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Advances in Pharmacological Sciences

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Therapeutic Potential of Selectively Targeting the $\alpha_2$C-adrenoreceptor in Depression and Schizophrenia

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$\alpha_2$A- and $\alpha_2$C-adrenoceptors (ARs) are the primary $\alpha_2$-AR subtypes involved in central nervous system (CNS) function. These receptors are implicated in the pathophysiology of psychiatric illness, particularly those associated with affective, psychotic, and cognitive symptoms. Indeed, non-selective $\alpha_2$-AR blockade is proposed to contribute toward antidepressant (e.g., mirtazapine) and atypical antipsychotic (e.g., clozapine) drug action. Both $\alpha_2$C- and $\alpha_2$A-AR share autoreceptor functions to exert negative feedback control on noradrenaline (NA) release, with $\alpha_2$C-AR heteroreceptors regulating non-noradrenergic transmission (e.g., serotonin, dopamine). While the $\alpha_2$A-AR is widely distributed throughout the CNS, $\alpha_2$C-AR expression is more restricted, suggesting the possibility of significant differences in how these two receptor subtypes modulate regional neurotransmission. However, the $\alpha_2$C-AR plays a more prominent role during states of low endogenous NA activity, while the $\alpha_2$A-AR is relatively more engaged during states of high noradrenergic tone. Although augmentation of conventional antidepressant and antipsychotic therapy with non-selective $\alpha_2$-AR antagonists may improve therapeutic outcome, animal studies report distinct yet often opposing roles for the $\alpha_2$A- and $\alpha_2$C-ARs on behavioural markers of mood and cognition, implying that non-selective $\alpha_2$-AR antagonism may compromise therapeutic utility both in terms of efficacy and side-effect liability. Recently, several highly selective $\alpha_2$C-AR antagonists have been identified that have allowed deeper investigation into the function and utility of the $\alpha_2$C-AR. ORM-13070 is a useful ligand for application in positron emission tomography (PET) studies, while ORM-10921 has demonstrated antipsychotic, antidepressant, and pro-cognitive actions in animals. ORM-12741 has demonstrated promise in clinical studies for the treatment of cognitive dysfunction and neuropsychiatric symptoms in patients with Alzheimer’s disease. This presentation will review the importance and relevance of the $\alpha_2$C-AR as a neuropsychiatric drug target in major depression, schizophrenia, and associated cognitive deficits.
Alzheimer’s Disease – Old Friends and New Promises

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Alzheimer's disease (AD) is the most common form of dementia, a general term for loss of memory and other intellectual abilities, serious enough to interfere with daily life. AD accounts for 60 to 80% of dementia cases. The greatest known risk factor is increasing age, and the majority of people with Alzheimer's are 65 and older.

Although AD has no current cure, symptomatic treatments are available. These can temporarily slow the worsening of symptoms and improve quality of life. Cholinesterase inhibitors used to treat AD include Donepezil and Galantamine. The latter is an alkaloid obtained from the bulbs and flowers of *Galanthus caucasicus*, a plant used in traditional medicine to treat memory loss.

While herbal remedies are widely used in developing countries, their use in treating diseases in developed countries is increasing dramatically. Although cholinesterase inhibitors are generally well tolerated, their effectiveness varies across populations and side-effects do occur. Efforts are under way to find improved treatments of AD, delay its onset, and even prevent it from developing. To this end the Department is involved in research to obtain safer and more efficacious treatments for the disease. Structure-activity guided analysis, based on the structure of Donepezil, has enabled the synthesis of novel compounds, which have shown promise in *in vitro* assays. Investigation of traditional remedies has led to the isolation of new compounds with promising activity. Currently compounds which may exhibit multi-target action against cholinesterase and β-secretase are being synthesised. Furthermore, *in vitro* models, which make use of neuroblastoma cells, are being optimized in order to be more representative of the *in vivo* environment, which would assist in drug development.
Mass Spectrometric Imaging as a Preclinical Tool for Medicinal Chemists

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Mass spectrometry imaging (MSI) allows for the label-free detection and mapping of a broad range of molecules from complex surfaces. It has become a fascinating molecular histology tool in pharmaceutical and medical research. The fundamental contributions of this method are for the rapid provision of molecular weight-specific maps or images at high resolution and sensitivity, offering a new powerful medical tool for pathology, chemotherapeutics, and discovery of disease biomarkers. Over the past decade this technique has been adopted for the investigation of TB, HIV and many other diseases.

Our group is currently involved in understanding the neuroprotective potential of current and pipeline anti TB drugs. None of the current frequently used anti TB drugs are capable of protecting against infection of the brain. Only a current pipeline drug, clofazamine, provides protection for this type of infection. This study which only took 3 years would not have been possible without this game changing preclinical tool.
Regulation and Promotion of Traditional Medicine - Indian Perspectives

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The Indian subcontinent, with the history of one of the oldest civilizations, harbours many traditional health care systems. Ayurveda, Yoga, Unani, Siddha and Homeopathy (AYUSH) are the major components of Indian traditional medicine being practiced in Indian culture for a long time. The development of traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only to preserve this traditional and cultural heritage but also to rationalize the use of TM in the healthcare. India has nearly 8000 herbal drug companies including the small and medium size. The regulatory documents offer the assessment of the safety, efficacy, and quality of herbs care and management of human health as prescribed in Drugs and Cosmetic Acts 1940 which has been amended several times based on requirements. The plant species mentioned in the ancient texts of these Indian systems of medicines (ISM) is being explored with the modern scientific approaches for better leads for healthcare.

Ministry of AYUSH has been established by Government of India for promotion and development of Indian traditional medicine. To ensure quality, safety, and efficacy of the herbal medicines, harmonizing efforts have been initiated on various issues i.e. Pharmacopoeial specifications, standardization, and classification of herbal drugs. Scientific validation and standardization of herbal medicine is needed for the globalization of traditional medicine. Appropriate use of herbal products of assured quality could also avoid the product associated risk or any toxicity. Globalization of TM is the need of today for harmonization in respect of its biomarker fingerprinting and metabolite profiling, chemical characterization, standardization, quality control, metabolomics study, documentation, and regulatory aspects in all contexts. Considering the widespread use and popularity of AYUSH, proper standardization and validation method are being developed for promoting Indian medicine including the Ayurvedic drugs. The existing knowledge of Ayurveda and other TM are being validated through newer guidelines of standardization, manufacture, quality control and modern techniques. In India, such efforts including administrative management and infrastructure facilities of AYUSH, standards for quality control and indigenous practices for integration of TM provides potential role in healthcare of the people.
Research and Development of African Traditional Medicines: Balancing Innovation and Academic Research

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Introduction: The South African government has integrated Indigenous African Knowledge Systems (IAKS) into the National System of Innovation. The IAKS offers South Africa a competitive advantage within the knowledge economy. The Bio-Economy Strategy recognises IAKS as a tool for holistic research, inclusive innovation, community-based technology transfer, underscored by an Ubuntu (humane) worldview. The Department of Science and Technology (DST) established an IKS-Bioprospecting and Product Development Programme with four platforms in African Traditional Medicines (ATM), Cosmeceuticals, Nutraceuticals, Health Beverages and Infusions. This paper seeks to present new models of interfacing IAKS within the NSI.

Discussion: The ATM Platform has research and innovation initiatives in priority health conditions like, HIV, TB, and Diabetes. The Cosmeceuticals Platform has developed various products on anti-aging, anti-hair loss, skin toning, and moistures that treat acne, eczema and other skin conditions. The Nutraceuticals Platform is in the process of developing nutraceuticals, functional foods and various value-added indigenous foods. The Health Beverages Platform is in the process of developing health teas from various indigenous crops based on IKS interfaced with modern science.

Conclusion: The context within which this programme is managed is inclusive of IK holders, grassroots communities, government, academia, science councils and industry. The Ubuntu-based models are aimed at transforming the National System of Innovation to invite common knowledge holders to participate directly in their knowledge economy. It is envisaged that innovation will lead to commercialisation for improved quality of file and sustainability.
The Role of Professional - learned Societies in Drug Research and Development

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The founding of academies or institutions of learning has been an important step in the history of the human race to allow for our educational advancement and research development. The Academy founded by Plato (c.428-347 B.C.) in the ancient Greek world is often regarded as the world’s first university. Plato expanded on the wealth of knowledge and insights of his teacher Socrates to teach amongst others his greatest student, the equally influential philosopher Aristotle. These early founded academies served as the basis for teachers, students and even the public to engage through informal gatherings and meetings to debate areas of mutual interest. These events led to the development of organised disciplined based entities. Learned and professional Societies, dating back to the fourteenth century, have proven to be of key importance to assist in the emergence and development of new disciplines or professions. The existence of thousands of Learned and professional Societies across the global is testimony of critical contributions these organisations make to world society. These organisations had also over time transformed to address the needs of its members, stakeholders and to adapt to the external changes such as the development of information technology and online virtual approaches to served even traditional activities of associations.

These important knowledge networks for research and education help to provide authoritative research information to assist not only in policy making but provide direction in innovation and offer a neural forum for universities, research institutes, government and industry to discuss evidence-based research. Increasingly, learned societies facilitate and create platforms for commercialisation and service based opportunities. Expanding their contributions beyond the traditional roles into these commercial areas further advances their income funding stream opportunities with improved sustainability. The importance for developing innovative non-due income streams for societies is critical for their survival not only in view of the current economic climate but to address the needs of its members and stakeholders. It is clear that several societies have been successful to make this transition and to venture into the potential opportunities.

In conclusion, societies are faced with global transformation and it is essential for societies to continuously assess their roles and their sustainability in order to reinvent themselves to meet the needs of the present and the future.
3-Phenylpyridin-4(1H)-one Derivatives Targeting the Cytochrome bc₁ Qi Site Shows Antimalarial Activity

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Introduction and Aim: *Plasmodium falciparum* is the causative agent of the common fatal form of malaria in Africa. Inhibitors of cytochrome bc₁, an essential inner mitochondrial membrane protein, are claimed to be lethal to apicomplexan species including *Plasmodium*. The Cyt bc₁ complex drives ATP synthesis in the mitochondria. Reports suggest that selectively inhibiting the parasite electron transport could be a potential multi-stage treatment. The emergence of resistance to atovaquone, which targets the Q₀ site of the Cyt bc₁ complex, casts doubt over new drugs targeting these mitochondrial proteins. Hence, many aspects must be investigated to assess the suitability of new emerging drugs on the mitochondrion. Target-based drug discovery has been used as a prominent and efficient tool to identify follower drugs. *In silico* target-based drug design methods using Autodock vina was used to design compounds that would theoretically bind to and inhibit the Qi site of cyt bc₁ of the *P. falciparum*. These compounds were selected from compounds defined by Gamo *et al.* (2010) and tested by *in silico* docking experiments. Homology models were developed and modified to improve their drug-likeness according to the Lipinski and QED parameters. The study objective is to assess the antiproliferative activity of six *in silico* compounds on *P. falciparum* parasites in vitro.

Methods: The SYBR green assay was used to screen the various stages of parasite development for potential antiproliferative activity and to determine the IC₅₀ values of the test compounds on *P. falciparum* sensitive (3D7), and multidrug-resistant (K1) strains during 96 hour exposure. The rate and stage effectiveness of the compounds in the ring and schizont stages were assessed. The sulforhodamine B (SRB) colorimetric assay was used to assess the cytotoxicity of the test compounds on human HepG2 cells to determine selectivity for the parasite.

Results: Data was obtained from initial *in vitro* screening of the test compounds at 1 µM and 5 µM and 96 hrs full dose response curves for compounds with >70% proliferation inhibition at 1 µM using *Pf*3D7 strain. Four of the test compounds, EE1, EE3, EE5 and EE7 gave IC₅₀ values of 89 nM, 664 nM, 64 nM and 249 nM respectively. The compounds showed a selectivity index >2 and a resistance index >120. Additionally, the compounds showed significant activity on both the schizont and the ring stages.

Conclusion: This study showed that *in silico* docking using software programs could be utilised as a potential tool for rapidly identifying feasible target-based antimalarial compounds while avoiding high throughput screening. Other possible target sites on the mitochondrion can be used to design new chemotypes. All the designed compounds did show significant antimalarial activity against the asexual stages of tested *Pf*3D7 strain and with a significant resistance index. However, these compounds showed no activity on the transmission blocking stage. Finally, compound EE5 showed to be the most potent, more selective and with higher resistance index, hence this can be used for further preclinical studies.
Comparison of Metabolome Attenuation in Monolayer and Three Dimensional Hepatocyte Cultures

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Introduction and Aim: Hepatotoxicity is one of the leading causes of drug withdrawal worldwide. Primary human hepatocytes (PHH) have for many years been considered the gold standard for hepatotoxicity testing but are no longer considered the most feasible source of hepatocytes. Immortalised cell lines such as HepG2 do not express the full complement of enzymes required for metabolism limiting their application in hepatotoxicity testing. In addition, classical monolayer culturing of hepatocytes is not a representative model of the architecture seen in vivo. However, this can be more closely approximated by the use of spheroid cultures. The aim of this study was to determine the degree to which the metabolic activity of selected phase I enzymes can be enhanced using a cytochrome P450-inducing drug cocktail in both monolayer and spheroid cultures of HepG2 cells.

Methods: A spheroid model, using an agarose based 3D Petri Dish® (12-series mold with 81 spheroid capacity) was optimised to compare induction in monolayer and spheroid HepG2 cells. Major drug metabolising CYP450 isoforms were targeted for induction using a four drug cocktail. Cell stocks were continuously exposed for three weeks to: Phenacetin (CYP 1A2, 2.5 µM), Dextromethorphan (CYP 2D6, 1.25 µM), Diclofenac (CYP 2C9, 5 µM) and Midazolam (CYP 3A4, 0.15 µM). Cells were then cultured and exposed to the same drug cocktail in monolayer or spheroid cultures for 72h. Cells were then exposed for 72h to the four drug cocktail along with Bupropion (CYP 2B6, 5 µM) which served as a control for cross-induction. Using liquid chromatography tandem mass spectrometry (LC-MS/MS) parent drug and metabolites were measured to determine the extent of metabolism achieved in differentially cultured HepG2 cells after induction.

Results: Optimal spheroid size was achieved when seeding 9.6x10⁵ cells/well resulting in spheroids of approximately 450 nm. Spheroid characterisation over a 7 day time course demonstrates an increase in protein content over time from seeding to day 5 (Day 0: 7.33 µg/spheroid; Day 5: 8.67µg/spheroid) with spheroids shown to be viable till day 6 using flow cytometry and FDA/PI live dead staining. An LC-MS/MS method for the detection of all five parent drugs and metabolites was developed and validated on an AB Sciex 4000 QTrap mass spectrometer. After induction, supernatants from HepG2 spheroid and monolayer samples were analysed. None of the drugs were able to induce an increase in metabolic activity as the parent compounds were present at similar concentrations between monolayer and spheroid samples. In addition, there was no increase in drug metabolites which were barely detected amongst the chromatographic noise.

Conclusion: This investigation shows that under long term exposure to sub-toxic concentrations of known inducers and the variable culture conditions described it was not possible to induce HepG2 cells to alter actively metabolising CYP450 enzymes. Therefore, the HepG2 cell line may remain limited for hepatotoxicity testing as induction in spheroid cultures does not confer an advantage.
A Comparative Proteomic and Glycoproteomic Study of Platelets from Patients with Diabetes and Non-Diabetic Healthy Individuals

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Introduction and Aim: In addition to alterations of platelet protein expression, post translational modifications, particularly glycosylation of functional proteins, have been implicated in the impaired wound healing of diabetic foot ulcers (DFUs). The impairment in healing of DFUs, in comparison to normal wounds, is a leading cause of leg amputations in diabetic patients which has a negative impact on healthcare financial resources. Characterizing these platelet protein modifications could identify novel therapeutic drug targets for the treatment of DFUs. SDS-PAGE coupled with selective visualization techniques is a useful screening tool to assess protein differences between samples prior to downstream proteomic applications such as mass spectrometry. SDS-PAGE gels can be imaged and visualized as “Stain free” images or stained with gel stains such as Coomassie blue, silver, Oriole (fluorescent stain) or periodic acid-Schiff stain (PAS), which are specific to certain protein residues and glycoproteins. Each visualization technique has its advantages and disadvantages and it is imperative to utilize the most sensitive and appropriate techniques to ensure successful subsequent proteomic analysis. The aim of this study is to qualitatively identify possible proteomic and glycoproteomic differences between platelets from diabetic patients and healthy individuals.

Methods: The HbA1c test was used as a screening tool to test for participant eligibility. After screening, non-stimulated platelets were isolated from diabetic patients and non-diabetic healthy individuals, washed and the total protein complement separated using SDS-PAGE. The gels were scanned using the BioRad™ Gel Doc EZ Imager to produce “Stain free” images and then separately stained with Coomassie blue, silver, Oriole or PAS. Gels were compared using BioRad™ Image Lab 5.2.1 software. In gel digestion of selected SDS PAGE bands and in solution digestion of total protein using trypsin was used to confirm the identity of proteins that were shown to be glycosylated.

Results: Coomassie blue visualization did not show significant differences, but when selective staining techniques were employed, differences could be seen between the two groups. The most important differences observed are related to glycosylation of the platelet proteins. Mass spectrum based proteomics could identify the dominant glycosylated proteins found in platelets despite the low abundance of these proteins.

Conclusion: Small glycosylation differences were observed between the two groups, making downstream mass spectrometry analysis important to identify which proteins are altered and their role in the wound healing process. Results highlighted the superior sensitivity of silver and Oriole staining compared to Coomassie blue stain. However, the silver stain is mass spectrometry incompatible and less suitable for downstream analysis, hence it is ideal for qualitative assessment of low abundance protein differences. Confirmation of glycosylation points still needs to be confirmed.
Kinetics of Acute Wound Healing Processes in the Porcine Model using MALDI-TOF Imaging of Proteomic, Metabolomic and Lipidomic Changes

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Introduction and Aim: Acute wounds undergo self-repair in a controlled and timely manner by sequentially proceeding through the normal and predictable phases of wound healing. This ultimately results in restoration of both functional and anatomical parameters at the wound site. Numerous studies have been done that have provided a general understanding and characterisation of the acute wound healing process, however this complex, highly integrated and dynamic process of tissue repair is yet to be completely characterised. The porcine model is the most reliable animal model for wound healing studies as pig skin displays many similarities to that of humans, both anatomically and physiologically, as well as in the way that wounds heal. The porcine model therefore allows for strictly controlled wound formation and healing studies to be conducted. The aim of this study was to better define the kinetic changes occurring during acute wound healing across proteomic, metabolomic and lipidomic bases, by using modern mass spectrometric techniques.

Methods: Full-skin thickness wounds were created via punch biopsy along each psoas muscle ridge of three healthy pigs. Complete wound areas were then surgically excised and snap frozen at 6 different time points over a total duration of 16 days. Mass spectrometric tissue imaging and profiling were performed on frozen wound sections prepared from wounds collected over the 16 days using MALDI-TOF. A triple analysis was performed across proteomic, lipidomic and metabolomic bases.

Results: Principle component analysis and heat maps clearly visualise the kinetic differences occurring in healthy tissue, the wound bed and wound edges as healing progresses. Wound healing progression is visually tracked by variation and different clustering patterns. Wound variance peaked 8 days post-wound creation within the proteomic bases and 5 days post-creation across the metabolomic and lipidomic bases, before retracting and more closely representing fully-restored healthy tissue. Minimal correlation of wound tissue to healthy tissue was seen 5 days post-wound creation across all ‘omic’ bases, in addition to other common trends being observed.

Conclusion: MALDI-TOF was used to spatially define the kinetic changes occurring during wound healing. By characterising changes during acute wound healing, one may develop a model for non-healing chronic wounds, such as diabetic foot ulcers. This could identify proteins, lipids or metabolites as potential drug targets or indicators of inappropriate metabolic processes. Such targets can be used as the basis to develop affordable and easily accessible treatment options for diabetic sufferers.
Screening of Actinobacteria for Novel Antimalarial Compounds

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Introduction and Aim: The success of first line antimalarial treatment is threatened by increased signs of drug resistance. Malaria is a severe disease responsible for nearly half a million deaths every year and threatens half the global population. This makes the development of novel replacements critical to continue the eradication of malaria. Historically, biological systems have been a source of novel antimalarial compounds such as quinine and artemisinin and thus are a useful source to search for replacements. The bacteria phylum, Actinobacteria, are well known antibiotic producers, however their antimalarial potential has not been thoroughly investigated. This makes the Actinobacteria a potential, valuable source for novel antimalarial compounds. The aim of this investigation was to screen Actinobacteria extracts for antimalarial activity and isolate any active compounds found.

Methods: Novel actinobacterial strains were selected for screening to increase the chance of discovering novel chemical compounds. Actinobacteria were cultured in liquid medium for 10 - 14 days. The cell mass and broth were separated by filtration and each was extracted with ethyl acetate. Crude extracts were redissolved in ethyl acetate and methanol, dried and weighed. Extracts were tested for in vitro antiplasmodial activity, using the pLDH assay against the chloroquine sensitive Plasmodium falciparum strain NF54 over a concentration range of 10 µg/ml – 20 ng/ml. Active extracts were separated by 2D thin layer chromatography (TLC), spots were excised, filtered and tested for antiplasmodial activity. Cytotoxicity and host selectivity of isolated compounds was determined against the Chinese Hamster Ovarian (CHO) cell line using the MTT assay. Novel actinobacterial strains were characterised by 16S rRNA sequencing.

Results: Three actinobacterial strains, PR3, UK1 and Streptomyces speibonae, were found to have extracts with antimalarial activity. PR3 and UK1 were shown to be Streptomyces by 16S rRNA sequencing. PR3 had the most active extracts and was thus selected for further study. Two compounds, DJW#1 and DJW#2, were isolated from PR3 by 2D TLC. DJW#1 has an IC50 of 27 ng/ml and DJW#2 has an IC50 of 407 ng/ml. Both compounds were found to be non-toxic to the CHO cell line.

Conclusion: Both DJW#1 and DJW#2 show excellent in vitro antiplasmodial activity and host selectivity making them candidates for in vivo studies and structural elucidation.
Percentage of Time in the Therapeutic Range (TTR) in Patients on Warfarin at a Dedicated Tertiary and District Level INR Clinic in the Western Cape, South Africa: A Retrospective Comparative Review from 2009 to 2014

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Background and Aim: South Africa has limited published data regarding time within the therapeutic range (TTR) for patients receiving long-term warfarin anticoagulation. The high risk and cost of warfarin failure and the lack of access to the direct oral anti-coagulants (DOACs) in the public health care sector, motivated this evaluation of TTR for warfarin therapy at a tertiary and secondary level facility in the Western Cape. The objective of the study was to describe the International Normalized Ratio (INR) adjusted-dose level of anticoagulation using the internationally recognized Rosendaal TTR method in a group of patients on long term warfarin therapy attending dedicated INR clinics at these two sites.

Methods: A retrospective folder review of patients attending the INR clinics at Groote Schuur Hospital (GSH) and Mitchells Plain (MPC) clinic between 2009 and 2014 was undertaken. 949 folders of patients attending the PI clinic from both sites were screened to achieve a sample size of 466. The included patients had a minimum of 27 months INR readings following initiation of warfarin therapy. Our primary outcome was to determine the median TTR across the cohort, over a period of 24 months excluding the first 3 months following therapy initiation. The secondary outcomes were to determine mean TTR by location, TTR by indication, mean number of INR readings over observation period and median TTR by time period. The TTR outcomes were calculated using the Rosendaal method, and a regression analysis was performed and expressed as an odds ratio (OR) with a 95% confidence interval (95% CI).

Results: 466 patients were included in the review. Valvular heart disease (VHD) followed by Atrial Fibrillation (AF), were the most common indications for warfarin therapy. The median TTR over the 24 month observation period was 48.1% (IQR 36.0 to 60.4%); with only 20.2% achieving a TTR above the recommended 65% level. TTR control between the two sites was not significantly different TTR at MPC 48.1% (IQR 34.8 to 66.7%) and at GSH 48.1% (IQR 36.4 to 58.8%). Poorly controlled patients (TTR <65%) on average had more INR readings (27.0 SD12.0) over 24months. For four different time durations of warfarin consumption: 1 to 3 months; 1 to 6 months; 3 to 9 months; 4 to 27 months, TTR was 32.4% IQR (12.5 to 54.4); 37.6% IQR (18.8 to 56.9); 43.9% IQR (25 to 66.6) and 48.1% IQR (36.0 to 60.4), respectively. When correcting for factors impacting TTR by means of regression analysis, MPC facility was associated with higher likelihood of having good control TTR >65%, odds ratio (OR) 0.86 (95% CI: 0.78 to 0.95). VHD patients were more likely to have a TTR <65%; OR 1.21 (95% CI: 1.05 to 1.39; p<0.01) and male gender barely reached statistical significance OR 0.90 (95% CI: 0.83 to 0.98).

