work includes qPCR to evaluate the expression levels of these genes within the parental and AICDA-expressing cell models, followed by pyrosequencing to investigate alterations in methylation profiles.

Polymorphisms in the IL-10 (-1082) and IFN-γ (+874) cytokine genes is associated with resistance or susceptibility to Schistosoma haematobium infection in children

Makwikwi T[1,2], Mduluza T[2]
[1]Human Metabolomics, North-west University, [2]Department of Biochemistry, University of Zimbabwe

Background
The promoter region of human interleukin-10 (IL-10) gene is highly polymorphic while the first intronic region of interferon gamma (IFN-y) gene is also highly polymorphic. These single nucleotide polymorphisms (SNPs) are associated with susceptibility or resistance to Schistosoma haematobium infection. Schistosomiasis is known to be a highly inflammatory disease that requires the delicate balance of pro- and anti-inflammatory cytokines. The SNPs are associated with low, moderate or high cytokine production resulting in exacerbation of the infection leading to pathological severity.
Methods
Urine filtration technique was used for diagnosis the S. haematobium. Whole blood samples were collected from 400 children aged between 6 to 13 years. Molecular determination of polymorphism related to resistance or susceptibility to infection was performed using the allele-specific polymerase chain reaction. Mutations in the IL-10 and IFN-y cytokine genes were examined in blood samples.
Results
Schistosomiasis was detected in 49.8% of the population. For IFN-y +874A/T, the distribution of TT, TA and AA was 7, 41 and 51% respectively. An analysis of the SNPs on IL-10 -1082G/A showed that most of the samples were heterozygous (47% GA) whereas AA (32%) and GG (21%) were monozygous. Mutations within the promoter region of IL-10 gene and in the intronic region of IFN-y have been associated with altered profiles of circulating IL-10 and IFN-y.
Conclusions
Our findings suggest that IL-10 and IFN-y polymorphisms participate in the progression of schistosomiasis rather than in its initial development in school aged children.

Establishment and biochemical evaluation of a human NDUFS4 knockout iPSC model

du Plessis C[1], Goolab S[2], Terburgh K[1], Scholefield J[2,3], Louw R[1]
[1]Human Metabolomics, Faculty of Natural and Agricultural Sciences, North-West University, [2]Bioengineering and Integrated Genomics Group, NextGen Health Cluster, Council for Scientific and Industrial Research, [3]Department of Human Biology, Faculty of Health Sciences, University of Cape Town

Background:
Mitochondrial diseases most frequently manifest through defects in the oxidative phosphorylation (OXPHOS) system's complex I (CI), with Leigh syndrome (LS) being a prevalent presentation in paediatric cases. The Ndufs4 knockout (KO) mouse model has significantly deepened our understanding of LS, despite challenges like embryonic and neonatal mortality that may limit the full representation of the disease's spectrum. These challenges highlight the necessity of alternative models like induced pluripotent stem cells (iPSCs), which are proving crucial for LS research by emulating the disease's pathology within the specific human cell types it affects.
Methods:
The genome editing tool CRISPR-Cas9 was employed to create a frameshift mutation in the NDUFS4 gene within a human iPSC line. Characterisation of the KO model involved (i) Sanger sequencing verification of the edited DNA region, (ii) confirming the absence of the NDUFS4 protein through semi-quantitative Western Blot analysis, (iii) measuring the activity levels of the respiratory chain enzymes (complexes I-IV) using kinetic spectrophotometric assays, and (iv) a comprehensive analysis of metabolic changes conducted via multi-platform metabolomics.
Results:
Analysis of Sanger sequencing data identified biallelic frameshift deletions in the NDUFS4 gene's coding region, whereas indel mutations were absent in the control cell line. The lack of NDUFS4 protein in the KO cell line was also verified through Western Blotting. Additionally, enzyme kinetic measurements showed that the NDUFS4 KO had only 38% of the control's CI activity. Comprehensive metabolomic studies across various platforms highlighted a significant metabolic disruption due to the absence of NDUFS4. This observation aligns with prior research, which has shown that both mouse models and individuals with LS exhibit similar metabolic changes.
Conclusion:
We have successfully developed a novel NDUFS4 KO model within a human iPSC line, leveraging the precision of CRISPR-Cas9 gene-editing technology. This significant breakthrough marks a critical step forward in developing a model that closely mirrors the clinical manifestations of neuropathological conditions, particularly those observed in LS. Our future research includes differentiating these iPSCs into neural progenitor populations, with the objective of establishing an advanced platform that could markedly improve the screening and assessment of innovative therapeutic strategies.

The effects of didox on oxidative stress in SARS-CoV-2 stimulated Raw 264.7 macrophage cells

Mangokoana D[1], Mbazima V[1], Matsebatlela T[1]
[1]University Of Limpopo

Background
Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) infections are characterised by inflammation-associated symptoms that are ultimately responsible for overall deterioration of health status in COVID-19 patients. The excessive innate inflammatory reactions generated by SARS-CoV-2 play a central role in disease severity observed in COVID-19 patients. It is therefore important to understand the macrophage-mediated innate immune response to SARS-CoV-2 as a crucial step in the design of rational therapeutic strategies. Thus, the study aimed at using the SARS-CoV2 spike-activated macrophage model to delineate the immunological mechanisms underlying the diverse clinical manifestations related to COVID-19 and establish the role didox (3,4-dihydroxybenzohydroxamic acid) can play in alleviating these inflammation-related clinical episodes.
Methods
The MTT and MuseTM Count viability assays were carried out to determine the cytotoxic effect of didox on macrophage cells challenged with SARS-CoV-2 spike proteins. Intracellular reactive oxidative species (ROS) and nitric oxide (NO) production was measured using H2DCF-DA and DAF-2-DA assays, respectively.
Results
Didox has shown not to have cytotoxic effect on SARS-CoV-2 spike proteins stimulated macrophage cells but suppressed the intracellular ROS and NO production. These results indicate that Didox exerts anti-inflammatory effects in SARS-CoV-2 activated macrophages without inducing cell cytotoxicity.
Conclusion
Further analysis of cytokine production in this model is underway to measure the extent to which Didox is able to modulate SARS-Cov-2-induced inflammation.

The immunomodulatory effects of vitamin D on cytokine homeostasis in monocytes and monocyte-derived macrophages

Naidoo K[1], Mol B[1], Gentle N[1], Meyer V[1]
[1]School of Molecular and Cell Biology, The University Of the Witwatersrand

1,25-dihydroxyvitamin D3 (1,25(OH)2D3), the biologically active form of vitamin D3, is a recognised immunomodulator that shows pronounced genomic and non-genomic effects in immune cells such as monocytes and macrophages. Despite this, there is relatively little information concerning the role of 1,25(OH)2D3 in monocyte-to-macrophage differentiation and macrophage biology. In this study, we differentiated the monocyte-like THP-1 cell line into macrophages in the presence and absence of 1,25(OH)2D3 to assess the effects of 1,25(OH)2D3 on macrophage biology using RNA-sequencing. Differential gene expression analysis confirmed that 1,25(OH)2D3 treatment of monocytes and monocyte-derived macrophages produced distinct transcriptomic profiles. Though 1,25(OH)2D3 treatment often demonstrated overlap in the over-represented biological processes identified in these two cell types, the differentially expressed genes implicated as contributing to these processes were often distinct. For example, in the macrophages, but not the monocytes, 1,25(OH)2D3 treatment altered the expression of cytokines and cytokine receptors, suggesting an immunomodulatory effect that may impact antiviral signalling. To assess the antiviral effects, these monocytes and macrophages were exposed to the 5 µM of motolimod, a TLR8 agonist, in the presence and absence of 1,25(OH)2D3 for 2 hours. The gene expression of IL1β, CCL2, CXCL10, NOS2, and IL10 was quantified by RT-qPCR, while IL6 and TNFα protein expression was quantified by ELISA. Preliminary results showed that TLR8-stimulated monocytes and macrophages both showed a significant ($p < 0.050$) increase in the expression of IL1β, CCL2, and CXCL10, with a significant reduction observed in TLR8-stimulated monocytes and macrophages treated with 1,25(OH)2D3. While IL6 protein level remained unchanged in both monocytes and macrophages, there was a significant increase in TNFα protein expression in TLR8-stimulated macrophages ($p < 0.050$), with a significant decrease in TNFα protein expression observed in TLR8-stimulated macrophages treated with 1,25(OH)2D3 ($p < 0.050$). The current data suggest a protective role for 1,25(OH)2D3 in macrophages where 1,25(OH)2D3 can prime an antimicrobial response, while still balancing inflammatory responses and protecting against autoinflammation induced by aberrant cytokine signalling in the absence of a microbial challenge.

Marathon-induced metabolome changes in males versus females using 1H-NMR metabolomics

Terblanche S[1], Loots D[1], Mason S[1]
[1]North-west University
Marathon-induced metabolome changes in males versus females using ^{1}H-NMR metabolomics

Stephan Terblanche, Shayne Mason, Du Toit Loots
Human Metabolomics, School for Physical and Chemical Sciences, North-West University, Potchefstroom, South-Africa

Background
Historically, females are generally excluded from sports research due to fluctuating sex hormones, pregnancy, and other physiological differences when compared to males that would complicate study designs. Additionally, there is a lack of metabolomic studies including both sexes participating in endurance exercise lasting longer than 2 hours. To this end, this study compared the metabolic changes in males and females induced by a marathon (duration > 2 hours) using untargeted proton nuclear magnetic resonance (^{1}H-NMR) metabolomics.

Methods
Blood samples were collected from male (n = 18) and female (n = 12) athletes 24 hours before- and immediately after the Druridge Bay Marathon. Plasma samples were analysed using untargeted ^{1}H-NMR metabolomics at the North-West University, South Africa.

Results
Males showed 1.8 – 2.4 fold greater post-marathon concentrations of the circulating ketone bodies (3-hydroxybutyrate, acetoacetate and acetone) than females did. Additionally, males showed a 1.5 – 2 fold greater marathon induced increase in the abovementioned ketone bodies than females did. Marathon-induced valine catabolism was higher in males vs females, with no significant leucine oxidation in either sex. Isoleucine oxidation during the marathon was similar in both sexes.

Conclusion
These results suggest that males rely less on the ketogenic amino acids, leucine and isoleucine, for energy production than they would during exercise of <2 hours, when compared to females, due to a greater lipid oxidation rate in males vs females during a marathon. Previous findings however indicate higher lipid oxidation rates in females vs males during endurance exercise of <2 hours. Considering this, our results suggest that after roughly 2 hours of endurance exercise, there is a sex-dependent shift in energy substrate utilization from comparatively higher lipid oxidation rates in females vs males, to comparatively higher lipid oxidation rates in males vs females. If the abovementioned hypothesis can be verified in larger population groups, it will lead to more specialized nutritional- and training programs for both sexes.

Key words: Endurance exercise, sex-dependent, metabolomics

Appraisal of anti-diabetic properties of polyphenolic compounds from Viscum combreticola Engl. through a combination of network pharmacology and molecular docking approach

Moyo N[1], Madala N[2]
[1]Department of Biochemistry and Microbiology, University Of Venda, [2]Department of Biochemistry and Microbiology, University Of Venda

Background
Viscum combreticola Engl. is currently used in African medicine for treating various ailments including diabetes. However, modernizing plant-based traditional medicine is a considerable challenge due to lack of scientific information linked to its therapeutic efficacy given the complexity of plant extracts.
Methods
Herein, metabolic profiling of V. combreticola methanolic extracts was conducted using the ultra-performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry. The identified chemically diverse polyphenols comprising of 19 diacylated chlorogenic acids (CGAs) and 12 flavonoids were explored for their mode of action in managing type 2 diabetes mellitus (T2DM) using network pharmacology.
Results
Systematic pharmacological analysis revealed that CGAs and flavonoids characterized by structural similarities or analogous spatial arrangements exhibited interactions with identical target proteins. Isomers of CGAs diacylated at carbon positions 3,4- and 4,5- of quinic acid were found to be hub therapeutic agents against T2DM related target proteins. The investigated CGAs and flavonoids could be playing a major role in the management of T2DM and its complications by synergistically regulating key protein targets. These include matrix metalloproteinase 2 (MMP2), caspase 3, protein kinase C alpha and delta, which are related to T2DM hub pathways such as the lipid and atherosclerosis, advanced glycation end products and their receptor signalling pathway for diabetes complications and diabetic cardiomyopathy signalling pathways. Finally, molecular docking studies revealed that viscutin 2 and 3,4-dicaffeoylquinic acid were key potential inhibitors of α-glucosidase and MMP2, respectively.
Conclusions
Therefore, the findings of this study suggest that isomerism in compounds might have pharmacological implications. Lastly, the anti-T2DM therapeutic effects of the polyphenols investigated in this study can serve as potential anti-diabetic agents for future experimental validation, particularly paying attention to isomerism.

The status and role of TBX2 in HPV-independent cervical cancer.

Rama A[1], Khan S[1], Burmeister C[1], Sinkala M[2], Prince S[1]
[1]University of Cape Town, Department of Human Biology, [2]University of Cape Town, Department of Integrative Biomedical Sciences

Background:
Cervical cancer (CC) is the fourth most prevalent female cancer globally and poses a significant health challenge accounting for 604,000 new cases and 342,000 deaths in 2020. The primary etiological agent of CC is persistent infection by high-risk human papillomavirus (HPV) strains. Although less prevalent than HPV positive (HPV+) types (95%), HPV negative (HPV-) CC is more invasive and aggressive with a poorer patient prognosis. Unlike HPV+ cancers, the mechanisms driving early transformation and progression in HPV- cases remain unclear. Preliminary data reveal there is increased expression of the oncogenic T-box transcription factor TBX2 in HPV- CC cells which is repressed in the presence of the HPV oncoproteins E6 and E7. We hypothesize that TBX2 may play an important role in HPV- CC which makes it a novel drug target.

Methods:
TBX2 status was determined in HPV- CC cell lines using the human protein atlas data base and western blotting. To determine if TBX2 contributes to HPV- CC it was depleted by siRNA in HPV- (C33A) CC cells and the impact on cell proliferation (trypan blue exclusion assay), invasion (transwell assay) and migration (2D scratch motility assay) was assessed. The effects of treating C33A cells with TBX2 targeting commercial drugs (niclosamide and pyrvinium pamoate) were assessed on TBX2 levels (western blotting), cell viability (MTT assays) and 3D spheroid formation (Calcein AM staining), long-term cell survival (clonogenic assay), as well as cell proliferation (trypan blue exclusion assay) and migration (2D scratch motility assay).

Results:
This study reveals that HPV- CC cells (1) express higher levels of TBX2 than HPV+ CC cells; (2) require TBX2 for their proliferation and migration but not for their invasive ability; (3) treated with niclosamide and pyrvinium pamoate for 48- and 72-hours have reduced TBX2 levels, cell proliferation, migration, and 3D spheroid formation.

Conclusion:
Results from this study reveal that TBX2 promotes HPV- cell proliferation and migration and is therefore an important oncogenic transcription factor in this aggressive malignancy. Furthermore, we show that the TBX2 targeting anthelmintics niclosamide and pyrvinium pamoate can be repurposed to treat this aggressive disease.

Phytochemical screening and assessment of antioxidant and antimicrobial activities of Geigeria aspera against bacterial pathogens.

Mphosi K[1], Masoko P[1]
[1]Department Of Biochemistry, Microbiology And Biotechnology, University of Limpopo

Background
Medicinal plants constitute of a wide range of phytochemicals that can be used in the pharmaceutical sector. The purpose of this study was to screen for phytochemicals and evaluate antibacterial and antioxidant properties of Geigeria aspera leaf extracts.
Methods
solvent used: Hexane, DCM, Acetone, Methanol and Water
Standard biochemical tests were used to assess the presence of phytoconstituents. The total phenolic, tannin and flavonoid contents was determined using colorimetric methods. The antioxidant activity of plant extracts was assessed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. Bio-autography and microbroth assay were used to evaluate the antimicrobial activity.
Results
Tannins, flavonoids, terpenes/terpenoids, cardiac glycosides, alkaloids, and steroids were found to be present in the plant extracts. Methanol and Acetone extracts had the highest total phenolic (253± 7,972583 GAE/g) and flavonoids content (423± 4,910464 QE/g) respectively. Furthermore, methanol extracts demonstrated noteworthy antioxidant activity with IC50 value of 0.69 µg/ml. The plant extracts were more active against S. aureas with MICs of 0.63 mg/ mL. Hexane and water extracts did not have any antibacterial activity.
Conclusion
The extracts of Geigeria aspera plant demonstrated good antioxidant activity however, very low antibacterial activity. Therefore, having potential in development of new antioxidant agents.

The role of MTHFD1L in proliferation, migration and mitochondrial function in lung cancer

Ackermann C[1], Labuschagne C[1]
[1]North-West University

Background
Lung cancer is the leading cause of cancer death worldwide and much research has been dedicated to developing new and more effective treatments. To sustain their rapid proliferation, cancer cells adapt their metabolism and rely heavily on folate metabolism for nucleotide synthesis, methylation reactions, and generation of reducing cofactors. Methylenetetrahydrofolate dehydrogenase 1-like (MTHFD1L) is a folate enzyme found in the mitochondria that catalyses formate synthesis and tetrahydrofuran (THF) regeneration. Studies have shown that MTHFD1L is overexpressed in several types of cancer tissue and that patients with high MTHFD1L levels have a lower survival rate. Furthermore, MTHFD1L silencing has been shown to inhibit cancer cell proliferation and increase apoptosis, indicating the therapeutic potential of targeting this enzyme. In this study, we examine the effect of MTHFD1L silencing on cell proliferation, migration, and mitochondrial function in lung cancer.

Methods
siRNA was used to suppress MTHFD1L expression in a lung cancer cell line, A549, to examine how the loss of this enzyme affects cell proliferation (using an automated cell counter) and migration (using scratch and Transwell migration assays). Since MTHFD1L is found in the mitochondria, we also examined mitochondrial morphology using fluorescence microscopy with MitoTrackerTM dye.

Results
Silencing of MTHFD1L by siRNA resulted in decreased migration and proliferation in lung cancer cells. Fluorescence microscopy revealed differences between the mitochondria of normal and knockdown cells.

Conclusions
These findings confirm the importance of MTHFD1L in lung cancer and its potential as a novel therapeutic target.

An ion channel antagonist inhibits Plasmodium falciparum asexual proliferation and perturbs K+ homeostasis

Muller J[1], Birkholtz L[1], Niemand J[1]
[1]Department of Biochemistry, Genetics and Microbiology, Institute for Sustainable Malaria Control, University of Pretoria

Background:
Plasmodium falciparum resistance against current antimalarials necessitates the development of new treatments with novel targets. One process that is both biologically important and targetable is the regulation of intracellular ion homeostasis. Both the V-type H+ ATPase, that maintains intracellular pH through the active export of H+, and PfATP4, a Na+/H+ ATPase that extrudes Na+ in exchange for H+, are promising drug targets. The influx of Na+ into the parasite, however, has not been investigated yet, but might play an essential role in ion homeostasis. Three classes of mammalian Na+ transport inhibitors, Na+/H+ exchanger-, voltage-gated Na+ channel- and epithelial Na+ channel inhibitors were used to probe this influx of Na+ into the parasites.
Methods:
The effect of these compounds on host erythrocyte integrity was determined by measuring the amount of haemoglobin released from erythrocytes. Total DNA was measured, as a proxy for parasite proliferation, using the SYBR Green I dye, to quantify the effect the compounds have on asexual parasite proliferation. Possible targets of the most active compound, BIII 890CL, were predicted using an in silico approach. Parasite cultures were treated with BIII 890CL and examined using light microscopy to determine the effect of the compound on parasite life cycle progression. The effect of BIII 890CL on intracellular [K+] and membrane potential were determined using the fluorescent dyes APG-1 and DiBAC4(3).
Results:
All of the compounds tested showed antiplasmodial activity in the low micromolar range, without affecting erythrocyte integrity. BIII 890CL was the most active compound with an IC50 of 2.06 ± 0.16 µM. Surprisingly, the K+ channel PfK1 was predicted as its most likely target. Treatment with BIII 890CL affects the trophozoite stage, consistent with the expression of PfK1. Furthermore, BIII 890CL caused a significant decrease in APG-1 signal, which is consistent with a decrease in intracellular [K+], while it had a negligible effect on the parasite membrane potential.
Conclusions:
Of the three classes of Na+ transport inhibitors, the voltage-gated Na+ channel inhibitor BIII 890CL had the largest inhibitory effect on P. falciparum parasite proliferation, possibly due to an interaction with a K+ channel.

Characterization of Plasmodium falciparum small heat shock proteins

Timothy F[1], Zininga T
[1]Stellenbosch University

Background
Plasmodium falciparum, the causative agent of malaria has developed mechanisms to resist the effects of existing antimalarial drugs. Heat shock proteins (Hsps) have emerged as promising candidates for drug development as they are implicated in facilitating the development of parasite drug resistance. Larger members of the Hsps family have been fairly studied in malaria, but little is known about the six parasite small Hsps of 20 kDa (sHsp20). In other species, sHsps have been reported to possess chaperone functions and ability to self-associate. The functions of P. falciparum sHsps is scanty, therefore this study aims to characterize P. falciparum sHsp20s.
Methods
We utilized multiple sequence alignment to compare the structure of six small Hsps of P. falciparum with their human homologs to predict domain organisation and structural conservation. Subsequently, we used an E. coli expression system to recombinantly produce three of the P. falciparum sHsps (PfHsp20a, b & c). The secondary and tertiary structure stability of the recombinant proteins were investigated using circular dichroism (CD), Differential scanning fluorimetry (DSF) and dynamic light scattering (DLS). Furthermore, Chaperone function was investigated by their ability to suppress heat-induced aggregation of malate dehydrogenase.
Results
The multiple sequence alignment and 3-D model match making analysis of PfsHsp20a, PfsHsp20b and PfsHsp20c revealed the presence of a conserved IXI motif which is important for their self-association. Moreover, homology modelling showed high conservation of the alpha crystallin domain (ACD) a signature domain of small Hsps. We expressed and purified the three P. falciparum sHsp20s for the first time. DLS analysis confirmed the ability of all three PfsHsp20s to self-associate. We further observed that PfsHsp20a and PfsHsp20b exhibited the capability to suppress thermal aggregation of MDH. Surprisingly, we observed that lower concentrations of small Hsps exhibited greater efficacy in preventing MDH aggregation compared to higher concentrations. Possibly due to the IXI motif interacting with ACD at higher concentrations, forming oligomers that make them ineffective as chaperones. However, Hsp20c followed a different stoichiometry as high concentrations inhibited better than lower concentrations.
Conclusions
Our study highlights the structural basis of PfsHsp20s' chaperone activity, emphasizing their role in maintaining cellular proteostasis.

Chemical composition, antibacterial, antibiofilm, antioxidant and mechanism of action of Tridentata riparia leaf extracts against bacterial pathogens.

Shekwa W[1], Masoko P[1]
[1]Department of Biochemistry, Microbiology and Biotechnology, University Of Limpopo

Background
Plant natural products have been an important part of medicine throughout human history. Scientific exploration of medicinal plants presents the possibility for the discovery of drugs that may combat the huge problem of antibiotic resistant strains. The study aims to evaluate the biological activities and mechanisms of action of Tridentata riparia leaf extracts against bacterial pathogens.
Methods
The phytochemicals were extracted using hexane, ethyl acetate and methanol. Polyphenols were quantified using spectrophotometric methods. Antioxidant activity was evaluated using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay. The antimicrobial activity against Staphylococcus aureus (ATCC), Enterococcus faecalis (ATCC), Pseudomonas aeruginosa (ATCC) and Escherichia coli (ATCC) was evaluated using the agar well diffusion and microbroth dilution assays.
Results
The methanol extract had the highest total phenolic (538 mg GAE/g), flavonoids (79 mg QE/g) and tannin contents (64 mg TAE/g). The methanol extract had an IC50 value of 179 µg/ml. Methanol extract had a 5 mm zone of inhibition against S. aureus and E. faecalis. The ethyl acetate and methanol extracts had a minimum inhibitory concentration of (0.15 -0.63 mg/ml) against E. faecalis and S. aureus. The same extracts had moderate activity against E. coli and P. aeruginosa (1.25- 2.5 mg/ml).
Conclusion
The ethyl acetate and methanol extract had promising antimicrobial activity against the tested pathogens. The study will further evaluate the mechanism of action employed by the bioactive extracts by assessing the leakage of cellular constituents (DNA and proteins) and mitochondrial dehydrogenase activity (iodonitrotetrazolium chloride method). Antibiofilm activity will also be evaluated using the crystal violet assay.

Investigating the effects of Commelina Benghalensis extracts in Breast Cancer cells

Mnisi H[1], Mbita Z[1], Morafo V[1]
[1]Department of Biochemistry Microbiology And Biotechnology at University of Limpopo
Investigating the effects of Commelina Benghalensis extracts in Breast Cancer cells
Mnisi HL1, Z Mbita2 and V Morafo1
University of Limpopo, Department of Biochemistry, Microbiology and Biotechnology
Private Bag X1106, Sovenga, 0727

Background: Female Breast cancer is the leading cause of global cancer incidence, with an estimated 2.3 million new cases. Treatments such as radiotherapy, chemotherapy and surgery are used to counteract the impact of this disease; however, conventional treatment approaches may, in some cases, be ineffective; thus the use of medicinal plants is enticing. Therefore, the aim of the study was to determine the immunomodulatory effect, and antioxidant activities of Commelina benghalensis (Cb) aqueous extract in breast cancer cells.
Methods: In this study, the MDA-MB 231 and MCF-7 breast cell lines were treated with Cb aqueous extract. Liquid Chromatography Mass Spectrometry (LC-MS) was utilised to identify potential metabolites with immunomodulatory activities. The 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) and Ferric ion reducing power FRAP assays were used to evaluate the antioxidant activity of the Cb aqueous extract. The cytotoxicity of the Cb leaf-aqueous extract was determined using the 3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide (MTT) and the Muse® cell count and viability assays. Annexin V assay was used to detect and quantify programmed cell death.
Results: The LCMS results revealed the presence of bioactive compound such as 3-(4-methoxyphenyl)-8,8-dimethylpyrano[2,3-f]chromen-4-one which have been documented to possess anti-inflammatory effects. The Cb leaf aqueous extract exhibited lower antioxidant activity scavenging more than 50% of free radicals at 1000 µg/ml compared to the higher antioxidant activity of ascorbic acid (positive control), implying that the Cb leaf aqueous extract influence cellular oxidative stress. The Cb leaf aqueous extract significantly (**P>0.01 and ****P>0.00001) reduced cell viability of MDA MB 231 cells (IC50 = 750µg/ml). Additionally, the treated breast cancer cells demonstrated an increase in apoptosis, indicating that the Cb leaf aqueous extract possess potent anticancer activities against breast cancer.
Conclusion: In conclusion Cb leaves aqueous extract possesses immunomodulatory effects and anticancer properties, potentially mediated by bioactive compounds. This may be a promising avenue for further exploration of Cb extract as a supplementary treatment for breast cancer.

Evaluating the effect of multiple substrates on the glycine conjugation rate of glycine N-acyltransferase

Botha C[1], Van Der Sluis R[2]
[1]North-West University, [2]North-West University

Christo L. Botha and Rencia Van Der Sluis
Focus Area for Human Metabolomics, North-West University, Potchefstroom, South Africa
Email: 31918530@mynwu.ac.za

Background

Glycine-N-acyltransferase (GLYAT) is an enzyme that forms part of the phase II glycine conjugation pathway that plays a key role in the detoxification of excess Acyl-CoA's in the liver. Studies have shown that GLYAT can possibly detoxify multiple acyl-CoAs involving differentamino acids. Unfortunately, there are a limited number of studies focusing on this variation and the role it can play on the pathway. Understanding the substrate preference of the enzyme could help us diagnose and better understand diseases that leads to acyl-CoA build-up like Isovaleric Acidemia which is caused by accumulation of Isovaleryl-CoA.

Methods

In this study, the objective was to identify the effect of substrate competition on the glycine conjugation rate of GLYAT using Nuclear Magnetic Resonance (NMR) spectroscopy. Substrates that were used include: Phenylacetyl-CoA, Benzoyl-CoA, Palmatoyl-CoA and Isovaleryl-CoA.

Results

The data generated from NMR spectroscopy included a chromatogram which shows individual spectra for each component in a mixture. These peaks allow for the quantification of products, substrates, amino acids etc. This makes it possible to compare the outcome of the enzymatic reaction and ultimately closely look at any potential substrate competition taking place.
Conclusions
From this study an effective method has been developed to quantify and analyse substrate preference and competition when looking at the conjugation rate of Glycine-N¬-acyltransferase.

Key words: GLYAT, detoxification, NMR, acyl-CoA

An in vitro model for autism spectrum disorder: Investigation of PCCB knockdown in neuronal cells

Cuttler K[1], O'Ryan C[1,2]
[1]Department of Molecular & Cellular Biology, University Of Cape Town, [2]Neuroscience Institute, University of Cape Town

Background: Autism Spectrum Disorder (ASD) is a complex neurodevelopmental condition characterized by impairments in language, social and communicative deficits, as well as repetitive behaviours. ASD has a significant genetic component, as it can segregate in families. Previous work in a South African ASD cohort implicated differential methylation (DM) of PCCB in mitochondrial dysfunction which contributed to the aetiology of ASD. PCCB was the most significantly DM gene in our cohort, and thus the molecular target of investigation using SH-SY5Y neuroblastoma cells as a cellular model of ASD.
Methods: PCCB was knocked down using siRNA in SH-SY5Y cells. Given that PCCB was DM in our cohort and is linked to mitochondrial dysfunction, we examined mitochondrial function. We compared cells with PCCB siRNA silenced compared to control cells using gene and protein expression changes using qPCR and western blotting respectively. Reactive oxygen species (ROS), reactive nitrogen species (RNS) and mitochondrial health were also assayed.
Results: PCCB siRNA silencing achieved a 75% reduction in PCCB gene expression levels; this indicated that the the silencing experiment was successful. PCCB silencing decreased cellular viability, altered oxidative state, and impairs mitochondrial health. Moreover, there was alterations to transcriptional regulation of mitochondrial dynamics. This data provides insight into mitochondrial stress in a cellular model of ASD.
Conclusions: Most research into ASD has been symptom based instead of trying to understand its molecular mechanisms. Since ASD is understudied in South Africa, the results of this study contributed to understanding the underlying mechanisms of ASD in South Africa. This research can hopefully inform future translatable and transformative molecular autism research.

Comparative Immune profiling of Pancreatic Ductal Adenocarcinoma Progression Among South African patients.

Elebo N[1,2], Abdel-Shafy E[2,3], Omoshoro-Jones J[1,4], Nsingwane Z[1], Hussein A[2,5], Smith M[1,4], Candy G[1], Cacciatore S[2], Fru P[2], Nweke E[1,6]

[1]Department of Surgery, Faculty of Health Sciences, University of Witwatersrand. Johannesburg 2193, South Africa., [2]Bioinformatics Unit, International Centre for Genetic Engineering and Biotechnology, Observatory 7925, Cape Town, South Africa., [3]National Research Centre, [4]Hepatopancreatobiliary Unit, Department of Surgery, Chris Hani-Baragwanath Academic Hospital, Soweto Johannesburg, South Africa. , [5]Theodore Bilharz Research Institute, [6]Department of Life and Consumer Sciences, College of Agriculture and Environmental Sciences, University of South Africa, Florida, Roodepoort

Background: Pancreatic Ductal Adenocarcinoma (PDAC) is an aggressive cancer characterised by an immunosuppressive microenvironment. Patients from specific ethnicities and population groups have poorer prognoses than others. Therefore, a better understanding of the immune landscape in such groups is necessary for disease elucidation, predicting patient outcomes and therapeutic targeting. This study investigated the expression of circulating key immune cell markers in South African PDAC patients of African ancestry.

Methods: Blood samples were obtained from a total of 6 healthy volunteers (HC), 6 Chronic Pancreatitis (CP) and 34 PDAC patients consisting of 22 resectable (RPC), 8 locally advanced (LAPC) and 4 metastatic (MPC). Real-time Polymerase Chain reactions (RT-PCR), Metabolomics, Enzyme-Linked Immunosorbent Assay (ELISA), Reactive Oxygen Species (ROS), and Immunophenotyping assays were conducted. Additional analysis of single-cell RNA data from 20 patients (16 PDAC and 4 controls) was conducted to interrogate the distribution of T-cell populations.

Results: Granulocyte and neutrophil levels were significantly elevated while lymphocytes decreased with PDAC severity. The total percentages of CD3 T-cell subpopulations (helper and double negative T-cells) decreased when compared to HC. Both NK ($p=0.014$) and NKT ($p<0.001$) cell levels increased as the disease progressed. Of note is the negative association of NK CD56dimCD16- ($p<0.001$) cell levels with survival time. The gene expression analyses showed CD3, CD4 and CD56 genes to be most upregulated in RPC and CD8 was highest in LAPC. The inflammatory status of PDAC was assessed by ROS levels of serum which were elevated in CP ($p = 0.025$), RPC ($p = 0.003$) and LAPC ($p = 0.008$) while no significant change was observed in MPC, compared to the HC group. ROS was shown to be positively correlated with GlycA ($R=0.45$, $p = 0.0096$). Single-cell analyses showed a significant difference in the ratio of NKT cells per total cell counts in LAPC ($p < 0.001$) and MPC ($p <0.001$) groups compared with HC, confirming observations from our sample group.

Conclusion: The expression of these immune cell markers observed in this pilot study provides insight into their potential roles in tumour progression and suggests their potential utility in the development of immunotherapeutic strategies.

Antimicrobial characterisation and optimisation of cellulose-type films and materials containing tyrocidine A and tryptocidine C.

Botha J[1,2], Rautenbach M[1,2]
[1]Biopep Peptide Group, [2]Stellenbosch University

Antimicrobial-resistance (AMR) is an ever-increasing threat to human-kind. Pathogens rapidly adapting to current antibiotics is resulting in more severe illnesses with few effective cures. Tyrocidines pose a potential solution to this rapidly growing problem. These cyclic decapeptides - first isolated in 1940 - are now getting their time in the spotlight due to their antimicrobial properties against various resistant pathogens. Naturally occurring in almost all organisms, these peptides have evolved alongside pathogens, making their mechanisms of action effective and incredibly difficult to develop resistance towards. Due to their haemolytic nature, the interaction and compatibility of tyrocidines with cellulose is now being harnessed to potentially synthesize externally viable antimicrobial products. Through semi-preparative high-performance chromatography, tyrocidine A/A1 (TrcA/A1) and tryptocidine C (TpcC) were isolated and their identities were confirmed through electrospray ionisation mass spectrometry (ESI-MS) and ultra-performance liquid chromatography linked to mass spectrometry (UPLC-MS). Once identified, the TrcA/A1-rich peptide complex and TpcC were inoculated into 10 different ethanol concentrations and dried onto cellulose filter paper. The peptide-treated cellulose was incubated with target organisms, and, through a specialized resazurin assay, the metabolic inhibition of Staphylococcus aureus, Escherichia coli, and Candida albicans was assessed. Further insight into TpcC and its structure was gained through fluorescence spectroscopy, exploiting the properties of Trp. The results of this study showed that both the TrcA/A1-rich peptide complex and TpcC were similarly active against C. albicans, while TpcC was more active against S. aureus. Both peptides were less active against E. coli, including when used in combination. Fluorescence studies showed that although TpcC fluorescence was low at 20% EtOH, this concentration was most active against E. coli and C. albicans. In contrast, 50% EtOH was the most active concentration against S. aureus and displayed the highest fluorescence. This highlights the importance of solvent polarity in the conformation and therefore activity of TpcC against various pathogens. The activity of these peptides, though not fully optimised yet, holds great antimicrobial surface potential in combination with cellulose.

Momordica balsamina aqueous leaf extract ameliorates cisplatin-induced nephrotoxicity via oxidative stress modulation in HEK-293 cells.

Kgakishe D[1], Poopedi K[1], Mbazima V[1]
[1]University Of Limpopo

Background
Nephrotoxicity is a major adverse effect reported in cancer patients undergoing cisplatin treatment. The pathological mechanisms of cisplatin-induced nephrotoxicity include oxidative stress-related inflammation and DNA damage. Momordica balsamina (M. balsamina) is considered a 'gift of nature' due to its vast and high nutritional content, as well as its rich phytochemistry. Plant parts are prepared and consumed as teas, decoctions, infusions, or poultices. Folkloric uses of Momordica balsamina in South Africa include treating diabetes, liver, and intestinal problems. This study explored the nephroprotective effects of cisplatin supplementation with M. balsamina aqueous extract.
Methods
The effect of cisplatin supplementation with M. balsamina extract on HEK-293 viability was evaluated using a cell counting kit-8 (CCK-8). The effects of extract supplementation on cisplatin-induced DNA damage and inflammation were assessed using the MultiColor DNA damage assay and human cycloxygenase-2 (COX-2) ELISA kit, respectively. Cisplatin-induced oxidative stress was examined using the oxidative stress assay and catalase (CAT) as well as superoxide dismutase (SOD) activity analysed using CAT assay and (SOD) activity assays.
Results
Momordica balsamina extract potentiated the viability of cisplatin-treated cells with increased concentrations and incubation periods. Combinatorial treatment of HEK-293 cells with cisplatin and M. balsamina extract further modulated cisplatin-induced DNA damage and decreased the expression of proinflammatory marker COX-2. Moreover, extract supplementation decreased the generation of ROS positive cells concomitant with increased levels of antioxidant markers CAT and SOD.
Conclusion
Supplementation of cisplatin with M. balsamina aqueous extract exerted beneficial nephroprotective effects by decreasing cisplatin-induced COX-2-mediated inflammation, leading to a marked decrease of oxidative stress which ultimately decreases DNA damage. This substantiates the continued use of M. balsamina as a plant with medicinal value and provides an avenue for its use as an adjuvant therapy for ameliorating nephrotoxicity associated with cisplatin administration.

Exploration of the interactome of Plasmodium falciparum Glucose regulated protein 170.

Muzenda F[1], Mthembu W[1], Zininga T[1]
[1]Stellenbosch University

Background

In the main malaria agent, Plasmodium falciparum, the endoplasmic reticulum (ER) facilitates protein secretion and export of folded parasite proteins. The exported proteins are important for host cell remodelling to provide a conducive environment for parasite development and survival. The glucose-regulated proteins (Grps) are implicated in facilitating ER protein folding, trafficking, and activating the ER stress response pathways. P. falciparum Grp170 (PfHsp70-y/PfGrp170), is an essential protein for parasite survival, however, there is limited experimental evidence on the interaction network partners of PfGrp170. Therefore, this study sought to identify and construct the interactome of PfGrp170 to elucidate its function in parasite survival and development.

Methods

This study used an E. coli protein expression system to produce recombinant PfGrp170 protein that was purified using affinity chromatography. The purified protein was adsorbed to trinitrophenylated acid treated naked Salmonella Minnesota R595 bacteria. The reaction mixture was used to immunise rabbits to generate polyclonal antibodies against PfGrp170. Subsequently, the antibodies were used for co-immunoprecipitation of PfGrp170 interacting partners from a mixed stage P. falciparum D10 parasite lysate. The pulldown cargo was analysed using LCMS to identify the interacting proteins.

Results

Our results show the expression and purification of PfGrp170 for the first time, and we generated antibodies that were specific to PfGrp170 protein. The proteomic analysis of the co-immunoprecipitation pull down cargo shows that the PfGrp170 antibody interacts with more than 300 proteins which are mainly involved in stress response (PfGrp78), signalling (cGMP-dependent protein) and translation (eIF2alpha kinase PK4) among others. Majority of the interactors are proteins that localise to the cell membrane (77), nucleus (28), mitochondrion (13), ER (14). This suggest that PfGrp170 is integral to several cellular processes as it facilitates the protein control of proteins to many cell compartments.

Conclusions

This study shows evidence for the association of PfGrp170 with a large complement of secreted proteins that are potentially processed through the ER. Further experimental studies on unravelling the role of PfGrp170 in these interactions are warranted to elucidate the essential role it plays in parasite survival mostly the development of schizonts as a potential drug target in malaria.

The in vitro antimycobacterial and antioxidant activity of selected traditional medicinal plants

Koma P[1]
[1]University Of Limpopo

Background
Tuberculosis (TB) is caused by bacteria, particularly, Mycobacterium tuberculosis that targets the lung parenchyma. Tuberculosis is resistant to first-line drugs which include isoniazid, rifampicin, ethambutol, and pyrazinamide. Medicinal plants, in particular, the secondary metabolites they produce, have been reported to have potent bioactive compounds that can be used as alternative sources of therapeutic agents. The study aimed to determine the antioxidant and antimycobacterial activity of selected medicinal plants against Mycobacterium smegmatis.
Methods
Plant leaves were collected, dried, and extracted using solvents of varying polarities. Qualitative phytochemical analysis was determined using Thin Layer Chromatography (TLC). The quantity of phytochemicals was determined using a colorimetric assay. The antioxidant activity of the plant extracts was determined using the 2,2-Diphenyl-1-picrylhydrazyl, while the quantitative antimycobacterial activity was determined using broth micro-dilution assay.
Results
Methanol had the highest extraction yield from all the plants except for C. glabrum (84 mg) and E. capensis (210 mg) which had high extraction yield from the water extracts. More diverse phytoconstituents were observed on TLC plates. All plants showed to have saponins, tannins, flavonoids, alkaloids, cardiac glycosides, terpenes, and steroids. D. cinerea had the highest phenolic (237.47±0.49 mg GAE/g of extract), and tannin content (73,99±1,00 mg GAE/g of extract). B. salviifolia had the highest total flavonoid content (94,29±9,08 mg QE/g extract) while C. apiculatum had the highest total flavanol content (116,30±1,07 mg QE/g extract). DPPH free radical scavenging activity showed that all the plants had antioxidant compounds except for C. glabrum. Antimycobacterial activity against M. smegmatis using bioatography showed that B. salviifolia, C. macrocarpa, C. apiculatum, and B. saligna were able to inhibit growth of M. smegmatis. C. apiculatum had the lowest minimum inhibitory concentration of 0.16 mg/mL from dichloromethane and acetone extracts.
Conclusions
Therefore, selected medicinal plants have potent antioxidant and antimycobacterial activity.

In vitro validation of chemo-transcriptomic profiles for drug mode of action classification

Venter N[1], van Heerden A[1], Birkholtz L[1]
[1]Department of Biochemistry, Genetics and Microbiology, Institute for Sustainable Malaria Control, University of Pretoria, Hatfield, Pretoria 0028, South Africa

Background
Malaria is a severe infectious disease brought on by Plasmodium parasites. The emergence of resistance to current treatment measures is a significant challenge to the progress made in the eradication of malaria. As a result, developing new drugs with novel targets and modes of action (MoAs) is of utmost importance to address this resistance. Traditional phenotypic screening is often used to identify active compounds, but it has a low success rate for identifying compound targets and MoAs. Several direct, indirect, and computational approaches can be employed to identify a compound's target and MoA, however, these methods are time-consuming and labour-intensive. Machine learning techniques have not yet been widely used in antimalarial drug discovery to aid in identifying compound MoA, but a recent study by van Heerden et al. used a classification model based on chemo-transcriptomic fingerprints to identify a set of 50 transcripts that were predicted as biomarkers that can accurately stratify compounds based on their MoA. To apply these biomarkers to rapidly and cost-effectively predict a new antimalarial candidate's MoA requires in vitro validation. Here, a streamlined real-time quantitative PCR (qPCR) platform was generated to use these biomarkers in drug MoA classification studies.
Methods
Primer sets for single, parallel amplification of all 50 transcripts under uniform conditions were identified. In vitro cultivated P. falciparum parasites were treated with compounds and incubated for the duration of their active window. RNA was isolated with a reduced-variability approach and cDNA was synthesised. qPCR was used to investigate the expression levels of the transcripts after compound treatment, and data were analysed using relative quantification and the △△CT method.
Results
Among the projected biomarkers, only 43 transcripts were suitable for uniform qPCR conditions. Nonetheless, the remaining transcripts still exhibited unique transcription profiles for each examined compound. Moreover, these transcripts displayed similar profiles for compounds that possess similar MoAs.
Conclusion
This data indicates that these transcripts identified using machine learning can serve as predictive biomarkers for compound MoA. This study provides a means to indicate compound MoA quickly and specifically and can therefore help to accelerate antimalarial drug discovery.

