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CONFERENCE ABSTRACTS

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New medicines

Assessment of starch isolated from bambara groundnut (*Vigna Subterranea* (l.) Verdc. as a potential excipient

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Background: Persistent droughts have led to increased costs of producing pharmaceutical-grade starches from conventional sources such as maize and potato[1]. Therefore, there is an increasing need for alternative sources of starch. Bambara groundnut (*Vigna subterranea* L. Verdc), an underutilised crop, is known to be drought, pest and disease resilient[2] and can be further evaluated for its potential as a source of high-grade starch (native and hydrothermally modified) for application in pharmaceutical formulations.

Purpose: The aim of this study is to test the quality and compliance of Bambara groundnut starch with European Pharmacopoeia criteria to determine its potential as a pharmaceutical excipient.

Materials and Methods: Bambara groundnuts were purchased from local markets in Pretoria. Seeds were washed, dried and reduced to flour. Different methods of starch extraction were evaluated (e.g. water, enzymes and alkaline extractions). The starch yield and quality were assessed by measuring the swelling power and solubility, as well as pH, water absorption capacity and starch molecule identification[3]. The pharmaceutical grade of the starch was evaluated as per the European Pharmacopoeia.

Results and Discussion: In this study, the enzyme extraction was found to be the most effective method, resulting in a higher yield and quality compared to the alkaline and water treatments [(56.19g vs 53.50g (NaOH) vs 45.46g (Water)]. Although the microbial evaluation highlighted a higher load of fungi compared to the literature, with a pH of 5.89, a moisture content of 9.79, a sulfated ash value of 0.01, and a loss of drying of 4.67, the starch extracted from Bambara ground nuts could therefore be accepted by the European pharmacopoeia. These values are in line with those reported for the currently used pharmaceutical-grade starch from potatoes and maize.

Conclusion: Based on the chemical characteristics of the starch produced in this study, Bambara groundnuts could be a source of high-grade starch for application in pharmaceutical formulations. However, further studies on the efficacy of starch on tablet quality should be conducted prior to manufacturing and commercialisation.

In silico exploration of RNA aptamers as inhibitors against HIV-1 protease

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Background: The Human Immunodeficiency Virus type 1 (HIV-1) protease poses a significant challenge in the treatment of HIV-infected individuals, and it is a primary target for antiretroviral therapy. The efficacy of protease inhibitors (PIs) is often limited by the emergence of protease mutations, ultimately leading to the development of

resistance against treatment. In this study, the authors explore RNA aptamers molecules as possible inhibitors against HIV-1 protease using computational approaches. RNA aptamers are typically selected through a process called systematic evolution of ligands by exponential enrichment (SELEX), where a large library of random RNA sequences is iteratively screened and enriched for those that bind to the target of interest. This process is costly, time-consuming, and labour-intensive. To address these challenges, the authors utilised the in-house T_SELEX programme, which is capable of identifying aptamers that are more likely to bind to the HIV protease target.

Method: An aptamer library consisting of 1100 sequences was randomly designed using the T_SELEX program. Predictions of both secondary and tertiary structures for these aptamers were made using T_SELEX, which incorporates RNAfold and RNAComposer algorithms. Subsequently, these RNA aptamers were screened against both normal HIV protease and mutant protease using T_SELEX.

Results: Certainly, aptamers exhibited enhanced binding activity towards the HIV-1 protease mutant as compared to the normal protease. Molecular Dynamics (MD) simulations were then utilised to demonstrate the stabilisation of these RNA aptamer-mutant complexes.

Conclusion: From a theoretical standpoint, RNA aptamers demonstrate promising inhibitory potential against HIV-1 mutant protease.

Preventive and curative effects of the α2δ1 subunit of voltage-dependent calcium channel in chemotherapy-induced peripheral neuropathy

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Background: Chemotherapy-induced Peripheral Neuropathy (CIPN) remained a major problem in cancer treatment. Although the chemotherapy agents are effective at killing cancer cells, CIPN downgrades the patient's quality of life and ruins the effectiveness of chemotherapy, particularly platinum-based chemotherapy. Modulation of α2δ1 subunit of voltage-dependent calcium channel is thought to have a crucial role in CIPN management. However, there is a lack of evidence describing this system modulation's role in preventive or curative action in the CIPN cases. Thus, this study aims to evaluate the preventive and curative effects of the α2δ1 voltage-dependent calcium channel subunit in CIPN.

Method: Oxaliplatin was used in this study to induce CIPN in mice. Pregabalin, an α2δ1 subunit of voltage-dependent calcium channel antagonist, co and post-administered with oxaliplatin treatment was used to evaluate the preventive and curative effects. The Von Frey filament test was used to evaluate the CIPN response.

Results: Oxaliplatin treatment induced CIPN in mice. Pregabalin co-administered with oxaliplatin treatment was successful in attenuating the CIPN response. Additionally, pregabalin post-administered with oxaliplatin treatment improved the CIPN response in mice significantly.

Conclusion: Modulation of α2δ1 subunit of voltage-dependent calcium channel exhibits preventive and curative action in the CIPN.

Fruit wine active compounds and its antioxidant and antiradical activity

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Background: Sweet cherry is a fruit which is mostly consumed as fresh and is a rich source of many natural beneficial compounds. Unfortunately, this fruit can be consumed during the short seasonal period. The problem can be solved by sweet cherry processing, and wine is a product that can be highlighted. During sweet cherry wine production, thermo labile compounds (such as phenolic compounds) are not destroyed, and their content is higher in comparison with other sweet cherry products such as juice, jam and many others.

Purpose: The aim of this study was to determine the phenolic profile and antioxidant and antiradical activity of sweet cherry fruit wines.

Method: Sweet cherry wines were produced by the application of different controlled microvinification procedures. Wines were produced with and without the addition of sugar and enzymatic preparation. The phenolic profile was evaluated by UPLC TQ-MS/MS, while total

phenolic content was determined by the Folin-Ciocalteu method expressed as mg GAE/L. Antioxidant activity was determined by FRAP (mmol/L Fe2+) and Briggs-Rauscher (expressed as inhibitory time) methods, while antiradical activity was determined by the DPPH method (expressed as IC50).

Results: Sweet cherry wine analysis conducted by UPLC TQ-MS/MS showed the presence of phenolic acids and flavonoids. The most dominant compound was chlorogenic acid, with a content of 232.77 to 317.34 µg/ml. Among other phenolic acids were detected: vanillic (7.62-11.58 µg/ml) and protocatehuic (12.81-22.37 µg/ml). Flavonoids detected in sweet cherry wines were catechin (11.23-21.83 µg/ml), epicatechin (75.81-110.43 µg/ml), quercetin (22.31-47.72 µg/ml) and kaempferol (2.87-7.53 µg/ml). Total phenolic content was in interval 1281.43-1721.52 mg GAE/L. Antioxidant activity detected by the FRAP method indicates that results were from 53.21-61.53 mmol/L Fe2+. Briggs-Rauscher and DPPH methods indicated significant inhibitory activity in sweet cherry wine. It is important to highlight that wines produced with the addition of sugar and enzymatic preparation showed higher contents of selected phenolic compounds and higher antioxidant and antiradical activity. Phenolic compounds have beneficial health effects on human organisms, so it is important to highlight fruit wine as their significant source.

Conclusion: Sweet cherry wine is a rich source of phenolic acids and flavonoids, which are important biologically active compounds that are beneficial for human health. The technological approaches during the production significantly increase the amount of phenolic compounds in sweet cherry wine. Besides compounds quantified in this study, sweet cherry wines are rich sources of many other active principles that are responsible for their antioxidant and antiradical properties.

Fruit wine antioxidant properties and its potential ability for hyperglycemia prevention

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Background: Fruit and derived products are rich sources of biologically active compounds which are essential for human organisms. Among fruits, it is possible to highlight berries, which showed many beneficial health effects. One of many representatives from this group of fruit is raspberry. Derived products from raspberry are numerous, and among them, wine stands out in particular.

Purpose: The aim of this study was to determinate phenolic profile, antioxidant and in vitro activity of analysed fruit wine. Especially it is possible to emphasise influence of raspberry wines on hyperglycemia prevention since it problem today is very common among the population.

Methods: Fruit wines were produced from raspberry, and different controlled conditions of microvinification were applied. Wines were produced with and without the addition of sugar and enzymatic preparation. Two different yeasts were used in separate fermentations. Identification and quantification of phenolic compounds were conducted by UPLC TQ-MS/MS, while antioxidant activity was detected by the FRAP method. Alpha-glucosidase inhibitory activity was measured in lyophilised raspberry wine dissolved in DMSO. In this method, alpha-glucosidase and substrate solution, p-nitrophenyl alpha-D-glucopyranoside.

