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Abstracts

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ORAL PRESENTATIONS

Biotechnology (BIOTECH)

Optimisation of an enzyme-linked immunosorbent assay for the biochemical characterisation of the plant cell wall and the automation of subsequent data processing

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The plant cell wall is a complex matrix that is largely comprised of polysaccharides. The properties and functioning of the cell wall are largely facilitated by the composition of these polysaccharides and interactions between them. Thus, there are great benefits in characterising the cell walls of plants, as this information may be able to predict the physiological status of plants and how well they respond to stress. One approach to cell wall characterisation entails the use of antibodies to target specific epitopes that are associated with cell wall-related polysaccharides and glycoproteins. This approach, based on the enzyme-linked immunosorbent assay (ELISA), allows for the detection and relative quantification of a broad spectrum of cell wall components. Despite the routine use of ELISA in plant cell wall analyses, current approaches lack standardisation in the processing and visualisation of the resulting data, leading to difficulties when comparing similar studies. In this study, an ELISA protocol for plant cell wall components was optimised in terms of throughput and cost, and an automated data processing pipeline was established to promote efficiency and reproducibility. Key parameters such as antibody concentration and incubation times were refined to reduce costs while improving statistical robustness. Additionally, VizELISA, an open-source software, was developed to automate data processing, statistical analysis, and visualisation of ELISA results. The optimised ELISA protocol demonstrated improved signal consistency and reproducibility across multiple experimental runs, and the protocol has been successfully used to characterise and compare various organs across several crop species. VizELISA enabled rapid data processing and improved approaches to visualisation through heatmaps and comparative statistical analyses. This optimised protocol, together with VizELISA, aids in reducing cost and minimising human error when routinely processing large ELISA datasets. Therefore, our optimised ELISA protocol has the potential to be a valuable tool for plant cell wall research.

Determining the molecular basis of the rare white-flowering *Greyia sutherlandii* phenotype

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Greyia is a near-endemic South African genus of trees or large shrubs, consisting of three species growing along the Great Escarpment. These species are valued ornamentally for their bright red inflorescences and have also attracted medicinal interest due to the anti-tyrosinase activity of their leaf extracts, which may be useful in treating skin hyper-pigmentation. The medicinal activity is conferred by a flavonoid, but the variability in activity and the lack of knowledge of flavonoid metabolism in this genus has impeded commercialisation. This study sought to gain an overview of flavonoid metabolism in *G. sutherlandii* by characterising the underlying mutation in a rare white-flowering phenotype, typically associated with a deficiency in anthocyanins, which form an end-product of the flavonoid biosynthesis pathway. Metabolite analysis with liquid chromatography-tandem mass spectrometry (LC-MS/MS) proved that the mutant phenotype is completely anthocyanin-deficient, while the wild-type accumulates both cyanidin and pelargonidin derivatives. In wild-type plants, cyanidin derivatives were on average five times more abundant than pelargonidin derivatives in autumnal leaves, whereas pelargonidin derivatives were over fifty times more abundant than cyanidin derivatives in flowers. Using a transcriptomics approach, a two-base deletion was identified in the anthocyanidin synthase (ANS) transcript, which would result in a truncated, non-functional protein lacking the iron-binding domain in the white-flowered variant. Sanger sequencing confirmed mutant individuals to be homozygous for this mutation, while heterozygotes displayed a wild-type phenotype, indicating a recessive mode of inheritance consistent with the nature of the mutation. This mutation provides a compelling explanation for the white-flowering *G. sutherlandii* by showing the drastic effects small mutations can have on a plant's phenotype.

Physiological and proteomic responses of contrasting soybean genotypes to combined drought and salinity stress

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Soybean (*Glycine max* L.) is a vital global crop valued for its high protein and oil content, contributing significantly to human diets, animal feed, and various industrial applications. However, its productivity is increasingly threatened by drought and salinity. The simultaneous occurrence of these two stresses is expected to become more frequent due to climate change, resulting in severe damage. Understanding how soybean responds to combined drought and salt stress is critical for developing stress-resilient cultivars. This study employed a comparative mass spectrometry-based proteomics approach to investigate the molecular responses of two soybean genotypes, HMC 202 (tolerant) and KCW (sensitive), under combined drought and salinity stress. Growth parameters (leaf and root length and weight), plant water status and soil water potential were assessed. Proteomic profiling revealed key differences in protein expression between the two genotypes under stress conditions, offering insights into the molecular mechanisms associated with combined drought and salt stress tolerance. These findings contribute to the knowledge on plant adaptation to combined abiotic stresses and provide a foundation for enhancing soybean resilience, thereby supporting sustainable agriculture and food security.

Decoding the morpho-physio-biochemical response mechanisms of *Phaseolus vulgaris* L. to waterlogging and *Fusarium oxysporum* infection

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South Africa's agricultural sector faces growing challenges from environmental stresses such as waterlogging and soilborne pathogens, which threaten crop productivity and food security. This study examined the effects of waterlogging stress and *Fusarium oxysporum* infection on common beans through a controlled greenhouse experiment, focusing on plant physiology, biomass, chlorophyll content, MDA content, antioxidant activity, and mineral uptake using ICP-AES. Two cultivars, Lazy Housewife (LHW) and Contender (CON), were assessed for their tolerance and susceptibility to these stressors. Waterlogging alone significantly reduced biomass at the V2 stage in both cultivars, with root biomass also declining. Affected plants exhibited smaller trifoliate leaves and chlorosis, while adventitious roots formed more prominently in CON. MDA levels varied, with waterlogging lowering leaf MDA in LHW but increasing it in roots. Under *F. oxysporum* infection and combined stress, both cultivars showed elevated MDA levels, indicating oxidative stress. Chlorophyll content declined significantly under waterlogging, further highlighting physiological impairment in both cultivars. Mineral uptake analysis revealed distinct cultivar responses. Antioxidant activity varied, with the highest increases in SOD and CAT in roots, suggesting a stress-adaptive response. In LHW, waterlogging increased potassium and calcium in leaves, while *F. oxysporum* infection reduced both. In CON, Ca levels increased across all stress treatments, while K remained stable under waterlogging. Root analysis showed LHW gained K under waterlogging but lost it under *F. oxysporum* infection, with K levels normalizing under dual stress. Ca followed a similar trend, while in CON roots, K and Ca increased across all treatments compared to controls. Overall, LHW exhibited greater resilience to waterlogging and pathogen stress than CON, mitigating damage across physiological, biochemical, and mineral parameters. Future studies will employ integrative omics approaches, such as proteomics and metabolomics, to further elucidate the mechanisms underlying this tolerance and its potential applications for crop improvement and agricultural sustainability.

Nanoparticle-Based Strategies for Controlling Alternaria alternata: A Step Towards Sustainable Crop Protection

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Fungal diseases are a major cause of crop losses worldwide, contributing significantly to global food insecurity. Among these, Alternaria alternata is particularly harmful, causing extensive damage to crops like sunflower, chili, and wheat, affecting both pre- and post-harvest yields. Current control methods for this pathogen are inadequate, highlighting the need for innovative solutions. This study explores the use of nanotechnology, specifically biogenic zinc oxide nanoparticles (ZnO-NPs) and silver-doped zinc oxide nanoparticles (Ag/ZnO-NPs), as potential control agents against A. alternata.

In a series of experiments, these nanoparticles exhibited concentration-dependent inhibition of A. alternata mycelial growth. Microscopic examination of treated fungal mycelia revealed notable alterations in cell wall structure and destruction of cytoplasmic organelles. Biochemical assays further demonstrated that the nanoparticles affected the fungus's polysaccharide and chitin content, along with reducing the activity of key enzymes such as β-1,4-glucanase and extracellular lipase. Moreover, the nanoparticles induced oxidative stress in A. alternata, leading to increased levels of hydrogen peroxide and malondialdehyde, which contributed to the fungus's deterioration.

In addition, proteomic analysis offered deeper insights, showing changes in the expression of essential proteins involved in fungal growth and development. The study concludes that ZnO-NPs and Ag/ZnO-NPs are promising tools for controlling A. alternata and could be applied in developing nanofungicides for sustainable agriculture. These findings lay the groundwork for future nanopesticide formulations to reduce crop pathogen damage, potentially transforming plant disease management strategies. This research marks a crucial step toward integrating nanotechnology into agriculture, offering a sustainable and effective approach to managing harmful plant-microbe interactions.

Optimizing *in vitro* propagation and agronomic performance of three ARC hemp (*Cannabis sativa*) lines for enhanced yield and cannabinoid production

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Hemp (*Cannabis sativa*) is a valuable crop with applications in fiber, seed, and cannabinoid production. Optimizing propagation methods is crucial for enhancing yield and maintaining genetic stability. This study investigates the *in vitro* propagation, acclimatization, and agronomic performance of three hemp lines (I-5, I-8, and I-27) under controlled conditions. *In vitro* propagation using various *meta*-topolin concentrations resulted in high multiplication rates, and 97% survival rate at acclimatization stage. Morphological assessments at flowering revealed significant variation among the lines. The I-5 line exhibited the highest node count and branching, while the I-8 line had the longest internodes and widest leaves. Rooting was best achieved when growth medium was supplemented with indole-butyric acid (IBA) and activated charcoal. The I-27 line showed superior floral traits, including the highest flower count per plant (187.5 ± 26.52), larger flower bud diameter (8.9 ± 0.39 mm), and the greatest number of stigmas per bud (12.0 ± 0.69). Principal component analysis highlighted distinct trait differentiation across the lines. Cannabinoid profiling indicated that tetrahydrocannabinol (THC) levels were lowest in I-27 at full bloom (0.15%), whereas I-5 and I-8 maintained levels below 0.9%. Variability in cannabidiol (CBD) and cannabinol (CBN) levels was observed, with I-5 and I-8 showing higher concentrations. Seed yield assessments demonstrated that I-27 produced a greater total seed number, but with smaller seed size compared to I-5 and I-8, which exhibited larger seeds. These findings provide valuable insights into optimizing hemp propagation for cannabinoid production and seed yield, with implications for commercial cultivation and breeding programs.

Improving *vitro* propagation of *Thymus vulgaris*: A step towards sustainable production

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Thyme, a versatile culinary and medicinal herb, present challenges in conventional propagation due to transplant shock, slow growth and low seed viability. *In vitro* propagation is a practical solution for the rapid multiplication of thyme, ensuring genetic uniformity and production of disease-free plants in a controlled environment. This study aims to enhance the *in vitro* propagation protocol of *Thymus vulgaris*. Through a series of controlled experiments, the effect of various concentrations (ranging from 0 to 15 µM) and types of plant growth regulators (PGRs) (6-benzylaminopurine (BA), kinetin (Kin), *meta*-topolin (*m*T) and indole-3-butyric acid (IBA)) was determined. Our findings showed that the use of PGRs significantly influenced the growth dynamics of thyme, particularly *m*T. The highest shoot number per explant was observed in treatment wiith 1 µM *m*T (7.25 ± 0.730). A decrease in shoot number per explant with increasing concentrations of BA and *m*T treatments was observed. The highest root number per explant was observed in 1 µM *m*T (3.38 ± 0.0748) and PGR-free treatment (3.00 ± 0.0298), while the highest mean height (cm) per regenerated shoot was observed in medium treated with 1 µM Kin (4.04 ± 0.27) and PGR free treatment (3.70 ± 0.11). Additionally, a decrease in height was observed with increasing cytokinin concentrations and a decrease in root number with increasing concentrations of *m*T. The study demonstrated that the application of 1 µM *m*T significantly enhances shoot proliferation compared to other cytokinins tested. Overall, this research highlights the importance of plant growth regulators (PGRs) as a valuable input for thyme growth, which can enhance its sustainable cultivation and use across various sectors, including pharmaceuticals, cosmetics, and food.

Improvement of sorghum tolerance to drought using rhizospheric bacteria

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Sorghum is an important source of food and animal feed. Given the increasing severity and frequency of drought due to climate change, it is prudent to enhance crops that are already tolerant such as sorghum because sensitive crops may not survive future conditions. Rhizospheric microbial communities play a crucial role in promoting plant health and mitigating environmental stresses. This study aimed to identify drought tolerant rhizospheric bacteria from *Cenchrus ciliaris*, a grass that thrives in the Kalahari Desert, and explore their potential for enhancing sorghum tolerance to water deficit stress. Additionally, the study sought to elucidate the role of proline and some of the antioxidant enzyme activities (APX, CAT and SOD) in rhizospheric bacteria-mediated drought tolerance in sorghum. Fifteen rhizospheric bacteria were isolated and screened for tolerance to PEG-induced osmotic stress (20 % and 30 % PEG 8000). The three most tolerant isolates, *Serratia* spp., *Corynebacterium* spp. and *Microbacterium* spp., were evaluated for tolerance enhancement of sorghum seedlings to water deficit stress. *Serratia* spp., which demonstrated the most significant tolerance enhancement in sorghum seedlings, was evaluated for improving sorghum tolerance at a later growth stage. The inoculation of sorghum with *Serratia* spp. significantly enhanced sorghum tolerance to water deficit. This tolerance enhancement was associated with increased proline accumulation in both leaves and roots under water deficit. In addition, analyses of antioxidant enzyme activities revealed a significant association between rhizospheric bacteria-mediated tolerance and improved activity of APX and CAT enzymes in the leaves, as well as increased SOD activity in the roots. These findings underscore the potential of rhizobacteria for enhancing sorghum resilience to drought, thus promoting sustainable agriculture and food security. Further multi-omics research aimed at understanding the molecular interactions between rhizobacteria and sorghum is crucial for more efficient and targeted application of these bacteria.

Mitigation of nutrient stress in *Hordeum marinum* via intercropping with alfalfa: Insights from physiological and metabolomic analyses

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Intercropping is a sustainable strategy for improving crop productivity and resilience, particularly under abiotic stress conditions. This study evaluates the impact of intercropping alfalfa (*Medicago sativa*) with sea barley (*Hordeum marinum*) on biomass production and metabolomic responses under nutrient-deficient and water-limited conditions in arid environments. The experimental design included monoculture and mixed cropping systems under optimal and low nutrient regimes, with water stress applied at 40% field capacity.

Metabolomic profiling was performed at selected harvests and nutrient levels. Results revealed that cropping mode significantly influenced plant performance, surpassing the effects of treatment and harvest timing. Intercropping consistently enhanced biomass production in both species, with the most significant improvements observed under nutrient stress during the second harvest. In *H. marinum*, intercropping improved ion uptake efficiency, increasing Cl^- and NO_3^- in leaves and Ca^{2+} and Na^+ in roots. Metabolomic analysis showed that intercropping altered primary metabolism. Fructose and pinitol levels were elevated in both leaves and roots, while myo-inositol was notably increased in alfalfa leaves. In *H. marinum*, intercropping significantly enhanced stress-related amino acids—proline, glycine, GABA, alanine, and serine—indicating improved stress resilience. Organic acids such as malate, quinate, citrate, and chloride were also differentially regulated, reflecting adaptive metabolic shifts under nutrient stress. These findings highlight the potential of alfalfa–sea barley intercropping systems to enhance physiological performance and metabolic adaptability in nutrient-poor arid regions, promoting sustainable crop production under climate-challenged conditions.

Evaluating the impact of deficit irrigation on bioactive compounds, UPLC-MS metabolites and antioxidant activities of *Artimesia afra* using a chemometric tool of analysis

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Drought conditions in semi-arid growing area can attribute to metabolites contents and antioxidant activities within plants. *Artemisia afra* Jacq. ex Wild. has known potential in the treatment of different aliments via the IKS system. Its aerial parts are rich source of biochemical compounds constituted by over 160 individual phenolic compounds contributing to antioxidant activities. The current study aimed to evaluate the impact of different levels of drought assimilation through deficit irrigation on bioactive compounds and antioxidant activities. Five treatments based on deficit irrigation levels including (i) Control: 100% (T1); ii) 75% (T2); iii) 50% (T3); iv). 25% (T4); and v) 10% (T5), were established from field capacity irrigation volume of 600 mL. Irrigation was performed on an eight-week-old seedling, on every 14th day from the first irrigation time. Total phenolic compounds (TPC), total carotenoids (TCC), untargeted Ultra High Performance Liquid Chromatography Mass Spectrometry (UHPLC-MS) metabolites, and antioxidants capacity were carried out in triplicate. Application of optimum and low deficit irrigation (T1 and T2) favoured the accumulation of TPC, 3,5-Dicaffeoylquinic methyl ester and Catechin 7-glucoside compounds, which further attributed to high scavenging activities IC50 (0.6 mg/mL). Moderate to severe drought conditions (T3, T4 and T5) showed higher reducing power activities (IC50: 1.55 mg/mL), Eruberin C, 4-coumaroylshikimic acid, which could be attributed to the chlorophyll regulation and PAL biosynthesis defence mechanism against drought. The PCA first cluster showed samples of T3; T4 and T5 to own similar metabolites which showed major heterogeneity variation with those exposed to T2 and T1. The current study describes the impact of different drought levels on *Artemisia afra*'s biochemical compounds and potential defence mechanisms during production.

Athrobacter sp. isolated from *Hordeum marinum* improves wheat tolerance to salinity by inducing ascorbate peroxidase and catalase activities

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Wheat (*Triticum aestivum*), the second most important cereal crop, provides 20% of the world energy and protein needs. Salinity, continuously exacerbated by climate change, poses a significant threat to wheat production. Developing eco-friendly, sustainable strategies to mitigate salt stress is essential. Salt-tolerant rhizospheric bacteria offer a promising solution. This study explores the potential of salt-tolerant rhizobacteria associated with sea barley (*Hordeum marinum*) to confer salt tolerance to wheat. 20 isolates were obtained from sea barley and screened for salt tolerance by growing the bacteria in LB supplemented with 900 mM NaCl. 5 best-performing isolates were selected for a greenhouse trial to assess their ability to confer salt tolerance in wheat. Shoot weight and length were evaluated as indicators of growth performance. Relative water content (RWC), soil water potential and proline accumulation were measured to evaluate plant water status and osmotic adjustment. Oxidative stress was assessed by measuring hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and malondialdehyde (MDA) contents. Antioxidant enzyme activities, including ascorbate peroxidase (APX), catalase (CAT) and superoxide dismutase (SOD) were determined using spectrophotometric assays. Our results showed that of the five isolates, *Arthrobacter* sp. exhibited the most pronounced tolerance improvement. Proline levels were lower in salt-treated plants inoculated with the *Arthrobacter* sp., suggesting alternative osmotic adjustment mechanisms. Additionally, salt-treated plants in the presence of the *Arthrobacter* sp. exhibited reduced lipid peroxidation and enhanced CAT and APX activities, with no significant change observed in SOD activity. These findings highlight the potential of *Arthrobacter* sp. to enhance salt tolerance in wheat, which could improve wheat production in salt-impacted soils, contributing to food security.

Mechanistic insight into the antifungal potential of gallic acid against *Fusarium oxysporum*

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Fusarium wilt, caused by *Fusarium oxysporum, is* a major threat to global food security, leading to significant soybean losses and mycotoxin contamination. The widespread use of chemical fungicides poses environmental and health risk, emphasising the urgent need for safer and more sustainable alternatives. Phenolic compounds such as gallic acid are ubiquitous in plant tissues and exhibit various properties including antioxidant, anti-inflammatory, and antimicrobial. However further research is required to elucidate its antifungal mechanisms. Therefore, the aim of this research is to investigate the efficacy of gallic acid in controlling *F. oxysporum*. The antifungal potential of gallic acid against *F. oxysporum* was evaluated using the food poison technique, while the effect of the treatment on the morphology of the fungi was monitored using high resolution scanning electron microscopy (HR-SEM). Additionally, changes in the polysaccharide production, chitin content and various biochemical activities of the treated *F. oxysporum* were evaluated. In addition, liquid chromatography mass spectrometry (LC/MS)-based protein profiling was performed to identify differentially expressed proteins related to stress response, metabolism, and pathogenicity. *In vitro* antifungal study as well as HR-SEM images of the treated fungus showed significant inhibition and changes in the cellular structure. The cell wall components of the fungi were greatly affected by gallic acid, causing significant reduction in the polysaccharide and chitin contents. The fungal mycelia treated with 1 mM gallic acid experienced the highest level of reactive oxygen species (ROS) induced oxidative damage. Additionally, gallic acid affected the pathogenesis of the *F. oxysporum*, by causing a significant decrease in its β-1-4-glucanase and lipase activity. Using LC/MS analysis, protein abundance of F. oxy was significantly altered in response to treatment with gallic acid. The results suggest that gallic acid may be a useful antifungal agent to manage and control the incidence of *Fusarium* wilt disease to enhance food security.

Mitigating nutrient stress in *Sutherlandia frutescens* (L.) R.Br. using cytokinin oxidase/dehydrogenase inhibitors

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Cytokinin oxidase/dehydrogenase (CKX) inhibitors are known to promote growth, nutrient signalling and yield in stressed and non-stressed model plants. Based on previous research, nitrogen (N) and phosphorus (P) stress have been shown to trigger phenotypic plasticity in *Sutherlandia frutescens*, a medicinal legume, which contributes to changes in plant metabolite profile. Hence, the aim of this study was to investigate the role of a new CKX inhibitor (pseudo-named MCKX) in mitigating the negative effect of reducing nitrogen and phosphorus levels in *S. frutescens* cultured in an *in vitro* system. Nitrogen and P stress was triggered by halving (½) or completely eliminating (-N/-P) essential nutrients (NH_4NO_3, KNO_3 or KH_2PO_4) in the growth medium. Nitrogen deficiency (-N) significantly reduced shoot production in plantlets. Whereas MCKX (0.05 and 1 nM) counteracted the effect of nitrogen deficiency by eliciting a two-fold increase in plant leaf number and shoot height. LC-MS analysis using targeted and non-targeted metabolites showed a significant decrease in biomarker compounds such as sutherlandins (C, D and derivatives), ermanin and soyasaponins, especially in treatments deficient of nitrogen. Phosphorus (-P and ½P) stress showed minimal effect on leaf number, shoot length and fresh weight. In comparison to the control treatment, glucose and pinitol synthesis increased by almost two-folds in phosphorus deficient plants. MCKX (1 nM) not only counteracted poor rooting caused by ½ N/P but also triggered a significant increase in pinitol, glucose, proline, GABA, L-canavanine and specialized metabolites (e.g., sutherlandins C, C/D derivatives, soyasaponins). The current findings demonstrate that MCKX is able to mitigate the impact of nutrient stress especially during plant growth and development by upregulating primary and secondary metabolites.

Controlling *Fusarium oxysporum*: Mechanistic investigation of caffeic acid against crown and root rot

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Crown and root rot, caused by *Fusarium oxysporum*, is a major agricultural challenge that leads to severe yield losses in economically important crops. The widespread use of chemical fungicides to manage this disease raises concerns about environmental sustainability, human health risks, and the emergence of resistant fungal strains. As a result, there is a growing need for eco-friendly and effective alternatives to conventional fungicides. A promising phenolic compound, caffeic acid (CA) has shown to have antioxidant, anti-inflammatory, and antimicrobial potential. This study aimed to evaluate the antifungal potential of CA as a sustainable biocontrol agent against *Fusarium oxysporum*. Using the food poison technique, we assessed the inhibitory effects of CA on fungal growth, while high resolution scanning electron microscopy was employed to examine morphological changes in fungal cells. Additionally, biochemical responses—including oxidative stress markers, antioxidant enzyme activity (superoxide dismutase and ascorbate peroxidase), and cell wall components (chitin and polysaccharides)—were analysed using spectrophotometric assays. The impact of CA on fungal pathogenicity was further investigated by measuring cellulase and lipase enzyme activity, and proteomic profiling was conducted to identify differentially expressed proteins associated with stress response, metabolism, and virulence. Our findings demonstrate that CA effectively inhibits the growth of *Fusarium oxysporum*, particularly at a higher concentration (1 mM). CA disrupts fungal cell membranes, induces oxidative stress, and increases cell death. Furthermore, it significantly reduces the activity of key fungal enzymes linked to pathogenicity, such as lipases and cellulases. Proteomic analysis displayed significantly altered proteins highlighting affected resistance mechanisms within fungal cells. These results suggest that CA possesses strong antifungal properties and holds promise as a natural alternative to synthetic fungicides. Its application in priming wheat seeds could enhance plant resistance to *Fusarium* crown and root rot infections, while providing practical benefits for sustainable agriculture.

Ecology (ECOL)

From marshes to mines: Assessing the distribution, germination and establishment of *Crinum bulbispermum* for restoration of gold mine tailings.

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This study investigates the distribution, ecological niche, and restoration potential of *Crinum bulbispermum* in disturbed environments, particularly those impacted by mining activities. The species is predominantly found in proximity to drainage systems, such as the Vaal River, in the eastern regions of South Africa, where it prefers high soil moisture and nutrient content. The primary objectives were to model the species' climatic niche to determine overlap with regions impacted by gold mines, assess germination success on tailings, and compare seedling survival and growth on tailings with other soil types. Species Distribution Modelling identified the Grassland Biome on the Highveld (1000+ m above sea level) as offering the most suitable conditions for its growth and survival. *Crinum bulbispermum* demonstrated resilience to less favourable environments, such as mine tailings, which are often seasonally dry, nutrient-poor and chemically harsh. The species exhibited over 85% germination success across various soil treatments, including mine soil, suggesting potential for use in restoration efforts involving seeding. However, longer-term survival (12 months) in mine tailings was hindered by several factors, including heavy metal toxicity (Co, Cu, Mn and Zn), low organic carbon content (0.11%), and high salinity (194.50 mS/m), all of which are chemically extreme conditions that hamper revegetation. Findings indicate that while *C. bulbispermum* could initially establish on mine tailings, its growth became stunted over time due to the harsh soil conditions. *C. bulbispermum* thrived in soil treatments rich in clay and organic matter, which is typical for natural grasslands and old fields. This highlights the importance of soil texture and organic content in the successful establishment and growth of the species. These findings hold significant potential for the restoration of seasonally waterlogged, chemically harsh mine tailings. Its ability to germinate and establish, without accumulating metals, suggests it could be an effective metal-excluding species for phytostabilisation. However, for restoration practices to be sustainable, further studies are needed to assess the long-term performance of this species, including the potential role of microbial communities, soil amendments, and other restoration strategies that might enhance its growth and reproductive success in harsh soil.

Spatial patterns and drivers of plant diversity in the Witsieshoek Valley, Drakensberg Mountains, South Africa

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Drivers of plant diversity remain poorly understood in Witsieshoek, a section of the Drakensberg mountains. This may be influenced by various factors, including elevation, soil types, climate change, and tourists. In this study, plant diversity was determined based on vegetation plots, morphological characteristics of plant specimens, field sampling and geographic information system (GIS) analysis of the mountains. A linear sampling at a range of 500-metre intervals, resulting in 30 sampling points, was employed to select sites across the Witsieshoek mountain. Species richness and phylogenetic diversity were evaluated within sampling points, as well as the differences in plant diversity between sampling areas. The results showed significant differences in species types and richness as elevation increases. However, the species *Leucosidea sericea*, members of the *Poaceae* (grasses) and *Asteraceae* families dominated the mountain, especially at high elevations. Our results also indicate that the lower elevation holds more plant diversity, especially around the camps and the Witsieshoek lodge, but no evidence for significant plant biomass differences between sampling points. This could be associated with higher carbon content found in the soils at the lower elevation and along the drainage, as revealed by soil analysis and GIS, respectively. This study contributes to the limited knowledge of plant species distribution in the Witsieshoek Mountains and examines how environmental, ecological, and indigenous values influence its vegetation.

Geographic distribution and environmental correlates of ploidy in the Cape *Oxalis hirta* L. (Oxalidaceae) species complex

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Whole genome duplication (WGD), or polyploidy is a key driver of plant diversification, yet its prevalence and adaptive significance in stable climates like the Greater Cape Floristic Region (GCFR) remain poorly understood. *Oxalis* L. (Oxalidaceae) is a speciose genus found in the GCFR that includes several polyploid species, many of them in the form of polyploid series within individual species. The high incidence of polyploidy within *Oxalis* and the presence of multiple species complexes renders the group ideal for exploring the evolutionary patterns and ecological consequences of WGD. The *Oxalis hirta* species complex, a widespread yet understudied group, is unique within the genus for its extensive variation in flower color and floral tube length and width. We investigated ploidy variation across 39 populations (289 individuals) of *O. hirta* and its close relatives across the Western Cape, South Africa, testing whether cytotypes are geographically and ecologically structured using generalised linear models (GLMs). Using flow cytometry (DAPI-staining with *O. articulata* as internal standard), we estimated ploidy levels and identified mixed-ploidy populations. Ploidy-level frequency (diploid vs. polyploid) was modelled against: (i) geographic coordinates to assess spatial structure; (ii) temperature seasonality to assess environmental variance; (iii) precipitation of the driest quarter to assess drought tolerance; and (iv) soil phosphorous (P) and nitrogen (N) to assess nutrient availability gradients. We show that *O. hirta* comprises multiple cytotypes with non-random spatial distributions and describe key environmental correlates of ploidy in the complex. Our findings highlight how cytotype variation influences tolerance to environmental variability, drought, and nutrient limitation. Our work provides new insights into the selective advantages conferred by polyploidy in the environmentally heterogenous GCFR.

Temporal and spatial distribution of vegetation fires in the Robertson Granite Renosterveld from 2000 to 2024

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The Fynbos biome is both fire-dependent and fire-adapted, making fire an important ecological process within Fynbos shrublands. The Robertson Granite Renosterveld (FRg3), nested between Robertson Karoo and Breede Shale Renosterveld to the south and Robertson Granite Fynbos to the north is an understudied vegetation type. We investigated a 25-year fire history (2000-2024) of the FRg3 and how fire influences the distribution of its plant communities. Using satellite imagery (MODIS/061/MCD64A1), fire data was obtained from Google Earth Engine, to map the extent of each fire. The FRg3 had three fires in 25 years (2000, 2006, and 2017), with 35.5%, 24.7% and 53.5% of the area burnt respectively. Some areas were burnt by multiple fires (2000 & 2017 = 23%; 2006 & 2017 = 22%), and almost half (46.49%) of the FRg3 vegetation type did not burn at all over the 25 years. Fire suppression by farm owners and livestock grazing, which reduced the fuel load, likely prevented the lower mountain slopes from burning. Four plant communities (*Muraltia heisteria – Restio capensis*, *Dodonea viscosa - Euryops tenuissimus, Passerina obtusifolia - Restio capensis*, and *Dicerothamnus rhinocerotis - Dodonaea viscosa*) were in fire affected areas, and only one community in the south, with many karroid species (*Pteronia paniculata - Dicerothamnus rhinocerotis*), did not burn in the 25 years. The FRg3 fire season is between January and March, the warmest and driest part of the year, providing favourable conditions for fire spread. The fires generally ignited on high mountain slopes from the adjacent fynbos type (probably ignited by lightning), and the low wind speeds (<13 km/h) during these fire periods (4-6 days/fire) sometimes resulted in back-burns towards the lower slopes.

Reduced reproductive output in small and isolated populations of the bird-pollinated *Aloe lettyae*, an endangered South African grassland endemic

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Large-scale habitat loss and fragmentation have reduced the formerly widespread *Aloe lettyae* to a few remnant populations in isolated, critically endangered grassland fragments. *Aloe lettyae* is likely self-incompatible and primarily pollinated by Amethyst sunbirds. The aim of this study was to determine the reproductive output in *A. lettyae* populations of different sizes. Reproductive output was quantified as number of flowers per aloe, percentage fruit set, number of seeds per fruit and per aloe in the large main population, one medium population and four small populations with ~6500, ~2200 and <350 aloes, respectively. Fruit set (percentage of flowers that produced fruit) varied from 29% in the large main population to 3% in one of the small populations. Overall, population size had a significant positive effect on fruit set. The number of intact/viable seeds produced per aloe tended to be higher in the large and medium *A. lettyae* populations than in the small ones. In conclusion, reduced reproductive output in terms of fruit set and seed production in small *A. lettyae* populations may suggest that an Allee effect is operating. Allee dynamics are difficult to study in natural populations, and more research into the dynamics of small *A. lettyae* populations which are based on multiple interactive demographic processes is needed to inform the conservation and management of remnant wild populations.

The future of savanna trees: Understanding the influence of elephants, fire and land use change on large tree demography

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Large trees are keystone structures in savannas. Through hydraulic lift, increased nutrient cycling and shading, trees alter microclimates beneath the canopy, creating resource hotspots for faunal and floral communities. Within the context of a global loss of large trees, conservation managers are concerned about the loss of large trees in the Kruger National Park (KNP). We compared sites in the open landscapes of the KNP, elephant-free sites in Eswatini and sites across a land use change gradient to assess the demographic resilience of two dominant tree species, *Senegalia nigrescens* and *Sclerocarya birrea*. Large trees have declined in KNP on account of elephant feeding, regular intense fires and drought. Although trees can resprout following top kill, for six years, we found no regeneration from seeds by *S. nigrescens*, a long-lived tree with limited dispersal. *S nigrescens* flowering and fruiting are largely determined by tree height; elephant feeding reduces tree height, consequently limiting regeneration potential. This is exacerbated by the reduction in tree density, which results in a decrease in propagule abundance. In contrast, we found significant germination of seeds of *S birrea*, likely dispersed by elephants, despite the local decline in adult trees in the KNP. In Eswatini, in the absence of elephants and irregular burns, we found significant germination of *S. nigrescens* but limited germination of *S. birrea*. In landscapes utilised by farmers, outside national parks, human consumption of *S. birrea* partially compensates for the loss of the elephant mutualist. In the long term, we predict a potential decline in *S nigrescens* in the Kruger Park due to the creation of sterile landscapes by elephants and fire, whilst *S birrea* may decline in Eswatini on account of the loss of key mutualists.

Invertebrate assemblages and vegetation dynamics in high elevation wetlands of the Eastern Escarpment, South Africa

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Wetlands offer vital ecological and economic services such as carbon storage, flood regulation, water purification and provision; however, they remain understudied and highly threatened in South Africa. A better understanding of the dynamics that structure and sustain wetlands can inform effective conservation strategies for both water resources and biodiversity. This study aimed to (1) assess spatial trends in macroinvertebrate diversity and abundance across 18 high-elevation wetlands of the Eastern Escarpment, South Africa (EESA), and (2) explore their relationships with vegetation dynamics (e.g., plant cover, dominant types, species richness, flowering/seeding) and key environmental variables (e.g., elevation, peat humification, water depth, wetland size, MAP, land use, management). Invertebrate richness, diversity, and abundance were assessed using timed sweep netting sessions. Community patterns were analysed using NMDS and PERMANOVA to detect significant environmental influences. Morphospecies invertebrate richness across 18 sites ranged from 5 to 25 species, and abundance from 10 to 175 individuals across 18 high-elevation wetlands. Among the 70 Coleoptera, 79 Diptera, and 34 Araneae morphospecies collected, the most diverse families were Chrysomelidae (21%) and Coccinellidae (12%) in Coleoptera, Muscidae (14%) and Chloropidae (13%) in Diptera, and Thomisidae (45.5%) and Tetragnathidae (22.3%) in Araneae. Key vegetation metrics—including plant species richness, flowering sedges, grasses and herbs, and vegetation height—showed significant relationships with invertebrate assemblages. This study enhances our understanding of high-altitude wetland ecology in the EESA, identifying key drivers of invertebrate communities to support the development of targeted strategies for the protection and restoration of these threatened ecosystems.

Early life stages matter to the rescue of a long-lived plant from the consequences of lethal root harvest in South Africa

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Anthropogenic pressures primarily drive population decline globally, prompting the urgent need to investigate sustainable harvesting of plant resources. Here, we collected demographic and environmental data on the long-lived species *Securidaca longepedunculata*, a highly valued medicinal plant in decline due to lethal harvesting in South Africa. To analyse these data, we first developed matrix projection models, then investigated how harvesting influences the population dynamics of this species through simulations and finally examined the effect of senescence on vital demographic parameters. We found that the sensitivity of the population growth rate λ to perturbation is habitat dependent. In mildly harvested habitats, λ is sensitive to adults, whereas it is most sensitive to early stages in heavily harvested habitats. Interestingly, we found consistent resilience of our species to harvesting, regardless of the habitats, proportions, and stages harvested. We also found evidence of positive senescence, particularly in heavily harvested habitats, suggesting that positive senescence might have driven low reproductive values and the low elasticity of λ to perturbation of adults in this habitat. We suggest a rotational harvest approach among different habitats, which is imperative to ensure the sustainability of this species.

Comparisons of mistletoe host species and densities between communal rangelands and protected areas in a semi-arid African savanna

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Mistletoes in Africa face growing biotic and anthropogenic threats, with impacts that vary across land-use types. This study compared mistletoe and host tree densities in protected areas (PA) and communal rangelands of Bushbuckridge, South Africa. We established 50 transects to survey woody plant densities, as well as mistletoe and host tree densities. Woody plant and mistletoe host species compositions differed significantly across land-use types. Communal rangelands had 59% higher tree densities, with 11%, 15%, and 23% greater tree abundance, Shannon diversity and richness, respectively, compared to PAs. Host tree and host species densities were 81% and 67% higher in communal rangelands than PAs, respectively, leading to 67%, 80%, and 92% greater host species richness, abundance, and diversity in communal rangelands. *Lannea discolor*, *Syzygium gerrardii, Sclerocarya birrea*, and *Albizia versicolor* were the preferred hosts. However, *S. birrea*, which had the highest availability (density) and acceptance as a host, had the most mistletoe infestation. Total mistletoe density was 81% higher in communal rangelands, with *Erianthemum dregei* the most dominant mistletoe species, infecting 12 host species. Differences between land-use types likely reflect the impacts of different disturbances. Elephant activity reduces large tree densities in PAs, compared with multiple uses in communal rangelands. Customary laws in communal rangelands may limit overharvesting, although signs of harvesting and bush encroachment were evident. These findings highlight how land-use types increase plant spatial heterogeneity in semi-arid savannas.

Long-term monitoring is essential to assess the effects of elephant fluctuations, harvesting and climate change on mistletoes and their hosts.

Potentially toxic metal(loid)s transfer in a tri-trophic ultramafic system

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Potentially toxic metal(loid)s (PTMs) occur both in natural and anthropogenically altered soils. There is a small contribution of studies from Africa and reduced focus on PTMs from natural sources worldwide. Hence, the present study unveiled the transfer of PTMs in a tri-trophic food chain using ultramafic (komatiite) and control soil as substrates from South Africa. The wild plant *Berkheya radula* and the crop plant *Brassica oleracea* var. *acephala* (comercial kale) were selected as primary producers, while the house cricket *Acheta domesticus* and the African twig mantis *Popa spurca* were the primary and secondary consumers, respectively. PTMs concentration were evaluated in each trophic level, along with bioaccumulation factors. Additionally, the effect of PTMs on house cricket feeding behaviour was assessed through olfaction tests, and the development of the African twig mantis was evaluated through the duration of their life cycle. Although higher concentrations of PTMs were found in the komatiite soil, the trophic levels did not reflect these differences. *Berkheya* and kale from control and komatiite soils bioaccumulated and excluded different PTMs, regardless of soil concentrations. Crickets bioaccumulated similar PTMs from *Berkheya* and kale leaves, grown in both soil types. Olfaction tests revealed that crickets didn't discriminate between plant leaves with different PTM levels. Mantids bioaccumulated different PTMs than those bioaccumulated by crickets overall. Although some differences were observed regarding mantid developmental duration in both trials, they were non-significant and therefore probably driven by other factors. Ultimately, the concentrations of PTMs in wild and crop plant ecosystem models did not reflect soil PTM concentrations. Thus, PTM bioaccumulation and subsequently availability of the next trophic level seemed to rather be a function of the biology of the trophic level than of soil content to which they are exposed.