Evaluating the role of the mitochondrial pyruvate carrier in Plasmodium falciparum parasites

Voges S[1], Da Rocha S[1], Birkholtz L[1], Niemand J[1]
[1]Department of Biochemistry, Genetics and Microbiology, Institute for Sustainable Malaria Control, University of Pretoria, Hatfield, Pretoria 0028, South Africa

Background
Pyruvate plays a key role in the energy metabolism of eukaryotic cells and acts as a metabolic switch between aerobic and anaerobic metabolism. This offers plasticity to energy metabolism, enabling the cell to switch to an alternative pathway when necessary. A causative agent of malaria, the Plasmodium falciparum parasite, exhibits divergent energy metabolism during the different life cycle stages. Anaerobic glycolysis is favoured during the rapidly proliferating asexual stages, while the transmissible sexual stages have a higher dependence on the TCA cycle. The mitochondrial pyruvate carrier (MPC) heterocomplex, composed of MPC1 and MPC2, has been identified as the transport complex responsible for the transport of pyruvate into the mitochondria. In P. falciparum, the putatively annotated genes mpc1 and mpc2 have yet to be characterised. In this study, we investigated the function of the MPC in P. falciparum.
Methods
A transgenic P. falciparum parasite line constitutively overexpressing the mpc1 and mpc2 genes was produced. The resulting transgenic MPC overexpression line was validated to confirm overexpression and then morphologically and functionally compared to the parental line.
Results
MPC overexpression did not affect asexual parasite proliferation, survival, or morphology when compared to the parental line. Similarly, the increased MPC expression did not affect mitochondrial viability. Chemical interrogation of the MPC overexpression line with an MPC inhibitor showed reduced sensitivity to the inhibitor, whereas interrogation with lactate transport inhibitors and inhibitors of mitochondrial function had no effect.
Conclusions
The observed differences between the transgenic and parental lines were due to the change in MPC expression rather than downstream metabolic effects and are consistent with an increase in MPC expression, confirming the presence of MPC function in P. falciparum parasites.

Prevalence and risk factors of drug-resistant Mycobacterium tuberculosis in rural communities of South Africa

Mashilo M[1], Kabue J[1], Potgieter N[1], Traore A[1]
[1]University Of Venda

Background: Globally, tuberculosis (TB) is the leading cause of mortality. TB is preventable and curable however drug-resistant TB (DR-TB) remains a menace to public health and health security. The study aimed to evaluate the prevalence and risk factors of DR-TB in rural Northern South Africa.
Methods: A cross-sectional study was conducted on 35 adult patients with active TB. Patient data were collected using a structured questionnaire including lifestyle behaviour, socioeconomic and environmental characteristics. A total of 70 samples including 35 blood and 35 sputum specimens were collected. A U-rapid test was performed on blood specimens to confirm HIV status. For sputum samples, DNA was extracted using an Allplex™ DNA extraction kit. The extracted DNA was subjected to multiplex real-time PCR using the Anyplex MTB/NTM kit and subsequently Allplex™ MTB/MDR/XDRe kit for the detection of MTB/NTM (Mycobacterium tuberculosis, Nontuberculous Mycobacteria) and MTB/MDR/XDR (Mycobacterium tuberculosis, Multidrug-Resistant, Extensively Drug-Resistant) tuberculosis respectively.
Results: Among the 35 participants, 54.3% (19/35) were females. The ages of the participants ranged from 23 to 72 years. The estimated prevalence of DR-MTB was 11.4% (4/35) in patients with ages ranging from 23 to 49 and all were unemployed. About 25% (1/4) of the DR-TB patients tested positive for HIV, whereas 75% (3/4) tested positive for co-infection of MDR-TB + NTM (50%, 2/4), and XDR-TB + NTM (25%, 1/35).
Conclusions: The prevalence and risk factors of DR-TB were evaluated, and co-infection appeared to be the major risk factor for DR-MTB infections followed by unemployment and mode of transport. Strategies for controlling DR-MTB should emphasize prioritizing MTB co-infection diagnosis/ treatment and encourage innovative or entrepreneurial knowledge/ thinking to mitigate unemployment in the study area.

Metabolomics and biochemical evaluation of heart tissue from a mitochondrial disease mouse model

Sweeney N[1], Terburgh K[1], Louw R[1]
[1]North-west University

Background:
Complex I (CI) deficiency is the most common defect associated with dysfunctional oxidative phosphorylation. The clinical presentations of this deficiency display a wide spectrum of variability, and considering its complexity, the exact mechanisms driving the disease pathology are not yet fully understood. As a critical component of the respiratory chain, CI plays an essential role in driving energy production. Cells with a high energy demand are thus more susceptible to this deficiency, for example cardiac cells. CI deficiency has been closely associated with a type of neurodegenerative disorder that mirrors aspects of Leigh syndrome, directing most research toward neurological manifestations. Consequently, our understanding of CI deficiency's impact on peripheral tissues remains sparse. The Ndufs4 knock-out (KO) mouse model is an established model to investigate CI deficiencies as it closely resembles Leigh Syndrome.

Methods:
A comprehensive biochemical evaluation, encompassing assays of respiratory chain complex activities (CI-IV) and an Oroboros respirometry analysis, coupled with a dual approach of targeted and untargeted metabolomics were employed to gain insight into the tissue-specific impact and mechanisms of the development of Leigh Syndrome. The multi-platform metabolic profiling of heart tissue from Ndufs4 KO and WT mice (n=22) included GC-TOFxMS, LC-MS/MS and 1H-NMR spectroscopy analyses.

Results:
The biochemical evaluation and respirometry analyses revealed that CI activity in the heart tissue of KO mice was reduced by 95% ($P<0.001$), while CI driven respiration was reduced by 66.1% ($P<0.001$), compared to the WT mice. The multi-platform metabolomics analyses revealed distinctive metabolic variances in the Ndufs4 KO group when compared to the WT group, highlighting numerous metabolites and their associated pathways that were significantly altered – several of which have been previously implicated in mitochondrial disease.

Conclusions:
Although cardiac symptoms are not typically emphasized as having a prominent involvement in CI deficiency and Leigh syndrome, our findings demonstrate that cardiac tissue is indeed affected by the consequences of CI deficiency, offering a broader perspective on the multi-systemic nature of the disorder and its potential wider physiological impact. These findings warrant further tissue-specific investigations, which could prove instrumental in the development of effective therapeutic strategies for this debilitating disease.

Investigating the antiproliferative effects of the novel flavonoid 5-methoxyjamaicin isolated from Erythrina abyssinica

Ntisa B[1], Kamdem M[2], Ndinteh D[2], Choene M[1]
[1]Department of Biochemistry, University Of Johannesburg, [2]Centre of Natural Product Research, Department of Chemical Sciences, University of Johannesburg

Background: Globally, cancer is regarded the second leading cause of mortality, with its burden increasing rapidly every year. Pancreatic and cervical cancer are among the top 10 deadliest types of cancer, with pancreatic ranking the 7th in both men and women, whereas cervical cancer ranks the 4th leading cause of mortality. Due to non-specificity of medicine, adverse effects on healthy cells, resistance and metastasis of cancer cells, current treatments are rendered ineffective. As such, it has become important to identify effective, bio-friendly, and affordable alternative cancer treatments.

Methods: The compound was isolated from a methanol extract of E. abyssinica. The extract was subjected to column and thin layer chromatography, and through a series of 1 and 2 dimensional spectroscopic techniques, the name and structure of the compound was determined. The anticancer activity of the compound was studied by conducting a series of cytotoxic, apoptotic, wound healing and gene expression analyses.

Results: The novel compound was established to be 5-methoxyjamaicin, and in vitro studies showed the compound had cytotoxic activity against pancreatic and cervical cancer, and showed no cytotoxicity against normal cells. This cytotoxic activity was accompanied by high caspase and low adenosine triphosphate levels in cancer cells, and inhibition of metastasis. Apoptosis was characterized morphologically by cell shrinkage, chromatin condensation, loss of epithelial morphology, nuclear fragmentation, and loss of cell-to-cell interactions. Furthermore, it was proposed that the compound utilized the intrinsic apoptotic pathway to exhibit its cytotoxic effect in cervical cancer cells, whereas the exact pathway in pancreatic cancer cells could not be determined.

Conclusion: The findings of this study suggest that 5-methoxyjamaicin may serve as a possible alternative cancer therapy that is toxic to cancer cells but not normal cells. However, further research is needed to identify and understand other molecular, cellular, and physiological processes that the compound may affect.

Phytochemical content, antioxidant and antimicrobial activities of some medicinal plants used for dental health care in Limpopo province

Mulaudzi L[1], Ndhlala A[2], Masoko P[1]
[1]University Of Limpopo, [2]University of Limpopo

Oral hygiene is critical for human health. Dental infections are a major health problem around the globe. The current study evaluated the phytochemical composition, antioxidant, and antimicrobial activities of five plants used for oral health care in a community in Limpopo Province. The five plant leaves (Dicerocaryum eriocarpum, Euclea divinorum Hiern, Erythrina lysistemon Hutch, Aloe falcata Baker and Drimia elata Jacq) were collected from Ga-Molepo community in Limpopo, South Africa, dried, and ground to powder. Extraction was done using four solvents with varying polarities. Biochemical tests were performed to detect phytoconstituents using colourimetric assays. Thin layer chromatography (TLC) with semi-automated high performance liquid chromatography (HPLC) was used to separate components in the plants, vanillin-sulphuric acid was sprayed on the plates and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) dye. The major constituents (Flavonoids, tannins and phenolics) were quantified using the folin-ciocalteau method. Antioxidants were evaluated using a DPPH free radical scavenging assay and Ferric-reducing power assay. The antimicrobial activity was evaluated using bioautography and micro-broth dilution assays. The HPTLC plate was visualised at three distinct UV radiation wavelengths (254 nm, 366 and white light). In contrast to white light and UV radiation at 254 nm, 366 nm was found to be the most effective for visualising and better separation of compounds in all mobile phases (Chloroform ethyl acetate) CEF and (Ethyl acetate methanol-water) EMW mobile phases. Euclea divinorum Hiern exhibited the best anti-radical activity on both methanol and water extracts in all mobile phases. DPPH-free radical scavenging assay water and methanol extracts exhibited the highest percentage of antiradical activity on the ferric-reducing power assay. Euclea divinorum Hiern leaves exhibited the highest total phenolics and flavonoids of all the extractants used. The methanol Aloe extracts had the best minimum inhibitory concentration (MIC) against P. aerugeinosa (0.31 mg/ml).

Evaluation of potent quinoxaline derivatives as potential anti-proliferative agents against lung cancer cells challenged with SARS-Cov-2 spike protein variants

Nyama M[1], Nxumalo W[2], Matsebatlela T[1]
[1]University Of Limpopo, [2]University of Limpopo

Background
Lung cancer is one of the leading causes of mortality in both men and women worldwide, and it accounts for most cancer-related deaths. The recent COVID-19 pandemic exacerbated the aetiology of the disease, resulting in prolonged alveolar damage and acute respiratory failure; hence, lung cancer patients were found to be at an increased risk of severe outcomes. Therefore, a novel cohort of quinoxaline derivatives designed to possess a wide spectrum of biological activities was synthesized with promising targeted and selective drug activity.

Methods
The quinoxaline derivatives, LAM21D and LAM29A, were screened for their antioxidant abilities using DPPH free radical scavenging activity and ferric reducing power assay. Their inhibitory effect on A549 lung cancer cells and Raw 264.7 cells was evaluated using MTT cell viability assay and Muse® count and viability assay. Muse® Annexin-V and dead cell assay, Muse® oxidative stress assay as well as Nitric oxide production assay were employed to evaluate the effect quinoxalines had on A549 lung cancer cells which were challenged with SARS-Cov-2 spike protein. The effect that quinoxaline derivatives had on cell cycle progression of A549 lung cancer cells was also evaluated. Furthermore, in silico ADMET and molecular docking studies were performed using the quinoxaline derivatives.

Results
Quinoxaline derivatives, LAM21D and LAM29A, showed impressive scavenging activity potential as well as good reduction potential activities. LAM21D had minimal inhibitory effect on both A549 cells and Raw 264.7 cells while LAM29A showed inhibitory effect on A549 cells in a time dependent manner and exerted cytotoxic effects on Raw 264.7 cells. An increase in ROS and NO production was observed using LAM21D which resulted in an increase in cell death at lower concentrations and was also shown to arrest the cell cycle of A548 cells at G2/M phase in Muse® cell cycle analysis. Molecular docking results also indicated potential MAPK13 p38 suppression by both LAM21D and LAM29A and potential SARS-Cov-2 S protein activity suppression.

Conclusions
Both quinoxaline derivatives can further be studied as potential anti-inflammatory as well as anticancer candidates. The results from this study could potentially provide an alternative targeted therapy possibility for both cancer and Covid-19.

Characterizing an essential Mycobacterium tuberculosis zinc metalloprotease Rv2017 as a potential novel drug target

Senzani S[1], Senzani S[1]
[1]University Of Kwa-zulu Natal (medical Campus)

Background: Mycobacterium tuberculosis (Mtb), the causative agent of tuberculosis (TB), causes almost two million deaths annually. In fact, TB is the single most infectious bacterial disease in the world. However, despite TB being curable, total eradication of it is difficult for many reasons such as its long treatment regimen and the spread of drug resistance strains. It is therefore imperative that new treatment strategies emerge. Zinc metalloproteases are virulence factors which contribute to the pathogenicity of bacterial species. Mtb possesses novel zinc metalloproteases which are hypothesized to represent promising pharmacological targets. This project aims to characterize the role of the essential Mtb Rv2017 zinc metalloprotease and to deduce it’s potential as a new target for drug discovery. The objectives of this project include down-regulating the target gene and thereafter examining the resultant mutant's phenotype.

Methods: The aims and objectives were accomplished by the creation of a gene knock-down mutant strain with reduced expression of the target gene by inserting a tetracycline regulated operator upstream the gene. Thereafter, examination of the mutant’s growth, physiology, antimicrobial tolerance and virulence was conducted.

Results: Bioinformatics analysis revealed that Rv2017 potentially regulates the repair of DNA damage. Additionally, the mutant strain displayed slow and stunted growth, impaired biofilm formation and exhibited increased susceptibility to antibiotics and UV radiation.

Conclusions: The results indicate that the Rv2017 gene does contribute to the growth and physiology of Mtb and therefore could serve as a new drug target: potentially revolutionizing TB treatment.

Evaluation of phytochemicals, antioxidant and antimycobacterial activity of selected plants used for treatment of tuberculosis and related symptoms

Mashilo M[1], Matotoka M[1], Masoko P[1]
[1]University Of Limpopo, Department of Biochemistry, Microbiology and Biotechnology

Background
The aim of the study was to investigate the phytochemical properties, antimycobacterial and antioxidant activity of eleven selected plants used for treatment of tuberculosis and related symptoms. Tuberculosis (TB) is a contagious disease caused by a pathogen called Mycobacterium tuberculosis complex. TB is treated with antitubercular drugs but due to emergence of multidrug-resistant and extensively drug-resistant TB cases this treatment strategy remains a challenge. Medicinal plants have proven to have potential in treating many diseases.
Methods
The major phytochemicals were quantified using reagent assays and analysed using standard curves. The antioxidant activity of the plants was screened using DPPH and further quantified. The antibacterial activity was determined using bioautography and serial broth micro dilution assay.
Results
Phytochemicals screening showed that all the plants had tannins, flavonoids and cardiac glycosides. The quantification results showed that Tarchonanthus camphoratus acetone extract had the highest total phenolic, tannin and flavonoid content while Tabernaemontana elegans acetone extract had the lowest total phenolic and tannin content and Lippia javanica had the lowest total flavonoid content. All the plant extracts had the antioxidant compounds in all the mobile system and the quantification results showed that the Tarchonanthus camphoratus extract had the highest percentage scavenging activity followed by Combretum hereroense extract while Tabernaemontana elegans extract had the lowest percentage scavenging activity. The bioautography results showed that some of the plants extract had the antimycobacterial compounds and the serial broth dilution assay showed Senecio macroglossus extracts (actone and water) and Tetradenia raparia extract (dichloromethane) had the lowest MIC value of 0.16 mg/mL while Tabernaemontana elegans extract (water) and Lippia javanica extracts (methanol and water) had the higher MIC value of greater than 2.5 mg/mL.
Conclusions
However, further studies should be done on Mycobacterium tuberculosis to validate the activity.

Optimisation of E. coli based complementation assay to screen Hsp70 inhibitors

Ncube H[1], Dali U[1], Dongola T[1], Shonhai A[1]
[1]Student

Background

Heat shock protein 70 (Hsp70) is a conserved and highly ubiquitous molecular chaperone whose induced expression plays an important role in the development of organisms. Hsp70 is important for the survival and pathogenicity of infectious agents including Mycobacterium tuberculosis (T.B agent) and Plasmodium falciparum (most lethal form of malaria). Hsp70 also plays an important in conferring both M. tuberculosis and P. falciparum with fitness to survive adverse conditions including drug pressure. As such, Hsp70 is implicated in drug resistance. For this reason, Hsp70 is a potential antimicrobial drug target. We optimised a high throughput in cellulo assay to screen Hsp70 inhibitors. E. coli Hsp70 (DnaK) is essential at high temperatures (>40 °C). As such, E. coli cells either deficient of DnaK or habouring a native mutated DnaK serve as a model for Hsp70 functional studies. By heterlogously expressing functional Hsp70 in these cells, the growth recovery at temperatures >40 °C serves as a useful complementation assay study. As such the inhibition of Hsp70 leads to cell death >40 °C. In the current study, we optimised the use of E. coli dnaK756 to screen Hsp70 inhibitors targeting P. falciparum Hsp70, E. coli DnaK and M. tuberculosis DnaK.

Methodology

E. coli dnaK756 competent cells were transformed with plasmid constructs encoding P. falciparum Hsp70, E. coli DnaK and M. tuberculosis DnaK. The cells were induced to express the Hsp70 proteins and incubated overnight at permissive growth temperature (30 °C) and non-permissive growth temperature (>40 °C). The growth assay was conducted using agar well plates and liquid broth assay (in 96 well plate system coupled to OD readings using a UV-Vis spectrometer)

Results: E. coli DnaK and M. tuberculosis DnaK were all able to grow at 30 °C even in the presence of Hsp70 inhibitors such as colistin sulphate. However, growth was abrogated at 43.5 °C when the cells were cultured in the presence of known Hsp70 inhibitors. Conclusions: The finding confirm the utility of E. coli complementation assay in screening Hsp70 inhibitors in cellulo. Since the assay was compatible with a 96 well plate system, it could be applied for inhibitor screening.

In vitro antimycobacterial activity and proteomic profile of selected medicinal plants extracts used traditionally to treat TB-related symptoms

Matlala M[1], Moganedi K[1], Masoko P[1]
[1]University of Limpopo

Mycobacterium tuberculosis is a causative agent of tuberculosis and uses specialized mechanisms to evade the immune system to ensure its survival and persistence. This study uses a bottom-up proteomic approach to trace proteomic changes correlated with exposure of Mycobacterium smegmatis ATCC 14468 to bioactive extracts from medicinal plants. The leaves and stem extracts of nine plants used traditionally to treat TB- related symptoms and which displayed antimycobacterial activity, were used in differential protein expression assays with treated and untreated M. smegmatis cells alongside the antibiotic rifampicin. The phyto-constituents were detected using biochemical tests. Distinct differences in the expressed protein pattern of plant extract treated M. smegmatis on SDS-PAGE were observed. Downregulation of several proteins was observed at 6 h, 12 h, and 18 h of the untreated bacterial cells and at 24 h and 48 h of rifampicin and plant extracts treated bacterial cells respectively. The upregulated proteins were observed at 6 h, 12 h and 18 h following rifampicin and plant extracts treatment of the bacterial cells. The band intensity of the differentially expressed proteins demonstrated that the extracts induced expression of some specific proteins at a high level compared to the positive control (rifampicin). This indicates that the plant extracts induced a different response mechanism in M. smegmatis. However, much work remains to be done on the systematic assessment of anti-TB efficacy of local plants against pathogenic Mycobacterium species, both in vitro and in vivo.

Adapting an HIV-1 subtype c plasmid for dolutegravir resistance studies

Matamela T[1], Matume N[2], Bessong P[1]
[1]University of Venda, [2]University of Kwazulu-Natal

Background
Human immunodeficiency virus is the ninth leading cause of death in most developing countries, with South Africa being one of the most affected countries. Antiretroviral treatment (ART) has reduced mortality and morbidity due to HIV. However, drug resistance is limiting the effectiveness of ART. Dolutegravir, a strand transfer inhibitor, was introduced in the standard first-line treatment regimen in South Africa in September 2019. This current study adapted an HIV-1 subtype C plasmid for Dolutegravir resistance studies.
Methodology
To document Integrase (IN) Surveillance Drug Resistance Mutations (SDRMs) and identify putative mutations of interest, HIV-1 subtype C IN sequences from ART naïve individuals in Africa were retrieved from the HIV database within Los Alamos Pathogen Research Databases and subjected to Calibrated Population Resistance (CPR) program within Stanford HIV drug resistance database. The T66S and S147R putative drug resistance mutations were selected and introduced in silico using Gibson assembly in Geneious prime version 2021.2.2. The mutations were also introduced in-vitro by Site-Directed Mutagenesis (SDM). The successful introduction of the mutations was verified by sanger sequencing.
Results
Out of 1206 integrase sequences from Africa that were retrieved, 12 (1.0%) of the sequences contained SDRM, and 22 (1.8%) had putative drug resistance mutation. The MJ4 subtype C plasmid was successfully adapted with T66S putative mutation and was verified by sequencing.
Conclusion
Mutations in positions 66 and 147 are known to cause resistance to other integrase strand transfer inhibitors (ISTIs) such as Raltegravir (RAL) and Elvitegravir (EVG); less is known about their impact on Dolutegravir. This study serves as a baseline for future phenotypic studies that will functionally characterize the impact of the T66S putative mutation on Dolutergravir resistance as well as the impact of the other identified putative mutations.

Functionalised Gold Nanoclusters for Targeted mRNA Delivery to Breast Cancer cells in vitro

Singh N[1], Singh M[1]
[1]University of KwaZulu-Natal

Background: Breast cancer is the leading cancer affecting South African women. Conventional treatments are limited by their invasivity, adverse health effects, and ineffectiveness at preventing recurrence. In recent years, mRNA therapies have emerged as a promising alternative cancer treatment. However, the delivery of therapeutic mRNA remains challenging due to mRNA's inherent instability and susceptibility to degradation. Gold nanoclusters (GNCs) have gained significant interest as potential delivery vectors owing to their ultrasmall size (< 2 nm), ease of synthesis, ability to be functionalised and low toxicity in biological systems. This study assessed the efficacy and cytotoxicity of functionalised folate-targeted and non-targeted GNCs in binding and delivering Fluc-mRNA to breast cancer cells in vitro.

Methods: GNCs were synthesised and encapsulated with chitosan (CGNCs) and folate (FCGNCs) to mediate mRNA binding and promote cellular uptake by folate-receptor targeting. Fluc-mRNA was bound to the functionalised GNCs, and all GNCs and mRNA-nanocomplexes were physico-chemically characterised. The compaction and protection abilities of the functionalised GNCs were evaluated using gel and fluorescence-based assays. The transgene expression and cytotoxicity profiles of the nanocomplexes were determined in the HEK293 (embryonic kidney) and MCF-7 (breast adenocarcinoma) cells.

Results: Successful formation and functionalisation of the GNCs were confirmed by UV-vis spectroscopy and Fourier-transform infrared spectroscopy. All GNCs displayed advantageous hydrodynamic sizes (ranging from 60 nm to 140 nm) and colloidal stability with narrow size distributions from the nanoparticle tracking analysis (NTA), and transmission electron microscopy (TEM) exhibited well-dispersed spherical GNCs. Functionalised GNCs favourably bound, compacted, and protected the Fluc-mRNA. All CGNCs were well tolerated in both cell lines with cell proliferation noted in the HEK293 cells. Gene expression by FCGNCs was greater than the non-targeted CGNCs in both cell lines, and significantly higher in the MCF-7 than in the HEK293 cells, confirming enhanced uptake by the folate-receptor positive MCF-7 cells.

Conclusion: Results indicate that this innovative approach holds immense promise in mRNA-based therapies for breast cancer as both GNCs are safe and effective delivery vehicles in vitro.

Phytochemical profiling and in silico evaluation of Atherixia phylicoides methanolic extracts antidiabetic potential through α-amylase and α-glucosidase inhibition

Ngoveni H[1], Madala N[1], Ramabulana A[1], Tshidino S[1]
[1]University Of Venda

Background:
Atherixia phylicoides DC (bush tea) is an herbal tea used as a natural remedy by South African communities to treat diverse illnesses including blood circulation problems and diarrhoea. Thus, A. phylicoides may contain several known and unknown phytochemicals with antidiabetic potential. This study was aimed at profiling the A. phylicoides phytocompounds and in silico evaluate its stem and leaf methanolic extracts, and antidiabetic potential through α-amylase and α-glucosidase molecular docking.

Methods:
Atherixia phylicoides were collected from the Mbhokota village mountainous area. The dried and ground stem and leaf samples were defatted using dichloromethane and extracted using absolute methanol. Phytochemicals in non-defatted and defatted methanolic extracts were profiled using DPPH, vanillin-sulfuric acid, and ferric chlorine reagents for the thin-layer chromatography (TLC), and Liquid Chromatography Mass Spectrometry (LC-MS) to verify and identify the presence of antioxidants, phenols, alkaloids, flavonoids, saponins, and terpenoids, using rutin and gallic acid as positive controls. A. phylicoides stem methanolic extracts selected compounds, kaempferol 3-O-(6-O-malonyl-beta-D-glucoside) and Silyhermin B were docked against α-amylase and α-glucosidase to evaluate their antidiabetic potential using acarbose as positive control.

Results:
Both non-defatted and defatted A. phylicoides leaf and stem methanolic extracts have phytochemicals such as phenols, alkaloids, and saponins with antioxidant activity. Kaempferol 3-O-(6-O-malonyl-beta-D-glucoside) and Silyhermin B in non-defatted and defatted stem methanolic extracts of A. phylicoides showed strong binding affinities against α-amylase (-9.0 and -9.9 Kcal/mol) and α-glucosidase (-7.8 and -8.8 Kcal/mol) respectively when compared with acarbose, a positive control with binding affinities of -8.2 and -7.8 Kcal/mol against both enzymes. Additionally, two compounds in the non-defatted and defatted leaf methanolic extracts also showed very strong binding affinities when docked against α-amylase (-10.6 and -12.4) and α-glucosidase (-9.9 and -10.9). Taken together, this study revealed that both non-defatted and defatted stem and leaf methanolic extracts contain phytochemicals with antidiabetic effect, suggesting their potential as novel antidiabetic agents.

Conclusion:
Defatted methanolic extracts of A. phylicoides leaves and stem contained phytochemicals with antidiabetic potential. Further characterization of the A. phylicoides phytocompounds may result in novel antidiabetic drug candidates

Progestins used in injectable contraception differentially downregulate the expression of hepatic sex hormone binding globulin

Simons M[1], Avenant C[2], Hapgood J[2,3], Africander D[1]
[1]Department of Biochemistry, Stellenbosch University, [2]Department of Molecular and Cell Biology, University of Cape Town, [3]Institute of Infectious Disease and Molecular Medicine, University of Cape Town

Background
Sex hormone binding globulin (SHBG) is a homodimeric glycoprotein primarily produced and secreted by the liver, and transports sex steroids to target tissues. Low SHBG levels have been associated with several diseases in women, including cardiovascular disease, polycystic ovary syndrome, and type 2 diabetes. Factors such as pro-inflammatory cytokines and hormonal contraception are known to affect SHBG levels. Previous studies have shown that the injectable progestin-only contraceptives, depo-medroxyprogesterone acetate intramuscular (DMPA-IM) and norethisterone enanthate (NET-EN) decrease SHBG levels. The mechanisms underlying SHBG regulation by these progestins are, however, unclear.

Methods
We investigated the regulation of SHBG by MPA and NET in the human HepG2 liver cell line using real-time quantitative PCR (qPCR) to evaluate effects on SHBG mRNA expression and ELISA to evaluate effects on SHBG protein secretion. The role of steroid receptors and two critical transcription factors in the regulation of SHBG expression, hepatocyte nuclear factor 4 alpha (HNF4α) and peroxisome proliferator activated receptor gamma (PPARγ), was assessed by using a combination of steroid receptor antagonists, an inhibitor of PPARγ, and qPCR.

Results
We found a greater decrease in hepatic SHBG mRNA and protein expression for NET than MPA, with multiple steroid receptors involved in the responses. MPA and NET both decreased HNF4α mRNA expression, suggesting that SHBG levels are decreased via a decrease in HNF4α levels. Although both MPA and NET increased the mRNA expression of PPARγ, the increase was greater with NET, suggesting that the greater decrease in SHBG levels by NET may be due to the greater increase in PPARγ expression.

Conclusions
These findings suggest that MPA and NET differentially regulate SHBG expression via an indirect mechanism involving multiple steroid receptors and the differential regulation of the key transcription factors HNF4α and PPARγ to downregulate SHBG transcription. These findings are relevant to female reproductive health as bioavailable androgen and estrogen levels are influenced by SHBG levels.

The resistome of the human gut is influenced by microbiome composition and E. coli in particular

Fri J[1,2], Raphalalani M[1,2], Mavhandu-Ramarumo L[1,2], Bessong P[1,2,3]
[1]SAMRC-UNIVEN Antimicrobial Resistance and Global Health Research Unit, University Of Venda, Thohoyandou, [2]HIV/AIDS and Global Health Research Programme, University of Venda, [3]School of Health Sciences, University of KwaZulu-Natal

Background
Antibiotic resistance is a global health problem. The human gut microbiome is implicated in antibiotic resistance acquisition and transmission dynamics. This study aimed to determine the potential influence of the human gut microbiome on the gut resistome. Considering E. coli as a proxy for studying gut microbiome characteristics, including antibiotic resistance in diseased and non-disease states, this study also determined the potential influence of the gut microbiome on E. coli resistome.
Methods
The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines was used to systematically review published studies that characterised gut microbiome and resistome using metagenomic analysis and/or those that reported gut E. coli resistance in healthy individuals. Changes in the diversity and abundance of the gut microbiome and resistome across different time points and participant groups and the microbial composition of the gut harbouring antibiotic-resistant E. coli were recorded.
Results
A higher diversity and abundance of microbial taxa were associated with a concurrent increase in the diversity and abundance of the overall gut resistome. The gut bacteriome of younger infants exhibited a greater diversity and abundance and was also higher in diversity and abundance of antibiotic-resistant genes (ARGs) compared to older infants. A strong positive correlation was observed between the compositional relative abundance of Proteobacteria and ARGs abundance. Enterobacteriaceae, particularly Escherichia coli, was the primary source of gut ARGs within this phylum. To determine the potential influence of gut microbiome on E. coli resistome, a high level of heterogeneity and a significant low level of granularity in the data sets prevented meaningful analysis. The findings of this study also revealed that human gut microbiome studies that explore the gut resistome using deep sequencing approaches in potentially healthy individuals are uncommon.
Conclusions
Microbiome composition influences the human gut resistome, especially members of the Enterobacteriaceae family, particularly E. coli. This implies that employing a strategy aimed at targeting Enterobacteriaceae, focusing on E. coli, could play a significant role in controlling the development and subsequent spread of antibiotic-resistant bacterial infections in humans.

Progestin-induced hypoestrogenism: A role for 17β-hydroxysteroid dehydrogenases?

Lalla J[1], Jackson H[1], Avenant C[2], Hapgood J[2], Storbeck K[1], Africander D[1]
[1]Stellenbosch University, [2]University of Cape Town

Background:
Clinical data have shown that women using the progestin-only injectable contraceptives depo-medroxyprogesterone acetate intramuscular (DMPA-IM) or norethisterone enanthate (NET-EN) have reduced serum 17β-estradiol (E_2) levels. Considering the protective effects of E_2 for viral infections in the female genital tract, the use of these progestins in contraception may be linked to increased risk of HIV-1 acquisition. As the family of 17β-hydroxysteroid dehydrogenase (17βHSD) enzymes is involved in the biosynthesis and metabolism of estrogens (E_2 and estrone (E_1) and androgens (testosterone and androstenedione; precursors for estrogen biosynthesis), we investigated the effects of MPA and NET on the expression and activity of the 17βHSD1, 17βHSD2, and 17βHSD5 isoforms.
Methods:
The mRNA expression of these isoforms was evaluated in the human Chub-S7 pre-adipocyte cell line, which endogenously expresses these enzymes, by real-time quantitative PCR. Steroid conversion assays followed by ultra-high performance liquid chromatography-tandem mass spectrometry were used to assess the activity of the enzymes in the pre-adipocytes as well as the non-steroidogenic HEK293 cell line in which the respective exogenous enzymes were transiently expressed.
Results:
Steroid conversion assays showed that MPA decreased the conversion of E_1 to E_2, while increasing the conversion of E_2 to E_1 in the Chub-S7 pre-adipocytes. Although this suggests a role for 17βHSD1 and 2, it is unlikely that these isoforms are involved as MPA did not have any effect on 17βHSD1 or 17βHSD2 overexpressed in HEK293 cells. NET increased the mRNA expression of 17βHSD2 and 17βHSD5 in the pre-adipocytes but did not affect substrate conversion in the Chub-S7 pre-adipocytes or the HEK293 cells. Both MPA and NET decreased 17βHSD5 activity in both cell models.
Conclusions:
Results suggest that MPA decrease systemic E_2 levels by reducing the conversion of E_1 to E_2 and promoting the conversion of E_2 back to E_1 in pre-adipocyte cells, independent of 17βHSD1 and 17βHSD2. The decreased activity of 17βHSD5 induced by both MPA and NET may result in lower testosterone levels, which may also contribute to decreased E_2 levels. Our findings suggest that the actions of both progestins in pre-adipocytes may contribute to the decrease in systemic E_2 levels in women on these contraceptives.

Characterization of Entamoeba spp. and Giardia lamblia

Netshivhazwaulu O[1], Samie A[2]
[1]University Of Venda, [2]University of Venda
Molecular Characterization of Entamoeba. Spp and Giardia lamblia

Netshivhazwaulu Ompilela1, Amidou Samie1
1. Department of Biochemistry and Microbiology, Faculty of Science, Engineering and Agriculture, University of Venda, Private Bag X5050, Thohoyandou, South Africa

Background: Entamoeba histolytica and Giardia lamblia are the causative agents of amoebiasis and giardiasis respectively. Infection by these two intestinal, protozoan parasites often result in diarrhoea mostly in children under the age of five and immune-compromised individuals around the world. The present study aims to characterise these protozoan species from diarrhoeal patients in Giyani and Pretoria, South Africa.
Materials and methods: One hundred and fifty (150) faecal samples that were previously collected and stored in the parasitology laboratory at the University of Venda were used for DNA extraction. Obtained DNA was used to screen Entamoeba and Giardia genus by primary Nested Polymerase Chain Reaction (PCR), and the second round of the Nested PCR was used to detect the different protozoan species followed by gel electrophoresis and the gel was visualised under ultraviolet light and all the amplicons with a clear bands were sent for sequencing.
Results: Out of all 150 samples tested, 36(24%) were positive for Entamoeba spp. From 36 positive samples, 18(50%) were positive for Entamoeba moshkovskii, 15 (42%) were positive for Entamoeba histolytica. Entamoeba dispar was not detected, however the present study identified Entamoeba muris which is a rare species in humans, interestingly this species was detected from 3(8%) samples. Of all 150, only 2 samples were positive for Giardia lamblia PCR and a prevalence of 1.13% was recorded.
Conclusion: From the present study, it can be concluded that the non -pathogenic E. moshkovskii is commonly associated with diarrhoeal stool than E. dispar. The findings in this study suggest the zoonotic potential of E. muris and raises questions about the pathogenicity and transmission routes of this species to humans. It can be further concluded that there is a decrease in the prevalence of Giardia lamblia.
Key words: Diarrhoea; Entamoeba histolytica; Giardia lamblia; Entamoeba moshkovskii, Molecular Biology, Epidemiology.

Pro-apoptotic effects of Didox on lung cancer cells challenged with SARS-CoV 2 recombinant spike proteins

Hlope K[1], Matsebatlela T[1]
[1]Department of Biochemistry, Microbiology and Biotechnology, University Of Limpopo

Lung cancer accounts for the high cancer related mortality in South Africa. The reduced lung function and immunosuppression in lung cancer patients increases their susceptibility to severe coronavirus diseases 2019 (COVID-19) fatalities. COVID-19 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a highly transmissible virus which causes a strong inflammatory response that may results in tissue damage. Various therapeutics have been researched for the development of treatments options for SARS-CoV-2 effects on lung cancer. Therefore, the aim of this study was to determine the effects of Didox on A549 lung cancer cells that were exposed to SARS-CoV-2 recombinant spike proteins. A549 cells were treated with SARS-CoV-2 spike proteins and Didox at various concentrations. Following treatment, the nitric oxide (NO) and reactive oxygen species (ROS) production of A549 cells was evaluated using the nitric oxide assay and the oxidative stress assay. The nuclear integrity and induction of apoptosis was also evaluated using the annexin V and PI staining assay. The production of NO and ROS induced by SARS-CoV-2 recombinant spike proteins was reduced when A549 cells were treated with Didox at various concentrations. Also, the population of apoptotic cells increased with exposure of A549 cells to SARS-CoV-2 spike proteins and Didox. It was hypothesized that in the presence of SARS-CoV-2 spike proteins, Didox accelerated cell death in A549 lung cancer cells. Didox exhibits potential anti-inflammatory and proapototic effects required for the treatment of SARS-CoV-2 in lung cancer thus further inflammation studies on Didox are recommended.

Progestins used in injectable contraception decrease aromatase expression and activity

Hanslow M[1], Janse van Vuuren M[1], Avenant C[2], Hapgood J[2,3], Louw-du Toit R[1], Africander D[1]
[1]Stellenbosch University, [2]University of Cape Town , [3]Institute of Infectious Disease and Molecular Medicine, University of Cape Town

Background: Progestins are synthetic compounds designed to mimic the effects of natural progesterone via the progesterone receptor and are commonly used in contraception. Data from the Women's Health, Injectable Contraceptive and HIV (WHICH) clinical trial revealed that serum 17β-estradiol (E_2) levels were significantly decreased in South African women randomized to the progestin-only injectable contraceptives intramuscular depo-medroxyprogesterone acetate (DMPA-IM) and norethisterone enanthate (NET-EN). Given that low E_2 levels are associated with an increased susceptibility to HIV, and the high prevalence of HIV in South Africa, especially in young women, it is crucial to understand the mechanism(s) whereby these progestins decrease E_2 levels. Since the aromatase enzyme is responsible for the biosynthesis of E_2 from testosterone, this study investigated whether the decrease in E_2 levels in response to MPA and NET may be due to these progestins decreasing the expression and/or activity of the aromatase enzyme.

Methods: Different model systems were optimized to investigate progestin effects on the expression and activity of the human aromatase enzyme. These included a cell line known to endogenously express aromatase (JEG-3 cells), a non-steroidogenic cell line model overexpressing aromatase (HEK293 cells), as well as a model of recombinant aromatase (CypExpress). A combination of real-time quantitative PCR and western blotting was used to measure aromatase expression, while ultra-high performance liquid chromatography tandem mass spectrophotometry was used to evaluate the activity of aromatase in steroid conversion assays.

Results: We showed that MPA and NET decreased aromatase activity in the human placental JEG-3 cell model, correlating to a decrease in endogenous mRNA and protein expression. MPA and NET also decreased the activity of aromatase overexpressed in the HEK293 cell model, as well as in the recombinant CypExpress model.

Conclusions: These findings showing decreased aromatase expression and activity provide at least one possible mechanism underlying the reduced systemic levels of E_2 observed in women using DMPA-IM and NET-EN as injectable contraceptives. Further kinetic analysis of the inhibition of aromatase activity by MPA and NET would provide insight into the effects of these progestins on steroidogenesis in vivo.

Using a 1H-NMR urine metabolomics approach to characterise HIV/TB coinfection and the effect of antiretroviral treatment

Ahumada Prado S, Luies L[1], Mason S[1]
[1]North West-University Focus Area Human Metabolomics
Title: Using a 1H-NMR urine metabolomics approach to characterise HIV/TB coinfection and the effect of antiretroviral treatment

Background: Tuberculosis (TB) and the human immunodeficiency virus (HIV) are the leading global infectious diseases, whose coinfection (HIV/TB) presents increased risks and complexities. Although progress has been made in understanding and treating each disease separately, the metabolic implications of their coinfection and the effects of antiretroviral therapy (ART) on these processes are not well understood. This study uses a standard urine-based 1H-NMR metabolomics approach to explore the unique metabolic alterations associated with HIV/TB coinfection and the effects of ART on these metabolomics profiles.

Methods: Urine samples were collected from participants in six groups: (1) treatment naïve HIV/TB coinfection (n=9), (2) HIV/TB coinfection on ART (n=18), (3) HIV only on ART (n=5), (4) untreated HIV only (n=9), (5) untreated TB only (n=41), and (6) healthy controls (n=32). HIV status was determined based on serum testing, and active pulmonary TB was identified through GeneXpert testing. The research utilised a fixed-dose combination ART regimen, consisting of Tenofovir (300 mg), Emtricitabine (200 mg), and Efavirenz (600 mg). Due to small sample cohorts, univariate statistical analysis was employed to evaluate metabolic patterns and implicated pathways across the cohorts.

Results: Our results show that the HIV/TB coinfection urinary profile differs significantly ($p < 0.1$) from the profiles of individuals with HIV or TB only, highlighting the intricate effects of HIV/TB coinfection on the metabolic pathways. We also shed light on the impact of ART.

Conclusion: This research enhances our understanding of the metabolic complexities of HIV/TB coinfection and the role of ART. As a proof-of-concept study, the identified metabolic fingerprint is a step towards improved diagnostic precision and targeted treatments. Although these initial findings require validation, they contribute valuable information for future investigations aimed at better management and outcomes for individuals with HIV/TB coinfection.

Establishing mtDNA haplogroup cell line models representing African populations.