Conclusion: Our study disappointingly revealed, despite patients attending a dedicated INR clinic, anti-coagulation control was poor. With only 20.2% of achieving a median TTR >65%. Future solutions to improve anticoagulation control will need to be considered and implemented in order to minimise warfarin therapy related risk.
Ascertaining the Reasons for Medication Returns at a Tertiary Academic Hospital in Gauteng

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Introduction and Aim: Medicine wastage is a global phenomenon that has a direct effect on budget estimation and allocation, often resulting in a reduced ability to provide optimal medical services to those dependent on a public health care system. Medicine shortage is a common occurrence in South African public health care facilities, compounded by large amounts of unused or expired pharmaceuticals being returned to the point of dispensing origin on a continuous basis. These returned medicines may not be re-used and are disposed of according to provisions made in Act 101 of 1965. Pharmacy returns are, however, overshadowed by various other improper disposal practices, which may result in environmental contamination, including accidental or intentional poisoning. The aim of this study was to identify the reasons patients returned unused or expired medicine to the Steve Biko Academic Hospital (SBAH) pharmacy. In addition, other non-formal methods and routes of medicine disposal were determined.

Methods: Institutional Research Ethics committee approval was obtained. A “Medicine Return” programme was initiated by displaying posters at strategic locations in SBAH. Daily institutional announcement of the take-back initiative, including several information sessions aired on various local radio stations one month prior to, and during the data collection period (1 March – 18 March 2017) was used as a recruitment tool for prospective participants. An interviewer-based semi-closed ended questionnaire was administered to 126 consenting adults with the assurance of anonymity. The multinomial proportion was derived to determine the reason for medication accumulation and orthodox discarding procedures for unused or expired medicine.

Results: More than a third (35%) of patients had expired medicine, of which only 15% of those returned it to the pharmacy. Other methods of disposal included discarding in the rubbish bin (31%), flushing down the toilet (45%), throwing down pit-toilets (7%), and storing it for future use (2%). The main reasons for accumulating expired medication were discontinuation of prescribed treatment (7.3%), condition improved and patient self-termination of treatment (4.9%), using only when necessary (2.5%), oversupply (3.3%), duplication from private health care providers (0.9%), improper use (0.8%) and the supply of incorrect medication (0.8%).

Conclusion: The medicine take-back study indicated that only a small proportion of patients returned unused or expired medication to the pharmacy. There is a general lack of knowledge on correct disposal practices where nearly 75% of all medication is discarded by various unsafe methods. The large proportion of patients in possession of unused or expired medication should raise concern to the prescribers and facilities issuing these items. Patients need to be educated on proper disposal practices, and significant emphasis should be placed on the importance of completing prescribed treatment. By informing health care providers on adherence related issues, significant funds could be saved, or redistributed, to other priority areas in the public pharmaceutical sector.
HIV Drug Resistance Patterns for Adult Patients on Third-Line ART in the North West Province since 2004-2017

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Introduction and Aim: South Africa has the largest human immunodeficiency virus (HIV) epidemic in the world with the total number of people living with HIV increasing from 4.02 in 2002 to 7.03 million in 2016. The number of patients that will experience virological failure and subsequently treatment failure in the future will increase due to improved access to antiretroviral therapy (ART) and a longer duration of exposure to ART. This will increase the need to access third-line ART. This study aimed to improve the knowledge on ART failure and the development of resistance in nucleoside reverse transcriptase inhibitors (NRTI’s), non-nucleoside reverse transcriptase inhibitors (NNRTI’s) and protease inhibitors (PI’s) in the North West province.

Methods: The study was an all-inclusive retrospective descriptive investigation of third-line approved patients in 3 districts in the North West Province performed during April-May 2017. Demographical and clinical variables were recorded from patient health records from 2004 until 2017. Captured data were statistically analysed to generate descriptive statistics (mean ± SD) of the third-line approved patients in the North West Province.

Results: Data of 21 patients were recorded and analysed. In this cohort, 17 females and 4 males had an average age of 45.19±7.26 years at the time of data capturing. The mean duration from HIV diagnosis to first-line initiation was 197±232 days. Mean duration on first-line ART was 1362±996 days and on second-line 1269±796 days. Alarmingl the average duration between second-line failure and third-line application approval was 91.38±73 days. The following resistance mutations were found to be the most prevalent for each drug class: NRTI’s (n=18); M184V (89.47%), D67N (31.58%), M41L (26.31%), Q151M and K65R (10.52%). Mutations for NNRTI’s (n=17) were; K103N (47.01%) and G190A (23.52%). Major PI’s (n=20); M46I (75%), V82A and I54V (65%). Antiretroviral drugs experiencing high level resistance in the NRTI class were; Emtricitabine (95.2%), Lamivudine (95.2%) and Zidovudine (52.4%). For the NNRTI’s; Nevirapine (76.2%) and Efavirenz (71.4%) and high level resistance for the PI’s; Nelvinavir/r (85.7%), Indinavir/r (81.0%), Lopinavir/r (76.2%), Fosamprenavir/r (66.7%) and Atazanavir/r (57.1%).

Conclusion: These HIV drug resistance patterns were also found in studies conducted in other parts of South Africa. The high resistance to PI’s, which was not used in treatment, is alarming and need further investigation. Non-adherence and ART side-effects can be highlighted as possible reasons for treatment failure. This study highlighted the importance of intensified support for adherence counselling and more frequent drug resistance testing.
Evaluation of Efavirenz on Addictive-Like Behaviours and Neurochemistry in Rats

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Introduction and Aim: HIV positive patients treated with the antiretroviral drug efavirenz have been observed to experience various neuropsychiatric symptoms. Moreover, efavirenz is regularly abused by HIV positive and non-infected people by crushing and smoking this lifesaving medication in a concoction of drugs known as Nyope. This study aimed to assess the addictive-like properties of efavirenz after acute and chronic exposure in rats.

Methods: The present study (ethics: NWU - 00267-16- S5) used a total of 84 male Sprague Dawley rats (12 per group), exposed to i.p injections of 5, 10 and 20 mg/kg efavirenz, 1 mg/kg methamphetamine (MA) (as a positive control) and vehicle for 6 days in an sub-acute treatment paradigm using the biased study design of the conditioned place preference (CPP) test. The chronic study was conducted using the most rewarding dose of efavirenz (5 mg/kg as established in the acute study), dosed as above for 14 days. CPP, sucrose preference and locomotor activity in the open field test were assessed in the chronic study. Furthermore, quantification of cortical and striatal dopamine (DA), serotonin (5-HT), and their metabolites (3,4-dihydroxyphenylacetic acid (DOPAC) and 5-hydroxyindoleacetic acid (5-HIAA)) as well as noradrenaline (NA) was performed using a high performance liquid chromatography system with electrochemical detection. One-way ANOVA with Dunnett’s post hoc test or unpaired student t-test were used for statistical analysis with a P value of 0.05 and smaller deemed significant.

Results: In the acute study it was observed that 5 mg/kg efavirenz induced a significant increase in the time spent in the drug-paired chamber compared to the control group in the CPP test. These results were comparable to the rewarding effects of MA in the same test. Efavirenz at 10 mg/kg showed no changes, while 20 mg/kg showed a significant decrease in the time spent in the drug paired chamber in comparison to the control group. Rats exposed chronically to efavirenz indicated a significant increase in the time spent in the drug paired chamber in comparison to the control group, although no changes in locomotion and sucrose preference were observed. In the chronically exposed efavirenz animals, a significant increase in cortical DA, DOPAC and 5-HT and striatal DA, 5-HT and NA was observed along with a significant decrease in cortical 5-HIAA and striatal DOPAC compared to the control group.

Conclusion: The findings in the acute and chronic study demonstrate a significant dose dependant rewarding effect of efavirenz in rats, with lower doses being most effective in this regard. The findings are in line with other studies showing that drugs of abuse increases regional brain DA, 5-HT and NA levels, driving motivational behaviour and reward, induce euphoria and arousal and cause relapse and craving. This study highlights the abuse potential of efavirenz in humans.
A Comparative Study of α-Mangostin and Raw Garcinia Mangostana Linn Pericarp vs. Haloperidol on Selected Behaviour in an Immune-Inflammatory Model of Schizophrenia

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Introduction and Aim: Schizophrenia is a severe brain disorder with complex pathological mechanisms. The disorder is associated with certain early life neurodevelopmental insults, such as pre-natal immune activation that can result in alterations in normal brain development and psychotic symptoms later in life. Abundant evidence implicates redox-immune-inflammatory dysfunction in the aetiology of schizophrenia. The aim of this study was to examine the therapeutic effects of the raw pericarp of Garcinia mangostana Linn (GML), known to contain a number of bioactive xanthones with antioxidant properties, and α-mangostin, a bioactive constituent of GML, on schizophrenia related behaviours in rats. For this purpose we used a maternal immune-activation (MIA) model recently established in our laboratory.

Methods: An MIA model was established by exposing 14 pregnant Sprague Dawley dams to lipopolysaccharide (LPS) on gestational days 15 and 16 (NWU-00376-16-A5). The male offspring from dams (n=55) were divided into four treatment groups (n=±14 per group). The groups received oral dosing of the following agents for 16 days: saline; haloperidol (2 mg/kg po); GML (50mg/kg po); α-mangostin (20mg/kg po). The respective drug treatments were dosed from postnatal day (PND) 51–66. After treatment, all groups were subjected to the following behavioural tests: (1) prepulse inhibition (PPI) on day 13 (PND 63); (2) open field test (OFT) on day 14 (PND 64); (3) forced swim test (FST) on day 14 (PND 64).

Results: A reduction in startle response was observed in all treatment groups, suggesting habituation across the test session. %PPI deficits in LPS offspring were significantly reversed in the haloperidol and GML treatment groups but not in α-mangostin-treated animals. Haloperidol and α-mangostin significantly decreased locomotor activity (OFT) in the LPS offspring, although GML treatment was without effect. All three treatment groups significantly reduced immobility in the FST in LPS offspring compared to saline-treated LPS offspring. α-Mangostin and GML treated animals displayed significantly increased swimming activity, with only GML increasing climbing behaviour, compared to saline treated LPS offspring.

Conclusion: Treatment with GML but not α-mangostin demonstrated effective reversal of %PPI deficits, although both were effective in the FST. Moreover, GML was comparable to the reference antipsychotic, haloperidol, in reversing behavioural changes such as %PPI following pre-natal inflammatory activation, but proved to be more effective in reversing depressive-like behaviour in the FST compared to haloperidol. GML was superior to α-mangostin in improving deficits in %PPI, suggesting that raw GML rind overs superior therapeutic benefits over its isolated constituent, α-mangostin. Overall, GML and α-mangostin have therapeutic potential in improving treatment outcome in an MIA model in rats, and possibly in schizophrenia. Further study is warranted.
Investigating Predator Scent Exposure to Model Posttraumatic Stress Disorder Related Anxiety in Rats

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Introduction and Aim: Posttraumatic stress disorder (PTSD) presents with symptoms of re-experiencing, avoidance, negative cognitions, arousal and depressed mood. Current treatment of PTSD is suboptimal. The predator scent exposure (PSE) model presents with good face and construct validity for PTSD, while it resembles a potentially life-threatening situation that is a more “natural” challenge than other types of stressors. Typically, only 25% of individuals exposed to a severe emotional trauma go on to eventually develop PTSD, i.e. are inherently at risk.

Methods: Male Wistar rats, known to be more stress-sensitive, were exposed to either a cat-exposed cloth (2 month exposure) or a clean odourless cloth for 10 minutes while under constant video surveillance (Ethical Approval NWU-00438-16-S5). The number of fecal boli was counted as an expression of inherent anxiety immediately post stress exposure, where after the rats were transferred to their home cages (4–5 rats/group). Seven days later the rats were tested for anxiety-like behaviours in the elevated plus maze (EPM). The number of entries and time spent in the open, closed and centre zones of the maze, as well as head dipping behaviour, was recorded. The latter is a reflection of risk assessment and exploratory activity.

Results: Rats exposed to PSE presented with significantly more fecal boli immediately post-stressor vs. the non-PSE group. Subsequently, PSE induced a significant reduction in the number of open arm entries and time in the centre zone on day 7 post-PSE vs. non-PSE animals. Furthermore, the lower quartile of distribution with respect to time spent in the open arms was lower in PSE vs. non-PSE rats. PSE rats also demonstrated more time in the closed arms vs. non-PSE animals, as indicated by the upper 75th percentile of the distributed values, implying that at least 25% of PSE animals preferred the closed arms of the EPM for longer periods than non-PSE rats. Finally, PSE animals engaged in significantly less head dipping episodes, suggesting reduced risk-assessment and exploratory behaviour.

Conclusion: A single 10-min exposure to predator scent elicits significant anxiety-like responses in rats during the time of PSE. Furthermore, PSE significantly increases post-exposure anxiety-like manifestations up to 7 days post PSE, suggesting a sustaining of aversive behaviour over time. That at least 25% of PSE animals demonstrated significantly more anxiety-like behaviour vs. non-PSE individuals is in agreement with the typical risk:resilience ratio described in clinical studies in PTSD. The PSE model therefore demonstrates robust face validity for PTSD, albeit requiring further study at the neuroendocrine level. The model holds promise as a useful research platform for application in novel drug discovery.
Immediate and Lasting Effects of Early-Life Escitalopram, Exercise and Omega-3 Supplementation on Depressive- and Anxiety-Like Behaviour in FSL Rats

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Introduction and Aim: Juvenile depression is a global concern with suicide being the second leading cause of adolescent mortalities worldwide. Pharmacotherapy for this age group is limited to only two serotonergic antidepressants, including escitalopram, partly due to the early maturation of this neurotransmitter system. Yet, juvenile antidepressant therapeutic outcome remains comparable to that of adult patients. Complementary treatment strategies, such as omega-3 fatty acid supplementation and exercise are gaining popularity as adjunctive treatment options, due to reported and postulated physiological, neurological and behavioural effects and perceived lower risk. These interventions hold to potential to augment pharmacotherapy and improve treatment outcome. The current study investigated the latter potential, and in particular whether early-life treatment had any lasting effects on the depressive- and anxiety-like behaviour in a stress-sensitive animal model of depression.

Methods: Male Flinders Sensitive Line (FSL) rats (±12 per group) received saline control (SAL) or escitalopram (ESC) (10 mg/kg/day sc) with various combinations of simultaneous normal (CRL) or omega-3 (OM3) supplemented rat chow, and/or no (SED) or low intensity exercise (EXE) (ethics approval # NWU-00148-14-A5 and NWU-00373-16-A5) from postnatal day 21 (PND21) to PND34 (pre-puberty). Thereafter rats were either subjected to behavioural testing on PND35 or normally housed until PND60 and then subjected to the open field (OFT) and the forced swim tests (FST), to assess immediate and lasting effects, respectively, on locomotor activity, anxiety- and depressive-like behaviour. The project was funded by the Centre of Excellence for Pharmaceutical Research of the NWU, the South African Medical Research Council and the National Research Foundation.

Results: Vivarium rat chow was successfully coated with OM3, containing double the amount of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) compared to uncoated chow. None of the pre-pubertal treatment strategies had any immediate, early-life effects on body weight, locomotor activity or centre zone/corner time ratio. However, a large effect size difference in FST immobility time was observed between ESC/CRL/SED and SAL/CRL/SED on PND35. On PND60, there were no significant three-way interaction effects on behaviour, yet distance moved in the OFT was significantly decreased by diet and exercise (irrespective of drug). A significant diet*exercise interaction also highlighted decreased centre zone/corner time ratios in CRL/EXE and OM3/SED groups, compared to CRL/SED (irrespective of drug). Finally, no significant long-term effects on depressive-like behaviour were identified on PND60.

Conclusion: Only pre-pubertal ESC mono-therapy induced a strong trend for an immediate, yet transient decrease in early-life depressive-like behaviour. Neither pre-pubertal OM3 supplementation nor EXE (irrespective of drug) induced an immediate effect on anxiety-like behaviour, yet both strategies increased anxiety-like behaviour later in life. Literature suggests OM3 supplementation to improve stress-induced coping responses, evinced by shorter environmental habituation times. This may explain the observed reduced ambulatory activity and consequent false-positive interpretation of enhanced anxiety-like behaviour, as well as possible masking of altered immobility in the FST. Therefore, EXE may induce similar adaptive responses, yet these observations will be confirmed with other behavioural models and neurochemical analyses in prospective studies. In conclusion, early-life complimentary interventions may induce long-term effects in stress-sensitive individuals.
An *In Vitro* Model for Drug Accumulation at the Target Site of Pulmonary Tuberculosis

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**Introduction and Aim:** The protracted duration of standard tuberculosis (TB) therapy reveals the inadequacy of current first-line TB drugs to eliminate *Mycobacterium tuberculosis* in its various host environments. This may be a result of poor distribution of anti-TB agents into the pulmonary lesions in which the bacilli reside. To address this possibility, the *M. tuberculosis*-infected macrophage was assessed as a model for TB drug penetration. Observations supporting the utility of this model in assessing intra-macrophage drug concentrations and efficacies of selected anti-TB agents at the target site are presented. The work forms part of the MRC flagship project led by Professor Richard K. Haynes, on “Development of Oxidant and Redox Drug Combinations for Treatment of Malaria, TB and Related Diseases.” Derivatives of phenoxazines, a group of N-heterocyclic compounds, were produced by Professor Chris Parkinson at the Charles Sturt University (Queensland, Australia). Phenoxazines and related compounds have shown diverse biological activities and were the series of compounds assessed in this study.

**Methods:** THP-1 macrophage-like cells were infected with *M. smegmatis*, treated with anti-TB agents (both known drugs as controls and experimental PHX compounds), and sampled at seven different time-points over 24 hours. Samples were analysed by liquid chromatography-tandem mass spectrometry (LCMS/MS) and quantitative estimations of drug concentrations were determined with reference to a standard curve. All analytical observations were complemented by flow cytometry and high resolution fluorescence microscopy.

**Results:** The anti-TB compounds showed marked differences in their ability to accumulate within the macrophage, especially following infection with *M. smegmatis*. This differential accumulation correlated with drug activity and, importantly, was consistent with the fluorescence intensity observed for the experimental compounds.

**Conclusion:** These results give insight into drug pharmacokinetics at the target site and support further studies, including investigations of the impact on drug levels of macrophage activation status and/or internalisation of *M. tuberculosis*. The dynamic system combining mycobacterial reporter strains with the inherently fluorescent phenoxazines offers the unique opportunity to complement LCMS/MS analyses with live cell imaging of intracellular *M. tuberculosis* under drug treatment.
The Development and Validation of a Direct LC-MS/MS Assay for the Determination of Tenofovir and Tenofovir-Diphosphate in Dried Blood Spots for the Analysis of Clinical Samples.

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Introduction and Aim: Tenofovir (TFV) and emtricitabine (FTC) are nucleoside reverse transcriptase inhibitors often used in pre-exposure prophylaxis (PrEP) trials, where antiretroviral drugs are administered to high risk HIV-negative individuals with the aim of preventing HIV infection should exposure occur. Both these drugs have been shown to be safe when taken either daily or intermittently, which is ideal for PrEP regimens where adherence may not be as high. The minimum number of doses estimated to confer high PrEP efficacy for a TFV/FTC regimen is four or more doses per week, resulting in a 95% lower risk of HIV acquisition. This is however highly dependent on various host factors of which adherence plays the largest role. The aim of the project is to develop a novel sensitive, specific and robust direct method for the measurement of adherence, utilising TFV and its metabolites in dry blood spots (DBS) through LC-MS/MS analysis. The difference in the half-life of the drugs will be used as a measure of recent and cumulative drug exposure. This will be used to elucidate adherence of patient samples. An indirect method is currently used, where tenofovir-diphosphate (TFV-DP) is dephosphorylated to TFV and subsequently quantified. This method is used, due to the polar nature of TFV and its metabolites, leading to difficulty in retaining and separating the analytes on a reversed phase analytical column. A direct method will be faster, less laborious and far less costly.

Methods: An ion-pairing reagent was used to increase the retention time and improve the chromatography of TFV and Tenofovir-diphosphate (TFV-DP). The method was optimised and validated using current FDA and EMA guidelines.

Results: The addition of dimethylhexylamine, as an ion-pairing reagent, resulted in a significant increase in retention time and separation of TFV, TFV-DP and tenofovir-monophosphate (TFV-MP). To allow the interaction of the analytes with the ion-pairing reagent, a pH of 9 was used.

Conclusion: In conclusion, the addition of dimethylhexylamine as an ion-pairing reagent allowed the direct quantification of TFV, TFV-MP and TFV-DP in DBS. Adequate baseline separation was achieved. This is essential for accurate quantification of TFV-DP, since it degrades to TFV in the source. The developed method is therefore a less laborious and time-consuming alternative to the indirect method.
Evaluation of Efficacies and Pharmacokinetics of a Novel Antimalarial Compound

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Introduction and Aim: Falciparum malaria-endemic areas present increasing symptoms of resistance to the current first line treatment comprising artemisinin combination therapies. New compounds that undergo redox cycling have been identified, which amplifies oxidative stress levels within the erythrocyte and generates ROS when oxidised back to the original molecule. Therefore we must develop new drugs based on these compounds for use in combination with existing therapies to combat resistance. In part of a PhD project and larger MRC Ship project, the aim of this study was to investigate in vitro ADME, as well as in vivo pharmacokinetics and efficacy of novel antimalarial compounds and assess candidates in new combinations.

Methods: In vitro ADME experiments are first conducted to predict physiological characteristics of compounds. Favourable ADME profiles of the screened group of compounds encourages further examination in vivo. Mice are administered orally or intravenously with the compounds at 20 mg/kg and 5 mg/kg, respectively to determine pharmacokinetic parameters. For efficacy studies, mice infected with P. berghei are dosed at 50 mg/kg daily for 4 days in order to determine percentage parasite suppression.

Results: ADME results warranted continuation into in vivo experiments. The pharmacokinetic experiments presented variable results whereby only a few compounds presented ideal properties. The in vitro efficacy experiments showed strong activity against Plasmodium falciparum but were less efficacious in in vivo tests against P. berghei.

Conclusion: It is evident that optimal pharmacokinetic parameters are critical to achieve significant efficacy levels for in vivo parasite suppression. Further investigation will determine the compound’s in vitro pharmacokinetic interactions in combinations with known antimalarials (such as artemisinin). Compounds will finally be tested in a SCID mouse model to test efficacies against the true parasite target.
Introduction and Aim: Biological medicines are substances derived from animal or other biological systems that are used to treat, diagnose or prevent diseases or disorders. The use of biological medicines has grown worldwide and has been paralleled with improved quality of life in patients due to better management of many disorders, particularly inflammatory diseases and cancer. However, in South Africa access to biological medicines remains limited. Moreover, the use of biological medicines poses challenges with regard to appreciating the minimum requirements for appropriate therapeutic response, and the difficulty in identifying their side-effects many of which are still unknown. Many biological medicines require preliminary tests for selection of patients who can benefit from them, and continuous monitoring of clinical response and adverse reactions during therapy is recommended. Also, there is wide variation in clinical response among patients largely due to patient and disease characteristics, which includes presence of autoantibodies, disease activity and severity, cytokine level, and immune cell phenotypes and genotypes, to mention but a few. These and many other factors should be taken into consideration before biological medicines can be used. Hence, it is envisaged that clinicians need to understand the major determinants of response and toxicity to biological medicines in their local population of patients to ensure cost-effective use of biological medicines. Therefore, the aim of this study was to investigate for factors that influence the utilization of biological medicines in South Africa.

Methods: Using a questionnaire, a prospective survey was conducted on newly qualified doctors (i.e., doctors with less than two years of practice) and still practicing in the Mangaung district (Bloemfontein) in the Free State. The study was approved by the University of the Free State Human ethics committee and the Free State department of health ethic committee. The doctors were identified at their point of work. Information sought included; the doctor’s particulars, experience in the use of biological medicines, available medical information resource on biological medicines, their role in patient’s care/management using biological medicines, their perception of biological medicines with regard to efficacy, toxicity or other, any problems with obtaining biological medicine, undergraduate training on biological medicines, procurement processes. Results were expressed as percentages.

Results: Out the 79 newly qualified doctors that were identified, 63 (80%) completed the questionnaire. From these, 63% did not know what biological medicines are, 70% indicated that biological medicines are not readily available to all clinicians, 70% suggested that there should be more lectures and seminars on biological medicines, 55% suggested that there should be more specific guidelines for use of biological medicines in textbooks; 17% suggested that more education is needed at undergraduate level, and 75% indicated that biological medicines are difficult to use because they do not have adequate on the pharmacology of biological medicines.

Conclusion: There is a general lack of knowledge on biological medicines among the newly qualified doctors, therefore, there is a need to educate these young doctors about biological medicines, and support in form of guidelines on the use of biological medicines to ensure that current patients benefit. Furthermore, there is a need for more emphasis on biological medicines during undergraduate training.
The Cardioprotective Potential of a Lanosteryl Triterpene from *Protorhus longifolia* on H9c2 Cardiomyocytes

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Introduction and Aim: Non-communicable diseases are attributed to 70 % of global deaths, with cardiovascular diseases contributing to over 33 % of the total disease burden. Cardiovascular diseases are the leading cause of morbidity and mortality in both diabetic and non-diabetic individuals. Importantly, diabetes mellitus is a major risk factor for the development of cardiomyopathies namely diabetic cardiomyopathy which is an understudied heart disease exclusive to people with diabetes. With the increasing prevalence of diabetes, the incidence of heart disease and in the poor quality of life are expected to increase. Furthermore, the lack of precise therapeutic strategies to protect the primary risk to the diabetic heart adds to the disease burden. Scientific evidence suggests that a lanosteryl triterpene (RA3) from the stem barks of *Protorhus longifolia*, which has anti-diabetic properties, could be beneficial in protecting the diabetic heart. In this study, we therefore aimed at investigating the aptitude of a lanosteryl triterpene (RA3) in reducing hyperglycaemic-induced shift in substrate preference, attenuating oxidative cell damage and apoptosis against diabetes-induced cardiomyopathy.