Assessing the effects of the invasive house mouse on the flora of Marion Island

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The house mouse (*Mus musculus*) is one of the most widespread, and harmful invaders of ecosystems worldwide, severely impacting the biodiversity of areas it invades. The house mouse has been introduced to several sub-Antarctic islands, including Marion Island, where it has detrimental impacts on the indigenous biota of the island. In comparison to Marion Island, the neighbouring Prince Edward Island has not been invaded by mice and provides a unique opportunity for assessing the ecological impacts of invasive species on the biodiversity of isolated ecosystems. Using detailed vegetation plot data, this study aimed to assess the ecological effects of the invasive house mouse on the vegetation of Marion Island compared to the mouse-free Prince Edward Island. Vascular species richness does not differ between the two islands; however, vascular plant cover is significantly higher on Prince Edward Island compared to Marion Island. Indeed, vascular species that are consumed by mice (e.g. *Carex dikei* and *Acaena magellanica*) or are affected by mouse burrowing (e.g. *Azorella selago*) have a lower cover on Marion Island than on Prince Edward Island. These results highlight the profound impact of invasive mice on vegetation, potentially reshaping plant communities, and thereby reinforcing the importance of invasive species as a major driver of ecosystem change.

Quantifying the effects of multiple disturbances on woody plant cover in a Marula-Knobthorn savanna

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Savannas are particularly susceptible to a rapidly changing climate, with woody plant densification and biome shifts occurring over a period of decades being documented globally. The National Department of Science & Innovation has identified the savanna biome as a research priority under the Global Change Grand Challenge. Current biodiversity conservation concerns in the Kruger National Park (KNP) include changes in woody plant cover, particularly the loss of large trees. This study investigates the single and interactive impacts of fire, herbivory, and drought on woody plant cover in KNP. Remote sensing and Geographic Information System techniques were employed to quantify woody plant dynamics in response to these stressors. The study utilised existing replicated, long-term fire manipulation trials and browser exclusion plots in Marula-Knobthorn savanna to determine woody plant density pre- (2015) and post- (2018) extreme drought. MultispectralSPOT6/7 pansharpened 1.5m resolution imagery sourced from the South Africa National Space Agency was processed using supervised classification models to categorize vegetation classes (woody vs. non-woody) and quantify change in cover over time. These changes were interpreted against species-level tree community data collected in 2016. The classification study results show a decline in woody cover across the fire manipulation trials. The annual fire trial indicates a significant decrease in woody plant cover, followed by the triennial fire trial, with the unburned trial showing the least decrease post-drought. Areas open to browsing show a significant reduction in woody cover which is attributed primarily to elephant-induced damage. Additionally, the data suggest that these elephant impacts are exacerbated during drought. Managing fire frequency and herbivore density are key tools for controlling woody vegetation in savannas. While their individual impacts are well understood, it is crucial to comprehend the interactive effects of multiple factors, as shown here, for effective management of woody plant density and savanna integrity.

The comprehensive assessment of plant species richness and habitat distribution in Cape Inland Salt Pans

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Cape Inland Salt Pans (Azi 9) are characterized by saline flats that serve as vital habitats, supporting uniquely adapted flora and fauna while attracting migratory birds. Azi 9 are unique to other inland salt pans as they are remnants of ancient paleolakes, shaped by climatic and geological processes over millennia, resulting in distinct soil chemistry and hydrological dynamics. Despite their ecological significance, these salt pans remain understudied, particularly in terms of plant species richness and habitat distribution. This study aimed to assess the plant diversity and habitat distribution associated with Azi 9 in South Africa. All known salt pans were visited throughout its distribution along the southeast, south and west coast, where species occurrences were georeferenced, and voucher specimens collected. Habitat composition was mapped using ArcGIS Pro from 2023 basemaps and South African land cover classes. A total of 34 plant species from 13 families were recorded, with Amaranthaceae and Asteraceae being the most abundant (7 species each). Rocherpan and Redhouse had the highest species richness (15 species) whereas Soetendalsvlei had the lowest species richness (5 species). The species *Salicornia meyeriana* occurred in 80 % of salt pans.

Species closer to the edges of salt pans also showed affinities to neighbouring terrestrial vegetation. Habitat mapping identified 59 CISPs, covering a total area of 1,924 hectares, with open water and vegetation as the dominant habitat types. These findings highlight the ecological heterogeneity of South African salt pans and provide crucial baseline data for conservation management. Given the increasing environmental pressures on these fragile ecosystems, further research is necessary to support their long-term preservation.

The South African Journal of Botany's (SAJB) contribution to botanical research in southern Africa: Bibliometric analysis

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This study presents a bibliometric analysis of the *South African Journal of Botany* (SAJB), evaluating its impact on botanical research in southern Africa from 2000–2025. Using a systematic literature search in the Web of Science database, a total of 5 812 relevant publications were identified and analyzed using Bibliometrix (R). The study examined publication trends, citation impact, authorship patterns, institutional contributions, and key research themes. Findings indicate an annual growth rate of 8.52%, with an average of 13.45 citations per document. Notable contributors include the University of KwaZulu-Natal. The study also highlights dominant research themes such as antioxidant studies, cytotoxicity, and taxonomy. Co-occurrence and network analysis reveal strong interdisciplinary collaborations and international partnerships, with 29.59% of publications involving cross-border research. This bibliometric review provides valuable insights into the evolution of botanical research in southern Africa and the role of SAJB in shaping scientific discourse in the region.

Using radiocarbon dating to assess maximum longevity in the arborescent monocot, *Aloidendron dichotomum* (Masson) Klopper & Gideon F.Sm.

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This study used radiocarbon dating to investigate the maximum longevity of the quiver tree (*Aloidendron dichotomum* (Masson) Klopper & Gideon F.Sm.) at 4 sites in the north of its range in Namibia. We found not only a remarkable extension to previous estimates of longevity of the species, but also a complex model of growth deviating unexpectedly from the typical radial expansion of dicotyledonous trees. Samples of dead quiver tree were taken in series from the core to the periphery of four trees, non-structural carbon removed chemically, and sample ages determined through radiocarbon dating using an accelerator mass spectrometer at the NRF iThemba labs in Pretoria. A range of possible ages was obtained for each sample. An oldest possible calibrated sample age of 746 years (recruited 1277-1452 cal yr BC/AD) was obtained for the tree at Blutkuppe in the northern Namib desert. A radial model of growth was not supported, as the ages of the stem samples from the centre and periphery were similarly young, while ages in between these positions were older in all trees sampled. This suggests material turnover at the core of the trees throughout their life, unlike in dicotyledonous trees, resulting in the loss of the oldest stem material. Maximum age was thus extrapolated using bayesian age-depth modelling selecting the most likely sample age of each sample which did not deviate from an assumed monotonic decline in age from center to periphery. While there is large uncertainty in these models around the oldest age due to exclusion of central samples, these models estimate the oldest age of the quiver tree found at Blutkuppe to be approximately 701 years old - double the age of previous estimates of longevity for this species. Despite the uncertainty, it can be assumed that errors in our assumptions would be biased towards an age under-estimation. This method of age estimation greatly improves the accuracy of population demographic models of this long-lived, slow-growing species which faces multiple compounding existential threats.

Ecological impact of *Leucosidea sericea* encroachment in high-altitude grassland of the Eastern Cape, South Africa

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Woody plant encroachment poses one of the most significant threats to biodiversity loss worldwide, with pronounced impacts in grasslands. *Leucosidea sericea*, a woody shrub or small tree native to southern Africa, is rapidly encroaching high-altitude grasslands potentially altering ecosystem functioning and community livelihoods. The aim of this study was to determine the ecological impact of *L. sericea* encroachment in the Vuvu communal area, Eastern Cape, South Africa. The impact of *L. sericea* was determined using community perceptions, field observations, and measurement of grazing capacity, species diversity assessments, and soil physicochemical analysis. Results highlighted that local perceptions are valuable in understanding the impacts of encroachment on rangelands. Community perceptions indicated that the main reasons for the spread of *L. sericea* were livestock movement, wind dispersal, and cessation of its use as firewood. Contrary to the perceptions, the findings of the study indicated that *L. sericea* encroachment may not reduce biodiversity or alter soil nutrient levels. However, *L. sericea* was associated with a noticeable reduction in grazing capacity, underscoring the economic effects of woody plants on livestock farming. The absence of *L. sericea* seeds in the soil seed bank was particularly intriguing, raising questions about its regeneration strategies and the conditions necessary for its germination. These findings highlight the unique ecological dynamics of *L. sericea* encroachment, where community perceptions and ecological field data provide contrasting but complementary insights, emphasising the need for integrated approaches to sustainably manage encroached rangelands. Further research is required to fully understand the regeneration mechanisms and long-term ecological implications of *L. sericea* in grasslands.

Kindling polyploidy: Experimental evidence that fire compounds induce diploid gametes in a geophyte

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Polyploidy is a key evolutionary process, yet the pathways through which it originates remain poorly understood. Recent research has proposed fire as a potential driver of recurrent polyploid formation, possibly through the mutagenic effects of heat and/or fire-derived chemicals in smoke and post-fire debris. While mechanistically plausible, this hypothesis requires empirical testing under ecologically relevant conditions. This study investigated whether fire promotes diploid (unreduced) gamete formation—an essential step in sexual polyploidization—in three varieties of *Rhodohypoxis baurii* (Baker) Nel. Flow cytometry was used to confirm diploidy in adult individuals, which were planted into five experimental units. Each unit contained 24 rhizomes (eight per variety) in modified stainless-steel bain-marie inserts, using sterilized potting mix and standardized planting depth. To assess the effects of fire, one unit was exposed to a controlled surface burn using *in situ* vegetation, with temperatures monitored via an infrared thermometer and high-temperature iButtons. To isolate the effects of heat, a second unit was divided into zones and sequentially heated to ~200 °C using an infrared heater. A third unit was exposed to cool smoke for 15 minutes using a smoke box, followed by application of cooled post-fire debris. A fourth unit was treated with acenaphthene, a fire product known to induce unreduced gametes. The fifth unit served as a negative control. Pollen diameter was assessed by fixing anthers in Carnoy's solution, staining pollen with modified Alexander's stain, and quantifying the size of viable grains using microscope images and ImageJ software. All treatments resulted in elevated unreduced gamete formation relative to the control, but only the cool smoke and acenaphthene treatments produced statistically significant increases. These findings provide the first empirical evidence that fire-released compounds can promote diploid gamete formation and suggest a novel ecological mechanism linking fire exposure to polyploid evolution.

Effects of mechanical control on *Pteridium aquilinum* and restoration of Woodbush Granite Grassland

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Pteridium aquilinum, commonly known as brackenfern, is an aggressive, opportunistic and resilient invader of grasslands and abandoned rangelands. Underground storage organs and rapid regrowth make bracken highly competitive for resource acquisition. Frond cover eventually shades out other vegetation, while its allelopathic exudates inhibit the germination of co-occurring plant species. This alters community composition and structure, posing significant ecological and management challenges. Bracken's dominance makes it difficult to eradicate, and there is limited knowledge of effective control methods. Current management strategies involve repeated slashing or herbicide use. This study investigated the effectiveness of mechanical clearing of bracken and biodiversity recovery in the threatened Woodbush Granite Grassland of South Africa. The study was conducted in the Swartbos grassland remnant (Magoebaskloof, Limpopo), comparing three site conditions: (1) bracken invaded areas, (2) areas where bracken has been mechanically removed, and (3) areas where bracken was absent. Field surveys were made with transect and quadrat sampling to measure frond density, aboveground biomass and species diversity, while germination studies and seedling pot trials examined the effects of allelopathy and shading on grassland species. Preliminary results indicate that bracken fern invasion significantly reduces species diversity, with diversity decreasing as frond density increases. Areas where bracken was cleared show signs of grassland diversity recovery, suggesting that mechanical removal may promote biodiversity restoration.
Allelopathy/Shading/Combination did not inhibit seed germination of grassland species but led to seedling deaths. These findings contribute to a better understanding of bracken invasion and management strategies for invaded grassland ecosystems.

Bud bank dynamics under fire-herbivory disturbances drive African savanna resilience

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Savanna ecosystems are important for carbon sequestration, biodiversity and sustaining livelihoods. Increasing disturbance intensities (e.g., fire frequency and herbivory pressure) due to a changing climate are amongst the largest threats to the sustainability of ecosystem services provided by savannas. Their ability to cope with recurrent aboveground biomass removal depends on their belowground storage and resprouting capacity. This study aimed to evaluate the individual and interaction effects of fire frequency and herbivory pressure on belowground bud bank traits in forbs and grasses. We hypothesized that different fire and herbivory treatments would differentially alter bud bank density and structural composition (e.g., rhizomes vs. root crowns) between these plant functional groups (grass and forb), reflecting their contrasting life history strategies. In each treatment: 10 plots (0.5 x 0.5 x 0.1 m) were erected across the different fire and herbivory treatments, and samples were collected. High fire frequency with herbivores had the highest bud bank density, whereas herbivores reduced bud bank density at lower fire frequencies. Grass and forb bud bank density responded differently to different treatments. Annual burns with herbivores caused a decline in the non-woody rhizome but promoted stoloniferous types. Triennial burns without herbivores supported bulbs. The results indicate that fire-herbivory interactions drive divergent bud bank strategies: grasses prioritize density (resistance), and forbs shift organs (resilience). These adaptive responses underpin savanna resilience, balancing recovery and persistence amid changing disturbance regimes. Managing fire-herbivory dynamics could stabilize ecosystem resilience.

Restoring old-growth grasslands with geophytes seems possible, but remains species-specific

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Natural recovery of floristic and functional diversity of old-growth grasslands after exogenous disturbances remains elusive. The need for trait-based approaches in grassland restoration has thus been highlighted. Grassland geophytes are considered potential candidates for trait-based restoration practices due to their belowground storage and resprouting capabilities, which allow them to persist in disturbance-prone ecosystems. Restoring old-growth grasslands with geophytic species requires a thorough understanding of their development before their establishment on disturbed soil can be maximised. Our study assessed germination success, survival and growth (i.e. development) of three native grassland geophyte species. Recalcitrant seeds of Amaryllidaceae species *Crinum bulbispermum*, *Haemanthus montanus*, and *Nerine krigei* were harvested from wild populations in dolomitic grasslands and sown in three soil treatments (i.e. non-transformed dolomite grassland soil, previously ploughed grassland soil and organic compost). Seedling emergence and productivity were recorded over 12 months in a greenhouse. Results indicated varying germination capacity, survival, and growth among the species, with the highest performance across treatments reported for *C. bulbispermum* and the lowest for *H. montanus*. In a subsequent field trial, the then 2-year-old individuals were transplanted directly from soil plugs into (i) a natural dolomitic grassland site, and (ii) an adjacent previously ploughed site to assess the establishment of seedlings from varying soil sources. Plants raised in and transplanted to dolomitic soil exhibited the highest survival of *N. krigei*, followed by *H. montanus*. *Crinum bulbispermum* survived best when raised in and transplanted to previously ploughed soil. *Haemanthus montanus* had the lowest seedling survival when dolomite soil plugs were transplanted in previously ploughed sites. *Nerine krigei* experienced a decline in productivity over time, thus casting doubt on its long-term suitability for restoring previously ploughed grasslands. Our results accentuate the complexities of grassland restoration, advocating a nuanced species-specific approach in grassland restoration.

Subtropical grassland root-to-shoot ratios and carbon estimates: why global averages fall short

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Grasslands are incredibly diverse ancient ecosystems that are often underestimated in their capacity to increase global carbon reserves. This paper aims to first evaluate the accuracy of the average root-to-shoot

(R:S) ratio in subtropical grasslands of South Africa, as a result of which the widely used 0.45 carbon conversion factor for subtropical grasslands will be evaluated based on actual data and derived data from the R:S ratio. These values will challenge the accuracy of global averages and standard conversion factors used for subtropical grasslands. Data collection occurred across various old-growth grassland (OGG) sites in two rainfall regions (>650 mm and <650 mm annually). Field sampling was conducted at 20 sites, where above- and belowground biomass was harvested, dried, weighed and analysed for R:S and organic carbon content in the laboratory. Belowground biomass was sampled to a depth of 20 cm, acknowledging that plant organic carbon may extend beyond this depth, which could lead to an underestimation of the total belowground carbon stock that we calculate. Our results show substantial variability in R:S ratios in the South African subtropical grasslands across the rainfall regions. The mean R:S ratio is 1: 15, which is about three times higher than the published average of approximately 1: 4.6. The R:S ratios were higher in wetter regions, with (1:19.6 ± 2.25). These findings challenge the R:S ratios that have been widely used for subtropical grasslands. Furthermore, the carbon conversion factor of 0.45 overestimated the plant organic carbon content in both aboveground and belowground biomass in subtropical grasslands. This discrepancy suggests that using a universal conversion factor may misrepresent carbon stock estimates. The high variability in the R:S ratios and the overestimation of the carbon factor highlight the limitation of applying global averages to local ecosystems. These findings have important implications for improving carbon modelling and land management strategies used in subtropical grassland ecosystems.

Fire frequency effects on germinability of grasses and forbs in a semi-arid Savanna

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Studies on soil seed banks in response to fire frequency in African savannas are limited. We assessed the impacts of three fire frequencies (annual, triennial, and fire exclusion) on seedling emergence from soil seed banks collected in a semi-arid savanna at the Satara experimental burn plots in central Kruger National Park. Results showed that frequent (annual) fire depleted the soil seed bank relative to less frequent fire treatments. Further, fire frequency influenced the grass-to-forb emergence ratio, with frequent fire resulting in the highest grass-to-forb ratio opposed to the lowest values observed in the fire exclusion treatment. Fire frequency had no significant effect on the richness of emerging grass species, although forb species richness increased with decreasing fire frequencies. The effects of fire frequency on seed viability were not significant and appeared to be highly species specific. Dormancy tests indicated that most species exhibited dormancy traits; forb seeds typically exhibited physical dormancy and grass seeds physiological dormancy. Germination of grass seed was higher than forbs after the application of different fire-related dormancy breaking treatments (i.e. heat shock and smoke/ash water exposure). Fire-related dormancy-breaking treatments failed to break physical dormancy observed in several forb species, particularly forbs from the Fabaceae and Malvaceae. Our results suggest that fire-related dormancy-breaking cues are suitable to enhance germination success in grasses, but not in forbs. Forb seeds with physical dormancy may require alternative mechanisms e.g. mechanical scarification, which implies herbivory-related dormancy-breaking cues. Our study provides insight into how fire frequency affects sexual recruitment attributes, which partly structure above-ground plant communities. The role of seedbanks in biodiversity conservation should therefore be considered.

Do nutrient poor soils select against large genome sizes?

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Genome size varies widely among plant species and is linked to key ecological and evolutionary traits. While genome size can differ across species and functional groups, it also varies within species, particularly through polyploidy, the condition of having more than two sets of chromosomes. Globally, both genome size and polyploid distribution are shaped by climate and latitude, with soil nutrients such as nitrogen and phosphorus thought to play an important role. Given that nitrogen and phosphorus are key structural elements in nucleic acids, plants with larger genomes should require higher levels of these nutrients to support their larger genomes. Consequently, nutrient-limited environments may impose selective pressures against organisms with large genomes, potentially constraining their establishment. However, the extent to which soil nutrients shape genome size distribution, especially at a local scale, remains unclear. This study tested whether soil nutrients predict genome size or ploidy level at two taxonomic scales. At a broader taxonomic scale, it was tested whether habitats with limited soil nutrients comprised species with small genome sizes. Genome size was measured for a range of perennial herbs, grasses, sedges, geophytes, and dwarf shrubs collected at three locations at Sentinel Peak. Across all models, none of the environmental variables (N, P, K, elevation) or functional group categories predicted genome size. This suggests that genome size variation in this dataset is not driven by the tested soil nutrients. Then to test the effect of nutrient limitation of ploidy level within a species, all known populations of *Hypoxis parvula var. parvula*, a native geophyte were sampled. Generalised linear mixed models showed that within species, polyploidy was positively associated with low nitrogen and phosphorus, suggesting that nutrient poor soils do not select against larger genomes. Collectively, these data suggest that factors other than soil nutrients may be responsible for shaping broader genome size distributions in the Drakensberg.

Over half of the phylogenetic diversity accumulated on the African plant tree of life may be eroded under current biodiversity crisis

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Although Africa contributes tremendously to global biodiversity, we have a poor understanding of how the African tree of life might be pruned owing to the ongoing biodiversity crisis. Here, we investigated this question integrating statistics and phylogenetics of ~24000 African vascular plants. We found that 54% of African plant families are hot nodes of threatened species, thus heightening the risk of losing entire clades. We also found that, if all threatened species go extinct, 59% of the evolutionary history of the African plant tree of life would be eroded, and this loss is more than expected at random, although threatened species are not evolutionarily unique. Unfortunately, ~72% of threatened species and ~79% of the top-1000-EDGE species are not found in any form of protected areas. Overall, our analysis reveals the extent of biodiversity crisis in Africa and the need for steadfast commitments to i) increased data collection efforts particularly in central African regions and ii) implementation of existing policy for an accelerated recovery.

Environmental heterogeneity as a driver of species and phylogenetic diversity in coastal salt marshes

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Studying the relationship between species diversity, phylogenetic diversity and environmental heterogeneity in coastal salt marshes will provide a more holistic understanding of the structure and functioning of these important habitats. This study aimed to determine how environmental heterogeneity drives species and phylogenetic diversity in coastal salt marshes. In eight estuaries along the South African coast, species composition, sediment and groundwater characteristics were measured. To determine phylogenetic diversity indices of transects and estuaries phylogenetic analysis was conducted. Environmental heterogeneity (EH) for each estuary was determined by Principal Component Analysis (PCA). To determine the influence of EH on macrophyte species richness, species diversity and phylogenetic diversity, a generalized linear model (GLM) was applied to identify significant variables. Results indicated a significant positive relationship between EH and species richness ($t = 2.19$; $p = 0.03$). Species richness significantly correlated with mean sediment moisture content ($t = 3.09$; $r^2 = 0.20$; $p = 0.00$). Simpson diversity showed a significant negative relationship with sediment electrical conductivity ($t = -2.86$; $r^2 = 0.16$; $p = 0.01$). There was a negative correlation between phylogenetic diversity and elevation (MPD: $t = -3.73$; $r^2 = 0.30$; $p = 0.00$) and sediment redox potential (MNTD: $t = -2.05$ $r^2 = 0.1$; $p = 0.05$). This suggests that elevation and the variation of the physicochemical variables are important predictors of salt marsh biodiversity. However, the greatest species diversity may arise not solely from larger salt marshes but from increased variation in elevation. It is expected that salt marshes along the west coast will experience the greatest loss of species richness and diversity under anticipated climate change because of lower rainfall and drier conditions. Restoration of salt marshes should be focused on the creation of habitat mosaics, where multiple habitat types are created along an elevation gradient.

Evolution and Systematics (EVOL&SYST)

Historical biogeography of *Acridocarpus* (Malpighiaceae) evidence the Miocene origins of Malagasy savannas

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Acridocarpus represents one of the seven past dispersal events from the Neotropics to the Paleotropical region in Malpighiaceae, being by far the most widely diversified and distributed genus of the family in Africa. We dated and calibrated a molecular phylogeny to reconstruct ancestral range distributions for biomes and continents. We sampled six genes, 21 species of *Acridocarpus* and three species of outgroups to test the monophyly of the infrageneric classification of the genus. Bayesian inference and Maximum Likelihood analyses were performed for the combined molecular dataset. Calibration points derived from a published chronogram for Malpighiaceae were used alongside a relaxed, uncorrelated molecular clock. Ancestral areas of *Acridocarpus* and its relatives were estimated for continental (South America, Africa, India, Madagascar, and New Caledonia) and biome (dry forests, humid forests, and savannas) ranges. The most recent common ancestor of *Acridocarpus* arose in the rainforests of East Africa + Madagascar 43.0 Ma and greatly diversified in this region, with a single long-distance dispersal event from Madagascar to New Caledonia (Oceania). The genus colonised African dry forests at least four different times, starting in the Oligocene and diversified a single time in Malagasy savannas throughout the Miocene. Different dated phylogenies corroborate the Miocene origin of Malagasy savannas on several lineages of woody plants native to these grassy ecosystems.

Molecular systematics of the endemic tree genus *Greyia* – novel DNA barcodes.

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The tree genus *Greyia* is endemic to southern Africa with three species described in the late 1800s. The Eastern Cape species *Greyia flanaganii* is confined to a limited range west of the Kei River. *Greyia radlkoferi* occurs in Limpopo, whereas *Greyia sutherlandii* is associated with KwaZulu-Natal, but the ranges of these two species overlap in Mpumalanga. They provide important ecosystem services along the Great Escarpment. *Greyia sutherlandii* leaf extracts have lower anti-tyrosinase activity used in herbal formulations for skin hyper-pigmentation, making reliable species identification important. Field observations report overlap in morphology (woolly vs glabrous leaves; compact vs. elongated inflorescences) for the latter two species. As a first step in resolving the molecular systematics of the genus *Greyia*, we sampled five trees from each species' centre of distribution, ensuring that morphology matched type specimen descriptions. Phylogenetic analysis with ITS, *trn*LF, *mat*K and *psb*A-*trn*H indicated (i) differentiation from other lineages in the order Geraniales, but (ii) poor discrimination between *Greyia* species, as these barcodes have two or less polymorphisms. To address this problem, a reduced-representation genome sequencing method, 3RAD, was applied to selected trees of each species.

Bioinformatics analysis filtered 47,727 SNPs down to 23 species-diagnostic SNPs. This was converted to a SNP assay on the microfluidics Biomark^{TM} HD platform and applied to 73 wild-growing *Greyia* trees across the distribution range. Genetic clustering analyses (PCA, UPGMA and ADMIXTURE) of the 23-SNP data differentiated most of the trees into the three species groups with high confidence. Furthermore, the assay identified 33 trees of unknown provenance in *Greyia* orchards grown for medicinal activity. This work (i) outlines a novel plant DNA marker discovery strategy; (ii) provides a useful 23-SNP assay for *Greyia* biodiversity conservation; and (iii) lays the groundwork for phylogenomics with genome-wide SNP markers to resolve the molecular systematics of the genus *Greyia*.

Systematic studies of *Argyrolobium* (Genisteae, Fabaceae)

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Argyrolobium Eckl. & Zeyh. is a genus in the tribe Genisteae (Fabaceae), comprising ca. 97 species distributed throughout tropical and northern Africa, Madagascar, the Mediterranean, southern Europe, and west into the highlands of India. The main centre of diversity for the genus is in southern Africa, where about 51 species occur. Previous molecular studies, based on a limited sampling of the genus, have indicated that the genus may not be monophyletic and that the generic concept needs to be revised. In addition, the South African species (47) have only been treated synoptically in recent years, based on an unpublished Ph.D. thesis, and the last published comprehensive taxonomic revision of the southern African species dates back to 1862. The present study is aimed at producing morphological and molecular data for *Argyrolobium* that will contribute towards resolving the generic circumscription and relationships to ultimately inform a revised taxonomy of the genus. It will also inform much needed future revisionary work on the genus. Phylogenetic analyses based on nuclear and plastid DNA sequences confirm the results of previous studies that *Argyrolobium* is not monophyletic. While a large clade of southern African, tropical African and Malagasy species is recovered, the remaining tropical African, Middle Eastern, and Eurasian species form at least three separate clades distributed among other genera of the Genisteae, indicating a need for a revised generic circumscription. An overview of the taxonomy of the genus will also be presented, including informal groups recognized as working groups for taxonomic studies, and also results from revisions already completed.

Toward a revision of the genus *Indigofera* in South Africa – six years of progress

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Indigofera L. is the third largest genus in the Fabaceae, with over 750 species distributed across much of the globe. South Africa has the highest diversity of *Indigofera* species, with over 300 species estimated. The Greater Cape Floristic Region (GCFR) is home to over 140 species, most of which belong to the Cape Clade, one of four globally recognized clades in the genus, and the only clade that has remained endemic to Africa. The summer rainfall region (SRR) is home to over 160 species, predominantly from the Tethyan, Pantropical and Paleotropical Clades. The two regions have long histories of taxonomic studies, but *Indigofera* has been neglected, with the last revision of GCFR species being in Flora Capensis (Harvey, 1862), while the SRR species were last revised by Gillett (1958). Decades of herbarium research by Schrire identified about 100 undescribed species from South Africa, laying the foundation for this multidisciplinary study. The past five years have included fieldwork in both regions, resulting in the collection of roughly 90% of *Indigofera* species in South Africa, which has further expanded our knowledge on new species and the role of habitat specificity that have resulted in the high species diversity. Taxonomic work in the GCFR is nearing completion, with three taxonomic revisions already published, and several more in preparation, resulting in the description of about 58 new species. A biogeographical study of the genus found that *Indigofera* originated in Africa, with multiple "Out-of-Africa" dispersal events during the Miocene leading to the colonization of all continents except Europe and Antarctica. New high-throughput phylogenomic sequencing has allowed for a detailed study of habitat preference and localized biogeography of GCFR *Indigofera*, and will play a crucial role going forward in understanding the evolutionary drivers that led to the radiation of the genus in South Africa and beyond.

The systematics of *Nemesia* (Scrophulariaceae)

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The genus *Nemesia* Vent. (Scrophulariaceae) comprises about 77 species, the majority of which are endemic to South Africa. *Nemesia* has garnered significant horticultural interest due to its diverse floral shapes, vibrant colours and ease of hybridization and cultivation. The genus was last revised in 1904 by Hiern and the only molecular phylogeny for *Nemesia* was published in 2008 by Datson and others. Datson *et al*'s phylogeny grouped the 23 included species into five distinct clades highlighting evolutionary relationships and potential adaptive traits. Their ancestral state reconstruction suggested that multiple transitions occurred between a perennial life history in species occurring in the summer rainfall areas to an annual life form in species adapting to winter rainfall or seasonally dry conditions in the Cape Floristic Region and parts of the Karoo. It was hypothesised that *Nemesia*'s diversification may have been driven by climate change in the Cape Region during the Miocene and Pliocene. Our research aimed to generate a more comprehensive phylogeny incorporating as many *Nemesia* species as possible, to deepen understanding of the genus's evolutionary history. This phylogeny was generated from gene regions ITS, ETS and trn*L*-intron, representing 48 species. The phylogeny is divided into two main clades, with five subclades that show significant geographic affiliations among species within clades. In this paper we review the phylogeographic patterns and morphological variation within the five clades of *Nemesia.* The phylogeny also contains several newly discovered, but as yet, undescribed species and their relationships to known species are explored.

New additions to the *Nemesia* lineage from the Succulent Karoo

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The genus *Nemesia* (Scrophulariaceae) is one of the most diverse in the Hemimerideae tribe, comprising approximately 77 species, with nearly all endemic to southern Africa. Three species have been recorded in Zimbabwe, but none are endemic to that country. Eleven species occur in Namibia, of which at least five are endemic or near-endemic. South Africa is home to most *Nemesia* species, with their centre of diversity located in the Cape Floristic Region (CFR) and Succulent Karoo. Between 1994 and 2010 Steiner published several new species, the majority primarily endemic to the winter rainfall region of South Africa. In his contribution to *Strelitzia 30*, Steiner published several brief, informal descriptions of new *Nemesia* species, the majority of which have never been formally described or named. During a taxonomic revision and phylogenetic analysis of *Nemesia* it became clear that the winter rainfall region of South Africa is likely to produce yet more new species of this genus. Here we review the morphology and phylogenetic relationships of *Nemesia* sp. E, as described by Steiner, and compare it to already known and putative new species from the Succulent Karoo.

Genetic diversity and population structure of *Strychnos madagascariensis*: Insights for conservation and sustainable utilization

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Strychnos madagascariensis Poir. is a highly valued indigenous fruit tree in South Africa with potential for domestication and commercialization. However, no study has reported the molecular diversity of *S. madagascariensis* morphotypes. This study assessed the genetic diversity among *S. madagascariensis* morphotypes using simple sequence repeat (SSR) markers. Newly sprouted leaves from 27 *S. madagascariensis* morphotypes were used for DNA extraction. Genotyping was performed using 17 SSR markers, followed by polymerase chain reaction (PCR) amplification and capillary electrophoresis for allele size determination. Genetic diversity and population structure were analyzed using PowerMarker, Cervus, STRUCTURE, GenAlEx, and XLSTAT software, revealing genetic variation and relationships among morphotypes. Seventeen amplified SSR markers produced 144 alleles, with a mean of 4.28 per locus. The polymorphic information content (PIC) values ranged from 0.00 to 0.72. The population structure had the highest delta value K = 2, thus dividing morphotypes into two subpopulations based on a Bayesian approach. Morphotypes were clustered according to genetic distance and phylogenetic relationship in the principal coordinate analysis where eight distinct sub-clusters were formed. The dendrogram based on genetic distance classified the morphotypes into two major clusters, where Cluster II was further subdivided into two sub-clusters. This study is the first to report on SSR marker development and their successful use for genetic diversity and population structure studies of *Strychnos madagascariensis*. These findings offer practical insights and understanding of the genetic diversity among various *S. madagascariensis* morphotypes, which could be beneficial for breeding programs and conservation efforts on behalf of this valuable species.

Lineage diversification and long-term preservation in the Greater Cape Floristic Region

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The Greater Cape Floristic Region (GCFR) is a globally recognized biodiversity hotspot, distinguished by its extraordinary species richness and fine-scale beta diversity. This unique flora has intrigued scientists for centuries, with numerous studies investigating the processes driving lineage diversification. Key mechanisms shaping this diversity include environmental heterogeneity, periodic fire, pollinator-driven divergence, range-limited dispersal, and phenological shifts. Another potentially significant yet underexplored driver is the impact of sea-level fluctuations during the Pleistocene (2.5 Ma – 11.7 ka) which likely played a crucial role in recent divergence of coastal specialist taxa endemic to coastal calcareous substrates. During glacial periods, lower sea levels expanded the GCFR's land area, exposing vast areas of calcareous substrata along the Palaeo-Agulhas Plain. Contemporary sea levels are approximately 130 m higher than at the Last Glacial Maximum, leaving modern coastal substrates spatially fragmented and significantly reduced in area. Coastal specialists endemic to these substrates consequently exhibit highly fragmented distributions. These fragmented distributions likely present barriers to gene flow, especially for species with limited dispersal abilities, as in the Cape-centred *Stoebe* daisies (Gnaphalieae: Asteraceae). In this study, we examine patterns of population genetic differentiation in eight *Stoebe* species to assess whether barriers presented by geographic isolation are driving incipient speciation. Phylogenetic reconstruction indicates coastal endemism has evolved at least three times independently within *Stoebe*, rendering the genus ideal for our study. Using genome-wide Single Nucleotide Polymorphism markers retrieved through Genotyping-by-Sequencing, we quantify genetic differentiation among multiple populations for coastal specialist and non-specialist *Stoebe* species using F_{st}, private alleles, and patterns of isolation-by-distance. Preliminary analyses reveal greater genetic diversity among coastal endemic lineages, suggesting that historic sea-level fluctuations may have contributed to their diversification. Our work is the first to assess Pleistocene marine transgression/regression effects on lineage diversification among Cape plants using genome-wide DNA markers. Our findings offer novel insights into the evolutionary processes underlying the assembly of the megadiverse Cape flora.

Historical biogeography of *Dianthus* (Caryophyllaceae) reveals several colonization events to Africa from a European ancestor

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Dianthus (Caryophyllaceae) is a diverse genus distributed across Europe, Asia, and Africa. To reconstruct its evolutionary and biogeographic history, we conducted a molecular phylogenetic analysis using nuclear ribosomal ITS and three plastid markers (*trnH-psbA*, *trnK-matK*, and *trnK-psbA*). We further inferred ancestral range distributions and colonization events using Reconstruct Ancestral State in Phylogenies (RASP). Our phylogenetic analysis supports a European origin for *Dianthus*, with clade 97 representing the most recent common ancestor (MRCA) of the genus, ca. 13 Ma. The first major divergence consists exclusively of European species, followed by a clade that also retains a European distribution. Clade 94 marks an important diversification event, giving rise to multiple lineages, including those that later colonized Africa. Biogeographic reconstructions reveal multiple independent colonization events from Europe to Africa. The first African lineage is represented by *Dianthus transvaalensis*, marking the genus's earliest dispersal to the continent. This was followed by three successive colonization events: clade 110, clade 109, clade 108, and finally, clade 144, which represents the most recent African radiation. Several recently evolved African taxa (*Dianthus cyr*, *D. kamisbergensis*, *D. serratifolius*, *D. pungens*, *D. cintrinus*, *D. serrulatus*, and *D. basuticus*) suggest ongoing diversification within Africa. The results indicate that *Dianthus* species reached Africa through multiple long-distance dispersals from Europe rather than a single colonization event, followed by *in situ* diversification starting in the Miocene. Additionally, the presence of a European recent common ancestor for both the ingroup and outgroup reinforces a shared evolutionary lineage within the genus. This study highlights the role of long-distance dispersal and repeated colonization events in shaping the current distribution of *Dianthus* and provides new insights into plant migration dynamics between Europe and Africa.

A phylogenomic study of southern African Members of *Silene* Section *Elisanthe* (Caryophyllaceae)

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Silene section *Elisanthe* (Caryophyllaceae) is represented in southern Africa by perennial herbaceous endemic species. The most recent taxonomic treatment recognized five taxa *viz*. *S. ornata*, *S. rigens*, *S. saldanhensis*, *S. undulata* subsp. *undulata* and *S. undulata* subsp. *polyantha*. Three (*S. ornata*, *S. rigens*, *S. saldanhensis*) of the five taxa are endemic to the Core Cape Subregion of South Africa, while *S. undulata* subsp. *polyantha* is restricted to the KwaZulu Natal–Swaziland region and *S. undulata* subsp. *undulata* is widely distributed across southern Africa. Although the phylogenetic position of the southern African section *Elisanthe* has recently been clarified, the species relationships within the section have been unclear. This study aims to reconstruct the phylogenetic relationships of southern African members of section *Elisanthe* focusing on inferring a species-level phylogeny and testing the current taxonomic classification. To achieve this, we collected tissue from multiple specimens of the currently recognized taxa across southern Africa. We generated target sequence capture data using custom taxon-specific baits targetting low copy nuclear genes together with ITS and *rps16*. We employed a Bayesian species-tree inference method to estimate the phylogenetic relationships under the multispecies coalescent model framework. Our results show that the Saldanha Bay endemic species *S. ornata* and *S. saldanhensis* are monophyletic sister species. This clade is subsequently sister to the monophyletic West Coast endemic *S. rigens*. The STACEY analysis recovered *Silene undulata* as monophyletic, and in most respects topologically in agreement with the unrooted ASTRAL-III tree. Our results show that the relationships in *Silene* section *Elisanthe* are strongly correlated to geographical distribution. Our analyses do not support the intraspecific classification of the widespread *S. undulata* but rather highlight that the taxon is diverse, and highlight the need for further investigation to better understand the intraspecific patterns observed in our results as well as other recent taxonomic studies.

