South African Journal of Botany 175 (2024) 574–586

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Extracts of southern African aquatic and wetland plant species as effective tyrosinase inhibitors

Namrita Lall[a,*,1], Karina Mariam Szuman[a], Bonani Madikizela[b], Anna-Mari Kok[a,c], Marco Nuno De Canha[a]

[a] Department of Plant and Soil Sciences, University of Pretoria, Pretoria 0002, South Africa
[b] Water Research Commission of South Africa, 4 Daventry Street, Lynnwood Manor, Pretoria 0001, South Africa
[c] South African International Maritime Institute (SAIMI), Nelson Mandela University, Gqeberha, 6019, South Africa

ARTICLE INFO

Article History:
Received 12 August 2024
Revised 20 September 2024
Accepted 17 October 2024
Available online 2 November 2024

Edited by: Dr S.C. Pendota

Keywords:
Medicinal aquatic plants
Antibacterial
Antimycobacterial
Tyrosinase inhibition
Molecular docking

ABSTRACT

South Africa harvests approximately 19 500 tonnes of medicinal plants annually for the treatment of various ailments and an additional 6000 tonnes is exported globally for the development and design of cosmetic and medicinal products. South Africa is home to 30 freshwater and marine ecological regions, however, medicinal plant species from these semi-aquatic and aquatic habitats are largely understudied in comparison to other biomes. The lack of knowledge and reported pharmacological activity of these species may be hindering the potential contribution to the herbal medicine and personal care markets. South Africa is Africa's largest market for cosmetic and personal care products and in 2018 this sector reported 3.2 billion US dollars in revenue. Eighteen (18) Southern African freshwater aquatic and wetland plant species were selected and screened for their potential as cosmeceutical ingredients by investigating their antibacterial, anti-tyrosinase and cytotoxicity. The extracts of *Erythrina zeyheri* and *Plantago longissima* exhibited antimycobaterial activity with MIC values of 125 μg/mL. Two extracts of the genus Cyperus, *C. marginatus* and *C. sexangularis* showed tyrosinase inhibition with IC_{50} values of 75.03 and 64.26 μg/mL, respectively. *Carex rhodesiaca* and *Gunnera perpensa* both exhibited dual antimicrobial activity against *Cutibacterium acnes* with MIC values of 250 and 125 μg/mL, respectively and anti-tyrosinase activity with IC_{50} values of 64.71 and 70.43 μg/mL. *Persicaria senegalensis* exhibited anti-tyrosinase activity with an IC_{50} of 71.59 μg/mL. Fractionation of *P. senegalensis* yielded F14 with an IC_{50} of 68.77 μg/mL against tyrosinase enzyme activity. Purification of F3 yielded (24S)-Stigmast-5,22(E)-dien-3-β-ol which is the first report of this compound from this species. This study provides scientific validation of the traditional use of the selected species and provides proof of concept for further investigations of these extracts as potential active ingredients in the personal care sector.
© 2024 The Author(s). Published by Elsevier B.V. on behalf of SAAB. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

1. Introduction

Wetlands are described as vital habitats providing crucial ecosystem services for both animal and plant biodiversity to thrive and impact the livelihoods of millions of people who inhabit their surrounds. Most human civilizations originated in and around wetland systems, providing drinking water, irrigation for agricultural lands and fishing. In addition, wetlands are important mitigators of climate change acting as carbon sinks and in their ability to store and regulate water. In order to protect people living close to and downstream from wetlands, particularly those reliant on water for their livelihoods, the conservation and restoration of wetlands is of vital importance. Historically, the flora found in wetlands were used as food, fodder and medicine, but with human advancement into terrestrial habitats the use of wetland plants has become less popular (Swapna et al., 2011). This is evident since, aquatic ecosystems such as mangroves, estuaries, lakes, rivers, streams and marshes contain medicinally important species, however, when compared to plants collected from terrestrial ecosystems including grasslands, savannas, forests and fynbos, there is a clear imbalance towards the latter with regards to scientific research. This suggests that plant species in aquatic habitats have little to no ethnobotanical information and are consequently neglected and understudied for their pharmacological activities. Some of the potential reasons for this are that the plants are not easily accessible and that wetland ecosystems are under more threat and less protection when compared to terrestrial ecosystems (Arya et al., 2022; Department of Environmental Affairs, 2016; Wang et al., 2014).

* Corresponding author.
E-mail address: namrita.lall@up.ac.za (N. Lall).
[1] Postal address: Office 3-39, Plant Science Complex, University of Pretoria, Hatfield Campus, Pretoria, 0002, South Africa.

https://doi.org/10.1016/j.sajb.2024.10.041
0254-6299/© 2024 The Author(s). Published by Elsevier B.V. on behalf of SAAB. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/)

Aquatic plants growing in freshwater or saltwater are classified into four categories based on their habitat and life cycles. Aquatic plants can have leaves floating on the surface of the water, completely submerged, semi-submerged, air-water (leaves are floating and the roots are submerged) or semi-aquatic (wetland) plants (Inelova et al., 2023). Major aquatic medicinal plants are defined as those which have high medicinal and economic value and are often grown commercially, while minor aquatic plants have a lack of sustainable source and have been rarely investigated for their pharmacological properties (Arya et al., 2022). Aquatic and semi-aquatic plants are highly adaptable to their environments, being exposed to both inorganic and biological stressors such as extreme water flow rates, differences in light (Arya et al., 2022) intensities, salinity, ice cover, temperature, nutrient availability, predation by aquatic animals and pollutants and therefore, it is no surprise that aquatic plant species produce secondary metabolites that include compounds such as flavones, flavonoids, flavonols, phenolic polyphenols, quinones, tannins, coumarins, terpenoids, essential oils, alkaloids, lectins, and polypeptides as a survival mechanism. Many of these plant compounds are used as antimicrobial, antiviral, anti-angiogenic and anticancer agents. While some research has been conducted on folkloric aquatic medicinal plants, the focus has been on the identification of their antioxidant activity, which has become redundant in modern medicinal plant research (Chai et al., 2015; Inelova et al., 2023; Osama et al., 2023; Saxena et al., 2021). (Alharthi et al., 2024) investigated the phytochemical composition and biological activities of 10 aquatic plants and mentions the justification of the study being due to the limited research conducted on aquatic plants for the development of pharmaceuticals.

Medicinal plant trade in South Africa is a growing industry with approximately 27 million consumers relying on traditional medicines for healthcare with traditional medicines having an estimated market value of around 2.9 billion rand. Contrary to many developed countries, where traditional medicine is considered an inferior alternative to western medicine, in South Africa, approximately 72 % of the black African population (varying in age, education level, religion and occupation) define traditional medicines as a desirable and necessary source of healthcare. A survey by Mander (1998) indicated that 97 % of patients who visit traditional health practitioners make a conscious choice to adopt this form of healthcare, which is independent of whether they have access to western medicine, or the cost implications associated with western healthcare practices (Mander et al., 2007). South Africa makes up a mere 2 % of the world's land mass but contains approximately 6 % of the world's plant and mammal species, likely due to a wealthy abundance of largely undisturbed ecosystems. The country has 9 terrestrial biomes and approximately 30 freshwater and marine ecological regions: including 3 global biodiversity hotspots (Department of Environmental Affairs, 2016). It is evident that potential therapeutic agents in wetland and other aquatic ecosystems are underutilized, not only resulting in limited scientific knowledge, but also identifying a potentially missed opportunity of the contribution of wetland plant species to healthcare and the bioeconomy through drug discovery. Therefore, this study aimed to screen selected plants from aquatic and semi-aquatic habitats with ethnobotanical medicinal uses, for their potential application as cosmeceuticals, which may increase conservation efforts by highlighting the potential benefits that these species may have as a source of active ingredients that are beneficial to people (Kotze, 2022).

2. Materials and methods

2.1. Reagents and chemicals

Reagents of analytical grade including l-tyrosine, kojic acid, chlorhexidine, tetracycline and tyrosinase from mushroom were purchased from Sigma-Aldrich (Johannesburg, SA). Culture media including brain heart infusion (BHI), tryptone soy (TS) agar, BHI liquid broth, TS liquid broth, Anaerocult chambers and Anaerocult A anaerobic generation strips were purchased from Merck (Pty) Ltd (Johannesburg, SA). The bacterial strains used in this study included *Cutibacterium acnes* (ATCC 6919), *Prevotella intemedia* (ATCC 25611) and *Streptococcus mutans* (ATCC 25175) and were acquired from Anatech Analytical Technologies (Johannesburg, SA). Cell viability reagent, PrestoBlue, was purchased from Life Technologies (Johannesburg, SA).

2.2. Plant collection and identification

Plant parts used for extraction were selected based on reported traditional use, impact on sustainability and quantity of available material. Eighteen (18) different aquatic or semi-aquatic plant species of differing morphological types were harvested from the Manie van der Schijff Botanical Garden, the L.C. de Villiers wetland restoration site, the rainwater harvesting garden and the Plant Sciences Complex pond at the University of Pretoria (South Africa). Plant species that were not available from the University of Pretoria sites were purchased from Wildflower Nursery (Plot 3M, Zilkaatsnek, Hartbeespoort, South Africa). Taxonomic identification was performed under the guidance of Mr. Jason Sampson (curator) and Ms. Magda Nel (herbarium technical assistant). Herbarium specimens were prepared for each of the collected or purchased species and deposited at the H.G. W.J. Schweickerdt Herbarium and voucher specimen numbers were then generated (PRU). The information pertaining to the selected species, their reported ethnobotanical uses, the plant parts used, collection sites, voucher specimen numbers and traditional uses have been recorded in Table 1.

2.3. Plant extraction

The collected plant material was rinsed with distilled water and subsequently frozen at -80 °C for 72 h. Plant material was then freeze-dried using a VirTis 4K Benchtop freeze dryer system (Vacutec, South Africa) until complete lyophilization. Dried plant material was then ground to a fine powder using an IKA grinder equipped with a MF 10.1 Head 2870900 and a 2 mm sieve (United Scientific, South Africa). The plant material (semi-aquatic species) was then extracted with 99 % ethanol in a ratio of 1:5 (w/v). Plant species that were collected from the Rainwater Harvesting Garden and the Plant Science Complex Pond (aquatic species) were rinsed then patted dry with laboratory paper towel to remove excess water. The fresh plant material was then weighed and added to a CB15E 4L blender (MRC Laboratory Instruments, South Africa) containing 1:5 (w/v) of 99 % ethanol. The aquatic and semi-aquatic macerations were then placed on a Labcon Shaker 3086U (Labotec, South Africa) for 7 days. The ethanol was separated from the ground/blended plant material using a vacuum filtration system consisting of a Buchner funnel fitted with a Whatmann No.1 filter paper. The crude extracts were prepared by concentration of the filtrates using a BUCHI Rotavapor B-480 rotary evaporator (Lasec, South Africa). Upon complete dryness, the crude extracts were then transferred to glass polytops and stored at 4 °C until further use.

2.4. Antibacterial activity against skin and oral pathogens

The minimum inhibitory concentration (MIC) for the plant extracts was determined using methods described by Lall et al. (2013). Pure cultures of each microorganism were purchased in the form of Microbiologics KWIK-STIKS (Anatech Analytical Technology, South Africa). *Cutibacterium acnes* (ATCC 6919) and *Streptococcus mutans* (ATCC 25175) were maintained on sterile brain heart infusion (BHI) agar. *Prevotella intermedia* (ATCC 25611) was grown on sterile tryptone soy (TS) agar plates supplemented with 1 % sucrose.

Table 1
Names, plant parts used, voucher specimen numbers, collection locality and traditional uses of the plants used.