Methods and Results: H9c2 cardiomyocytes were exposed to, either normal (5.5 mM) or high (33 mM) glucose concentrations for 24 hrs. Subsequently, cells exposed to 33 mM glucose were treated with RA3 (0.1 µM) or metformin (0.1µM), as well as a combination of RA3 and metformin for a further 24 hrs. In vitro studies revealed that RA3 improved glucose utilization by decreasing myocardial fatty acid uptake and subsequent cell damage through reduced expression of MDA and increase in ATP metabolic activity. Furthermore, RA3 inhibited loss of membrane potential in high glucose-induced H9c2 cardiomyocytes as observed by an increase in 5’,6,6’-tetrachloro-1,1’,3,3’-tetraethylbenzimidazolyl-carbocyanine iodide (JC-1) ratio (orange:red fluorescence). RA3 was also able to reduce early and late apoptosis by reducing intracellular reactive oxygen species and DNA fragmentation, while increasing glutathione content.

Conclusion: This study offers scientific substantiation that RA3 increases glucose oxidation, attenuates β-oxidation and reduces oxidative stress damage and apoptosis in high glucose-induced H9c2 cardiomyocytes. The latter is especially important in protecting the primary risk to ‘diabetic heart’.
Dietary Phytomolecules Significantly Reduce Oxidative Stress of Mononuclear Cells of Patients with Rheumatoid Arthritis: An Ex Vivo Study

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Introduction and Aim: Rheumatoid arthritis (RA) is an autoimmune disease responsible for significant human morbidity in modern life. Oxidative stress is the ultimate potential biomarker for determining disease activity in patients with RA. Different numbers of dietary phytomolecules are already being established as significant antioxidant activity in isolated synovial cellular infiltrate or peripheral blood neutrophils and lymphocytes. The present study scientifically validated the effectiveness of two dietary phytomolecules to neutralize the free radical mediated oxidative stress in isolated peripheral blood mononuclear lymphocytes (PBML) of patients with RA.

Methods: The study population included patients with Rheumatoid arthritis, RA (n =15) who fulfilled the American College of Rheumatology criteria for RA. Peripheral blood was collected and isolated mononuclear lymphocyte cells (PBML) were pretreated with phorbol myristate acetate (PMS) and further incubated with different concentrations of two selected phytomolecules Naringenin and β-carotene; and also with synthetic N-acetyl cysteine (NAC) in an ex-vivo condition. Resultant cell lysate were used for further studies and validated for determination of other oxidative biomarkers.

Results: An increase in superoxide (O2-) and nitric oxide (NO) production (p<0.01) was observed when PBML were treated with PMS. Importantly, the increased oxidative stress was effectively decreased by the selected plant derived compounds i.e. β-carotene and naringenin. However, no significant changes were observed in SOD content in cells treated with both the phytomolecules.

Conclusion: The study scientifically evaluated the efficacy of two phytomolecules as compared to synthetic antioxidant NAC. Collectively, our results indicate that both β-carotene and naringenin may be a promising non-toxic food supplement in attenuating the oxidative stress associated pathology in RA, meriting further pharmacological studies on others inflammatory cells like neutrophils.
**Immunostimulatory Properties of Fructans Derived from Raw Garlic (Allium sativum L.)**

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**Background and Aim:** Garlic (Allium sativum L.), commonly used as a spice and traditional medicine has innumerable biological activities including immunomodulation. Fructans are present in abundance in garlic. Although fructans from aged garlic extract have been shown to exert immunomodulatory properties, such information is lacking for fructans from raw garlic. The main objective of this study was to isolate raw garlic fructans and investigate its effects on the cells of the immune system in vitro.

**Methods:** Raw garlic fructans (detected by cold anthrone assay) was isolated, in a yield of ~14 g/100 g (fresh weight) garlic, from the flow-through pool of Q-Sepharose chromatography of raw garlic extract. Proliferation of murine splenocytes by raw garlic fructans was assessed by MTT assay. Activation of macrophages in peritoneal exudates cells was followed by the release of nitric oxide (NO) and phagocytosis of yeast cells.

**Results:** Raw garlic fructans stimulated murine splenocytes to a similar extent as seen in the case of established polysaccharide immunostimulators (galactan, arabinogalactan, mannan and zymosan); 4-5 fold stimulation was seen compared to untreated cells (p<0.01). Further, raw garlic fructans activated macrophages present in rat peritoneal exudates to release NO which was significant at both 24 h and 48 h (p<0.01). Yeast cell phagocytosis by activated macrophages was significantly enhanced (p<0.05) by raw garlic fructans in comparison to untreated cells.

**Conclusion:** Raw garlic fructans causes significant immunostimulation of murine lymphocytes and macrophages in vitro. Since fructans are present in abundance in raw garlic, it appears to be promising for the development of functional foods for immunomodulation by its inclusion in processed foods.
Nephroprotective Effects of a Lanosteryl Triterpene Isolated from Stem Bark of *Protorhus longifolia*

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Introduction and Aim: Metabolic disorders and drug-induced nephrotoxicity are among the most common causes of nephropathy. Plant-derived triterpenes could become potential targets in the development of new generations of pharmacological active nephroprotective drugs. This study evaluated the nephroprotective effect of a lanosteryl triterpene (RA-3) isolated from stem bark of *Protorhus longifolia*.

Methods: The nephroprotective effect of RA-3 was investigated in gentamicin-adenine induced kidney injury and in isoproterenol (ISO)-induced myocardial infarction in rat models. The rats received gentamicin (40 mg/kg, intraperitoneal) and adenine (150 mg/kg, oral) daily for 14 days to induce nephropathy. To evaluate the nephroprotective effect of RA-3 against the secondary cause of nephropathy, other groups of rats were subcutaneously injected with ISO (85 mg/kg) daily for two consecutive days at 24 h intervals to induce myocardial injury. Animals in the experimental groups were orally administered with RA-3 at 50 and 100 mg/kg daily for 14 days. Blood and kidney samples were then collected and analysed for some renal dysfunction biomarkers and histopathological changes.

Results: Elevated levels of blood urea nitrogen (BUN, 22.5 ± 1.11 mg/dL), serum creatinine (Scr, 48 ± 1.78 mg/dL), uric acid (UA, 0.61 ± 0.10 mg/dL) and angiotensin-converting enzyme (ACE, 138 ± 39.61) were observed in the untreated group of nephropathy; BUN (7.4 ± 1.16 mg/dL), Scr (23.3 ± 4.37), and UA (0.40 ± 0.05) were observed in the untreated myocardial infarction group. However, treatment of the animals with RA-3, especially at 100 mg/kg, effectively lowered these parameters; BUN (4.00 ± 0.29 mg/dL; 4.56 ± 0.38 mg/dL), Scr (8.25 ± 2.28 mg/dL; 13 ± 2.31) and UA (0.16 ± 0.02 mg/dL; 0.18 ± 0.04) in both the animal models. Improved histomorphological changes of the kidneys from the triterpene-treated groups were also observed when compared to the untreated groups.

Conclusion: Although molecular mechanisms involved remain to be established, the results indicate that the lanosteryl triterpene possesses nephroprotective potential.
Acute and Subchronic Oral Toxicity Evaluation of Aqueous Root Extract of *Dicoma anomala* (Sond.) in Wistar Rats

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**Introduction and Aim:** The present study evaluated the safety of aqueous root extract of *Dicoma anomala* (AQRED) through acute and subchronic toxicity studies.

**Methods:** Single oral dose of AQRED at the concentration of 0, 5, 300, and 2000 mg/kg as well as 125, 250, and 500 mg/kg/day was administered to rats for 14-day acute and 90-day subchronic oral toxicity studies.

**Results:** The results revealed no mortalities or observed clinical signs of toxicity in all the rats during both investigation periods. In subchronic toxicity testing, administration of AQRED also did not cause any changes in body weight as well as food and water consumption patterns. The haematological parameters and blood chemistry revealed no significant difference (*p* > 0.05) between the treatment and the control except in platelet count, alkaline phosphatase, and sodium levels where there was a significant increase (*p* < 0.05), although there was also a significant reduction (*p* < 0.05) in alanine transaminase, aspartate transaminase, and creatinine when compared to control. However, these changes were not reflecting the results from histology.

**Conclusion:** Conclusively, the obtained results suggested that the LD$_{50}$ of AQRED is in excess of 2000mg/kg and its oral administration for 90 days revealed that it is unlikely to be toxic, hence, safe.
Erythritol Reduces Small Intestinal Glucose Absorption, Increases Muscle Glucose Uptake, Improves Glucose Metabolic Enzymes Activities and Increases Expression of Glut-4 and IRS-1 in Type 2 Diabetic Rats

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Introduction and Aim: Studies have reported that erythritol, a low glycemic sugar alcohol possesses anti-hyperglycemic and anti-diabetic potentials but the underlying modes of action is not clear. This study investigated underlying modes of action behind the anti-hyperglycemic and anti-diabetic potentials of erythritol in different experimental models (experiment 1, 2 and 3).

Methods: Experiment 1 examined the effects of increasing concentrations (2.5 – 20%) of erythritol on glucose absorption and uptake in isolated rat jejunum and psoas muscle respectively. Experiments 2 and 3 examined the effects of a single dose of erythritol (1 g/kg bw) on intestinal glucose absorption, gastric emptying and postprandial blood glucose increase, glucose tolerance, serum insulin level, muscle/liver hexokinase and liver glucose-6 phosphatase activities, liver and muscle glycogen contents and mRNA expression of muscle Glut-4 and IRS-1 in normal and type 2 diabetic animals.

Results: Experiment 1 revealed that erythritol dose-dependently enhanced muscle glucose uptake ex vivo. Experiment 2 demonstrated that erythritol feeding delayed gastric emptying and reduced small intestinal glucose absorption as well as postprandial blood glucose rise, especially in diabetic animals. Experiment 3 showed that erythritol feeding improved glucose tolerance, muscle/liver hexokinase and liver glucose-6 phosphatase activities, glycogen storage and also modulated expression of muscle Glut-4 and IRS-1 in diabetic animals.

Conclusion: Data suggest that erythritol may exert anti-hyperglycemic effects not only via reducing small intestinal glucose absorption, but also by increasing muscle glucose uptake, improving glucose metabolic enzymes activity and modulating muscle Glut-4 and IRS-1 mRNA and protein expression. Hence, erythritol may be a useful dietary supplement for managing hyperglycemia, particularly for type 2 diabetes mellitus.
Introduction and Aim: Type 2 diabetes remains one of the leading causes of death worldwide. Persistent hyperglycemia within a diabetic state is implicated in the generation of oxidative stress and aggravated inflammation that is responsible for accelerated modification of pancreatic beta cell structure. Here we investigated whether a lanosteryl triterpene, methyl-3β-hydroxylanosta-9,24-dien-21-oate (RA-3), isolated from Protorhus longifolia can improve glucose tolerance and pancreatic beta cell ultrastructure by reducing oxidative stress and inflammation in high fat diet and streptozotocin-induced type 2 diabetes in rats.

Methods: the anti-hyperglycemic activity of the triterpene was evaluated in high-fat diet (HFD) fed and streptozotocin (STZ) induced diabetes in rats. The hyperlipidemic rats received a single intraperitoneal injection of STZ (30 mg/kg body weight) to induced diabetes. The experimental animals were orally administered with a single dose of RA-3 (100 mg/kg body) daily for 28 days. At the end of the experimental period an oral glucose tolerance test was also performed to the overnight fasted rats. Blood and pancreas were then collected for analysis of some biochemical parameters and histopathology, respectively.

Results: the untreated diabetic rats showed increased fasting plasma glucose and C-peptide levels. These untreated diabetic rats further demonstrated raised cholesterol, interleukin-6 (IL-6), and lipid peroxidation levels as well as destructed beta cell ultrastructure. Treatment with RA-3 was effective as metformin in improving glucose tolerance and antioxidant effect of the diabetic rats. Interestingly, RA-3 displayed a slightly enhanced effect than metformin in reducing elevated IL-6 levels and improving beta cell ultrastructure.

Conclusion: Although involved molecular mechanisms remain to be established, RA-3 demonstrates a strong potential to improve pancreatic beta cell ultrastructure by attenuating impaired glucose tolerance, reducing oxidative stress as well as inflammation.
Effects of *Aloe ferox* Leaf Powder on Glucose Biomarkers in Rodents

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**Introduction and Aim:** In 2015, there were 415 million adults with diabetes globally and the prevalence is projected to increase to 642 million by 2040, of which type 2 (obesity related diabetes) accounts for 91%. Both obesity and diabetes therefore contribute largely to the increase in mortality rate globally. There is a therapeutic need for new, safer anti-diabetic and anti-obesity therapies. Fructosamine determines the glycosylation of protein in the blood which is a biomarker for diabetes. The aim of the study was to determine the effects of *Aloe ferox* leaf powder on glucose biomarkers in rodents.

**Methods:** Sprague Dawley rats were fed with either a high fat diet (HFD), or a high fat diet containing *Aloe ferox* leaf powder (HFD-AL) over a period of 12 weeks to induce obesity and glucose intolerance. The animals were weighed weekly and their food consumption was monitored three times a week. At the end of the study, the animals were sacrificed and blood samples were collected for analysis of fructosamine, liver function, total cholesterol, triglycerides, LDL and HDL. Histopathology was also performed.

**Results:** Body weight gain was lower in animals on high fat diet with *Aloe ferox* leaf than the control. The final weight observed was 337.13 ± 6.37 g for HFD-AL compared to 349.50 ± 7.64 g of HFD. Fructosamine level was significantly lower in HFD-AL than HFD at p≤0.05. HFD-A showed fructosamine level of 145.00 ± 6.32 μmol/L compared to 154.50 ± 7.03 μmol/L of HFD. HFD-AL also presented with lower cholesterol and triglycerides levels than HFD. ALP was lower in HFD-AL than in the control, with ALT and AST comparable to the control. Less adipose tissue was obtained in HFD-AL as compared to the control. Histology examination did not reveal any lesions as a result of toxic effects in any of the organs in both groups.

**Conclusion:** *Aloe ferox* leaf powder showed a possible hypoglycaemic effect in animals fed with high fat diet.
Specific Bioactive Compounds from Ginger, Tea, and Apple Prevent Diabetes-Related Cataract via Inhibition of Aldose Reductase

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Introduction and Aim: Diabetes and its secondary complications are of major concern worldwide. Hyperglycemic stress activates polyol pathway and aldose reductase (AR) is one of the key enzymes responsible for generating secondary complications during diabetes. Aldose reductase inhibitors from dietary sources are gaining interest as potential therapeutic agents for diabetics. In this study the therapeutic potential of phloretin, a dihydrochalone, a type of natural phenol found in apple tree leaves and apricots, epigallocatechin 3-gallate (EGCG) from green tea (Camellia sinensis) and [6]-gingerol from the ginger plant (Zingiber officinale), were evaluated for their AR inhibitory activity in vitro and in vivo.

Methods: In vitro screening of bioactive compounds on cell viability and AR activity in high glucose induced human retinal pigment epithelial (HRPE) cells were evaluated. Male C57BL/6J mice were randomly assigned to one of the different treatments (3 bioactive compounds at 2 concentrations each) with either a low fat diet (LFD) or high fat diet (HFD). Bioactive compounds were administered intraperitoneal (i. p.) three times a week. After sixteen weeks, AR activity was determined in the heart, eyes and kidney of the test mice.

Results: Under high glucose conditions, cell viability decreased compared to the untreated cells and aldose reductase activity increased 2 - 5 folds from 24 to 96 h. Pre-treatment of cells with phloretin, EGCG and [6]-gingerol improved cell viability and inhibited AR activity. The enzyme inhibition kinetics followed a non-competitive mode of inhibition for phloretin and EGCG whereas [6]-gingerol indicated non-competitive type of inhibition against AR. These in vitro findings were further validated in the in vivo mouse model where HFD group developed diabetes over time, with higher blood glucose levels 260 ± 27 mg/dL compared to 130 ± 20 mg/dL glucose level in LFD group. Furthermore, the eye lens of the mice from HFD group had developed cataract and the AR activity increased four folds compared to the group fed with a normal diet. Administration of EGCG, phloretin and [6]-gingerol significantly reduced both blood sugar levels and AR activity.

Conclusion: This study supports the antidiabetic potential of the tested bioactive dietary compounds, which were found to provide cytoprotection and inhibit aldose reductase, a key enzyme associated with the onset of diabetes-related complications.
Investigating the Glucose Modulation Activity of Herbal African Traditional Preparations (ATPs) Used to Manage Diabetes

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Introduction and Aim: Type 2 diabetes (T2D) is the most prevalent form of diabetes mellitus. It is characterized by a chronic hyperglycemic condition resulting from a defect in insulin secretion and insulin resistance or both. Some of the antidiabetic therapeutic approaches used are to inhibit carbohydrate-digesting enzyme such as α-amylase and α-glucosidase, to delay absorption of glucose in the gastrointestinal fluid and to improve glucose uptake by mammalian cells. The aim of the study was to investigate the types of ATPs administered by Traditional Health Practitioners (THPs), with alleged antidiabetic properties for α-amylase and α-glucosidase inhibition activity and enhancing glucose uptake.

Methods: An ethnomedicinal survey was conducted in four locations Thohoyandou, Makhado, Malamulela and Pretoria where ATPs were collected. Pre-tested semi-structured questionnaire and interviews were conducted with 12 THPs and a list of medicinal plant used to prepare ATPs was compiled. ATPs were filtered, the aqueous was freeze-dried and tested for ability to inhibit porcine pancreatic α-amylase and rat intestinal α-glucosidase. ATPS were screened against C2C12 muscle cell measuring utilization of glucose.

Results: Twenty one medicinal plants were documented belonging to 8 different families. ATPs 3, ATPs 10, ATPS 11 and ATPs 12 inhibited rat intestinal α-glucosidase with an IC50 of 0.54mg/ml, 0.21mg/ml, 0.38mg/ml and 0.17mg/ml, respectively. While cells treated with ATPs 3 and P11 were able to utilized glucose by 71.7% and 91.7%, respectively.

Conclusion: In summary, all the preparations did not show inhibition of α-amylase activity above 25% at highest concentration. ATPs 3 and ATPs 11 were most active in inhibiting α-glucosidase and enhancing the uptake of glucose.
Lansoprazole-Sulfide, Pharmacokinetics of this Promising Anti-Tuberculous Agent

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Introduction and Aim: This study is based on evaluating the plasma and tissue concentration and distribution of the FDA approved proton pump inhibitor (PPI) lansoprazole (LPZ). This is a well-known PPI that reduces gastric acid secretions through the inhibition of H+K+-ATPASE pump in parietal cells. It was also reported to be a prodrug, since its major metabolite lansoprazole sulphide (LPZS) has in vitro (IC50 values of 0.59 µM) and in vivo activity against Mycobacterium tuberculosis (M. tb). However, high oral dosage of LPZS (up to 300 mg/kg) was recently reported to be necessary to reach in vivo therapeutic concentration against tuberculosis (TB). This drug is known to target cytochrome bc1 within the cytoplasm of M. tb host cells, which leads to the breakdown of its respiratory chain followed by rapid ATP depletion. Due to the crisis of M. tb resistance prevalence, there is an emphasis on the continuous need for new antibiotics targeting this pathogen. South Africa is not only in the midst of the tuberculosis (TB) burden but also suffers from Human Immune Deficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS). The purpose of this study was to investigate if a low dose of 15mg/kg LPZ administered to a healthy rat model could result in sufficient conversion to LPZS, for reaching the required in vivo therapeutic concentration. Repurposing of existing drugs into antibiotics capable of inhibiting multidrug resistant strains is urgently needed and the approval of novel antibiotic drugs requires extensive effort and long time periods from development to approval.

Methods: In order to reach the goals of this study, Sprague-Dawley healthy female rats were orally dosed (n=3) with LPZ (15 mg/kg), followed by the same dose of LPZS through intraperitoneal administration. LPZ and its metabolite, LPZS, concentrations were quantified time intervals of 0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0 and 24 hours after the treatment. The evaluation of LPZ and LPZS were simultaneously conducted by means of a sensitive, accurate, robust and selective liquid chromatography tandem mass spectrometry method. The validated method complied with the European Medicines Agency guidelines and the mean percentage recoveries and relative standard deviations were within ±15% deviation in all analyses and there were no significant matrix effects.

Results: Through intraperitoneal dose of LPZS, high concentrations were found in plasma and lung, 7841.1 and 9761.2 ng/mL respectively, which were significantly higher than the published MICs for M. tb. Oral administration of LPZ did not result in the sufficient metabolic conversion of LPZ to its anti-TB metabolite. However, LPZS itself reached higher plasma and tissue concentrations with intraperitoneal administration, when compared to a previous study, which used oral administration.

Conclusion: This finding warrants further studies investigating on M. tb infected animal models and most importantly to investigate if intravenous administration of LPZS could potentially be used in clinical practice.
The Downfall of TBA-354 – A Possible Explanation for its Neurotoxicity via Mass Spectrometric Imaging

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Introduction and Aim: Tuberculosis (TB) is one of the well-recognized ancient human diseases in the history of mankind and it remains the major cause of human death amongst the transmittable diseases despite the use of antitubercular antibiotics. TB is caused by a pathogenic bacterium known as Mycobacterium tuberculosis (M. tb). The M. tb bacteria primary site of infection is the human lungs (resulting in pulmonary TB) but it can also affect other body parts (extrapulmonary TB) such as bones, central nervous system (CNS), liver and many more others. Present-day TB research is focused on the development of more effective anti-TB drugs that can help shorten the treatment period. One of the major set-backs in TB drug development is to find the balance between the potential drug’s side effects and its activity. TBA-354 is recognized to have an excellent activity against M. tb strains but also known to have mild signs of neurotoxicity. The present study demonstrates the potential of liquid chromatography-tandem mass spectrometry (LC-MS/MS) and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) techniques in the evaluation of the fundamental in-vivo pharmacokinetics and tissue distribution properties of a bicyclic nitroimidazole derivative, TBA-354.

Methods: The study was conducted on healthy female Sprague-Dawley rats by administering 20 mg/kg of the drug, via an intraperitoneal route. After dosing the biological samples (plasma, lungs and brain) were collected at different time points for analysis. A validated LC-MS/MS method was used to quantify TBA-354 in rat plasma, lung and brain homogenate samples. LC-MS/MS cannot provide enough information regarding the drug localization and where it accumulates in the brain, therefore, MSI was then used to study the accumulation of the drug in different regions of the brain.

Results: As per LC-MS/MS results, the drug showed significant pharmacokinetic and distribution properties in the rat model with the highest levels in plasma compared to lung and brain. MSI results showed that the drug was effectively able cross the blood-brain barrier (BBB) resulting in toxic accumulation in the neocortical regions of the brain. The use of MSI in this study shows the exact localization and accumulation of the drug in the brain, providing evidence as to why it showed certain neurotoxic signs during clinical trials.

Conclusion: This study has proven the efficacy of MSI as a suitable analytical technique that can be used in future preclinical studies to evaluate the neurotoxicity of drugs targeting the brain, thus minimizing possible side effects.
Clofazimine Protects Against *Mycobacterium tuberculosis* Dissemination in the CNS Following an Aerosol Challenge in a Murine Model

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**Introduction and Aim:** Tuberculosis (TB) has been the scourge of the human race for many decades, claiming countless number of lives along the way. This is further complicated by the ability of *Mycobacterium tuberculosis* (*M. tb*) to infect extra-pulmonary sites, more specifically the brain. These forms of TB are difficult to treat due to the problems associated with drug delivery across the blood brain barrier (BBB). Linezolid (LZD) and clofazimine (CFZ) are two of the more promising anti-TB antibiotics in recent times. Therefore, the aim of this study was to evaluate the brain penetration of LZD and CFZ, using LC-MS and mass spectrometry imaging (MSI) in a murine model of TB.

**Methods:** In this study Balb/c mice were aerosol infected with *M.tb* H37Rv and treated for four weeks with both LZD (100mg/kg.b.w) and CFZ (100mg/kg.b.w). Animals were sacrificed weekly to allow for drug quantitation in serum and for drug distribution via MSI. Concurrently, we investigated if an aerosol TB infection would lead to the dissemination of TB bacilli into the brain by determining post-treatment CFU counts in tissue.