An exploration of the abiotic and biotic drivers associated with geographic structuring of floral colour variation in *Pauridia capensis*

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Pauridia capensis (Hypoxidaceae) demonstrates remarkable floral colour variation at the population level, with three distinct forms occurring across the Western Cape. The most striking form, colloquially dubbed “iridescens”, exhibits flowers, typically white, sometimes yellow and occasionally pink, marked with distinctive iridescent centres. The other two forms, “lutea” and “alba”, have yellow and white flowers, respectively, often with small maroon tepal markings but lacking iridescence. Floral colour polymorphisms within a species are frequently attributed to divergent selection by biotic agents, such as pollinators, but can also be associated with abiotic variables and the interplay between biotic and abiotic drivers across heterogenous landscapes. I documented pollinator interactions within the generalised pollination system of *P. capensis* and assessed population-level variation thereof. I show that pollinator assemblages are broadly dominated by beetles, while bees are also frequent visitors to lutea and alba but almost never interact with flowers displaying iridescent colouration, suggesting a potential role for iridescence in the partitioning of available pollinator communities. Subsequently, I used MaxEnt modelling to investigate the predicted distribution ranges of iridescens and lutea with respect to underlying environmental variables and found a pattern of geographic structuring of floral forms. Using ecological niche models, I then assessed differences in the occupied abiotic niches of iridescens and lutea in multivariate environmental space ("E-space") and found that the two forms occupy significantly different niches. Finally, by limiting the analysis to only the portion of E-space that is present and accessible within the ranges of both forms, I demonstrate statistical evidence that niche differences directly result from divergent adaptation to abiotic conditions. This represents a first step towards understanding the ecological mechanisms linked to the formation and maintenance of floral colour variation within *P. capensis.*

Commelinales Working Group: turning a new taxonomic leaf

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Commelinales are a small order of Monocots composed of five families: Commelinaceae, Haemodoraceae, Hanguanaceae, Philydraceae, and Pontederiaceae. These families were historically placed in distinct orders and were studied as unrelated groups. However, molecular data strongly supports these families as forming a monophyletic assembly despite their superficial morphological incongruence. Luckily, all families are morphologically and molecularly well-delimited and supported. Commelinales are economically relevant due to their large number of weedy and ornamental species, with some species also being used as a source of biofuel, fibres, and natural dyes. The group is very diverse in the tropics, especially in Africa, continental Asia, South America, and Australia. Despite the order's small size, the group has been historically plagued by subpar taxonomy, primarily due to their complex and ephemerous flowers, which tend to make poor herbarium specimens. The Taxonomic Expertise Network (TEN) for Commelinales, the Commelinales Working Group, was founded in 2016 around the need to integrate and coordinate the taxonomic, systematic, and nomenclatural studies of the order. Our aim is to provide an updated classification for Commelinales based on the integration of morphological, molecular, ecological, cytological, and phytochemical data. This mammoth task has only been made possible through the collaboration and cooperation of botanists worldwide, with representatives from all continents. Pontederiaceae is the family with the least amount of systematic issues, with recent studies clarifying generic limits. Haemodoraceae and Commelinaceae, the two largest families in the order, are in similar situations, having been given a considerable amount of attention but requiring further finessing of their classification and generic limits. Philydraceae still require the most attention, with persistent classification and taxonomic issues. Finally, the monogeneric Hanguanaceae does not require an infrafamilial classification. However, the enormous number of newly described and still undescribed species prompts the need for an infrageneric classification.

A first DNA barcoding attempt on the African cycad pollinator Erotylidae reveals more than a single species pollinate the cycad genus *Encephalartos* in South Africa

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Although interest in the pollination biology of cycads is growing, our knowledge of the diversity of cycad pollinators is extremely poor. What is known is that cycad pollinators belong to the beetle family Erotylidae, and that cycads in the northern hemisphere are pollinated by three groups of beetles; however there is a knowledge gap in the southern hemisphere. Our aim was to uncover the extent of the diversity of pollinators of the African cycad genus *Encephalartos*. To this end, we collected 100 specimens of Erotylidae pollinating *Encephalartos natalensis* in the Umtamvuna Nature Reserve, Kwazulu-Natal, South Africa. First, we sequenced the CO1 marker for all beetle specimens collected, following the DNA barcoding procedure. We then collected morphological data on the shape of abdomen and thorax, status of insect head (attached or not to the body), body and antenna sizes. These data were analysed in three ways: i) by BLAST-ing the CO1 sequencing on GenBank and BOLD databases, ii) by reconstructing a phylogenetic tree based on all CO1 sequences, and iii) by mapping all morphological features onto the phylogenetic tree. We had a poor sequencing success rate (21%) despite multiple sequencing repetitions, suggesting difficulties in CO1 sequencing of African Erotylidae. Our BLAST analysis revealed a similarity index lower than 90%, suggesting that our CO1 sequences are new to these two databases: the highest similarity is 87.80% on GenBank corresponding to *Menesia albifrons* and 89.92% on BOLD corresponding to *Aleochara tristis*. Furthermore, our phylogenetic tree revealed at least nine potential beetle species, some of which are supported by the mapping exercises of morphological features onto the phylogeny. Our study reveals that the pollinators of African cycads comprise multiple species and there is an urgent need to accelerate taxonomic studies to identify and describe these beetles.

Phylogeny Inference Under Time-Decaying Migration

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Post-speciation gene flow is widespread across the Tree of Life but is ignored as a cause of gene tree discordance under the standard multispecies coalescent. Failing to account for migration can mislead estimates of effective population sizes, divergence times, and topology. Isolation-with-migration models accommodate migration but involve additional parameters that frequently limit their computational viability with even moderate sized molecular datasets. Problematically, reliable parameter estimation under these models may require large datasets. This study evaluates IM-based phylogenetic inference using simulated and empirical data with a focus on incorporating gradually time-decaying migration, reflecting realistic expectations of reduced gene flow among more deeply diverged lineages. We compare the performance of

DENIM (an IM model) and StarBeast3 (an MSC model) using sequences simulated with continuous low-level migration and strict isolation on a ten-taxon ultrametric tree. Our results show that DENIM improves phylogenetic estimation despite incomplete lineage sorting and time-decaying migration, achieving improved estimates with relatively few loci and single-core runtimes of several days. Unlike some IM implementations, DENIM achieves these improvements without requiring a predefined topology. Both DENIM and StarBeast3 perform best where migration is effectively limited to closely related lineages, but DENIM achieves better phylogenetic accuracy and convergence for all simulated datasets including migration despite its higher parameter complexity. While varying locus length and number had limited detectable effects on phylogenetic accuracy, longer sequences improved convergence. Empirical validation using transcriptomic data for *Silene* (Caryophyllaceae) reflects similar results. These findings affirm DENIM as a computationally efficient approach for mitigating migration-induced distortions in species tree inference.

The evolution of the *Satyrium longicauda* species complex: combining field observations and phylogenetic inference to study pollinator-driven diversification.

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Phylogenetic analyses have been critical for macroevolutionary studies and can also be used to study population-level divergence, though the latter application is still uncommon. Here we present pollinator observations and trait data acquired over several decades for members of the *Satyrium longicauda* (Orchidaceae) species complex and analyse these data in a phylogenetic context to reveal how various evolutionary processes have shaped phenotypic diversity and speciation. Communities of *S. longicauda* include a large number of morphologically and genetically distinct forms, which often co-occur and regularly co-flower in sympatry. The length of the nectar spurs is one of the most variable traits and most forms are pollinated by moth species, which often have proboscides that match the length of the nectar spur. Two exceptions occur in a clade that comprises forms characterized by a single erect leaf. One of these forms, which differs primarily from the moth-pollinated forms in the absence of nectar in the spurs and unusual floral scent chemistry, is pollinated diurnally by oil-collecting bees. Optimization of floral traits suggests that the presence of spurs is a vestigial trait, and that presence of diacetin in closely related moth-pollinated forms was likely a preadaptation for bee pollination. Another form with unscented red flowers has shifted towards pollination by sunbirds. Both these forms have acquired separate species status. A third form that is part of a different clade, is similar in most traits to several other forms, but differs in floral scent bouquet. This form appears to have lost the biochemical pathway involved in the production of cinnamyl-alcohol derivatives, but is nevertheless, like its close relatives, pollinated by hawkmoths, which respond to the dominant scent compounds in both blends. This suggests that not all changes in floral traits are associated with a pollinator shift. We conclude that the use of phylogenetic tools, especially those involving next-generation sequencing approaches, offer great potential to study pollinator-driven diversification among populations.

Invasion Biology (INV BIOL)

Fleshy-fruited invasive alien shrub populations change over time along roadsides of South African grasslands

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The expansion of woody, fleshy-fruited invasive alien plants pose significant ecological risks to South African grasslands, necessitating effective, long-term monitoring. This study investigates the use of Google Street View to monitor invasive plant populations along South African roadsides. Using Google Street View derived repeat photography, we assessed changes in abundance over 12 years for multiple invasive species. Historical images of populations dating back to 2010 were accessed from Google Street View and recent images of the same populations were taken using a digital camera in 2022. Plant species recorded included *Cotoneaster pannosus* Franch. (Rosaceae) (n = 15 points), *Lantana camara* L. (Verbanaceae) (n = 1), *Ligustrum lucidum* W.T.Aiton (Oleaceae) (n = 1), *Melia azedarach* L. (Meliaceae) (n = 6), *Opuntia ficus-indica* (L.) Mill. (Cactaceae) (n = 35), *Prunus persica* (L.) Batsch (Rosaceae) (n = 18), *Pyracantha angustifolia* (Franch.) C.K.Schneid. (Rosaceae) (n = 74), *Pyracantha crenulata* (D.Don) M.Roem. (Rosaceae) (n = 3), *Rosa rubiginosa* L. (Rosaceae) (n = 28), *Schinus molle* L. (Anacardiaceae) (n = 2), and *Solanum mauritianum* Scop. (Solanaceae) (n = 9). The study identified population increases in several species like *Pyracantha angustifolia*, *Solanum mauritianum,* and *Prunus persica*, while others like *Opuntia ficus-indica* decreased. Our findings showed that, while some species exhibit increases in abundance, most trends were not statistically significant. This study provides a foundation for using Google Street View in large-scale monitoring of invasive plant species.

Evaluating the water use of *Arundo donax* and *Phragmites australis* from leaf to stand level

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Tall-statured grasses are globally recognised among the most transformative functional plant groups, with considerable potential to disrupt hydrological regimes through waterway constriction and channel flow reduction. The invasive giant reed, *Arundo donax* L. (Poaceae) and its native analogue, a wetland encroacher, the common reed, *Phragmites australis* (Cav.) Trin. ex Steud. are among two of the most conspicuous and widespread herbaceous species in South Africa. While both species have been prioritised for management due to their potential impacts on ecosystem hydrology, quantitative estimates of the water resources impacted by *A. donax* and *P. australis* are unavailable in South Africa. To quantify the water lost through stands of both species in the field, models of *A. donax* and *P. australis* water use were developed in the laboratory by measuring the soil water content of individuals grown in gravel-lined pots, and leaf-level transpiration using a portable infrared gas analyser. Leaf-level transpiration was measured throughout the canopy along with plant traits for *A. donax* and *P. australis* individuals across four field sites in Johannesburg, using the laboratory models for calibration. Whole plant water use measurements were upscaled to the stand level using the allometric relationships developed using plant traits. Analyses suggest no differences in leaf-level transpiration between *A. donax* and *P. australis*, while whole plant water use is higher for *A. donax* due to its higher total leaf area. We anticipate that the invasive *A. donax* will have higher stand-level water consumption compared to the native *P. australis*. This ongoing study will provide insights into the water resources impacted by *A. donax* invasion and *P. australis* encroachment in Gauteng, quantifying water losses that can be converted into economic costs and assist the prioritisation of stands for control efforts based on the spatial extent of invasion or encroachment.

Mapping the current and future distribution of invasive alien shrubs in south Africa's high-elevation grasslands

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Understanding the future distribution of invasive plant species under changing climatic conditions is essential for biodiversity conservation and management. This study models the potential range shifts of two common alien invasive Rosaceae shrubs, *Cotoneaster pannosus* Franch. and *Pyracantha angustifolia* Franch. that have become naturalised in South Africa's high-elevation grasslands. These shrubs alter native ecosystems by forming dense monocultures and outcompeting endemic flora. The models aim to identify future invasion hotspots and assess whether climate change may facilitate or restrict the spread of these species. Using bioclimatic niche modeling, we combined species occurrence data with climatic variables from the WorldClim and CMIP6 datasets to predict suitable habitats for each species. We assessed the current and projected future ranges of these two species, based on key climatic variables influencing their germination and establishment. Variables including annual mean temperature, precipitation seasonality, and temperature of the coldest quarter were selected based on ecological relevance and low collinearity. Species distribution models were evaluated using cross-validation and performance metrics such as Area Under Curve (AUC). Projections were made using the Shared Socioeconomic Pathways (SSPs), representing the low-emission scenario (SSP1-2.6), for the period 2041 to 2060. Our results indicate that suitable habitats for both species will decrease but remain restricted to the Drakensberg escarpment along the eastern part of the country. This result aligns with projections of regional warming, as these species require cold stratification for successful germination and are likely to shift to cooler climates found at higher elevations. Given the unique biodiversity of these high-elevation ecosystems, the encroachment of invasive shrubs could lead to the competitive displacement of native flora, thus altering plant community composition and potentially impacting dependent fauna. This study highlights the need for integrating ecological forecasting into conservation planning to mitigate the spread of these invasive species in vulnerable montane ecosystems.

Reproductive and seed-germination ecology of the invasive alien woody *Calotropis procera* (Aiton) Dryand, in the Vhembe Biosphere Reserve, South Africa

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Invasive alien plant species (IAPS) threaten biodiversity and ecosystem function, particularly in sensitive areas such as the Vhembe Biosphere Reserve (VBR) in South Africa, where *Calotropis procera* has become a widespread woody invader. Understanding the reproductive mechanisms of *C. procera* is crucial for developing effective management strategies to mitigate its spread and ecological impacts. This study revealed that *C. procera* exhibits significant variation in seed production across different sites within the VBR, indicating adaptation to local environmental conditions. To determine seed production, the number of fruits and seeds per fruit were counted at three sites, and seed production variation across sites was analysed using one-way ANOVA and Tukey HSD multiple comparison analysis. The mean number of seeds per fruit averaged 401.4 ± 6.52, with seed production varying significantly across sites ($F = 9.585$, $p < 0.001$); Musina showed the highest seed production, and Sanari the lowest, while the water table level did not significantly influence seed production ($p = 0.899$). This higher seed production relative to populations in its native range supports the evolution of the increased competitive ability (EICA) hypothesis. *Calotropis procera* prolific seed production and adaptation to varying environmental conditions likely contribute to its invasiveness. These findings highlight the importance of considering local environmental factors in managing invasive species and addressing the broader issues of biodiversity conservation and ecosystem resilience in the face of plant invasions, informing targeted management strategies to prevent further spread in biodiversity hotspots.

In vivo management of fungal diseases of carrot using seed extracts of *Azadirachta indica, Ricinus communis* and *Eucalyptus camaldulensis*

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Carrot production is severely threatened by fungal diseases such as root rot, leaf blight, and crown decay, causing significant yield losses and economic hardship for farmers. Current management strategies rely heavily on synthetic fungicides, which pose environmental and health risk. Meanwhile, plant extracts have shown promise as eco-friendly and sustainable alternatives for managing fungal diseases. This study investigated the in vivo efficacy of n-hexane seed extracts of *Azadirachta indica*, *Ricinus communis*, and *Eucalyptus camaldulensis* on fungal diseases of carrot. Carrot plant samples were collected using purposive sampling and was taken to the mycology lab for isolation and identification. Fungal pathogens (*Fusarium oxysporum*, *Mucor hiemalis* and *Alternaria altanata*) are treated with varying concentrations of the seed extracts. Disease severity and plant growth were assessed using some growth parameters. Results showed that the seed extracts significantly reduced disease severity, and improved plant growth. Seed extract of *Azadirachta indica* at higher concentration exhibited the highest efficacy, followed by *Ricinus communis* and *Eucalyptus camaldulensis*. The study demonstrates the potential of n-hexane seed extracts as a sustainable and eco-friendly approach for managing fungal diseases of carrot, offering a promising alternative to synthetic fungicides.

Random Forest machine learning and field spectroscopy to discriminate *Equisetum hyemale* L. (Snake grass) from morphologically similar species and co-existing species

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Alien invasive species can negatively affect recipient ecosystems, and this severity may be promoted by failing early detection of these problematic species due to morphological similarities between alien species and native species. Our aim was to test the discrimination ability of spectral reflectance between *Equisetum hyemale* L. (snakegrass) and selected morphologically similar species (*Equisetum ramosissimum*, *Elegia* *tectorum*, and *Juncus effusus*) using a Random Forest Classification model. We found an overall classification accuracy of 93.44% and a Cohen's Kappa statistic (k = 0.91), which indicates that each species shows a distinct spectral signature which likely reflects the structural and physiological differences of these species. The robustness of the model reinforces the potential of hyperspectral data in informing the control measures of alien species.

Cryptic invasion of *Rosa* species in the grassland biome of South Africa

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Grasslands are among the world's most vital biomes, covering roughly 40% of the Earth's surface. They are critically important due to the essential ecosystem services and functions they provide. Unfortunately, they are threatened by biological invasions. The South African Grassland Biome is not immune as it has been invaded by a number of invasive Rosaceae shrubs including *Rosa rubiginosa* L. Research into the impacts and spread of *R. rubiginosa* in these grasslands revealed interesting morphological variations within the populations. This study aimed to determine if these varieties actually represent cryptic invasions of previously undetected *Rosa* species in the biome. Morphological and genetic differences of these varieties were determined by investigating several plant traits, such as the presence or absence of trichomes on leaves and fruits, fruit and flower colour, fruit and leaf shape and size, in combination with analysis of differences in ITS gene sequences. As *R. rubiginosa* is impacted by an associated seed predator, *Megastigmus aculeatus*, the impact of this wasp was also compared between different varieties, by assessing wasp emergence frequencies from fruits of different varieties. Four plant varieties were identified, each showing a distinct variation in flower, fruit, and leaf traits. For example, one variety had white flowers, smooth red fruits, and hairless leaves with no fragrance. The others had pinkish flowers, spiny fruits with glandular bases (small spines on the pedicels), and hairy, sweet-scented leaves when crushed. Preliminary genetic analyses have revealed the presence of two *Rosa* species. Moreover, level of seed predation by the wasp varied between different fruit morphologies. This project will provide invaluable information regarding the detection of an additional invasive *Rosa* species in South Africa, apart from *R. rubiginosa*, informing appropriate management interventions.

Control options of chestnut blight disease caused by *Cryphonectria parasitica* in Hungary

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European chestnut (*Castanea sativa*) is an important tree species. It is most threatened by chestnut blight disease caused by the fungus *Cryphonectria parasitica*. The pathogen is able to infect other tree species from the Fagaceae (beech, oaks). Control of the fungus is very difficult. An effective biocontrol method used in Europe is with hypovirulent fungal strains of *C. parasitica* with reduced virulence caused by a mycovirus encoded in the RNA of their cytoplasm. This virus-like particle (VLP) causes significant changes in the virulence of the fungus. Artificial spreading of hypovirulent fungal strains can be used for biological control actions. In Hungary, we have been researching the topic for several decades. This method for biological control can only be used in a way that is adapted to the growing site. We assessed the prevalence of the disease in the country and identified its prevalent Vegetative Compatibility Groups (VCGs) by laboratory tests. We have developed and established the practical biological control of *C. parasitica* using its hypovirulent strains. This method has also been applied on sweet chestnuts in Hungary, Slovakia, and, based on recent results, also in Romania. However, it is not enough to base the control of the pathogen on only one method. It is necessary to use all methods of integrated control options for effective control. Carrying out mechanical control helps to slow down the spread of the disease. In our work, this was also developed. *Trichoderma* species as hyper-parasitic control agents were also examined against *C. parasitica.* We also tested *in vitro* chemical fungicides and examined their effect on the development of the pathogen. According to our results, each of the control options tested showed positive results, and each may have significance and a role in practical integrated control.

Ensemble model-guided survey of *Populus alba* L. in Limpopo Province, South Africa

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Invasive alien woody trees can negatively affect the environment and the economy by altering ecosystem services. *Populus alba* (Salicaceae) native to Europe, North Africa, and Asia was intentionally introduced to South Africa for ornamental purposes. This study determines the current distribution of *P. alba* and its likelihood of invasion in Limpopo Province, which is crucial for effective planning and management. An ensemble model was developed using Maximum Entropy, Generalised Additive Models, and Random Forest algorithms. The model performance was validated using the Area under the curve (AUC) and Pearson's correlation (COR) tests. Model predictions were validated by selecting fifty random points across varying suitability levels of the model output. Confusion matrix, Kappa statistics, and Kruskal-Wallis chi-squared test were used to evaluate the model performance using the true presence and absence data. The AUC mean model performance was 0.91 for Maximum Entropy, 0.9 for Generalised Additive Model, and 0.94 for Random Forest. The mean performance of the model under COR for Maximum Entropy, Generalised Additive Model, and Random Forest were 0.64, 0.64, and 0.67, respectively. The model predicted a larger geographic area of unsuitable conditions compared to suitable areas. The survey revealed *P. alba* presence at thirty-three sampling points (66%). The model predictions validations were 0.147 for Kappa statistics and 4.2768 for the Kruskal-Wallis chi-squared test with a p-value of 0.03864. The model-directed field survey proved to be a valuable tool for detecting the presence of *P. alba*, yielding twenty-six new occurrence records, primarily in areas of medium to high suitability.

Medicinal Plants/Ethnobotany (MED PLNT)

Ethnobotanical survey of medicinal plants used for managing mental health conditions in Mahikeng, South Africa

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Medicinal plants play a crucial role in Africa's healthcare, especially in rural areas. However, environmental degradation and societal changes have led to a loss of many medicinal plants and their associated indigenous knowledge. This study explored the use of indigenous knowledge and medicinal plants for managing mental health in Masutlhe 2 and Ramatlabama 600, Mahikeng, South Africa. The ethnobotanical survey was carried out through interviews using a semi-structured interview with 10 knowledge holders. The participants were purposefully selected via snowball technique. Data was analysed using thematic analysis and ethnobotanical indices. The study revealed traditional healthcare practitioners had detailed knowledge on mental health conditions and medicinal plants used for their management. The study documented 45 plants belonging to 23 families that were dominated by Fabaceae (5) and Asteraceae (4). The Relative Frequency of Citations (RFC) ranged from 0.1 to 0.5 and the five most popular plants for mental health management were *Cadaba aphylla* (Thunb.) Wild (0.5), *Cannabis sativa* L. (0.3), *Dysphania ambrosioides* (L.) Mosyakin & Clemants (0.3), *Osyris lanceolata* Hochst. & Steud. (0.3) and *Gompocarpus fruticosus* (L) Aiton.f.sudsp. *fruticosus* (0.2). Additionally, the most used plant parts were the roots (9) and leaves (8), which were usually prepared by infusion, decoction, or boiling. The preparation of medicinal plants is often governed by cultural taboos. The mental health conditions treated were mainly madness (15) and headaches (10) as well as the less reported ones such as amnesia, depression, and epilepsy. This research underscores the importance of preserving indigenous knowledge on medicinal plants and ultimately adds to the limited body of work on African indigenous health practices for mental health care.

Quantitative ethnobotanical survey of medicinal plants used to manage andrological conditions in the North-West Province, South Africa

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Andrological conditions remain a global and significant health concern. Although conventional drugs are available to treat some of these conditions, their adverse side effects, limited access in global south and high cost have prompted seeking alternative solution in natural remedies. Thus, this study explored the medicinal plants used to manage andrological conditions in the North-West Province of South Africa. A quantitative ethnobotanical study was conducted from December 2024 to May 2025 in four district municipalities of the North West Province. Data was obtained primarily from the Traditional Health Practitioners using snowball and purposive sampling techniques, and interviews were conducted using a semi-structured interview guide. Quantitative data analysis was performed using ethnobotanical indices, while descriptive statistics were employed to present the data. The results revealed that Fabaceae (30 sp.) and Asteraceae (21 sp.) were the plant families with the highest number of species reported. Plant parts such as roots (45%) and leaves (20%)

were the most used parts in the preparations of herbal medicines. The frequently used method for preparation was decoction (50%), and oral route (56%) was the commonly used administration mode. Sexual and erectile dysfunction (30%), reproductive challenges (25%), and urinary tract infections (15%) recorded the highest use reports and the highest taxa. The current study highlights the continued reliance on plants for primary health care, particularly for andrological conditions in the study area amongst the local communities. However, the phytochemical profiles and safety of these identified plants remain crucial aspects for future research.

In vitro **antibacterial and antioxidant activity of the leaves of indigenous fruit species from Mpumalanga province, South Africa**

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Antimicrobial drug resistance and unwanted side effects of conventional medicines necessitate research on medicinal plants as alternatives. This study investigated the antibacterial and antioxidant properties of *Carissa spinarum* L., *Diospyros mespiliformis* Hochst. ex A.DC, *Euclea crispa* (Thunb.) Gürke, *Ficus thonningii* Blume., *Strychnos madagascariensis* Poir, and *Strychnos spinosa* Lam. Hexane, acetone, and methanol leaf extracts were tested for antibacterial activity against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* using disc diffusion and microdilution assays. The methanol extracts of selected plant species were evaluated for antioxidant activity using 2,2-diphenyl-1-picryhydrazyl (DPPH) and ferric-reducing power assays. The extracts of *D. mespiliformis* and *E. crispa* showed strong antimicrobial activity against *P. aeruginosa* with inhibition zones up to 28 mm and minimum inhibitory concentrations (MICs) from 0.781–1.563 mg/mL. The acetone extract of *D. mespiliformis* also exhibited activity against *K. pneumoniae* (MIC: 3.125 mg/mL). The methanol extract of *E. crispa* displayed potent antioxidant activity, achieving a half-maximal inhibitory concentration (IC_{50}) of 1.42 µg/mL, which was comparable to ascorbic acid at concentrations of 62.5–250 µg/mL. Methanol extracts of *S. madagascariensis* and *S. spinosa* demonstrated good ferric-reducing power at 250 µg/mL. The leaves of indigenous fruit species exhibited significant antimicrobial and antioxidant activities, making them possible sources of novel compounds for combating infections and oxidative stress.

Phenolic metabolite profiling of *Moringa oleifera* waste extracts: influence of drying methods and solvent extraction

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Moringa *(Moringa oleifera Lam.*) processing for its extracts has gained popularity for the abundance of bioactive compounds, making it suitable for application in various industries. This has resulted in the generation of a substantial amount of waste rich in phytochemicals such as phenolic acids and flavonoids. However, the challenge lies in effectively recovering these bioactive compounds. This study explored the influence of drying techniques and extraction solvents on phenolic metabolites of moringa waste. Moringa wastes (twigs) were collected, sorted, washed in distilled water and exposed to three drying techniques: freeze drying (FD), sun drying (SD) and oven drying (OD). The dried materials were then ground into powder and extracted with various solvents: distilled water (H_2O), 50% ethanol (EtOH50%), and 100% ethanol (EtOH100%) in a sonication bath. Ultra-perfomance liquid chromatography-mass spectrometry (UPLC-MS/MS) was used for phenolic metabolites profiling. Key phenolic compounds, including chlorogenic acid, quercetin-4'-glucoside, kaempferol, rutin, and caffeoylquinic acid derivatives were identified, with variations in their concentrations depending on the drying method and solvent used. SD-EtOH50% and OD-EtOH50% treatments contributed significantly to the phenolic metabolite profile, with these treatments being particularly effective in extracting bioactive flavonoids and phenolic acids. In conclusion, OD-EtOH50% demonstrated superior efficiency and reliability, establishing itself as the preferred method for preserving the bioactive potential of moringa waste.

Characterization of phytochemical constituents and phytopharmacological activities of the South African freshwater medicinal plant *Cyperus laevigatus* (Cyperaceae)

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Medicinal plants are the oldest and most prevalent form of healthcare utilized globally; however, scientific profiling is lacking. *Cyperus laevigatus* L. is an aquatic medicinal plant used as an antimicrobial, antioxidant, liver tonic and to relieve fatigue. This study aimed to investigate the phytochemical constituents and phytopharmacological activities of the aerial and root biomass of the South African *C. laevigatus* plant extracted with solvents of varying polarity. The qualitative tests showed that all parts contained phytochemicals but were more prevalent in the aerial than in root extracts (17 and 12 out of 20 phytochemicals tested, respectively). The quantitative tests confirmed the presence of phenolic compounds, flavonoids, tannins, and proanthocyanidins in the plant extracts with the greatest contents in the methanolic extracts. The *C. laevigatus* plant extracts exhibit moderate antioxidant activities assessed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide (H_2O_2) scavenging assays, and metal chelating activity. The ethyl acetate aerial extract had the best antioxidant activity with IC_{50} of 34.29±0.36 mg/mL and 36.67±0.38 mg/mL for DPPH and H_2O_2, respectively. In contrast, the n-hexane aerial and root extracts were most potent in the metal chelating test with IC_{50} of 0.66±0.01 mg/mL and 0.59±0.01 mg/mL, respectively. The antibacterial tests revealed that the *C. laevigatus* extracts had moderate activity against *Staphylococcus aureus* and *Streptococcus pyogenes* and very slight inhibition against *Escherichia coli* where only the ethyl acetate and n-hexane aerial extracts showed inhibition with ZOI of 11.9±0.15 mm and 12.62±0.19 mm, respectively. *Cyperus laevigatus* is a potent medicinal plant with diverse medicinal applications and investing resources into its research can greatly improve public health.

What orangutans can teach us: plants used for self-medication as a potential source of bioactive substances with antiparasitic activity

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The main aim of this work is the isolation, identification and examination of bioactive plant fractions with potential antiparasitic activity. These extracts are obtained from plants that are routinely eaten by orangutans. Our previous research suggests that the purpose of this consumption is self-medication. The main criteria for the plant selection are the behavioral data of the orangutans and the parasite infections thereof. The effect of these extracts on the progression of significant waterborne protozoal diseases (WBD), namely amoebiasis and giardiasis, is tested using both *in vitro* and *in vivo* methods. Moreover, anthelmintic properties of selected extract are investigated *in vivo* in a model nematode, *Caenorhabditis elegans*. Subsequently, activity-guided fractionation is performed and active substances from these extracts are isolated and their chemical structure characterized using HPLC-HR MS/MS as well as 1D and 2D NMR methods. Their identity and biological activity is subsequently compared with authentic standards. Project is supported by Grant Agency of the Czech Republic (23-06571S) and by the “UMI – Saving of Pongidae” Foundation project “Parasites and Natural Antiparasitics in the Orangutan”.

Effect of propagation techniques on the seedling establishment of medicinal cannabis (*Cannabis sativa*) found in Lusikisiki, Eastern Cape, South Africa

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Availability of adequate propagation techniques is a barrier in the production of high-quality medicinal cannabis extensively found in rural communities, particularly in an era where commercialization of this plant is critical for medicinal applications and product development. This study investigated the effect of propagation techniques on the establishment of medicinal cannabis (*Cannabis sativa*) seedlings. We set up a factorial experiment laid out in a randomized complete design with three replications and treatments: i) herbaceous shoot cutting with 50% of leaf area trimmed, ii) herbaceous shoot cutting with 100% leaf area, and iii) basal stem cutting. The experiment was conducted at Dohne Agricultural Development Institute, Stutterheim, Eastern Cape. We collected cuttings from wild cannabis plants in the vicinity of Lusikisiki using secateurs. Sixty 15cm cuttings per treatment were excised at 45^0 below the node of actively growing vegetative branches. Cutting bases were dipped into Indole-3-Butyric acid, planted into a peat-based commercial rooting medium, inserted into 60-cell propagation incubators, and placed in the propagation chambers at 15-20 °C under T8 fluorescent illumination for 24 hours. The level of mist in the incubator was checked every morning and adjusted accordingly with distilled water. Growth parameters, including the number of rooted and unrooted propagules, time to 50% (T50) rooting, height and number of new leaves per propagule, weights of fresh propagule, shoot and root, shoot and root length, dry shoot and root weight, root-shoot ratio and the success percentage, were collected every second week between two and six weeks after planting. The results showed that herbaceous shoots with 100% untrimmed leaf area exhibited the best performance in all measured rooting, growth, and success parameters, followed by herbaceous shoots with 50% trimmed leaf area. However, propagation of *Cannabis sativa* using the basal stem technique did not yield favorable results across any of the measured parameters. This study demonstrates that herbaceous shoots with 100% untrimmed leaf area propagation is a better technique used in enhancing growth attributes for successful and seedling establishment option in vegetative propagation of medicinal cannabis.

Nutraceutical potential of underutilized *Trianthema portulacastrum* L. (Aizoaceae)

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This study aimed at characterizing and quantifying index anticancer, anti-HIV and antioxidant metabolites in *T. portulacastrum* using the Ultra-High Performance Liquid Chromatography Mass Spectroscopy (UHP-LCMS) while minerals and proximate nutrients of the plant were assessed following the Association of Organic and Analytical Chemists (AOAC) methods. The cytotoxicity of the tested samples was monitored on the xCELLigence Real Time Cell Analyser as a function of cell index at a specific time post dose using the concentration range of 0.16 - 2.5%. Ethanolic extracts of *T. portulacastrum* induced the inhibition of at least 50% of the HIV-1 viral load at considerably low IC_{50} values of 1.757 mg/mL which is comparable to the AZT standard. Based on the responses observed 48 hours post exposure, the tested extracts showed high levels of cytotoxicity as determined by the xCELLigence. These biological activities may be attributed to different anticancer, anti-HIV and antioxidant metabolites such as epigallocatechin, glucaric acids, byakangelicin, xanthotoxin, apaensin, acetoxy-6-gingerol among other compounds of significant therapeutic benefits that were quantified with UHP-LCMS. The protein composition ranged between 8.63-22.69%; fat (1.84-4.33%); moisture (7.89 - 9.04%); fibre (23.84-49.98%); and carbohydrate content (38.54-70.14%). Mineral contents of tested T. portulacastrum varied considerably in different parts of the plant. Nitrogen mineral ranged between 13.8-36.3 mg/g; sodium (2.0-14.0 mg/g); potassium (14.0-82.0 mg/g); magnesium (2.8-7.1 mg/g); calcium (9.1-24.7 mg/g); phosphorus (1.3-3.6 mg/g); iron (193.5-984.0 ppm); zinc (42.5-96.0 ppm); manganese (28.5-167.5 ppm); and copper (2.0-8.5 ppm). These mineral values are comparable or higher than values reported for common vegetables, suggesting that *T. portulacastrum* is a nutrient-dense vegetable that could provide alternative sources of anticancer and antiviral nutrients to HIV-infected individuals. These findings suggest that *T. portulacastrum* contains diverse nutrients, anticancer and anti-HIV metabolites with extended therapeutic utility that could provide alternative sources of anticancer and antiviral nutrients to HIV-infected individuals.

Roots with riches: Unveiling the chemical complexity of South African *Withania somnifera*.

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Withania somnifera (in the Solanaceae family, and commonly referred to as Ashwagandha) is a commercially important species that has not received much attention in South Africa, despite its use in local traditional medicine. The plant occurs in drier regions of tropical and subtropical zones across Asia, southern Europe and Africa. Indian plants have been well studied and are cultivated for the adaptogenic and anxiolytic effects of their roots. The species is known to have a high degree of genetic diversity, with several chemotypes. A semi-targeted investigation of South African plants was conducted, with a focus on withanolides and their glycosides. Roots were collected from several populations across the country and subjected to analysis by ultra performance liquid chromatography high resolution mass spectrometry (UPLC-HRMS). Travelling wave ion mobility spectrometry (TWIMS) was utilized as an orthogonal analytical technique to aid in the separation of coeluting isomers. The South African and Indian root material analyzed by UPLC-IM-MS showed highly complex chemical profiles, with many isobaric constituents. The withanolides were identified using standards where available and literature sources for additional compounds. Most previous chromatographic studies conducted on *W. somnifera* involve targeted analysis on the same handful of withanolides, so when neither standards nor literature sources were available for a compound, structural elucidation was performed through fragmentation analysis. The roots of South African plants were found to possess various withanolides, but little to no withaferin A – a characteristic that is highly sought after. This work reported the electrospray ionization and fragmentation behavior of many withanolides and could provide valuable insight for future untargeted and semi-targeted analyses of *W. somnifera*. It also demonstrated the suitability of South African plants as natural products and could provide an incentive for companies to obtain material locally instead of importing material from India.

Investigating the *in-vitro* anti-inflammatory and anti-cancer activity of the tuber of *Dioscorea sylvatica*

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Inflammation is often linked to cancer growth or its progression, and cancer is described as a group of diseases caused by the loss of cell cycle. Recent studies have highlighted that inflammation is seen as a hallmark of cancer, with researchers aiming to determine the potential role of inflammation in various aspects of cancer. The study focused on investigating both the *in-vitro* anti-inflammatory and anti-cancer activity of tuber of *Dioscorea sylvatica* plant extract and fractions. The conducted ethnobotanical survey suggested that the tuber of *D. sylvatica* possesses both the anti-inflammatory and anti-cancer properties. *D. sylvatica* plant materials were subjected to the *in-vitro* anti-inflammatory activity using the 5-lipoxigenage (5-LOX) model assay, as well as anti-cancer studies using MTT assay. The results from the 5-LOX model, showed that ethanol extract exhibited the best inhibitory activity with an IC_{50} value of 3.063 ± 0.040 µg/µL, followed by hydro-ethanol extract with 4.042 ± 6.993 µg/µL, and hydro-ethanol fraction also yielded good activity with an IC_{50} value of 2.218 ± 0.002 µg/µL. Anti-cancer studies showed that acetone extract depicted low cytotoxicity level at its highest concentration of 17.9 mg/mL with cell viability above 80%, and weak cytotoxicity level with cell vitality of 69% at a concentration of 8.9 mg/mL was also recorded, indicating the possibility of a good anti-cancer agent. Additionally, hydro-ethanol fraction depicted good anti-cancer activity against HuTu-80 and H4IIE-*luc* cell lines. Moreover, this study has provided a very useful baseline for medicinal properties of *D. sylvatica* in reference to inflammation and cancer, and also validated the information obtained from the conducted ethnobotanical survey.

In vitro antiplasmodial, antigonococcal and cytotoxicity activity of South African *Ziziphus* Mill. (Rhamnaceae) species

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For centuries, *Ziziphus* species have been used in African traditional medicine as remedies for human ailments. In this study, South Africa's indigenous *Ziziphus* species (*Z. mucronata*, *Z. rivularis*, and *Z. zeyheriana*) are evaluated for their antiplasmodial, antigonococcal, and cytotoxicity activity. The plant parts were air-dried, and sequentially extracted using *n*-hexane, dichloromethane, ethyl acetate, ethyl acetate: methanol (1:1), and methanol. Forty-eight extracts were obtained. Further ^{1}H NMR-based metabolomics to identify bioactive constituent classes was conducted. The Principal Component Analysis (PCA) and Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) analyses were applied to reduce dimensionality and observe discrimination according to observed bioactivity. Of the 48 extracts tested, 31% showed no activity against malaria ($IC_{50} > 20$ µg/ml), while 54% were inactive against gonorrhea (MIC > 6.25 mg/ml). For malaria, 13% exhibited high activity ($IC_{50} \leq 5$ µg/ml), and 23% showed moderate activity (5 µg/ml < $IC_{50} \leq 20$ µg/ml). Against gonorrhea, 12.5% of extracts demonstrated high activity (MIC < 1 mg/ml), while 33.3% had moderate activity (1 mg/ml < MIC < 6.25 mg/ml). *Ziziphus rivularis* leaves exhibited the strongest antigonococcal activity, while *Z. zeyheriana* roots had the highest antiplasmodial activity. None of the extracts showed cytotoxicity when tested on rat skeletal cell lines. ^{1}H NMR-based metabolomics successfully predicted antiplasmodial and antigonococcal activity, with OPLS-DA analysis yielding *P*-values < 0.05. Aliphatic, allylic, methyl ketone, and carboxylic-based phytoconstituents were tentatively attributed to the observed antiplasmodial activity. Antigonococcal compound classes were aliphatic, alcohol, aldehyde, and carboxylic acid-based classes. The study demonstrates the potential of bioprospecting *Ziziphus* species for antiplasmodial and antigonococcal activity. It marks a significant milestone as the first to report *in vitro* antiplasmodial and antigonococcal activity in *Z. rivularis* and *Z. zeyheriana*. Further isolation of bioactive constituents is currently underway.