Mokoena D[1], Pretorius M[1]
[1]North West University, Faculty of Natural and Agricultural Sciences, Focus Area for Human Metabolomics

Background
Evidence shows that life began in Africa, however, there is very little research representing the African mitochondrial DNA (mtDNA) haplogroups. While European populations are well represented in in vitro studies, African populations are lacking, thus leading to reduced understanding of health and disease in these populations. Therefore, this study aimed to establish cytoplasmic hybrid (cybrid) cell lines representing African haplogroups.
Methods
This was achieved by isolating blood platelets from blood samples donated by healthy participants representing African populations in South Africa, North West Province. Before generating cybrids, haplogroup screening and a full mtDNA next generation sequencing was performed on all donor samples to scan for and exclude deleterious mtDNA variants. Based on the sequencing results, The isolated blood platelets from the selected samples were fused with 143B cell lines devoid of mtDNA (143B ρ0 cells).
Results
The new cell lines had the same 143B nuclear DNA (nDNA) background, but different donor mtDNA representing different African haplogroups. To confirm the success of mtDNA transfer from platelets into the 143B ρ0 cells, the relative mtDNA copy number and basal respiration in new cell lines was determined, cells with the mtDNA had an increased basal respiration compared to the ρ0 cells. Further confirmation was done by checking the haplogroups of the newly formed cybrids.
Conclusion
The addition of African haplogroup cell lines has the potential to bridge existing gap in health research and move towards population specific treatment interventions. These cybrid cell lines will be utilized in future studies that investigate the impact of mtDNA haplogroups on cellular function in health and disease state.
Key words: African haplogroup, mtDNA, cybrid cell line

In silico evaluation of the novel 2-(chloropropyl-1-ynyl-quinoxaline) (LA59E4) derivative as a potential inhibitor of (Severe Acute Respiratory Syndrome Coronavirus 2) SARS-COV-2 viral entry, assembly and replication

Mogodi M[1], Nxumalo W[1], Matsebatlela T[1]
[1]University of Limpopo, [2]University of Limpopo

In silico evaluation of the novel 2-(chloropropyl-1-ynyl-quinoxaline) (LA59E4) derivative as a potential inhibitor of SARS-COV-2 viral entry, assembly and replication.

Mahlatse Mogodi[1], Winston Nxumalo[2]and Thabe Matsebatlela[1]

[1]University of Limpopo, Department of Biochemistry, Microbiology and Biotechnology, Sovenga, 0727, South Africa.

[2]University of Limpopo, Department of Chemistry, Sovenga, 0727, South Africa.

Email: 202025303@keyaka.ul.ac.za

Background

The landscape for (Coronavirus disease 2019) COVID-19 is drastically altering because of vaccine-driven prevention. Despite these developments, COVID-19 survivors and newly diagnosed patients still require ongoing evidence-based care. In South Africa only, COVID-19 is responsible for 102,595 deaths out of 4,055,656 positive cases. Treatment for COVID-19 and related respiratory syndromes remains a struggle, complicated by the frequent emergence of variants and delayed production of suitable vaccines. Hence, the main aim of this study was to develop and identify a suitable quinoxaline derivative for inhibiting (Severe Acute Respiratory Syndrome CoronaVirus 2) SARS-Cov-2 viral entrance, replication, and assembly in the host cell.

Methods

Molecular docking of the selected quinoxaline derivatives was performed with spike, RNA-dependent RNA polymerase (RdRp), and Main protease (Mpro). The estimation of pharmacokinetic characteristics and the oral bioavailability of quinoxaline derivatives along with an (Food and Drug Administration) FDA- antiviral compound was performed using Absorption, Distribution, Metabolism, Excretion and Toxicity (ADMET) and Lipinski's rule of five analyses.

Results

Molecular docking analysis revealed that LA59E4 possessed the highest binding affinity with the spike protein, indicating its potential to form a stable protein-ligand complex. The physicochemical properties of LA59E4, such as its low molecular weight, balanced hydrophilic-hydrophobic profile, and favourable oral bioavailability, suggested its suitability for further exploration as an antiviral agent against SARS-Cov-2. The ADMET results showed that quinoxaline derivative is predicted to be non-toxic and safe for clinical use.

Conclusions

These results suggest that quinoxaline derivatives could be probable candidates for inhibiting SARS-Cov-2 entry, replication and assembly.

Keywords: COVID-19, quinoxaline derivative, molecular docking, ADMET, Lipinski rule of 5 ,SARS-COV-2

Comparison and optimisation of extraction methods for non-targeted GC-MS urine metabolomics

Allen B[1], Olivier C[1], Luies L[1]
[1]Focus Area: Human Metabolomics, North-West University (Potchefstroom Campus)
Background: Urine is an optimal biological sample for non-targeted metabolomics, offering insights into cellular functions under normal and pathological conditions. The diversity of compound classes analysed in non-targeted metabolomics necessitates optimal extraction methodologies for accuracy.
Methods: We refined a low-volume urine preparation procedure suitable for non-targeted GC-MS. Our study evaluated five extraction methods, including four organic acid (OA) variations and a "direct analysis" (DA) approach, focusing on repeatability, metabolome coverage, and metabolite recovery.
Results: The DA outperformed OA variations in repeatability and metabolome coverage, identifying 91 unique metabolites across multiple compound classes. Conversely, OA methods may not be universally suitable for all non-targeted metabolomics investigations due to inherent bias towards a specific compound class. The OA methods exhibited limitations, such as reduced compound recovery and a higher incidence of undetected compounds. Further enhancement to the DA method included an additional drying step between two-step derivatization, which improved outcomes. However, sample pre-treatment with urease did not yield significant benefits.
Conclusion: This study establishes an optimised low-volume urine preparation technique for future non-targeted metabolomics studies employing GC-MS. Our findings propel metabolomics research forward by facilitating a more efficient and comprehensive analysis of urinary metabolites. This could significantly improve disease diagnosis and biomarker discovery.

Genetic diversity of human herpesvirus type 8 in northern South Africa

Raphalalani M[1,2], Mavhandu-Ramarumo L[1], Bessong P[1,3]
[1]HIV/AIDS & Global Health Research Programme, University of Venda, [2]Department of Biochemistry and Microbiology, University of Venda, [3]Centre for Global Health Equity, School of Medicine, University of Virginia

Background: Human herpesvirus type 8 (HHV-8) is an oncogenic virus responsible for causing all forms of Kaposi`s sarcoma (KS). It is prevalent in African countries, with South Africa being the third country with the highest number of HHV-8 infections. The viral genetic landscape of any geographical area is of paramount importance in vaccine development and diagnostics. However, data on HHV-8 genotypes is scarce in northern South Africa. Therefore, this study aimed to describe the genetic diversity of HHV-8 in northern South Africa.

Methodology: Deoxyribonucleic acid (DNA) was extracted from mouthwash samples collected from five healthcare facilities in northern South Africa. Partial K1 gene was amplified in a conventional two-round PCR. The band of interest was extracted by phenol-freeze protocol and enriched using conventional PCR. Enriched amplicons were purified and sequenced in an Illumina MiniSeq platform. The quality of the generated sequences was evaluated using FastQC programme. K1 genotypes were inferred using BioAfrica HHV-8 subtyping tool and confirmed by computing a phylogenetic tree. Intra-genetic diversity among HHV-8 genotypes was analysed using Geneious software and SNAP tool.

Results: K1 genotype assignment was available in 68% (24/35) of the study sequences that span partial or complete K1 gene. Two major genotypes (A and B) were detected; genotype B (19/24; 79%) had a higher prevalence than genotype A (5/24; 21%). Genotype A sequences were further classified as subtype A5. Interestingly, sequences that were classified as genotype B did not cluster with any of the B subtypes. Genotype A and B sequences displayed a 16.67% and 7.41% variation at the amino acid level, respectively. Several amino acid polymorphisms were observed in the immunoreceptor tyrosine-based activation motif (ITAM) region of genotype A sequences, while the ITAM region of genotype B sequence was conserved.

Conclusion: A predominance of HHV-8 genotype B was observed in northern South Africa. A higher frequency of nonsynonymous mutations was observed at the ITAM region of A5 sequences, and these mutations might negatively impact the function of ITAM in the K1 gene.

Investigating the effect of a therapeutic cocktail on mitochondrial respiration in Complex I deficient Caenorhabditis elegans.

Welgemoed C[1], Lindeque Z
[1]Human Metabolomics, Natural And Agricultural Sciences, North-west University, Potchefstroom, South Africa.

Background: Mitochondrial diseases caused by Complex I (CI) defects such as Leigh syndrome has a tremendous impact on brain, heart, and muscle health. Therapeutic cocktails have been invented to counteract the effects associated with a CI deficiency, thus improving the wide variety of symptoms associated with low ATP production, redox imbalance, oxidative stress, and inflammation. In this sub-study, the effect of a developed cocktail on respiration will be investigated in CI deficient - (MQ1333) and wild-type C. elegans (N2) with the help of a standardized Oroboros method.

Methods: C. elegans strains were cultured on agar plates containing Escherichia coli (E.coli). After age synchronization, the nematodes were collected for respiratory analysis. A standardized Oroboros (O2k) method was used to measure baseline and maximum respiration (with the addition of specific substrates and uncouplers) in the strains.

Results: A moderate difference between the two strains' residual oxygen consumption were seen throughout the analysis, albeit not statistically significant. The therapeutic cocktail did not increase the residual oxygen consumption of the MQ1333 strain as hypothesized.

Conclusion: In conclusion our study did not find any significant findings when comparing the mitochondrial respiration of the MQ1333 and N2 strains before and after treatment. Although the therapeutic cocktail is aimed at promoting membrane potential and thus ATP production, it could not be confirmed with respiratory analysis (oxygen consumption). A follow-up study should focus on membrane potential.

Parameterizing the covalent intermediates of the nitrilase superfamily

van Heerden D[1], Sewell T[1]
[1]University Of Cape Town

The nitrilase superfamily, which includes the amidases, are thiol enzymes that catalyze the hydrolysis and condensation of non-peptide, carbon-nitrogen linkages. There are several possible applications for nitrilases in biotechnology, including the chemo-, regio- and enantioselective synthesis of carboxylic acids from nitriles. One of the most intensively studied nitrilases was originally isolated from the bacterium Pseudomonas fluorescens EBC191. This enzyme has been shown to convert a broad range of aromatic and aliphatic nitriles and the enantioselective synthesis of various α-substituted arylacetic acids by this enzyme has been described. Although a wide range of variants of this enzyme have been generated, the molecular basis of its reaction specificity and enantioselectivity is still largely unclear. In this poster, the molecular dynamics (MD) simulation-based minimization of various docked substrates and their covalent intermediates are described. Sequence homology with other superfamily members identifies the residues equivalent to a cysteine (Cys-164), two glutamates (Glu-48 and Glu-137), and a lysine (Lys-130) as being the grouping that qualifies as the “the catalytic tetrad”. Various nitriles and their amide equivalents were docked into the wild type, A165F, and W188K variants using the Interactive Structure Optimization by Local Direct Exploration (ISOLDE) package incorporated in UCSF ChimeraX. In addition to coordinates, ISOLDE employs electron density to minimize protein structures. Furthermore, the AmberTools23 suit of programs was used to generate Amber force field parameters for the thioester and thioimidate intermediates, enabling their minimization in ISOLDE.

WARBURGIA SALUTARIS MODULATES OXIDATIVE STRESS TO INDUCE CELL DEATH IN HEPG2 CELLS

Maruma L[1], Khan R
[1]School of laboratory Medicine and Medical sciences

Introduction: Liver cancer is the third leading cause of cancer-related deaths globally. Despite several treatment options available, the five-year survival rate is low. Medicinal plants are prescribed for liver diseases by indigenous healers, but little is known about the anti-cancer potential of these remedies for liver cancer.

Aim: To clarify the anti-proliferative effects of Warburgia salutaris commercial extract in HepG2 cells.

Methods: Viability of HepG2 cells treated with Warburgia salutaris (0-5000µg/ml) assessed by the MTT assay was used to extrapolate the half maximum inhibitory concentration (IC50). Thereafter cell proliferation, cell death and oxidative stress were evaluated in IC50-treated cells. The Hoechst assay assessed cell growth and apoptosis, and the comet assay assessed DNA fragmentation. Externalised phosphatidylserine, caspase activity and western blotting (p53) further evaluated apoptosis. Oxidative stress was evaluated by quantifying reactive oxygen species (ROS) and reactive nitrogen species (RNS), while GSH and stress response proteins (western blotting and/or qPCR) were used to evaluate the antioxidant response.

Results: The MTT assay and decreased ATP indicated decreased cell viability. Cell death was executed by apoptosis since caspase 3/7 increased. Cell debris (Hoechst assay) also suggested cell death, possibly by DNA damage as DNA fragmentation was evident and p53 decreased. Furthermore, chromatin changes indicated decreased cell growth, also alluded by decreased NFkB and cMyc. Oxidative stress was implied by increased ROS and RNS accompanied by decreased SOD2 and GSH. Interestingly, HSP70, Nrf2, catalase and GPx were increased.

Conclusion: HepG2 cell death induced by Warburgia salutaris may be a consequence of oxidative stress.

Wastewater-Based Epidemiology for Upstream SARS-CoV-2 Variant Monitoring in Breede Valley Municipality

Malemela-Mabuda K[1], Johnson R[1,2]
[1]Biomedical Research and Innovation Platform, South African Medical Research Council, [2]Division of Medical Physiology, Faculty of Medicine and Health Sciences, Centre for Cardio-metabolic Research in Africa, Stellenbosch University

Background
The COVID-19 pandemic has significantly damaged economies and health systems globally, especially in developing countries. As a result, wastewater-based epidemiology (WBE) has emerged as a popular method for monitoring the disease's spread within communities. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been detected in multiple wastewater-treatment plants (WWTPs) in the Breede Valley Municipality, Western Cape, South Africa. Notably, the Rawsonville WWTP that unceasingly had a high viral load with the Delta and Omicron (Deltacron) variants continuously co-circulating, even during the predominance of the Omicron strain in the Western Cape. Hence, this study examined multiple pump stations and manholes upstream of the primary WWTPs to determine the specific sewer line responsible for the emergence of the highly virulent Deltacron variant.
Methods
Wastewater samples were collected between January 2022 and March 2023 from the main WWTPs and May 2022, and March 2023 from upstream locations. SARS-CoV-2 viral RNA copy numbers were quantified by reverse transcription–quantitative polymerase chain reaction (RT-qPCR).
Results
The study showed that, of the four major towns in the Breede Valley Municipality, Rawsonville maintained the highest overall median viral load (6395 gc/mL) surpassing that of Touwsriver (4356.3 gc/mL), De Doorns (4314.7 gc/mL), and Worcester (4037.5 gc/mL). This study confirmed the continued resurgence of the Delta variant (AY.32 and B1.617.2) in Rawsonville. Data from upstream sites of the Rawsonville WWTP showed that the suburban (RV_G) and peri-urban (RV_R) neighbourhoods significantly contributed to the high SARS-CoV-2 viral load (5962.2 gc/mL and 10527.2 gc/mL, respectively) detected in Rawsonville. Furthermore, the public toilets (RV_P) exhibited the lowest contribution to the viral load detected in Rawsonville, with an overall average of 2174.1 gc/mL. Additionally, as RV_G had the highest overall median RNA signal (10527.2 gc/mL) coupled with the high infectivity rate of Deltacron, it is probable that this line served as the entry point for the Deltacron variant into Rawsonville.
Conclusion
This study has demonstrated the importance of upstream surveillance of the main WWTPs to track and identify the origin and emergence of circulating variants.

Monsonia burkeana induces caspase-dependent apoptosis in colorectal adenocarcinoma (Caco-2) cells and nitrosative stress-induced necroptosis in hepatocellular carcinoma (HepG2) cells.

Naicker M[1]
[1]University Of Kwazulu Natal

Background
Cancer is a major global cause of death, prompting interest in plant-based alternatives due to side effects from current treatments. Monsonia burkeana Planch. Ex Harv is a medicinal plant native to southern Africa. This study explored its potential anticancer effects using its crude aqueous extract on Caco-2 and HepG2 cell lines.

Methods
The study subjected target cells to M. burkeana plant extract ranging from 0 to 5000µg/ml for 48 hours to determine IC20 and IC50 concentrations through the MTT assay. Cytotoxic effects were assessed via LDH and CYP3A4 activity assays, evaluating oxidative breakdown and mitochondrial integrity with ATP and JC-10 levels. Antioxidant response was measured through TBARS/NOS assay for reactive oxygen and nitrogen species, GSH quantification, and protein expression analysis via western blotting (SOD, NRF2, iNOS). The mRNA gene expression of Gpx and OGG1 was assessed using qPCR. Cell death mechanisms were investigated with Annexin-V assay for apoptosis, caspase activation assays, and western blotting (p53, p-p53, BAX, NFκB, cIAP2, cleaved caspase 3, BCL-2). Gene expression of MLKL, RIP1, RIP3, NFκB, and TNF-α was also evaluated.

Results
A dose-dependent decrease in cell viability in Caco-2 cells and HepG2 cells, with an IC50 value of 293.8 µg/ml and an IC20 value of 169.8 µg/ml in Caco-2 cells. In HepG2 cells, the IC50 value was 335.4 µg/ml of M. burkeana extract, and the IC20 value was 154.9 µg/ml. M. burkeana decreased cell viability in Caco-2 cells, which was associated with mitochondrial toxicity but attenuated oxidative and nitrosative stress. The study provides evidence supporting the role of M. burkeana in causing caspase-dependent apoptosis in Caco-2 cells for both treatment concentrations. Still, the intrinsic apoptotic pathway was only stimulated for the IC20 treatment. Nitrosative stress-induced necroptosis was the mechanism by which M. burkeana exerted its cytotoxic effect in HepG2 cells.

Conclusions
The results showed promise of M. burkeana potentially being used as an anticancer treatment for colorectal and hepatocellular carcinomas.

The cyclic antimicrobial peptides Gramicidin S and Tyrocidine A inhibit the chaperone function of Plasmodium falciparum Grp78

Mthembu W[1], Rautenbach M[1], Zininga T[1]
[1]Department of Biochemistry, Stellenbosch University

Background
The emerging antimalarial drug resistance is limiting the achievements in malaria therapy. The resistance to artemisinin the current first line of treatment targeting Plasmodium falciparum (Pf), is facilitated by the protein folding system in the endoplasmic reticulum (ER). Artemisinin sensitivity is directly linked to an ER stress response activating the unfolded protein response (UPR). The molecular chaperone PfGrp78 is the main signaling protein that initiates the UPR that makes it an attractive drug target. Inhibiting PfGrp78 from binding its protein substrates could result in parasite protein accumulation in the ER. This inhibition abrogates their export and secretion resulting in proteopathy. In addition, PfGrp78 inhibition may also reverse the ER mediated resistance to artemisinin. This study aims to investigate the effect of known anti-Pf peptides, for example, gramicidin S (GS) and tyrocidine A (TrcA), on the chaperone function and protein folding activity of PfGrp78.
Methods
Computational molecular docking studies of PfGrp78 were conducted to screen for the most potent peptide inhibitor based on binding affinities. Aggregation suppression assays were used to confirm the effect of the selected peptide inhibitors.
Results
Based on the in-silico predictions PfGrp78 bound the peptide inhibitors in the substrate binding region. TrcA had a higher binding affinity at -7.434 kcal/mol when compared to that of GS at -6.617 kcal/mol. To confirm these findings, we expressed and purified the full length PfGrp78 protein, and confirmed its predominantly α-helical secondary structure using circular dichroism spectroscopy. Using a model substrate malate dehydrogenase (MDH) aggregation suppression assay, we observed that GS had lower inhibitory activity on the chaperone function of PfGrp78 compared to TrcA. Therefore, TrcA shows a greater potential of inhibiting the activity of PfGrp78 which may partly explain the promising antimalarial activity previously reported.
Conclusion
Our study findings show that GS and TrcA selectively affect the activity of PfGrp78 chaperone function. These results suggest that further research need to be conducted to develop GS and TrcA scaffold as potential selective antimalarial drugs.

Does the diversity of amino acid sequences in HIV-1 viral proteins influence the pathogenesis and neuropathogenesis of HIV-1?

Williams M[1], Asia L[1], Gotora P[1], Brown K[2], Martin D[2,3], Cloete R[2], van der Sluis R[1], Ruhanya V[4], Paul R[5], Ipser J[6,7], Stein D[6,7,8], Joska J[7,9], Naude P[6,7], Jansen Van Vuren E[10,11]
[1]Human Metabolomics, Biochemistry, North-West University, [2]South African Medical Research Council Bioinformatics Unit, South African National Bioinformatics Institute, University of the Western Cape, [3]Department of Science and Innovation/Mintek Nanotechnology Innovation Centre, Biolabels Node, University of the Western Cape, [4]Division of Medical Virology, Stellenbosch University. , [5]Department of Psychological Sciences, Missouri Institute of Mental Health, University of Missouri-Saint Louis. , [6]Department of Psychiatry and Mental Health, University of Cape Town, [7]Neuroscience Institute, University of Cape Town, [8]SAMRC Unit on Risk and Resilience in Mental Disorders, Department of Psychiatry, University of Cape Town, [9]HIV Mental Health Research Unit, Division of Neuropsychiatry, Department of Psychiatry and Mental Health, Faculty of Health Sciences, University of Cape Town, [10]Hypertension in Africa Research Team (HART), North-West University, , [11]South African Medical Research Council: Unit for Hypertension and Cardiovascular Disease, North-West University

Background: Despite extensive investigation, HIV-1 continues to pose a global challenge, exhibiting variations in its progression and effects on the nervous system across diverse regions and HIV-1 subtypes. This diversity is evident in the amino acid sequences of crucial viral proteins like transactivator of transcription (Tat), Viral protein R (Vpr), and Viral infectivity factor (Vif). Previous research has shown that variations in these proteins' amino acid sequences play a role in influencing pathways relevant to HIV-1 progression and its impact on the nervous system. However, the majority of studies exploring sequence diversity in Tat, Vpr, and Vif have primarily focused on HIV-1 subtype B, prevalent in North America and Europe. Consequently, there are significant knowledge gaps, particularly concerning HIV-1 subtype C regions such as South Africa. To address this gap, our study aimed to investigate the sequence diversity of these proteins in a South African cohort and evaluate its effects on protein structure and function, inflammation, and metabolism.

Methodology: We employed computational molecular modelling and docking, along with viral protein sequence diversity analysis using Sanger sequencing, immune marker measurements via enzyme-linked immunosorbent assay, and metabolomics analysis utilizing liquid chromatography-mass spectrometry.

Results: Our study revealed amino acid sequence diversity in Tat, Vpr, and Vif within the South African context. These sequence variations were found to impact the underlying mechanisms associated with HIV-1 progression and its effects on the nervous system.

Conclusions: Our findings offer insights into the factors contributing to diverse clinical outcomes among HIV-infected individuals in different global regions. Future research should delve deeper into these identified amino acids to gain a thorough understanding of their significance within the context of South African HIV-1 subtypes.

Molecular mechanism of follicle stimulating hormone regulation by medroxyprogesterone acetate and norethisterone in vitro.

Van Eck S[1], Capitaine C[1], Bick A[1], Africander D[2], Avenant C[1], Hapgood J[1]
[1]Department of Molecular and Cell Biology, University of Cape Town, [2]Department of Biochemistry, Stellenbosch University

The Women's Health, Injectable Contraceptive and HIV (WHICH) clinical trial recently showed that the use of depo-medroxyprogesterone acetate intramuscular (DMPA-IM) and norethisterone enanthate (NET-EN) both resulted in hypoestrogenism. The biosynthesis of 17β-Estradiol (E2) is regulated by various hormones involved in the hypothalamic-pituitary-ovarian axis, including gonadotropin releasing hormone (GnRH), which regulates the production of luteinizing hormone (LH) and follicle stimulating hormone (FSH). LH is required to produce androgens, while FSH increases the conversion of androgens to E2. However, the mechanism(s) whereby these contraceptives regulate LH, FSH and GnRH to cause hypoestrogenism is not known. We aimed to investigate the regulation of FSHβ, LHβ and the GnRH receptor (GnRHR) by MPA or NET in a pituitary gonadotrope cell line model. Regulation of LH, FSH and GnRHR by medroxyprogesterone acetate (MPA) and norethisterone (NET) was investigated using promoter-reporter assays and quantitative real-time PCR. MPA and the glucocorticoid dexamethasone (DEX), but not NET, increased FSHβ mRNA and promoter-reporter activity (in the absence and presence of GnRH), in a glucocorticoid receptor (GR)-dependent manner. MPA and DEX also decreased GnRH-induced LHβ promoter-reporter activity, but not LHβ mRNA or protein. Furthermore, MPA and DEX increased GnRHR mRNA and promoter-reporter activity, whereas NET had no effect. Data show that MPA acts on pituitary gonadotrope cells to increase FSH in a GR-dependent manner in vitro. Furthermore, the MPA-induced increase in GnRHR mRNA suggests an increased sensitivity to GnRH, even in the absence of a change in GnRH levels, thereby possibly accounting for the increase in FSH expression. Taken together, these results suggest that hypoestrogenism reported for DMPA-IM users can, at least in part, be mediated via direct action of MPA on the pituitary to decrease LHβ promoter activity. The physiological significance of increased FSH by MPA, as well as the mechanism of hypoestrogenism reported for NET-EN users remain unclear. The data offer mechanistic understanding of hypoestrogenism, which could impact various physiological and health outcomes.

Potential Matrix Metalloproteinase 2 and 9 inhibitors identified from Ehretia species for the treatment of chronic wounds - Computational drug discovery approaches.

Oselusi S[1,2], Martin D[2], Sibuyi N[2], Meyer M[2], Madiehe A[1,2]
[1]Nanobiotechnology Research Group, Department of Biotechnology, University of the Western Cape, Private Bag X17, [2]DSI/Mintek Nanotechnology Innovation Centre (NIC), Biolabels Node, Department of Biotechnology, University of the Western Cape, Private Bag X17

Background
The dysregulation and overexpression of matrix metalloproteinases (MMPs), particularly, MMP2 and MMP9 have been used as prognostic factors in several pathophysiological conditions, including chronic wounds. As a result, these MMPs are considered important therapeutic targets in the intervention and treatment of chronic wounds. Leaf extracts of Ehretia species possess strong anti-inflammatory and wound-healing properties in phytomedicine.
Methods
In this study, Ehretia species phytoconstituents were curated from the literature to search for potential inhibitors of MMPs using computational drug design tools. The predicted MMP inhibitors were subjected to molecular docking to model their interactions in the binding sites of MMP2 and MMP9 protein targets. The top-scoring phytoconstituents were screened for synthetic accessibility. Molecular dynamic (MD) simulations, principal component analyses (PCA), and Molecular Mechanics Poisson-Boltzmann Surface Area (MMPBSA) were further performed to gain insight into their dynamic behaviour, and stability under physiological conditions for 500 ns.
Results
A total of 74 phytoconstituents were curated from the literature, and 46 were identified as potential inhibitors of at least one type of MMP. Molecular docking revealed promising dual inhibitors, such as DB11, DB13, and DB03 as they showed greater docking score energies than the reference co-crystalized ligands. DB70 had a stronger docking score for MMP2, while DB09, and DB02, were more selective for MMP9 compared to the reference ligand. Apart from their binding with the zinc ions at the catalytic domain of the proteins, Glu121, Asp72, and His120 residues were key interactions of MMP2 with the phytoconstituents, while Glu227, Try245, and Pro246 were prominent in the complex formed with MMP9. The structural moieties of these phytoconstituents were found to be accessible and moderately easy to synthesize. MD simulations and PCA analyses revealed stable, and non-fluctuating binding interactions between the molecules and their respective protein targets. MMPBSA calculations also showed that the overall change in energy of the systems was favourable throughout the simulation.
Conclusion
Based on the aforementioned results, it is concluded that Ehretia species could be of benefit to the search for novel therapeutic agents for the treatment of chronic wounds.

Synthesis and Characterization of Medicinal Plant Extracts-Based Nanoparticles and their Evaluation against Antibiotic-Resistant Bacterial Pathogens

Nemaguvhuni M[1], Samie A[2]
[1]Faculty Of Science, Engineering, And Agriculture, University of Venda, Department Of Biochemistry And Microbiology, University Of Venda, [2]Faculty Of Science, Engineering, And Agriculture, University of Venda, Department Of Biochemistry And Microbiology, University Of Venda

Background: The rise of antibiotic-resistant (ABR) bacterial pathogens is a major global health concern, especially in the case of respiratory tract infections (RTIs), which can make treatment more difficult. According to statistics, more than 2 million lives were lost due to ABR bacterial infections in 2019, highlighting the seriousness of the situation. This study aims to propose a novel approach to combating ABR bacteria by synthesizing metallic nanoparticles (MNPs) from medicinal plants. Integrating nanoparticle-based strategies with medicinal plants demonstrates considerable promise in addressing RTIs.

Methodology: We prepared extracts of Spirostachys Africana using methanol, ethanol, acetone, and distilled water, then synthesized silver nanoparticles (AgNPs) and gold nanoparticles (AuNPs) from these extracts. The nanoparticles were characterized using advanced techniques, including Ultraviolet-Visible spectrophotometry (UV-Vis), X-ray diffraction (XRD), Dynamic light scattering (DLS), Transmission electron microscopy (TEM), Fourier-transform infrared spectroscopy (FTIR), and Liquid chromatography-mass spectroscopy (LC/MS).

Results: The study revealed peaks at 480 nm for AgNPs and 541 nm for AuNPs in UV-Vis analysis, with XRD showing face-centered cubic structures and crystalline sizes of 9-19 nm for AgNPs and 9-10 nm for SA-AuNPs. DLS indicated polydisperse distribution for AgNPs and monodisperse distribution for SA-AuNPs, while TEM images depicted particle core sizes of 5-49.5 nm for AgNPs and 6-32 nm for SA-AuNPs, predominantly spherical and spheroidal, respectively. FTIR analysis revealed various functional groups in extracts and NPs, with LCMS identifying 24 bioactive compounds from S. Africana extracts, predominantly flavonoids. Antimicrobial assays demonstrated AgNPs' efficacy against ABR bacteria, especially with acetone extracts conjugated AgNPs showing the highest zone of inhibition (22 mm) against P. aeruginosa. Methanol and ethanol extracts conjugated AgNPs exhibited potent antimicrobial activity (MIC = 0.05 mg/mL) against E. coli, P. aeruginosa, and A. baumannii. Acetone and ethanol extract-mediated SA-AuNPs showed significant cytotoxicity at concentrations of 0.078 and 0.16 mg/mL, respectively. Ethanol extracts conjugated with AgNPs demonstrated the most potent anti-inflammatory activity (0.01 mg/mL), while acetone extract displayed excellent antioxidant activity (IC50 = 0.000335 mg/mL).

Conclusions: The MNPs synthesized from S. Africana extracts show promise in combating ABR bacterial pathogens causing RTIs, with significant cytotoxic, anti-inflammatory, and antioxidant activities, highlighting their potential for biomedical applications.

Clinical and genetic characterization of Emery-Dreifuss Muscular Dystrophy: insights from three cases with LMNA mutations: an ICGNMD study

Schoonen M[1], van der Westhuizen F[1], Bisschoff [1], Human R[2], Lubbe E[2], Nonyane M[2], McFarland R[3], Taylor R[3], Fassad M[3], Patel K[3], Wilson L[4], Hanna M[4], Vandrovcova J[4], ICGNMD Consortium I[5], Smuts I[2]
[1]Human Metabolomics, North-West University, [2]Department of Paediatrics, Steve Biko Academic Hospital, University of Pretoria, [3]Wellcome Centre for Mitochondrial Research, Faculty of Medical Sciences, Newcastle University, [4]Centre for Neuromuscular Diseases, UCL Queen Square Institute of Neurology, [5]For list of Consortium Members see https://www.ucl.ac.uk/genomic-medicine-neuromuscular-diseases/global-contributor-list

Background:
Emery-Dreifuss muscular dystrophy (EDMD) is a rare genetic disorder that falls under the group of muscular dystrophies. It is clinically heterogeneous, although skeletal and cardiac muscle are mostly affected. EDMD is caused by mutations in the Lamin A/C (LMNA) gene. Inheritance of EDMD can be autosomal dominant or recessive, usually presenting in early childhood. Here we present three cases of EDMD: the index patient presenting with a likely pathogenic novel homozygous deletion (LMNA:c.1095_1112Del) and two cases presenting with heterozygous de-novo pathogenic mutations (LMNA:c.1357C>T and LMNA:c.746G>A).

Methods:
Clinical evaluations with informed consent and assent were done at Steve Biko Academic Hospital in Pretoria. Whole exome sequencing was performed on the three cases, which clinically all presented with EDMD.

Results:
The index case, a 10-year-old male, presented with Gower's sign, a waddling gait, proximal muscle weakness, and neck flexor weakness. Metabolic analysis revealed elevated serum creatinine levels. Segregation analysis revealed a heterozygous mutation in the unaffected mother, suggesting an autosomal recessive inheritance pattern. The second and third cases, both females, presented with proximal muscle weakness, Gower’s sign, and intellectual disability. Both presented with a known pathogenic mutation in a de-novo state, as segregation analysis showed no variants in the unaffected parents, suggesting an autosomal dominant inheritance pattern.

Conclusions:
EDMD is a rare, disabling muscular dystrophy that poses a diagnostic challenge. There is currently no cure, and early diagnosis is essential to recognizing cardiac complications, improving the clinical management and prognosis of the disease. This work forms part of a larger neuromuscular disease study, The International Centre for Genomic Medicine in Neuromuscular Diseases.

N, N bis (2-pyridylmethyl)-1, 2-ethylenediamine tetrahydrochloride (H2pmen) stimulates intrinsic apoptosis mediated by oxidative and nitrosative stress induction of the Nf-kB/Stat3 pathway in human hepatocellular carcinoma (HepG2) cells.

Ntanzi N[1], Khan R[1], Nxumalo M[1], Shunmugam L[1], Kumalo H[1]
[1]University Of Kwa-Zulu Natal

Introduction
Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related deaths worldwide, its incidence is rising, and this trend is expected to continue for decades. Several cancer therapeutics have already been discovered and are being used to treat HCC. However, most of them cause severe side effects, which decrease the treatment's effectiveness. N, N-bis (2-pyridylmethyl)-ethylenediamine tetrahydrochloride (H2pmen) is a tetradentate ligand that forms stable complexes with iron, chromium, copper, and zinc, and it has been shown to be a potentially effective reagent for metal chelation. This study investigated the antiproliferative and cytotoxic effects of H2pmen in the HepG2 cell line.

Methods
The cell viability was determined by treating HepG2 cells with different concentrations (0–1000 µM) of H2pmen over 24 hours. MTT assay was used to obtain an IC50, which was then used in all subsequent assays. The cells were then assayed for oxidative stress and membrane damage (TBARS, NOS, GSH, and LDH cytotoxicity), apoptotic induction (ATP assay, JC-10 assay, Annexin v, Caspases), cytochrome P450 3A4 activity (Luminometry). Protein expression of iNOS, SOD2, Bax, Caspase-2, and STAT3 was identified using western blot analysis. The gene expression of GPx1, Nrf2, NF-κB, p53, and OGG1 was determined using qPCR.

Results
H2pmen reduced cell viability of HepG2 cells, exerting a cytotoxic effect associated with decreased mitochondrial membrane potential, ATP and increased LDH leakage. The ROS-associated membrane damage was induced by an increase in lipid peroxidation and RNS production. Oxidative stress occurred due to a decrease in antioxidant levels. H2pmen initiated and executed caspase-dependent apoptosis indicated by decreased initiator caspases, upregulation in Bax and p53 expression, and increased caspase-3/7, LDH, and STAT3.

Conclusion
H2pmen is cytotoxic to HepG2 cells, and this was revealed by the induction of oxidative and nitrosative stress that affected lipids and DNA and eventually led to apoptosis via the intrinsic pathway.

The effect of dipeptidyl aminopeptidase inhibitors on Plasmodium falciparum asexual stage parasites

Sanders N[1], Birkholtz L[1], van der Watt M[2]
[1]Department of Biochemistry, Genetics and Microbiology, University of Pretoria, [2]Institute for Sustainable Malaria Control, School of Health Systems and Public Health, University of Pretoria

Background
Plasmodium falciparum parasites are resistant to multiple frontline antimalarials, emphasising the need to identify new compounds with novel modes of action to treat malaria and impede the development of resistant parasites. Dipeptidyl aminopeptidase (DPAP) 1 is involved in haemoglobin degradation, while DPAP3 functions in merozoite invasion of erythrocytes, warranting these biological processes as potential targets. Given the homology between P. falciparum DPAPs and human cathepsins, known human cathepsin inhibitors were selected from the Boehringer Ingelheim opnMe library and evaluated for their in vitro inhibitory and in silico properties in asexual P. falciparum parasites.
Methods
We first evaluated the inhibitory effect of the BI compounds on in vitro parasite proliferation using a SYBR Green I DNA proliferation assay. To confirm that inhibition of parasite proliferation was due to compound activity and not erythrocyte lysis, a haemolysis assay was used. The phenotypic effect of BI-1124, the most active compound, on ring and trophozoite-stage parasites was evaluated using light microscopy 24 h post-treatment. Lastly, potential binding modes of BI-1124 to its proposed target was investigated using in silico docking studies in Schrödinger.
Results
BI-2051 and BI-1124 inhibit parasite proliferation with IC50 values of 2.93 and 1.62 µM, respectively. The haemolysis assay confirmed that these compounds are not toxic to human erythrocytes. Morphology studies of the most active compound, BI-1124, showed a delay in parasite progression, with parasites showing aberrant morphology and decreased parasitaemia in compound-treated parasite cultures. Lastly, docking studies revealed a possible interaction between BI-1124 and Falcipain 3, with a docking score of – 6.576.
Conclusions
Our findings show that BI-1124 inhibits in vitro parasite proliferation. This study therefore provides valuable insight into the targetability of haemoglobin degradation and erythrocyte invasion for future drug discovery studies.

Screening of antimicrobial and anticancer activities of Kirkia acuminata-related endophytic fungi extracts

Magagula M[1], Motaung T[2,3], Mbita Z[1], Dithebe K[1]
[1]Department of Biochemistry, Microbiology and Biotechnology, [2]Department of Biochemistry, Genetics and Microbiology, [3]Forestry and Agricultural Biotechnology Institute

Background
Fungal endophytes of medicinal plants produce structurally and chemically diverse secondary metabolites with notable biological activities. However, the fungal endophytes of medicinal plants used in South Africa are relatively under-explored for their ability to produce secondary metabolites with therapeutic potential. In this study, 18 morphologically distinct fungal endophytes previously isolated from the stems of the overharvested medicinal plant, Kirkia acuminata, were screened for their antimicrobial and anticancer potential.
Methods
The antimicrobial activity of the ethyl acetate (EtOAc) crude extracts was assessed using the broth micro-dilution assay to determine their minimum inhibitory concentrations (MIC). The cytotoxicity of the EtOAc crude extracts against the non-cancerous cell line HEK-293 was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The anticancer activity of four safe crude extracts was tested against ME-180 cervical cancer and A375 melanoma cells using the Alamar blue assay. Furthermore, the mycochemical composition of the four crude extracts was assessed using quantification assays to determine the total phenolic, tannin and flavonoid contents of the crude extracts.
Results
Inhibitory activity against clinical pathogens was exhibited by 17 of the 18 crude extracts, with MIC values ranging from 0.31 to 2.5 mg/mL. Only 4 of the crude extracts displayed low cytotoxicity against the noncancerous HEK-293 cells. The four extracts exhibited variable anticancer activity against the ME-180 and A375 cancer cell lines. The mycochemical screening revealed that all four crude extracts had higher total phenolic content.
Conclusions
The study highlights that the fungal endophytes of K. acuminata are sustainable sources of potential potent antimicrobial and selective anticancer agents.

Profiling the metabolic products of a polymer therapeutic nanomedicine of the antimalarial lumefantrine

Matshe W[1,2], Balogun M[2], Williams A[1]
[1]Human Metabolomics, North-west University, [2]Biopolymer Modification and Advanced Therapeutics laboratory, Centre for Nanostructured and Advanced Materials, CSIR

Background:
Recently, a water-soluble polymer therapeutic of the antimalarial lumefantrine (PTL) was developed as an intravenous (I.V) therapeutic to treat severe malaria (SM). PTL is proposed as a potential partner drug to the water-soluble antimalarial, artesunate, to meet the unmet need for an intravenous combination therapy to treat SM. PTL showed >50% efficacy in an artificial rodent model. However, pharmacokinetic evaluations of PTL in healthy mice showed it to be rapidly cleared from the blood. This quick decline in the drug-plasma concentrations is speculated to be as a result of metabolic processes in the blood and surrounding tissue. The increase of the major metabolite of lumefantrine, desbutyl-lumefantrine, in the blood-plasma after I.V administration of PTL compared to the control, further motivates the assumption of the metabolism of PTL. Understanding the presumed mechanism by which PTL is cleared from the blood is critical to its development as an effective antimalarial drug and enhancing the knowledge of metabolomics for polymer therapeutics.

Methods:
Synthesis of PTL involved the conjugation of lumefantrine to a water-soluble polymer. The drug was linked to the polymeric carrier via a pH-sensitive linker, which is also cleavable by enzymatic hydrolysis. PTL was incubated in selected simulated physical and biochemical solutions, i.e. pH buffers and soluble enzyme fractions, to investigate its drug release and subsequent metabolite profile. Spectrophotometric and other analytical techniques was used to characterize the conjugate product and its metabolites.

Results:
NMR and UV/Vis techniques confirmed the successful synthesis of the PTL and its drug content. Incubation of PTL with different physical and biochemical solutions informed on its stability and conversion to metabolite-products.

Conclusions:
PTL's biological stability is essential to its development as a candidate drug for the treatment of SM. PTL's drug release and conversion to various metabolites could offer a plausible explanation to the rapid clearance of PTL and the occurrence of significant metabolites in the blood, as well as the potential of these metabolites in reducing parasite levels. These results could potentially advance the application of polymer therapeutics particularly for infectious disease like malaria and enhance the available treatment strategies.

High-throughput microarray analysis of IgG and IgA autoantibody responses in pancreatic ductal adenocarcinoma

Maimela P[1], Smith M[1], Nel A[1], Jonas E[2], Blackburn J[1]
[1]Department of Integrative Biomedical Sciences, Faculty of Health Sciences, University of Cape Town, [2]Department of Surgery, Gastroenterology Unit, Groote Schuur Hospital, University of Cape Town

Background: Pancreatic ductal adenocarcinoma (PDAC) is considered to be a 'cold' tumour due to a lack of immune cell infiltration and limited response to therapy, typically exhibiting an immunosuppressive tumour microenvironment. Consequently, PDAC has a poor diagnosis and prognosis and is highly malignant. As part of early immune surveillance, the adaptive immune response can mark disease-associated changes in cancer proteomes through the production of autoantibodies that are specifically able to recognise new or aberrantly expressed proteins.
Methods: In this study, we applied a proteomic approach and used a high-throughput multiplexed in-house cancer testis (CT100+) protein microarray platform to assess IgG and IgA autoantibody responses against 117 tumour-associated and cancer testis antigens in homogenised bulk tissue lysates of patient samples with matched PDAC tumour (n = 8), normal adjacent (n = 8) and chronic pancreatitis (n = 5).
Results: We observed significantly higher ($p<0.0001$) levels of IgA-positive antigens in tumour samples compared to IgG. Moreover, we identified a unique autoantibody profile within the diseased tissue which distinguished PDAC tumour samples from normal-adjacent samples, as well as from CP controls, resulting in distinct clustering of autoantibody-positive antigens between the pancreatic tissue samples. Our findings suggest that there may be local autoantibody production at the site of disease.
Conclusion: Since cancer antigens are produced de novo at the site of disease, determining the local autoantibody response and identifying specific antibodies that drive the immune response against these antigens would aid in tumour characterisation, and understanding PDAC immunity.

Rapid detection of Mycobacterium tuberculosis directly from sputum samples of patients from healthcare facilities in Venda, South Africa.