Results: Raspberry wine samples were the source of phenolic acids and flavonoids. Among phenolic acids, the most dominant were protocatehuic, gallic, ellagic and caffeic. Catechin was observed as the most dominant flavonoid. Antioxidant activity determined by the FRAP method showed that raspberry wine was in the range of 57.43 to 72.27 mmol/L Fe2+. Raspberry wine showed the ability to inhibit alpha-glucosidase activity. The obtained results for this activity were in the range of 38.23 to 55.47 µg/ml. Acarbose, whose inhibitory activity was 72.53 µg/ml, was used as a control in this method. Wines which were produced with the addition of sugar and enzymatic preparation showed a higher content of phenolic compounds and better antioxidant activity. Higher inhibitory activity against alpha-glucosidase was detected for some wine samples.

Conclusion: Raspberry wine showed as a good inhibitor of alpha-glucosidase compared to acarbose. This activity of, raspberry, wine is responsible for detected compounds, but also many other biologically active principles. All those compounds and their synergistic and antagonistic effects are responsible for antioxidant and inhibitory activity against alpha-glucosidase.

Impact of winemaking techniques on phenolic content and antioxidant activity of cabernet sauvignon wines

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Background: Polyphenols are a diverse group of very important molecules in red wine that have a positive effect on human health due to their antioxidant properties. Those compounds could prevent non-communicable diseases, so

their intake should be part of everyday nutrition. Winemaking techniques have a major influence on polyphenols, as they can lead to very different final polyphenol contents in the wine.

Purpose: The aim of this study was to evaluate the impact of different maceration periods applied during the winemaking on the total phenolic content and antioxidant activity of wine.

Methods: The wine samples were obtained after five maceration periods (3, 5, 7, 14 and 21 days). The alcoholic fermentation of the Cabernet Sauvignon grape variety took place spontaneously. No enzymatic preparation or selected yeast strain was added. After grape pressing it was sulfitised by K2S2O5 in amount of 10 g/100 kg. The total phenolic content was determined using the Foli-Ciocalteu method and expressed in mg GAE/L. Antioxidant activity was measured using the TEAC test and expressed in mmol TE/L of wine.

Results: The highest total phenol content was determined on the 21st day of maceration and amounted to 1963.40 mg GAE/L. During spontaneous fermentation, a wine whose maceration lasted 14 days showed the highest value of antioxidant activity (15.7 mmol TE/L). Longer maceration periods are responsible for higher total phenolic content since phenolic compounds are extracted during alcoholic fermentation from skin and grape seeds. After calculations, it was shown that antioxidant activity reached its peak on the 15th day of maceration and then started to decrease.

Conclusion: An important step in the production of red wine is the maceration process, which significantly influenced on the quality of wine. The content of phenolic compounds in red wine is strongly affected by the duration of maceration. Longer maceration leads to a higher total phenol content and higher antioxidant activity.

Structural elucidation of potential anticancer compounds from *Ficus nota* Blanco Merr. and its cytotoxic effect on Human Embryonic Kidney (HEK293) cells

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Background: Cancer is a leading cause of mortality worldwide and ranks as the third leading cause of morbidity in the Philippines. Currently, approximately 50% of anticancer drugs originate from plant sources. Despite the benefits of conventional chemotherapy drugs, they often come with undesirable side effects that limit their efficacy as treatments. Phytochemicals derived from natural sources offer a promising avenue for cancer treatment with minimal adverse effects. The rich biodiversity of Philippine flora includes numerous plants of therapeutic importance, among which *Ficus nota* Blanco Merr., locally known in the Philippines as Tibig, stands out for its valuable properties and rich compounds with potential bioactivities.

Purpose: The present study was aimed to structurally elucidate and identify the potential anticancer compounds from F. *nota* and confirm its cytotoxic effects on human embryonic kidney (HEK293) cells.

Methods: The methanol leaf extract of F. *nota* underwent solvent partitioning to yield a dichloromethane solvent fraction. This fraction was further analysed using Ultra Performance Liquid Chromatography with Mass Analysis (Waters Xevo G2-XS QTof, MSE mode) to detect, identify, and elucidate the compounds present. To confirm these activities, a cell viability assay (MTT assay) was conducted on human embryonic kidney (HEK293) cells.

Results: The identified compounds revealed the presence of 3-tert-butyl-4-methoxyphenol, 5,7,4’-trihydroxy-8,3’-diprenylflavone, and kuwanon S as the predominant flavonoids and related flavones; gracillin, picrasinoside D, and azedarachin C were present as the abundant terpenes and saponin-related structures, which are all known for their potent cytotoxic and anticancer activities. In the cell cytotoxicity test to establish the activities of the identified compounds, the dichloromethane fraction of F. *nota* demonstrated significant cytotoxic activity, with an IC50 of 13.174±0.002 mcg/mL. against the human embryonic kidney (HEK293) cells.

Conclusion: The identified compounds, particularly flavones and terpenes, synergistically interact to demonstrate potent cytotoxic activities within the dichloromethane solvent fraction of F. *nota*.

These findings highlight the promising potential of compounds derived from F. *nota* as a novel source of new anticancer agents.

Multi-stakeholder engagement in early phase tuberculosis drug development – Regulators, HTA organisations and the TB community

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Introduction: ERA4TB, one of Europe's largest tuberculosis (TB) research consortia, aims to accelerate the development of new TB treatment regimens. ERA4TB works in the early phases of research, from pre-clinical to first-in-human trials. To ensure new treatments can be brought to market efficiently and are acceptable to patients, it is important to have multi-stakeholder engagement, including with community representatives, regulators and Health Technology Assessment (HTA) organisations. Opportunities to engage these stakeholders in earlier phases of drug development are often not as apparent or actively sought as for later phases of clinical development. Additionally, not all countries have the same capacity and infrastructure for regulatory and HTA processes. Thus, there is a need to understand and explore relevant engagement routes, particularly in countries with high TB burdens.

Purpose: To describe the work undertaken and planned within ERA4TB to engage with key stakeholders, including people affected by TB/TB community representatives and regulatory and HTA organisations, and to show the potential routes for engagement with these audiences and the challenges associated with this.

Method: During the initial stages of the project, whilst building research infrastructure and capacity, there was a focus on informing key stakeholder groups about who ERA4TB is and the work being conducted in the project. This included building a social media presence across multiple platforms, developing promotional videos and setting up an external webinar series. To date, ERA4TB has conducted five webinars, including one on the challenges to TB drug development and access and one on community involvement in early-stage TB research.

Going forward, the authors will engage with TB community representatives through a dedicated workshop at the ERA4TB consortium meeting in May 2024 and involvement in the ERA4TB advisory boards. Mapping of current regulatory and HTA processes regarding new antibiotics and/or antimicrobials will be conducted for a select number of countries covering both Europe (where the ERA4TB project is funded) and countries with a high TB burden. This work will aim to understand who is responsible for the regulatory and HTA routes of new antibiotics/antimicrobials across countries and regions and what information is currently available to drug developers around regulatory and HTA engagement routes.

Results: This presentation will summarise the work undertaken to engage with the key stakeholder groups (TB community, regulators and HTA). It will discuss the current status of the mapping of regulatory/HTA engagement routes for TB drug development across countries. The presentation will discuss the lessons learnt on routes for engagement with these stakeholder groups that are suited for early-stage preclinical research and the associated challenges to provide transferable lessons for others working in drug development, particularly in the field of antimicrobials.

Conclusion: Engagement of multi- stakeholders in early phase research for new TB treatments, is important to ensure treatment relevance and that later stage research is designed effectively to meet the evidence requirements for market access stakeholders. Engagement routes are often not well-known in early-phase research, so it is important to share learnings from projects working in this area, such as ERA4TB.

Exploring the potential of antibacterial peptides: A promising approach to address antibiotic-resistant *Pseudomonas aeruginosa*

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Background: The health risks associated with *Pseudomonas aeruginosa* are severe and are made worse by the rise in antibiotic resistance. It is essential to use creative approaches to solve this problem. The goal of this work is to create a peptide (PP) that targets P. *aeruginosa* and evaluate its antibacterial activity against P. *aeruginosa,* which is resistant to antibiotics. It also looks at possible synergies between PP and traditional antibiotics to provide new therapeutic strategies to fight this hardy Gram-negative bacterium.

Methods: The peptide (PP), which consists of three tryptophan and three lysine amino acid subunits, was thoroughly characterised using mass spectrometry, electrospray ionisation mass spectrometry, and reverse-phase high-performance liquid chromatography. Minimum Inhibitory Concentration (MIC) values were determined using broth microdilution assays against both control and multidrug-resistant P. *aeruginosa*. Toxicity was assessed through erythrocyte hemolytic assays. The time-kill curve method was employed to evaluate PP bactericidal effects over time. Combinations of PP with conventional antibiotics were examined for potential synergistic effects.

Results: Findings showed that PP exhibited potent antibacterial activity against both control and multidrug-

resistant P. *aeruginosa*, with MIC values of 4.5 micrograms per mL and 20 micrograms per mL, respectively. Minimal hemolytic effects (3%) at higher concentrations underscored its safety profile. The cytotoxicity assay further supported its safety. The time-kill curve demonstrated rapid and sustained bactericidal effects. Combinations with conventional antibiotics, particularly gentamicin, showed synergistic effects with low Fractional Inhibitory Concentration Index (FICI) values (0.07 and 0.27 against control and resistant P. *aeruginosa*, respectively).

