

Investigating the cosmeceutical potential of *Spirostachys africana* Sond. bark extracts used traditionally for child skincare by *isiXhosa* communities in South Africa

By

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**Submitted in fulfilment of the requirements of the degree
of Doctor of Philosophy**

Research Centre for Plant Growth and Development

School of Life Sciences

University of KwaZulu-Natal

Pietermaritzburg Campus

December 2024

DECLARATION BY STUDENT

**Investigating the cosmeceutical potential of *Spirostachys africana* Sond.
bark extracts used traditionally for child skincare by isiXhosa communities
in South Africa**

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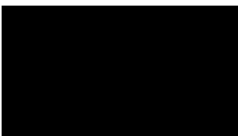
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Regular consultation took place between the student and ourselves throughout the investigation.

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DECLARATION 2-PUBLICATIONS

Publication 1:

Rofhiwa Mulaudzi, Sogolo L. Lebelo, Kholosa Maqolo, Rosemary C. Erhabor, Thulani Tshabalala, Lyndy J. McGaw, Ashwell R. Ndhlala and Johannes van Staden. Antibacterial, antioxidant, anti-inflammatory activities and skin irritation side effects of *Spirostachys africana* stem-bark extracts used traditionally for child skincare by *isiXhosa* communities in South Africa (Accepted; South African Journal of Botany).

Contribution: **Rofhiwa Mulaudzi:** Writing – original draft, Resources, Investigation, Formal analysis, Data curation, Conceptualization. **Sogolo L. Lebelo:** Supervision, Project administration. **Kholosa Maqolo:** Helped with data curation. **Rosemary C. Erhabor:** Helped with data curation. **Thulani Tshabalala:** Helped with data curation. **Lyndy J. McGaw:** Review & editing. **Ashwell R. Ndhlala:** Supervision, Project administration. **Johannes van Staden:** Writing – review & editing, Supervision.

Publication 2:

R. Mulaudzi, S.L. Lebelo, A.R. Ndhlala, J. Van Staden. Anti-tyrosinase activity of *Spirostachys africana* stem-bark extracts towards natural solutions for hyperpigmentation in South Africa (Manuscript in preparation; Journal of Herbal Medicine).

Contribution: The first author did the experimental work and wrote the manuscript under the supervision of the last three authors.

CONFERENCE CONTRIBUTION

Conference 1: International

R. Mulaudzi, S.L. Lebelo, A.R. Ndhlala, J. Van Staden. Antibacterial and antioxidant activity of *Spirostachys africana* stem-bark extracts used traditionally for child skincare by *isiXhosa* communities in South Africa. Training course on production and utilization of tropical medicinal plants for developing countries. 7-26 September 2023. Chinese Academy of Tropical Agricultural Sciences, Haiko China. Oral presentation.

Conference 2: Local

R. Mulaudzi, S.L. Lebelo, A.R. Ndhlala, J. Van Staden. Antibacterial activity of *Spirostachys africana* stem-bark extracts used traditionally for child skincare by *isiXhosa* communities in South Africa. 3rd Postgraduate Student Symposium (PSS 2024). 10-11 July 2024. University of South Africa, College of Agriculture and Environmental Sciences, Gauteng, South Africa. Poster presentation.

Conference 3: Local

R. Mulaudzi, S.L. Lebelo, A.R. Ndhlala, J. Van Staden. Evaluation of antibacterial and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activities of *Spirostachys africana* stem-bark extracts traditionally used for child skincare by *isiXhosa* communities in South Africa. Innovation Festival 2024. Research, Postgraduate Studies, Innovation and Commercialisation. 25-29 November 2024. University of South Africa. Poster presentation.

ACKNOWLEDGEMENTS

I am very grateful to:

- The Omnipotent God for providing me with the tenacity and strength to persevere during this research. Without God's spiritual guidance, support, and illumination, I would not have been able to finish my thesis.
- My Supervisor, Professor J. Van Staden for providing me with an excellent environment to never stop learning, supporting me, encouraging me, his constructive comments, invaluable advice, understanding and supervision throughout this study.
- My Co-supervisor and mentor, Professor A.R. Ndhlala, for his supervision, support, advice, contributions, patience, and amazing attention to detail during this journey.
- My Co-supervisor, Professor S.L. Lebelo for his supervision throughout this study.
- Mrs L.A. Warren (Administrative Assistant) of the Research Centre for Plant Growth and Development (RCPGD) for her unwavering support, enormous contributions, and readiness to support me throughout my academic career.
- My family for their unwavering support during my studies, which made it possible for me to reach this academic milestone. I can never thank you enough for your concern, compassion, and belief in my ability to succeed.
- My parents and siblings for their prayers, encouragement, and unrelenting love.
- My spiritual parents from the Ignitehouse Ministries International for all their prayers and support throughout my journey.
- All the staff and students at the Research Centre for Plant Growth and Development for their insightful recommendations and support. I truly enjoyed our working relationship.
- The University of Kwa-Zulu Natal for providing adequate financial assistance for my studies.
- The University of Sefako Makgatho Health Sciences (SMU) for giving me an opportunity to conduct my experiment at their laboratory.
- The Departments of Paraclinical Sciences (UP-PS) and Plant and Soil Sciences laboratory (UP-PSS) from the University of Pretoria, for the collaboration through Prof Lyndy McGaw.

- The University of KwaZulu-Natal, Research Centre for Plant Growth and Development (UKZN-RCPGD), University of Limpopo Green Biotechnologies Research Centre of Excellence (UL-GBRCE) and University of South Africa, Department of Life and Consumer Sciences (UNISA-LCS) for their wonderful collaboration and financial assistance.

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ABBREVIATIONS

ABTS	– 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA	– Analysis of variance
ANT	– Antioxidant Activity
CAT	– Catalase
CLAMS	– β -carotene-linoleic acid model system
DCM	– Dichloromethane
DPPH	– 2,2-diphenyl-1-picrylhydrazyl
DMEM	– Dulbecco's Modified Eagle Medium
DMSO	– Dimethyl sulfoxide
GBD	– Global Burden of Diseases
GBRCE	– Green Biotechnologies Research Centre of Excellence
EC ₅₀	– Half maximal effective concentration
EtOH	– Ethanol
FBS	– Foetal bovine serum
FRAP	– Ferric reducing antioxidant power
IC ₅₀	– Half maximal inhibitory concentration
KA	– Kojic acid
LCS	– Life and Consumer Sciences
L-DOPA	– 3,4-dihydroxy-L-phenylalanine
LOX	– Lipoxygenase
LPS	– Lipopolysaccharide
MeOH	– Methanol
MIC	– Minimum inhibitory concentration
NO	– Nitric Oxide
ORR	– Oxidation rate ratio
PG	– Turfloop Research Ethics Committees
PS	– Paraclinical Sciences
PSS	– Plant and Soil Sciences
RCPGD	– Research Centre for Plant Growth and Development
ROS	– Reactive oxygen species
RSA	– Radical scavenging activity
SOD	– Superoxide dismutase
SD	– Standard deviation

SE – Standard error
SPSS – Statistical package for social science
SMU – Sefako Makgatho Health Sciences University
TPC – Total phenolic content
TREC – Turfloop Research Ethics Committees
UKZN – University of KwaZulu-Natal
UL – University of Limpopo
UNISA – University of South Africa
UP– University of Pretoria

ABSTRACT

Traditional medicine derived from medicinal plants has been very critical in dealing health issues across many cultures, particularly in rural and underserved communities. In South Africa, the prevalence of skin disorders has necessitated the exploration of natural, culturally grounded remedies to complement modern dermatological practices. The study investigated the cosmeceutical potential of *Spirostachys africana*, an indigenous medicinal plant found in South Africa and is usually used by *isiXhosa* communities for curing skin conditions, particularly in children. To scientifically validate its ethnomedicinal applications, stem-bark extracts were evaluated for antibacterial activity against skin-relevant pathogens (*Klebsiella pneumoniae*, *Escherichia coli*, and *Staphylococcus aureus*) using the microdilution method. Results revealed potent efficacy, with dichloromethane and ethanol extracts exhibiting minimal inhibitory concentrations (MICs) ranging from 0.19 to 1.56 µg/mL. Petroleum ether extract showed modest inhibitory action with a MIC of 0.78 µg/mL. The aqueous extract displayed the highest MIC value of 3.12 µg/mL, indicating the least inhibitory activity against *E. coli* among the tested extracts. *In vitro* assays, including DPPH radical scavenging and β -carotene-linoleic acid model system (CLAMS), revealed significant free radical scavenging and lipid peroxidation inhibition capabilities. The DPPH assay showed a half maximal effective concentration (EC₅₀) value of 83.26 ± 1.22 µg/mL and a maximum inhibition of $72.60 \pm 0.24\%$ at the highest tested concentrations. Additionally, anti-inflammatory assays demonstrated that ethanol and distilled water extracts exhibited strong 15-lipoxygenase inhibition (IC₅₀ values of 0.421 ± 0.079 µg/mL and 0.447 ± 0.057 µg/mL, respectively), while ethanol and petroleum ether extractsshowed moderate nitric oxide inhibition, further supporting the plant's potential therapeutic value. A rat model study demonstrated that the water extract demonstrated the least overall irritation potential, with a maximum visual score of 0.62 ± 0.05 at 72 h, indicating its suitability for topical applications. Ethanol and methanol extracts showed moderate concentration-dependent tyrosinase inhibition, reaching approximately 40-45% enzyme activity reduction at 2.5×10^{-6} mol/L, suggesting potential for skin-lightening applications. This study also evaluated the anti-tyrosinase activity of *Spirostachys africana* stem-bark extracts and found moderate enzyme inhibition compared to the potent effect of kojic acid. These findings suggested the potential of *S. africana* as a natural source of tyrosinase inhibitors for managing hyperpigmentation disorders, warranting further phytochemical investigation and compound isolation. These conclusions scientifically support the traditional application of *S. africana* in treating skin conditions and demonstrate its potential in addressing various dermatological concerns. The study emphasized the importance of integrating indigenous knowledge with

modern scientific approaches in healthcare, particularly in resource-limited settings. Further research is recommended to extract and identify specific bioactive compounds, conduct *in vivo* and clinical studies, develop formulations, and ensure sustainable sourcing of this valuable medicinal plant resource. This comprehensive evaluation of *S. africana* contributes to the preservation of cultural heritage, biodiversity conservation, and the development of effective, accessible, and culturally appropriate therapeutic solutions for skin disorders.

Keywords: Ethnomedicine; Antibacterial activity; Antioxidant properties; Skin irritation; Tyrosinase inhibition; Anti-inflammatory; Cosmeceutical potential

CHAPTER ONE

General Introduction



South African woman applying traditional medicine powder for skin protection. Source: <https://www.cnnindonesia.com>.

1.1 Background

Sufficient evidence exists that shows the vital role medicinal plants have played in healthcare systems worldwide, serving as rich sources of bioactive compounds for pharmaceutical, cosmeceutical, and nutraceutical applications. In many developing countries, traditional medicine derived from plants remains the primary form of healthcare for most of the population due to cultural importance, accessibility, and economic factors. According to **Oyebode et al. (2016)**, almost 80% of the people in developing countries rely on traditional plant-based medicines for their basic medical needs. This reliance highlights the immense potential of exploring medicinal plants for novel therapeutic and cosmeceutical agents. Furthermore, **Sofowora et al. (2013)** wrote that the worth of medicinal plant items on the world market each year surpasses \$100 billion. As a consequence, South Africa sees the utilization of over 70,000 metric tons of plant matter annually (**Williams et al., 2013**). **Twilley et al. (2020)** further estimated that at least 27 million South Africans depend on traditional medicine to meet their healthcare needs.

Communities in Africa have a rich heritage of utilizing indigenous plant species for various medicinal and cosmetic purposes (**Ndlovu et al., 2019; Thothela et al., 2025**). Knowledge of medicinal plant use is intrinsically linked to the livelihoods, social cohesion, and cultural identity of many African societies. However, growing concerns have been raised over the depletion of this priceless traditional knowledge together with the potential extinction of many important medicinal plant species due to factors such as urbanization, deforestation, and climate change (**Groner et al., 2022**).

Traditional medicine, which heavily relies on medicinal plants, has been the primary source of treatment for skin conditions in many African communities (**Mabona and Van Vuuren, 2013**). Utilizing plant-based remedies is deeply rooted in the cultural heritage and traditional knowledge systems of these communities. The skin is among the body's most vital organs, protecting it from both external and internal environmental factors. It aids in protection, thermoregulation, percutaneous adsorption, and sensory functions. For many years, numerous dermatological problems and skin ailments have been treated with natural therapies (**Mabona and Van Vuuren, 2013**).

Skin ailments affect a substantial population of children, ranging from 21% to 87% in developing nations across Africa (**Wenk and Itin, 2003**). The frequency of skin conditions like

eczema, psoriasis, acne, fungal infections, and hyperpigmentation in Africa is compounded by factors such as limited access to modern healthcare facilities, socioeconomic challenges, and cultural practices **(Mabona and Van Vuuren, 2013; Vashi et al., 2018; Oninla et al., 2019)**. In response to these challenges, African communities have developed a rich repertoire of traditional plant-based remedies for curing different skin conditions **(Mabona and Van Vuuren, 2013)**. Plants with antimicrobial, anti-inflammatory, and antioxidant properties are frequently utilized when making topical ointments, powders, and infusions. These remedies are often prepared and administered by traditional healers or sometimes by the ordinary public.

While medicine derived from traditional plants continues to play a crucial role in healthcare systems across many African nations, particularly in the treatment of skin conditions, there are significant challenges that must be addressed. Despite the long-standing cultural heritage and rich traditional knowledge surrounding the utilization of medicinal plants, thorough scientific validation and standardization of such remedies is desperately needed. Concerted efforts are currently underway to record and safeguard the invaluable indigenous knowledge related to the therapeutic applications of medicinal flora. However, these initiatives must be complemented by extensive research endeavors aimed at exploring the full potential of these natural resources for the development of safe novel pharmaceuticals and cosmeceutical products.

Among many different primary challenges hindering the extensive use of conventional plant-based therapies into mainstream healthcare practices is the lack of comprehensive scientific evaluation and quality control measures. Many of these remedies, passed down through generations of oral tradition, lack detailed documentation of their precise formulations, dosages, and mechanisms of action. Furthermore, there is a need to address concerns regarding the effectiveness, safety and possible side effects or interactions of these plant-based treatments, especially when used in conjunction with modern medications. Rigorous clinical trials and toxicological studies are essential to establish evidence-based guidelines for their responsible and effective use **(Mandrioli and Silbergeld, 2016)**.

Additionally, the viable supply and preservation of medicinal plant species pose significant challenges. Overexploitation, climate change and habitat loss pose a threat to the long-term availability of these natural resources, underscoring the urgency of implementing sustainable

harvesting practices and exploring alternative methods such as cultivation and domestication (Shivanna, 2022).

