Cosmeceutical Products from Indigenous South African Wetland Plants

Report to the
Water Research Commission

by

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Executive summary

RATIONALE

South Africans are becoming more aware of the products they are using. Close to a quarter of South African have indicated that natural, organic and environmentally friendly considerations influence the type of product they purchase. Over the years there has been an increase in demand for alternative hyperpigmentation or even tone and anti-wrinkle-based treatment. This is because existing products often pose deleterious effects on the skin, which include toxicity and inflammation. The present study identified five indigenous South African wetland plants to be further developed into commercial products for the treatment of wrinkles and skin hyperpigmentation. The current research project also focused on the identification of the active compounds present in the plant for the treatment of wrinkles and skin hyperpigmentation. The study demonstrated the importance of wetland species, the need to protect these biomes and why more research should be done. This study linked to water security as wetlands act as a natural filter for nearby streams and lakes as well as water adaptation since native plants found in this biome could be utilised in cosmetics.

AIMS AND OBJECTIVES

The project aimed to bridge the gap between knowledge holders, farmers, researchers and consumers. Currently, there are no products available on the market from indigenous South African aquatic and wetland plants for the above-mentioned skin disorders. The present study moved one step further into the commercialisation phase, providing the industry with information that enables them to take these products directly for commercialisation. This further entailed moving towards the high-end international pharmaceutical and cosmeceutical market. The expected results and product from this study identified two anti-wrinkle and one even skin tone prototype that will be available for commercialisation along with all the required data as per the objectives stated below:

1. Identification of compounds for standardization
   - 1.1 Expert phytochemist: Prof. Oyedeji (WSU). Biological investigation of compounds isolated from *C. marginatus* and *C. sexangularis* for anti-elastase and cytotoxicity.
   - 1.2 Gas chromatography-mass spectrometry (GC-MS) – Identify phytochemicals present and create a chemical fingerprint and standard that can be used in commercializing the actives.
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   5.1 Application for bio-prospecting permit
   5.2 Training on sustainable propagation and cultivation of identified indigenous South African wetland plants.

METHODOLOGY

The following methods were used to achieve the abovementioned objectives:

1. Plant collection and extraction

Each plant was collected from the University of Pretoria in Gauteng and Botanica Natural Products situated in Limpopo. Extracts were prepared by blending either the whole, stems or aerial parts of the plants with absolute ethanol. These extracts were used throughout the study.

2. Heavy metal and microbial analysis

In collaboration with Botanica, water samples were collected and sent to Capricorn Veterinary Laboratories for heavy metal and microbial analysis (Chapters 4 and 5).

3. Stability and Material safety data sheet (MSDS)

The ethanolic extracts of *Cyperus marginatus* (CM), *C. sexangular* (CS), *Juncus lomatophyllus* (JL), *Elegia tectorum* (ET) and *Persicaria senegalensis* (PS) were submitted to Botanichem where some of the extract was sent to Bioindustrial Services to generate an MSDS for each plant. Thereafter, Botanichem evaluated the stability of the extracts by exposing them to various storage temperatures for 12 weeks and measured the sample's appearance, odour, pH and specific gravity (Chapters 4 and 5).

4. Marketing sheet and technical file

During the study, JL, ET and PS displayed the most significant activity thus the project focused on developing prototypes using these plants. A marketing sheet and technical file was

generated for PS, ET and JL to ensure that these plant actives met all the requirements needed to commercialise the final product. This was compiled based on all the information that was obtained during the course of the study (Chapter 6).

5. Compound identification

Compound identification was conducted on CM, CS, ET, PS and JL whereby liquid-liquid partitioning was conducted to isolate different solvent partitions. In the case of CM and CS these partitions were further purified. In the case of JL, ET and PS, bioactive partitions were identified and a column was used to collected fractions, which were pulled together if they displayed similar TLC fingerprints. Thereafter, these pulled fractions were evaluated for their anti-elastase or anti-tyrosinase activity. Furthermore, a gas chromatography-mass spectrometry (GC-MS) spectra was generated for PS, ET and JL to identify volatile compounds present in the extracts (Chapters 4 and 5).

6. Anti-elastase activity

An anti-wrinkle assay was conducted on CM, CS, ET, PS and PSF. Briefly, the extracts were dissolved in DMSO, serially diluted to(?) two-fold and exposed to elastase enzyme and *N-succinyl-Ala-Ala-Ala-p-nitroanilide* substrate. Thereafter, the 50% inhibitory concentration (IC_{50}) of the extracts were measured using GraphPad Prism version 4 and recorded. The positive control used was ursolic acid (Chapters 4 and 5).

7. Tyrosinase inhibition

An anti-tyrosinase assay was conducted on JL whereby the ethanolic extract was dissolved in DMSO, serially diluted(?) two-fold and exposed to mushroom tyrosinase enzyme and *L-tyrosine* substrate. The IC_{50} values were measured using GraphPad Prism and recorded with kojic acid used as the positive control (Chapters 4 and 5).

8. Fermentation

The ethanolic extracts of JL, ET and PS were fermented using *Bifidobacterium bifidum* as previous papers have reported that fermentation enhance the biological activity. Once fermented, the extracts activity was evaluated whereby the fermented PS (PSF) enhanced the anti-elastase potential of PS (Chapters 4 and 5).

9. Metal nanoparticle formation

Metal nanoparticles, specifically gold nanoparticles due to the use of gold salt, were synthesised using the ethanolic extracts of ET, JL and PS. The extracts were dissolved in water, combined with gum arabic (stabiliser) and heated. Once heated to the required temperature gold salt was added which converted the green coloured solution into a wine colour. Thereafter, different characterisation methods were conducted to identify how stable and active the gold nanoparticles were (Chapters 4 and 5).

10. Cytotoxicity

Human keratinocyte cells (HaCaT) were cultured using Dulbecco's modified Eagle's Medium (DMEM) and were sub-cultured when a confluent layer was formed. These cells were seeded

into a 96 well plate, incubated overnight to prevent stress and exposed to CM, CS, ET, PS, PSF and JL. Thereafter, a viability reagent known as PrestoBlue was added to the cells and the fluorescence was measured. Using GraphPad Prism the fluorescence was measured and a IC_{50} value was determined (Chapters 4 and 5).

11. *In vivo* irritancy

An irritancy patch test was conducted at Future Cosmetics once ethical clearance was obtained. Volunteers of different age groups was selected and the neat extracts were applied directly to the inner forearm of the patients. After 24-, 48- and 72 hours the reaction was graded and a mean score was determined (Chapters 4 and 5).

12. Gel-based cream formulation

An oil in water emulsion was prepared using heat to formulate a gel-based cream. Once prepared, the samples that displayed the most significant anti-wrinkle (PS, PSF and ET) and anti-tyrosinase (JL) activity were dissolved in 40% ethanol and 10% of this stock was added to the cream. A placebo control was prepared by adding 10% of a 40% ethanol stock solution to the cream (Chapters 4 and 5).

13. Efficacy study

A double-blind efficacy study was conducted at Future cosmetics once ethical clearance was obtained. The prepared gel-based formulations were applied topically to the patients and the extract's effect on wrinkle topography and depth or reduction in dark spots was evaluated using a chromameter and visioscan. Both of these aspects were measured after 14- and 28 days and a statistical analysis was performed in comparison to the baseline established on the first day for both the extract and the placebo (Chapters 4 and 5).

RESULTS

Heavy metal, microbial analysis and stability

None of the plants nor the water displayed significant heavy metal readings and microbial count. Furthermore, MSDS sheets were generated for all five wetland plants and were found to be stable for 24 months.

Biological assays

CM, CS, PS, PSF and ET displayed significant anti-elastase activity and showed no significant cytotoxic effect against human keratinocyte cells (HaCaT) at 400 µg/mL. Furthermore, compounds and bioactive fractions were isolated from CM, CS, PS and JL. The irritancy and efficacy of ET, PS and PSF was evaluated whereby ET and PS displayed a mild irritancy with a significant reduction in wrinkle depth after 28 days while PFS showed no irritancy with a significant effect after 14 days. JL displayed significant anti-tyrosinase activity and showed no significant cytotoxic effect against HaCaT cells at 400 µg/mL. Furthermore, JL displayed no irritancy and significantly reduced the formation of dark spots after 14 days when combined with 10% niacinamide.

Marketing

Prototypes have been designed along with the marketing sheet and technical file. The BABS permits have been obtained for PS, JL and ET so that the license of these actives may be sold to interested formulators (Appendix A). Furthermore, the registration process of PS, JL and ET INCI names are in progress and will be completed soon. Study team will be responsible.

Conferences, awards and publications

Throughout the study numerous conferences were attended in order to share the work that has been completed with other scientists and to spread awareness of the importance of wetland biomes. Furthermore, conferences like The Society of Cosmetic Chemists South Africa (Coschem) were attended to locate interested formulators who will take the prototype onto the shelf. Lastly, a few publications are in progress regarding the work done during the study (Appendix C).

DISCUSSION

During this study JL, ET and PS displayed significant anti-wrinkle and anti-tyrosinase activity thus were selected for further analysis and prototype development. Nanotechnology and fermentation were utilised on JL, ET and PS to enhance the biological activity, however, only PSF displayed a significant difference. Previous studies have indicated that fermentation enhances anti-photoaging by reducing the expression of matrix metalloproteinase (MMP). Thus, this specific mode of action may be applicable to PS but not to ET, which could be one of the reasons as to why PSF displayed significant activity. In the case of JL, the gold nanoparticles did display some activity, which could be due to the presence of gold salt that may have enhanced the active compounds present in the extract. The fermented extract displayed no activity, which could be due to the duration the extract was fermented for or the preparation method used. With regard to the irritancy response of JL, the combination of niacinamide may have helped to lower the irritancy effect of JL, however, further evaluation is required. PSF displayed no irritation in comparison to PS, which could be due to the chemical alteration caused by the fermentation. This alteration may have shifted the presence of phenolic groups, organic acids and other compounds that may have caused an irritancy response when exposed to skin.

SHORT SUMMARY RESULTS

In summary, five wetland species were selected due to their anti-elastase and anti-tyrosinase activity. The heavy metal, microbial count and stability of these ethanolic extracts were assessed and were found to contain negligible amounts of heavy metals and microbes. Furthermore, the extracts were found to be stable for 24 months. Compound identification was conducted whereby a compound known as M1 was isolated from CM and CS while bioactive fractions were identified in PS and JL. Significant *in vivo* results were obtained from PS, PSF, ET and JL. The results obtained for JL, ET and PS could be used by industry members to commercialise these plant actives in their anti-wrinkle and even skin tone formulations. Since the BABS permits have been obtained for all three, the commercialisation process will be easier for industry members making these actives more favourable.

As for CM and CS, these results could be used to identify the potential mode of action and to promote research on other wetland species. Furthermore, the data obtained during this study can be used to motivate the conservation of wetlands throughout South Africa as these water resource types are becoming endangered due to urbanisation and deforestation.

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER RESEARCH

In conclusion, wetland species hold many secretes that need to be explored and the conservation of these water resources are imperative. PS, JL and ET are effective anti-wrinkle and even skin tone treatments that will one day reach the market once an interested formulator has taken the license with the University of Pretoria. Recommendations of this study is to further explore CM and CS by identifying their potential anti-elastase mode of action and to commercialise these as anti-wrinkle treatments. Furthermore, to identify the optimal growth phase and season when JL, PS and ET should be harvested to ensure that the supply remains consistent.

ACKNOWLEDGEMENTS

We would like to extend special thanks to the review group chairperson, Mr Bonani Madikizela, as well as a number of collaborators and Steering Committee members whose questions, discussions and suggestions have the work delivered in this report.

Collaborators:

Person	**Affiliation**	**Respective projects (where applicable): C2019/2020-00132**
Marizé Cuyler	Department of Plant and Soil Sciences, University of Pretoria	Assisted with running the project and was involved with all the aspects associated with JL and ET
Bianca D. Payne		Was involved with all the aspects associated with PS
Prof. Adebola Oyedeli	Walter Sisulu University	Isolated and identified compounds from CM and CS as well as prepared the extract and analysed their biological activity
Dr Gugulethu Miya		

Steering Committee Members (alphabetic by surname):

Person	**Affiliation**
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Mr Josua le Roux	Owner of Future Cosmetics
Ms Portia Mngomezulu	Owner of Portia M Skin Solutions
Ms Sinah Mosehla	Director at Department of Trade and Industry
Prof. Hendrick Motswaledi	Department of Dermatology, Sefako Makgatho Health and Sciences University
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CHAPTER 1: INTRODUCTION

South Africans are becoming more aware of the products they are using. Close to a quarter of South African have indicated that natural, organic and environmentally friendly considerations influence the type of product they purchase. Over the years there has been an increase in demand for alternative hyperpigmentation or even tone and anti-wrinkle-based treatment. This is because existing products often pose deleterious effects on the skin, which include toxicity and inflammation (Panico et al., 2019).

Hyperpigmentation is a pigmentation disorder that is due to the overexpression of tyrosinase (Figure 1.1). Tyrosinase is a copper-containing glycoprotein that functions as the rate-limiting enzyme within the pathway of melanogenesis. Since tyrosinase is the first enzyme in this process, by targeting its degradation or inhibition, there could be a drastic decline in the abundance of melanin resulting in more evened skin tone, thus it is a common target to alleviate cutaneous hyperpigmentation (Ando et al., 2007).

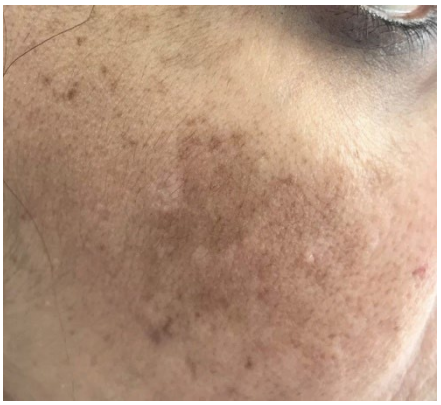

Figure 1.1. Illustration of hyperpigmentation (Thames Skin Clinic, 2022).

Ageing can be defined as the accumulation of diverse deleterious changes occurring in cells and tissues with advancing age that is responsible for the increased risk of disease and health (Harman, 2003). It is a multifactorial and very complex process with many theories surrounding it (Kowald and Kirkwood, 1996; Weinert and Timiras, 2003). The dermal layer of the skin contains the structural components of the skin, which provide it with its structural integrity and elasticity. One of these structural components, elastin, is an insoluble elastic fibrous protein, which forms the main component of connective tissues and tendons (Figure 1.2) (Halper and Kjaer, 2014). It is an essential part of various human tissues, which depend on elasticity, including the arteries, skin and lungs (Daamen et al., 2007; Fulop et al., 2012). The presence of elastin within these tissues is what affords them the ability to stretch and recoil, thus is the main contributor to skin elasticity and firmness (Daamen et al., 2007; Yuan and Walsh, 2006). The problem, however, is that the skin exhibits a drastic declined in elastin levels with ageing, which results in a loss of strength and flexibility materialising as visible wrinkles (Ndlovu et al., 2013).

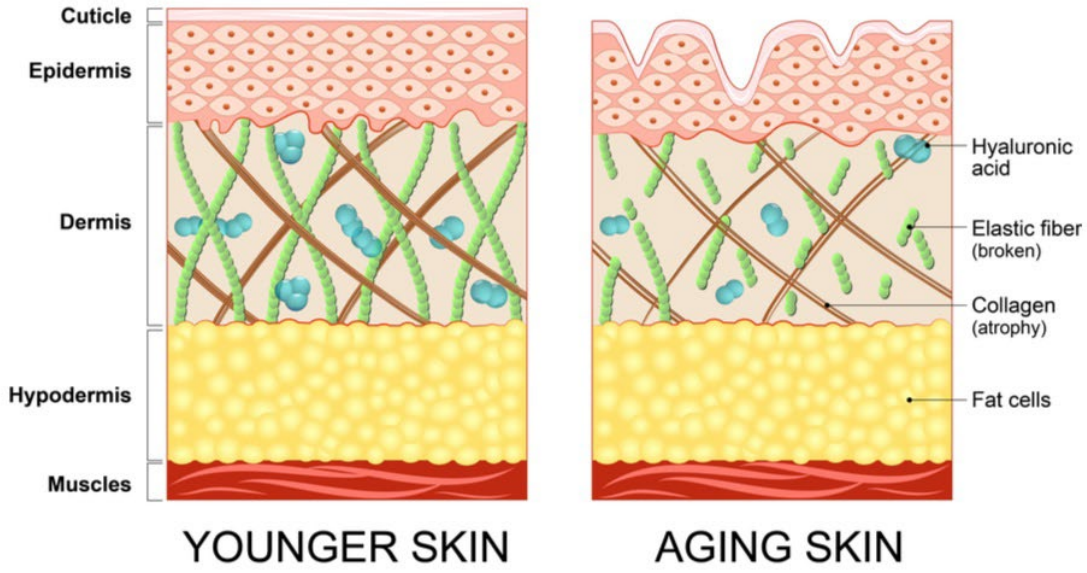

Figure 1.2. In young skin, elastin fibres are abundant within the dermis. However, as a person ages, elastic fibres are broken down resulting in wrinkle formation (Chandra, 2019).

The flexibility and strength of elastin are increasingly perturbed with time as the activity of elastase, the key degrader of elastin is enhanced with age and external factors (Nar et al., 2001). In addition to this, elastin is thought to be produced at a slower rate with time. The exclusivity of the elastase-elastin interaction and the decreased quality of the elastin network as a consequence of this interaction has resulted in a recent peak of interest in degradative enzymes such as elastase, for its role in skin ageing (Maity et al., 2011).

Elastase is naturally required by the skin to degrade dysfunctional proteins that remain within the extracellular matrix after a wounding event such that they can be eliminated for repair to occur. This problem is further compounded by the fact that the number of epidermal stem cells (responsible for allowing the skin to differentiate and regenerate itself) declines with age and with it the skin's ability to regenerate important structural components, such as elastin and collagen, declines as well (Nar et al., 2001). For that reason, inhibitors capable of inhibiting the enzyme elastase can be used in the successful treatment of wrinkles to fight the appearance of ageing.

1.1 Skin care market

The skincare market is the third-largest revenue segment in South Africa. Market studies have shown that the South Africa skincare market is expected to grow at a Compound annual growth rate (CAGR) of 8.61% between 2023 and 2028 (Mordor Intelligence, 2023). Studies have confirmed that people are becoming more aware of their skin and skin problems such as hyperpigmentation and wrinkles affect their quality of life. This is reflected in the sales of skincare products, with the market valued at 581.7 million U.S. dollars (R8.418 billion) in 2017 and is estimated to reach 839.2 million dollars (R15.767 billion) by 2023. Natural products from medicinal plants are of particular interest in the treatment of hyperpigmentation and wrinkles. Cosmetic houses that create skincare products from African plants widely use

terrestrial plants such as Marula and rooibos in their formulations (Petruzzi, 2022). Currently, there is no skincare product from an indigenous South African wetland plant on the market.

1.2 Purpose of this study

A previous research project supported and funded by the Water Research Commission of South Africa, project number K5/2540, aimed to investigate the potential of 30 indigenous South African wetland plants that are both traditionally used and unknown for the treatment of acne, skin hyperpigmentation, wrinkles, periodontal diseases, tuberculosis and cancer. The study identified five indigenous South African wetland plants to be further developed into commercial products for the treatment of wrinkles and skin hyperpigmentation. The *in vitro* results for wrinkles and skin hyperpigmentation are as follows:

Table 1.1. *In vitro* elastase, tyrosinase and human keratinocyte (HaCaT) inhibition by five indigenous South African wetland plants

Samples	Elastase (IC_{50}: (µg/mL)	Tyrosinase IC_{50} ± SD (µg/mL)	Cytotoxicity HaCaT (IC_{50} ± SD (µg/mL)
Cyperus marginatus	20.90	75.00 ± 5.00	> 400
Cyperus sexangularis	38.80	64.30 ± 5.10	> 400
Elegia tectorum	13.50 ± 1.50	65.26 ± 8.38	102.15 ± 1.77
Juncus lomatophyllus	-	31.64 ± 6.91	> 400
Persicaria senegalensis	29.50 ± 3.40	71.59 ± 4.23	> 400

The current research project focused on the development of previously identified indigenous South African wetland plants into novel commercial products and the identification of the active compounds present in the plant for the treatment of wrinkles and skin hyperpigmentation.

1.3 Aims and objectives.

The project aimed to bridge the gap between knowledge holders, farmers, researchers and consumers. Currently, there are no products available on the market from indigenous South African wetland plants for the above-mentioned skin disorders. The present study will move one step further into the commercialisation phase, providing the industry with information that enables them to take these products directly for commercialisation. This will further entail moving towards the high-end international pharmaceutical and cosmeceutical market.

Table 1.2. Objectives for the current project (Take them to executive summary and declare all were achieved, if so, if not what was not achieved and why).

OBJECTIVES
1. Identification of compounds for standardization 1.1 Expert phytochemist: Prof Oyedeji (WSU). Biological investigation of compounds isolated from *C. marginatus* and *C. sexangularis* for anti-elastase and cytotoxicity. 1.2 Gas chromatography-mass spectrometry (GC-MS) – Identify phytochemicals present and create a chemical fingerprint and standard that can be used in commercialising the actives.
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4. Marketing strategy 4.1. Market research & business model 4.2. Marketing sheet 4.3. INCI name
5. Community involvement 5.1. Application for bio-prospecting permit 5.2. Training on sustainable propagation and cultivation of identified indigenous South African wetland plants.

CHAPTER 2: PLANT SELECTION

2.1 *Cyperus*

Cyperus is the second largest genus in the *Cyperaceae* family. It grows fast and covers the topsoil with its roots and rhizomes (Browning et al., 1999; Gamal et al., 2015a; Gamal et al., 2015b). This genus has been reported to have about 700 species and is widely distributed throughout Asia, Africa, and Europe (Gambo and Da'u, 2014; Sharma and Singh, 2011; Srivastava et al., 2014). This study focused on two species that are growing in the Limpopo province of South Africa, namely *C. marginatus* and *C. sexangularis*.

Cyperus sexangularis Nees (Figure 2.1A) commonly known as bushveld sedge is a tufted, firm, lasting sedge that grows in wetlands. It is perfect for rehabilitation work, but not for a lesser pool, except it can be contained. It has a clump-forming shape, is very hardy, grows fast, needs to be kept moist, evergreen, flowering in summer with red flowers, and grows up to 1.5 m high (Raimondo et al., 2009). While *C. marginatus* Thunb. (Figure 2.1B) commonly known as desert sedge is a common taxon found reported from all areas of southern Africa except Botswana.

In the Northern province of South Africa, they are described as perennial herbs of temporary wet habitats. However, in KwaZulu-Natal, they are frequently rooted in shallow, permanently flowing water, often among rocks. It has long green leaves with rounded stems (Browning et al., 1999). To the best of our knowledge, these two species have not been intensely studied in literature compared to other *Cyperus* genus species such as *Cyperus rotundu, Cyperus articulates, Cyperus scariosus, Cyperus papyrus* and *Cyperus esculentus.*

Figure 2.1. Visual depiction of *Cyperus sexangularis* (A) and the inflorescence of *Cyperus marginatus*.

Numerous compounds have been isolated from *Cyperus* genus; however, none have been isolated from *C. sexangularis* and *C. marginatus*. *Cyperus rotuntdus* tubers from Iran essential oils had 0.2% (w/w), which contained a total of sixty identified compounds, where sesquiterpene compounds had the most quantities in the oil, and constituents included cyperene

(16.9%), caryophyllene oxide (8.9%), α-longipinane (8.4%) and β-elinene (6.6%) (Ghannadi et al., 2012). The percentage of essential oils in *C. rotundus* nut sedge was (0.19%), 52 components were isolated from Egypt, (+) oxo-α-ylangene (9.35%), (+) a-cyperone (9.07%) trans-pinocarveol (7.92%) and cyperene (7.83%) were the major compounds in the oil (Al-Snafi, 2016).

The essential oil extracted from *C. longus* growing in Morocco had 1% (w/w) yield, and they are very rich in sesquiterpene hydrocarbons where β-himachalene (46.6%), α-humulene (16.9%), and γ-himachalene (10.1%) were the prominent constituents (Ait-Ouazzou et al., 2012). A study on the volatile constituents of *Cyperus esculentus* using smoke trapped by methanol and hexane in Ethiopia had about 40 compounds identified using GC/MS. Cyperene (35.94%), *p*-vinylguaiacol (11.57%), α-copaene (9.49%), limonene (8.98%), coumaran (7.84%), cymene (7.73%) and β-pinene (6.70%) were identified as the most prominent compounds for both hexane and methanol (Gugsa and Yaya, 2018). Studies from Mostafa et al. in 2018 showed that essential oils obtained from Egyptian *Cyperus articulatus* had three major components, namely 4-terpinenol (8.00%), (-)-cyperene (7.08%) and lb,7a,10b H-guaia-4,11(13)-dien-3-one (5.47%) (Mostafa et al., 2018).

In the same manner, studies from Kakarla et al. (2016) reported the isolation of ten different compounds from *C. rotundus* and *C. scariosus* rhizomes from India. The compounds include stigmasterol, β- amyrin acetate, 4- hydroxy butyl cinnamate, β- sitosterol, Lupeol, gallic acid, quercetin, β- amyrin, Oleanolic acid, 4- hydroxy cinnamic acid, kaempferol and Caffeic acid. The isolation of tryptophan, α-D-fructofuranoside, uridine and adenosine have also been reported isolated from *C. rotundus* aerial part (Gamal et al., 2015a). Octopamine 6, 7-Dihydro-2, 3- dimethyl-5- cyclopentapyrazine were isolated from *C. rotundus*, *C. papyrus* and *C. esculentus* rhizomes. While, rotundine B, rotundine C (6-pi-rotundine B) and rotundine A has been reported isolated from *C. rotundus* rhizomes (Gamal et al., 2015a).

Generally, *Cypreus* species are known to be used in traditional medicine. Rhizomes of *C. rotundus* are traditionally used as an astringent, diaphoretic, diuretic, analgesic, antispasmodic, carminative, antitussive, emmenagogue, litholytic, sedative, stimulant, stomachic, vermifuge, tonic and antibacterial medicine (Al-Snafi, 2016). While *C. scariosus* is known to have the following ethnomedicinal uses to treat fever, arthritis, diuretic, nervine tonic, treatment of diarrhoea and dysentery, leprosy, bronchitis, amenorrhea and blood disorders (Rahmatullah et al., 2012). The fruits of *C. scariosus* are used as carminative, diuretic tonic and have stomachic (Srivastava et al., 2014). In South Africa, the nutsedge of *C. sexangularis* has also been used as a herb pounded and taken orally with warm water three times a day to treat asthma, fatigue and tuberculosis in the Limpopo province (Mosina et al., 2015; Semenya and Maroyi, 2018). The stem of *C. marginatus* are traditionally used to make hats, ropes, traditional mats, baskets and tools such as beer strainers while the stems of *C. sexangularis* were used in reed mat manufacturing in South Africa (Moteetee et al., 2019; Shackleton, 2005).

2.2 *Elegia tectorum*

Elegia tectorum (L. f.) Moline & H. P. Linder (ET), previously known as *Chondropetalum tectorum* and commonly known as Cape thatching reed or dakriet belongs to the family

Restionaceae (Figure 2.2). This plant is mainly used as an attractive garden plant and is traditionally used for thatching roofs, and weaving baskets and brooms (SA-Venues, 2020; Turner and Jamieson, 2016). This native perennial evergreen is located in marshes and wet areas throughout the Western Cape (Immediate Media Co, 2020; Turner and Jamieson, 2016). Secondary metabolites within the family include flavones (C-glycosides and luteolin), flavonols (myricetin, quercetin, laricitrin, syringetin) and proanthocyanidins, however, no compounds have recently been isolated from ET (Harborne, 2000).

Figure 2.2. Depiction of the stems of *Elegia tectorum* (Plantinfo, 2022).

2.3 *Juncus lomatophyllus*

Juncus lomatophyllus Spreng. (JL) commonly known as Leafy Juncus or Aalwynbiesies is part of the Juncaceae family that consists of eight genera of which this genus is the most well-known (Figure 2.3) (El-Shamy et al., 2015). Most species within the *Juncus* genus are found throughout the world, mainly in salty marshes. Secondary metabolites that have been isolated from plants within the Juncaceae family include terpenes, sterols, carotenoids, stilbenes, phenolic acid derivatives, coumarins, phenanthrenoids and flavonoids, however, as of current no compounds have been isolated from JL (Bús et al., 2018; El-Shamy et al., 2015).

Figure 2.3. Depiction of the stems of *Juncus lomatophyllus* (Wentzel and Wentzel, 2022).

2.4 *Persicaria senegalensis*

Persicaria senegalensis (Meisn.) Soják (PS) commonly known as silver snake root is, an indigenous South African plant (Figure 2.4). It is distributed throughout Africa and South Africa in many provinces, namely the Eastern Cape, Gauteng, KwaZulu-Natal, Limpopo, Mpumalanga, and the North-West Province. Phytochemical studies have revealed the presence of anthocyanin pigments, flavonols, flavones, and quinones and tannins. A few potential toxins have been identified within the genera including calcium oxalate, gallic acid, hydrocyanic acid, indicant, quercetin, rutin and tannic acid.

Midiwo et al. (1990) isolated 12 flavonoids of the chalcone and flavanone type from the exudate (Midiwo et al., 1990). Twenty-nine polylipids were analysed using GC-MS in *P. senegalensis,* including hexadecenoic acid, oleic acid, 1-[(2-aminoethoxy) hyroxyphosphinyl [oxy] methyl]-1, 2-ethanediylester, octadecanoic acid, 2, 3-dihydroxypropyl ester, luteolin, apigenin, kaempferol, quercetin, rutin, gallic acid, caffeic acid, and lucenin (Youssef and El-Swaify, 2018).

Figure 2.4. Depiction of the flowers of *Persicaria senegalensis* (Mifsud, 2022).

CHAPTER 3: COMMUNITY INVOLVEMENT

3.1 Bioprospecting, Access and Benefit Sharing (BABS) Regulations

The Bioprospecting, Access and Benefit Sharing (BABS) Regulations, 2008 made under the National Environmental Management Biodiversity Act (NEMBA), Act 10 of 2004, entered into force on 1 April 2008, which have since been amended. The BABS Amendment Regulations 2015 came into force on 19 May 2015 and prescribe the notification process for the discovery phase of bioprospecting involving any indigenous genetic and biological resources contemplated in section 81A (2) of the Act and further prescribe the permit system set out in Chapter 7 of the Act insofar as that system applies to bioprospecting involving any indigenous genetic and biological resources or export from the Republic of any indigenous genetic and biological resources for the purpose of bioprospecting or any other kind of research.

In addition, the BABS Amendment Regulations set out the form and content of, and requirements and criteria for benefit-sharing and material transfer agreements and the administration process of the Bioprospecting Trust Fund. Permits were required and obtained for the commercialisation of ET, JL and PS. The acceptance letter for ET and JL has been included in the Appendix A of the document. However, PS has been submitted and is undergoing review, thus we are still awaiting the acceptance letter.

3.2 Propagation of plant material at Botanica Natural Products

Botanica Natural Products have been identified as the leading supplier of the raw plant material. Botanica Natural Products is a farm-based in the Limpopo province on the banks of the Mogalakwena river that is also the primary water source of the farm where they grow medicinal plants for natural products. Botanica Natural Products has agreed to use a portion of their land for the waterlogging system to cultivate the identified wetland plants (Figure 3.1, 3.2 and 3.3). This was to prevent the overharvesting of the plant material in the wild and to promote wetland conservation within the area. Furthermore, as South Africa is considered an arid country, this was seen as the most cost-effective option as the waterlogged system uses stored rainwater during the winter and collects rainwater during the summer.

Figure 3.1. Preparation of the waterlogging system at Botanica Natural Products

Figure 3.2. Lead wetland plants growing at Botanica Natural Products.

Figure 3.3. Maintenance of the waterlogging system at Botanica Natural Products (TT 817/20).

In the present study, the water used in the waterlogging systems at Botanica was analysed for microbial and heavy metal contamination. However, to ensure minimal amounts of heavy metal and microbial contamination an analysis was conducted. Furthermore, to ensure the plant material harvested from Botanica Natural Products met with regulations, a material safety data sheet (MSDS) was generated.

3.3 Collaboration between Watler Sisulu University (WSU) and University of Pretoria (UP)

In collaboration with the Walter Sisulu University (WSU) a PhD student was selected to profile the chemical composition of *C. sexangularis* (CS) and *C. marginatus* (CM). As a result, the University of Pretoria contributed to his travels, accommodation, dietary means and access onto campus to learn how to prepare extracts, conduct an anti-elastase assay and learn cell culture techniques. These techniques will be taught to other students at WSU to assist them with their projects. Images of Gugulethu Miya trip to UP can be found in Appendix B.

3.4 Online course

Phytomedicine and Natural Products is an online course hosted by Enterprise to try and inform the public of the vital importance plant have within the industry and to bridge the gap traditional healers may face when trying to commercialize their products. There are two components to the course namely a theory component that is conducted online and a practical component that is done at the University of Pretoria.

A female employee from Botanica Natural Products was selected to attend both the online theory and practical contact sessions of the course (Figure 3.4). This was done so that members of the community in Limpopo may learn from her experience and be taught how to prepare the different formulations. This will support the community as products that are prepared can be sold and the money obtained can be used for necessities that are required.

Furthermore, since the ingredients used to prepare the formulations are not expensive, Botanica may be able to assist the community to obtain them reducing the cost. Lastly, this will provide members of the community jobs as they will prepare the plant extracts and formulations that will be sold while others market the products to tourists and nearby villages. Furthermore, the information that was learnt during the theory section of the course can be taught to other communities expanding their knowledge on traditional plants and their potential uses.

Figure 3.4. Depiction of the practical aspect of the online course.

CHAPTER 4: BIOLOGICAL ACTIVITY

4.1 Plant collection and extraction

A similar extraction method was used as reported in TT 817/20 WRC report. Fresh plant materials of *C. marginatus* and *C. sexangularis* were collected from Limpopo Province at Botanica Natural products in November 2020. *Cyperus marginatus* (CM) and *C. sexangularis* (CS) collected from Limpopo province were rinsed with distilled water, cut into small pieces, dried at room temperature for two weeks, and then grounded into a powder. The powdered samples were used for ethanolic and sequential solvent extraction.

About 200 g of CM and CS powdered material were weighed, poured into 2 L conical flasks and soaked with 99% ethanol until the plant material was covered. The ethanolic solution of CM and CS was continuously shaken for 48 hours. After 48 hours, the mixtures were filtered using the gravitational filtration method. The filtrates were concentrated using a rotary-evaporator instrument, and the concentrated extracts were left to dry at room temperature. The above process was repeated five times for CM and CS. The crude extracts were stored at 4ºC.

The whole plant of *J. lomatophyllus* (JL), stems of *E. tectorum* (ET) were collected from Manie van der Schijff Botanical Gardens at the University of Pretoria, rinsed with distilled water and placed in a -80˚C freezer for three days. Afterwards, the samples were freeze-dried/ oven-dried for a week. Aerial parts of *P. senegalensis* were collected from the rehabilitated wetland at the University of Pretoria and oven dried. Once dry, the samples will be ground into a fine powder using a grinder and weighed. The powder was mixed with 96% ethanol at a ratio of 1:5 and placed in a shaker for seven days. Afterwards, the solution will be filtered using a Whatman number three filter paper and dried using a rotary evaporator. The final plant extracts will be stored in a 4˚C walk-in fridge.

4.2 Heavy metal and microbial analysis

Water samples were collected from Botanical Natural Products, whereby the total heterotrophic count, coliforms and *E. coli* per 100 mL of water was measured. These samples were sent to Capricorn Veterinary Laboratories, an accredited facility, for analysis.

4.3 Material safety data sheet

Plant material was obtained from Botanica, and ethanolic extracts were prepared and submitted to Bioindustrial Services through Botanichem for heavy metal and microbial testing. Furthermore, a database of the potential hazards and how to handle the plant material was generated.