Conclusion: The synthesised PP represents a significant advancement in combating MDR P. *aeruginosa*. Its robust antibacterial activity, coupled with minimal cytotoxic effects and synergies with conventional antibiotics, positions PP as a promising alternative therapeutic agent. This peptide holds promise for addressing the challenges posed by multidrug-resistant bacteria, contributing substantially to the ongoing efforts against antibiotic resistance in clinical settings.

A randomised phase 2a clinical trial to assess the efficacy of KAND567 in ST-segment elevation myocardial infarction patients undergoing primary percutaneous coronary intervention

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Background: ST-elevation myocardial infarction (STEMI) is the most dramatic form of myocardial infarction (MI) and is associated with a high morbidity and mortality rate worldwide. Acute treatment is critical to restore blood flow to the heart in the coronary occlusion artery, limit infarct size, reduce the risk of post-STEMI complications, and improve long-term survival. However, STEMI patients are treated with primary percutaneous coronary intervention, a form of reperfusion therapy that causes further damage to the myocardium, known as reperfusion injury, that triggers the inflammatory response. This immune response can lead to progressive myocarditis and adverse left ventricular remodelling of the heart following recovery. Therefore, specific treatment targets are required to reduce excessive inflammation while maintaining immune defence. A study of more than 1,300 heart attack patients showed that T cells significantly decrease within minutes of reperfusion therapy, which may correlate closely with pathophysiological conditions. This significant reduction was observed in cells expressing CX3CR1, the receptor of fractalkine. Fractalkine (FKN, CX3CL1) is a member of the chemokine family that facilitates the extravasation and recruitment of CX3CR1-expressing lymphocytes to the inflammation site. Thus, blocking fractalkine/CX3CR1 signalling is suggested as a promising anti-inflammatory strategy for the treatment of both acute and chronic cardiovascular disease.

Purpose: The study aims to evaluate the efficacy of intravenous and oral administration of KAND567 for safety, tolerability, anti-inflammatory and cardioprotective effects.

Method: This is a randomised, 2-arm, parallel-group, placebo-controlled, double-blind, multi-centre Phase IIa clinical trial, FRACTAL; research registry number SRCTN18402242, which enrolled 71 STEMI patients. Blood sampling was at nine time points, including baseline (before KAND567 administration and reperfusion), bolus (after KAND567 administration), 90 min, 180 min, day 1, day 3, day 6, day 30 and day 90. Leukocyte kinetics was assessed by flow cytometry. LV remodelling was assessed by cardiovascular magnetic resonance (CMR) imaging and was defined as left ventricular ejection fraction (LVEF) at day three vs day 90. Fractalkine level was measured at baseline, 5 min, 90 min, 180 min, day 1, day 3, day 6, day 30 and day 90.

Results: No comparable changes were seen in leukocyte counts between the placebo and KAND567 groups. Baseline analysis revealed the highest CX3CR1 receptor density in CD8+TEMRA and NK cells. During KAND567 administration, a notable decrease in CX3CR1 relative expression on CD8+TEMRA and NK cells was observed ($p < 0.001$), with a rapid recovery observed up to day 6. Fraktaline serum levels increased during the drug administration compared to the placebo group. ($p < 0.01$ at 90 min, 180 min, day 1). Comparison of CMR imaging at day 90 to day 3 in both groups showed a decrease in infarct size at day 90 ($p < 0.01$ for placebo, $p < 0.0001$ for KAND567). KAND567 did not appear to affect changes in cell numbers during treatment. However, the observed effects of KAND567 on CX3CR1-expressing effector T-cell subsets and NK cells suggest a potential role in mitigating the inflammatory response associated with myocardial infarction.

Conclusion: Future research is warranted to elucidate further the precise mechanisms underlying the protective effects of KAND567 in acute myocardial infarction.

Application of traditional Chinese nano-medicine in the treatment of acute myeloid leukaemia

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Introduction: Acute myeloid leukaemia (AML) is an invasive hematopoietic malignancy caused by the excessive proliferation of myeloblasts. The incidence increases dramatically with age, from 1.8 cases per

100,000 people younger than 65 to 13.7 cases per 100,000 people older than 65. Because chemotherapy drugs are often unbearable, leading to many complications and poor quality of life. It was reported that phytonanomedicine was found to be effective against resistant AML cells and had become a research focus for nanomedicine forms (nanocrystals, nanoemulsion, nanoparticles, nanoliposome, and nano micelles et al.). Therefore, it is urgent to discuss the phytonanomedicine and mineral application in AML treatment with nanotechnology. It is important to study phytomedicine and mineral medicine in AML therapeutic applications and to offer ideal nanomedicine for AML patients.

Methods: A systematic literature search was conducted to retrieve literature published between January 1978 and January 2023 in many databases(Embase, PubMed, NCBI and CNKI) using the following keywords: (nano-drug delivery system OR nanomedicine delivery system OR nanoparticle OR nanocrystal OR nanoemulsion OR nanoliposome OR nanomicelle) AND (traditional Chinese medicine OR traditional herbal medicine OR phytomedicine OR mineral medicine OR Curcumin OR Alkaloids OR Parthenolide OR Triptolide OR berberine OR Arsenic) AND (Acute Myeloid Leukemia OR AML OR leukaemia) AND (pharmacokinetics OR regulation effect OR treatment). The search string was informed by the results of a preliminary landscape analysis of the global nano-drug delivery system of phytomedicine and mineral medicine within the peer-reviewed literature.

Results: Of the 168 articles published between 1978 and 2023, ~50% (n = 86) were published within the last five years (2018-2023). Articles were predominately focused on China (n=122). The authors found that the mineral medicine containing arsenic and its derivatives (such as arsenic trioxide and arsenic tetrasulfide), phytomedicine including berberine, gambogic acid, parthenolide, ordonin, grinsenside, and rubesidin had been applied in AML treatment. Also, the authors found that there were various nanomedicine delivery systems of phytomedicine and minerals such as nanocrystals, nanoemulsions, PLGA nanoparticles, Fe3O4 nanoparticles, nanoliposomes and nano micelles in the AML preclinical research.

Conclusion: Nanomedicine containing phytomedicine ingredients can improve the drug physicochemical characteristics, affect the pharmacokinetics, increase the stability, promote the permeability, and improve the bioavailability. Compared with free phytomedicine and mineral medicine, it also showed great therapeutic effects such as prolonged release, avoidance of drug resistance, reduced toxicity and enhanced targeting. This research may provide useful guidance for the use of phytomedicine constituents in AML treatment.

Investigation of bioactive products from *Centella asiatica*, L. urban leaf extract as acetylcholinesterase inhibitor and cognitive enhancer in stunted animal models

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Background: *Centella asiatica* L. urban (C. *asiatica*) is a plant widely used as a traditional medicine because it has many benefits. C. *asiatica* improves memory, intelligence, and neural protection in vitro and in vivo. Compounds in C. *asiatica* can be used as an alternative to prevent cognitive function decline in stunted children. Stunting is the impaired growth and development that children experience from poor nutrition, repeated infection, and inadequate psychosocial stimulation. According to the Indonesia Basic National Health Survey 2022, Indonesia's stunting prevalence reached 21.2%. Acetylcholine plays a crucial role in cognitive function. It is one of the most important neurotransmitters in the central cholinergic system, binding to muscarinic and nicotinic receptors and degrading by acetylcholinesterase (AChE). AChE increases central AChE levels, enhancing cognitive ability and overall brain function. Thus, the present study aimed to examine the bioactive products from C. *asiatica* as an AChE inhibitor cognitive enhancer in stunted animal models

Methods: Using the Multiple Ligand Simultaneous Docking (MLSD) approach, investigating the bioactive products from C. *asiatica* as an AChE inhibitor. The MLSD simulation parameters were validated using the re-docking method. In identifying the effect of the overall macromolecules of C. *asiatica* leaf, single compound molecules and a comparator molecule to AChE receptor macromolecules can be observed based on several parameters of the Multiple-Ligand-Mapping

Molecular Dynamics (MMLD) simulation. Moreover, the Molecular Mechanics Poisson-Boltzmann Surface Area (MM/PBSA) method was used to calculate the binding free energy values during the MLMMD simulation. In vivo studies were carried out by developing stunted animals. A stunted mouse model was developed by administering antithyroid agents from gestational day (GD) 18 to postnatal day (PND) 21 to pregnant mice. The stunted mice were administered either donepezil 5 mg/kg BW/mL (positive control) or treatment group (ethanol extract of C. *asiatica* (EEC) 2 mg/kg BW/mL) from PND 21 to PND 35 (14 days). The light-dark test (LDT) and memory tests of offspring were conducted on PND 36. At the end of the test, the mouse was decapitated, and the hippocampus and cerebellum were sampled for molecular analysis.