Ultimately, the integration of validated and standardized traditional plant-based therapies into mainstream healthcare practices holds immense potential for improving access to affordable and culturally appropriate treatments for skin conditions and other ailments, particularly in underserved and resource-limited areas of the African continent.

This research aims to explore the cosmeceutical potential of stem-bark extracts of *Spirostachys africana* Sond., a plant traditionally used for child skincare by *isiXhosa* communities. By exploring the bioactive compounds and therapeutic properties of this indigenous species, this study seeks to contribute to the biodiversity conservation, and the development of novel, plant-based cosmeceutical products.

1.2 Medicinal plants used for skin treatments

In recent years, there has been a sharp increase in the market for skincare products that are organic. This has been driven by the understanding that medicinal herbs are essential for treating a variety of skin conditions. Historically, such plant species were utilized for skincare reasons across numerous cultures and countries worldwide (De Wet et al., 2013). Notably, Mabona and Van Vuuren (2013) recently documented the extensive application of herbal medicines in the traditional medicinal systems of southern Africa for addressing dermatological disorders. On a global scale, skin infections and diseases are the fourth most common cause of health problems worldwide, impacting nearly 33% of the global populace. However, their importance is frequently undervalued, despite their wide use (Flohr and Hay, 2021).

While the human body uses natural antioxidant systems to offset the impact of free radicals, dietary sources provide exogenous antioxidants like β -carotene, vitamin E and ascorbic acid. Plant sources have gained public interest due to their natural antioxidant production capabilities, in contrast to synthetic antioxidants, which can exhibit side effects such as toxicity and carcinogenicity (Jadid et al., 2016). The primary requirements for herbal remedies are that they be safe for the skin, widely accessible, and free of side effects (Kadam Vaishali et al., 2013).

Despite the Eastern Cape Province's abundant natural resources and the *isiXhosa* people's deep-rooted knowledge of traditional medicine, few studies have looked into the particular applications of native flora for cosmetic and skincare applications. Researchers such as **Grierson and Afolayan (1999)**, **Dold and Cocks (2005)**, **Afolayan et al. (2014)** and **Thibane et al. (2019)** have noted this knowledge gap, highlighting the need for further exploration in this area.

It is well recognized that plant extracts have a plethora of phytochemicals and antioxidants, which make them promising candidates for incorporation into cosmetic formulations (**Lim et al., 2009**). Among the *isiXhosa* community and other South African ethnic groups, plants have been widely utilized not only for maintaining general health but also for enhancing or preserving beauty and addressing various skin-related concerns (**Mwinga et al., 2019; Fisher et al, 2025**).

While the traditional use of herbal remedies for skin conditions is prevalent, comprehensive scientific studies are desperately needed to confirm the ethnopharmacological uses of numerous South African medicinal plants. For these natural resources to be responsibly and sustainably incorporated into contemporary skincare products and treatments, thorough research is necessary to ascertain their efficacy, safety, and potential therapeutic benefits. Numerous plants have the potential to treat skin conditions, such as *Aloe vera* (**Hekmatpou et al., 2019**), *Acacia erioloba* (**Smith, 1996**), *Bridelia micranthai* (**Samie et al., 2005**), *Erythrina lysistemon* (**Van Wyk, 2013**) and *Spirostachys africana* (**Singh et al., 2020**).

1.3 *Spirostachys africana*

Spirostachys africana Sond. is a versatile and widely utilized plant species that holds significant cultural and medicinal value across various regions of South Africa (**Singh et al., 2020**). The plant is a member of the Euphorbiaceae family and goes by a number of names like “*tamboti*”, “*tambotie*”, “*umThombothi*”, “*Modiba*”, “*umThombotsi*”, “*Ndzopfori*”, “*Morukuru*”, and “*Muonze*” in different languages (**TreeSA, 2024**). *Spirostachys africana* has the capability of reaching heights of up to 18 m. It is renowned for its milky latex, which is toxic and comes from every area of the tree (**Random Harvest, 2024**). Its bark is typically dark brown to black and is distinguished by its rough, thick texture, and orderly cracking into rectangular blocks arranged in longitudinal rows (**Figure 1.1**) (**Random Harvest, 2024**).



Figure 1.1: The bark of *Spirostachys africana*. Source: **Random Harvest (2024)**

As per **Van Wyk and Gericke (2000)**, *Spirostachys africana* is naturally found on the African continent spanning from KwaZulu-Natal (South Africa) in the South to Tanzania in the North. It is prevalent in the Lowveld region and thrives in various soil types. Typically, it is observed in clusters of several large trees along riverbanks or streams, although it can also form extensive groups of smaller trees. Additionally, this species can be encountered throughout all southern African countries, with the exception of Lesotho (**Van Wyk and Gericke, 2000**).

Spirostachys africana is a versatile tree with diverse applications including its smoke being used for fumigation, used as traditional medicine and woodcraft. Its sap can cure wounds that are infested with maggots, treat stomach ulcers, diarrhea and even drive bad spirits away (**Shackleton and Steenkamp, 2004; Mukandiwa et al., 2012; Saxena et al., 2013; Lennox and Bamford, 2015; Nyahangare et al., 2015**). Furthermore, the leaves of this versatile plant are believed to provide therapeutic benefits for periodontal disease, dental caries, and oral thrush (**Akhalwaya et al., 2018**).

In addition, among the *isiXhosa*-speaking people from the province of the Eastern Cape, *S. africana* is applied topically to treat infantile cradle cap, which is locally referred to as *ishimca* (Beach et al., 2010). Numerous ethnobotanical researches have reported the use of the stem-bark for treating a range of ailments, including infantile body rashes, diarrhea, stomach pain, and dysentery (Mabogo, 1990; Cocks and Dold, 2000; McGaw et al., 2000; Verschaeve et al., 2004; Mathabe et al., 2006).

The widespread application of *S. africana* in traditional medicine practices across southern Africa underscores its cultural significance and the depth of indigenous knowledge surrounding its applications. However, it is crucial to conduct rigorous scientific research to validate and standardize the traditional uses of this plant, ensuring its safe and effective incorporation into modern healthcare practices while preserving the rich cultural heritage associated with its use.

1.4 Phytochemical properties of *Spirostachys africana*

Through the synthesis of several secondary metabolites, plants have evolved complex defense mechanisms to combat external stressors such as temperature fluctuations, water stress, light intensity variations, herbivory, and microbial attacks (Ncube et al., 2012). *Spirostachys africana*, an indigenous tree species of Southern Africa, is renowned for its rich phytochemical profile, which is central to its resilience and therapeutic properties.

According to Singh et al. (2020), *S. africana* comprise seven different groups of phytochemicals, which include, terpenoids, flavonoids, phenols, tannins, alkaloids, steroids and saponins. These are available from all parts of the plant, including the root, leaves, stem and bark. The presence of alkaloids makes *S. africana* a rare species within the Euphorbiaceae family to contain this class of phytochemicals (Salatino et al., 2007). It is well known that alkaloids generated from plants have both antifungal and antibacterial properties (Augusto et al., 2011), justifying the traditional use of *S. africana* materials in eastern Africa to treat malarial infections, as there have been reports of alkaloids having antimalarial qualities (Lemmens et al., 2012; Dua et al., 2013). However, alkaloids are also commonly recognized as potent toxins, accounting for the plant's toxicity to humans (Harborne, 1973; Iqbal et al., 2015).

The Euphorbiaceae family is phytochemically diverse and particularly contains abundant terpenoids and alkaloids (**Webster, 1986**). Previous research on the latex of *S. africana* showed that beyerene derivatives, acid metabolites, α -ketols, diosphenols, and stachynones were present (**Baarschers et al., 1962; Duri et al., 1992; Munkombwe et al., 1997**). According to reports, terpenoids have the ability to prevent bacterial growth (**Drewes et al., 2005**), protozoa (**Ghoshal et al., 1996**) and viruses (**Jassim and Naji, 2003; Cho, 2006**).

There are reports on the phytochemical studies conducted on *S. africana* latex including the tree's bark that displayed antibacterial properties against a range of bacteria that cause diarrhea, such as *Escherichia coli*, *Shigella typhi*, *Shigella sonnei*, *Shigella dysenteriae*, *Shigella flexneri*, and *Shigella boydii*, with the lowest possible inhibitory amounts (MIC) ranging from 0.156 to 0.625 mg/ml (**Mathabe et al., 2006**).

Given the therapeutic potential and diverse phytochemical profile of *S. africana*, interest in creating quick and reliable techniques to measure its biological characteristics is rising, which could benefit researchers, farmers, and pharmaceutical companies in exploring and optimizing the utilization of this valuable medicinal plant.

1.5 Skin disorders

Many African countries are significantly affected by various skin conditions, and traditional medicine derived from plants plays a crucial role in their treatment (**Naidoo and Cooposamy, 2011**). Skin ailments affect a substantial population of children, ranging from 21% to 87% in developing nations across Africa (**Wenk and Itin, 2003**). The prevalence of skin diseases in Africa is compounded by factors such as restricted availability of present-day medical facilities, socioeconomic challenges, and cultural customs (**Mabona and Van Vuuren, 2013**).

Skin ailments like eczema, psoriasis, fungal infections, acne, and hyperpigmentation are prevalent in many African nations like South Africa, Nigeria, Ghana and Congo-Brazzaville (**Hartshorne, 2003; Hay et al., 2006; Oninla et al., 2019**). These conditions can be exacerbated by environment-related factors, like high temperatures, humidity and being among different allergens and irritants. Additionally, certain cultural practices, such as the use of traditional

cosmetics and the application of plant-based products, can occasionally cause skin issues to develop or worsen **(Vashi et al., 2018)**.

Skin disorders represent a significant health burden among children in the African continent. Common dermatological conditions affecting this pediatric population include eczematous disorders, bacterial skin infections like impetigo, fungal infections, scabies infestations, insect bite reactions, and pigmentation abnormalities **(Ayanlowo et al., 2018)**. Poor hygiene habits are among the factors contributing to the high occurrence of these illnesses, together with restricted availability of contemporary medical facilities, cramped living arrangements and malnutrition, especially in rural and underserved communities **(Cooten et al., 2018)**.

To address these dermatological issues, African traditional medicine systems have historically relied on medicinal plants and natural resources as therapeutic interventions **(Mahomoodally, 2013; Tyavambiza et al., 2021)**. Indigenous people in diverse locations have evolved a rich tradition of using plant materials, including leaves, barks, roots, and other components, to make topical formulations such as ointments, pastes, powders, and washes for skincare purposes **(McMullen and Dell’Acqua, 2023)**. These traditional remedies often contain bioactive phytochemicals with wound-healing, antimicrobial, anti-inflammatory and antioxidant properties, which are beneficial in managing various skin disorders **(Mahachi, 2013; Alliya, 2019)**. In response to these challenges, African communities have developed a rich repertoire of traditional plant-based remedies for curing numerous skin ailments **(Mabona and Van Vuuren, 2013)**. Plants with antimicrobial, anti-inflammatory, and antioxidant qualities are frequently utilized in preparing topical ointments, powders, and infusions. These remedies are often prepared and administered by traditional healers or sometimes by the ordinary public.

In South Africa, the *isiXhosa* communities, renowned for their extensive traditional knowledge, have a long-standing practice of utilizing indigenous plant species for child skincare **(Bhat, 2013; Bhat, 2014)**. One particularly notable example is the “*tamboti*” tree (*S. africana*), whose leaves and bark are highly regarded for treating conditions like rashes, burns, and eczema in children **(Grace et al., 2003)**. Other plant species, such as those from the *Scilla* and *Bulbine* genera, are also used as moisturizers and for curing minor wounds and skin irritations in paediatric populations **(Mudau and Ledwaba, 2023)**.

While traditional plant-based remedies for child skincare are widely practiced among the *isiXhosa* communities of South Africa, reflecting their deep-rooted cultural heritage and indigenous knowledge systems, there remains a significant gap in scientific validation and standardization of these formulations. The absence of thorough studies to assess the effectiveness, safety, and quality control procedures for promising traditional remedies highlights the need for integrating traditional knowledge with modern dermatological practices. By fostering collaborative efforts between traditional healers, researchers, and healthcare professionals, there exists a remarkable opportunity to develop culturally acceptable, affordable, and effective solutions that can improve child skin health outcomes, particularly in resource-limited settings where access to modern dermatological care is limited. Moreover, the conservation of medicinal plant resources is crucial to make sure that these traditional practices are sustainable, as reliance on plant-based remedies persists owing to limited access to contemporary medical services in many remote and underserved areas. Through the harmonious synergy of indigenous knowledge systems and scientific advancements, the exploration of plant-based skincare solutions for children in South Africa and other African regions holds great potential for developing innovative and culturally relevant approaches that can positively impact child health, while conserving the rich legacy of conventional medical procedures **(Mulaudzi, 2001; Hartshorne, 2003; Department: Science and Technology, 2008; Lall, 2017).**

1.6 General cosmeceutical uses

Cosmeceuticals, a portmanteau of "cosmetics" and "pharmaceuticals", have emerged as the personal care sector with the quickest rate of growth **(Draelos, 2008)**. These products bridge the gap between cosmetics and therapeutics, designed to deliver medical benefits while improving physical appearance and overall attractiveness **(Martin and Glaser 2011)**. According to **Alnuqaydan (2024)**, cosmeceuticals encompass a wide range of formulations, including skin cleansers, lipsticks, creams, hair shampoos, body lotions, face packs, ointments, fairness formulations, eyeliners, face powders, and beauty soaps. Their active ingredients promote collagen formation, keep skin and keratin healthy by neutralizing free radicals **(Joshi, 2012)**.

Herbal cosmetics, a subcategory of cosmeceuticals, are natural preparations that harness the power of uncontaminated herbs or acceptable cosmetic herbal elements in extract or raw form

(Devi et al., 2018). These products are gaining popularity due to their skin compatibility, widespread availability, and absence of adverse effects. A wide variety of herbs are commercially accessible as cosmetics for skincare purposes, antioxidant benefits and hair treatment qualities **(Kadam et al., 2013).**

Plants make a great source of cosmetic products because of their calming, soothing, restoring, healing, and skin protecting properties through their herbal extracts **(Patel et al., 2013).** Cosmetic formulations are designed for topical application, serving purposes such as cleansing, beauty enhancement, and appearance modification. A person's general health and well-being are strongly correlated with how they look, according to research **(Dlova et al., 2015a).** Globally, plants are used for a variety of cosmetic purposes, such as moisturizing, UV protection, masking pimples and blemishes, depigmentation, treating and preventing acne and eczema, lightening, antimicrobial activity, among other things **(Van Wyk and Gericke, 2000; Mapunya et al., 2012; Patel et al., 2012; Sharma and Kumar, 2013; Lall and Kishore, 2014).**

As cosmeceuticals gain popularity, many cosmetic products are being enriched with natural ingredients, driving the rapid growth of this sector **(Draelos, 2008).** In South Africa, the *IsiXhosa* people have long utilized plants to improve their appearance and preserve their health **(Dold and Cocks, 2005).** The increasing demand for herbal cosmetics has created new opportunities in the cosmeceutical industry **(Chintamunnee and Mahomoodally, 2012; Suroowan and Mahomoodally, 2013).** Consumers often prefer herbal products for personal care, as they are thought to have less adverse effects and to be devoid of artificial ingredients **(Ekor, 2014).** In the Eastern Cape Province, herbal cosmetics are commonly purchased from herbal shops, although some are still prepared at home.