4.4 Stability of extracts

Stability studies were conducted at Botanichem, whereby the samples underwent 12 weeks of various analysis to measure the effect storage temperatures at 5ºC, ± 23ºC, 40ºC and 50ºC. Furthermore, the samples' appearance, odour, pH, and specific gravity (SG) were measured after 1, 2, 4, 8 and 12 weeks.

4.5 CM and CS sequential solvent extraction.

About 700 g of both CS and CM dried powdered material were poured into 2x 5 L of conical flasks, and they were firstly soaked in n-hexane covering the whole plant material; each mixture was continuously shaken for 48 hours. Afterward, it was filtered using the gravitational filtration method, and the filtrate was concentrated using a rotary-evaporator instrument. The concentrated sample was then left to dry at room temperature. The soaking and concentrating process with n-hexane was repeated until n-hexane could not extract anything in the plant material. The final product of this process was then called CM or CS Hexane fraction. The material was then soaked in dichloromethane (DCM). The same process followed for the n-hexane extraction was repeated to give the CM or CS DCM fraction. After DCM extraction, the same material was soaked with ethyl acetate leading to the CM or CS ethyl acetate fraction, and after ethyl acetate, it was lastly soaked into ethanol. The final product of this was the CM or CS ethanolic fraction.

4.5.1 Isolation and Purification of CS n-hexane fraction.

The method described by Cyril-Olutayo et al. (2020) was followed for the isolation and purification of the n-hexane fraction with some modifications. The fraction was dissolved in 50 g of silica gel and dry-packed into a 200 g silica gel column using gradient elution profile of solvent system starting from 100% n-hexane ending with 100% ethyl acetate. The samples were evaluated for their phytochemical fingerprint by dissolving 2 mg of the ethanolic extracts in 100 µL of ethanol. Each sample was spotted on a TLC plate 20 times. The mobile phase was a ratio of 6.5 MeOH: 3.5 Chloroform: 2 H_2O.

4.6 Bioassay-guided fractionation of ET

4.6.1 Liquid partitioning

Crude ethanol extract (100 mg) was dissolved in 300 ml dH_2O until it completely dissolved. It was then added in the separating funnel; ethyl acetate (250 ml x3) was added, mixed and allowed to settle resulting in two layers. The water fraction was at the bottom, the ethyl acetate fraction was at the top. The water fraction was collected then *n*-butanol (250 ml x3) was added to it in a separating funnel. Then the water fraction was collected at the bottom while the *n*-butanol fraction was at the top. There were three fractions in total after this, ethyl acetate, butanol and water. These were concentrated using a rotary evaporator apparatus (BUCHI Rotavapor B-480). The water and butanol fractions were concentrated by making an azeotropic mixture (butanol-water, 60:40, v/v). The fractions were further tested for elastase inhibition.

4.6.2 Column chromatography

A slurry of 500 g of silica in *n*-hexane was placed into the column and allowed to settle. Thereafter, a dried slurry of the water fraction and silica was added into the column. The column was then eluted with 100% hexane. Thereafter, the column was eluted in the following manner: fraction 2-11 with ethyl acetate, fraction 12-40 with 2% methanol in ethyl-acetate, fraction 41-62 with 5% methanol in ethyl-acetate, fraction 63-181 with 8% methanol in ethyl-acetate, fraction 182-215 with 10% methanol in ethyl-acetate, fraction 216-237 with 15% methanol in ethyl-acetate, fraction 238-250 with 20% methanol in ethyl-acetate, fraction 251-260 with 30% methanol in ethyl-acetate and fraction 261-265 with 50% methanol in ethyl-

acetate. TLC plates were saturated using a mixture consisting of 6.5 ml ethyl acetate, 3.5 ml methanol and 1 ml water.

In total 265 fractions were collected and pooled based on their TLC profile. Six major fractions consisting of 1: F1-12 (82.4 mg), 2: F13-21 (71 mg), 3: F22-62 (541.4 mg), 4: F63-175 (1.54 g), 5: F175-250 (617.9 mg) and 6: F251-265 (280.9 mg) were collected. Fraction 3 displayed a major compound, which was purified using precipitation. Briefly, fraction 3 was dissolved in minimum amounts of methanol and ethyl acetate using the drop method. This was repeated five times to allow the compound to precipitate. Thereafter, the precipitated compound was dissolved in methanol and examined for its TLC profile. The TLC showed a single spot, which confirmed the purity of the compound. This purified compound was then submitted for 1 dimensional (D) and 2D nuclear magnetic resonance (NMR) analysis.

4.7 Bioassay-guided fractionation of JL

4.7.1 Liquid-liquid partition

Twenty grams of *J. lomatophyllus* ethanolic extract (JL-EtOH) was dissolved in 500 mL of distilled water (dH_2O) and partitioned using *n*-hexane, ethyl acetate and *n*-butanol. Briefly, 500 mL of *n*-hexane was added to the solution (1:1), thoroughly mixed and left to stand. Once two distinct layers were seen, the top layer (*n*-hexane) was collected by removing the bottom layer. Thereafter, the bottom layer, consisting of the water solution, was added to the funnel and the process was repeated three times. Using the remaining water solution in the funnel, the same method was used to collect the ethyl-acetate and butanol partition. A sublayer between *n*-butanol and ethyl acetate was formed which was collected separately and labelled as sub-1. The collected partitions were concentrated using a rotary evaporator and left to dry overnight in a fume hood. The water partition (consisting of the remaining water solution) was left overnight in a -80˚C freezer and freeze-dried for a week. The partition that showed the highest anti-tyrosinase activity was further purified.

4.7.2 Column chromatography

A slurry consisting of 2.45 g of the butanol partition (JLB) was dissolved in minimal amounts of methanol and combined with silica. Once dry, the slurry was added to a column containing silica that was saturated with *n*-hexane. A total of 165 fractions were collected and the following solvent systems were used to elute the column: fraction 1 with 100% hexane, fraction 2-3 with 100% dichloromethane, 3-4 with 80% chloroform in hexane, 5-109 with 80% chloroform in methanol, 110-136 with 80% ethyl acetate in methanol, 137-157 with 70% ethyl acetate in methanol and 158-164 with 50% ethyl acetate in methanol. Fractions that displayed similar TLC profiles were pooled together. A total of seven main fractions were collected and were evaluated for anti-tyrosinase activity.

4.8 Bioassay-guided fractionation of PS

4.8.1 Liquid partitioning

Persicaria senegalensis (PS) was selected for silica gel column chromatography to isolate and identify pure compounds responsible for the significant elastase inhibitory activity of the extract. PS ethanolic extract (40 g) was dissolved in 500 mL dH_2O and transferred to a

separating funnel, to which 500 mL hexane was added and mixed rigorously. The mixture formed two layers, the hexane layer was collected, and the water layer was mixed again with 500 mL hexane and allowed to separate. The hexane fraction was collected once again, and the water layer was re-extracted with 400 mL hexane one final time. This was repeated using the water layer, ethyl acetate, and saturated n-butanol. Sodium sulphate was used to wash the hexane and ethyl acetate partitions, and sodium chloride solution was used to wash the *n*-butanol partition. The entire process was repeated such that the total mass of PS ethanolic extract, which was partitioned, was 40 g. The four partitions, hexane, ethyl acetate, *n*-butanol and water, were dried by rotor evaporation, and the dried extracts were investigated for their elastase inhibitory potential. Based on this, the most noteworthy activity was observed for the n-butanol partition, which was then used for further purification.

4.8.2 Column chromatography

A silica gel-G column was prepared, and 12 g of the butanol partition was packed at the top. Fractions of ~250-300 mL were collected for the following solvents: DCM, 1:1 Chloroform:DCM, Chloroform, 2% MeOH in Chloroform, 5% MeOH in Chloroform, 10% MeOH in Chloroform, 15% MeOH in DCM, 20% MeOH in DCM, 25% MeOH in DCM, 30% MeOH in DCM, 35% MeOH in DCM, 40% MeOH in DCM, 45% MeOH in DCM, 50% MeOH in DCM, 55% MeOH in DCM,60% MeOH in DCM, 100% MeOH. A total of 235 fractions were collected, and the silica was washed with 100% MeOH to produce fraction 236. These fractions were pooled to yield 13 major fractions which were investigated for their elastase inhibitory potential.

4.9 Gas chromatography-mass spectrometry (GC-MS)

GC-MS of bioactive fractions of PS and JL as well as PS, ET and JL crude extracts was performed using a LECO Pegasus 4D GC-TOFMS (LECO Africa (Pty) Ltd., Kempton Park, South Africa) that was equipped with a capillary column (Rxi-5SiMS (30 m × 0.25 mm ID with a film thickness of 0.2 mm)) (Restek, Bellefonte, PA, USA). The carrier gas used consisted of high-grade helium (99.999%) (Afrox, Gauteng, South Africa) that was flowing at a constant rate of 1 mL/min. Furthermore, the injector was maintained at a constant temperature (250°C) and a splitless mode set at every 30 s was used to operate the inlet. The temperature program that was set for the GC oven was 40°C for three minutes with an increase of 10°C per minute to reach a final temperature of 300°C for five minutes. The MS transfer line and ion source were set at a temperature of 280 and 230°C, respectively. Spectroscopic detection was operated in electron impact ionization mode (EI^+) with an electron energy of 70 eV with a data acquisition rate of 10 spectra per second. The total running time of the analysis was 35 minutes with a solvent delay of five minutes. The national institute of standards and technology (NIST) database was used to compare and identify the phytochemical constituent of each peak.

4.10 Synthesis of gold nanoparticle

The gold nanoparticles were synthesised using the ethanolic extract of JL, ET and PS. To prepare *J. lomatophyllus* nanoparticle extract (JLAuNP), the crude extract was dissolved in distilled water (dH_2O; 2 mg/mL), boiled and centrifuged. The collected supernatant was

combined with gum arabic acid which was used as a stabiliser. Thereafter, the mixture was heated to 60˚C, whereby 100 mM of gold salt was added.

A similar method was used to prepare *E. tectorum* nanoparticle extract (ETAuNP). However, 0.1 M of gold salt was added once the solution was heated to 70˚C. To characterise the synthesised ETAuNPs and JLAuNPs, an ultraviolet-visible spectrometry (UV-Vis), phenolic content, dynamic light scattering (DLS), zeta-potential and Fourier-transform infrared spectroscopy (FTIR) was conducted.

The synthesis of gold nanoparticles using *P. senegalensis* (PSAuNP) was achieved by dissolving 125 mg of *P. senegalensis* ethanolic extract in 70 mL ethanol (EtOH) to ensure complete dissolution and topped up to 500 mL using sterile dH_2O. This was then heated to 60°C. The stock (210 mL) was decanted and maintained at 60°C, to which 14 mL of gold salt was added dropwise with continuous mixing using a magnetic stirrer. Synthesis was allowed to continue for a further 10 min. A negative control was also prepared exactly as the nanoparticles, except the gold salt, was replaced with sterile dH_2O. A vehicle control was also prepared, exactly as the nanoparticles were, except the plant extract was substituted with 14% EtOH in dH_2O. All samples were centrifuged at 10 000 rpm for 10 min and the supernatant discarded. The remaining pellet was resuspended in sterile dH_2O.

4.11 Characterisation of synthesised gold nanoparticle

4.11.1 Ultraviolet visible (UV-Vis) spectrometry

To confirm the formation of gold nanoparticles, a full spectral scan was conducted using ultraviolet-visible spectrometry (UV-Vis) to determine if the surface plasmon resonance (SPR) was similar to gold metal (Au). In a 96-well plate, 100 µL of the synthesized gold nanoparticles solutions were added and the absorbance was read between 450-800 nm at 50 nm increments using a Victor Nivo plate reader (PerkinElmer, Midrand, South Africa).

4.11.2 *In vitro* stability

In vitro stability of the AuNP solutions were evaluated in various mediums consisting of buffer solutions and cell culture mediums, which included 0.5% bovine serum albumin (BSA), 5% sodium chloride (NaCl), pH of 4, 7 and 10, phosphate buffer (pH 6.5), Dulbecco's Modified Eagles medium (DMEM) and Roswell Park Memorial Institute (RPMI-1640) medium. ETAuNPs were added to the abovementioned solutions at a 1:1 ratio with a final volume of 1.5 mL and were incubated at 37˚C. To confirm whether the nanoparticles were stable, the SPR peaks (λ_{max}) between 450 and 800 nm were measured using a Victor Nivo plate reader at 0, 2, 24 (Day 1), 48 (Day 2), 72 (Day 3), 96 (Day 4) and 120 hours (Day 5).

4.11.3 High-resolution transmission electron microscopy (HRTEM)

High-resolution transmission electron microscopy was used to identify the particle size and shape of AuNP solutions. Furthermore, the crystallinity was identified through selected area electron diffraction (SAED). Five microlitres of AuNP solutions were loaded onto a carbon-coated copper TEM grid and allowed to dry. Thereafter, the grids were loaded into a JEOL JEM-ARM200F double Cs-corrected transmission electron microscope equipped with a large solid angle energy dispersive spectrometer (EDS) (Akishima, Tokyo, Japan) and images were captured.

4.11.4 Quantification of the total phenolic content present in the synthesised nanoparticle

The total phenolic content was quantified using the Folin Cioalteau technique as described by De Canha et al. (2021). A standard curve was prepared from the crude extracts of JL, ET and PS that was serially diluted two-fold, in dH_2O, resulting in a final concentration range of 4000-31.25 µg/mL. In a 2 mL Eppendorf tube, 125 µL of 7.5% (w/v) sodium bicarbonate solution (Na_2CO_3) and 125 µL 10% (v/v) Folin Cioalteau reagent (1 in 10 mL dH_2O) were added to 250 µL of each dilution and 250 µL of AuNP solutions. Thereafter, 100 µL of each solution was transferred into a 96-well plate and incubated at 30˚C for 30 minutes in the dark. Blanks of either crude extracts or AuNPs, 7.5% Na_2CO_3 and dH_2O in place of 10% Folic Cioalteau were included. The absorbance was measured at 765 nm using a Victor Nivo plate reader and the phenolic content of AuNP solutions was determined using the equation generated from the standard curve. The quantified phenolic content was used as the highest stock concentration in each of the bioassays that were conducted.

4.11.5 Dynamic light scattering (DLS)

To determine the hydrodynamic size of the AuNPs, 1 mL of the AuNP solutions was transferred into a zeta cell and read using a Zetasizer Nano ZS instrument (Malvern Instruments Ltd., Malvern, Worcestershire, UK). Three reads were performed, and the average was obtained.

4.11.6 Zeta potential

The electrostatic charge of AuNPs was identified by transferring 1 mL of solution into a cuvette, which was read three times using a Zetasizer Nano ZS instrument and the average of three reads was recorded.

4.11.7 Fourier transform infrared spectrometry (FTIR)

To identify potential phytochemical groups, present in AuNPs, a Fourier transform infrared spectrometry analysis was conducted using 9 mg of ET-EtOH as a blank. The percentage transmittance was detected over an infrared range of 550-4000 cm^{1} using a Perkin Elmer spectrum 100 FTIR spectrometer (Perkin Elmer, Midrand, South Africa).

4.12 Fermentation

The ethanolic extract of PS, ET and JL was fermented using *Bifidobacterium bifidum* (ATCC 11863) according to the method of Park and Bae (2016) with slight modifications. To complete this, the bacteria were cultured from Kwik Sticks on Bifidus Selective Medium Agar (Sigma Aldrich) supplemented with BSM-Supplement prepared to a stock solution of 23.2 g/L, specifically for the selective isolation, identification, and enumeration of *Bifidobacteria*. The *Bifidobacterium* colonies are purple/brown and easy to differentiate from other organisms. Single colonies were then inoculated in BSM-Broth supplemented with BSM supplement. The bacteria were then cultured for 48 hours and prepared to an 8 McFarland.

To prepare the McFarland standard, 800 µl 1% Barium Chloride and 9.2 mL 1% Sulphuric acid were mixed, and the OD at 600 nm was measured to be 1.210. The fermentation was completed by dissolving 500 mg of the ethanolic extract in 0.5 mL EtOH. To this, warmed supplemented BSM broth was added and 2 mL of the 8 McFarland bacterial suspension to a total volume of 50 mL. The fermentation samples were incubated in an anaerobic jar with an Anaerocult® A

at 37℃ for six weeks, with a vortex for 20 seconds weekly agitations. The fermented samples were then sonicated at 45℃ for 5 minutes, frozen and freeze-dried to remove any remaining bacteria by desiccation. Negative control was included for each sample that contained all the fermentation components excluding the bacteria, and vehicle control in which the samples were substituted with 0.5 mL EtOH.

4.13 Anti-elastase assay

The method used to determine elastase inhibition was described by Lall et al. (2017) with slight modifications. *Elegia tectorum* (ET) crude extract as well as ursolic acid (positive control), was dissolved in DMSO to a stock concentration of 20 mg/mL. Fractions obtained from ET were dissolved in DMSO to a stock concentration of 10 mg/mL while PS, CM and CS crude, fractions and PS fermented extracts stock concentration was 40 mg/mL. Compounds isolated from CS were dissolved in DMSO to a stock concentration of 0.2 mg/mL. A final concentration range from 60-0.94 µg/mL was prepared from the ursolic stock solution to prevent precipitation from occurring. The extracts were serially diluted two-fold, resulting in a 500-7.81 µg/mL concentration range for ET, 400-6.25 µg/mL for PS, CM and CS, 250-3.91 µg/mL for ET fractions and 50-0.78 µg/mL for CS isolated compounds.

In a 96-well plate, 100 mM Trizma base (pH 8) was added to all the wells, where after, each extract dilution was added. Afterwards, 4.942 mU of the elastase enzyme was added to the respected wells and incubated at 37˚C for 5 minutes. Following incubation, the reaction was terminated by adding 4.4 mM *N*-succinyl-Ala-Ala-Ala-*ρ*-nitroanilide substrate. A vehicle control (1% DMSO) was prepared in the same manner as the crude extract. The blank control contained all the reagents, including 1% DMSO; however, no enzyme was added. The absorbance values were determined using a BIO-TEK Power-Wave XS plate reader at a wavelength of $OD_{405\ nm}$ at a temperature of 37˚C for 15 minutes. To calculate the percentage inhibition, the following equation was used:

$$\%\ inhibition = 100 - \left(\frac{Absorbance\ sample}{Absorbance\ control}\right) \text{ x } 100$$

The absorbance $_{control}$ was determined by subtracting the absorbance value of enzyme control at 0 minutes from the absorbance value of enzyme control at 15 min. The absorbance $_{sample}$ was determined by subtracting the absorbance of the extract or positive control at 15 min to the absorbance of the extract or positive control once the substrate was added. GraphPad Prism 4 was used to calculate the sample's concentration, whereby 50% of tyrosinase enzyme was inhibited (IC_{50}).

4.14 Tyrosinase inhibition assay

The method used to determine tyrosinase inhibition was described by Lall et al. (2019). In 24 well-plates, JL crude extract and fractions were dissolved in DMSO (20 mg/mL), added to potassium phosphate buffer (pH 6.5) and serially diluted two-fold to achieve a concentration range of 200-1.56 µg/mL. Kojic acid (positive control) and 1% DMSO (negative control) were prepared the same manner as the crude extract. To determine the inhibitory effect of the plant extracts, each dilution was added to the tyrosinase enzyme (333 units/mL in phosphate buffer)

in a 96-well plate in triplicates. After incubating for 5 minutes at 25˚C, the reaction was started with the addition of 44 mg/mL *L*-tyrosine substrate.

Using a BIO-TEK Power-Wave XS plate reader (Analytical and Diagnostic Products CC, Roodepoort, South Africa), the absorbance values were determined at a wavelength of $OD_{492\ nm}$ for 30 min. To calculate the percentage inhibition, the following equation was used:

$$\%\ inhibition = 100 - \left(\frac{Absorbance\ sample}{Absorbance\ control}\right) \text{ x } 100$$

The absorbance $_{control}$ was determined by subtracting the absorbance of DMSO at 30 min with the absorbance of DMSO at 0 min. The absorbance $_{sample}$ was determined by subtracting the absorbance of the extract or positive control at 30 min to the absorbance of the extract or positive control at 0 min. GraphPad Prism 4 was used to calculate the sample's IC_{50}.

4.15 Cell culture

Human keratinocyte (HaCaT) cell line was used to determine the cytotoxicity. To maintain the HaCaT cell line Dulbecco's modified Eagle's Medium (DMEM) media was used supplemented with 1% gentamicin and 10% foetal bovine serum. The cells were incubated at 5% CO_2 and 37˚C until a confluent monolayer was obtained. The cells were sub-cultured using 0.25% trypsin-EDTA once the monolayer had formed.

4.16 Antiproliferative activity

The method used to determine cytotoxicity was described by Lall et al. (2019), which uses PrestoBlue cell viability reagent to measure the cytotoxicity, following the treatment of HaCaT with the plant extract. Within a microtiter culture plate, cells were seeded into each well at a concentration of $1x10^5$ cells/mL, and the plate was incubated overnight at 37˚C and 5% CO_2. A stock solution of the plant samples was prepared at a 40 mg/mL concentration in DMSO while the prepared AuNP's was used. Two positive controls were used for each cell line, with actinomycin D used against HaCaT. A stock concentration of actinomycin D at 1 mg/mL in distilled water was prepared. These stock solutions were diluted two-fold. Once the cells adhered, the plant extracts, AuNP's and the respected positive controls were added in triplicates. The final concentration of the plant samples ranged between 3.125-400 µg/mL and actinomycin D ranging from 3.9×10^{-4} - 0.05 µg/mL in a 96-well plate. Media control and a 1% DMSO control were added; however, the media control contained no cells. After 72 hours, PrestoBlue reagent was added to all the wells and incubated for two hours. Thereafter, the fluorescence was measured at an excitation/emission wavelength of 560/590 nm using a Victor Nivo plate reader. Cell viability was calculated using the following equation, which was used to determine the IC_{50} values using GraphPad Prism 4 software.

$$\%\ viability = \frac{Fluorescence\ \ sample - Fluorescence\ 0\%\ control}{(Fluorescence\ vehicle\ control - Fluorescence\ \ 0\%\ control)} \text{ x } 100$$

4.17 *In vivo* irritancy

An irritancy test was conducted at Future Cosmetics using the Patch test method. Before the study could commence, ethical approval through Pharma-Ethics (Lyttleton Manor, South Africa) (reference number 200523309). Briefly, 20-22 volunteers aged between 21-65 years

were selected and patch discs (Finn chambers) of 25 x 10 mm in diameter were drawn on the inner forearm. Thereafter, the 15 µl of the neat extracts (solution containing extract dissolved in 40% ethanol and distilled water) were applied on one of the patch discs using a 1CC syringe. Visual assessments were conducted at 24, 48 and 72 hours with 24-hour intervals and the reaction was graded. A sample is considered to be an irritant if the mean irritancy score (average + standard deviation) falls above that of the positive control; mild-irritant when the mean irritancy score falls above that of the negative control, but lower than that of the positive control; and a non-irritant when the mean irritancy score is below the negative control.

4.18 Formulation of gel-based cream

A gel-based cream was prepared, which was used as the bases of the formulation. To prepare the cream two different phases were prepared including a water and oil phase. The water phase consisted of demineralized water that was heated to 75ºC. Thereafter, ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA NA_2), glycerin and Carbopol 934 were added. Once dissolved, triethanolamine-99% (TEA 99%) was added and mixed for 20 minutes until the mixture was lump free, thick and clear. The oil phase consisted of article 165, ceto stearyl alcohol, BHT, mineral oil, kotilen S1, kosteran S1, lanolin and silicone 200/100, which were heated to 75ºC. Once dissolved, the oil and water phase were combined using a hand blender. Once the mixture cooled to 40ºC, kemaben, moist 24, vitamin E acetate and witch hazel distillate were added. Using a hand blender, the formulation was mixed until cooled to room temperature.

Thereafter, the formulations of each plant were prepared. Briefly, 300 mg of the ethanolic extract of JL, ET and PS as well as the fermented extract of PS was dissolved in 50 mL of diluted ethanol consisting of 60% distilled water with a final concentration of 6 mg/mL. Thereafter, 10% of this stock solution was added to the prepared gel-based cream with a final concentration of 600 µg/mL. Furthermore, to protect the skin from environmental damage which can cause hyperpigmentation, 10% niacinamide was added to the JL formulation.

4.19 Efficacy study for even skin tone

A double-blind efficacy study was conducted at Future Cosmetics to ensure that JL depigmented dark spots when applied topically. Before the study could commence, ethical approval through Pharma-ethics (reference number 200523299). Briefly, 26 patients suffering from pigmentation disorders, a Fitzpatrick skin photo type of V-VI, female and between the ages of 21-66 were selected. Roughly, 0.6 mg of a gel-based cream containing 10% JL and 10% niacinamide was applied to one half of the face for a duration of 28 days. As a baseline, the side whereby the cream was to be applied on was photographed using a chromameter and Visioscan. Thereafter, the area was assessed after 14 and 28 days. A placebo gel-based cream was used as a negative control. To prepare this a solution of 40% ethanol and distilled water was prepared and 10% of this solution was added to the cream.

4.20 Efficacy study for wrinkle reduction

Efficacy trials were conducted at Future Cosmetics to ensure that ET, PS crude and fermented extract reduced wrinkle formation when applied topically. Before the study could commence, ethical approval through Pharma-ethics (reference number 200523309). Briefly, 27 patients

with predominant wrinkles, a Fitzpatrick skin type of I-III, female and between the ages of 38-64 were selected. Roughly, 0.6 mg of a gel-based cream containing 10% PS, PSF or ET was applied to one half of the face for a duration of 28 days. As a baseline, the side whereby the cream was to be applied on was photographed using a chromameter and visioscan. Thereafter, the area was assessed after 14 and 28 days. A placebo gel-based cream was used as a negative control. To prepare this a solution of 40% ethanol and distilled water was made and 10% of this solution was added to the cream.

4.21 Statistical analysis

Experiments were performed in triplicate, and results are presented as mean ± SEM. Results are reported as mean ± standard deviation as displayed in the results section. To obtain the IC_{50} values, a nonlinear regression analysis of the sigmoidal dose-response curves (4-parameter logistic) using GraphPad Prism 4 was conducted.

CHAPTER 5: RESULTS AND DISCUSSION

5.1 Heavy metal and microbial analysis

The reports confirmed that the ethanolic extracts from all five plants had no bacterial or heavy metal contamination. Acceptable levels of bacteria, yeast and moulds in cosmetic products are not more than 100 cfu/g, with the samples tested displaying levels below 10 cfu/g (Kim et al., 2020). All the samples contained no heavy metals of arsenic, cadmium, mercury and lead at the highest concentrations tested.

5.2 Material Safety Data Sheet

Furthermore, material safety data sheets have been developed for all five products. This provides information on the characteristics of each product, their stability and potential hazardous information that will form part of the commercialisation and licensing information to be provided to future licensees of the technologies.

5.3 Stability studies

The stability studies of all samples were investigated. All extracts were confirmed to have a 24-month shelf life. This information will form part of the commercialisation and licensing information to be provided to future licensees of the technologies. Below are the comments made by Botanichem for each extract.

5.3.1 *Cyperus marginatus*

The stability results are good. Sedimentation was recorded after the first week of testing. The 40°C and 50°C samples became very slightly darker after the 4 weeks of testing. The odour of the samples remained unchanged throughout the 12-week testing period. The pH and specific gravity values remained within acceptable limits. Filtration is recommended to reduce the amount of sedimentation in the product. A 24-month shelf life can be expected (Figure 5.1).

Figure 5.1. Depict of *Cyperus marginatus* when exposed to different temperatures.

5.3.2 *Cyperus sexangularis*

The stability results are good. The appearance and odour of the samples remained unchanged throughout the 12-week testing period. The pH values remained within acceptable limits. The specific gravity value of the 50 °C sample increased which may be attributed to the extreme conditions of storage. A 24-month shelf life can be expected (Figure 5.2).

Figure 5.2. Depict of *Cyperus sexangularis* when exposed to different temperatures.

5.3.3 *Elegia tectorum*

The stability results are good. Sedimentation was recorded after the fourth week of testing. The 50°C sample became slightly darker after the last week of testing which may be attributed to the extreme conditions of testing. The odour of the samples remained unchanged throughout the 12-week testing period. The pH and specific gravity values remained within acceptable limits. Filtration is recommended to reduce the amount of sedimentation in the product. A 24-month shelf life can be expected (Figure 5.3).

Figure 5.3. Depict of *Elegia tectorum* when exposed to different temperatures.

5.3.4 *Juncus lomatophyllus*

The stability results are good. Sedimentation was recorded after the first week of testing. The 50°C sample became very slightly darker after the last week of testing. The odour of the samples remained unchanged throughout the 12-week testing period. The pH and specific gravity values remained within acceptable limits. Filtration is recommended to reduce the amount of sedimentation in the product. A 24-month shelf life can be expected (Figure 5.4).

Figure 5.4. Depict of *Juncus lomatophyllus* when exposed to different temperatures.

5.3.5 *Persicaria senegalensis*

The stability results are good. Sedimentation was recorded after the fourth week of testing. The odour of the 50°C sample became slightly weaker after the first week of testing which may be

attributed to the extreme conditions of storage. The pH values remained within acceptable limits. A slight increase in the specific gravity values of the samples was recorded and should be noted for packaging purposes. Filtration is recommended to reduce the amount of sedimentation in the product. A 24-month shelf life can be expected (Figure 5.5).

Figure 5.5. Depict of *Persicaria senegalensis* when exposed to different temperatures.

5.4 *Cyperus marginatus* and *C. sexangularis*

5.4.1 Compound isolation

The sequential solvent extraction of all the four solvents was conducted successfully, with n-hexane having the highest percentage yield while ethyl acetate had the lowest percentage yield (Table 5.1). The formula used to calculate percentage yields was:

$$Yield = \frac{\text{Mass of Starting material}}{\text{Recovered mass}} \times 100\%$$

Each solvent extracted all the compounds present in its polar portion because each solvent was extracted until the mixture looked clear before moving to the next solvent. The collected fractions were kept for further use in biological studies and purification to isolate pure compounds.

Table 5.1. Sequential solvent extraction of *Cyperus sexangularis* (CS) and *Cyperus marginatus* (CM).

Fraction	Starting material (g)		Weight recovered (g)		w/w %	
	CM	CS	CM	CS	CM	CS
Hexane	786.07	723.98	83.10	84.10	10.57	11.62
DCM	-	-	23.06	19.43	2.93	2.68
ETOAC	-	-	9.67	7.75	1.23	1.07
ETOH	-	-	68.48	68.63	8.63	9.48

5.4.2 Isolation of pure compound M1

Fraction 80-89 afforded a whitish solid deposable. It was washed twice with 50ml of 100% n-hexane and filtered to give filtrate (H6a) and whitish amorphous powder (H6b). Nuclear Magnetic Resonance (NMR) analysis of (H6b) confirmed it to be a pure compound, and it was named M1.

5.4.3 Structural elucidation of compound M1

The ^{1}H NMR spectrum of compound **M1** showed the presence of six methyl signals that appeared as two angular methyl singlets at δ 0.65, and 0.99 ppm; three methyl doublets at δ 0.84, 0.86, and 0.91 ppm; and a methyl triplet at δ 0.80 ppm. The spectrum showed one hydroxyl group as a multiplet at δ 3.54 ppm, and one olefinic proton at δ 5.34 ppm. The ^{13}C NMR spectrum showed twenty-nine carbon signals, which comprised six methyls (CH_3), eleven methylenes (CH_2), nine methines (CH) and three quaternary (CQ) carbon signals. The deshielded signal at 71.70 ppm represented a carbon directly linked to a hydroxyl group, while the more downfield signals at 121.71 and 140.71 ppm were those of the olefinic carbon atoms. The full C-H assignments were based on the 1D- and 2D NMR experiments, as indicated in Table 5.2. Based on the result obtained, and in comparison with spectra data reported in the literature, **M1** was characterised as Stigmast-5-en-3β-ol, also known as β-Sitosterol (Aliba et al., 2018).

Table 5.2. The observed ^{1}H and ^{13}C NMR spectra data in CDCl3 on a 300 MHz VARIAN NMR Spectrometer.

Position	Type of Carbon atom	M1 – Chemical Shift δ (ppm) value		β-Sitosterol (Arjun *et al*., 2010)	
		^{1}H NMR (multiplicity)	^{13}C NMR	^{1}H NMR (multiplicity)	^{13}C NMR
1	CH_2		37.18		37.28
2	CH_2		31.59		31.69
3	CH	3.54 (*m*, 1H)	71.77	3.53 (*m,* 1H)	71.82
4	CH_2		42.23		42.33
5	QC=	5.34 (*d*, 1H)	140.70	5.36 (*d,* 1H)	140.70
6	HC=		121.71		121.72
7	CH_2		31.84		31.69
8	CH		31.87		31.93
9	CH		50.04		50.17
10	CQ		36.45		36.52
11	CH_2		21.02		21.10
12	CH_2		37.18		39.80
13	CQ		39.70		42.33
14	CH		56.70		56.79
15	CH_2		24.26		24.37
16	CH_2		28.23		28.25
17	CH		55.96		56.09
18	CH_3	0.65 (*s*, 3H)	11.83	0.63 (*s,* 3H)	11.86
19	CH_3	0.99 (*s*, 3H)	19.37	1.01 (*s*, 3H)	19.40
20	CH		36.10		36.52
21	CH_3	0.91 (*d*, 3H)	18.97	0.93 (*d*, 3H)	18.97
22	CH_2		33.86		33.98
23	CH_2		25.92		26.14
24	CH		45.74		45.88
25	CH		29.03		28.91
26	CH_3	0.86 (*d*, 3H)	19.81	0.84 (*t*, 3H)	19.80

Position	Type of Carbon atom	M1 – Chemical Shift δ (ppm) value		β-Sitosterol (Arjun *et al*., 2010)	
		^{1}H NMR (multiplicity)	^{13}C NMR	^{1}H NMR (multiplicity)	^{13}C NMR
27	CH_3	0.84 (*d*, 3H)	18.73	0.83 (*d*, 3H)	18.79
28	CH_2		22.98		23.10
29	CH_3	0.80 (*t,* 3H)	11.94	0.81 (*d*, 3H)	11.99

M1 – isolated compound, *s*-singlet, *d*-doublet, *t*-triplet, *m*-multiplet, CQ- quaternary carbon, CH- methine, CH2- methylene, CH3- methyl

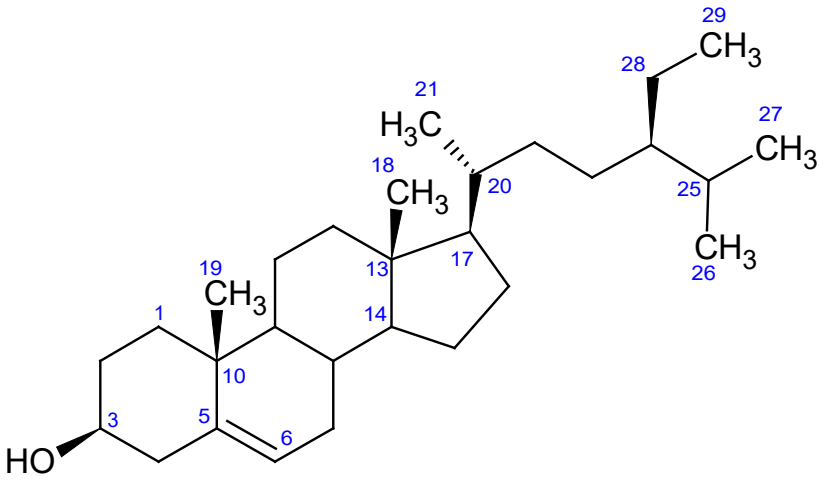


Figure 5.6. Chemical Structure of β-Sitosterol (M1) Isolated from *Cyperus* spp.

5.4.4 Thin-layer chromatography

Thin-layer chromatography was used to determine the difference in the chemical fingerprint of CS and CM extracts. The extract prepared in March 2016 at the University of Pretoria from plant material collected from campus and plant material collected at Botanica Natural Products in November 2020 and prepared at Walter Sisulu University were analysed. A difference in the phytochemical profile of CM and CS was observed between samples of 2016 and 2021 (Figure 5.7). This difference could explain the elastase inhibitory activity observed for these samples. Furthermore, this could indicate the compounds responsible for elastase inhibitory activity in the extracts of CS and CM.