Banda N[1], Traore A[1], Potgieter N[1]
[1]University of Venda
Email: ntshunxeko@gmail.com

Background
Tuberculosis caused by Mycobacterium tuberculosis (MTB) its curable; however, it remains a major killer disease worldwide. In 2019, statistical records show 10 million people falling ill, and about 1.4 million people died from TB. The disease was previously controlled with the development of Rifampicin; however, the emergence of anti-tuberculosis drug resistance is currently a major public health. To successfully achieve sustainable development foal (SDG) in beating TB in our communities by the year 2030; rapid detection techniques that can simultaneously detect drug resistant strains of MTB.
Methodology
A total of 30 sputum samples were collected from TB active patients that are currently under treatment. The samples were pretreated using 4% NaOH and sterilized water. The Allplex TM and Anyplex TM assay were conducted using the manufacturer's instructions.
Results
A total of 15 (50%) samples were MTB positive, 5 (17%) were NTM and 10 (33%) were a co-infection of MTB and NTM. 1 (10%) sample with MTB and NTM co-infection was INH-R, 1(10%) was FQ-R resistant and 1 (10%) was RIF-R resistant. A patient was diagnosed as a multidrug resistant through the assay since due to detection of RIF-R, INH-R, FQ-R.
Conclusion
The finding suggests that Allplex TM and Anyplex TM techniques can be of good implementation in low-income countries since it has the capability of distinguishing MTB and NTM as well as providing a highlight on more than one resistant drugs in one test run faster than culturing methods. This allows healthcare practitioners to provide correct treatment regimen from initiation of treatment to patients and prevents misdiagnoses.

The effect of rooibos tea extracts on the expression of the estrogen receptor subtypes in the MCF7-BUS breast cancer cell line.

Averia G[1], Verhoog N[1]
[1]Stellenbosch University

Background:
Binding of estrogen (E_2), the natural ligand to the estrogen receptors (ERs), ERα and ERβ, mediates the transcriptional regulation of genes involved in the development and metastasis of ER-positive (ER+) breast cancer. While E_2-ERα-induced signalling has been identified to promote breast cancer cell proliferation, studies have shown that ERβ acts as an ERα antagonist.
ER+ breast cancer is often treated with ER-targeting endocrine therapy agents such as fulvestrant, a complete anti-estrogen that is classified as a selective estrogen receptor downregulator (SERD). Once intravenously administered, fulvestrant competitively binds to, and decreases the stability of the ER via proteasomal degradation. However, prolonged use of fulvestrant is associated with an array of adverse side-effects such as vasodilation and muscle weakness. For this reason, there is an increasing demand for developing novel endocrine therapy agents with more favourable properties.
Plant polyphenols, contained in Aspalathus linearis (rooibos), have been shown to provide health benefits such increased antioxidants and potential anti-cancer activity. These characteristics can be attributed to plant compounds known as phytoestrogens, which are structurally similar to E_2 and therefore present a promising alternative to current endocrine therapy agents.
This study therefore aims to evaluate the effect of rooibos extracts on the ER subtypes in a human breast cancer cell line.

Methods:
Fermented and unfermented rooibos extracts were prepared by the Agricultural Research Council using three plant batches and three different extraction methods. Along with E_2 and fulvestrant, MCF7-BUS cells were treated with the rooibos extracts to determine the effect on ER subtype protein expression.

Results:
The rooibos extracts were shown to specifically downregulate ERα protein expression in the MCF7-BUS breast cancer cell line, therefore displaying SERD activity. However, proteasomal inhibition via MG132 abrogated the observed ERα protein degradation in response to the rooibos extracts, suggesting that the mechanism of the selective protein degradation occurs via the proteasome.

Conclusions:
The findings of this study suggest that rooibos could potentially limit E_2-induced breast cancer development by selectively targeting ERα for proteasomal degradation and therefore provides valuable insight into the possibility of using plant polyphenols as a foundation in novel drug design targeting the ER.

Cytotoxic effect of Euryops floribundus acetone leaf extract against cervical cancer cells

Makwaana K[1], Laka K[1], Masibonge G[1], Mbita Z[1]
[1]University of Limpopo

Cancer remains a significant public health concern, with cervical cancer ranking as the fourth most prevalent cancer in women, worldwide. These soaring numbers can be accredited to inadequacies of the currently used treatment strategies, which are associated with adverse side effect; thus, novel anticancer drugs are required, with medicinal plants attracting a lot of interest. Many species of Euryops have been used for medicinal purposes and only a few have been studied for anticancer activities. Therefore, this study aimed at investigating the potential anticancer activity of Euryops floribundus acetone leaf extract against cervical cancer HeLa cells, which was assessed using the cell viability assays (MTT and Muse® Count and Viability) and apoptosis analysis. Screening of phytochemicals revealed the presence of coumarins, carbohydrates, phenolic compounds, flavonoids, and tannins in the Euryops floribundus acetone leaf extract. This extract (IC50 = 250 µg/mL) significantly (***P<0.001) reduced the viability of the cervical HeLa cells, which was confirmed using the Muse® Count and Viability assay. The extract also induced apoptosis in the same cells. Therefore, it can be concluded that the Euryops floribundus acetone leaf extract has potential anticancer activities against cervical cancer.

Cytotoxic analysis of the Carpobrotus edulis leaf acetone extract against cervical cancer cells

Madaba B[1], Makgoo L[1], Mbita Z[1]
[1]University Of Limpopo

Cancer is a disease characterized by uncontrolled cell division, with cervical cancer ranking as the second and fourth most diagnosed cancer in South Africa and worldwide, respectively. These high numbers can be attributed to inefficacies associated with the currently used cancer treatments, which include chemotherapy, radiotherapy, and surgery. Contrary, medicinal plants have shown great potential for cancer treatment due to their specificity, efficacy and lower toxicity. Carpobrotus edulis (C. edulis), a succulent medicinal plant native to South Africa, has been used by traditional healers to treat various ailments; however, its anticancer activities remain unclear. Thus, this study aimed to determine the anticancer potential of C. edulis leaf acetone extract against cervical cancer cells. Thin-layer chromatography (TLC) and phytochemical screening were performed to identify phytochemicals in the extract. Cytotoxicity was evaluated using the MTT assay on HeLa and Caski cells treated with varying extract concentrations for 24, 48, and 72 hours. Cell viability was confirmed by flow cytometry using the Muse ® Cell Analyzer. TLC revealed the presence of phenolic compounds and tannins in the extract. The MTT assay showed that the extract induced proliferation in a dose-dependent manner. Cell count, viability assay, and ki67 proliferation marker assay confirmed active cell proliferation, as indicated by ki67 protein expression, suggesting that this extract may not have anticancer activities against cervical cancer.

A biocomputational approach to unveil the potential benefits of flavonoids in COVID-19 disease and diabetes by targeting AMPK.

Tata F[1]
[1]University of Kwazulu-Natal

Background
Hyperglycemia induces damage and escalates complications in several tissues and organs involved in COVID-19. Flavonoids widely found in natural products have demonstrated diverse therapeutic activities on metabolic and infectious diseases by activating the 5' adenosine monophosphate-activated protein kinase (AMPK). The molecular mechanisms of these pharmacological activities remain very unclear. Therefore, the study evaluated the molecular mechanisms involved in the flavonoid activation of AMPK.
Methods
Molecular docking and molecular dynamics simulation using Maestro Schrodinger were used for in silico screening, selecting, and analyzing the flavonoids in Artemisia afra and Catharanthus roseus. The in vitro cytotoxicity of the aqueous leaf extracts of Artemisia afra and Catharanthus roseus in HepG2, Hek293, and CaCo-2 cells, was carried out at the cell densities of 2 × 104 cells/ml in the MTT assay. IC20, which corresponds to 80% cell viability was used for the cytotoxicity on ATP, MMP, and LDH assays.
Results
An observed decline in ATP, MMP and increased LDH concentrations of HepG2 cells by Catharanthus roseus suggest compromised cellular energy status and potential cell death. The computational analysis unveiled the binding preferences and interactions of the flavonoids with the AMPK compared to metformin and the co-crystallized ligand. Metformin maintained an interesting correlation and consistent pattern when observing the RMSD, MMGBSA, and H–bond occupancy, indicating a high degree of stability and favourable binding interaction despite its relatively low van der Waals contribution (ΔGvdw).
Conclusions
These findings unveiled the higher binding energy demonstrated by rutin which suggests superior activity and biological effects. The cytotoxicity assays present Artemisia afra and Catharanthus roseus as relatively safe and have potential anticancer effects on colorectal.

A computational study on the influence of HIV-1 Tat subtype C-specific amino acid substitutions on TAR binding: implication for HIV-associated neurocognitive disorders

Gotora P[1], Brown K[2], Martin D[2,3], van der Sluis R[1], Cloete R[2], Williams M[1]
[1]Human Metabolomics, North-West University,, [2]South African Medical Research Council Bioinformatics Unit, South African National Bioinformatics Institute, University of the Western Cape, [3]Department of Science and Innovation/Mintek Nanotechnology Innovation Centre, Biolabels Node, University of the Western Cape

Background
HIV-1 produces Transactivation of transcription (Tat), a regulatory protein crucial for viral transcriptional elongation and central nervous system neurotoxicity. Tat interacts with the Trans-activation response element (TAR), altering its structure to enhance HIV reverse transcription initiation. Notably, certain amino acid substitutions in Tat subtype C, including C31S, R57S, and Q63E, have been associated with reduced transactivation and neuropathogenesis compared to subtype B. However, the precise influence of these neuropathogenic-related amino acids on Tat-TAR binding remains unclear. This study aimed to elucidate how individual Tat-C amino acid substitutions (C31S, R57S, and Q63E) affect Tat-TAR binding.
Methods
Molecular modelling techniques, including MODELLER, were employed to generate precise three-dimensional structures of HIV-1 Tat protein variants. Tat subtype B (TatWt) was the reference, with Tat variants created to mimic the amino acid variants present in Tat subtype C. Subsequently, molecular docking of each Tat protein variant to TAR was conducted using HDOCK, followed by molecular dynamic simulations.
Results
Molecular docking results revealed that TatWt exhibited highest affinity for TAR (-262.07), followed by TatC31S (-261.61), TatQ63E (-256.43), TatC31S/R57S/Q63E (-238.92), and TatR57S (-222.24). Analyzing binding free energy indicated higher affinities for single variants TatQ63E (-349.2 ± 10.4 kcal/mol) and TatR57S (-290.0 ± 9.6 kcal/mol) compared to TatWt (-247.9 ± 27.7 kcal/mol), while TatC31S and TatC31S/R57SQ/63E showed lower values. Interactions observed over the protein trajectory were also higher for TatQ63E and TatR57S compared to TatWt, TatC31S, and TatC31S/R57S/Q63E, suggesting that modifications within Arginine/Glutamine-rich region notably impact TAR interaction. Single amino acid substitutions TatR57S and TatQ63E had significant effects, whereas TatC31S had minimal impact. Introducing single amino acid variants from TatWt to a more representative Tat-C (TatC31S/R57S/Q63E) lowered the predicted binding affinity, consistent with previous findings.
Conclusions
These findings suggest specific amino acid positions are important in Tat-TAR interaction and contribute to the differential pathogenesis and neuropathogenesis observed between subtype B and subtype C. Further experimental investigations are warranted to explore the influence of these amino acid signatures on TAR binding, potentially identifying them as therapeutic targets.

EGFR in different cancers: Potential for Novel diagnostic targets

Gama N[1], Mbita Z
[1]Department of Biochemistry, Microbiology & Biotechnology, University of Limpopo, Private bag X1106, Sovenga 0727, South Africa

Breast cancer remains a major health concern, and it has surpassed lung cancer as the most prevalent cancer, worldwide. It is also the leading cancer amongst women in South Africa. These rocketing breast cancer numbers can be attributed to late lack of specific and effective diagnostic and therapeutic tools for early detection and elimination, respectively. Therefore, novel diagnostic and therapeutic targets are required. The Epidermal Growth Factor Receptor (EGFR) signalling pathway has emerged as a critical important role player in breast cancer pathogenesis, offering potential diagnostic marker and therapeutic targets. Alternative splicing of EGFR generates various splice variants, contributing to the complexity of EGFR signalling and impacting cancer biology breast cancer. This research aims to comprehensively analyse the expression of EGFR splice variants in breast cancer by utilizing the Next Generation Sequencing Catalog to correlate novel EGFR splice variants with various breast cancer subtypes. This work is envisaged to yield novel breast cancer diagnostic and therapeutic targets.

Treating redox imbalance in mitochondrial complex I deficient Caenorhabditis elegans with developed therapeutic cocktail.

Steenekamp S[1], Lindeque J[1]
[1]North-west University

Background:
Mitochondrial disease is a group of genetically heterogeneous, often fatal diseases with an estimate incidence of one in 5000 live births. It is a complex disease state, and the biochemical consequences include: an imbalance of redox metabolism due to low NAD+ levels; loss of membrane potential resulting in a lowered cellular ATP:ADP ratio; electron leakage which causes increased ROS production and Inflammation. Current approaches to treat mitochondrial dysfunction range from symptomatic management to advanced interventions like stem cell and gene replacement therapies, exogenous mitochondrial supplementation, and promotion of mitochondrial biogenesis. Recently, trends gravitated towards treatment with small molecules and endogenous metabolites. A pilot study was designed to evaluate the supplementation of a cocktail of compounds specifically chosen to mitigate the aforementioned problems in a mitochondrial Complex I (CI) deficient C. elegans strain. The aim of this sub-study was to investigate the effects of this intervention on redox metabolism (NADH/NAD+ equilibrium) in CI deficient and wild-type (WT) C. elegans.

Methods:
The novel treatment cocktail consisted of compounds with the following properties: anti-inflammatory, antioxidant, NAD+ precursors, alternative electron acceptors and proton shuttles (across the membrane). CI deficient and WT nematodes were treated and compared with untreated worms. Redox metabolites were quantified with a targeted GC-MS/MS method. Briefly, nematodes were collected, metabolites extracted and derivatized with MTBSTFA before analysis.

Results:
The treatment lowered several redox ratios in the treated CI deficient strain, indicating a lower NADH/NAD+ ratio comparable with WT worms. The ratios of the following reduced vs oxidized metabolites were lowered: 3-hydroxybutyric acid/acetoacetate; lactate/pyruvate; pyruvate/citrate; 2-hydroxyglutarate/2-ketoglutarate; 2-hydroxy-adipate/2-keto-adipate; 3-hydroxydecanoate/3-ketodecanoate; 2-hydroxy-isovalerate/2-keto-isovalerate.

Conclusions:
The accumulation of NADH with mitochondrial dysfunction affects most oxidation-reduction reactions catalyzed by dehydrogenases, leading to the accumulation of reduced intermediates like lactate. The therapeutic cocktail seemingly restored redox balance in the CI deficient nematodes which allowed the dehydrogenase reactions in different pathways to run.

Epizoic diatoms as a novel source of anti-infectious compounds

de Swardt G[1], Helloin E[2], Bezuidenhout J[3], Venter M[1], Majewska R[4,5]
[1]Focus Area for Human Metabolomics, Mitochondria Research Group, North-West University, [2]Centre de recherche INRAE Val de Loire, 37380, [3]Unit for Environmental Science and Management: Microbiology, North-West University, [4]Unit for Environmental Sciences and Management, Faculty of Natural and Agricultural Sciences, North-West University, [5]Division of Algae and Microbial Biotechnology, Faculty of Biosciences and Aquaculture, Nord University

Background

Diatoms occur in a wide variety of aquatic, humid, and moist habitats, including the body surfaces of aquatic animals. The animal-associated (i.e. epizoic) diatoms form symbiotic relationships with, for example, sea turtles and sea snakes, being an essential component of the animal epi-microbiome. Due, to the scarcity of hard-surfaced substrata within the photic zone of the opened ocean, these carapace- and skin-associated microbes are in continuous fierce competition with other microbes. This can lead to the production of novel bioactive compounds, including anti-infectious compounds, which are used against both opportunistic and pathogenic microbes. Thus, the current study aimed to screen metabolites of several epizoic diatom species for the presence of anti-infectious compounds.

Methods

Diatom species (Craspedostauros danayanus, Chelonicola costaricensis, and Tursiocola yinyangii) used in this study were isolated from sea turtles and cultured in f/2 medium. Endo- and exo-metabolites were evaluated for potential bioactive compounds. Following cell lysis, proteins were precipitated using acetonitrile, and polar and non-polar compounds were extracted. The various fractions were combined and dried under nitrogen gas, followed by reconstitution with water and 2% dimethyl sulfoxide. To assess the potential antibacterial, antiviral and antiparasitic activity of the extracts, the minimum inhibitory concentrations were determined.

Results

Low-yield diatom extracts were obtained. No inhibition was found against the selected bacteria, viruses, or larvae of Haemonchus contortus. However, all tested extracts showed strong antiprotozoic activity against a parasite Eimeria tenella.

Conclusions

Epizoic diatoms have the potential to produce either known or novel bioactive compounds that inhibit Eimeria tenella parasite. Future planned studies will use high-concentration extracts obtained from a larger number of epizoic diatom species and strains, which will be tested against additional viruses, bacteria and parasites, as well as pathogenic fungi.

Investigating the Impact of HIV-1 Tat Amino acid Sequence Variation on Microglia-

Associated Neuroinflammation

Muvenda T[1], Williams M[1], Williams A[1]
[1]North West University

Investigating the Impact of HIV-1 Tat Amino acid Sequence Variation on Microglia- Associated Neuroinflammation

Tshengedzeni Muvenda 1 , Aurelia A. Williams 1 , Monray E. Williams 1

1 Focus Area Human Metabolomics, North-West University, Potchefstroom, South Africa.

Email; muvendachenge@gmail.com

Background
Even in the current era of antiretroviral therapy (ART), HIV-1 within the central nervous system (CNS) remains accountable for the onset of HIV-associated neurocognitive disorders (HAND). However, persistent, and chronic inflammation orchestrated by CNS-resident macrophages and microglia plays a pivotal role in the pathogenesis of HAND. Furthermore, the degree of inflammation is influenced by specific amino acid sequence variations within the HIV-1 viral protein, transactivator of transcription (Tat). Subtype C specific amino acid substitutions including R57S, Q63E, and TatR57SQ63E has shown to influence neuroinflammation, a key mechanism in the onset of HAND. However, it is not clear which amino acid variant is responsible for the most significant neuroinflammatory response. Therefore, our study investigated the impact of these Tat subtype C mutations in comparison to subtype B (Wt)Tat on microglia-associated neuroinflammation.

Methods
Immortalized human microglia cells (IMhu-SV40) were cultured and exposed to various Tat peptide variants for 0h,12h, and 24h at 100ng/ml, 200ng/ml and 1000ng/ml, respectively. Culture supernatants were collected, and the levels of several inflammatory markers were measured using human RnD systems DuoSet ELISA kits. Student's t-test were conducted to determine the differences in inflammatory markers between the groups.

Results
Using these optimal conditions, our findings demonstrate differential levels of cytokine production by microglia in response to specific Tat variants. Preliminary data shows a time and concentration dependent effect on the neuroinflammatory response of human microglia cells.

Conclusions
These results emphasize the role of Tat variants in facilitating neuroinflammation in microglia cells, highlighting their potential as crucial mediators in the pathogenesis of HAND.

Key words: Tat protein, HAND, Immortalized human microglia cells (IMhu-SV40), neuroinflammation, cytokines, pathogenesis.

In vitro and in silico evaluation for phytochemicals and anti-diabetic potentials of extracts from different organs of Trichodesma zeylanicum

Manyuwa M[1]
[1]University Of Venda

Background: Trichodesma zeylanicum (Burm. f) R.Br. belongs to the Boraginaceae family. It is an annual shrub native to Australia, Africa, and Asia. This plant is known to contain phytochemicals with antioxidant and anti-diabetic properties in people with type 2 diabetes. The present study was aimed at an in vitro and in silico evaluation of phytochemicals and anti-diabetic potentials of leaves, stems, and roots ethyl acetate extracts of T. zeylanicum.

Materials and methods: Trichodesma zeylanicum was collected from Maungani village located near the University of Venda. Its leaves, roots, and stems were washed, dried under the shade, and ground to powder. Plant part powders were weighed separately and extracted using absolute ethyl acetate. Plant extract filtrates were concentrated using the rotary evaporator. Qualitative biochemical methods including thin layer chromatography (TLC) were used for phytochemical and antioxidant analyses using ascorbic acid, gallic acid, and rutin as standards. Liquid chromatography quadrupole time of flight mass spectrometry (LC-qTOF-MS) technique was used for phytochemical analyses and identifications. In silico α-amylase, α-glucosidase, and vascular endothelial growth factor receptor (VEGFR2) inhibitory potentials of selected identified phytochemicals were screened using the Protein Data Bank available α-amylase, α-glucosidase, and VEGFR2 by molecular docking methods using acarbose and sorafenib as standard inhibitors. In vitro α-amylase inhibitory assay was conducted to validate the active plants extracts with anti-diabetic potentials using acarbose as control.

Results: Known and unknown phytochemicals in ethyl acetate extracts of all of T. zeylanicum parts were analysed and identified. Among the selected compounds docked against two enzyme markers for diabetes and one protein for wound development, ligand 1 [CHEMBL1170880] in roots ethyl acetate extract of T. zeylanicum showed the highest binding affinities when docked against α-amylase (-11,0), α-glucosidase (-10,2), and VEGFR2 (-9,6) in comparison with acarbose (positive control) for α-amylase (-7.4) and α-glucosidase (-7.8), and sorafenib (positive control) for VEGFR2 (-8.1) as inhibitors.

Conclusion: Findings in this study demonstrated that ethyl acetate extracts of all different organs of T. zeylanicum contain phytochemicals with wound healing, antioxidant, and anti-diabetic potentials, suggesting that roots of this plant can serve as sources of phytochemicals with antidiabetic and wound healing properties.

Regulation of the lymphomagenic factor AICDA by miRNA-181b in HIV-associated aggressive B-cell Lymphomas

Kubheka T[1], Alves de Souza Rios L[1], Mowla S[1]
[1]University Of Cape Town

Background
Non-Hodgkin lymphoma (NHL) constitutes a diverse group of aggressive malignancies, with the subtypes Diffuse Large B-cell Lymphoma (DLBCL) and Burkitt Lymphoma (BL) being associated with Human Immunodeficiency Virus (HIV) infection. Overexpression of Activation-induced cytidine deaminase (AICDA/AID), a DNA-modifying enzyme, is a driving factor of these cancers. Investigations of the dysregulation of AICDA/AID in NHL may provide useful insights into disease aetiology. MicroRNAs (miRNAs), which are epigenetic modulators of gene expression, are also dysregulated in cancer. In the current research, the potential regulation of AID, by miRNA-181b, is investigated.

Methods
In silico analyses were used to identify miRNA-181b binding sites within the AICDA 3`-UTR promoter, and luciferase reporter assays, as well as site-directed mutagenesis, were used to study direct regulation of the promoter by this miRNA. Transfection with a miRNA-181b mimic and western blotting were used to establish a relationship between AID and miRNA-181b expression.

Results
Luciferase reporter assays show that the AICDA 3`-UTR is a direct target of miRNA-181b, and in silico analysis identified three putative miRNA-181b binding elements (namely site 1, site 2, and site 3) of varying similarity scores. Multiple alignments show that these sites are highly conserved within the human, bonobo, gorilla, and mouse genomes. Using site-directed mutagenesis, site 2 (highest similarity score) was disrupted and used in luciferase assays for the comparative analysis between the wildtype and site2-mutated AICDA 3`-UTR promoters. No significant difference in repression by miRNA-181b was observed. Currently, the remaining two putative sites are being investigated using a similar approach. In parallel, the effect of a miRNA-181b mimic, on AID protein expression was investigated in a panel of three DLBCL cell lines (SU-DHL4, U2932, and HBL-1). The expression of the AID protein was reduced, relative to controls, in all three cell lines, in the presence of miRNA-181b.

Conclusions
The results reveal that the 3`-UTR promoter of the human AICDA gene is a direct target of miRNA-181b and that AID protein expression is reduced in DLBCL cells that ectopically express a miRNA-181b mimic. Future work includes experiments aimed at fully defining this regulatory axis within the context of lymphoma.

Exploring the avian semi-synthetic phage-display library as a source for high binders targeting selected essential P. falciparum protein

Gasa N[1], Zuma L[2], Kwezi L[3], Chiliza T[4], Pooe O[1]
[1]University Of Kwazulu-natal, School Of Life Sciences, Discipline Of Biochemistry, Westville, Kwazulu-natal, South Africa, [2]University Of Kwazulu-natal, School Of Health Sciences, Discipline Of pharmacology, Westville, Kwazulu-natal, South Africa, [3]Future Production: Chemicals Cluster, Council for Scientific and Industrial Research, Pretoria 0001, South Africa, [4]University Of Kwazulu-natal, School Of Life Sciences, Discipline Of Microbiology, Westville, Kwazulu-natal, South Africa

Background

To date malaria, caused by Plasmodium falciparum is still regarded as the leading cause of malaria related deaths. P. falciparum heat shock protein 70 (PfHsp70) and Lactate dehydrogenase (PfLDH) have not only attracted attention due to their respective biological roles and also regarded as potential diagnostic targets. In the study we sought to use the phage-display library technology as a tool for selection of highly specific, and effective alternative binders that can be used in the development of diagnostic tools, imaging and immunohistochemistry studies.

Methods

Using highly purified recombinant PfHSP70 and PfLDH, we respectively screened the avian semi synthetic phage display library (Nkuku library) to select for anti-PfHSP70 and anti-PfLDH phage. Four rounds of panning were used to enrich highly specific binders and confirmed by ELISA. TEM images of bacteriophages were taken for shape confirmation of pooled bacteriophage. Fluorescent spectra and microscale thermophoresis (MST) analysis were conducted to investigate the binding between target proteins and their generated phage.

Results and Discussion

As expected, TEM imaging displayed a filamentous shaped bacteriophage. Using the selected phage, a concentration dependant killing effect was observed against PfHSP70 or PfLDH expressing E. coli BL21 cells.. A hypochromic shift, indicating direct phage binding to PfHSP70 protein was observed. While, a hyperchromic shift, was observed for PfLDH incubated with their pooled phages indicating their specific binding implying direct interaction . Futhermore, MST data confirmed superior binding affinity between pooled phages targeting PfHsp70 (KD ± 6.1839E-06) and for PfLDH (KD ± 1.2843E-06), respectively.

Conclusions

The results illustrate that these Ig-Y antibodies generated on these phages can potentially be used in the development of new ScfVs for studying the malaria biology, diagnostic bioassays and antibody imaging studies for malaria related studies.

Interaction Studies of iso-mukaadial acetate and betulinic acid compounds against Plasmodium falciparum glycolytic pathway proteins

Zuma L[1], Mabaso N[2], A-Lake J[1], Obakachi V[1], Simelane M[3], Ayeni G[2], Karpoormath R[1], Pooe O[2]
[1]Department of Pharmaceutical Chemistry, Discipline of Pharmaceutical Sciences, College of Health Sciences, University of KwaZulu-Natal, Durban 4000, South Africa, [2]Discipline of Biochemistry, School of Life Sciences, University of KwaZulu-Natal, Westville Campus, Private Bag X54001, Durban 4000, South Africa, [3]Department of Biochemistry, Faculty of Science, University of Johannesburg, Johannesburg 2006, South Africa

Background
Medicinal plants vitally provide therapeutic effects against several diseases with lower systemic toxicity. The emergence of drug resistance in P. falciparum argues for the discovery and exploration of potent antimalarial drugs. Thus, this study aimed to effectively explore the interaction capabilities of the IMA and BA compound against the glycolytic pathway proteins (PfLDH and PfHk).
Methods
The two compounds were subjected to an in-silico ADMET tool for drug-likeness and toxicity assessments. The inhibition activity was performed against NF54 strain of the P. falciparum parasite. Both proteins, PfLDH and PfHk, were expressed in E. coli BL21 (DE3) cells and protein purification was conducted using affinity chromatography. Protein-ligand interaction studies were conducted using the FTIR, Microscale Thermophoresis (MST), molecular docking and molecular dynamics simulation studies to measure and confirm the interaction strength of complexes.
Results
BA and IMA had IC50 values of 1.27 µg/ml and 1.03 µg/ml against the asexual stage of P. falciparum, respectively. Furthermore, the ADMET analysis reported drug-likeness and no toxicity of the compounds. The FTIR experiments showed slight interactions of the compounds with secondary structure proteins. Molecular docking reported -1.155 kcal/mol binding score for PfLDH-BA complex and -3.200 kcal/mol binding score for PfLDH-IMA. The PfHk-BA complex had -2.871 kcal/mol and PfHk-IMA complex had -4.225 kcal/mol binding score. The MST analysis showed a KD value of 0.1036 ± 0.6001 µM for PfLDH-BA complex and 0.7473 ± 0.3554 µM KD value for PfLDH-IMA. Meanwhile, PfHk-IMA had 0.39701± 0.16298 KD value, while PfHk-BA complex had no interaction detected.
Conclusion
The compounds showed potential as possible anti-P. falciparum molecules and inhibitors of glycolytic pathway proteins.
Keywords: Malaria, P. falciparum, medicinal compounds, in silico analysis

Mass-spectrometry based investigation of fibrosis in tuberculous pericarditis (TBP)

Smit-wright C[1], Fikamva S[1], Ramasamy V[1], Nstekhe M[2], Sturrock E[1]
[1]UCT, [2]Groote Schuur

Background
Tuberculous (TB) pericardial effusion is a pro-fibrotic condition that results from the extrapulmonary spread of TB to the pericardium which can lead to constrictive pericarditis; with various mechanisms, peptides, proteins, and pathways being dysregulated. However, not much is known about the pathophysiological processes arising in TBP, especially those leading to fibrosis. In the present study, we investigated protein dysregulation at the pericardial fluid (PF) level in TBP patients, to identify proteins which could be used as predictive biomarkers for pericardial constriction and changes in the TBP proteome which could warrant potential treatment.

Methods
TB pericardial fluid samples were collected during therapeutic pericardiocentesis at Groote Schuur Hospital (GSH), and TB negative PF controls were collected from patients undergoing coronary artery bypass graft surgeries. PF samples were centrifuged and filtered, before undergoing in solution digestion for mass spectrometry. The raw data was analysed using MaxQuant and statistical analyses were performed in Perseus. To assess the biological significance of the resulting identified proteins, STRING-DB was used to determine protein interactions and perform gene ontology (GO) analysis.

Results
In this study, a total of 180 proteins were identified in the pericardial fluid. There were five potentially dysregulated proteins in the diseased (TB+) group in comparison to the control (TB-) group, with each dysregulated protein showing a fold change ≥2. The proteins include ceruloplasmin, Ig alpha-2- chain C region, Alpha-2-macroglobulin (A2M), Ig heavy chain V-III, and Ig kappa V-III region VG.

Conclusion
These results enhance our understanding of fibrosis in TBP by introducing established fibrotic markers previously unexplored in the pathophysiology of TBP. Enrichment analysis further validates the notion that the progression towards constriction in TBP arises from intricate interactions between the innate and humoral immune systems, accompanied by exaggerated wound healing from various pathways.

Keywords
Fibrosis, tubercular pericarditis, mass-spectrometry

Characterizing the gut microbiome: Method development to Unravel Microbial Complexity in the Gut for Metaproteomic studies.

Makaka J[1], Ganief T[1], Blackburn J[1], Miller T[1]
[1]Department of Integrative Biomedical Sciences, Division Chemical and Systems Biology, University Of Cape Town

Background
Stool metaproteomics facilitates the simultaneous measurement of host and microbe proteins, and post-translational modifications, offering insights into complex interactions that nucleic acid sequencing alone cannot capture.Standard proteomic techniques can be adapted for microbial proteins, but extracting proteins from the diverse gut microbial community presents greater challenges due to variations in bacterial cell wall structures. Existing lysis methods were designed for single-tube processing allowing for variability in the data, prompting the need for high-throughput methodologies. A workflow combining mechanical and chemical lysis methods was proposed to fit a 96-well plate format, facilitating effective protein isolation and cleanup for metaproteomic studies aimed at characterizing the gut microbiome.
Methods
Stool samples underwent a wash step to produce a microbial pellet followed by just chemical lysis or a combination of chemical and mechanical lysis processes. Chemical lysis involved trifluoroacetic acid (TFA) treatment followed by neutralization. Mechanical lysis utilized ultrasonication. Protein quantification was performed using Bradford assay. Trypsin digestion and desalting using a previously described method. These samples were analysed using Data-Independent Acquisition -Sequential Window Acquisition of all Theoretical Mass Spectra LC/MS-MS mass spectrometry. Protein sequence databases were generated based on microorganism identifications from 16S rRNA studies found in gut microbiome repositories and constructed from Uniprot data. These were used in conjunction with DIA-NN software to identify proteins.
Results
Both methods identified around the same number of microorganisms, proteins, and peptides, suggesting that chemical lysis alone would provide an efficient way for lysing faecal microbial cells. Both methods also yielded high concentrations of protein from the sample. It is also more time-efficient and highly reproducible.
Conclusions
A workflow consisting of chemical lysis alone in a 96-well plate is deemed efficient for protein isolation from faecal microbiome samples while simultaneously eliminating bias and variation that existing methods introduce.

Cyclen tetrahydrochloride induces necroptosis via oxidative and nitrosative stress in MCF-7 and MDA-MB breast cancer cells respectively

Munsamy M[1]
[1]University Of Kwazulu-natal - Howard

Introduction: Breast cancer (BC) is a heterogeneous disease categorised based on the availability of specific female hormone receptors and is thus predominantly associated with female mortality and morbidity globally. The progression of BC and conventional treatments are subtype-specific, producing immunocompromising effects often linked to oxidative stress and cell death pathways, including necroptosis. This study aims to determine the anti-proliferative and antioxidant mechanisms of cyclen tetrahydrochloride in MCF-7 and MDA-MB-231 human breast cancer cells.

Methods: The MTT assay assessed the cell viability of MCF-7 and MDA-MB-231 cells following exposure to cyclen tetrahydrochloride (0-1000µM) for 48 hours. Luminometric analysis of ATP and Δψm ascertained mitochondrial integrity. Cells were assayed for free radical production (TBARS and NOS assays), while RNS were verified by western blotting for iNOS. The antioxidant response was evaluated luminometrically (GSH) and by western blotting (Nrf2 and SOD2). Cell death by necroptosis was validated by qPCR analysis of RIPK1, RIPK3, MLKL, TNF-α, NF-κB, Gpx-1 and OGG1 gene expression.

Results: Cell viability decreased with increasing doses of cyclen tetrahydrochloride treatments for MCF-7 (IC50 = 168.4µM, IC20 = 41.69µM) and MDA-MB-231 (IC50 = 561µM, IC20 = 302.9µM) cells. This was associated with non-significant changes in CYP34A activity, coupled with a dissipated ΔΨm and decreased ATP production ($p<0.05$). The MCF-7 cells experienced oxidative stress, implied by significant increases in MDA concentration ($p<0.05$), increased OGG1 gene expression suggesting lipid peroxidation in correlation with ROS production, and diminished antioxidant defence; GSH, SOD2, and Nrf2 were decreased. Also, nitrosative stress was not evident since iNOS and RNS was reduced. However, cyclen tetrahydrochloride upregulated iNOS to facilitate RNS production in MDA-MB-231 cells ($p<0.05$) and was associated with increased NF-κB gene expression ($p<0.05$) that correlates with nitrosative stress. Interestingly, evidence of necrotic cell death in both cell lines was presented by increased DNA fluorescence and LDH leakage ($p<0.05$). Thus, necroptosis was investigated as an alternate mode of cell death. Significant increases in gene expression of TNF-α ($p<0.05$), RIPK1 ($p<0.05$), RIPK3 ($p<0.05$), and MLKL ($p<0.05$) demonstrated that necroptosis was executed.

Conclusion: Cyclen tetrahydrochloride induced ROS-mediated necroptosis in MCF-7 cells, but ROS and RNS facilitated necroptosis in MDA-MB-231 cells.

Deciphering Immune Differences Among 3 Clinical BCG Vaccine Types Using Mass Spectrometry

Iradukunda J[1]
[1]University Of Cape Town

Tuberculosis (TB) is a communicable disease caused by Mycobacterium tuberculosis (Mtb) that is of global importance, particularly in low-income countries. It spreads through the nasal pharyngeal route and localizes in the alveolar macrophages of the lung, with 5-10% of infected individuals developing active TB disease. To combat this chronic airborne disease, there is an urgent need for better therapeutic prophylaxis and measures that are both effective and affordable. Vaccines are a cost-effective and safe prophylaxis, and Mycobacterium bovis bacillus Calmette-Guerin (M. Bovis BCG) is the only anti-tuberculosis vaccine available. There are 13 BCG strains in use today, but it is unclear why they elicit different immunological responses following vaccination.

To better understand the immunological differences between the BCG vaccine strains, this study aims to use proteomics, Phosphoproteomics, Lipidomics and Immunopeptidomics, to investigate the extent in which three clinical M. Bovis BCG vaccine strains (BCG Danish, BCG Pasteur, and BCG Russia) differ from each other. We will analyze the molecular complexity of BCG - mammalian systems through mass spectrometry and correlate the observed changes with the differing immunological responses found in infants vaccinated with the different BCG strains.

We hypothesize that the BCG strains will induce distinct immune responses and that these differences can be measured with various mass spectrometric techniques. We observe differential protein expression in macrophage cells infected with the different BCG strains; Enriched pathways highlight various lipid classes, and differential histone modifications as possible drivers for the differing macrophage immune responses. This research has the potential to inform decision-making around which BCG strain to use and to inform future vaccine development efforts.

INVESTIGATING THE BIOSTIMULATION AND BIOREMEDIATION POTENTIAL OF PHOSPHATE SOLUBILISING BACTERIUM, AGROBACTERIUM PUSENSE PS2.

Sekwadi K[1], Livhuwani M[1], Phasha N[1]
[1]University Of Limpopo

Phosphorus is considered among most vital macro-elements, which is mandatory for successful plant growth. Phosphate solubilising bacteria are beneficial bacteria capable enhancing phosphorous availability in soils through dissolving inorganic P pools into a form that can be assimilated by plant. These organisms provide a promising alternative to the use of chemical fertilisers, while providing other soil health benefits. The aim of the study was to investigate the, biostimulant and bioremediation potential of phosphate solubilising bacterium Agrobacterium pusense PS2. The bacterium was isolated on Pikovskaya Agar media from soil collected from a dumping site of a phosphate mine in Phalaborwa. The ability to solubilise phosphate was confirmed on National Botanical Research Institute's Phosphate broth. The isolated strain was tested for plant growth promotion abilities and the ability to degrade phenol, a toxic compound that can be found in irrigation water and agricultural soils, with adverse effects on crops and human health. The results show that the bacterium, Agrobacterium pusense PS2 can solubilise phosphate at a PSI value of 4.3 and a PSE value of 86.96. The strain can utilize L-tryptophan and secreted 75.30 g·mL-1 IAA. Phenol degradation efficiency was recorded at a rate of 93.88% removal for 200 mg/L of phenol. The results of the study indicate that Agrobacterium pusense PS2 is a promising candidate for use as a biofertiliser due to the plant growth promotion traits observed and can be used to remediate phenol-contaminated agricultural soils.

Determining the role of microbiomes in modulating mucosal immunity in a human lung challenge model of tuberculosis disease.

Phaweni D[1]
[1]University Of Cape Town

Background:
Tuberculosis (TB) manifests a nuanced spectrum of susceptibility that transcends simplistic binary distinctions. This investigation delves into the immunopathogenesis of Latent TB Infection (LTBI), elucidating host immune responses and potential links to host alveolar microbiomes, thereby broadening our comprehension of TB susceptibility.

Methods:
Nested within the TB-HART study, bronchoalveolar fluid was collected from LTBI participants pre- and post-BCG rechallenge. Samples underwent filtration, protein precipitation, digestion, and quantification. A protein sequence database was constructed using 16S rDNA and metagenomic data. A spectral library representing the study cohort's protein content was generated using a Triple TOF 6600 mass spectrometer (Sciex, Redwood, USA) in positive SWATH data-independent acquisition (DIA) mode. We employed MS1-based label-free quantification (LFQ) within DIA-NN for quantification. Differential protein identification and statistical analysis were performed using MetaboAnalyst (Xia Lab, version 5.0) for human proteins and MicrobiomeAnalyst (Xia Lab, version 2.0) for microbial proteins. Functional analysis employed STRING (version 12.0) and KEGG (version 108) software.

Results:
We identified two LTBI subgroups with distinct immunological profiles. Group 1 showed a classical immune response with complement and coagulation activation, while Group 2 exhibited heightened neutrophil activity alongside dysregulated complement dynamics. Analysis of dysregulated proteins associated with TB revealed insights: CD14 impairment hindered proinflammatory cytokine release, CTSS disruption compromised antigen presentation, and C3 dysregulation contributed to a neutrophil-dominated response in Group 2. Integration of immunological and alveolar microbiome data suggested Proteobacteria and Bacteroidetes prevalence might contribute to proinflammatory responses, potentially involving microbial elements like lipopolysaccharides (LPS).

Conclusion:
Our study sheds light on the complex immunological landscape of LTBI, emphasizing the necessity for comprehensive approaches in TB research and intervention, while laying the groundwork for exploring the intricate interplay between host immunity, microbial factors, and TB pathogenesis to enhance TB management strategies.

Establishing a cell culture model to evaluate glycine conjugation.

Visagie J[1], van der Sluis R[1], Gouws C[2]
[1]Focus Area for Human Metabolomics, North-West University, [2]Centre of Excellence for Pharmaceutical Sciences (Pharmacen™), North-West University

Background: In the past couple of decades great strides have been made with the use of recombinant proteins to evaluate the capacity of the phase II detoxification pathway, glycine conjugation. However, the further study of this pathway calls for a physiologically relevant in vitro model. A three-dimensional (3D) human hepatocellular carcinoma cell (HepG2/C3A) system has shown significant resemblance to in vivo cell behaviour when cultured using the clinostat bioreactor technology by CelVivo. This 3D spheroid model serves as a suitable candidate and would be verified for its use to study glycine conjugation.

Methods: A single cell (HepG2/C3A) suspension cultured as 3D spheroids in the ClinoStar™ system were matured for 21 days. The spheroids were then harvested for gene expression analysis (qPCR) to determine the change in expression of selected genes involved in glycine conjugation between 2D (monolayer) and 3D cultures.

Results: It is anticipated that the HepG2/C3A 3D spheroid model is suitable for glycine conjugation studies based on the upregulation of the selected genes compared to 2D cultures.

Conclusions: The mature 3D spheroid model is suitable for glycine conjugation studies and deemed more physiologically relevant than recombinant proteins. Further work can implement this model to evaluate the detoxification of endogenous and xenobiotic carboxylic acids keeping real-world scenarios in mind i.e. organic acidaemias, increased use of benzoate as preservative, or salicylism from aspirin overdose.