Cosmeceuticals are believed to improve skin tone, texture, and radiance, leading to wrinkle reduction. They are designed to suppress acne, exhibit anti-wrinkle effects, protect against sunburn, enhance complexion, delay the effects of aging, and improve skin texture. The wealth of plants and natural ingredients available offers an enormous potential for cosmeceutical applications. Natural herbs are essential for preserving and improving one's look and general character, contributing to a holistic concept of natural beauty that encompasses all aspects of body preparation, conditioning, and beautification **(Joshi, 2012).**

Medicinal plant extracts possess an high vitamin content, making them promising candidates for incorporation into cosmetic formulations (**Lim et al., 2009**). The active ingredients within plants may provide corrective benefits as they can function as antioxidants, inhibitors of oxidative chemicals, metal chelators and UV radiation shields (**Pisoschi and Pop, 2015**).

1.7 *isiXhosa* community of South Africa

The *isiXhosa* are an indigenous South African ethnic group, primarily residing in the province of the Eastern Cape. They have a rich cultural tradition deeply combined with using of therapeutic plants for a range of objectives. Indigenous medicine is critical in the healthcare system of the *isiXhosa* community, with many households relying on indigenous plant species to treat common ailments and maintain overall well-being (**Bhat, 2013; Bhat, 2014; Petersen et al., 2014**). This custom has been carried down for many generations, preserving an abundance of traditional ecological knowledge about the medicinal qualities and applications of various native plants.

This resilient tree species, native to the semi-arid regions of southern Africa, has been extensively used by the *isiXhosa* for its diverse therapeutic properties (**Singh et al., 2020**). The *isiXhosa* speaking people have traditionally utilized the fruits, roots, leaves and all other components of the *S. africana* tree to cure different ailments and conditions. The bark and leaves have been used to manage skin conditions, respiratory infections, and as a general tonic, while the roots possess antimicrobial and anti-inflammatory qualities (**Mathabe et al., 2006; Singh et al., 2020**).

1.8 Aim

The overall aim of the present research was to evaluate the cosmeceutical potential of *S. africana* extracts traditionally used for child skincare among South Africa's *isiXhosa* communities in the province of the Eastern Cape.

1.9 Objectives

The specific objectives of the study were to:

- i. Evaluate the antimicrobial activity of *S. africana* extracts against a panel of skin-relevant bacterial strains,
- ii. Determine the antioxidant capacity of *S. africana* extracts through various *in vitro* antioxidant assays, assessing their potential to scavenge free radicals and safeguard against oxidative stress,
- iii. Conduct skin irritation tests using appropriate *in vitro* models to evaluate the safety and non-irritant nature of *S. africana* extracts, ensuring their suitability for topical applications,
- iv. Examine the anti-tyrosinase activity of *S. africana* extracts, exploring their potential to inhibit the enzyme tyrosinase, which produces melanin and is implicated in hyperpigmentation disorders, and
- v. Determine the anti-inflammatory potential of *S. africana* extracts, specifically evaluating their ability to inhibit nitric oxide (NO) production and 15-lipoxygenase (15-LOX) activity.

1.10 Hypotheses

The study tested the following hypotheses,

- i. H₁: *Spirostachys Africana* extracts exhibit broad-spectrum antimicrobial activity against skin pathogens (e.g. *S. aureus*), with efficacy comparable to standard antibiotics.
- ii. H₂: The extracts demonstrate significant free radical scavenging activity *in vitro* indicating their potential to mitigate oxidative stress.
- iii. H₃: *Spirostachys africana* extracts are non-irritating in skin models, showing their traditional suitability for pediatric skincare.
- iv. H₄: The *Spirostachys africana* extracts inhibit tyrosinase activity, suggesting utility in managing hyperpigmentation disorders in humans.
- v. H₅: The extracts suppress pro-inflammatory markers (NO, 15-LOX), aligning with their traditional use for inflammatory skin conditions.

1.11 Scope of the study

The aim of the present study was to explore the cosmeceutical potential of *S. africana* extracts used traditionally for child skincare by *isiXhosa* communities found within the province of the Eastern Cape in South Africa. Firstly, the study highlights the ethnobotanical significance and traditional uses of *S. africana* among the *isiXhosa* people, particularly in the context of child skincare practices.

The study evaluated the antibacterial efficacy of *S. africana* extracts against a panel of skin-relevant bacterial and fungal strains, exploring their potential as natural antimicrobial agents for topical applications. Furthermore, the antioxidant capacity of these extracts was evaluated by using different *in vitro* antioxidant assays, looking into how well they can scavenge free radicals and protect against oxidative stress.

Additionally, the study includes skin irritation tests using appropriate *in vitro* models to ensure the safety and non-irritant nature of these extracts for topical use.

Moreover, the anti-tyrosinase activity of *S. africana* extracts was evaluated, exploring their potential to inhibit the enzyme tyrosinase, which produces melanin and is implicated in hyperpigmentation disorders. This aspect of the study is particularly relevant for cosmeceutical applications targeting skin lightening or pigmentation regulation.

This information is crucial for understanding the underlying mechanisms and developing standardized, quality-controlled cosmeceutical formulations.

1.12 Outline of the thesis

The thesis resulting from this study comprises seven Chapters. The general introduction and study synthesis are found in the first and last Chapters, respectively. The in-between five Chapters are presented as independent papers, each with introduction, methods, findings, and discussion sections. These chapters that have either been submitted, published in a one or more peer-reviewed journals, or are in the process of being submitted.

Chapter 1 presents the motivation for the study, aim and objectives, hypotheses, and the organization of the thesis.

Chapter 2 provides an overview of the traditional uses and ethnobotanical significance of *S. africana*, particularly regarding child skincare practices. In this chapter existing literature on the phytochemistry, medicinal properties, and skin-related applications of *S. africana* was reviewed.

Chapter 3 is an experimental Chapter focusing on the antimicrobial activity of *S. africana* stem-bark extracts against a panel of skin-relevant bacterial strains. Its goal was to determine how well these extracts work as antimicrobials.

Chapter 4 investigates the antioxidant capacity effect of *S. africana* stem-bark extracts through various *in vitro* assays. This Chapter explored these extracts' ability to scavenge free radicals, protect against oxidative stress, and offer potential sun protection benefits for skincare applications.

Chapter 5 examines the skin irritation potential activity of *S. africana* stem-bark extracts using appropriate *in vitro* models. This chapter looked at skin irritation properties of the plant extract only, assessed by a visual score.

Chapter 6 evaluates the anti-tyrosinase activity of *S. africana* stem-bark extracts and compared to the potent effect of kojic acid. It investigates the potential of *S. africana* as a natural source of tyrosinase inhibitors for managing hyperpigmentation.

Chapter 7 addresses the anti-inflammatory capabilities of *S. africana* extracts, specifically evaluating their ability to inhibit nitric oxide (NO) production and 15-lipoxygenase (15-LOX) activity. These assays aimed to identify bioactive extracts capable of modulating inflammatory pathways, a critical feature in managing inflammatory-related disorders.

Chapter 8 provides the overall synthesis of the thesis, highlighting the key findings and their implications for the development of natural cosmeceutical products based on *S. africana* stem-bark extracts. This Chapter also outlines the conclusions, limitations, and future research recommendations.

CHAPTER TWO

***Spirostachys africana* in South African Traditional Medicine: A Comprehensive Review of its Phytochemistry, Medicinal Properties, and Potential for Skincare**



A woman from the Eastern Cape Province preparing traditional medicine.

(Source: www.poemsearcher.com)

2.1 Introduction

Plant-based traditional medicine has been a crucial component of healthcare practices across numerous cultures globally. For a large percentage of the population in developing nations like South Africa, herbal treatments constitute their main source of healthcare, especially in remote and underserved communities **(Naidoo and Coopooosamy, 2011; Mabona and Van Vuuren, 2013)**. It is estimated that approximately 80% of South Africans rely on medicinal herbs to treat a variety of illnesses **(Street and Prinsloo, 2012)**. The traditional understanding of using native plant species for medicinal purposes has seen a resurgence in recent decades across the African continent. While modern pharmaceuticals are generally considered more effective, some patients may be cautious about potential side effects and prefer natural alternatives from their cultural traditions. Traditional healers and rural communities often turn to herbal medicine, drawing upon centuries of accumulated wisdom about the healing properties of local flora. The limited access to modern healthcare facilities in many areas further contributes to the reliance on traditional plant-based remedies.

In South Africa, the prevalence of skin conditions is on the rise, with acne, eczema, pigmentation disorders, atopic dermatitis, viral warts and superficial fungal infections being among the most common **(Dlova et al., 2015b; Katibi et al., 2016)**. The country's diverse climatic regions, ranging from hot and humid to arid and semi-arid, can exacerbate or trigger various skin ailments. Many South Africans may have reservations about Western medicine due to concerns over side effects, antibiotic resistance, or cultural beliefs, leading them to seek out traditional remedies. Traditional plant-based remedies have the potential to provide affordable, culturally acceptable, and effective solutions, especially in rural and underserved areas. However, rigorous scientific validation and standardization of these remedies are crucial to ensure their safety, efficacy, and quality.

These plant-based treatments, rooted in indigenous knowledge systems, usually thought of as kinder substitutes that have less negative consequences. However, in South Africa, there is still little scientific proof and documentation of the herbal remedies utilized for curing skin conditions. This research aims to explore types of plants that are utilized by traditional rural communities to address skin ailments, with a focus on preserving this valuable knowledge and exploring its potential for future drug discovery efforts.

Given the widespread use of traditional plant-based remedies for skin conditions among South African communities (**Dlova et al., 2015b; Katibi et al., 2016**), it is critical to review the existing literature on the plants used for this purpose. In particular, it is crucial to examine both the potential benefits and associated risks of these medicinal plants. By delving into the therapeutic qualities, phytochemistry and ecological implications of these indigenous species, we are able to acquire a thorough comprehension of their suitability for addressing skin disorders. This chapter aims to collate and synthesize the significant research findings reported to date on *S. africana* traditionally used for skincare in South Africa. By consolidating this knowledge, researchers can identify existing gaps and opportunities for further exploration, while also enabling the cosmeceutical and pharmaceutical industries to leverage this collated information in creation of innovative plant-based solutions designed to meet the various skincare requirements of South African communities.

2.2 *Spirostachys africana*

Belonging to the order Malpighiales and the family Euphorbiaceae, *S. africana* is native to various regions in southern Africa, naturally occurring from South Africa's KwaZulu-Natal province and Tanzania (**Hutchings and Scott, 1996; Coates Palgrave, 2002**). The Euphorbiaceae family comprises about 275 genera and 7,500 species, including notable genera such as *Alchornea*, *Croton*, *Euphorbia*, *Macaranga*, and *Spirostachys* (**Palgrave, 1990; Simpson, 2010; TreeSA, 2024**). *Spirostachys africana* is commonly known by various names, including “*tamboti*” (English), “*tambotie*” (Afrikaans), “*Umthombothi*” (Zulu and Xhosa), “*Umthombotsi*” (siSwati), “*Modiba*” (Northern Sotho), “*Morukuru*” (Tswana), “*Muonze*” (Tshivenda), and “*Ndzopfori*” (Tsonga) (**TreeSA, 2024**).

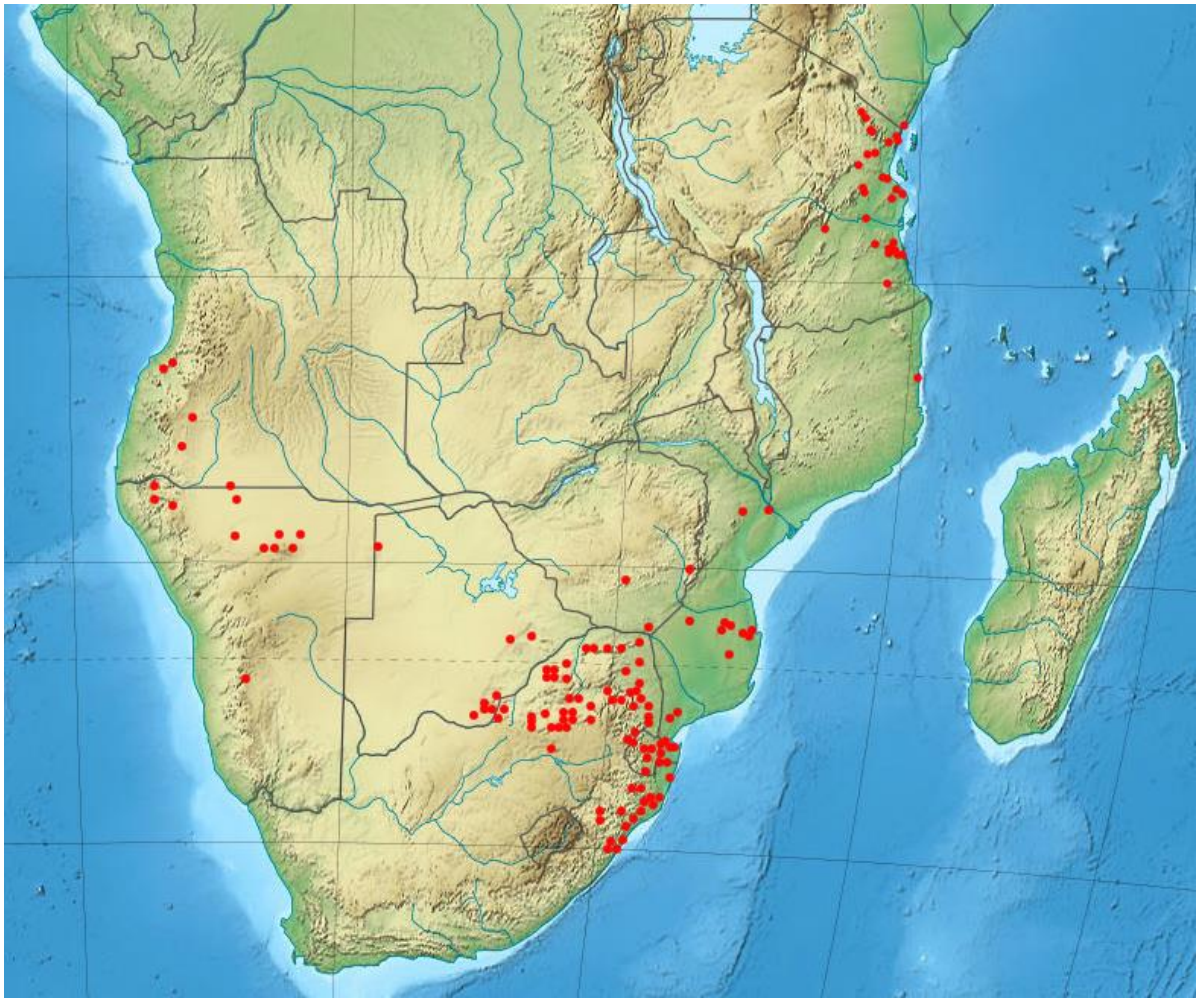


Figure 2.1: Distribution of *Spirostachys africana*. Source: <https://one-namibia.com/en/tamboti-insights/>

This hardy tree species is widely distributed across southern Africa and can include various growth forms, including herbs, shrubs, or trees (**Figure 2.1**). The Euphorbiaceae family is known for the presence of latex, and *S. africana* is no exception, with its fine wood being recognized as toxic and capable of causing nausea and headaches (**Van Wyk and Gericke, 2000**). It is characterized by a dense, round crown and distinctive bark that is dark, thick, rough, and distinctively broken into regular longitudinal columns (**Arnold et al., 2002**). The tree's simple (**Figure 2.2**), alternating leaves have finely toothed margins and emerge with a striking red colour in spring, contrasting with the older green foliage (**Arnold et al., 2002**). Flowering occurs in

August before the new leaves emerge, and the blooms are occasionally visited by bees (**Arnold et al., 2002**).