**Results:** CFZ displayed a strong bactericidal effect in the lung, while LZD had a bacteriostatic effect. *M.tb* appeared after one week post-infection in the untreated group (2.38±0.43 log_{10}CFU) and more surprisingly after two weeks’ post-infection in the LZD (1.14±0.99 log_{10}CFU). TB bacilli could not be detected in the brains of the CFZ group. Each of the drugs displayed different pharmacokinetic profiles, with CFZ increasing gradually over time and LZD peaking within the first week and maintaining a steady state throughout the study. MSI analysis showed that CFZ was widely distributed throughout the brain while LZD localized in the brainstem. This difference in distribution may explain the absence of TB bacilli in the brain of the CFZ treated group.

**Conclusion:** This is the first study to show the appearance of *M.tb* in the brain after an aerosol TB infection in a mouse. This study may advocate for the use of CFZ as a prophylactic treatment to prevent the development of extra-pulmonary TB of the CNS, using a two-pronged approach. Firstly, CFZ maintains low bacterial numbers in the lung, preventing entry into the systemic circulation and secondly its widespread distribution in the brain may protect against the entry of bacterial entities.
Comparison of Different Reverse Phase Columns on the Separation of the Geneva Phenotyping Cocktail by High Performance Liquid Chromatography-Tandem Mass Spectrometry

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Introduction and Aim: Phenotyping cocktails are used to assess metabolic and transport phenotypes in vivo and consist of a number of probe drugs for simultaneous quantitation in different biological matrices. The most commonly used reversed phase column in high performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) method development are the alkyl C18 phase. Multidrug cocktails often consist of related substituted aromatic and polycyclic compounds that may require more selective separation. The objective of this study was to compare the selectivity of a Kinetex Biphenyl column to a Kinetex C18 column on the separation of the 7 probe drug Geneva phenotyping cocktail.

Methods: After tuning each compound to optimise source detection parameters, known concentrations of each probe drug, including internal standards, were prepared in 50% methanol. Separation was achieved by HPLC on a triple quadrupole LC-MS/MS system consisting of an Agilent combination 1100 & 1200 series LC system coupled to an AB Scie 4000 Qtrap equipped with a Turbo “V” electrospray ionization source. Isocratic runs with 60:40 methanol/water and 40:60 acetonitrile/water with similar elution strength were used as mobile phases on both the Kinetex C18 and Kinetex Biphenyl columns with similar silica backbones. Scatter plots were drawn comparing the logarithm of the retention factors (log k’) for all compounds on the biphenyl column against their respective log k’ values on the C18 column for both mobile phase conditions. The slopes and correlation coefficients were determined from linear regression analysis.

Results: Results showed that the alternative selectivity of the Kinetex Biphenyl column using methanol as co-eluent mostly resolved the separation of similarly structured aromatic compounds. Lower correlation coefficients and slope values gave an indication of the differences in selectivity. Better peak resolution with methanol was attributed to increased non polar interaction of compounds with the biphenyl stationary phase as opposed to acetonitrile that suppressed the π-π interactions of the aromatic compounds with the biphenyl stationary phase by competitive interaction with both solute and stationary phase phenyl groups.

Conclusion: In this study the selectivity of the Geneva phenotyping cocktail was altered by pairing a biphenyl stationary phase with methanol taking advantage of the increased non-hydrophobic π-π interactions on the biphenyl column in addition to hydrophobic interactions by using methanol as the mobile eluent. This combination mostly resolved co-elution of similarly structured aromatic compounds.
Introduction and Aim: The prevalence of diabetes is increasing annually, affecting more than 150 million (about 4.6%) people globally and the projection is pegged at well above 300 million before 2025. With this projection, someone dies from its complications every 10 sec and 1 in every 5 individuals may be diabetic by 2025. The continuous search for new lead compounds as viable inhibitors of specific enzymes linked to carbohydrate metabolism has intensified. *Cyperus esculentus* L. is one of the therapeutically implicated botanicals against several degenerative diseases including diabetes mellitus. This study evaluated the membrane stabilization and mechanisms of inhibitory potential of aqueous extract of *C. esculentus* on α-amylase and α-glucosidase in vitro.

Methods: The extract was evaluated for its membrane stabilization effect against bovine serum erythrocytes. The α-amylase inhibitory potential of the extract was investigated by reacting its varying concentrations with the enzyme and starch solution, while the α-glucosidase inhibition was determined by pre-incubating α-glucosidase with different concentrations of the extract followed by addition of *p*-nitrophenylglucopyranoside. Lineweaver-Burke plot was used to predict the manner in which the enzymes were inhibited.

Results: The data obtained revealed that the extract moderately and potently inhibited the specific activities of α-amylase and α-glucosidase, respectively. The inhibition was concentration-related with respective IC50 values of 5.19 and 0.78 mg/mL relative to that of the control (3.72 and 3.55 mg/mL). The extract also significantly stabilized erythrocyte membrane, scavenged free radicals and the effects elicited could be ascribed to its phytoconstituents.

Conclusion: The respective competitive and uncompetitive mode of action of the extract is due to its inhibitory potentials on the activities of α-amylase and α-glucosidase which is crucial to modulating glucose metabolism. Going forward, in addition to completely characterize the exact compounds responsible for the elicited activity in this study, pertinent attention will be given to the *in vivo* evaluation of the identified constituents.
An Ethnobotanical Survey and Anti-Plasmodial Activity of Medicinal Plants Indigenous to Namibia and South Africa

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Introduction and Aim: The recent malaria outbreak that affected 6 500 people in Namibia earlier this year shows that malaria is still a serious health hazard in Southern Africa. Although introduction of artemisinin-based combination therapy (ACT) has effectively reduced the prevalence of malaria, the use of ACTs is limited by the small number of available and affordable co-formulated anti-malarial drugs. The search for new lead compounds from medicinal plants used traditionally in the treatment for malaria remains necessary. The aim of this study was to investigate medicinal plants used traditionally for treatment of malaria by local communities in Namibia and South Africa, for anti-plasmodial activity in vitro and in vivo, and to identify chemical constituents responsible of the activity.

Methods: An ethnobotanical survey conducted in the Northern regions of Namibia resulted in collection of 14 plant species. A few other plants were collected in the Western Cape, South Africa. The plants were extracted sequentially with organic solvents. The resultant extracts were subjected to anti-plasmodial activity testing in vitro against chloroquine sensitive and resistant strains of Plasmodium falciparum; 3D7, D10 and K1 respectively. Active extracts were evaluated for cytotoxicity against CHO cell lines. Selected extracts were further evaluated for acute toxicity in mice at concentration of 500mg/ml over a period of 30 days, and for parasite suppression activity in a Plasmodium berghei-mouse model. Bio-guided fractionation and application of various chromatographic techniques on selected extracts lead to purification and identification of compounds responsible for the anti-plasmodial activity.

Results: The study reports on the in vitro and in vivo anti-plasmodial activity of Achillea millefolium and Eriocephalus africanus extracts, their selectivity indexes, and their chemical constituents responsible for activity. The DCM leaf extracts of E. africanus and A. millefolium exhibited anti-plasmodial activity with IC₅₀ values of 8.5 ± 0.5 μg/ml and 3.1 ± 0.4 μg/ml respectively against the D10 and K1 strains, and less cytotoxicity against CHO cells with IC₅₀ values of 89.1 ± 5.2 μg/ml and 69.6 ± 3.5 μg/ml respectively. The purified fractions of A. millefolium had enhanced activity (1.3 ± 0.16 μg/ml) and no cytotoxic effect (>100μg/ml) in the tested concentration range. The study lead to isolation of three chemical compounds from E. africanus and one sesquitopene from A. millefolium. When evaluated for acute toxicity, DCM extract of E. africanus produced physical signs of toxicity and mortality while the DCM extract of A. millefolium was well tolerated. The active fraction of A. millefolium suppressed the parasitemia by 67% on day 6, and throughout the duration of the treatment phase.

Conclusion: Sesquiterpenes isolated from A. millefolium are responsible for the in vitro and in vivo anti-plasmodium activity observed. Medicinal plants continue to be a promising reservoir for discovery of new lead compounds that can be developed further into malaria treatments.
A Prospective Strategy to Advance Age-Old Anti-Malarial Agent: Approach with a Biocompatible Nanoparticle

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Introduction and Aim: Biocompatible nanoparticles are receiving an escalating significance as drug delivery mediators to depict justification against a variety of diseases. Chitosan, is a biocompatible polysaccharide obtained by deacetylation of chitin. It has drawn a growing scientific interest due to its unique physicochemical and biological characteristics, and its safety and efficacy in drug delivery systems. This study highlights the efficacy potentiation of an age-old antimalarial drug, chloroquine (CQ) after delivery with chitosan-tripolyphosphate (CS-TPP) nanoparticle against \textit{Plasmodium berghei} NK65.

Methods: The CS-TPP particle was prepared through ion tropic gelation method. After successive infection development by intra-peritoneal infected blood injection in Swiss mice, a number of parameters were measured and these included the parasitemia, ROS generation in peripheral blood lymphocyte (PBMC), mitochondrial membrane potential, anti apoptotic and pro apoptotic protein levels in liver and spleen.

Results: The results showed that CS-TPP conjugated CQ efficacy improved and that host organ damage were comparatively less compared to those of animals treated with CQ alone. This study suggests that, \textit{P. berghei} NK65 induces oxidative imbalance in blood lymphocytes and causes programmed cell death by reducing the mitochondrial membrane potential. CS-TPP conjugated chloroquine has capability to reduce the parasitemia as well as oxidative stress and tissue damage.

Conclusion: The application of CS-TPP nanoparticles conjugated CQ against rodent malaria has encouraging results in protecting the host’s system and maintaining normal homeostasis than when CQ is used alone. More research needs to be further done to conclusively confirm these preliminary results.
Efficacy, Safety and Tolerability Study of Arterolane Maleate and Piperaquine Phosphate Combination (Synriam) versus Artemether-Lumefantrine (Co-Artem) for the Treatment of Uncomplicated *Plasmodium falciparum* Malaria in Olabisi Onabanjo University Health Services in Nigeria

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**Introduction and Aim:** Artemisinin based combination therapy (ACT) has become the standard of care for the treatment of uncomplicated *Plasmodium falciparum* malaria in the world. Although several ACT regimens are approved by the World Health Organisation (WHO), data guiding optimal choices of ACTs are limited. Artemisinin-based combination therapy of artemether-lumefantrine is currently used for the first line treatment of uncomplicated *P. falciparum* malaria. However, limited efficacy and tolerability data are available on alternative forms of ACT. There has been reported cases of reduction in the susceptibility of artemether-lumefantrine (Coartem®) to malaria treatment. This study was conducted to compare the efficacy and tolerability of two fixed-dose formulation of ACT, arterolane maleate and piperaquine phosphate (Synriam®) a 72 hours complete dosage regimen with dearth of information about its pharmacology and artemether-lumefantrine (Coartem®) for the treatment of *P. falciparum* in Olabisi Onabanjo Health Services in Nigeria.

**Methods:** A randomized, open-label trial was conducted comparing the efficacy of a three day regimen of Synriam® one tablet of 1000 mg daily for three days (2.2mg/kg arterolane plus 10.7mg/kg of Piperaquine Phosphate per day), and Coartem® eight tablets per day over three days (equivalent to the total adult dosage of 3360 mg), for the treatment of adults with uncomplicated falciparum malaria. The primary endpoint was at day 42, PCR-corrected, parasitological cure rate, and the secondary endpoints were parasite and fever clearance time and tolerability. Of 64 patients enrolled, 31 were treated with Synriam® and 33 with Coartem®.

**Results:** Of the patients who completed the 42 days follow-up period or had a recurrence of malaria, 28 were on Synriam® and 29 were on Coartem®. Recrudescence parasitaemia was PCR-confirmed for all patients in each treatment group, with cure rates at day 42 of 98% (98% CL: 90-100) for both forms of ACT. The median parasite clearance time was significantly slower in the Coartem® group compared with the Synriam® group (48h vs 36h, P<0.005) and fever clearance times shorter in Synriam® group (12h vs 24h, P<0.05). The two forms of ACT were well tolerated with no serious adverse events.

**Conclusion:** The two forms of ACT were highly efficacious in the treatment of uncomplicated *P. falciparum* malaria. Although the 3 tablets 72 hours dosage regimen of Synriam® was equally as effective as the three-day course of Coartem®, parasite clearance time and fever clearance times were shorter in the Synriam® group. Further studies are warranted in different regions of the world to determine the nationwide efficacy of Synriam®.
Twenty years’ (1996-2015) Trends in Deaths Caused by Poisoning in the Transkei Sub-Region of South Africa

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Background and Aim: Poisoning is a serious public health problem worldwide. Acute poisoning from traditional medicine is a known cause of death in South Africa. Members of the Xhosa tribe in the Transkei region frequently consult traditional healers and use herbal medicine, when they fall ill. The objective was to study the trends in deaths caused by poisoning in the Transkei sub-region of South Africa from 1996 to 2015.

Methods: An autopsy record review study at the Forensic Pathology Laboratory at Mthatha for a period of 20 years (1996-2015).

Results: Over a period of 20 years, 24,693 autopsies were conducted. In 1,139 (4.61%) of these cases, death was caused by poisoning. Male victims numbered 609 (53.46%). The male-to-female ratio was 1.1:1. More than one third of causes 360 (32.78%) were aged between 21 and 30 years of age. The average number of poisoning-related deaths was 8.9 per 100,000 of the population per year. The highest rate of death, 16.6 per 100,000, was recorded in 2012.

Conclusion: There has been an increasing trend in death as a result of poisoning in the Transkei sub-region of South Africa.
Comparative Neuropharmacological Activities of Oral and Intraperitoneal Administration of Sesquiterpene-rich Essential Oil of *Lantana camara* L. Fresh Leaf from Southwest Nigeria in Mice

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**Introduction and Aim:** *Lantana camara* L. (family: Verbenaceae) is a well-known medicinal plant found in several regions of the world and is well distributed in southwestern states of Nigeria where it is used in the treatment of various diseases including malaria, asthma, epilepsy, toothache, rheumatism, snake bite, fever, cough and malaria. The plant has been reported to be poisonous to grazing animals in some countries including South Africa and India. The acute toxicity profile of the essential oil of the plant has not been documented nor its central activities in laboratory animals, hence this study evaluated the essential oil of fresh leaf of *L. camara* (EOLC) for acute toxicity profile and some neuropharmacological activities.

**Methods:** Fresh leaf and fruit of the plant were collected and hydro-distilled, the essential oils obtained were thereafter analysed to determine their chemical components. The fresh leaf oil was further evaluated for acute toxicity (LD₅₀) effect, novelty-induced behaviour, anxiolytic, sedative and anticonvulsant properties using appropriate standard models.

**Results:** The results obtained indicate that the LD₅₀ of the fresh oil were 4472 and 1789 mg/kg orally and intraperitoneally respectively. The oil comparatively through the oral and intraperitoneal routes caused significant (p<0.01) reduction in novelty-induced behaviours of rearing, grooming and locomotion signifying CNS depressant activity; decreased head dips indicating sedative or anxiogenic effect, significantly (p<0.01) shortened sleep latency and increased total sleeping time when compared to the vehicle suggesting a sedative effect. Finally, the oil prolonged latency to convulsion and prolonged death time caused by pentylenetetrazol-induced convulsions, signifying anticonvulsant activities. Chemical analysis showed that thirty nine compounds were detected in the fresh leaf oil and the major ones identified include β-caryophyllene (30.4%), α-humulene (16.3%) and caryophyllene oxide; while twenty six compounds were detected in the fresh fruit oil with major ones being caryophyllene oxide (16.9%), β-caryophyllene (9.4%), α-humulene (7.6%), γ-murolene (7.6%) and (-)-humulene epoxide II (6.9%).

**Conclusion:** It is hereby concluded that the essential oil of *L. camara* fresh leaf was composed mainly of sesquiterpene hydrocarbons, showed slight-moderate toxicity, displayed significant CNS depression; and possessed sedative and anticonvulsant activities in mice.
The In Vitro Effects of Herbal Medicines on a Rapid Urine Screening Test

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Introduction and Aim: The prevalent use of African traditional medicine (ATM) by the general public has been reported, as well as the non-disclosure behaviour of the users thereof. With commercialisation and marketing, some of the herbal medicines used are readily available over the counter, most of them promoted as immune boosters. These commercial herbal medicines have not been taken through clinical trials and other tests where their composition, safety and other properties are validated. Generally there are concerns regarding possible adverse or toxic effects and herb-drug interactions if the ATMs are used concurrently with conventional medicines. However because these herbal medicines are mixtures with multitudes of compounds, they could also affect the quality of laboratory diagnostic tests used for various conditions. Hence, the aim of the study was to investigate the cross-reactivity of selected herbal mixtures with commonly tested substances of abuse using a qualitative rapid urinalysis assay.

Methods: Six commercial herbal mixtures (HMs) commonly used as immune-boosters were selected for the study. The rapid urine screening test was performed with the Instant view® Multi-Drug of Abuse Test kit form Lab-stix Diagnostics (Pty) Ltd. Drug-free urine (DFU) was pooled from urine samples donated by healthy adult volunteers. Urine samples that had tested positive for drugs of abuse were obtained from the Pharmacology Laboratory at Sefako Makgatho Health Sciences University. Aliquots of the urine samples were spiked with the HMs in neat and dilute form, and tested at various time intervals. A dipstick test was done on all the urine samples for pH and specific gravity (SG).

Results: The results for the DFU samples spiked with the HMs remained negative. There were no significant changes in pH and specific gravity of the samples. The results of samples that had tested positive for tetrahydrocannabinol (THC) were not altered by five of the HMs when spiked at low concentrations as well as at 40% v/v of the HMs. The HM, Ngoma herbal tonic immune booster® caused false negative results for THC when samples were spiked at 40% v/v. Conclusion: An important finding from this study is that the herbal mixture, Ngoma herbal tonic immune booster® caused false negative results for THC when present at high concentration. It adds up on the list of substances that are potential adulterants of urine for screening tests. The findings therefore facilitate the route for further studies investigating whether the use of ATMs could interfere with diagnostic tests.
Antioxidant Capacity, Phytochemical Screening and Identification of Active Compounds in Anchomanes difformis

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Introduction and Aim: The therapeutic effects of numerous medicinal plants have been associated with the phytochemicals (bioactive compounds naturally occurring in plants) present in them. These compounds have enormous health benefit in many pathological conditions and exhibit their effect through their anti-oxidative, anti-diabetes, anti-cancer, anti-inflammatory, antimicrobial abilities among others. Anchomanes difformis (AD) is specie of flowering plants in the family Araceae. It is commonly reported for ameliorating diabetes, inflammation, ulcer, asthma and inhibiting microbial growth. The present study therefore aim to screen for phytochemicals present in the ethanol, ethyl acetate and aqueous extract of the leaves and rhizome of AD and to further evaluate the antioxidant capacities of these extracts.

Methods: In vitro quantitative, phytochemical analysis and antioxidant capacities were carried out on ethylacetate, ethanol and aqueous extracts of A. difformis leaf and rhizome, using combined principles of spectrophotometry and colorimetry. The phytochemicals assayed for were; total alkaloids, total polyphenols, flavonols, and total carotenoids. ORAC, TEAC and FRAP were carried out to determine antioxidant capacity of these extracts. Identification of specific polyphenolic compounds in Chloroform, ethylacetate, ethanol and aqueous extract of A. difformis leaves was done using high performance liquid chromatography (HPLC).

Results: Polyphenols, flavonols, flavanols and alkaloids were present in all the extracts in varying concentrations. All extracts exhibited free radical scavenging ability, ferric reducing power and oxygen radical absorbance capacity. Aqueous leaves extract exhibited highest antioxidant properties (1225.82 ± 7.95 µM TE/g; 257.69 ±1.71 mgTE/mg and 198.01 ±2.00 mgAAE/mg sample) for ORAC, TEAC and FRAP respectively. Aqueous Leaves extract also had the highest concentration of polyphenols (47.58 ± 1.36 mgGAE/mg sample). Ethanol leaves extracts contained the highest concentration of alkaloids (9891 ± 0.28 µgAtropine/g sample), and flavonols (1.373 ±0.11 mgCatechin/mg). Ethylacetate extract of the rhizome had highest concentration of flavonols (156.67 ± 1.17 mgQuercetin/mg). HPLC analysis results identified kaempferol, quercetin, rutin, cinnamic acid, chlorogenic acid, ferulic acid, catechin and coumaric acid.

Conclusion: A. difformis leaves and rhizome are potential sources of natural antioxidants. Leaf extracts (aqueous and ethanol) of AD contained higher concentrations of polyphenols, alkaloids and flavonols and may have more medicinal potentials when compared to its respective rhizome extracts. The aqueous leaves extract demonstrated, free-radical scavenging and inhibition capacity, hence, can possess more potential as a therapeutic agent against diseases linked with oxidative stress when compared with ethanol and ethylacetate extracts. Presence of health-promoting, active compounds in the leaf extracts indicates possible preventive and curative potentials of A. difformis.
Inhibition of HIV-1 Infection of Cells with the South African Medicinal Plant coded BP36

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Introduction and Aim: The HIV-1 pandemic affected about 33 million of people worldwide with approximately 70% of those affected residing in sub-Saharan Africa. The CSIR’s collaboration with Traditional Healers (THs) on the use of medicinal plants in South Africa led to the identification of a plant (BP36) that has anti-HIV-1 activities. The objective of this study is to isolate the active protein(s) and/or small molecules in the BP36 plant that can be developed into a drug against HIV-1. This is based on the fact that the precipitate from the crude extract of BP36 showed potent inhibitory activities against the virus in different assay formats.

Methods: The goal was purification to homogeneity of active proteins or peptides from the initial proteins mixture. This was achieved through a combination of fractional precipitation and chromatography steps that would separate the protein(s) based on variations in size, charge and hydrophobicity. Neutralization assay in TZM-bl cells was performed to determine HIV-1 inhibitory activities of the isolated proteins or peptides. The TZM-bl neutralization assay is widely used to study molecules, especially entry inhibitors, inhibitory activity against HIV-1. Sodium dodecyl sulphate-poly acrylamide gel electrophoresis (SDS-PAGE) was also used to assess the size and integrity of the isolated protein(s).

Results: From the three different extraction methods utilized, only seven protein bands were visualized on the SDS PAGE gel. The isolated proteins were at a low concentration hence the low yields and moderate anti HIV-1 activity. Further physical characterization of the proteins is underway including amino acid sequence analysis. Once the complete sequence of the protein(s) has been elucidated, it will then be used to create a synthetic gene using the E. coli codon preference table. The gene will then be inserted into a plasmid for inducible expression in the bacterium. Various growth and induction conditions will be evaluated for optimal protein(s) production and the crude protein extracts from the recombinant E. coli will be tested for activity against HIV-1.

Conclusion: Fractionation through FPLC needs to be repeated simple because the generated protein fractions were low in terms of yield but showed better anti-HIV-1 activity compared to SDS PAGE gel pieces. Gel filtration and affinity chromatography will also be utilized to separate proteins based on their binding abilities and charge. Mass spectra for the isolated proteins for sequencing and identification are underway.
Introduction and Aim: Poultry is the most popular source of meat in South Africa. The country consumes 60% of 2.9 million tonnes of poultry, beef and pork meat per annum. According to a recent USDA GAINS report, broiler meat has grown to be the most important protein source in the diet of the majority of South Africans because it is relatively inexpensive and ubiquitous. The consumption of poultry meat (of which most is broiler meat) increased by almost 80%, from 25.1kg per person per year in 2000 to 38.5kg per person per year in 2014. This study was conducted to evaluate the effects of using alternative source of protein and energy ingredients in chickens.

Method: Hundred day old chicks (n=100) were purchased from a commercial chicken farm and were randomly allotted into three groups (33 birds in each for group one and two and 34 in group three). Group 1 served as the control group (i.e. chicks fed on a commercial poultry feed). Chicks in group 2 were fed with a feed formulated by replacing the existing protein with insect protein (MW), while chicks in group 3 were fed with a formulation in which the protein and the carbohydrate source have been replaced with insect protein (MW) and finger millet (FM). The effects of the formulated feeds on various physiological parameters in the chicks were investigated.

Results: It was observed that the chicks in groups 2 and 3 recorded lower body weight compared to group 1. Physical activity and feeding rate of the chicks in groups 2 and 3 decreased compared to the control group 1. Moreover, an increase in mortality was observed in these groups (30%) as compared to the control group whose death rate was only 1%. Microbial analysis of the insect protein ingredient (MW) in the feed components in group 2 and 3 feed formulation showed the presence of *E.coli*. Post-mortem results of the chicks in groups 2 and 3 showed the presence of yolk sac infection which is also caused by *E.coli*. Histopathology results showed that the livers of chicks from the three groups had fine vacuolation of hepatic cytoplasm which is normal. Thus the cells around the livers were fine, and no particular infection was found around the liver cells and membrane. Organs of chicks from all three groups were also tested for possible Newcastle disease and result showed that three of ten in group 2 tested positive for Virulent Newcastle disease in the brain tissue.