Potential benefits of the dietary compounds, aspalathin, and sulforaphane, in protecting against lipid-induced cardiotoxicity

Mthembu S[1,2], Mazibuko-Mbeje S[1], Silvestri S[3], Orlando P[3], Nkambule B[4], Muller C[2,5,6], Tiano L[3], Dludla P[6,7]
[1]Department of Biochemistry, Mafikeng Campus, [2]Biomedical Research and Innovation Platform, [3]Department of Life and Environmental Sciences, [4]School of Laboratory Medicine and Medical Sciences, [5]Centre for Cardiometabolic Research Africa (CARMA), [6]Department of Biochemistry and Microbiology, [7]Cochrane South Africa

Background
Dyslipidemia remains the prominent pathological feature responsible for oxidative stress-induced cardiac damage. Currently used lipid-lowering agents like statins are effective, however, these therapies fail to protect against oxidative stress-induced insult. Nutritional supplements, due to their abundant antioxidant properties, are increasingly explored for their potential benefits to protect against dyslipidemia-induced cardiotoxicity.
Methods
Cultured H9c2 cardiomyoblasts, as a routinely used experimental model to assess cardiotoxicity, were treated for 24 hrs with the dietary compounds, aspalathin (1 µM) and sulforaphane (10 µM), after exposure to palmitic acid (0.25 mM) for 24 hrs. Simvastatin (2.5 µM) and reduced coenzyme Q10 (2.5 µg/ml) were used as comparative controls. To assess the protective effects of dietary compounds against palmitate-induced cardiac toxicity, the following assays were performed, including cellular metabolic activity, cholesterol content, mitochondrial respiration, intracellular levels of reactive oxygen species (ROS), antioxidant responses, and markers of cellular damage.
Results
The dietary compounds, aspalathin, and sulforaphane, improved the cellular metabolic activity and reduced cholesterol content, while also attenuating palmitic acid-induced alterations in mitochondrial respiration. These compounds showed similar levels or performed even better than the known lipid-lowering agent, simvastatin, in attenuating palmitic acid-induced cardiotoxicity. This included reducing ROS production, enhancing intracellular antioxidant levels like superoxide dismutase and total glutathione, and gene expression of nuclear factor erythroid 2-related factor 2. The benefits were accompanied by reduced lipid peroxidation and cellular damage, as measured by assessing malonaldehyde levels and the apoptosis rate.
Conclusion
This preclinical study supports the potential benefits of dietary compounds or foods rich in aspalathin or sulforaphane in protecting against lipid-induced oxidative damage within the myocardium. However, such preliminary results require confirmation in well-established animal or clinical studies.

Evaluation of the Ziziphus mucronata and Ximenia caffra plant extracts, for anti-viral and anti-cancerous activity

Ncembu V[1]
[1]University of Johannesburg Student

The Human Immunodeficiency Virus-1 continues to be a severe hazard to public health, with millions of death cases worldwide. The combination antiretroviral therapy (cART) has however been seen to be effective in suppressing viral progression and restoring immune function. Nevertheless, cART has been linked to a number of side effects, including an increased risk of developing opportunistic infections such as cervical cancer (through the persistence of the Human Papillomavirus). The objective of this study was to investigate the anti-viral and anti-cancerous activity of Ziziphus mucronata (hexane and methanolic (bark and leaf) extracts) and Ximenia caffra. (ethanolic extract). The Reverse Transcriptase (RT) calorimetric assay was used to determine the direct inhibition of HIV-1 reverse transcriptase induced by the plant extracts. Using the Alamar Blue assay, the crude extracts were screened for cytotoxicity against the malignant cervical cancer (ME180) and non-malignant human embryonic kidney (HEK293) cell lines. Light microscopy was used to determine any apoptotic-related morphological changes. Caspase 3/7 was further used to confirm the mode of cell death induced by the plant extracts. From the 5 crude extracts that were tested, none of them inhibited HIV-1 reverse transcriptase. For the anticancer evaluation, the Ziziphus mucronata methanolic extract from both the leaves and the bark yielded an inhibitory concentration (IC50) of 88 ± 2.01 µg/mL and 87 ± 8.94 µg/mL against the ME180 cells. In contrast, the hexane extract from both the leaves and the bark yielded IC50 of 31 ± 2.25 µg/mL and 28 ± 0.97 µg/mL respectively. Against HEK293, the Ziziphus mucronata hexane extract from both the leaves and bark yielded IC50 of 39.45 ± 3.4 µg/mL and 30.18 ± 2.4 µg/mL respectively. The Ximenia caffra ethanolic extract yielded an IC50 of 63.8 ± 1.73 µg/mL against the ME180 cells. Cell blebbing was seen through light microscopy. Caspase 3/7 showed that the Ziziphus mucronata hexane extracts (bark and leaves) had the highest caspase activity, which was higher than the positive control at 100uM. The methanolic and hexane extracts from Ziziphus mucronata as well as the ethanolic extract of Ximenia caffra exerted antiproliferative effects in cancerous ME180 cells.

Analysing the virulence factors of Listeria monocytogenes across phenotypically diverse listeriosis cases to identify contributors to disease severity

Smith I[1], Schubert W[1]
[1]University Of Pretoria
Imke Smith, Wolf-Dieter Schubert
University of Pretoria, Faculty of Natural Sciences, Department of Biochemistry, Genetics and Microbiology

Background
Listeria monocytogenes is the causative agent of the foodborne illness known as listeriosis. Manifestations of the diseases vary from mild gastrointestinal distress and fever to severe cases of meningitis and spontaneous abortions in pregnant women. A range of virulence factors, collectively creating the virulome, drive and modulate listerial pathogenicity through a multitude of host interactions. Here, we aim to analyse the virulome of L. monocytogenes across phenotypically diverse listeriosis cases to identify and classify contributors to disease severity.

Methods
The research involves analysing clinical strains and categorising them based on diverse disease outcomes. Virulence factor genes are annotated for each strain, creating a comprehensive database. Strains are then assigned to genomically related sequence types. A phylogenomic tree is being derived to visualise the multitude of evolutionary relationships.

Results
Correlation studies help explore the interplay between virulence factors, sequence types, and phylogenomic relatedness with phenotypic disease manifestations. This will help draw conclusions on the relationship between the genotype of the bacterium and the severity of listeriosis cases.

Conclusions
By leveraging the power of computational tools, this research aims to expand our understanding of the biological roles and pathogenic mechanisms of the bacterium. The results obtained have the potential to make a valuable contribution to the wider realm of bacterial pathogenesis and serve as a significant resource for guiding forthcoming studies on virulence factors in L. monocytogenes.

Key words: Infectious disease, listeriosis, phylogenomic analysis

Antidiabetic potential: In silico molecular interactions and ligand-protein binding of compounds isolated from the ethyl acetate fraction of Ficus lutea acetone extract

Olaokun O[1]
[1]Sefako Makgatho Health Sciences University

Background:
Despite significant scientific advancements in understanding and managing diabetes, its prevalence continues to rise. No single therapeutic agent has effectively treated and prevented the progression of this condition. Diabetes involves multiple defects and has a multifactorial pathogenesis, necessitating a multi-modal therapeutic approach rather than targeting a single drug. A combination of multiple therapeutic agents may offer a holistic approach to managing this disease. Medicinal plant extracts contain a variety of bioactive phytochemicals that possess synergistic, potentiating, agonistic, and antagonistic properties, that can exert diverse therapeutic actions.
Method:
The extract of Ficus lutea was previously shown to possess antidiabetic potential, leading to its fractionation using solvents of increasing polarity. This process yielded an ethyl acetate fraction with potent antidiabetic properties that was subjected to a silica gel column chromatography, leading to the identification of five compounds (lupeol, stigmasterol, α-amyrin acetate, epicatechin, and epiafzelechin) using nuclear magnetic resonance. These compounds were then investigated at the molecular level to understand their interactions and binding with selected diabetic-related protein receptors (1NOI, 3G9E, 2P8S, 5EQG, 4RCH, 5T19, 1OSE, and 2QMJ) using in silico techniques.
Results:
All the isolated compounds demonstrated antidiabetic potential in the in silico study, with their binding energies for glycogen phosphorylase (1NOI) and α-amylase (1OSE) being stronger (< -8.1) than those of the positive controls. Stigmasterol exhibited higher binding affinity for five out of the eight selected receptors involved in glucose metabolism. Also, the amino acid residues involved in the interactions and the types of interactions varied among the different compounds. While stigmasterol interacted with four amino acids (HIS571, TYR573, ALA383, and HIS341) of 1NOI receptor with one hydrogen bonding, α-amyrin acetate interacted with three (HIS571, ALA383, and HIS341) without any hydrogen bonding.
Conclusion:
The isolated compounds exhibited characteristics indicative of their potential as antidiabetic agents. The ethyl acetate fraction and its compounds, especially stigmasterol, demonstrate potential as effective antidiabetic agents. However, further comprehensive studies are necessary to validate these findings.

The effect of plastic pollution on the gut microbiota of the major malaria vector, Anopheles arabiensis Patton (Diptera: Culicidae)

Misser S[1,2], Chen C[1,2], Oliver S[2,1]
[1]WITS Research Institute for Malaria, University Of The Witwatersrand, [2]Vector Control Reference Laboratory, National Institute for Communicable Diseases

Background
The microbiota of the midgut of mosquitoes plays a significant role in determining vector competency and is essential for development, reproduction, and immunity. The gut microbiota of Anopheles arabiensis is influenced by the larval environment. Therefore, it is important to understand the impact of pollutants on the gut microbiota. In this study, we determined the effect of microplastic and plastic additive exposure on the gut microbiota of adult females of two laboratory strains of An. arabiensis.
Methods
An insecticide-susceptible (SENN) and an insecticide-resistant (SENN-DDT) strain were used. Larvae were reared in four plastic treatments: artificially degraded disposable nappies, phthalic acid, bisphenol-A (BPA) and fluorescently labelled latex beads. Larvae reared in untreated water served as the control. Metagenome sequencing of the 16S rRNA gene was performed on the midgut of plastic-treated and untreated three-day-old adult females. The hypervariable V3 and V4 regions were amplified and sequenced. The library preparation was performed according to the protocol.
Results
In terms of alpha-diversity, SENN showed significant differences in species richness and evenness. Conversely, only a significant difference in species evenness was observed in BPA-treated SENN-DDT. In beta-diversity analysis, all plastic treatments cluster separately from the untreated group. A significant difference in the beta diversity between the treatment groups was observed. Plastic treatment increased the number of differentially abundant genera in SENN but not SENN-DDT. The nappy-treated group had the greatest number of unique genera in SENN. However, in SENN-DDT the untreated group consisted of the most unique number of genera. The most abundant genera in both strains were Acinetobacter, Citrobacter, Elizabethkingia, Rahnella and Yersinia.
Conclusion
The impact of plastic on the microbiota was more significant in the insecticide susceptible strain whereas the resistant strain better managed the effects. The findings of this study indicate that plastic pollution does impact the midgut microbiota of An. arabiensis however insecticide resistance buffers the effect of plastic pollution on the microbiota.

Investigating the status and regulation of the Suppressor of Cytokine Signalling 1 (SOCS1) gene in DLBCL

Ramorola B[1], Sinkala M[2], Mowla S[1]
[1]Division of Haematology, Department of Pathology, Faculty of Health Sciences, University of Cape Town, [2]Division of Computational Biology, Department of Integrative Biomedical Sciences, and Institute of Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town

Background
Diffuse large B-cell lymphoma (DLBCL) is the most common aggressive B-cell lymphoma globally and is also the most prevalent subtype of lymphoma among people living with HIV. Approximately 40% of DLBCL of patients undergoing standard first-line therapy eventually relapse or are refractory to treatment, highlighting a need to identify novel targets for the development of targeted therapy. The Suppressor of cytokine signalling protein 1 (SOCS1) has been identified as a frequently altered gene in DLBCL, with recent studies associating it with resistance to treatment. However, its role in the pathogenesis of DLBCL remains largely undefined.

Methods
A Cancer Genome Atlas DLBCL dataset was analysed for SOCS1 expression and stratified into two groups, namely, “low-SOS1” and “high-SOCS1”. The Kaplan-Meier method was used to estimate survival, and enrichment analyses were performed to identify overrepresented genes and related pathways. Furthermore, SOCS1 expression levels were assessed in a panel of cell lines representing the two molecular DLBCL subtypes (Germinal center B-cell (GCB)-DLBCL: SUDHL-4, OCI-LY1 and WSU-DLCL2; and Activated B-cell (ABC)-DLBCL: HBL-1 and U2932) and a control lymphoblastoid cell line (LCL) using qPCR and western blotting. These were correlated with mRNA expression levels of methylation-related genes (DNMT1, DNMT3A, DNMT3B and MeCP2).

Results and Conclusions
In silico analysis revealed that the low-SOCS1 expression group had better survival estimates, relative to high-SOCS1 expressors. Interestingly, SOCS1 was found to be most frequently mutated in the early-stage phase of disease. This indicates that SOCS1 may have a dual role in DLBCL; where its reduced expression is advantageous in the initial stages of disease, while its increased expression in late-stage disease promotes a more aggressive disease phenotype. This was further corroborated by enrichment analyses, showing association with cancer-promoting processes including proliferation, migration, invasion, and metastasis. In DLBCL cell lines, SOCS1 expression was lower compared to the LCL control. This was inversely correlated with expression of the methylation-related genes, including DNMT1 and DNMT3A. This therefore indicates that the modulating of SOCS1 expression in DLBCL may be via methylation. In future work, a SOCS1-overexpressing DLBCL cell model will be used to study its impact on specific cancer phenotypes and pathogenic pathways.

Structure of African Horse Sickness virus VP2

Britz H[1], Potgieter C[2], Schubert W[1]
[1]University of Pretoria, [2]Deltamune

Background

African Horse Sickness virus (AHSV) causes exceptionally high mortality rates in horses and severe disease in other non-native equines. It is closely related to Bluetongue virus (BTV), another Orbivirus from Africa, and is spread by Culicoides midges. A midge blood meal transfers the virus to the blood stream from where it infects and damages endothelial cells. VP2 is a receptor binding protein on the outer surface of the non-enveloped virus. It is responsible for both tissue tropism and antibody specificity. Current live-administered vaccines while effective have limitations that could be overcome if the structure and function of VP2 were better understood.

Methods

Theoretical structural models of VP2 for both AHSV and BTV were generated using RoseTTAfold. BV VP2 was docked into published CryoEM electron density maps and conformationally adjusted. AHSV VP2 protein was produced in tissue culture by transient expression and purified by Strep-tag affinity chromatography. The purified protein was used for sitting-drop crystallization experiments and electron microscopy. 2D classification of protein images from negative stain electron microscopy allowed a low-resolution 3D reconstruction of VP2. CryoEM images were also analysed.

Results

Confidence in protein structure prediction for AHSV VP2 by RoseTTAfold was only 0.54 compared to 0.86 for BTV VP2. Correspondingly, predicted BTV VP2 agreed well with a previous electron density map. SDS-PAGE analysis for AHSV VP2 indicated highly pure samples but yields were low at 0.10, 0.06 and 0.04 mg protein from three production cycles. AHSV VP2 crystallised into hexagonal prisms of ~20 x 20 x 40 µm3. Negative stain microscopy confirmed AHSV VP2 to form propeller-shaped trimers. 3D reconstruction from negative stain EM yielded a 23 Å resolution map while CryoEM molecular snapshots were insufficiently resolved to allow a 3D reconstruction.

Conclusion

Initial structural analyses are promising though low proteins yields need to be improved. Better data collection by cryoEM or X-ray crystallography are required to improve the current resolutions of 23 (negative stain data) and 14 Å (previously published map).

Investigation of Drug Repurposing Studies in the Identification of Plasmodium falciparum Hexokinase Inhibitors

Magibile Y[1], Zuma L[1], Gasa N[1], Yakobi S[1], Pooe O[1]
[1]School of Life Sciences, Biochemistry, University of KwaZulu-Natal

Background

The emergence of drug-resistant malaria underscores the necessity identify novel targets for antimalarial drugs. During erythrocyte infection, the malaria parasite relies on glycolysis for energy. P. falciparum hexokinase, (PfHK) is an essential enzyme that plays a crucial role in the beginning of glycolysis. According to the literature, PfHK inhibition decreases ATP production and generates NADPH that P. falciparum utilises to safeguard itself against oxidation and facilitate nucleotide production. In our present investigation, we utilised a combination of computational analysis and laboratory experiments to evaluate the effectiveness of five categories of antibiotics (cephalosporin, penicillin, macrolide, fluoroquinolone, and sulfonamide) as prospective inhibitors of PfHK, to identify potential antimalarial drugs.

Methods

The 3D conformation of PfHK was acquired from RCSB Protein Data Bank (PDB ID: 7ZZI) and prepared using UCSF Chimaera. The chemical structures of antibiotics were acquired from the PubChem compound database. The process of molecular docking was performed using PyRx V.0.8 graphical user interface (GUI) AutodockVina. Molecular dynamics simulation studies were done using Schrödinger Maestro and the binding affinity for each ligand was calculated based on their interaction energy. Additional in vitro experimentation was carried out to validate the identified relationship.

Results

Azithromycin (macrolide) had a binding affinity of –8.3 kcal/mol and established hydrogen bond interactions with ALA-314, TRP-311, TYR-312, and LYS-35 residues located in the substrate-binding site showing its affinity with the catalytic domain of PfHK. Confirmatory studies are still in progress to elucidate the potential of hexokinase inhibitors in antimalarial drug discovery.

Conclusion

This study presents a comprehensive drug repurposing approach combining computational analysis and laboratory experimentation to explore potential inhibitors of PfHK. Through molecular docking and dynamics simulations, we identified azithromycin as a promising candidate with a strong binding affinity. Our findings suggest that azithromycin may inhibit PfHK activity by forming hydrogen bond interactions with key residues in its catalytic domain.

Breaking Barriers in Early Cancer Diagnosis: The Role of Biosensing Platforms

Saasa V[1]
[1]University Of South Africa

One of the key challenges in disease control and prevention, especially for cancer, is early detection. Better clinical outcomes are directly related to early detection of diseases, enabling effective treatment to reduce the suffering and cost to society associated with the diseases. However, traditional screening methods, such as biopsy and clinical imaging, are currently not very powerful at very early stages, quite costly, and not available to many patients. The earlier cancer can be detected, the better the chance of a cure. Currently, many cancers are diagnosed only after they have metastasised to the entire body. Effective and accurate methods of cancer detection and clinical diagnosis are urgently needed. The development of biosensing platforms provides a non-invasive, portable, easy-to-use, cost-effective detection tool with high specificity, sensitivity, and reliability for cancer markers. In this poster, we explore the barriers to early cancer detection and explore the fields of biosensor technologies, including immunosensors, aptasensors, enzyme biosensors, and non- enzymetic biosensors, and highlight their potential for detecting cancer biomarkers.

Phytochemical contents and antioxidant potential of the methanolic extract of Eriosema montanum Baker f. (Fabaceae) and its main fractions.

Tabakam Tchangou G[1,2], Nfotie Njoya E[1], Awouafack M[2], Mashele S[1], Makhafola T[1]
[1]Faculty of Health and Environmental Science, Central University of Technology, Free State. Bloemfontein 9300, South Africa , [2]Natural Product Research Unit, Department of Chemistry, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon

Background
Eriosema montanum Baker f. a member of the Fabaceae family, is an African medicinal plant used in folk medicine to treat several diseases such as conjunctivitis, snake-bites, cough and asthma. These diseases are commonly associated with free radical production or inflammation. Therefore, the aim of this study was to evaluate the phenolic and flavonoid contents as well as antioxidant potentials of methanolic extract of E. montanum and its main fractions.
Methods
Column chromatography techniques were used to obtain ten main fractions (A-J) whereas the total phenolic and total flavonoid contents were measured using the Folin-Ciocalteu reagent and aluminium chloride colorimetric methods, respectively; the antioxidative potential was evaluated using 2,2-dyphenyl-1-picrylhydrazyl (DPPH), [2,2'-Azino-bis(3-ethylbenzothiazoline-6-Sulfonic acid)] (ABTS), and ferric reducing antioxidant power (FRAP) scavenging assays.
Results
Among the isolated compounds from the different fractions, two are identified as Genistin (1) and hexadecanoic acid (2), The highest total phenolic and flavonoid contents were recorded with fractions E and D (115.95 ± 1.51 mg GAE/g and 84.84 ± 3.30 mg QE/g, respectively) whereas the methanolic extract displayed 86.77 ± 6.24 mg GAE/g and 82.11 ± 4.60 mg QE/g, respectively. Fraction F and D had the highest DPPH and ABTS radical scavenging capacities with IC50 values 34.64 ± 1.75 and 23.12 ± 1.07 µg/mL and were more potent than the methanolic extract (IC50 values 141.40 ± 4.66 and 41.28 ± 1.03 µg/mL), respectively. Ascorbic acid, used as a positive control, showed IC50 values of 4.08 ± 1.04 and 1.28 ± 0.81µg/mL in DPPH and ABTS assays, respectively. Fractions D and F had the highest antioxidant potentials as well as total flavonoids contents. The recording of the NMR spectra of others compounds as well as their biological tests are still going on.
Conclusion
A positive linear correlation was observed between total flavonoid and phenolic contents with the antioxidant activities of the plant extract and main fractions. This indicates that the highest the extract or fraction contains flavonoids or phenolics, the highest was the antioxidant potential. Our results support that E. montanum could be a potential source of natural antioxidants.

Keywords: Eriosema montanum, Fabaceae, radical scavenging, phytochemicals.

The effect of cholesterol depletion on TGF-β-induced epithelial-mesenchymal transition in pancreatic cancer cells

Breytenbach A[1], Kaur M[1]
[1]University Of The Witwatersrand

Background
Pancreatic ductal adenocarcinoma (PDAC) is a highly metastatic cancer that relies on the epithelial-to-mesenchymal transition (EMT) program for its spread. EMT is a cell plasticity program that involves the reorganisation of cell structure as cells transition from an epithelial to a mesenchymal phenotype. The dysregulated cholesterol metabolism resulting from metabolic reprogramming in PDAC is thought to play a role in EMT by affecting EMT-related signalling pathways. However, no publication has yet investigated the impact of EMT on cholesterol content in PDAC.
Methods
EMT was induced in PANC-1 cells using 10 ng/mL TGF-β1, followed by investigation of the effects of cholesterol-depleting agents (KS-01 and methyl-β cyclodextrin) alone or with chemotherapeutic agents (Gemcitabine (GEM) and 5-Fluorouracil (5-FU)) on cholesterol content, EMT state, drug resistance, and invasion. Cholesterol content was assessed using Vybrant Alexa Fluor® Lipid Raft labelling kit, Cholesteryl BODIPY™ FL C12 stain, and Filipin III stain. Evaluation of the EMT state involved immunocytochemistry of E-cadherin, vimentin, and Ki-67 alongside growth curve analysis. Drug resistance and invasive capabilities were determined using the Vybrant® MDR Assay Kit and Transwell® Cell invasion assay. Lastly, Real Time-Quantitative PCR was utilised to complement and validate the findings from the aforementioned assays.
Results
Mesenchymal cells rely on reduced membrane cholesterol levels, synthesis, and uptake but store more cholesterol and promote efflux. EMT also promoted drug resistance via increased ABCB1 functionality and expression and reduced hENT1 expression. Cyclodextrin treatment promoted a cholesterol compensatory mechanism, leading to the promotion of a hybrid EMT state, drug resistance, and metastatic potential. GEM or 5-FU monotherapy in mesenchymal cells induced EMT transcription factors and cholesterol efflux, synthesis, and import. Combining GEM with KS-01 promoted a hybrid EMT state, while combining KS-01 with 5-FU mitigated EMT-promoting effects, possibly through translational mechanisms.
Conclusions
The exact mechanism linking the cholesterol compensatory mechanism to EMT remains complex and unknown. Therefore, targeting cellular cholesterol, particularly with cholesterol-depleting agents, warrants continued exploration, with implications for understanding the treatment repercussions of the use of cholesterol-depleting agents for the treatment of other disorders in patients with PDAC.

The ameliorative effects of curcumin on key enzymes that play a role in hepatic glucose output

Mdutyana M[1], Ibrahim K[2], Matumba M[1]
[1]North-west University Mafikeng Campus, [2]University of Witwaterstrand

Background
Diabetes mellitus (DM) is a prevalent and consequential disorder. Currently, it affects more than 463 million people globally with the numbers expected to rise, owing to the growing consumption of high-fructose diet. The most common type of diabetes is type-2 diabetes (T2D), making up 90% of all diabetes cases. The hallmark of T2D diabetes is insulin resistance, affecting tissues like the liver. The liver is responsible for 60% of glucose released into the blood. During glucose metabolism, two critical mechanisms occur in the liver: gluconeogenesis and glycogenolysis. In a diabetic state, these mechanisms release excessive glucose into the bloodstream, promoting hyperglycaemia, a symptom of DM. In this study, natural polyphenol curcumin is administered to T2D-induced rats to observe the ameliorative effects of curcumin on crucial enzymes involved in hepatic glucose output.

Method
Forty male Sprague Dawley rats were fed a high-fructose diet (HFD) to induce metabolic syndrome model. The rats were treated with curcumin (CU). After 116 days the experiment was terminated, and hepatic tissues were collected for molecular analysis. Firstly, RT-qPCR was conducted to analyse the gene expression of the four rate-limiting enzymes, PEPCK, G6Pase, GP, and FBPase, which play a significant role in hepatic glucose output. Additionally, western blot analysis was conducted to determine the protein expression of these enzymes. Lastly, an enzyme activity assay was performed to analyse the activity of the PEPCK enzyme.

Results
Increased PEPCK gene expression in the high-fructose diet group compared to the control (CON) group was observed in the results. However, it was downregulated in the group treated with curcumin alone and in the HFD group treated with curcumin. Additionally, when checking the activity of PEPCK, the CON group showed increased activity, and the CU group showed reduced PEPCK activity compared to CON. HFD shows elevated levels of PEPCK enzyme activity compared to the group treated with HFD+CU.

Conclusion
These findings indicate that curcumin can be a potential treatment for T2D and reduces excessive hepatic glucose output by downregulating the activity and gene expression of key enzymes involved in gluconeogenesis and glycogenolysis.

Evaluation of ethnobotanical uses, biological activities, and phytochemical properties of selected Gomphocarpus and Pachycarpus species.

Mdaka A[1]
[1]University Of Johannesburg

Medicinal plants have been used for centuries for their effectiveness in treating and preventing different microbial ailments in southern Africa. For example, many species of the family Apocynaceae have been widely used as they have continuously played a significant role in the well-being of the southern African population. An earlier study showed that several species belonging to the closely related Gomphocarpus and Pachycarpus in the family are used for medicinal purposes in the region. The aim of this study was to record the known ethnobotanical uses, phytochemistry, and antimicrobial activity of Gomphocarpus and Pachycarpus species, thereafter, analyse the phytochemical composition and evaluate the antimicrobial activity of G. fruticosus subsp. fruticosus against Staphylococcus aureus and Klebsiella pnuemoniae . An ethnobotanical review was conducted using databases such as Science Direct, Google Scholar, Springer Link, and book chapters. The knowledge gaps were identified. Gomphocarpus fruticosus subsp. fruticosus antimicrobial activities and phytochemical properties were evaluated in this study. Possible phytochemical compounds were assessed using thin-layer chromatography. Thereafter, antimicrobial activity was evaluated using a minimum inhibitory concentration assay. Ten species have records of ethnobotanical uses whereby the most common medicinal uses of evaluated plants (6 species) were in respiratory tract infections, followed by skin inflammations and wounds (4 species). Only one species has been previously evaluated for antibacterial activity. Four species have been evaluated for phytochemical constituents, and the most common constituents are cardiac glycosides and terpenoids. In preliminary phytochemical screening of G. fruticosus subsp. fruticosus leaves were found to contain terpenes, phenols, alkaloids, and flavonoids, whereas the stems were found to have terpenes, alkaloids, and phenols. The antimicrobial test of G. fruticosus subsp. fruticosus stems and leaves extracts showed weak inhibitory activity against S. aureus and K. pneumoniae pathogens.

Unravelling the Molecular Basis of Isoniazid Resistance in Mycobacterium tuberculosis Clinical Isolates in Cameroon with Unknown Resistance Mechanisms

Nono V[1], Edouard N[2], Donfack Valerie D[3], Mutshembele A[1]
[1]South African Medical Research Council, [2]The University of Bamenda, [3]Centre Pasteur du Cameroun

Background: Isoniazid (INH) resistance poses a significant challenge in the effective treatment of tuberculosis. While mutations in the katG and inhA genes have been identified as the primary cause of INH resistance in many strains, there remain a subset of clinical isolates in Cameroon with unknown resistance mechanisms. This study aims to investigate the molecular basis of INH resistance in these strains and shed light on alternative pathways leading to resistance.
Methodology: After performing drug susceptibility testing using the GenoType MTBDRplus assay on 432 INH-resistant strains of Mycobacterium tuberculosis clinical isolates collected from the National Tuberculosis Reference Laboratory in Cameroon, a 13.66% phenotypically confirmed INH-resistant strains with an unknown mechanism of resistance was obtained. Whole-genome sequencing will be employed to analyze the 13.66% of strains to identify novel genetic variations conferring resistance. Also, comparative gene expression analysis will be conducted to quantify the level of INH-activating enzyme and associated efflux pumps between INH-resistant strains with no detected mutation and INH-susceptible strains.
Expected results: It is anticipated that novel genetic variants and modified or differentially expressed genes/proteins will be identified in INH-resistant strains compared to susceptible strains. These differentially expressed genes/proteins can serve as potential biomarkers for resistance.
Conclusion: This research will improve our understanding of drug resistance dynamics and identify potential drug targets to facilitate the development of more effective targeted diagnostic tools and therapeutic strategies. The findings from this study will have implications for both clinical management and public health interventions, ultimately contributing to global efforts to combat tuberculosis.

In vitro transcribed designer epigenome modifiers targeted against hepatitis B virus to induce transcriptional silencing

Singh P[1], Bloom K[1], Cathomen T[2], Mussolino C[2], Arbuthnot P[1], Ely A[1]
[1]Wits/SAMRC Antiviral Gene Therapy Research Unit, Infectious Diseases And Oncology Research Institute (IDORI), Faculty of Health Sciences, University of the Witwatersrand, [2] Institute for Cell and Gene Therapy, Medical Center - University of Freiburg

Approximately two thirds of the world's population has been exposed to the hepatitis B virus (HBV). Covalently closed circular DNA (cccDNA) is responsible for persistence of infection that results in development of hepatocellular carcinoma (HCC) and cirrhosis. Current treatments do not impact nor completely remove the stably maintained cccDNA reservoir. Gene editing technologies target cccDNA directly and offer the possibility of eliminating or disabling this reservoir. Altering or modifying cccDNA epigenetically by de novo methylation using designer epigenome modifiers (DEMs) is a safer and less genotoxic alternative to designer nucleases. Anti-HBV DEMs have been generated using by fusing repression domains to TALE-DNA binding domains that bind the surface (S), core (C) and polymerase (P) ORFs of HBV, and this study focuses on expressing DEMs as in vitro transcribed (IVTed) mRNA.

To generate DEMs and reporter mRNA plasmids, sequences encoding existing DEMs and reporter genes were cloned into an expression vector specifically designed for the in vitro transcription (IVT) of mRNA. Anti-HBV DEMs and reporters were successfully IVTed. To enhance expression and decrease immune stimulation, modified nucleotides was incorporated during IVT process and cellulose based chromatography was used during purification of IVTed mRNA. Immunofluorescence staining and GFP fluorescence imaging confirmed expression of IVTed anti-HBV DEMs and reporter mRNA in vitro. Toxicity of mRNA was assessed using 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. IVTed anti-HBV DEM-encoding mRNA was transfected in vitro in liver-derived cells (Huh 7 and HepG2-hNTCP cells (HBV infection model))

Preliminary results indicate anti-HBV DEMs-encoding mRNA are capable of transcriptional repression of HBV surface antigen in liver-derived cells with no observable toxicity. Future work will entail in depth analyses on the efficacy of anti-HBV DEMs-encoding mRNA assessing markers of viral replication.

Targeted transcriptional silencing of HBV using RNA based technology represents a promising strategy to target cccDNA unlike conventional treatments.

Deciphering Host-Mycobacterium tuberculosis Interactions through Integrated Proteomics and Antibody Profiling

Mullins M[1,2], Hermann C[1,2], Phaweni D[1], Ganief T[1,2], Dheda K[3], Blackburn J[1,2]
[1]Department of Integrative Biomedical Sciences, Division of Chemical and Systems Biology, Proteomics Lab, University of Cape Town, [2]Institute of Infectious Disease and Molecular Medicine, University of Cape Town, [3]Centre for Lung Infection and Immunity, Lung Institute, University of Cape Town

Tuberculosis (TB) remains a global health threat, with millions of new cases reported annually. Understanding the complex interactions between Mycobacterium tuberculosis (Mtb) and the host immune system is crucial for developing effective TB control strategies.

In this study, we aimed to unravel the intricate interplay between Mtb and the host immune system, leveraging state-of-the-art proteomics techniques. Our study cohort consisted of 70 South African individuals with varying TB histories, providing a unique opportunity to dissect immune responses and proteomic signatures across different TB scenarios. An intra-lung rechallenge was carried out by instilling 10^4 CFU of live M. Bovis Bacillus Calmette–Guérin (BCG) into a distinct lung lobe, followed by repeat bronchoscopy and bronchoalveolar lavage fluid (BALF) collection after 3 days, stimulating memory immune responses in individuals with immunological memory against Mtb.

Using BALF samples collected pre- and post-BCG rechallenge, we employed advanced mass spectrometry technology, utilizing a Sciex 6600 Triple TOF with data-independent SWATH acquisition, to conduct comprehensive proteomic analyses.

Our results revealed significantly different patterns of proteomic response within the latent TB infection (LTBI) group following BCG restimulation, allowing us to define two distinct sub-groups. The one subgroup shows robust upregulation of proteins involved in neutrophil degranulation and B-cell receptor signaling. However, the way M. tb is taken up into a neutrophil greatly influences what happens next. Neutrophil activation and outcome depends which Fc receptor is utilised. Antibody data suggests IgA response to surface antigens on the bacterium at the site of disease in LTBI 2, and uptake via FcαRI more potently activates neutrophils in comparison to FcγRI.

This strongly suggests that we should be looking at the antibody component of BAL to gain deeper insights into the immunological dynamics and molecular mechanisms underlying TB infection, thereby informing innovative vaccination strategies and bolstering global TB control endeavors.

Exploring Optogenetic Approaches for Gene Expression Control in Mycobacteria

Razak Y[1], Chengalroyen M[1], Jacobs C[1], Warner D[1]
[1]UCT

Mycobacterium tuberculosis, the etiological agent of Tuberculosis, is responsible for the most deaths by a single infectious agent barring SARS-COV-2. Increasing drug resistance poses a threat to global health and the current repertoire of drugs available for treatment. Chemically induced genetic switches such as the tetracycline-inducible promoter system transformed the study of mycobacterial gene function and pathogenesis. Key investigations enabled include of gene function, protein overexpression for structural and other analyses, and control of gene expression in cellular and animal infection models, thereby providing invaluable tools for drug target identification and validation. In contrast, light-based control systems have been less extensively applied in (myco)bacteria; yet offer several benefits, including that they are fast, non-invasive, and often reversible.

Many optogenetic systems have been studied, offering various advantages and challenges for use depending on the host and application. The Opto-T7RNAP system developed and optimized in E coli has great potential for use in mycobacteria. A one-component system increases the likelihood of successful porting; however challenges such as high GC content in mycobacteria remain.

Here, we evaluate baseline characteristics including the potential for phototoxicity in Mycobacterium smegmatis. We describe the use of flow cytometry and microscopy to determine system performance in moving towards optimization of experimental conditions. Our results support the potential development of optogenetic systems in mycobacteria and might provide a guide to the future adaptation of similar systems.

Investigating the Role of KS-01 as an Anti-Cancer Agent in Colorectal Cancer

Pillay R[1], Kaur M[1]
[1]Student

Background
CRC (CRC) ranks as the third most prevalent cancer globally, presenting significant challenges in treatment. Various cancers, including CRC maintain a high cholesterol phenotype, essential for cell proliferation and survival and this has led to the exploration of targeting cholesterol as a promising therapeutic avenue for cancer treatment. The current study explored the use of KS-01, a proprietary cyclodextrin with membranous cholesterol depletion properties, in combination with conventional chemotherapeutic drugs 5-Fluoruracil (5FU) and Oxaliplatin (OXAL) in the treatment of CRC.

Methods
HT-29, a CRC cell-line was treated with various concentrations of KS-01, 5FU, OXAL and combinational therapies of KS-01 with 5FU or OXAL. In vitro assays that were performed included cell viability assays, apoptosis assays, multi-drug resistance assays and lastly cholesterol staining using Filipin, BODIPY and Alexa Fluor. NOD/SCID mice were inoculated with HT-29 cells, tumours were allowed to develop, mice were then treated with KS-01, 5FU, OXAL and combinational therapies and lastly monitored foe tumour growth.

Results
In vitro results displayed a decrease in lipid raft and lipid droplet content in combination therapies with KS-01. Lipid rafts and lipid droplets enriched with cholesterol have been implicated in CRC aggressiveness and drug resistance. Cholesterol depletion further enhanced the cytotoxic effects of 5FU and OXAL, rendering HT-29 cells more susceptible to apoptosis. Furthermore, KS-01 reduced multi-drug resistance, possibly through downregulation of drug efflux pumps, DNA synthesis and repair mechanisms. In vivo findings indicated that cholesterol depletion significantly reduced tumour sizes by as much as 75% when KS-01 was co-administered with 5FU or OXAL. Mechanistically, KS-01 exerts its cholesterol depletion properties through downregulation of cholesterol synthesis and uptake genes. However, an intricate interplay between cholesterol import, export and synthesis is required for KS-01 to reduce CRC pathogenesis.

Conclusions
In conclusion, this study emphasizes the potential of cholesterol depletion using KS-01 in combination with conventional chemotherapeutic drugs like 5FU and OXAL as a novel strategy for treating CRC. This approach is not only more cost- and time-effective but also helps limit drug resistance and toxicity while enhancing the cytotoxic effects of these drugs.

Investigating the role of iron acquisition in Mycobacterium abscessus infection

Mogapi K[1], Williams M[1]
[1]Department of Molecular and Cell Biology, University of Cape Town

Background

Pulmonary infection caused by Mycobacterium abscessus (Mab) pose a significant threat to individuals with lung abnormalities like cystic fibrosis, mainly due to its intrinsic antibiotic resistance. Despite its clinical relevance, little is known about the genetic determinants driving Mab pathogenicity. To elucidate genes responsible for pathogenicity relies on the generation of isogenic mutants which has often been challenging in Mab. In this study we have generated Mab deletion mutant deficient in one of the genes involved in iron acquisition, namely mbtB to assess its growth in epithelial cells.

Methods

A deletion mutant lacking mbtB gene was generated by using homologous recombination. To employ a two-step selection strategy for homologous recombination we generated an allelic exchange substrate containing a zeocin resistance cassette to select for the first cross-over event and the Mycobacterium tuberculosis katG gene as a negative selection marker (isoniazid susceptibility) for the second cross-over event. A complementation strain was generated by re-introducing the mbtB gene under the control of its native promoter into the deletion mutant using an episomal plasmid. To closely mimic in vivo conditions of the airway epithelium, we are developing an air-liquid interface (ALI) model using A549 epithelial cell line. Growth of the epithelial cells over 14 days was monitored using florescent microscopy.

Results

Electroporation of 1ug allelic exchange substrate into Mab and selection on zeocin resulted in 5 colonies. Patching of potential double cross-over mutants revealed that only 3 of the 27 isoniazid-resistant colonies were zeocin susceptible. PCR screening confirmed that two of these colonies contained the mbtB deletion. Preliminary in vitro growth analysis suggested that there is no significant difference between the mutant and wild type, regardless of iron availability. Fluorescence microscopy showed consistent A549 cell density over 14 days during ALI culture.

Conclusions

Although homologous recombination was successful, katG does not function very efficiently as a negative selection marker in Mab. A549 cells can be maintained at ALI for 14 days and can therefore be used to study Mab infection.

The effects of tumour acidosis on cancer metabolism, mitochondrial morphology and function, metastasis and drug resistance

Gerber J[1], Labuschagne S[1]
[1]1Department of Biochemistry, North-West University

Background
Cancer is a growing global concern as one of the leading causes of death worldwide. Its increasing presence, profound lethality and ineffective treatment methods is pressuring scientists to explore the internal biochemical mechanisms involved and come up with more effective treatment methods, especially in developing countries with less readily available healthcare. Tumour acidosis is regarded as an emerging hallmark of the tumour microenvironment where certain areas in the tumour become increasingly acidic. This causes therapy resistance and increased metastasis. Literature regarding the effects of tumor acidosis remains lacking, hence the need for research to study the effects and mechanisms involved more carefully to exploit any potential therapeutic opportunities.
Methods
We are growing A549 and MDA-MB-231 cells in varying acidity including pH 6.4, pH 6.8 and pH 7.4. We are using a combination of NMR, GC-MS, LC-MS and the Seahorse XF Mito Stress Test to investigate any metabolic differences induced by acidosis. Proliferation assays are used to portray the differences in growth rate as well as to examine the effects of therapy resistance to chemotherapy drugs. Migration assays are used to measure differences in metastatic potential. Confocal imaging is used to portray differences in mitochondrial morphology and lipid droplet accumulation.
Results
We observed that cells grown in extreme acidic conditions (pH 6.4) exhibited increased lipid droplet accumulation within the cells and goes into a state of senescence when grown chronically. These two observations might be linked to each other. Metabolic differences have also been identified through NMR and Seahorse experiments including utilization of different pathways such as differential use of oxidative phosphorylation and glycolysis. Cells grown in acidic media was also more fibroblastic in shape with less cell-to-cell contact.
Conclusion
These findings confirm the hypothesis that tumour acidosis is responsible for furthering metastasis and therapy resistance. It was also found that there are clear morphological and metabolic differences induced by acidosis which could be seen as a potential intervening opportunity to combat the effects of tumour acidosis, thereby furthering the chances to provide more effective treatment.
Keywords: lung cancer, tumour acidosis, breast cancer

Development of a screening toolkit for antimalarial mode-of-action determination in Plasmodium falciparum gametocytes

Kok M[1], Birkholtz L[1]
[1]Department of Biochemistry, Genetics and Microbiology, Institute for Sustainable Malaria Control, University of Pretoria

Presently, malaria control and preventive strategies rely primarily on vector control methods and clearing the disease-causing asexual Plasmodium falciparum parasites in an infected patient. However, the ability of drugs to target the transmittable stages of the parasites, and thereby blocking human-to-vector transmission, is important in eliminating the disease. To streamline the drug development of novel antimalarial agents, their drug targets need to be elucidated and validated to ensure their potential therapeutic benefit thereby ensuring their progression in the drug development pipeline. Mature gametocytes have distinctive metabolic processes that differ markedly from immature gametocytes and asexual stage parasites. Identifying whether novel gametocytocidal compounds influence these specific processes in mature gametocytes will enable their progression in the drug development pipeline.

Since most of the work done to date focused on targeting the highly proliferative asexual blood stages of the parasites; the methods used for mode-of-action (MoA) determination cannot be used for non-replicative gametocytes. This research project centres on the development of useful tools to investigate the MoA of novel transmission-blocking compounds, active against the mature gametocytes. One tool being developed is the use of morphological profiles to distinguish biologically relevant similarities and differences among parasites treated with gametocytocidal compounds. Morphological fingerprint profiles are obtained through the use of rich multiplexed confocal images and machine-learning tools to stratify compounds with specific MoA into morphological classes. Cell Painting is a relatively novel technique successfully used for high-content morphological profiling in cancer research, however, this project is the first to utilise the technique on P. falciparum parasites.

The regulation of AMPK and adipokines on visceral fats of rats treated with curcumin

Kgasago K[1], Ibrahim K[2], Matumba M[1]
[1]North West University, Mafikeng campus, [2]University ofWitswaterstrand

Background

AMP-activated protein kinase (AMPK) is a master regulator of glucose and lipid metabolism. Moreover, in a state of obesity, the expression of AMPK is found to be down regulated. Curcumin is being recognized and used worldwide in many different forms for multiple potential health benefits. Curcumin supplementation may promote weight loss and ameliorate obesity-related complications through its antioxidative and anti-inflammatory properties. Hence, the current study investigated the effect of curcumin on the regulation of adipokines such as adiponectin, TNF-alpha, and Interleukin 6 as well as the regulation of AMPK and its subsequent genes such as CPT-1 and ACC-1 in the lipid metabolism.