Effects of the meter square ultra-high density planting technology on yield and phyto-nutritional composition of *Moringa oleifera*

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Moringa oleifera Lam. is a highly nutritious and fast-growing tree widely recognized for its medicinal, nutritional, and economic benefits. However, optimizing agronomic practices, particularly planting density, is crucial for enhancing nutrient yield while maintaining sustainable production. This study aimed to determine the effects of planting density and leaf age on the phyto-nutritional composition of *M. oleifera* to provide insights into improving its cultivation for food and industrial applications. The experiment was conducted using four planting densities including ultra-high density planting (10 x 10 cm), high density (30 x 30 cm), medium density (50 x 50 cm), and conventional density (100 x 100 cm), with young and mature leaves collected from each treatment. A factorial experimental design was used, and the nutritional composition, including protein, zinc content, and secondary metabolites, were analyzed using standard AOAC laboratory procedures. The leaves were also analyzed for biochemical compounds (total phenols and total carotenoids), and antioxidant activity, including 2,2-diphenyl-1-picrylhydrazyl, Ferric Reducing Antioxidant Power, and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid). Results revealed significant interactions between planting density and leaf age, influencing biomass distribution, nutrient allocation, and secondary metabolite accumulation. Ultra-high density planting enhanced zinc (34.35 ± 1.0 mg/kg) and proline content (1.40 ± 0.03%), particularly in young leaves, but reduced total biomass per plant (93.23 ± 0.72 g). Conversely, conventional density increased protein content (29.11 ± 0.3%) in young leaves but reduced yield per unit area (1562 ± 1.02 g). Ultra-high density planting also promoted stress-induced secondary metabolite accumulation, particularly total phenols (1260.9 ± 3.12 mg GAE/100 g DW), carotenoids (0.06 ± 0.01 mg β-carotene/100 g DW), and antioxidant activity in young leaves, suggesting an adaptive response to resource competition. Principal component analysis revealed distinct clustering patterns, highlighting metabolic shifts influenced by planting density and leaf age. These findings suggest that optimizing planting density can enhance *M. oleifera*'s nutrient and phytochemical profile while maintaining sustainable leaf production. Further research is needed to explore long-term physiological adaptations under different planting configurations.

Livestock husbandry in the Batswana communities: Evidence-based research on medicinal plants used in livestock practices

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Livestock husbandry is essential in Batswana communities for economic support, cultural significance and sustenance. Due to limited accessibility, and high costs of conventional veterinary services, the Batswana communities have maintained their ancient traditional practices by using medicinal plants to manage livestock health. The current review highlights ethnobotanical uses and pharmacological properties of medicinal plants used in livestock husbandry among the Batswana communities in southern Africa. Different scientific databases were explored to extract data from articles published in January 1997 to June 2024. The review integrates information on the traditional knowledge associated with the use of plants, their preparation and administration methods, and biological efficacy. A total of 116 plant species (belonging to 44 families) were documented as being traditionally used plants for managing nine categories of livestock conditions. The most prominent plant species were *Senna italica* Mill. (10 citations, for treating 16 conditions), *Terminalia sericea* Burch. ex DC. (8 citations, for treating 14 conditions), and *Ziziphus mucronata* Willd. (8 citations, for treating 12 conditions). Based on the ethnobotanical data retrieved from surveys, 52% of the plant species have empirical data on their biological effect, safety, and phytochemicals. The pharmacological activities of the plants were mainly generated using *in vitro*-based assays relative to findings from *in vivo* test systems. Given the identified research gaps, there is a further need for well-planned ethnobotanical surveys in some specific areas and evidence-based pharmacological studies to create a comprehensive inventory of environmental-friendly botanicals with potential for livestock management.

Investigating the use of *Hermannia geniculata* leaves for the presence of anticancer induced inflammation

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This investigation focuses on *Hermannia geniculata* that is found within the Maluti-a-Phofung municipality, located in Thabo Mofutsanayane district in the eastern Free State and is surrounded by part of the Drakensburg mountains as well as Maluti mountains. The roots and leaves of *H. geniculata* have been used in traditional medicine for a long time as treatment for illnesses such as ulcers, sores, heartburn, colic and others. The primary aim of this project was to investigate the phytochemical constituents present in the leaves of *H. geniculata*, assess their potential anti-inflammatory and anticancer activities. Fresh leaves were collected, dried and subjected to solvents extraction using distilled water, acetone, ethanol, methanol and hexane respectively. The leaf extracts are anticipated to contain various phytochemical compounds, including alkaloids, flavonoids, phenols, and triterpenes, which exhibit biological activities such as antioxidant, antidiabetic, anti-inflammatory, and antimicrobial properties. Tests were made using the 5-lipoxygenase (5-LOX) enzyme to measure the anti-inflammatory effects of the extract by assessing reductions in inflammatory markers. The extracts were subsequently tested against various cancer cell lines through the MTT assay, to evaluate cytotoxicity level and the cell viability of *H. geniculata* in relation to its ability to prevent cell growth and induce apoptosis, or programmed cell death. This assay measures the metabolic activity of viable cells by converting MTT, a yellow tetrazolium salt, into a purple formazan product through cellular enzymes. A decrease in formazan formation indicates reduced cell viability and potential cytotoxicity of the extracts. This study contributes to the growing body of research on natural products, particularly those with roots in traditional medicine. If proven effective, the findings will confirm the traditional use of *H. geniculata* and provide a scientific foundation for developing new anticancer and anti-inflammatory drugs from this plant.

Traditional medicinal plants used in livestock health management in Namahadi District, Free State, South Africa

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Livestock production is essential for rural livelihoods and food security despite the farming sector facing major challenges caused by infectious diseases that lead to significant losses. Up to date, rural communities in South Africa still rely on traditional medicinal plants for the treatment and management of livestock conditions due to limited veterinary access and high costs associated with the consultation. Therefore, the study aimed to document indigenous knowledge and practices in the Namahadi District, Free State, South Africa. The conducted surveys used structured interviews involving traditional healers, livestock farmers, and community members with livestock knowledge. From the survey, a total of 34 plant species were recorded. The cited species were used for the treatment of diarrhoea (12 plant species), skin ailments (11 plant species), and parasitic infections (7 plant species). Amongst the documented livestock, a number of plants were used to manage conditions affecting cattle (50%), sheep (31%), and chickens (11%) while donkeys and horses accounted for 8%. The most commonly cited families included Asphodelaceae (21%), Convolvulaceae (16%), Rhamnaceae (16%), and Thymelaeaceae (11%). The preferred administrative route was oral (85%) compared to topical (15%). The findings highlight the importance of traditional knowledge in livestock management and the documentation of such valuable information can ensure sustainable and cost-effective animal healthcare solutions if developed into commercial products.

Ethnobotanical practices of medicinal plant use for skin issues in limpopo's rural village

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Skin diseases remain a significant health concern for humans of different ages. Healthy skin is directly proportional to one's high confidence level. Skin can also drop self-esteem from teenage acne to fine lines in middle-aged humans. On the other hand, it is seriously costly to rely on dermatological lotions and creams to deal with skin problems. It even becomes difficult for the inhabitants of the rural villages due to the high unemployment rate. This study focused on documenting ethnomedicinal uses of plants to treat skin diseases. Semi-structured interviews were conducted with 60 participants to document the plants used, the specific parts, the taste of the parts used, the plant forms, and the application of the plant parts for dealing with the ailments. The descriptive data analysis approach assisted with the knowledge of the Frequency of Citation (FC), Use value (UV), and Relative Frequency of Citation (RFC). In total, 23 plant species of 18 different botanical families were recorded. *Aloe arborescens* Mill. had the highest frequency of citation, followed by *Bidens pilosa* L. and *Psidium guajava* L. Treatment of skin ailments was by application of pastes and decoctions of the leaves, seeds, and roots. These species and application methods were mostly used based on their availability and level of efficacy. This shows the ongoing usage of medicinal plants by rural villagers. The study highly recommends biological bioassay experiments and expansion of the study group and area to ensure the validity of the outcomes.

Exploring the antidepressant mechanisms of *Leonotis leonurus* (L.) R. Br through network pharmacology and molecular docking

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Depression is a prevalent mental health disorder characterized by a complex pathophysiology, often requiring alternative therapeutic strategies. *Leonotis leonurus* (L.) R. Br has long been used in traditional medicine to manage mental health conditions, yet its antidepressant potential has limited scientific evidence and an unclear mechanism of action. This study explored the antidepressant mechanisms of *L. leonurus* through network pharmacology and molecular docking to identify its active compounds, target proteins/genes, and predict possible signaling pathways. Bioactive compounds were retrieved from various online databases, and potential antidepressant targets were identified using network pharmacology analysis, including protein–protein interaction (PPI) networks and gene ontology (GO) enrichment analysis. KEGG pathway enrichment was used to examine critical biological pathways involved in the pathophysiology of major depression. Furthermore, molecular docking was assessed via the binding affinity between major active compounds and key depression-related targets. A total of 36 active *L. leonurus* compounds, 88 targets associated with *L. leonurus* and 3079 targets linked to depression were identified. While serotonergic synapse, neuroactive ligand-receptor interactions and dopaminergic synapse pathways were profiled as the hub pathways, PTGS1, SCL6A4, and MAOB occurred as the most prominent therapeutic targets of interest. An insight into the mechanism of interactions between the prominent targets and the datamined constituents of the plant revealed marrubin (-9.1 kcal/mol), and calamenene (-9.0 kcal/mol), as putative leads against prostaglandin-endoperoxide synthase 1 (PTGS1), solute carrier family 6 member 4 (SCL6A4), and monoamine oxidase B (MAOB), respectively. These observations suggest that *L. leonurus* could exert its antidepressant effects by regulating SLC6A4, PTGS1, MAOB and other targets through serotonergic synapses, neuroactive ligand-receptor interactions and dopaminergic synapse signaling pathways. While the findings from the study provide credence to the primary pharmacological action of *L. leonurus* in alleviating depression, further experimental studies are required to validate and fully understand its therapeutic potential.

Medicinal plants used for the management of various ailments and disorders in the Eastern Cape, South Africa: A review

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Aboriginal people in the Eastern Cape Province had, for many years, relied on the knowledge and use of medicinal plants to meet their healthcare and nutritional needs. In these communities, the use of traditional medicine is mainly associated with spiritual practices and cultural beliefs. Therefore, identification and documentation of medicinal plants that are predominantly used in these communities are crucial for both research and conservation of biodiversity. A comprehensive review was conducted to gather information using various scholarly search engines such as Google Scholar, Science Direct, Scopus and Web Science. The literature review revealed that 62.4% of plants in the province are used as herbal medicine, 30.4% for food, 18.4% for ethnoveterinary medicine, while 11.2% are used for construction (i.e. as timber and for thatching). Species in the Asteraceae, Solanaceae, and Asparagaceae plant families are essential in the management of various ailments. Forty-eight species were listed and used for the management of diabetes mellitus. Other diseases and disorders that are managed with the use of medicinal plants include stomach ailments, skin disorders, fever, fertility, TB, and cancer. Despite the historical use of medicinal plants, their adoption in the health care system is minimised due to unclear dosages. There are also toxicity concerns, which result in hesitation against the use of herbal products. Further, potential herbal-drug interactions have been reported as a safety concern. Since the onset of the “Covid era”, there has been an exponential growth in the use of herbal medicine and a gain in popularity amongst people irrespective of their educational status and religion. Most of the medicinal plants used are collected from the wild, and therefore, research on cultivation protocols should be prioritised for sustainable biodiversity.

Indigenous knowledge on the uses and morphological variation among *Strychnos gerrardii* N.E.Br. morphotypes at Emkhandlwini area, KwaZulu-Natal, South Africa

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Strychnos gerrardii N.E.Br., a member of the Loganiaceae family, is primarily known in rural communities for its edible fruits. However, indigenous knowledge on the uses and morphological diversity of native fruit trees such as *S. gerrardii* has not received much attention. This study aimed to document the existing ethnobotanical knowledge on local uses and morphological diversity of *S. gerrardii* morphotypes growing in the Emkhandlwini area. The existing indigenous knowledge was collected from 100 randomly selected participants using a structured questionnaire. A simple random sampling method was used to select participants. Different food uses of *S. gerrardii* include direct fruit consumption as well as fruit processing into fermented beverages and food products called *umBhantshi*, *uJwembe*, *amaHewu*, *umNkwankwa* in isiZulu local language, and alcohol. Therapeutic reports of *S. gerrardii* include its use to treat stomach disorders, influenza virus, high blood pressure, diabetes, and respiratory problems, as well as increasing milk production in females and cleansing of the body system. Other uses of *S. gerrardii* include its importance as a homestead defense against thunderstorms and lightning, treatment of livestock diseases, ornament making, and controlling traditional spiritual problems. *Strychnos gerrardii* varied morphologically according to its leaf, fruit, and organoleptic traits. Leaf colour varied from light-green, green, dark green, and grey. Most leaves were roundish followed by elongated, round and heart-shaped, and elongated and heart-shaped leaves. Fruit shapes ranged from roundish, wedged, and pear-shaped, with the wedge-shaped fruits being reported sweeter than other shapes. Indigenous knowledge will benefit future breeding initiatives and the selection of desired traits for domesticating the species as a food crop.

Assessing the antifungal activity of selected medicinal plants against economically important plant pathogens

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Phytopathogenic diseases, caused by pathogenic microbes, result in significant production losses in several commercially important crops. The continuous use of chemical pesticides to manage these diseases results in environmental contamination and the development of resistance in microorganisms. In contrast, medicinal plants present an environmentally friendly, easily accessible, and sustainable alternative for managing pathogenic diseases. This study aimed to assess the *in vitro* antifungal activity of different individual and combined extracts of medicinal plants traditionally used to treat plant diseases against twelve economically important pathogens, including *Aspergillus niger, A. flavus, Alternaria solani, Fusarium semitectum, F. graminearum, F. oxysporum, F. verticillioides, F. chlamydosporum, F. proliferatum, F. subglutinans, F. solani* and *F. equiseti*. Powdered plant samples were extracted with water and acetone. A microplate dilution assay was used to determine the minimum inhibitory concentration (MIC) of the plant extracts. Results showed that acetone extracts exhibited stronger antifungal activity (MIC < 1 mg/mL) than aqueous extracts. Nine acetone extracts showed activity against at least one pathogen, whereas only four aqueous extracts showed activity. The acetone extract of *Solanum campylacanthum* was the most active, with strong activity against *A. niger*, *F. semitectum*, *F. graminearum*, *F. oxysporum*, and *F. chlamydosporum* (MIC of 0.2 mg/ml for *A. niger* and 0.78 mg/ml for the rest). In conclusion, the results of this study demonstrate the potential of the selected medicinal plants as natural and environmentally friendly fungicides against economically important plant pathogens.

A survey of botanicals used by subsistence farmers to manage plant diseases in Madibeng Local Municipality, South Africa

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Annually, high food commodity losses are reported worldwide due to pests and plant diseases. Due to high cost, most subsistence farmers cannot afford chemical treatments, and hence often rely on botanical extracts as an alternative. This study aimed at documenting the ethnobotanical methods used for plant disease management in Madibeng Local Municipality, South Africa. An ethnobotanical survey was conducted; purposive sampling and snowball techniques were used to recruit 32 consenting participants. The data emanating from the survey was subjected to descriptive statistical and ethnobotanical analyses. The participants identified 14 plants belonging to 8 families that are used to manage plant diseases in various

crops. Amaryllidaceae and Fabaceae were the most represented families, each represented by three plant species. The most utilized species were *Capsicum annuum*, *Allium cepa* and *Aloe greatheadii* var. *davyana*, indicated with relative frequency of citations of 0.63, 0.19, and 0.19, respectively. Fruit (35%) and leaves (28%) were the frequently used plant parts in preparing remedies for managing different diseases, such as bacterial leaf spot, downy mildew, and powdery mildew, which appeared to be very common, affecting crops and causing production losses. Results obtained from this on-going study demonstrate the potential of botanicals in managing plant diseases, and could be significant in addressing the issue of food insecurity. However, further scientific evaluation is imperative to authenticate the efficacy of the botanical-based remedies and promote their integration into modern agricultural practices.

In vitro evaluation of antibacterial activity and associated cytotoxicity of Biogenic Silver Nanoparticles using various extracts of *Tabernaemontana ventricosa*

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The development of innovative nanocomposites and their applications to a range of goods and technologies with antimicrobial properties has received increased interest. However, recently researchers have been concerned about the toxic effects of silver nanoparticles (AgNPs) on cellular systems. Hence, this study aimed to evaluate biogenic silver nanoparticles' antibacterial activity and associated cytotoxic effects using various extracts of *Tabernaemontana ventricosa*. Synthesized AgNPs using leaf and stem extracts were subjected to the disc diffusion technique for testing against gram-negative and gram-positive strains, and the antibacterial efficacy of the produced AgNPs was evaluated. Conversely, the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) test was used to assess the nanoparticles' cytotoxic potential on several cell lines, including HeLa, MCF-7, and human embryonic kidney (HEK293) cells. The most potent antibacterial suppression against *Bacillus subtilis*, *Escherichia coli*, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus*, and *Pseudomonas aeruginosa* was demonstrated by the AgNPs that were made utilizing both powdered and fresh leaf extracts, with the highest zone of inhibition at 15.33 ± 0.58 mm. Whereas, in the human cervical cancer (HeLa) cell line, the AgNPs produced using fresh leaf extracts showed high cytotoxic effects (IC_{50} value 0.39 µg/mL). These findings suggest that the synthesized AgNPs using *T. ventricosa* extracts display adequate antibacterial activity but also some toxic effects.

Antifungal activity of the endophytic fungi associated with the medicinal plant *Nicotiana tabacum* from South Africa

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Endophytes are microorganisms that inhabit plant tissues asymptomatically for at least part of their life cycle, forming mutualistic relationships that enhance plant resilience by improving pathogen resistance, promoting growth, and aiding in nutrient uptake. The aims of this study were to optimize the endophytic fungi isolation method for extracting endophytic fungi from *Nicotiana tabacum* collected in South Africa, and to screen and characterize the endophyte's anti-fungal activity against four crop pathogens. Out of the twelve endophytic fungi that were isolated from the *N. tabacum* plant leaves, five were identified and seven remained unknown. The known endophytics were from two predominant genera, *Penicillium* and

Alternaria. Endophytic fungi Nt12 exhibited the greatest inhibitory effect against all pathogenic fungi, followed by Nt11. Endophytic fungi Nt5, which was identified as *Penicillium*, had the lowest inhibitory effect against all pathogenic fungi. These findings are significant in the context of plant pathology as they demonstrate the potential of endophytic fungi as biopesticides, offering a safer and more sustainable alternative to chemical fungicides. In conclusion, microbial agents such as biopesticides can be used for biological control of plant diseases.

Indigenous monkey orange (*Strychnos spinosa* Lam.) fruit as an ingredient to develop a healthy fermented wine beverage

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The importance of indigenous fruit trees as valuable natural resources with a potential to meet the nutritional, food, health, and socio-economic needs is increasing globally. The development of fermented beverages (wines) from underutilized African fruits presents opportunities for local value addition and economic development. This study was aimed at investigating the feasibility of fermenting monkey orange (*Strychnos spinosa* Lam.), a wild fruit known for its nutritional value and cultural significance. Fruits were collected from Bonamanzi Game Reserve, KwaZulu-Natal, South Africa. The pulp of the ripe fruits was carefully separated from the seeds manually and the juice was extracted by squeezing the pulp. The mixture was then filtered through a sterile cheesecloth to remove the remaining seeds and fibres. An equal amount of the filtered juice was transferred into sterile glass fermenters. The juice's initial Total Soluble Solids (TSS) was 3.6 Brix%. Microbial analysis of the juice showed high aerobic bacterial counts of 490,000 CFU/ml but very low levels of lactic acid bacteria, *E. coli*, and yeast/moulds (<10-100 CFU/ml). The total coliform counts were less than 10 CFU/ml. Fermentation was initiated by inoculating *Saccharomyces cerevisiae*, with an inoculum size of 170.4 CFU/ml. After eight days, 300 ml of fermenting juice was aseptically sampled for analysis. The antioxidant activity the beverage showed significant levels of activity in 2,2-picrylhy-1-drazyl (DPPH), Ferric Reducing Antioxidant Power assay (FRAP) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS), with the fermented beverage (wine) exhibiting higher levels than the juice. Similarly, the fermented beverage exhibited higher total phenolic compound value of 1.55 as compared to 1.47 mg TE/100 g DW of the juice. This study demonstrates the potential of monkey orange fruit as an ingredient for fermented beverages which can be further developed into wines upon more research. This further offers a sustainable way to utilize this indigenous fruit while creating value-added products.

Anti-inflammatory, anti-neuraminidase and cytotoxicity evaluation of selected medicinal plants traditionally used for the treatment of respiratory viral infections

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The incidence, morbidity, and mortality caused by respiratory viral infections remains a major global challenge. Respiratory viruses can be targeted directly or indirectly. One of the indirect viral reduction targeting looks to address the host response rather than solely focusing on the virus, this is done by the plant's metabolites restoring the balance of the inflammatory response that can cause organ damage if left unchecked. While the inhibition of neuraminidase is an attractive direct target for anti-influenza drug design as it halts viral propagation by interfering with effective shedding. Traditionally different plant parts can be used to combat different diseases or to treat one disease but using different mechanisms to target the disease,

however there is little to no scientific evidence of the mechanisms to how these anti-viral medicinal effects are achieved. The study aims to do an anti-inflammatory and anti-neuraminidase evaluation of the selected plants and check for their cytotoxic effects. An aqueous and ethanol cold maceration extraction was performed to get the extracts. The anti-inflammatory effect of the plant extracts and the formulated concoction against cyclooxygenase-2 (COX-2) inhibition was determined using colorimetric COX-2 inhibitor screening assay kit. For the neuraminidase inhibition activity, the Amplex Red Neuraminidase Assay Kit was employed to test the effects of the plant extracts on the influenza viral neuraminidase as per the manufacturer's instructions. An MTT assay was employed to check for the extract's toxicity using the Vero and raw macrophage cell lines. The aqueous root extract of *Lippia javanica* showed the highest inhibitory activity of COX-2 anti-inflammatory activity at 5µg/ml compared to all other extracts. The aqueous root extract of *Lippia javanica* also showed the lowest IC50 at 0,05mg/ml for the inhibitory activity of viral neuraminidase. The aqueous extracts of all the plant parts for the medicinal plants showed higher anti-neuraminidase activity when compared to ethanolic extracts, this could mean that the active components against neuraminidase activity could be extremely polar in nature. The results obtained from this study suggest that the medicinal plants *Artemisia afra*, *Lippia javanica*, *Tetradenia riparia* and *Tulbaghia violacea* with the reported antiviral properties could be a potential source for new antiviral drugs that target neuraminidase and uncontrolled inflammatory inhibition in respiratory viruses.

An investigation of ethnobotanical utilization of indigenous plants of Tshivhilidulu Village in Limpopo Province, South Africa

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This ethnobotanical study examines the traditional knowledge and practices related to the use of indigenous plants by the Tshivhilidulu Village community. Such studies provide critical insights into the cultural, medicinal, and economic roles of plants, especially in rural areas where traditional knowledge is passed down through generations. Indigenous plants play an essential role in Limpopo Province and have been utilized by local communities for generations. The study aimed to document, categorize plant parts, categorize their utilization and determine the conservation status of indigenous plants utilized. Of the 30 participants interviewed, females (n = 25) were dominant gender compared to males (n = 5). The most dominant ages were middle and old age with the same frequency (n = 12), while the youth (n = 6) were the least represented. This was because the youth were at school during the interviews. Data was collected using semi-structured questionnaires and field observation. Data were analysed using descriptive statistics. A total of 51 indigenous plants were documented from 32 families, with Fabaceae being the dominant family. Leaves, bark, roots, and wood were used for various purposes. These plants were classified into trees, herbs, shrubs, and climber life forms. They were utilized as medicine, food, vegetable, shade, needle, birdlime, pesticide, banana ripening agents and firewood. The dominant conservation status of these plats was 'Least Concern'. The study shows that the community of Tshivhilidulu continues to depend on indigenous plants for some of their daily activities. However, the sustainability of these plant resources is seriously threatened by overharvesting. The study emphasizes the necessity of sustainable harvesting methods, plant conservation education, and the need for additional research to ensure future use.

Establishing the use-pattern of wild edible plants among the Batswana in Ngaka Modiri Molema District, South Africa

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Wild edible plants (WEPs) have long been an essential part of the cultural heritage of the Batswana in the North-West Province of South Africa. However, the knowledge surrounding WEPs and their traditional uses is at risk of disappearing due to limited systematic documentation. This study aimed to record the indigenous knowledge and practices associated with WEPs used by the Batswana in Ngaka Modiri Molema District. Through purposive sampling and snowballing methods, 60 participants were recruited, leading to the identification of 103 WEPs belonging to 34 botanical families. The most frequently utilised plants were *Amaranthus thunbergii* Moq. (66.6%), *Grewia flava* DC. (65%), and *Ziziphus mucronata* Willd. (53.3%). The most represented plant families included Asteraceae (42%), Solanaceae (30%), and Malvaceae (19%). In addition to their use as food, 75% of the recorded WEPs were considered as medicine by the participants. Fruits (42.2%) and leaves (28.8%) were the most consumed plant parts. Overall, the study highlights the rich and diverse knowledge of WEPs among the Batswana. Preserving these practices remain crucial for protecting biocultural diversity and maintaining indigenous knowledge on plant utilisation. Furthermore, WEPs hold significant potential in enhancing food security and promoting future economic opportunities.

Phytochemical variability in *Laggera alata* and *Laggera crispata* forms from Southern Africa

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Laggera species have a wide range of therapeutic activities including antimicrobial, antiviral, antibacterial, and anti-inflammatory. However, the identification of the specific phytochemicals responsible for these activities are limited. The aim of this study was to identify and quantify the phytochemical profile of *Laggera alata* and *L. crispata* forms and assess variability in their phytochemical content using Gas Chromatography-Mass Spectrometry (GC-MS). The leaf extracts of *Laggera* forms were rich in monoterpenes, sesquiterpenes, diterpenes, hemiterpenes, and ketones. A total of 32 phytochemicals were common across all studied *Laggera* forms. Among these compounds, Benzene, 1,3-dimethyl-; O-xylene; Ethylbenzene, and Decane were the most abundant phytochemicals. *Laggera alata* with a small capitulum (LA-SC), *L. crispata* from South Africa (LC-SA), *L. alata* with a big capitulum (LA-BC), and *L. crispata* from Zimbabwe (LC-ZIM) had 72, 66, 46, and 35 unique phytochemicals, respectively. The first three principal components accounted for 100% of the total variance, signifying heterogeneity in the phytochemical profiles of *Laggera* forms. The biplot highlighted the phytochemical diversity across various forms of *Laggera*, with specific sets of compounds accounting for the observed chemical variations among the different forms. In the dendrogram, *Laggera* forms were clustered based on their geographic origin. Specifically, LA-SC, LA-BC, and LC-SA grouped together while LC-ZIM formed a singleton. This study provides a foundation for future research aimed at isolating these compounds for potential applications in the pharmaceutical, cosmetic and food industries.

Preliminary phytochemical screening and antioxidant potential of *Bulbine inflata* (Asphodelaceae)

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This study aimed to investigate the phytochemical profile and antioxidant potential of *Bulbine inflata*, a species within the *Bulbine* genus with a paucity of documentation for its medicinal uses. Phytochemicals from the leaves were extracted with different solvents (water, methanol, ethyl acetate, and hexane) after air- or freeze-drying. The extracts were used to carry out qualitative and quantitative analyses of phytochemicals as well as various antioxidant assays. The qualitative screening revealed the presence of tannins, flavonoids, phenols, glycosides, steroids, coumarins, quinones, saponins, and terpenoids in *B. inflata* leaves. Most phytochemicals were detected in extracts using polar solvents, especially water, and in freeze-dried samples. Quantitative analysis showed high total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC), particularly in the water extracts. The highest TPC was found in air-dried water extract, while freeze-dried water extract had the highest TFC. *Bulbine inflata* exhibited moderate to high antioxidant activities against 2, 2 diphenyl-1-picryhydrazyl (DPPH), hydrogen peroxide (H_2O_2), and in metal chelating assays. The water extract showed the highest DPPH inhibition capacity. Ethyl acetate extract from air-dried leaves had the highest antioxidant activity against H_2O_2. Freeze-dried samples generally presented higher antioxidant results compared to air-dried samples. The study concluded that the presence of significant phytochemicals and high antioxidant capacity suggests the potential of *B. inflata* for medicinal applications, potentially expanding ethnomedicinal resources. The findings also indicate that polar solvents are more effective for extracting phytochemicals from *B. inflata*.

Metabolomics-guided screening of chosen southern African plants for antifungal candidates with a specific focus on the FBA-II covalent inhibitor

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Ethnopharmacological research serves as a link between indigenous knowledge and modern drug discovery, offering insights into the mechanisms and therapeutic potential of plant-derived compounds. This study aimed to investigate the antifungal efficacy of three southern African medicinal plants—*Aloe ferox*, *Salvia aurea*, and *Tulbaghia violacea*—against the azole-resistant pathogen *Candida albicans*, alongside selected bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus epidermidis*, and *Bacillus subtilis*) to assess further antimicrobial properties. Given the declining effectiveness of existing antifungal drugs, there is an urgent need to identify novel synergistic and/or isolated bioactive agents- particularly against the FBA-II covalent inhibitor. Three plant species were collected and extracted following green extraction methods. Untargeted Liquid Chromatography-Mass Spectrometry (LC-MS) was used to characterise the metabolite composition of each plant. In parallel, we assessed the profiles of three solvents, three plant parts, and two drying methods. Thereafter, the solvent containing the highest metabolite abundance amongst the plants were used to complete antimicrobial assays. A diverse range of metabolites, including campherol (*Tulbaghia violacea*), aloein (*Aloe ferox*), and rosmarinic acid (*Salvia aurea*), was evident. Varying metabolites were isolated amongst different drying methods, plant parts, and solvents. The 50% methanol extracts showed the richest profiles via LC-MS and was thus selected for testing using the minimum inhibitory concentration (MIC) bioassay. Freeze-drying generally resulted in higher antifungal activity than sun-drying, particularly evident in *T. violacea* and *A. ferox* extracts. Freeze-dried roots of *T. violacea* achieved nearly complete inhibition at 5 mg/mL. Sun-dried extracts were less potent at lower concentrations, suggesting phytochemical degradation or reduced concentration. These findings highlight the importance of optimising extraction methods when evaluating natural products. The selected indigenous plants thus perform as promising candidates for developing alternative antifungal therapies and produce chemical isolates that target the FBA-II covalent inhibitor.

Ethnobotanical knowledge and cross-cultural utilisation of plants in Southwestern Nigeria: Insights from Ogun, Oyo, and Ondo States

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Ethnobotanical knowledge plays a vital role in conserving biodiversity and its sustainable uses. This study explored the ethnobotanical practices and uses of plants across diverse cultures in southwestern Nigeria, specifically in Ogun, Oyo, and Ondo States. Through structured ethnobotanical surveys, key informant interviews, and field observations, a comprehensive record of medicinal, nutritional, cultural, and economic plant species was generated. In total, 520 semi-structured questionnaires were distributed to indigenous participants from various ethnic backgrounds, resulting in the identification of 239 plant species belonging to 63 botanical families. The findings revealed significant overlap in plant uses among the three states, with plants such as *Vernonia amygdalina*, *Ocimum gratissimum*, and *Mangifera indica* frequently indicated for medicinal, nutritional, and culinary uses. Notably, the Fabaceae family, comprising 42% of the identified plant species, is recognised for its medicinal value and crucial role in maintaining ecological balance. In addition, Malvaceae, Asteraceae, and Euphorbiaceae families accounted for 30.15%, 20.63%, and 19.05%, respectively. Cross-cultural comparisons indicated that 68% of the plants were common to all the three states, while 20% were unique to specific states, highlighting cross-cultural variations in ethnobotanical knowledge and ecological factors influencing plant availability and use. Additionally, this research emphasises the urgent need for sustainable practices to conserve biodiversity and preserve traditional knowledge, as 75% of participants expressed concerns about the loss of plant species due to urbanisation and climate change. Overall, the insights gained from this study deepen our understanding of the intricate relationship between cultural identity and environmental stewardship in southwestern Nigeria.

Fungal intruders meet floral enforcers: The *Pelargonium* showdown

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Fungal infections caused by *Candida albicans* are a major public health concern, specifically in immuno-compromised individuals. The growing resistance to azole-based antifungal drugs underscores the urgent need for novel therapeutics with alternative mechanisms of action. In this study, four *Pelargonium* species (*P. elongatum*, *P. quercifolium*, *P. ribifolium*, and *P. odoratissimum*) endemic to the Greater Cape Floristic Region were assessed for their antifungal activity and metabolomic composition. Using various solvents (acetone, acetonitrile, methanol, and hydro-methanol with 1% formic acid) and different plant parts (leaves and roots), crude extracts were initially screened for any antifungal activity using a solid surface assay against *C. albicans*. Acetone-based extracts displayed the most promising antifungal activity with a growth inhibition range of 3.125 to 6.25 mg/mL. To better understand the metabolite profiles underlying this activity, a Liquid Chromatography Mass Spectrometry (LC-MS) metabolomics approach was used to annotate and cluster significant metabolites across the extracts. The metabolomic data revealed rich profiles enriched with coumarins, benzoic acid derivatives, organic acids, fatty acids, and phospholipids. Principal Component Analysis (PCA) of the leaf extracts showed 53.4% variance, with some overlapping metabolite patterns indicating shared chemical traits across species. In contrast, root extracts had more distinct clustering, suggesting clearer chemical differentiation among species. Complementary *in silico* studies involving molecular docking and dynamics simulations were conducted to evaluate compound interactions with the fungal protein Fructose 1,6-bisphosphate aldolase (FBA). The findings of this study provide valuable insights into the chemical diversity of *Pelargonium* species and highlight their potential as sources of novel antifungal agents. This work not only helped identify promising candidates for the development of antifungals but also underscores the broader therapeutic potential of these species. This study informs future isolation, characterisation, and possible pharmaceutical application of bioactive compounds from selected *Pelargonium* species.

Exploring plant-derived alternatives for sustainable bovine mastitis management: Compound isolation and nanotechnology application

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Bovine mastitis is a major problem in dairy industry, causing financial losses and animal welfare concerns. The use of antibiotics is complicated by pathogen resistance and drug residues, leading to increased interest in alternative therapies. Medicinal plants, with bioactive compounds possessing antibacterial, anti-inflammatory, and immunomodulatory properties, offer promising solutions for sustainable mastitis management. This study investigates natural plant-derived compounds as alternatives for bovine mastitis treatment. The species studied, (*Searsia* sp.) was extracted and fractionated through liquid-liquid partitioning, with the dominant bioactive compound isolated using column chromatography. Bioactivity testing included anti-inflammatory activity, cytotoxicity assay, and antibacterial activity. Tentative compound identification was performed through Ultra-Performance Liquid Chromatography-Mass Spectrometry (UPLC-MS) whereby the crude extract yield revealed 10 major compounds, with the dominant bioactive compound successfully isolated. Additionally, the crude extract was used to biologically synthesize silver nanoparticles (AgNPs), which were characterized using UV-Vis spectroscopy and Dynamic Light Scattering (DLS) to determine size distribution, zeta potential, and polydispersity index (PDI). AgNPs depicted an average hydrodynamic size of 170 nm, a zeta potential of -31.6 mV, a PDI of 0.253, and maximum absorbance at 375 nm. The nanoparticles remained stable for over 4 weeks. All tested treatments (extract, fractions and nanoparticles) showed efficacy against bovine mastitis bacterial strains. The crude extract showed good antiinflammatory (IC_{50} 44.19±1.49 µg/ml), antioxidant (IC_{50} 44.90±5.42 µg/ml and 35.63±3.93 µg/ml in ABTS and DPPH radical reduction assays, respectively), and cytotoxicity (IC_{50} 0.79± 0.09 mg/ml) observed in mammalian cell tests. In conclusion, these results underscore the potential of *Searsia* species as effective alternatives for treatment of bovine mastitis in addressing the challenges of antibiotic resistance and drug residues in dairy farming.

Aromatic plants of the cape fynbos: A neglected resource in essential oil research

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The Fynbos of the Cape Region is one of the most biodiverse and highly endemic floristic regions in the world, with an estimated 9,600 recorded plant species. Among these are a remarkable number of aromatic taxa traditionally used for their fragrance, flavour, and medicinal properties. Despite this richness, only about 69 species from eight plant families have been chemically investigated for their essential oil profiles. These include Asteraceae (36 species), Rutaceae (12), Lamiaceae (8), Geraniaceae (6), and Apiaceae (4), a surprisingly low number considering the size, diversity, and known presence of essential oil-bearing species in these families within the Cape flora. Several well-known genera are reported in the literature as aromatic, such as *Adenandra*, *Agathosma*, *Diosma*, *Helichrysum*, and *Pelargonium*, but remain chemically poorly known. Moreover, while some species exhibit aromatic traits, they may yield only trace amounts of volatile oil, a factor that is rarely addressed but carries important implications for the sustainable use and commercial viability of some of these species. This paper provides an overview of the known aromatic flora of the Cape Fynbos and highlights the substantial gaps in phytochemical data. It argues for renewed and systematic research into the essential oil profiles and chemistry of this underexplored flora, both to advance our scientific understanding and potentially unlock new opportunities for local economic development through the responsible and sustainable use of our indigenous biodiversity.