Plant species	Part collected	Voucher specimen number (PRU)	Location and time of collection	Traditional uses
***Afrosciadium magalismontanum* (Sond.) P.J.D.Winter**	Leaves and stems	123068	Wildflower Nursery October 2016	The bark is used to make medicines and dyes and the leaves are used as a tea (Selina Wamucii, 2024a) Thesium sp., and the roots of *Afrosciadium magalismontanum* (formerly *Peucedanum magalismontanum*) and *Polygala rarifolia* are used as a hygiene wash (Lombard et al., 2020).
***Carex rhodesiaca* Nelmes**	Leaves and inflorescence	122250	Rainwater Harvesting Garden March 2016	This species is used for the treatment of skin conditions including eczema and psoriasis (Selina Wamucii, 2024b).
***Crinum campanulatum* Herb.**	Leaves	122648	Plant Science Complex Pond October 2016	Numerous species in the genus treat bodily swelling and urinary tract ailments (Fennell and Van Staden, 2001).
***Cyperus marginatus* Thunb.**	Stems and inflorescence	122256	Rainwater Harvesting Garden March 2016	A warm poultice prepared from the roots is wrapped around the throat to treat sore throats and mumps (Fern, 2014a).
***Cyperus sexangularis* Nees**	Stems and inflorescence	122258	Rainwater Harvesting Garden March 2016	The plant is used medicinally to treat fever, headaches and skin diseases (Selina Wamucii, 2024c)
***Cyrtanthus breviflorus* Harv.**	Leaves	123074	Wildflower Nursery October 2016	Bulbs are used to treat intestinal worms (Crouch et al., 2005; Machete and Masila, 2023).
***Dracoscirpoides ficinioides* (Kunth) Muasya**	Leaves	123,070	Wildflower Nursery October 2016	A medicinal plant used to treat a variety of ailments (not specified) (Selina Wamucii, 2024d)
***Erythrina zeyheri* Harv.**	Leaves	123065	Wildflower Nursery October 2016	Seeds treat asthma. Leaves are drunk as a tea are for relief of tuberculosis. Powdered bark treats rheumatism and made into a tea for blood disorders (Buwa and Van Staden, 2006; Hennessy, 1991; Pillay et al., 2001).
***Gunnera perpensa* L.**	Leaves and stems	122254	Rainwater Harvesting Garden March 2016	Roots treats skin disorders (pimples, eruptions and wounds). Leaves are crushed and smoked to relieve headaches. Other parts of the plant treat gonorrhoea, syphilis and urinary tract infections (Moteetee and Van Wyk, 2011; Ríos and Recio, 2005)
***Kniphofia pauciflora* Baker**	Leaves	123991	Wildflower Nursery October 2016	No reported medicinal uses
***Marsilea schelpeana* Launert**	Leaves and stems	122251	Rainwater Harvesting Garden March 2016	The whole plant of *Marsilea* species possess anti-inflammatory and detoxifying properties. Leaf juice is applied to treat snakebites and is used as a diuretic (urine production stimulant) and febrifuge (fever reducing agent) (Mabona and Van Vuuren, 2013).
***Nymphaea nouchali* Burm.f. var. *caerulea* (Savigny) Verdc.**	Leaves and stems	122252	Rainwater Harvesting Garden March 2016	Whole plant is applied as a poultice to heal wounds. Seeds are a remedy for diabetes. Flowers (possessing narcotic and aphrodisiac effects) treat dysuria and coughing. An infusion of the roots and stems infusions are taken for gonorrhoea and urinary tract infections (Fern, 2014b; Ríos and Recio, 2005).
***Nymphoides thunbergiana* (Griseb.) Kuntze**	Leaves, stems and flowers	122253	Rainwater Harvesting Garden March 2016	Emollient plaster from the stems, leaves and flowers is applied to wounds (Fern, 2014c).
***Persicaria senegalensis* (Meisn.) Soják**	Leaves, stems and inflorescence	122180	LC de Villiers Wetland Restoration May 2016	The pounded leaves and roots are applied to reduce skin swelling (syphilis and infections) (Fern, 2014d).
***Plantago longissima* Decne.**	Leaves, stems and inflorescence	122181	LC de Villiers Wetland Restoration May 2016	Astringent, anti-toxic, anti-histamine, demulcent (reduces pain and inflammation in membranes), styptic (blood coagulant agent), antimicrobial and diuretic properties. Poultice of leaves treat inflammation associated with insect bites, minor sores, boils and rashes caused by poison ivy. Tea, tincture or syrup of the leaves treat respiratory tract problems (Heyman et al., 2009; Watt and Breyer-Brandwijk, 1962).
***Scadoxus multiflorus* Raf. subsp. *katherinae* (Baker) Friis & Nordal**	Leaves	123069	Wildflower Nursery October 2016	Bulbs are used to as a fish poison or to treat scabies and long healing wounds (Notten, 2001).
***Tulbaghia leucantha* Baker**	Leaves	123071	Wildflower Nursery October 2016	Milk infusion treat intestinal worms, fever, influenza, high blood pressure, tuberculosis (Ranglová et al., 2015; van Wyk, 2008; Watt and Breyer-Brandwijk, 1962).

Cultured plates were then incubated anaerobically in Anaerocult chambers with Anaerocult A strips until colonies were visible on the agar plates. For the oral pathogens (*P. intermedia* and *S. mutans*), plant extracts were dissolved in 10 % dimethyl sulfoxide (DMSO – in TS broth for *P. intermedia* and BHI for *S. mutans*) to a stock concentration of 40 mg/mL and serially diluted to final concentrations of 0.078–10.00 mg/mL. The positive control, chlorhexidine gluconate was prepared to a stock concentration of 50 mg/mL with final test concentrations ranging from 0.098 - 12.50 mg/mL. The plates were inoculated with 100 μL of 48-hour bacterial subcultures of the oral pathogens prepared to a 1.0 McFarland standard (3×10^8 CFU/mL) and incubated for 24 h anaerobically. For the skin pathogen *C. acnes*, the stock concentration of the plant extracts (prepared in 10 % DMSO – DMSO in BHI broth) was 2 mg/mL and final test concentrations ranged from 3.91 μg/mL-500 μg/mL and the positive control, tetracycline, prepared to a stock concentration of 400 μg/mL was tested from 0.78 μg/mL - 100 μg/mL. Serially diluted plates were inoculated with 100 μL of a 72-hour subculture of *C. acnes* prepared to a 0.5 McFarland standard (1.5×10^8 CFU/mL) and incubated for 72 h anaerobically. Additional controls included a vehicle control (10 % v/v DMSO in BHI and TS broth), *P. intermedia* in TS broth (untreated), *S. mutans* in BHI broth (untreated), *C. acnes* in BHI broth (untreated), BHI broth only (no bacteria) and TS broth only (no bacteria). After incubation, the minimum inhibitory concentrations (MICs) were determined for each microorganism. For the determination of the MICs, 20 μL of PrestoBlue was added to each well containing plant

extract dilutions and controls, after 1 hour the MICs were determined visually and defined as the lowest concentration where no colour change was observed from blue to pink.

2.5. Antimycobacterial activity

A cryopreserved (−80 °C) glycerol stock of *Mycobacterium smegmatis* (MC^2 155), was thawed and 100 μL was plated on sterile 7H11 agar plates and incubated at 37 °C until colonies were observed. A single colony was then transferred to a sterile Erlenmeyer flask containing Middlebrook 7H9 liquid broth media, which was supplemented with glycerol (2 %) and Tween-80 (0.5 %). The inoculated flask was then incubated for 24 h at 37 °C. The antimycobacterial test was performed according to Lall et al. (2013). Briefly, 100 μL of plant extracts (prepared to a stock concentration of 4 mg/mL) were serially diluted to test concentrations of 7.81 μg/mL - 1000 μg/mL, thereafter, 100 μL of a *Mycobacterium smegmatis* inoculum (adjusted to 1.5×10^6 CFU/mL) was added to the wells of a 96-well plate and incubated at 37 °C for 24 h. The minimum inhibitory concentration (MIC) was determined by the addition of 20 μL of PrestoBlue, followed by an additional incubation period of 2 h Ciprofloxacin, the positive control, was prepared to a stock concentration of 400 μg/mL and serially diluted to final test concentrations between 0.15625 μg/mL - 20 μg/mL, the negative control and vehicle controls were untreated *M. smegmatis* culture in 7H9 media and DMSO at 2.5 % v/v respectively. The 7H9 media with no inoculum was included as an indication of sterility.

2.6. Colorimetric tyrosinase inhibition assay

The colorimetric tyrosinase inhibition assay was performed according to the method described by Mapunya et al. (2011). The plant extracts and the positive control, kojic acid, were dissolved in 100 % DMSO (20 mg/mL stock concentration) and then diluted in 50 mM potassium phosphate buffer (pH 6.5) to obtain a concentration range of 4.6875 - 600 μg/mL in a 24-well plate. In a 96-well microtiter plate, which was placed on ice, 70 μL of each serial dilution of each plant extract and kojic acid (from the 24-well) was added to 30 μL of a solution of 333 U/mL of tyrosinase enzyme (in phosphate buffer pH 6.5). The final test concentration range of the plant extracts and kojic acid in the 96-well plate was 1.5625 – 200 μg/mL. After 5 min of incubation at 37 °C, 110 μL of a substrate solution containing 2 mM of l-tyrosine was added to all the wells. Control wells included enzyme and substrate only (100 % activity) and enzyme without substrate (0 % activity) were also included. The optical density was measured over a period of 30 minutes at a wavelength of 492 nm using BIO-TEK power Wave XS multi-well plate reader (A.D. P., Weltevreden Park, South Africa). The fifty percent inhibitory concentration (IC_{50}) was the determined using GraphPad Prism 4.0 software (San Diego, California, USA).

2.7. Column chromatography of Persicaria senegalensis

The dried ethanolic extract (10.26 g) of *P. senegalensis* was used in a 10 × 70 cm silica column of gradient hexane and dichloromethane (DCM) of increasing polarity (0 % to 100 % DCM) as the eluent. One hundred and fifty-three fractions were collected as follows: fractions 1–5 (100 % hexane); fractions 6–18 (95 % hexane and 5 % DCM); fractions 19–21 (90 % hexane and 10 % DCM); fractions 22–70 (75 % hexane and 25 % DCM); fractions 71–147 (50 % hexane and 50 % DCM); fractions 148–190 (25 % hexane and 75 % DCM); fractions 190–200 (100 % DCM). Fractions were analysed using thin layer chromatography (TLC) with the respective solvent ratios as eluents. The TLC plates were developed using vanillin (7.5 g) and sulphuric acid (5 ml) in 250 ml ethanol, the plates were then heated to 100 °C for 3 min. Fractions with similar TLC profiles and presence of compounds were pooled together resulting in seventeen (17) major fractions. The combined fractions (F1–F17) were tested for tyrosinase inhibition.

2.8. Molecular docking of previously isolated Persicaria senegalensis compounds

The computational analysis was performed using a combination of GOLD and PyMol Molecular Graphics System according to the methods described by Lall et al. (2016). The compounds selected for computational analysis were previously isolated compounds from *P. senegalensis* which were reported by Hussein et al. (2017). Prior to the computational analysis the chemical structures were constructed in Chemdraw 3D, minimised using the MM2 function (RMSD cut-off of < 0.1 Å) in Avogadro. Molecular docking was performed using the crystal structure of the mushroom tyrosinase enzyme (from *Agaricus bisporus*) PDB ID: 2Y9X accessed from the Protein Data Bank. This enzyme contains two Cu^{2+} (copper) ions bound to an inhibitor ligand, tropolone, in the enzyme active site and a crystal structure resolution of 2.68 Å. Default parameters were employed: 100 000 genetic operations, 100 individuals, mutation rate of 95. For the molecular docking at the active site (defined as a 6 Å region around the tropolone ligand), tropolone was redocked into the crystal structure with all the selected compounds and the positive control for the tyrosinase inhibition assay, kojic acid. PyMol Molecular Graphics System was employed to evaluate the types of interactions of the docked molecules within the enzyme active site.

2.9. Cytotoxicity on human keratinocyte cells (HaCat)

Cryopreserved vials of human skin keratinocytes (HaCat) were thawed into a T-75 cell culture flask and maintained in Dulbecco's modified Eagle's medium (DMEM) complete media (addition of 10 % fetal bovine serum (FBS), 100 μg/ml penicillin, 100 μg/ml streptomycin, and 250 μg/ml fungizone) in a cell culture incubator set at 37 °C and equilibrated with 5 % CO_2. Culture flasks were observed under a light microscope until cells were attached and reached 80 % confluency. Once confluent the cells were sub-cultured by cell detachment using trypsin-ethylenediaminetetraacetic acid (EDTA) followed by the addition of fresh media into new growing flasks. Once enough cells were acquired, cells were detached (as described above), counted using a hematocytometer and plated at a cell density of 1×10^5 cells/mL. The antiproliferative effect of the extracts was determined in a 96-well plate, by adding 100 μL of the cell suspension in each well and incubating for 24 h, to allow for cell attachment. Samples were then added (100 μL) at a concentration range of 3.125 – 400 μg/mL and the toxic positive control, Actinomycin D was tested at 3.91×10^{-4} - 0.05 μg/mL. Plates were then incubated for 72 h after which the XTT Cell Proliferation Kit II was used to quantify cell viability. After 72 h, 50 μL of activated XTT was added to all the test and control wells and further incubated for 2 h The optical density was read at 490 nm with a reference wavelength set at 690 nm. The antiproliferative assay was performed in triplicate and the percentage viability was then calculated and used to calculate the 50 % inhibitory concentration (IC_{50}) by analysing data in GraphPad Prism 4.0 software.