One of the most notable features of *S. africana* is its poisonous, smooth latex, which emanates from all parts of the tree. Throughout the region, traditional medical practices have made extensive use of this latex due to its unique qualities. The latex from the leaves is milky, while the heartwood produces a brown, viscous latex (**Van Wyk, 2013**). The latex can be renowned for its poisonous properties, even being used as an arrow poison for stunning fish when mixed with water (**Arnold et al., 2002**). When mixed with porridge, the latex acts as a purgative or emetic and is taken orally to alleviate stomach aches (**Arnold et al., 2002**). It is also applied topically to boils to prevent infection and is believed to drive away bad spirits when inhaled as smoke (**Arnold et al., 2002**). Additionally, the latex has been traditionally used as a malaria treatment in Mozambique and Tanzania (**Arnold et al., 2002**).

According to (**Beach et al., 2010**), among the *isiXhosa*-speaking people from the province of the Eastern Cape, *S. africana* is applied topically to treat infantile cradle cap, which is locally referred to as “*ishimca*”. While *S. africana* offers numerous benefits, it is crucial to take cognisance of the fact that the extract of the plant from its twigs and bark has been found to be genotoxic to micronuclei (**Arnold et al., 2002**). Additionally, consuming meat that has been smoked or roasted using the tree's wood can cause diarrhea, and continuous use of ox-yokes made from the wood can induce uncontrollable bruising (**Arnold et al., 2002**).



Figure 2.2: *Spirostachys africana* tree (<https://plantinfo.co.za/plant/spirostachys-africana/>).

2.3 Phytochemical Composition

Plants have evolved sophisticated defense mechanisms characterized by the synthesis of diverse secondary metabolites aimed at counteracting a range of external stressors, including fluctuations in temperature, water availability, variations in light intensity, herbivore predation, and microbial assaults (Ncube et al., 2012). These secondary metabolites encompass numerous compounds, including phenolics, alkaloids, saponins, terpenoids, and flavonoids, each serving specific functions in defence and adaptation processes (Kumar, et al. 2023; Zandavar et al., 2023). According to Singh et al. (2020), saponins, alkaloids, tannins, steroids, flavonoids, phenols, and terpenoids are among the seven types of phytochemicals found in *S. africana*. These compounds are found in the plant's roots, bark, leaves, and stems.

2.3.1 Alkaloids

Alkaloids comprise a heterocyclic nitrogen atom in their chemical structure and are among the many types of organic chemicals found in nature (Marutescu et al., 2017). In order to defend themselves against herbivores and other dangers, plants mainly manufacture these substances,

which serve as natural toxins **(Mohan et al., 2016)**. Alkaloids are widely distributed across various plant families within the angiosperms (flowering plants), with approximately 15% of plant species known to produce these compounds **(Wink, 2016)**.

The structural diversity of alkaloids allows them to be categorized into multiple classes according to their biological precursors and heterocyclic ring structure. These classes consist of imidazoles, indoles, pyrrolidines, piperidines, pyrrolizidines, tropanes, isoquinolines, purines, and quinolizidines **(Thawabteh et al., 2019)**. One factor contributing to the broad spectrum of biological activity displayed by alkaloids is the diversity of their chemical structures **(Bhambhani et al., 2021)**. Through *in vitro* research, a few alkaloids have been identified and their antibacterial qualities investigated. Antibacterial action against both Gram-negative and Gram-positive and microorganisms has been shown by alkaloid compounds such as sanguinarine, berberine, tomatidine, piperine, and cinchona **(Barbieri et al., 2017)**. These conclusions demonstrate how alkaloids may be used to create new antibacterial compounds. Alkaloids exert their biological effects through mechanisms such as DNA intercalation and topoisomerase inhibition **(Baikar and Malpathak, 2010)**.

The presence of alkaloids in *S. africana* is a significant characteristic that sets it apart from most other genera within the Euphorbiaceae family **(Salatino et al., 2007)**. These alkaloid compounds add to the medicinal qualities and toxicity of the plant **(Singh et al., 2020)**. The alkaloids found in *S. africana* exhibit various biological activities that support the traditional medicinal uses of this plant in different parts of Africa, including South Africa, Mozambique, and Tanzania **(Sighn et al., 2020)**. Notably, compounds like spiramine have demonstrated promising antimalarial effects, justifying the plant's use in treating malaria **(Lemmens et al., 2012; Dua et al., 2013)**. It is believed that alkaloids generated from plants have antifungal and antibacterial qualities **(Mathabe et al., 2006; Souto et al., 2011)**. However, it is critical to appreciate that some alkaloids are highly toxic and add to the plant's overall toxicity to both humans and animals **(Iqbal et al., 2015)**.

Future research prospects include further investigating the bioactivities, mechanisms of action, and potential applications of the isolated alkaloids from *S. africana*. This could pave the way for developing new pharmaceutical or cosmeceutical products based on these bioactive compounds. Additionally, efforts are needed to document and preserve the traditional knowledge associated

with the plant's utilization for medicinal purposes, including promoting the sustainable utilization of its natural resources.

2.3.2 Flavonoids

Flavonoids are another significant class of phytochemicals found in *S. africana*. Flavonoids are secondary metabolites that are mostly made up of a benzopyrone ring with phenolic or polyphenolic groups attached to it in different places (**Cavalcante et al., 2018**). According to **Kozłowska and Szostak-Wegierek (2014)** more than 10,000 flavonoids have been found. Based on how unsaturated they are, chemical structure and oxidation of the carbon ring are divided into several categories. As reported by **Ullah et al. (2020)** these include flavanones, flavans, anthocyanidins, isoflavonoids, anthoxanthins (flavanone and flavanol) and flavanonols, (**Figure 2.3**). Several studies have reported the presence and potential significance of flavonoids in different parts of *S. africana* (**Amoo et al., 2012; Mulaudzi et al., 2012**). For instance, **Mulaudzi et al. (2012)** reported that *S. africana* bark contains about 11.57 µg/g catechin equivalents (CTE) of flavonoids.

In plants, flavonoids play a variety of functions, such as regulating cell division, attracting pollinating insects, and offering protection from biotic and abiotic stresses. Due to their strong antioxidant qualities, flavonoids shield plants from cellular peroxidation and aid in their ability to withstand oxidative stressors by scavenging free radicals (**Schroeter et al., 2002**). Flavonoids are linked to numerous health benefits, including antioxidant, anticancer and antiviral properties. Furthermore, they possess cardio- and neuro-protective qualities. These biological processes are influenced by the type of flavonoid, its potential mode of action, and its bioavailability (**Ullah et al., 2020**). **Allemann and Baumann (2009)** reported the use of flavonoids in skincare products. Additionally, these polyphenolic compounds exhibit a range of biological activities, including antioxidant, antibacterial, and anti-inflammatory properties (**Singh et al., 2020**).

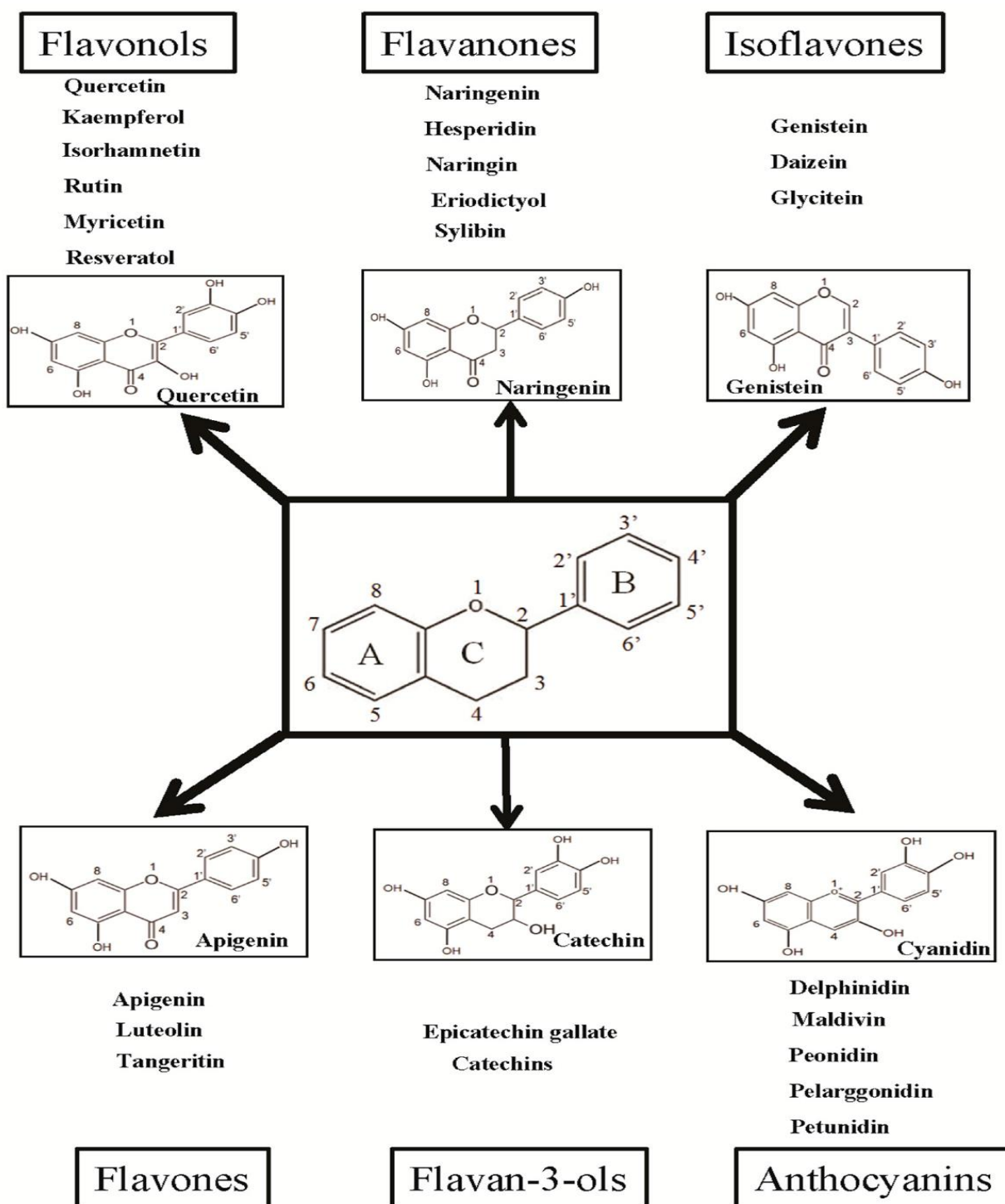


Figure 2.3: Flavonoids are categorized into six distinct categories based on their basic chemical composition: flavan-3-ols, isoflavones, flavanones, flavones, flavonols and anthocyanins (**Kawser Hossain et al., 2016**).

2.3.3 Saponins

Many different plant species contain saponins, a broad class of naturally formed glycosides. These substances are distinguished by their capacity to dissolve in aqueous solutions and produce stable, soap-like foams, a property that derives its name from the word "*sapo*", which is Latin for "soap" (Vincken et al., 2007). Lanostanes, dammaranes, cucurbitanes, cycloartanes, tirucallanes, hopanes, oleananes, taraxasteranes, lupanes, ursanes, and steroids are among the 11 major classes of saponins (Vincken et al., 2007).

Saponins have foaming properties, hence they are added in washing detergents, beverages and toothpaste as emulsifiers (Chen et al., 2010). Plant saponins have some form of pharmacological effects, for instance molluscicidal properties (Ibrahim et al., 2023), anti-inflammatory (Passos et al., 2022), antimicrobial (Arabski et al., 2012; Khan et al., 2018), antitussives and cytotoxic activities (Sparg et al., 2004). While saponins offer promising therapeutic potential, it is imperative to thoroughly assess their safety and toxicity profiles, as some saponins can exhibit cytotoxic or hemolytic effects at high concentrations (Sparg et al., 2004). Furthermore, there is adequate evidence suggesting that some saponins might not be able to pass through bacterial strains' cell membranes, especially Gram-negative bacteria (Desai et al., 2009). Saponins' ability to alter cell membranes' permeability, disturb and destabilize bacterial cell membranes, cause oxidative stress, DNA damage, protein malfunction, and ultimately death of the cell is believed to be the mechanism behind their antibacterial effect (Gurunathan et al., 2014).

A number of research studies have shown the presence of saponins in various parts of the *S. africana* plant, which includes the roots, leaves, and stem-bark (Alliya, 2019; Singh et al., 2020). The exact saponin profile and quantities may differ based on the plant's component and the geographic region (Kregiel et al., 2017). In conventional medicine practices, saponins are thought to contribute to the medicinal applications of *S. africana*, particularly in curing skin ailments, wounds and infections (Alliya, 2019). The antimicrobial action of these saponins supports the traditional use for treating infections and skin conditions (Okello et al., 2020).

2.3.4 Terpenoids

Terpenes are important basic biosynthetic chemicals found in all living organisms. When terpenes undergo chemical alteration, such as oxidation or carbon skeleton rearrangement, the

subsequent combinations form terpenoids (Yang et al., 2020). Currently, nature contains more than 50,000 terpenoids (Sun et al., 2017). They contain isoprene units ($\text{CH}_2=\text{C}(\text{CH}_3)$ and $\text{CH}=\text{CH}_2$, C_5H_8), with eight hydrogen atoms and five carbons, making them the building block of all terpenes. Two isoprene units (C_{10}) are found in monoterpenes, three in sesquiterpenes (C_{15}), four in diterpenes (C_{20}), five in sesterpenes (C_{25}), six in triterpenes (C_{30}), and eight in tetraterpenes (C_{40}) (Perveen, 2018). The classes are shown in Figure 2.4.