An appraisal of the South African antibacterial plants and their computational bioprospection for putative leads against *Staphylococcus aureus* penicillin-binding protein 2a and beta-lactamase

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The penicillin-binding protein 2a (PBP2a) and beta-lactamase play important roles in the resistance of *Staphylococcus aureus* to beta-lactam antibiotics, making them vital targets for the discovery of new chemical entities. A vast majority of South African medicinal plants (SAMP) with reported antibacterial properties have not been sufficiently explored to date. This study documented SAMP with reported antibacterial activities for putative leads against *S. aureus* PBP2a and beta-lactamase using computational studies. A total of 187 SAMPs with antibacterial activity were identified, spanning 67 families, with Fabaceae (18), Asteraceae (19), and Lamiaceae (11) being the most prominently represented. A library of 4230 secondary metabolites (SM), predominantly identified from leaves of the plants, was constructed and docked against the active site of *S. aureus* PBP2a. The top-ranked SM were further docked to the allosteric site of *S. aureus* PBP2a, beta-lactamase and their ensembles obtained from conformational clustering and investigated for druggable pharmacokinetic and synthetic features. Thermodynamic stability analysis of the resulting complexes of the leads over a 120-ns MD simulation period revealed astragalin 60’-gallate (-62.11 and -39.70 kcal/mol) and 20(29)-lupene-3b-isoferulate (-55.66 and -42.88 kcal/mol) as the most potent against *S. aureus* PBP2a and beta-lactamase compared to reference standards, ceftaroline (-46.06 kcal/mol) and clavulanate (-21.40 kcal/mol), respectively, with an allosteric modulatory effect on Tyr446, the active site gatekeeper residue in *S. aureus* PBP2a. Astragalin 60’-gallate and 20(29)-lupene-3b-isoferulate, identified in *Myrothamnus flabellifolia* and *Euclea natalensis*, respectively, are likely contributors to the reported antibacterial activity of these plants. The potential dual-target inhibitory properties and the ability to modulate the active site conformation of *S. aureus* PBP2a position these metabolites as promising candidates for further investigation in combating *S. aureus* infections. Efforts are underway in this direction.

Phytochemical and antifungal effect of botanicals used traditionally for managing fungal pathogens in Mpumalanga Province, South Africa

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Crop losses due to phytopathogenic diseases contribute to food insecurity. This study aimed to characterise the phytochemical profiles and evaluate the *in vitro* antifungal efficacy of eleven plants used in traditional crop disease management. Different parts of the eleven plant species were extracted with acetone, water and hexane. The phytochemical characterisation was undertaken using a spectrophotometric method, while the antifungal activity was investigated using micro-plate dilution assay against 11 fungal phytopathogens, including *Aspergillus flavus*, *Fusarium chlamydosporum*, *F. equiseti*, *F. graminearum*, *F. oxysporum*, *F. proliferatum*, *F. solani*, *F. subglutinans* and *F. verticillioides*. The plants with the highest total phenolic content (TPC) for the water extraction were *C. sativa* (34.79 mg GAE/g), *S. birrea* (44.50 mg GAE/g), *M. esculenta* (66.74 mg GAE/g), *E. diversicolor* (81.39 mg GAE/g) and *P. pinaster* (94.15 mg GAE/g). The highest concentrations for *C. aconitifolius* (10.62 mg CE/g DW), *C. sativa* (12.84 mg CE/g DW), C. annuum (14.10 mg CE/g DW), *E. diversicolor* (14.28 mg CE/g DW), and *D. cinerea* (15.02 mg CE/g DW) were recorded when hexane was used as the extraction solvent. Good antifungal activity with minimum inhibitory concentration (MIC) ≤ 0.1 mg/ml against some tested pathogens was demonstrated by *D. cinerea*, *E. diversicolor*, *M. esculenta*, *Capsicum annuum*, *Bidens pilosa*, *P. pinaster*, *and Annona cacans*. Compared to individual extracts, a synergistic effect was observed when combining *D. cinerea* and *E. diversicolor,* as well as *E. diversicolor* and *M. esculenta*. The phytochemical and antifungal activity results of the plant species in the current study underscore the potential efficacy of these plant extracts for sustainable disease control, aligning with the broader goals of promoting healthy crop production and supporting sustainable agriculture. Further research is needed to isolate the active compounds in plant species with antifungal properties, potentially leading to the discovery of new natural antifungal agents.

Conserving South Africa's iconic and critically endangered African wild ginger, *Siphonochilus aethiopicus*

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In South Africa, millions of people rely on medicinal plants for primary healthcare. Unfortunately, such plants are under constant threat due to unsustainable harvesting. One such species, *Siphonochilus aethiopicus* (Schweif.) B.L. Burt has been overharvested for traditional medicine, leaving extremely low numbers in the wild and resulting in the plant being given the critically endangered conservation status. Its rhizomes and roots are highly valued for the principal bioactive compound, siphonochilone. Poor seed production and viability make long-term conservation through seed banking unfeasible. Conventionally, rhizome buds are vegetatively propagated, but only one plant is produced per bud. Furthermore, previous micropropagation attempts yielded only 3 to 4 plants per bud. Therefore, we aimed to develop effective conservation strategies through improved micropropagation and cryopreservation. Furthermore, we quantified the siphonochilone in micropropagated clones. Plant yield was improved to 13 plants per bud by optimizing plant growth regulators at the shoot multiplication (5 mg L benzylaminopurine [BAP]) and rooting (0.1 mg/L BAP + 1 mg/L indole-3-acetic acid) stages, using very small (±1 mm) in vitro-derived shoot apices as explants following in vitro bud culture. Gas Chromatography-Mass Spectroscopy confirmed the presence of siphonochilone in micropropagated clones, with mature plants exhibiting higher rhizome (36 g) and root (16 g) biomasses, and root siphonochilone yield (0.5 g/g extract), than conventionally propagated plants (4 and 1 g, and 0.04 g/g extract, respectively). Rhizomes had similar siphonochilone content in the comparative populations. Shoot apices were successfully cryopreserved using a droplet-vitrification method, which resulted in 67% regrowth following storage in liquid nitrogen. Successful recovery is a crucial step towards long-term preservation of *S. aethiopicus*; however, additional research is required to improve recovery rates and to quantify siphonochilone in cryopreserved regenerants. Further research on improving micropropagation yields with the use of bioreactor culture is also needed. Lastly, successful cell suspension cultures could also provide a means of sustainable siphonochilone production.

Antimicrobial activity and anti-inflammatory activity of medicinal plants used in ethnoveterinary remedies for gastrointestinal illnesses

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Ethnoveterinary medicine is important for rural communities with limited access to veterinary services. Gastrointestinal infections can result in losses to livestock farmers by compromising the health of the livestock, and affect the safety of meat and dairy products. Plant bioactive compounds are effective in managing gastrointestinal illnesses in animals. The study aims to evaluate the efficacy of medicinal plants identified from a survey conducted in the North West Province, South Africa, to manage gastrointestinal infections in livestock. *Artemisia afra*, *Peltophorum africanum*, *Bulbine frutescenc, Ziziphus mucronata* and *Z. zehriana* were selected for screening due to their reported use in traditional practices. Extracts were prepared using polar and non-polar solvents. The serial dilution microplate assay was used to determine the species' *in vitro* antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, *Mycobacterium smegmatis* and *Mycobacterium fortuitum.* The 15-lipoxygenase assay was performed to analyse potential anti-inflammatory activity. *Artemisia afra* stem methanol extract showed good activity against *E. coli* and *S. aureus* [minimum inhibitory concentration (MIC) = 0.313 mg/ml]. *Zanthoxylum capense* leaf methanol extract also exhibited good activity against *M. smegmatis* (MIC = 0.313 mg/ml). The *Artemisia afra* leaf extract (ethanol) inhibited 92% 15-lipoxygenase enzymatic activity at 0.5 mg/ml while *A. afra* stem extracts (ethanol) inhibited 59% enzymatic activity at 0.063 mg/ml. Further research is ongoing to determine the safety and metabolite profiles of the reported plant species.

Phytochemical and proximate analysis of the leaves of *Morinda citrifolia*

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Morinda citrifolia Linn (Rubiaceae), commonly known as Noni, is a traditional and Polynesian medicinal plant that has been used for more than 2000 years. When the ancestors of Polynesians migrated from South East Asia they brought many plants with them for food and medicinal purposes. Among them, Noni was one of the most popular herbal medicinal plants that was used to treat many types of diseases. The aim of this study was to determine the phytochemicals, and conduct a proximate analysis of *Morinda citrifolia* leaves. Fresh leaves of *Morinda citrifolia* were collected at the biological garden. The plant was identified and authenticated at the Department of Biological Sciences, Sokoto State University. Results of phytochemical analysis of *Morinda citrifolia* showed the presence of phytochemicals like steroids, cardiac glycosides, terpenoids, carbohydrates and flavanoids in elevated amounts. The proximate analysis indicated that the leaves has a high carbohydrate content (76.53 ± 0.13 units), making it a potential source of energy. Additionally, the crude protein content (12.46 ± 0.22 units) suggest that it could also serve as a dietary protein source. The low crude lipid (0.6 ± 0.2 units) and moderate fiber content (2.75 ± 0.30 units) further highlight its nutritional value, indicating that it can be included in a balanced diet. Mineral analysis showed significant levels of potassium (345.17 ± 0.04 units) and calcium (213.15 ± 0.14 units); essential for various physiological functions, including muscle contraction and bone health.

An updated review of ethnobotanical studies in South Africa

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In several published reviews, the need for a more complete inventory of the traditional uses of plants in South Africa has been highlighted. This has been particularly relevant to the traditional Khoe and San regions of the Cape and Karoo, with their high levels of endemic species. Much progress has been made in the last two decades, especially in terms of medicinal ethnobotany, as summarized in two recent papers by Cornelius and Van Wyk (2023, 2024). Several publications and unpublished dissertations and theses are now available, covering almost all the cultural groups and regions of South Africa. Inventories of medicinal plants are available, or are nearing completion, not only for southern Africa in its entirety, but also for several regions in the Western and Northern Cape provinces, as well as the Eastern Cape, Free State, Limpopo, Mpumalanga, North-West, and KwaZulu-Natal provinces (as well as Lesotho and Eswatini). Most of the studies are concentrating on medicinal ethnobotany, but food plants have also been receiving considerable research attention. There is a conspicuous paucity of information on craft plants and other miscellaneous applications. To maximize the socio-economic benefits of the rich South African flora, it is important to document all the various traditional uses of all South African plant species, so that this precious knowledge remains available for future generations. Future innovations of scientific and commercial importance will not only rely on new technologies but also on the indigenous wisdom about the uses of plants that was gained through millennia of trial and error. Once lost, such knowledge can never be regained.

Natural Resource Management and Conservation, Ecology (NRMC)

Poaching exacerbates the effects of climate change on the long-term viability of an endemic South African succulent plant species

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Natural populations often face multiple threats that jeopardize their viability, yet most population forecasts consider only a single threat. To better address ecological and conservation challenges, it is crucial to account for multiple threats. South African dwarf succulents are at risk from both climate change and illegal harvesting for the ornamental plant trade. To assess these combined impacts on dwarf succulents, we developed a stochastic integral projection model (IPM), using demographic data from 1999-2003 for *Argyroderma pearsonii*. Our IPM was used to evaluate short-term (<40 years) extinction risk and long-term viability under 90 simulation scenarios combining climate conditions (historical, mild climate change [SSP126], and moderate climate change [SSP245]), harvesting methods (fruit harvesting, non-selective plant harvesting, or size-selective plant harvesting), and harvest frequencies (annual to once every decade). Additionally, we used perturbation analyses to identify critical demographic components influencing population persistence. We show that historically *A. pearsonii* populations have remained stable or growing. Our projections suggest that populations will remain viable under mild climate change, but moderate climate change leads to declines in approximately 50% of simulated populations. However, extinctions are not projected within 40 years unless harvesting is introduced. Plant harvesting significantly increases extinction risk, especially size-selective harvesting of mature individuals, which disrupts critical demographic processes. Even harvesting plants as infrequently as once per decade leads to declines under historical conditions, with climate change exacerbating these effects. However, fruit collection emerges as a potentially sustainable alternative to meet the ornamental plant trade's demands. Our study highlights the importance of incorporating multiple threats into population forecasts, providing valuable tools for ecologists and conservation managers.

Best practice for mapping aboveground carbon in Afrotemperate forests

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Under the Paris Agreement, signatories are obliged to reduce carbon emissions and enhance carbon sinks. Multiple global aboveground biomass products are now available; however, they still require additional calibration and validation datasets at the local scale. I use South Africa's Afrotemperate forests as a case study to investigate best practice for remote sensing aboveground carbon for improving the accuracy of reporting under the Paris Agreement. I collected both field and LiDAR data for three forests in KwaZulu-Natal, South Africa, and tested three different allometric equations for calculating AGC. I used linear models to predict AGC from LiDAR data for both local and multi-site models and then compared the results with two global biomass products. The locally derived allometric produced intermediate AGC values to the temperate and pan-tropical equations and is recommended for use in South African forests. Local LiDAR models for each forest varied in performance with R2 values ranging from 0.16 to 0.75, and RMSE% of 7.3 to 50%. Overall, the multi-site LiDAR model performed better than two of the three local models with a conditional pseudo-R2 of 0.82 and RMSE on 24% (30 MgC.ha-1) and is recommended in the absence of field data. However, since the forest physiognomy is diverse, it is important to take local context into account when interpreting AGC results. Although global biomass products tend to overestimate the carbon in these forests, they can still be used for the UNFCCC’s 2023 global carbon stock take until further calibration and validation data have been included. While generalized mapping techniques may produce some errors in AGC estimates, it's important to use the data currently available and report the uncertainty in the estimates as part of mitigating anthropogenic changes.

Observations of demographic changes in eight populations of *Aloidendron dichotomum* over a 14-year continuous survey period

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The tree aloes of southern Africa are enigmatic species with value to society as emblems of arid ecosystems and the associated tourism value that they garner. The plants also have ecosystem service value through the provision of shade and nesting sites to animals, food sources and pollination services to birds and other wildlife, as well as refuge and perching spots. Recent research has indicated the potential impact of climate change on arid region tree aloes and their potential as climate change indicators, although other environmental drivers are also at play. Most species are currently cultivated for the ornamental plant market but have also been considered for sustainable harvesting. In light of the paucity of data on the long-term dynamics of tree aloes and how these dynamics may relate to climate impacts and harvesting potential, the Northern Cape DALRRD undertook a long-term monitoring project of the common Quiver Tree, *Aloidendron dichotomum*, in 2010 to augment the current research on the plant group. The aim is to track the size and health of specific individuals in designated monitoring plots in each of eight large populations that represent the range extent of the species in the Northern Cape. Plots were set up in 2010 – 2013 and resurveyed in 2015, 2018, 2021 and 2024. Demographic variation in the different populations was evident and several biotic drivers were identified that were unique to specific populations. An analysis of (a) population demographic structure (curve type), (b) Centroid vs. Median size classes and (c) linear regression slope of size classes, points to populations that are mostly stable but unsuited to harvesting.

Mitigating microbial dysbiosis under environmental stress: Engineering plant-microbe interactions for sustainable remediation

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Microbial interactions within the rhizosphere and endosphere are partially regulated by plant signalling molecules, notably root exudates, which play a crucial role in shaping microbial communities under conditions of abiotic stress. Such stressors trigger specific shifts in the soybean microbiome that correlate with changes in root exudate composition and the physiological responses of the plant. This study investigates the impacts of nutrient stress on the dynamics between soybean plants and their associated microbes, utilising both culturedependent (e.g., Biolog assay) and culture-independent (e.g., metagenomics) techniques to analyse shifts in microbial community structure within rhizosphere soil and the root endosphere following stress induction. Screening tests for plant growth-promoting traits were conducted to identify beneficial microbial isolates with the potential to mitigate stress. Our results indicate that nutrient stress leads to changes in the composition and diversity of microbial communities in both the rhizosphere and endosphere, driven by alterations in root exudate profiles. Through a combined metagenomic and functional screening approach, we identified key bacterial isolates with notable plant growth-promoting traits, including phosphate solubilisation. Our findings highlight beneficial microbes with the potential to address significant agricultural challenges in South Africa, providing a framework for creating crop-specific, and sustainable microbiome-based solutions to boost crop productivity in nutrient-poor environments worldwide.

Life after plantations. Long-term vegetation dynamics reveal passive restoration of montane grasslands in South Africa

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Large areas of montane grasslands have been afforested with timber plantations or transformed to croplands. Some of these are now being abandoned, leading to the potential for restoration and additions to grassland conservation. To study the determinants of vegetation change in abandoned plantations, a long-term research project was established on the slopes of Mariepskop Mountain (Limpopo), where approximately 11 000 ha of *Pinus* and *Eucalyptus* plantations were decommissioned in the early 2000s. A patchwork of alien plant removals, informal timber harvesting and wildfires, combined with the natural variation of soils and rainfall across the site, have created a mosaic of vegetation types in varying stages of transition, providing a rare opportunity for a large-scale natural experiment. 60 permanent vegetation plots were established from 2018 to 2022 encompassing a variety of management histories and abiotic conditions. Historical aerial photography was used to establish the land use history for each plot, and to describe larger-scale changes. Isotopic analysis of soil carbon was used to confirm that all sites were dominated by C_4 grasses prior to afforestation, and to provide baseline data on soil organic carbon stocks. Secondary grasslands have developed on many of the former plantations, with estimated gamma plant species richness of 231 for herbaceous species and 45 for savanna species. These are high values considering the size of the study area (4000 ha), and comparable to estimates from primary grassland sites in the broader region. However, at the scale of 1 m^2 sampling quadrats, species richness was below that recorded in the last remaining patches of primary grassland (means of 2.6 versus 4.7 for graminoid species, and 1.9 versus 2.9 for forbs). Some of the former *Eucalyptus* plantations had transformed into novel savannas dominated by fire-tolerant, resprouting trees, and may require active restoration.

Non-timber forest products of Hamutsha Village in Makhado Local Municipality, Limpopo Province

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Non-timber forest products (NTFPs) are products other than timber produced naturally in the forest. These products are harvested for their different purposes within the homesteads of local communities surrounded by those forests. The study aimed to investigate the utilization of non-timber forest products by the community of Hamutsha Village, Limpopo Province, South Africa. Thirty research participants were interviewed on non-timber forest products they utilized from the surrounding forests. A total of 22 plant species was documented, with parts such as leaves (37%), fruits (26%), bark (22.2%), roots (7.4%), and whole plant (7.4%) harvested for various purposes. These plants were classified into three life forms namely, trees (n=11), herbs (n=9), and sedges (n=2). Their utilization categories included food (42%), medicine (38%), artifacts production (8%), smoking (4%), termite bait (4%), and insect repellent (4%). The conservation status of the species recorded revealed that most of the plants were either Least Concern (n=15) or Not Evaluated (n=7). However, local perceptions of availability varied, with most species being considered either very lowly (n=4), low (n=6), moderately (n=6), highly (n=3), or very highly (n=3) available. These findings emphasize the critical role of NTFPs in the community and highlight the importance of sustainable practices that should ensure long-term availability of these resources.

Recommendations include promoting sustainable harvesting, conservation awareness campaigns, and ongoing monitoring of plant populations to safeguard these valuable resources.

Whilst the gymnosperm tree of life may survive the ongoing biodiversity crisis, the angiosperm tree of life may not

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How would the tree of life be affected by the ongoing biodiversity crisis? We know that the vertebrate tree of life may survive this crisis. However, due to the lack of comprehensive risk assessment for angiosperms, this question could not be fully investigated for vascular plants. Here, I took advantage of the first ever comprehensive risk predictions for 328 565 angiosperm species to investigate, using simulations, whether high-risk vascular plant species represent a disproportionate amount of total evolutionary history in the plant tree of life. The findings showed significant association between risk status and evolutionary distinctness but only for angiosperms and not for gymnosperms, suggesting that future extinctions may result in a disproportionate pruning of angiosperm tree of life whilst most of the evolutionary history of gymnosperm might persist. To aid bending the curve of loss, I identified most over-threatened clades (e.g., Balsaminaceae) and priority species (e.g., *Hymenophyllum exquisitum*) for conservation.

Physiology (PHYS)

The growth potential of Kenaf (*Hibiscus cannabinus*) on coal tailings in South Africa

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Coal mining remains a fundamental component of South Africa's energy sector; however, mine closures result in the accumulation of substantial volumes of coal tailings, which have significant environmental and socio-economic implications. Sustainable remediation strategies are therefore essential, though the elevated concentrations of heavy metals in coal tailings pose considerable challenges. Phytoremediation has emerged as a potential solution, with kenaf (*Hibiscus cannabinus*) being a particularly promising candidate due to its high tolerance to diverse soil conditions and pH levels. Its extensive root system and high biomass enhance its capacity for metal uptake and soil stabilization. This study aimed to evaluate the feasibility of cultivating kenaf on coal tailings in South Africa by assessing the physiological responses of the plants. Kenaf seeds were sown in five distinct soil types: a sandy loam soil serving as the control, two raw coal tailings obtained from different mining sites, and two mixtures of coal tailings and soil combined in a 1:2 ratio. Over a two-month period, plant physiology was monitored through weekly prompt- and modulated fluorescence measurements using an MPEA Fluorimeter, chlorophyll content analysis with a CCM-300 Chlorophyll Content Meter, and subsequent soil and plant analyses at the termination of the experiment. The results indicated minimal significant differences in physiological performance across the soil treatments, with the exception of Coal B. Plants grown in Coal B exhibited a reduction in specific physiological parameters, although overall physiological performance remained largely unaffected. Soil analysis revealed that Coal B contained elevated concentrations of heavy metals known to inhibit chlorophyll synthesis, which likely contributed to the observed physiological decline. Despite this, kenaf successfully germinated and established in all five soil types, demonstrating its potential for growth on coal tailings in South Africa and supporting its viability as a candidate for phytoremediation in post-mining landscapes.

Investigating the effect of cysteine derivatives on the physio-biochemical responses of edamame under drought stress

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Edamame (*Glycine max* L. Merr.) is highly sensitive to drought, especially during reproductive stages, making it vulnerable in South Africa's unpredictable climate. With crop production negatively affected by drought and the population rising, there is an urgent need for innovative solutions to improve plant resilience and food production. One such strategy includes using environmentally friendly cysteine derivatives like N-acetylcysteine (NAC) and its derivative N-acetylcysteine trisulfide (NAC-TS). This study aimed to examine the efficacy of NAC and NAC-TS in improving the physio-biochemical responses of two edamame cultivars: drought- susceptible UVE17 and drought-tolerant AGS429. The individual compounds were applied foliarly at 1.5 mg/mL during vegetative, flowering, and pod-filling stages. In addition, another treatment included the application of these compounds throughout all three growth stages (i.e. 2x (vegetative and flowering) and 3x (vegetative, flowering and pod-filling). The assessed parameters included oxidative stress indicators, and enzymatic and non-enzymatic antioxidants. Both NAC and NAC-TS markedly improved the activities enzymatic antioxidants as well as total phenolics and flavonoids during the one-time application at pod-filling. Additionally, the application of these compounds led to a significant reduction of hydrogen peroxide levels and membrane destruction marker, malondialdehyde during the 3X application and the one-time application during pod-filling. These improvements were observed across both cultivars, especially under UVE17 cultivar. The findings of this study suggest that applying NAC and NAC-TS to both drought-tolerant and susceptible edamame cultivars has bio-stimulatory effects, enhancing both enzymatic and non-enzymatic antioxidant responses. This improvement helps stabilize cellular integrity, reducing cell membrane damage, which in turn lowers electron leakage and prevents the excessive buildup of reactive oxygen species. As a result, the treated plants showed an improved resilience to oxidative stress induced by drought stress.

Short-term effects on non-photochemical quenching of a combined light and chilling stress in lichens

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While light is essential for photosynthesis in lichens, when the light that is absorbed exceeds that which can be used in carbon fixation, the excess energy can instead activate oxygen. This will result in the formation of a range of reactive oxygen species (ROS), which can damage photosystem II (PSII), eventually reducing growth rates. One of the ways that photosynthetic organisms reduce the harmful effects of light stress is to convert any excess energy to heat in a process termed non- photochemical quenching (NPQ). It has often been observed that treatment of lichens with moderate light stress increases NPQ. In the field, lichens are subject to multiple abiotic stresses at the same time, e.g., desiccation and temperature stress. The effects of multiple stresses can be synergistic. For example, it seems likely that damage caused by excess light will be exacerbated by chilling stress. This is because while rates of photophosphorylation are almost temperature-independent, low temperatures will decrease carbon fixation, which is dependent on enzyme activity. The aim of this work was to test the hypothesis that lichens treated with a combination of moderate light and chilling stress will display greater induction of NPQ than lichens treated with moderate light stress alone. Five lichen species were used to test this hypothesis (*Ramalina celastri*, *Usnea undulata*, *Crocodia aurata*, *Lobaria pulmonaria*, and *Roccella fuciformis*). The effect of combinations of stress on PSII activity was measured using chlorophyll fluorescence. FV/FM (maximum efficiency of PSII) and rETR (relative electron transport rate) were measured to test for photoinhibition and NPQ to test for photoprotection. Treatments used were 100 µmol m^{-2} s^{-1} at both cold (5 °C), and room temperature (22-25 °C). Measurements were made after 0, 3, 6h and then following recovery for 24h at 15 °C under dim light. Results suggested that all lichen species increase NPQ in response to moderate light stress. For some species, the induction of NPQ was greater under chilling stress, but other species appeared to upregulate other mechanisms when a combination of light and chilling stress occurs.

Inhibition of key carbohydrate metabolism enzymes improves wheat acclimatisation to osmotic stress

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Studying germinating wheat seeds and seedling development under osmotic stress could improve our understanding of their adaptation during drought conditions. The current study aimed to elucidate the effects of osmotic stress on carbohydrate metabolism during wheat germination. Wheat (Tsholoha cultivar) seeds were germinated in 5, 10 and 20% (w/v) concentrations of sorbitol, mannitol and polyethene glycol (PEG) to induce osmotic stress. Seeds germinated for five days, and seedling tissue harvested at three leaf stage was crushed using a mortar and pestle to extract soluble sugars, starch, chloroplast pigments, proline, and total the protein used as a source of enzymes. In addition, the inhibition assays of sorbitol, mannitol and PEG and their kinetic inhibition constant (Ki) were determined for amylase, invertase, amyloglucosidase and β-glucosidase. Results showed that the germination percentage, and root and coleoptile length decreased in seeds treated with sorbitol, mannitol and PEG. Compared to PEG and control samples, the 10% and 20% sorbitol and mannitol treatments strongly reduced the root and coleoptile length. However, starch and sucrose content increased by more than 2-fold in all treated seeds relative to the control. The amylase, amyloglucosidase and invertase enzyme activities were reduced in the treated germinated seeds and seedlings; moreover, seeds displayed more than 30% reduction in amylase activity, followed by more than 90% reduction of invertase and amyloglucosidase activity. All treatments significantly increased proline and pigment contents in the seedling samples, but PEG increased their trehalose and sucrose relative to control. Kinetic analysis showed that treatments competitively inhibited invertase and amyloglucosidase. This study shows that during germination and seedling development, Tsholoha cultivar acclimatised to osmotic stress by reducing the activity of enzymes involved in carbohydrate metabolism, leading to increased starch and osmolytes, i.e., sucrose and trehalose and proline confirming osmotic adjustments that improved wheat survival to osmotic stress.

Plant Defence Plasticity: Investigating changes in chemical defence under varying abiotic stress conditions in African savanna grass species

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This project explores plant responses towards abiotic and biotic factors and the mechanism behind the response. This will be done by investigating the chemical defences produced by grasses as a response against stressors such as herbivory, drought and fire. There are multiple theories that investigate the mechanisms behind plant response which depend on the availability of resources or the vulnerability of the plant part. These theories include the carbon nutrient balance which suggests that the expression of defences is dependent on carbon to nitrogen ratio, altered by herbivory. This theory has been investigated in tree species against insect herbivores. However, there are a few studies that investigate the impact of abiotic factors that have the same effect of herbivores on plants (removal of biomass), this includes fire and drought. Grasses are a major part of the ecology of African savannas, yet limited research has been conducted on their chemical/physical defences against herbivores and other abiotic stressors. This study aims to investigate how the carbon to nitrogen ratio changes when exposed to herbivory, fire and drought, and the response to the alteration of carbon to nitrogen impacts the production of chemical defences, i.e. chemical diversity and chemical concentration in grass species found in African savannas Chemical analysis will be done to profile grasses, this includes chemical concentrations of chemical groups found in grass species. In addition, soil analysis will be done to get the stoichiometric ratio of carbon and nitrogen and investigate the correlation between carbon: nitrogen ratio and chemical defences produced in grass species.

Evaluating the combined efficacy of *Tagetes minuta* and *Urtica urens* in controlling *Diuraphis noxia* in wheat

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Russian Wheat Aphids (*Diuraphis noxia; RWA*), a major pest of wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*) threaten cereal production due to virulence and rapid adaptation to chemical treatments and resistant plant genes. This adaptation compromises food security by reducing wheat yield and the effectiveness of pest management strategies. The study evaluates the bio-insecticide activity from *Tagetes minuta* and *Urtica urens* against RWA. Phytochemical crude extracts were prepared from dried *T. minuta* and *U. urens* using 70% methanol and concentrated in a rotary evaporator at 40°C. Phytochemical crude extract analysis was involved, the IRAC leaf dip test (antibiosis) measured direct effects on *D. noxia* mortality, and antixenosis assay was measured using choice chamber aphid preference and response. Tolerance assessment measured the symptomatic leaf damage or response over 15 days at 5-day intervals. *Urtica urens* exhibited strong antibiosis activity compared to *T. minuta*, along with significant antixenotic effects at 50% and 75% concentrations. In contrast, *T. minuta* showed moderate antixenotic activity but contributed significantly to tolerance mechanisms, resulting in minimal plant damage. While *T. minuta* enhanced tolerance in the susceptible wheat cultivar, the combined application of both plant extracts demonstrated superior efficacy, outperforming individual treatments in antibiosis, antixenosis, and tolerance. These findings highlight the bio-insecticidal potential of *U. urens* and *T. minuta* in managing *D. noxia*, emphasizing their synergistic role in pest control.

Effects of seaweed *Ecklonia maxima* (Osbeck) Papenfuss and *Ascophyllum nodosum* (L.) Le Jolis-based biostimulants on maize seed germination and seedling growth under salinity stress

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Climate change poses significant challenges to agriculture through abiotic factors like increased atmospheric carbon dioxide, higher temperatures, drought and soil salinity, negatively affecting crop growth and yield. Soil salinity, a major threat to global food security, affects a significant portion of arable land and endangers staple crops such as maize. Salinity stress disrupts plant growth and yields through osmotic, ionic and oxidative damage. This study explored the use of seaweed-based biostimulants as a potential solution for addressing salinity in the germination of maize (*Zea mays* L.) seeds. The study employed the application of liquid seaweed biostimulants, Kelpak (*Ecklonia maxima*) and Acadian (*Ascophyllum nodosum*). These seaweed biostimulants have been reported to enhance growth and increase yield by aiding plants in absorbing additional soil nutrients. Additionally, they enhance seed germination, photosynthetic pigments, and abiotic and biotic resistance of crops. In this study, four salinity concentrations (50, 100, 150 and 200 mM NaCl) and four seaweed extract concentrations (0.5, 1.0, 1.5 and 2%) were used to assess the efficacy of seaweed extracts in enhancing the seed germination and seedling growth of maize under saline conditions. The key physiological parameters, germination rate, germination percentage, seedling growth (shoot length,

root length, biomass accumulation), chlorophyll content, proline, protein and carbohydrate levels were determined in 14-day-old treated and untreated seedlings. Kelpak was most effective at 200 mM salt concentration, significantly enhancing seedling growth (shoot and root length and number of roots) and improving root length as salinity concentrations increased. Acadian was also effective in improving these parameters at 100 mM salt concentration. The changes in proline, protein and carbohydrate levels were evident in treated maize seeds. Overall, both seaweed extracts significantly improved root growth under saline conditions.

Invertase mediates *Puccinia triticina* proliferation, leading to cell wall collapse in susceptible wheat

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Wheat is a vital grain crop and one of the leading staple food sources globally. *Puccinia triticina* (*Pt*) is one of three rust fungi that significantly reduce wheat yield and threaten food security. During the wheat-*Pt* interaction, haustorial mother cells and haustoria secrete effector molecules, including cell wall degrading enzymes (CWDEs). CWDEs facilitate host cell wall degradation, enabling *Pt* nutrient acquisition. The study aimed to elucidate *Pt* capacity to degrade cell walls during wheat infection and enzymes facilitating *Pt* colonisation. Histochemical, scanning electron microscope (SEM), and extracted cello-, manno- and xylo-oligosaccharides analyses were employed to assess cell wall damage. β-glucanase and invertase were assayed in *Pt*-infected susceptible (Thatcher) and resistant (Thatcher+*Lr9*) varieties, followed by xylosidase, mannosidase, and β-glucosidase activity assays. Results showed that Thatcher and Thatcher+*Lr9* were successfully infected with *Pt*. However, *Pt* uredinia and urediniospores were only formed in Thatcher, with Thatcher+*Lr9* inducing a hypersensitive response (HR) that stopped further fungal growth. High lignification and cell wall strengthening around infection sites in Thatcher+*Lr9* validated the HR, indicating localised defence responses. Thatcher's cell wall did not show any resistance modification toward *Pt* growth, to the point that cell walls collapsed over time. The *Pt* growth was facilitated by invertase activity, which was significantly higher in Thatcher than in Thatcher+*Lr9*. Biochemical characterisation of invertase revealed secretion during *Pt* infection, regulating the release of hexose-sugars from sucrose and fuelling fungal growth and development. Xylosidase and β-glucosidase activity levels were comparable to the controls. However, high mannosidase enzyme activity was only detected in the *Pt*-infected Thatcher, signifying that this enzyme functions as a virulence effector. The findings provide evidence that *Pt* degrades and collapses the cell walls of infected leaf cells of susceptible wheat, and its development and colony formation are facilitated by secreted invertase enzymes.

Mitigating Soil sodicity in irrigated sugarcane plantations with *Canavalia rosea*: a pot experiment

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To promote the use of legumes for the alleviation of sodicity in small-scale irrigated sugarcane plantations to reduce the cost of soil amendments for small-scale sugarcane farmers. A greenhouse pot experiment was conducted by growing *Canavalia rosea* (Sw.) DC. in high sodium (Na^+) concentration/sodic sugarcane plantation soils over a period of seven months. Plant material and soil characteristics, including salinity indicators, were analysed pre- and post- *C. rosea* harvest. *Canavalia rosea* hyper accumulated more sodium (Na^+) with age significantly decreasing soil Na^+ concentrations from 271.870 to 51.573 µmol Na g^{-1} post- *C. rosea* harvesting. The electrical conductivity (EC), sodium adsorption ratio (SAR), and exchangeable sodium percentage (ESP) were significantly reduced after three months and remained statistically similar over the period to the seventh month. *Pseudomonas putida* and various species in the *Bacillus* genus including *B. amyloliquefaciens*, *B. safensis*, *B. pumulis*, *B. subtilis*, and *B. zhangzhouensis* were isolated from *C. rosea* plants, revealing associations that likely assisted *C. rosea* in Na^+ tolerance and improved nutrient availability. The increased abundance of Flavobacterium species in rhizosphere soils was attributed to the ability of *C. rosea* to reduce soil Na^+ concentrations, creating favourable conditions for microbial proliferation. Consecutive cultivation of *C. rosea* reduced Na^+ concentration by five-folds and improved soil characteristics in sugarcane plantation soils from Sikhwahlane, Mpumalanga. However, field studies are recommended to study the soil Na^+/ sodicity reduction, microbial interactions and growth of C. rosea at irrigated sugarcane plantations. Pot size and irrigation protocols in controlled experiments may lead to pot binding, potentially influencing the response of *C. rosea* in sodic soils.

Can insights from plant histology reveal a role of nutrient supplementation in promoting plant chloride tolerance?

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Soil salinity poses stress to plants leading to a decline in agricultural yield, compromising economic welfare and environmental health. Salinisation changes ionic composition and physicochemical properties of the soil, threatening both soil and plant health. Salinity changes osmotic pressure in the root zone, invokes ion toxicity, induces oxidative damage, and interferes with water and nutrient acquisition due to their antagonism with sodium, chloride, and sulfates. Chloride, a vital micronutrient for plants, facilitates key physiological functions necessary for the healthy growth of plants. It forms part of the oxygen-evolving complex of photosystem II (PSII) and regulates enzyme function, however, inhibits growth when deficient and becomes toxic when excessive. According to the Adaptive Plant uptake theory, plants adapt to changing environmental conditions by modifying multiple physiological, biochemical, and molecular processes, reflected in their morphology. In this study, elevated CO_2 and nitrates are applied to plants as additional nutrition to overcome the nutrient limitations, set by salinity, through different pathways. The aim is to investigate the physiological and morphological responses of crop plants grown under macronutrient concentrations of chloride, combined with CO_2 and nitrate supplementation. It is predicted that plants receiving higher nitrate and CO_2 levels will perform better and exhibit increased tolerance to chloride. The objectives are to assess the effect of nitrate supplementation on plants under chloride stress and to study the impact of elevated CO2 on plant development under chloride stress. Although results are pending, this study will provide insights into the potential benefits of CO_2 and nitrate supplementation in mitigating chloride-induced stress.

Soil legacy drives drought resilience in *Lessertia frutescens* and *Glycine max*: Metabolomic and functional insights from fynbos microbiomes

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Soil legacy plays a significant role in enhancing drought resilience in plants through metabolomic and functional adaptations. This project compares the adaptive contributions of varying microbial communities from heterogenous environments to the plant-extended phenotype. Our study species *Lessertia* (syn. *Sutherlandia) frutescens* (cancer bush), a medicinal plant native to southern Africa, and *Glycine max* (soybean), a globally cultivated nutrient-rich crop are both leguminous and ideal for comparing holobiont drought responses. *Lessertia frutescens* displays distinct regional profiles in its metabolite accumulation, indicating the soil microbiome may influence chemotypes and pharmacological activity. Fynbos soil microbiomes were isolated and applied as inoculum to drought-stressed plants. We hypothesised that fynbos soil microbiome sampled from two different microenvironmental regions would confer varying adaptive traits and extended phenotype. The metabolite fingerprints ascertained through Eco-Plate inoculation and principal component analyses (PCA) established that the Clanwilliam microbiome displayed greater functional diversity compared the Wellington microbiome, evident in microbial communities' utilisation of metabolites such as L-Arginine, D-Xylose and Pyruvic Acid Methyl Ester. The response to drought stress in each inoculation treatment was quantified through oxidative stress assays and eco-physiological measures which showed a contribution to a 20% increase in emergence rate, improved root elongation and biomass accumulation by the Clanwilliam inoculum. For the overall impact on productivity, protein content in *G. max* and accumulation of metabolites in *L. frutescens* (methanolic extractions and liquid chromatography coupled with mass spectrometry) were assessed. Amplicon sequencing showed differences in microbial communities and identified plant growth promoting rhizobacteria that could potentially be used to develop a bio-fertiliser. This project has the potential to mitigate climate change impact, improve crop quality and yield, and standardise the production of medicinal metabolites, all while promoting sustainable water use.

Comprehensive assessment of variation in physicochemical properties and bioactive compounds among *Strychnos spinosa* Lam. fruit accessions: A Multivariate Statistical Analysis Approach

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Monkey orange (*Strychnos spinosa* Lam) fruit, belongs to the Loganiaceae family. It is rich in bioactive compounds including phenols, β-carotenes and antioxidant metabolites. Recent evidence showed that there are different accessions of *S. spinosa* based on its morphological, nutritional and genetic traits. Given this background, the current study assessed a link between morphological aspects and bioactive compounds using a multivariate analysis approach. A total of 32 fruit accessions were collected from Bonamanzi Game Reserve, KwaZulu-Natal province of South Africa. Various morphological, physiological and bioactive components including fruit weight, length (L), width, total soluble solids (TSS), titratable acidity (TA), pH, colour, total carotenoid content (TCC), total phenolic content (TTP) and antioxidant metabolites were measured. Principal Component Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) demonstrated clear distinctions in the studied variables across the accessions. PC1 and PC2 collectively explained 83.7% of the total variation suggesting that fresh weight, TCC, TPC, L*, TA, TSS and antioxidant metabolites were the primary contributors to the variation. Fresh weight was highly negatively correlated with PC1. Colour showed the strongest influence on PC1, followed by TSS and TTP, while TA contributed least to the observed variation. The morphological attributes were clustered predominantly in the negative PC1 region, creating distinct groupings among accessions, with GRP accessions associating with size. GSxCR-DGRO, PRxCP-DGEO, and GRxCR-DGEF formed distinct clusters based on their chemical composition. These findings reveal substantial variability among the accessions, and indicates the need profile untargeted secondary metabolites and carotenoids to guide future directions in product development for functional food products.