3. Results and discussion

3.1. Antimicrobial activity against skin and oral pathogens

The ethanolic extracts of *Carex rhodesiaca* and *Gunnera perpensa* exhibited antibacterial activity against the skin pathogen *Cutibacterium acnes* with minimum inhibitory concentration (MIC) values of 250 μg/mL and 125 μg/mL, respectively, while tetracycline, the positive control had an MIC of 1.56 μg/mL. Drewes et al. (2005) reported the antimicrobial activity of the aqueous extracts of the leaves and

stems of *Gunnera perpensa* which had MIC values of 100 μg/mL and 120 μg/mL against *Staphylococcus aureus* (ATCC 6538) and *Staphylococcus epidermidus* (ATCC 2223), respectively. The results reported in 2005 study suggest that the extracts of *G. perpensa* are more active against Gram-positive microorganisms and this could explain why similar activity is observed against *Cutibacterium acnes* where comparative activity is observed for *G. perpensa* with an MIC of 125 μg/mL. This study also associates the antimicrobial activity with the compound, 2-methyl-6-(3-methyl-2-butenyl)benzo-1,4quinone which is present in the leaf and stem extracts of *G. perpensa*. A similar trend was observed for the hexane, ethanol and water extracts prepared from the root/rhizome of *G. perpensa* which showed no activity against the Gram-negative microorganisms *Escherichia coli* and *Klebsiella pneumoniae*. The ethanolic and aqueous root/rhizome extracts did, however, show activity against the Gram-positive microbe, *S. aureus* with MIC values of 3.13 and 0.78 mg/mL, respectively (Mcgaw et al., 2000). Gram-positive bacteria are reported to be more susceptible to antimicrobial agents, when compared to their Gram-negative counterparts as they do not contain an additional outer lipopolysaccharide membrane (De Angelis et al., 2014) The antimicrobial results for *G. perpensa* validate its traditional use to manage skin diseases including pimples, skin eruptions and skin infections (Ríos and Recio, 2005) (Table 1.). The leaves and rhizomes of *G. perpensa* are traditionally used by communities to help treat dermatologically related problems including psoriasis and stimulate wound healing (Watt and Breyer-Brandwijk, 1962) and could be linked to its antibacterial activity against Gram-positive opportunistic skin microorganisms. The antibacterial activity of *Carex rhodesiaca* has not previously been reported and there is also limited knowledge on the traditional uses of this species, however, there are studies that report the antibacterial activity of other species in the *Carex* genus which have been tested against microorganisms. The ethanolic extracts of the leaves, stems and fruits of *Carex baccans* were tested against a methicillin susceptible *Staphylococcus aureus* (MSSA) strain and three Methicillin Resistant *S. aureus* (MRSA 1-3) strains. The leaf and stem extracts showed no activity, while the ethanolic fruit extract exhibited an MIC of 50 μg/mL. The study also reported the compound curcusinol, as the bioactive marker, with an MIC of 4 μg/mL against the MSSA, MRSA-1, MRSA-2 and MRSA-3 strains (Liu et al., 2024). The ethyl acetate partition of the methanolic root extract of *Carex humilis* resulted in the isolation of a stilbene oligomer known as α-viniferin, which exhibited antibacterial against MSSA, MRSA and Methicillin Resistant *Staphylococcus epidermidis* (MRSE) with MIC values of 9.2–18.4 μM (6.25–12.5 μg/mL) (Seo et al., 2017). In both the studies by Liu et al. (2024) and Seo et al. (2017) the isolated constituents show better activity than the crude extracts, therefore isolating compounds from *Carex rhodesiaca* should be considered in future. None of the plant extracts inhibited the growth *Prevotella intermedia* and *Streptococcus mutans* at the highest concentration tested (10 mg/mL). Chlorhexidine the positive control had an MIC of 0.78 mg/mL and 0.39 mg/mL against *P. intermedia* and *S. mutans*, respectively. While there are numerous studies that report the antibacterial activity of terrestrial plants against these oral pathogens, however, there is not much data reported for species from semi-aquatic and aquatic habitats. More et al. (2008) reports the activity of six South African terrestrial plants against *P. intermedia* and *S. mutans* with 5 of the species (83.33 %) showing no activity at the highest tested concentration of 25 mg/mL, indicating that higher concentrations may be required to inhibit the bacterial growth of this species. Similarly, four of the six species investigated against *S. mutans* showed an MIC of 12.5 mg/mL – 25 mg/mL. Castillo et al. (2022) reports that 0 %, 10 % and 90 % of *P. intermedia* isolates are found in healthy individuals, individuals with gingivitis and individuals with periodontitis respectively. This suggests that this microorganism is involved only in the progression of oral diseases since it is absent in healthy individuals. The study also reported that 20 % of the isolates were resistant to amoxicillin and around 4 % were resistant to metronidazole due to the presence of the resistance genes blaTEMF, tetMF, tetQF nimABF (%). Constant subculturing of *P. intermedia* could potentially result in resistance of the bacteria and explain the lack of activity shown by the tested plant extracts. Another possible explanation for the tested extracts not showing activity is the selection of ethanol as the extraction solvent. Studies by Didry et al. (1998) and Iauk et al. (2003), tested the chloroform and methanol extracts of some terrestrial plant extracts which exhibited potent antibacterial activity against *Prevotella* species and *S. mutans* showing activity in the μg/mL range. A study by Akhalwaya et al. (2018), thirty-one South African plant species with reported traditional uses in oral care were tested for their ability to inhibit the growth of different oral pathogens. The study concluded that dicholoromethane: methanol extracts were the most effective, with six DCM: MeOH extracts exhibiting noteworthy antibacterial activity against *S. mutans* with MICs below 1.00 mg/mL including that of a South African semi-aquatic plant, *Berula erecta*, MIC of 0.50 mg/mL (500 μg/mL). *Streptococcus mutans* is a known acidogenic bacteria and produces acid to aid in demineralization of the tooth enamel when pH levels reach between 5.0 and 5.5 (Henley-Smith et al., 2018). Chandrabhan et al. (2012) showed that isolates from dental plaques which were characterised as high acid producers also showed resistance to antibiotics, erythromycin, chloramphenicol and vancomycin. The effect of microenvironments including low pH and anaerobic conditions can reduce the activity of some antimicrobial agents and could possibly explain why none of the extracts exhibited inhibition of *S. mutans* (Kim et al., 2022).

3.2. Antimycobacterial activity

The extracts of *Erythrina zeyheri* and *Plantago longissima* both exhibited antimycobacterial activity against *Mycobacterium smegmatis* with an MIC value of 125 μg/mL. *Mycobacterium smegmatis* has been characterised as a model microorganism for the prediction of drugs with antimycobacterial activity against *M. tuberculosis* as it enters dormancy after a 14-day period resulting resistance to most antimycobacterial treatments (Lelovic et al., 2020). This suggests that the antimycobacterial activity of *E. zeyheri* and *P. longissima* against *M. smegmatis* may translate to the same activity against *M. tuberculosis*. There are no reports of *Erythrina zeyheri* against *Mycobacterium smegmatis*, however, the crude methanolic, ether and chloroform root bark extracts of *E. abyssinica* have been tested against *Mycobacterium tuberculosis* (MDR strain and non-pathogenic strain) and *Mycobacterium avium*. The MIC values ranged from 0.30 mg/mL to 4.69 mg/mL with the best activity being observed against *M. avium* (Bunalema et al., 2011). Furthermore, the fractions of the dichloromethane bark extract of *Erythrina verna* exhibited better activity than the crude extract (MIC > 100 μg/mL) varying in MIC values between 2.50 – 99.10 μg/mL against *Mycobacterium bovis, Mycobacterium tuberculosis* susceptible strain (H37Rv) and a resistant *M. tuberculosis* strain (M299). Three compounds were also isolated from *E. verna*; erythratidinone, alpinumisoflavone and erisenegalensein M which showed inhibition of the H37Rv (MIC all < 10 μg/mL) and the M299 strain (MIC between 2.60 μg/mL –18.30 μg/mL) (Simao et al., 2022). Fractionation of *E. zeyheri* should be considered in addition to testing the fractions against *M. tuberculosis* strains. There is no antimycobacterial data published for *Plantago longissima*, however, Heyman et al. (2009) reported the antimicrobial activity of the ethanolic root extract with an MIC of 12.5 mg/mL against methicillin-sensitive *Staphylococcus aureus*. Another species of the genus, *Plantago lanceolata* has shown antitubercular activity with the leaf extracts prepared using petroleum ether, chloroform:methanol (1:1) and methanol showed MIC values of 12.5 μg/mL, 6.25 μg/mL and 1.25 μg/mL respectively, against *Mycobacterium tuberculosis* (Fayera, 2022)

Table 2
Biological activity of the ethanolic plant extracts.

Species name	Anti-proliferative activity on HaCaT cells	Tyrosinase inhibition	Antibacterial activity			
			Cutibacterium acnes (ATCC 6919)	*Mycobacterium smegmatis* (MC[2] 155)	*Prevotella intermedia* (ATCC 25,611)	*Streptococcus mutans* (ATCC 25,175)
	IC_{50} ± SD (μg/mL)		MIC (μg/mL)		MIC (mg/mL)	
Afrosciadium magalismontanum	> 400	> 200	> 500	Not tested	> 10	> 10
Carex rhodesiaca	> 400	**64.71 ± 5.361**	**250**	> 1000	> 10	> 10
Cyrtanthus breviflorus	> 400	> 200	> 500	> 1000	> 10	> 10
Crinum campanulatum	205.05 ± 16.33	> 200	> 500	> 1000	> 10	> 10
Cyperus marginatus	> 400	**75.03 ± 4.980**	> 500	> 1000	> 10	> 10
Crocosmia paniculata	> 400	> 200	> 500	> 1000	> 10	> 10
Cyperus sexangularis	> 400	**64.26 ± 5.120**	> 500	> 1000	> 10	> 10
Dracoscirpoides ficinioides	> 400	> 200	> 500	> 1000	> 10	> 10
Erythrina zeyheri	> 400	> 200	> 500	**125**	> 10	> 10
Gunnera perpensa	> 400	**70.43 ± 3.594**	**125**	> 1000	> 10	> 10
Kniphofia pauciflora	> 400	> 200	> 500	> 1000	> 10	> 10
Marsilea schelpeana	> 400	> 200	> 500	> 1000	> 10	> 10
Nymphaea nouchali var. *caerulea*	199.10 ± 7.01	> 200	> 500	> 1000	> 10	> 10
Nymphoides thunbergiana	> 400	> 200	> 500	> 1000	> 10	> 10
Persicaria senegalensis	> 400	**71.59 ± 4.229**	> 500	> 1000	> 10	> 10
Plantago longissima	> 400	> 200	> 500	**125**	> 10	> 10
Scadoxus multiflorus subsp. *katherinae*	> 400	> 200	> 500	> 1000	> 10	> 10
Tulbaghia leucantha	> 400	> 200	> 500	> 1000	> 10	> 10
Persicaria senegalensis F13	–	177.65 ± 6.765	–	–	–	–
Persicaria senegalensis F14	–	**68.77 ± 2.796**	–	–	–	–
Persicaria senegalensis F15	–	79.82 ± 3.404	–	–	–	–
* Kojic acid	–	2.908 ± 1.267	–	–	–	–
* Actinomycin D	< 0.005	–	–	–	–	–
* Tetracycline	–	–	1.56	–	–	–
* Ciprofloxacin	–	–	–	0.156	–	–
* Chlorhexidine	–	–	–	–	0.78	0.39

* Positive controls for respective assays and microorganisms tested

3.3. Inhibition of tyrosinase enzyme activity

The ability of each plant extract to inhibit the activity of tyrosinase varied depending on the species with *Carex rhodesiaca, Cyperus marginatus, Cyperus sexangularis, Gunnera perpensa* and *Persicaria senegalensis* showing the best inhibitory activity with IC_{50} values of 64.71 μg/mL, 75.03 μg/mL, 64.26 μg/mL, 70.43 μg/mL and 71.59 μg/mL respectively (Table 2).