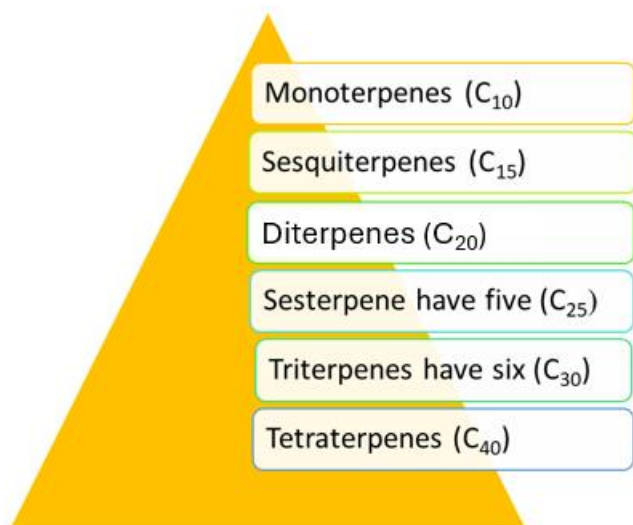


Figure 2.4: Classification of terpenes and terpenoids (Vincken et al., 2007).

Perveen (2018) reported that human and other animal bodies contain terpenoids, a varied group of secondary metabolites with antioxidant properties. Due to its potent antibacterial properties like *Escherichia coli*, *Vibrio cholera* and *Staphylococcus aureus*, the plant *Vitex peduncularis*, which contains the triterpenoid Epifriedelinol (C_{30}), has long been utilized to cure dysentery and diarrhea in several countries (Kannathasan et al., 2019). Despite their important medicinal antioxidant qualities, terpenoids are toxic to animals and can be lethal if ingested (Lennox and Bamford, 2015). Research done by Mathabe et al. (2006) found four terpenoids in *S. africana*, including two triterpenoids (compound 1: d-Friedoolean-14-en-oic acid, also called 3-acetyl aleuritolic acid), compound 2: Lupeol and two diterpenes, compound 3: ent-2,6 α -dihydroxy-

norbeyer-1,4,15-trien-3-one, also called diosphenol **2**, and compound **4**: ent-3 β -hydroxy-beyer-15-ene-2-one). These substances demonstrated cytotoxic and antimicrobial qualities, highlighting the potential benefits of *S. africana*. However, it's important to exercise caution, as several authors, such as **Wink and Van Wyk (2008)** and **Lennox and Bamford (2015)**, have classified *S. africana* as highly poisonous. It is categorized as a Class IA cellular poison, indicating its extreme toxicity (**Lennox and Bamford (2015)**). Therefore, despite its potential usefulness, precautions must be taken when handling this plant.

2.3.5 Tannins

Many diverse plant components, including the bark, leaves, fruits, and roots, contain tannins, a large family of naturally generated polyphenolic compounds (**Mikail et al., 2022**). Their capacity to precipitate and bind proteins to form solid structures is what distinguishes them (**Sieniawska and Baj, 2017**). The process of tanning animal hides and skins to produce leather gave rise to the word 'tannin' (**Sieniawska and Baj, 2017**). There are two broad categories of tannins, namely: hydrolysable and condensed tannins (also referred to as proanthocyanidins) (**Molino et al., 2023**). Proanthocyanidins are the most prevalent polyphenols and are almost universally present in plant groups (**Dai et al., 2010; Nie and Stürzenbaum, 2019**). Esters of gallic or ellagic acid are hydrolyzable tannins combined with glucose or other polyols. They are also divided into two groups: ellagitannins, which include ellagic acid, and gallotannins, which contain gallic acid (**Macáková et al., 2014**). On the other hand, condensed tannins are flavan-3-ols polymers like epicatechin and catechin, and are distinguished by their capacity to create insoluble compounds (**Molino et al., 2023**). The four types of tannins - complicated tannins, ellagitannins, gallotannins and condensed tannins - are depicted in **Figure 2.5 (Besharati et al., 2022)**.

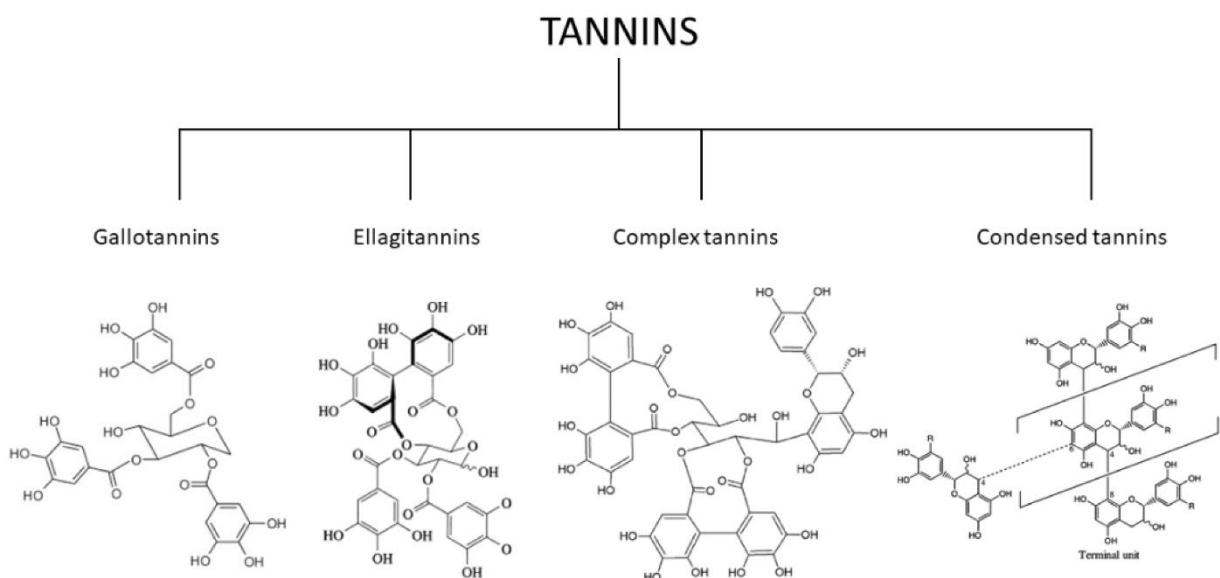


Figure 2.5: Tannins classification. Source: **(Besharati et al. (2022)).**

Tannins are used in many different fields, including food, medicine, and industry. Numerous illnesses are treatable with their astringent properties, such as diarrhea, inflammation and mild skin irritations **(Mikail et al., 2022)**. Because tannins can attach to proteins and form a robust substance, they are frequently used in the industrial sector for tanning leather **(Sieniawska and Baj, 2017)**. They are also used in the manufacturing of inks, dyes, and adhesives **(Das et al., 2020)**.

In *S. africana*, various types of tannins are found. The *S. africana*'s leaves and bark have condensed tannins, which contribute to its medicinal properties and traditional uses **(Mulaudzi et al., 2012; Singh et al., 2020)**. Additionally, **Mulaudzi et al. (2012)** reported that the bark of *S. africana* contains about 0.587 of leucocyanidin equivalents (LCE) of condensed tannins. In the realm of skincare issues, tannins assume a notable role owing to their astringent qualities and antioxidant attributes. In addressing dermatitis, topically applied tannins function by coagulating the surface proteins found in cells and exudates, thereby diminishing permeability and secretion. Additionally, the proteins that have precipitated create a protective barrier on the skin **(Shenefelt, 2011)**.

2.4 Conclusion

With a resurgence of interest in indigenous knowledge systems and traditional medicine, there is a growing recognition of the efficacy and cultural relevance of herbal treatments, especially in regions with limited access to modern healthcare facilities. *Spirostachys africana*, a tree native to southern Africa, possesses a rich phytochemical profile, including alkaloids, flavonoids, saponins, and terpenoids, each with distinct medicinal properties. These compounds have demonstrated antibacterial, antifungal, and anti-inflammatory activities, suggesting their potential therapeutic benefits in treating various skin ailments.

However, acknowledging the likely toxicity linked to certain compounds found in *S. africana*, such as alkaloids and terpenoids, cautious handling and dosage considerations is important. Despite the plant's historic uses and intriguing medical properties, care must be taken, especially because it is classified as a Class IA cellular toxin.

Moving forward, there is a need for further research to explore the bioactive components of *S. africana* and their mechanisms of action, as well as to document and preserve traditional knowledge associated with its medicinal use. Rigorous scientific validation and standardization of plant-based remedies are imperative to ensure their safety, efficacy, and quality. By consolidating existing knowledge and fostering interdisciplinary collaborations between traditional healers, scientists and healthcare practitioners, the potential of *S. africana* and other medicinal herbs can be harnessed to develop novel, culturally appropriate skincare solutions. This holistic approach not only respects indigenous knowledge systems but also addresses the healthcare needs of diverse communities while supporting the ecological utilization of resources found in nature.

CHAPTER THREE

The Antibacterial Potential of *Spirostachys africana* Extracts against Common Dermatological Pathogens: Implications for Traditional Medicine



Local woman using traditional medicine as sunscreen. Source: www.theafricangourmet.com

3.1 Introduction

Despite the advancement of modern pharmaceuticals, some individuals harbour reservations regarding potential side effects or cultural beliefs, leading them to prefer natural remedies rooted in their traditions (**Ozioma and Chinwe, 2019**). This reliance on herbal medicine is further reinforced by limited access to modern healthcare facilities, particularly prevalent in remote areas. In South Africa, the prevalence of skin conditions is escalating, encompassing numerous conditions such as acne, eczema, pigmentation ailments, and superficial fungal infections (**Dlova et al., 2015b; Katibi et al., 2016**). Such conditions pose considerable challenges, exacerbated by reservations about Western medicine and the limited documentation and validation of traditional plant-based treatments (**Abdullahi, 2011**).

This particular research endeavours to fill this void by investigating the plant species used by rural communities to address skin ailments, with a specific focus on bacterial skin infections. As per **Ärnlöv and Larsson (2015)**, skin conditions rank as the fourth most common cause of disability worldwide. A great proportion of skin diseases is caused by bacterial infections (**Nawas et al., 2016**). In keeping with (**Brueton et al., 2010**), in South Africa's Eastern Cape region, skin infections rank as the second most common ailment observed in clinics and health facilities. However, a recent study in South Africa conducted by **Naidoo et al. (2023)** demonstrated that many traditional healers' consultations were from people with skin conditions. The reliance on traditional healing practices is deeply embedded within the cultural fabric, with a predominant consultation preference for traditional healers in black African communities (**Nyakudya et al., 2020**).

South Africa's rich biodiversity of plants, notably in the province of the Eastern Cape, offers a vast repository of medicinal plants, albeit under threat due to unsustainable harvesting practices as well as climate challenges (**Van Wyk and Prinsloo, 2018; Tshabalala et al., 2022**). The Eastern Cape Province is characterised by a total of 6,611 floral species, of which 12% of these are endemic to this region (**Bredenkamp, 2019**). Plants possessing antibacterial properties are particularly prized for their ability to combat bacterial skin infections. Among these indigenous plants, *Spirostachys africana*, widely referred to as the “*tamboti*” or “*umthombothi*” tree, emerges as a promising candidate authority with a rich history of traditional medicinal use among *isiXhosa* communities (**Thibane et al., 2019**). In South Africa, *Spirostachys africana*, which is a member

of the Euphorbiaceae family, occurs naturally (**Hutchings and Scott, 1996; Coates Palgrave, 2002**). The search for effective antibacterial agents remains a global endeavour, with scientists exploring natural sources for novel compounds (**Silver, 2011**). Medicinal plants, with their vast array of secondary metabolites, represent a valuable reservoir of therapeutic molecules, both historically and contemporarily (**Nyakudya et al., 2020; Bittner Fialová et al., 2021**).

Given the accessibility of bacterial skin lesions for topical treatment, investigating the antibacterial potential of *S. africana* holds particular significance. This indigenous plant, deeply entrenched in *isiXhosa* culture, presents a potentially sustainable solution for combating bacterial skin infections in South Africa's province of the Eastern Cape.

3.2 Material and methods

3.2.1 Plant material

The current study used specimens from another study done by **Thibane et al. (2019)**. Stem-bark from *S. africana* was collected from South Africa's Eastern Cape region. Guided by the plant's traditional usage for child skincare among the local communities, collected samples were from the Raymond Mhlaba local municipality, located in South Africa's Amathole district municipality (**Figure 3.1**). Mr Tony Dold, botanist and curator at Rhodes University, Eastern Cape province, properly identified the collected plant specimens. Following proper documentation procedures, voucher specimens were made and placed at the Bews Herbarium, Pietermaritzburg Campus of the University of KwaZulu-Natal (NU-Herbarium), under the accession number THIBANE 6-NU (**Thibane et al., 2019**).

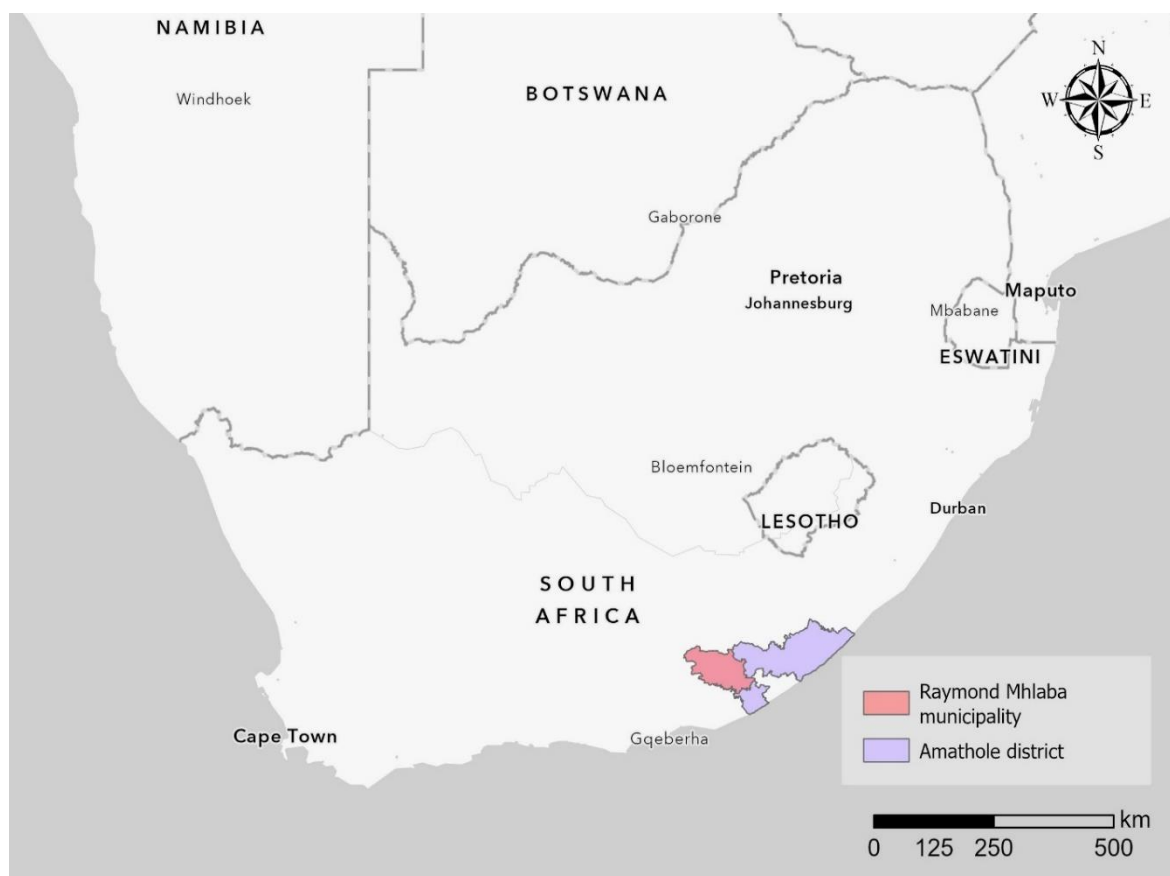


Figure 3.1: Raymond Mhlaba municipality map (Thibane et al., 2019).