Conclusion: The potential for toxicological effects was found, which could be attributed to bacterial contamination of the feed ingredients and yolk sac of chickens. Thus in developing an alternative chicken feed these should be kept in mind and can be avoided by sterilizing feed ingredients before formulation as well as vaccination of chicks.
Adverse Drug Reactions to Antiretroviral Therapy (ART): Prospective Study in HIV Infected Children in Bamako (Mali)

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Introduction and Aim: The adverse effects of ARV drugs (ADRs) are indeed a common reason for discontinuing treatment in infected patients. More than 25% of these patients discontinue treatment in the first year because of these effects. These adverse effects result in changes in the biological parameters resulting in clinical manifestations. All of these adverse effects can have a significant impact on patients' quality of life and adherence to treatment. Adverse events in HIV-infected patients are well documented in developed countries. In Mali, very few studies on adverse effects and their management have been carried out in children with the aim of evaluating the adverse effects linked to the use of antiretrovirals (ARV) in HIV+ USAC of Bamako.

Methods: A cross-sectional descriptive study was conducted at USAC Common V in Bamako from May 1, 2014 to July 31, 2014. We included children aged 2 to 14 with at least 6 months of ARV treatment, undesirable (EI) or not. The assent of the parents was obtained. The WHO graduation method was used.

Results: In total, 146 children were included in the cohort. The age group under 36 months was the most represented (52.5%). The male sex was predominant (54.7%). The clinical stages WHO 2 and 3 were the most observed with respectively (35.6% and 36.3%). Poor adherence at the time of the study was observed (15.0%). The CD4 count was less than 350 cells / mm3 in 52.05% of cases at the time of adverse events. A lower plasma viral load of less than 100,000 copies / mm3 was present in 47.3% of cases at the time of the adverse reactions. We observe any clinical adverse events in approximately 80% of children. There was a statistically significant difference in the degree of toxicity in clinical adverse events (Fisher exact test P <10-3). Grade 2 toxicity was the most common clinical event. Biological adverse effects were absent in about 20% of children. There was a statistically significant difference in the degree of toxicity in biological events (Fisher exact test P <10-3). The toxicity of WHO grade 4 was observed mainly in 17 cases of anemia. The ‘certain’ accountability score was found in the majority, i.e. 27.4% of the reported cases.

Conclusion: The nature of these ADR was gastrointestinal disorders (Nausea). Regular monitoring of children receiving ARVs is essential to detect and treat complications associated with these therapies. We recommend active surveillance of antiretroviral therapy to strengthen Pharmacovigilance in Mali.
Lopinavir Concentrations in HIV-infected Malian Women and Their Nursing Infants

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Introduction and Aim: Breastfeeding increases the risk of HIV transmission by 14%, with an additional 1% risk per month for the first six months of breastfeeding. Currently, available data are limited on antiretroviral pharmacokinetics in breast milk, as well as plasma in breast-fed infants. In this work, we measured plasma and milk plasma concentrations of HIV-infected mothers and their infants during lactation. The second objective was to evaluate the correlation between plasma concentrations and plasma viral load.

Methods: Included patients who were HIV-positive pregnant women receiving antiretroviral prophylaxis from at least 25 weeks of gestation up to 6 months after delivery and their breast-fed infants. Blood samples were taken at delivery and at month 1, 3 and 6 postpartum. Lopinavir concentrations were measured by tandem liquid chromatography mass spectrometry. The quantification detection limit was 0.264 mg/L for lopinavir. Plasma viral load was measured on M2000rt (Abbott). The quantification detection limit was 40 copies/ml. The viral load was determined at delivery and at 6 months postpartum for mothers and at 3 and 6 months postpartum for children. All children received nevirapine for 6 weeks after birth.

Results: A total of 9 couples (mothers and newborns breastfed) were included. All the mothers were all on zidovudine (AZT), 3TC and lopinavir/ritonavir (LPV/r). The median mother age was 29 years (19-40 years). The mean duration of treatment for mothers was 84 months. The median maternal plasma LPV concentration (IQR) was 1870 ng / mL (586, 4190) at month 1; 10900 ng/mL (5495, 15750) at 3 months; 5790 ng/mL (1230, 10600) at month 6. The median maternal breast milk concentration was 530 ng/mL (150-890ng /mL) at month 1; 650ng/mL (160-940ng/mL) at month 3 and 590ng/mL (200-770ng/mL) at month 6. Infant plasma concentrations of LPV were undetectable. No adverse reactions or ARV-related toxicity were observed in children. Two mothers had a viral load> 50 copies / mL at 6 months, showed plasma levels of the drug undetectable at the same time.

Conclusion: LPV was undetectable in breast-fed infants in this study in Mali.
Application of the CASE Adherence Index to HIV Patients on Single-Dose Regime of HAART in Standfort Terrace Clinic in Mthatha, Eastern Cape, South Africa

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Introduction and Aim: The adherence to ARV treatment is widely recognized as critical for achieving therapeutic success in the treatment of HIV infection. It has been shown to correlate strongly with both biologic markers of HIV and clinical outcomes, including HIV progression and death. Although the measurement of adherence is still problematic, the Center for Adherence Support Evaluation (CASE) in the USA created an index of ARV adherence treatment (CASE Adherence Index) using 3 unique adherence questions. The CASE Adherence index uses three standard measures of self-reported adherence, is simple to apply, and can be used by both researchers and clinicians in the field. The objective of the study was to apply the CASE Adherence Index to patients on a single-dose regimen of the highly active antiretroviral therapy (HAART) in the Standfort Terrace Clinic in Mthatha, Eastern Cape Province, South Africa.

Methods: The CASE Adherence Index Questionnaire was applied to patients in single-dose regimen of HAART attending the clinic for checkup and collection of their monthly treatment.

Results: 90 patients (77.58%) scored a CASE Index >10 (GOOD Adherence). 73 were female (62.93%) and 17 were male (14.65%). 26 patients (22.41%) scored a CASE Index ≤ 10 (POOR Adherence). 18 were female and 8 were male.

Conclusion: 1-The CASE Adherence Index was successfully applied in a health facility in a rural area for the first time in South Africa. 2-The study population has a relatively GOOD Adherence level of 77.58% to HAART (female 80.21% and male 68.00%). The GOOD Adherence pattern is possibly due to the large number of female patients attending the clinic regularly. 3-The POOR adherence in female patients was 19.78% and in male patients 32.00%. 4-Although the single (or fixed) dose regimen has contributed to increase adherence more is needed to be done for further improvement.
Effects of Anti-Retroviral (ARV) Drugs on CD-4 Counts and Viral Loads in HIV-AIDS Patients from a Rural Area in South Africa: An Inferential Study

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Background and Aim: It is well documented that ARV drugs decrease the viral load and increases the CD-4 counts in patients with HIV-AIDS. However the effects vary in different populations due to many factors, e.g., sex, age, diet, genetic, etc. The present study was done to assess the effect of ARV drugs on the CD-4 counts and the viral load (VL) in HIV-AIDS patients in Qumbu Clinic, Eastern Cape Province, South Africa.

Methods: All the data were collected from the clinical records (files) of patients on ARV treatment. The Medical Ethics rules and confidentiality protocol were followed. The files of all patients on ARVs drugs at Qumbu Clinic in the Eastern Cape Province were checked and those ones that fulfilled the inclusion criteria were selected for the study. The gender, age, type of treatment (at initial, 6 months and 12 months), CD-4 counts and viral load values were recorded. The descriptive and inferential statistical analysis of the data was done using the SPSS program.

Results: 950 files were checked and 173 were chosen for the study (39 male and 134 female). A significant statistical increase occurred in the CD-4 count at 3 months (mean=221) and 6 months (mean=377) of treatment compared with the initial values (mean=103). Females responded better than males. The importance of the CD-4 initial value and the ages of the patients on the ARVs drugs response was observed. A significant statistical difference was also seen in the CD-4 counts between patients with detectable and non-detectable viral loads.

Conclusion: 1-ARVs are effective in treating HIV/AIDS in both males and females. Females have an initial response (1st 3 months), that is better than in males. 2-Even elder patients with very low (<50) CD4 counts benefit from treatment. 3-The higher the initial CD4 value, the higher the earlier response to treatment is. 4-As the CD4 value increases (>300) the viral load decreases to undetectable limits.
Investigation of a Marine Natural Product, Manayimmune, for its Claimed Use in the Traditional Treatment and Management of HIV

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Introduction and Aim: Traditional medicine and natural products are used as primary treatments for a variety of ailments and diseases including Human Immunodefiency Virus (HIV) and Acquired Immunodefiency Syndrome (AIDS). Traditional medicine preparations in Africa and other countries are mainly extemporaneous preparations complex that are prepared at the time of the patient consultation and are patient specific. Manayimmune is a marine natural product that has been used to treat and manage people with HIV and AIDS. Manayimmune is a family owned treatment that has been disclosed under contract with the University of the Free State. Manayimmune has been used for over a decade and the family to treats only patients who are confirmed HIV positive. Anecdotal evidence from the HIV and AIDS patients who are using this product, claim to receive some health benefits and an improved quality of life. This study aimed at investigating the traditional use claims of Manayimmune and the possible scientific basis of its health claims on HIV through the inhibition of the integrase enzyme.

Methods: Traditionally Manayimmune is prepared by cooking the raw product in water and the filtrate and the dried retentate are dispensed to patients. However, for the purposes of the scientific research, both the cooked and uncooked Manayimmune were extracted separately methanol and water. The aqueous and methanolic extracts were used to do phytochemical analysis to test for the presence or absence of groups of chemical compounds to include proteins, glycosides, phenol, tannins, saponins, gums and flavonoids. The method of Harbone (1984) were used for the phytochemical analysis. For in vitro cytotoxicity, in vitro efficacy testing, spectroscopic chemical characterization and for the isolation of bioactive molecules, Manayimmune was extracted sequentially using hexane, chloroform, dichloromethane and butanol in a sample solvent ration of 1:5 (m/v). A colometric kit for the efficacy evaluation based on the in vitro inhibition of HIV-1 reverse transcriptase (Roche) and inhibition of integrase enzymes (XpressBio) was used. Absorbance readings at 450nm using PheraSTAR FS were used to calculate % inhibition by the extracts for the integrase inhibition activity whereas for the reverse transcriptase assays the absorbance readings were measured at 405 reference wavelength. The extracts concentrations used for the in vitro efficacy tests ranged from 100µg/ml to 1µg/ml and IC50 were calculated.

Results: The results have shown that none of Manayimmune extracts had inhibitory activity on HIV-1 Reverse Transcriptase, whereas the hexane and chloroform extracts inhibited in vitro, the activity of the integrase enzyme by 99.4% and 93.6% each at 50µg/ml, respectively. The qualitative phytochemical analysis of the extracts indicated the presence of proteins, glycosides, phenols, tannins, saponins, gums and flavonoids. Further studies are required to chemically identify the possible chemical compounds responsible for the inhibition of the in vitro inhibition integrase enzymes by the hexane and chloroform.

Conclusion: The preliminary results indicate the possible mechanism of anti HIV effects of Manayimmune could be through the inhibition of the integrase enzyme although more studies are need to conclusive make this judgement.
Introduction and Aim: Cytochrome P450 monooxygenases (CYPs/P450s) are heme-thiolate proteins found in species belonging to different biological kingdoms. All P450s need electrons to perform their enzymatic reactions. It is logical that if one can inhibit P450 redox proteins, it will eventually result not only in loss of all P450s functions but also lead to the death of an organism. Mycobacterium tuberculosis, the deadly pathogen to humans, was found to have 20 P450s in its genome. Studies revealed that M. tuberculosis P450s can serve as novel drug targets. This suggests P450 redox proteins are very important in keeping M. tuberculosis P450s physiological function. With the exception of few studies on M. tuberculosis P450 redox proteins nothing is known about the P450 redox content in species of Mycobacterium. Therefore, the aim of this study is to perform comparative genomics of P450 redox partners in 81 mycobacterial species genomes.

Methods: 81 mycobacterial genomes that are available for public use at KEGG database were data-mined for P450 redox proteins. Two methods were used to identify redox proteins. First, proteins were collected using the term “redox protein”. Second M. tuberculosis redox protein (total 9) was individually blasted against different mycobacterial species and hit proteins were collected. Conserved protein domains (matched to InterPro and Pfam database entries) were identified using the InterPro Scan plugin in Geneious in the hit proteins. Based on characteristic conserved domains the hit proteins were classified into different P450 redox protein categories. Phylogenetic analysis was performed using Maximum likelihood method using PHYML as implemented in geneious, each with 1000 bootstrap replicates.

Results: Genome data-mining of 81 mycobacterial species revealed presence of 1063 P450 redox proteins grouped into ferredoxins (662) and ferredoxin reductases (401). Phylogenetic analysis of ferredoxins revealed presence of two major clades of ferredoxins with characteristic Pfam/InterPro protein domains. The clade with domains Fer4 (PF00037), Fer4_7 (PF12838) and Fer4_9 (PF13187) was associated with the same InterPro entry (IPR17896) and it was classified under group 2 and the clade supported with 83% bootstrap proportion with domains Fer4_13 (PF13370), Fer4_15 (PF13459) and Fer4_19 (PF06902) shared similar HMM-motifs which was classified under as group 1. Phylogenetic analyses indicated the presence of two major divergent clades of ferredoxin reductases (FdRs) in mycobacteria; the Bacterial-type, which belong to the Plant-type FdRs, and the Glutathione Reductase (GR)-type. Within the GR-type FdRs, two different clades were identified: the adrenodoxin (Adr)-like clade and the oxygenase-coupled NADH-ferredoxin reductase (ONFR)-like clade. Mycobacterial tuberculosis complex species showed 0-8 FdRs in their genomes. MCL species contained two FdRs. NTM species contained 2-7 FdRs in their genome. Saprophytes showed a high number of FdRs with the range of 0-12. Mycobacterium avium complex species showed 3-7 FdRs in their genomes. Mycobacterium chelonae-abscessus complex ranged from 1-6 FdRs.

Conclusion: In conclusion, genome data mining was performed in 81 mycobacterial species and identified possible redox proteins and then these proteins were annotated into ferredoxins and ferredoxin reductases. Furthermore, based on the phylogenetic analysis ferredoxins and ferredoxin reductases were grouped into different groups.
Genome-wide Annotation, Gene-Cluster, and Structural-Analysis and Substrate Prediction of Drug Target *Mycobacterium Leprae* P450 CYP164

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**Introduction and Aim:** Leprosy is a chronic infectious disease that is caused by bacterium *Mycobacterium leprae*. During evolution, *M. leprae* went through a massive decay of genes. However, it retained only one cytochrome P450 monooxygenase CYP164A1 in its genome. Cytochrome P450 monooxygenases (CYPs/P450s) are mixed function oxidoreductases and well-known drug target against pathogenic microorganism. Study revealed that CYP164A1 can act as a novel drug target against *M. leprae*. However, to date, no information is available on its function, distribution and structural organization across mycobacterial species. This study is aimed to perform genome-wide annotation, gene-cluster, and structural-analysis and substrate prediction of drug target CYP164.

**Methods:** (i) Data mining and annotation: 95 Mycobacterial species genomes available at KEGG database for public use were used in this study. CYP164 of *M. leprae* were blasted against each of the mycobacterial genome and hit proteins were selected and named based on >40% amino acid identity as CYP164 family and >55% amino acid identity as CYP164 subfamily as set by the International P450 Nomenclature Committee. (ii) Phylogenetic analysis: For phylogenetic analysis the CYP164 protein sequences were aligned by HMMER package 3.1 through adjusting them to the P450 profile hidden Markov model PF00067 downloaded from the Pfam protein families’ database. Then, the phylogenetic trees from alignments were inferred by FastTree version 2.1.4 with the maximum-likelihood method. (iii) *In silico* structural analysis: Structural analysis of CYP164 members was carried out using methods described by our laboratory. Briefly, template selections for modelling were performed using the PSI-BLAST algorithm over Protein Data Bank. The three best templates were used to build sequence alignment with I-TASSER and T-Coffee, with default parameters and subsequent manual editing. The constructed models were subjected to structural quality evaluation with I-TASSER and PROCHECK. Volumes and surface areas of the cavities were calculated with CASTp. The final 3D model of P450s and active-site cavities were rendered using PyMol.

**Results:** Genome-wide identification and annotation of CYP164 P450s revealed presence of 32 P450s in 31 mycobacterial species. *Mycobacterium abscesses* 4529 species showed two CYP164 P450s in its genome. This study revealed that CYP164 is absent from species belonging to the *Mycobacterium tuberculosis* complex (MTBC) category. *Mycobacterium chelonae*-abscessus complex (MCAC) showed highest number of CYP164 P450s. Phylogenetic analysis revealed grouping of CYP164 P450s as per different categories suggesting that after speciation specific changes happened in CYP164 primary structure. However, a saprophyte species, *Mycobacterium neoarum*, retained CYP164 P450s that are closely related to the group MCAC. Gene-cluster analysis revealed that CYP164 is located in a highly conserved operon across mycobacterial species. Structural analysis of selected CYP164 P450 members revealed the presence of distinctive, enlarged hydrophobic active site that extends above the porphyrin ring towards the access channels.

**Conclusion:** In conclusion, this study revealed a low number of CYP164 across mycobacterial species with absence of this P450 in MTBC category. *In silico* structural analysis revealed a large active site cavity. Gene-cluster analysis indicated a possible role of CYP164 in *M. leprae* and this role is under investigation.
**In silico Analysis of Cholesterol Catabolic Genes/Proteins in the Genus Mycobacterium**

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**Introduction and Aim:** It is well-known that *Mycobacterium tuberculosis*, the causative agent of deadliest human disease tuberculosis, uses human cholesterol as a carbon source both in latent and active phases of its lifestyle. The discovery of the ability of *M. tuberculosis* to degrade and use cholesterol as a sole source of carbon and energy, has opened up the possibility of using genes/proteins involved in cholesterol degradation as novel drug targets. If one can find the highly conserved genes/proteins across the mycobacteria that are capable of degrading cholesterol, then in future these genes can possibly be used as universal drug targets against mycobacterial infections. However, to date, data on how many mycobacterial species uses cholesterol is not reported. Furthermore, performing laboratory experiments is laborious and time consuming considering each of the mycobacterial species has different lifestyle and culture conditions. The proposed study is aimed to utilize the available genomic data and perform comparative genomic studies to unravel the nature of cholesterol catabolic genes/proteins in the genus *Mycobacterium* to determine which mycobacterial species are capable of degrading cholesterol.

**Methods:** Ninety-three mycobacterial species whose genomes are available for public use at KEGG database were used in this study. Literature on cholesterol degradation by bacteria was collected and the cholesterol degradation pathway was deduced. The intermediate metabolites and enzymes involved in each of the steps were identified and mapped using ChemDraw software. A software program that automatically identifies possible cholesterol catabolic proteins/genes across 93 mycobacterial species was developed. The hit proteins identified using a software program were subjected to functional annotation using the NCBI Batch Web CD-search tool and Pfam. Based on the sequence identity, functional motifs and functional data, if available, the hit proteins will be sorted into specific enzymatic reactions of cholesterol degradation. Based on the data generated, a cholesterol degradation pathway map with genes involved in each of the reactions was deduced and presented using ChemDraw software.

**Results:** After thorough literature analysis, a total of 152 genes/proteins were identified as cholesterol catabolic genes/proteins and grouped into 4 different categories. The four categories includes: (i) Genes predicted to be specifically required for growth on cholesterol (ii) Cholesterol catabolic genes that proven to be or predicted to be essential for survival of *M. tuberculosis* in macrophage cells and in murine infection (iii) Genes/proteins that up-regulated during growth on cholesterol (iv) Genes involved in cholesterol degradation by *M. tuberculosis* H37Rv, but not confirmed or predicted to be essential. *In silico* analysis of 152 genes across 93 mycobacterial species revealed that 23 mycobacterial species are unable to degrade cholesterol as essential genes/proteins are missing from these species. *Mycobacterium leprae* species lacked most of the cholesterol degrading genes.

**Conclusion:** In conclusion, using the latest bioinformatic techniques this study revealed that 23 mycobacterial species are unable to degrade cholesterol as essential genes required for cholestrol degradation are not present in these species.
Comprehensive Comparative Modelling and Substrate Binding Analysis of Novel Common Alternative Anti-Fungal Drug Target CYP53 family P450s

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Introduction and Aim: Cytochrome P450 monooxygenases (P450s) are heme-thiolate proteins responsible for the biotransformation of several drugs. Recently, our laboratory reported that P450 family CYP53 can act as a common alternative anti-fungal drug target. In the current study we performed in silico structural analysis of twenty-two CYP53 family members to identify common amino acids involved in different substrate binding in order to design a common inhibitor against this family P450s to be used as an antifungal drug.

Methods: (i) Homology modelling: Template for construction of 3D models was selected at PDB database. 3D structure of CYP53 members were generated by homology modelling using MODELLER 9.17. Both the template and the modelled structure of CYP53 P450s were aligned using SPDBV 4.1.0 with CA (Carbon-alpha) backbone. The 3D structure of CYP53 P450s obtained was visualized by Pymol. The generated 3D structure of model CYP53 P450s were submitted to PDBsum database (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum). Thus quality of CYP53 P450s models were analysed by PROCHECK which uses Ramachandran map. (ii) Active site analysis: The active site was obtained by sequence alignment of model CYP53 P450s with template. The active site residues were used to generate the grid box which describes the active site of model CYP53 P450s for docking. (iii) Ligand Database: The 11 ligands (substrates) were retrieved from Pubchem and ligand database was built using Avogadro for conversion of SDF files to PDB file. The selected ligands were further prepared for docking in Autodock tools 1.5.6. (iv) Molecular docking: The CYP53 P450s models were prepared for docking in Autodock tools 1.5.6. Molecular docking of CYP53 P450 models with respective ligands was carried out using Autodock Vina. In Autodock Vina the ligands are docked in the active site cavity. All structures were docked successfully and the results were visualized in Pymol and Discovery Studio 3.5.

Results: High quality 3D models were generated for 22 CYP53 members and performed comprehensive comparative structural analysis and binding studies with 11 different substrates. The study revealed conservation of P450 characteristics motifs and high conservation of active site cavity and its amino acids in 22 CYP53 P450s. Docking of 22 CYP53 P450s with 11 different substrates revealed that amino acids involved in binding of different substrates are highly conserved across 22 CYP53 members suggesting inhibitors aimed at any of these CYP53 P450s can act as a common alternative anti-fungal inhibitor against different ascomycete pathogens.

Conclusion: In conclusion, comprehensive comparative modeling of 22 CYP53 P450s and substrate binding analysis revealed high conservation of active site cavity and its amino acids in all CYP53 P450s, re-instating our hypothesis that this family can act as a common alternative anti-fungal drug target. Work is in progress to identify novel inhibitor(s) using virtual screening and chemical libraries.
Genome-Wide Annotation and Genome Mapping of Essential P450 CYP125 in the Genus *Mycobacterium*

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**Introduction and Aim:** Tuberculosis, an infectious lung disease and leading cause of death worldwide, is caused by *Mycobacterium tuberculosis*. Genome-wide screening for genes essential for the survival of *M. tuberculosis* has revealed that cytochrome P450 monoxygenase CYP125A1 is critical for *M. tuberculosis* survival. CYP125A1 play key role in oxidation of cholesterol and help *M. tuberculosis* to utilize cholesterol as a carbon source during its inhabitant in host organism. Despite this great importance, to date, genome-wide identification, annotation and phylogenetic analysis of CYP125A1 and its genome mapping with respect to gene-cluster analysis across mycobacterial species has not been performed. Also, to date, P450s from prokaryote organisms has not been subjected to evolutionary analysis. Thus this study is aimed to addresses these two research gaps.

**Methods:** Mycobacterial genomes that are publicly available were data mined for CYP125 and its genome mapping. In this study, 77 mycobacterial species belonging to six different categories includes, *M. tuberculosis* complex (MTBC) (36 species); *M. chelonae-abscessus* complex (MCAC) (7 species); *M. avium* complex (MAC) (14 species); Mycobacteria causing Leprosy (MCL) (2 species); Non-tuberculous mycobacteria (NTM) (7 species) and Saprophytes (SAP) (11 species) were used. CYP125 hits were then assigned to their respective families and subfamilies based on P450 Nomenclature criteria. Upstream and downstream genes of CYP125 were accessed and then mapped using pDRAW32. The genes were then grouped into different clusters based on the function/characteristic motif of the corresponding proteins.

**Results:** Analysis of CYP125 P450s in 77 mycobacterial species revealed presence of 136 CYP125 P450s in their genomes. These CYP125 P450s were grouped into four different subfamilies such as A, D, E and F. The range of CYP125 P450s ranged from 0-5 in mycobacterial species. *Mycobacterium abscessus subsp. bolletii* CCUG48898=JCM15300 and *Mycobacterium* sp. JDM601 has the highest number of CYP125 P450’s (5 CYP125 each). Among six different mycobacterial categories, SAP had the highest number of CYP125 count with 35 CYP125 P450’s. Analysis of CYP125 gene clusters in the genus *Mycobacterium* revealed the presence of 28 CYP125 gene-clusters. Gene clusters 1 to 20 comprised of quite a number of CYP125 P450s ranging from 2 to 23 and gene clusters 21 – 28 named as unique gene clusters considering each of the CYP125 P450 in this cluster have different genes both in the upstream and downstream of CYP125. Overall, SAP species showed the highest CYP125 gene cluster diversity (10 clusters including 1 unique cluster) followed by MAC (8 clusters including 3 unique clusters), NTM (5 clusters including 3 unique clusters), MCAC (4 clusters) and MTBC (2 clusters including one unique cluster).