There are no previous reports on the tyrosinase inhibitory activity of *Carex rhodesiaca*, however, several species within the *Carex* genus have been reported from previously published data. Masuda et al. (2005) reported the activity of the methanolic leaf extract of *Carex pumila* which exhibited a 58.30 % reduction in tyrosinase enzyme activity at a concentration of 500 μg/mL. The acetone seed extract of *Carex vulpinoidea* was fractionated to yield vulpinoideol A (stilbenoid) and B (chalcone), hopeaphenol, α-hydroxychalcone and tested for their tyrosinase inhibitory activity. The IC_{50} values of the compounds were 151 μM, 49.4 μM, 6.1 μM and 29.1 μM, respectively (Niesen et al., 2015). *Cyperus rotundus* is the most well-studied species in the *Cyperaceae* family. Many of the species in this plant family are natural sources of stilbenes and oligostilbenes which have various biological activities, including tyrosinase inhibitory activity (Dávid et al., 2021). This could explain the activity of *C. marginatus* and *C. sexangularis*. The oxygen-containing functional groups are critical structural components which have been shown to be critical for effective tyrosinase inhibition (Likhitwitayawuid et al., 2006)

Several species in the genus *Persicaria* have been investigated for their anti-tyrosinase activity. Phan (2021) tested the ethanolic leaf extract and aqueous leaf extract of *Persicaria odorata* which showed 54.20 ± 2.90 % and 49.62 ± 0.77 % inhibition at 100 μg/mL, respectively. The ethanol and aqueous extracts that were processed to remove chlorophyll were also tested and showed 40.90 ± 1.60 % and 41.45 ± 1.90 % inhibition at 100 μg/mL, respectively. Methanolic extracts prepared from the roots and aerial parts of *P. maritimum* exhibited anti-tyrosinase activity with IC_{50} values of 590.00 and 600.00 μg/mL, respectively (Seimandi et al., 2021). Choi et al. (2020) tested several species including *P. hydropiper, P. tinctoria, P. dissitiflora, P. thunbergii, P. nepalensis, P. longiseta, P. chinensis, P. japonica, P. viscofera, P. conspicua, P. sieboldi, P. perfoliata* and *P. lapathifolia* for anti-tyrosinase activity. The methanolic extracts prepared from the whole plant of *P. dissitiflora, P. chinensis, P. japonica, P. viscofera* exhibited anti-tyrosinase activity with IC_{50} values of 411.20 ± 4.80 μg/mL, 726.40 ± 6.90 μg/mL, 431.10 ± 7.80 μg/mL and 694.90 ± 6.80 μg/mL, respectively. The methanolic flower extract of *Persicaria tinctoria* exhibited an IC_{50} value of 70.80 ± 2.20 μg/mL against mushroom tyrosinase activity (Woo et al., 2011). Masum et al. (2019) reported the tyrosinase inhibitory effects of the methanolic root extract of *P. orientalis* which exhibited an IC_{50} of 13.00 and 17.00 μg/mL, respectively when using l-tyrosine and l-DOPA as substrates.

The major fractions (F1–17) collected through column chromatography were also evaluated for their inhibitory tyrosinase activity. Major fractions showing tyrosinase inhibition were F13, F14 and F15 with IC_{50} values of 177.65 ± 6.77, 68.77 ± 2.80 and 79.82 ± 3.40, respectively. Fraction 14 exhibited a lower IC_{50} than the crude extract, indicating better activity (Table 2). Statistical analysis of the IC_{50} values indicate that F13 and F15 were significantly different ($p < 0.01$) from the crude ethanolic extract, indicating that they contain less effective tyrosinase inhibitors than the crude extract. F14 was not significantly different ($p > 0.05$) from the crude ethanolic extract and therefore shows similar activity (Fig. 1). Purification of the ethanolic extract of *Persicaria senegalensis* is therefore not worthwhile as no improved activity of the purified fractions was observed.

The compound isolated from F3, β-stigmasterol was tested for tyrosinase inhibitory activity but exhibited an IC_{50} > 200 μg/mL. This observation was expected as the pooled F3 fraction showed an IC_{50} > 200 μg/mL, and the compound was elucidated purely based on the

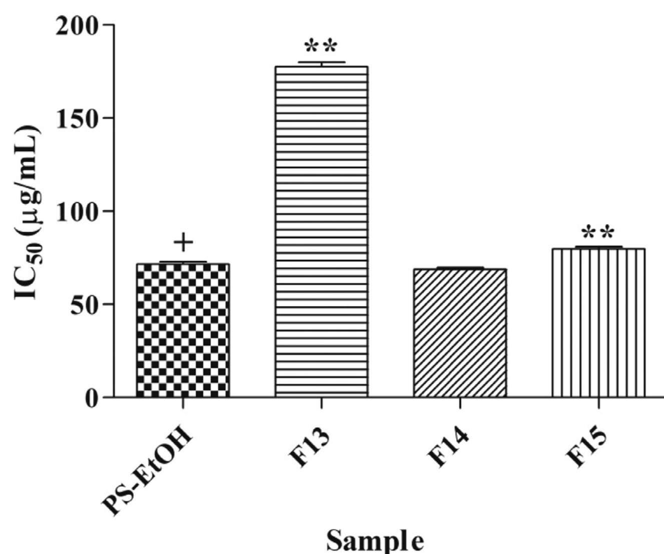

Fig. 1. The fifty percent inhibitory concentration (IC_{50}; μg/ml) values against tyrosinase for each of the bioactive major fractions (F13–15) compared with that of *Persicaria senegalensis*. Results obtained for each of the fractions were compared to the results of the whole extract. Statistically significance is indicated with an asterisk (**) where $p < 0.01$ using a One-Way ANOVA with Dunnett's multiple comparisons test.

appearance of a single band on the developed TLC plates. A study by Khan et al. (2014), showed similar results with β-stigmasterol, which exhibited no tyrosinase inhibitory activity in comparison with other steroid compounds (betulinic acid 3-β-caprylate, β-amyrin acetate, ursolic acid, erythrodiol 3-β-caprylate, oleanolic acid 3-β-caprylate and madhucic acid) isolated from the fruit pulp and seeds of *Madhuca latifolia* which showed better activity. The presence or absence of specific functional groups at the C3 position can enhance or reduce tyrosinase inhibition. In addition, Chang (2009) reported that for tyrosinase inhibitors the presence of the bulky and hydrophilic moieties interferes with the ability of molecules to interact with the active site of the enzyme and therefore exhibit no inhibitory effect on tyrosinase enzyme activity.

3.4. *Bioassay guided fractionation of* Persicaria senegalensis

Generally, tyrosinase inhibition is defined by the following criteria: strong inhibition ($IC_{50} < 50$ μg/mL), moderate inhibition (50 μg/mL $\geq IC_{50} \leq$ 100 μg/mL) and weak inhibition ($IC_{50} >$ 100 μg/mL (Hassan et al., 2023; Murata et al., 2022). While *P. senegalensis* exhibited an IC_{50} of 71.59 $\pm$ 4.23 μg/mL, which was lower than that of some of the other extracts, the extract yield was of significant quantity to perform bioassay-guided fractionation. Column chromatography yielded 17 major fractions (F1-F17). Major fraction 3 (F3 – 13.9 mg) was developed using silica gel TLC plates with 80 % hexane: 20 % dichloromethane as the mobile phase and yielded a single blue band after derivatization with acidified vanillin. Analysis of F3 (5.8 mg) included ^{1}H NMR spectroscopy and electrospray ionisation mass spectrometry (ESI-MS). The results obtained from the ^{1}H NMR spectroscopy and mass spectroscopy are provided in (Fig. 2). The ^{1}H NMR spectrum depicts a typical steroidal pattern comprising of a mixture of two steroidal compounds. The ESI-MS provided the molecular weights of 413.2699 $[M + H]^+$ and 393.2976 $[M + H]^+$for the proposed steroidal mixture (Fig. 3). Comparison with data from literature showed similar fragmentation peaks at *m/z* 395 and 413 which identified the presence of (24S)-Stigmast-5,22(E)-dien-3-β-ol (β-sitosterol), however, the identity of the second steroidal compound could not be elucidated (Rangra et al., 2021) (Fig. 4). Similar ^{1}H NMR spectrum was observed by Ekhuemelo et al. (2019), who reported mixture of sitosterol, stigmasterol and cycloeucalenol from *Erythrophleum suaveolens* could indicate a mixture of these steroidal compounds in the ethanolic extract of *P. senegalensis.* In plants these two phytosterols are often identified together particularly since β-sitosterol is converted to β-stigmasterol in plants as a response to pathogen infection. Stigmasterol is synthesized from β-sitosterol by the cytochrome P450 CYP710A1 by desaturation of C22. Since the only difference in structure between these two phytosterols is the double bond between C22 and C23, separation is difficult as they possess largely similar properties (Ayaz et al., 2019; Griebel and Zeier, 2010; Xu et al., 2005) isolated both β-sitosterol and stigmasterol from *Persicaria hydropiper* and Rodrigues et al. (2017) also identified both phytosterols in the leaf extracts of *Polygonum maritimum,* which supports the theory that these phytosterols are present in species of the Polygonaceae family.

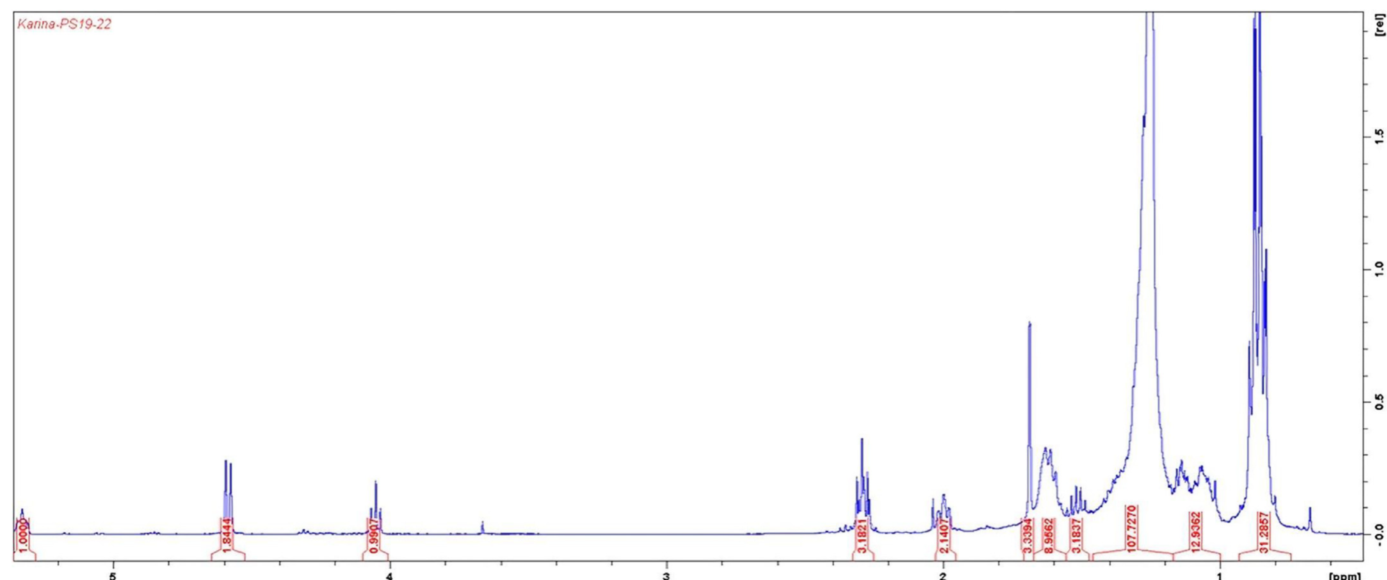

Fig. 2. ^{1}H NMR spectrum (CDCl3, 500 MHz) of F3 collected from *Persicaria senegaenlsis*, representing a typical pattern of a mixture of two steroids.