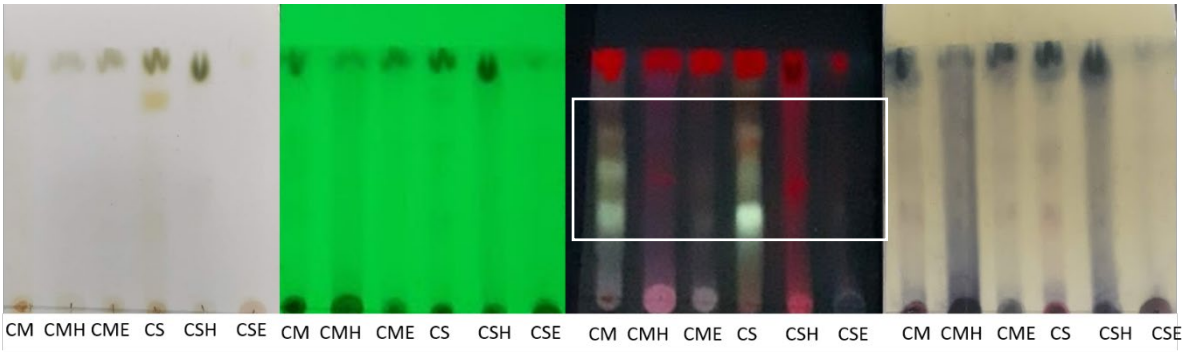


Figure 5.7. Thin-layer chromatography comparison of *Cyperus sexangularis* and *Cyperus marginatus* prepared at Walter Sisulu University in November 2020 and the University of Pretoria in 2016. CM: *C. marginatus* prepared in March 2016 from plant material collected at the University of Pretoria Botanical Garden; CS: *C. sexangularis* prepared in March 2016 from plant material collected at the University of Pretoria Botanical Garden; CMH: *C. marginatus* hexane extract prepared in November 2020 from plant material collected at Botanica Natural products; CME: *C. marginatus* ethanolic extract prepared in November 2020 from plant material collected at Botanica Natural products; CSH: *C. sexangularis* hexane extract prepared in November 2020 from plant material collected at Botanica Natural products; CME: *C. sexangularis* ethanolic extract prepared in November 2020 from plant material collected at Botanica Natural products.

5.4.5 Anti-elastase activity

Samples prepared from sequential extraction did not display elastase inhibition at the highest concentration tested of 400 µg/ml. The ethanolic extracts prepared from the leaves purchased and collected at Botanica Natural Products in November 2020 prepared by the Walter Sisulu University displayed no elastase inhibitory activity. Ethanol extracts prepared at the University of Pretoria from plant material purchased and collected at Botanica Natural Products in November 2020 and 2021 displayed elastase inhibitory activity (Table 5.3). The ethanolic extract of CM had a lower elastase inhibitory activity compared to an ethanolic extract prepared in March 2016 that could be due to seasonal variation or the presence of inflorescence in the 2016 plant material. No inflorescence was available in November 2021 to determine if the activity of CM could be related to the presence of the inflorescence. Ethanolic extracts will be prepared from the inflorescence that could indicate if the activity observed in 2016 could be attributed to flowering or seasonal variation.

The ethanolic extract of CS prepared at the University of Pretoria from plant material collected at Botanica Natural Products in 2021 that contained the inflorescence displayed an IC_{50} value significantly similar to the sample prepared in March 2016. Therefore, the significant elastase inhibitory activity observed is due to the presence of the plant's inflorescence and not due to seasonal variation (Table 5.3).

Table 5.3. Anti-elastase activities of *Cyperus sexangularis* and *Cyperus marginatus*.

Sample		IC_{50}[a] ± SD [b] (µg/mL)
Cyperus marginatus	n-Hexane[c]	NI_{400}
	Ethyl acetate[c]	NI_{400}
	DCM[c]	NI_{400}
	Ethanol: Leaves + inflorescence (March 2016) [c]	39.82 ± 3.03
	Ethanol: Leaves (prepared at WSU; Nov 2020) [d]	NI_{400}
	Ethanol: Leaves (inflorescence unknown; Nov 2020) [e]	93.56 ± 31.45
	Ethanol: Leaves (Nov 2021) [e]	74.96 ± 10.55
Cyperus sexangularis	n-Hexane[c]	NI_{400}
	Ethyl acetate[c]	NI_{400}
	DCM[c]	NI_{400}
	Ethanol: Leaves + inflorescence (March 2016) [c]	20.28 ± 1.71
	Ethanol: Leaves (prepared at WSU; Nov 2020) [d]	124.65 ± 38.54
	Ethanol: Leaves (inflorescence unknown; Nov 2020) [e]	32.43 ± 6.65
	Ethanol: Leaves (Nov 2021) [e]	165.50 ± 24.61
	Ethanol: Leaves + inflorescence (Nov 2021) [e]	26.36 ±6.44
Ursolic acid		17.37 ± 1.11

a Fifty percent inhibitory concentration, b Standard deviation, c Samples prepared at the University of Pretoria. Plant material collected at the University of Pretoria Botanical Garden in March 2016; d Samples prepared at the Walter Sisulu University. Plant material collected at Botanica Natural products in November 2020, e Samples prepared at the University of Pretoria. Plant material collected at Botanica Natural products in November 2020, NI400: No inhibition at the highest concentration tested of 400 µg/mL.

5.4.6 *In vitro* cytotoxicity activity

The eight solvent extract samples were evaluated for cytotoxicity using HaCaT cells. No cytotoxicity was observed for the n-hexane fractions of CS and CM and the ethanolic fraction of CM. The IC_{50} values for CM_{EA} and CM_{DCM} were 160.45 ± 24.82 µg/mL and 213.2 ± 3.68 µg/mL respectively. The samples CS_{EA} and CS_{DCM} had IC_{50} values of 223.25 ± 74.46 µg/mL and 196.8 ± 1.70 µg/mL, respectively (Table 5.4). The samples can be considered to have low cytotoxic activity.

Table 5.4. Cytotoxicity evaluation of *Cyperus sexangularis* and *Cyperus marginatus* fractions.

Sample		IC_{50}[a] ± SD [b] (µg/mL)
Cyperus marginatus	n-Hexane	NI_{400}
	Ethyl acetate	160.45 ± 24.82
	DCM	213.2 ± 3.68
	Ethanol	NI_{400}
Cyperus sexangularis	n-Hexane	NI_{400}
	Ethyl acetate	223.25 ±74.46
	DCM	196.8 ± 1.70
	Ethanol	157.4 ± 8.77
Actinomycin D		0.025 ± 0.007

a Fifty percent inhibitory concentration, b Standard deviation, NI400: No inhibition at the highest concentration tested of 400 µg/mL.

5.5 *Elegia tectorum*

5.5.1 Bioassay-guided fractionation

Of the three partitions that were prepared from ET, the water partition displayed the lowest IC_{50} value against elastase production (Table 5.5). Thereafter, column chromatography on the water fraction was conducted, whereby six major fractions were pooled. Of these fractions none displayed anti-elastase activity (Table 5.6). According to Abu-Lafi et al. (2018), such observation shows that crude extracts sometimes surpass the efficacy of isolated compounds due to synergistic effects (Abu-Lafi et al., 2018).

Table 5.5. Elastase inhibition of liquid-liquid partitioning collected from *Elegia tectorum*.

***Elegia tectorum* partitions**	**IC_{50} (µg/mL ± SD)**
ET crude extract	13.50 ± 1.50
Water	34.44 ± 3.55
n-butanol	63.51 ± 3.52
Ethyl acetate	110.2 6 ± 5.94
Ursolic acid (positive control)	4.42 ± 2.55

Table 5.6. Elastase inhibition of the six pooled fractions collected from *Elegia tectorum*.

***Elegia tectorum* water fractions**	**IC_{50} (µg/mL ± SD)**
1	>250
2	>250
3	>250
4	>250
5	>250
6	>250
Ursolic acid	4.63 ± 1.33

Fraction 3 was further purified via precipitation and 1D and 2D NMR analysis of the purified compound was conducted. This indicated that the compound was an oligosaccharide containing two aldose and two ketose sugars (Figure 5.8). The anomeric proton and carbon NMR signals indicated that both sugars had one alpha and one beta anomer. However, the linkage of the sugar residues could not be confirmed due to the overlapping of NMR peaks as displayed below:

^{1}H NMR (400MHz, CD_3OD): δ_H, 3.14-3.76 (26H, m), 4.51 (1H, d, J = 6.2 Hz), 5.14 (1H, d, J = 2.9 Hz). **^{13}C NMR (400MHz, CD_3OD):** δ_C, 62.8 (CH_2), 62.9 (CH_2), 64.2 (CH_2), 64.6 (CH_2), 64.7 (CH_2), 65.9 (CH_2), 69.43 (CH), 71.28 (CH), 71.81 (CH), 71.90 (CH), 71.92 (CH), 73.08 (CH), 73.87 (CH), 74.93 (CH), 76.35 (CH), 76.84 (CH), 77.68 (CH), 78.09 (CH), 78.13 (CH), 83.33 (CH), 94.02 (CH), 98.26 (C), 99.34 (CH), 103.22 (C).

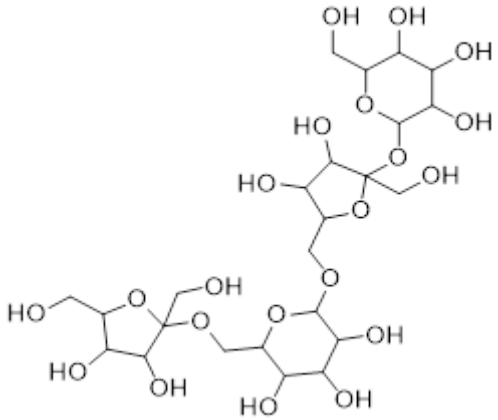


Figure 5.8. Tentative structure of the purified compound obtained from *Elegia tectorum* fraction 3.

5.5.2 GC-MS analysis

A total of 47 constituents in ET were identified as displayed in Figure 5.9 and Table 5.7. Major constituents present in ET above a peak area of 2% included benzothiazole (12.81%), acetate, 4-hydroxyl-3-methyl-2-butenyl- (9.26%), 2,7-octanedione (7.45%), hentriacontane (6.03%), Dl-α-tocopherol (4.89%), hexanedioic acid, bis (2-ethylhexyl) ester (4.02%), n-hexadecanoic acid (3.30%), 1-hepatnol, 2,4-demethyl-, (2S, 4R)-(-)- (2.65%), α-sitosterol (2.59%), 2,3-diozabicyclo[2.2.1]heptane, 1-methyl-1 (2.57%), stigmasterol (2.52%), octadecanoic acid (2.32%), 2,2-dimethyl-4-octenal (2.16%) and hexadecanoic acid, butyl ester (2.03%). Phytosterols are plant sterols that are similar to cholesterol including stigmasterol.

In a study conducted by Tu et al. (2014), *Clinacanthus nutans* (Burm.f) Lindau previously displayed an inhibitory effect of 68.33% against elastase at 10 µg/mL (Tu et al., 2014). Furthermore, stigmasterol has previously been isolated from this plant, which could contribute to the anti-elastase activity, however, further investigation is required (Alam et al., 2016).

Table 5.7. Constituents present in the ethanolic extract of *Elegia tectorum* (ET) were identified using gas chromatography-mass spectrometry (GC-MS).

No.	Name	Molecular weight	Formula
1	1,2,4-Butanetriol	106	$C_4H_{10}O_3$
2	1,2-Dioxolan-3-one, 5-ethyl-5-methyl-4-methylene-	142	$C_7H_{10}O_3$
3	1-Heptanol, 2,4-dimethyl-, (2S,4R)-(-)-	144	$C_9H_{20}O$
4	1-Hepten-6-one, 2-methyl-	126	$C_8H_{14}O$
5	1-Hexanol, 2-(hydroxymethyl)-	132	$C_7H_{16}O_2$
6	2(3H)-Furanone, 5-ethenyldihydro-5-methyl-	126	$C_7H_{10}O_2$
7	2(3H)-Furanone, dihydro-5-methyl-	100	$C_5H_8O_2$
8	2,2-Dimethyl-4-octenal	154	$C_{10}H_{18}O$
9	2,3-Dioxabicyclo[2.2.1]heptane, 1-methyl-	114	$C_6H_{10}O_2$
10	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one	144	$C_6H_8O_4$
11	2,7-Octanedione	142	$C_8H_{14}O_2$
12	2-Butenoic acid, 2-methoxy-3-methyl-, methyl ester	144	$C_7H_{12}O_3$
13	2-Butenoic acid, 2-methyl-, 2-methylpropyl ester, (E)-	156	$C_9H_{16}O_2$
14	2-Cyclohexen-1-one, 3-methyl-	110	$C_7H_{10}O$
15	2-Hexadecyl-5-methylpyrrolidine	309	$C_{21}H_{43}N$
16	2-Hexanone, 6-bromo-	178	$C_6H_{11}BrO$
17	3-(5-Methylfuryl)-N-furamidopropionamide	262	$C_{13}H_{14}N_2O_4$
18	3-Hexen-2-one, 3,4-dimethyl-	126	$C_8H_{14}O$
19	5,6-Dihydropyran-2-one, 5-acetoxy-6-(1,2-epoxypropyl)-	212	$C_{10}H_{12}O_5$
20	5-Nonen-2-one	140	$C_9H_{16}O$
21	5-Octen-2-one, 3,6-dimethyl-	154	$C_{10}H_{18}O$
22	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	278	$C_{18}H_{30}O_2$
23	Acetate, 4-hydroxy-3-methyl-2-butenyl-	144	$C_7H_{12}O_3$
24	α-Sitosterol	414	$C_{29}H_{50}O$
25	Benzothiazole	135	C_7H_5NS
26	Bis(2-ethylhexyl) phthalate	390	$C_{24}H_{38}O_4$
27	Decane	142	$C_{10}H_{22}$
28	Decanoic acid, ethyl ester	200	$C_{12}H_{24}O_2$
29	Dibutyl phthalate	278	$C_{16}H_{22}O_4$
30	dl-α-Tocopherol	430	$C_{29}H_{50}O_2$
31	Hentriacontane	436	$C_{31}H_{64}$
32	Heptacosane	380	$C_{27}H_{56}$
33	Heptadecane, 2-methyl-	254	$C_{18}H_{38}$
34	Hexadecane	226	$C_{16}H_{34}$
35	Hexadecanoic acid, butyl ester	312	$C_{20}H_{40}O_2$
36	Hexanedioic acid, bis(2-ethylhexyl) ester	370	$C_{22}H_{42}O_4$
37	Hexanoic acid, anhydride	214	$C_{12}H_{22}O_3$
38	Neophytadiene	278	$C_{20}H_{38}$
39	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$

No.	Name	Molecular weight	Formula
40	Octacosane	394	$C_{28}H_{58}$
41	Octadecanoic acid	284	$C_{18}H_{36}O_2$
42	Octanoic acid, 6,6-dimethoxy-, methyl ester	218	$C_{11}H_{22}O_4$
43	Oxepine, 2,7-dimethyl-	122	$C_8H_{10}O$
44	p-Nitrophenyl hexanoate	237	$C_{12}H_{15}NO_4$
45	Squalene	410	$C_{30}H_{50}$
46	Stigmasterol	412	$C_{29}H_{48}O$
47	Undecane	156	$C_{11}H_{24}$

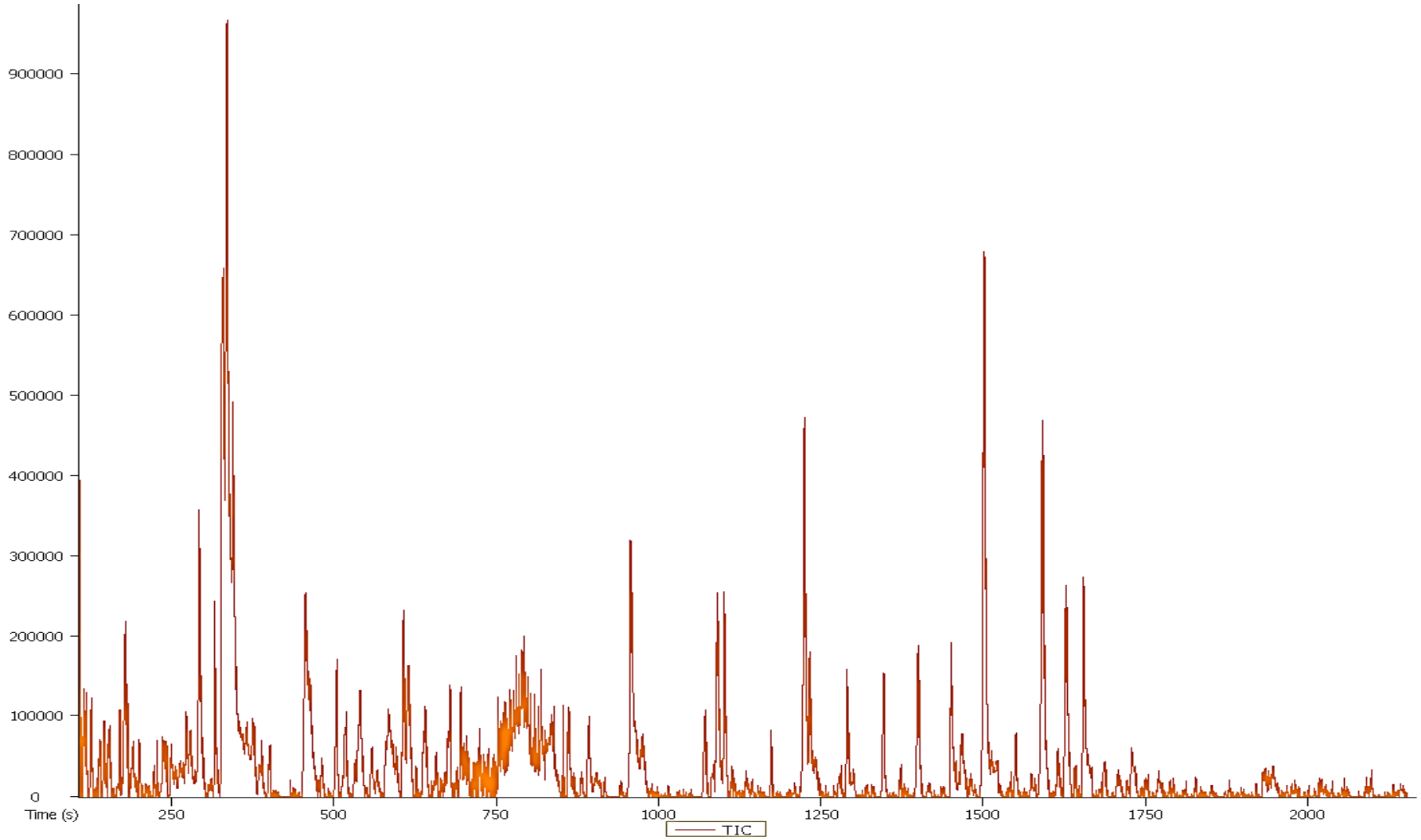


Figure 5.9. Gas chromatography-mass spectrometry (GC-MS) spectrometry of constituents present in the ethanolic extract of *Elegia tectorum* (ET).

5.5.3 Gold nanoparticle synthesis

During the preparation of the ETAuNP, an immediate colour change was observed whereby the green tinge of the solution converted to a wine colour once exposed to the gold salt, which indicated the presence of reducing compounds within ET-EtOH. This was confirmed with UV-Vis, as ETAuNP displayed a wavelength of 535 nm (Figure 5.10A) with a total phenolic content of 3.04 mg/mL. Furthermore, the functional groups present in ET-EtOH and ETAuNP that were identified were tabulated (Figure 5.10B, Table 5.8).

The average diameter and zeta potential of the synthesized nanoparticles were measured and found to be 115 ± 77.74 nm and -9.42 mV, respectively. Furthermore, the morphology of ETAuNP was determined using high resolution transmission electron microscopy (HRTEM), which consisted mostly of round shapes (Figure 5.10C). A selected area diffraction pattern (SAED) was used to characterize whether ETAuNP was similar to gold metal. The face centred lattice planes of ETAuNP displayed a diffraction index of (111), (200), (220) and (311) (Figure 5.10D). Lastly, ETAuNP displayed minimal shifts in the surface plasmon resonance peak (λ_{max}) when exposed to the mediums except for BSA whereby low λ_{max} peaks were observed (Figure 5.11G).

Table 5.8. Potential functional groups were identified using Fourier transform infrared spectrometry (FTIR) in the ethanolic extract (ET-EtOH) and synthesized gold nanoparticles (ETAuNP) of *Elegia tectorum*.

Functional groups	ET-EtOH transmittance (cm^{-1})	ETAuNP transmittance (cm^{-1})
O-H	3321	3353
C-H	2924	-
C-O	1058	-
Aromatic ring (C-C)	-	1637
C=O	1734	-

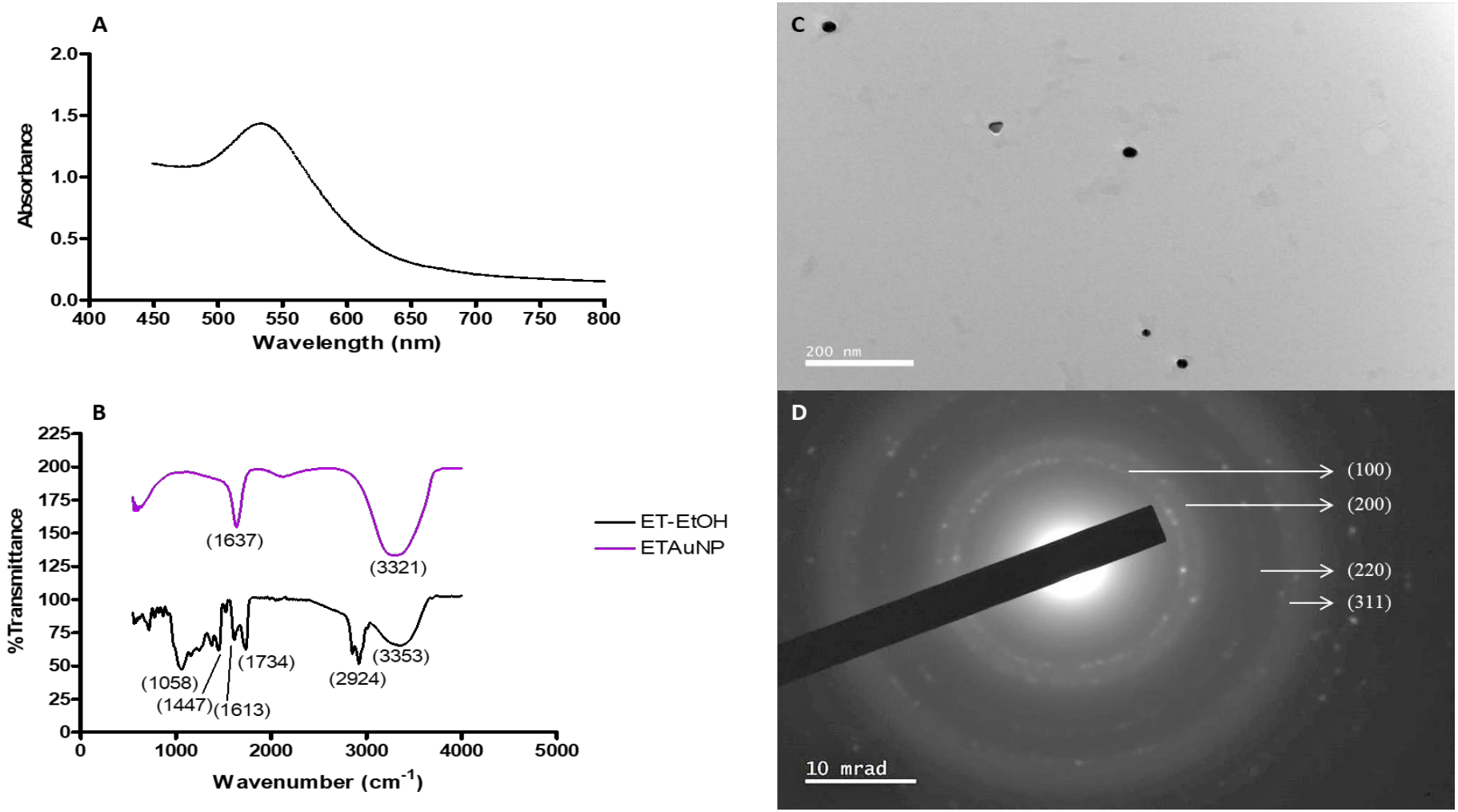


Figure 5.10. Gold nanoparticle characterization including an ultraviolet-visible (UV-Vis) spectroscopy (A), Fourier-transform infrared spectrometry (FTIR) of the ethanolic extract (ET-EtOH) and synthesized gold nanoparticles (ETAuNP) of *Elegia tectorum* (B), high-resolution transmission electron microscopy (HRTEM) at 200 nm (C) and selected area diffraction pattern (SAED) at 10 mrad (D).

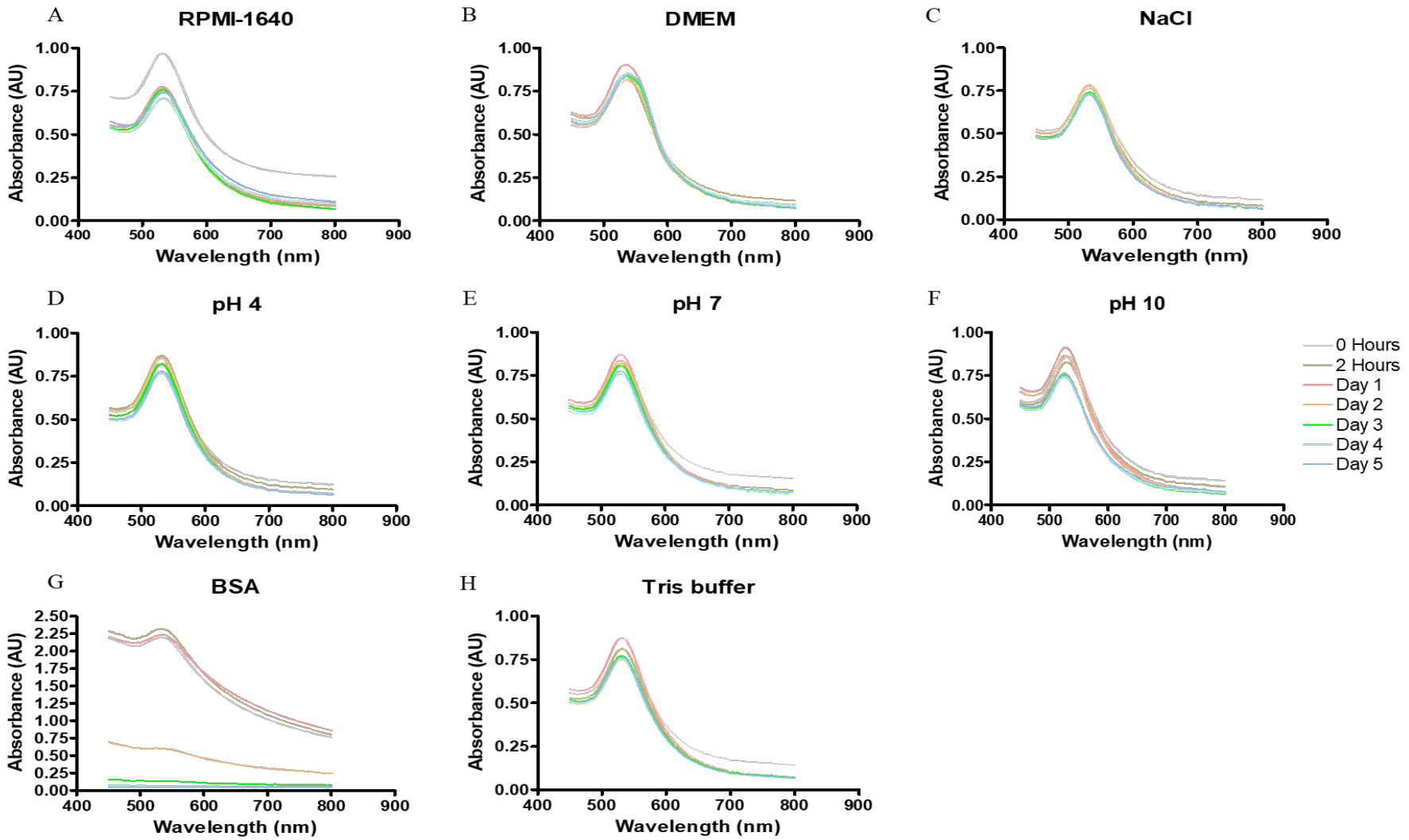


Figure 5.11. ***In vitro*** **stability of** ***Elegia tectorum*** **synthesized gold nanoparticles (ETAuNP) in different mediums. These solutions include Roswell Park Memorial Institution (RPMI-1640) medium (A) and Dulbecco's modified Eagle's Medium (DMEM) (B), 5% sodium chloride (NaCl) (C), pH level of 4 (D), 7 (E) and 10 (F), 0.5% bovine serum albumin (BSA) (G) and tris buffer (pH 8.1) (H).**

5.5.4 Elastase inhibition

An elastase inhibition assay was conducted on the crude, fermented, and synthesised nanoparticles of ET. Of these extracts, only the crude extract of ET showed inhibition towards elastase with an IC_{50} of 28.27 ± 2.02 µg/mL while the positive control (ursolic acid) displayed an IC_{50} of 22.30 ± 2.79 µg/mL, respectively. Little to no studies could be found on the biological activity of ET nor on the effects gold nanoparticles have on elastase inhibition. Ali et al. (2017) indicated that an increase in the concentration of zinc peroxide nanoparticles (ZnO_2-NP) significantly decreased elastase activity (Ali et al., 2017). This suggests that the gold salt may affect the crude extract's active compounds, resulting in ETAuNP displaying no inhibition towards elastase at the highest testing concentration (500 µg/mL). However, further investigation into the effects of the gold salt against elastase inhibition is required.

A study done by Park and Bae (2016) demonstrated that *B. bifidum* fermented *Acanthopanax koreanum* root extract displayed greater antioxidant and anti-photoaging activity than the crude extract due to the repression of specific signalling pathways. These pathways include the UVB- or H_2O_2-induced activities of matrix metalloproteinase (MMP)-1 and -3, the overexpression of MMP-1 and the activation of nuclear factor kappa beta (NF-κB). This study indicated that fermented plant extracts might produce anti-wrinkle effects; however, the effect these extracts may have on the release of elastase was not mentioned (Park and Bae, 2016). Thus, further investigation into the anti-photoaging activity of ET fermented extract may indicate as to why this extract displayed no inhibition towards elastase at the highest concentration (500 µg/mL).

5.5.5 Antiproliferative activity

The antiproliferative activity of ET and it's respective fermented and nanoparticle synthesised extracts were evaluated against HaCaT. The extracts displayed no antiproliferative activity at the highest testing concentration (400 µg/mL), while the positive control, actinomycin D, displayed antiproliferative properties with an IC_{50} of 0.01 ± 0.005 µg/mL. Currently, no information regarding the antiproliferative activity of ET could be found.

5.5.6 Irritancy response

A patch test was used to evaluate the irritancy response of ET when applied to the forearm of a patient (Figure 5.12). ET (Table 5.9) displayed a larger mean score than the negative control and thus was considered a mild irritant.

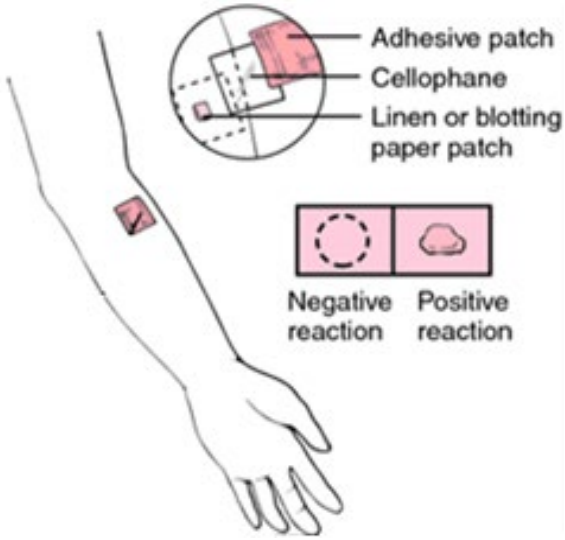


Figure 5.12. Depiction of how an irritancy patch test is performed on the forearm of the skin.

Table 5.9. Irritancy response of *Elegia tectorum* ethanolic extract dissolved in 40% ethanol using an *in vivo* patch test.

Product name	Average value	Mean score	Number of subjects with reactions after 48 hours	Skin compatibility Potential %	Skin Compatibility
Positive control sodium lauryl sulphate solution (1%)	0.59	1.25	8	100	Irritant
ET ethanol extract (neat)	0.12	0.33	0	3.06	Mild irritant
Negative control-demineralized water	0.13	0.30	0	0.00	Non-irritant

Skin irritation is defined as a minor injury or inflammation when exposed to a substance resulting in itching and redness. This response, however, does not initiate an immune response and only causes slight discomfort until the irritant is removed (Chris, 2022). According to healthcare workers, products that contain ethanol causes skin irritation when used, however, a study conducted by Loffler et al. (2007) demonstrated this to be inaccurate. However, when applied to damaged or irritated skin, patients reported experiencing a burning sensation (Löffler et al., 2007). This indicates that compounds present in ET may play a role, however, further research is required to confirm this.

5.5.7 Wrinkle reduction efficacy study

To evaluate the efficacy of ET, a wrinkle reduction efficacy study was conducted. After 28 days, ET significantly reduced the topography or depth of the wrinkles with a confidence level of 5% in comparison to the placebo.

5.6 *Juncus lomatophyllus*

5.6.1 Bioassay-guided fractionation

JL displayed an IC_{50} of 31.64 ± 6.91 µg/mL while the butanol partition (JLB) (40.4 ± 2.31 µg/mL) displayed the lowest IC_{50} value against tyrosinase in comparison to the other partitions. Column chromatography was conducted on JLB of which seven major fractions were pooled. When evaluated against tyrosinase, P1 (155.70 ± 4.95), P4 (105.55 ± 7.28) and P5 (125.60 ± 3.68 µg/mL) displayed inhibition.

Chiou et al. (2015) indicated that the butanol partition of both the fruit and seed shells of *Camellia tenuifloria* (Hayata) Cohen-Stuart displayed the highest inhibition against tyrosinase with IC_{50} values of 70.0 ± 5.5 and 32.7 ± 0.4 µg/mL, respectively. This could be due to the hydroxyl groups of flavonoids and the presence of terpenes and steroids which have previously shown tyrosinase inhibitory activity and have been isolated from *Juncus* genus (Chiou et al., 2015; El-Shamy et al., 2015). An increase in IC_{50} value for P1 (155.70 ± 4.95), P4 (105.55 ± 7.28) and P5 (125.60 ± 3.68 µg/mL) were observed compared to JLB (40.4 ± 2.31 µg/mL), suggesting that JLB anti-tyrosinase properties may rely on synergistic effects. These effects have been examined in previous studies whereby the combination of more than one compound lowers the IC_{50} value (Rasoanaivo et al., 2011).

5.6.2 GC-MS analysis

A total of 77 constituents in JL were identified as displayed in Figure 5.13 and Table 5.10. Major constituents present in JL above a peak area of 2% included 1,2-benzenedicarboxylic acid, butyl 2-ethylhexyl ester (10.38%), 1-docosene (5.75%), dodecane, 1,1-dimethoxy- (5.72%), acrolein, dimethyl acetal (3.33%), pyrimidine-2,4,6-trione, 1-butyl-5-[(2-piperazin-1-yl-ethylamino)methylene]- (3.33%), tridecanoic acid, methyl ester (3.30%), butanedioic acid, dimethyl ester (3.09%), hexanoic acid, 2-ethyl-, methyl ester (3.05%), 2(3H)-furanone, 5-ethenyldihydro-5-methyl- (2.97%), diisooctyl phthalate (2.75%), butyric acid, 4-isopropoxy-, methyl ester (2.63%), hexanal dimethyl acetal (2.62%), acetamide, N,N'-ethylenebis(N-nitro- (2.62%), p-nitrophenyl hexanoate (2.60%) and 2-propen-1-ol, 2-bromo-, acetate (2.21%).