**Conclusion:** Analysis of genome-wide data mining and annotation and gene-cluster analysis of CYP125 in mycobacterial species revealed CYP125 may have another function apart from cholesterol degradation. Assessing the novel function is under investigation.
In silico Analysis of Cytochrome P450 Monooxygenases in the largest antibiotic-producing genus Streptomyces

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Introduction and Aim: Streptomyces is the largest genus in the phylum Actinobacteria. Streptomyces is a prominent source for natural products, secondary metabolites especially antibiotics; accounting for an estimated two thirds of the clinically used antibiotics. Many of the secondary metabolites are synthesized by polyketide synthases, and they are structurally modified by numerous biosynthetic enzymes, including cytochrome P450 monooxygenases (CYPs/P450s). Recent study from our laboratory revealed presence of quite a large number of P450s in the genus Mycobacterium and led to the discovery of a novel P450 fused family, suggesting that much remains to be explored and understood about the evolution of these enzymes in prokaryotes. In this direction, this study is first of its kind on genome-wide data mining, annotation and comparative analyses of P450s in Streptomyces with an aim to identify P450s involved in secondary metabolites including antibiotic production.

Methods: Forty-eight Streptomyces species genomes available for public use at KEGG database was data mined for P450s. The proteomes were subjected to NCBI CDD search and proteins grouped under P450 superfamily were selected and assigned to different P450 families and subfamilies based on P450 nomenclature criteria i.e. >40% identity as family and >55% identity as a subfamily. P450s showed less than 40% identity assigned to a new P450 family. Secondary metabolite clusters were identified based on the presence and location of non-ribosomal peptide synthases compared to P450s. The genomes maps were generated using pDRAW software. Mycobacterial species P450s generated previously from our laboratory were used for comparative analysis.

Results: Genome-wide data mining and annotation of P450s revealed presence of 1163 P450s in 48 Streptomyces species. The P450 count in the genus Streptomyces ranged from 3-63 P450s where S. albulus ZPM showed highest number of P450s in its genome. These P450s were grouped into 106 P450 families and 269 P450 subfamilies. This study revealed presence of 56 new P450 families and 2 new P450 subfamilies in Streptomyces species. Analysis of P450 families revealed that the CYP107 family was the dominant P450 family with 272 members (23.4%) of the total P450s found, followed by CYP105 (14.8%), CYP157 (7.9%) and CYP152 (7.2%); suggesting an important role of these P450s in the Streptomyces physiology. Comparative analysis of P450s between the genera Streptomyces and Mycobacterium revealed 19 P450 families are common between these two genera and 87 and 58 P450 families were uniquely present in Streptomyces and Mycobacterium respectively. Overall, Streptomyces showed lowest number of P450s compared to Mycobacterium. Presence of different P450s in both genera reflects their different lifestyle or adaptation to different ecological niches such as Streptomyces species primarily involved in production of secondary metabolite whereas Mycobacterium species involved in causing infections. Gene-cluster analysis revealed presence of secondary metabolite producing clusters with quite a large number of P450s involved in these gene clusters with CYP107 and CYP105 were involved in production of secondary metabolites.

Conclusion: In conclusion, study unravelled P450 contingent in Streptomyces and future study involves assessing the role of these gene-clusters in production of different secondary metabolites including identifying novel antibiotics.
Structure Analysis of Cytochrome P450 Monooxygenase CYP123A1 from *Mycobacterium tuberculosis* H37Rv

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**Introduction and Aim:** Cytochrome P450 monooxygenase (CYPs/P450s) are heme-thiolate enzymes distributed across the biological kingdoms. P450s in the deadly human pathogen *Mycobacterium tuberculosis* H37Rv, the causative agent of Tuberculosis, play a key role in its physiology and was found to be novel drug target against this pathogen. Among 20 *M. tuberculosis* P450s, CYP123A1 was found to be a good drug target for both active and latent phase of *M. tuberculosis*. Despite this greater importance that CYP123A1 can act as a novel drug target no study has been carried out to understand this P450 role in *M. tuberculosis* physiology. In this direction, this study is aimed to construct a 3D-model of CYP123A1 using homology modelling and perform binding analysis with different azole drugs.

**Methods:** (i) Homology modeling: The model of CYP123A1 was generated by homology modeling using MODELLER 9.17 based on the template selected using BLASTp at PDB data bank. Both the template and the modelled structure of CYP123A1 were aligned using SPDBV 4.1.0 with CA (Carbon-alpha) backbone with RMS value 1.81 Å. The 3D structure of CYP123A1 obtained was visualized by Pymol. The quality of the model was analysed by PROCHECK and Ramachandran map. (ii) Active site cavity: Active site cavity residues were identified based on the template structure using PDBSUM and used to generate the active site grid box. (iii) Ligand preparation: The 8 ligands (azole drugs) were retrieved from Pubchem and ligand database was build using Avogadro for conversion of SDF files to PDB file. (iv) Molecular docking: Docking of CYP123A1 model with different ligands was carried out using Autodock Vina. In Autodock Vina ligands were docking in the active site cavity. All structures were docked successfully and the results were visualized in Pymol and Discovery Studio 3.5.

**Results:** A 3D-model of CYP123A1 was built based on the template 3A4G. The protein 3A4G was selected on the basis of maximum identity of 38% with query cover 96%. Amino acids, Asn 65, His 94, Arg 98, Arg 291, His 348 and Cys 350 were found to be part of the active site cavity. The interaction of model CYP123A1 with the eight azole drug ligands clotrimazole, econazole, fluconazole, itraconazole, ketoconazole, miconazole, posaconazole and voriconazole was performed in Autodock vina. The results showed ligand interactions with active site residues Tyr 282, Asn 241, Gln 287 and Glu 306. Tyr 282 is the most interacting amino acid residue within the cavity. Clotrimazole, itraconazole and miconazole showed no interactions within the active site cavity of CYP123A1. Itraconazole showed the lowest binding at -9.6kcal/mol and had no interaction within the cavity. Posaconazole had -8.8 kcal/mol binding energy with 1 interaction with Glu 306 with bond distance of 2.32 Å, and can be considered as the best ligand for model CYP123A1.

**Conclusion:** In conclusion, CYP123A1 of *M. tuberculosis* H37Rv 3D-model was successfully constructed and binding analysis with different azole drugs were carried out. It is noted that clotrimazole, itraconazole and miconazole show no interactions within the active site cavity of CYP123A1.
Aqueous Root Extracts of \textit{Dicoma anomala} (Sond.) Extenuates Postprandial Hyperglycaemia \textit{In Vitro} and Normalizes the Activity of Carbohydrate-Metabolizing Enzymes in Streptozotocin-Induced Diabetic Wistar Rats

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Introduction and Aim: \textit{Dicoma anomala} Sond. (Asteraceae) is widely incorporated as a treatment for diabetes mellitus among the Basotho tribe of the eastern Free State Province, South Africa. The study examined the antidiabetic effect of the plant extracts via \textit{in vitro} inhibition of $\alpha$-amylase, and $\alpha$-glucosidase as well as against streptozotocin (STZ)-induced diabetic Wistar rats.

Methods: The effect of administration of aqueous root extract of \textit{Dicoma anomala} (AQRED) at 125, 250, and 500 mg/kg bodyweight (b.w.) was investigated on water consumption, feed intake, body-weight, blood glucose, carbohydrate-metabolizing enzymes, antioxidant enzymes, glycosylated haemoglobin and lipid profiles were determined in STZ (60 mg/kg b.w.) -induced diabetic rats in comparison with glibenclamide (5 mg/kg b.w.) which is the standard prescribed orthodox antidiabetic medication.

Results: While all the extracts of \textit{D. anomala} showed activity against $\alpha$-amylase and $\alpha$-glucosidase, water extract revealed the most effective inhibition with an IC$_{50}$ of 101.90 and 27.41 $\mu$g/mL respectively. The water extract displayed competitive and non-competitive inhibition of $\alpha$-amylase and $\alpha$-glucosidase respectively. AQRED reversed towards normal control the elevated food/water intake, blood glucose levels, lipid peroxidation, lipid profiles, glycosylated haemoglobin and activities of gluconeogenesis enzymes with a concomitant decrease in body-weight, activities of enzymatic antioxidants, glycolytic enzymes as well as the high-density lipoprotein-cholesterol level brought-about by STZ administration.

Conclusion: The result of our findings proved the anti-hyperglycaemic activity of the plant and therefore validates the folkloric usage of the herb.
Introduction and Aim: The continued use and popularity of plant-based traditional medicines demands scientific validation of their claimed therapeutic use in disease management and treatment. A number of herbal products are sold to the public for serious disease and health conditions without sufficient or no scientific data in support of their disease claims. This is contrary to the regulations by medicine regulatory authority such as the South African Medicines Control Council (MCC) or SAPHIRA (South African Health Products Regulatory Authority) as it is now known as. One such product is the Antidiabetic Tea of Sing Fefur Herbs in McGregor in the Western Cape that is sold through the Fruit & Veg chain stores. The Antidiabetic Tea is a combination of 8 medicinal herbal plants. The project aimed at testing the effect of the Antidiabetic Tea on its inhibition of α-amylase and α-glucosidase enzymes in vitro, to determine the antioxidant properties of the product, to determine which of the plants has α-glucosidase inhibition activity and to attempt the purification and chemical characterization of the bioactive compound with α-glucosidase inhibition effects.

Methods: The finely powdered commercial product and the individual plant constituents of the product were sequentially extracted at room temperature with hexane, DCM, a 1:1 DCM:MeOH solvent mixture and MeOH. The water extraction was done separately also at room temperature. In all the extractions, the plant solvent ratio was kept at 1:5 (m/v) and each solvent extraction was done over 24hrs. Qualitative phytochemical analysis of the plants was done as per the methods of Trease & Evans (1989) and Tiwari (2011). The Oxygen Radical Absorbance Capacity Assay (ORAC) to determine antioxidant capacity of the plants was performed using a fluorescence spectrophotometer. The isolations and purifications were achieved through the use of solid phase extraction (SPE), TLC and HPLC. The α-amylase and the α-glucosidase assays were performed as the modified methods of Keerthana et al. (2013) and Sindhu et al. (2013) respectively. The flash chromatography for the isolations were done through step gradients of hexane:EtOAc:MeOH. 10 g of the extract was loaded onto a 50 g silica column and eluted on the flash chromatography system using a step gradient of (1) hexane:EtOAc (5:1), (2) hexane:EtOAc (3:1), (3) hexane:EtOAc (4:1), (4) 100% EtOAc, (5) EtOAc:MeOH (5:1), (6) EtOAc:MeOH (3:1) and (7) EtOAc:MeOH (1:1).

Results: The antioxidant capacity of the Antidiabetic Tea and the individual plants were compared with that of the rooibos tea. The Antidiabetic Tea and its plant component had relatively higher antioxidant capacity. The ORAC assay results of the water extracts of the Antidiabetic Tea and the individual plants were significantly higher than that of rooibos tea. The plants and the Antidiabetic Tea had no inhibitory effects on α-amylase enzyme whereas they inhibited α-glucosidase enzyme. The highest inhibitory activity towards alpha-glucosidase was found in the U. urens hexane extract and the T. vulgaris hexane extract (69.66% and 68.43%, respectively). This observation suggests that α-glucosidase enzyme is inhibited mostly by the less polar or medium polarity chemical components of the plant extracts. Phytochemical screening of selected methanolic and aqueous extracts of the diabetes tea and the S. africana-caerulea showed the presence of alkaloids, sugars, flavonoids, glycosides, proteins and amino acids, phenolics and tannins and saponins. Thin layer chromatography (TLC), flash chromatography and column chromatography resulted in the generation of 29 fractions. Of all the fractions generated, DM 23 was the purest and its structural elucidation was attempted.

Conclusion: The Antidiabetic Tea inhibited α-glucosidase enzyme. The α-glucosidase enzyme inhibition appears to be attributed to the hexane extract of U. urens and the T. vulgaris plants.
Effect of Valproate, Sodium Benzoate and Dextromethorphan in Hyperglycinemic Captive-Bred Vervet Monkeys (*Chlorocebus aethiops*)

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Introduction and Aim: The Primate Unit and Delft Animal Centre (PUDAC) of the SAMRC maintains the only research colony of captive bred vervet monkeys in South Africa. A small percentage (8%) of the cataract affected colony has high levels of glycine in their plasma (457-795 µmol/L) and cerebrospinal fluid (CSF) (7.5-12.7 µmol/L). Human patients with such high glycine levels (CSF/Plasma ratio >0.08), are diagnosed with nonketotic hyperglycinemia (NKH). Although there is a controversy with regards to the effectiveness of the available NKH treatment, sodium benzoate and dextromethorphan are used to normalise glycine levels. Therefore, the purpose of this intervention was to investigate the effectiveness of sodium benzoate and dextromethorphan in reducing glycine levels in spontaneous and valproate induced hyperglycinemia.

Methods: Twelve animals (4 controls, 4 induced and 4 spontaneous hyperglycinemic/cataract) were selected for the duration of three months. Blood, urine and CSF were collected in order to determine glycine levels for baseline, phase 1, phase 2 and washout period. The induction was achieved by therapeutic drugs such as valproate; whereas sodium benzoate together with dextromethorphan were used as treatment to normalise glycine in both induced and hyperglycinemic monkeys.

Results: The glycine levels before the induction and after treatment varied within the selected animals. Induction with 50 mg/kg valproate was not significant, however, changes in biochemistry findings for alkaline phosphatase, phosphate and platelet count were observed. Additionally, treatment with sodium benzoate and dextromethorphan significantly reduced glycine levels in CSF (p=0.04) and plasma (p=0.007) of the spontaneous group.

Conclusion: The treatment with sodium benzoate and dextromethorphan normalised glycine in the spontaneous group. It can be concluded that the NKH treatment was effective in selected spontaneous vervet monkeys and can be considered as alternative treatment in vervet colony.
A Comparison of New Column Technologies for Rapid Quantification of Current Antiretroviral Therapy

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Introduction and Aim: HIV remains a burden in developing countries and sub-Saharan Africa, including South Africa which remains the most severely affected, with approximately 1 in every 25 adults (4.2%) living with HIV. Atripla is the first line approved antiretroviral combination which incorporates tenofovir disoproxil fumarate (TFV-DP), emtricitabine (FTC) and efavirenz (EFV). It is a one-day-pill that was approved in July 2006 by the US Food and Drug Administration (FDA). It is considered to be the most affordable, yet effective anti-HIV regimen. ARV therapy can be very expensive, and therefore (therapeutic drug monitoring) TDM is essential, resulting in to select the criteria of individuals chosen for specific treatment regimens personalized medicine and accurate dosing which also decreases the prevalence of adverse treatment outcomes. TDM is commonly conducted by using LC-MS/MS to quantify drug levels in plasma. Currently available methods use old column technology that requires longer runtimes and is not feasible in a point of care environment. Therefore, the aim of this study was to compare the new column technologies in the development of an LC-MS/MS method for the rapid quantification of the current first-line ART.

Methods: This study was conducted to evaluate the efficiency of four columns (F5, C18, Biphenyl and RP-Amide) from the same manufacturer. Resolution, number of theoretical plates and asymmetry factor were the three parameters that were evaluated for determination of column efficiency in addition to other method validation parameters such as accuracy and precision. Each of the columns was used for LC-MS/MS quantification of Atripla together with their respective internal standards. All columns have the same dimensions of 5 cm x 2.1 mm, 2.7 µm and pore size of 90 Å. Different gradients were used for each column, however they all used the same mobile phase A (H2O + 0.1% FA) and mobile phase B (MeOH + 0.1% FA).

Results: The LODs ranged from 5 to 15, 5 to 10, and 1 to 5 for EFV, TFC and TFV-DP, respectively. Correlation coefficients were above 0.99 for all the analytes irrespective of column. Accuracy and precision for all the analytes in all columns met the +/-15% acceptable range as outlined by the EMA. Recoveries ranged from 26.82 to 97.94, 21.36 to 304.47, 40.37 to 109.68, and 35.86 to 86.74 on F5, C18, biphenyl, and RP-Amide, respectively. Resolution was within the acceptable range of 1.5 and above except for the resolution between EFV and TFV-DP which was 1.14 and 1.29 on the F5 and C18 columns, respectively. The number of theoretical plates ranged from 940.80 to 31710.40, 2616.44 to 428262.36, 2228.94 to 463542.04, and 1320.34 to 379502 for F5, C18, biphenyl, and RP-Amide, respectively. Lastly, the asymmetry factors ranged from 1.11 to 1.74, 1.03 to 1.52, 0.97 to1.47, and 1.03 to 1.61 on F5, C18, biphenyl, and RP-Amide, respectively.

Conclusion: With the exception of emtricitabine and its internal standard, lamivudine, which were out of the acceptable range for all of the tested parameters, the other drugs performed extremely well under all column conditions. However, the RP-Amide performed better than the rest of the column technologies tested.
Simultaneous Determination of Isoniazid, Nevirapine and Paracetamol in Plasma by High Performance Liquid Chromatography

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Introduction and Aim: Isoniazid, nevirapine and paracetamol are associated with drug-induced liver injury. Patients suffering from AIDS/TB may be on nevirapine and isoniazid treatment, and their pain managed with an analgesic such as paracetamol. Thus, concurrent use of the three drugs increases the potential for development of hepatotoxicity and, as such, plasma drug monitoring would be appropriate. Therefore, a high performance liquid chromatography (HPLC) assay was developed for the simultaneous determination of isoniazid, nevirapine and paracetamol.

Methodology: To 100 µl of plasma spiked with isoniazid, nevirapine and paracetamol, sulfapyridine (internal standard) was added. After protein precipitation with zinc sulphate, followed by methanol, the sample was centrifuged and supernatant purified by solid phase extraction. Of the eluent, 50 µl was injected into the HPLC. A mobile phase of 0.06% trifluoroacetic acid (A) and acetonitrile (B) at a flow rate of 1 ml/min, run with gradient, was used. The compounds were separated on a C18 analytical column with a run time of 13 minutes. UV detection was achieved at 260 nm. Retention times of isoniazid, paracetamol, sulfapyridine and nevirapine were 3.1, 9.9, 10.6 and 11.6 minutes, respectively.

Results: The average 5 days calibration curve was linear for isoniazid (y = 0.029x + 0.025; r = 0.9977), nevirapine (y = 0.043x + 0.127; r = 0.9984) and paracetamol (y = 0.097x + 0.070; r = 0.9998) with a CV% <20%. Accuracy at low, medium and high concentrations was 102%, 98% and 101% (isoniazid), 94%, 96% and 100% (nevirapine), and 99%, 97% and 99% (paracetamol), respectively. Paracetamol was most stable at ambient temperature, 4ºC and -20ºC. The method was used successfully in animal experiments to monitor isoniazid, nevirapine and paracetamol in the plasma of treated rats.

Conclusion: A robust and accurate HPLC method for simultaneous determination of isoniazid, nevirapine and paracetamol in plasma was successfully developed and applied.

(ENCORE ABSTRACT)
The *In Vitro* Diffusion Characteristics across Exercised Porcine Skin of Various Compounds Used Topically in the Treatment of Adiposis Edematosa

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Introduction and Aim: Adiposis edematosa, also known as cellulite, is characterized as the alteration of skin surface which gives rise to an orange peel or mattress effect. More than 85% of the female population suffers from this undesirable aesthetic problem as it usually begins at puberty and progresses throughout life. Aesthetic and cosmetic industries have used techniques such as vigorous massage or surgical methods to attenuate the progression of cellulite, however these techniques are known to be invasive and not cost effective. Topical creams contain active ingredients such as methylxanthines (caffeine and theophylline) and retinol. The methylxanthines may act by increasing lipolysis in adipose cells and increasing blood flow while retinol may improve skin texture. The purpose of this study was to determine the effectiveness of caffeine, theophylline and retinol in various formulations in penetrating porcine skin.

Methods: Method validation (linearity, accuracy, precision, LOQ, LOD, robustness) was performed for all three compounds according to ICT guidelines. A Flexar Perkin Elmer HPLC system containing a Flexar LC binary pump, autosampler and an UV-Vis detector as well as a Kinetex RP C18 (5µm, 150 x 4.6mm) column were used for all analyses. Mobile phases: caffeine and theophylline: 40% methanol: 60% water, 272 nm; retinol: 95% methanol: 5% water, 325nm. All running conditions: 1 ml/min, 20°C, 20 µl injection. The *in vitro* diffusion of all compounds across exercised porcine skin was determined using a Perme Gear flow-through diffusion system with seven in-line flow-through cells. 1 ml of caffeine (2.5%), theophylline (2%) or retinol (0.3%) in formulation (15% ethanol (70%), water and 34% polypropylene glycol) was loaded in each donor compartment and PBS (pH 7.4) pumped through the receptor compartments at 1.5ml/h and at 32°C. Samples were collected every 30 min over a 4.5 hour time period and analysed via HPLC for the presence of the three compounds.

Results: Linearity was obtained for all three compounds; caffeine: Rt = 2.5 min; 5 – 20 µg/ml ($R^2 = 0.9872$); theophylline: Rt = 2.1min; 1 - 25µg/ml ($R^2 = 0.9945$); retinol: Rt = 2.9min ($R^2 = 0.9992$). All other validity results indicated that the methods were precise, accurate and sensitive for the determination of the compounds. *In vitro* diffusion results indicated that both caffeine and theophylline diffused rapidly across the skin (30 min) after which diffusion decreased with limited detection observed after 120 min. Diffusion of theophylline resulted in the detection of two compounds (Rts = 2.1 and 2.5 min) indicating possible metabolism. Retinol diffusion was not detected in the time period tested indicating possible accumulation within the skin.

Conclusion: Both caffeine and theophylline penetrated rapidly across porcine skin as compared to retinol that seemed to accumulate within the skin due to its very lipophilic characteristics.
The Development of a Rapid LC-MS/MS Method for Simultaneous Quantification of First-Line HIV and TB Drugs

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Introduction and Aim: The human immunodeficiency virus (HIV) is a rapidly proliferating infection occurring in the body, which further develops into acquired immune deficiency syndrome (AIDS) causing destruction to the immune system. To date, there is no known cure to exist for the virus; however, medication available on the market hinders the damage caused by the viral disease, allowing the immune system to improve, thus increasing patient’s lifespan. The 2015 statistics on UNAIDS, reports that approximately 6 700 000 to 7 400 000 people in South Africa are living with HIV/AIDS. Due to the HIV virus supressing the immune system, co-morbidities may occur, one of the most common opportunistic infection being Tuberculosis (TB). TB is caused by Mycobacterium tuberculosis (M. tb), a parasitic bactericidal species belonging to the family Mycobacteriaceae. It is categorized as an airborne communicable disease and therefore uses the lung as its point of infection, causing pulmonary TB. However, it may also cause extra-pulmonary TB by targeting other regions of the body, further complicating treatment. Roughly nine million new TB cases are reported each year, with nearly two million deaths as a cause of the disease. Thus far, there has been no rapid method developed to quantify both HIV and TB drugs concurrently, therefore the aim of this study was to develop and validate a rapid and sensitive LC-MS/MS method for the simultaneous quantification of HIV and TB drugs, in order to provide a possible point of care solution to therapeutic drug monitoring.

Methods: First-line HIV drug combination Atripla (emtricitabine, efavirenz, and tenofovir disoproxil), together with first-line TB drugs (rifampicin, pyrazinamide, ethambutol, and isoniazid) was investigated. Methanol was used to prepare the appropriate serial dilutions for working standards and calibration and quality control standard solutions (LLOQ, LQC, MQC and HQC) of targets and their appropriate internal standards. The liquid chromatography tandem mass spectrometry (LC–MS-MS) system encompassed a Shimadzu LC-20 AD series HPLC system (Shimadzu Corporation, Kyoto, Japan) joined to a MicroTOF-Q II electrospray ionization (ESI) time-of-flight-mass spectrometry (TOF-MS) instrument (Bruker Daltonics, Bremen, Germany). Results were then analysed using Data Analysis 4.0 SP 5 (Bruker Daltonics).

Results: Results obtained from LC-MS/MS conditions presented recoveries ranging from 50-85% for first line TB and HIV drugs. Validity of the method and analytical parameters were investigated by analysing blank samples (rat plasma) which were spiked at different concentration levels. All of the data created was well within the prescribed EMA and FDA guidelines.

Conclusion: In summary, an LC-MS/MS with the ability to simultaneously quantify both HIV and TB first line drugs is both practical and cost-effective and is beneficial for public health as it would be applicable in a point of care environment providing personalised medicine.
The Effect of *Cannabis Sativa* L. Aerial Parts Extracts on Glucose Uptake and Growth Differentiation Factor-15 on Mouse 3T3-L1 Adipocytes *In Vitro*

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**Introduction and Aim:** Elevated levels of growth differentiation factor-15 (GDF-15) in patients are associated with the development of diabetes mellitus and its complications. Hyperglycaemia and insulin resistance are still observed in many diabetic patients on these treatments. *Cannabis sativa* L. extracts have been claimed to be used in diabetes treatment. These stories on *C. sativa* L. extracts have insufficient scientific data to justify their efficacy and the mechanism. Thus, the aim of this study is to test for the effect of *C. sativa* L. extracts on GDF-15 and glucose uptake on mouse 3T3-L1 adipocytes cells, *in vitro* as one of the many mechanism to control diabetes.