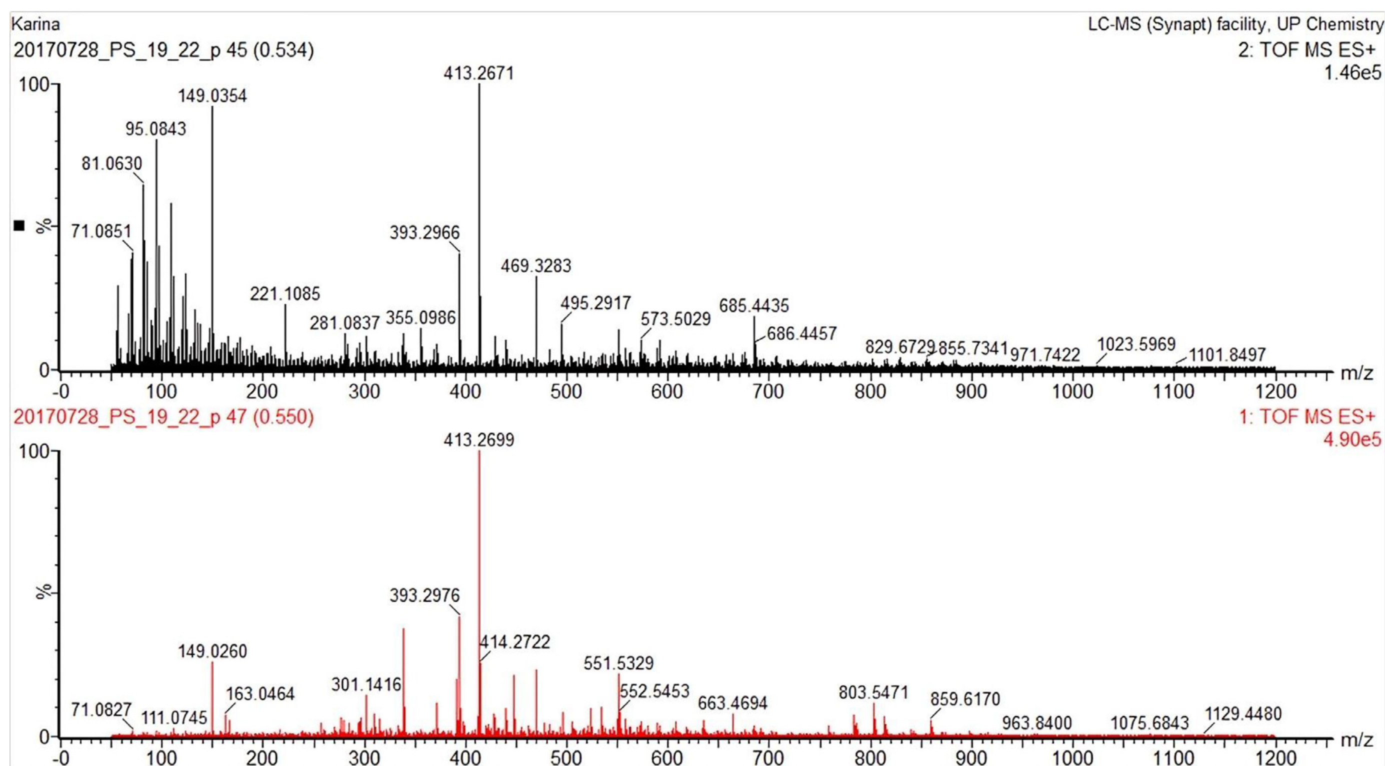

Fig. 3. ESI-MS (Electrospray Ionisation Mass Spectrometry) of a mixture of two steroidal compounds collected from major fraction 3 of *Persicaria senegalensis* fractionation using column chromatography.

3.5. *Molecular docking of previously isolated* Persicaria senegalensis *compounds*

Molecular docking is an effective tool used for *in silico* screening and prediction of interactions between molecules and target proteins, playing an important role in the design and development of novel drug treatments. Docking relies on computational algorithms which predict various ligand poses within the active site of the target protein and ranks these poses with a specific score. This tool provides crucial information of how ligands bind both energetically and geometrically (location, orientation and conformation) into the active site of an enzyme and depicts the optimal fit herein, predicting possible interactions that may be associated with inhibitory effects (Azam and Abbasi, 2013; Li et al., 2010). Due to the lack of improved biological activity in the fractionated crude extract of *Persicaria senegalensis*, compounds from this species which were previously identified by Hussein et al. (2017), were docked into the active site of the *Agaricus bisporus* (cultivated mushroom) tyrosinase enzyme (PDB ID: 2Y9X).

This enzyme was selected based on the similarity with the enzyme which was used in the *in vitro* evaluation of the eighteen extracts used on this study. The crystal structure of the enzyme has been elucidated by Ismaya et al. (2011). The three-dimensional structure of the enzyme provides an opportunity for the evaluation of

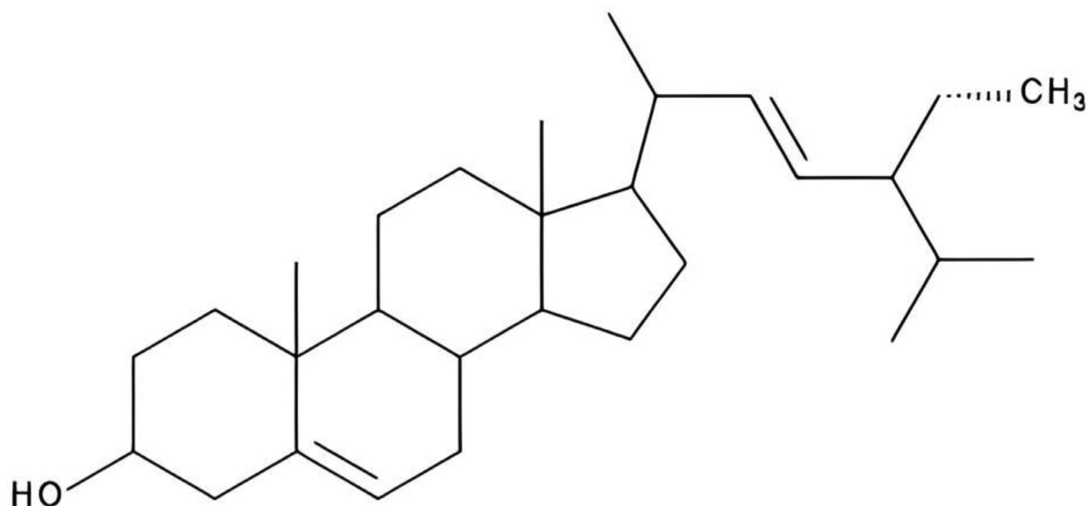

Fig. 4. Chemical structure of (24S)-Stigmast-5,22(E)-dien-3-β-ol.

Table 3
Compounds from *Persicaria senegalensis* previously isolated by Hussein et al (2017) and their docking scores and interactions with Cu^{2+} ions in the active site of *Agaricus bisporus* tyrosinase enzyme (PDB ID: 2Y9X).

Compound Number	Name of molecule	GOLD fitness score	van der Waals distances (Å)
C1	3,7,4′-trimethoxy-kaempferol	53.72	2.15; 2.37
C2	3,6-dimethoxy-kaempferol	55.47	2.17; 2.33
C3	5-hydroxy-7-methoxy-isoflavone	53.41	2.15; 2.41
C4	Apigenin (5,7,4′-trihydroxyflavone)	60.05	2.20; 2.31
C5	Calycopterin (5,4′-dihydroxy-3,6,7,8-tetramethoxy-flavone)	56.59	2.40
C6	Gentisic acid-5-O-(2′-O-glucopyranosyl)-rhamnoside	63.13	2.27, 2.83
C7	Gentisic acid-5-O-(6′-O-galloyl)-glucopyranoside	49.79	1.78
C8	Myricetin-3-O-rhamnoside	62.56	1.83; 3.12,
C9	Quercetin (3,5,7,3′,4′-pentahydroxy-flavone)	61.48	2.16; 2.38;
C10	Quercetin-3-O-glucopyranoside	20.90	2.19, 2.54
C11	Quercetin-3-O-rhamnoside	63.08	2.31; 2.38
PC	Kojic acid	47.95	2.17; 2.53

potential inhibitor ligands binding affinity to the active site of the enzyme and can be a powerful predictive tool when used in combination with *in vitro* experimental evaluation of potential inhibitors.

The GOLD program was used to dock the positive control (kojic acid) and twelve flavonoid compounds previously isolated from *P. senegalensis* by Hussein et al. (2017), into the active site of the enzyme. The compounds were analysed to obtain a GOLD fitness score and identify possible van der Waals interactions with the Cu^{2+}ions. The results for each of the docked compounds is given in angstroms (Å) and is summarised in Table 3.

Compound 6 (Gentisic acid-5-O-(2′-O-glucopyranosyl)-rhamnoside) had the highest fitness score of 63.13 which was closely followed by compound 8 with a fitness score of 62.56. These scores differ in relation to the fitness score obtained by the positive control, kojic acid by approximately 15. This indicates that the binding pose of these molecules allows for the functional groups to interact differently within the active site when compared with kojic acid. Fitness scores similar to that of kojic acid suggest that the molecules take up a similar position within the active site to that of kojic acid. Compounds with low scores molecules such as compound 10 (Quercetin-3-O-glucopyranoside) with a fitness score of 20.90, suggests that the interaction of this molecule with the tyrosinase enzyme differs remarkably from both the positive control and the other docked molecules. The 3D optimal binding position of each molecule was assessed using PyMOL. The position and orientation of the molecules and the distance of interactions with the Cu^{2+} ions within the active site of the tyrosinase enzyme are depicted in Fig. 2. The analysis of the binding position of the molecules revealed that each molecule was able to position itself deep within the active site of tyrosinase and exhibited polar interactions with the Cu^{2+} ions through interactions with oxygen (red) atoms located on the functional groups of the compounds. The copper (Cu^{2+}) ions located within the active site of the enzyme are essential for its catalytic activity, therefore any close interactions with both ions indicate a potential candidate for effective tyrosinase inhibition (Claus and Decker, 2006). Interactions of around 2 Å or less are considered noteworthy and indicate strong interactions (Nokinsee et al., 2015).

Within the active site of the tyrosinase enzyme, two copper (Cu) ions are located within the hydrophobic pocket. The presence of these two ions allows for interactions with molecules containing oxygen atoms, since the enzyme has monooxygenase (incorporation of a hydroxyl group) and oxidase (oxidation–reduction) activity. Interference with either of the copper (Cu^{2+}) ions causes the substrate to change oxidation state leading to enzyme inactivation. Molecules that interact with both the copper ions provide more effective pathway of inactivation (Ramsden and Riley, 2014). Taking this into account compound 5 (5,4′-dihydroxy-3,6,7,8-tetramethoxy-flavone) and compound 7 (Gentisic acid-5-O-(6′-O-galloyl)-glucopyranoside) were not considered effective tyrosinase inhibitors as they each interacted with only one of the Cu^{2+} ions. Although each interaction was ~2 Å in distance, interacting with only one copper ion does not successfully hinder the catalytic activity of the enzyme. However, one must consider that compounds which exhibit high docking scores, but do not interact with both copper ions, may exhibit enzyme inhibitory effects through non-competitive inhibition by interacting at another site on the enzyme, other than active site.

Results for compound 2 (3,6-dimethoxy-kaempferol) exhibited the lowest distance for van der Waals interactions (2.17 and 2.33 Å) between the oxygen atom and both the copper ions (Fig. 5). However, part of the molecule protrudes outside of the active site, *in vitro* studies coupled with computational biology suggest that molecules found to protrude outside of the cavity of the active site, could diminish the biological activity (Lall et al., 2016). The same observation was made for compound 6 (Gentisic acid-5-O-(2′-O-glucopyranosyl)-rhamnoside), compound 11 (Quercetin-3-O-rhamnoside) and compound 8 (Myricetin-3-O-rhamnoside). Although all three compounds showed the highest fitness scores (63.13, 63.08 and 62.56, respectively), the moieties of these molecules were found to protrude into the crystal structure of the enzyme, therefore making them unlikely candidates to be effective inhibitors of tyrosinase. All three of these molecules contain a rhamnoside moiety, the presence of the rhamnoside moiety could increase the number of polar interaction between the compounds (hydroxyl groups) and the residues within the active site of the enzyme providing the rhamnoside-containing molecules with a higher fitness score. However, the presence of this sugar moiety increases the distance of interaction between the oxygen atom and the Cu^{2+} ions to approximately 3 Å. The increased distance along with the protrusion renders these molecules unlikely inhibitors of tyrosinase. It has been suggested that the glycosylation of flavonoids reduces the inhibitory ability due to the increased stereo-hindrance effects of the bulky glycoside moiety resulting in the inadequate placement of the molecule into the binding site of the enzyme (Karioti et al., 2007).

The largest variation in fitness scores was observed between kojic acid and compound 10 (Quercetin-3-O-glucopyranoside) which can be explained by variation in binding pose (position, orientation and conformation) within the active site. Quercetin-3-O-glucopyranoside is a much larger molecule and must undergo a greater deal of conformational change in order to fit into the active site. Unfortunately, this low fitness score suggests that this molecule would not naturally be found within the active site as the conformational changes required to fit into the active site requires a large expense of energy. Therefore, biologically this molecule is not considered to be a potential inhibitor of tyrosinase as the affinity for its pose is low.