Table 5.10. Constituents present in the ethanolic extract of *Juncus lomatophyllus* (JL) were identified using gas chromatography-mass spectrometry (GC-MS).

No.	Name	Molecular weight	Formula
1	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	334	$C_{20}H_{30}O_4$
2	1,3-Octanediol	146	$C_8H_{18}O_2$
3	1-Docosene	308	$C_{22}H_{44}$
4	1-Nonen-3-ol	142	$C_9H_{18}O$
5	2(3H)-Furanone, 5-ethenyldihydro-5-methyl-	126	$C_7H_{10}O_2$
6	2(3H)-Furanone, dihydro-5-methyl-	100	$C_5H_8O_2$
7	2,2-Dimethyl-4-octenal	154	$C_{10}H_{18}O$
8	2,3-Dimethyl-5-oxohexanethioic acid, S-t-butyl ester	230	$C_{12}H_{22}O_2S$
9	2,3-Octanedione	142	$C_8H_{14}O_2$
10	2,5-Dimethyl-2-(2-tetrahydrofuryl)tetrahydrofuran	170	$C_{10}H_{18}O_2$
11	2,5-Hexanedione	114	$C_6H_{10}O_2$

No.	Name	Molecular weight	Formula
12	2-Cyclohexen-1-one, 3-methyl-	110	$C_7H_{10}O$
13	2-Furanol, tetrahydro-2,3-dimethyl-, trans-	116	$C_6H_{12}O_2$
14	2-Octanol, 8,8-dimethoxy-	190	$C_{10}H_{22}O_3$
15	2-Propen-1-ol, 2-bromo-, acetate	178	$C_5H_7BrO_2$
16	3-Octanone	128	$C_8H_{16}O$
17	4-Propoxy-2-butanone	130	$C_7H_{14}O_2$
18	5-Undecanone	170	$C_{11}H_{22}O$
19	à-Amino-2,5-dihydro-5-methyl-2-furanaceticacid	157	$C_7H_{11}NO_3$
20	Acetamide, N,N'-ethylenebis(N-nitro-	234	$C_6H_{10}N_4O_6$
21	Acetate, 4-hydroxy-3-methyl-2-butenyl-	144	$C_7H_{12}O_3$
22	Acetic acid, dimethoxy-, methyl ester	134	$C_5H_{10}O_4$
23	Acetophenone	120	C_8H_8O
24	Acetoxyacetic acid, 2-dimethylaminoethyl ester	189	$C_8H_{15}NO_4$
25	Acrolein, dimethyl acetal	102	$C_5H_{10}O_2$
26	Allyl(methoxy)dimethylsilane	130	$C_6H_{14}OSi$
27	Benzaldehyde dimethyl acetal	152	$C_9H_{12}O_2$
28	Benzene, 1,3-dichloro-	146	$C_6H_4C_{l2}$
29	Benzene, 1-ethyl-2-methyl-	120	C_9H_{12}
30	Benzothiazole	135	C_7H_5NS
31	Benzyl butyl phthalate	312	$C_{19}H_{20}O_4$
32	Butanedioic acid, dimethyl ester	146	$C_6H_{10}O_4$
33	Butyric acid, 4-isopropoxy-, methyl ester	160	$C_8H_{16}O_3$
34	Cyclohexane, 1,1-dimethoxy-	144	$C_8H_{16}O_2$
35	Cyclopentanone ethylene ketal	128	$C_7H_{12}O_2$
36	Decane	142	$C_{10}H_{22}$
37	Decanoic acid, 2-methyl-	186	$C_{11}H_{22}O_2$
38	Diethyl Phthalate	222	$C_{12}H_{14}O_4$
39	Diisooctyl phthalate	390	$C_{24}H_{38}O_4$
40	dl-à-Tocopherol	430	$C_{29}H_{50}O_2$
41	Dodecane, 1,1-dimethoxy-	230	$C_{14}H_{30}O_2$
42	Ethanamine, 2,2'-oxybis-	104	$C_4H_{12}N_2O$
43	Glycine	75	$C_2H_5NO_2$
44	Heptane, 1,1-dimethoxy-	160	$C_9H_{20}O_2$
45	Heptanoic acid, 7,7-dimethoxy-	190	$C_9H_{18}O_4$
46	Heptanoic acid, methyl ester	144	$C_8H_{16}O_2$
47	Hexadecane, 1,1-dimethoxy-	286	$C_{18}H_{38}O_2$
48	Hexadecanoic acid, 15-methyl-, methyl ester	284	$C_{18}H_{36}O_2$
49	Hexanal dimethyl acetal	146	$C_8H_{18}O_2$
50	Hexanedioic acid, bis(2-ethylhexyl) ester	370	$C_{22}H_{42}O_4$
51	Hexanethioic acid, S-ethyl ester	160	$C_8H_{16}OS$
52	Hexanoic acid, 2-ethyl-, methyl ester	158	$C_9H_{18}O_2$
53	Hexanoic acid, methyl ester	130	$C_7H_{14}O_2$
54	Isopropyl myristate	270	$C_{17}H_{34}O_2$
55	Methane, trimethoxy-	106	$C_4H_{10}O_3$

No.	Name	Molecular weight	Formula
56	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
57	Nonanal dimethyl acetal	188	$C_{11}H_{24}O_2$
58	Nonanoic acid, methyl ester	172	$C_{10}H_{20}O_2$
59	Octadecane, 1,1-dimethoxy-	314	$C_{20}H_{42}O_2$
60	Octan-2-one, 3,6-dimethyl-	156	$C_{10}H_{20}O$
61	Octanal dimethyl acetal	174	$C_{10}H_{22}O_2$
62	Octanoic acid, 6,6-dimethoxy-, methyl ester	218	$C_{11}H_{22}O_4$
63	Octanoic acid, methyl ester	158	$C_9H_{18}O_2$
64	Pentanedioic acid, dimethyl ester	160	$C_7H_{12}O_4$
65	Pentanoic acid, 4-oxo-, methyl ester	130	$C_6H_{10}O_3$
66	Pentanoic acid, 5,5-dimethoxy-, methyl ester	176	$C_8H_{16}O_4$
67	Phthalic acid, butyl undecyl ester	376	$C_{23}H_{36}O_4$
68	Phthalic acid, hexyl 2-methoxyethyl ester	308	$C_{17}H_{24}O_5$
69	p-Nitrophenyl hexanoate	237	$C_{12}H_{15}NO_4$
70	Propanedioic acid, dimethyl ester	132	$C_5H_8O_4$
71	Pyrimidine-2,4,6-trione, 1-butyl-5-[(2-piperazin-1-yl-ethylamino)methylene]-	323	$C_{15}H_{25}N_5O_3$
72	Spiro(1,3-dioxolane)-2,3'-(5'-androsten-16'-ol), TMS derivative	404	$C_{24}H_{40}O_3Si$
73	Thiazole, tetrahydro-	89	C_3H_7NS
74	Tridecanedioic acid, dimethyl ester	272	$C_{15}H_{28}O_4$
75	Tridecanoic acid, methyl ester	228	$C_{14}H_{28}O_2$
76	Undecanoic acid, 10-methyl-, methyl ester	214	$C_{13}H_{26}O_2$
77	Undecanoic acid, methyl ester	200	$C_{12}H_{24}O_2$

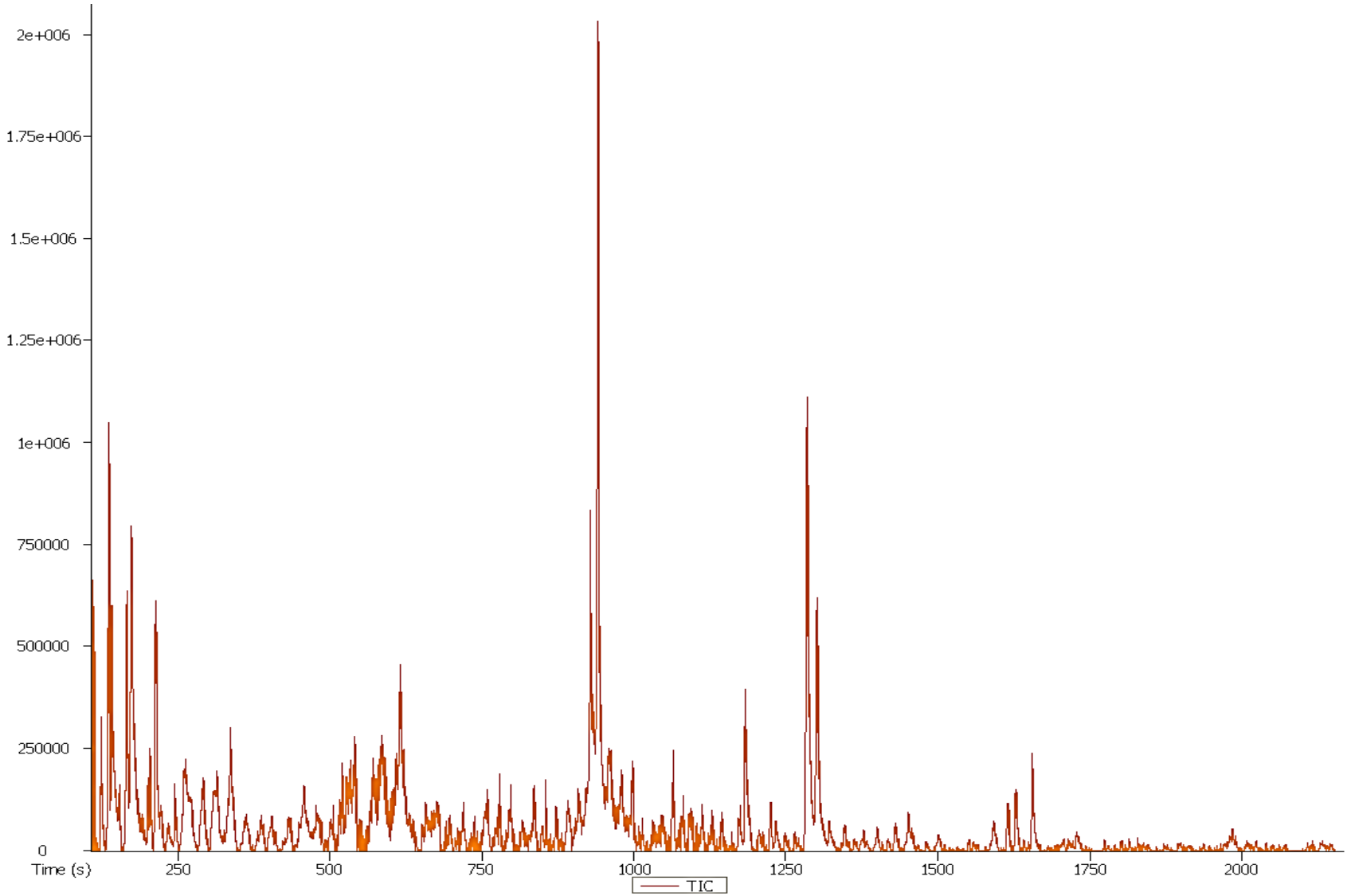


Figure 5.13. Gas chromatography-mass spectrometry (GC-MS) spectrometry of constituents present in the ethanolic extract of *Juncus lomatophyllus* (JL).

Furthermore, GC-MS analysis was conducted on P4 and P5 of JLB. A total of 78 and 92 peaks in P4 (Table 5.11) and P5 (Table 5.12) were observed using GC-MS (Figures 5.14 and 5.15). Major constituents present in P4, above a peak area of 2%, included 9-octadecenamide (34.89%), dodecanamide (25.32%), [1,1'-biphenyl]-4,4'-diamine, N, N'-diphenyl- (4.16%), octadecanoic acid (4.09%), hexasiloxane, tetradecamethyl- (3.75%) and n-hexadecanoic acid (2.81%). Furthermore, major constituents discovered in P5 above a peak area of 5% included diisooctyl phthalate (20.50%), 9-octadecenamide (13.43%), 1-octanamine (8.01%), sulfurous acid, butyl octyl ester (7.80%) and dodecanamide (5.99%). Furthermore, it was noted that P4 and P5 shared similar constituents with varying peak area percentages.

It was observed that P4 contained n-hexadecanoic acid (2.81%), which has previously displayed anti-tyrosinase properties. According to Panda et al. (2018), n-hexadecanoic acid (peak area of 10.15%) was one of the saturated fatty acids responsible for the anti-tyrosinase activity of *Bauhinia vahlii* Wight & Arn. with an IC_{50} of 98.70 ± 0.70 µg/mL (Panda et al., 2018).

Table 5.11. Chemical composition of P4 isolated from the butanol partition of *Juncus lomatophyllus*.

Peak#	Name	Molecular weight	Formula	Similarity [A]	Area % [B]
1	Benzene, 1,3-dimethyl-	106	C_8H_{10}	924	0.12
2	o-Xylene	106	C_8H_{10}	938	0.49
3	p-Xylene	106	C_8H_{10}	951	0.25
4	Ethane, 1,1,2,2-tetrachloro-	166	$C_2H_2Cl_4$	844	0.62
5	Ethane, pentachloro-	200	C_2HCl_5	748	0.39
6	Decane	142	$C_{10}H_{22}$	929	0.80
7	Ethane, hexachloro-	234	C_2Cl_6	883	0.18
8	Octane, 2,3,6,7-tetramethyl-	170	$C_{12}H_{26}$	866	0.27
9	Undecane	156	$C_{11}H_{24}$	930	1.00
10	Silane, cyclohexyldimethoxymethyl-	188	$C_9H_{20}O_2Si$	869	0.05
11	1,4:3,6-Dianhydro-à-d-glucopyranose	144	$C_6H_8O_4$	850	1.44
12	Benzothiazole	135	C_7H_5NS	864	0.27
13	Hexadecane	226	$C_{16}H_{34}$	894	0.59
14	p-Nitrophenyl hexanoate	237	$C_{12}H_{15}NO_4$	830	0.16
15	D-Allose	180	$C_6H_{12}O_6$	898	1.05
16	4H-Imidazol-4-one, 2-amino-1,5-dihydro-	99	$C_3H_5N_3O$	809	0.57
17	Cyclobutanol, TMS derivative	144	$C_7H_{16}OSi$	618	0.57
18	2,4-Di-tert-butylphenol	206	$C_{14}H_{22}O$	856	0.13
19	Eicosane	282	$C_{20}H_{42}$	870	0.27
20	Hydrazinecarboxamide, N,N-diphenyl-	227	$C_{13}H_{13}N_3O$	930	2.00
21	Heneicosane	296	$C_{21}H_{44}$	904	0.26
22	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	278	$C_{16}H_{22}O_4$	870	0.15

Peak#	Name	Molecular weight	Formula	Similarity [A]	Area % [B]
23	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	444	$C_{13}H_{40}O_5Si_6$	725	0.30
24	Dibutyl phthalate	278	$C_{16}H_{22}O_4$	937	0.53
25	Nonanamide	157	$C_9H_{19}NO$	905	0.35
26	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	914	2.81
27	Eicosane	282	$C_{20}H_{42}$	916	0.30
28	Silane, tetramethyl-	88	$C_4H_{12}Si$	693	0.53
29	9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	851	1.03
30	Dodecanamide	199	$C_{12}H_{25}NO$	926	15.30
31	Octadecanoic acid	284	$C_{18}H_{36}O_2$	909	4.09
32	Hexadecanoic acid, butyl ester	312	$C_{20}H_{40}O_2$	892	0.48
33	Hexasiloxane, tetradecamethyl-	458	$C_{14}H_{42}O_5Si_6$	706	0.65
34	Heptadecane, 2-methyl-	254	$C_{18}H_{38}$	922	0.32
35	Dodecanamide	199	$C_{12}H_{25}NO$	906	0.30
36	Benzenecarbothioic acid, 2,6-dichloro-, S-methyl ester	220	$C_8H_6Cl_2OS$	830	0.01
37	Heptacosane	380	$C_{27}H_{56}$	915	0.26
38	9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	772	0.95
39	9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	927	34.89
40	[1,1'-Biphenyl]-4,4'-diamine, N,N'-diphenyl-	336	$C_{24}H_{20}N_2$	566	3.82
41	Hexasiloxane, tetradecamethyl-	458	$C_{14}H_{42}O_5Si_6$	685	1.87
42	6H-Dibenzo[b,d]pyran-1-ol, 6,6,9-trimethyl-3-propyl-	282	$C_{19}H_{22}O_2$	702	0.01
43	Dodecanamide	199	$C_{12}H_{25}NO$	909	9.72
44	Hexadecanoic acid, 1,1-dimethylethyl ester	312	$C_{20}H_{40}O_2$	713	0.46
45	Cyclohexanecarboxylic acid, octyl ester	240	$C_{15}H_{28}O_2$	614	0.46
46	Tetracosane	338	$C_{24}H_{50}$	924	0.41
47	Dicyclohexyl phthalate	330	$C_{20}H_{26}O_4$	846	0.08
48	Hexasiloxane, tetradecamethyl-	458	$C_{14}H_{42}O_5Si_6$	710	0.48
49	Heptadecane, 2-methyl-	254	$C_{18}H_{38}$	902	0.34
50	Diisooctyl phthalate	390	$C_{24}H_{38}O_4$	911	0.44
51	Cannabinol	310	$C_{21}H_{26}O_2$	876	0.14
52	Dotriacontane	450	$C_{32}H_{66}$	921	0.31
53	Hexasiloxane, tetradecamethyl-	458	$C_{14}H_{42}O_5Si_6$	720	0.42
54	Heptacosane	380	$C_{27}H_{56}$	894	0.34
55	Hexasiloxane, tetradecamethyl-	458	$C_{14}H_{42}O_5Si_6$	699	0.33
56	Heptacosane	380	$C_{27}H_{56}$	880	0.30
57	Dotriacontane	450	$C_{32}H_{66}$	897	0.31
58	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	430	$C_{12}H_{38}O_5Si_6$	676	0.46

Peak#	Name	Molecular weight	Formula	Similarity [A]	Area % [B]
59	2,5-Dihydroxybenzoic acid, 3TMS derivative	370	$C_{16}H_{30}O_4Si_3$	610	0.46
60	Unknown 1	236	$C_8H_{24}O_2Si_3$	386	0.46
61	2-methyloctacosane	408	$C_{29}H_{60}$	864	0.24
62	[1,1'-Biphenyl]-4,4'-diamine, N,N'-diphenyl-	336	$C_{24}H_{20}N_2$	742	0.34
63	Unknown 2	222	$C_6H_{18}O_3Si_3$	428	0.05
64	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	430	$C_{12}H_{38}O_5Si_6$	731	0.09
65	1-Iodo-2-methylundecane	296	$C_{12}H_{25}I$	842	0.53
66	Unknown 3	222	$C_6H_{18}O_3Si_3$	476	0.03
67	Unknown 4	222	$C_6H_{18}O_3Si_3$	441	0.05
68	Acetic acid, bis[(trimethylsilyl)oxyl]-, trimethylsilyl ester	308	$C_{11}H_{28}O_4Si_3$	552	0.48
69	Sulfurous acid, decyl 2-propyl ester	264	$C_{13}H_{28}O_3S$	527	0.48
70	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	444	$C_{13}H_{40}O_5Si_6$	596	0.22
71	Tris(tert-butyldimethylsilyloxy)arsane	468	$C_{18}H_{45}AsO_3Si_3$	561	0.00
72	4,4'-bi-4H-pyran, 2,2',6,6'-tetrakis(1,1-dimethylethyl)-4,4'-dimethyl-	414	$C_{28}H_{46}O_2$	519	0
73	Nonadecane, 1-chloro-	302	$C_{19}H_{39}C_l$	653	0.73
74	Unknown 5	458	$C_{14}H_{42}O_5Si_6$	475	0.17
75	4,4'-bi-4H-pyran, 2,2',6,6'-tetrakis(1,1-dimethylethyl)-4,4'-dimethyl-	414	$C_{28}H_{46}O_2$	541	0.12
76	Unknown 6	412	$C_{24}H_{36}O_2Si_2$	488	0.02
77	Unknown 7	222	$C_6H_{18}O_3Si_3$	444	0.05
78	Unknown 8	430	$C_{12}H_{38}O_5Si_6$	470	0.05
Total					**100**

A: Mass spectral similarity to NIST08 library, B: Relative peak area

Table 5.12. Chemical composition of P5 isolated from the butanol partition of *Juncus lomatophyllus*.

Peak #	Name	Molecular weight	Formula	Similarity [A]	Area % [B]
1	Hexane, 2,3,4-trimethyl-	128	C_9H_{20}	900	0.27
2	Benzene, 1,3-dimethyl-	106	C_8H_{10}	897	0.06
3	o-Xylene	106	C_8H_{10}	950	0.22
4	o-Xylene	106	C_8H_{10}	931	0.12
5	Ethane, 1,1,2,2-tetrachloro-	166	$C_2H_2Cl_4$	851	0.27
6	Ethane, pentachloro-	200	C_2HCl_5	733	0.24
7	Decane	142	$C_{10}H_{22}$	926	0.36
8	Ethane, hexachloro-	234	C_2Cl_6	884	0.26
9	Decane, 2,3,5,8-tetramethyl-	198	$C_{14}H_{30}$	872	0.41
10	Undecane	156	$C_{11}H_{24}$	944	0.38
11	Silane, cyclohexyldimethoxymethyl-	188	$C_9H_{20}O_2Si$	897	0.10
12	Hexadecane	226	$C_{16}H_{34}$	876	0.32
13	2,4-Di-tert-butylphenol	206	$C_{14}H_{22}O$	896	0.37
14	Eicosane	282	$C_{20}H_{42}$	875	0.38
15	Hexadecane	226	$C_{16}H_{34}$	928	0.83
16	Eicosane	282	$C_{20}H_{42}$	921	0.93
17	Heptadecane, 2,6,10,14-tetramethyl-	296	$C_{21}H_{44}$	895	0.48
18	Eicosane	282	$C_{20}H_{42}$	898	0.33
19	Cetene	224	$C_{16}H_{32}$	915	0.29
20	Eicosane	282	$C_{20}H_{42}$	920	2.67
21	Hexadecane, 2,6,10,14-tetramethyl-	282	$C_{20}H_{42}$	904	0.60
22	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	278	$C_{16}H_{22}O_4$	878	0.03
23	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	276	$C_{17}H_{24}O_3$	905	0.23
24	Heneicosane	296	$C_{21}H_{44}$	924	1.35
25	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester	292	$C_{18}H_{28}O_3$	799	0.53
26	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$	864	0.53
27	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	334	$C_{20}H_{30}O_4$	927	0.39
28	Pentanal, oxime	101	$C_5H_{11}NO$	729	0.07
29	Sulfurous acid, butyl octyl ester	250	$C_{12}H_{26}O_3S$	810	7.80
30	1-Octanamine	129	$C_8H_{19}N$	759	8.01
31	Dodecanoic acid, ethyl ester	228	$C_{14}H_{28}O_2$	704	0.19
32	Heptadecane, 2-methyl-	254	$C_{18}H_{38}$	933	1.73
33	Phenylpyruvic acid oxime, 2TMS derivative	323	$C_{15}H_{25}NO_3Si_2$	672	0.14

Peak #	Name	Molecular weight	Formula	Similarity [A]	Area % [B]
34	1-Hexadecanol	242	$C_{16}H_{34}O$	928	0.68
35	2-Ethylhexyl methyl isophthalate	292	$C_{17}H_{24}O_4$	805	0.02
36	Heptadecane, 2-methyl-	254	$C_{18}H_{38}$	937	0.70
37	9,12-Octadecadienoic acid (Z,Z)-	280	$C_{18}H_{32}O_2$	867	0.55
38	Hexadecanoic acid, 15-methyl-, methyl ester	284	$C_{18}H_{36}O_2$	765	0.22
39	Oleic Acid	282	$C_{18}H_{34}O_2$	926	1.62
40	Hexanamide	115	$C_6H_{13}NO$	640	1.62
41	1-Ethylsulfanylmethyl-2,8,9-trioxa-5-aza-1-sila-bicyclo[3.3.3]undecane	249	$C_9H_{19}NO_3SSi$	733	0.15
42	Dodecanamide	199	$C_{12}H_{25}NO$	932	5.99
43	Octadecanoic acid	284	$C_{18}H_{36}O_2$	877	1.34
44	Hexasiloxane, tetradecamethyl-	458	$C_{14}H_{42}O_5Si_6$	708	0.27
45	1-Acetoxynonadecane	326	$C_{21}H_{42}O_2$	861	0.29
46	Heptadecane, 2-methyl-	254	$C_{18}H_{38}$	922	0.94
47	Nonanamide	157	$C_9H_{19}NO$	673	0.34
48	Dodecyl acrylate	240	$C_{15}H_{28}O_2$	899	0.82
49	Benzyl butyl phthalate	312	$C_{19}H_{20}O_4$	878	0.09
50	Heptacosane	380	$C_{27}H_{56}$	920	0.63
51	9,12-Octadecadienoic acid, methyl ester, (E,E)-	294	$C_{19}H_{34}O_2$	762	0.31
52	9-Octadecenamide, (Z)-	281	$C_{18}H_{35}NO$	928	13.43
53	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	430	$C_{12}H_{38}O_5Si_6$	711	0.74
54	Tetradecanamide	227	$C_{14}H_{29}NO$	910	3.82
55	Hexanedioic acid, bis(2-ethylhexyl) ester	370	$C_{22}H_{42}O_4$	752	0.17
56	Heptacosane	380	$C_{27}H_{56}$	916	0.76
57	Diisooctyl phthalate	390	$C_{24}H_{38}O_4$	807	0.06
58	1H-Indene, 1-hexadecyl-2,3-dihydro-	342	$C_{25}H_{42}$	556	0.09
59	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	430	$C_{12}H_{38}O_5Si_6$	707	0.65
60	Decanoic acid, 2-ethylhexyl ester	284	$C_{18}H_{36}O_2$	815	0.73
61	Heptacosane	380	$C_{27}H_{56}$	919	0.44
62	Diisooctyl phthalate	390	$C_{24}H_{38}O_4$	913	20.44
63	Dotriacontane	450	$C_{32}H_{66}$	921	0.56
64	Hexasiloxane, tetradecamethyl-	458	$C_{14}H_{42}O_5Si_6$	709	0.46
65	1H-Indene, 1-hexadecyl-2,3-dihydro-	342	$C_{25}H_{42}$	667	0.09
66	Decanoic acid, 2-ethylhexyl ester	284	$C_{18}H_{36}O_2$	806	0.66
67	Dotriacontane	450	$C_{32}H_{66}$	923	0.56

Peak #	Name	Molecular weight	Formula	Similarity [A]	Area % [B]
68	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	390	$C_{24}H_{38}O_4$	828	0.19
69	Hexasiloxane, tetradecamethyl-	458	$C_{14}H_{42}O_5Si_6$	723	0.48
70	Decanedioic acid, bis(2-ethylhexyl) ester	426	$C_{26}H_{50}O_4$	634	0.09
71	Heptacosane	380	$C_{27}H_{56}$	870	0.43
72	1-Iodo-2-methylundecane	296	$C_{12}H_{25}I$	882	0.37
73	8,10-Undecadiene-3,7-dione, 6,6,10-trimethyl-, (E)-	222	$C_{14}H_{22}O_2$	543	0.56
74	Benzenamine, 4-octyl-N-(4-octylphenyl)-	393	$C_{28}H_{43}N$	548	0.56
75	Hexasiloxane, tetradecamethyl-	458	$C_{14}H_{42}O_5Si_6$	723	0.56
76	Heptacosane	380	$C_{27}H_{56}$	923	0.24
77	Unknown 1	336	$C_{24}H_{20}N_2$	486	0.02
78	(-)-Neoclovene-(II), dihydro-	206	$C_{15}H_{26}$	729	0.10
79	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	430	$C_{12}H_{38}O_5Si_6$	679	0.87
80	Unknown 2	454	$C_{23}H_{35}BrO_4$	485	0.05
81	1-Iodo-2-methylundecane	296	$C_{12}H_{25}I$	862	0.63
82	Unknown 3	142	$C_7H_{14}OSi$	472	0.63
83	4,8,12,16-Octadecatetraen-1-ol, 4,9,13,17-tetramethyl-	318	$C_{22}H_{38}O$	699	0.20
84	Hexasiloxane, tetradecamethyl-	458	$C_{14}H_{42}O_5Si_6$	700	0.58
85	(7a-Isopropenyl-4,5-dimethyloctahydroinden-4-yl)methanol	222	$C_{15}H_{26}O$	689	0.20
86	Methanol, [4-(1,1-dimethylethyl)phenoxy]-, acetate	222	$C_{13}H_{18}O_3$	582	0.03
87	1-Octadecanesulphonyl chloride	352	$C_{18}H_{37}ClO_2S$	751	0.68
88	1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane	444	$C_{13}H_{40}O_5Si_6$	690	0.54
89	Unknown 4	468	$C_{18}H_{45}AsO_3Si_3$	449	0.01
90	Hexasiloxane, tetradecamethyl-	458	$C_{14}H_{42}O_5Si6$	710	0.4261
91	4-tert-Amylphenol, TMS derivative	236	$C_{14}H_{24}OSi$	613	0.01
92	3,5-Decadien-7-yne, 6-t-butyl-2,2,9,9-tetramethyl-	246	$C_{18}H_30$	594	0.42
Total					**100**

A: Mass spectral similarity to NIST08 library, B: Relative peak area

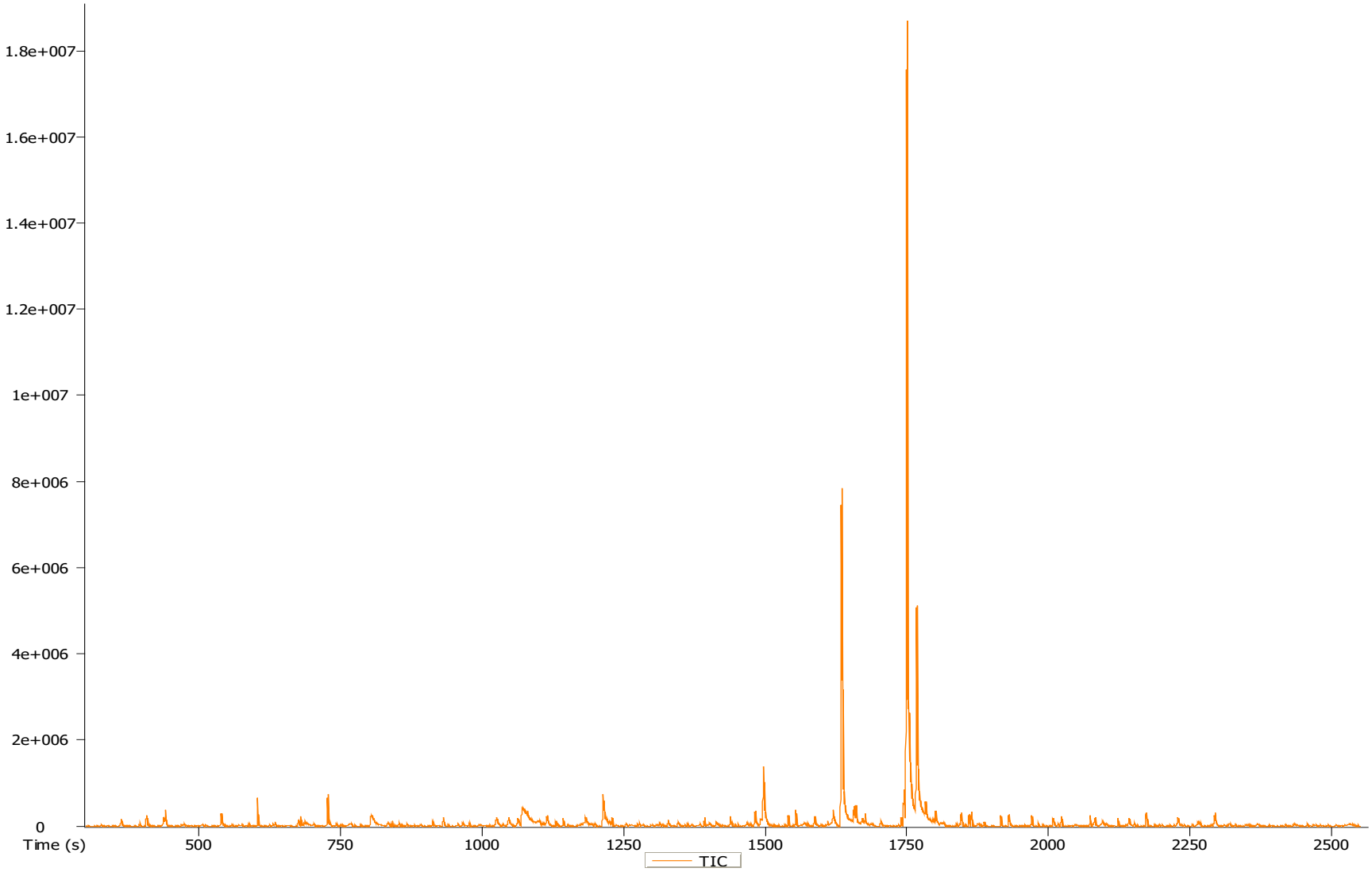


Figure 5.14. GC-MS chromatogram of a semi-pure bioactive fraction (P4) isolated from the butanol partition of *Juncus lomatophyllus*.

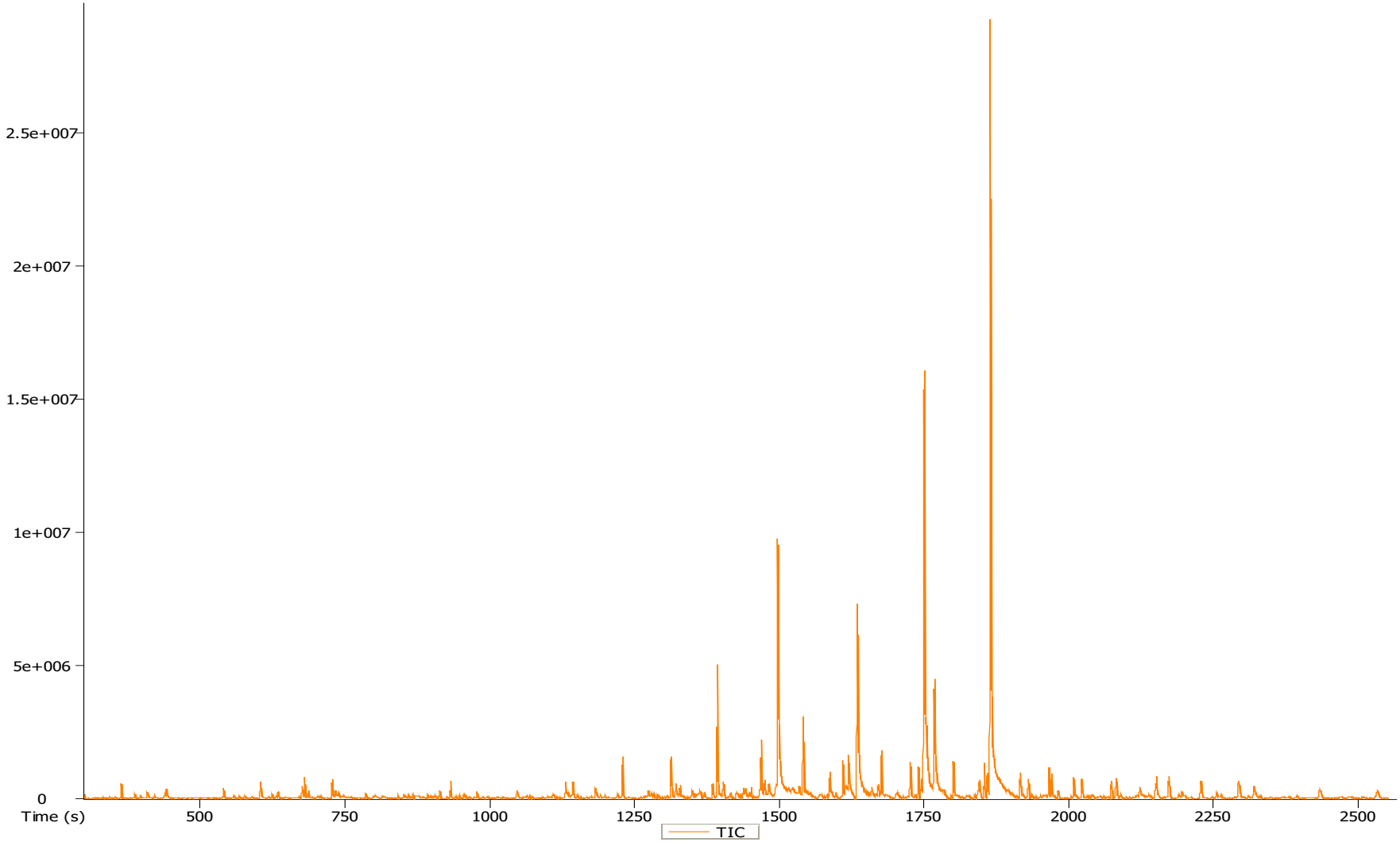


Figure 5.15. GC-MS chromatogram of a semi-pure bioactive fraction (P5) isolated from the butanol partition of *Juncus lomatophyllus*.