Results: Based on the analysis, the graphs of Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Solvent Accessible Surface Area (SASA), Radius of Gyration (Rg), Radial Distribution Function (RDF), and Hydrogen Bonds (H- bonds), it can be predicted that the macromolecule of C. *asiatica* leaf extract tends to fluctuate at the active site of AChE receptor binding domain (RBD). Furthermore, the authors found that EEC improved the cognition and memory of stunted mice and increased mRNA and protein levels in the brain.

Conclusion: In silico results are supported by the loss of some molecular interactions, especially hydrogen bonds, in the final conformation of the MLMMD simulation. Thus, further studies are needed to confirm the results of the MLSD and MLMMD simulations. Moreover, this study may be used for basic research for developing memory-enhancer supplement preparations, especially for stunted children.

Pinocembroside exerts anti-neuroinflammatory effects by attenuating MAPK / NF-κB signalling pathway, NLRP3 inflammasome activation and ROS production in LPS-stimulated BV2 microglia

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Purpose: Neurodegenerative diseases are associated with neuroinflammation along with overactive microglia and excessive oxidative stress; therefore, ameliorating neuroinflammation has been considered an effective strategy for attenuating neurodegenerative disease progression. Pinocembroside is a natural flavonoid compound isolated from Penthorum Chinese and has been indicated to have protective effects on acute liver injury, as well as anti-fungal and anti-bacterial effects. However, the effects of pinocembroside on neuroinflammation remain unclear. Hence, this study aimed to investigate the effect of pinocembroside on neuroinflammation and explore its regulatory mechanisms.

Methods: BV2 cells were pre-treated with pinocembroside following LPS treatment. Secretion of nitric oxide (NO) and the production of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β and PGE_2) were analysed by Griess reagent and ELISA, respectively. Expressions of iNOS, COX-2, STAT-3, and MAP kinases (ERK, JNK, and p38) were analysed by using Western blotting. Production of IL-1β and NLRP3 inflammasome NLRP3 inflammasome-related proteins and phospho IκBα/IκB were also examined using Western blotting. Moreover, the production of intracellular and mitochondrial ROS was analysed by flow cytometry.

Results: These experimental results showed that pinocembroside attenuated the secretion of pro-inflammatory mediators and cytokines, including NO, IL-1β, IL-6, PGE_2, and decreased the expression of iNOS and COX-2 by LPS-induced BV2 cells. In addition, pinocembroside also inhibited the activation of the MAPK signalling pathway, reduced NF-κB activity and attenuated the activation of NLRP3 inflammasome in LPS-induced BV2 cells. Moreover, pinocembroside significantly decreased the production of intracellular and mitochondrial ROS by LPS-induced BV2 cells.

Conclusion: In summary, these experimental results demonstrated that pinocembroside exhibits anti-inflammatory and anti-oxidative properties, suggesting it might have the potential to ameliorate neurodegenerative disease progression.

The role of antipsychotic drugs in the modulation of mitochondrial functions

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Introduction: The role of mitochondria in the pathophysiology of psychiatric disorders is supported by studies investigating genomic differences, changes in energy metabolism and mitochondrial changes. Variability in intracellular processes probably participates in interindividual differences in the response to treatment with drugs. Mitochondria have been linked to the aetiology of schizophrenia (SZ). Also, mitochondria in SZ might be confounded by the effects of pharmacological treatment with antipsychotic drugs (APDs) and other common medications. Method: Systematic review methods.

Results: The authors have analysed the relevant literature available so far regarding the mechanism of action of antipsychotic drugs on mitochondrial function. Studies have shown that mitochondrial dysfunctions lead to impaired energy metabolism, perturbed calcium homeostasis, increased ROS, oxidative stress and apoptosis. Also, impaired functions of mitochondria contribute to a wide range of diseases, including different psychiatric disorders. Ongoing research into mitochondrial dysfunction in schizophrenia may pave the way for the creation of innovative therapeutic approaches that can treat symptoms that existing antipsychotic drugs cannot sufficiently treat because studies examining the impact of these drugs on mitochondrial dysfunction have shown conflicting results.

Conclusions: The information on the molecular basis of antipsychotic action provided a more complete picture of how antipsychotic drugs modulate cellular outputs and mitochondrial processes. The results of the studies suggest that mitochondrial dysfunction is a key component in the neurobiology of neuropsychiatric disorders and a target of psychotropic drugs.

Lessons learned working in a consortium: An IK-based drug development for COVID-19

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Background: Indigenous knowledge systems (IKS) about plant-based remedies have been the basis of primary healthcare for a vast majority of South African ethnic communities. The anecdotal evidence on the therapeutic properties of plant-based remedies is a source of knowledge that inspires IKS-driven innovations towards drug development systems.

Purpose: The paper aims to draw on the experiences and lessons learned from a collaborative consortium in developing a COVID-19 drug informed by IKS.

Method: In support of the Small, Medium, and Micro Enterprises (SMMEs) for IK-based product development and optimisation to enhance biosurfactant technology, the South African government, through the Department of Science and Innovation, implemented a strategic biotechnology policy for funding a four-member consortium that comprised an SMME, Science Council, and two academic institutions. This consortium provided a platform for the SMME-owned IK-based biosurfactant to access tools to generate scientific data. The consortium also incorporated a post-doctoral research fellow (PDRF) as part of human capacity-building to participate in different institutional activities. The respective consortium partners played different work package roles as follows: SMMEs provided the IK-based biosurfactant product and led the market analysis for the current drugs used for the treatment of COVID-19; the science council was the project manager for the consortium, and provided the scientific techniques for up-scaling and development of a biosurfactant prototype product; whereas, the academic institutions were mandated to conduct the preclinical safety testing of the candidate biosurfactant product upon which the findings would be relayed for the subsequent clinical studies.

Results: The consortium produced the IK-based biosurfactant laboratory scale batch subjected to preclinical safety investigations. A market research analysis report was issued to inform the market strategies for the product. The recipient of the post-doctoral fellowship was a black female who was

successfully trained and mentored by the consortium partners as part of the South African government's strategy to promote gender balance in the national systems of human research, development, and innovation capacity. The PDRF effectively contributed to all the project deliverables.

Conclusion: Government-led and funded consortia with multi-sectoral partners are good for developing a support model for innovative IK-based products. As they gain further attention, this will add to the global knowledge of biosurfactants and their novelty, contributing to socio-economic growth.

The effect of boric acid sugar bait on *Anopheles gambiae* Sensu Stricto

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Introduction: Malaria is a life-threatening disease that poses a major health concern globally. The disease burden has been attributed to several challenges encountered in the carrying out of vector control to decimate mosquito populations, including insecticide resistance and changes in anthropophagic mosquito feeding behaviour from endophagic to exophagic. This laboratory-based study was done to determine the potential of boric acid incorporated in sugar bait to decimate adult mosquito populations by exploiting their sugar-feeding behaviour.

Method: This was an experimental study that was conducted at the Tropical Pesticides Research Institute (TPRI) in Arusha, Tanzania, from May to June 2018. Fresh mango juice was prepared, diluted with distilled water into 10% mango juice and then mixed with boric acid to obtain 2%, 1%, 0.5%, 0.25% and 0.175% concentrations, which were each put into separate cages containing 45 (20 males and 25 females) laboratory-reared adult *Anopheles gambiae* Sensu Stricto. Mortality was recorded after 24-hour intervals from 2 replicates per boric acid concentration.

Results:

(i) Mortality was calculated using Abbot's Formula and found to be concentration-dependent. After 24 hours, 48%, 55%, 60%, 80% and 96% mortalities were recorded for 0.175%, 0.25%, 0.5%, 1.0% and 2% boric acid, respectively. After 48 hours, 89%, 89%, 95%, 98% and 100% mortalities were recorded for 0.175%, 0.25%, 0.5%, 1.0% and 2% boric acid, respectively. After 72 hours, 100% mortalities were recorded for all concentrations ranging from 0.175%, 0.25%, 0.5%, and 1.0% boric acid. Thus, boric acid sugar bait was proved effective in mosquito control.

(ii) Probit analysis at 95% Confidence Interval (CI) to obtain Lethal Dose 50% (LD50) and LD95 of boric acid for 24 hours and 48 hours intervals from SPSS software version 20 resulted into the following; LD50 for 24 hours and 48 hours intervals were 0.683% and 0.196% respectively, LD95 for 24 hours and 48 hours intervals were 2.733% and 0.490% respectively.

(iii) Kaplan-Meir survival analysis from Excel software resulted in the pattern, which was interpreted as follows: The lowest survival probability was observed at a boric acid concentration of 2%, with a maximum of two days, while the highest survival probability was observed at 0%, which served the control, with a maximum of five days.