Methods

Visceral tissues were collected from forty male Sprague Dawley rats for molecular analysis. The rats were divided into four experimental groups, namely, Control (CON), Curcumin (CU, 500 mg/kg), high fructose diet (HFD, 20%) and high fructose diet + curcumin (HFD, 20% + CU, 500 mg/kg). Quantitative polymerase chain reaction (qPCR) was used to analyze the gene expression of AMPK, CPT-1, ACC-1, adiponectin and TNF-α. Furthermore, western blot was performed to analyze the protein expression of the above-mentioned genes.

Results

In our study, we observed that curcumin induced the gene expression of AMPK in HFD + CU group compared to the HFD group alone. Additionally, the gene expression of CPT-1 was increased in the HFD + CU group compared to the HFD group alone and ACC-1 gene expression was reduced in HFD + CU group. Subsequently, adiponectin gene expression was increased and the gene expression of TNF-α was successfully reduced in HFD + CU group.

Conclusion

Therefore, our findings suggest that curcumin could be used as a potential therapeutic agent against metabolic disease such as obesity and T2D through expression of AMPK.

Chemical resistance of soybean against Helicoverpa armigera

Nengovhela N[1]
[1]Fabi, University Of Pretoria, [2]FABI, University of pretoria

Background

The African bollworm, Helicoverpa armigera, is a Lepidopteran pest that causes significant damage on a wide variety of agricultural crops in South Africa and throughout the world. The most important host species are legumes, most notably cowpea, chickpea, and soybean. Control of H. armigera is difficult, due to its high reproductive rate and widespread tolerance to BT CRY toxins and pyrethroids. For this reason, plant resistance is thought to be the most effective strategy for controlling this pest. However, not many studies have been conducted specifically on the defences of soybean to H. armigera, and it is not clear which defencse mechanisms in this species are most effective against the insect pest. Therefore, we aimed to study the chemical defence mechanisms of soybean in response to feeding by H. armigera in order to identify plant traits that can be used for breeding more resistant cultivars.

Methods

Twenty soybean varieties were treated with the plant hormone, methyl jasmonate (MeJa), to induce defences. Two weeks after treatment, leaves were harvested and prepared for analysis. The metabolites in the leaves were analyzed using liquid chromatography mass spectrometry. We also characterized the resistance of the varieties towards H. armigera by assessing larval feeding damage on MeJa-treated and untreated plants.

Results

The metabolic profiles did not differ significantly across the twenty soybean cultivars that were used. We also observed no significant difference between feeding of the insects on the different soybean cultivars, with only a few cultivars exhibiting lower feeding by the insect. However, MeJa treated plants exhibited less feeding than the untreated controls.

Conclusions

Our findings suggest that constitutive and MeJa-induced chemical resistance mechanisms between the soybean cultivars used in this study were similar. Further studies will focus on studying the MeJa-induced defence mechanisms of soybean against insect feeding.

Novel mass spectrometry workflows for small molecule omics research

Kruft V[1]
[1]Sciex

Novel mass spectrometry workflows for small molecule omics research

Mass spectrometry (MS) allows us to capture and analyze the complex array of small molecule metabolites within a biological sample. There are several types of metabolomics experiments including both targeted and untargeted analyses.

A consistent demand on the performance of all mass spectrometers is high sensitivity and high speed. The ZenoTOF 7600 system includes a novel trapping/releasing technology, the Zeno trap, which offers >90% ion yield over the classical QTOF design, resulting in a boost to analysis sensitivity by 4- to 20-fold. The dramatically increased sensitivity allows for higher acquisitions speeds – the instrument is capable of 133 Hz acquisition without sacrificing resolution or sensitivity.

Collision-induced dissociation (CID) in nominal mass workflows is the gold standard for MRM-based quantitation. However, the quantitation might be compromised by unpredictable interferences. In addition, for full structure elucidation of unknowns or metabolites, nominal mass instruments lack full scan capabilities and resolution. Also, with CID being the standard fragmentation pathway on the majority of high resolution MS, diagnostic fragments or those to determine the structure unequivocally are often missing. The ZenoTOF has an additional collision cell that allows for fast and tunable dissociation driven by electron interactions (electron activated dissociation – EAD).

We have applied the combination of EAD and Zeno trapping to a number of molecule classes that are difficult to fully characterize and quantitate and we will be presenting the results.
Lipid structures can be fully analyzed on an LC time scale (i.e. 50 ms spectral accumulation time), capturing structural details including not only the position but the stoichiometry of double bonds.
We will show the qualitative and quantitative analysis of steroid hormones – including the definitive identification of isomers that do yield identical fragments on CID fragmentation, in the CID approach making a chromatographic separation of the isomers essential.
Bile acids, lipid mediators, and drug metabolites are more examples showing that the combination of EAD fragmentation with the increased sensitivity offered by the Zeno trap allow unprecedented levels of structural characterization and MS/MS quantitation accuracy.

Advancing mass spectrometry performance to increase the depth of coverage of post-translational modifications and optimizing protein characterization

Kruft V[1]
[1]Sciex

Advancing mass spectrometry performance to increase the depth of coverage of post-translational modifications and optimizing protein characterization

A novel hybrid collision cell is at the heart of the technological innovations introduced with the SCIEX ZenoTOF 7600 system.

In the past, QTOF mass spectrometers have suffered from duty cycle losses; that is, losses in ion transmission in the ion path. This was mainly due to the mating the continuous beam coming from the quadrupole ion path with time-of-flight (TOF) analysis, a pulsed, discontinuous measurement technique. A series of ion-staging events and reverse-mass sequential ion release, with high-capacity ion traps, have been introduced just after the CID collision cell (Q2) and before the pusher region of the TOF. This allows the duty cycle losses to be minimized leading to MS/MS sensitivity gains of 4- to 20-fold.

The newly engineered collision cell also has the ability to perform both collision induced dissociation (CID) and electron activated dissociation (EAD) experiments for high-resolution, high sensitivity MS/MS flexibility. Electron kinetic energies can be tuned from 0-25 eV without the use of chemical transfer reagents. This precise tunability means EAD can be performed on a wide range of analytes, from multiply charged peptides to singly-charged small molecules.

The presentation will demonstrate a wide range of unique mass spectrometric results only possible with the sensitivity and fragmentation regime of ZenoTOF technology. Amongst others, intact post-translational modifications of peptides can directly be analyzed and quantified such as phosphorylations including those of histidines. Isoleucine and Leucine can be unambiguously distinguished, as can aspartic acid and iso-aspartic acid – all impossible with traditional CID fragmentation workflows. We will finish the presentation demonstrating the system performance on proteomics analysis, covering both the numbers of protein Ids on complex samples and middle-down (top-down) analysis of purified protein samples.

The effect of Bauhinia bowkeri extracts on hypercholesteremia: Insite from in vitro and in silico investigations

Thethwayo S[1], Thethwayo S[1], Madoroba E[1], Opoku A[1]
[1]Biochemistry

Background: Among the current cholesterol-lowering drugs present, the persistent surge of hypercholesterolemic related complications ignites a fascinating search for the discovery of novel therapeutics. This study aimed at investigating the anti-hypercholesterolemic effect of Bauhinia bowkeri extracts.
Methods: The plant material was sequentially extracted with n-hexane, DCM and 70% ethanol. The extracts were screened through GC-MS analysis and tested against wide range of free radicals (ABTS, DPPH, metal iron chelating, hydroxyl radical, nitric oxide and reducing power). Thereafter, the enzyme inhibitory activity of the extracts on pancreatic lipase, cholesterol esterase and HMG-CoA as well as bile binding capacity were evaluated. Molecular docking on HMG-CoA reductase, cyclooxygenase, and hormone-sensitive lipase was also investigated.
Results: A total of number of 122 compounds were detected in all three extracts, Only seven common compounds (E-15-Heptadecenal, Diethyl Phthalate, Hexadecanoic acid ethyl ester, 9,12,15-Octadecatrienoic acid ethyl ester, (Z,Z) Tetradecane 5-methyl, Octadecane 5-methyl, etc.) were found to be common in all extracts. The extract displayed a varying degree of efficiency on free radicals with IC50 values ranging from 0.42 mg/ml to 1.62 mg/ml. It worth noting a concentration-dependent inhibition of pancreatic lipase and cholesterol esterase, along with a reduction in bile binding capacity exhibited by the extracts. In silico investigations revealed significant inhibition of HMG-CoA reductase, cyclooxygenase, and hormone-sensitive lipase with binding affinity ranged between –4.4 kcal/mol to –7.7 kcal/mol.
Conclusions: These findings suggest that Bauhinia bowkeri extracts possesses some antioxidant and anti-hypercholesterolemic properties.

Biochemical and structural comparisons of Plasmodium falciparum heat shock protein 90 isoforms

Makatsela L[1], Gildenhuys S[2], Nxumalo W[3], Mokoena F[1]
[1]Department of Biochemistry, Faculty of Natural and Agricultural Science, North-West University, [2]Department of Life and Consumer Sciences, School of Agriculture and Life Sciences, College of Agriculture and Environmental Science, University of South Africa, [3]Department of Chemistry, School of Physical and Mineral Science, Faculty of Science and Agriculture, University of Limpopo

Background
The 608 000 malaria-related deaths in 2022 emphasize the critical need to explore novel therapeutic targets. Plasmodium falciparum heat shock protein 90 (PfHsp90) plays a vital role, as inhibition halts parasite growth. The P. falciparum genome encodes for four Hsp90 isoforms—PfHsp90, PfGRP94, PfTRAP1 and PfHsp90_A—localized to the cytoplasm, endoplasmic reticulum, mitochondria, and apicoplast, respectively. This study investigates the similarities between PfHsp90 isoforms, particularly in the N-Terminal ATP binding domain, to inform the future development of isoform-specific inhibitors.

Methods
In silico tools including PROMALS3D assessed the amino acid sequence similarities and identities of PfHsp90 isoforms in a multiple sequence alignment using sequences retrieved from PlasmoDB. Molecular docking and dynamics simulations evaluated ligand binding interactions and dynamic behaviours of isoforms. Ligand-induced variations in the structure and stability of PfHsp90 isoforms were determined using thermal shift assay and a limited proteolysis assay.

Results
PfHsp90 shares some amino acid conservation with PfGRP94 (56.5%) and PfHsp90_A (48.8%), with weaker conservation in PfTRAP1 (37.1%). Interestingly, ATP binding residues were conserved except for Arg98, substituted by Lys100, Lys98 and Glu134 in PfGRP94, PfTRAP1 and PfHsp90_A, respectively. Molecular dynamics simulations revealed diverse ADP and ATP interactions between isoforms. Interactions with Arg98, Lys46 and Lys44 are the most prominent. Limited proteolysis revealed similar fragment profiles of the apo state and ADP-bound PfHsp90, which is completely digested after 15 minutes. In contrast, the ADP-bound PfTRAP1 may assume a different conformation. Preliminary data indicates greater thermal stability in PfGRP94 than PfHsp90. ADP and ATP stabilize PfHsp90 isoforms against thermal denaturation differently, as higher melting temperatures (Tm) are observed in the ATP-bound PfHsp90 than the ADP-bound form, contrasting the higher Tm of ADP-bound PfGRP94 and PfTRAP1.

Conclusions
Despite some conservation of ATP binding residues in PfHsp90, the divergence of the selectivity-conferring residue Arg98, compared to other isoforms, may offer valuable insights into the preferential binding of inhibitors. Also noted were distinct biochemical differences between the PfHsp90 isoforms. These include variations in thermal stabilities influenced by natural ligands. Additionally, ATP offers some degree resistance against digestion by trypsin, compared to ADP-bound proteins.

Linking BRCA1/Olaparib interaction with LINC00511 in breast cancer using in silico bioinformatics analysis

Fadebi O[1,2], Kandhavelu J[3], Penny C[4], Dlamini Z[2], Marima R[2]
[1]Department of Medical Oncology, Steve Biko Academic Hospital and University of Pretoria, Hatfield, [2]SAMRC Precision Oncology Research Unit (PORU), DSI/NRF SARChI Chair in Precision Oncology and Cancer Prevention, Pan African Cancer Research Institute (PACRI), University of Pretoria, Hatfield, 0028, [3]Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC, USA, [4]Department of Internal Medicine, Faculty of Health Sciences, School of Clinical Medicine, University of the Witwatersrand, Parktown 2193

Background
Breast cancer (BCa) remains a significant global health concern, necessitating the continuous development and optimisation of targeted therapeutic approaches. Breast cancer gene 1 (BRCA1) mutations are associated with BCa, while Olaparib (a PARP inhibitor) targets BRCA1-linked BCa. Neighbours of BRCA1 long intergenic non-coding ribonucleic acids (lincRNAs), such as LINC00511, are involved in BRCA1-associated BCa. This study aimed at mapping biological pathways associated with Olaparib/BRCA1 interaction using in silico analysis.
Methods and results
To achieve this, the molecular docking MCULE platform was used. Next, LncSEA, a lncRNA analysis platform, was used to gain insights into the potential roles and functions of the LINC00511/BRCA1 molecular mechanisms. The molecular mechanisms of these interactions were further analysed using the KEGG pathway.
The docking analysis revealed that Olaparib forms two conventional hydrogen bonds with critical amino acid residues Lys 1711 and Arg 1753; one carbon and hydrogen bond with Gln 1756; one alkyl and π–alkyl interaction with Arg 1753 in the BRCA1 binding pocket, which are crucial for stabilising the drug-protein complex. The calculated binding energy of -6.3284 kcal/mol suggests a favourable interaction between Olaparib and BRCA1, supporting its therapeutic efficacy, particularly BRCA1-associated BCa. LncSEA analysis verified the BRCA1/LINC00511 interaction through the LINC00511/miR-185-3p/E2F1/Nanog axis, which plays a role in breast cancer stemness and malignancy. Interestingly, E2F Transcription Factor 1 (E2F1), which plays a critical role in regulating cell cycle progression, DNA repair, and apoptosis in response to DNA damage or cellular stress, was also shown to be targeted by LINC00511.
Furthermore, KEGG pathway analysis revealed that Olaparib inhibits the DNA repair pathway mediated by BRCA1, leading to the accumulation of DNA damage and apoptosis in BCa cells with BRCA1 mutations. DNA repair mechanisms are essential to BRCA1/LINC00511 mediated BCa and its response to Olaparib treatment.
Conclusion
Our findings reveal insights into the Olaparib/BRCA1 molecular interaction. BRCA1 BCa pathogenesis was also elucidated through LINC00511, as lncRNAs are multifaceted in cancer, including BCa. A deeper biological understanding of these complex drug/target interactions associated with ncRNAs may provide improved BCa drug therapeutic efficacy.
Keywords: Breast cancer; BRCA1, Bioinformatics; DNA repair; E2F1; LINC00511; Olaparib

Prevalence And Molecular Profiling Of Plasmodium Falciparum Kelch 13 In Ha-Lambani Area, Limpopo Province, South Africa

Munzhedzi M[1,2], Guler J[3], Operario D[4], Shifflett P[4], Krivacsy S[4], Dillingham R[4], McQuade E[6], Bessong P[1,5]

[1]HIV/AIDS & Global Health Research Programme, Faculty of Science, Engineering and Agriculture, University of Venda, [2]Department of Biochemistry and Microbiology, Faculty of Science, Engineering and Agriculture, University of Venda, [3]Department of Biology, University of Virginia, [4]Department of Public Health Sciences, University of Virginia, [5]Center for Global Health Equity, Department of Infectious Diseases and International Health, University of Virginia, [6]Rollins School of Public Health, Emory University

Background: Identification and monitoring of the Plasmodium falciparum Kelch13 (pfk13) gene associated with artemisinin resistance is critical to understanding the emergence and spread of drug-resistant malaria in endemic areas. This study analyzed the nucleotide sequences of the pfk13 gene for P. falciparum drug resistance-associated mutations in the Ha-Lambani area, Limpopo Province, South Africa.

Methods: Genomic DNA was extracted from 985 dried blood spot samples collected from three Ha-Lambani villages. All samples were subjected to P. falciparum-specific Rapid diagnostic test (RDT) and high-resolution melting (HRM) analysis for speciation. The samples positive for P. falciparum were subjected to nested PCR for the amplification of the pfk13 gene. Amplicons of sufficient concentrations were sequenced using Illumina Next Generation Sequencing (NGS) and analyzed for polymorphisms across the gene compared to the PF3D7_1343700 reference genome. Geneious was used for sequence analysis.

Results:

The prevalence of Plasmodium infection was 7.1% (70/985) (either by RDT or HRM). Of these, 3.95% (n=39), 2.64% (n=26), 0.51% (n=5) accounted for P. ovale, P. falciparum, and mixed infections, respectively. A total of 16% (5/30) P. falciparum samples were successfully amplified for pfk13 analysis. Two samples were sequenced by NGS and a total of 57 SNPs were detected across the pfk13 gene (≥20% minority level, mean coverage of 60697.7 and 87257.1). More than half of the detected SNPs (70.1%, 39/57) were non-synonymous and none were previously associated with artemisinin resistance. However, we detected several novel SNPs in the propeller domain (H719Q, P701T, M472I, I526R, and P443S).

Conclusion: The detection of novel SNPs in the propeller domain of pfk13 indicates that this region may be under selective pressure with the use of artemisinin in this region of Africa. Therefore, further research is required to better understand the role of novel SNPs in artemisinin resistance and combined with additional SNP monitoring in Limpopo, South Africa.

Antimicrobial effects of Piper betel compounds on resistant strains of Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, and Klebsiella pneumoniae.

Motlhoki T[1]
[1]South African Medical Research Council, [2]University of Pretoria, [3]Tshwane University of Technology
Background: Antimicrobial resistance (AMR) may lead to an estimated 10 million/year mortality and a $100-210 trillion global gross domestic product loss by 2050. According to the World Health Organization (WHO) in 2017, AMR strains of Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Klebsiella pneumoniae (ESPK) are priority pathogens for which new antibiotics are urgently needed. In previous studies, it has been shown that (i) specific compounds from Piper betel species highly inhibit the growth of multidrug-resistant (MDR) strains of several pathogens, including Vibrio cholera, and Escherichia coli. (ii) Acetate kinase (AcK) could be a novel MDR inhibitory target protein for these Piper betel compounds.
Methods: The Piper betel compounds (piperidine, eugenyl acetate, chlorogenic acid and pinoresinol) will be evaluated for their growth inhibition and antimicrobial effects against (ESPK) using minimal inhibitory concentration broth dilution tests. The determination of resistant acetate kinase gene and other MRD genes will be conducted using conventional polymerase chain reaction and ribonucleic acid transcription assays. Drug Affinity Responsive Target Stability (DARTS)-Western blotting assays identify novel protein targets binding interaction with natural products (Piper betel compounds).
Results: These are preliminary results. We are anticipating the Piper betel compounds to exhibit antimicrobial activities by inhibiting the growth of MDR-ESPK strains through the interruption of regulatory mechanisms of the targeted AcK protein of the pathogen by binding it to the Piper betel compounds.
Conclusion: MDR-ESPK strains have a highly resistant prevalence in South Africa and globally. The use of Piper betel compounds with proven antimicrobial properties against WHO-priority-AMR-ESPK strains will have a significant impact on combating AMR prevalence. This study poses an advantage for the bio-economy, which will provide scientific growth in drug discovery, treatment strategies and public health advancements.
Keywords: Multidrug resistant-ESPK; Drug discovery; Piper betel compounds.

Bioinformatics identification of antituberculosis resistance and associated lineages using whole-genome sequence data.

Matodzi U[1], Mutshembele A[2], Makhado N[3], Reva O[1]
[1]University of Pretoria, [2]South African Medical Research Council, [3]Sefako Makgatho Health Sciences University, Pretoria, South Africa.

BACKGROUND
Whole-genome sequencing (WGS) has a potential to expedite the detection of drug resistance (DR). The drawback of WGS is associated with limited technical experience and clinical interpretation of data. This study aims to develop a user-friendly Bioinformatics tool that rapidly analyse WGS data without the need of expertise.
METHODS
Culture-based DST of anti-TB drugs was conducted on a total of 127 MTB isolates. WGS was performed on 10 genomic DNA using Illumina platform. WGS-based DST analysis was performed using TB-Profiler and bioinformatics pipeline to detect DR and lineages.
RESULTS
Culture-based DST yielded 10 susceptible strain and 117 DR constituting 64 MDR and 53 mono resistance strains. This study reveals that clofazimine resistant and bedaquiline-clofazimine co-resistant were prevalent amongst other second line drugs tested. WGS-based and culture-based DST results for the 10 sequenced samples were compared, resulting in 4 samples in agreement and 6 discordant results. Our study detected East-Africa Indian, Beijing and Euro-American which are the most predominant lineages in South Africa. Mutation determinants consisting of single nucleotide polymorphisms and insertion-deletion were identified and deposited in the mutation catalog. These recorded mutations will be utilized to develop bioinformatics tools with robust analytical capabilities. The study is ongoing, with further analysis planned to identify additional mutations.
CONCLUSION
This study anticipates that WGS can significantly accelerate the detection of DR profiles as compared to phenotypic and other genotypic methods. Our end goal is to have a significant impact on the global TB diagnosis and treatment as well as reducing tuberculosis transmission.

The metabolomics profiles of two accessions of Amaranthus cruentus cultivated in KwaZulu-Natal.

Khumalo E[1]
[1]University Of South Africa

Amaranthus crops are significant for their medicinal properties in addition to their value as sustenance and nutritional sources. Due to the fact that cultivation of Amaranthus species is still uncommon and the majority of them are harvested in the wild, efforts are being made to commercialise and market this vital crop. This study investigated the chemical profile of accessions of Amaranthus cruentus by multivariate statistical analysis of spectral data deduced by Nuclear Magnetic Resonance (NMR). The preliminary results indicate that the principal component analysis (PCA) failed to identify a clear differentiation between the two Amaranthus cruentus accessions. Conversely, the utilisation of the Orthogonal partial least squares discriminant analysis (OPLS-DA) model revealed a clear differentiation between the samples derived from the two accessions. The future analysis will be done to determine which compounds are responsible for the separation that was observed between the two accessions of Amaranthus cruentus.

Development of strategies to comprehending seriniquinone anti-melanoma mechanism of action and enabling its in vivo administration

Miguel R[1,2], Hirata A[1], Khan S[2], Serala K[2], Burmeister C[2], de Jesus D[1], Garnique A[1], Ishida K[1], Prince S[2], Lopes L[1], Lotufo L[1,2]
[1]University Of São Paulo, [2]University of Cape Town

Background
Seriniquinone (SQ) is a cytotoxic substance that is selective towards melanoma cells and has a unique target: dermcidin (DCD). There are, however, two drawbacks when envisioning the future application of SQ and these include (i) its poor water solubility and (ii) the link between DCD modulation and cell death. This study aimed at addressing these drawbacks by, respectively, encapsulating SQ into poly (lactic-co-glycolic) acid (PLGA) nanoparticles (NPs) and developing DCD-overexpressed and DCD-silenced cell models.
Methods
SQ was encapsulated in PLGA-NPs by a single emulsion-solvent evaporation method. Size and morphology were depicted by scanning electron microscopy (SEM). SQ encapsulation efficiency and in vitro release kinetics were determined by SQ quantification using high performance liquid chromatography. Anti-melanoma activity of encapsulated SQ was assessed upon treatment (6-48h) of melanoma cells (SK-MEL-28 and SK-MEL-147), followed by sulforhodamine B and clonogenic assays. NP uptake was observed during 20h by phase contrast optical microscopy. Spheroid models were standardized, and drug treatments performed. Toxicity evaluation in vivo was conducted by treating Galleria mellonella larvae with SQ-loaded NPs at doses up to 80 mg/kg.
Results
SEM images revealed spherical NPs with 120-800 nm, and most of them were ~210-280 nm. Encapsulation efficiency of ~83% was obtained and only ~16% was released in vitro after 96h. When compared to free SQ, anti-melanoma activity of SQ-loaded NPs was maintained; even a short exposure (6h) to NPs demonstrated similar effects in clonogenic assays. We were able to observe NP uptake by cells, followed by their detachment from culture dishes in a similar manner to free SQ. Preliminary tests demonstrated ~15-fold increase in SQ inhibitory concentration values against spheroid models developed when compared to monolayer. No in vivo toxicity was observed upon nanocarriers treatment in G. mellonella, even in the highest dose, resulting in 95% of survival and high health indexes.
Conclusions
PLGA-NPs increased SQ solubility by enabling its dispersion in aqueous-based vehicles, sustaining its release and allowing its in vivo administration, while maintaining its biological activity. Melanoma cells are being transfected with either FLAG-tagged DCD overexpression or DCD shRNA knockdown plasmids, for which validated models are expected to be obtained.

Characterisation of the PFF1010c, a type IV J domain protein of Plasmodium falciparum

Netshiheni T[1], Masoga T[1], Mudau T[1], Zininga T[2]
[1]University of Venda, [2]Department of Biochemistry, Stellenbosch University
Characterisation of the PFF1010c, a type IV J domain protein of Plasmodium falciparum
Thabo Masoga1, Thendo Pertunia Mudau1, Hygon Mutavhatsindi1, Tawanda Zininga2, Addmore Shonhai1

1Department of Biochemistry and Microbiology, University of Venda
2Biochemistry Department, Stellenbosch University
Email: thabo.masoga0618@gmail.com

Background
J domain proteins (JDPs) of Plasmodium falciparum are implicated in the development and pathogenicity of the malaria parasite. Type I and type II JDPs are well characterized and are known to regulate the protein folding Hsp70 machinery of the parasite thus fulfilling a co-chaperone function. On the other hand, the function of type III and IV JDPs remains generally unknown. PFF1010c is an essential type IV JDP of P. falciparum. The role of PFF1010c, a type IV JDP is unknown. The current study sought to characterise the structure-function features of PFF1010c.

Methods
We expressed and characterised the recombinant form of PFF1010c. We studied its structural features using circular dichroism and fluorescence spectrometry. We investigated association of PFF1010c with P. falciparum Hsp70-1 (PfHsp70-1), a well-known chaperone of the parasite. Furthermore, we explored the co-expression of PFF1010c with PfHsp70-1, PfHsp90, and a co-chaperone, Hsp70-Hsp90 organizing protein (PfHop). In silico assays were conducted to further explore its possible function.

Results
We employed Western blot analysis to demonstrate the co-expression of PFF1010c with PfHsp70-1, PfHsp90 and PfHop at the gametocyte stage. We further observed that PFF1010c directly interacts with PfHsp70-1. In addition, we observed that PFF1010c is stable to heat stress. Substitution of the SVN motif of PFF1010c with HPD residues enhanced the stability of the protein.
Conclusions
Our findings constitute the first report on the possible role of PFF1010c as a co-chaperone of PfHsp70-1.

Key words: Malaria, Plasmodium falciparum, PFF1010c, gametocyte, chaperone

Characterisation of Mycobacterium tuberculosis DnaK: towards antitubercular drug design

Netshiheni T[1], Netshiheni T[1], Dali U[1], Dongola T[1], Shonhai A[1]
[1]University of Venda

Background
In 2022, tuberculosis (TB) caused an estimated 1.13 million deaths. Of these, 54 200 were accounted for by South Africa representing one death every 10 minutes. Mycobacterium tuberculosis is the agent of human TB. Treatment interventions against TB are frustrated by drug resistance. M. tuberculosis DnaK (MtbDnaK)/Hsp70 is an essential chaperone the bacterium that is implicated in drug resistance. For this reason, MtbDnaK presents a prospective antitubercular drug target. Here we characterized MtbDnaK towards developing an assay for screening inhibitors targeting this chaperone.

Methods
We conducted in silico assays to map out the structure-function features of MtbDnaK. We further cloned, expressed, and purified recombinant MtDnaK protein followed by conducting biochemical assays on the protein. We further conducted a complementation assay to study the function of this protein by expressing it in E. coli dnaK103 cells which express a truncated and non-functional native DnaK. These cells are unable to grow at high temperatures (>40° C). Heterologous expression of functional DnaK restores their growth at high temperatures.

Results
In silico studies demonstrated that MtbDnaK shares high sequence identity to E. coli DnaK. In addition, MtbDnaK was able to restore the growth of E. coli dnaK103 cells at temperatures >40° C. We further optimized the complementation using both agar plate- and a liquid broth-based assays. We intend to employ the liquid broth assay to screen possible inhibitors targeting MtbDnaK.

Conclusions
MtbDnaK is functionally equivalent to E. coli DnaK. The E. coli complementation assay we developed provides a platform for screening antitubercular compounds targeting MtbDnaK.

Key words: Tuberculosis; MtbDnaK; Complementation assay; drug screening

Comparative Genomics of the Listeria monocytogenes ST204 Subgroup using Whole-Genome Sequencing - A 4IR study

Makhubela R[1]
[1]University Of Johannesburg

Listeria monocytogenes is a rod-shaped, gram-positive, facultative anaerobic, bacteria belonging to the genus Listeria, and was responsible for the 2017-2018 listeriosis outbreak in South African and set the record as the worst listeriosis outbreak in the history of the disease, the Subgroup ST204 is among the most isolated from Food processing industries in South Africa, and knowing the genetic diversity of this bacterium can help solve problems it causes and prevent unnecessary death. This study aims to make use of Bioinformatics Software like Python, Linux, R, C, and knowledge to perform comparative genomics of the Subgroup ST204 of L. monocytogenes. Using software such as FastQC, SPAde, and ABRicate to scan for genes that code Virulence factor, antibiotic resistance, track evolutionary pattern, and build a phylogenetic tree. All major antibiotic genes and plasmids, including Listeria pathogenicity islands(LIPI-1 and LIPI-3), bcrABC cassette, and plasmids (rep26_2_repA(pLGUG1 and rep25_2_M640p00130). This study shows that the subgroup ST204 has adapted well to the food processing environment and houses all essential virulence factors and stress resistance genes to cope with the existing measures, and threaten public health.the obtained results will aid in developing new approaches to assess the virulence potential of L. monocytogenes isolates and the efficacy of using BC disinfectants in food-processing facilities in SA.

The potential of plastic biofilms as a reservoir of Vibrio sp. and antibiotic resistance genes in selected urban rivers in the Eastern Cape, South Africa.

Nnadozie H[1], Nnadozie C[1]
[1]Institute for Water Research, Rhodes University

Background
The genus Vibrio encompasses a group of pathogenic bacteria that cause cholera and vibriosis. Periodic cholera outbreaks and other vibriosis have been linked to direct and indirect exposures to contaminated freshwater environments. Plastic biofilms have been suggested as reservoirs for pathogenic bacteria such as Vibrio sp. and their antibiotic-resistant genes (ARGs). The potential of plastic biofilms as reservoirs of Vibrio sp. in selected urban rivers in the Eastern Cape, South Africa was investigated.
Methods
2L surface water was collected from the water column. Concurrently, megaplastics and microplastic samples were collected. Water temperature, salinity, turbidity, pH, and dissolved oxygen were measured on-site. 250 mL of water was collected from each sampling site to measure nitrates, nitrites, phosphates, and ammonia concentrations. Genomic DNA was extracted from sampled water, microplastics and megaplastics biofilms using the Qiagen DNAEasy Powersoil Pro kit. Quantitative PCR (qPCR) was used to measure the relative abundance of the Vibrio 16S rRNA and tetB genes in the samples. Student t-test was used to test for the significant differences between samples. A p-value < 0.05 was regarded as statistically significant.
Results
Water temperature, salinity, pH, dissolved oxygen, turbidity, total nitrates, nitrites, total phosphates and ammonium ranged from 9.36 - 25.18 °C, 0 – 2.45 mg/L, 7.18 - 14 0 – 6.42 mg/L, 3.59 – 89 NTU, 0 – 28.1 mg/L, 0 – 1.1 mg/L, and 0 -9.8 mg/L 0 – 6.1 mg/L respectively. Vibrio sp. 16S rRNA and tetB were detected in 100% of the water, microplastic and megaplastic samples. Preliminary qPCR results show higher Vibrio 16S rRNA and tetB concentrations in microplastic and megaplastic samples. The mean normalized relative abundance of the Vibrio 16S gene was 0.22 and 0.65 times higher in microplastics and megaplastics samples, respectively, than in water. For tetB, the relative abundance was 0.45 and 3.83 higher in microplastic and megaplastic samples, respectively, than in water.
Conclusions
Although not statistically significant ($p>0.05$), the higher abundance of Vibrio sp. and the tetB genes in microplastic and megaplastics in comparison to water, indicate that plastic in rivers are reservoirs for pathogens such as Vibrio sp. and their ARGs.

Identified novel colon cancer subtypes with different gene expression patterns and unique molecular biomarkers for potential targeted therapy.

Kehinde C[1]
[1]University Of Capetown

Background: Colon cancer is an extremely heterogeneous disease, with significant intra-tumour and inter-tumour heterogeneity. Understanding the heterogeneity can be facilitated by investigating transcriptomic profiles. Genome-transcriptome research on heterogeneity is restricted to examining primary tumours and normal matched tissues; without accounting for the multi-stage cancer development from early to advanced stages. This study will reveal the diverse progression (subtypes) in multi-stage colon cancer development by analysing transcriptomic profiles. Additionally, the colon subtypes exposed unique differentially expressed genes and driver genes.
Methods: RNA-sequenced gene expression of 572 colon cancer patients was downloaded from the UCSC Xena database. The heterogeneity in colon cancer was captured by implementing a recently developed normalisation method by our research group. Hierarchical clustering was applied to identify clusters (colon subtypes) with varied progression. Limma was utilised to identify differentially expressed genes (DEGs) between the clusters. The DEGs were subjected to OncodriveClustL which further identified mutated driver genes. Lastly, the Cox regression model based on the Lasso algorithm was used to analyse the driver genes for a significant prognostic value. Kaplan-Meier was displayed to estimate the significant differences in overall survival between the novel subtypes.
Results: The heterogeneous gene expression accumulation, shown by normalisation during multi-stage cancer progression led to uniquely separated cancer samples. Hierarchical clustering revealed three new colon cancer subtypes with heterogeneous transcriptome profiles and survivals. A total of 895 DEGs were identified between the three subtypes. Genes SYNE1, JAM3, and ZIK1 were identified as driver genes in which the latter showed that upregulation is associated with a favourable prognosis.
Conclusion: Keeping track of the progression of colon cancer helps to comprehend the characteristics of heterogeneity and their extent in colon cancer, this enables the discovery of new subtypes. The discovered driver genes will help simplify the process of screening and identifying patients susceptible to developing colon cancer, hence enhancing prognosis.

Assessing the selectivity of FM6 analogues as potential N-terminal inhibitors of Plasmodium falciparum Hsp90

Mafethe O[1], Mokoena F[1], Aucamp J[2], N'Da D[2]
[1]North-west University, [2]North-west University

Background

Plasmodium falciparum causes the deadliest form of malaria, and contains the heat shock protein (PfHsp90), a possible drug target for malaria. Because PfHsp90 is essential for the parasite's survival, its disruption results in parasite mortality. We previously used pharmacophore and Auto-quantitative structure-activity (Auto-QSAR) modelling to identify chemically diverse PfHsp90 inhibitors. FM6 bound tightly to PfHsp90, displayed selectivity and moderate activity against drug-sensitive strains of P. falciparum. However, FM6 had low solubility and toxicity to HepG2 cancer cells, the toxicity was likely due to an off-target interaction between FM6 and human Hsp90 (HsHsp90), which is abundantly expressed in cancer cells. We reasoned that examining the relationship between PfHsp90 and FM6 analogues could lead to obtaining information about functional groups needed for selectivity and increased potency.

Methods

Nine FM6 analogues and known inhibitors of Hsp90 were subjected to induced-fit docking and molecular dynamics simulations to understand their binding affinities and possible mode of action. R98 on the N-terminal domain of PfHsp90 was shown to confer selectivity, we therefore generated R98K-PfHsp90 mutant and human Hsp90 and used them in comparison to PfHsp90. Fluorescence polarization assays were conducted to assess the ability of the analogues to compete for binding with geldanamycin a known Hsp90 inhibitor.

Results

The study found that FM6-1, FM6-2, and FM6-5 bind to PfHsp90 with a binding affinity of -7.7 to -8.7Kcal/mol, while weak to no binding was observed towards HsHsp90. FM6-1 and FM6-2 were selective due to hydrogen bond with Arg98, and the bond distances of 2.13Å and 1.99Å respectively. This interaction in R98K-PfHsp90 mutant was lost. FM6-1 was the most selective and stable in the PfHsp90 pocket and the complex equilibrated after 3ns with root mean square deviation (RMSD) value of 1.25Å and maintained stable fluctuations. Using 20µM FM6-1 and FM6-2 inhibited PfHsp90 at 60% and 51%, respectively, while FM6-5 was the most potent at 86%, showing no inhibition towards HsHsp90.

Conclusions

This study reports three analogues inhibiting and binding to PfHsp90 with improved affinity and selectivity.

Spectral molecular networking digital lenses to evaluate the effects of pre-analytical and analytical parameters on the metabolome coverage of Momordica species

Ramabulana A[1], Madala N[2], Petras D[3], Tugizimana F[1]
[1]University Of Johannesburg, Department of Biochemistry, [2]University of Venda, Department of Biochemistry and Microbiology, [3]University of Tubingen, CMFI Cluster of Excellence, Interfaculty Institute of Microbiology and Medicine

Background
Recent advancements in capturing and mining metabolomics spectral data are transforming (plant-derived) natural product (NP) research. However, due to the inherent complexity of the metabolome (e.g., highly dynamic, and chemically diverse), the adage “what you see is what you extract and measure” remains valid. This defines the captured metabolomics spectral data, limiting the insights on the phytochemistry and natural product space of the plant under consideration.
Methods
Reported in this study is a multi-layered evaluation of both pre-analytical (solvent extractions) and analytical (data-dependent acquisition, DDA) parameters and methodologies to investigate the metabolome of Momordica plant species. The effects of these parameters were assessed through computational strategies such as molecular networking methods and related workflows (classical molecular networking, MS2LDA, network annotation propagation and MolNetEnhancer).
Results
Molecular networking (MN) emerged as a key strategy to organise and annotate untargeted tandem mass spectrometry (MS/MS) data. The results obtained from exploring datasets acquired from Momordica plant samples extracted with solvents of varying polarities (dichloromethane, ethanol, ethyl acetate and methanol) indicated a biased investigation of metabolomes when a single solvent is utilised. Methanol, dichloromethane and ethanol were observed to extract compounds of a wider chemical class diversity relatively. The solvents utilized also had an impact on the relative abundance of extracted metabolites. Computationally, combining datasets acquired using multiple solvents offers a broader metabolome coverage. Furthermore, exploring various DDA parameters demonstrated that the lowest predefined intensity thresholds and collision energies favoured generation more MS/MS spectra subsequently benefiting a broader view into the chemical diversity of Momordica species. Comparatively, higher intensity thresholds and collision energies resulted in fewer MS2 spectra acquisition, subsequently fewer nodes, and a limited exploration of the metabolome through MN.
Conclusions
This study proposes a methodological framework where multiple solvents and DDA parameters are utilised to increase the coverage of ions acquired, improving the global metabolome coverage.

Key words: computational metabolomics, molecular networking, natural products

Investigating the role of Hop in v-Cyclin-CDK6-NPM complex in KSHV-associated malignancies

Ndabeni A[1], Edkins A[1], Mhlanga R[1]
[1]Rhodes University, Biomedical Biotechnology Research Unit (BioBRU)
Investigating the role of Hop in v-Cyclin-CDK6-NPM complex in KSHV-associated malignancies

Anita Ndabeni, Richwell Mhlanga and Adrienne L. Edkins
The Biomedical Biotechnology Research Unit, Department of Biochemistry and Microbiology, Rhodes University, Grahamstown 6139, South Africa

Background
Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8 (HHV8) is a carcinogen that is required for the development of Kaposi's sarcoma (KS). KS is a highly vascular tumor of endothelial origin and is considered an AIDS-defining cancer contributing to ~ 15% of cancers worldwide. Despite increased access to anti-retroviral therapy (ART) leading to improved HIV infection management, KS remains a substantial cause of morbidity and mortality worldwide. Apart from the ART, there are still no available KSHV-specific antivirals or vaccines, hence therapeutic approaches to inhibit both KSHV latent and lytic replication cycles and KSHV-mediated tumorigenesis are still needed. Molecular chaperones, Hsp70 and Hsp90 play an important role in both KSHV latent and lytic replication and Hsp90/Hsp70 organizing protein (Hop) facilitates the function of these chaperones. Virus-encoded cyclin (v-Cyclin) is a viral cyclin D homolog expressed during KSHV latent and lytic replication cycles. v-Cyclin forms a complex with cyclin-dependent kinase 6 (CDK6), which phosphorylates various substrate proteins, including nucleophosmin (NPM). The binding of the phosphorylated NPM to latently associated nuclear antigen (LANA1) inhibits KSHV lytic gene expression by promoting LANA1 de-acetylation. Proteomic analysis suggested that v-Cyclin was significantly enriched in the Hop interactome during KSHV lytic replication, hence we focused on the mechanistic analysis of the association of Hop with v-Cyclin, CDK6, and NPM and examined the potential of Hop as a novel antiviral target.

Methods
We used SDS-PAGE, western blot, and resazurin assay.

Results
In this project, we demonstrate that Hop overexpression or knockdown does not affect the levels of v-Cyclin, CDK6, and nucleophosmin. We also demonstrate that the knockdown of Hop decreased cell viability during KSHV lytic replication, indicating the importance of Hop during KSHV lytic replication. Interestingly, we have observed that CDK6 and nucleophosmin interact with Hop.

Conclusion
These initial data suggest that Hop plays a role in the lytic replication of KSHV.

Keywords: KSHV, Hop, v-Cyclin, CDK6, nucleophosmin

Distribution of rutin metabolite from domesticated Moringa oleifera foliage in Vhembe District of Limpopo Province using ultra high performance liquid chromatography quadrupole time of flight mass spectrometry (UHPLC-qTOF-MS)

Ndou D[1], Ndhlala A[2], Tawanda N[1], Madala N[3]
[1]University of Venda - Chemistry, [2]University of Limpopo, [3]University of Venda - Biochemistry and Microbiology

Background
Moringa oleifera, recognized for its diverse nutritional and medicinal advantages, is categorized as a functional crop because of its diverse metabolic composition. Among the numerous flavonoid glycosides present in M. oleifera, flavonoids attached to a rutinoside disaccharide have also been reported, although conflicting reports exist regarding their levels. The current study focused on analyzing Moringa plants from various households in the Vhembe District of Limpopo Province, South Africa, specifically for the presence of rutin (quercetin rutinoside).
Methods
A modified method for the extraction of metabolites from M. oleifera was used. Briefly, ground leaf powder was extracted with 80% aqueous methanol (MeOH) with the aid of a dragon shaker overnight. The resulting extract was analyzed for rutin using an ultra high performance liquid chromatography quadrupole time of flight mass spectrometry (UHPLC-qTOF-MS).
Results
The study results reveal that out of the 135 samples analyzed, only 15 household plants are capable of producing rutin. The presence of rutin was confirmed through the fragmentation pattern of rutin. Rutin was also observed to have different concentrations. The highest concentrations were found to be 4088, 4908, 8444, and 14701 mg.kg-1. This indicates the existence of different cultivars in different households, potentially impacting the perception of this species.
Conclusions
This study reaffirms that the accumulation of rutin in M. oleifera is cultivar specific and not all Moringa plants have the same metabolite distribution patterns and, as such, differences in pharmacological properties should be expected.

Antioxidant, antidiabetic, and anti-obesity potentials of Macrocytis pyrifera in vitro

Chabalala V[1], Islam S
[1]University Of Kwazulu-natal
Antioxidant, antidiabetic, and anti-obesity potentials of Macrocytis pyrifera in vitro
Chabalala, Vunene N. and Islam, Md. Shahidul
Department of Biochemistry, School of Life Science, University of Kwazulu-Natal, Westville campus, Durban 4000, South Africa.
Email: 222045806@stu.ukzn.ac.za

Background
Macrocystis pyrifera, commonly known as giant kelp, is a brown seaweed that is abundantly found along coastlines worldwide. This study examines the emerging potential therapeutic effects of Macrocystis pyrifera, a brown seaweed, in the management of type 2 diabetes and obesity.