5.6.3 Gold nanoparticle synthesis

During the biosynthesis of JLAuNPs, the solution converted immediately from a green to wine colour once exposed to the gold salt. This visual colour change was confirmed with UV-Vis, as JLAuNPs displayed a spectral peak at 549 nm (Figure 5.16A). To determine functional groups, present in JL-EtOH and JLAuNPs, FTIR was conducted at a range of 550 to 4000 cm^{-1} (Figure 5.16B, Table 5.13). The total phenolic content of JLAuNPs was evaluated and was found to be 2.55 mg/mL, while the average diameter and zeta-potential were 166.9 ± 79.64 nm and -23.9 mV, respectively. Furthermore, the morphology of JLAuNPs was determined using HRTEM, which consisted mostly of irregular and round shapes with a few triangular nanoparticles (Figure 5.16C). A selected area diffraction pattern (SAED) was used to characterize whether JLAuNPs were similar to gold metal. The face-centred lattice of JLAuNP displayed a Bragg reflection of (111), (200), (220) and (311) (Figure 5.16D). *In vitro* stability indicated that JLAuNPs displayed minimal shifts in the surface plasmon resonance peak (λ_{max}) when exposed to various mediums (Figure 5.17).

Table 5.13. Potential functional groups were identified using Fourier transform infrared spectrometry (FTIR) in the ethanolic extract (JL-EtOH) and synthesized gold nanoparticles (JLAuNPs) of *Juncus lomatophyllus*.

Functional groups	JL-EtOH transmittance (cm^{-1})	JLAuNPs transmittance (cm^{-1})
O-H	3305	3321
C-H	2922	-
C-O	1257	-
Aromatic ring (C-C)	-	1637

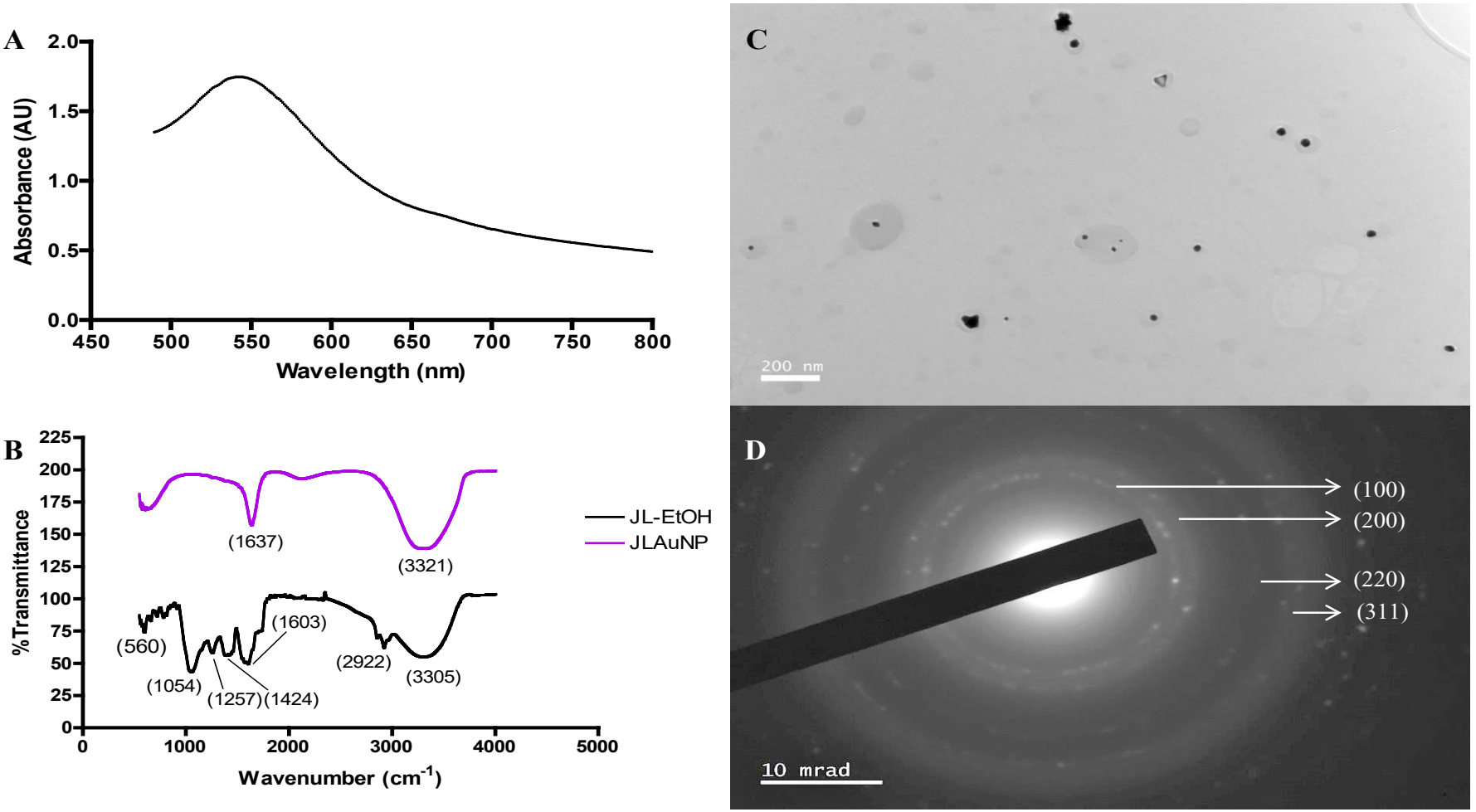


Figure 5.16. Gold nanoparticle characterization including ultraviolet-visible (UV-Vis) spectroscopy (A), Fourier-transform infrared spectrometry (FTIR) of the ethanolic extract (JL-EtOH) and synthesized gold nanoparticles (JLAuNPs) of *Juncus lomatophyllus* (B), high-resolution transmission electron microscopy (HRTEM) at 200 nm (C) and selected area diffraction pattern (SAED) at 10 mrad (D).

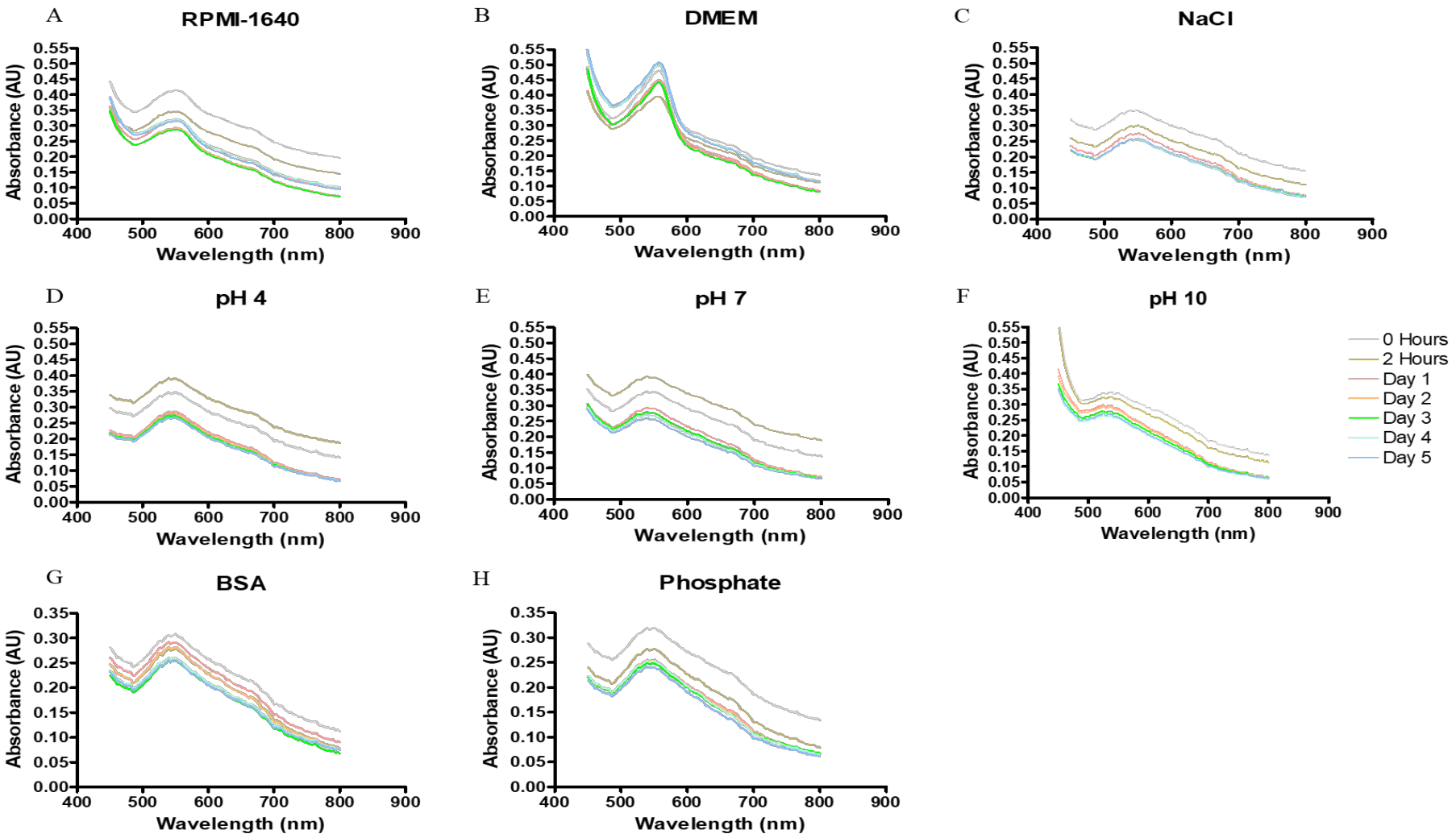


Figure 5.17. ***In vitro*** **stability of** ***Juncus lomatophyllus*** **synthesized gold nanoparticles (JLAuNPs) in different mediums. These solutions include Roswell Park Memorial Institution (RPMI-1640) medium (A), Dulbecco's modified Eagle's Medium (DMEM) (B), 5% sodium chloride (NaCl) (C), pH level of 4 (D), 7 (E) and 10 (F), 0.5% bovine serum albumin (BSA) (G) and phosphate buffer (pH 6.5) (H).**

5.6.4 Tyrosinase inhibition

Anti-tyrosinase assay was conducted on the crude, fermented and synthesised nanoparticle extracts of *Juncus lomatophyllus* (JL). Of these, only JLAuNP displayed anti-tyrosinase activity with an IC_{50} of 268.8 ± 5.64 µg/mL. The positive control used in this assay (kojic acid) displayed an IC_{50} value of 5.60 ± 1.68 µg/mL. As of current, no biological assays have been conducted on *J. lomatophyllus*. A study by Pérez et al. (2017) suggested that using nanotechnology enhances the biological activity of the crude extract and was demonstrated by the authors using ginseng berries (Pérez et al., 2017). Therefore, the anti-tyrosinase properties exhibited by the synthesised nanoparticles (JLAuNP) could be due to the presence of the gold salt, which may enhance the active compounds present in the crude extract (JL) at the highest testing concentration (400 µg/mL); however, further investigation is needed.

In a study conducted by Wang et al. (2016), Chinese herbs were selected and inoculated with *B. bifidum,* demonstrating a higher percentage inhibition towards tyrosinase than their crude counterparts (Wang et al., 2016). This could be due to the preparation method used, allowing these selected plants' biological activity to be enhanced as the concentration of the fermented extracts was higher and the fermentation period was shorter. Thus, further evaluation of the effects of different cultivation methods should be considered in future studies.

5.6.5 Antiproliferative activity

The extracts displayed no antiproliferative activity at the highest testing concentration (400 µg/mL), while the positive control, actinomycin D, displayed antiproliferative properties with an IC_{50} of 0.01 ± 0.005 µg/mL. Currently, no information regarding the antiproliferative activity of JL could be found. Antiproliferative studies on synthesised gold nanoparticles against HaCaT cells are limited. However, one study on the effects of gold salt against this cell line corresponds with the results found in this study (Dasari et al., 2015).

5.6.6 Irritancy response

A patch test was used to evaluate the irritancy response of JL when applied to the forearm of a patient. The mean score of JL (Table 5.14) was lower than the positive (1% sodium lauryl sulphate solution) and negative control (demineralized water) indicating that JL is considered a non-irritant.

Table 5.14. Irritancy response of *Juncus lomatophyllus* ethanolic extract dissolved in 40% ethanol using an *in vivo* patch test.

Product name	Average value	Mean score	Number of subjects with reactions after 48 hours	Skin compatibility Potential %	Skin Compatibility
Positive control sodium lauryl sulphate solution (1%)	0.93	1.47	12	100	Irritant
JL ethanol extract (neat)	0.16	0.32	0	-2.0	Non-irritant
Negative control-demineralized water	0.13	0.34	0	0.00	Non-irritant

5.6.7 Even tone efficacy study

An *in vivo* efficacy study was conducted on JL to confirm that the *in vitro* response that was obtained reflects when applied to a patient. As of current, no clinical data for any of the selected wetland plants has been conducted. An even skin efficacy study was conducted on the prepared formulation of JL. After 14 and 28 days, a significant reduction in dark spots could be observed and a confidence level of 5% was given in comparison to the placebo. This indicated that JL significantly reduces dark spots when applied topically, confirming the *in vitro* results that were obtained.

5.7 *Persicaria senegalensis*

5.7.1 Bioassay-guided fractionation

The butanol partition collected from PS crude extract displayed the lowest IC_{50} value against elastase production. Thereafter, column chromatography on the butanol fraction was conducted, whereby 13 major fractions were pooled. Of these fractions, F13 displayed anti-elastase activity with an IC_{50} of 39.02 ± 0.14 µg/mL (Table 5.15).

Table 5.15. Anti-elastase activity of *Persicaria senegalensis* fractions and crude extracts.

***Persicaria senegalensis* major fractions and extracts**	**Elastase inhibition IC_{50} (µg/mL ± SD)**
F1	NI_{400}
F2	NI_{400}
F3	NI_{400}
F4	NI_{400}
F5	NI_{400}
F6	NI_{400}
F7	NI_{400}
F8	NI_{400}
F9	NI_{400}
F10	NI_{400}
F11	NI_{400}
F12	NI_{400}
F13	39.02 ± 0.14
PS Fermented	10.86 ± 2.15
PS EtOH	20.24 ± 0.84
Hexane	NI_{400}
Ethyl acetate	10.04 ± 0.09
Butanol	0.62 ± 0.06
Water	NI_{400}
Butanol fermented	NI_{400}
Butanol fermented control	NI_{400}
Ethyl acetate fermented	161.3 ± 5.23
Ethyl acetate fermented control	NI_{400}
Water fermented	NI_{400}
Water fermented control	NI_{400}
Hexane fermented	NI_{400}

Persicaria senegalensis major fractions and extracts	Elastase inhibition IC_{50} (µg/mL ± SD)
Hexane fermented control	NI_{400}
Ursolic acid	22.30 ± 2.79

5.7.2 GC-MS analysis

A total of 71 constituents in PS were identified as displayed in Figure 5.18 and Table 5.16. Major constituents present in PS above a peak area of 2% included á-sitosterol (9.73%), neophytadiene (6.18%), acetate, 4-hydroxy-3-methyl-2-butenyl- (4.04%), cis-3-butyl-4-vinyl-cyclopentene (3.69%), heptacosane (3.67%), pentanoic acid, 2-methyl-, anhydride (3.61%), n-hexadecanoic acid (3.60%), 2,2-dimethyl-4-octenal (3.38%), octadecanoic acid (3.03%), benzothiazole (2.80%), decane, 2,4-dimethyl- (2.78%), adipic acid, cyclobutyl isohexyl ester (2.50%), 9,12-octadecadienoic acid, methyl ester, (E,E)- (2.50%), 2,3-dioxabicyclo[2.2.1]heptane, 1-methyl- (2.40%), stigmasterol (2.34%), lupeol (2.20%), urs-12-en-3-ol, acetate, (3á)- (2.20%) and 2,5-dimethyl-2-(2-tetrahydrofuryl)tetrahydrofuran (2.14%).

In a study conducted by Tu et al. (2014), *Clinacanthus nutans* (Burm.f) Lindau previously displayed an inhibitory effect of 68.33% against elastase at 10 µg/mL (Tu et al., 2014). Furthermore, stigmasterol has previously been isolated from this plant, which could contribute to the anti-elastase activity, however, further investigation is required (Alam et al., 2016).

Table 5.16. Constituents present in the ethanolic extract of *Persicaria senegalensis* (PS) were identified using gas chromatography-mass spectrometry (GC-MS).

No.	Name	Molecular weight	Formula
1	1-(7-Hydroxy-1,6,6-trimethyl-10-oxatricyclo[5.2.1.0(2,4)]dec-9-yl)ethanone	238	$C_{14}H_{22}O_3$
2	1,10-Undecadiene	152	$C_{11}H_{20}$
3	1,2-Butanediol	90	$C_4H_{10}O_2$
4	1,3-Dioxolane-2-propanoic acid, 2,4-dimethyl-, ethyl ester	202	$C_{10}H_{18}O_4$
5	1-Hexanol, 2-(hydroxymethyl)-	132	$C_7H_{16}O_2$
6	1-Hexene, 3,4,5-trimethyl-	126	C_9H_{18}
7	1-Iodo-2-methylundecane	296	$C_{12}H_{25}I$
8	2(3H)-Furanone, 5-ethenyldihydro-5-methyl-	126	$C_7H_{10}O_2$
9	2(3H)-Furanone, dihydro-5-methyl-	100	$C_5H_8O_2$
10	2,2-Dimethyl-4-octenal	154	$C_{10}H_{18}O$
11	2,3-Dioxabicyclo[2.2.1]heptane, 1-methyl-	114	$C_6H_{10}O_2$
12	2,5-Dimethyl-2-(2-tetrahydrofuryl)tetrahydrofuran	170	$C_{10}H_{18}O_2$
13	2,6-Heptadien-1-ol, 2,4-dimethyl-	140	$C_9H_{16}O$
14	2-Butanone, 3,4-epoxy-3-ethyl-	114	$C_6H_{10}O_2$
15	2-Cyclohexen-1-one, 6-(1-hydroxy-1-methylethyl)-3-methyl-	168	$C_{10}H_{16}O_2$
16	2-Heptene, 5-ethyl-2,4-dimethyl-	154	$C_{11}H_{22}$
17	2-Hexanone, 6-bromo-	178	$C_6H_{11}BrO$

No.	Name	Molecular weight	Formula
18	2-Methyl-4-octenal	140	$C_9H_{16}O$
19	2-methyloctacosane	408	$C_{29}H_{60}$
20	2-Pentenoic acid, 2-methoxy-3-methyl-, methyl ester	158	$C_8H_{14}O_3$
21	3-(5-Methylfuryl)-N-furamidopropionamide	262	$C_{13}H_{14}N_2O_4$
22	3,4-Diacetylfurazan	154	$C_6H_6N_2O_3$
23	3-Acetoxytridecane	242	$C_{15}H_{30}O_2$
24	4H-Imidazol-4-one, 2-amino-1,5-dihydro-	99	$C_3H_5N_3O$
25	4-Nonanone, 7-ethyl-	170	$C_{11}H_{22}O$
26	5-Decen-3-one, 9-hydroxy-2,2,9-trimethyl	212	$C_{13}H_{24}O_2$
27	6,7-Dodecanedione	198	$C_{12}H_{22}O_2$
28	6-Butyl-1,4-cycloheptadiene	150	$C_{11}H_{18}$
29	8-Nonen-2-one	140	$C_9H_{16}O$
30	9,12-Octadecadienoic acid, methyl ester, (E,E)-	294	$C_{19}H_{34}O_2$
31	á-Amyrin	426	$C_{30}H_{50}O$
32	Acetate, 4-hydroxy-3-methyl-2-butenyl-	144	$C_7H_{12}O_3$
33	Adipic acid, cyclobutyl isohexyl ester	284	$C_{16}H_{28}O_4$
34	á-Sitosterol	414	$C_{29}H_{50}O$
35	Benzothiazole	135	C_7H_5NS
36	Bicyclo[3.1.1]heptan-2-one, 3,6,6-trimethyl-	152	$C_{10}H_{16}O$
37	Carbonic acid, eicosyl vinyl ester	368	$C_{23}H_{44}O_3$
38	cis-3-Butyl-4-vinyl-cyclopentene	150	$C_{11}H_{18}$
39	ç-Tocopherol	416	$C_{28}H_{48}O_2$
40	Cyclopentane, 1-acetyl-1,2-epoxy-	126	$C_7H_{10}O_2$
41	Cyclopentanol, 2-methyl-, acetate, cis-	142	$C_8H_{14}O_2$
42	Decane	142	$C_{10}H_{22}$
43	Decane, 2,4-dimethyl-	170	$C_{12}H_{26}$
44	dl-à-Tocopherol	430	$C_{29}H_{50}O_2$
45	Eicosane	282	$C_{20}H_{42}$
46	Heptacosane	380	$C_{27}H_{56}$
47	Hexadecane	226	$C_{16}H_{34}$
48	Hexadecanoic acid, butyl ester	312	$C_{20}H_{40}O_2$
49	Hexanedioic acid, bis(2-ethylhexyl) ester	370	$C_{22}H_{42}O_4$
50	Hexanoic acid, anhydride	214	$C_{12}H_{22}O_3$
51	Ledol	222	$C_{15}H_{26}O$
52	Lilac aldehyde A	168	$C_{10}H_{16}O_2$
53	Lupeol	426	$C_{30}H_{50}O$
54	Neophytadiene	278	$C_{20}H_{38}$
55	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$
56	Octadecanoic acid	284	$C_{18}H_{36}O_2$
57	Octadecanoic acid, ethyl ester	312	$C_{20}H_{40}O_2$
58	Octahydrobenzo[b]pyran, 4a-acetoxy-5,5,8a-trimethyl-	240	$C_{14}H_{24}O_3$
59	Pentanoic acid, 2-methyl-, anhydride	214	$C_{12}H_{22}O_3$
60	Phthalic acid, butyl hex-3-yl ester	306	$C_{18}H_{26}O_4$
61	Phthalic acid, di(6-methylhept-2-yl) ester	390	$C_{24}H_{38}O_4$

No.	Name	Molecular weight	Formula
62	Phytol	296	$C_{20}H_{40}O$
63	p-Nitrophenyl hexanoate	237	$C_{12}H_{15}NO_4$
64	Pyrrolidin-2-one, 5-heptyl-	183	$C_{11}H_{21}NO$
65	Silane, tetraethenyl-	136	$C_8H_{12}Si$
66	Squalene	410	$C_{30}H_{50}$
67	Stigmasterol	412	$C_{29}H_{48}O$
68	Tetracosane	338	$C_{24}H_{50}$
69	Undecane	156	$C_{11}H_{24}$
70	Undecanoic acid, ethyl ester	214	$C_{13}H_{26}O_2$
71	Urs-12-en-3-ol, acetate, (3á)-	468	$C_{32}H_{52}O_2$

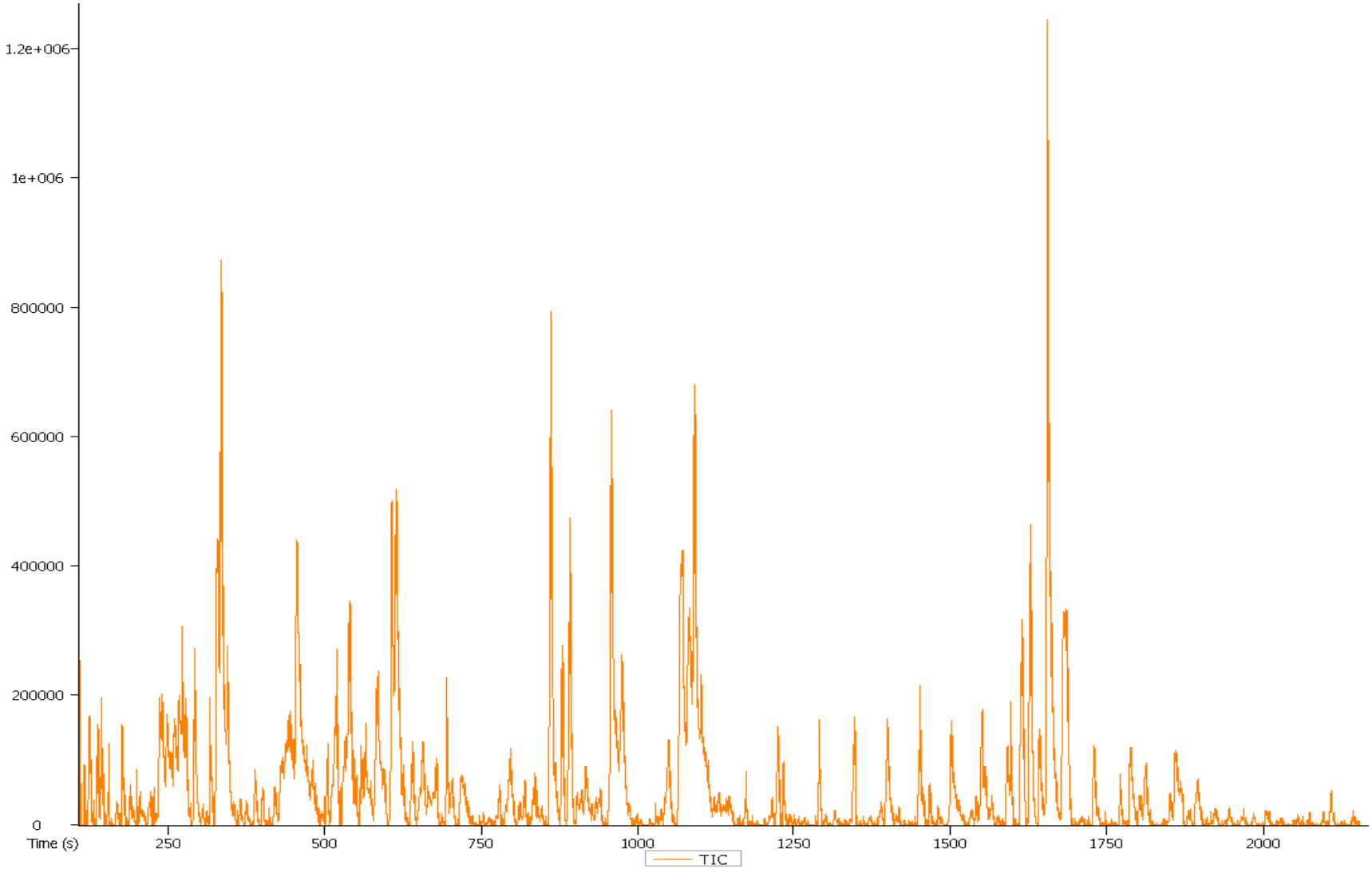


Figure 5.18. Gas chromatography-mass spectrometry (GC-MS) spectrometry of constituents present in the ethanolic extract of *Persicaria senegalensis* (PS).

The fermented extract of *Persicaria senegalensis* has been submitted for GC-MS analysis at the University of Pretoria's Department of Chemistry in order to conduct a multivariate analysis and to better distinguish the phytochemical differences that may be responsible for the observed enhanced bioactivity.

5.7.3 Gold nanoparticle synthesis

During the preparation of the gold nanoparticles, the crude extract solution of PS converted from a green to wine colour once exposed to the gold salt. This visual colour change indicated the gold salt bound to the plant extract, which was confirmed spectrometrically using UV-Vis. The diameter and zeta-potential of the synthesised nanoparticles were measured. Using DLS and zeta potential, the average diameter and charge of PSAuNP was found (Figure 5.19 C and D). In a study conducted by Salopek et al. (1992) the authors assessed the stability of different zeta-potential sizes. In accordance with this, PSAuNP display peptization due to the higher binding affinity towards the stabilising agent (gum arabic) (Salopek et al., 1992). This indicates that the synthesised nanoparticles are unstable, leading to the aggregation of particles resulting in a larger diameter, as observed with the DLS results. This correlates with a study conducted by Tantra et al. (2010), which measured an increase in diameter size within the unstable region of the nanoparticles (Tantra et al., 2010).

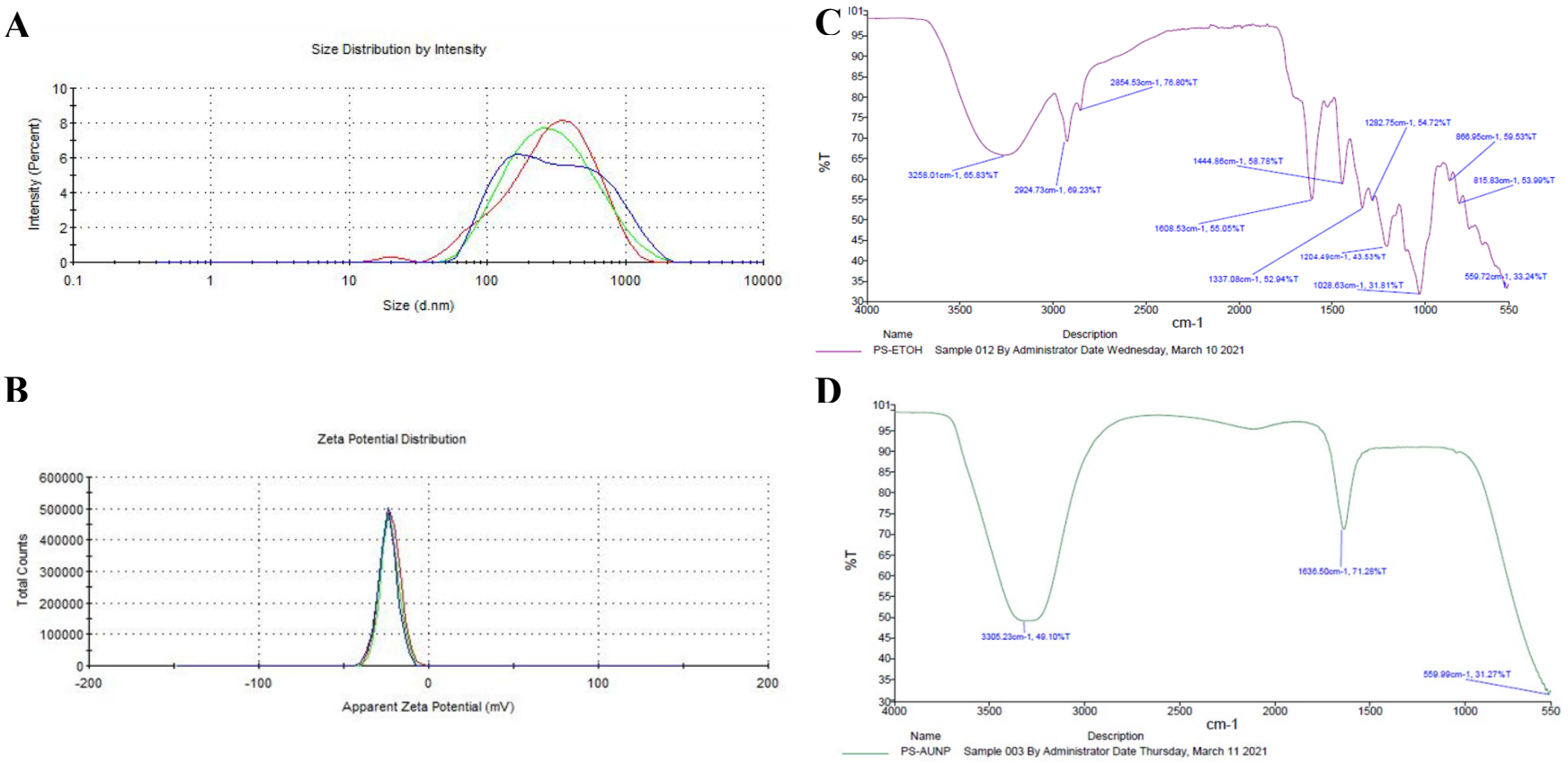


Figure 5.19. Nanoparticle characterisation including (A) dynamic light scattering, (B) zeta-potential, (C and D) Fourier-transform infrared spectroscopy of *Persicaria senegalensis.*

5.7.4 *In vitro* stability

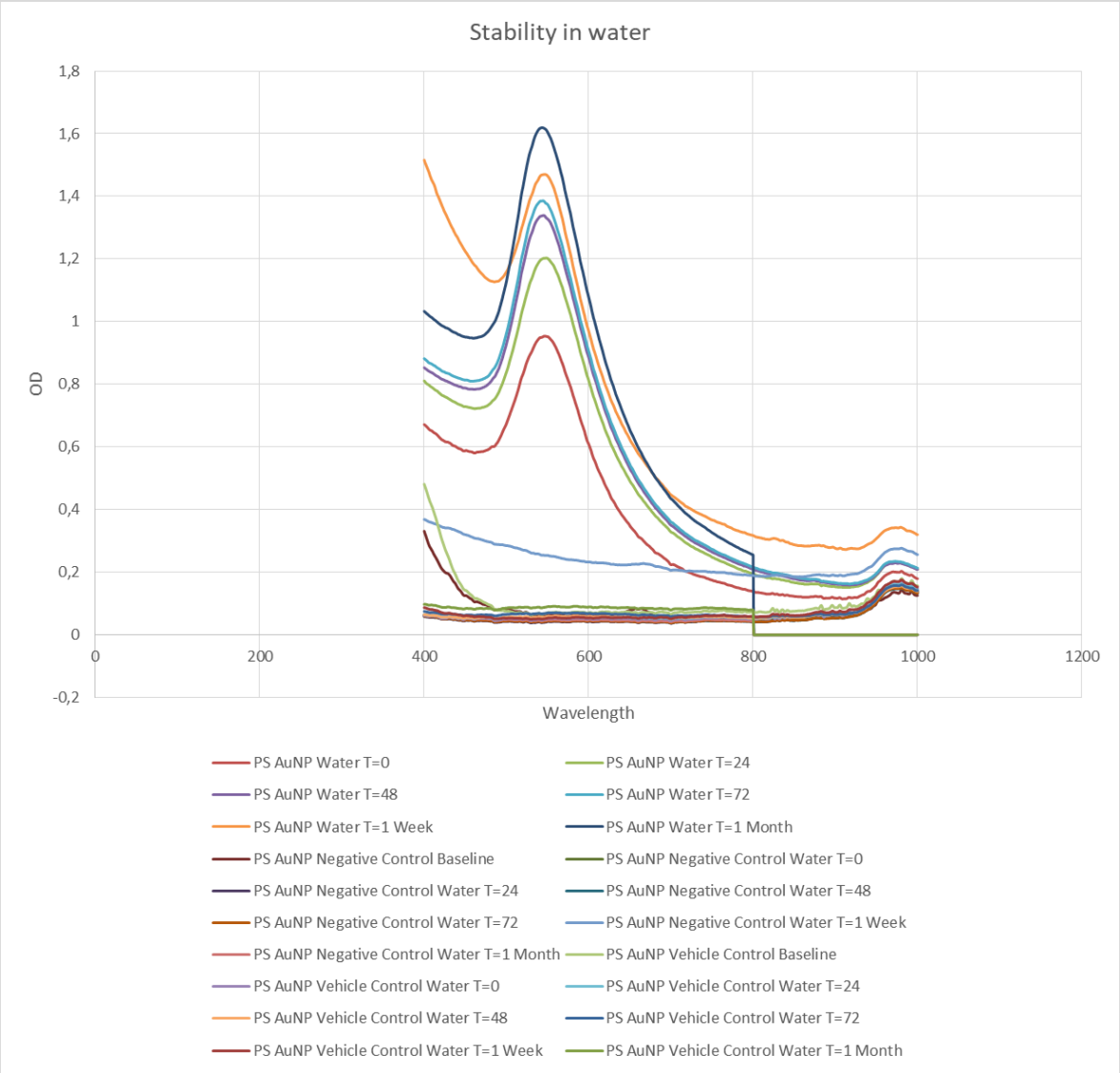


Figure 5.20. Stability of *Persicaria senegalensis* gold nanoparticles, negative and vehicle controls in water at T=0, T=24h, T=48h, T=72h, T=1 week and T=1 month.