Conclusion: The findings of this study have shown that the boric acid sugar-baiting method is effective in mosquito control. The exploitation of this intervention, which uses attractive toxic sugar bait, is important in malaria vector control as it may circumvent the insecticide resistance problem, and it is cheap to prepare. It should thus be incorporated into malaria vector control by utilising boric acid-soaked fruit peels and other plant parts which attract mosquitoes, like blossoms of *Acacia saligna*, *Tamarix jordanis* and *Polygonum equisetiforme*. Further studies should be done on more vegetational mosquito attractants and the range of boric acid's safety for the environment. Boric acid sugar bait's applicability to control other disease vectors, such as house flies and tsetse flies, should also be evaluated.

Antiplasmodial activity of a novel quinoline-urea-benzothiazole hybrid compound in a mouse model

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Background: Despite decades of research, malaria remains a major global public health problem, especially in sub-Saharan Africa. The main cause of malaria prevalence is the ever-increasing occurrence of resistance of malaria-causing Plasmodium parasites to currently used antimalarial drugs. This calls for the development of alternative antimalarial drug compounds that have different modes of action from those that are currently used. Therefore, compounds with promising antiplasmodial activities are continuously explored.

Purpose: This research aims to evaluate the in vivo antiplasmodial activity of Chloroquine-urea-benzothiazole. This compound has been reported to have potential antiplasmodial activity in vitro against a mouse model infected with *Plasmodium berghei* (ANKA).

Method: The antiplasmodial activity of the chloroquine-urea-benzothiazole (hybrid compound) will be assessed based on the Peters' 4-day suppressive test against male and female BALB/C mice (6- 8 weeks) infected with chloroquine-sensitive *Plasmodium berghei* Murine malaria parasite. The treatment groups are the untreated group, Chloroquine, urea, benzothiazole, and the hybrid compound (high and low dose), all dissolved in polyethylene glycol (PEG) will be administered orally for four days. The antiplasmodial efficacy of the compound will be evaluated by determining parasitemia, packed cell volume, survival time, body weight, and temperature. All mice will be euthanised on day 35 of the experiment and subject to a postmortem evaluation. Histopathology of the spleen, liver, brain, and kidney will be performed using haematoxylin and eosin (H and E) staining. Means (parasitaemia) will be used to determine the percentage of chemosuppression and compared using analysis of variance (ANOVA) (P = 0.05).

Results and conclusion: Considering the adverse malaria burden posed by antimalarial drug resistance, the findings of this study will provide valuable insight into the antiplasmodial efficacy of this compound. This will, in turn, lead to antimalarial drug development, therefore contributing to combating this global health challenge.

Formulation and characterisation of chitosan-loaded *Guiera senegalensis* Nanoparticles for the treatment of Malaria

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Background: Despite the eradication of malaria in several countries around the globe, malaria remains a deadly disease in Sub-Saharan Africa, killing over half a million people every year, most of whom are young children. Nanomedicine has truly evolved in the field of research. Plants have played crucial roles in drug discovery and development by providing lead compounds for drug development. In the quest to find other effective and more reliable alternatives to schistosomiasis, natural products and nanomedicine have been explored, and they show great potential for treating and eliminating malaria and other tropical diseases. Africa is rich in medicinal plants, which have been employed for decades to treat many health conditions and infectious diseases, including malaria. *Guiera senegalensis* is a plant that has antimalarial properties.

Methods: The leaves of *Guiera senegalensis* were bought, dried, blended, and extracted using a mixture of 70 % ethanol and 30 % water. The mixture and the extract were left for 22 hours in a shaker to ensure even distribution and further drying. The UV analysis was done after serial dilution of the leaf extract using phosphate buffer. Three different concentrations of the chitosan nanoparticles were prepared, centrifuged, and weighed.

Results/ Discussion: The dried leaves totalled 230.54g before synthesising the nanoparticles. The wavelength of the *Guiera senegalensis* extract is 265.4nm. The various absorbances for the different concentrations were recorded and had an average of 0.985. Upon further analysis of the chitosan nanoparticles, the pH for the three samples was 4. The disc calorimetry was 354. This research is ongoing because in vivo studies on lab animals are being conducted.

Conclusion: As the use of chitosan nanoparticles and natural products continues to evolve, further studies are needed to assess the effect of this *Guiera senegalensis* chitosan nanoparticles on animals before it is tried on humans.

Anti-diabetic potential of *Bulbine frutescens* root extracts in diabetic rats

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Background: Metabolic disorders disrupt normal bodily metabolic processes, with diabetes mellitus being a common example, characterised by elevated blood glucose levels. In South Africa, type 2 diabetes mellitus (T2DM) is particularly prevalent, accounting for over 80% of diabetic patients. This is largely due to unhealthy lifestyles and exacerbated by the high cost of medications and limited access to healthcare. Consequently, there is a rising demand for natural alternatives, such as the *Bulbine frutescens* plant, which has shown therapeutic potential. However, research on its anti-diabetic properties is limited, highlighting the need to explore its effectiveness and safety in managing metabolic disease.

Purpose: This study aimed to investigate the potential of an aqueous root extract of *Bulbine frutescens* in managing type 2 diabetes mellitus (T2DM) in a diabetes-induced rodent model.

Methods: An aqueous extract of B. *frutescens* plant root was prepared by decoction in distilled water, filtered, and then freeze-dried. A T2DM rat model was established in 32 male

Sprague-Dawley rats by administering multiple low-dose streptozotocin intraperitoneally and fed a high-fat diet rat chow for 13 weeks. The rats (N=32) were randomly divided into four groups (n=8): untreated diabetic control, a metformin-treated (70 mg/kg BM) control, and two *Bulbine frutescens*-treated groups (25 and 50 mg/kg BM). Treatments were done daily over four weeks using dosed gelatine cubes. Body mass and health checks were done daily. Blood samples were collected weekly for blood glucose levels, and terminal blood and liver were collected at the end of the study. Biochemical assessments were conducted on the liver samples for oxidative stress and diabetes-relevant enzymes and biomarkers.

Results: Throughout the four weeks of treatment, the blood glucose levels in *Bulbine frutescens*-treated groups gradually decreased to significantly lower ($p < 0.05$) levels at the end of the study compared to the untreated diabetic control. The reduction in blood glucose levels was similar to that of the metformin-treated control. Moreover, the high-dose *Bulbine frutescens*-treated group exhibited significantly ($p < 0.05$) lower lipid profile levels and lower α-glucosidase activity compared to the diabetic control group. No notable changes in terms of oxidative stress were observed relative to the controls.

Conclusion: This study's findings suggest the potential of *Bulbine frutescens* root extract as an anti-diabetic agent in Sprague-Dawley rats. However, further research is essential to elucidate its mechanisms of action and validate its efficacy and safety for human use in managing type 2 diabetes mellitus.

Investigating the therapeutic potential of topical 2% CBD application in atopic dermatitis: A prospective cohort study

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Introduction: Atopic dermatitis is a frequently diagnosed chronic inflammatory skin condition characterised by Th2-type inflammation. Post-diagnosis treatment focuses on moisturising the skin, controlling itching, and enhancing appearance. Commonly used treatments, such as topical corticosteroids or calcineurin inhibitors, have adverse effects and are unsuitable for long-term use. Recently, cannabidiol (CBD) has gained attention in dermatology due to its anti-inflammatory properties, showing the potential to halt Th2 immune responses when applied topically. However, further research is needed to determine its clinical efficacy. This study aims to establish the therapeutic benefits of topical CBD application in eczema, potentially introducing a therapeutic regimen. The study intends to explore the potential therapeutic effects of 2% CBD topical application in individuals diagnosed with atopic dermatitis. The hypothesis posits that CBD will effectively alleviate the common symptoms of atopic dermatitis, thereby enhancing the overall quality of life for the patients.

Method: In a 4-week observational prospective cohort study (n=18), patients diagnosed with moderate-to-severe eczema, aged 18 and above, were assessed. Inclusion criteria required participants not currently using steroids or biologics, no use of other topical products, and absence of pregnancy or allergies to listed formulation ingredients. Factors such as appearance and patient satisfaction were evaluated. Baseline and post-treatment photo-measurements were compared. Patients applied the topical CBD moisturiser as needed, with assessments at intake, week 1, week 2, and week 4, followed by a subsequent follow-up appointment at week 8.

Results: The majority of participants (59%) reported enhanced hydration by the end of Week 1, and this trend continued at Week 2 (71%) and Week 4 (71%). Participants experienced a soothing or comforting feeling on irritated skin, with substantial relief reported at Week 1 (67%), Week 2 (81%), and Week 4 (61%). In terms of the duration of itch/scratch-free periods upon affected area healing, the majority of participants reported periods at or exceeding 6 hours. Additionally, responses to immediate relief from skin inflammation were predominant, with 67% in Week 1, 75% in Week 2, and 61% in Week 4. Improvements in skin condition appearance were observed, with 67%, 71%, and 67% of participants expressing positive changes in Weeks 1, 2, and 4, respectively. Flare-up frequencies prior to the study revealed varying responses, with weekly occurrences (27.8%) and once-a-month (33.3%) being prominent. Regarding changes in flare-up frequency since the study, a substantial 72% reported a decline. Moreover, 100% expressed the intention to continue using the product, with 88% expressing a preference for CBD topical usage.