Investigating the binding position of compound 1 (3,7,4′-trimethoxy-kaempferol), compound 3 (5-hydroxy-7-methoxy-isoflavone), compound 4 (5,7,4′-trihydroxyflavone) and compound 9 (3,5,7,3′,4′-pentahydroxy-flavone) revealed that all four molecules fit deep

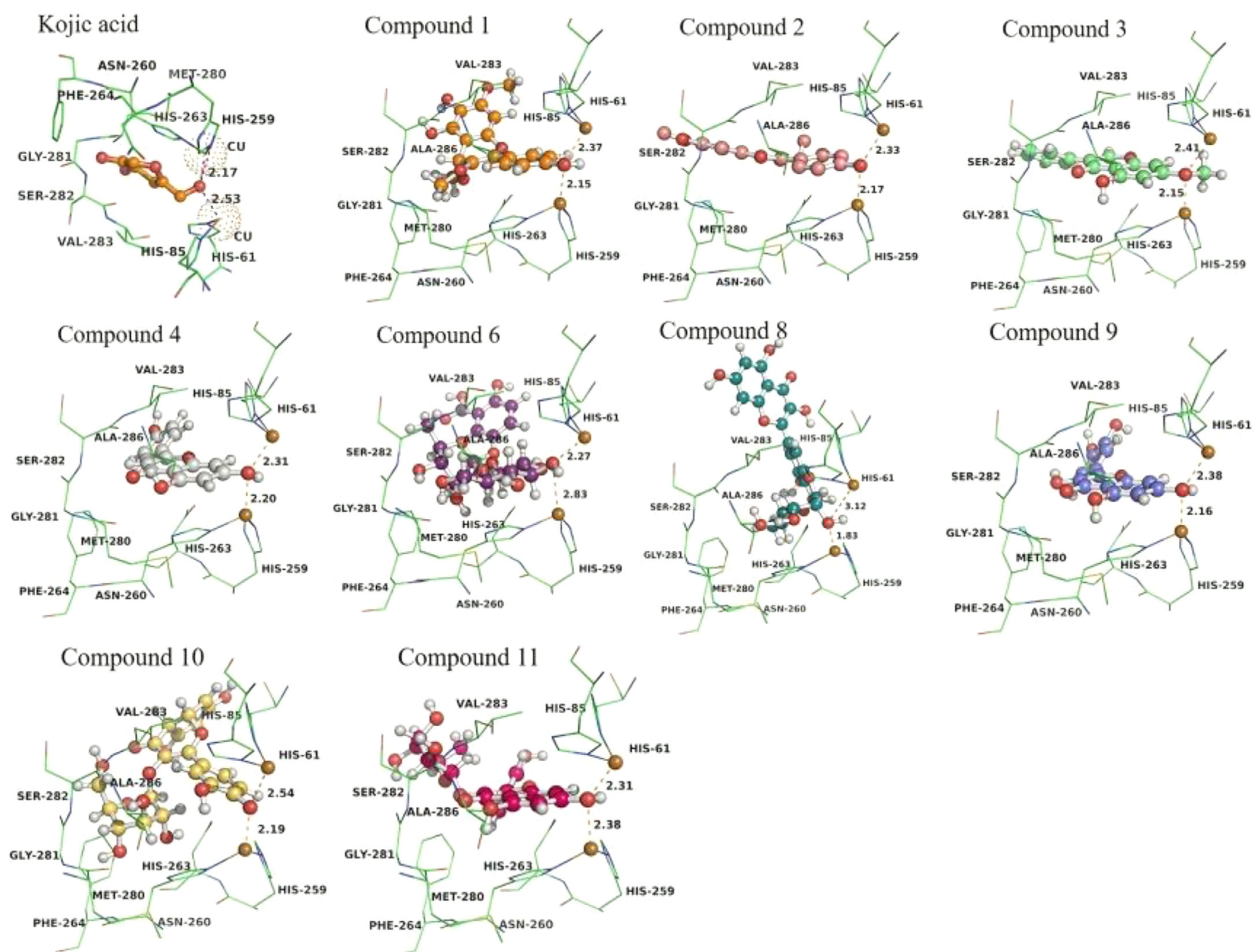

Fig. 5. The molecular docking poses of compounds previously isolated from *Persicaria* senegalensis in the active site of tyrosinase (PDB ID: 2Y9X) showing interactions with Cu^{2+} ions (dashed lines). The distance of the oxygen (red) atom from Cu^{2+} ions is given in angstrom (Å).

within the active site of the tyrosinase enzyme, comparable to that of kojic acid. The conformation of each of the molecules allows for effective interactions with both the Cu^{2+} ions and some additional interactions between the amino acid residues. The insertion of these molecules into the active site of tyrosinase allows each of the molecules to effectively occupy the catalytic centre of the enzyme. The potential of additional interactions with the enzyme amino acid residues allows the molecules to induce conformational changes in the enzyme (Wang et al., 2014). By doing this, these molecules prevent the substrates (L-tyrosinase and l-DOPA) from entering the active site, signifying potential competitive inhibition (Chang, 2009).

3.6. Cytotoxicity on human skin keratinocytes (HaCat cells)

Cytotoxicity evaluation as an initial step in drug discovery determines potential toxicity of a test substance, which includes plant extracts or biologically active compounds that are isolated from plants. Minimal toxicity is essential for the successful development of active ingredients or lead molecules with cosmetic or pharmaceutical applications and therefore cellular toxicity studies play a crucial role in determining safety profiles of such potentially therapeutic agents. In addition, determining cytotoxicity together with biological activity allows for determination of selectivity indices, which often determine whether further research and development of a potential treatment is worthwhile (McGaw et al., 2014). Of the 18 ethanolic plant extracts that were tested only two showed potential cytotoxic effects on human keratinocyte cells (HaCat). The extracts of *Crinum campanulatum* and *Nymphaea nouchali* var. *caerulea* exhibited IC_{50} values of 205.05 ± 16.33 μg/mL and 199.10 ± 7.01 μg/mL, respectively. Kuete and Efferth (2015) define the cut-off values for toxicity profiles when testing on normal cell lines (non-cancerous) as the following: significant or strong cytotoxicity (IC_{50} < 100 μg/mL); moderately cytotoxic (100 μg/mL < IC_{50} < 300 μg/mL), low cytotoxicity (300 μg/mL < IC_{50} < 1000 μg/mL) and non-toxic (IC_{50} > 1000 μg/mL). Following these guidelines, indicates that the extracts of *C. campanulatum* and *N. nouchali* var. *caerulea* are moderately toxic to human keratinocyte cells. All the other tested extracts show low cytotoxicity according to these cut-offs as their IC_{50} values were above the highest tested concentration of 400 μg/mL. Alam et al. (2018) reported the cytotoxicity of the ethyl acetate partition prepared from the methanolic flower extract of *N. nouchali* against Melan-a cells (embryonic mouse melanocyte cell line) and reported the IC_{50} to be between 50 and 100 μg/mL. However, the difference could be due to the use of the flower as the raw material for the preparation of the extract. The evaluation of the ultrasonic-assisted cellulase extract of the flowers of *N. hybrid* for cytotoxicity against HaCat cells showed a reduction of viability from 100 % to 88.79 % ± 2.64 % and 81.14 % ± 3.66 % after 24 h exposure at concentrations of 160 μg/mL and 200 μg/mL, respectively. Cell

viability decreased to levels as low as 72.85 % ± 3.58 % at 200 μg/mL when exposure time was increased to 48 h (Liu et al., 2022). Considering that the cytotoxicity assay in our study was performed for 72 hrs, this could explain the IC_{50} value. There were no reports on the cytotoxic effects of *C. campanulatum* when assessing literature for this species, Refaat et al. (2013) reports various *Crinum* species with antiproliferative activity against a variety of cancer cells. However, since cancer cells have characteristics of continuous growth and increased survival times it is difficult to compare these effects to that of the extracts tested on non-cancerous cell lines (Chandra et al., 2023). The compound Crinamine (Crinum alkaloid), isolated from the bulbs of *Crinum asiaticum* and present in numerous other *Crinum* species exhibited a lower IC_{50} on HaCat cells (85.45 μM ~ 25.75 μg/mL) when compared to the cancerous cervical cell line HeLa (IC_{50} > 100 μM ~ > 30.34 μg/mL) (Khumkhrong et al., 2019). However, this is a single compound rather than a mixture of compounds that is present in a crude extract and makes it difficult to compare. The methanolic extract of *Crinum papillosum* was tested at 100 μg/mL and 10 μg/mL against human epidermoid carcinoma cells (skin cancer cells) which showed cell viability of 25−50 % and 100 %, respectively when compared to the control (Kamuhabwa et al., 2000).

4. Conclusion

This study emphasises the need for more focussed research on South African aquatic and wetland plants with traditional uses and identifies a number of species with potential as active ingredients with cosmeceutical applications, not including generalised antioxidant activity. Seven of the eighteen plant extracts showed noteworthy biological activity with two extracts (*Erythrina zeyheri* and *Plantago lingissima*) exhibiting potential as antimycobacterial agents. While South Africa has made significant progress in the fight against tuberculosis since 2010, this disease still poses a significant burden on healthcare with 468 cases per 100 000 people in the population. This is largely due to the prevalence of undernutrition, overcrowding and biosocial risk factors such as HIV co-infection, alcohol-related disorders, smoking and diabetes. For this reason, new treatments for tuberculosis should be made a research priority (Mizrahi, 2024). Dual anti-acne and anti-tyrosinase activity was observed for two extracts *Carex rhodesiaca* and *Gunnera perpensa*. The dual inhibition exhibited by *Gunnera perspensa* shows promise as an anti-acne agent with Wamba et al. (2023) describing botanicals with MIC values of 40 < MIC ≤ 128 μg/mL against Gram-positive microorganisms (*Cutibacterium acnes*) as showing very good antibacterial activity. In addition, the inhibition of tyrosinase enzyme activity suggests that the ethanolic extract could potentially be used to prevent both the progression of acne and post-inflammatory skin hyper-pigmentation, particularly in individuals with Fitzpatrick type IV-VI skin types. Inhibitors of tyrosinase *C. marginatus, C. sexangularis* and *P. senegalensis* are particularly important since there is an increased demand from consumers for naturally derived ingredients for skincare, that are both safe and effective. Individuals affected by skin hyperpigmentation and uneven skin tone, particularly those with darker skin types, are particularly interested in active ingredients to replace hydroquinone (Murphy and Dow, 2021). *Persicaria senegalensis* is amongst one of the more understudied plants in this study, however, the anti-tyrosinase activity of the extract *in vitro* and the results from the *in silico* data suggest that this extract should be further explored as a source of natural tyrosinase inhibitors for the development of new molecules against skin hyperpigmentation and products for the personal care market. According to the National Biodiversity Assessment of 2018, estuarine and inland wetland ecosystems are amongst the least protected and most highly threatened ecosystems (99 % and 88 %, respectively), this study provides crucial insights into the potential benefits and importance of plants outside of terrestrial habitats for communities and emphasises the need to increase conservation efforts for the protection and restoration of wetlands in South Africa.

Funding

This study was supported by the South African Water Research Commission (WRC) [Project K5/2540].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Namrita Lall: Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Karina Mariam Szuman:** Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. **Bonani Madikizela:** Writing – review & editing, Project administration, Funding acquisition. **Anna-Mari Kok:** Writing – review & editing, Investigation, Formal analysis. **Marco Nuno De Canha:** Writing – review & editing, Writing – original draft, Project administration, Formal analysis.

References

Akhalwaya, S., van Vuuren, S., Patel, M., 2018. An *in vitro* investigation of indigenous South African medicinal plants used to treat oral infections. J. Ethnopharmacol. 210, 359–371. https://doi.org/10.1016/j.jep.2017.09.002.

Alam, M.B., Ahmed, A., Motin, M.A., Kim, S., Lee, S.H., 2018. Attenuation of melanogenesis by Nymphaea nouchali (Burm. f) flower extract through the regulation of cAMP/CREB/MAPKs/MITF and proteasomal degradation of tyrosinase. Sci. Rep. 8. https://doi.org/10.1038/s41598-018-32303-7.