Differential responses of edamame cultivars to high temperature: Insights into photosynthesis and chloroplast ultrastructure

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High temperatures reduce photosynthesis and yield in many crop plants. This study investigated how heat stress affects the photosynthetic efficiency and chloroplast ultrastructure in three edamame (*Glycine max* L. Merr.) cultivars (AGS354, UVE17, and UVE14) during the reproductive stages (flowering and pod filling). Plants were grown under controlled conditions at three temperature regimes: 25/18 °C (control), 30/23 °C (moderate heat stress: HS-I), and 35/28 °C (severe heat stress: HS-II). AGS354 was the most heat-sensitive cultivar. Under HS-II, it showed a marked decline in the photochemical reactions, lower chlorophyll-a and carotenoid levels, and disrupted chloroplast ultrastructure. The ultrastructural changes included increased starch grains, and reduced plastoglobule and grana area, suggesting damage to the photosynthetic machinery. In contrast, UVE14 and UVE17 coped better under heat stress. Both cultivars showed increased chlorophyll-a and chlorophyll-b levels under HS-II, along with elevated carotenoids, which likely protected chlorophyll from breakdown and reduced photooxidative stress. The chloroplasts of UVE14 and UVE17 also maintained or improved grana structures under HS-II, supporting continued light-harvesting under stress conditions. Although heat stress reduced key photosynthetic parameters such as the quantum efficiency of photosystem II and performance indices in all cultivars, the impact was less severe in UVE14 and UVE17. Importantly, plastoglobules, which are key for carotenoid biosynthesis remained stable or increased in UVE14 and UVE17 under HS-II, indicating enhanced stress tolerance. These structural and biochemical adjustments suggest that UVE14 and UVE17 have mechanisms to better withstand high temperatures compared to AGS354. In conclusion, UVE14 and UVE17 showed greater tolerance to heat stress at the reproductive stage, making them promising candidates for breeding heat-resilient edamame cultivars. These findings contribute to a better understanding of how heat affects photosynthesis and offer strategies for improving crop performance under changing climate.

Protection of photosystem II from photoinhibition in the mosses *Atrigum androgynum* and *Dicranella subsulata*

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Bryophytes often grow in microhabitats where they receive more light than they can use to fix carbon. Unless regulated, this excess energy can end up activating oxygen, thereby forming reactive oxygen species (ROS). These ROS can damage the photosynthetic apparatus and other cellular components, causing photoinhibition and photo-oxidative stress. Tolerance to high light can be achieved in a variety of ways, but for Bryophytes, our knowledge of the precise mechanisms involved is rather fragmentary. Here, we used chlorophyll fluorescence to study mechanisms of tolerance to light stress in two common KwaZulu-Natal, South Africa Afromontane species of mosses, *Atrichum androgynum* and *Dicranella subsubulata*. Specifically, we tested whether exposure to a moderate light intensity can increase the tolerance of the mosses to a second exposure to high light i.e., to test whether mosses can be "hardened" to light stress. To achieve this we exposed mosses to light at 1200 and 1300 µmoles m^2.s and assessed their subsequent photosynthetic performance under exposure to high light conditions. Mechanisms of tolerance were further probed using lincomycin, an inhibitor of the photosystem II repair cycle. Tolerance against photoinhibition showed a decline in photosynthetic indicators ((Fv/Fm - maximum quantum yield of Photosystem II (PSII), Electron transport rate (ETR) - indicator of photosynthetic activity, reflecting the rate at which electrons move through the photosynthetic machinery and non-photochemical quenching (NPQ – mechanisms used to dissipate excess energy in their photosynthetic machinery to prevent damage from high light level) in both species, measured using chlorophyll fluorescence techniques, with good recovery except for NPQ in *A. androgynum*. Results from photoinhibition and hardening showed that NPQ induction and relaxation was high in samples kept in the dark (control samples) in both species compared to samples that were exposed to light. The absence of lincomycin under light stress showed high NPQ induction and quick relaxation in both species. Conclusion can be made that moss can be hardened using high light intensities treatments and that the presence of lincomycin lowers induction in the presence of light.

Comparative photosynthesis and gas exchange traits of seedlings of selected *Eucalyptus* species: A South African perspective

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One of the most common forestry species in South Africa and across the globe are those found in the *Eucalyptus* genus, due to their fast growth, high wood quality, and ability to tolerate a large variety of climatic and environmental conditions. In South Africa, indigenous forests only cover approximately 0.3% of land which creates a demand for plantation forestry to meet the need for timber products. This makes *Eucalyptus* important for the country's socioeconomic wellbeing, and for the preservation of natural forest ecosystems. The current study focuses on seedlings of a variety of *Eucalyptus* species, natively distributed along distinct temperature and rainfall bioclimatic niches. Leaf-level measurements were investigated with the aim of interrogating differences in leaf physiology, specifically chlorophyll fluorescence, photosynthetic efficiency and gas exchange traits. We found that species from natively xeric environments had greater gas exchange and photosynthetic capacity, whilst species from warm and humid climates exhibited notably lower performance in these traits, and that both of these trends intensified over time. Additionally, gas exchange traits showed considerable diurnal variability with a general trend of decreasing performance as the day progressed. This finding contrasts that of chlorophyll fluorescence, which stayed comparatively constant throughout the day, within all species. These results underline how understanding the mechanisms by which seedlings survive can help to inform decisions on species to site matching, and increasing survivorship and wood production per plantation area, thereby minimising habitat transformation of native South African ecosystems.

Phytochemical analysis of South African *Crocosmia* species for Type 2 Diabetes treatment

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Natural-product based drug discovery faces additional complexity due to the variable nature of secondary metabolites in the face of varying environmental conditions, as accumulation of medicinal bioactives can vary across species and populations. Montbretin A (MbA), a secondary metabolite only found in the corms of *Crocosmia* species, has shown anti-diabetic pharmacological activity and is thus being developed as a new treatment for Diabetes type 2. This study set out to find a chemo-elite population that produced significantly higher levels of MbA amongst different species of *Crocosmia*. As *C. crocosmiiflora* is hybrid variety, it was thus hypothesized that the metabolomic profile of this species would display shared chemistry with both *C. pottsii* and *C. aurea*, and that *C. paniculata* would be the most distinct out of all of them. A liquid chromatography-mass spectrometry-based metabolomic approach was used to assess phytochemical differences. There was significant metabolite variation between the different species and populations, but *C. crocosmiiflora* was more similar to *C. aurea* than *C. pottsii* and was insignificantly different from *C. aurea* ($p \geq 0.05$) for all the main metabolites. It was evident that *C. paniculata* was the most distinct of all the species, displaying the lowest MbA-Rhamnose concentration while *C. pottsii* had the highest production of MbA-Rhamnose, but not significantly different from *C. crocosmiiflora*. Metabolite production appeared more determined by genetic variation than environmental variation. Although there were no species with superior MbA levels or chemo-elite populations, biotechnological approaches may provide alternative routes to access MbA for commercial markets, and there is ongoing research investigating and comparing further populations of *C. aurea* and *C. pottsii,* as well as the species *C. pearsei* and *C. mathewsiana*. *In Vitro* abiotic stress experiments on *C. aurea* plantlets are also underway, testing stressors such as salinity, drought, and heat.

The influence of selenium soil application on osmolytes and their relationship with photosynthetic efficiency in edamame under severe water stress

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Drought, exacerbated by anthropogenic climate change, is a prevalent stressor that results in considerable agricultural losses globally. In water-scarce South Africa, drought threatens food security and economic stability, making drought-sensitive crops like edamame (*Glycine max* L. Merrill) vulnerable. Recently, selenium soil drench has proven to increase photosynthetic efficiency, antioxidant activity and yield of drought stressed edamame in comparison to foliar and seed application methods. However, there is no evidence regarding the impact of selenium soil drench application on osmolyte accumulation in edamame during drought stress. We investigate how selenium soil drenching influences osmolyte accumulation and its connection to photosynthetic efficiency in two edamame cultivars, UVE17 (drought-susceptible) and UVE14 (drought tolerant). The study was conducted under controlled greenhouse conditions (25/18 °C day/night under natural light). At the fourth vegetative stage (V4), selenium was applied to the soil before drought stress was induced by maintaining soil moisture at 30% water holding capacity (WHC), while control plants remained at 100% WHC. The photosynthetic efficiency traits and osmolytes were measured at the flowering and pod filling stages. Under drought stress, selenium increased carbohydrate levels, including sucrose and starch in UVE14, as well as glucose and raffinose in UVE17 at both developmental stages. Selenium application increased free amino acid levels in both cultivars under drought stress at the flowering stage, with UVE17 showing a specific increase in osmolyte-associated amino acids, including arginine and proline. Sucrose positively correlated with the maximum photosystem II (PSII) quantum yield ratio of variable to maximum fluorescence (Fv/Fm), total performance index (PI_{total}) in UVE14 at the flowering stage, indicating that improved photosynthetic efficiency contributed to higher sucrose production under drought conditions. The findings of this study show that selenium application enhances UVE14 drought tolerance by improving photosynthetic performance, as demonstrated by starch and sucrose accumulation, and stable photosystems performance. However, in cultivar UVE17, selenium treatment appeared to accelerate metabolic processes, as evidenced by elevated levels of glucose, raffinose, and arginine.

Assessing crop responses to stressors in a changing climate

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Any unfavorable conditions that impact a plant's metabolism, growth, and development are considered stress factors. These stresses can be categorized as natural or anthropogenic, as well as biotic or abiotic. The goal of this study is to summarize the diverse nature of plant stress physiology research through my team's research activities. Over the past few years, we have examined the effects of various abiotic and biotic stressors. Among the most critical abiotic stresses, low pH and aluminum (Al) stress are closely linked. Aluminium stress does not occur without an acidic growing medium. Soil pH and Al toxicity significantly affect plant metabolism, induces oxidative stress and reduces growth and yield quality. We examined the effects of low soil pH on two South African bread wheat cultivars' parameters. Our findings indicate that growth stages influence the rate of lipid peroxidation and antioxidative capacity, with the most significant effects observed during grain filling. This makes grain filling a crucial stage for studying acidity stress at the biochemical level. Aluminium stress was examined in an acidic nutrient solution with 25 bean cultivars. Our results identified two cultivars with potential for future production in aluminum-toxic soils. These cultivars could also be valuable for Al tolerance breeding programs. The effects of biotic stressors on plants' morphological, physiological, biochemical, and quality parameters are equally important to study. Additionally, the application of various fertilizers - such as those containing amino acids or microelements - and industrial by-products like sewage sludge or compost can also act as stressors. High nutrient concentrations can become a stress factor shortly after foliar fertilizer application. In some cases, stress does not produce visible symptoms, and its effects are only evident in yield parameters. This phenomenon can be attributed to the correlation between increased antioxidant enzyme activity and yield quality and quantity.

Pollination Biology (POLL BIOL)

Development of a colorimetric stigma receptivity assay and the evaluation of stigma receptivity across various plants

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Pollination sustains plant biodiversity and ensures global food security, as most crops depend on it for successful reproduction and yield. Stigma receptivity refers to a stigma's ability to receive, hydrate, and germinate viable pollen. Successful pollination depends on the synchronization of pollen viability and stigma receptivity. Receptive stigmas contain key antioxidant enzymes, peroxidase and esterase, the exact biological function of peroxidase within the stigma remains unclear. The commonly used 3% hydrogen peroxide (H_2O_2) assay uses peroxidases to degrade the H_2O_2 into water and oxygen bubbles, the number of bubbles formed is then counted and represents the receptivity of the stigma. However, this method lacks sensitivity and can produce false negatives due to bubble migration or bursting. Alternate assays, such as benzidine and α-naphthyl acetate, are qualitative, microscopy-based assays that are not suitable in the field. We aimed to develop a robust, sensitive, high throughput and field adapted stigma receptivity assay. In this novel assay, 4-aminoantipyrine (4-AAP) and vanillic acid act as secondary substrates, they undergo oxidation and coupling to form red quinoneimine dye in the presence of H_2O_2 and peroxidase. This assay is able to indicate the peroxidase concentration as a proxy for receptivity and visualizes the receptive area of the stigma, which is stained red. The 4-AAP assay accurately indicates stigma receptivity in wet, dry, and semi-dry stigma types and successfully indicated receptivity across different plant orders including Poales (Maize), Astrids (Sunflower), Proteales (Macadamia) and Fragales (Pecan). This 4-AAP protocol is effective and can serve as a valuable tool for exploring pollination strategies across plant families and can aid in crop development or production, which is essential for global food security.

Using cut flowers in pollination experiments: an investigation of the effects on volatile emissions and pollinator attraction

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Pollination biologists employ diverse experimental approaches, including the use of cut flowers. I explored the impact of cutting flower stems on volatile floral emissions and insect visitation in *Satyrium longicauda* (moth-pollinated orchid) and *Stapelia gigantea* (fly-pollinated milkweed). Using gas chromatography–mass spectrometry, I investigated changes in floral scent chemistry over time since excision. Using field observations, I also monitored the effects of excision on insect visitation and rate of floral anthesis. Results revealed minimal effects of cutting on scent emissions in both species, challenging the assumption that cutting mimics herbivory-induced changes in volatile compounds or results in immediate impairment of the physiological functioning of flowers. Additionally, cutting did not significantly affect insect visitation to flowers of either species over a period of several days. Further research is needed to validate these observations across a broader range of plant species and to explore potential implications for ecological experiments involving cut flowers. Overall, our study contributes new insights into the reliability of using cut flowers in ecological research.

Different strokes for different folks: Floral trait differences among pollination ecotypes reflect characteristics of contrasting pollinators

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Pollination ecotypes, in which among-population differences in floral traits of species are associated with differences in pollination system, are ideal to study initial stages of floral divergence and identify traits that are important for adaptation to particular pollinators. I use three examples of pollination ecotypes from the South African flora to illustrate how initial changes in floral traits among populations reflect the characteristics of the pollinators involved. In pollination ecotypes of *Hesperantha coccinea* (Iridaceae), red and pink flowers of ecotypes also differ in flower orientation and tube length, reflecting the importance of colour for attraction as well as differences in feeding mechanics and behavior of the respective butterfly and fly pollinators. Flowers of two subspecies of the rare *Nerine bowdennii* (Amaryllidaceae) are similar in colour and form, but differ in size, corresponding to dimensions of the nemestrinid fly pollinators. Finally, in pollination chemotypes of *Guthriea capensis* (Achariaceae), flowers are similar in morphology and appearance, but exhibit very distinct scent chemistry, consistent with the importance of odour for attraction of lizard and rodent pollinators. These studies add to the evidence that differences in morphology and advertising traits among flowers reflect adaptations to the characteristic behavior, morphology and sensory perception of pollinators.

Hue's pollinating who? Floral variation and pollinator efficacy in three grass aloes

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Plant-pollinator interactions are among the most vital relationships for plants, underlying the reproductive success of many angiosperms. Various aspects of pollination biology have been extensively studied in aloes, however grass aloes have received little attention. Few studies have investigated the pollination biology of grass aloes and the floral features facilitating successful pollination, despite their concerning conservation status and the wide variation in floral characteristics and pollinator systems. In this study, we investigate and compare the pollination systems of three differently coloured grass aloe species and the floral traits that facilitate effective pollination. Individuals of *Aloe albida*, *A. craibii*, and *A. chortolirioides* represented the three main colour groups in grass aloes, having white, yellow, and red flowers, respectively. Floral measurements, nectar volume and sucrose concentration, tepal colour reflectance, as well as pollinator observation and exclusion trial data were collected for each species. *Aloe albida* and *A. craibii* are effectively bee pollinated, corroborated by their nectar characteristics, floral traits and pollinator observations and exclusion trials. *Aloe chortolirioides* is effectively pollinated by both bees and sunbirds. Despite being visited by a similar range of bee functional groups, there is variation in their pollinating effectiveness due to floral traits. Anther exsertion and corolla tube length influence pollen placement and effective pollen transfer across the various insect visitors. Principal Coordinate Analysis reveal that 88% of the morphological variation between the three species can be mainly attributed to differences in the floral traits, nectar sucrose concentration, nectar volume, and stamen length. Furthermore, Canonical Correspondence Analysis showed that colour explains 44% of variation in insect visitation patterns across the three species. These findings highlight the intricate relationships between floral traits and pollinator efficiency, emphasising the importance of pollinators in the reproduction, and hence persistence of grass aloes.

Sex-specific negative frequency-dependent selection maintains equal morph ratios of mirror-image flowers

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Floral diversity is often shaped by selection pressures that influence pollination success, yet the mechanisms maintaining polymorphisms are sometimes poorly studied. Species with mirror-image flowers have reproductive structures positioned in opposite orientations, pointing either to the left or to the right. This striking arrangement is thought to promote mating between morphs and reduce mating within morphs, thus increasing cross-pollination and reducing self-pollination. Similar to the forces that maintain equal sex ratios in animals, negative frequency-dependent selection is thought to maintain equal morph ratios in mirror-image plants. Here, the rarer morph gains a reproductive advantage over the more abundant morph because it has more access to mates. However, empirical evidence for this process, particularly considering reproductive success through the lens of both seed production and pollen export remains limited. Here, we tested whether reproductive success varies with relative morph frequency in the South African endemic *Wachendorfia paniculata*. We experimentally altered the ratios of left- and right-styled flowers and assessed patterns of pollen transfer (male fitness) and seed production (female fitness) using quantum-dot pollen tracking. Our results show that female reproductive success was not influenced by morph ratio. In contrast, male reproductive success increased as a morph became rarer, following a negative exponential relationship. These findings provide strong evidence that negative frequency- dependent selection in this species acts primarily through pollen export. This underscores the importance of considering both male and female reproductive roles when investigating the evolutionary maintenance of floral form and diversity.

Pollinators, floral traits, and timing: Unravelling plant community dynamics

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Community assembly processes are driven by plant-pollinator interactions. The interaction between these two groups is mutualistic, and a decline in either of them could affect the other and result in biodiversity loss. Despite this, floral traits have often been overlooked in plant community studies. This study investigates how floral trait variation and phenology influence plant community assembly, the extent of niche partitioning among co-flowering species, and overall community fitness in a montane grassland in Mountainlands Nature Reserve, Barberton, Mpumalanga. Floral traits considered important for pollination were measured, viz. anther and stigma height and the difference between them, corolla tube depth, and floral diameter. Trait variation was assessed using Rao's Quadratic Index, while phenological overlap was quantified using the Czechanowski index. Pollinator observations and pollen load assessments were used to classify pollinators into functional groups. Fitness estimates were obtained by counting the number of pollen grains and germinated pollen grains on the stigma. Linear models showed that in early summer, greater trait diversity correlated with increased fitness, whereas in late summer, high trait diversity resulted in decreased fitness. This suggests that competition is beneficial during early summer, whereas facilitation benefits plant species in late summer. Floral abundance showed weak correlations with fitness in both seasons, suggesting that it had minimal influence on pollinator visitation patterns. A chi-square test indicated a significant shift in floral colour composition between early and late summer ($\chi2 = 19.204$, df = 6, $p = 0.0038$), which may be linked to the colour preferences of the abundant pollinator functional groups in both periods. This study highlights that the structure of flowering communities is dynamic and varies at different points in the season.

Interfamilial floral colour preferences amongst South African butterfly families: Are butterfly pollination syndromes consistent across families?

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Butterflies are important generalist and specialist pollinators, yet their floral colour preferences at the family level remain understudied. This study aims to investigate whether interspecific floral colour preferences differ amongst the five butterfly families found in South Africa. Using the citizen science platforms LepiMap and iNaturalist, butterfly-flower colour interactions were scored and analysed. Our findings show that floral colour preferences differ across the butterfly families. For example, the Papilionidae show an overall preference for red and pink flowers, while the Lycaenidae show a preference for yellow flowers. Additionally, a bipartite network analysis shows that white flowers are visited by either family relatively equally. The observed differences in floral colour preferences suggest colour is a key trait influencing butterfly-flower visitation interactions and that floral colour plays a functional role in shaping butterfly-flower visitation networks. These differences in floral colour preferences could suggest niche partitioning amongst the butterfly families as a means to decrease competition amongst members of the families.

Plant-pollinator networks along an altitudinal gradient in the Drakensberg Mountain Centre – observation and pollen-based networks

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Pollination is essential for ecosystem stability and biodiversity maintenance, yet pollination systems face increasing pressures including habitat degradation and climate change. The dynamic and often locally specific responses of pollination systems to these pressures require local data to be able to monitor for future changes in these systems, especially in areas of high diversity and endemism as seen in the Maloti-Drakensberg Mountains. This study establishes baseline data for plant-pollinator interactions in the northern region of the Maloti-Drakensberg in three altitudinal zones — lower montane (~1350 m a.s.l.), upper montane (~2100 m a.s.l) and alpine (~3000 m a.s.l) during early, mid and late summer (2023/2024). Through observation and pollen analysis of insect visitors, we assessed interaction network properties (connectance, nestedness, dependence asymmetry, modularity and specialisation), as well as (flowering) plant species and insect functional group richness and Shannon-Hill diversity. Networks shifted from bee- to fly-dominated with increasing altitude. Low-altitude sites were characterized by low plant species richness, presence of invasive species, and network structures that indicate habitat degradation, making them the most immediately vulnerable to further anthropogenic disturbances. The high species richness and network structures at mid-altitude may act as temporary buffers against adverse effects, but progressive species losses over time are still expected. While high altitude networks currently appear to be the most resilient, with high nestedness and species richness scores, the future colonisation by more competitive species and absence of higher altitudes for upslope movement may eventually lead to ecological tipping points in the community.

Myths about moths: recent advances in our understanding of hawkmoth pollination in South Africa

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Hawkmoth pollination is thought to involve crepuscular visits on balmy evenings to flowers displaying a typical floral syndrome of white, fragrant, long-tubed flowers, especially in areas with host plants for both the larval and adult phases. These ideas, which stem from findings of numerous studies, may influence the planning and execution of field observations and bias these towards observing likely candidates in particular regions and at particular times of day, thereby potentially underestimating hawkmoth visitation to flowers and misrepresenting hawkmoth-flower associations. Here we use chemical analyses and observations, both by observers and obtained using motion-activated cameras, to reveal unexpected aspects of hawkmoth pollination of multiple plant species across South Africa. First, contrary to expectations based on rarity of larval host plants, several plant species that occur in the fynbos of the Cape Floristic Region, including in the mountain cloud zone, are visited and pollinated by hawkmoths and these visits may occur during inclement weather. Second, hawkmoths visit flowers that appear to be adapted for different pollinators, including flowers that are (virtually) unscented and brightly coloured. Third, hawkmoths are prolific flower feeders in highly transformed habitats, including plantations and urban gardens. Fourth, while the peak activity time of many hawkmoth species is around dusk, some are also active at dawn, while others are active throughout the night. These results suggest that hawkmoths are underestimated flower visitors, including to flowers that do not conform to the typical hawkmoth pollination syndrome, and that greater consideration of the temporal and spatial scope of this interaction in South Africa is warranted.

POSTER PRESENTATIONS

Category: Evolution & Systematics

Taxonomic synopsis of Malpighiaceae (Malpighiales, Rosids) in Madagascar

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Madagascar is the home of most genera of African Malpighiaceae, a mostly Neotropical family of flowering plants. French botanist Jean Arenes was the only researcher to properly study the diversity of Malpighiaceae in this island, publishing several taxonomic revisions for most Malagasy genera from 1943 to 1955. However, no study has ever provided a taxonomic synoptic account for the Malagasy Malpighiaceae, providing a proper overview of this family in the light of scientific progress in the past eight decades. Thus, the goal of this study is to provide a taxonomic synopsis comprising a key to the genera of Malagasy Malpighiaceae, generic descriptions, a checklist of accepted species, and photographic illustrations of all genera included in this study. This study was based on the analysis of type specimens, protologues, field photographs and herbarium sheets of all currently accepted species for Malagasy Malpighiaceae by Word Flora Online. Malpighiaceae is represented by seven genera and 67 accepted species. The most species-rich genera are: *Microsteira* (27 ssp.), *Tristellateia* (19 ssp.), *Philgamia* (12 ssp.), *Acridocarpus* (5 ssp.), *Rhynchophora* (2 ssp.), *Digoniopterys* (1 sp.), and *Madagasikaria* (1 sp.). Lianas are the most common habit in Malagasy Malpighiaceae (49 ssp.), followed by shrubs (13 ssp.), and trees (5 ssp.). Most Malpighiaceae species, 49 out 67, are only found in open biomes of Madagascar (i.e., dry forests, grasslands, and savannas). And most herbarium specimens for this family are old specimens collected over 50 years ago. All Malagasy Malpighiaceae genera are found in open biomes of Madagascar. These very understudied biomes of Madagascar are also home to most species of Malpighiaceae from this island. A comprehensive fieldwork effort is urgently needed for Malagasy Malpighiaceae, since most herbarium specimens for this family on this island are represented by over 50 year-old specimens.

Category: Medicinal Plants/Ethnobotany

Ethnobotanical study and economic impact of medicinal plants with potential of modulating SARS-CoV-2- related inflammatory response in the upper and lower respiratory system

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In the wake of the global crisis initiated by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak, South Africans explored alternative therapeutic strategies. This exploration was driven by vaccine hesitancy, the emergence of diverse SARS-CoV-2 variants, and the enduring challenges posed by the virus. The current study aims to document medicinal plants with antiviral and anti-inflammatory properties and further report on their economic impact during the pandemic. A comprehensive desktop study utilizing search engines such as Google Scholar, PubMed, ScienceDirect, and Scopus was utilised for documentation of these plants. Data gathered included plant species, family, parts used, preparation methods, administration routes, and conservation status. The study identified 23 plant species from 18 different families that exhibit dual antiviral and anti-inflammatory properties. The study identified 23 plants from 18 different families that exhibit dual antiviral and anti-inflammatory properties. The study revealed a predominant utilization of the Lamiaceae family (14.8%), with leaves being the most used plant part (31.0%). Medicinal plants were primarily administered orally (75.0%) following preparation by decoction (24.0%). In addition to their reported pharmacological potential, these plants have significant economic value, specifically in rural communities. Challenges such as the overharvesting of endangered species highlight the need for sustainable practices. The limited data on their immunomodulatory properties also calls for further research to fully validate their therapeutic significance.

Category: Medicinal plants/Ethnobotany

Bridging tradition and science: Ethnobotanical perspectives on medicinal plants for venereal disease treatment in Sub-Saharan Africa

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Sub-Saharan Africa (SSA) faces a critical intersection between its disproportionate venereal disease (VD) burden—representing 40% of global cases—and its underutilised Indigenous knowledge (IK) and medicinal flora. A PRISMA-structured systematic review of ethnobotanical surveys (2008 to May 2024) identified 20 studies meeting rigorous ethnobotanical indices, revealing Nigeria and South Africa as primary contributors (25% each). The synthesis documented 445 medicinal plants across 99 botanical families, drawing on insights from 872 IK contributors. *Cassia abbreviata*, *Ziziphus mucronata*, *Ximenia caffra*, *Catharanthus roseus*, and *Terminalia prunioides* emerged as the most frequently cited species across VD treatments. Leading plant families encompassing multiple species included Fabaceae (15.8%), Cucurbitaceae (5.9%), and Solanaceae (5.9%). Roots (41.5%) and leaves (26.3%) were the prioritised plant parts for ethnomedicinal (anti-venereal) uses, while decoctions (36.7%) and infusions (12.2%) predominated, potentially reflecting traditional efficacy in bioactive compound extraction. Oral administration constituted the primary delivery method (72.9%). Critical gaps persist, with 80% of SSA regions lacking VD-focused ethnobotanical research and trichomoniasis remaining unstudied. This review underscored urgent conservation needs for medicinal flora, identifying urbanisation, deforestation, and overharvesting as existential threats. Cultivation initiatives and conservation education for traditional medicine practitioners are proposed to safeguard these biocultural resources.

Category: Medicinal plants/Ethnobotany

Oxidative stress responses of *Ceratophyllum demersum* L. against metals: A case study

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Environmental pollution is a major problem in urban areas, and high concentrations of metals in water can be bioaccumulated in aquatic organisms. Metals, depending on their oxidation state, can be toxic to most organisms. *Ceratophyllum demersum* L. (coontail) is a submerged, free-floating aquatic plant, with cosmopolitan distribution, native to all continents except Antarctica, and is a rootless macrophyte suitable for laboratory bioassays. This plant could be a suitable candidate to determine whether antioxidant responses can be applied as possible biomarkers for metal exposure. Thus, antioxidant capacity and oxidative stress parameters were investigated in *C. demersum* during exposure to a metal cocktail "pollution event". Plants were divided into four experimental treatment groups, each with different exposure concentrations of aluminium (AlSO4), copper (CuSO4) and zinc (ZnSO4) in mixtures. A fifth uncontaminated group of plants served as the control. Total polyphenol content (TP), oxygen radical absorbance capacity (ORAC), thiobarbituric acid reactive substances (TBARS), catalase (CAT) and total glutathione (GSHt) were measured. The results suggested oxidative stress responses to the toxicity of Al, Cu and Zn in combination, and also slightly different adaptive strategies of plants in response to different concentrations the tested metals. This study demonstrated that *C. demersum* shows tolerance to different metal induced oxidative stress and can survive under high concentrations of these metals by adapting its antioxidant defence strategies. It will be important to test the field application of these biomarkers.

Category: Medicinal plants/Ethnobotany

Cheminformatics identification of phenolics Against L,D-Transpeptidases (LDT) of *Acinetobacter baumannii*

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Inhibition of L,D-Transpeptidases (LDTs) activity, an enzyme crucial for bacterial cell wall synthesis similar to D,D-trans-peptidases (DDTs), has emerged as a promising therapeutic strategy against *Acinetobacter baumannii*. Additionally, inactivating LDT of *A. baumannii* (Ldt_{Ab}) and penicillin binding protein 1a (PBP1a) in *A. baumannii* is lethal, underscoring their potential as valuable drug targets. In this study, phenolic compounds with high binding affinity and better stability with PBP1a were screened for the first time against Ldt_{Ab} using molecular docking techniques. The top-hit phenolics' ability to inhibit the LysM and PG domains of Ldt_{Ab} was further refined through 200 ns molecular dynamic simulations. Interestingly, the top six profiled phenolics demonstrated superior Ldt_{Ab} binding, with ZINC12659587 displaying strongest affinity ($\Delta G_{\text{bind}} = -51$ kcal/mol) relative to the reference standards [carbapenem (–17 kcal/mol) and imipenem (–24 kcal/mol)]. Upon further analysis of the post-dynamic data, it was observed that ZINC12659587 exhibited superior stability and was well accommodated within the active site of Ldt_{Ab}. This was characterized by a stable thermodynamic entropy and minimal fluctuations around the important amino acid residues (His352, Gly350, Thr368, and Try311) of the resulting complexes. These findings underscore the importance of the profiled phenolics, particularly ZINC12659587, as potential inhibitors for effective management of *A. baumannii* infections due to its ability to modulate Ldt_{Ab} in *A. baumannii*. Further in vitro research is ongoing to validate the efficacy and the exact mode of action of the compounds against *A. baumannii.*

Category: Physiology

Comparative metabolomics between maize and pearl millet genotypes contrasting in their responses to water deficit

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Drought continues to threaten food production across Sub-Saharan Africa, where maize plays a central role in both human diets and animal feed. However, maize sensitivity to drought presents a major challenge to food security in the region. Pearl millet, an indigenous crop well adapted to arid environments, offers valuable insight into mechanisms of drought tolerance in cereal crops. In this study, we investigated the metabolic responses of contrasting genotypes of maize and pearl millet under water-deficit conditions. Using mass spectrometry-based metabolomics, we generated and analyzed metabolic profiles to gain a deeper understanding of their molecular responses to drought. Cross-species and genotype-level comparisons are key to identify differentially accumulated metabolites associated with crop tolerance to stresses. These metabolites are being functionally annotated and mapped to known pathways using KEGG and related databases to uncover metabolic shifts associated with drought response. Our findings contribute to a broader understanding of drought adaptation strategies employed by pearl millet, which support future efforts to enhance resilience in maize and other cereals, advancing sustainable agriculture in water-limited regions.

Category: Ecology

Grass-forb interaction dynamics through a dry, cold season

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Grass-forb interactions are crucial in the functioning and resilience of grasslands systems. However, the influence of seasonal stressors on these interactions is not well understood. Highveld grasslands of South-Africa are characterized by cold and dry winter seasons; therefore, it is essential to understand the influence of seasonal stressors on the grass-forb coexistence dynamics. This study aims to investigate the potential (i) facilitating effects of grasses on forb communities during the cold and dry months and (ii) competitive advantage of forbs over grasses at the onset of spring in a Highveld grassland. Twenty paired plots (quadrats of 50 cm x 50 cm each) were established within similar plant communities inside a wildlife estate in the North-West Province. At the end of the growing season, all above-ground grass biomass was removed from one of the paired plots to monitor the change in above-ground cover of forb species throughout the non-growing season in plots with- and without aboveground protection from grass. At the onset of spring, both paired plots will receive equal quantities of water for one month to test whether water-stimulated resprouting of perennial forbs will be faster in plots where grass biomass was removed. We expect that in grass-removed plots, the above-ground forb cover will (i) reduce faster during the winter, but (ii) increase faster in aboveground biomass production after post-winter watering because of reduced light competition and resources stored in underground storage organs. Gaining knowledge on grass-forb interactions across seasonal changes in a highveld grassland is considered as a first step towards an improved understanding of some mechanisms that drive the coexistence of these dominant growth forms.

Category: Physiology

Investigating the effects of heat stress on the photosynthetic machinery of *Hedera helix* through chlorophyll *a* fluorescence analysis

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The study of heat stress in plants has gained increasing importance as climate change and global warming intensify. The rising frequency of heat stress conditions places significant strain on plants' ability to acclimate. Previous research has shown that heat stress-induced damage to chloroplasts inhibits Rubisco activase, leading to reduced carbon fixation and assimilation, ultimately lowering photosynthetic efficiency. *Hedera helix* is known for its resilience to environmental fluctuations and its ability to withstand a broad range of temperatures. Investigating its photosynthetic responses provides valuable insights into its capacity to tolerate high-temperature stress. This study examined the effects of temperature stress on the photochemical performance of *H. helix* by subjecting plants to temperatures of 4°C, 20°C, 30°C, 40°C, and 50°C. Photochemical efficiency was assessed using chlorophyll *a* fluorescence measurement, which allowed for the evaluation of electron transport dynamics and energy flow modifications under heat stress. Results revealed temperature-dependent adaptations in light absorption, excitation energy retention, and electron transport efficiency. A comparison of heat stress transients with the 20°C reference transient indicated significant alterations in photosystem II (PSII) activity. At 50°C, a marked decrease in fluorescence intensity at 0.3 ms (K-step) suggested damage to the oxygen-evolving complex (OEC). Further evidence of disrupted electron flow was observed through the formation of ΔJ- and ΔI-bands, indicating impaired electron transport. Interestingly, leaves exposed to 40°C exhibited considerably less damage to PSII; however, the efficiency of final electron acceptor reduction was reduced at this temperature. These findings suggest that chlorophyll *a* fluorescence can be used as a non-destructive diagnostic tool to assess plant health and evaluate stress responses to extreme temperatures.

Category: Invasion Biology

The siege of *Solanums*: Cataloguing the exotic and invasive *Solanum* species in South Africa

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In South Africa, the Solanaceae represents a notably important family when considering invasive species, particularly those found within the genus *Solanum*. Of the 14 exotic *Solanum* species known to be present in South Africa, seven are currently listed as invasive as per NEMBA, namely *S. betaceum, S. chrysotrichum, S. elaeagnifolium, S. mauritianum, S. pseudocapsicum, S. seaforthianum* and *S. sisymbriifolium*, with the remaining unlisted exotic species, namely, *S. aculeatissimum, S. capsicoides, S. laciniatum, S. nigrum, S. rostratum, S. torvum* and *S. viarum*, also considered in this assessment. Using a combination of databases and repositories, including the South African Plant Invader Atlas (SAPIA), Plants of Southern Africa (NEWPOSA) as well as the Global Biodiversity Information Facility (GBIF), the distribution of all 14 of these invasive / exotic *Solanum* species were mapped at a quarter degree square (QDS) resolution. Troublingly, over the past 25 years, all *Solanum* species appear to have increased drastically in the number of QDS they are now recorded within, with an average increase of ~660%. This research provides a useful update on the status of many of the high-profile listed invasive *Solanums* in South Africa, ultimately aiding in their continued management. Furthermore, this assessment offers insights into newer invasions by species not currently listed as invasive but requiring management interventions, such as rapid response efforts targeting *S. laciniatum* and the initiation of potential biological control programmes against *S. torvum* and *S. viarum*.

Category: Evolution and Systematics

Alive and well in the wild: Rediscovery of *Nerine bowdenii* subsp. *bowdenii* in its native range and its implications for a taxonomic revision of the *Nerine bowdenii* species complex

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Within the biodiverse flora of South Africa, almost a quarter of species are considered of conservation concern. *Nerine bowdenii* is native to the summer rainfall region of South Africa, where its Red List status was recently changed from Rare to Vulnerable due to possible collecting threat, as it is a valuable horticultural species internationally. Within its native range in South Africa, two subspecies, each, for most of the last century, known from only a single locality, are currently recognised: *N. bowdenii bowdenii* with larger flowers, flowers in late autumn in the Eastern Cape, and *Nerine bowdenii wellsii*, with slightly smaller flowers, flowers in late summer below Mont aux Sources in the Orange Free State Drakensberg. In 2015 a Nerine and Amaryllid Society expedition targeting *Nerine bowdenii* in its native range failed to relocate any of the historical herbarium collection localities and concluded that climate change, habitat loss and over-collecting had possibly led to local extinction in most of the species' original range. We report on rediscovery of five of the six historically known populations of *N. bowdenii*. and on quantitative analysis of floral and vegetative variation across the now seven currently known populations. Analysis of trait variation in parallel with molecular evidence indicates that recognition of a third subspecies is warranted. While the population at the type locality appears not to have recovered from intensive harvesting for horticultural export in the first half of the last century, most populations include many individuals, with low levels of current threat due to land use change and alien encroachment. This study substantially increases knowledge of the phenology, habitat preferences, elevation range, and floral traits in the *N. bowdenii* species complex, and confirms that the species is still present across its original range, a century after some of these populations were last recorded.