Conclusion: In conclusion, this study regarding the therapeutic effects of 2% CBD topical application for atopic dermatitis yielded consistently positive outcomes, including increased skin hydration, soothing relief, and extended itch/scratch-free periods. This highlights the potential of 2% CBD moisturiser in managing symptoms and enhancing overall quality of life. While valuable, future research could benefit from a larger sample and an extended follow-up. Integrating objective measures alongside subjective assessments would enhance the intervention's evaluation. This study contributes to broader research on CBD in

dermatology, paving the way for further exploration and integration of CBD-based products.

Investigating the effect of combining atovaquone with radiotherapy in head and neck squamous cell carcinoma

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Background: Radiotherapy is commonly used as adjuvant or palliative treatment in Head and neck squamous cell carcinoma. However, its effect is often diminished by tumour hypoxia, which is an adverse factor in head and neck squamous cell carcinoma treatment. Thus, the authors assumed the combination of hypoxia modifiers might enhance the effect of radiotherapy. One of the hypoxia modifiers, nimorazole, has been used with radiotherapy in Denmark. However, a clinical trial in 2023 showed that the combination treatment has no improvement in tumour region control and patient survival. As a result, the authors used another hypoxia modifier – atovaquone to investigate its synergistic effect with radiotherapy.

Purpose: The present study deciphers the effect and mechanism of atovaquone combined radiotherapy in Head and neck squamous cell carcinoma. It will then complement the syngeneic mouse model experiments done in Dr. Kong's lab.

Method: Mouse oral squamous cell carcinoma cell lines were treated with atovaquone (10-30μM) +/- radiotherapy (2-8Gry). The effect of the combination treatment was evaluated by cell viability and clonogenic assay. Besides, the effect of combination treatment on the epidermal growth factor receptor (EGFR) signalling pathway, AMP-activated protein kinase (AMPK) signalling pathway, as well as programmed cell death ligand 1 (PD-L1) protein expression was conducted by western blot analysis.

Result: Radiotherapy alone increased the expression of EGFR and pEGFR. While combined with atovaquone, the western blot result showed a reduction of EGFR and pEGFR expression but an evolution of pAMPK expression. As for the PD-L1 expression, the authors observed a decrease in PD-L1 expression as the concentration of atovaquone increased. Furthermore, the clonogenic assay found that combination treatment was more effective than radiotherapy alone.

Conclusion: This result showed that combining atovaquone with radiation enhanced the efficacy of radiotherapy. The synergistic response is likely to be regulated through the effect on EGFR and AMPK pathways. Additionally, as indicated by these results, further experiments are required to investigate the effect of atovaquone on PD-L1 expression.

Uses and benefits of immortelle essential oil for skin care

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Introduction: Immortelle is a plant that belongs to the Asteraceae family and includes over 600 species of herbaceous plants, semi-shrubs, and shrubs spread all over the world. In the European area, there is a mixture of two types of immortelle, Mediterranean and Asian. The Mediterranean immortelle plant has long been known as a plant whose oil brings many benefits to the skin. In this research, the authors described the benefits of immortelle essential oil from the Montenegro region. Immortelle essential oil is known for its medicinal properties that promote the healing of skin, fight infection, and reduce inflammation and redness. This essential oil has long been known to help smooth the appearance of fine lines and wrinkles. It also helps to even out skin tone and restore sun-damaged skin. Since immortelle oil is loaded with antibacterial and anti-inflammation properties, it is a great wound healer. It can help wounds heal quicker and can help stop skin infections. In this research, the authors focused on this essential oil's anti-infective and anti-inflammatory pharmacological effects.

Method: The authors analysed 50 patients with inflammatory skin processes and symptoms indicating a skin infection. The authors treated the problematic region for 21 days with the essential oil of immortelle or oily bases with the addition of 1% essential oil of immortelle.

Results: In 80% of cases, the authors noticed an improvement after seven days of oil application, and in 90% of cases, they noticed an improvement after 21 days of application. 10% of cases were without improvement.

Conclusion: Helichrysum essential oil comes from a natural medicinal plant used to make a beneficial essential oil that boasts many different full-body benefits due to its anti-inflammatory, antioxidant, antimicrobial, antifungal and antibacterial properties.

Helichrysum essential oil, typically from the Helichrysum italicum plant from Montenegro region, has been established in various experimental studies to have strong abilities to lower inflammation due to several mechanisms:

inflammatory enzyme inhibition, free radical scavenging activity and corticoid-like effects.

A tannin tale of triumph in a pre-clinical model of Alzheimer's Disease

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Background: Despite some advances in treatment, a cure for Alzheimer's disease (AD) remains elusive. Disease hallmarks include heightened neuroinflammation and oxidative stress, associated with progressive decline in mobility and cognitive functions. Natural compounds provide a valuable reservoir of novel bioactive substances with therapeutic potential, fewer side effects, and increased affordability. A hydrolysable tannin, 1,2,3,4,6-Penta-O-Galloyl-β-D-Glucose (β-PGG) displays potent antioxidant, anti-inflammatory, and neuroprotective properties in vitro, but in vivo evidence is limited.

Purpose: This study aims at assessing the efficacy of β-PGG in mitigating age-dependent mobility deficits and conferring antioxidant protection in a pre-clinical model of AD.

Methods: A fruit fly line overexpressing the human amyloid precursor protein (hAPP) and β-site APP-cleaving enzyme (hBACE), in neurons was used as AD model. Newly eclosed flies were supplemented with 0, 5, or 10μM β-PGG, and locomotion was assessed in young (7- and 14-days old) and aged (21- and 30-days old) flies via a negative geotaxis assay. The number of flies passing 2-, 4-, and 8 cm marks in 10 seconds was recorded by genotype, sex, age, and treatment. Oxidative stress sensitivity was measured by quantifying 48-hour survival to paraquat exposure. Virgin flies were randomised to receive 0 or 10μM β-PGG for 14 days. On day 15, flies were exposed to either 20 mM paraquat (PQ) dissolved in sucrose or sucrose alone for 48 hours. The number of surviving flies was recorded at 24 and 48 hours. Data were analysed using a two-way ANOVA to assess the effects of genotype, treatment, and interaction.

Results: Young non-supplemented AD females ($p < 0.0001$) and males ($p < 0.0001$) moved significantly slower than controls. β-PGG significantly ameliorated locomotion deficits in young AD flies. AD females ($p < 0.0001$) and AD males ($p < 0.0001$) supplemented with 10μM β-PGG climbed significantly faster than their non-treated counterparts. On the other hand, old females supplemented with 10μM β-PGG were significantly faster than untreated cohorts ($p < 0.05$), regardless of genotype. Conversely, β-PGG supplementation in old AD but not control males conferred significant locomotion advantages compared to untreated AD males ($p < 0.0001$). Locomotion in young and aged flies was unaffected by 5μM β-PGG supplementation. This preliminary oxidative stress sensitivity data showed that at 48 hours, β-PGG-supplemented AD males exhibited a survival rate ~3.8 times higher than supplemented controls ($p = 0.0167$) and ~13 times higher than non-supplemented AD males ($p = 0.0184$).

Conclusions: These findings provide strong evidence that β-PGG supplementation mitigates age-associated mobility deficits and confers a survival advantage to paraquat exposure in this pre-clinical model of AD. Benefits from this supplementation may delay physiological ageing in a sex-specific manner.

An open-label feasibility study of the efficacy of remdesivir for Long-COVID

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Background: Approximately 65 million people globally live with persistent and disabling symptoms following a COVID-19 infection. Termed Long-COVID, this post-viral condition is likely to remain a challenge for healthcare systems for many years. Three possible targets for intervention are immune dysregulation, disordered anticoagulation, and viral persistence/ reactivation. Remdesivir is currently licensed for the treatment of COVID-19 during acute admission to the hospital and has shown improved long-term outcomes. Remdesivir, a nucleotide prodrug of adenosine analogue, binds to the viral RNA-dependent RNA polymerase. It terminates RNA transcription prematurely and thus inhibits viral replication. However, data is currently lacking on any potential role of remdesivir in people living with Long-COVID.

Prior to any double-blind multi-centre randomised control trial to determine effectiveness, there is a need to assess the feasibility of using an anti-viral administered intravenously for Long-COVID. There is also a need to assess acceptability and test study procedures and processes to ensure participants follow the study protocol.

Purpose: To test the feasibility of using a 5-day course of intravenous Remdesivir for patients with Long Covid.

Method: Phase IV, open-label, single-arm proof of concept study of patients ≥ 18 years of age with previously confirmed SARS-CoV-2 infection and confirmed or suspected diagnosis of Long COVID. This feasibility study is being conducted at two sites in the UK (Derby and Exeter).