Alharthi, F., Althagafi, H.A., Jafri, I., Oyouni, A.A.A., Althaqafi, M.M., Al-Hijab, L.Y.A., Al-Hazmi, N.E., Elagib, S.M., Naguib, D.M., 2024. Phytochemical composition and bioactivities of some hydrophytes: antioxidant, antiparasitic, antibacterial, and anticancer properties and mechanisms. Plants 13. https://doi.org/10.3390/plants13152148.

Arya, A.K., Durgapal, M., Bachheti, A., Deepti, Joshi, K.K., Gonfa, Y.H., Bachheti, R.K., Husen, A., 2022. Ethnomedicinal use, phytochemistry, and other potential application of aquatic and semiaquatic medicinal plants. Evid.-based Complement. Alternat. Med. 2022. https://doi.org/10.1155/2022/4931556.

Ayaz, M., Sadiq, A., Wadood, A., Junaid, M., Ullah, F., Zaman Khan, N., 2019. Cytotoxicity and molecular docking studies on phytosterols isolated from Polygonum hydropiper L. Steroids 141, 30–35. https://doi.org/10.1016/j.steroids.2018.11.005.

Azam, S.S., Abbasi, S.W., 2013. Molecular docking studies for the identification of novel melatoninergic inhibitors for acetylserotonin-O-methyltransferase using different docking routines. Theor. Biol. Med. Model. 10. https://doi.org/10.1186/1742-4682-10-63.

Bunalema, L., Kirimuhuzya, C., Tabuti, J.R.S., Waako, P., Magadula, J.J., Otieno, N., Orodho, J.A., Okemo, P., 2011. The efficacy of the crude root bark extracts of Erythrina abyssinica on rifampicin resistant Mycobacterium tuberculosis. Afr. Health Sci. 11, 587–593.

Buwa, L.V., Van Staden, J., 2006. Antibacterial and antifungal activity of traditional medicinal plants used against venereal diseases in South Africa. J. Ethnopharmacol. 103, 139–142. https://doi.org/10.1016/j.jep.2005.09.020.

Castillo, Y., Delgadillo, N.A., Neuta, Y., Hernández, A., Acevedo, T., Cárdenas, E., Montaño, A., Lafaurie, G.I., Castillo, D.M., 2022. Antibiotic susceptibility and resistance genes in oral clinical isolates of Prevotella intermedia, Prevotella nigrescens, and Prevotella melaninogenica. Antibiotics 11. https://doi.org/10.3390/antibiotics11070888.

Chai, T.T., Ooh, K.F., Quah, Y., Wong, F.C., 2015. Edible freshwater macrophytes: a source of anticancer and antioxidative natural products—a mini-review. Phytochem. Rev.. https://doi.org/10.1007/s11101-015-9399-z.

Chandra, S., Gahlot, M., Choudhary, A.N., Palai, S., de Almeida, R.S., de Vasconcelos, J.E.L., dos Santos, F.A.V., de Farias, P.A.M., Coutinho, H.D.M., 2023. Scientific evidences of anticancer potential of medicinal plants. Food Chem. Adv.. https://doi.org/10.1016/j.focha.2023.100239.

Chandrabhan, D., Hemlata, R., Renu, B., Pradeep, V., 2012. Isolation of dental caries bacteria from dental plaque and effect of tooth pastes on acidogenic bacteria. Open J. Med. Microbiol. 02, 65–69. https://doi.org/10.4236/ojmm.2012.23009.

Chang, T.S., 2009. An updated review of tyrosinase inhibitors. Int. J. Mol. Sci.. https://doi.org/10.3390/ijms10062440.

Choi, Y.H., Lee, J.Y., Lee, J.E., Jung, Y.W., Jeong, W., Hong, S.S., Cho, Y.R., Choi, C.W., 2020. Skin-related properties and constituents from the aerial parts extract of Persicaria senticosa. Oxid. Med. Cell Longev. 2020. https://doi.org/10.1155/2020/6627752.

Claus, H., Decker, H., 2006. Bacterial tyrosinases. Syst. Appl. Microbiol. 29 (1), 3–14. https://doi.org/10.1016/j.syapm.2005.07.012.

Crouch, N.R., Mulholland, D.A., Chetty, J., van Staden, J., 2005. Lupane triterpenoid and alkaloid isolates from Cyrtanthus breviflorus (Amaryllidaceae). South Afr. J. Bot. 71, 104–106. https://doi.org/10.1016/S0254-6299(15)30157-5.

Dávid, C.Z., Hohmann, J., Vasas, A., 2021. Chemistry and pharmacology of cyperaceae stilbenoids: a review. Molecules. https://doi.org/10.3390/molecules26092794.

De Angelis, G., D'Inzeo, T., Fiori, B., Spanu, T., Sganga, G., 2014. Burden of antibiotic resistant gram negative bacterial infections: evidence and limits. J. Med. Microbiol. Diagn. 03, 1–1152. https://doi.org/10.4172/2161-0703.1000132.

Department of Environmental Affairs, 2016. 2nd South Africa Environment Outlook: a report on the state of the environment.

Didry, N., Dubreuil, L., Trotin, F., Pinkas, M., 1998. Antimicrobial activity of aerial parts of Drosera peltata Smith on oral bacteria. J. Ethnopharmacol. 60, 91–96. https://doi.org/10.1016/S0378-8741(97)00129-3.

Drewes, S.E., Khan, F., Van Vuuren, S.F., Viljoen, A.M., 2005. Simple 1,4-benzoquinones with antibacterial activity from stems and leaves of Gunnera perpensa. Phytochemistry 66, 1812–1816. https://doi.org/10.1016/j.phytochem.2005.05.024.

Ekhuemelo, D.O., Agbidye, F.S., Anyam, J.V., Ekhuemelo, C., Igoli, J.O., 2019. Antibacterial activity of triterpenes from the stem bark and heartwood of *Erythrophleum suaveolens* (Guill. & Perr.) Brenan. J. Appl. Sci. Environ. Manage. 23, 783. https://doi.org/10.4314/jasem.v23i5.2.

Fayera, S., 2022. Anti-tuberculosis activity and GC−MS Analysis of leaf extract of Plantago lanceolata L. Nat. Prod. Chem. Res. 10, 1–4. https://doi.org/10.37532/npcr.22.10.3.01-04.

Fennell, C.W., Van Staden, J., 2001. Crinum species in traditional and modern medicine. J. Ethnopharmacol..

Fern, K., 2014a. Useful tropical plants Cyperus marginatus Thunb. [WWW Document]. Cyperus marginatus Thunb. https://tropical.theferns.info/viewtropical.php?id=Cyperus+marginatus (accessed 8.3.24).

Fern, K., 2014b. Useful Tropical Plants - Nymphaea nouchali Burm.f. [WWW Document]. Nymphaea nouchali Burm.f. https://tropical.theferns.info/viewtropical.php?id=Nymphaea+nouchali#:∼:text=It%20grows%20from%20the%20bottom,commonly%20cultivated%20as%20an%20ornamental. (accessed 8.3.24).

Fern, K., 2014c. Useful tropical plants - Nymphoides indica [WWW Document]. Nymphoides indica (L.) Kuntze. https://tropical.theferns.info/viewtropical.php?id=nymphoides+indica. (accessed 8.3.24).

Fern, K., 2014d. Useful tropical plants - Persicaria senegalensis [WWW Document]. Persicaria senegalensis (Meisn.) Soják. https://tropical.theferns.info/viewtropical.php?id=Persicaria+senegalensis. (accessed 8.3.24).

Griebel, T., Zeier, J., 2010. A role for β-sitosterol to stigmasterol conversion in plant-pathogen interactions. Plant J. 63, 254–268. https://doi.org/10.1111/j.1365-313X.2010.04235.x.

Hassan, M., Shahzadi, S., Kloczkowski, A., 2023. Tyrosinase inhibitors naturally present in plants and synthetic modifications of these natural products as anti-melanogenic agents: a review. Molecules. https://doi.org/10.3390/molecules28010378.

Henley-Smith, C.J., Botha, F.S., Hussein, A.A., Nkomo, M., Meyer, D., Lall, N., 2018. Biological activities of Heteropyxis natalensis against micro-organisms involved in oral infections. Front. Pharmacol. 9. https://doi.org/10.3389/fphar.2018.00291.

Hennessy, E.F.F., 1991. Erythrineae (Fabaceae) in southern Africa. Bothalia 21, 1–2.

Heyman, H.M., Hussein, A.A., Meyer, J.J.M., Lall, N., 2009. Antibacterial activity of South African medicinal plants against methicillin resistant Staphylococcus aureus. Pharm. Biol. 47, 67–71. https://doi.org/10.1080/13880200802434096.

Hussein, S., EL-Magly, U., Tantawy, M., Kawashty, S., Saleh, N., 2017. Phenolics of selected species of Persicaria and Polygonum (Polygonaceae) in Egypt. Arab. J. Chem. 10, 76–81. https://doi.org/10.1016/j.arabjc.2012.06.002.

Iauk, L., Lo Bue, A.M., Milazzo, I., Rapisarda, A., Blandino, G., 2003. Antibacterial activity of medicinal plant extracts against periodontopathic bacteria. Phytother. Res. 17, 599–604. https://doi.org/10.1002/ptr.1188.

Inelova, Z., Zayadan, B., Zaparina, Y., Aitzhan, M., Boros, E., 2023. Perspectives for the application of aquatic and semi-aquatic plants in biomonitoring of freshwater, saline and soda aquatic ecosystems. Pak. J. Bot. 55, 1099–1115. https://doi.org/10.30848/PJB2023-3(33.

Ismaya, W.T., Rozeboom, H.J., Weijn, A., Mes, J.J., Fusetti, F., Wichers, H.J., Dijkstra, B.W., 2011. Crystal structure of agaricus bisporus mushroom tyrosinase: identity of the tetramer subunits and interaction with tropolone. Biochemistry 50, 5477–5486. https://doi.org/10.1021/bi200395t.

Kamuhabwa, A., Nshimo, C., De Witte, P., 2000. Cytotoxicity of some medicinal plant extracts used in Tanzanian traditional medicine. J. Ethnopharmacol..

Karioti, A., Protopappa, A., Megoulas, N., Skaltsa, H., 2007. Identification of tyrosinase inhibitors from Marrubium velutinum and Marrubium cylleneum. Bioorg. Med. Chem. 15, 2708–2714. https://doi.org/10.1016/j.bmc.2007.01.035.

Khan, S., Tareq Hassan Khan, M., Kardar, M.N., 2014. Tyrosinase Inhibitors from the Fruits of Madhuca latifolia. Curr. Bioact. Compd. 2014, 31–36.

Khumkhrong, P., Piboonprai, K., Chaichompoo, W., Pimtong, W., Khongkow, M., Namdee, K., Jantimaporn, A., Japrung, D., Asawapirom, U., Suksamrarn, A., Iempridee, T., 2019. Crinamine induces apoptosis and inhibits proliferation, migration, and angiogenesis in cervical cancer siHa cells. Biomolecules 9. https://doi.org/10.3390/biom9090494.

Kim, S.M., Zou, G., Kim, H., Kang, M., Ahn, S., Heo, H.Y., Kim, J.S., Lim, K.M., Ausubel, F.M., Mylonakis, E., Gao, H., Kim, W., 2022. Antimicrobial activity of the membrane-active compound nTZDpa is enhanced at low pH. Biomed. Pharmacother. 150. https://doi.org/10.1016/j.biopha.2022.112977.

Kotze, P., 2022. Protecting nature's medicine cabinet: how research is upping the value of endangered aquatic ecosystems. Water Wheel 21, 1–39.

Kuete, V., Efferth, T., 2015. African flora has the potential to fight multidrug resistance of cancer. Biomed. Res. Int.. https://doi.org/10.1155/2015/914813.

Lall, N., Henley-Smith, C.J., De Canha, M.N., Oosthuizen, C.B., Berrington, D., 2013. Viability reagent, prestoblue, in comparison with other available reagents, utilized in cytotoxicity and antimicrobial assays. Int. J. Microbiol. 2013. https://doi.org/10.1155/2013/420601.

Lall, N., Mogapi, E., de Canha, M.N., Crampton, B., Nqephe, M., Hussein, A.A., Kumar, V., 2016. Insights into tyrosinase inhibition by compounds isolated from Greyia radlkoferi Szyszyl using biological activity, molecular docking and gene expression analysis. Bioorg. Med. Chem. 24, 5953–5959. https://doi.org/10.1016/j.bmc.2016.09.054.