Category: Evolution and Systematics

Biogeography and diversification of *Dierama* (Iridaceae: Crocoideae)

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The genus *Dierama* K.Koch (Iridaceae: Crocoideae), the 'hairbells of Africa', comprises c. 44 species – most occurring in the eastern mountain regions of southern Africa. Its distribution extends from Ethiopia to Knysna in the Western Cape of South Africa, across a wide range of elevations. In this study, we explored biogeographic patterns and phylogenetic relationships among species of *Dierama* and tested the hypothesis that pollinators may have played a role in the diversification of the genus. Species distributions were sourced from GBIF and mapped, excluding introduced cultivated specimens, and weighted endemism was calculated and mapped across *Dierama*'s distribution. Phylogenies comprising 22 *Dierama* species were constructed using Parsimony and Maximum Likelihood (ML), based on the plastid *mat*K and nuclear ITS regions. *Dierama*'s centre of diversity, the region of its highest species richness, and highest weighted endemism, is in the KwaZulu-Natal Midlands with surrounding regions having a declining species richness towards the extremes of *Dierama*'s distribution. Nuclear markers showed more variation than plastid markers at the species level, however, *Dierama* is highly conserved for most of the markers considered in this study, suggesting a relatively recent origin for the genus. As a result, the reconstructed phylogenies have relatively low support. Nonetheless, based on the ITS ML phylogeny, it is possible to hypothesise that *Dierama* may have originated in its centre of diversity and migrated northwards (more than once) and southwards towards the extremes of its distribution. Optimising the floral characters, perianth length and tepal colour, onto the Maximum Likelihood ITS phylogeny, it appears that pollinators are potential drivers of diversification in *Dierama*.

Category: Medicinal Plants/Ethnobotany

The use of traditional medicine in neonates and infants in KwaZulu-Natal

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African Traditional Medicine (ATM), a type of traditional medicine native to African cultures, is believed to be available, accessible, and affordable and to have been in practice for the past thousands of years and used currently by approximately 80% of Africans. Unfortunately, ATM is not well documented and information on its use on neonates and infants is extremely scarce. Furthermore, there is a lack of safety and efficacy data as well as quality controls on the medicine. This is unfortunate as neonate and infant primary health care is essential, and their morbidity and mortality rates are significantly high in Africa with expected increases in the upcoming years, therefore, ATM could be a good alternative healthcare to consider. Taking into consideration that KwaZulu-Natal (KZN) has a rich traditional history and is amongst the top three provinces with many neonate and infant deaths, this study aimed to determine and document commonly used medicinal plants to treat neonates and infants in KZN. Survey interviews were conducted in various areas and regions in the province, collecting 40 respondents, including parents, herbalists, and traditional healers. The survey interviews were analyzed using ethnobotanical parameters, such as Frequency Citation, Use Value, and Informed Consensus Factor, to determine the commonly used medicinal plants and common illnesses and diseases being treated for neonates and infants in KZN. Results show *Senecio serratuloides*, *Tetradenia riparia*, *Artemisia afra*, *Lippia javanica*, and *Leucas lavandulifolia* amongst the commonly used medicinal plants, whilst the common illnesses and diseases are in the categories of skin-related, respiratory-related, and gastro-intestinal. Using the mentioned results and other information collected from the survey interviews, the medicinal plants are being evaluated for their phytochemical properties, antioxidant activities, and pharmacological activities before computational modelling of compounds identified within the plant species against important proteins that confer infections or diseases that are highlighted in the survey.

Category: Plant Biotechnology

New phytohormone derivatives as a tool to optimize micropropagation of recalcitrant plant species

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Cytokinins (CKs), in concert with auxin, are critical for successful plant regeneration in tissue culture, where the most frequently used CK is 6-benzylaminopurine (BAP). However, due its fast in-situ N9-glucosylation, it can also generate diverse negative effects, which complicate micropropagation processes, especially in recalcitrant species. To solve this problem, N9-glucosylation could be suppressed by appropriate N9 purine substitution of BAP or hydroxylation of its benzyl ring. Series of CK derivatives substituted at N9-position by various sugars and protective groups have been recently prepared to improve CK specific biological activity and are already routinely used in plant micropropagation. Moreover, by small change in cytokinin structure, a potent cytokinin antagonists and/or inhibitors of their inactivation have been obtained, including their isotopically and fluorescently labelled analogues. Phytohormones auxins, thanks to their capacity to determine plant architecture, also have been successfully employed to obtain more economically advantageous plants in vitro. The ratio between activity of auxin and cytokinin needs to be tightly controlled to achieve proper shoot generation as well as rooting and acclimation. Recently we prepared a set of new PEO-IAA-inspired anti-auxins capable of antagonizing auxin responses in vivo and successfully used them to facilitate hemp micropropagation. Here, recent results of synthesis, characterization and biological activity testing of several new phytohormone derivatives will be presented and demonstrated that they can be used as an interesting new tool for plant biotechnology and agriculture. Plant hormones are typically found at minute concentrations in plant tissues containing very complicated biological matrices. We recently developed solid-phase microextraction and an ultra-high-sensitivity UPLC–ESI(+)– MS/MS methods, useful for studying their endogenous levels as well as metabolism, crosstalk (between different groups of exogenous and endogenous phytohormones) and modes of action in plants.

Category: Evolution & Systematics

First record of *Ceratium brachyceros* Daday in southern Africa: Morphology, taxonomy, and geographical distribution

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Until now, the freshwater dinoflagellate *Ceratium hirundinella* was the only species of this genus documented in South African freshwater systems. Recently another species, *Ceratium brachyceros*, was recorded for the first time in the Limpopo Province of South Africa. *C. brachyceros* is an exceptionally rare species, previously observed only five times across three continents - once in North America and Southeast Asia, and three records known in Africa. The species is characterised by a robust theca, a single elongated apical horn, and two short, stubby antapical horns. Due to its rarity, little is known about its ecological preferences, and no environmental variables have been documented in association with its occurrences. In this study, we present a detailed morphological and taxonomical description of *C. brachyceros*, supported by high-resolution light and scanning electron microscopy. Additionally, we provide an updated account of its global distribution.

Category: Evolution & Systematics

Systematics of the indigenous *Hibiscus* L. (Malvaceae) in South Africa

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Hibiscus L. belongs to the mallow family (Malvaceae), with more than 400 species, predominantly found in pantropical and sub-tropic regions, with some presence in temperate zones. Within South Africa, there are approximately 43 *Hibiscus* species, of which 38 species are indigenous with four endemics (*H. aridus*, *H. coddii* subsp. *barnardii*, *H. marlothianus* and *H. waterbergensis*). The taxonomic circumscription of *Hibiscus* has been a subject of ongoing debate and instability. Phylogenetic analyses of both nuclear and chloroplast genes have provided evidence that *Hibiscus* is a paraphyletic group with members of several other genera in the Hibisceae tribe intricately interwoven within *Hibiscus*. This caused the need to either broaden the definition of the genus *Hibiscus* to encompass several related genera or to divide it into ten or more distinct genera. In 2001 and 2024, two name changes were proposed, which included two indigenous South African species (*H. tiliaceus* and *H. caesius*). A comprehensive taxonomic revision of the South African *Hibiscus* has yet to be conducted since the last revision was in 1999 for Africa and Asia but only included five South African *Hibiscus* species. The present revision will address the challenges associated with circumscribing the species by combining traditional morphology, micro-morphology and molecular phylogenetics. Diagnostic characters will be identified based on the morphological descriptions, while the leaf trichome complement will be described by using a scanning electron microscope to identify new characters that may aid in the proper identification of *Hibiscus* species. The phylogeny of the indigenous South African *Hibiscus* will be analysed by using chloroplast (*mat*K, *ndh*F, *trn*Q-*rps*16) and ribosomal (ITS) gene regions. This systematic analysis will also contribute to a natural classification that accurately reflects evolutionary relationships between species, thereby enhancing our understanding of the indigenous South African *Hibiscus* diversity and evolutionary history.

Category: Physiology

The role of plant biostimulants in promoting lateral roots and root architecture

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Crop productivity and abiotic stress tolerance of crop plants depend on a well-developed root system. A better understanding of root architecture, anatomy, and physiology may aid in sustained productivity in agriculture and horticulture. An increase in the vascular system and enhanced lateral root production may lead to an improved water status of the crop plants under drought conditions, since the potential for additional water uptake and utilisation might be possible. Root system architecture can influence the capacity of roots towards more efficient uptake of nutrients and better nutrient availability. Environment-friendly biostimulants have been suggested to promote lateral root growth and sustainably improve root architecture. Though biostimulant products have no defined mode of action, they indirectly influence root development, including lateral roots, as they may contain plant growth-promoting substances, such as auxins, cytokinins and gibberellins. The review emphasises the utilisation of environmentally friendly biostimulants in promoting the lateral root system and its architecture, its role in nutrient foraging, and crosstalk with other phytohormones.

Category: Plant Biotechnology

Synergistic interaction between microbial biocontrol agents and commercial mycorrhiza enhances pathogen tolerance and growth in *Phaseolus vulgaris*.

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Although there is an increase in research regarding microbial biocontrol in commodity crops, agricultural implementation remains limited, primarily due to inconsistent field performance influenced by existing agricultural practices. One such practice is the application of commercial mycorrhizal inoculant. This study investigated the interaction between microbial biocontrol agents and commercial mycorrhizae, and how this relationship influenced the pathogen tolerance of *Phaseolus vulgaris* (common bean). To determine this interaction, in vitro co-culture was done. The impact of this interaction on hydrolytic enzyme activities (protease and amylase) and nutrient solubilizing characteristics (siderophore and zinc solubilization), which are critical for biocontrol efficacy were also evaluated. The results demonstrated enhanced enzyme activity and nutrient solubilizing when biocontrol agents were combined with mycorrhizae, compared to the individual treatments. Furthermore, the combined application significantly suppressed the *in vitro* growth of *Fusarium oxysporum*, a major soil borne pathogen. Subsequent in vivo experiments assessed both physiological and biochemical response of *P. vulgaris* under pathogen stress. Plants treated with the combined biocontrol and mycorrhizal inoculation demonstrated, improved root architecture, increased leaf and root dry biomass, decreased lipid peroxidation and ROS content, even in the presence of pathogen infection. These findings suggest a synergistic relationship between microbial biocontrol agents and commercial mycorrhizae, wherein the growth promoting properties attributed to mycorrhizae area preserved while the biocontrol efficacy against *F. oxysporum* is retained. This synergism holds promise for integrated disease management strategies and sustainable yield enhancement in leguminous crops.

Category: Physiology

Relationships between physio-biochemical, yield, and morphological traits in edamame under combined drought and heat stress

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The impact of combined drought and heat (DH) stress on the physiological, metabolic, growth, yield, and seed quality of crops such as edamame (*Glycine max* L.) is more severe and unique than when the stresses are applied individually. The study aimed to investigate the effects of DH stress on the morphology and yield in three edamame cultivars (AGS429, UVE14, and UVE17) and to determine their associations with the biochemical and physiological responses. Physiological and biochemical responses of the three edamame cultivars were determined using destructive and non-destructive methods. The yield indices were measured manually at full maturity (R8) after the seeds have completely dried. A laboratory scale was used to measure total seed mass per plant (TSMP) and 100 seed mass (100 SM). A leaf area meter was used to measure the leaf area (LA). Correlations and principal component analysis (PCA) were determined using

Genstat. The poor rate of photosynthesis, membrane damage and reduced cellulose synthesis (resulting in high number of flat and shattered pods) led to the highest yield reduction and poor seed quality in UVE17, contributing to its susceptibility to DH stress. The PCAs showed that LA and yield indices [total pods per plant (TPP), total seeds per plant (TSP), TSMP, and 100 SM] were strongly positively associated with AGS429. In contrast, UVE14 was associated with high carbohydrate accumulation (sucrose, trehalose, cellulose, and hemicellulose) at both growth stages. The higher rate of photosynthesis, antioxidant response systems, stable plasma membranes and cell wall re-enforcements in AGS429 and UVE14 contributed to yield enhancement and tolerance to DH stress. Therefore, AGS429 and UVE14 can be suggested for breeding programs due to moderate yield reduction (AGS429) and low yield reduction (UVE14) under DH stress. Future studies could explore the nutritional quality of the edamame cultivars under DH stress.

Category: Medicinal plants/Ethnobotany

Possible indigenous medicinal uses of *Ziziphus Mucronata* Willd. from Mphambo Village, Limpopo Province, South Africa

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Most of rural African people depend on indigenous medicinal plants for healthcare. In South Africa many people are still struggling to find medical help due to distance and high cost, hence they opt for indigenous use of medicinal plants. *Ziziphus mucronata* is a is one of the plants with potential medicinal uses in Southern Africa. The study aimed at investigating the indigenous medicinal uses of *Z. mucronata* from Mphambo village, Limpopo Province, South Africa. This study has hypothesised that people in Mphambo village have the indigenous knowledge of *Z. mucronate.* A survey was conducted with 50 participants by using purposive sampling and semi-structured interviews (face-to-face). Ethnobotanical indices such as Relative Frequency of citation and Use-value were used for data analysis. About 18 diseases and different 9 categories of diseases were recorded. Plants parts which are mostly used were leaves (35%), followed by the roots (32%), whole plant (15%), bark (12%) and fruits (6%). Maceration (44%) and decoction (33%) were the most main preparation methods, and plant remedies were mainly administered orally (68%). This study is in agreement with some previous studies were indigenous medicinal plants can be used to treat and manage some diseases. Therefore, this study might bring hope to people of Mphambo village to continue using and conserve *Z. mucronata*.

Category: Medicinal Plants/Ethnobotany

In vitro antimicrobial and antioxidant evaluation of *Corchorus tridens* L extracts

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Medicinal plants have traditionally been used worldwide for therapeutic purposes, including the treatment of microbial infections. Among these is *Corchorus tridens* L. (Malvaceae), a wild plant species widely distributed in 29 African countries. The leaves of this plant are used both a vegetable and for ethnomedical applications, including treatment of sexually transmitted infections, diarrhoea, stomach disorders, back pain, fever, and inflammation. Antimicrobial resistance is now considered one of the most serious global health threats and as a result, there is an increasing scientific interest in identifying natural and effective plant-based antibacterial agents. Given the accessibility and ethnomedicinal potential of *C. tridens* L, this study therefore investigated the antimicrobial, antioxidant activities, and cytotoxic effects of the leaf extracts of

Corchorus tridens L using micro-broth dilution assays, DPPH, and Ferric reducing power assay, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay and Real-time cell analysis assay (RTCA) respectively. The study findings revealed the presence of antimicrobial compounds with MIC values ranging from 80 to 2500 µg/ml with the methanolic leaf extract showing the highest antioxidant activity (IC_{50} = 167.01 ±15.56 µg/ml). The extracts exhibited low to no toxic effects on HEK 293 and Vero CCL-81 cells with LC_{50} ranging from 113.72 ± 46.28 µg/ml to 274.73 ± 65.65 µg/ml. Furthermore, RTCA results revealed that hot water extracts had the highest cell index on both cell lines. These findings as well as the LC-MS chemical profile analysis currently underway provide preliminary reference for guiding future research fort alternative therapeutic options to combat antimicrobial resistance.

Category: Medicinal Plants/Ethnobotany

Roots with riches: Unveiling the chemical complexity of South African *Withania somnifera*

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Withania somnifera (in the Solanaceae family, and commonly referred to as Ashwagandha) is a commercially important species that has not received much attention in South Africa, despite its use in local traditional medicine. The plant occurs in drier regions of tropical and subtropical zones across Asia, southern Europe and Africa. Indian plants have been well studied and are cultivated for the adaptogenic and anxiolytic effects of their roots. The species is known to have a high degree of genetic diversity, with several chemotypes. A semi-targeted investigation of South African plants was conducted, with a focus on withanolides and their glycosides. Roots were collected from several populations across the country and subjected to analysis by ultra performance liquid chromatography using high resolution mass spectrometry (UPLC-HRMS). Travelling wave ion mobility spectrometry (TWIMS) was utilized as an orthogonal analytical technique to aid in the separation of coeluting isomers. The South African and Indian root material analyzed by UPLC-IM-MS showed highly complex chemical profiles, with many isobaric constituents. The withanolides were identified using standards where available and literature sources for additional compounds. Most previous chromatographic studies conducted on *W. somnifera* involve targeted analysis on the same handful of withanolides, so when neither standards nor literature sources were available for a compound, structural elucidation was performed through fragmentation analysis. The roots of South African plants were found to possess various withanolides, but little to no withaferin A – a characteristic that is highly sought after. This work reported the electrospray ionization and fragmentation behavior of a large number of withanolides and could provide valuable insight for future untargeted and semi-targeted analyses of *W. somnifera*. It also demonstrated the suitability of South African plants as natural products and could provide an incentive for companies to obtain material locally instead of importing material from India.

Category: Medicinal plants/Ethnobotany

Analysis of phytochemical content and antioxidant properties of selected medicinal plants with evaluation of their antiviral activity against H1N1 and SARS-CoV-2.

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Respiratory viruses such SARS-CoV-2 (causing COVID-19) and influenza virus both infect the respiratory system, leading to symptoms such as fever, cough, and shortness of breath. While COVID-19 may no longer be classified as a global pandemic, the virus remains present and continues to pose a public health threat. Medicinal plants which have been reported to be used as traditional remedies through steaming to alleviate colds and flu symptoms could provide relief against neighboring respiratory viruses. The study is aimed at evaluating the antiviral activity of three medicinal plants commonly used to treat colds and flu symptoms through steaming. The leaves samples of *E. globulus*, *S. molle* and *S. lancea* were screened for phytochemical content and their antioxidant activity assessed through DPPH, FRAP and H_2O_2. The samples were further assessed through *in vitro* and *in silico* studies for their antiviral potential at selected targets of H1N1 and SARS-CoV-2. The three medicinal plants demonstrated similar quantities in flavonoid and phenolic content between *S. lancea* and *E. globulus*, however, the findings were significantly lower for *S. molle* in their phytochemical quantities. The plant samples exhibited high scavenging potential at varying levels with 50% inhibitory concentrations ranging from 17.747 ± 0.37 µg/mL to 826 ± 42.69 µg/mL. All three hexane plant extracts demonstrated dual inhibitory activity against both neuraminidase and PLpro enzyme. The selected medicinal plants proved to possess secondary metabolite constituents which were responsible for the observed antioxidant and antiviral activity.

Category: Evolution & Systematics

Rediscovery of *Penium margaritaceum* var. *incognitum* Claassen (Desmidiales, Zygnematophyceae) in South Africa 64 years after its description)

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During a routine study of South African freshwater algal diversity, we discovered unusual desmids in fresh samples collected from sediments of Klerkspruit in Golden Gate Highlands National Park, Free State province. The dominant alga was identified as *Penium margaritaceum* var. *incognitum* described by Martha Isabella Claassen in 1961 from Doornfontein Spruit about one mile north-east of Leeuport Dam, between Nylstroom (now Modimolle) and Vaalwater, Limpopo Province. This variety differs from the typical form in possessing of basal inflation in each semicell similar to that found in the genus *Pleurotaenium* Nägeli. These morphological characteristics are illustrated in our presentation by high-resolution LM images of newly discovered population. Sexual reproductive stages (formation of zygotes) were not observed. Other algal genera associated with *P. margaritaceum* var. *incognitum* included: Closterium Nitzsch ex Ralfs, *Cosmarium* Corda ex Ralfs, *Staurastrum* Meyen ex Ralfs, *Lepocinclis* Perty, *Phacus* Dujardin, *Trachelomonas* Ehrenberg. This species is polymorphic and has at least 14 accepted intraspecific taxa, including the type, but some of them are indistinguishable. *P. margaritaceum* var. *incognitum* is an endemic alga for South Africa and our record is the second one after 64 years since its description.

Category: Evolution & Systematics

Systematic position and biogeography of *Trachelomonas afra* Rino (Euglenophyta)

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In 1972 Almeida Rino described the loricate euglenid *Trachelomonas afra* from freshwaters near Lourenço Marques (now Maputo), Mozambique. Based on lorica dimensions, apical view, and the shape and arrangement of the spines, he noted its close resemblance to *T. lismorensis* var. *mirabilis* Playfair 1915.

However, Playfair's protologue lacks precision in distinguishing *T. lismorensis* var. *mirabilis* from the type variety, leaving no clear diagnostic features for reliable identification. Despite their similarities, several morphological differences distinguish *T. afra* from *T. lismorensis* var. *mirabilis*: i) The lorica of *T. lismorensis* var. *mirabilis* is subglobose/depressed, while *T. afra* has a spherical lorica; ii) *T. afra* possesses a collar around the pore, whereas *T. lismorensis* var. *mirabilis* only has short spines; iii) the anterior polar row of spines in Playfair's taxon contains only seven spines, compared to ten spines consistently present in all rows of *T. afra*. Whether these differences are significant enough to justify species distinction remains debatable. Playfair acknowledged that he did not observe the frontal view of the lorica and assumed it to be flattened, similar to the type variety. We propose that minor morphological differences, such as the slightly flattened anterior shape, variations in spine number and position, and differences in collar structure, are likely influenced by ontogenetic development. Additionally, we observed that the lorica surface changes with age: in juvenile specimens it exhibits delicate, net-like ornamentations, which later become smooth as the cell matures. These morphological characteristics are illustrated through high-resolution SEM images of African specimens. Reports of *T. lismorensis* var. *mirabilis* (= *T. afra*) are scarce in literature. In addition to its original records from Australia and Mozambique, it has been documented in Côte d'Ivoire (SEM images), Bangladesh (LM) and India (LM). Our findings expand its known distribution to several new locations in Angola and South Africa.

Category: Plant Biotechnology

Exogenous IBA stimulatory effects on root formation of Actinidia deliciosa rootstock and Actinidia arguta male scion stem cuttings.

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Recalcitrance to root of kiwifruit (Actinidia spp.) stem cuttings (SCs) is a restriction for its commercial production. Exogenous hormones is the key factor to regulate root formation, but the research on exogenous hormone stimulatory effects on Actinidia spp. is still lagging. A study was conducted to investigate the potential responses of kiwifruit SCs from *Actinidia deliciosa* rootstock and *Actinidia arguta* male scion SCs treated with IBA concentrations at low (10, 100 ppm), medium (1,000, 10,000 ppm) and high (100,000 ppm) levels. Parallel experiments for *A. deliciosa* and *A. arguta*'s treatments were arranged in a randomized complete block design (RCBD), with 12 replications. Treatments comprised different IBA concentrations, namely, 0 (control), 10, 100, 1,000, 10,000 and 100,000 ppm. At 103 days, rooting percentage, number of roots, root length, dry root mass, size of callus formation and callus percentage were determined. In *A. deliciosa* rootstock SCs, the highest (42%) rooting percentage and lengthy (0.3 cm) roots were observed at 10,000 ppm IBA, whereas the most (0.29) number of roots were produced at the highest (100,000 ppm) IBA, when compared to the control. Calli percentage (94%) was highest at 100 ppm IBA, while the biggest (2.8) size of callus formation was at the highest IBA of 100,000 ppm. In *A. arguta* male scion SCs, the greatest number of roots (1.08) and root mass (0.07 g) were attained at 10,000 ppm IBA. The longest (0.94 cm) root length was achieved at 100 ppm IBA. No callus formation was observed. In conclusion, the SCs of *A. arguta* male scion required low to medium (100 to 10,000 ppm) concentrations of IBA for proper roots stimulation and development, whereas in *A. deliciosa SCs*, a medium (10,000 ppm) to highest (100,000 ppm) IBA concentrations achieved highest rooting percentage and lengthy roots, paired with callus formation.

Category: Medicinal Plants/Ethnobotany

Evaluation of the cytotoxic and antibacterial effects of medicinal plants commonly used in South African traditional medicine

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The increasing number of antibiotic-resistant bacteria has highlighted the need to innovate and find effective solutions regarding our healthcare. Despite the alleged therapeutic benefits of medicinal plants, a more thorough examination may reveal the existence of poisonous elements that are damaging to the human body when consumed. In this study, five plants were tested for antibacterial properties using the minimum inhibitory concentration (MIC) and cytotoxic properties using 3-(4,5-dimethyl-thiazol- 2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay: *Lantana rugosa, Lippia javanica, Lippia wilmsii, Hilliardiella elaeagnoides,* and *Withania somnifera.* Four species and liver cells were employed to test the efficacy and safety of these plant extracts. The extracts demonstrated antibacterial activity, with MIC values ranging from 0.04 ± 0.04 mg/mL to 11.46 ± 9.66 mg/mL. The acetonic extract of *L. javanica* showed the best antibacterial activity, with MIC values of 0.04 ± 0.04 mg/mL against *P. aeruginosa* and 0.28 ± 0.35 mg/mL against *S. aureus*. The aqueous and acetonic extracts of *L. wilmsii* (LC_{50} > 1000 µg/mL) and the hexane extract of *L. javanica* (LC_{50} > 1000 µg/mL) were the least hazardous. While acetonic extract of *L. javanica* (0.01 ± 0.64 µg/mL) and hexane extract of *L. wilmsii* (0.03 ± 0.31 µg/mL) were the most cytotoxic. The findings of this study confirm that the medicinal plants tested possess antibacterial activity, supporting their traditional use in South Africa for treating various bacterial infections and illnesses.

Category: Medicinal Plants/Ethnobotany

In vitro therapeutic bioactivity testing of *Medicago sativa l.* (alfalfa/lucerne)

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As a response to the persisting global burden of diseases, the United Nation's SGD3 aims to ensure healthy lives and to promote well-being for all people at all ages by the year 2030. The urgent need for the discovery and development of novel therapeutics is motivated by emerging infectious diseases, resistance to drugs by superbugs, the high cost of medicines and clinical care, the negative side effects, and the unequal access to healthcare and medicines. The therapeutic potential/activity of *Medicago sativa* L. was measured using the reliable Sulforhodamine B Assay, Broth Micro-Dilution Assay, the colorimetric Microplate Alamar Blue Assay (MABA), The 3,4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) assay, anti-inflammatory assay towards the macrophage cell line RAW 264.7, and the Glucose Utilization Activity using L6 muroid skeletal muscle and C3A/HEPG2 liver cells. *Medicagos sativa* L. is a well-used animal fodder crop globally, and this paper aimed at measuring therapeutic potential against human diseases. *Medicago sativa L.* is a rich source of therapeutic and nutritional compounds that includes carbs, proteins, tannins, saponins, alkaloids, carotenoids, flavonoids, and phenolics. In our experiments, *Medicago sativa L.* demonstrated considerable anti-microbial, anti-cancer, anti- inflammatory, antioxidant, and anti-diabetic healing activity. The use of water infused with herbs (tea) is a reliable non-drug therapeutic concoction that is supported by our experimental results, therefore further fractionation of alfalfa to identify active compounds is recommended.

Category: Plant Biotechnology

Detecting the biochemical activity of cassava (*Manihot esculenta* Crantz) in response to drought stress

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The global significance of cassava and the impact of drought stress have prompted studies on the metabolic alterations under varying drought conditions in its cassava host. However, drought prevention measures remain inadequate. This study used untargeted metabolomics to investigate the metabolic responses of two cassava genotypes to drought stress, namely: UKF4 (moderately tolerant) and P4/10 (highly tolerant). Leaf samples were collected and analyzed by LC-MS qTOF at the early and late stages of tuber bulking, followed by multivariate statistical and pathway analysis. The findings revealed distinct metabolic reprogramming between the two genotypes. UKF4's rapid but temporary response was characterized by an early increase in flavonoids and phospholipids, indicating that a stress relief mechanism was first activated. In contrast, P4/10 maintained metabolic balance and levels of key metabolites despite extended stress, indicating greater long-term tolerance. The pathway analysis revealed that alpha-linolenic acid metabolism was strongly associated with early drought response, whereas the glycerophospholipid and anthocyanin biosynthesis pathways were associated with late drought response, emphasizing the importance of membrane integrity and oxidative defence. In line with this, the link between P4/10, pantothenate, and CoA biosynthesis suggested an energy-saving mode during stress. Flavonoids and lipids are important biomarkers for drought tolerance, and P4/10 could be a promising choice for breeding efforts. These findings give light on the mechanisms of metabolic adaptive plasticity in cassava, potentially serving as a foundation for enhancing drought tolerance in cassava and other crops. Further research with multi-omics data integration and field experiments is suggested to allow the application of these findings in agriculture.

Category: Plant Biotechnology

Integrative proteomic and metabolomic profiling reveals distinct response mechanisms in drought-tolerant and drought-susceptible maize varieties

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Maize is a crucial crop, and its productivity is greatly threatened by drought stress, particularly in regions with variable rainfall patterns. Thus, understanding the molecular mechanisms underlying drought tolerance is important for developing resilient maize varieties. This study employed integrative proteomic and metabolomic approaches to compare the molecular mechanisms of a drought-tolerant maize variety (QN 701) and a drought-susceptible variety (QS 7646 QPM). Proteomic profiling analysis using SDS-PAGE, followed by functional annotation through Panther GO and protein interaction mapping via STRING, revealed that the tolerant variety exhibited highly interconnected protein clusters related to osmotic regulation, oxidative stress defense, and cellular repair mechanisms that enhance its resilience. In contrast, the susceptible variety displayed weaker and less connected protein networks, indicating limited activation of adaptive pathways. Additionally, metabolomic profiling via LC-MS, analyzed through Sirius, MetaboAnalyst and GNPS, identified key drought-associated metabolites, including increased accumulation

of lipids in the tolerant variety. These lipids play an important role in membrane stabilization and stress mitigation. These datasets demonstrated a coordinated regulation between proteins and metabolites involved in drought adaptation, providing a comprehensive view of the molecular mechanism in maize. These findings provide insights into the biochemical basis of drought tolerance and identify potential target biomarkers for breeding drought-resilient maize varieties.

Category: Physiology

Investigating the impact of seaweed biostimulants in alleviating heat stress for improved crop resilience

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Many plants have suffered heat stress due to continuous rising temperatures. Heat stress is over-exposure of plants to temperatures above their threshold, resulting in permanent damage to plant growth and development. When the temperature increases between 10–15°C above the usual temperature, it is denoted as heat stress. Increased temperature impairs several crop development processes, including general plant growth, reproduction, photosynthesis, and pollen development. These adverse effects have resulted in the urgency to discover strategies to counteract the effects of heat stress to sustain food security and production. Biostimulants hold the potential to bring sustainable and cost-effective solutions to the impact of heat stress for improved agriculture and crop production. They are highly favourable because, unlike many chemically synthesized fertilizers, they do not have adverse side effects on the environment, and they fit in with organic farming, which has been gaining popularity in the agriculture industry. They are also believed to possess opportunities for developing novel strategies to control and alter the physiological processes of plants to stimulate plant growth, increase crop production, and ease the effects of abiotic stress on crops. Many studies have testified to the effectiveness of plant biostimulants against abiotic stresses in plants. However, more research is needed to focus on the effects of biostimulants on plants exposed to heat stress. This study addresses that need by investigating the potential of the seaweed extracts biostimulant (Kelpak, 1%) to alleviate the effects of heat stress on three crops (Amaranthus spinosus L., Helianthus annuus L., and Phaseolus vulgaris L) where open-top chambers have been placed over crops planted in the field to elevate the temperature. The study considers physiological responses, metabolomics, nutritional profile, and phyto-hormone profiles of these crop species under heat stress.

Category: Medicinal Plants/Ethnobotany

Assessing the phytoremediation potential & extract utility of *Carpobrotus* species *in vitro*

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This study examined the phytoremediation capability and medicinal extract efficacy of seven South African *Carpobrotus* species when exposed to copper-contaminated substrates, simulating mine tailing conditions. Phytoremediation, which uses plants to extract or stabilise toxins in impacted soils, is a sustainable approach to the environmental issues that result from mine tailing dust and metal contamination. . Importantly, if plants retain metals primarily within their roots, their aerial parts may still hold value for medicinal applications, even when grown under contaminated conditions. The aim of the study was to assess how seven South African Carpobrotus species*, C. deliciosus, C. dimidiatus, C. edulis, C. mellei, C. muirii, C. acinaciformis, and C. quadrifidus,* respond to controlled copper exposure by measuring tolerance indices, phytochemical profile, and palatability. Cuttings of each species were propagated in river sand within a greenhouse and acclimatised for four weeks before receiving one of two treatments, tap water or contaminated solution, for 6 weeks. The contaminated solution was prepared using leachate from Palabora Copper Mine tailings, supplemented with a copper salt. Both groups were fertilised biweekly with a nutrient solution. All species survived under copper stress, although varying degrees of senescence were observed, particularly in *C. dimidiatus, C. edulis, C. mellei*, and *C. muirii*. Most species maintained comparable biomass across treatments; however, *C. muirii* demonstrated a significant reduction in dry mass under contamination ($p = 0.039$). Copper exposure caused a stronger flavonoid expression in all species, while tannin and phenolic presence remained largely unaffected. Notably, sucrose concentration, a measure for palatability, decreased significantly in *C. muirii* and *C. acinaciformis* under contamination ($p = 0.049$ and $p = 0.034$, respectively). Overall, these results suggested that *Carpobrotus* have a promising tolerance to metal-contaminated environments and retain considerable phytochemical value, supporting their potential role in both mine tailings rehabilitation and local medicinal applications.

Category: Physiology

Investigating The Effects of Organic Fertilizers on Radish and Cabbage Health

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This study aimed to evaluate the potential of organic fertilizers to enhance photosynthetic efficiency and crop yield. The effects of two organic fertilizers - Fertilizer 1, derived from gelatine extraction byproducts, and Fertilizer 2, derived from processed cattle blood and bones - on the growth and photosynthetic efficiency of cabbages (*Brassica oleracea*) and radishes (*Raphanus sativus*) were investigated. Plants were treated weekly with two fertilizer dilutions (1:100 and 1:200) over the course of five weeks, during which the control plants only received water. Chlorophyll fluorescence was measured using a Handy-PEA. By the final week, radishes treated with Fertilizer 1 showed a significantly higher potential to reduce $NADP^+$ to NADPH compared to all other treatments, which shows that Fertilizer 1 significantly increased the availability of NADPH to be used in the carbon reduction cycle. In cabbages, the same trend was observed, though the differences were not statistically significant. In contrast, Fertilizer 2 either resulted in a lower potential for the reduction of $NADP^+$ to NADPH, or had no notable effects compared to the control groups. Thus, in both plants the results suggest that these fertilizers most notably affect the potential for electrons to be transported beyond PSII to reduce $NADP^+$ to NADPH. These findings suggest that organic fertilizers could potentially increase crop yield by providing higher availability of NADPH that can be used during the carbon reduction cycle during the synthesis of sugars and other essential biomolecules needed for the growth and photosynthetic efficiency of the plant. Future research could explore the potential for more optimised application procedures to further improve crop yield and photosynthetic efficiency.

Category: Evolution and Systematics

Why plant names change: Assessing reasons for name changes of dicotyledons on the Witwatersrand from 1990 to the current time

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The long-awaited Volume 2 of the Flora of the Witwatersrand covering the Dicotyledons is currently in press after many years of preparation. It includes 61 families and more than 450 genera and covers the region from Midrand in the north to Heidelberg in the south and from beyond Krugersdorp in the west to Springs in the east. In preparing for the volumes on the Dicotyledons of the Witwatersrand, a list of plants collected in the area was compiled from specimens housed in the C.E. Moss Herbarium as well as lists from the National Herbarium. This list dates to the late 1980s and includes 1041 species. Comparing this list to currently accepted names give an insight into the proportion of name changes arising from each of the situations where names require changing. The taxonomic history of taxa that were previously considered to be widely distributed globally often differs markedly from those taxa endemic to southern Africa. Circumscriptional changes to large and well-known genera create the perception that taxonomy in the past was somehow deficient. This is often refuted by cases where older names have been revived when modern approaches (e.g. use of molecular phylogenies to assess monophyly) are applied. The relative proportions of reasons for changes are provided, with some selected examples.

Category: Physiology

Coal tailings as a soil conditioner for flax (*Linum usitatissimum*): impacts on growth and photosynthesis

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Coal tailings, a by-product of mining activities, pose major environmental challenges due to their acidity and heavy metal contamination, which degrade soil quality. However, depending on their composition, coal tailings may contain certain macro- and micronutrients that, if managed appropriately, could potentially benefit plant growth and improve soil fertility. In this study, coal tailings were investigated for their potential as a soil conditioner for flax (*Linum usitatissimum*) when amended with potting mix soil (1:1) and organic fertilizer. The impact of these amendments on soil properties, seed germination, seedling survival, photosynthetic efficiency [OJIP transient analysis, maximal quantum efficiency of PSII (Fv/Fm), Performance Index ($\mathrm{PI}_{\mathrm{total}}$)], and biomass production was assessed. Pure coal tailings inhibited flax germination and seedling survival, possibly due to acidic pH (Coal A: 6.11, Coal B: 6.81) and elevated heavy metals (Fe, Pb, As). Soil amendment significantly improved germination (Coal A+Soil: 70%, Coal B+Soil: 80%), suggesting a mitigation of adverse conditions through improved soil structure and dilution of potential toxins. Photosynthetic performance in fertilized plants varied. Coal A+Soil initially enhanced the $\mathrm{PI}_{\mathrm{total}}$, while Coal B+Soil decreased it without fertilizer. Fertilization significantly increased $\mathrm{PI}_{\mathrm{total}}$ in both amended soils, with fertilized Coal B+Soil showing the highest $\mathrm{PI}_{\mathrm{total}}$ and Fv/Fm. Biomass production followed a similar trend, with no significant differences observed in unfertilized treatments, but significant increases in both Coal A+Soil and Coal B+Soil upon fertilization compared to fertilized control soil. These results indicate that while raw coal tailings are detrimental, their amendment with soil and fertilizer can enhance flax photosynthesis and biomass. However, the variability in coal tailing composition, particularly pH and heavy metals, requires careful characterization before application. Further research is crucial to evaluate long-term impacts on soil health and potential risks of heavy metal accumulation in plants.

Category: Medicinal Plants/Ethnobotany

Antibacterial and antibiofilm activity of *Argemone ochroleuca* Sweet. and *Argemone mexicana* L. extracts against phytopathogenic microorganisms of Solanaceae crops

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Plant diseases pose a serious threat to agriculture and food security globally, with the potential to lower yields by 21- 30% in several major crops worldwide. Synthetic pesticides are effective in managing plant diseases, however their excessive usage have resulted in most pathogens developing resistance. Plants such as *Argemone ochroleuca* and *A. mexicana* have bioactive compounds that can be explored as eco-friendly alternatives. However, there is limited information on the biological potential of these plants against phytopathogens. Hence, the study aimed at evaluating the inhibitory potential of *A. ochroleuca* and *A. mexicana* extracts on the growth of bacterial pathogens through antibacterial and anti-biofilm activities. Dried shoots and roots of *A. ochroleuca* and *A. mexicana* were extracted with water, acetone, methanol and dichloromethane. The antibacterial activity was assessed using microdilution method by determining the minimum inhibitory concentration (MIC) while the biofilm inhibition was assessed at three stages: initial cell attachment (0 hours), preformed biofilm (8 hours), and mature biofilm (24 hours). All extracts demonstrated broad-spectrum antibacterial and antibiofilm activity, however methanol extracts had the lowest activity on all antibiofilm stages. The good MIC values ranged from 0.06 to 0.16 mg/ml, with acetone extracts of *A. ochroleuca* roots and *A. mexicana* shoot and root having the best activity with MIC of 0.06 mg/mL against *Xanthomonas gardneri.* Most of the extracts inhibited the adhesion of biofilm with inhibition percentages of over 50 % at 0 h. The highest inhibition at 0 h was recorded by *A. mexicana* root water extracts against *Ralstonia solanacearum* at 87.21%. In mature biofilms, most extracts showed activity less than 50%. The obtained preliminary results highlighted the antibacterial potential of these plant extracts, with further studies underway to characterize their bioactive compounds.