Methods
The seaweed extracts (cold-water and ethanol) were screened for antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH), Nitric oxide (NO), and non-site-specific hydroxyl radical (HO•) scavenging activity. The extracts were also evaluated for α-amylase, α-glucosidase, pancreatic lipase inhibitory activities, as well as on glucose uptake using yeast cells. Furthermore, the extracts were subjected to Liquid Chromatography-Mass Spectroscopy (LC-MS) analysis to elucidate their possible bioactive compounds.

Results
The cold-water extract exhibited highest phenolic & flavonoid content (50.61 mg/GAE/g and 63.13 mg/QAE/g, respectively) and antioxidant activity through DPPH and OH• scavenging assays (IC50 = 0.8 mg/mL and 0.97 mg/mL, respectively). The ethanol extract displayed the higher scavenging activity in NO assay (IC50= 22.33 mg/mL) compared to the cold-water extract. Moreover, the ethanol extract significantly inhibited the carbohydrates (α-amylase & α-glucosidase) and lipase enzymes (IC50 values of 63.85 mg/mL, 0.89 mg/mL, and 0.05 mg/mL, respectively) and displayed good potentials in the ability to uptake glucose by yeast cells (IC50 value of 1.84 mg/mL) than the cold-water extract. The LC-MS analysis revealed the presence of phenolic compounds including p-hydroxybenzoic acid (4- Hydroxybenzoic acid), Cyanidin-3-glucoside, and Quercetin which are potentially responsible for the activity observed.

Conclusion
The results of this study suggest the possible antioxidant, antidiabetic, and anti-obesity potentials of Macrocystis pyrifera however, further studies in animal model are warranted to ascertain the results of these experiments.

Key words: α-Amylase, α-glucosidase, Antioxidant, Lipase, Macrocystis pyrifera, Obesity, Type 2 diabetes.

In silico analysis of a small molecule inhibitor as a therapeutic approach in high-risk Human Papilloma Virus infected cervical cancer cells

Basera A[1], Kandhavelu J[2], Moela P[3], Penny C[4], Dlamini Z[5], Marima R[5]
[1]Department of Medical Oncology, Faculty of Health Sciences, Steve Biko Academic Hospital, University Of Pretoria, [2]Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, [3]Biochemistry, Genetics, Microbiology, University of Pretoria, [4]Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences, [5]SAMRC Precision Oncology Research Unit (PORU), DSI/NRF SARChI Chair in Precision Oncology and Cancer Prevention, Pan African Cancer Research Institute (PACRI)

Introduction
Cervical cancer (CCa) presents a formidable global health challenge. The Human papillomavirus (HPV) is a cause of CCa, with its viral oncoproteins E6 and E7 promoting and maintaining CCa. Alternative splicing of viral transcripts E6 and E7 results in isoforms linked to late-stage CCa. SRPK1, involved in the phosphorylation of splicing factors, is upregulated in CCa. Despite the increased understanding of viral and host interactions in cervical carcinogenesis, and existing therapies, CCa remains a burden. Furthermore, the splicing inhibitory effect of small molecules is understudied in CCa. The study aimed to assess the potential of an SRPK1 small molecule inhibitor (Sphinx31) as a therapeutic agent against CCa.

Method
In silico analysis was used to assess the interaction of proteins with Sphinx31. The structure of sphinx31 was visualized on PubChem and drawn using Marvin sketch. High throughput docking was used to assess proteins that interact with Sphinx31, and the strength of the interactions. Proteins with high docking scores above 8 were selected, their Uniprot numbers were identified using the Uniprot database, and their involvement in Cca was determined. The pathways associated with the proteins were identified using the KEGG pathway.

Results
EGFR, PKM2, and CYP1B1 exhibited docking scores of 12.3, 10.9, and 10.3, respectively, with EGFR showing the strongest interaction with the inhibitor. Pathway analysis indicated EGFR and PKM2 involvement in HPV infection and viral carcinogenesis. Upregulated EGFR correlates with increased HPV E7 levels, enhancing cell division, survival, and proliferation, while HPV E6 boosts PKM2 expression, promoting cell proliferation. CP1B1, prevalent in HPV 16/18 patients, induces carcinogenesis through Wnt/β-catenin signaling activation.

Conclusion
Our findings suggest that EGFR, CP1B1, and PKM2 may serve as therapeutic targets for Sphinx31 in HPV-associated CCa.

The synthesis and evaluation of 3-benzoylbenzofurans and their pyrazole derivatives against HIV-1 infections and cancer.

Khuzwayo S[1]
[1]University Of Pretoria, Biochemistry Department, [2]University of Pretoria, Chemistry Department, [3]Sol Plaatje University, School of Natural and Applied Sciences

Background
The human immunodeficiency virus-1 (HIV-1) remains one of the leading global epidemics in the world. The HIV/AIDS epidemic is particularly dominant in Eastern and Southern Africa, as is evident by the 670 000 annual new infections in this region alone, compared to 1.5 million global annual new infections (UNAIDS, 2022). HAART is an effective treatment for controlling HIV-1 infections; however, severe adverse events and the emergence of drug resistance have been major challenges (Amblard et al., 2022). Lenacapavir, a pyrazole-based drug, was FDA-approved in 2022 and has been proven to be the most potent HIV-1 inhibitor thus far (Segal-Maurer et al., 2022). Numerous experimental pyrazole compounds have demonstrated high potency against HIV-1 infections and have been shown to have minimal cytotoxicity, thus making them attractive candidates for HIV-1 treatment (Cherne et al., 2019).

Methods
Novel 3-benzoylbenzofurans and pyrazole derivatives were synthesized and characterized using mass spectrometry (LC-MS and HRMS), NMR, and melting points. Our synthesis method involved methylating the 5-hydroxy group of 3-benzoylbenzofurans, which produced the intermediates from which the pyrazoles were derived. The 3-benzoylbenzofuran precursors and derivatives were assessed for cytotoxicity in TZM-bl cells and for anti-HIV-1 activities in Q23 and CAP210 pseudoviruses. The cytotoxic 3-benzoylbenzofurans were assessed against cervical cancer and hepatic cancer cells using both MTT assays and real-time cell electronic sensing (RT-CES) and for antioxidant activities using DPPH assays.

Results
The methylated 3-benzoylbenzofurans were discovered to be cytotoxic in TZM-bl cells, while the pyrazole derivatives showed mild cytotoxicity. The most active compounds included a non-methylated (2,5-dimethoxyphenyl)(5-hydroxy-4,7-dimethyl-1-benzofuran-3-yl)methanone, a methylated benzofuran (2,5-dimethoxyphenyl)(5-methoxy-4,7-dimethyl-1-benzofuran-3-yl)methanone, and a pyrazole derivative (2-[3-(4-fluoro-2-methoxyphenyl)-1H-pyrazol-4-yl]benzene-1,4-diol) with IC50 values that were comparable to nevirapine in both Q23 and CAP210 pseudoviruses. The cytotoxic benzofurans demonstrated high potency against cervical cancer and hepatic cancer cells and were also demonstrated to be potent antioxidants using a DPPH assay.

Conclusions
Together, these results demonstrate the potential of benzofurans and pyrazole derivatives for further development as antiretrovirals and anti-cancer treatment.

Investigating theophylline interactions with human proteins in cervical cancer using molecular docking

Nyalambisa A[1], Kandhavelu J[2], Penny C[3], Dlamini Z[4], Marima R[4]
[1]Department Of Medical Oncology, Faculty Of Health Sciences, Steve Biko Academic Hospital, University Of Pretoria, [2]Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University Medical Cnter, [3]Department of Internal Medicine, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, [4]SAMRC Precision Oncology Research Unit (PORU), DSI/NRF SARChI Chair in Precision Oncology and Cancer Prevention, Pan African Cancer Research Institute (PACRI), University of Pretoria

Background
Cervical cancer (CCa) is the fourth most common cause of cancer-related death in women worldwide. Despite advances in screening and treatment strategies, CCa continues to be a significant worldwide health problem with high morbidity and mortality rates. As a result, there is an urgent need to explore novel therapeutic avenues to address this challenge. Therefore, this study aims to identify novel therapeutic avenues for CCa through in silico analysis, focusing on evaluating the interactions between a Serine/arginine-rich splicing factor 3 (SRSF3) inhibitor Theophylline and its target proteins associated with the disease, and elucidating pathways implicated in its progression. Theophylline is a drug that is used to treat airway diseases. It has been shown to have anti-inflammatory effects. A recent study revealed that theophylline down-regulated SRSF3 expression and switched p53 from alpha into a beta isoform in CCa cell lines. Additionally, studies demonstrated that theophylline induced cellular apoptosis, senescence, and decreased colony formation. Interestingly, it was found that theophylline had a suppressive effect on cellular proliferation. SRSF3 is highly expressed in CCa, significantly contributing to tumorigenesis.
Method
In this study, an in silico analysis was conducted to evaluate interactions between Theophylline and its target protein, particularly those linked to CCa. The Chemical Entities of Biological Interest/ChEBI database provided the chemical structure of Theophylline for analysis. High-throughput docking was employed to assess protein-ligand interactions, identifying potential targets with strong interactions. The UniProt database was then used to obtain protein IDs, which were further analyzed for pathway involvement using KEGG and WIKI databases.
Results
Notably, Theophylline exhibited interactions with several target proteins, including Tankyrase-2, Cytochrome P450 1B1, ADAM 17, Cytochrome P450 1A2, and Islet amyloid polypeptide. Remarkably, two of the top proteins identified for Theophylline; Cytochrome P450 1B1, and ADAM 17 were previously reported to be linked to CCa. ADAM 17 was found to directly target EGFR leading to increased EGFR signaling and promoting tumor progression. Additionally, Cytochrome P450 1B1 induces carcinogenic processes in cells following Wnt/β-catenin signaling activation, further implicating its role in CCa pathogenesis.
Conclusion
the findings highlight the potential of targeting these proteins as novel therapeutic strategies for CCa.

Prevalence and genetic characterization of cervical Alphapapillomaviruses in a cohort of HIV infected and HIV uninfected women in Limpopo Province, South Africa

Rikhotso R[1], Mitchell E[2], Bessong P[1,2,3]
[1]University Of Venda, [2]University of Virginia, [3]University of KwaZulu-Natal

Background: High-risk (hr) alpha human papillomaviruses (HPV) are associated with cervical cancer. The current study investigated HPV prevalence and types from women in Limpopo Province, South Africa.
Methods: The prevalence of HPV in cervical specimens was determined in 450 women by a double nested polymerase chain reaction (PCR), targeting the L1 gene of the virus. The first nested PCR products (about 450 bp) were sequenced on an Illumina MiniSeq. Sequence reads of acceptable quality were analyzed for viral types.
Results: An overall HPV prevalence of 32.7% (147/450) was observed in the study population. HPV DNA was more frequently detected in HIV infected individuals compared to HIV uninfected individuals (p=0.002). Forty-one HPV types were identified from 48 samples with acceptable sequence quality. Overall, HPV 45 was the most predominant type. HPV 81 (18.8%, 6/32) was the most common type in HIV infected women, while HPV 56 (25.0%, 3/12) was the most common type among HIV uninfected individuals (p=0.713). Of note, HIV infected individuals were more prone to be infected with hrHPV types.
Conclusion: The study showed a relatively high rate of HPV infection in the study population. HIV infected women were more likely than HIV un-infected women to test positive for HPV DNA. HIV infected persons also harbored more hrHPV types. This suggests that HIV infected women are in most need of HPV vaccination.

The identification of DNA aptamers for their application in the rapid diagnosis of soil-transmitted helminthiases

Van Der Walt J[1], Meyer M[1], Martin D[1], Madiehe A[1,2]
[1]Department of Science and Innovation/Mintek Nanotechnology Innovation Centre, Biolabels Node, Department of Biotechnology, Faculty of Natural Sciences, University of the Western Cape, Bellville, South Africa, [2]Nanobiotechnology Research Group, Department of Biotechnology, Faculty of Natural Sciences, University of the Western Cape, Private Bag X17, Bellville, South Africa

Soil-transmitted helminthiases (STH) is a neglected tropical disease which can be caused by several parasitic tapeworms such as Ascaris lumbricoides (roundworms). It is mostly transmitted by contact with contaminated soil or water. STH is prevalent in regions with warm tropical climates such as China, South America, Asia, and sub-Saharan Africa and is more prevalent in poorer communities. Mass deworming using chemotherapeutic drugs, has not been very effective due to lack of discrimination between infected and healthy individuals, leading to drug resistance. Effective treatment starts with effective diagnosis. Current diagnostic techniques involve the examination of faecal or other gastrointestinal tract specimens for the presence of eggs, larvae, or adult worms. These methods are costly and time consuming. Nucleic acid lateral flow assays (LFAs) utilising DNA aptamers, which are short synthetic oligonucleotides easier to produce and use compared to antibodies, to recognize disease biomarker proteins will be ideal for the rapid detection of STH. Therefore, the aim of this study was to identify DNA aptamers that could be used to develop a rapid diagnostic test for STH.

Recently, protein J was identified as a suitable biomarker. Protein J is secreted by parasitic worms and affects the host immune system. We employed in silico tools to select candidate aptamers against protein J. Briefly, a suitable model of the protein J was selected from Alphafold, its quality validated using various SAVES web server tools, and the modelled protein J was virtual screened to the proprietary aptamer database using AutodockVinaXB. The selected aptamer-protein J complexes underwent stability testing prior to molecular dynamic simulations (MDS) to access their root mean square deviations, fluctuations, radius of gyration, non-bonded interaction energies and molecular mechanics Poisson-Boltzmann surface area.

A suitable model of the protein J was successfully produced. Virtual screening identified about 20 potential aptamers with increasing docking scores (-8.0 to -13.0 kcal/mol) of which 8 were stable after stability testing. Three potential aptamers with higher binding affinities were selected (Apt 1, 2 and 3). The binding of these aptamers to protein J will be characterised using microscale thermophoresis and applied in the development of a lateral flow assay.

SYNTHESIS, CHARACTERIZATION AND ANTINEOPLASTIC ACTIVITY INVESTIGATION OF DIRUTHENIUM (II, III) COMPLEXES.

Medupe T[1], Prince S[1], Ngubane S[1]
[1]University Of Cape Town

Introduction: Breast cancer poses a significant health burden in South Africa, as it is the most diagnosed and second most deadly cancer in women. Conventional chemotherapeutics used in the management of breast cancers are often associated with debilitating side effects which can affect vital organs, fuelling the need for novel chemotherapeutic options. Ruthenium complexes or metallodrugs, which incorporate ruthenium metal centres through coordination with organic compounds or ligands, have received great attention in cancer research. This study sheds light on the potential anticancer properties of the bimetallic ruthenium(II, III) complexes of interest against human breast cancer cells.

Methodology: Human breast MCF-7 cancer and non-malignant MCF12A mammary epithelial cells were treated with an array of [Ru2(O2CCH3)3(R-ap)Cl] (C1 - C5) complexes of interest. Various molecular and cell biology techniques were employed to determine the anti-cancer activity and selectivity of the complexes including MTT cell viability, colony formation assays, and western blotting with antibodies to markers of DNA damage (ɣ-H2AX) and apoptosis (caspase-9 and its downstream substrate, PARP).

Results: [Ru2(O2CCH3)3(R-ap)Cl] complexes (C1 - C5) show promising cytotoxicity and inhibited MCF-7 cell viability significantly after 72-hours. The metallodrug lead complexes, [Ru2(O2CCH3)3(4-CH3ap)Cl] (C3) and [Ru2(O2CCH3)3(4-Fap)Cl] (C5) showed favourable selectivity towards the MCF-7 human breast cancer over the mammary non-malignant MCF-12A breast epithelial cells. Results from colony formation assays showed that C3 inhibited the growth and survival of MCF-7 cells after 7 days of monitoring cell colonies. Western blot analysis suggests that C3 induces double-stranded DNA breaks, as observed by an increase in levels of ɣ-H2AX. Furthermore, C3 activates the intrinsic apoptotic cell death pathway as demonstrated by an increase in cleaved caspase-9 and its downstream substrate, PARP.

Conclusion: Altogether, the results obtained suggest that [Ru2(O2CCH3)3(R-ap)Cl] type complexes show promising anticancer properties against MCF-7 human breast cancer cells. Additionally, the possible mechanisms of action of [Ru2(O2CCH3)3(R-ap)Cl] complexes were highlighted. The results provide evidence to support the use of ruthenium metallodrugs in cancer therapy.

Cervicovaginal virome and bacteriome in HIV infected women in northern South Africa

Ratshilindela M[1], Mavhandu-Ramarumo L[1], Madala N[2], Tebit D[1,3], Bessong P[1,4,5]
[1]HIV/AIDS & Global Health Research Programme, University of Venda, [2]Department of Microbiology and Biochemistry, University of Venda, [3]Global Biomed Scientific LLC, P.O. Box 2368, Forest, VA, 24551, USA, [4]Center for Global Health Equity, Department of Infectious Diseases and International Health, School of Medicine, University of Virginia, Charlottesville, VA, USA, [5]School of Health Sciences, Kwazulu-Natal, Durban, South Africa
BACKGROUND: Highly diverse bacterial species in the cervicovaginal niche is linked to a higher risk of contracting human immunodeficiency virus (HIV), and persistent infection with human papillomavirus (HPV). This study characterized cervicovaginal virome and bacteriome in HIV-infected women from selected health care facilities in Northern South Africa.

METHODOLOGY: Total DNA was extracted from 150 cervical swabs obtained from 50 HIV/HPV co-infected women; 50 HIV-infected, HPV-noninfected women; and 50 HPV-infected HIV-noninfected women. Cervicovaginal virome and bacteriome were determined through a metagenomic approach and analysed with Dragen metagenomic tool. Chi square test and Partial Least Square-Discriminant Analysis were used for statistical analysis.

RESULTS: A diverse group of viral and bacterial families was observed among the HIV/HPV co-infected group ($P<0.0001$). The least diverse group was observed among the HIV-infected HPV-noninfected group. Majority of sequence reads were assigned to Papillomaviridae and Myoviridae families irrespective of HIV status. HSV-2, and members of Myoviridae, Siphoviridae and Podoviridae families were detected in all three study groups. Bacterial community state types one, three, four and five were detected among the study participants.

CONCLUSION: It appears that there is a high diversity of cervicovaginal virome and bacteriome in women who are HIV/HPV co-infected than in those infected with either HIV or HPV alone.

Evaluating the effect of synthetic curcumin derivatives on skeletal muscle metabolism

Ramashia R[1,2], Pheiffer C[1,2,3], Titinchi S[4], Ramharack P[1,5], Windvogel S[2]
[1]South African Medical Research Council (SAMRC), Biomedical Research and Innovation Platform, [2]Centre for Cardio-Metabolic Research in Africa (CARMA), Division of Medical Physiology, Faculty of Medicine and Health Sciences, University of Stellenbosch, [3]Department of Obstetrics and Gynaecology, Faculty of Health Sciences, University of Pretoria, [4]Department of Chemistry, Faculty of Natural Science, University of the Western Cape, [5]Discipline of Pharmaceutical Sciences, School of Health Sciences, University of KwaZulu-Natal

Background: Curcumin, a polyphenolic compound from turmeric, has potential therapeutic benefits against metabolic diseases like skeletal muscle disorders. However, it's poor bioavailability prompts the development of synthetic curcumin derivatives. The study aims to compare the efficacy of three synthetic curcumin derivatives with curcumin on skeletal muscle metabolism using in silico and in vitro approaches.
Methods: Two chloro-curcumin derivatives (1A6 and 1A8) and an asymmetric curcumin derivative (1B8) were synthesized through a chemical reaction of 3-chloroacetylacetone (1A6 and 1A8) or acetylacetone (1B8) with boric oxide. HR-MS-ESI, FT-IR spectroscopy, and 1H-NMR spectroscopy were used to characterize the derivatives. Molecular docking was conducted to predict the molecular interactions and binding affinities of curcumin, and the derivatives with the predicted biological targets. The bioactivity of curcumin, and the derivatives was assessed in vitro in differentiating C2C12 myoblasts and in TNFα-treated C2C12 myotubes, by assessing cell viability using the MTT and the ATP assays, oxidative stress was quantified by TAC and lipid peroxidation. The expression of genes involved in insulin signaling, oxidative stress, inflammation, myogenesis and muscle atrophy was measured using quantitative real-time PCR.
Results: Curcumin derivatives were synthesized with high purity and the chemical structures were elucidated by spectroscopic techniques. In silico prediction showed that curcumin and the derivatives exhibited favourable pharmacokinetic profiles, low toxicity, and improved drug-likeness. 1A8 had the highest binding affinity and 1A6 had the lowest binding affinity for the majority of predicted biological targets when compared to curcumin. In differentiating C2C12 myoblasts, curcumin and the derivatives reduced cell viability at higher doses (10 and 20 µM), while treatment had no effect on TNFα-induced C2C12 myotubes. In TNFα-treated myotubes, 1A6 at 5 µM increased TAC. The expression of Pi3kr1 was upregulated in C2C12 myoblasts treated with curcumin, and the derivatives (5 µM). In contrast, Myf6 expression was downregulated by curcumin, and the derivatives (5 µM) in differentiating C2C12 myoblasts, while curcumin and 1A8 also decreased the expression of Myod1 at 5 µM.
Conclusion: Curcumin and the derivatives present a potential to be further explored for their efficacy to improve skeletal muscle metabolism including myogenesis and insulin signaling.

In silico analysis of SRPIN340 splicing disruptor drug in cervical cancer cells.

Mphahlele R[1]
[1]Department Of Medical Oncology, Faculty Of Health Sciences, Steve Biko Academic Hospital, University Of Pretoria, Hatfield, [2]Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington, DC, USA , [3]4Department of Internal Medicine, Faculty of Health Sciences, School of Clinical Medicine, University of the Witwatersrand, Parktown 2193, South Africa , [4]SAMRC Precision Oncology Research Unit (PORU), DSI/NRF SARChI Chair in Precision Oncology and Cancer Prevention, Pan African Cancer Research Institute (PACRI), University of Pretoria, Hatfield, Pretoria 0028, South Africa, [5]SAMRC Precision Oncology Research Unit (PORU), DSI/NRF SARChI Chair in Precision Oncology and Cancer Prevention, Pan African Cancer Research Institute (PACRI), University of Pretoria, Hatfield, Pretoria 0028, South Africa

Background
Cervical Cancer (CCa) remains a global health concern with approximately 90% mortality in low-income and middle-income countries (LMICs). Aberrant Alternative splicing (AS) is implicated in CCa. SRPIN340 is a small molecule compound known for its inhibitory effect on proteins involved in AS. The effect of SRPIN340 in CCa is understudied. This study aimed to assess the effect of SRPIN340 on the CCa progression and evaluate its therapeutic efficacy.
Method
The interaction of proteins with SRPIN340 was evaluated using in silico analysis. The structure of the disruptor drug was uploaded onto the High Throughput docking software and the interaction between the proteins and the drug was evaluated. The docking scores of the interactions were compared, proteins exhibiting docking scores of 8 and above were selected and their Uniprot numbers were determined using the Uniprot database. A literature search was conducted to determine the protein involvement in CCa. KEGG pathway analysis was used to identify the pathways in which the proteins are involved.
Results
Estrogen receptor beta (ESR2), Epidermal growth factor receptor (EGFR), and RAC-beta serine/threonine-protein kinase Glycogen synthesiskinase-3 beta (AKT2) were identified with docking scores of 9.8, 8.9, and 8.7, respectively. The association between EGFR, ESR2, and AKT2 involves complex signaling pathways. EGFR have the potential to activate AKT2 through the PIK3-AKT pathway, leading to the promotion of cell survival and proliferation whilst ESR2 prevents the proliferative effects of EGFR and AKT by inhibiting cell proliferation and promoting apoptosis in CCa cells.
Conclusion
The results from this study suggest that ESR2, EGFR and AKT2 serve as potential therapeutic targets for SRPIN340 in CCa cells. Therefore, more research is needed to elucidate the underlying mechanisms of SRPIN340 in CCa.

Isolation and identification of compounds from Trichilia emetica

Mugayi L[1], Tagwireyi D[1], Mukanganyama S[1]
[1]University Of Zimbabwe

Lydia Chenesai Mugayi*1, Dexter Tagwireyi*2 and Stanley Mukanganyama*1
1Department of Biotechnology and Biochemistry, University of Zimbabwe
2Department of Pharmacy and Pharmaceutical Sciences, University of Zimbabwe
E-mail: lydiachenesai@gmail.com

Background The genus Trichilia belongs to the Meliaceae family, and only two of these species, Trichilia emetica and T. dregeana, produce seeds with high oil content. Trichilia emetica has previously been reported as a valuable source of limonoids have been found in Maliaceae and are good chemotaxonomic markers.
Methodology The Gas Chromatography–Mass Spectrometry analysis of the hydroethanolic and the ethyl acetate extracts was performed using a capillary column called HP-5 MS, which had dimensions of 25 m length and 250 µm diameter, with a thickness of 0.25 µm. Liquid-to-liquid partitioning of the hydroethanolic extract was carried out using hexane, ethyl acetate, and n-butanol. The ethyl acetate fraction was then subjected to gradient column chromatography for the isolation of compounds using solvents of different polarities. Fractions were collected and grouped based on their thin-layer chromatography profiles to obtain the compounds. Results
At a polarity of hexane:ethyl acetate 40:60, a white flaky compound, TEL1, was obtained. At a polarity of hexane:ethyl acetate 50, TEL2, a brown crystal that dissolves in methanol, was obtained. TEL3 was obtained as a white flaky compound at the elution system hexane:ethyl acetate 25:75. Upon comparing the TLC profile of TEL3 with a glucoside, it was observed that TEL3 was a glucoside. Chemical profiling of T. emetica leaf extracts revealed the presence of a total of seven phytochemicals in both the ethyl acetate and 70% ethanol extracts. Gamabufotalin, octadecanoic acid, rescinnamine, and 9-octadecen-1-ol (Z) were identified from the ethyl acetate extract. Toulene and 2.4-di-tert-butylphenol was identified from the hydroethanolic leaf extract.
Conclusion The compounds identified showed that this plant contains a lot of fatty acids as it has high oil content. Further work will be done on the identification and structure elucidation of TEL1, TEL2, and TEL3. Liquid Chromatography Mass Spectrometry analysis will also be carried out on the leaf extracts. The compounds will then be tested for anti- inflammatory and wound healing activity.
Key words T. emetica, isolation, compounds, chromatography

The compendium of metabolites – At the confluence of metabolomics and genomics

Mundembe R[1], Nkqenkqa V[1,2], Jackson V[1]
[1]Department of Biotechnology & Consumer Science, Cape Peninsula University of Technology,
[2]Department of Environmental Health and Occupational Studies, Cape Peninsula University of Technology

Background

The fields of metabolomics and genomics including transcriptomics are rapidly growing with even greater potential for future growth if the two fields are studied together. Since most practitioners have expertise in only one or the other of these fields, establishment of a platform or repository that brings together metabolomics and transcriptomics has the potential to make these emerging technologies more accessible.

Aim

The aim of the project was to initiate a repository that brings together metabolomics profiles and genomics data for any given species.

Methods

The initial efforts were focussed on Warburgia salutaris. A literature search was carried out to list all secondary metabolites and biomolecular resources that have been reported for the species.

Results and Discussion

For W. salutaris, the compendium lists 89 metabolites and gives the internet links to the online sources of the information. The link to the transcriptome data is also provided. The compendium is currently hosted at https://www.cput.ac.za/academic/faculties/appliedsciences/departments/biotechnology-consumer-studies/compendiumofmetabolites.

Conclusion and way forward

In its envisaged format, any researcher will be able to create a page for each species they work with and populate it with all relevant metabolomic and genomics data. Researchers are encouraged to register, add, and update pages of their species of interest.

Arg- and Trp-rich peptide candidates for antifungal drug development: Activity and structural characterisation

Mamhende G[1], Rautenbach M[1]
[1]Stellenbosch University

Background

A burgeoning population of immunocompromised individuals combined with a drug arsenal whose efficacy has been curtailed by antifungal drug resistance have culminated into an unprecedent rise in fungal infection mortalities. To deal with this challenge, it is imperative that alternative antifungal agents with novel mechanisms of action are developed. In view of this, we elucidated the antifungal activity and structural properties of synthetic antifungal peptides with immense potential for development into novel antifungal drugs.

Methods

Antifungal activity of Arg and Trp-rich synthetic hexapeptides was characterised by determining their metabolic inhibitory capacity against Candida albicans through a fluorometric microdilution broth dose response assay. Circular dichroism (CD) spectroscopy was employed to determine the secondary structure of 100 µM peptide analogues in aqueous and 50% trifluoroethanol (TFE) solutions. The TFE solution was representative of a membrane mimicking environment.

Results

While the antifungal activity characterisation revealed that cyclic analogues had greater antifungal activity (lower IC_{50}) in comparison to the linear analogues, the peptide sequences of the most active peptide in each group differed. The most active cyclic peptide was cyclo(RRRWWW) (IC_{50} = 5.2 µM) whereas the most active linear analogue was Acetyl-KRKWWW-amide (IC_{50} = 38.2 µM). CD analysis of the secondary structure of the cyclic peptides showed the existence of two minima at 203 nm and 220 nm indicative of a β-turn and β-sheet motifs, respectively. Altering the peptide sequence in the polar region did not cause huge structural differences whereas alterations in the hydrophobic region reversed the intensities of the minima which indicates structural changes in the peptide backbone. However, no clear corelation between antifungal activity and peptide secondary structure was observed.

Conclusions

Antifungal activity is not simply a function of the secondary structure of the peptide backbone; more parameters such as volume, surface area and hydrophobicity may play a role in antifungal activity and their involvement should be investigated.

Biophysical and biochemical characterization of Tn5 transposase

Oscar K[1]
[1]University Of Venda

Tn5 transposase is an enzyme that mediates the fragmentation of dsDNA and ligates synthetic oligonucleotides at both ends. Transposase 5 (Tn5) is the key reagent in library preparation for Illumina platforms, The most rapid and scalable Next-generation sequencing library preparation strategy available to date is based on a hyperactive version of the Tn5 transposase. determining its three-dimensional structure, identifying key functional domains, and elucidating its mechanism of action
in transposition. Characterization efforts may also aim to investigate the protein's interactions with DNA and other molecules and its stability under different conditions. The goal of the proposed project is to produce, biophysically, and biochemically characterize Tn5 transposase, determine its three-dimensional structure, identify key functional domains, and investigate the protein's interactions with DNA and other
molecules, its stability under different conditions, and its activity. Tn5 transposase will be expressed in E.coli cells and purified using Nickel affinity chromatography. Biochemical (Activity) assay and biophysical assays (intrinsic/extrinsic fluorescence spectroscopy, Far UV circular dichroism, SPR) will be used to explore the structural uniqueness of Tn5 transposase and protein’s interaction. Limited proteolysis will be used to determine the structural features of TN5 transposase (N-terminal domain, C-terminal domain). Peptide Mass Fingerprinting will be used to determine the mass of each peptide. Tn5 transposase interaction with DNA/ other molecules will be determined by Surface plasmon resonance (SPR). Moreover, Tn5 transposase activity will be determined by the fragmentation of viral DNA. Upon the completion of this study, the Biophysical & biochemical characteristics of a Tn5 transposase will be determined.

Keywords: Tn5 transposase, HIV, Circular dichroism, Peptide mass fingerprinting, SPR, E.coli BL21 (DE3) cells.

Pathway analysis of Sphinx31 small molecule inhibitor interacting with serine/threonine protein kinases in Prostate Cancer cells: An in-silico approach.

Dlamini Z[4], Gabada L[1,2], Kandhavelu J[3], Marima R[2]
[1]Department of Medical Oncology, Steve Biko Academic Hospital and University of Pretoria, [2]2SAMRC Precision Oncology Research Unit (PORU), DSI/NRF SARChI Chair in Precision Oncology and Cancer Prevention, Pan African Cancer Research Institute (PACRI), University of Pretoria, Hatfield, [3]3Department of Oncology, Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, [4]4Department of Internal Medicine, Faculty of Health Sciences, School of Clinical Medicine, University of the Witwatersrand

Background
Prostate cancer (PCa) is the leading cause of significant mortality and morbidity globally. Diagnoses are expected to rise from 1.4 million in 2020 to 2.9 million by 2040, hence the rising need for innovative treatment approaches to address this growing health challenge. Serine/Threonine protein kinases are overexpressed in PCa, and their inhibition poses a promising therapeutic approach. Moreover, metastasis-associated lung adenocarcinoma transcript-1 (MALAT1) oncogenic lncRNA has been reported to be linked to these kinases' overexpression in cancer. This study uses in silico analysis to map biological pathways associated with Serine/Threonine protein kinases by Sphinx31 small molecule inhibitor.
Methods
High-Throughput Docking website was employed to examine the interactions between sphinx31 and various proteins, determining the strength of these interactions. The sdf. file of sphinx31 2D-structure was downloaded from PubChem and analysed on the HT-docking site. This process identified multiple proteins that could interact with sphinx31, with the docking score indicating the strength of the interaction between the inhibitor and the protein. A higher docking score suggests a stronger ligand and protein interaction. The UniProt database was utilised to acquire the protein ID for each protein with a docking score greater than 9, and they were utilised to identify the pathway(s) in which each protein participates. Furthermore, LncSEA lncRNA database identify MALATA1 targets and KEGG pathway was used to study molecular mechanisms.
Results
Serine/threonine protein kinase (SGK_Human), demonstrated a high docking score of 10.3. These results suggest that the binding of sphinx31 to the ATP-binding site of SGK1 may inhibit its kinase activity; SGK is upregulated in PCa. LncSEA and KEGG pathway analysis also revealed that MALAT1 has also been shown to interact with SRPK1 and SRSF1, which are involved in splicing and transcriptional regulation. SRPK1 interacts with MALAT1 and SRSF1, which are involved in the Notch pathway, suggesting a potential link between SRPK1 and the Notch signalling cascade. SRSF1 plays a role in the Notch pathway by regulating MAML2 expression, highlighting its involvement in cell proliferation and cancer progression processes.
Conclusion
Sphinx31 small molecule inhibitor is a potential PCa therapeutic agent.

Keywords: Prostate Cancer, Serine/Threonine Protein Kinases, Sphinx31, In-silico Analysis, Notch Signalling Cascade.

Associations of anaemia with blood pressure in women of reproductive age: a cross-sectional study in Johannesburg, South Africa

Nxele X[1], Symingtom E[2]
[1]University Of The Western Cape, [2]University of South Africa

About 39 million people are infected with human immunodeficiency virus (HIV) globally. Anaemia occurrence has been shown to be proportional to HIV severity and a predictor for cardiovascular diseases, and hypertension.
Premenopausal women (pregnant and non-pregnant) were recruited from a primary healthcare facility in Roodepoort, Johannesburg. Socio-demographic information and lifestyle behaviours were obtained using questionnaires and medical files for medical history, including HIV status. Mid-upper arm circumference (MUAC) was measured using standard methods. Blood pressure (BP) was obtained using an automated monitor, and haemoglobin (Hb) determined using a handheld meter from venous blood. Multiple- and logistic- regression analyses were performed to determine association between Hb, HIV and BP.
Of 228 women enrolled in study,165 (72%) were pregnant and 22% HIV positive. Pregnant women were younger (28.8±6.1 vs 31.2±7.0 years, p=0.013), had smaller MUAC (30±5.0 vs 32±4.0, p=0.040) and lower BP (SPB: 104±11 vs 115±11 mmHg, p<0.001; DBP: 68±8 vs 80±10 mmHg, p<0.001) compared to non-pregnant women. Non-pregnant women were prehypertensive and hypertensive compared to pregnant women (52% vs 13%, p<0.001 and 19% vs 1%, p<0.001, respectively). Hb levels were lower among HIV positive compared to HIV negative participants (11.4±1.6 vs 12.1 ±1.4 g/dL, p=0.010). HIV positive women were classified as anaemic (37% vs 16%, p=0.003). In unadjusted multiple linear models, Hb levels were associated with SBP (β 1.20 [95% CI, 0.28,2.33], p=0.013) and DBP (β 1.94 [95% CI, 1.08,2.80] p <0.001) and similarly in unadjusted logistic regression models, anaemia was associated with hypertension (OR 1.18 [95% CI, 1.20,2.80], p = 0.006). However, in both cases, significance was lost in adjusted models. These results suggest anaemia may be a risk factor for hypertension and should be investigated in larger, homogenous samples.

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Abel G[1]
[1]Durban University Of Technology
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Developing a Universal Continuous Assay for Glycosyltransferases: Illustrating Mechanistic Studies with ST3GAL1, C1GAlT1, and FUT1 for Kinetics and Inhibition Database Development

Nashed A[1], Naidoo K[1]
[1]Department of Chemistry, University of Cape Town, The Scientific Computing Research Unit.
Chemical systems glycobiology requires experimental and computational tools for big data analytics, benefiting genomics and proteomics. However, the unconventional nature of glycan construction, driven by collaborative glycosyltransferase (GT) catalysis, presents challenges for tool development. Addressing this, we introduce a universal glycosyltransferase continuous (UGC) assay for accurate kinetics and inhibition studies, particularly overcoming limitations in processing nucleoside phosphate UDP, GDP, and CMP donor-based glycosylation reactions. This assay, based on monitoring NADH concentration reduction via fluorescence spectrophotometry, offers standardized measurements of reaction rate parameters. Our studies on representative GTs demonstrate the UGC assay's efficacy in determining initial rates compared to traditional end-point assays. Additionally, we use the UGC assay to investigate time-dependent inhibition of ST3GAL1 by the natural product soyasaponin1 inhibitor, revealing insights into enzyme sensitivity. The UGC assay's standardized Km values establish ST3GAL1 as the most responsive to soyasaponin1 inhibition, followed by FUT1 and C1GALT1.

Establishing and Characterizing Colorectal Cancer Organoid and CD8+ T-lymphocyte Co-Cultures from South African Patients

Addulla N[1], Du Plessis T[1], Aronson R[1], Bebington B[2], Kaur M[1]
[1]University of the Witwatersrand, [2]Wits Donald Gordon Medical Centre

Background: The progression from a healthy colonic epithelium to an adenomatous polyp, which eventually leads to metastatic disease, is not solely determined by genetic abnormalities but also influenced by the dynamic interactions within the tumor microenvironment (TME). Subsequently, the lack of physiologically relevant pre-clinical models to study disease pathogenesis is often associated with poor patient prognosis. By focusing on the specific biology of South African patients and employing advanced 3D cell culturing techniques, we aim to employ these models to improve therapeutic outcome.

Methods: South African patient-derived colorectal adenocarcinomas were obtained, along with matched healthy controls (Ethics Clearance Number: M210233). These specimens were dissociated into crypts, a critical step in generating patient-derived organoids. Reverse transcription quantitative polymerase chain reaction (RT-PCR), immunofluorescence microscopy and Hematoxylin and Eosin staining was completed to characterize these organoids. Following successful validation, a protocol facilitating co-culture of these organoids with CD8+ T-lymphocyte has been established.

Results: Employing the current protocol proved efficient in yielding organoids that demonstrate stable maintenance and mimic the physiological composition of human tissue. This is evidenced by the expression of principal genes (CHGA, EPCAM, LGR5, LYZ, MUC2, MKI67, and VIL1) and characteristic proteins. Furthermore, morphology of the organoids recapitulate that of the host tissue. Subsequently, organoid CD8+ T-lymphocyte co-culture experiments have been optimized for further downstream experimentation.

Conclusion: With the successful establishment of CRC organoid co-cultures, these models serve as a valuable tool to elucidate the molecular mechanisms driving CRC disease progression within the unique context of a South African patient cohort. Utilizing this model, our future objectives is to comprehensively delineate the significance of cholesterol within the tumor microenvironment, with a specific emphasis on its contribution to T-cell exhaustion. This will be completed with the ultimate aim of exploring cholesterol depletion as a potential therapeutic strategy for CRC.

Electrostatic potential and 3D molecular shape screening for high-throughput virtual screening and drug discovery

Dilsook K[1], Naidoo K[1]
[1]University Of Cape Town, Department of Chemistry

Background
In recent years, fewer and fewer drugs have been approved despite increased funds invested. This has prompted an emphasis on a different approach to drug discovery, including virtual screening. Virtual screening includes searching large chemical databases for potential hits which bind protein targets. This data-driven approach needs to be highly accessible, reproducible and shareable. For these reasons, the Biomolecular Reaction and Interaction Dynamics Global Environment (BRIDGE) was developed as a flavour of Galaxy. Galaxy is a widely used open-source workflow management system that allows rapid analysis of a large amount of data.

Methods
A BRIDGE workflow enabling high throughput virtual screening, and thereby aiding in-silico drug discovery strategies, was developed. The workflow begins with a tool that rapidly screens large databases of molecules based on machine learning-generated electrostatic potential (ESP) and shape similarities. Additional tools were developed to fully automate the setup, running and analysis of GPU-accelerated molecular docking and free energy simulations. The ESP and shape screening tool can then be used in conjunction with these tools in an automated workflow, which narrows down the large database to potential drugs.

Results
A large database was spiked with two known inhibitors which share a high structural similarity but possess varied activity in inhibiting the ST3Gal-1 enzyme. The proposed workflow and tools identified these inhibitors in the top 3% of the ~300000 molecules and accurately distinguished which was the better inhibitor. Three additional molecules were identified as potential inhibitors with activities similar to or greater than the weaker known inhibitor.

Conclusion
This work presents a publicly available, highly reproducible, user-friendly, automated workflow enabling high throughput virtual screening accelerated by GPUs and cloud resources via the BRIDGE Galaxy platform.

Clinical strains of Mycobacterium tuberculosis complex exhibit lineage-specific growth patterns during early infection of macrophages.

Cebani L[1], Mvubu N
[1]School of Laboratory Medicine and Medical Sciences, College of Health Sciences, University of KwaZulu-Natal

BACKGROUND
Tuberculosis (TB), caused by the members of the Mycobacterium tuberculosis complex (MTBC) remains a significant health concern globally. MTBC members belong to well characterized ancient (L1, L5, L6, L7) and modern lineages (L2, L3, L4), with the recently identified MTBC lineage 8 and Mycobacterium africanum lineage 9. During infection, MTBC enter and replicate within innate cells lining the alveoli, including resident alveolar macrophages. Understanding the growth patterns of MTBC lineages during early infection of macrophages may provide insights of pathogen manipulation strategies exploited by these strains.
METHODS
The current study was aimed at elucidating the replication capacity of drug sensitive ancient and modern MTBC lineages with lineage 8 in THP-1 macrophage like cell lines for 5 days post infection at an MOI of 10:1.
RESULTS
Modern lineages of MTBC had significantly high growth rates compared to ancient lineages at all time intervals, with the exception of lineage 4 that was variable at 48 and 72 hours post infections. Furthermore, lineage 8 showed slow growth rate compared to modern lineages with patterns that were similar to ancient lineages.
CONCLUSIONS
The high invasiveness and growth rates exhibited by modern lineages may be linked to their general high transmission patterns as shown by global dominance of lineage 2 and 4 in the population. Furthermore, differences in behaviour of the MTBC strains during early growth on macrophages as well as their distribution and transmission rates among the population alludes to complex molecular mechanisms that are “uniquely” regulated among these clinically relevant pathogens.