Dosing will follow the licensed regimen for patients admitted to acute hospital environments, and participants will receive the following treatment:

Day 1: single loading dose of 200 milligrams of Remdesivir in 250ml sodium chloride 0.9% bag via intravenous infusion over 60 minutes.

Days 2 to 5: 100 milligrams of Remdesivir in a 250ml sodium chloride 0.9% bag will be administered via intravenous infusion once daily over 30 minutes.

Primary outcome measures are related to feasibility and include recruitment, retention, fidelity to treatment regimen and outcome measures, and acceptability. Secondary outcomes include quality of life, the burden of symptoms, functional status, biomarker and inflammatory profiles, cognitive, physical and psychological symptoms, and clinical safety measures, including adverse events.

Results: The results from this proof-of-concept study can inform the development of effective treatments for Long-COVID. Remdesivir has shown in vitro and in vivo activity against SARS-CoV-2. Prior to a full clinical trial, the authors need to assess the feasibility of conducting the trial. This study's feasibility and clinical and safety results will inform the development process.

Conclusion: Developing effective treatments for Long-COVID can improve patient outcomes and quality of life.

Essential oils combatting microbial resistance: insights into anti-quorum sensing, anti-biofilm, and resistance induction

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Background: Bacterial resistance to antibiotics is a pressing global concern. Mechanisms of resistance include quorum sensing (QS) influencing virulence and biofilm formation. Essential oils are commonly used for infections and have demonstrated antimicrobial activity against drug-resistant bacteria such as methicillin-resistant *Staphylococcus aureus*. Although essential oils are presented as an option against antimicrobial resistance, their ability to withstand resistance mechanisms requires further investigation. Thus, this study sought to investigate the antimicrobial activity of five essential oils by determining their anti-QS and anti-biofilm activity. Furthermore, the essential oils were exposed to S. *aureus* at sub-inhibitory concentrations to explore the development of resistance.

Methods: The broth microdilution method was undertaken to quantify the five essential oils' minimum inhibitory concentration (MIC). The anti-AQ activity was evaluated using *Chromobacterium violaceum* (ATCC 12472) as the biomonitor strain. The crystal violet method was used to quantify the biofilm inhibition percentage for the antibiofilm assay. The induction of resistance assay involved exposure of S. *aureus* ATCC 6538 to five essential oils (*Origanum vulgare*, *Thymus vulgaris*, *Carum carvi*, *Matricaria recutita*, *Commiphora myrrha*) at sub-inhibitory concentrations, over a total of 20 cycles.

Results: All five essential oils exhibited considerable (≥ 70%) QS-inhibition at concentrations ranging from 0.02 - 1.00 mg/mL. Notably, O. vulgare and T. vulgaris inhibited biofilm formation (≥ 70%, concentration ≤ 1.00 mg/mL). Origanum vulgare and T. vulgaris consistently maintained their antimicrobial activity, with minimal variations in the MIC (standard deviation ± 0.22 and ± 0.32, respectively) when exposed to 20 cycles in the resistance induction assay. Although C. *carvi* displayed greater MIC variability, no instances of resistance were detected. Furthermore, exposure to essential oils altered the growth kinetics of S. *aureus*. Compared to exposure to antibiotics, the repeated sub-inhibitory concentrations led to a pattern of heteroresistance in S. *aureus*.

Conclusion: This study highlighted the notable anti-resistance properties of O. vulgare and T. vulgaris as these essential oils demonstrated considerable antimicrobial activity in the AQ sensing and antibiofilm assays, operating within a narrow concentration range (0.03 - 0.75 mg/mL and 0.02 - 2.00 mg/mL, respectively). Furthermore, this study expanded the repertoire of resistance induction assays, demonstrating the ability to withstand repeated exposure. The cumulative findings reinforce the hypothesis that essential oils hold substantial potential in combating antimicrobial resistance.

The ameliorating effects of antioxidant compounds to treat the risk of nicotine addiction and withdrawal

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Background: Nicotine is a compound found in tobacco plants that can cause addiction by producing a rewarding effect. It is suggested that nicotine addiction is linked with the production of reactive oxygen species in the nucleus accumbens, which has a deteriorating effect on the body's defence system against harmful agents, thereby resulting in an increased urge to smoke. When someone goes through nicotine withdrawal, their brain undergoes changes that lead to increased eating habits, weight gain, and heightened anxiety-like behaviour. This is caused by increased CRF in the amygdala and melanocortin signalling in the hypothalamus, which affects the individual's physical and emotional aspects.

Purpose: The present study aims to analyse the effect of antioxidant compounds on the risk of nicotine addiction and withdrawal. It also investigated the molecular mechanisms underlying these conditions and the effects of several antioxidants.

Methods: Balb/c male mice were used in this study. An experiment was conducted using a biased design method called conditioned place preference (CPP) to measure the reward effects caused by nicotine. A behavioural evaluation was performed to measure somatic, affective, and motoric changes in nicotine withdrawal. At the end of the experimental timeline, the animals were sacrificed, and several areas of the brain were collected to measure the gene expression that contributed to the behavioural changes.

Results: Based on the CPP score, andrographolide and epigallocatechin gallate (EGCG) significantly decreased the nicotine-induced reward effect. For nicotine withdrawal, the administration of EGCG resulted in a significant reduction in food intake, body weight, and anxiety-like behaviour. EGCG also modulates the CRF and POMC mRNA expression.

Conclusion: Antioxidant compounds are promising to lower the risk of nicotine addiction and improve nicotine withdrawal responses.

Insight into the potential mechanism of antiproliferative activity of harmiprims, harmine-primaquine hybrids: Cell localisation and cell cycle analysis

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Background: Cancer, a devastating disease characterised by abnormal cell growth and proliferation, remains a leading cause of morbidity and mortality worldwide. Existing treatment modalities often face limitations such as drug resistance, toxic side effects and insufficient effectiveness, emphasising the urgent need for alternative therapeutic options. Molecular hybridisation has emerged as a popular approach in the pursuit of novel anticancer agents as it combines diverse pharmacological properties, leading to more effective agents that can overcome drug resistance. Recently, the authors have prepared harmiprims, hybrid molecules comprised of harmine, a β-carboline alkaloid with pronounced anticancer properties, and primaquine, a well-known antimalarial agent which repurposes for cancer treatment, has been extensively investigated. Harmiprims have shown significant and selective antiproliferative activity in vitro on a panel of human tumour cell lines, prompting further exploration into their potential mechanism of action.

Purpose: The authors investigate the intracellular localisation of harmiprims and their impact on the cell cycle of the most sensitive MCF-7 cell line.

Methods: Cells were treated with 1-4 for 30 min for the intracellular localisation experiments. The samples were then subjected to fluorescence microscopy analysis, exploiting the intrinsic fluorescence of harmiprims to observe their distribution within the cells. Compounds 1, 3 and 4 entered cells and accumulated in cellular organelles other than mitochondria, which could be correlated to the observed cytotoxicity towards human cancer cell lines. In contrast, the fluorescence of compound 2 could not be detected inside the cells even after prolonged exposure (1 h). The lack of cellular uptake of 2 is consistent with its non-cytotoxic nature in the MTT assay. To determine harmiprims' impact on the cell cycle, the cells were treated with 0.5 IC50 concentrations of compounds 3 (selective activity against MCF-7) and 4 (non-selective, highly cytotoxic) for 24 and 48 hours. Afterwards, DNA was stained with propidium iodide and subjected to flow cytometry analysis.

Results: Harmiprims 3 and 4 showed a significant increase in cells in the G1 phase after 24 h. Compound 4 induced a stronger G1 cell cycle arrest, accompanied by a significant

decrease in cells in the S phase. This trend persisted after 48 hours, indicating a sustained effect on the G1 phase.

Conclusion: In this future work, the authors will focus on unveiling the molecular mechanisms responsible for the selective antiproliferative activity of harmiprims.

Natural remedies for pain management: An evidence-based consensus from global experts

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Background: Pain is one of the most common reasons affected individuals seek medical care. Natural remedies have shown promise as alternatives to modern medicines in the management of pain. However, while the use of natural remedies for pain relief has increased markedly among consumers across the globe, there is a lack of evidence-based recommendations on indications, dosing, and duration of use of natural remedies for healthcare professionals (HCPs) to guide treatment choices.

Purpose: This study aimed to use published literature and expert opinion to develop recommendation statements on using natural remedies for five common pain conditions, including osteoarthritis (OA), dysmenorrhea, back pain, tension-type headache (TTH), migraine, and sleep.

Method: Twenty-two natural remedies were identified from a scoping review of global clinical practice guidelines for the six conditions. Available literature (including monographs, clinical studies, and guidelines) on indications, dosage, and duration of use for each identified ingredient in younger adults (18–65 years) and older adults (≥65 years) was searched and compiled to develop draft recommendations. An international, multidisciplinary panel of experts in naturopathy, Ayurvedic medicine, traditional Chinese medicine, pharmacy, pain medicine, physiotherapy, and academics was convened for this modified Delphi exercise by Haleon in 2022. Experts were identified according to published research and professional expertise in natural medicines. Draft recommendations were subjected to two rounds of review of voting with refinements based on feedback received at round 1. Consensus was achieved when there was >70% agreement on a statement after round 2.