**Methods:** Five *C. Sativa* L extracts, HEX, DCM, DCM:MEOH (1:1), MEOH and water were generated by 24 h of maceration. Qualitative phytochemical analysis of the plant drug was performed to determine the presence or absence of certain groups of chemical compounds. Solid phase extraction (SPE) of four extracts (HEX, DCM, DCM:MEOH (1:1) and MEOH) were generated as a means for fractionating and purification of the extracts. Concentration of 0.15 g/mL of MEOH and 0.05 g/mL of HEX, DCM, and DCM:MEOH (1:1) in acetonitrile (ACN) was used. This was achieved using solvent volume of 200 mL Acetonitrile:H2O at different ratios of H2O (25-100%) on a silica-based C18 10g/60mL. The chemical fingerprint of fractions obtained by SPE was generated using an LC/MS. In summary, auto sampler system with the injection volume of 20 µl was used for LC/MS analysis. The scan collected in the Orbitrap at a resolution of 30,000 in an m/z range of 0-800. Cytotoxicity of these extracts was carried out on 3T3-L1 cell line using the MTT assay.

**Results:** *C. Sativa* L tested positive for terpenoids, glycosides, and flavonoids; it tested negative for carbohydrates. The gram yields were as follows, for 100 H2O fractions: HEX = 119 mg, DCM = 981 mg, DCM:MEOH = 502 mg, and MEOH = 688 mg and 100% ACN fractions: HEX = 352 mg, DCM = 4001 mg, DCM:MEOH = 565 mg, and MEOH = 213mg. The LC/MS fingerprint was obtained for all fractions. In this presentation the results will be presented on the work done thus far on the *C. sativa* L. extracts. This shall include the LC/MS chemical finger prints, the TLC of the extracts and preceding antidiabetic data produced thus far.

**Conclusion:** Phytochemical analysis of the extracts indicated the presence of terpenoids, glycosides, and flavonoids. The SPE purification produced 15 fractions. LC/MS analysis for all fractions were done. These fractions are currently being tested for their antidiabetic effect (glucose uptake) and the effect on GDF-15 *in vitro* using 3T3-L1 adipocyte cells.
The Potential of Cannabis Sativa L. Aerial Plant Parts Extracts to Reverse Drug Resistance in Selected Lung and Colon Cancer Cells

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Introduction and Aim: Multidrug resistance (MDR) in cancer is characterized by the overexpression of ABC transporters, which facilitates the active efflux of chemotherapeutic drugs from cells. The current mode of treatment is aimed at reducing the overexpression of ABC transporters but still presents intolerable signs of toxicity. Cannabis sativa L. has been widely used traditionally in most parts of the world to treat a number of ailments such as diabetes, cancer and malaria. Cancer patients use it therapeutically for its anti-emetic, analgesic and appetite stimulant properties. Numerous pharmacological studies have shown that Δ9-tetrahydrocannabinoidal (THC) and cannabidiol (CBD), known major cannabinoids, exerts antitumoural actions both in vitro and in vivo. However, few reports of its anticancer resistance reversal properties could be traced. The present study was therefore designed to demonstrate the potential resistance reversal effect of Cannabis sativa L. extracts using selected MDR lung and colon cancer cells. The aim of this study was to investigate the potential anticancer drug resistance reversal effect of Cannabis sativa L. extracts in vitro.

Methods: Firstly, the pulverized plant material was sequentially extracted with four organic solvents in order of increasing polarity starting with hexane, dichloromethane (DCM), DCM: methanol (1:1; v/v) and methanol, respectively every 24 hours for 2 days; whereas the water extract was performed in 3 days. The crude extracts were further fractionated by means of solid phase extraction using the following solvent concentrations; 100% H2O, 75% H2O, 50% H2O, 25% H2O and 100% acetonitrile. Thereafter, characterizations of the crude extracts and fractions of the plant was performed. It involved subjecting the pulverized plant to phytochemical analysis. Secondly, a high performance liquid chromatography tandem mass spectrometry (HPLC-MS) was applied to DCM and methanol crude extracts; and fractions for the identification and quantification of available compounds in the plant. Thirdly, fingerprinting the crude extracts using thin layer chromatography was done. Furthermore, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay was used to evaluate the antiproliferative effect of Cannabis sativa L. crude extracts, doxorubicin, docetaxel, verapamil, gemcitabine and 5-fluorouracil against the following cell lines: CCD-18C0, HT-29, NL20, NCI-H146 [H146], HCT-15 MDR cells, LS513 MDR cells and H69AR MDR cells.

Results: Seemingly, hexane followed by DCM delivered a higher yield of crude extracts than DCM:methanol (1:1; v/v) and methanol. Methanol and distilled water delivered equal amounts of yields respectively. About 5 fractions from each crude extract were obtained using SPE. Phytochemical analysis revealed the presence of glycosides, tannins, saponins, tannins, terpenoids and phytosterols. The following cannabinoids [tetrahydrocannabinolic acid (THCA-C4), THC, CBD, cannabichromenic acid (CBCA), cannabigerolic acid monomethylether (CBGAM), cannabivarin (CBV) and ethyl-3, 10-dimethyl-undecanoate (Palmitic acid)] were identified and verified by means of a liquid chromatography coupled to a quadrupole-time-of-flight (Q-ToF) detector. Several more unknown compounds were quantified and observed in both DCM and methanol crude extracts.

Conclusion: In conclusion, sufficient quantities of crude extracts and fractions were obtained; and successful characterization of extracts from the aerial plant parts of Cannabis sativa L. was achieved.
Evaluation of the Effect of *Cannabis Sativa Lennaeus* Aerial Parts Extracts on Cholinesterase Enzyme Activity *In Vitro*

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**Introduction and Aim:** Alzheimer’s disease (AD) is one of the most common neurodegenerative disorders characterized by progressive loss of cholinergic neurons in the brain. The worldwide prevalence of AD is 44 million, and in South Africa 350 000 elderly individuals are affected by the disease. Current AD treatment is through the inhibition of the activity of cholinesterase enzymes, however, the drugs used in this treatment are associated with side effects and limitations such as, nausea and vomiting. There is a need therefore to evaluate African Traditional Medicines (ATM) as potential therapeutics to be developed for the management and treatment of AD. There are anecdotal claims of the use of *Cannabis sativa L.* in the treatment of AD. There is lack of scientific data to support the efficacy and safety of these extracts in AD treatment. The study therefore aims to evaluate the effect of *C. sativa L.* extracts on acetyl cholinesterase (AChE) and butyryl cholinesterase (BChE) activities.

**Methods:** *C. sativa L.* aerial plant parts were collected and extracted in solvents, sequentially using hexane, dichloromethane (DCM), DCM: methanol, methanol and water, respectively, over 72 hours, after which they were concentrated by evaporation and freeze-drying. Phytochemical analysis of the crude drug was performed to determine the presence and absence of alkaloids, flavonoids, glycosides, saponins, and terpenoids. Thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) analysis to determine the chemical fingerprints of the extracts were performed. TLC bio-autographic and Ellman’s colorimetric methods were successfully developed. The purification of the bioactive compounds was achieved through the use of preparative TLC.

**Results:** Crude extracts of hexane, DCM, DCM: methanol, and methanol were obtained and their percentage yields were 23%; 0.80%; 1.75%; 3% and 16.7%, respectively. Different phytochemicals, *i.e.*, alkaloids, flavonoids, glycosides, saponins, and terpenoids were present in the ground aerial parts of *C. sativa L.*. The hexane and DCM extracts showed inhibitory activity of the AChE, while the water and MeOH extracts were devoid of any inhibitory activity. The TLC purification of the active hexane and DCM extract produced eight fractions. The Rf values of 0.19, 0.35, 0.39, and 0.44 cm were identified from the hexane extract, and 0.20, 0.35, 0.39 and 0.44 cm for DCM, inhibited AChE and BChE enzyme activity, respectively. These spots are indicative of the same compounds.

**Conclusion:** The hexane and DCM crude extracts inhibited the AChE and BChE as proven in the TLC auto-biography. These fraction or compounds need further analyses for their inhibitory effects on the AChE and BChE enzymes. More chromatographic analyses are needed to determine the chemical structure of the active compounds and any similarities that may exist. There is need for a dose response analysis of the active compounds using the 96 well spectroscopic AChE and BChE methods.
Determination of the Effects of *Cannabis sativa* L. Aerial Parts Extracts on Inhibition of Alpha Glucosidase and DPP-4 Enzymes and on Glucose Uptake in Chang Liver Cells *In Vitro*

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**Introduction and Aim:** According to the World Health Organisation, in 2014, there were 422 million people living with diabetes globally, as compared to 108 million people in 1980 (www.who.int). Several therapeutic approaches exist in treating diabetes, however, most treatments have undesirable side effects, and drug failure and insulin resistance to the drugs may occur. As such, there is a need to evaluate medicinal plants for potential in the treatment of diabetes. *Cannabis sativa* is a well-known medicinal plant. It is used traditionally to treat a variety of ailments including diabetes. To date, the efficacy of *C. sativa* L. against diabetes has not been scientifically evaluated. Thus the aim of this study is to investigate the effects of *C. sativa* L. extracts on 3 diabetes type II drug targets *in vitro*.

**Methods:** The aerial parts of *C. sativa* L. were extracted using different solvents. To test the *C. sativa* *in vitro*, 3 bioactivity assays were performed namely; α-glucosidase inhibition, DPP-4 enzyme inhibition and glucose uptake in the Chang liver cells. Qualitative phytochemical analysis of the crude plant extract was done. The plant extracts were then separated into fractions using column chromatography. The chemical fingerprint of the fractions was done using chromatographic analyses including TLC, HPLC and LCMS.

**Results:** To date, five *C. sativa* L. extracts were obtained namely; methanol, hexane, dichloromethane, water and a mixture of dichloromethane and methanol. The crude plant drug tested positive for the presence of tannins, saponins, glycosides and alkaloids as per the qualitative phytochemical analyses. All the organic *C. sativa* L. extracts showed inhibitory activity towards the alpha glucosidase enzyme with the methanol:dichloromethane extract exhibiting the highest percentage inhibition of 89.1% at 100µg/ml.

**Conclusion:** The *C. sativa* L. showed promising results in terms of inhibiting α-glucosidase. More efficacy tests will be conducted to answer some of the questions on the use of *C. sativa* L. extracts in the treatment and management of diabetes. This study is ongoing.
The Effect of *Phela* on *P*-Glycoprotein and Multidrug Resistance-Associated Protein-2 Transporters

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**Introduction and Aim:** Membrane transporters play an integral role in the determination of the pharmacokinetic, safety and efficacy profiles of drugs. Even so, little is known about the role of membrane transporters in herb-drug interactions. Therefore, the effect of *Phela* on intestinal *P*-glycoprotein (*P*-gp) and multidrug resistance-associated protein 2 (MRP2) was investigated in a rat model. Paclitaxel (PTX) and cyclosporin A (CyA) were used as the respective substrate and inhibitor of *P*-gp, while methotrexate (MTX) and probenecid (PRO) were those of MRP2.

**Methods:** Ethical approval was obtained and male Sprague-Dawley (SD) rats (200 – 250 g) were used. The animal experiment was divided into two parts. In Part I, three groups of 40 rats each received a once-off oral dose of PTX-only (10 mg/kg), PTX & CyA (10 mg/kg) or PTX & *Phela* (15.4 mg/kg), while in Part II, three groups of 40 rats each received a once-off oral dose of MTX-only (10 mg/kg), MTX & PRO (20 mg/kg), or MTX & *Phela* (15.4 mg/kg). For each group, 5 rats were sacrificed after 0.5, 1, 2, 4, 6, 8, 10, and 12 hours. Blood was analysed for full blood count, liver function, and PTX and MTX concentrations.

**Results:** CyA and PRO increased the area under the plasma concentration-time curve (AUC) of PTX and MTX, respectively, whereas *Phela* had no effect on the AUC of PTX or MTX.

**Conclusion:** Therefore, *Phela* did not inhibit *P*-gp or MRP2, and this implies that *Phela* will most probably not be involved in herb-drug interactions of membrane transporter origin.

(ENCORE ABSTRACT)
The Use of the ‘Queuing Theory’ and Patient Based Characteristics to Assess the Performance of the Paediatric Intensive Care Unit (PICU) at Universitas Academic Hospital in South Africa

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Introduction and Aim: The performance of an intensive care unit (ICU) is the prompt admission of patients with specific conditions, and institution of appropriate management leading to expected outcomes within the expected time. Such performance is influenced by patient, institutional and environmental based characteristics. Patient based characteristics include the severity of the condition, complications developed, disease pattern, number of patients, and age, etc. Institutional based characteristics include human resources, structure of the health facility, equipment, supplies, and the nature of the ICU, while environmental based characteristics refer to the social-economic status and the health system of the country. Assessment of ICU performance generally involves selection of appropriate indicators in patient, institutional and environmental based characteristics, and their application in relevant mathematical models. Unfortunately, these models have tedious requirements and are more accurate in settings similar to those they were developed. Here, a simple method using the ‘queuing theory’ and patient based characteristics to gauge the performance of the Paediatric Intensive Care Unit (PICU) is described.

Methods: This was a ten year retrospective study to determine the queuing nature and patient based characteristics in the PICU using records of patients who were prescribed antibiotics from January 1998 to December 2007. The daily arrival rates and length of stay were used in the queuing simulation formula to derive the appropriate parameters.

Results: Sixty-three percent of the PICU was utilised by patients who stayed for 7.48 ± 6.77 days. It admitted mainly children and infants who presented with low body weight, a variety of medical and surgical problems, and experienced many complications while in the PICU. These patients were successfully managed, whereby most patients improved, leading to a low mortality rate.

Conclusion: The queuing theory was successfully used to evaluate the performance of the PICU and to recommend appropriate remedial measures, and this should be applicable to other ICUs.

(ENCORE ABSTRACT)
Introduction and Aim: Despite the existence of prescribing guidelines, appropriate antibiotic use remains a major challenge to all intensive care units. Knowledge of the pattern of antibiotic use is important to develop better strategies for rational antibiotic use in a particular setting. Therefore, the aim of this study was to describe the pattern of antibiotic use in the Paediatric Intensive Care Unit of Universitas Academic Hospital (PICU-UAH).

Methods: This was a retrospective study of patients admitted to the PICU-UAH from 1998 to 2007. Data collected included admission information, patient demography, problems on admission and during stay in the PICU-UAH, culture and sensitivity, and antibiotics used.

Results: There were 685 patients in whom 38 different antibiotics were prescribed at an average rate of 24.1±2.5 per year. Broad-spectrum bactericidal antibiotics were more preferred and narrow-spectrum bactericidal antibiotics were used for specific indications. The top ten antibiotics accounted for 81.2% of antibiotic usage, of which 52.6% was for the top three (cefotaxime, amikacin and vancomycin). Regarding the phases of admission, 29±5.8% patients were on antibiotics on admission, 79.9±3.3% used antibiotics within the first three days, and 23.2±4.6% had their antibiotics modified after three days. The top antibiotics used on admission and within the first three days were similar, but differed from those used after three days.

Conclusion: It has been shown that the pattern of antibiotic use in the PICU-UAH depicted a disproportionate utilization of a few older broad-spectrum and narrow-spectrum bactericidal antibiotics, mostly as combination regimens and according to the phase of admission.

(ENCORE ABSTRACT)
The Antibiotic Resistance Pattern in the Paediatric Intensive Care Unit of Universitas Academic Hospital (PICU-UAH) in South Africa

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Introduction and Aim: Antibiotic resistance is a major problem in all settings where antibiotics are regularly used, especially in intensive care units. Therefore, the aim of this study was to describe the pattern of antibiotic resistance in the Paediatric Intensive Care Unit of Universitas Academic Hospital (PICU-UAH).

Methods: This was a retrospective study of patients admitted to the PICU-UAH from 1998 to 2007. Data collected included admission information, patient demography, problems on admission and during stay in the PICU-UAH, culture and sensitivity, and antibiotics used.

Results: The top ten bacteria accounted for 91.8%, viz; Staphylococcus (29.3%), Klebsiella (11.8%), Acinetobacter (11.7%), Pseudomonas (11.2%), Escherichia (8.5%), Enterococcus (5.9%), Streptococcus (4.1%), Enterobacter (4.1%), Stenotrophomonas (3.4%) and Haemophilus (2%). The majority (58.7%) of the bacteria cultured were Gram-negative and these were mainly from tracheal aspirates (90.1%), while Gram-positive bacteria were mainly from blood (73.2%). Staphylococcus exhibited high resistance to all penicillins with no resistance to vancomycin. Klebsiella and Pseudomonas exhibited resistance to some aminoglycosides, cephalosporins and penicillin, but Klebsiella remained sensitive to imipenem. Acinetobacter and Stenotrophomonas were highly resistant (>70%) to almost all antibiotics, except tobramycin for Acinetobacter and co-trimoxazole for Stenotrophomonas.

Conclusion: It has been shown that the pattern of bacterial resistance in the PICU-UAH depicts increasing resistance by well-known bacteria to the most commonly prescribed antibiotics, implying that acquired resistance is the most prevalent mode of resistance.

(ENCORE ABSTRACT)
In silico Analysis of Ketoconazole Resistance by Chronic Granulomatous Infectious Fungus Sporothrix schenckii

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Introduction and Aim: Sporotrichosis is an emerging chronic, granulomatous, subcutaneous, mycotic infection caused by Sporothrix species. Sporotrichosis is treated with the azole drug itraconazole as ketoconazole is ineffective. It is a well-known fact that azole drugs act by inhibiting cytochrome P450 monooxygenases (P450s), the heme-thiolate proteins. To date, nothing is known about P450s in Sporothrix schenckii and the molecular basis of its resistance to ketoconazole. Here we present genome-wide identification, annotation, phylogenetic analysis and comprehensive P450 family-level comparative analysis of S. schenckii P450s with pathogenic fungi P450s, along with a rationale for ketoconazole resistance by S. schenckii based on in silico structural analysis of CYP51.

Methods: The whole proteome of S. schenckii was subjected to NCBI CDD search and proteins grouped under P450s were assigned to different P450 families and subfamilies based on P450 nomenclature committee rules. P450 diversity analysis was estimated based on number of P450 families and number of P450s. Template selection for CYP51 modeling was performed using PSI-BLAST algorithm over Protein Data Bank. Alignment of the sequence was used as the input to generate a homology model of CYP51 using I-TASSER and Modeller followed by final refinement with molecular dynamics [GROMACS]. Coordinates for heme were obtained from the closest template 4K0F and positioned accordingly in the model. The constructed models were subjected to structural quality evaluation with I-TASSER and PROCHECK. The best two models were used for docking simulations. All docking experiments were carried out with AutoDock Smina. CYP51 model was prepared for docking with AutoDockTools. A three dimensional grid box that narrows probing space was set over the active site (24 x 16 x 16 Å). Best poses selected by AutoDock Smina scoring function were superposed with known CYP51 P450s co-crystalized with azole inhibitors to discard outliers.

Results: Genome data-mining of S. schenckii revealed 40 P450s in its genome that can be grouped into 32 P450 families and 39 P450 subfamilies. Comprehensive comparative analysis of P450s revealed S. schenckii share 11 P450 families with plant pathogenic fungi and have three unique P450 families: CYP5077, CYP5386 and CYP5696 (novel family). Among these P450s, CYP51, the main target of azole drugs was also found in S. schenckii. 3D modeling of S. schenckii CYP51 revealed the presence of characteristic P450 motifs with an exceptionally large reductase interaction site 2. In silico analysis revealed a number of mutations that can be associated with ketoconazole resistance, especially at the channel entrance to the active site. In silico CYP51 docking with azole drugs revealed that itraconazole, as a more extended molecule, can form a hydrogen bond with ASN-230 and thus a better stabilization compared to ketoconazole, hence it is effective against S. schenckii vis-a-vis resistant to ketoconazole.

Conclusion: To our knowledge, this study is the first report on S. schenckii P450s and in silico analysis of CYP51-based ketoconazole resistance by this fungus. Overall, the study results opened new vistas for the future unravelling of the long standing mystery of ketoconazole resistance by this fungus.
Understanding the Regulatory Framework and Requirements for Clinical Trial Authorization in Africa

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Introduction and Aim: Medicinal products are amongst the most strictly regulated products in the world. Health authorities’ mandates include protecting and promoting public health. Health authorities protect the public by ensuring only medicinal products of good quality, and by which clinical trials have shown to have acceptable safety and efficacy are approved. Africa bears a huge burden of the world’s communicable and non-communicable diseases, yet only 2% of global clinical trials are carried out in Africa. Importantly conducting clinical trials in Africa is projected to increase; however, the lack of clarity on the ever changing regulatory environment may delay and/or hinder this projection. Thus, understanding the evolving regulatory landscape may help sponsors and investigators to comply with requirements, better plan and efficiently execute clinical trials, while maintaining best practices in meeting the unmet patient medical needs. The aim of this study was to evaluate the regulatory framework and requirements for clinical trials authorization (CTA) in Africa.

Methods: We conducted a descriptive study and collected information to answer the study objectives from the following: latest guidelines from the health authorities’ websites or direct enquiry from the health authorities, contract research organizations, and our past and ongoing experience with application for CTA in Africa. Additional information were obtained by doing a literature search using PubMed, Google Scholar, the Cochrane Library, and Cortellis database. The data were entered into excel sheet, and categorized on the basis of similarities and differences in both the regulatory framework and the requirements for CTA. Thirteen (13) African countries were selected based on ongoing applications for CTA or for possible future CTA applications. The countries were grouped into English East Africa, English West Africa, French West Africa, and Southern African countries.

Results: We found that requirements to apply for CTA were similar in all countries with respect to the following: compliance with ICH-GCP, Declaration of Helsinki, protocol, investigator brochure, insurance certificate, sponsor and investigator declarations, informed consent form (ICF) and patient information leaflets (PIL) in respective official and local languages. The requirements between countries differed widely with respect to IMP Dossier (IMPD), import license (IL), Certificate of Analysis, Good Manufacturing Practice (GMP), local and central ethics committees’ requirements, material transfer agreement (MTA), clinical trials registry, safety reporting, and translations of some documents from English to French and Portuguese, etc. There have been several efforts at harmonization of CTA and inspections involving various African countries under the auspices of African Vaccine Regulatory Forum (AVAREF) facilitated by WHO, and supported by non-governmental organizations, and competent health authorities with stringent requirements. In parallel to AVAREF is the African Medicine Regulatory Harmonization (AMRH) aimed at promoting regional harmonization for registration of medicinal products, and ultimately the formation of an African Medicine Agency.

Conclusion: The regulatory landscape in Africa is evolving with structure similar to what is obtainable from international guidelines such as ICH-GCP etc. The regulatory framework and requirements for CTA vary across countries, and in some instances even within the same country. Harmonization of CTA in Africa is needed, which may allow more clinical trials to be done more efficiently.
Pilot Study - A Survey on the Awareness and Use of Complementary Medicines among Staff Members of the National Department of Health (NDoH) Living in Pretoria and Surrounding Areas

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Introduction and Aim: There is a growing concern within the South African medicines regulatory authority on certain class of products known as complementary medicines (CM). These products are sold to the public and consumed without their safety, efficacy and quality being evaluated and approved by the Medicines Control Council (MCC). The generally accepted perception that complementary medicines being natural medicines are safe is wrong and misleading. The MCC has established a regulatory framework on complementary medicines as a way of ensuring that these products are evaluated and registered accordingly. However, there is still inadequate information on the awareness and use of such products by the South African public. The aim of this pilot study was to investigate the awareness and use of complementary medicines among staff members of the National Department of Health living in Pretoria and surrounding areas.

Methods: The study was approved by Health Research Ethics Committee (HREC) of Stellenbosch University before commencement. Random sampling was used to select participants from NDoH employees (n = 200). Participants were asked to complete an informed consent form and study questionnaire. The categorical data of the responses from the participants were analysed by Fischer’s exact test at 95 % confidence interval using SANTA 14 statistical programme. A p-value of < 0.05 was considered to be statistically significant where applicable.

Results: Of the 200 participants, 70.5 % were aware of complementary medicines and 23.0 % were not aware. There was 66 % of the younger participants who were aware of CM compared to 75 % of older participants (p = 0.041) and 79 % of female participants who were aware of CM compared to 62 % of male participants (p = 0.029). A proportion of 29.5 % of participants have used CM during the past 12 month prior to the study and 58.0 % never used CM. There was 42 % overall usage of CM among the female participants compared to 17 % usage among male participants (p = 0.000) and 66 % CM usage among younger participants compared to 75 % usage among older participants (p = 0.807). There was 23.5 % use of Western Herbal Medicines, 3 % use of Unani-Tibb and 3 % use of Ayurveda among the participants. A proportion of 36.5 % of participants used CM for the maintenance of good health.