Optimization of short fragment DNA sequencing for rapid diagnostics of Amitraz resistance in Rhipicephalus tick species.

van Barkenhuizen R[1], Zipfel R[2], Maritz-Olivier C[1]
[1]Department of Biochemistry, Genetics and Microbiology, University of Pretoria, [2]DNA Sanger Sequencing Facility, University of Pretoria, Pretoria, South Africa

Background
Amitraz, a formamidine used as an acaricide, faces decreasing efficiency due to target site mutations and subsequent development of resistance. In the cattle ticks, Rhipicephalus microplus and Rhipicephalus decoloratus, two SNPs linked to amitraz resistance in the OCT/Tyr receptor gene is known as well as 3 novel SNPs in linkage disequilibrium, all occurring in a short 100bp region. As homozygous resistant ticks are fully resistant, and heterozygous individuals remain susceptible, a genotyping tool for rapid diagnostics is in dire need. This project aims to develop a cost-effective, short fragment DNA sequencing method for SNP detection and genotyping, to aid livestock famers in selecting protective acaricides.
Methods
Firstly, primer pairs were evaluated. PCR amplification of the OCT/Tyr receptor gene region using several gene specific PCR primers (GSPs) and GSPs modified with universal 5' M13 tail sequences were tested. PCR amplification was performed using R. microplus genomic DNA samples and the different primer combinations. Secondly, PCR product purification through various salt/ethanol precipitations versus commercial purification kits was evaluated. Thirdly, the BigDye sequencing reactions, were optimised. Once the optimized methodology was determined, based on sequence data quality and cost-effectiveness, it was validated using additional Rhipicephalus field samples.
Results
The addition of M13-tail sequences successfully allowed sequencing of the gene region of interest. Despite lower yields, the salt/ethanol precipitation purification provided sequence data of identical quality to that of a commercial kit. Optimization of the BigDye sequencing parameters successfully minimized dye blobs obscuring sequence data, and finally the method developed was used with success for both R. microplus and R. decoloratus ticks.
Conclusions
Current published methods only allowed for genotyping of 2 SNPs in the target OCT/Tyr gene region. With the new optimized method, we can now reliably genotype 4 SNPs (1 validated SNP and 3 in linkage). We were able to cut costs by optimization of sodium and ammonium-based salt precipitation, reduced BigDye by half and performing DNA sequencing in a single direction and achieving reliable genotyping. Upscaling of the methodology is now in progress to support farmers in selecting the correct acaricide(s) for tick control.

Confirmation of Hsp70 inhibition by pifthrin and colistin sulphate using complementation assay

Ncube H[1], Dali U[1], Dongola T[1], Shonhai A[1]
[1]University Of Venda

Hsp70 is not essential in E. coli. However, this molecular chaperone is essential at elevated temperatures (>40 °C). E. coli dnaK756 habour a native mutated DnaK and as such they do not grow at temperatures >40 °C. By heterologously expressing a functional Hsp70 in these cells, it is possible to recover growth at at temperatures >40 °C. These cells could serve as a model for screening Hsp70 inhibitors. To test this hypothesis, we subjected E. coli dnaK756 cells heterologously expressing Plasmodium falciparum Hsp70-1 (PfHsp70-1), DnaK and a chimeric protein, KPf (made of nucleotide binding domain of DnaK fused to the substrate binding domain of PfHsp70-1).

Methodology

E. coli dnaK756 competent cells were transformed with plasmid constructs encoding PfHsp70-1, DnaK and KPf. The cells were induced to express the Hsp70 proteins and incubated overnight at permissive growth temperature (30 °C) and non-permissive growth temperature (>40 °C). The growth assay was conducted using agar well plates and liquid broth assay (in 96 well plate system coupled to OD readings using a UV-Vis spectrometer). In addition, we conducted solubility studies on the proteome of cells grown in the presence of inhibitors versus control cells.

Results

E. coli dnaK756 heterologously expressing PfHsp70-1, DnaK and KPf were all able to grow at 37 °C even in the presence of the two inhibitors. However, growth was abrogated at 43.5 °C when the cells were cultured in the presence of the two inhibitors. We further observed general aggregation of cells cultured in the presence of inhibitors confirming that inhibition of the Hsp70 chaperone led to protein misfolding and aggregation.

Conclusions

Pifithrin and colistin sulphate are known Hsp70 inhibitors. Here we showed that the E. coli complementation serves as an ideal tool for screening Hsp70 inhibitors.

Antiproliferative Activities of Phytochemicals Isolated from the Leaves of Dolichos kilimandischaricus (Harms) ex Taub. (Fabaceae) on Jurkat-T and HL-60 Leukemic Cells

Sithole S[1]
[1]Marondera University Of Agricultural Sciences And Technology

Background
Natural plant products represent one of the most productive sources of innovative lead drugs for use in the treatment of a wide range of ailments. Dolichos kilimandischaricus (Harms) ex Taub. (Fabaceae) root extracts have been reported to be traditionally used for the treatment of HPV-related cancers. The extracts from this plant have been previously shown to have antiproliferative effects on Jurkat and HL-60 cells. The aim of this study, therefore, was to isolate, purify and analyze phytochemicals from the leaves of D. kilimandscharicus.

Methods
Phytochemicals in the ethanol leaf extract were separated by column chromatography on silica gel. The structures of the compounds were elucidated using spectroscopic techniques, and then compared with the reported spectral data. Cytotoxicity of the phytochemicals on HL-60 and Jurkat cells was assessed by the sulforhodamine B (SRB) assay with chlorambucil as a positive control.

Results
The isolated compounds were identified as 3β-stigmasterol (1) and α-spinasterol (2). Isolated compounds exhibited dose-dependent antiproliferative effects in the human cancer cell lines, Jurkat-T and HL-60 cells. The results showed that 3β-stigmasterol and α-spinasterol (2) had more potent antiproliferative activity against HL-60 cells than Jurkat cells with IC50 values of 9.49 and 4.62 µg/mL respectively.

Conclusions
Therefore, this study has identified some of the phytochemicals that may be responsible for the antiproliferative activity in D. kilimandascharicus. These phytochemicals may provide leads in the development of compounds for treating cancer and related neoplastic diseases.

Potato peel as carbon source inductive for gut inhabiting fungal cellulase and xylanase production

Phasha N[1]
[1]University Of Limpopo

Potato peel as carbon source inductive for gut inhabiting fungal cellulase and xylanase production

Nkateko N. Phasha1 and Livhuwani Makulana1
1Department of Biochemistry, Microbiology and Biotechnology. University of Limpopo, Limpopo, Mankweng Sovenga 0727, South Africa.
nkateko.phasha@ul.ac.za

Background
The lack of proper disposal methods for the tons of biodegradable wastes such as potato peels, generated by industries using potatoes constitutes a nuisance in the environment and contributes to global warming. In recent years, the exploitation of wastes generated by these industries as cheap substrates for the production of high-value products such as microbial enzymes gained keen interest. The purpose of this study was to evaluate potato peels generated by local quick-service restaurants at the University of Limpopo as an inductive carbon source for fungal xylanase and endoglucanase production using gut-inhabiting fungi.
Methods
The preliminary screening for endoglucanase and xylanase was done using carboxymethyl cellulose-Congo Red agar plates and Remazol Brilliant blue-xylan agar plates, respectively. The fungal isolates that showed positive results from preliminary screening were grown in media with pre-treated potato peels as carbon substrate in submerged and solid-state fermentation. These fungal isolates were identified using ITS1/2 sequences.
Results
The highest endoglucanase and xylanase activities were obtained during submerged fermentation. AB3A1-P. expansum produced the highest xylanase activity (106 nkat/ml) and the highest endoglucanase activity was produced by AB1A3-A. flavus (4,7 nkat/ml) after 120 hours of fermentation.
Conclusion
In Conclusion, potato peels are a good inducer carbon for the economical production of fungal endoglucanase and xylanase from gut-inhabiting fungi. Furthermore, AB3A1-P. expansum and AB1A3-A. flavus can be used for efficient production of xylanase and endoglucanase.
Keywords: Endoglucanase, Xylanase, Potato peels waste, Solid state fermentation, Submerged fermentation

Thatch grass (Hyparrhenia hirta) as carbon source inductive for fungi isolated from the gut of Scarabaeidae dung beetle for xylanase production

Makulana L[1]
[1]University Of Limpopo, [2]University of Limpopo
Thatch grass (Hyparrhenia hirta) as carbon source inductive for fungi isolated from the gut of Scarabaeidae dung beetle for xylanase production

1. Livhuwani Makulana, Nkateko N Phasha, Danie C La Grange and Elbert L Jansen van Rensburg

1. Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo, Limpopo, Private Bag X1106, Sovenga 0727, South Africa.

Abstract
Background
Xylanase enzymes play an important role in different industries. However, its use is restricted by high production costs, which can be reduced by using plant-based waste. In this study, acid-pretreated thatch grass was evaluated as an inducer substrate for xylanase production by fungi isolated from two dung beetles (Anachalcos convexus and Pachylomerus femoralis) and dung beetle larvae (Euoniticellus intermedius).
Methods
The xylanase production was further evaluated in submerged and solid-state fermentation using acid/base and untreated thatch grass. Fungi from Aspergillus, Trichoderma, Hypocrea, Neosartorya, Talaromyces, and Rhizopus were grown in xylan and acid-pretreated thatch grass in submerged fermentation.
Results
Six of the fungal strains showed higher xylanase activity on acid-pretreated thatch grass than pure xylan. Furthermore, three filamentous fungi (Aspergillus fumigatus L1XYL9, Hypocrea lixii AB2A3 and Neosartorya sp AB2XYL20) that showed higher xylanase activity on acid-pretreated thatch grass were used for comparative studies in submerged and solid-state fermentation. Aspergillus fumigatus L1XYL9, Hypocrea lixii AB2A3 and Neosartorya sp AB2XYL20 produced a higher xylanase activity of 304, 526 and 549 nkat/ml in submerged fermentation, respectively. These strains were further evaluated for endoglucanase production in submerged and solid-state fermentation. Neosartorya sp AB2XYL20 produced a higher endoglucanase activity in solid-state fermentation.
Conclusion
In conclusion, instead of disposing of unused thatch grass by burning it causing pollution, it could be used as an inducer substrate for the production of xylanase enzymes that will reduce production costs.
Keywords
Thatch grass, submerged fermentation, Solid-state fermentation, dung beetles, xylanase enzyme

MicroRNAs from the wheat-Russian wheat aphid interaction

Sibisi P[1]
[1]UNIVERSITY OF SOUTH AFRICA

Wheat (Triticum aestivum L.) is one of the most dominant crops for human and livestock feed. Yields of wheat have declined worldwide due to pathogens and pests. Diuraphis noxia (Russian wheat aphid, RWA) is the most devastating aphid pest affecting wheat cultivation in South Africa and other regions. Feeding by this insect causes the appearance of severe symptoms, including necrosis, streaking and trapping of the heads of the wheat plant. This reduces crop yield and can lead to the death of susceptible cultivars. The use of resistant cultivars against the RWA is being negated by the emergence of resistance-breaking biotypes. Feeding by the RWA on wheat induces differential expression of microRNA genes. Thus, this study aimed to use next-generation sequencing to identify a larger pool of microRNAs and to further characterize them and their putative targets. In this study, 12 microRNA libraries (3 bioreps) from Tugela uninfested, Tugela Dn uninfested, Tugela infested and Tugela Dn infested were constructed respectively. The expression of candidate miRNAs and their targets was determined by quantitative real-time PCR. The predicted target genes were analysed for their gene ontology placement to determine their biological roles in plants. A total of 503 miRNA candidates were obtained, and only 87 of these matched the known Triticum aestivum miRNA. The identified miRNAs seem to target known resistance gene family members and previously identified resistance responses from wheat after infestation by the RWA. The gene ontology results indicated that most of the identified targets in this study play a role to regulate some biological pathways that are known to be regulated during wheat-RWA interaction.

An indirect co-culturing model system that links lithium anti-inflammatory effects and endothelial integrity in RVFV inoculated Raw 264.7 cells

Makola R[1], More G[2], Paweska J[3], Matsebatlela T[1]
[1]University of Limpopo, [2]University of South Africa, [3]National Institute of Communicable Disease

Background
Rift Valley fever virus (RVFV) is a mosquito born zoonotic viral infection that has resulted in several outbreaks in Africa and Arabian Peninsula. RVFV elicits flue like symptoms and clinical complications such as haemorrhagic fever, however, the link between this viral infection and haemorrhagic fever is not well elucidated. Viral haemorrhagic fever is characterised by altered endothelial integrity from damaged endothelial cells that results in septic shock and multiple organ failure leading to death.
Methods
Therefore, this study hypothesises the use of lithium as prophylaxis for oxidative stress-linked endothelial leakage post-RVFV infection. Co-inoculation model system was used to measure the effects of inflammatory mediators on endothelial integrity. The xCelligence system together with the Transwell assay were used to measure endothelial integrity.
Results
Results from the xCelligence system showed that endothelial cells exposed to RVFV-infected, lithium-treated Raw 264.7 cells supernatant showed a cell integrity index of above 4.0, displaying similar protective properties as compared to RVFV-inoculated Raw 264.7 cells supernatant not treated with lithium. Moreover, lithium-treated cells supernatant upregulated the expression of Adherence junction genes.
Conclusion
The endothelial integrity observed in the permeability assays correlates with the expression of the molecules involved in keeping the cell-to-cell junction intact. Thus, this study links the anti-inflammatory properties of lithium with cytoprotective ability of endothelial integrity.

Modelling charge-transfer states in phycobilisomes

Alem M
[1]University of Pretoria, [2]University of Pisa, [3]University of Pretoria
Modelling charge-transfer states in phycobilisomes
Mamaru B. Alem1, Lorenzo Cupellini2 and Tjaart P. J. Krüger1

1Department of Physics, University of Pretoria, Pretoria, South Africa
2Department of Chemistry, University of Pisa, Pisa, Italy
Email: mamaru2005@gmail.com
Background
Phycobilisomes are the giant (~6 MDa) main light-harvesting pigment-protein complexes of cyanobacteria and certain algae, responsible for harvesting sunlight and regulating the flow of absorbed energy to provide the photochemical reaction centres with a constant energy throughput. Single-molecule spectroscopy studies have disclosed an intrinsic light-driven excited-state quenching mechanism in phycobilisomes that likely involves charge-transfer states in a single phycocyanobilin pigment.
Methods
The ground-state geometries of the trimeric core units of phycobilisome retrieved from Synechocystis PCC 6803 and Synechococcus PCC 7002 were optimized using Density Functional Theory (DFT) employing the CAM-B3LYP exchange-correlation functional together with the 6-31+G* basis set. Linear response time-dependent DFT (TD-DFT) was used to calculate the excited-state geometries. All calculations were performed in Gaussian 16 without any symmetry constraints. A natural transition orbitals (NTOs) analysis was performed on the excited-state geometries. Visualization of the electron densities, potential energy surface, and molecular parameters was conducted in GaussView 6.0.
Results
Although phycocyanobilins from different trimers possess the same number of atoms and electrons, we found that they have different excitation energies, ranging from 2.286 to 2.398 eV. Even phycocyanobilins in the same trimer have varying excited-state and charge-transfer state energies, which we could associate with torsional differences in the tetrapyrrole rings due to changes in the protein microenvironment. The energy and localization of the charge-transfer states were found to be influenced by the background charge of the protein microenvironment. The charge-transfer state modelling results showed the presence of holes and electrons at distances ranging from 2.57 to 3.21 Å on single phycocyanobilins.
Conclusions
Small geometrical variations in the phycocyanobilin pigments of phycobilisomes give rise to different charge-transfer state energies amongst the trimers, resulting in varying photophysical properties that determine their finely-tuned functions within these giant light-harvesting complexes.

Keywords: Charge transfer states, TD-DFT, Torsion, Phycobilisomes, Phycocyanobilins

Characteristics and antimicrobial resistance of both methicillin-resistant and methicillin-susceptible non-aureus staphylococci (NAS) and Mammaliicoccus species (formerly Staphylococcus sciuri group) from animals and meat in different countries worldwide: A review

Thwala T[1], Madoroba E[1], Magwedere K[2], Butaye P[3,4]
[1]Department of Biochemistry and Microbiology, University Of Zululand, [2]Directorate of Veterinary Public Health, Department of Agriculture, Land Reform and Rural Development, [3]Department of Pathology, Bacteriology and Poultry Diseases, Faculty of Veterinary Medicine, [4]Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences,
Non-aureus staphylococci and mammaliicocci are part of the commensal flora of diverse animals and have been isolated from the environment. Rarely have they been involved in disease in animals and humans. The Mammaliicoccus sciuri group (formerly Staphylococcus sciuri) has gained special attention after a mecA homologue was found in all Mammaliicoccus strains and identified as the evolutionary precursor of the methicillin-resistant mecA gene carried by methicillin-resistant Staphylococcus aureus (MRSA) and other Staphylococcal species. Recently, the mecC gene has been found in staphylococci and mammaliicocci as part of the SCCmec type XI, and even more recently, an SCCmec-mecC hybrid element has been described in mammaliicocci. However, the epidemiology of methicillin resistance in staphylococci and mammaliicocci differs in different continents, with the mecC gene instead of the mecA gene on the African continent. Therefore, this manuscript aims to discuss the current situation on the characteristics of methicillin resistance in staphylococci and mammaliicocci from food-producing animals and food of animal origin in different continents.

Exploring Iso-mukaadial acetates and other small compounds as inhibitors of recombinant Plasmodium falciparum lactate dehydrogenase.

Mabaso N[1], Pooe O[2], Mhlongo N[2]
[1]North-west University, [2]University of KwaZulu-Natal

Background
Malaria is a major killer disease in Sub-Saharan Africa; a protozoan parasite of the genus Plasmodium causes this disease. It's an urgent health problem facing the public, and the emergence of parasite resistance to drugs jeopardises effectiveness of treatments developed to date. This calls for new antiprotozoal drugs that require novel approaches to ensure long-term efficacy. The investigation of compounds such as Iso-mukaadial acetate (IMA), Betulinic acid (BA), Ursolic acid (UA), and Oleanolic acid (OEA) which are isolated from plants shows to possess antimalarial activity. IMA is isolated from a pepper bark tree, BA from the bark of a plant species, UA from leaves of (lavender and rosemary), and OEA is found in leaves and Olea europaea fruit. This study aims to investigate the inhibitory properties of these compounds against Plasmodium falciparum lactate dehydrogenase (PFLDH). PFLDH is an enzyme in the parasite glycolytic pathway on the parasite that converts pyruvate into lactate, thus providing the energy required for the survival of the malaria parasite.

Methods
Recombinant PFLDH was expressed and purified for further analysis, including colony PCR, expression, purification, interaction studies including Fourier transform infrared (FTIR) analysis and Ultraviolet-visible spectroscopy (UV-Vis), antimicrobial activity, and in silico analysis were conducted.

Results
Colony PCR confirmed the presence of a 951bp insert in the PKK223 plasmid. Metal affinity chromatography successfully purified PFLDH protein sized 34.9 kDa. The following results were obtained from isolated compounds (BA and IMA) screened for IC50 to demonstrate overall activity against the asexual P. falciparum. BA and IMA had IC50 values of 1.27 and 1.03µg/ml against asexual P. falciparum, respectively. When compounds were incubated with protein, FTIR analysis showed a clear shift in the curve, indicative of an interaction between IMA and BA with PFLDH. UV-Vis showed that structural conformational change was induced, resulting in an interaction of the compounds with the aromatic side chains of PFLDH. In-silico analysis showed where these interactions occurred, highlighting the ligand atoms responsible for the interaction.

Conclusion
Based on these findings, the investigated compounds could be effective PFLDH inhibitors, as they have binding affinities similar to those of the standard drug chloroquine.

Synthesis of Cytotoxic Cr(III), Cu(II) and Zn(II) Complexes: A Combined Experimental and Computational Study

Alem M[1]
[1]University of Pretoria

In this work, Cr(III), Cu(II) and Zn(II) heteroleptic complexes of metformin and oxo-ligands were synthesized and subjected to cytotoxicity,
antibacterial and antioxidant activity evaluations. All the synthesized complexes were confirmed to possess polycrystalline natures with average
crystallite sizes ranging from 18 – 36 nm. Promising results from the in-vitro cytotoxic activity with IC50 value ranging 3.72 – 44.88 µM compared
to the positive control cisplatin (18.62 µM) were found. Among the synthesized complexes three Cu(II) complexes that consists of 1,10-
phenathroline and chrysin (55), 1,10-phenathroline and metformin (60) and, 1,10-phenathroline and ciprofloxacin (61) were found to show unique
cytotoxicity profile with IC50 values of 3.72,4.29 and 7.58 µM, respectively. A Cr(III) complex (54) of 1,10-phenanthroline and chrysin was found
to be the fourth cytotoxic complex against MCF-7 cell lines with IC50 8.08 µM. The overall in vitro results analysis showed that 54 (Cr(III)),
55(Cu(II)), 60(Cu(II)), and 61(Cu(II)) metal complexes have better cytotoxicity compared to the standard drug cisplatin. Moreover, the
antibacterial activity of the complexes showed that all cultured bacteria were susceptible to the synthesized metal complexes (54 – 61) with
inhibition diameter zone ranging from 8.00 mm to 17.33 mm and percent activity index ranging from 33 to 86%. The biological activity and
quantum chemical descriptor findings showed the structure-activity and ligand-type dependence of the complexes. Computationally, the biological
significance of the synthesized metal complexes was inferred from the small HOMO-LUMO band gap energy, large binding affinity, small
inhibition constant, and inability to permeate the blood-brain barrier.

Antimicrobial resistance characteristics of Coagulase-Negative Staphylococci Recovered from Beef

Nene N[1]
[1]University Of Zululand

Antimicrobial resistance characteristics of coagulase negative staphylococci recovered from beef.

By N.Nene, Prof.E Madoroba, Prof P. Butaye.

Background

Coagulase negative staphylococci cause infections that are challenging to treat due to resistant towards prescribed antimicrobials. While methicillin and Staphylococcus aureus are well characterized, there is little in formation on resistance in other staphylococci, particularly those strains found in beef. Therefore, the aim of this study is to determine antimicrobial resistance characteristics of coagulase negative staphylococci recovered from beef.

Methodology

A total of 35 confirmed isolates of coagulase negative staphylococci preserved in glycerol were resuscitated. They were then screened for purity using Gram stain. DNA extraction was performed by boiling method and. the Quick-DNA Fungal/Bacterial Miniprep Kit(Zymo Research, Catalogue No.D6005). The antimicrobial resistant genes; mecA,mecC, tetA, tetB, tetC, CatA1, aadA2, Aac(3)IV, aadB ,blaTE, blaPSE, nfsA, parC, gyrA, dfrA1, dfrB, dfrA14 were assessed by PCR.

Results

The overall proportion of samples that tested positive for mecC was 53% (13/53) and 47% was for the negative.

Conclusion.

The samples that were mecC positive emphasize the methicillin resistance of CoNs in beef, which makes them resistance to most Beta-lactams antibiotics such as Penicillin, Oxacillin and methicillin to name but a few. This study provides valuable insight into the genetic diversity of antimicrobial resistance among CoNS from beef, thus giving basic knowledge on techniques for preserving efficacy of antimicrobial therapy and safeguarding public health. For in-depth analysis Whole Genome Sequence analysis is recommended to better understand their antimicrobial resistance genes.

An investigative study of differences in root metabolite profiles of Senna plant species

Makhado R[1], Madala N[1], Mathomu L[1]
[1]University of Venda

Background
Senna species are medicinal plants used and prescribed by traditional medicine practitioners and herbalists for the management and treatment of various ailments. Bioactive compounds responsible for the therapeutic effects of Senna species are found in extracts derived from their roots, bark, seeds, and leaves, which include alkaloids, quinines, and anthraquinones. This study aimed to investigate the root metabolite profiles of two Senna species, namely, Senna alexandrina and Senna obtusifolia which grow vastly in many communities around the world.
Methods
The root compounds of S. alexandrina and S. obtusifolia were extracted using methanol and were validated using ultraviolet visible light spectroscopy. The extracts were analysed using ultra-high-performance liquid chromatography hyphenated to quadrupole time of flight mass spectrometry (UHPLC-QTOF-MS). The observed profiles were then explored with multivariate statistical analysis tools and Global Natural Products Social (GNPS) molecular network to summarize and identify important phytochemicals.
Results
The use of multivariate statistical analysis tools was able to simplify visualization of the data. Principal Component Analysis (PCA) showed that the two species are different, and this was further investigated with Hierarchical Cluster Analysis (HCA) which showed no relationship between them. Molecular network was able to identify classes of metabolites such as a range of flavonoids and anthraquinones.
Conclusions
This study provided a comprehensive and comparative analysis of the root metabolite profiles of the Senna species, which may facilitate the identification and quality control of Senna-based herbal medicines. Although both species root metabolites constitute important concoction for use and casual prescription within traditional communities, S. alexandrina emerged as superior to S. obtusfolia.

Antiatherogenic potential of two triterpenoids from the stem bark extract of Protorhus longifolia (Benrh.) Engl

Mosa R[1], Ramasia N[1], Ndlovu M[1]
[1]University Of Pretoria

Background
Despite the use of the current hypolipidemic drugs, the continuous increase in morbidity and mortality rates associated with atherocardiovascular events indicate limitations of the current treatment regime. This study investigated the antiatherogenic potential of two triterpenoids (ARM-2 and RA-5) isolated from the stem bark of Protorhus longifolia.
Methods
The in silico molecular docking of the compounds against some proteins involved in cholesterol trafficking was performed. The oxygen radical absorbance capacity and copper sulfate-mediated low-density lipoprotein (LDL) oxidation in vitro assays were used to determine the antioxidant activity of the compounds. The potential of the compounds to inhibit foam cell formation was determined in the RAW 264.7 cell line. The RAW 264.7 cells were exposed to 50 µg/mL of an oxidized LDL (oxLDL) in the presence and absence of the plant compounds at 1, 10 and 25 µM for 48 hours. Oil red O and Nile red staining were used to quantify the amount of lipid accumulated in the cells.
Results
Molecular docking revealed low binding affinity of the compounds against CD-36, SR-A1, and ABCA-1 compared to their high binding affinities to ABCG-1, ACAT-1 and LOX-1. Both compounds showed, at varying degrees of efficacy the capacity to absorb oxygen radicals. The compounds also inhibited conjugated diene and malondialdehyde (MDA) formation in the copper sulfate-induced LDL oxidation. ARM-2 exhibited the highest inhibition of MDA formation by up to 62% compared to 45% for RA-5. An increase in intracellular lipid accumulation was observed in the cells exposed to the oxLDL only. However, lipid accumulation was decreased in the cells exposed to the oxLDL in the presence of ARM-2 (25 µM) or RA-5 (10 µM).
Conclusion
The results suggest that both triterpenoids possess properties to inhibit oxidative modification of LDL and decrease foam cell formation, indicating their antiatherogenic potential.

The effect of acute phthalates exposure on insulin secretion

Makhubela S[1], Kgopa A[1], Mokgotho M[2], Shai L[2]
[1]Sefako Makgatho Health Sciences University, [2]Tshwane University of Technology
Sebolaishi Makhubela1, Ananias Kgopa1, Matlou Mokgotho2 and Leshweni Shai2
1Department of Biochemistry and Biotechnology, Sefako Makgatho Health Sciences University, Ga-Rankuwa, Pretoria, South Africa
2Department of Biomedical Sciences, Faculty of Science, Tshwane University of Technology, Pretoria, South Africa
Email: Sebolaishi.kolobe@smu.ac.za

Background:
Epidemiological studies have associated phthalates exposure with metabolic conditions such as diabetes. Experimental data is necessary to support and understand these associations. This study investigated the effect of selected phthalates/phthalate metabolites on glucose stimulated insulin secretion in a rat β cell line.

Methods
Cell viability assay was carried out to determine the toxicity of the selected phthalates on liver cells. The concentrations tested were 10, 50, 100, 300, 500 µM and were comparable to those used in similar studies. To measure insulin secretion, β cells were seeded in culture media and incubated for 48 hours at 37°C in 5% CO2. Cells were then treated with various concentrations of phthalates in the presence of 5.5 mM or 16.7 mM glucose. Insulin secretion was measured using an ELISA kit.

Results
All the phthalates did not have a significant effect on cell viability in the concentration range tested compared to untreated cells, except one phthalate at higher concentrations (300 and 500 µM) ($p<0.001$). The three phthalate affected glucose stimulated insulin secretion in different ways, however the changes were not significant.

Conclusion
The preliminary data suggests that acute treatment of the rat β cell line with the selected phthalates induces different responses with regards to insulin secretion, nonetheless, the responses did not reach significant levels.

Key words: diabetes, phthalates, insulin secretion

Expression and purification of sterol 24-C-methyltransferases from opportunistic pathogenic fungi for structural studies

Mpontshane N[1], Opperman D[1], Hart R[2], Breda V[2], Tolmie C[1]
[1]Dept Microbiology And Biochemistry, University Of The Free State, [2]Agricultural Research Council

Background:
Opportunistic pathogenic fungi cause infectious fungal disease with extremely high mortality rates, especially in immune-compromised patients. Antifungal resistance and multi-drug resistance have emerged against the limited number of clinically used antifungals and pose a global threat to human health and well-being. Because of the high HIV/AIDS incidence in Sub-Saharan Africa, antifungal drug resistance is specifically of concern; therefore, there is an urgent need to develop novel therapies. Ergosterol biosynthesis is a target for existing antifungal drug classes such as azoles and allylamines; however, many enzymes in the pathway remain unexplored as novel targets. We have identified sterol 24-C-methyltransferase (SMT) as a novel antifungal target. SMT catalyses the transfer of a methyl group from S-adenosyl-methionine to the C-24 of zymosterol to form fecosterol in the ergosterol biosynthesis pathway. The aim of the study is to recombinantly express and purify fungal SMTs for structural studies, specifically X-ray crystallography, as a basis for structure-based drug discovery.

Methods:
An expression vector library of SMT genes from Candida albicans, Candida auris, Aspergillus fumigatus and Cryptococcus neoformans was prepared and expressed in Escherichia coli using auto-induction media. The recombinantly produced SMTs were purified with immobilised metal-affinity chromatography (IMAC) and the purity analysed with SDS-PAGE.

Results:
Expression of the optimised SMT genes from all four fungi was successful. During IMAC purification, the SMT proteins bound to and could be eluted from the column; however, SDS-PAGE analysis indicated multiple prominent smaller bands, possibly due to degradation or truncation of the target protein. Expression construct libraries with N-terminal truncations and an affinity tag at either terminus aim to address the truncation or degradation.

Conclusions:
Expression in E. coli is effective for producing SMTs from pathogenic fungi. However, expression construct optimisation is required to obtain pure and stable protein, which would be suitable for downstream crystallization trials.

Keywords: Sterol 24-C-methyltransferase, antifungals, ergosterol biosynthesis, pathogenic fungi

Characterization of archaeal methanogens found in fresh cow dung and digestate in anaerobic biodigester.

Nemaridili N[1]
[1]University Of Venda

Background:
Methanogens are archaeal anaerobic prokaryotes that uses hydrogen to convert carbon dioxide,acetate and range of methyl compounds to methane. These organisms differ from bacteria in terms if 16s rRNA sequences.The 16s rRNA and mcrA gene sequences are used to determine molecular diversity of archaeal methanogens.
Methodology:
Fresh cow dung and digestive slurry for a biodigester will be collected and cultured in an anaerobic environment for 72 hours. The colonies will be observed under an electron microscope to confirm the cell of methanogens. The confirmed cells will be pure cultured for DNA extraction and sequencing. The whole genome sequencing will be done for comparison of altered genes during the process of methanogenesis.
Expected results:
The genes of methanogens found in fresh cow dung won't be the same with those in digestate due to the process they undergo in the absence of oxygen. However,the methanogens will still be able to produce same,less or more methane than before.

In-silico design and expression of recombinant multi-epitope TB vaccine in E.coli system

Masondo N[1], Chiliza T, Mvubu N
[1]University of KwaZulu-Natal

Tuberculosis (TB), a chronic disease that continues to be a threat to humankind due to increasing emergence of extensive drug resistance strains is caused by Mycobacterium tuberculosis. Bacillus Calmette Guérin (BCG) is the only available TB vaccine, with its drawbacks and limitations such as the different efficacy for different people, lack of protection from pulmonary TB in adults and subsequent reactivation which prompt the research for novel TB vaccine approach. Vaccination is considered an effective way of protecting the host from pathogens. Multi-epitopes vaccines are a selective combination of epitopes from different candidate proteins, and they have found to enhance immunity to diseases. This study aimed to design a multi-epitope TB vaccine using immuno-informatics approach and express in vitro using E. coli system. In our previous study, we identified 9 (Rv2896c, Rv1566c, Rv1980c(Mpt64), Rv1811(mgtC), Rv0834c, Rv2972c, Rv2875(Mpt70), Rv2578c, and Rv3021(PPE47)) extracellular proteins secreted by Mtb with some involvement in TB pathogenesis. Reverse vaccinology method was used to select vaccine candidate epitopes. B- and T-cell epitopes were predicted from all nine proteins, analysed, and in silico joined together using appropriate linkers to form a candidate multi-epitope TB vaccine, named TBNMVacc. Moreover, the tertiary structure was predicted. The predicted antigenicity score was 0.6228 with 0.8055 probable solubility of peptides upon expression. These results suggested that the vaccine sequence is potentially antigenic in nature. NMVacc gene candidate was cloned into pET-22b (+) vector and transformed into E. coli BL21 (D3) strain. The recombinant TBNMVacc protein expression was induced with 0.5 mM IPTG, and expression analysed using SDS-PAGE method. The expressed recombinant TBNMVacc protein was about 46 kDa in size. In conclusion, the TBNMVacc candidate was constructed successfully and expressed in E. coli BL21 (D3) strain. Future work should include in vivo testing of candidate vaccine ability to elicit humoral and cellular mediated immune responses to determine its potency and efficacy in controlling TB. This contribute toward END TB initiatives and diversify pipeline portfolio of new TB vaccines.

Methicillin-resistant coagulase-negative staphylococci recovered from beef in KwaZulu Natal Province, South Africa

Madoroba E[1], Nene N[1]
[1]University Of Zululand

Background
Coagulase-negative staphylococci (CoNS) have been linked to infections such as bovine mastitis in veterinary medicine where the bacteria pose economic challenges for livestock producers. In human medicine, CoNS may be associated with nosocomial infections including urinary tract infections and infections associated with medical invasive devices. People at risk include those who are hospitalized for long periods, immunocompromised patients, and individuals with medical devices that are invasive. The challenge is exacerbated by the global increase in antibiotic resistance, including the rise in methicillin-resistant coagulase-negative staphylococci (MRCoNS). While methicillin and Staphylococcus aureus are well characterized, there is little information on MRCoNS in South Africa.. Therefore, the aim of this study was to determine the presence of mecC gene from 53 CoNS recovered from retail beef.
Methodology
A total of 53 confirmed coagulase negative staphylococci preserved in glycerol were resuscitated, and the purity was confirmed. Total genomic DNA was extracted, followed by PCR targeting the mecC gene.
Results
Interestingly, the majority of CoNS (52,83%; n = 28/53; Confidence interval 38,6 - 67) from retail beef tested positive for the mecC gene. These findings are in harmony with studies that show an increase in methicillin-resistant coagulase-negative staphylococci. However, the mechanism of methicillin resistance for the majority of MRCoNS in other parts of the world has been associated with production of penicillin-binding protein (PBP 2a) encoded by the mecA gene, whereas in this study the mecC gene was predominant.
Conclusion
Surprisingly, the majority of CoNS from this study tested positive for the mecC gene. The presence of mecC positive MRCoNS indicates their potential pathogenicity fitness, which implies that if a person is infected, the treatment options are limited. This study provides valuable insight into the genetic diversity of antimicrobial resistance among CoNS from beef in KwaZulu Natal province. Whole Genome Sequence analysis is recommended to better understand the antimicrobial resistance gene of the mecC positive MRCoNS in the context of “One Health”.
Key words: Coagulase negative staphylococci; Methicillin Resistant Coagulase Negative staphylococci; beef; mecC gene; PCR

Antimicrobial Resistance Factors and Molecular Characteristics of Methicillin Resistant Staphylococcus aureus isolated from beef

Myawe S[1], Madoroba E[1]
[1]University Of Zululand

Background
Methicillin resistant Staphylococcus aureus (MRSA) is a causative agent of diverse infections including life-threatening septicaemia. MRSA can present public health challenges in communities, hospitals, and animals. MRSA are diverse and they evolve, which may be linked to multidrug resistance. Detailed information about MRSA from beef in South Africa is limited. Therefore, the aim of this study is to confirm mec genes from MRSA recovered from beef in KwaZulu Natal South Africa.
Methods
MRSA isolates that were preserved in 50% glycerol were revived using nutrient broth, followed by nutrient agar cultivation overnight at 37 °C. DNA was extracted using a commercial kit, followed by PCR targeting mecA and mecC genes.
Results
The mecC gene was confirmed in the MRSA isolates that were revived. The mecC gene is considered to be a mecA homologue, and is emerging among MRSA. The mecA gene was not detected in the MRSA isolates. The other genomic characteristics will be discussed.
Conclusion
The revived S. aureus from beef harbor the mecC gene. It is important to perform extensive epidemiological surveillance of mec genes that are present in MRSA from food production and wild animals in South Africa because mecC positive MRSA may be a potential zoonotic risk to humans. Whole genome sequence analysis will be done in future to provide details about molecular epidemiological characteristics of the mecC positive MRSA from this study in the context of “One Health”.

Key words: Methicillin Resistant Staphylococcus aureus; mecC gene; mecA gene; beef

Cytotoxic effects of Leptospermum petersonii extracts and its isolate on melanoma

Choene M[1], Motadi L[1], Rahlano J[1]
[1]University of Johannesburg
Cytotoxic effects of Leptospermum petersonii extracts and its
isolate on melanoma
Johannes Rahlanoa, Mpho Choenea, Lesetja Motadib
aUniversity of Johannesburg
bUniversity of Johannesburg
Cancer is one of the leading causes of death worldwide. According to WHO data in collaboration with GLOBOCAN, cancer mortality in 2018 was more than 50% in a South African sample of 107 467 cancer cases. Melanoma is the most aggressive kind of skin cancer and most common among fair-skinned people, and it accounts for about 80% of fatalities from skin lesions. The rise in deaths from melanoma worldwide has been related to a lack of access to healthcare mostly in low-income nations. Melanoma therapy using current treatments such as cisplatin has been beneficial. However, it was linked to irreversible DNA damage, whereas most current treatments are either ineffective or have intolerable side effects. The purpose of this study was to investigate the cytotoxic effects of Leptospermum petersonii extracts on a375 cells, and further distinguish at least one putative bioactive component. L. petersonii is a well established medicinal plant for its antimicrobial activities. Its leaves are reported to contain essential oils that suppress fungus development. However, no previous research on the effects of this herb on cancer was reported before. The sequential extraction was done by soaking the leaves in hexane, ethyl acetate, and methanol. The chemical 6-methyltectochrysin was effectively isolated from L. petersonii and showed potency against melanoma cells. Caspase Glo 3/7 and annexin-V FITC - PI apoptotic tests were performed following 6-methyltectochrysin administration. The findings showed cytotoxicity and possible apoptosis induction in a375 cells. Further examination using relative expression analysis revealed an increase in the pro-apoptotic Bax and a decrease in BCL2.
Keywords:
Melanoma 1; Leptospermum petersonii 2; caspase Glo 3/7; 6-Methyltectochrysin; Apoptosis

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Molecular Insights into the Functions of Cholesteryl Ester Transfer Protein as a Marker for Drug Resistance and Survival in Breast Cancer

Gu L[1], Pillay R[1], Aronson R[1], Kaur M[1]
[1]University Of The Witwatersrand

Background
Cancer aggressiveness and resistance is directly proportional to intracellular cholesterol levels. Cholesteryl ester transfer protein (CETP) maintains cellular homeostasis and it has been previously reported to be a cell survival gene. In the present study, we knocked down CETP in breast cancer (BC) cells to reduce resistance towards current therapies and furthermore to explore the molecular pathways involved.
Methods
Subsequent to CETP knock-down, cholesterol quantification and cellular proliferation and viability assays were performed in MCF-7 and MDA-MB 231 cells. Thereafter, cellular apoptosis was measured post Tamoxifen (TAM) and acetyl-plumbagin treatments, pre and post CETP knock-down. RT² Profiler™ qPCR arrays were used to determine the gene expression profiles in the absence of CETP. Kaplan-Meier plots were also generated in different types of cancers. A mice xenograft study was performed to measure tumour growth rate in CETP knocked-down MDA-MB 231 cells.
Results
The preliminary results showed that knocking-down CETP resulted in an increase in apoptosis in MCF-7 cells when treated with TAM (by 10-40%). Furthermore, CETP knock-down with the addition of a cholesterol-depleting agent increased apoptosis by 10-fold. Similar results were observed in MDA-MB-231 cells. In MCF-7 cells, CETP knock-down decreased cancer resistance through a decrease in cholesterol synthesis genes and in MDA-MB 231 cells; cholesterol efflux increased with increased estrogen receptors. Lastly, high levels of CETP in estrogen positive BC cells reduces survival rate up to 100-150 days by 40% and 50% in triple negative BC cells. The mice xenograft study showed that there was an 86.45% reduction in tumour growth in the absence of CETP.
Conclusion
In conclusion, CETP could thus serve as a potential drug-resistance marker in cancer cells, more specifically BC.

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Metabolic response to drought stress in ten bread wheat (Triticum aestivum L.) genotypes

Makhubu F[1], Mutanda M[1,2], Chaplot V[3,2], Shimelis H[2], Madala N[4], Figlan S[1]
[1]University of South Africa, [2]University of KwaZulu-Natal, [3]Institut de Recherche pour le Dévelopement (IRD), [4]University of Venda

Background
Bread wheat (Triticum aestivum L.) is a globally significant crop, extensively cultivated and consumed worldwide. Drought severely restricts its development, growth, and production, resulting in significant economic losses globally, including in the sub-Saharan region. For the development of an improved drought-tolerant wheat, it is important to understand the adaptive responses of different bread wheat genotypes. Omics technologies, such as metabolomics, have been employed to elucidate metabolic pathways that could be manipulated to mitigate the impact of drought stress. In this study, the drought-induced changes were compared in ten bread wheat genotypes. Our aim was to present differences in the metabolites that play important role in metabolic responses to drought using an untargeted metabolomic approach.
Methods
Ten different genotypes of bread wheat (Triticum aestivum L.) from CIMMYT's heat and drought nurseries were used in the experiment. The genotypes were evaluated under field conditions during the 2021/2022 growing season at UKZN's Ukulinga Research Farm (29° 40’ S, 30° 24’ E). The trials were laid out in a 10 x 10 Alpha lattice design with 2 replicates per treatment (ten wheat lines under non-drought and drought stress). Grain samples from 10 bread wheat genotypes were collected under control and drought conditions. Samples were analysed for metabolite change using ultra-performance liquid chromatography-mass spectrometry (UPLC-MS).
Results
Using chemometrics analysis, total of 58 metabolites were tentatively identified from primary and secondary metabolisms; 52 of these were up-regulated and 6 of them down-regulated during drought stress. Metabolite concentration varied in different genotypes, and their redistribution was highly concentrated in drought treated wheat genotypes. Phenolic compounds, carbohydrates and lipids formed the largest group and represented the most discriminant metabolites.
Conclusion
The findings from the present study suggest that the tested wheat genotypes use different metabolic strategies to adapt to drought stress conditions. The production of specific metabolites, in specific genotypes during drought can be used to understand the responsive mechanisms wheat uses for defence and can be used to identify metabolic markers. Markers identified may serve as valuable tools in future breeding programmes as they can be used to develop or select drought-tolerant wheat genotypes.