3.2.2 Sample preparation for antibacterial bioassay

Stem-bark material was dried at 50 °C for 72 h and ground into fine powder. Several solvents (dichloromethane, petroleum ether, ethanol, and distilled water) were used separately to extract the powdered matter for 1 h with ultrasound assistance. These solvents were selected to ensure a broad polarity range, allowing the extraction of diverse phytochemical classes with varying solubility profiles. Following extraction, the mixtures underwent filtering, and the filtrates were dried under vacuum until they were almost completely dry. The dried residues were then resuspended in their respective extraction solvents, or solvents specified for the assays, adjusting the concentrations as required. Whatman No. 1 filter paper was used to filter the extract preparations (200 ml) before being vacuum-dried until almost dry. Weighing and resuspending the dry substance in sterile water was then done to obtain a stock concentration of 50 mg/ml. Sterilization of this stock solution was done by passing it through a 0.22 µm Millex® GV filter unit (Molsheim, France). Subsequent

dilutions were made from the sterile stock solution to achieve the desired concentrations for the antimicrobial assays. The residues obtained after the initial filtration were resuspended in their respective extraction solvents or assay-specific solvents. The resuspended extracts were then subjected to the respective biological activity assays, following the appropriate protocols for each assay.

3.2.3 Antibacterial activity

The microdilution method was performed in 96-well microtiter plates (Greiner Bio-one GmbH, Germany) to estimate the minimum inhibitory concentrations (MIC) for antibacterial activity, following the protocol described by **Eloff (1998)**. For every bacterial strain examined, a two-fold serial dilution of neomycin (Sigma) at 0.1 mg/ml served as a positive control. Sterile distilled water served as both a solvent blank and a negative control.

Overnight cultures of three bacterial strains known to cause skin infections were prepared in Mueller-Hinton (MH) broth and then incubated at a temperature of 37 °C in a shaking water bath. These included one Gram-positive strain, *Staphylococcus aureus* (ATCC 12600), and two Gram-negative strains, *Escherichia coli* (ATCC 11775) and *Klebsiella pneumoniae* (ATCC 13883). Aqueous extracts were reconstituted in sterile distilled water, and plant extracts made using organic solvents were reconstituted in 80% ethanol. The analytical procedure for MIC determination was done in accordance with **Thibane et al. (2019)**. Every assay was carried out in triplicate and triple-tested to ensure repeatability.

Bacterial strains were diluted in sterile MH broth for the MIC assays. This resulted in an inoculum concentration of approximately 10^6 cfu/ml. Each bacterial culture was added into the respective wells on the microtiter plates in quantities of 100 µl. The plates were covered with parafilm and incubated overnight (approximately 14hrs) at 37 °C.

Each well received 50 µl of 0.2 mg/ml p-iodonitrotetrazolium chloride (INT), which was then incubated for 1 h at 37 °C to determine the bacterial growth.

Clear wells indicated that the extract had inhibited growth, while reddish-pink wells suggested bacterial development. Utilizing the lowest extract concentration that showed a clean well, signifying total suppression of bacterial growth, the MIC values were computed. Each experiment was conducted twice, with two duplicates.

3.2.4 Statistical analysis

The outcomes of the findings were determined as means $\pm$ standard error (SE). The mean differences were separated using one-way analysis of variance (ANOVA). The Fisher's multiple range tests was used, where $p < 0.05$ indicated statistical significance.

3.3 Results

Table 3.1 indicates the antibacterial activity of different extracts of *S. africana* stem-bark against three bacterial strains: *K. pneumoniae*, *E. coli*, and *S. aureus*. The antibacterial activity was measured in terms of MIC, expressed in micrograms per millilitre ($\mu\text{g/mL}$). Neomycin, a known antibiotic, was used as a reference for comparison.

Table 3.1: Minimum inhibitory concentrations ($\mu\text{g/mL}$) of extracts from *Spirostachys africana* stem-bark against bacteria associated with skin infections.

Bacteria tested	MIC ($\mu\text{g/mL}$)				
	PE extract	DCM extract	EtOH extract	DH ₂ O extract	Neomycin
<i>Klebsiella pneumoniae</i>	0.78	0.19	0.19	1.56	0.8×10^{-3}
<i>Escherichia coli</i>	0.78	0.39	1.56	3.12	9.77×10^{-3}
<i>Staphylococcus aureus</i>	0.78	0.19	1.56	1.56	1.60×10^{-3}

Minimum inhibitory concentration, MIC; Petroleum ether, PE; Dichloromethane, DCM; Ethanol, EtOH; Distilled water, DH₂O; Positive control for neomycin solution. Bold values indicate high activity (MIC $< 1 \mu\text{g/mL}$).

The DCM extract had the lowest MIC for *K. pneumoniae*, measuring $0.19 \mu\text{g/mL}$. The petroleum ether (PE) extract came in second, with a MIC of $0.78 \mu\text{g/mL}$. Similarly, for *S. aureus*, the dichloromethane (DCM) extract and distilled water (DH₂O) extract exhibited the least MIC of $1.56 \mu\text{g/mL}$, whereas the PE extract obtained a MIC value of $0.78 \mu\text{g/mL}$.

The ethanol (EtOH) and DH₂O extracts generally exhibited higher MIC values compared to the DCM and PE extracts for all tested bacterial strains. For instance, the DCM extract yielded an

MIC value of 0.19 µg/mL against *K. pneumoniae* and *S. aureus*, indicating high antibacterial activity. Similarly, the DH₂O extract demonstrated an MIC of 1.56 µg/mL against both *K. pneumoniae* and *S. aureus*. Notably, the MIC values of the EtOH and DH₂O extracts against *E. coli* were higher compared to the DCM and PE extracts, suggesting reduced efficacy against this bacterial strain.

Furthermore, comparisons were made with neomycin, a reference antibiotic, to evaluate the relative potency of the *S. africana* extracts. Neomycin exhibited MIC values with a range between 0.8×10^{-3} µg/mL and 9.77×10^{-3} µg/mL against the tested bacterial strains, indicating its strong antibacterial activity. Neomycin exhibited MIC values in the nanogram range (0.8×10^{-3} to 9.77×10^{-3} µg/mL), which is approximately 20 to 1,000 times more potent than the plant extracts, whose MICs ranged from 0.19 to 3.12 µg/mL. Among the extracts, dichloromethane (DCM) showed the strongest activity (0.19 µg/mL), but it was still significantly less potent than neomycin. This indicates that while the plant extracts have promising antibacterial properties, particularly the DCM and PE extracts, they have low efficacy of a conventional antibiotic like neomycin.

3.4 Discussion

Bacterial skin infections pose a significant health challenge worldwide, with pathogens such as *E. coli*, *K. pneumonia* and *S. aureus* being among the most prevalent causes (**Mancuso et al., 2021**). These infections can range from localized conditions like cellulitis and folliculitis to more severe forms like abscesses and wound infections (**Aly, 2011**). Conventional antibiotic treatments are often associated with resistance mechanisms and adverse effects, emphasizing the need for alternative and sustainable therapeutic approaches (**Nwobodo et al., 2022**). Given this, natural compounds from medicinal plants have drawn more attention because of their ability to serve as safe and effective antibacterial agents (**Vaou et al., 2021**).

The goal of the current study was to examine the antibacterial properties of several solvent extracts, including DCM, PE, EtOH, and DH₂O, obtained from the bark of *S. africana* against three clinically relevant bacterial strains: *E. coli*, *K. pneumonia* and *S. aureus*. The study aimed to confirm the traditional usage of *S. africana* in treating infections caused by bacteria and investigate whether it could serve as an origin of new antibacterial agents by assessing the MICs of these extracts.

The MIC values obtained for *K. pneumoniae* demonstrated the potent antibacterial activity of *S. africana* extracts, particularly the DCM and EtOH extracts, which exhibited the highest active MIC value (0.19 µg/mL). This indicates a robust inhibitory effect against *K. pneumoniae*. On the other hand, the PE extract showed modest inhibitory action with a MIC of 0.78 µg/mL.

Against *E. coli*, the DCM extract of *S. africana* exhibited an MIC of 0.39 µg/mL, indicating strong inhibitory activity. However, the MIC values for the PE and EtOH extracts were 0.78 µg/mL and 1.56 µg/mL, respectively, suggesting moderate inhibitory effects. The aqueous extract displayed the highest MIC value of 3.12 µg/mL, indicating the least inhibitory activity against *E. coli* among the tested extracts. These findings align with those of earlier research, indicating *S. africana*'s efficacy against *E. coli*. The antibacterial activity that was detected is consistent with the results of earlier studies by **Mathabe et al. (2008)**, which also showed that *S. africana* was efficient against *E. coli*.

For *S. aureus*, the DCM extract of *S. africana* exhibited the most potent antibacterial activity, with an MIC value of 0.19 µg/mL. The PE extract displayed moderate inhibitory activity with an MIC of 0.78 µg/mL. Like the findings with *K. pneumoniae*, the highest MIC value of 1.56 µg/mL was found in the aqueous extract, indicating weaker inhibitory activity against *S. aureus*. These results are consistent with prior research, supporting the efficacy of *S. africana* against *S. aureus* (**Mathabe et al., 2008**).

The consistent pattern of higher inhibitory activity observed with DCM extracts, and to a lesser extent with EtOH extracts, compared to PE and aqueous extracts, suggests the presence of non-polar bioactive compounds in *S. africana* stem-bark. The utilization of a panel of solvents for extraction, as opposed to water alone, allowed for more comprehensive extraction of *S. africana*'s antibacterial phytochemicals, contributing to the observed range of inhibitory activities against the tested bacterial strains.

The strong antibacterial activity shown by *S. africana* extracts against *S. aureus*, *E. coli*, and *K. pneumoniae* holds proof of implications for combating bacterial skin infections. These three bacterial pathogens are commonly implicated in many different types of skin disorders, underscoring the potential clinical relevance of the findings. *Klebsiella pneumoniae* and *E. coli* are notorious causative agents of various skin infections, including cellulitis, folliculitis, and abscesses. **Paczosa and Meccas (2016)** reported that there has been a recent rise of antibiotic resistance to *K.*

pneumoniae. However, the antibacterial potency demonstrated by *S. africana* extracts against these Gram-negative bacteria suggests their potential as natural and effective alternatives for managing such infections. Incorporating these extracts into topical formulations or skincare products could provide a valuable treatment option, particularly in scenarios where conventional antibiotics may be compromised by resistance mechanisms or associated with adverse effects.

Furthermore, the observed antibacterial activity against *S. aureus*, a Gram-positive bacterium, highlights the broad-spectrum potential of *S. africana* extracts in addressing various skin infections. *Staphylococcus aureus* is a common culprit in conditions such as impetigo, folliculitis, cellulitis, and more severe forms like furuncles and carbuncles **(Del Giudice, 2020)**. Additionally, wound infections caused by this bacterium are common and can seriously slow down the process of healing process and result in complications **(Bowler et al., 2001)**. The potent inhibition of *S. aureus* growth by PE and DCM extracts of *S. africana* underscores their potential application in wound care and management strategies.

DNA intercalation and topoisomerase inhibition are two examples of alkaloid phytochemicals' antibacterial actions **(Baikar and Malpathak, 2010)**. Topoisomerases are enzymes that control the structure and function of DNA, and inhibition of these enzymes can disrupt DNA replication and repair processes in bacteria, ultimately resulting in the death of cells **(Thirumaran et al., 2007)**. DNA intercalation refers to the insertion of a molecule, such as an alkaloid, between DNA base pairs, which may obstruct transcription and DNA replication **(Mukherjee and Sasikala, 2013)**. By targeting these essential processes, alkaloid phytochemicals contribute to their antibacterial activity, providing potential avenues for the creation of novel antimicrobial compounds. Additionally, the presence of flavonoids can impact various bacterial functions, such as gene expression, enzyme activity, and cell membrane integrity **(Panche et al., 2016; Górniak et al., 2019)**.

Collectively, these findings suggest that *S. africana* extracts possess promising antibacterial properties that could be harnessed for creating novel and effective cures for bacterial skin ailments. However, further research is warranted, including clinical trials and *in vivo* studies to corroborate these findings and explore the potential therapeutic applications of *S. africana* extracts in dermatology and wound care settings. Additionally, efforts to optimize extraction methods and

formulation techniques could enhance the efficacy, safety, and bioavailability of *S. africana*-derived treatments, paving the way for their integration into clinical practice.

Importantly, in addition to meeting the increasing need for environmentally friendly and sustainable healthcare solutions, the investigation of natural goods like *S. africana* extracts aids in biodiversity conservation and the safeguarding of traditional knowledge (Xaba et al., 2022). This study's conclusions have important ramifications for both traditional medicine and modern drug discovery efforts. Firstly, the demonstrated antibacterial efficacy of *S. africana* extracts validates its traditional use in indigenous medicine for treating various skin ailments, providing scientific support for the therapeutic claims of traditional healers. This not only enhances the credibility of traditional medicinal practices but also underscores the importance of preserving indigenous knowledge systems. Furthermore, the identification of potent antibacterial compounds in *S. africana* extracts presents promising opportunities for drug discovery. New antibacterial medications may be developed using the bioactive compounds that produce the observed antibacterial activity as lead molecules, addressing the urgent need for new therapeutic agents, especially considering the growing prevalence of resistance to antibiotics. By establishing a connection between conventional knowledge and present pharmacology, this study emphasizes how natural products from *S. africana* can be used in contributing to both traditional and allopathic healthcare systems, ultimately with the potential of benefiting public health on a global scale.

3.5 Conclusions

The results demonstrate that *S. africana* extracts, particularly the DCM extract, exhibited potent antibacterial efficacy against the bacterial strains that were examined, including *K. pneumoniae*, *E. coli*, and *S. aureus*. These findings raise the possibility that *S. africana* is a naturally occurring source of antibacterial compounds that can be used to treat bacterial illnesses.