Stability studies were conducted on PSAuNP in various biological reagents used in biological assays. These studies were done to determine if the nanoparticles are stable during various biological assays and potentially in the human body. The absence of a peak within the 500-600 nm range for the negative and vehicle controls confirms that gold nanoparticle formation was due to the interaction between the phytochemical extract and gold salt.

The presence of the peak in this region confirms the formation of gold nanoparticles and is accompanied by a visual red wine colour. The increasing OD for the peaks observed for each time point in Figure 5.20 indicates that the gold nanoparticles continue to synthesise until the final measurement of 1 month, indicating that at T=0, synthesis was incomplete. There were

no noteworthy red/blue shifts. The results indicate the nanoparticles to be stable in water over the observed period.

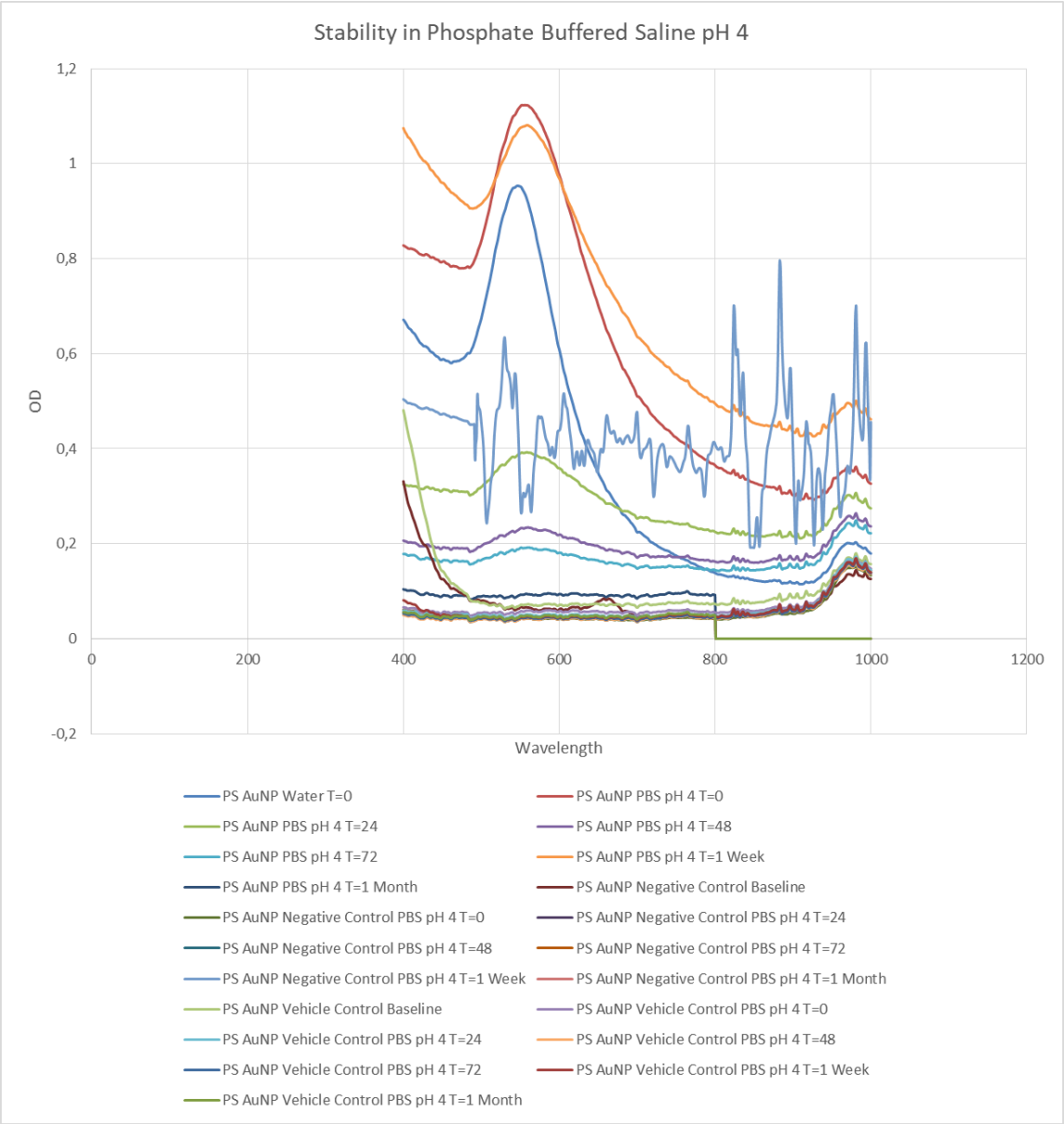


Figure 5.21. Stability of *Persicaria senegalensis* gold nanoparticles, negative and vehicle controls in phosphate-buffered saline (pH 4) at T=0, T=24h, T=48h, T=72h, T=1 week and T=1 month.

The concentration of the gold nanoparticles declines with the increasing time points in PBS at a pH 4 with the peak intensities observed in descending order as follows: t=0 >1 week >24h > 48h >72h > 1 month (Figure 5.21). This general trend indicates that extended periods in PBS at a pH4 deteriorate the gold nanoparticles resulting in a lower concentration and that the nanoparticles are sensitive to a low pH. Interestingly, the maximum peak absorbance observed for t=o is higher than that observed for water at t=0, indicating that at the time of the baseline reading, the gold nanoparticles were more concentrated in the PBS (pH 4), and the same is true for t=1 week in PBS (pH 4) and t=0 in water. However, between one week and one month, the concentration declines. The trend is not linear in that the concentration of the gold nanoparticles

Between t=0 and t=72 h declines with each measured time point but then increases at one week and proceeds to decline once again. This may indicate that synthesis is not stable, which may result from continuous reduction/oxidation reactions between the phytochemicals and colloidal gold. This may also result from the low pH in which compounds such as phenolics are unstable.

Compared to the stability results obtained in water, the gold nanoparticles in PBS (pH 4) exhibited a moderate redshift of 10-15 nm. It is unlikely that ligand binding is the reason for this shift, as when comparing the peak absorbance within the results obtained only in PBS (pH 4), there is none. This indicates the local refractive index change may instead be due to further enhancement of the electromagnetic field at points of the unevenness of the particle structure (which remains to be confirmed in HRTEM studies). Alternatively, this shift may result from particle intercoupling (aggregation).

Suspended in PBS at a pH of 7 (Figure 5.22), the synthesis of gold nanoparticles generally appears to increase over the time points observed as observed by the increasing peak intensities for each time point. This is the opposite of the results observed for PBS in pH 4, indicating that the gold nanoparticles are more stable at a higher pH similar to that of physiological conditions or even higher, more basic conditions as observed in PBS pH 10 (Figure 5.23). Despite the increase in gold nanoparticle concentration over time, the maximum concentration reached at one month remained lower than the result obtained for water at one month. The maximum concentrations in descending order are as follows: water 1 month > PBS pH 7 1 month > PBS pH 7 48h > PBS pH 7 24h > PBS pH 7 0h > water 0h. Interestingly, when comparing the baseline concentrations for water and PBS, the gold nanoparticles were more concentrated in PBS (pH 7). The peaks at 72 h and one month showed a moderate blue shift of ~20 nm, again indicating unstable ligands transitioning between conjugated and unconjugated states, ultimately reverting to a more reduced spheroid state.

Similarly, in PBS pH 10 (Figure 5.23), the gold nanoparticles continue synthesising over time to reach a maximum concentration at one month, with these nanoparticles also exhibiting a moderate blue shift of 10 nm. It is also interesting to note that the final concentration of gold nanoparticles in this buffer system was greater than that obtained in water and PBS pH 4 or 7, indicating that more alkaline conditions are better suited for synthesis.

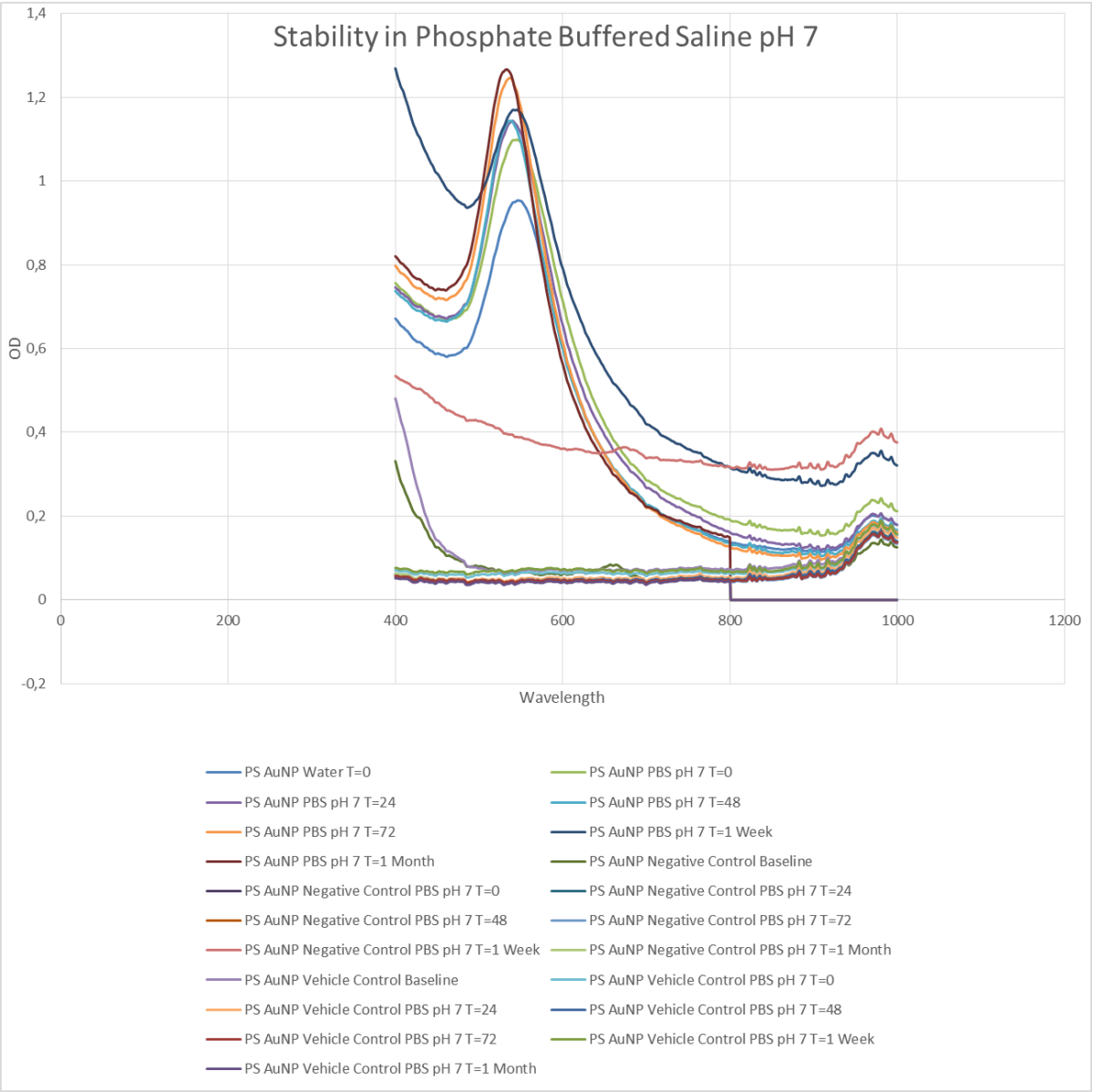


Figure 5.22. Stability of *Persicaria senegalensis* gold nanoparticles, negative and vehicle controls in phosphate-buffered saline (pH 7) at T=0, T=24h, T=48h, T=72h, T=1 week and T=1 month.

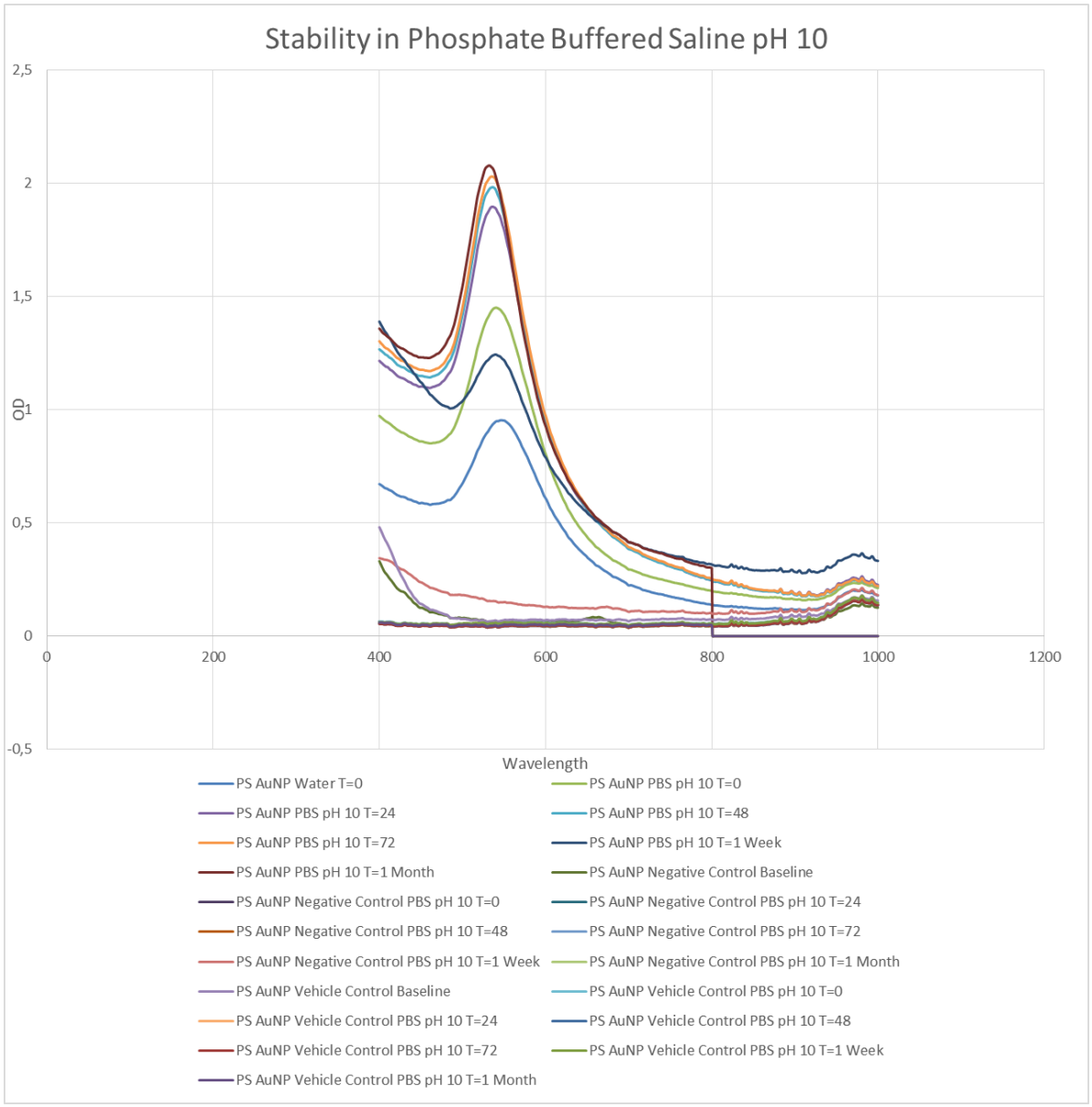


Figure 5.23. Stability of *Persicaria senegalensis* gold nanoparticles, negative and vehicle controls in phosphate-buffered saline (pH 10) at T=0, T=24h, T=48h, T=72h, T=1 week and T=1 month.

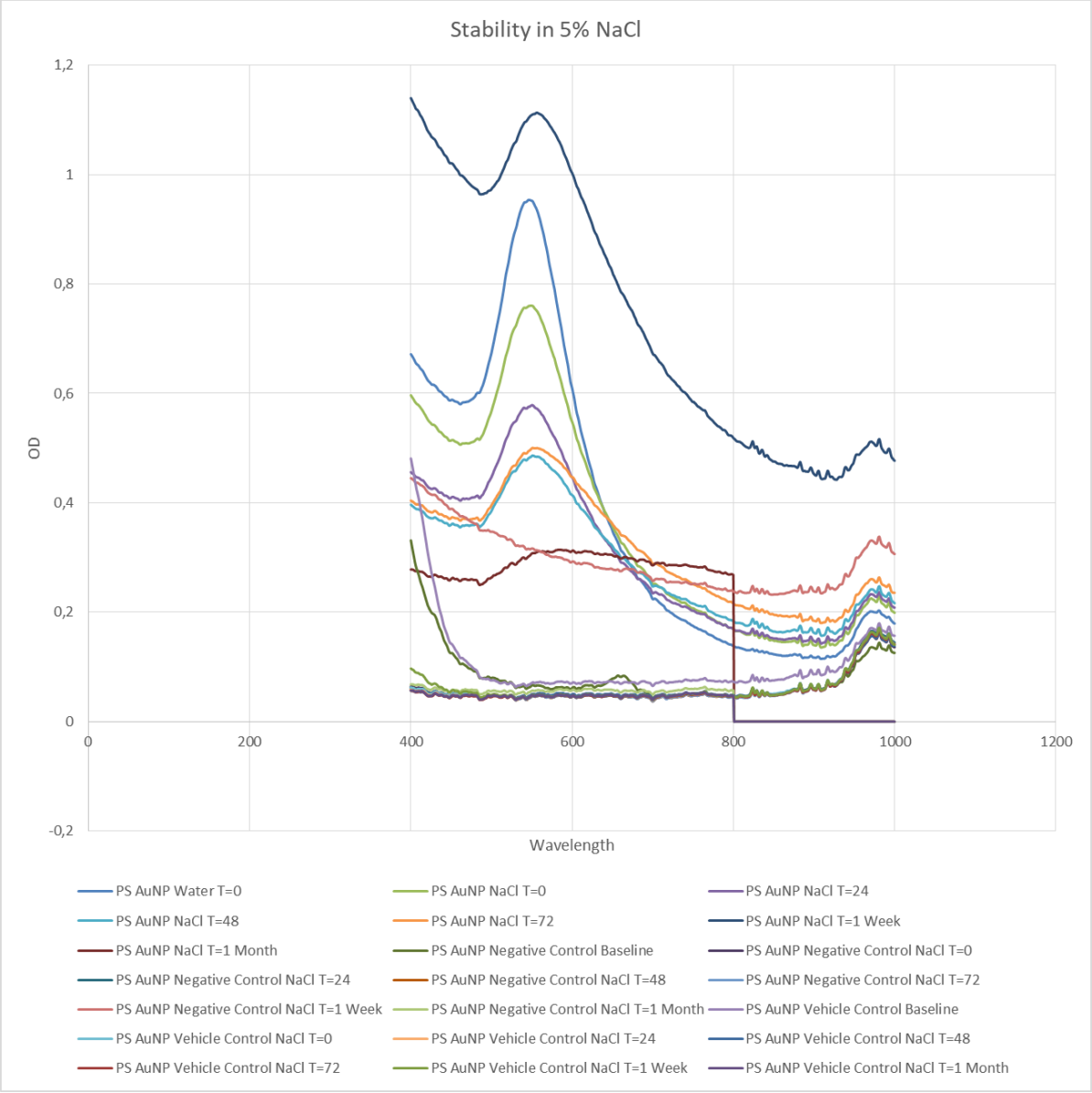


Figure 5.24. Stability of *Persicaria senegalensis* gold nanoparticles, negative and vehicle controls in 5% sodium chloride (NaCl) at T=0, T=24h, T=48h, T=72h, T=1 week and T=1 month.

The most concentrated gold nanoparticles were obtained at one week; however, this maximum absorbance remains lower than the findings obtained for the stability of the gold nanoparticles in water. The peak width is also wider than for the graphs obtained for water stability, which indicated a larger range in the average particle diameter and a less uniform distribution of gold nanoparticles. The order of highest to lowest concentration is as follows: Water one month > NaCl one week > water 0h > NaCl 0h > NaCl 24h > NaCl 72h > NaCl 48h (Figure 5.24).

Literature indicates that non-functionalised gold nanoparticles aggregate in sodium chloride; however, conjugation of these nanoparticles prevents this. Thus, the absence of a redshift indicates that these nanoparticles are conjugated.

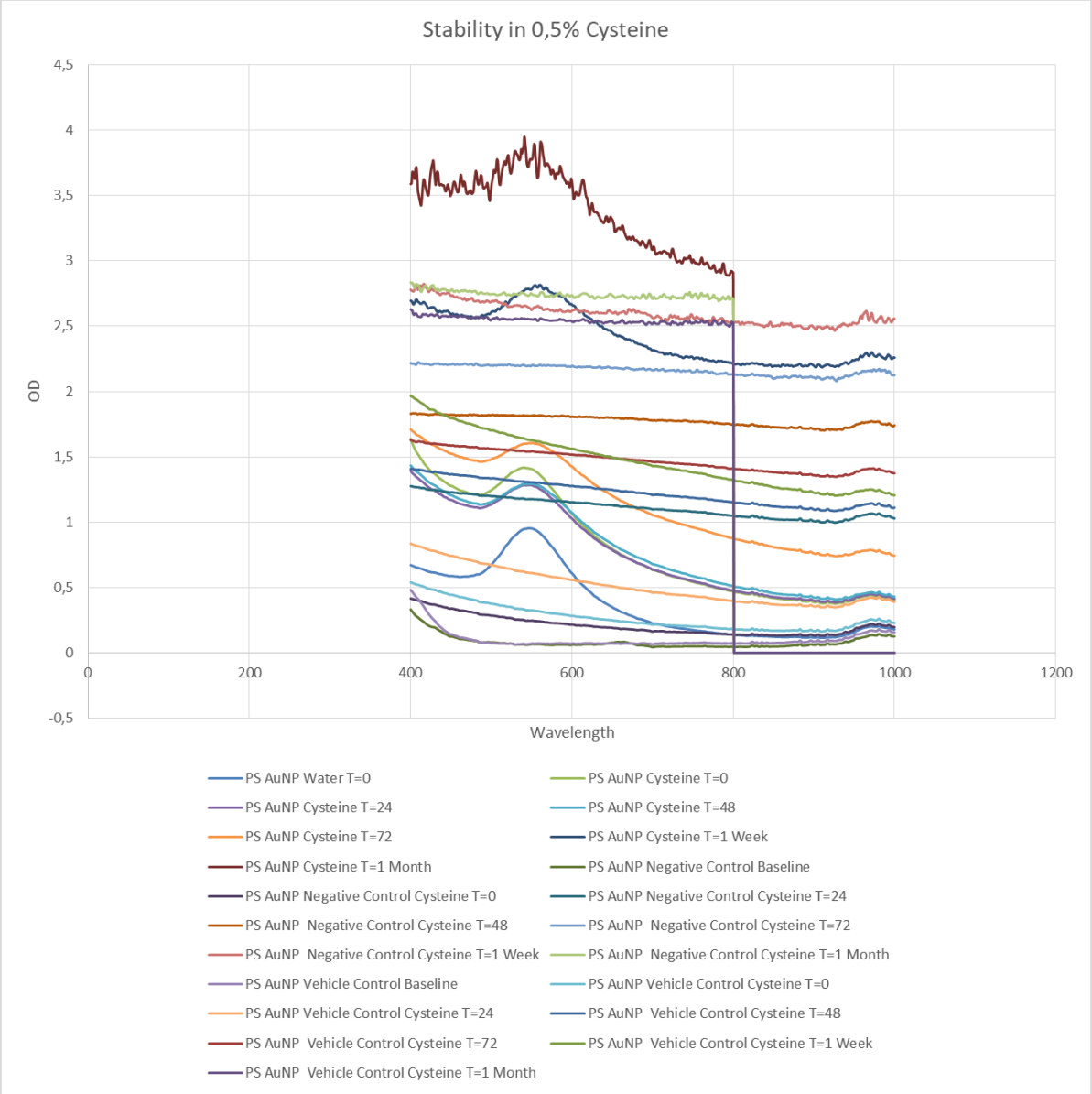


Figure 5.25. Stability of *Persicaria senegalensis* gold nanoparticles, negative and vehicle controls in 0.5% cysteine at T=0, T=24h, T=48h, T=72h, T=1 week and T=1 month.

The maximum peak absorbance was obtained at one month for the stability results of the gold nanoparticles in 0.5% cysteine; however, the broad and flattened nature of the cure indicates that a wide range of particle sizes and shapes were obtained. This peak absorbance, which is significantly higher than the maximum peaks obtained for any other stability results, is because cysteine is often used as a capping/ reducing agent in the synthesis of gold nanoparticles and, as such, is the result of the interaction between the gold nanoparticles and cysteine, and not the gold nanoparticles formed by the plant extract (Figure 5.26).

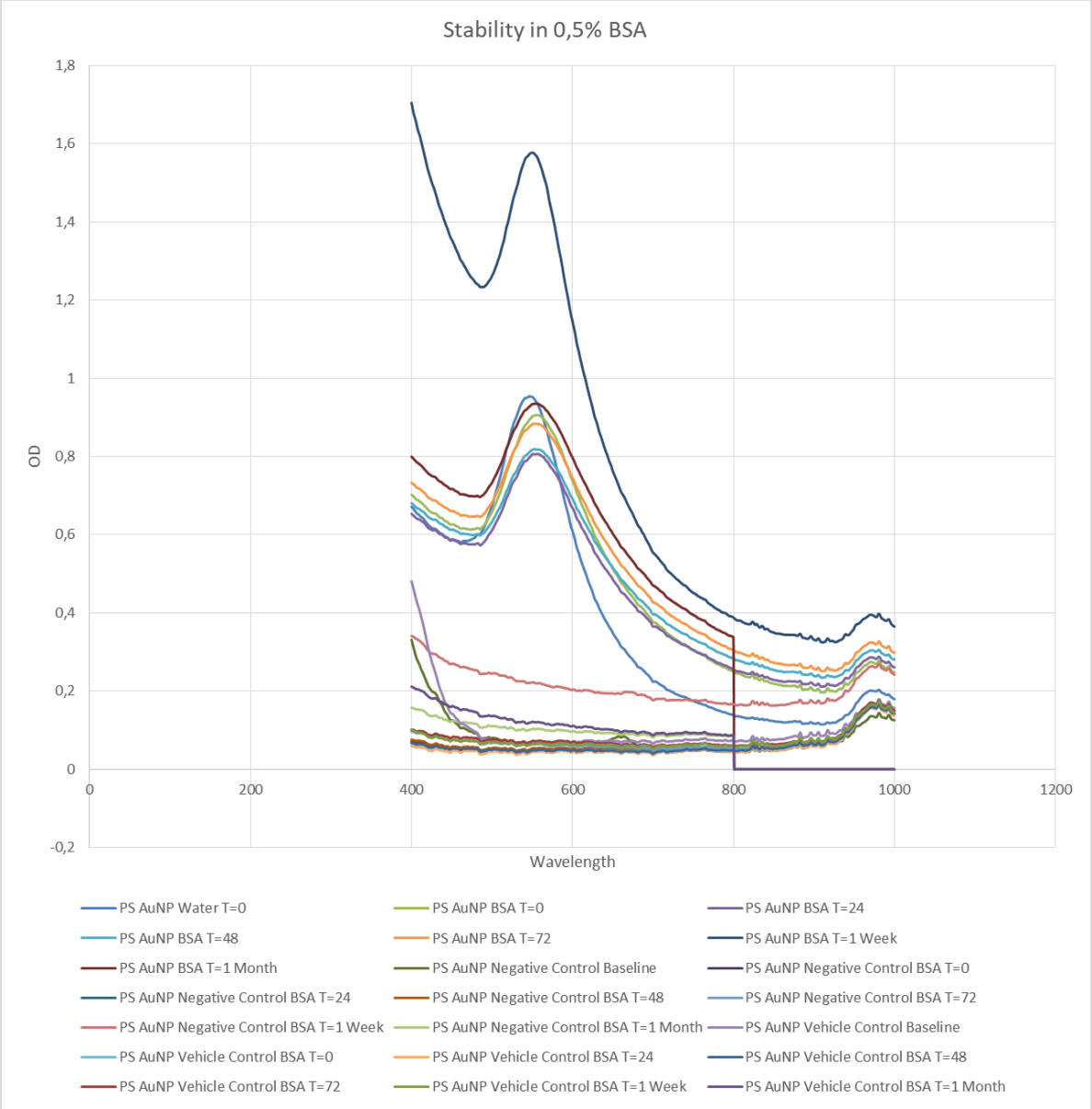


Figure 5.26. Stability of *Persicaria senegalensis* gold nanoparticles, negative and vehicle controls in 0.5% bovine serum albumin (BSA) at T=0, T=24h, T=48h, T=72h, T=1 week and T=1 month.

The fluctuations of the maximum absorbance peaks observed for each time point indicate that the gold nanoparticles are not stable in 0.5% BSA.

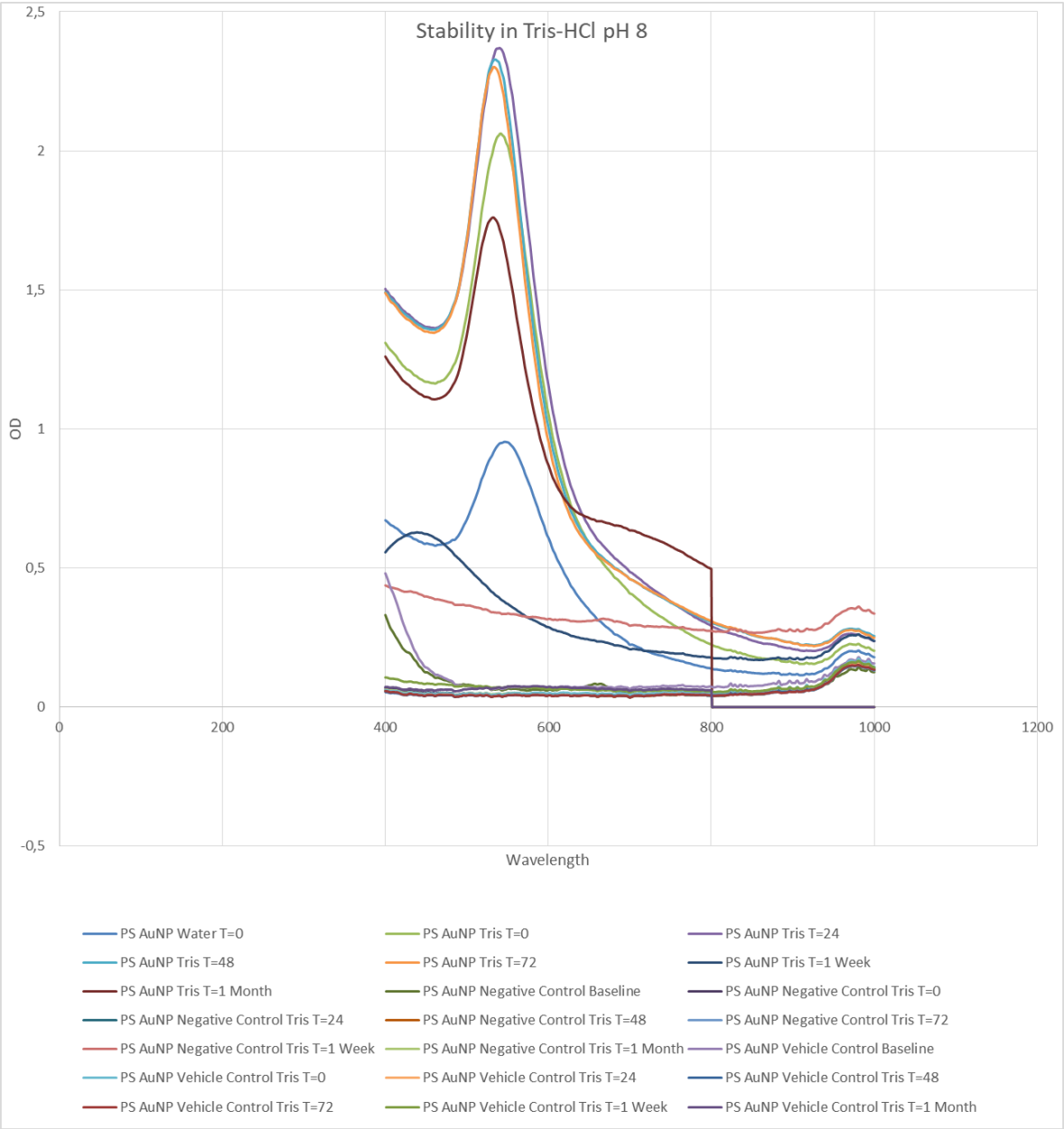


Figure 5.27. Stability of *Persicaria senegalensis* gold nanoparticles, negative and vehicle controls in Tris-HCl at T=0, T=24h, T=48h, T=72h, T=1 week and T=1 month.

For the stability results recorded in the Tris-HCl buffer system, unlike the others, the highest concentration of gold nanoparticles was observed for the 24h period, followed by 48h, 72h, 0h, one month and one week. After one week, a major blue shift (~100 nm) was observed and a flattening of the peak; however, the sample appears to have reached a more stable form at one month with an improved and acceptable peak within the expected limits. Further, the remaining time points all exhibit red/blue shifts relative to one another, indicating the instability of the gold nanoparticles and fluctuation between conjugated and unconjugated states.

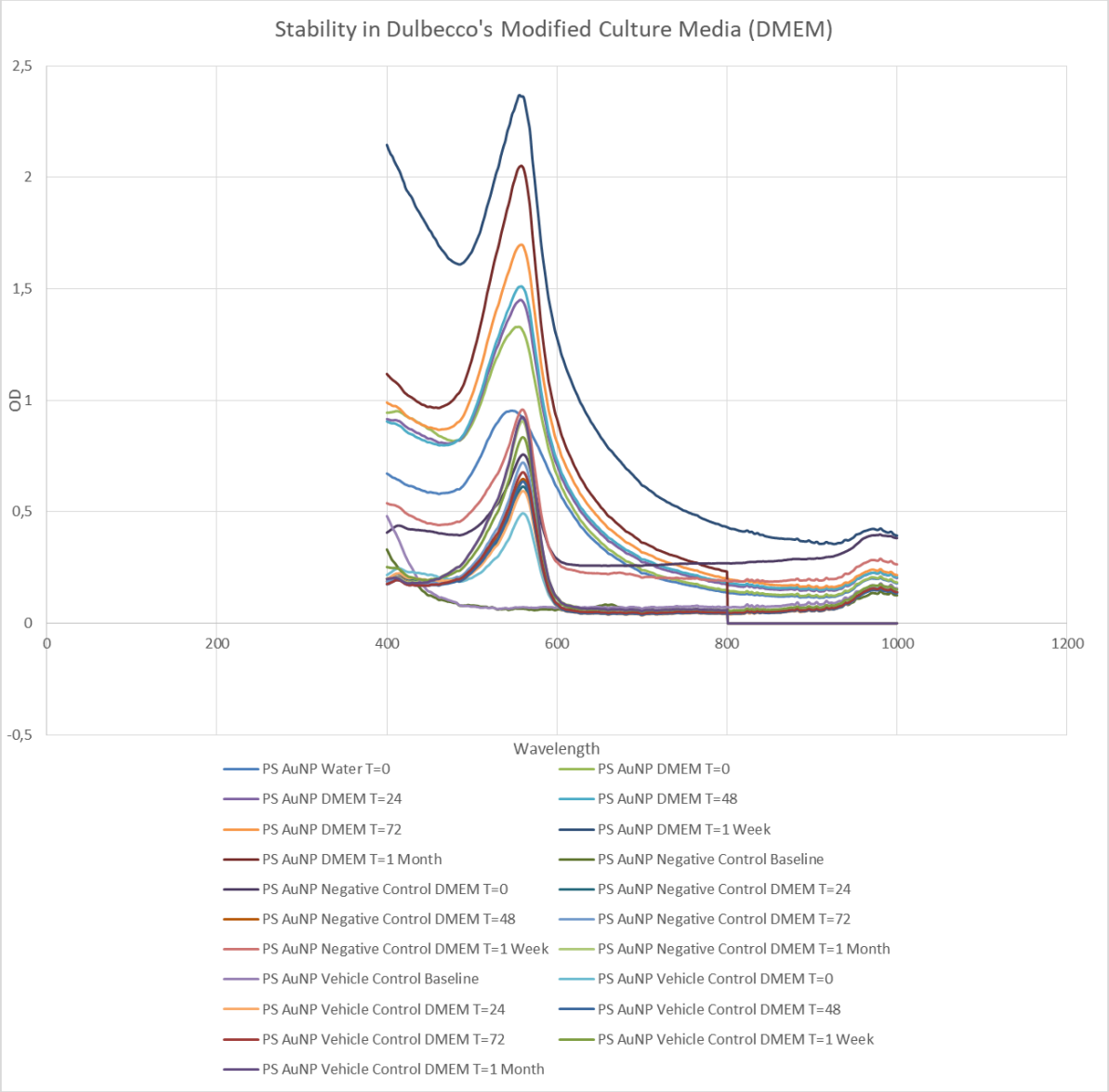


Figure 5.28. Stability of *Persicaria senegalensis* gold nanoparticles, negative and vehicle controls in Dulbecco's Modified Culture Media (DMEM) at T=0, T=24h, T=48h, T=72h, T=1 week and T=1 month.