Conclusion: The results obtained in this pilot study will serve as preliminary findings for the much broader future surveys on the awareness and use of complementary medicines in South Africa. The findings will also prompt more extensive campaigns on the awareness, knowledge and use of complementary medicines in South Africa.
Comparison of Oral Paracetamol to Amitriptyline as an Adjunct to Morphine in the Management of Head and Neck Cancer Pain in Dr George Mukhari Hospital, Pretoria, South Africa

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Background and Aim: Pain is the most common symptom reported by patient with head and neck cancer to physicians. The majority patients with head and cancer have problem of chronic pain even if they are on analgesics. To compare the effectiveness and side effects of paracetamol to amitriptyline as an adjuvant to morphine for the head and neck cancer patients.

Methods: This was a randomized double-blinded controlled trial amongst head and neck cancer patients at Dr George Mukhari Hospital, South Africa. The study participants were divided into two groups: group A (given paracetamol 1 g and morphine orally (3) three times a day), while group B (given 25 mg amitriptyline and morphine orally (3) three times a day). The participants scored their pain using a verbal 0–10 pain scale. The data was analysed using Stata version 9.

Results: A total of 99 patients were enrolled in the study of which two died. The mean age of the patients was 55.3±13.7 years (range: 15 to 86 years). Majority (32%) were in the age group 50-59 years and (81%) were males. A greater proportion of (35%) of cancer types were larynx cancer. The mean duration of pain treatment was 13.7±4.1 days (range 1 to 14 days). Sixty percent (58/97) suffered from pain during various time and most (82%) were patients with oral cavity cancer. Mean duration of pain was 4.5±3.6 days (range: 1 -14 days). The level of pain for both treatment groups decreases with time with paracetamol shows a lower mean pain score in the first week of the study. Nausea, constipation and itching were the most common side effect in both groups.

Conclusion: Our results indicate that paracetamol and amitriptyline are clinically effective and equivalent for pain control in the treatment of pain for head and neck cancer patients. Adjuvant analgesics are not effective when used alone however in combination with opioids improve the level of analgesia and reduce side-effects in head and neck cancer patients.
Cardiovascular Disease Risk Assessment in Healthy Volunteers Recruited to the Study: The Effects of Oral Hypoglycaemic Drugs on Arterial Elasticity and Pro-Inflammatory Markers in Type 2 Diabetic Mellitus Patients at Dr George Mukhari Academic Hospital, Pretoria, South Africa

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Background and Aim: Cardiovascular disease (CVD) is the leading cause of death worldwide. According to WHO in 2008 30% (17.3 million) of all deaths worldwide could be attributed to CVD. In South Africa in 2009 CVD was responsible for 14% of total deaths and mortality rates are expected to increase as unhealthy lifestyle tendencies linked with urbanisation spread to the countryside. Increased arterial stiffness is a determinant of cardiovascular mortality and an independent marker of cardiovascular disease. Arterial stiffness can be assessed non-invasively by measurements of pulse wave velocity (PWV) and augmentation index (Aix). Increased PWV and Aix indicates damage to the elastic tissue of the arteries and has been shown to be an integrated index of cardiovascular risk factors. The objective of this study was to assess cardiovascular disease risk in healthy volunteers recruited to the study.

Methods: This was an open label cross sectional longitudinal study. The study was approved by Sefako Makgatho University Research and Ethics Committee (SMUREC/M/112/2016: PG). Forty healthy volunteers were recruited. The eligibility criteria were male or female, aged 18-70 years with no abnormalities in physical examination, no chronic or acute diseases and not on any chronic medication. After signing a consent form and filling in a questionnaire, vital signs, BMI, and fasting glucose were measured. Elasticity of the arterial tissue was assessed by determination of PWV and Aix using the AtCor SphygmoCor®. Blood was collected via vacupuncture and determination of HBA1c levels and the lipid profiles were done by the National Health Laboratory Services. The Framingham risk assessment scale for CVD, Aix and PWV were used to determine CVD risk.

Results: 35% of the study participants were pre-obese (24-30 kg/m²), 33% were obese (30-40 kg/m²) and 10% were very severe obese (>40 kg/m²). 27% of the study population below 30 years had PWV and Aix above the 90 percentile, 30% of those between 30-40 years, 40% of those between 40-49 and 43% of those between 50-59 also had PWV and Aix above the 90 percentile. There was a statistically significant correlation between PWV, Aix and age (p=0.01; p=0.06 respectively) and aortic SBP (p=0.05; p=0.05 respectively). There was a statistically significant correlation between PWV and BMI (p=0.01), radial SBP (p=0.02).

Conclusion: There is a statistically significant correlation between CVD risk factors, PWV and Aix, indicating that an increase in age, aortic SBP and BMI is associated with an increase stiffness of the arteries.

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Introduction and Aim: Many epidemiological studies have shown relation between high plasma cholesterol, particularly of LDL-cholesterol and the risk of cardiovascular diseases (CVD). Lipoproteins and their constituents (apolipoproteins) play a major role in atherosclerosis. Cholesterol metabolism to bile acids represents a major pathway for elimination of excess cholesterol from the body. Dietary fibre, spice adjuncts, plant sterols and polyunsaturated fatty acids play a very important role in the regulation of body’s cholesterol level. The present study evaluated the mRNA expression of CYP7A1, CYP27A, Apo A, Apo B and HMG-CoA reductase which are key enzymes that regulate the bile acid synthesis and play an important role in the elimination of cholesterol from body during induction of hypercholesterolemia experimentally.

Methods: Myocardial infarction was induced by isoproterenol administration in experimental animal groups. After the treatment period liver tissue was excised from representative animals from each group were immediately stored at – 80°C in RNA later until further analyses. RNA was extracted from liver tissue and cDNA was synthesized from total RNA using m-MULV reverse transcriptase. Finally, mRNA expression was analyzed by quantitative real-time RT-PCR.

Results: mRNA expression of CYP7A in normal as well as HCD-fed rats was up-regulated by dietary fenugreek+garlic; this combination also up regulated the same in isoproterenol administered rats in HCD fed group. Similar effect was observed in mRNA expression of CYP27A in HCD-fed group in both normal as well as infracted situation. These spices upregulated the mRNA expression of HMG-CoA reductase in the groups fed with combination of these in normal and in myocardial infarcted animals. Dietary fenugreek+garlic, upregulated the mRNA expression of Apo-A and downregulated the mRNA expression of Apo-B in both normal and infarcted situation as well.

Conclusion: This gene expression study indicates the favorable upregulation of Apo A1 which is responsible for elevated levels of HDL (meant for excretion of cholesterol from the body) and downregulation of Apo-B which is responsible for lowered levels of LDL (meant for deposition of cholesterol in tissues). These results on mRNA expression supported the significant increase observed in the bile acid pool as a result of dietary interventions with fenugreek, garlic or their combination, as a mechanism contributing to cardio protection.
A Comparative Study for the Topical Treatment of Atopic Dermatitis with *Aloe ferox* and *Aloe vera* in Balb/c Mice

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Introduction and Aim: Atopic dermatitis (AD) typically develops in patients with a history of allergic ailments, and is characterised by an itchy, inflammatory skin condition with scaling, lichenification, papules, excoriations and pruritus. In AD patients a chronic relapsing inflammatory condition is seen, associated with IgE hyper production. AD flares are largely triggered by environmental factors. However, the exact aetiology of AD is unclear and there is a pressing need for new treatment regimens as AD is a chronic condition and requires long-term treatment. Historically *Aloe* has been used to treat skin conditions as well as a variety of other diseases.

Method: The aim of this study was to investigate and confirm the therapeutic efficacy of *Aloe ferox* versus a comparator *Aloe vera* as a topical treatment for Dinitrofluorobenzene (DNFB)-induced atopic eczema/dermatitis syndrome (AEDS) in Balb/c mice. To explore the pathogenesis and treatment of AD, Balb/c mice were sensitized and challenged with 2,4-dinitrochlorobenzene (DNCB) for atopic dermatitis induction. Thereafter, mice were treated with either *Aloe ferox* or *Aloe vera* applied daily on the dorsal skin for 10 consecutive days. A placebo gel was used for the control mice. Blood was collected at the end of the treatment period and serum IgE levels measured.

Results: Serum IgE levels were significantly lower in the *Aloe ferox* group and the *Aloe vera* group compared to the placebo group. Superiority of *Aloe ferox* vs. *Aloe vera* was demonstrated following application of the appropriate pair-wise t-test (p=0.002) as well as from the fact that the upper limit of the 95% confidence interval *(-.090; -.028)* for the difference between *Aloe ferox* and *Aloe vera* was less than zero.

Conclusion: These Balb/c mouse models have highlighted the role of allergic sensitization to epicutaneously introduced allergens and taken together, the present data indicated that topical application of *Aloe ferox* and *Aloe vera* can suppress chronic AD by a comparatively selective reduction of Th2 responses. Furthermore, it was shown that not only is *Aloe ferox* effective in treating AD but is also superior to *Aloe vera* for its effectiveness in treating AD and achieved a good cosmetic result without any side-effects such as alopecia, telangiectasia and skin atrophy. Therefore, *Aloe ferox* may be useful as an alternative or intermittent treatment for the management of patients with recurring or chronic AD who require long-term therapy.
Blockchain Technology in Improving Patient-Centered Care Delivery and Supply Chain Effectiveness in Africa

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Background and Aim: The rapid growth in blockchain (BC) applications in industries from capital markets to public healthcare and supply chains performance is still widely misunderstood in strengthening health services delivery in the absence of standard definition and resource capacity. Understanding how and what BC technology linked internet of a things and social media discovery did for information on health decision-making policy, patient access and utilization of care delivery services regionally and globally. The prospective paper aims at assessing the value of BC technology and its applications and integration in improving patient-centered care delivery and supply chain efficiency and cost-effectiveness in health system strengthening in Africa.

Methods: A retrospective survey was conducted in assessing the potential value of blockchain (BC) technology and applications implications from 2010-2017, in order in improving patient-centered services, transactions and mutual benefits amongst public health, pharmaceutical and biotechnology to financial management and society cohesion.

Results: BC emerging technologies and cross-industry powerful properties adoption and integration is crucial for scale health systems strengthening and stakeholders’ networks. Importantly, blockchain-enabled pharmaceutical systems and collaboration in data sharing needed in decentralized and encrypted distribution, supply chain management and procurement ramifications benefits. Moreover, it allows improved pharmacy and clinical epidemiological practice crucial in enhancing health informatics workflows, patient-centered data confidentiality, transparency, reliability and scalability. The emerging and fast-growing care solution with highly interconnected and robust digital blockchain-secured applications implementation model or ecosystem enables digital or mobile quality care delivery, safe and transparent supply chains, risk management, digital rights and precision-medicine quality assurance and management at all levels. This is essential in strengthening appropriate goods production and service delivery, supply chain and empowering patient data safety, integrity and others additional functionalities (collaboration, data sharing, claim adjudication and insurance payment, communication and interoperability). Improving the efficiency and cost effective local and international electronic pharmaceutical and biotechnology goods productions, sourcing and supply chain to medication prescriptions and pharmacist-professionals service delivery. Moreover, fostering Patient- health professional-provider collaboration in empowering patients, logistical information tracking hurdles and reliability-centered maintenance laboratory, supply chain to socio-demographic data sources discrete, security and segregation of vulnerability in frontline and hospitals own vital data analysis or readily identify and respond to threats/outbreaks and cyber-attacks challenges and ethical patient data/information issues.

Conclusion: Fostering local and global BC integrated public health ecosystem leadership and partnerships commitment and investment, standard BC and frameworks development and implementation is imperative in optimizing patient-centered care delivery and lifestyle diseases preventive measures and access to and uptake of supply chain interventions. This is important and requires for robust, sustainable and effective health and information sharing and communication, need-based stakeholders transactions in Africa and worldwide.
Preventive Effect of Cactus (*Opuntia Ficus-Indica*) Cladodes on Methotrexate Induced Oxidative Damage of the Small Intestine

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Introduction and Aim: Methotrexate (MTX) is one of the most widely prescribed anticancer agents which is used in the treatment of leukemia, and other malignancies. It is a cytotoxic chemotherapeutic element for various inflammatory diseases such as psoriasis dermatomyositis and rheumatoid arthritis. The cytotoxic effect of MTX is also seen in normal tissues having a high proliferation rate, including gastrointestinal and bone marrow.

Methods: Adult, healthy male Wistar rats (200-250g) were pre-treated by an intraperitoneal administration of an ethanol fraction of cactus cladode. Control animals were treated for 10 days by an intraperitoneal administration of saline or ethanol fraction of cactus cladode (0.4 g/kg). Following a single dose of methotrexate (20 mg/kg) given intraperitoneally, either vehicle (saline) or ethanol fraction (400 mg/kg) was administered intraperitoneally. Treatments were continued daily for 10 days consecutive. All animals were killed 24 hours after the intraperitoneal injection of MTX. Small intestine samples were collected for MDA level, protein carbonyl generation and Peroxidase and Catalase activity measurement. Small intestine was also collected for histopathology analysis.

Results: Our results showed that MTX induced significant alterations in oxidative stress markers noticed in the form of intestinal tissues damage, MDA level increase and protein carbonyls generation. Catalase and Peroxidase activities decreased with MTX administration. The combined treatment of MTX with cactus extracts showed a reduction of MTX induced oxidative damage.

Conclusion: It could be concluded that cactus cladodes extract was effective in the protection of the small intestine against MTX-induced damage.
Grapefruit Juice Prevented the Rise in Liver Enzymes after Paracetamol Overdose in Rats

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Introduction and Aim: Paracetamol (PARA) is a widely used analgesic and antipyretic agent. While it is generally safe for use at recommended doses, acute overdose of PARA can cause fatal liver damage. Despite the understanding that some cytochrome P450 isoforms are responsible for activation of PARA to the hepatotoxic metabolite, N-acetyl-p-benzoquinoneimine (NAPQI), the use of enzyme inhibitors for prevention and/or treatment of PARA hepatotoxicity is still not well researched. Therefore, grapefruit juice (GFJ), a well-known enzyme inhibitor, was investigated for the prevention of hepatotoxicity after PARA overdose in rats.

Methods: The study was approved by the animal ethics committee, which also imposed dosage limitations for this experiment. Twelve groups of 5 Sprague-Dawley rats each were treated with single oral dose of either saline, PARA only 1000 mg/kg, PARA + GFJ low dose (2 ml) and PARA + GFJ high dose (3 ml). The remaining six groups were treated with bergamottin, a GFJ derivative and known enzyme inhibitor, instead. Thereafter, 5 rats from each group were sacrificed after 24, 48 and 72 hours and, on each occasion, blood samples were collected for determination of liver and renal function, electrolytes, FBC and PARA concentration. A piece of liver was sent for histopathology.

Results: By 48 hrs the liver enzymes in the PARA only group were significantly (P<0.05) higher than in the GFJ + PARA and bergamottin + PARA groups. The concentrations, median (range), were: alkaline phosphate (ALP) 319 [71-328] u/L and alanine transaminase (ALT) 56 [50-59] u/L for PARA only, versus ALP 44 [39-58] u/L, ALT 40 [36-49] u/L for GFJ, ALP 34 [24-401] u/L, ALT 46 [41-49] for bergamottin, and ALP 96 [75 -238] u/L and ALT 38 [37-43] u/L for the control group.

Conclusion: GFJ and bergamottin prevented PARA induced increase in liver enzymes after paracetamol overdose. Although the increase in liver enzymes did not meet the criteria for clinical hepatotoxicity, the results are promising, and call for use of a higher dose.

(ENCORE ABSTRACT)
Amelioration of CCl₄-induced hepatotoxicity in male rats treated with methanol extract of *Piper guineense*

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Introduction and Aim: The current increase in the incidence and prevalence of liver diseases remain a major globally health concern. Chronic liver diseases have been identified as the fifth most common cause of death in the United Kingdom, as well as accounting for about 4.8 % deaths among American Indians and Alaska Natives. The primary role of the liver is in metabolism and subsequent removal of various therapeutic agents and xenobiotics from the body. There has been no definite therapeutic agent for total cure of liver diseases; documented scientific evidence suggests that most of the available therapeutic agents facilitate the healing or regeneration of the liver. To ascertain the hepatoprotective effect of drugs/molecules during drug screening, Carbon tetrachloride (CCl₄) induced hepatic injury remains an excellent commonly used model. Therefore, this study seeks to investigate the possible protective role of *Piper guineense* against CCl₄-induced hepatotoxicity in male rats.

Methods: Hepatotoxicity was induced by administering oral dose of CCl₄ (1.2 g/kg bw) three times a week for 3 weeks. In the modulatory experiment, *Piper guineense* (PG), (400 mg/kg bw) was administered by oral gavage for 14 days prior to the administration of CCl₄ and simultaneously with CCl₄ in the pre-treatment group; PG (400 mg/kg bw) was administered simultaneously with CCl₄ in the post-treatment group while Livolin forte (20 mg/kg bw) was administered simultaneously with CCl₄ in the standard drug group.

Results: Administration of CCl₄ induces histopathological alteration in the liver with concomitant increased activities of serum hepatic marker enzymes associated with increased level of lipid peroxidation. Similarly, there was decrease in non-enzymatic (reduced glutathione; GSH) and enzymatic antioxidants (glutathione S-transferase; GST), superoxide dismutase (SOD) and catalase; CAT). Elevation in serum triglyceride (TG) and total cholesterol (TC) levels was noticed along with decreased level of serum total protein (TP). Treatment with PG 400 mg/kg bw exhibited excellent modulatory activity with respect to all the parameters studied by reversing the above-mentioned biochemical changes significantly in experimental animals.

Conclusion: These results suggest that PG offered protection comparable to that of Livolin forte with better efficacy when pre-treated with 400 mg/kg bw 14 days prior to CCl₄-exposure.
Larvicidal Effects of Ethanolic Leaf Extract of *Chanca piedra* (*Phyllantus niruri*) Against Mosquito Larva

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**Introduction and Aim:** Mosquitoes act as a vector for many life threatening diseases like malaria, yellow fever, dengue fever, filariasis, etc. The integrated Mosquito Management laid emphasis on the application of alternative strategies in mosquito control. Application of active agents from plant extracts can serve as an alternative to mosquito control since they are in most cases nontoxic and easily available at affordable prices. They are also biodegradable and show broad spectrum target-specific activities against different species of mosquitoes’ larva thereby controlling the vector mosquitoes and the spread of malaria. This study is aimed at evaluating the mosquito’s larvicidal activity of dried leaves Part of *Chanca Piedra* against the larvae of Anopheles Mosquitoes which develops to mosquitoes the vector of Malaria.

**Method:** Fully developed leaves of the *Chanca Piedra* were collected from Moshood Abiola Polytechnic Campus field, identified by the Forest research institute of Nigeria FRIN 2016/17. They were washed, and dried at room temperature 25°C for 2 weeks, pulverized and 80g weighed into a beaker. 600 ml of ethanol was measured and added to 80 g of pulverized leaf of the plant and left for a period of 72 hours at room temperature. The mixture was shaken vigorously and filtered, the filtrate was then concentrated using a rotary evaporator. The concentrates were evaporated to semi solid concentrate in a water bath at temperature of 40°C and transferred to a labeled specimen bottle. Different concentrates was made from this extracts 0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml, 0.5 mg/ml. Anopheles mosquito larvae were reared in covered plastic and 10 mosquitoes’ larva were transferred into a beaker containing 10 ml distilled water. They were fed with fresh food containing mixture of cabin biscuits and dried yeast for 2 weeks until they reached the 4th instars larvae. Bioassay were performed with the 4th larvae stages using 1 ml of ethanol and 1 ml of distil water as control. Larvae mortality was assessed after every hour of exposure.

**Results:** After 1 hour of exposure with the aid of a hand lens, all the 10 mosquitos’ larva died and stopped moving in the beaker #3 added with 0.3 g/ml of the concentrate at this same time 2 died from beaker 1 and 3 from beaker 2. While at 45 minutes and 30 minutes respectfully, beakers 4 and 5 containing the mosquitos’ larva added with 0.4 g/ml and 0.5 g/ml of the concentrates had all the mosquitos’ larva died. In the control group, all the mosquitos’ larvae are still active even at day 2 of the experiment i.e. after 48 hours.

**Conclusion:** Ethanolic extract of *Chanca piedra* showed an activity against wriggler, the vector of mosquitoes at all the concentration tested but the optimum concentration of 0.3 mg/ml at exactly 1 hour after exposure. All the wriggler eventually died in all the test column as the concentration increases, the time taken to kill the wriggler decreases. Hence, ethanolic leaf extract of *Chanca piedra* has an activity against mosquitoes’ larva. Future research might want to investigate formulation of the extract into aerosol and evaluating the effects on mosquitoes.
Standardization and Safety Assessment of *Carissa spinarum* Extract through CYP450 Inhibition Study

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**Introduction and Aim:** *Carissa spinarum* L. (CS) (Family: Apocynaceae) a food plant commonly known as ‘Conker berry’ or ‘Wild Karanda’ widely found in tropical deciduous fields of Indian sub-continent. CS is a small spinous and evergreen shrub potentially used in Indian systems of medicine (ISM). CS is reported to be effective against several ailments such as wound, antibacterial, antiviral, maternity bleeding, muscle cramps, malaria, diabetes, male and female sexual weaknesses, skin disease and as a snake poison antidote. The present study was performed to evaluate the interaction potential of methanolic extract of *Carissa spinarum* (CS) and its active phyto-constituent ursolic acid (UA) through CYP450 inhibition study.

**Methods:** Standardization of the same was performed by RP-HPLC and Heavy metal content in the selected medicinal plants was determined by Atomic Absorption Spectroscopy. Standardization was carried out using UA as the standard phyto marker by an isocratic RP-HPLC method (Shimadzu SPD-M20A) where methanol: water (0.1 M acetic acid, pH 3.3) was used as the mobile phase. Safety assessment of the extract was carried out through CYP450-CO and fluorimetric assay.

**Results:** Results showed that the UA content in the CS extract was found to be 1.14 ± 0.03 % (w/w). CYP450-CO assay result stated that the interaction potential between the extract and pooled liver microsomes and it was found to be less than the standard inhibitor ketoconazole. In the fluorimetric assay, CYP450 enzyme inhibition assay revealed that extract showed highest interaction potential with CYP2D6 (IC$_{50}$ 101.60 ± 1.37 μg/mL) and UA illustrated diminutive interactions with CYP3A4 (IC$_{50}$ 223.95 ± 2.15 μg/mL). The heavy metals content in the plant extracts were within the permissible limit.

**Conclusion:** The findings suggested that selected food plants and bioactive compounds contributed negligible interaction potential with CYP isozymes and may not possess any harmful effect upon their therapeutic benefits.
Comparison of Total Flavonoid Content (TFC) in South African Indigenous Tea Samples and Commercial Tea Products Available in South Africa

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Introduction and Aim: The eventual biological activity as well as anti-oxidant activity of tea extract is dependent on the presence of flavonoid type of compounds. Flavonoid are well known for their powerful anti-cancer, anti-bacteria, and antifungal properties and health benefit. This study was designed to compare Total Flavonoid Content (TFC) in different South African local commercial teas extracts as compared with some of local South African indigenous tea samples.

Methods: Identified currently UFS research indigenous teas and select commercial teas were collected from different regions of South Africa and local market of Bloemfontein, South Africa respectively. The extraction was performed by 24 h of maceration in a mechanical shaker with double distilled water and using different organic solvent/mixture at normal temperature using ratio of 1:3 (W/V). The percentage yield was calculated as W/W consideration. The filtrates of the extracts were concentrated using a vacuum evaporator, and the dried extracts were then stored at cold temperature for further use of Total Flavonoid Content (TFC) estimation as quercetin equivalents (QE) mg dry mass$^{-1}$.

Results: The percentage yield of the extract differs tremendously among all similar extracts. The average TFC found as around 170 µg/mg of dried water extract in branded tea however in collected tea average TFC was found to be more than 180 µg/mg of dried water extract. Among all commercial branded teas, significantly (*$p<0.0001$) more amount of TFC found in Rooibos tea (T-9) marketed by “Enyce Beverage Ltd”. All other brands other than T-1 & T-5 contain below the average value of TFC. Interestingly in indigenous tea samples, 5 out of 7 samples are above the average value limit. However of the commercial teas, except T-9, and T-5 none contain above the average level of TFC in them. It was found that in n-hexane and DCM fraction, average TFC is much higher (Approx. 30-35 times) in collected tea samples as compared to branded one. Approximately 3-4 times higher amount of average quantity of TFC in DCM:Methanol and methanol fraction found in collected indigenous tea as compared to branded tea.

Conclusion: Tea is the most popular refreshing drink now a day. The biological importance of tea depends on source, processing, quality and extraction technique. The TFC in indigenous teas was found higher that the commercially available branded tea. From the study it is assumed that the individual preparation/processing methods of tea in industry may alter their biological importance. Flavonoid enriched tea ensured the maximum benefit so consumer consciousness on quality of tea is demanded. However, the biological importance of flavonoid may interact with direct or indirect interaction with polyphenol, globular proteins or other bio compounds.
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