The study revealed variability in antibacterial activity among different extracts of *S. africana*. The DCM extract consistently exhibited the highest antibacterial potency, while the PE, EtOH and DH₂O extracts showed moderate to weak inhibitory effects against the tested bacterial strains. This variation emphasizes how crucial extraction techniques and solvent choice are to optimizing *S. africana*'s antibacterial potency.

The observed antibacterial activity of *S. africana* extracts aligns with its traditional use in indigenous medicine for treating various ailments, including skin infections. These findings provide scientific validation for the traditional medicinal practices involving *S. africana* and underscore its potential as a valuable potential resource in conventional systems of medicine. To identify the precise bioactive substances causing the noted antibacterial activity of *S. africana* extracts, more investigation is necessary. Additionally, clinical trials and *in vivo* research studies are needed to evaluate the safety and effectiveness of *S. africana*-derived treatments for bacterial infections. Efforts to optimize extraction techniques and formulate *S. africana*-based products may enhance their therapeutic potential and facilitate their integration into modern healthcare practices.

In conclusion, the study underscores the *S. africana*'s potential as an indigenous origin of antibacterial agents for curing bacterial infections, particularly those associated with skin diseases. To fully utilize *S. africana*'s therapeutic potential in the fight against bacterial infections and the enhancement of public health outcomes, additional studies and development action are required.

CHAPTER FOUR

Traditional Knowledge and Modern Science: Investigating the Antioxidant Capacity of *Spirostachys africana* for Treating Childhood Skin Diseases



Infantile cradle cap known as *ishimca* in South Africa.

Source: <https://za.pinterest.com/pin/434808539017914744/>

4.1 Introduction

Childhood skin conditions represent a serious public health concern, particularly in underdeveloped nations where access to contemporary medical care is sometimes restricted (García et al., 2020). Skin conditions were the fourth most common cause of non-fatal disability worldwide in 2013, according to the Global Burden of Diseases (GBD) study (Karimkhani et al., 2017). Traditional medicinal practices have long played a crucial role in treating various ailments, including skin conditions, among indigenous communities (Shankar et al., 2012; Mothibe and

Sibanda, 2019). The great biodiversity of medicinal plants in South Africa has shown to be a valuable resource for treating illnesses, including those that impact children.

One such plant, *Spirostachys africana*, locally known as "tamboti" or "umthombothi," has attracted attention for its potential therapeutic properties (**Singh et al., 2020**). This tree, belonging to the Euphorbiaceae family, has been used by the *isiXhosa* people of South Africa's Eastern Cape Province to treat cradle cap (*ishimca*) in infants through the topical application of its stem-bark extracts (**Beach et al., 2010**). Cradle cap, a scaly, inflammatory skin condition affecting the scalps of newborns, can persist for weeks or months, causing discomfort and potential complications if left untreated (**Nobles et al., 2023**).

Plant antioxidants are essential substances that aid in shielding cells from oxidative stress, which happens when the body's levels of antioxidants and free radicals have an imbalance (**Lobo et al., 2010**). Free radicals are very reactive chemicals that may damage DNA, proteins, and lipids in cells, resulting in several health problems, including skin conditions. Numerous antioxidants, such as phenolic compounds, flavonoids, vitamins (such as C and E), and carotenoids, are produced by plants and function in concert to scavenge free radicals and stop cellular damage (**Kasote et al., 2015**). These natural antioxidants not only protect the plants themselves from environmental stressors like UV radiation, pollution, and pathogens but also offer significant health benefits when consumed or applied therapeutically by humans (**Maury et al., 2020**). Antioxidants are essential for skin health because they reduce inflammation, promote healing, and improve the skin's general appearance and resiliency (**Pullar et al., 2017; Comino-Sanz et al., 2021**). By scavenging free radicals, antioxidants can help mitigate oxidative stress-related skin conditions, making plant-derived antioxidants valuable in developing treatments for skin diseases (**Michalak, 2022**).

Previous research has showed the antibacterial activity of *S. africana* extracts against numerous pathogenic bacteria, including *Escherichia coli*, *Salmonella typhi*, *Shigella dysenteriae*, *Staphylococcus aureus* and *Vibrio cholerae* (**Mathabe et al., 2008**). These findings lend scientific support to the plant's ethnomedical applications and highlight its capacity to be a source of novel anti-infective agents. Additionally, preliminary studies have shown that *S. africana* extracts possess promising antioxidant capacity, suggesting their potential in combating free radical-induced damage and promoting skin health (**Amoo et al., 2012**).

The aim of this research was to examine the antioxidant capacity of *S. africana* extracts using a variety of *in vitro* antioxidant tests, evaluating their ability to scavenge free radicals and safeguard against oxidative stress. By investigating the antioxidant properties of this medicinal plant, the study seeks to provide scientific validation for its traditional use in treating pediatric skin diseases, particularly cradle cap, and aid in the creation of efficient and accessible therapeutic options for underprivileged people in resource-constrained environments.

4.2 Material and methods

4.2.1 Plant material

Stem-bark from *S. africana* was collected from the Eastern cape province, South Africa in the Raymond Mhlaba municipality in the Amathole district, as described in Chapter 3.

Stem-bark materials of *S. africana* were dried in an oven at 50 °C for 72 h, or until constant dryness was achieved. Dried plant material was ground into fine powder using a grinder (MF 10, Merck Chemical (Pty) Ltd). Ten grams of stem-bark powder was extracted separately in 100 mL of 50% methanol solvent and macerated for 24 h at ambient temperature (25 °C). After passing the mixture through Whatman No. 1 filter paper, the organic solvents were dried at 40 °C by evaporating them in a rotary evaporator. Plant extracts for the antioxidant activity assay were prepared as per standard by extracting with 50% aqueous methanol (v/v) for 30 min in a sonication bath. Ascorbic acid, Butylated hydroxytoluene (BHT) and Trolox served as a positive control.

4.3 Chemical assays

4.3.1 DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging assay

The antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay following the method described by **Karioti et al. (2004)**. The test measured how effectively plant extracts could decolorize a DPPH radical solution. The experiment used ascorbic acid as a positive control to validate the assay, while 50% methanol solution without any plant extract served as the negative control.

To conduct the experiment, 15 μL of the plant extract was diluted with 735 μL of methanol and added to a DPPH solution (750 μL , 0.1 mM) to create a final DPPH solution reaction mixture (1.5 mL, 500 μM). The reaction mixtures were made in dim lighting and incubated at room temperature for 30 min. After incubation, the absorbance of the reaction mixture was measured at 517 nm using a microplate reader (Optic Iveyemen® System 2100-C) according to Equation 1:

$$\text{RSA (\%)} = \left\{ 1 - \left(\frac{\text{Abs}_{517 \text{ nm}} \text{ Sample}}{\text{Abs}_{517 \text{ nm}} \text{ Neg Control}} \right) \right\} \times 100 \quad \text{Equation (1)}$$

The Radical Scavenging Activity (RSA) percentage was calculated using the absorbance values, where A_{sample} represents the absorbance of the mixture containing either the sample extract or standard antioxidant, and A_{control} represents the absorbance of the DPPH solution alone. To determine the EC_{50} (half maximal effective concentration), the RSA percentage was plotted against extract concentration using a normalized logarithmic regression curve. The entire experiment was performed in duplicate and repeated three times.

4.3.2 The 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay

The ABTS radical scavenging ability of the methanolic extract was assessed using a modified version of **Re et al. (1999)**'s method, as adapted by Dzoyem and Eloff in 2015. The test solution was prepared by combining equal volumes of 7 mM ABTS and 2.45 mM potassium persulfate (both from Sigma Aldrich, USA) in the methanol extract (Minema, SA). This mixture was stored in darkness for a minimum of 16 h to allow the reaction to complete. Trolox (Fluka, UK) and ascorbic acid (Merk, Germany) were used as positive controls, and 50% methanol was used as a negative control. The absorbance was measured at 734 nm using an Epoch BioTek Reader (Agilent Technologies, USA). The experiment was performed in triplicate. The percentage of ABTS inhibition was calculated using the formula:

$$\text{Scavenging activity (\%)} = \frac{AB - AA}{AB} \times 100, \quad \text{Equation (2)}$$

Where AB = absorbance of control and AA = absorbance of sample (extract and positive controls). The graphs were plotted using non-linear regression and the IC_{50} values were calculated to determine the concentration value at which the extract reduces scavenging activity by 50%.

4.3.3 Carotene-linoleic model system (CLAMS)

The plant extracts' antioxidant ability was assessed using the β -carotene-linoleic acid bleaching experiment, modified by **Amarowicz et al. (2004)**. This assay evaluates the test solution's ability to prevent oxidation of β -carotene and linoleic acid in an emulsified aqueous environment. Firstly, 10 mg of β -carotene was dissolved in 10 mL of chloroform in a brown Schott bottle. The surplus chloroform was evaporated under vacuum, leaving a thin layer of β -carotene nearly dry. The β -carotene film was then treated with 200 μ L of linoleic acid and 2 mL of Tween-20. After diluting the mixture with 497.8 mL of aerated distilled water, the final β -carotene concentration was 20 μ g/mL. The mixture was vigorously agitated to saturate it with oxygen, resulting in an orange emulsion.

Butylated hydroxytoluene (BHT), a well-known antioxidant, served as a positive control. After dispensing the emulsion (4.8 mL) into test tubes, 200 μ L of resuspended herbal preparation or BHT (6.25 mg/mL) was added to achieve a final concentration of 250 μ g/mL in the reaction mixtures. The absorbance of each reaction mixture was immediately measured at 470 nm ($t = 0$) and then incubated at 50 °C. The absorbance measurements were conducted every 30 min for a total of 180 min. Tween-20 solution was used to blank the spectrophotometer, and the negative control was 50% methanol instead of the sample extract. The rate of β -carotene bleaching was determined using the formula below (Equation 1):

$$\text{Rate of bleaching (R)} = \left\{ \ln \left(\frac{A_{t=0}}{A_{t=t}} \right) \right\} \times \frac{1}{t} \quad \text{Equation (1)}$$

Where: $A_{t=0}$ represents the initial absorbance of the emulsion (at time zero), while $A_t=t$ represents the absorbance at any specific time point t during the 180-minute period (measured at 30-minute intervals).

The rate of β -carotene bleaching was determined using measurements at 30, 60, and 90 min. These average rates were then used to calculate the antioxidant activity (ANT). The extract's effectiveness was expressed as a percentage, showing how much it inhibited β -carotene bleaching, calculated as in Equation 2:

$$\% \text{ ANT} = \left(\frac{R_{\text{control}} - R_{\text{sample}}}{R_{\text{control}}} \right) \times 100 \quad \text{Equation (2)}$$

Where R_{control} is the average bleaching rate of the control and R_{sample} is the average bleaching rate of the extract. The antioxidant activity was also expressed as an oxidation rate ratio (ORR), calculated as in Equation 3:

$$\text{ORR} = \frac{R_{\text{sample}}}{R_{\text{control}}} \quad \text{Equation (3)}$$

4.3.4 Statistical analysis

The findings were reported as mean $\pm$ standard error (SE). The mean differences were determined using one-way ANOVA. Fisher's multiple range tests were performed to determine statistical significance ($p < 0.05$).

4.4 Results

4.4.1 DPPH radical scavenging

The DPPH radical scavenging assay was used to evaluate the antioxidant potential of 50% methanol extracts from *S. africana* stem-bark (**Table 4.1**). The extracts showed antioxidant activity, achieving a $72.60 \pm 0.24\%$ DPPH scavenging rate at maximum test concentrations, with an EC_{50} value of $83.26 \pm 1.22 \mu\text{g/mL}$. While this indicates strong antioxidant activity, it was less potent than the ascorbic acid positive control, which had an EC_{50} of $0.39 \pm 0.23 \mu\text{g/mL}$.

Table 4.1: Antioxidant activity of methanol (50%) extracts from *Spirostachys africana* stem-bark as determined by the DPPH scavenging assay as percentage and estimated as EC₅₀ values.

Sample	DPPH Scavenging Activity (%)	DPPH Scavenging Activity EC ₅₀ (µg/mL)
<i>S. africana</i>	72.60 ± 0.24	83.26 ± 1.22
Ascorbic Acid	81.45 ± 1.72	0.39 ± 0.23

Methanol extracts with EC₅₀ values of (< 0.39 µg/mL) are considered potent DPPH radical scavengers. Ascorbic acid (positive control). n = 5.

The dose-dependent nature of the antioxidant activity is evident from **Figure 4.1**, which illustrates the relationship between the extract concentration and the corresponding percentage of DPPH radical scavenging.

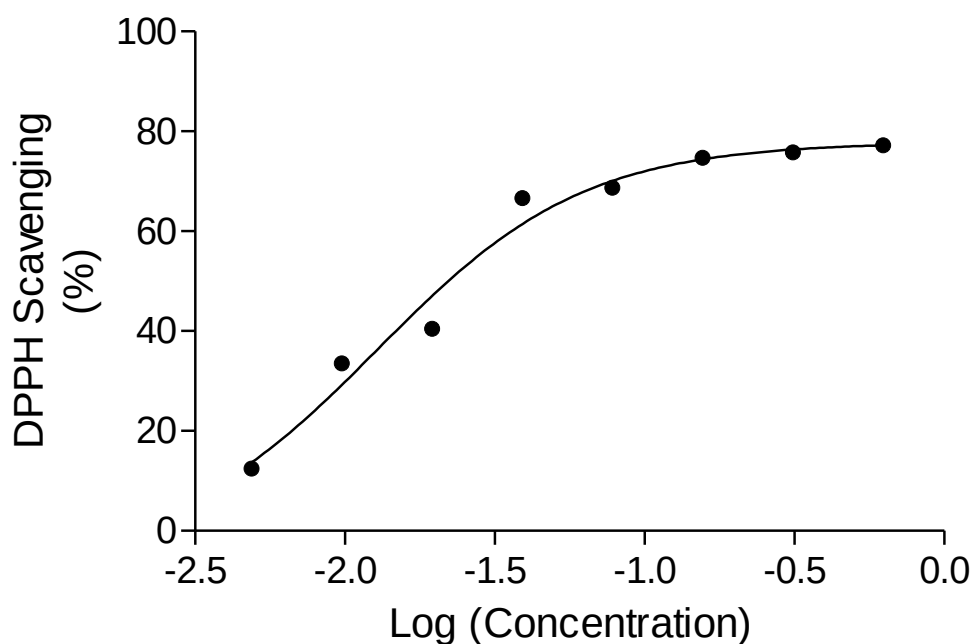


Figure 4.1: Antioxidant activity of methanol extract from *Spirostachys africana* stem-bark as determined by the DPPH percentage radical scavenging and the concentration for the reported percentage scavenging activity.

4.4.2 The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay

The results presented in **Figure 4.2** shows the ABTS radical scavenging capacity methanol extract from *S. africana* stem-bark. Ascorbic acid and trolox served as positive controls.