Finally, the stability of the gold nanoparticles in Dulbecco's Modified Culture media (Figure 5.28) revealed the lowest concentration at t=0, which increased with each timepoint up until one week, and which decreased at one month, indicating that the optimal time for use in a culture media is one week. The peaks also appear consistently narrow and uniform with minimal blue/redshifts.

The gold nanoparticles exhibited varying stability according to each buffer system and pH and exhibited a general tendency to remain more stable or continue synthesising optimally in those with a higher pH than those at a lower pH. The synthesis of the gold nanoparticles also generally appears to reach a peak after one week and may not remain stable for periods longer than that. Generally, Tris-HCl appeared to exhibit the best buffer for the preservation and stability of the gold nanoparticles, while suitable stability was observed in the culture media.

These findings provide valuable insights into the stability of gold nanoparticles which can be applied to various *in vitro* experiments.

5.7.5 Elastase inhibition

An elastase inhibition assay was conducted on the crude, fermented, and synthesised nanoparticles of PS. An increase in activity was observed for PS fermented sample compared to the ethanolic extract with IC_{50} values of 14.56 ± 1.27 µg/mL and 20.24 ± 0.84 µg/mL, respectively. The positive control (ursolic acid) displayed an IC_{50} of 22.30 ± 2.79 µg/mL. A study done by Park and Bae (2016) demonstrated that *B. bifidum* fermented *Acanthopanax koreanum* root extract displayed greater antioxidant and anti-photoaging activity than the crude extract due to the repression of specific signalling pathways. These pathways include the UVB- or H_2O_2-induced activities of matrix metalloproteinase (MMP)-1 and -3, the overexpression of MMP-1 and the activation of nuclear factor kappa beta (NF-κB). This study indicated that fermented plant extracts might produce anti-wrinkle effects; however, the effect these extracts may have on the release of elastase was not mentioned (Park and Bae, 2016).

5.7.6 Antiproliferative activity

The extracts displayed no antiproliferative activity at the highest testing concentration (400 µg/mL), while the positive control, actinomycin D, displayed antiproliferative properties with an IC_{50} of 0.01 ± 0.005 µg/mL. Currently, no information regarding the antiproliferative activity of PS could be found. Antiproliferative studies on fermented extracts against HaCaT cells are limited.

5.7.7 Irritancy response

A patch test was used to evaluate the irritancy response of PS when applied to the forearm of a patient. The ethanolic PS (Table 5.17) displayed a larger mean score than the negative control and thus was considered a mild irritant. Due to the significant anti-elastase properties of the fermented PS extract, an irritancy test was conducted. Interestingly, the irritancy response of PS decreased when fermented resulting in a lower mean score indicating that PS fermented was considered a non-irritant.

Table 5.17. Irritancy response of *Persicaria senegalensis* ethanolic and fermented extract dissolved in 40% ethanol using an *in vivo* patch test.

Product name	Average value	Mean score	Number of subjects with reactions after 48 hours	Skin compatibility Potential %	Skin Compatibility
Positive control sodium lauryl sulphate solution (1%)	0.59	1.25	8	100	Irritant
PS ethanol extract (neat)	0.23	0.48	0	18.66	Mild irritant
PS fermented extract (neat)			0		Non-irritant
Negative control-demineralized water	0.13	0.30	0	0.00	Non-irritant

Skin irritation is defined as a minor injury or inflammation when exposed to a substance resulting in itching and redness. This response, however, does not initiate an immune response and only causes slight discomfort until the irritant is removed (Chris, 2022). According to healthcare workers, products that contain ethanol causes skin irritation when used, however, a study conducted by Loffler et al. (2007) demonstrated this to be inaccurate. However, when applied to damaged or irritated skin, patients reported experiencing a burning sensation (Löffler et al., 2007). This indicates that compounds present in PS may play a role, however, further research is required to confirm this.

This supports the non-irritant response observed for PSF since fermenting the ethanolic extract may alter the chemical composition. In a study conducted by Wu et al. (2021), blueberries (*Vaccinium caesariense* Mack.) and blackberries (*Rubus fruticosus* G.N. Jones) were fermented with either *Lactobacillus plantarum* and *Streptococcus thermophilus* or *Bifidobacterium bifidum*. This study showed that by fermenting the berries the chemical composition was altered as there was a shift in the presence of organic acids, anthocyanin and some phenolic acids in comparison to the fresh material (Wu et al., 2021).

5.7.8 Wrinkle reduction efficacy study

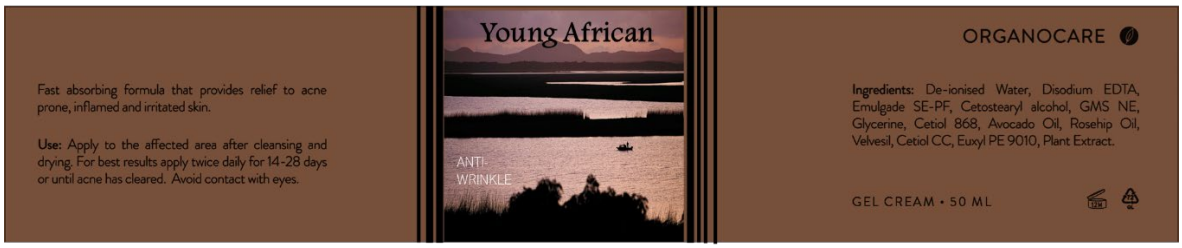
To evaluate the efficacy of PS, a wrinkle reduction efficacy study was conducted. After 28 days, the fermented extracts of PS significantly reduced the topography or depth of the wrinkles with a confidence level of 5% in comparison to the placebo. However, the ethanolic extract of PS reduced wrinkle formation after 14 days with a confidence level of 5% in comparison to the placebo. This shift in the significant response duration between PS ethanolic and fermented extract could be due to the presence or abundance of compounds once fermented. As shown in a study conducted by Wu et al. (2021) by fermenting the blue and blackberries, shifts in the abundance of compounds were observed indicating that fermentation could potentially alter the chemical composition (Wu et al., 2021).

CHAPTER 6: MARKETING

Due to the significant biological activity of JL, ET and PS prototypes were designed. To market these prototypes to formulators and manufacturers a marketing sheet and technical file was generated. Furthermore, the international nomenclature cosmetic ingredient (INCI) name of the plants was registered.

6.1 Marketing sheet

A market or product data sheet contains a summary of the performance of the product (Figure 6.1-6.3) as well as certain characteristics associated with it. These characteristics form part of the technical file. The purpose behind generating a product sheet is to provide information to the public, beneficial use of the product, what it is comprised of, how to use the product and mentions any concerns associated with it (Corrosionpedia, 2019).



Indications:

Young African is indicated to assist in the reduction of the appearance of wrinkles.

How does it work?

This product contains, among other ingredients, an active ingredient that has the ability to reduce the amount of elastase protein in the skin which in return reduces the appearance of wrinkles.

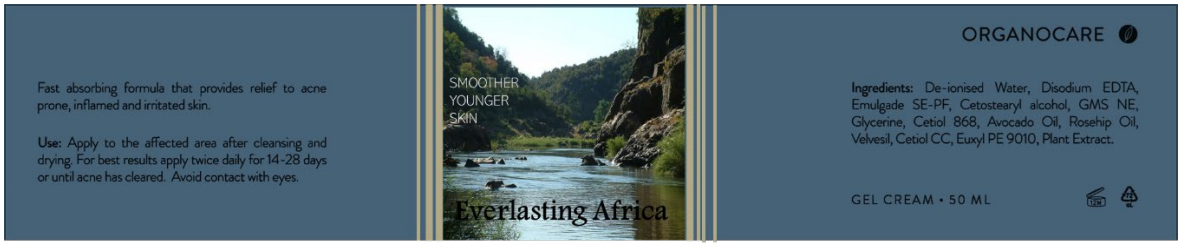
INCI name

Persicaria senegalensis aerial plant part extract

Funders

This product development was funded by the Water Research Commission and the University of Pretoria.

Figure 6.1. Visual of the packaging of Young African containing *Persicaria senegalensis* and a summary on the performance of the product



Indications:

Everlasting Africa is indicated to assist in the reduction of the appearance of wrinkles.

How does it work?

This product contains, among other ingredients, an active ingredient that has the ability to reduce the amount of elastase protein in the skin which in return reduces the appearance of wrinkles.

INCI name

Elegia tectorum stem extract

Funders

This product development was funded by the Water Research Commission and the University of Pretoria.

Figure 6.2. Visual of the packaging of Everlasting Africa containing *Elegia tectorum* and a summary on the performance of the product

Indications:

Golden Complexity is indicated to assist in the management of even toning of the skin.

How does it work?

This product contains, among other ingredients, an active plant ingredient that inhibits the tyrosinase enzyme which then evens skin tone.

INCI name

Juncus lomatophyllus aerial plant part extract

Funders

This product development was funded by the Water Research Commission and the University of Pretoria.

Figure 6.3. Visual of the packaging of Golden Complexity containing *Juncus lomatophyllus* and a summary on the performance of the product

6.2 Technical file

Contains all the characteristics of the product such as the physical, chemical, bacteriological and toxicological characteristics, the mutagenicity of the ingredients and the optimal storage conditions. This provides consumers and companies with information that may help identify whether these products are suitable for their or the target market's type of skin.

6.2.1 Physical characteristics

The physical characteristics are any distinct attribute of the product that can be seen as listed below. These characteristics provide the consumer insight as to whether the product is suitable for their type of skin (pH) and whether the smell of the product is favourable to them.

- Form: How the product looks
- Colour: The distinct colour of the product
- Odor: What kind of smell the consumer can expect when applying the product
- Solubility: Different solutions (mainly water) that the product dissolve in
- pH: The pH range of the product

These physical characteristics differ for each prototype due to the active ingredient. Below are the different characteristics for each prototype:

1. *Persicaria senegalensis*

- Form: Liquid

- Colour: Dark brown
- Odor: Floral berry-like
- Solubility: Soluble in water
- pH: 4-6

2. *Elegia tectorum*
- Form: Cream
- Colour: Pale/light green
- Odor: Odourless
- Solubility: Soluble in water
- pH: 5-7

3. *Juncus lomatophyllus*
- Form: Liquid
- Colour: Dark green
- Odor: Sweet herb
- Solubility: Soluble in water
- pH: 4-6

6.2.2 Chemical characteristics

The chemical characteristics are the chemical composition as well as any preservatives that may have been used to extend the shelf life of the product. This provides the consumers with an indication as to the percentage concentration of each chemical that was used and whether it will be suitable for their type of skin. For all three of the prototypes, the chemical composition consisted of 60% distilled water and 40% alcohol with no preservative agents.

6.2.3 Bacteriological characteristics

The bacteriological characteristics inform the reader whether this product contains any bacterial contamination due to overexposure when harvesting the plant material. None of the prepared prototypes contained any microbial activity.

6.2.4 Toxicological characteristics

Toxicological characteristics inform consumers whether any heavy metals specifically arsenic, cadmium, mercury and lead are present in the product and if there are any known side effects such as irritancy. None of the prepared prototypes contained heavy metals.

6.2.5 Mutagenicity

Mutagenicity is whether any mutagens or carcinogens are present in the product which could pose a health risk. None of the prepared prototypes displayed mutagenicity.

6.2.6 Storage conditions

The storage conditions are based on the stability of the product which is conducted over a period of time at different temperatures. In most cases, the optimal storage condition is at room temperature away from the sunlight.

6.3 INCI name registration

An international nomenclature cosmetic ingredient (INCI) name is an internationally recognized systematic name that is used to identify cosmetic ingredients. This uniform system benefits the medical community as it provides the necessary scientific information regarding the composition of the products which allows medical practitioners to identify agents that are responsible for adverse reactions. Though obtaining INCI names for natural products does not imply that the ingredients used are approved or safe for cosmetic use, it does provide consumers transparency and allows cosmetic industries to track the safety and efficacy of the product (Personal Care Products Council, 2022).

To apply for an INCI name, an application form must be completed which requires the following information:

- Trade name
- Chemical structure (if applicable)
- Chemical abstract service (CAS) number
- Chemical synonyms
- Composition statement
- Manufacturing methods
- Diluents
- Botanicals
- Minerals and inorganics
- Polymers
- Ferments
- Peptides

Thereafter the application is processed and if accepted goes through a lengthy review process. The INCI names that were applied for are displayed in Table 6.1. The application for the plants has been submitted and will undergo review beginning of 2024.

Table 6.1. INCI names of *Persicaria senegalensis*, *Elegia tectorum* and *Juncus lomatophyllus*.

Plant name	Product name	INCI name
Persicaria senegalensis	Young African	Persicaria senegalensis aerial plant part extract
Elegia tectorum	Everlasting Africa	Elegia tectorum stem extract
Juncus lomatophyllus	Golden Complexity	Juncus lomatophyllus aerial plant part extract

CHAPTER 7: CONCLUSION AND RECOMMENDATIONS

In conclusion, the project focused on commercialising five wetland species that previously displayed significant anti-wrinkle or tyrosinase activity. This was done by cultivating the plant material at Botanica Natural Products and conducting a series of analyses including heavy metals, microbial count and MSDS sheet. None of the samples or the water system used to cultivate the plants showed significant levels of heavy metals or microbials. Furthermore, the stability of the extracts was evaluated, whereby the shelf life of each extract was found to be 24 hours.

Of the five extracts, PS, ET and JL displayed the most significant results, thus prototypes were designed and prepared. Market sheets and technical files were prepared to enhance the marketability of the extracts. However, in accordance with the National Environmental Management Biodiversity Act (NEMBA), Act 10 of 2004 a BABS permit was required before the actives could be licenced out to potential formulators. All three BABS permits were successful and the letter of acceptance was received.

Due to all the information obtained during this study, it is recommendations to further explore CM and CS by generating viable anti-wrinkle prototypes for commercialisation. Furthermore, it is recommended to generate a prototype for PSF due to its significant anti-wrinkle activity and low irritancy potential. It is also recommended to identify the optimal growth phase and season as to when JL, PS and ET should be harvested to ensure that the activity remains consistent during commercialisation.

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APPENDIX A: BABS permits

Elegia tectorum

MINISTER
FORESTRY, FISHERIES AND THE ENVIRONMENT
REPUBLIC OF SOUTH AFRICA

Private Bag X447, Pretoria, 0001, Environment House, 473 Steve Biko Road, Tel: (012) 399 8743
Private Bag X9002, Cape Town, 8000, Tel: (021) 469 1500, Fax: (021) 465 3362

Ref: EDMS 228666 (BABS/001820P)

Professor Namrita Lall
Plant Science Complex
Lynwood Road
Private Bag X20
HATFIELD
0028

Email: namrita.lall@up.ac.za
Cc: nigel.barker@up.ac.za

Dear Professor Lall

APPLICATION FOR BIOPROSPECTING PERMIT BY THE UNIVERSITY OF PRETORIA, REFERENCE NUMBER BABS/001820P

The bioprospecting permit application was submitted on 17 November 2020 with reference number: BABS/001820P.

Please be informed that the bioprospecting permit application with reference number: BABS/001820P has been approved for a period of five years (2023–2028) with certain conditions. You may apply for the amendment of the permit after the first annual project status report. The official permit document is enclosed.

Please note that Regulation 33(3) of the Bioprospecting, Access, and Benefit-Sharing Amendment Regulations, published under General Notice No. 447 in Government Gazette No. 38809 of 19 May 2015, in terms of the National Environmental Management: Biodiversity Act, 2004 (Act No.10 of 2004) (NEM:BA) provides that the following standard permit conditions apply to all bioprospecting permits:

(i) All money due to stakeholders in terms of a benefit-sharing agreement must be paid into the Bioprospecting Trust Fund (Suspense Account), as required by section 85(1) of NEM:BA. The banking details of the Bioprospecting Trust Fund (Suspense Account) are provided below:
The banking details of the Bioprospecting Trust Fund (Suspense Account):
Department of Forestry, Fisheries and the Environment
ABSA Bank
Account number: 1044240072
Swift Account: ABSA ZAJJ CPT

Pretoria
South Africa
Branch code: 632005

(ii) The permit holder must notify the Department of Forestry, Fisheries and the Environment (DFFE) when the money due to stakeholders, as specified in the benefit-sharing agreement, will be transferred or paid into the Bioprospecting Trust Fund (Suspense Account).

(iii) The permit holder must notify the stakeholder or stakeholders entitled to a monetary benefit, in terms of the benefit-sharing agreement, that money was transferred or paid into the Bioprospecting Trust Fund (Suspense Account).

(iv) The permit holder must, on an annual basis, or such alternative timeframe as determined by the Issuing Authority, submit a status report to the Issuing Authority in a format determined by the Issuing Authority.

(v) The indigenous genetic and biological resources to which a permit relates may not be sold, donated or transferred to a third party without written consent of the Issuing Authority.

(vi) The permit holder will be liable for the costs of mitigation or remedying the impact of bioprospecting on the environment in terms of section 28 of the National Environmental Management Act, 1998 (Act No. 107 of 1998).

Additional specific permit conditions imposed are as follows:

(i) Note that the DFFE and the Department of Science and Innovation are currently working together to identify legitimate traditional knowledge holder(s) associated with the use of *Elegia tectorum* species. Once this process has been finalised, you will be advised to negotiate a benefit-sharing agreement with the legitimate traditional knowledge holder(s).

For any queries regarding the bioprospecting permit and its conditions, please contact Ms Natalie Feltman, Director: Bioprospecting and Biodiversity Economy on 012 399 8917 or at NFeltman@dffe.gov.za; or Mr Ntambudzeni Nepfumembe, Biodiversity Officer Control Grade B: BABS Policy Development and Implementation, on 012 399 9612/066 430 4385 or at NNepfumembe@dffe.gov.za

Yours sincerely

MS B D CREECY, MP
MINISTER OF FORESTRY, FISHERIES AND THE ENVIRONMENT

DATE: 10/6/2023

Juncus lomatophyllus

MINISTER
FORESTRY, FISHERIES AND THE ENVIRONMENT
REPUBLIC OF SOUTH AFRICA

Private Bag X447, Pretoria, 0001, Environment House, 473 Steve Biko Road, Tel: (012) 399 8743
Private Bag X9052, Cape Town, 8000, Tel: (021) 469 1500, Fax: (021) 465 3362

Ref: EDMS 228886 (BABS/001720P)

Prof. Namrita Lall
Plant Science Complex
Lynwood Road
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0028

Email: namrita.lall@up.ac.za / nigel.barker@up.ac.za

Dear Prof. Lall

APPLICATION FOR A BIOPROSPECTING PERMIT BY THE UNIVERSITY OF PRETORIA, REFERENCE NUMBER BABS/001720P

The bioprospecting permit application submitted on 17 November 2020, reference number: BABS/001720P, has reference.

Kindly be informed that the bioprospecting permit application, reference number: BABS/001720P, has been approved for a period of five (5) years (2023–2028). You may apply for an amendment of the permit after the first annual project status report. The official permit documents are enclosed.

Please note that Regulation 33(3) of the Bioprospecting, Access and Benefit-Sharing Amendment Regulations, published under General Notice No. 447 in Government Gazette No. 38609 of 19 May 2015, in terms of the National Environmental Management: Biodiversity Act, 2004 (Act No. 10 of 2004) (NEMBA), provides that the following standard permit conditions apply to all bioprospecting permits:

(i) All money due to stakeholders in terms of a benefit sharing agreement must be paid into the Bioprospecting Trust Fund (Suspense Account), as required by section 85(1) of NEMBA. The banking details of the Bioprospecting Trust Fund (Suspense Account) are provided below:
Department of Forestry, Fisheries and the Environment
ABSA Bank
Account number: 1044240072
Swift Account: ABSA ZAJJ CPT
Pretoria
South Africa
Branch code: 632005

(ii) The permit holder must notify the department when the money due to stakeholders, as specified in the benefit sharing agreement, will be transferred or paid into the Bioprospecting Trust Fund (Suspense Account);
(iii) The permit holder must notify the stakeholder or stakeholders entitled to a monetary benefit, in terms of the benefit sharing agreement, that money was transferred or paid into the Bioprospecting Trust Fund (Suspense Account);
(iv) The permit holder must, on an annual basis, or such alternative timeframe as determined by the issuing authority, submit a status report to the issuing authority in a format determined by the issuing authority;
(v) The indigenous genetic and biological resources to which a permit relates may not be sold, donated or transferred to a third party without written consent of the issuing authority; and
(vi) The permit holder will be liable for the costs of mitigation or remedying the impact of the bioprospecting on the environment, in accordance with section 28 of the National Environmental Management Act, 1998 (Act No. 107 of 1998).

Additional specific permit conditions imposed are as follows:
(i) Note that the Department of Forestry, Fisheries and the Environment and the Department of Science and Innovation are currently working together to identify legitimate traditional knowledge holder(s) associated with the use of *Juncus lomatophyllus* species. Once this process has been finalised, you will be advised to negotiate a Benefit Sharing Agreement with the legitimate Traditional Knowledge holder(s).

For any queries on the discovery phase export permit and its conditions, please contact Ms Natalie Feltman, Director: Bioprospecting and Biodiversity Economy, on 012 399 8917, or through email: NFeltman@dffe.gov.za; or Mr Ntambudzeni Nepfumembe, Biodiversity Officer Control: Grade B: BABS Policy Development and Implementation, on 012 399 9612 / Cell: 066 430 4365, or through email: NNepfumembe@dffe.gov.za.

Yours sincerely

MS B D CREECY, MP
MINISTER OF FORESTRY, FISHERIES AND THE ENVIRONMENT

DATE: 25/6/2023

Persicaria senegalensis

MINISTER
FORESTRY, FISHERIES AND THE ENVIRONMENT
REPUBLIC OF SOUTH AFRICA

Private Bag X447, Pretoria, 0001, Environment House, 473 Steve Biko Road, Tel: (012) 399 8743
Private Bag X9052, Cape Town, 8000, Tel: (021) 469 1500, Fax: (021) 465 3362

Ref: EDMS 235214 (BABS/001620P)

Prof. Namrita Lall
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Email: namrita.lall@up.ac.za
Cc: nigel.barker@up.ac.za

Dear Professor Lall

APPLICATION FOR A BIOPROSPECTING PERMIT BY THE UNIVERSITY OF PRETORIA, REFERENCE NUMBER BABS/001620P

The bioprospecting permit application submitted on 17 November 2020, reference number: BABS/001620P, has reference.

Kindly be informed that the bioprospecting permit application, reference number: BABS/001620P, has been approved for a period of five (5) years (2023–2028) subject to the conditions set out in this letter and the permit. You may apply for an amendment of the permit after the first annual project status report. The official permit documents are enclosed.

Please note that Regulation 33(3) of the Bioprospecting, Access and Benefit-Sharing Amendment Regulations, published under General Notice No. 447 in Government Gazette No. 38809 of 19 May 2015, in terms of the National Environmental Management: Biodiversity Act, 2004 (Act No. 10 of 2004) (NEM: BA), provides that the following standard permit conditions apply to all bioprospecting permits:

(i) All money due to stakeholders in terms of a Benefit Sharing Agreement must be paid into the Bioprospecting Trust Fund (Suspense Account), as required by section 85(1) of NEM: BA.

APPLICATION FOR A BIOPROSPECTING PERMIT BY THE UNIVERSITY OF PRETORIA, REFERENCE NUMBER BABS/001620P

The banking details of the Bioprospecting Trust Fund (Suspense Account) are provided below:

Department of Forestry, Fisheries and the Environment
ABSA Bank
Account number: 1044240072
Swift Account: ABSA ZAJJ CPT
Pretoria
South Africa
Branch code: 632005

(ii) The permit holder must notify the department when the money due to stakeholders, as specified in the Benefit Sharing Agreement, will be transferred or paid into the Bioprospecting Trust Fund (Suspense Account);
(iii) The permit holder must notify the stakeholder or stakeholders entitled to a monetary benefit, in terms of the Benefit Sharing Agreement, that money was transferred or paid into the Bioprospecting Trust Fund (Suspense Account);
(iv) The permit holder must, on an annual basis, or such alternative timeframe as determined by the issuing authority, submit a status report to the issuing authority in a format determined by the issuing authority;
(v) The indigenous genetic and biological resources to which a permit relates may not be sold, donated or transferred to a third party without written consent of the issuing authority; and
(vi) The permit holder will be liable for the costs of mitigation or remedying the impact of the bioprospecting on the environment, in accordance with section 28 of the National Environmental Management Act, 1998 (Act No. 107 of 1998).

Additional specific permit conditions imposed are as follows:
(i) Note that the Department of Forestry, Fisheries and the Environment and the Department of Science and Innovation are currently working together to identify legitimate traditional knowledge holder(s) associated with the use of *Persicaria senegalensis* species. Once this process has been finalised, you will be advised to negotiate a Benefit Sharing Agreement with the legitimate Traditional Knowledge holder(s).

For any queries you might have on the bioprospecting permit and its conditions, please contact Ms Natalie Feltman, Director: Bioprospecting and Biodiversity Economy, on 012 399 8917, or through email: NFeltman@dffe.gov.za; or Mr Ntambudzeni Nepfumembe, Biodiversity Officer Control: Grade B: BABS Policy Development and Implementation, on 012 399 9612 / Cell: 066 430 4365, or through email: NNepfumembe@dffe.gov.za.

Yours sincerely

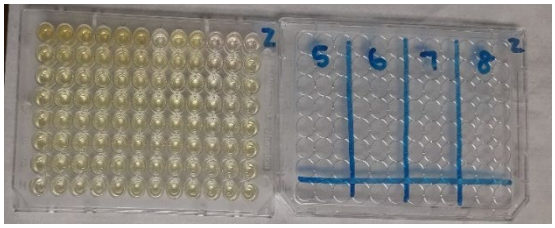
MS B D CREECY, MP
MINISTER OF FORESTRY, FISHERIES AND THE ENVIRONMENT

DATE: 8/11/2023

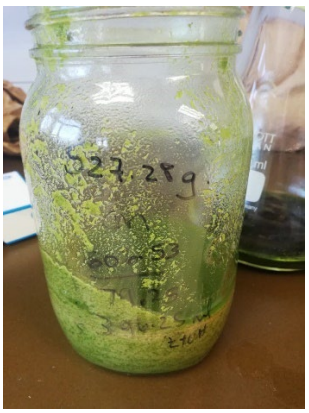
APPENDIX B: Gugulethu Miya visit to UP

Cytotoxicity – Cell culture training


Elastase training

Extraction training

APPENDIX C: Conferences, awards and publications

During the course of this project numerous conferences were attended, awards were given and publications were submitted and accepted. Below is a list of all the aforementioned items:

1. Conferences

1.1 The Society of Cosmetic Chemists South Africa (Coschem)

The Society of Cosmetic Chemists South Africa (Coschem) was formed in 1977 and in the past 48 years Coschem has grown exponentially from being affiliated with The International Federation of Societies of Cosmetic Chemists (IFSCC) in 1979, to obtaining 270 members in 1991, being registered as a non-profit organization in 2005 and holding its 40th anniversary in 2017.

The society is associated with other foundations including the Cosmetic Toiletry & Fragrance Association of South Africa (ctfa), South African Association for the Flavour & Fragrance Industry (SAAFFI), Pharmaceutical & Cosmetic (P & C) review and Kosmet. Each year the society holds their annual conference, which were attended in 2019 by Bianca Payne and 2023 by both Bianca and Marizé Cuyler.

1.1.1 Beauty Kaleidoscope 2023: The new dawn

The first in-person conference since the pandemic was held on the 13-14th September 2023 in Linbro Park South Africa. This conference was attended by Bianca Payne who presented her talk on "The holistic anti-aging potential of *Persicaria senegalensis* (Meisn.) Sojak and its probiotic fermented counterpart" and Marizé Cuyler who presented on "Anti-elastase potential of *Elegia tectorum* (L.f) Molin and H.P Linder". Furthermore, to promote the protypes that were formulated during the course of the study, Professor Namrita Lall exhibited the prototypes during the duration of the conference.

The Holistic Anti-Aging Potential of *Persicaria Senegalensis* (Meisn.) Sojak and its Probiotic Fermented Counterpart

Bianca Payne
University of Pretoria, South Africa

Bianca obtained her Bachelor of Science Degree from the University of Pretoria in Microbiology, followed by her Honours and Masters at the same institution in specializing in Medicinal Plant Science. She is currently enrolled for her PhD which investigates tools such as fermentation and gold nanoparticle synthesis on enhancing the potential bioactivity of South African plants, specifically in relation to maintenance of the dermal matrisome as for the development of anti-ageing candidates. Since 2016 she has also worked as a research assistant at the University of Pretoria and co-founded a start-up company, "Blyde Botanics" alongside a fellow PhD student from Prof Namrita Lall's research group, in 2019.

In 2018 she was awarded the prize for best Masters Student at the 44th Annual Conference for the South African Association of Botanists and Best Young Scientist. Later that year she was awarded the DST Albertina Sisulu Fellowship for Women in Science. She has published 17 research articles in peer-reviewed journals and 9 book chapters.

ABSTRACT

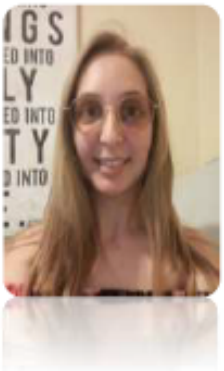
Skin health and beauty have long been considered to reflect the overall well-being of an individual. While literature is scant regarding the use of plants for their anti-ageing potential, the use of plants for skin related ailments is abundant and can be applied to anti-ageing research. This can be done as many aspects of the molecular pathways responsible for the formation of wrinkles and an aged phenotype are common to stress responses experienced by the skin under duress. *Persicaria* is one of fifty genera in the Polygonaceae family of mono- and dioecious herbs, small trees, and shrubs. It is abundantly distributed throughout the Northern hemisphere but can be found throughout Africa growing along streams, rivers, pools, and swamps in freshwater systems. Traditionally, it has been used for a range of skin troubles and was therefore selected for the current study. A semi pure sample form this plant has been confirmed to exhibit significant antiaging effects in a human validation study.

In vitro assessment using elastase inhibition revealed promising activity against key contributors to the ageing pathway including elastase, collagenase, and inflammatory mediators. Based on the noteworthy elastase inhibitory potential of this extract, bioassay guided liquid-liquid partitioning was used to determine the most bioactive partition which was selected for further compound isolation using column and flash chromatography, respectively. From this a possible novel compound has been isolated and structure elucidation is underway.

Fermentation has long been used in the food and beverage industry, and with the range of benefits offered by probiotic bacteria, it is becoming an increasingly interesting tool in cosmetics. The ethanolic crude ethanolic extract was fermented using *Bifidobacterium bifidum* and investigated for its elastase inhibitory potential. The fermented extract was found to have a five-fold lower IC50 value of 10.86 ± 2.15 µg/mL compared to 50.59±4.36 µg/mL. Furthermore, the fermented extract was also found to have improved irritancy potential in a human irritancy study compared to the ethanolic extract, while eradicating the appearance of wrinkles after 28 days of application in a human efficacy study.

These results support the use of extracts of *P. senegalensis* for use in the development of anti-ageing technologies. Multiple mechanisms of action provide a multi-faceted approach to natural well-ageing strategies that will progress the value that scientifically proven natural ingredients can contribute to the cosmetic industry and growing demand for natural products.

Marize' Cuyler
University of Pretoria, South Africa



Marizé is currently enrolled for a PhD degree in Medicinal Plant Science at the University of Pretoria. She completed her undergraduate degree in Plant Science and Biochemistry and completed her BSc Hons and MSc in Medicinal Plant Sciences.

The aim of her PhD is to determine whether South African plant extracts reduce symptoms associated with eczema including the intolerable itching sensation, rehydration of the skin, formation of dark patches and wrinkles. Furthermore, her study focuses on the inhibitory effect of these extracts on tumor necrosis factor alpha (TNF-α), one of the main cytokines involved in the development of acute and chronic eczema.

Marizé has published several peer-reviewed articles. She also served as the project manager of a book published by Taylor and Francis titled "Medicinal Plants for Cosmetics, Health and Diseases" and has also contributed a chapter to the book. She was awarded the third best paper presentation in a young scientist category at the 2022 Indigenous Plant Use Form (IPUF) conference, South Africa. She has been awarded the UP Postgraduate Bursary for Masters and Doctoral studies.

ABSTRACT

The prevalence of atopic dermatitis, commonly referred to as eczema, is an inflammatory skin condition that has increased in prevalence worldwide. One of the proposed causes for the development of eczema is the outside-in hypothesis, which states that eczema is caused by a disruption within the skin barrier. This results in the overexpression of histamine leading to an intolerable itching sensation. The increase in histamine levels further stimulates the overexpression of proteases such as elastase, which promotes wrinkle formation. In addition, histamine levels enhance the expression of cell migration-inducing proteins (CEMIP) resulting in inflammation.

Elegia tectorum (L.f) Moline & H. P. Linder is a native perennial evergreen plant that is mainly used as an attractive garden plant and is traditionally used for thatching roofs, weaving baskets and booms. Previously, a study conducted by Lymperis et al. (2021) reported that an ethanolic of *E. tectorum* displayed anti-elastase activity with a 50% inhibitory concentration (IC_{50}) of 13.50 ± 1.50 µg/mL. Therefore, this study aimed to confirm the anti-elastase activity of *E. tectorum* and to determine whether the ethanolic extract (ET-EtOH) could inhibit the production of cell-migration induced protein (CEMIP) and histamine. Furthermore, this study focused on determining whether the activity of ET-EtOH was enhanced when fermented (ETF) or used to synthesize gold nanoparticles (ETAuNP). Lastly, the potential mutagenic properties, irritancy potential and *in vivo* efficacy of ET-EtOH were evaluated.

The extract's anti-elastase activity was evaluated, where ET-EtOH displayed an IC_{50} value of 14.58 ± 2.00 µg/mL, while ETF and ETAuNP showed IC_{50} values > 500 µg/mL. Thus, ET-EtOH was further evaluated for its effect on CEMIP and histamine production using human adenocarcinoma cells (HT-29) and phorbol 12-myristate 13-acetate (PMA) stimulated granulocytes, respectively. Thereafter, the effect of ET-EtOH on histamine and CEMIP production was evaluated. ET-EtOH at a concentration of 6 µg/mL (0.10 ± 0.01 ng/mL) and 3 µg/mL (0.11 ± 0.01 ng/mL) significantly inhibited ($p < 0.05$) histamine production compared to the 0.25% DMSO vehicle control (0.26 ± 0.02 ng/mL). Furthermore, compared to the untreated control (0.31 ± 0.04 ng/mL), ET-EtOH at a concentration of 240 µg/mL (0.12 ± 0.01 ng/mL, $p < 0.01$) and 60 µg/mL (0.18 ± 0.02 ng/mL, $p < 0.05$) significantly reduced CEMIP production.