Results: The literature search yielded a total of 56 guidelines, 362 monographs, and 899 journal publications for the 22 natural ingredients across the six conditions. Evidence on the use of natural remedies was lower for older adults compared with younger adults. Twenty-six statements were initially developed and proposed for expert appraisal. Eleven experts (Europe (n = 6), Asia (n = 4), and Australia (n = 1)) participated in the exercise and completed both rounds. After refinement of statements over two rounds of review, consensus was reached on 25 of the 26 statements. Overall, recommendations were developed for a total of 22 indication-ingredient combinations. The panel formulated recommendations on the use of Boswellia serrata, curcumin, chondroitin sulfate, collagen, and glucosamine for OA pain. For back pain, recommendations were developed for devil's claw, palmitoylethanolamide, and willow bark. Additionally, consensus was achieved on using ginger, magnesium, and raspberry leaf for dysmenorrhea. Further, recommendations for butterbur, coenzyme Q10, feverfew, magnesium, and riboflavin for migraine and peppermint oil for TTH were developed. Lastly, recommendations were developed for Ashwagandha, L-tryptophan, melatonin, and valerian in the management of sleep. However, the one statement on which consensus could not be achieved involved the use of valerian for dysmenorrhea due to disagreement on the upper dosage limit.

Conclusion: This study develops evidence-based expert consensus recommendations to help HCPs provide consumers with practical guidance on the indication, dosage, and duration of use of key natural remedies for common pain conditions to promote their suitable and efficacious use for optimal outcomes.

Spectrophotometric characterisation of harmiprims, harmine-primaquine hybrids and their interaction with nucleic acids

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Background: Harmine is a naturally occurring β-carboline alkaloid that has gained considerable attention due to its diverse range of biological activities. In this previous work, the authors prepared several hybrids in which harmine and related β-carbolines were covalently linked to other bioactive molecules such as cinnamic acid, ferrocene, coumarin, chloroquine, and primaquine. In doing so, the authors have shown that molecular hybridisation is indeed a valuable strategy to obtain compounds with pronounced activities.

Purpose: To get insight into harmiprims – harmine/β-carboline and primaquine hybrids' possible mechanism of antiproliferative action.

Methods: Based on the results of an extensive in vitro screening of harmiprims' antiproliferative activities, four hybrids: 1) the most selective, 2) the most potent, 3) non-toxic, and 4) a monomeric analogue of non-toxic harmiprime were selected for further studies of their spectrophotometric properties and interactions with both ds-DNA and ds-RNA. Spectrophotometric characterisations were performed in aqueous buffer solution. Spectrophotometric titrations were performed at pH 7.0 by adding portions of the polynucleotide solution to the compound under study. UV/vis and circular dichroism (CD) experiments were performed by adding portions of the compound's stock solution to the polynucleotide solution. Thermal denaturation curves for DNA, RNA, and their complexes with investigated hybrids were determined by following the absorbance change at 260 nm as a function of temperature.

Results: Spectrophotometric characterisation in an aqueous medium showed that absorbances and emissions of selected harmiprims were proportional to their concentrations up to 2 × 10-5 (absorbance) and 5 × 10-6 M (emission). After titrations with ds-DNA and ds-RNA, quenching of emission was observed, which was rather weak after titrations with ds-DNA and ds-RNA, rather weak for non-toxic but more pronounced for the most selective and the most active harmiprims. At variance to others, the monomeric analogue of non-toxic harmiprime exhibited emission quenching only close to an excess of compound over DNA/RNA binding sites. In contrast, emission strongly increased at the excess of DNA/RNA over the compound. Thermal denaturation results showed that adding selected harmiprims did not stabilise ct-DNA or ds-RNA against thermal denaturation. No induced CD bands were observed in CD spectra above > 300 nm.

Conclusion: According to the calculated binding constants, the selected harmiprims have a moderate but biorelevant affinity for ds-DNA/RNA, with the exception of the monomeric analogue of non-toxic harmiprime. Its significantly lower affinity could be attributed to its tendency to aggregate. These results support the hypothesis that the selected harmiprims bind primarily to the grooves of DNA/RNA and not by intercalation.

Identifying new tool compounds targeting dengue Rdrp using open science and virtual screening

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Introduction: Dengue infections are rising fast around the world, with the WHO estimating as many as 400 million infections yearly, a 10-fold increase over the past two decades that is not stopping; this year saw a big surge in infections in South America, with Brazil reporting over 600.000 cases in a few weeks. This is primarily driven by climate change that broadens the habitat for the virus-carrying mosquitoes. While most cases result in flu-like symptoms that require minimal healthcare interventions, severe cases can result in hemorrhagic fever that requires hospitalisation. The societal cost of Dengue is growing, as is the strain the increasing number of infections places on healthcare systems. Battling Dengue is complex and will require multiple tools, impacting the vector and treatments such as vaccines and drugs. There are no drugs on the market to date, and with an ever-increasing need for new therapies, efforts to identify new starting points for drug development are highly relevant. In this project, the authors take an open-science approach to identify new possible drug candidates and tool compounds to assist with continued research on Dengue infections. These efforts aim to identify a new hit compound for the RNA-dependent RNA polymerase (RdRp), a key protein in the viral replication machinery.

Method: Using mutational studies, new sites for drug binding were explored on the Dengue RdRp. These results were used to guide a virtual screen of the Enamine REAL space to identify hits that could bind to this new site. For these hits, small libraries of chemical entities were generated to explore the structure-activity relationship (SAR) of these early hits and further explore their binding mode and stability. In this open science project, input from the scientific community has been sourced to support development.

Results: Mutational studies identified a new pocket on the protein that has previously not been reported in the literature. Using this site in the virtual screening cascade generated 1000 candidates, and 65 of these were selected as representative compounds sent for assay testing. This resulted in three top compounds that showed antiviral activity in the low micro-molar range, and further studies

showed binding to Rdrp in a surface plasmon resonance assay. The authors identified that solubility was moderate, and thus, our continued chemical exploration is aimed at improving potency, solubility, and metabolic stability. These efforts involve both identifying commercially available compounds to go into our SAR libraries and synthetic chemistry. Further biological studies are focused on confirming the binding pocket by applying our hit compound and other iterations on the mutated strains used to identify the site, in addition to crystal structure generation.

Conclusion: In this project the authors have used mutational studies and combined that with a virtual screening campaign to generate new starting points for developing a chemical probe that binds to Dengue RdRp in a site previously not reported.

The application of classic and modern pharmacognosy in monographing African traditional medicines

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Background: Southern Africa harbours a unique flora comprising over 22,000 species of flowering plants. Woven within this tapestry of botanical diversity is the traditional use of indigenous plants as ethnomedicines. Developing official monographs, establishing a national repository of botanical standards, and producing validated analytical methods are fundamentally important prerequisites to encouraging research and commercialisation of Africa's medicinal flora. Furthermore, the availability of reference standards, analytical methods and comprehensive monographs would be highly beneficial to the regulator, consumer and nascent industries. The unfortunate underrepresentation of pharmacognosy in the curricula of many pharmacy schools has left a void of expertise, which has hampered the development of a comprehensive herbal Pharmacopoeia. A further challenge involves the inherent complexity of medicinal plants, exacerbated by extensive chemotypic variation. Chemical fingerprinting is a crucial component in characterising plant material and requires a dedicated approach to develop analytical methods for the profiling of complex herbal extracts.

Purpose: Funding from the National Research Foundation (DSI-NRF SARChI Initiative) and the South African Medical Research Council has catalysed initiatives at the Tshwane University of Technology to develop herbal monographs that aid in the identification and quality control of important South African herbal medicines.

Method: Selected examples will be presented to illustrate the daunting workflow, which includes extensive sampling, the development of analytical methods to profile volatile and non-volatile compounds using gas chromatography coupled to mass spectrometry (GC-MS) and liquid chromatography coupled to mass spectrometry (LC-MS) respectively, high-performance thin layer chromatography (HPTLC), vibrational spectroscopy, as well as the use of preparative chromatography to isolate biomarkers. The powerful tandem application of analytical chemistry and chemometric modelling will be highlighted.

Results: Comprehensive chemical profiles were developed for selected indigenous medicinal plants and documented in comprehensive monographs, as well as the identification and isolation of biomarker compounds, which are important in the quality control of herbal products. Chemometric modelling using large sample sizes enabled the recording of chemotypic variation in wild-harvested medicinal plant species, which is also an important quality parameter to monitor in herbal products.

Conclusion: Developing comprehensive species monographs requires a multidisciplinary collaborative effort that will inevitably contribute to the safety, efficacy, and quality of African traditional medicines and commercial product development.