Lelovic, N., Mitachi, K., Yang, J., Lemieux, M.R., Ji, Y., Kurosu, M., 2020. Application of Mycobacterium smegmatis as a surrogate to evaluate drug leads against Mycobacterium tuberculosis. J. Antibiotics 73, 780–789. https://doi.org/10.1038/s41429-020-0320-7.

Li, X., Li, Y., Cheng, T., Liu, Z., Wang, R., 2010. Evaluation of the performance of four molecular docking programs on a diverse set of protein-ligand complexes. J. Comput. Chem. 31, 2109–2125. https://doi.org/10.1002/jcc.21498.

Likhitwitayawuid, K., Sornsute, A., Sritularak, B., Ploypradith, P., 2006. Chemical transformations of oxyresveratrol (trans-2,4,3′,5′-tetrahydroxystilbene) into a potent tyrosinase inhibitor and a strong cytotoxic agent. Bioorg. Med. Chem. Lett. 16, 5650–5653. https://doi.org/10.1016/j.bmcl.2006.08.018.

Liu, H.M., Lei, S.N., Tang, W., Xun, M.H., Zhao, Z.W., Cheng, M.Y., Zhang, X.D., Wang, W., 2022. Optimization of ultrasound-assisted cellulase extraction from Nymphaea hybrid flower and biological activities: antioxidant activity, protective effect against ROS oxidative damage in HaCaT cells and inhibition of melanin production in B16 cells. Molecules 27. https://doi.org/10.3390/molecules27061914.

Liu, T., Wang, Z.J., Shi, Y.Z., Tao, R., Huang, H., Zhao, Y.L., Luo, X.D., 2024. Curcusinol from the fruit of Carex baccans with antibacterial activity against multidrug-resistant strains. J. Ethnopharmacol. 318. https://doi.org/10.1016/j.jep.2023.116892.

Lombard, N., van Wyk, B.E., Marianne le Roux, M., 2020. A review of the ethnobotany, contemporary uses, chemistry and pharmacology of the genus Thesium (Santalaceae). J. Ethnopharmacol.. https://doi.org/10.1016/j.jep.2020.112745.

Mabona, U., Van Vuuren, S.F., 2013. Southern African medicinal plants used to treat skin diseases. South Afr. J. Bot.. https://doi.org/10.1016/j.sajb.2013.04.002.

Machete, I., Masila, A., 2023. PlantZAfrica [WWW Document]. Cyrtanthus breviflorus Harv. https://pza.sanbi.org/cyrtanthus-breviflorus (accessed 8.3.24).

Mander, M., 1998. Marketing of indigenous medicinal plants in South Africa : a case study in Kwazulu-Natal. Rome. https://doi.org/10.13140/2.1.1073.4084

Mander, M., Ntuli, L., Diederichs, N., Mavundla, K., 2007. Economics of the traditional medicine trade in South Africa. S. Afr. Health Rev. 2001, 189–196.

Mapunya, M.B., Hussein, A.A., Rodriguez, B., Lall, N., 2011. Tyrosinase activity of Greyia flanaganii (Bolus) constituents. Phytomedicine 18, 1006–1012. https://doi.org/10.1016/j.phymed.2011.03.013.

Masuda, T., Yamashita, D., Takeda, Y., Yonemori, S., 2005. Screening for tyrosinase inhibitors among extracts of seashore plants and identification of potent inhibitors from Garcinia subelliptica note. Biosci. Biotechnol. Biochem..

Masum, M.N., Choodej, S., Yamauchi, K., Mitsunaga, T., 2019. Isolation of phenylpropanoid sucrose esters from the roots of Persicaria orientalis and their potential as inhibitors of melanogenesis. Med. Chem. Res. 28, 623–632. https://doi.org/10.1007/s00044-019-02312-w.

McGaw, L.J., Elgorashi, E.E., Eloff, J.N., 2014. Cytotoxicity of African medicinal plants against normal animal and human cells. Toxicological Survey of African Medicinal Plants. Elsevier, pp. 181–233. https://doi.org/10.1016/B978-0-12-800018-2.00008-X.

Mcgaw, L.J., Jäger, A.K., Van Staden, J., 2000. Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. J. Ethnopharmacol..

Mizrahi, V., 2024. A spotlight on the tuberculosis epidemic in South Africa. Nat. Commun. 15, 1–4. https://doi.org/10.1038/s41467-024-45491-w.

More, G., Tshikalange, T.E., Lall, N., Botha, F., Meyer, J.J.M., 2008. Antimicrobial activity of medicinal plants against oral microorganisms. J. Ethnopharmacol. 119, 473–477.

Moteetee, A., Van Wyk, B.-E., 2011. The medical ethnobotany of Lesotho: a review. Bothalia 41, 209–228.

Murata, K., Suzuki, S., Miyamoto, A., Horimoto, M., Nanko, S., Mori, D., Kanamaru, H., Endo, Y., 2022. Tyrosinase inhibitory activity of extracts from Prunus persica. Separations. 9. https://doi.org/10.3390/separations9050107.

Murphy, M.J., Dow, A.A., 2021. Natural cosmeceutical ingredients for the management of hyperpigmentation in Hispanic and Latino women. J. Clin. Aesthet. Dermatol. 14, 52–56.

Niesen, D.B., Ma, H., Yuan, T., Bach Ii, A.C., Henry, G.E., Seeram, N.P., 2015. Phenolic Constituents of Carex vulpinoidea Seeds and their Tyrosinase Inhibitory Activities. NPC Natural Product Communications.

Notten, A., 2001. PlantZAfrica [WWW Document]. Scadoxus multiflorus (Martyn) Raf. subsp. katharinae. URL http://pza.sanbi.org/scadoxus-multiflorus-subsp-katharinae (accessed 8.3.24).

Nokinsee, D., Shank, L., Lee, V.S., Nimmanpipug, P., 2015. Estimation of inhibitory effect against tyrosinase activity through homology modeling and molecular docking. Enzyme Res. 2015 (1), 1–12. https://doi.org/10.1155/2015/262364.

Osama, M., Wei, C.R., Saleem, R., Unar, A.A., Unar, K., Siyal, F.J., Shaikh, B., Baig, S.G., Siddiqu, A., 2023. View of aquatic plants with anti-inflammatory and anti-oxidant activities. J. Surv. Fish Sci. 10, 3802–3806.

Phan, D.T.A., 2021. Screening of antioxidant and tyrosinase inhibition activities of spicy vegetables in Vietnam and application of persicaria odorata leaf extract to preservative white leg shrimp (Litopenaus vannamei). An. Acad. Bras. Cienc. 93. https://doi.org/10.1590/0001-3765202120191341.
Pillay, C.C.N., Jäger, A.K., Mulholland, D.A., van Staden, J., 2001. Cyclooxygenase inhibiting and anti-bacterial activities of South African Erythrina species. J. Ethnopharmacol. 74, 231–237. https://doi.org/10.1016/S0378-8741(00)00366-4.
Ramsden, C.A., Riley, P.A., 2014. Tyrosinase: the four oxidation states of the active site and their relevance to enzymatic activation, oxidation and inactivation. Bioorg. Med. Chem.. https://doi.org/10.1016/j.bmc.2014.02.048.
Ranglová, K., Krejčová, P., Kubec, R., 2015. The effect of storage and processing on antimicrobial activity of Tulbaghia violacea. South Afr. J. Bot. 97, 159–164. https://doi.org/10.1016/j.sajb.2015.01.011.
Rangra, N., Samanta, S., Pradhan, K., 2021. Evaluation of Acacia auriculiformis Benth. leaves for wound healing activity in type 2 diabetic rats. Pharmacogn. Mag. 17, 129 . https://doi.org/10.4103/pm.pm_496_20.
Refaat, J., Kamel, M.S., Ramadan, A., 2013. Crinum; an endless source of bioactive principles: a review. Part V: biological profile. Int. J. Pharm. Sci. Res. 4, 1239–1252.
Ríos, J.L., Recio, M.C., 2005. Medicinal plants and antimicrobial activity. J. Ethnopharmacol. 100, 80–84. https://doi.org/10.1016/j.jep.2005.04.025.
Rodrigues, M.J., Custódio, L., Lopes, A., Oliveira, M., Neng, N.R., Nogueira, J.M.F., Martins, A., Rauter, A.P., Varela, J., Barreira, L., 2017. Unlocking the *in vitro* anti-inflammatory and antidiabetic potential of Polygonum maritimum. Pharm. Biol. 55, 1348–1357. https://doi.org/10.1080/13880209.2017.1301493.
Saxena, M.K., Singh, N., Kumar, S., Dobhal, M.P., Datta, S., 2021. Potent pharmaceutical products from aquatic plants – review. Asian J. Pharmaceut. Clin. Res. 14, 48–63. https://doi.org/10.22159/ajpcr.2021.v14i1.39992.
Seimandi, G., Álvarez, N., Stegmayer, M.I., Fernández, L., Ruiz, V., Favaro, M.A., Derita, M., 2021. An update on phytochemicals and pharmacological activities of the genus persicaria and polygonum. Molecules 26. https://doi.org/10.3390/molecules26195956.
Selina Wamucii, 2024a. Afrosciadium magalismontanum - uses, benefits & care [WWW Document]. https://www.selinawamucii.com/plants/apiaceae/afrosciadium-magalismontanum/(accessed 8.3.24).
Selina Wamucii, 2024b. Carex rhodesiaca - uses, benefits & care [WWW Document]. https://www.selinawamucii.com/plants/cyperaceae/carex-rhodesiaca/. (accessed 8.3.24).
Selina Wamucii, 2024c. Cyperus sexangularis - uses, benefits & care [WWW Document]. https://www.selinawamucii.com/plants/cyperaceae/cyperus-sexangularis/ (accessed 8.3.24).
Selina Wamucii, 2024d. Dracoscirpoides ficinioides - uses, benefits & care [WWW Document]. https://www.selinawamucii.com/plants/cyperaceae/dracoscirpoides-ficinioides/(accessed 8.3.24).
Seo, H., Kim, M., Kim, S., Mahmud, H.Al, Islam, M.I., Nam, K.-W., Cho, M.-L., Kwon, H.-S., Song, H.-Y., 2017. *In vitro* activity of alpha-viniferin isolated from the roots of Carex humilis against Mycobacterium tuberculosis. Pulm. Pharmacol. Ther. 46, 41–47. https://doi.org/10.1016/j.pupt.2017.08.003.
Simao, T.L.B.V., Aguiar, G.V., Ramos, A.C., da Silva, G.P., Muzitano, M.F., Lassounskaia, E., De Oliveira, R.R., 2022. Antimycobacterial and anti-inflammatory activities of fractions and substances from Erythrina verna Vell focusing on dual severe TB treatment approach. Ann. Br. Acad. Sci. 94 (3), 1–15. https://doi.org/10.1590/0001-3765202220211032.
Swapna, M.M., Prakashkumar, R., Anoop, K.P., Manju, C.N., Rajith, N.P., 2011. A review on the medicinal and edible aspects of aquatic and wetland plants of India. J. Med. Plant Res. 5, 7163–7176. https://doi.org/10.5897/JMPRx11.005.
van Wyk, B.E., 2008. A review of Khoi-San and Cape Dutch medical ethnobotany. J. Ethnopharmacol.. https://doi.org/10.1016/j.jep.2008.07.021.
Wamba, B.E.N., Mbaveng, A.T., Kuete, V., 2023. Fighting Gram-positive bacteria with African medicinal plants: cut-off values for the classification of the activity of natural products. pp. 413–522. https://doi.org/10.1016/bs.abr.2022.08.008
Wang, Y., Zhang, G., Yan, J., Gong, D., 2014. Inhibitory effect of morin on tyrosinase: insights from spectroscopic and molecular docking studies. Food Chem. 163, 226–233. https://doi.org/10.1016/j.foodchem.2014.04.106.
Watt, J.M., Breyer-Brandwijk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa: Being an Account of Their Medicinal and Other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal, 2nd ed. E. & S. Livingstone Ltd., Edinburgh.
Woo, Y.-M., Kim, A.-J., Kim, J.-Y., Lee, C.-H., 2011. Tyrosinase inhibitory compounds isolated from Persicaria tinctoria flower. J. Appl. Biol. Chem. 54, 47–50. https://doi.org/10.3839/jabc.2011.008.
Xu, W.L., Huang, Y.B., Qian, J.H., Sha, O., Wang, Y.Q., 2005. Separation and purification of stigmasterol and ß-sitosterol from phytosterol mixtures by solvent crystallization method. Sep. Purif. Technol. 41, 173–178. https://doi.org/10.1016/j.seppur.2004.02.005.