The potential mutagenic properties of ET-EtOH using *Salmonella typhimurium* was evaluated, where ET-EtOH at 5 mg/mL (131.33 ± 10.84) displayed no significant difference in the number of revertant colonies compared to the 10% DMSO vehicle control (100.00 ± 8.52). Thereafter, ET-EtOH was evaluated for its *in vivo* irritancy and efficacy, which indicated that ET-EtOH displayed mild irritancy and significantly reduced wrinkle formation after 28 days. Further investigation into identifying bioactive compounds and the effect of ET-EtOH on mast cell histamine-associated receptors should be considered.

1.2 Indigenous Plant Use Forum (IPUF)

The Indigenous Plant Use Forum, or better known as IPUF, was started to "promote the cultural, socio-economic and scientific benefits derived from the sustainable use of the Southern African flora". Since 1993, the National Research Foundation (NRF) has funded the forum to allow all walks of life to attend their annual symposium. The current chair of the form is Professor Ben-Erik van Wyk, a research professor at the University of Johannesburg.

Furthermore, the number of attendances grew from 65 delegates in 1998 to 200 in 2007 and 250 in 2015.

Since IPUF's start up, the conference has attracted enthusiastic support from a diversity of

interest groups and has been especially successful in promoting scientific research amongst young students. The annual symposia are unique, multicultural and multi-disciplinary events that serve as a meeting point for academics, anthropologists, resource managers, conservationists, policy makers and anyone interested in the sustainable use of the Southern African flora (Wyk, 2018). These symposia were attended in 2022 and 2023.

1.2.1 24th Indigenous Plant Use Forum (IPUF)

The 24th IPUF conference was held virtually from the 4-7th July 2022. The theme of the conference was “Local and global contributions to ethnobotany and natural products development”. This conference was attended by Marizé Cuyler who presented her talk on “Effects of South African plants against symptoms associated with eczema”.

Effects of South African plants against symptoms associated with eczema

Marizé Nel[a], Danielle Twilley[a], Velaphi C. Thipe[b], Kattesh V. Katti[b], Vusani Mandiwana[c], Michel L. Kalombo[c], Suprakas S. Ray[d], Rirhandzu Rikhotso[d], Arno Janse van Vuuren[e] and Namrita Lall[a]

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b Department of Radiology, Institute of Green Nanotechnology, University of Missouri, Columbia, MO, United States
c Chemical Cluster, Centre for Nanostructures and Advanced Materials, Council for Scientific and Industrial Research, Pretoria, South Africa
d DST/CSIR National Centre for Nanostructured Materials, Council for Scientific and Industrial Research, Pretoria, South Africa
e Centre for High Transmission Electron Microscopy, Nelson Mandela University, Port Elizabeth, South Africa
f School of Natural Resources, University of Missouri, Columbia, MO, United States
g College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru, India
h Bio-Tech R&D Institute, University of the West Indies, Kingston, Jamaica

Abstract: Atopic dermatitis has been increasing in prevalence, with the cause uncertain, however, two major hypotheses have been acknowledged. Atopic dermatitis is associated with an immunological defect including the overexpression of tumour necrosis factor-alpha (*TNF-α*), which in turn causes post-inflammatory hyperpigmentation. Existing clinical treatments have adverse side effects, such as skin atrophy and tachyphylaxis, leading to a need for alternative treatments. The aim of this study was to evaluate whether the ethanolic extract of JL (JL-EtOH) exhibits anti-tyrosinase activity and can downregulate *TNF-α* expression. Furthermore, this study also investigated whether the biological activity of JL-EtOH was enhanced when fermented using *Bifidobacterium bifidum* (JLF) or utilized to synthesize gold nanoparticles (JL-AuNPs). JL-AuNPs exhibited enhanced anti-tyrosinase activity compared to JL-EtOH and JLF with IC50 values of 268.8 ± 5.64 μg/mL and > 400 μg/mL, respectively. JL-AuNPs and JL-EtOH were selected for further evaluation since neither exhibited antiproliferative activity against human keratinocytes (HaCaT) nor peripheral blood mononuclear cells (PBMCs) were observed (IC50 > 400 μg/mL). Furthermore, the effect of JL-EtOH and JL-AuNPs on *TNF-α* production using PBMCs was evaluated. Compared to the untreated control (42.40 ± 4.17 pg/mL), JL-AuNPs (23.59 ± 1.95 pg/mL) significantly inhibited ($p < 0.05$) the production of TNF-α, while JL-EtOH (25.48 ± 7.56 pg/mL), compared to the vehicle control (38.41 ± 0.98 pg/mL), showed no effect at a concentration of 200 μg/mL. Further investigation into JL-AuNP's effect on the translation of *TNF-α* as a potential mode of action should be considered.

1.2.2 25th Indigenous Plant Use Forum (IPUF)

The 25th IPUF conference was held in Kruger National Park from the 27-31st August 2023. The theme of the conference was "are we maximising the potential benefits of a rich endemic flora and associated indigenous knowledge?". This conference was attended by Bianca Payne who presented her talk on "The holistic anti-aging potential of plant extracts used traditionally for skin trouble". Furthermore, Prof Namrita Lall was invited as a keynote and presented a talk titled "Methodology and Ethics of Ethnobotany: The journey of Natural Product development".

Methodology and Ethics of Ethnobotany: The journey of Natural Product development

Namrita Lall[a,b,c,d]

[a] Department of Plant and Soil Sciences, University of Pretoria, Pretoria 0002, South Africa
[b] School of Natural Resources, University of Missouri, Columbia, MO, United States
[c] College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru, Karnataka, India
[d] Senior Research fellow, Bio-Tech R&D Institute, University of the West Indies, Kingston, Jamaica

Abstract: plants hold untouched potential for the identification and development of novel therapeutics and natural products. However, there are many steps and obstacles which should be overcome to ensure the production of ethically sourced and effective plant-derived products. This seminar will cover various pre-developmental stages encountered within the natural product development journey, including the scientific and research process (including in-laboratory case studies), consisting of; problem identification, plant selection, pre-clinical efficacy and safety testing, formulation, characterisation and stability testing, and optimisation of novel therapeutics derived thereof. The talk will discuss the identification of medicinal plants, based on traditional use, and acquiring of source material in collaboration with traditional healers. Furthermore, this discussion will address the process and challenges which occur when acquiring bioprospecting permits and licensing agreements, as well as the importance and ethical need for establishing Benefit Sharing Agreements, Material Transfer Agreements, and the Indigenous Knowledge Systems (IKS) Policies involved to ensure IKS preservation and respect, and adequate royalty sharing to the relevant parties involved. The challenges faced during the establishment of patented technologies, and identifying appropriate manufacturers and initiation of licencing agreements for product development will also be discussed. The seminar further reviews the different types of complementary and natural product medicines, and will elaborate on the regulation, establishment, and appropriate labelling and marketing of plant-derived cosmeceuticals and pharmaceuticals in Southern Africa, and how it compares to natural product regulations set in place by the U. S. Food and Drug Administration (FDA).

The holistic anti-ageing potential of plant extracts used traditionally for skin troubles

Bianca Payne[a] **and Namrita Lall**[a,b]

[a] Medicinal Plant Science, Department of Plant and Soil Sciences, University of Pretoria, Pretoria, 0002, South Africa
[b] School of Natural Resources, University of Missouri, Columbia, Missouri, USA

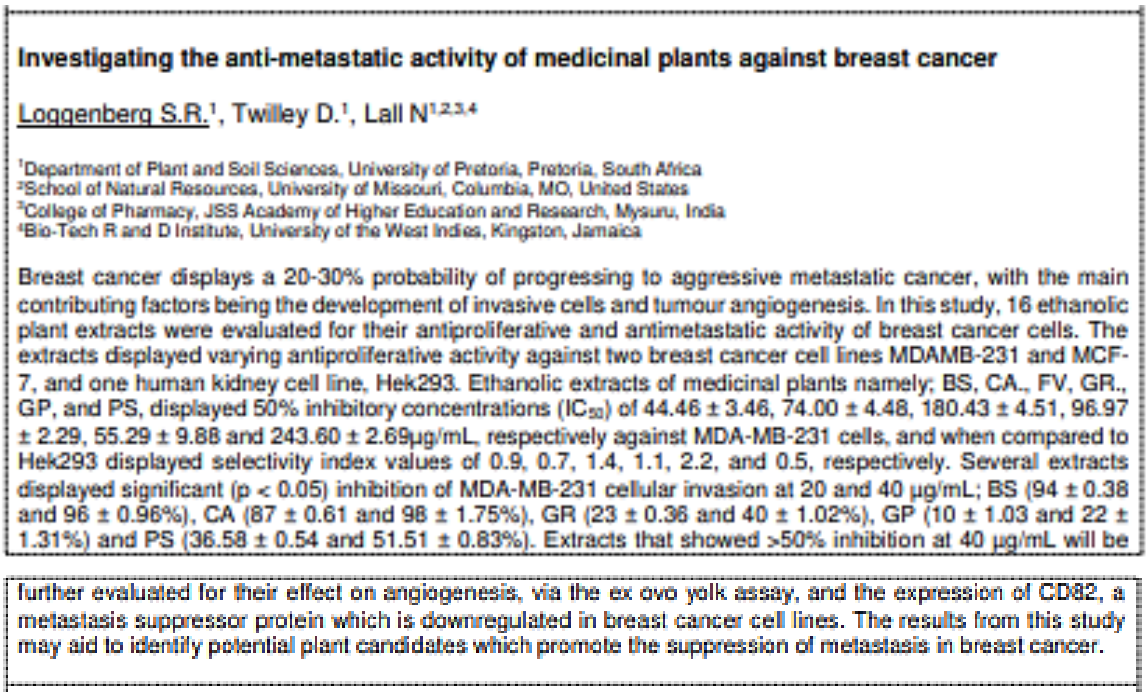
Abstract: Skin health and beauty have long been considered principle factors reflecting overall well-being in society. While literature is scant regarding the use of plants for their anti-ageing potential, the use of plants for skin-related ailments is abundant and can be applied to anti-ageing research. This can be done as many of the molecular pathways responsible for the formation of wrinkles and an aged phenotype are common to stress responses experienced by the skin under duress. This talk will discuss the use of *Persicaria senegalensis* (Meisn.) Sojak, *Greyia radlkoferi* Szyszyl. and *Myrsine africana* L., traditionally reported for the treatment of skin ailments, as potential anti-wrinkle candidates. *In vitro* assessment using elastase inhibition, collagenase inhibition, and anti-inflammatory potential revealed *P. senegalensis* to be the most promising candidate. *Persicaria* is one of the 50 genera in the Polygonaceae family of mono- and dioecious herbs, small trees, and shrubs. It is abundantly distributed throughout the Northern hemisphere but can be found throughout Africa, growing along streams, rivers, pools, and swamps in freshwater systems. Traditionally, it has been used for a range of skin troubles and was therefore selected for the current study. Based on the noteworthy elastase inhibitory potential of this extract, bioassay-guided liquid-liquid partitioning was used to determine the most bioactive partition which was selected for further compound isolation using column and flash chromatography, respectively. Phytochemical characterisation of five semi-pure fractions is underway. These results support the use of extracts of *P. senegalensis* for use in the development of anti-ageing technologies.

1.3 South African Association of Botanists (SAAB)

The South African Association of Botanists (SAAB) was established to promote and uphold the status of the Botany profession and to incorporate all walks of life that are interested in the profession. The association is comprised of 9 members, an active group of student participants and an email network for their members. Furthermore, the members of the association can be located throughout different South African universities, academics and research institutions (South African Association of Botanists, 2018).

1.3.1 48th Annual SAAB conference

The 48th annual SAAB conference was held on the 17-20th January 2023 in Polokwane. The theme of the conference was "Plants, Health and Prosperity". The conference was attended by Sam Loggenberg, one of Prof. Lalls master students who presented a talk on "Investigating the anti-metastatic activity of medicinal plants against breast cancer" using *Persicaria senegalensis* as one of her selected plants.



Investigating the anti-metastatic activity of medicinal plants against breast cancer

Loggenberg S.R.[1], Twilley D.[1], Lall N[1,2,3,4]

[1]Department of Plant and Soil Sciences, University of Pretoria, Pretoria, South Africa
[2]School of Natural Resources, University of Missouri, Columbia, MO, United States
[3]College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru, India
[4]Bio-Tech R and D Institute, University of the West Indies, Kingston, Jamaica

Breast cancer displays a 20-30% probability of progressing to aggressive metastatic cancer, with the main contributing factors being the development of invasive cells and tumour angiogenesis. In this study, 16 ethanolic plant extracts were evaluated for their antiproliferative and antimetastatic activity of breast cancer cells. The extracts displayed varying antiproliferative activity against two breast cancer cell lines MDAMB-231 and MCF-7, and one human kidney cell line, Hek293. Ethanolic extracts of medicinal plants namely; BS, CA., FV, GR., GP, and PS, displayed 50% inhibitory concentrations (IC_{50}) of 44.46 ± 3.46, 74.00 ± 4.48, 180.43 ± 4.51, 96.97 ± 2.29, 55.29 ± 9.88 and 243.60 ± 2.69µg/mL, respectively against MDA-MB-231 cells, and when compared to Hek293 displayed selectivity index values of 0.9, 0.7, 1.4, 1.1, 2.2, and 0.5, respectively. Several extracts displayed significant ($p < 0.05$) inhibition of MDA-MB-231 cellular invasion at 20 and 40 µg/mL; BS (94 ± 0.38 and 96 ± 0.96%), CA (87 ± 0.61 and 98 ± 1.75%), GR (23 ± 0.36 and 40 ± 1.02%), GP (10 ± 1.03 and 22 ± 1.31%) and PS (36.58 ± 0.54 and 51.51 ± 0.83%). Extracts that showed >50% inhibition at 40 µg/mL will be further evaluated for their effect on angiogenesis, via the ex ovo yolk assay, and the expression of CD82, a metastasis suppressor protein which is downregulated in breast cancer cell lines. The results from this study may aid to identify potential plant candidates which promote the suppression of metastasis in breast cancer.

1.4 Society for Medicinal Plant and Natural Product Research (GA)

The Society for Medicinal Plant and Natural Product Research (GA) was founded in 1953 by a small group of medicinal plant researchers located in Camberg/Taunus Germany. In 1969, the society became internationally recognised (Society for Medicinal Plant and Natural Product Research, 2023c). GA was formed to promote a healthier lifestyle for humans and animals through evidence-based use of medicinal plants. Furthermore, the society is a platform that exchanges knowledge and ideas regarding medicinal plant products (Society for Medicinal Plant and Natural Product Research, 2023a). The society aims to promote the cultivation of medicinal plants, utilising them in pharmacognosy, identify the biological and pharmacological activity of the plant material and formulate plant-based products for the market that can be used in animal care, veterinary medicine and phytotherapy (Society for Medicinal Plant and Natural Product Research, 2023b).

1.4.1 70th international congress and annual meeting

The 70th international congress and annual meetings for GA was held in Thessaloniki Greece from the 28-31st August 2022 and was attended by Dr Marco N. De Cahna, a postdoctoral fellow at the University of Pretoria. Dr De Cahna presented a talk titled "Investigating the potential of *Elegia tectorum* as an anti-ageing agent". Furthermore, the abstracts were

published in Planta Medica. During this congress, Prof Namrita Lall was invited to chair a session titled “African Research Pre-Congress Symposium” due to her significant contribution and knowledge in this field.

Authors De Canha MN[1], Radebe PG[1], Payne BD[1], Oosthuizen CB[1], Twilley D[1], Verma S[1], Skaltsa H[2], Lymperis P[2], Tomou E-M[2], McGaw L[3], Lall N[1]

Institutes 1 University Of Pretoria, Department of Plant and Soil Sciences, Pretoria, South Africa; 2 National and Kapodistrian University of Athens, Department of Pharmacognosy and Chemistry of Natural Products, Panepistimiopolis, Greece; 3 University of Pretoria, Department of Paraclinical Sciences, Pretoria, South Africa.

DOI 10.1055/s-0042-1758967

Elegia tectorum, also known as the Cape Thatching Reed (English) is a plant belonging to the Restionaceae family. In South Africa, it is distributed in the Eastern, Western and Northern Cape provinces, populating marshes and deep sandy soils along coastal or lowland flats at altitudes between 10–600 m. The aim of this study was to investigate the anti-ageing potential of E. tectorum (ET) due to the lack of pharmacological activity and ethnobotanical uses available in literature. The ethanolic extract of ET (ETEtOH) inhibited elastase enzyme activity with an IC_{50} of 13.50 ± 1.53 µg/mL. Cytotoxicity of ETEtOH was determined on HT29 cells (human colorectal adenocarcinoma) due to their expression of the KIAA1199 protein, responsible for hyaluronic acid degradation. No toxicity was observed at 400 µg/mL. There was significant reduction of KIAA1199 protein production levels in cells treated with 60 µg/mL and 240 µg/mL ETEtOH, when compared to the untreated HT29 cell control. To determine safety of the ETEtOH, the mutagenic potential of the extract was determined, with 50, 500 and 5000 µg/mL showing no mutagenicity using the TA 98 strain of *Salmonella typhimurium*. A clinical study for irritancy determined the irritancy potential of ETEtOH to be − 30.83, which was less than the negative control (distilled water) characterizing the extract as a non-irritant. An antiwrinkle efficacy study showed that ETEtOH at 10 % (w/w) in aqueous cream reduced the appearance of wrinkles after 28 days. The extract of ET is a strong lead for the development of a botanical anti-ageing ingredient.

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[2] Turner S, Jamieson H. Elegia tectorum (L.f.) Moline & H.P.Linder. 2001; South African National Biodiversity Institute. Internet: https://www.plantzafrica.com/plantefg/elegiatectorum.html

[3] Lymperis P, Tomou EM, De Canha MN et al. Traditional Uses, Phytochemistry, and Pharmacology of Elegia Species: A Review. Scientia Pharmaceutica 2022; 90(4): 1–11

1.5 The Society for Ethnobotany (SEB) and the Society of Ethnobiology (SOE)

The Society for Ethnobotany (SEB), previously known as the Society of Economic Botany was established in 1959 with the aim to encourage scientific research into past, present and future use of plants, their relationships with people and how to interpret this information for the general public to understand when mentioned during meetings or published. Over the years SEB has grown across the 50 USA states and members can be found over 64 countries throughout the world indicating that SEB serves as one of the largest societies (The Society for Ethnobotany, 2023).

The Society of Ethnobiology (SOE) was founded in 1981 by two graduate students, Steven A. Weber and Steven D. Emslie. The society was formed specifically for academic and professionals that work in the field of ethnobiology regardless of their ethnicity, gender, religion, rank and other manners. The society also focuses on other professions including ethnobotany, ethnozoology, ethnoecology and linguistics to name a few (Society of Ethnobiology, 2023).

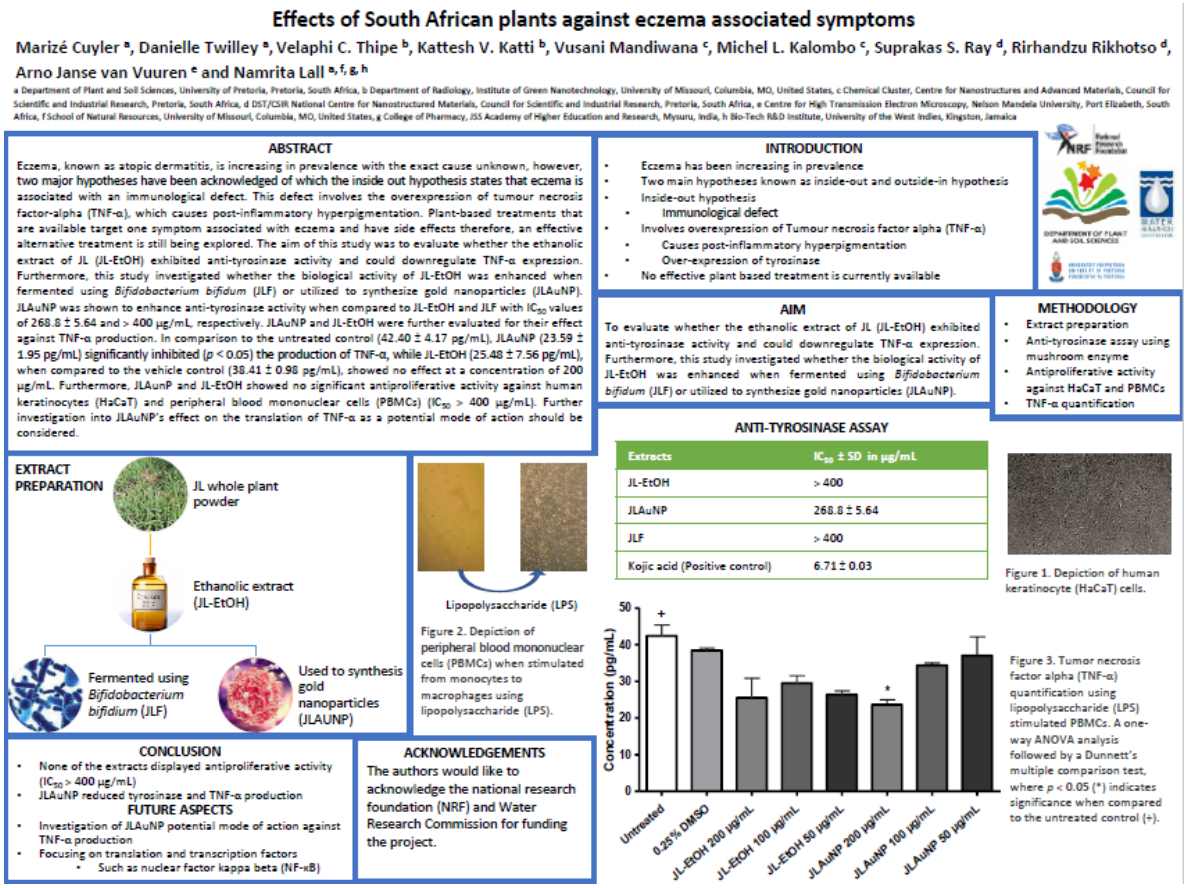
1.5.1 SEB/SOE conference

The Society of Ethnobotany and Society of Ethnobiology held a joint conference in 2023 in Atlanta, Georgia from the 4-9th June. This conference was attended virtually by Marizé Cuyler who presented her talk on “Effects of South African plants against eczema associated symptoms”.

Effects of South African plants against eczema associated symptoms

Marize' Cuyler marizenel7@gmail.com, Danielle Twilley berrington.danielle@gmail.com, Velaphi C. Thipe vcthipe@gmail.com, Kattesh V. Katti kattik@health.missouri.edu, Vusani Mandiwana vusy.mandi@gmail.com, Michel L. Kalombo lkalombo@csir.co.za, Suprakas S. Ray rsuprakas@csir.co.za, Arno Janse van Vuuren arno.jansevanvuuren@mandela.ac.za, Rirhandzu Rikhotso rrikhotso@csir.co.za, Namrita Lall namrita.lall@up.ac.za
University of Pretoria

Eczema, known as atopic dermatitis, is increasing in prevalence with the exact cause unknown, however, two major hypotheses have been acknowledged of which the inside-out hypothesis states that eczema is associated with an immunological defect. This defect involves the overexpression of tumour necrosis factor-alpha (TNF-α), which causes post-inflammatory hyperpigmentation. Plant-based treatments that are available target one symptom associated with eczema and have side effects therefore, an effective alternative treatment is still being explored. The aim of this study was to evaluate whether the ethanolic extract of JL (JL-EtOH) exhibited anti-tyrosinase activity and could downregulate TNF-α expression. Furthermore, this study investigated whether the biological activity of JL-EtOH was enhanced when fermented using *Bifidobacterium bifidum* (JLF) or utilized to synthesize gold nanoparticles (JLAuNP). JLAuNP was shown to enhance anti-tyrosinase activity when compared to JL-EtOH and JLF with IC50 values of 268.8 ± 5.64 and > 400 µg/mL, respectively. JLAuNP and JL-EtOH were further evaluated for their effect against TNF-α production. In comparison to the untreated control (42.40 ± 4.17 pg/mL), JLAuNP (23.59 ± 1.95 pg/mL) significantly inhibited ($p < 0.05$) the production of TNF-α, while JL-EtOH (25.48 ± 7.56 pg/mL), when compared to the vehicle control (38.41 ± 0.98 pg/mL), showed no effect at a concentration of 200 µg/mL. Furthermore, JLAunP and JL-EtOH showed no significant antiproliferative activity against human keratinocytes (HaCaT) and peripheral blood mononuclear cells (PBMCs) (IC50 > 400 µg/mL). Further investigation into JLAuNP's effect on the translation of TNF-α as a potential mode of action should be considered.



1.6 WRC symposium

In 2021, the Water Research Commission (WRC) held their fifth biennial symposium from the 20-22nd September. Due to the pandemic, this symposium was held virtually and was attended

by Marizé Cuyler who presented her talk on "From Wetland to counter: developing cosmeceutical prototypes from hydrophytes". The theme of the symposium was "Re-imagine, re-build and repeat: Future proofing water", which focused on ways to improve future proofing water within the sector. Furthermore, WRC celebrated their 50th anniversary during this event by reflecting on South Africa's water research, development and journey.

Abstract – From Wetland to counter: developing cosmeceutical prototypes from hydrophytes.

Cosmeceutical products have been around for centuries to enhance the beauty of people and provide comfort to those suffering from chronic conditions. Initially, these products were prepared traditionally using different plant materials and preparation methods. Nowadays, these products are manufactured in companies that use synthetic or semi-synthetic versions of the plant-based active ingredient. However, due to the overuse of these commercial ingredients, people are once again resorting to natural-based products. Though favourable due to their minimal side effects, many plant-based treatments are ineffective and lack scientific data.

Thus, to combat this, our research group focused on analysing the biological activity of wetland plants against common skin conditions. Wetland plants or hydrophytes are species of plants that survive and thrive in wetland biomes and play a vital role in ensuring the ecosystem remains stable. Though diverse, little to no research on the biological activity of these species have been conducted. Through extensive analyses, five aquatic plants were found to possess biological activity against acne, wrinkle formation and pigmentation disorders.

Acne, caused by a bacteria known as *Cutibacterium acnes*, is located on the torso, neck and face of patients either as non-inflammatory, inflammatory acne or both. The severity of the condition ranges from mild to severe and varies between each patient. Wrinkle formation, which can be caused by numerous factors such as aging, ultraviolet (UV) radiation and pollution, occurs on visible skin and is due to the loss of elasticity and the degradation of collagen. This formation can be exhilarated by overexposing the skin to harmful UV rays causing elastin and collagen to degrade exponentially. Lastly, pigmentation disorders are caused by the over or under-production of melanin via a process known as melanogenesis. Further analysis is currently being conducted to determine the effectiveness of these plants against eczema-associated symptoms, which include an intolerable itching sensation and inflammation. Future aspects of the research group are to develop natural products from these selected hydrophytes, which can be sold on the market as effective cosmetic treatments. Currently, two prototypes have been developed with three in progress.

1.7 The African Phytomedicine Scientific Society (APSS)

The African Phytomedicine Scientific Society (APSS) is a non-profit organisation that was founded by Prof Namrita Lall in 2022. Since then, the society has gained roughly 40 members and hosted its first international conference on the 9-10th November 2023. The society aims to promote networking not just within the African continent but internationally as well. Furthermore, the society focuses on expanding research capabilities, scientific development and communication in Africa. The conference was attended by Bianca Payne who presented her talk on "The fountain of youth might be a plant! The effect of *P. senegalensis* (Meisn.)

Soják on the maintenance of the dermal matrisome in photoageing skin and its commercialization".

The fountain of youth might be a plant! The effect of *P. senegalensis* (Meisn.) Soják on the maintenance of the dermal matrisome in photoageing skin and its commercialisation

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Abstract

Skin health and beauty are considered to reflect the overall well-being of an individual. While literature is scant regarding the use of plants for their anti-ageing potential, the use of plants for skin related ailments is abundant and can be applied to anti-ageing research. This can be done as many of the molecular pathways responsible for the formation of wrinkles are common to stress responses experienced by the skin under duress. *Persicaria* is one of fifty genera in the Polygonaceae family of mono- and dioecious herbs, small trees, and shrubs. abundantly distributed throughout Africa. Traditionally, it has been used for a range of skin troubles and was therefore selected for the current study. *In vitro* assessment revealed promising activity against key contributors to the ageing pathway including regulation of proteolytic degradation, inflammatory mediators and aging free-radicals. Bioassay guided liquid-liquid partitioning was used to determine the most bioactive partition which was selected for further compound isolation and from this a possible novel compound has been isolated and structure elucidation is underway. To support the commercialisation of *P. senegalensis*, *in vivo* human irritancy and anti-wrinkle trials were conducted. The findings showed a significant reduction in the appearance of fine lines and wrinkles after 28 days of application.

These results support the use of extracts of *P. senegalensis* for use in the development of anti-ageing technologies. Multiple mechanisms of action provide a multi-faceted and holistic approach to natural well-ageing strategies for the cosmetic industry and growing demand for natural products.

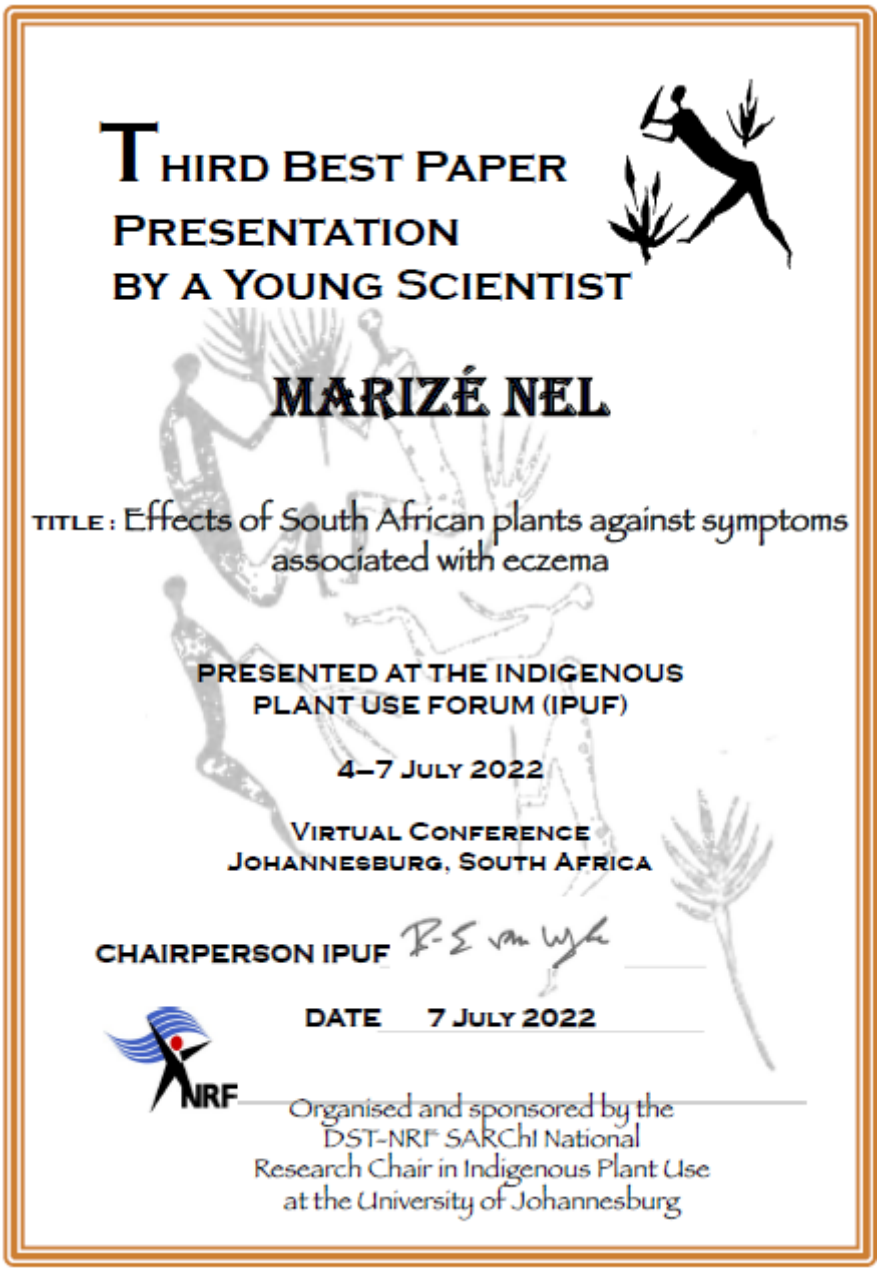
2. Future conferences

2.1 International Society for Ethnopharmacology (ISE)

The International Society for Ethnopharmacology was established in 1990, whereby its first congress was held in Strasbourg. Since then, the society has been registered as a charity under the law of Michigan. The society aims to promote the discussion of local and traditional knowledge on the medicinal, food aspect and toxic effects of plants both in the past, present and their future potential (International Society for Ethnopharmacology, 2023). ISE will be hosting its 23rd International Congress in collaboration with APSS in Cape Town from the 23-26th October 2024. The theme of the congress is "The footprint of ethnopharmacology in drug discovery". Once the abstract submission has been made available Marizé Cuyler will be submitting in hopes that she will be able to attend.

3. Awards

Marizé Cuyler was awarded the third best paper presentation by a young scientist at the 24th IPUF conference for her presentation titled "Effects of South African plants against symptoms associated with eczema".



Furthermore, Bianca D. Payne was awarded the APSS Innovation award for her presentation titled "The fountain of youth might be a plant! The effect of *P. senegalensis* (Meisn.) Soják on the maintenance of the dermal matrisome in photoageing skin and its commercialization" at the 1st International Congress of APSS, which was held on the 9th and 10th of November 2023.

4. Publications

4.1 Published

Petro Kotzé, 2022. "Wetlands and Society Protecting nature's medicine cabinet: How research is upping the value of endangered aquatic ecosystems", The Water Wheel May/June edition, pg 8-11. WW May-June 2022_web.pdf (wrc.org.za)

Gugulethu Mathews Miya, Ayodeji Oluwabunmi Oriola, Bianca Payne, Marizé Cuyler, Namrita Lall, Adebola Omowunmi Oyedeji, 2023. "Steroids and Fatty Acid Esters from *Cyperus sexangularis* Leaf and Their Antioxidant, Anti-Inflammatory and Anti-Elastase Properties", Molecules 28 (8), pg. 3434. DOI: https://doi.org/10.3390/molecules28083434

4.2 In progress

Marizé Cuyler, Danielle Twilley, Bianca D. Payne, Marco N. De Canha, Carel B. Oosthuizen, Princess G. Radebe, Balungile Madikizela, Lyndy L. McGaw, Velaphi C. Thipe, Vusani Mandiwana, Michel L. Kalombo, Suprakas S. Ray, Rirhandzu Rikhotso-Mbungela, Arno Janse van Vuuren, Kattesh V. Katti and Namrita Lall, In progress. "Anti-elastase potential of *Elegia tectorum* (L. f.) Moline & H. P. Linder". Target journal: Nanotechnology, Science and Applications (IF: 4.9)

Panagiotis Lymperis, Ekaterina-Michaela Tomou, Bianca D. Payne, Marizé Cuyler, Namrita Lall and Eleni Skaltsa, In progress. "Chemical constituents from *Elegia tectorum* and their chemophenetics importance". Target journal: Biochemical Systematics and Ecology (IF: 1.6)

Bianca D. Payne and Namrita Lall, Submitted to Journal of Ethnopharmacology Women Scientists in Ethnopharmacological Research special edition. "*Persicaria senegalensis* Meisn. (Sojak): A review on identifying features, traditional uses, and biological activities." (IF: 5.4)