On the Phylogeny and Species Delimitation of the Cape Endemic *Wiborgia* Thunb.

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Wiborgia Thunb. is a legume genus comprising ten species endemic to the Greater Cape Floristic Region. Species in the genus are generally shrubs, with distinct ascending to an erect habit with shoots terminating in rigid thorns, and are distinguished from other Crotalarieae by their indehiscent one-seeded fruits. The genus was recently revised in 1975, and to date, phylogenetic relationships within the genus are poorly understood. Recent studies inferred phylogenetic relationships based on two DNA markers and included seven out of ten *Wiborgia* species, resulting in unresolved species relationships. Therefore, this study aimed to explore relationships within the genus using five fast-evolving DNA markers and sampling multiple accessions representing the morphological and ecological variability, enabling testing hypotheses on the utility of Dahlgren's key hypotheses for infrageneric classification. Phylogenetic relationships based on morphology and genealogy were inferred using coalescent-based methods. Within the *Wiborgia* clade, two strongly supported clades were observed. Clade 1, comprised a novel, well-supported sister relationship between the widely-distributed *W. mucronata* and the narrowly distributed *W. tenuifolia*, both characterized by thorny glabrous branches. Clade 2, comprised of *W. tetraptera*, identified sister to *W. fusca*, whilst *W. monoptera* and *W*. incurvata formed part of a strongly supported clade. *Wiborgia obcordata*, the only species in Dahlgren's subgenus *Wiborgia*, was found to be embedded within subgenus *Pterocarpia*, thus, the subgeneric classification of Dahlgren was not supported. Key morphological characters (including inflated/compressed fruit walls, the presence of lateral wings, and the presence of thorns) were identified to derive independently across the phylogeny, and thus unsuitable to delineate subgenera. Our reconstructions have shown that these characters, together with other important characters such as branch pubescence and flower colour, could be used in delineating species, but not subgenera. Sister pairs overlap geographically, but in most cases showed differences in floral characters.

Category: Ecology

A test of the Grant–Stebbins pollinator-shift model of floral evolution

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Pollinators are thought to play a key role in driving incipient speciation within the angiosperms. However, the mechanisms underlying floral divergence in plants with generalist pollination systems remains understudied. *Brunsvigia gregaria* displays significant geographical variation in floral traits and is visited by diverse pollinator communities. Because pollinators are often shared between populations, we investigated whether specific pollinators are responsible for driving floral divergence between them. Three distinct ecotypes were identified, each dominated by three different pollinators: bees, swallowtail butterflies, and long-proboscid flies. Across seven populations, we found a pattern of association between style length and the morphology of pollinators that visit the flowers most frequently and contact the reproductive parts most often. Furthermore, we found significant linear, quadratic and correlational selection on flower number, tepal length and style length within the butterfly- and bee-dominated populations. We also found partial evidence for divergent selection on these traits between experimental sites. Our findings suggest that a handful of key pollinators that vary in their importance have the potential to drive population-level divergence in floral traits, which may lead to pollination ecotype formation.

Category: Pollination biology

No Boundaries? Weak ethological isolation in co-flowering milkweeds

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The subfamily Asclepiadoideae encompasses an array of specialised pollination syndromes, presumably due to their complex floral morphology; however, few studies have considered how these specialised plant-pollinator interactions and floral morphology maintain reproductive isolation in co-flowering assemblages of asclepiads. There is also a deficit of studies on the Asclepiadoideae in Gauteng, with most studies limited to KwaZulu-Natal and Western Cape. The present study aimed to assess the role of ethological and floral isolation in maintaining species boundaries in co-flowering asclepiads. The study was conducted at Melville Koppies Nature Reserve, Gauteng, during the summer months of 2024–2025. Six species co-flowered during that period: *Asclepias curassavica, A. albens, Gomphocarpus fruticosus, G. physocarpus, G. tomentosus* var. *tomentosus*, and *Xysmalobium undulatum*. For each species, we measured the amount and concentration of nectar produced, observed and collected pollinators, harvested flowers for morphometric measurements, obtained scent samples, and estimated pollinator transfer efficiency. Honeybees visited all six

species, however they removed pollinia from only three: *G. fruticosus*, *G. physocarpus*, and *X. undulatum*. Notably, pollinia were consistently attached to the same locations on the bees' hind- and forelimbs across these species. In contrast, wasps and beetles carried pollinia from only one species, even when the beetles were non-specific in their visits. The pollination niche breadth was lowest for *A. albens* and highest for *G. fruticosus*, with the former only being visited by one pollinator functional group. This pattern suggests that ethological isolation may be weak or context-dependent, as honeybees do not exhibit species- specific foraging behaviours that would reinforce isolation. Nectar volume and concentration were similar amongst species, however, floral measurements differed significantly. These findings suggest that floral structural differences may play a stronger role than pollinator behaviour in maintaining species boundaries among sympatric asclepiads.

Category: Medicinal Plants/Ethnobotany

Comprehensive untargeted metabolomic profiling, antimicrobial, and cytotoxicity evaluation of *Artemisia afra* leaf extracts collected from Pretoria

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Investigation of natural resources has contributed to the identification of medicinal plants with a wide range of properties. The pursuit of effective antimicrobial agents remains an important focus. This study explored the potential of identifying compounds with antimicrobial activity in *Artemisia afra* extracts. Samples of *A. afra* were obtained from three different vendors in Pretoria and they were extracted using butanol, chloroform, and water. Extracts were evaluated for antimicrobial and cytotoxic activity using standardized methods and characterized for antimicrobial compounds using untargeted metabolomics. Among all the extracts, chloroform extract from vendor B exhibited the highest cytotoxicity, with an LC_{50} value of 414.56 ± 4.81 mg/mL, while other extracts were less cytotoxic. All extracts exhibited antibacterial properties. The butanol extract from vendor B was effective against *Pseudomonas aeruginosa*, while the water extract from vendor A showed activity against *Staphylococcus aureus*, *Escherichia coli*, and *Enterococcus faecalis*, as compared to vendor B's butanol extract. A total of 436 compounds were detected using GC×GC-TOF-MS, including 34 compounds known for antimicrobial properties. The compound concentrations were different amongst the vendors' samples with the highest from vendor C (14.85 mg/mg) being Bicyclo[2.2.1]heptan-2-one 1,7,7-trimethyl-(1S)-, an antimicrobial compound. Two compounds were undetectable in vendor A sample, four were undetected in vendor C sample, and eleven compounds in vendor B sample. The antibacterial activity of extracts could not be related to metabolite variations and concentrations. In conclusion, *A. afra* obtained from traditional medicinal markets demonstrated notable antibacterial activity.

Category: Physiology

Cajanus cajan enhances nutrient cycling and supports *Eragrostis curvula* growth

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Cajanus cajan enhances soil nutrition by increasing bacterial communities and their associated enzyme activities, creating optimal conditions for the growth of succeeding crops like *Eragrostis curvula*. This study explored the viability of rotating *C. cajan* and *E. curvula* and assessed their effects on soil nutrient cycling. *Cajanus cajan* and *E. curvula* were monocropped and rotated in soils collected from Jameson Park, Kaydale, and Rensburg, Gauteng, South Africa. Pre-planting and post-harvest soil analyses were done to determine the nutrient concentrations, pH, total cation concentrations, exchange acidity, enzyme activities, and bacterial identification. Furthermore, the growth kinetics and nitrogen (N) source reliance of *C. cajan* and *E. curvula* were determined. *Cajanus cajan* and *E. curvula* selected for different bacterial genera in their rhizosphere soils. *Cajanus cajan* selected for *Pseudomonas, Novosphingobium, Paraburkholderia, Burkholderia,* and *Croceiccocus* genera, while *E. curvula* selected for *Pseudomonas, Flavobacterium, Pedobacter, Arthrobacter, Pseudoarthrobacter,* and *Luteimonas* species. The rotation of *C. cajan* and *E. curvula* selected for *Pseudomonas, Caulobacter*, and *Pedobacter* genera. Monocropped *C. cajan* significantly increased nitrate reductase, β-glucosidase, and acid and alkaline phosphatase activities compared to rotation treatments and *E. curvula* monocropping. *Cajanus cajan* primarily derived N from the atmosphere (NDFA), with 62.67%, 63.38%, and 62.04% in Jameson Park, Kaydale, and Rensburg soils, respectively. Rotated *E. curvula* showed higher reliance on NDFA and had higher plant N and P concentrations than in monocropping. This study highlights the role of *C. cajan* in enhancing soil nutrient dynamics, suggesting its rotation with *E. curvula* can yield multiple benefits for subsistence farmers in impoverished communities.

Category: Physiology

Growth responses to warming and soil moisture provide insights into vulnerability thresholds of key plant species of Southern Africa

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The study tested whether common species of different plant functional groups of Southern Africa, namely bulbous plants (*Eucomis automnalis, Bulbine natalensis, Agapanthus praecox*), herbaceous perennials (*Pelargonium sp.*), palms (*Dypsis lutescens*, D*ypsis decaryi*), shrubs (*Barleria greenii, Carissa macrocarpa*) and succulents (*Portulacaria cafra*, *Crassula ovata*) significantly differ in their biomass responses under future warmer climate scenario (2˚ above ambient) and soil moisture levels, in ways that favour functional groups with better drought and temperature escape strategies. The hypothesis was tested using a factorial arrangement (species x warming x soil moisture) in a completely randomised design, with five replicates of each treatment combination. A warmer microclimate increased plant stress, which was more intense at low soil moisture, this suggesting that the predicted warmer drier climatic scenarios for Southern Africa may impact the physiology of the represented species or their functional groups. Although the effects of warming were not discernible after 150 d, in bulbous plants the biomass significantly dropped. At low soil moisture, significant reductions in total biomass were only detected in bulbous and herbaceous plants. Under a warmer microclimate, shrubs and herbaceous plants prioritised their biomass towards belowground organs. Thus, the ambient thermal conditions are approaching the tolerable limits for the represented species within the bulbous and herbaceous functional groups. Long-term field monitoring studies of these common functional groups are therefore recommended.

Category: Medicinal plants

Systematic review on traditional medicinal plants used for the treatment of livestock diseases in the Eastern Cape Province

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Many of the plant species found in South Africa’s Eastern Cape province have been used in traditional medicine, yet no systematic review has been conducted to synthesize this information. The aim of the study was to conduct an overview of the medicinal plants used for the treatment of livestock diseases in the Eastern Cape province. Data for this review were generated from published peer reviewed journals, conference proceedings and theses found using scholarly search engines, including Pub Med, Google Scholar, Research Gate, and Open Access Journals. No restriction was placed on the period of publication and no discrimination was made in relation to methodologies applied when collecting data. Focus was only on flora found in the Eastern Cape Province. Publications were searched. Key words such as “medicinal plants to treat livestock”, “plant extracts to treat livestock”, and a total of 19 articles were found. From the literature analysed, most studies were done within the radius of 200 km around Fort Hare University. The results showed that a total of 56 plant species belonging to 33 families have been used. Helminths was the most prevalent disease. The most used plant families were *Asphodelaceae (*10.7%*),* *Fabaceae (*8.9%), *Lamiaceae (*8.9%), *Rubiaceae (*5.4%), *Rutaceae (*5.4%), *Asteraceae (*3.6%), *Apiaceae (*3.6%), *Hypoxidaceae* (3.6%) and *Asparagaceae (*3.6%). The most widely used plant parts for the preparations of remedy were leaves (38.7%), followed by roots (24.2%) and bark (19.4%) and used in solution form by using water as a most cited solvent. Information obtained from this review serves as a guide to discover novel drugs from plants. There is an urgent need to conduct further research to document, screen and perform clinical trials on medicinal plants at larger scale.

Category: Medicinal Plants/Ethnobotany

A study of medicinal plants used for paediatric skin infections in rural South Africa

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In rural communities, traditional medicine remains crucial to primary healthcare due to the accessibility of Indigenous Knowledge Holders (IKHs). This study investigated the phytochemical composition and

antimicrobial activity of medicinal plants traditionally used for treating skin diseases in children. Data were collected through ethnobotanical surveys, and the most frequently cited plants were selected for further laboratory analysis. Plant extracts were prepared using hexane, acetone, and methanol, and were subjected to phytochemical screening to identify major classes of secondary metabolites. Quantitative analyses of total phenolic and flavonoid contents were performed. The antimicrobial potential of the extracts was evaluated against two Gram-negative bacterial strains, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. These strains were chosen for their clinical relevance and high prevalence in skin and wound infections in environmentally polluted areas. *Commelina diffusa*, *Euphorbia prostrata*, and *Aptosinum elongatum* were most frequently cited. Leaves and stems were the most commonly used plant parts, with maceration being the preferred method of preparation. Phytochemical screening revealed the presence of multiple bioactive compounds. The total phenolic content of the plant extracts was relatively high, ranging from 20 mg Gallic acid equivalents (GAE)/g in *E.prostrata* to 35 mg GAE/g in *C. diffusa*. Similarly, the flavonoid content varied significantly, from 2.99 mg Quercetin equivalents (QE)/g in *E. prostrata* to 91.11 mg QE/g in *C. diffusa*. Both *C. diffusa* and *E. prostrata* exhibited antimicrobial activity against *Pseudomonas aeruginosa*, suggesting a potential link between their phytochemical composition and antimicrobial efficacy. These findings warrant further investigation to identify the active compounds responsible for the observed activity, which could potentially lead to the development of novel therapeutic agents

Category: Ethnobotany

Utilisation of medicinal plants in treating livestock ailments at Amathole District Municipality.

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The study was conducted to document various medicinal plants used and their application in the treatment of livestock ailments at Amathole District Municipality. Data was collected from 17 respondents through semi-structure questionnaire, supplemented with filed visit and personal observations. Descriptive statistics were used in data analysis. A total of 45 medicinal plant species from 22 families were used for different disease control, with most plants belonging to the family *Asphodelaceae*. For various ailments, these plants were utilized either alone or in combination. Decoction was the predominant herbal formulation used. Out of the 11 diseases target for control, the predominant one were warts (30%) followed by brucellosis (15%) and prolapse 13%. Leaves (50%) were the most part utilized. The main route of remedies administration was oral. More than 70% of the plant species used were collected from the wild. The study's conclusions demonstrate that Amathole District Municipality's plant resources continue to have a significant socioeconomic impact. To shed additional light on the matter, more research should be done on the distribution, usage patterns, and effectiveness of these involved plant species.

Category: Evolution & Systematics

Botanical Gold Rush: Investigating taxonomic boundaries in yellow-flowered *Commelina africana* species L. complex (Commelinaceae, Commelinales)

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Commelina Plum. ex L. is the largest genus of Commelinaceae, comprising ca. 210 species. It is easily differentiated from the remaining genera of the family by its inflorescences with a spathaceous basal bract and reduced to (1–2) fasciculate cincinni, strongly zygomorphic and enantiostylous flowers, petals unequal, paired petals clawed and mostly sky blue (but sometimes white or lilac, rarely yellow, apricot, orange, or pink), three posterior staminodes with X-shaped antherodes, and three dimorphic anterior stamens. Despite being an easily recognised genus, species delimitation represents a recurring issue in *Commelina*, with an overreliance on the colour of their complex and ephemerous flowers. This is perfectly illustrated by the *C. africana* complex, currently circumscribed to include most perennial *Commelina* species with solitary inflorescences, spathes free at base, yellow to orange flowers, filaments connate at base, and capsules with ventral locule generally aborted during fruit development. Our study aims to investigate the distribution, ecological characteristics and morphological variation of all taxa and morphotypes included in the *C. africana* complex. This requires the combination of traditional alpha taxonomy and nomenclature, extensive field expeditions, and cultivation studies coupled with a molecular and morphological phylogenetic investigation of these taxa. Our preliminary results indicate extensive taxonomic and nomenclatural confusion regarding the application of the existing names, which has been historically aggravated by the group having been primarily studied by botanists from the Global North with inadequate to no access to living specimens and limited access to herbarium species from several regions in Africa. Our results highlight the vast importance of fieldwork and how imperative it is for local botanists to spearhead the study of their local biodiversity.

Category: Medicinal Plants/Ethnobotany

Assessing the pharmacological activities of *Pterocelastrus echinatus* extracts as a potential source of new drugs

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Pterocelastrus echinatus N.E. Br. (Celastraceae) is used to treat microbial infection such as tuberculosis in the Eastern Cape, South Africa. This study was aimed at evaluating antimicrobial, antioxidant activities, and cytotoxic effects of the leaf and stem extracts of *Pterocelastrus echinatus* using micro-broth dilution assays, DPPH, Ferric reducing power assay, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay and Real-time cell analysis assay (RTCA) using xCELLigence system. The results revealed the presence of antimicrobial compounds with MIC values ranging from 310 ± 0.001 to 2500 ± 0.001 µg/ml. Meanwhile the methanolic leaf extract had the highest antioxidant activity (IC_{50} = 14.97 ± 6.53 µg/ml). The extracts had less to no toxic effects on HEK 293 and Vero CCL-81 cells with LC_{50} ranging from 114.82 ± 22.11 µg/ml to 582.73 ± 37.42 µg/ml. RTCA results revealed that hot and cold-water extracts had the highest cell index on both cell lines these are consistent with the MTT assay results. These findings could contribute to ethnobotanic antimicrobial drug discovery, moreover, further preclinical studies such as isolation of bioactive compounds, mechanism of action assays, and *in vivo* studies in animal models still need to be done. Studies are currently underway to identify secondary metabolites from plant *P. echinatus* extracts using an UPLC-HD-QTOF MS system

Category: Natural Resource Management and Conservation

Effect of organic and inorganic fertilization on the growth and yield of two *Amaranthus* accessions

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The production of indigenous leafy vegetables such as *Amaranthus* could address malnutrition and food insecurity experienced globally by providing the necessary nutrients needed in a balanced diet. However, escalating prices of chemical fertilizers make them unaffordable to small-holder farmers, aggravating the threat to food security. This is because an adequate nutrient supply through fertilization is essential to achieve better growth of these vegetables. Based on this, a study was established to evaluate the effects of organic and inorganic fertilizers on growth and yield of two *Amaranthus* accessions (*Amaranthus hypochondriacus* L. and *Amaranthus retroflexus*). The study consists of eight treatments, *A. hypochondriacus* L. + control (T1), cattle manure (T2), goat manure (T3), NPK (T4), and *A. retroflexus* + control (T5), cattle manure (T6), goat manure (T7), and NPK (T8). The experiment was laid out in a Completely Randomized Design (CRD) with four replications. Goat manure application increased the growth, fresh and dry shoot biomass yield of both *A. retroflexus* and *A. hypochondriacus* L. Goat manure application resulted in considerable results in growth and yield parameters and thus serves as a good alternative to small-scale farmers who may not be able to purchase inorganic fertilizer.

Category: Medicinal Plants

Evaluation of the phytochemistry and biological activity of the leaves and stem of *Rhoicissus tomentosa* (Lam.) Wild & Drummond

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Rhoicissus tomentosa is indigenous to South Africa. The genus *Rhoicissus* represents several species that are reported to be of great phyto-medicinal and ethnopharmacological value. However, *R. tomentosa* is relatively understudied. This study aimed to investigate the phytochemical composition and biological activity of the leaf and stem crude extracts of *R. tomentosa*. Results revealed that leaf extracts had a greater phytochemical yield (%) than stem. Phytochemical screening of the extracts showed positive results for various phytochemicals: alkaloids, tannins, phenols, naphthoquinones, flavonoids, saponins, steroids, proteins, carbohydrates, mucilage, gum and resin. The agar well diffusion method was used to evaluate the antibacterial activity against *Escherichia coli* and *Staphylococcus aureus.* The leaf extract was distinguished for its potential antibacterial activity against both bacterial strains with an inhibition zone (mm) of 8.17± 1.04 and 6.83 ± 0.58 at 10 mg/ mL. The 2, 2′-diphenyl-1-picrylhydrazyl (DPPH) assay was used to evaluate the antioxidant activity. The percentage scavenging activity of the extracts were significantly greater than the control. Furthermore, at 15, 30, 60,120 and 240 µg/ mL, the percentage scavenging activity of leaf extract was 74.65, 78.31, 85.45, 90.02, 95.68% and for stem was 71.66, 73.57, 84.05, 88.22, 96.28% respectively, indicating that the leaf extracts had greater scavenging activity (%) than stem. Also, the IC_{50} value for the leaf extracts (0.67µg/ mL) was lower than the ascorbic acid (8.26µg/ mL) (control). Results from this study suggest that extracts of *R. tomentosa* have great medicinal potential, thus these results are expected to stimulate interest and open the possibility of clinically effective drugs from this plant species.

Category: Medicinal Plants/Ethnobotany

Green Synthesis of Gold and Copper Nanoparticles by *Lannea discolor*: Characterization and Antibacterial Activity

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Green synthesis using plant extracts has emerged as an eco-friendly and viable alternative to chemical and physical approaches. The leaf, stem, and root extracts of *Lannea discolor* were employed as a reducing and stabilising agent in synthesizing gold (AuNPs) and copper (CuNPs) nanoparticles. The formation of AuNPs and CuNPs, confirmed by colour change, was characterised by UV-Vis spectroscopy (UV-Vis), scanning electron microscopy analysis, and energy-dispersive X-ray (SEM-EDX), transmission electron microscopy (TEM), and Fourier-transform infrared spectroscopy (FTIR). Furthermore, a minimum inhibitory concentration (MIC) antibacterial assay was carried out. Gold nanoflowers (AuNFs), NPs, and CuNPs peaked at wavelengths of 316, 544, and 564 nm, respectively. TEM showed unexpected nanoflowers (30-97 nm) in the leaf extracts and spherical NPs (10–33 nm; 9.3–37.5 nm) from stem and root extracts, while spherical CuNPs (20–104 nm) were observed in all extracts. EDX confirmed the presence of metal salts, and FTIR revealed stable capping agents. AuNPs and NFs from *L. discolor* extracts showed antibacterial activity against *Staphylococcus aureus, Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Bacillus subtilis* when compared to the plant extracts, while none was observed from the CuNPs. These AuNPs and CuNPs are appealing for use in various biomedical and manufacturing applications due to their properties and environmentally friendly production. To our knowledge, this is the first study of the synthesis of gold and copper nanoparticles from *L. discolor*.

Category: Medicinal Plants/Ethnobotany

Characterisation and screening of secondary metabolites from *Commiphora viminea* for anti-fungal against *Candida auris*

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Candida auris, a pathogenic yeast-like fungi, is an emerging pathogen that has garnered significant attention in recent years due to its ability to cause a range of infections and its resistance to antifungal medications. This study aimed at screening and characterizing secondary metabolites from *Commiphora viminea* for antifungal properties against *Candida auris.* The preliminary qualitative phytochemical analysis was carried out on crude (water, ethyl acetate, and acetone) to determine the presence of different phytochemical groups, while Liquid Chromatography Mass Spectrometry was conducted to obtain a detailed phytochemical profile of *C. viminea*. To determine antioxidant activity, 2, 2 diphenyl-1-picrylhydrazyl (DPPH) was used as a free radical scavenger. The minimum inhibitory concentration was conducted to evaluate the effectiveness of the plant extracts against *Candida auris*. Phytochemical testing and LC-MS profiling showed that the plant had the presence of various secondary metabolites, such as flavonoids, phenols, glycosides, saponins, and alkaloids. Antioxidant and antifungal testing showed that the compounds found in *C. viminea* may confer the ability to neutralise free radicals, thereby protecting against oxidative damage, and may also efficiently treat various fungal infections.

Category: Plant Biotechnology

Evaluating the antifungal potential of 8-Hydroxyquinoline against *Fusarium proliferatum*

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Fusarium proliferatum, a mycotoxin producing phytopathogen, affects a wide range of crop plants, thus negatively impacting sustainable crop production and food safety. This fungus causes diseases such as Fusarium crown and ear rot, which reduce both crop quality and yield. Disease control of *Fusarium proliferatum* are limited and often ineffective, relying primarily on chemical fungicides that impacts human health and the environment. In addition, the fungus has developed resistance to several commonly used fungicides, underscoring the need for alternative management strategies. The used of quinoline derivatives are receiving widespread attention given its broad-spectrum antifungal potential against economically important phytopathogens. 8-hydroxyquinoline (HQ) has garnered significant interest in agriculture for its broad-spectrum antifungal efficacy against phytopathogens, disrupting critical cellular functions like DNA replication and protein synthesis that is essential for fungal growth. This study aims to assess the in vitro antifungal activity of 8-hydroxyquinoline against *Fusarium proliferatum* through antagonistic dual culture plate assays, scanning electron microscopy analysis coupled with biochemical profiling and enzymatic characterization.

Category: Plant Physiology

Exploring the dynamic response mechanisms of *Pennisetum glaucum*: enhancing resilience to dual drought and salt stress

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Agriculture is pivotal in sustaining the world's growing population through extensive food production. However, crop plants face numerous challenges in their natural environment, including abiotic stresses such as drought and salt stress. Plants have developed complex survival mechanisms. Understanding the response mechanisms of Pennisetum glaucum (pearl millet) to these stresses is vital for improving resilience in water-deficit and saline environments. Our study subjected pearl millet to separate and combined drought and salt stress for 25 days. The results showed significant reductions in morphological traits, such as plant height, fresh weights and lengths of both shoots and roots, and the number of leaves observed. Furthermore, key physiological parameters, including chlorophyll content, stomatal conductance, photosynthesis, and transpiration rates notably declined, indicating a complex interaction between stress factors and water regulation mechanisms. Proteomic analysis revealed altered protein expression in response to stress, suggesting potential upregulation or downregulation of proteins. Gene ontology analysis identified various unclassified proteins involved in essential biological processes, molecular functions, and cellular components. This study provides a comprehensive understanding of the detrimental effects of drought and salinity on pearl millet at the morphological, physiological, and proteomic levels, uncovering previously unexplored proteomic responses. It also offers valuable insights for researchers and agricultural experts studying stress responses in pearl millet and related crops, serving as a reference tool for understanding drought and salt stress pathways.

Category: Plant Biotechnology

Isolation of endophytic fungi from *Helichrysum petiolare* Hilliard & B.L.Burtt and GC-MS analysis of lipid metabolites fermented using a shake-flask bioreactor for their anti-cancer potential

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Despite the established link between polycystic ovary syndrome (PCOS) and an increased risk of obesity and endometrial cancer, there is still a lack of effective treatments. Insulin resistance is a consequence of extremely abnormal hormonal and metabolic activity which is a distinguished hallmark of both type 2 diabetes (T2D) and PCOS-related obesity. Moreover, the presence of insulin receptors in the endometrium has the potential of exerting adverse mutagenic effects on the endometrial lining. The discovery of bioactive compounds from natural products remains a key focus in phytochemistry, especially in the search for novel anticancer agents. Analytical techniques, such as Gas Chromatography-Mass Spectrometry (GC-MS), provide a powerful tool for identifying these metabolites. The aim of the study was to isolate endophytic fungi from leaves of *Helichrysum petiolare* and analyze their lipid profile fermented using a shake-flask bioreactor and assess them for potential to serve as anticancer agents. Fresh plant material of *Helichrysum petiolare* was surface-sterilized, and plant material cultured on Potato Dextrose Agar (PDA) to isolate culturable fungal endophytes. Purified fungal colonies belonging to the genus *Diaporthe*, *Botryosphaeria*, *Aspergillus* and *Fusarium* were isolated from the leaves of *Helichrysum petiolare*. The isolated fungal endophytes were then fermented in Potato Dextrose Broth (PDB) using a benchtop shake-flask bioreactor system. After 7 days of fermentation, lipids were extracted using a liquid-liquid extraction technique with the lipid profile analyzed using GC-MS. The lipid profile indicated the presence of fatty acid alcohols, hydrocarbons and esters, with Octadecane-6-methyl being the most abundant with a base peak of 18%. Qualitative phytochemical content analysis of the lipid extract showed the presence of alkaloids, tannins, phenols, flavonoids and terpenoids at moderate levels suggesting that the lipid extracts might have anti-cancer potential.

Category: Physiology

Analysis of the effects of non-composted and composted sewage sludge on a fodder corn variety

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Sewage sludge (SS) has been widely applied to arable land as a nutrient source, though its use is regulated. This study examined the effects of increasing concentrations of non-composted and composted SS (0%, 25%, 50%, and 75% as m/m%) from two Hungarian cities on maize (*Zea mays* L. cv. Armagnac). Conducted in a greenhouse under controlled conditions, the experiment measured plant characteristics 21 and 35 days after sowing. Results showed that photosynthetic pigment content increased significantly in all treatments compared to the control. Non-composted SS treatments resulted in a greater chlorophyll (Chl) a/b ratio,

indicating a stress response. Enzyme activities, including ascorbate peroxidase (APX) and guaiacol peroxidase (GXD), increased with higher concentrations of non-composted SS but showed no correlation with composted SS treatments. Superoxide dismutase (SOD) activity was significantly higher under all non-composted SS treatments. Malondialdehyde (MDA) content showed a significant positive correlation with Chl-b, Fo, Fm, and Fv, while a significant negative correlation was observed for Chl a/b, Fv/Fm, and Fv/Fo. Proline content significantly positively correlated with Chl-a, Chl-b, Fv/Fm, Fv/Fo, and MDA. A highly significant interaction (**$p \leq 0.01$) between origin and concentration was observed for carotenoids, Fo, Fv/Fo, and proline. Additionally, the interaction between origin and sampling time was significant (**$p \leq 0.05$) for Fm, Fv, Fv/Fm, MDA, and SOD, and highly significant for photosynthetic pigments. Multivariate analysis of variance (MANOVA) confirmed a highly significant effect on photosynthetic pigments, Fo, Fv/Fm, Fv/Fo, and SOD. Composted SS improved chlorophyll content but had a weaker effect on biochemical responses compared to non-composted SS. Non-composted SS may enhance short-term growth, its potential long-term environmental risks warrant caution. Future studies should improve composting techniques, explore cost-effective large-scale applications, and conduct extensive field research to assess SS impacts on soil and crops. Composted SS offers promising potential for sustainable agriculture by balancing productivity and environmental protection.

Category: Physiology

Seed production and seedling establishment of wild watermelon (*Citrullus lanatus* var. citroides) in response to various growing media

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The objective of this study was to evaluate various growth media for the proper establishment and emergence of wild watermelon seedlings, *Cucumis lanatus* var. citroides, grown in a shade house. Four different growth media were used: Hutton soil (control), Hygromix, Compost, and Culterra seedling mix, which served as treatments. The experiment was set up in a randomized complete block design (RCBD) with three replicates ($n = 12$). Fourteen days after establishing the treatments, emergence percentage (EP%), mean emergence time (MET) in days, and emergence rate index (ERI in seeds/d) were determined. The treatments had highly significant ($P \leq 0.01$) effects on MET and ERI; however, no significant effect ($P \leq 0.05$) was observed in EP%. Relative to Hutton soil (EP: 84%), the highest EP% was achieved in seeds planted in Hygromix (93.3%), followed closely by Culterra seedling mix (93.0%) and Compost (92.3%). The earliest seedling emergence (MET: 1.58 days) was recorded in seeds planted in the Culterra seedling mix, while the latest emergence (MET: 1.80 days) occurred in seeds planted in Compost growth medium compared to Hutton soil. Similarly, the fastest seedling emergence was observed in the Culterra seedling mix, which had the highest ERI of 26.6 seeds/d compared to the slower emergence rate of 21.7 seeds/d in Compost. Therefore, the Culterra seedling mix proved to be effective across all tested parameters, demonstrating it as a suitable growth medium for the seedling establishment of wild watermelon, *C. lanatus* var. citroides, in this study.

Category: Invasion Plant Biology

Advancements in the eradication of *Cistus ladanifer* in South Africa

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Cistus ladanifer is a woody, perennial shrub native to the Mediterranean region. In 2012, three small, naturalized populations of *C. ladanifer* (each smaller than 0.1 ha) were discovered in the native heathland vegetation ("Fynbos") of the Western Cape Province, South Africa. Following surveys and increased awareness efforts, three additional populations were identified in the Western Cape. *Cistus ladanifer* poses a significant threat to local endemic vegetation due to its adaptation to similar environmental conditions, and if left unmanaged, it could spread further. *Cistus ladanifer* is not yet listed under South African legislation (i.e., the National Environmental Management: Biodiversity Act 10 of 2004 [NEMBA] Alien and Invasive Species Regulations, 2020). We conducted delimitation surveys, monitored the species' response to both mechanical and chemical control methods, and tracked its spread since 2012. A risk analysis was also completed using the Risk Analysis for Alien Taxa framework. Here we present findings on the extent and density of *Cistus ladanifer* and offer insights into the feasibility of eradicating the species after 13 years of control and monitoring. Furthermore, we recommend that *C. ladanifer* be classified as category 1a under the NEM:BA regulations.

Category: Medicinal Plants/Ethnobotany

Investigating the bioactivity of medicinal plants used in traditional remedies for men's sexual health

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Male sexual health disorders are a significant global concern, often affecting quality of life and reproductive health, particularly in regions with limited access to conventional medical care. In such contexts, medicinal plants play a crucial role as accessible and culturally accepted alternatives for the treatment and management of these conditions. This study evaluated the biological activities of ten ethnobotanically selected medicinal plants traditionally used to support male sexual health. Acetone extracts were investigated for their antioxidant activity, anti-inflammatory activity against *15-lipoxygenase*, inhibition of nitric oxide production on RAW 264.7 macrophages and antimicrobial activity against *Neisseria gonorrhoeae* and *Candida albicans*. Phytochemical analysis was performed by determination of total flavonoid and total phenolic content. All the extracts investigated showed moderate to high content of flavonoids and total phenolic content. Cytotoxicity of the extracts was assessed using the XTT reduction assay against Vero monkey kidney cells. *Diospyros mespiliformis* extract was less toxic and showed significant antioxidant (IC_{50} = 8.34 µg/mL), anti-inflammatory (IC_{50} = 63 µg/mL) and antigonococcal (MIC = 0.39 mg/mL) activities. Extracts from *Garcinia livingstonei* and *Rhoicissus tridentata* also exhibited promising bioactivity. While these three extracts seem to inhibit the production of nitric oxide, the rest of the extracts seem to promote its production. The findings of the study revealed that the selected plants possess biological activities directly and indirectly associated with men's sexual health. The plants investigated have potential therapeutic effects and can be used as leads in the development of new pharmaceuticals to manage men's sexual disorders.

Category: Ecology

Structure and species composition of a disturbed Afrotemperate forest in the KwaZulu-Natal Midlands

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Inland natural forests in the KwaZulu-Natal Midlands were logged for timber in the historical past and currently experience low intensity disturbances. These forests, surrounded by grasslands and timber plantations, are expected to progress to secondary succession through natural regeneration after the disturbance. However, many forests have not recovered and show evidence of arrested succession. The aim of the study was to examine the structure and species composition in the edge and the interior of an Afrotemperate forest (~ 5.46 ha) at Enon Estate near Richmond in the KwaZulu-Natal Midlands. Ten plots (25 m × 10 m) were established in 2018 at each of the edges and the interior of the forest and used to mark, identify and measure the diameter at breast height of trees. Trees were identified and measured in 2024 to determine the diversity, basal area and distribution of tree size classes. Between the edge and the interior, tree density was similar, at 17.7 ± 2.32 trees/ha vs. 21.6 ± 7.25 trees/ha in 2024, respectively. The interior (0.53 ± 0.07 m^2/ha) had a significantly ($t = - 3.184$, df = 285, $p < 0.002$) greater mean tree basal area than the edge (0.26 ± 0.04 m^2/ha). *Allophylus dregeanus* (60 trees/ha) and *Xymalos monospora* (92 trees/ha) were predominantly found at the edge, while *Rinorea angustifolia* (172 trees/ha) and *Xymalos monospora* (116 trees/ha) dominated the interior. Small trees were more abundant in the edge (84 trees/ha) than in the interior (48 trees/ha). Medium (232 trees/ha) and large (292 trees/ha) trees were more abundant in the interior than in the edge (204 and 288 trees/ha, respectively). The higher mean tree density, mean basal area and abundant medium and large trees in the interior of the forest suggest greater growth as trees are less exposed to the edge effects, such as high temperatures, low humidity and strong winds.

Category: Ecology

Ecophysiological responses and projected climatic suitability of Podocarpaceae in South Africa

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Podocarpaceae (podocarps) are ancient gymnosperms that persist in fragmented Afromontane forests across southern Africa, yet their future remains uncertain under rapid climate change. In this study, we combined experimental ecophysiology and species distribution modelling (SDM) to assess the persistence potential of four South African podocarp species (*Afrocarpus falcatus*, *Podocarpus henkelii*, *P. elongatus*, and *P. latifolius*). In controlled conditions, seedlings of *A. falcatus* and *P. henkelii* were exposed to factorial combinations of heat and drought stress to evaluate species-specific responses. Both species exhibited physiological and morphological traits consistent with stress avoidance strategies, such as reductions in chlorophyll content and increased leaf shedding. *Podocarpus henkelii* maintained stable photosystem II activity and water use efficiency under drought, indicating a degree of resilience at the seedling stage. These findings were contextualised using ensemble SDMs under RCP 4.5 and 8.5 scenarios. While *A. falcatus* and *P. latifolius* showed relatively stable or expanding suitable habitat, *P. henkelii* and *P. elongatus* were predicted to lose substantial portions of their current range under RCP 4.5, with limited expansion under RCP 8.5. The integration of physiological performance with spatial projections reveals a mismatch between short-term stress tolerance and long-term habitat availability in some species. This highlights the importance of incorporating mechanistic traits into predictive models to understand podocarp responses to environmental change. This research informs us of the responses of podocarp species to environmental stress and how this may influence their distribution and potential persistence under current and future conditions. Mapping the distribution of podocarps and understanding the differences in their climate niches provides valuable insights into the potential climate drivers of podocarp persistence and their capacity for adaptation.

Category: Plant Biotechnology

CenGen's journey sequencing indigenous South African plants

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To build capacity for designing and carrying out biodiversity genomics experiments in the country, a South African Biodiversity genomics program called 1KSA (www.1ksa.org.za) was launched in 2024 by DIPLOMICS, a DSTI-SARIR Research Infrastructure Road Map Programme (www.diplomics.org.za). CenGen, a private plant genetics laboratory, has been a DIPLOMICS partner lab (https://cengen.co.za/diplomics-partner) since 2018. CenGen's sequencing capacity was established in 2021 when they obtained their first in-house next-generation sequencing instrument (Mk1C) from Oxford Nanopore Technologies through a research grant. When they joined the 1KSA initiative, it was upgraded to a PromethION 2 instrument. CenGen's current focus centres around the whole-genome sequencing of indigenous South African plants with historical, ecological, medicinal and alternative food-source value. The plant types are diverse and range from fynbos to trees, succulents and grasses supplied by sample contributors from across South Africa. The notoriously complicated nature of plants when performing DNA extractions is amplified when it comes to high molecular weight (HMW) DNA extractions which are needed for long-read sequencing. Since the first sequencing run, many valuable lessons have been learnt with regards to HMW DNA extractions, post-extraction DNA purification, genome size predictions, data analysis and the bioinformatics of assembling plant genomes with varying ploidy levels. This endeavour stands as a significant stride towards harnessing the rich genetic potential within South African indigenous plants, with the additional gain of bringing scientists from different disciplines together. The assemblies produced through the 1KSA initiative serve as a starting point to unlock the genomes for downstream research and industry applications.