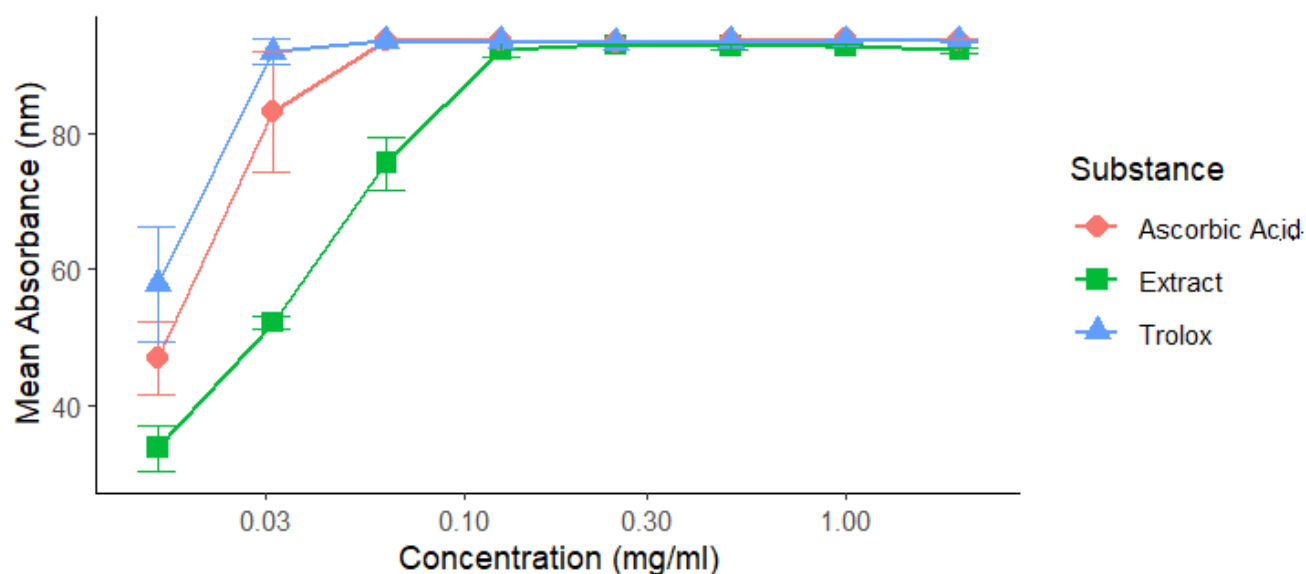


Figure 4.2: The 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging activity of methanol extract from *Spirostachys africana* stem-bark. Ascorbic acid and trolox served as positive controls. The colour orange represents ascorbic acid, green colour represents methanol extract from *S. africana* stem-bark and blue represents trolox.

4.4.3 β -Carotene-linoleic model system (CLAMS)

The antioxidant activity of *S. africana* extracts was further evaluated using the β -carotene-linoleic acid model system (CLAMS), which mimics lipid peroxidation in biological systems. **Figure 4.3** depicts the rate of β -carotene bleaching at $t = 60$ min, a measure of the extract's ability to inhibit oxidation. Notably, *S. africana* extracts with oxidation rate ratios (ORR) ≤ 0.05 were considered potent antioxidants, effectively protecting β -carotene from oxidative degradation. The

lower the ORR value, the higher the antioxidant capacity of the extract, as it indicates a slower rate of β -carotene oxidation relative to the control.

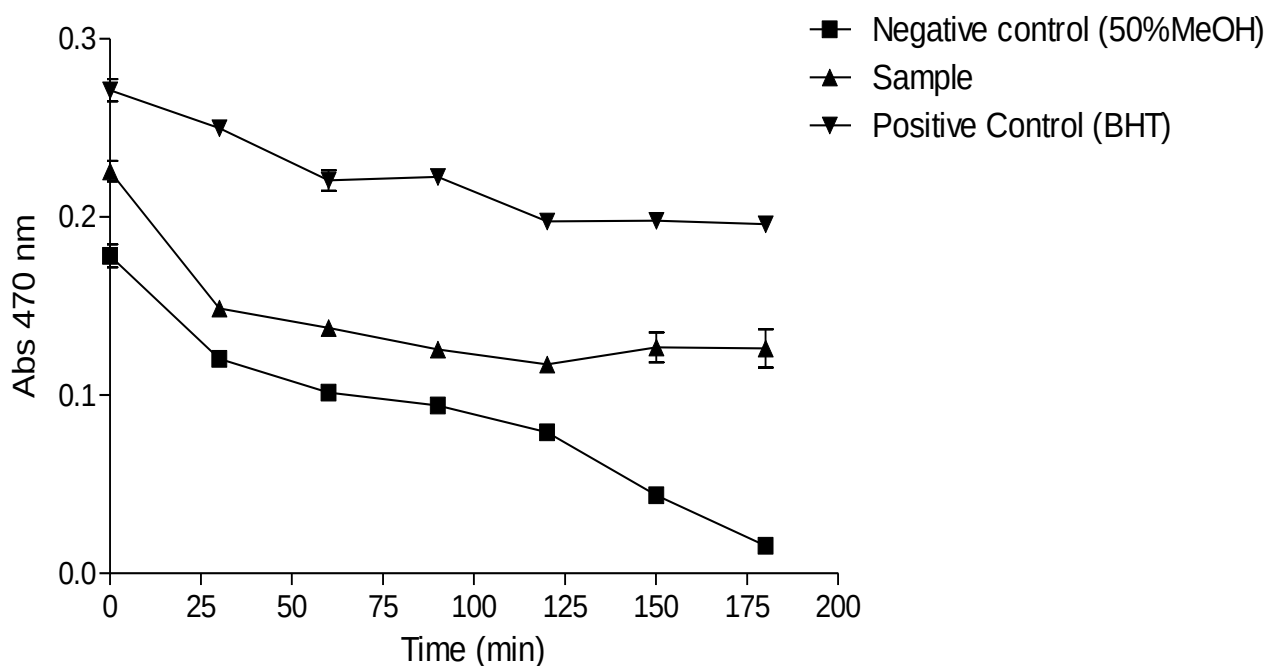


Figure 4.3: β -Carotene-linoleic acid model system (CLAMS) activity of *Spirostachys africana* and the rate of β -carotene bleaching at $t = 60$ min. The extract has greater protection against β -carotene bleaching. Extracts with oxidation rate ratios (ORR) values ≤ 0.05 are considered potent antioxidants.

4.5 Discussion

The current study investigated the antioxidant activities of stem-bark extracts of *S. africana*, an indigenous South African medicinal plant with a long history of traditional use. The results obtained from a comprehensive array of an *in vitro* assay provides compelling evidence for the therapeutic potential of this plant, substantiating its ethnobotanical applications and warrants further exploration as a source of bioactive compounds.

4.5.1 DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay provides insights into the possible antioxidant capacity of substances or extracts against free radicals, which play an important role in the development of numerous skin diseases (**Gulcin and Alwasel, 2023**). Unpaired electrons make up the very reactive chemical entities known as free radicals. These can participate in redox reactions that cause oxidative damage to other molecules (**Lobo et al., 2010**).

In the context of skin health, free radicals can be generated both endogenously through metabolic processes and exogenously from environmental factors like UV radiation, air pollution, and cigarette smoke (**Lobo et al., 2010**). The oxidative stress induced by these free radicals is a major contributing factor to skin aging, inflammation, and the development of numerous dermatological ailments, including photoaging, psoriasis, and skin cancer (**Rinnerthaler et al., 2015; Liu et al., 2023**). Antioxidants combat this oxidative stress by neutralizing free radicals and avoiding further oxidative damage (**Lobo et al., 2010**). The DPPH assay analyzes an antioxidant's ability to transfer a hydrogen atom or an electron to the stable DPPH radical, prompting a color shift that can be detected spectrophotometrically (**Gulcin and Alwasel, 2023**). The EC₅₀ value represents the concentration of the test sample required to reduce the initial DPPH concentration by 50%, with lower values indicating higher antioxidant potency (**Gulcin and Alwasel, 2023**).

The lower the EC₅₀, the faster the colour of free radical scavenging bleaches and the stronger the antioxidant. The *S. africana* extracts had a higher estimated EC₅₀ value of 83.26 ± 1.22 compared to the ascorbic acid which slowed down the colour change. **Table 4.1** shows that ascorbic acid had a low EC₅₀ value of 0.39 ± 0.23 µg/mL and acted as a potent radical scavenger compared to *S. africana* extract. The antioxidant activity of the *S. africana* extract was concentration-dependent, with the highest concentration of $72.60 \pm 0.24\%$ of scavenging activity, preventing the formation of free radicals and lipid peroxides, as indicated in **Figure 4.1**. Free radicals and lipid peroxides play an important role in the development of skin disorders (**Bickers and Athar, 2006**). As a result, based on the DPPH concentration, it appears that *S. africana* extract had moderate antioxidant activity preventing the formation of the oxidants and as such may have a positive impact on skin beauty. Since oxidation is a dynamic process with multiple pathways, no single method can fully assess the antioxidant property of a plant extract. DPPH is a stable free radical

assay that yields results in the form of producing a lower EC₅₀ value indicating stronger antioxidant activity.

The current study assessed the DPPH radical scavenging activity of *S. africana* extracts, and the findings suggest that these extracts have significant antioxidant potential. The extracts exhibited an EC₅₀ value of 83.26 ± 1.22 µg/mL, which, although higher than the potent antioxidant ascorbic acid, still demonstrates a substantial ability to scavenge free radicals in a concentration-dependent manner (**Figure 4.1**).

Notably, the antioxidant activity of the *S. africana* stem-bark extracts has implications for skin health, as they may help prevent the formation of free radicals and lipid peroxides, that are key contributors to the development of various skin diseases (**Michalak, 2022**). By mitigating oxidative stress, these extracts could potentially be incorporated into skincare formulations or anti-aging products to combat skin aging, inflammation, and other dermatological conditions associated with oxidative damage (**Michalak, 2022**).

The antioxidant and potential skin-protective effects of *S. africana* extracts can be accredited to the existence of various phytochemicals, including saponins, tannins, and flavonoids (**Mathabe et al., 2008; Mulaudzi et al., 2012; Alliya, 2019; Singh et al., 2020**). Saponins, which are natural glycosides with surfactant properties, have been stated to have anti-inflammatory, antioxidant, and wound-healing qualities that could be beneficial for skin health (**Timilsena et al., 2023**). Tannins, particularly condensed tannins, found in the bark of *S. africana*, contain multiple hydroxyl groups that can participate in antioxidant reactions, scavenging free radicals and preventing oxidative damage to skin cells (**Vuolo et al., 2019**). Flavonoids, another class of polyphenolic compound present in *S. africana*, have been extensively studied for their antioxidant, anti-inflammatory, and photoprotective effects on the skin (**Ullah et al., 2020; Al-Khayri et al., 2022; Ma and Khachemoune, 2023**). These compounds can neutralize reactive oxygen species, inhibit enzymes involved in inflammation, and absorb harmful UV radiation, thereby mitigating the effects of oxidative stress and photoaging (**Ma and Khachemoune, 2023**). The synergistic actions of these phytochemicals could contribute to the potential of *S. africana* stem-bark extracts in preventing or alleviating various skin disorders associated with oxidative stress, inflammation, and photodamage.

While the DPPH assay provides valuable insights into the antioxidant potential of *S. africana* extracts, it is imperative to realize that oxidation is a complex and dynamic process involving

multiple pathways. Therefore, a comprehensive assessment of the extracts' antioxidant qualities and their specific mechanisms of action in relation to skin health should use complementary assays that assess different aspects of antioxidant activity.

4.5.2 The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging

The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging results are represented in **Figure 4.2** for the tested extracts. Ascorbic acid and Trolox exhibited high antioxidant action at low concentrations, as evidenced by a rapid drop in absorbance at concentrations as low as 0.0625 mg/ml. This shows that they are strong antioxidants, capable of neutralising ABTS radicals in modest doses. While the extract does exhibit antioxidant activity, it requires a larger concentration (about 1 mg/ml) to achieve a comparable impact to the standards, showing that it is less effective than ascorbic acid and Trolox.

4.5.3 β -Carotene-linoleic model system (CLAMS)

The β -carotene-linoleic acid model system (CLAMS) assay is a valuable tool for assessing the antioxidant potential of various compounds or extracts against lipid peroxidation. This assay is premised on the principle that β -carotene, a lipophilic antioxidant with a characteristic orange colour, undergoes bleaching or discoloration when its conjugated double bond system is disrupted by free radicals produced in the course of the oxidation of linoleic acid in an emulsified system (**Amarowicz et al., 2004; Lu et al., 2014**). In the presence of an antioxidant, the rate of β -carotene bleaching is slowed down or inhibited, indicating the antioxidant's capability to nullify the free radicals and prevent lipid peroxidation (**Lu et al., 2014**).

The CLAMS test was used in the current study to determine the antioxidant capacity of *S. africana* stem-bark extracts. The rate of β -carotene bleaching was determined spectrophotometrically, and the ORR was derived by comparing sample extracts' bleaching rates to the control. Lower ORR values suggest stronger antioxidant activity, as they represent slower β -carotene oxidation compared to the control. The results, as depicted in **Figure 4.3**, revealed that *S. africana* stem-bark extracts with ORR values ≤ 0.05 were potent antioxidants, effectively protecting β -carotene from oxidative degradation. These conclusions show that the extracts possess

the aptitude to prevent lipid peroxidation, a crucial mechanism in mitigating oxidative stress and associated disorders, including skin diseases.

The CLAMS assay's antioxidant activity can be linked to the presence of different phytochemicals in *S. africana* stem-bark extracts, including as saponins, tannins, and flavonoids, which have been widely reported to display antioxidant and free radical scavenging capabilities (Mathabe et al., 2008; Mulaudzi et al., 2012; Alliya, 2019; Singh et al., 2020). These compounds can directly neutralize free radicals or chelate metal ions involved in the creation of reactive oxygen species, thereby preventing the initiation and propagation of lipid peroxidation chain reactions.

It is important to note that while the CLAMS assay offers valuable comprehensions into the potential antioxidant activity of *S. africana* extracts against lipid peroxidation, a comprehensive evaluation should incorporate complementary assays that assess different mechanisms of antioxidant action. The synergistic effects of various phytochemicals existing in the extracts may add to their overall antioxidant and potentially skin-protective properties.

4.6 Conclusions

The current study provides compelling evidence for the antioxidant potential of stem-bark extracts from the medicinal plant *S. africana*. The findings from *in vitro* antioxidant assays, including the DPPH radical scavenging assay and the CLAMS, demonstrate the extracts' ability to scavenge free radicals and inhibit lipid peroxidation, two key mechanisms underlying oxidative stress and associated disorders.

These antioxidant properties can be accredited to the existence of numerous phytochemicals in *S. africana* extracts, such as saponins, tannins, and flavonoids, which are known to have antioxidant, anti-inflammatory, and photoprotective properties beneficial for skin health. The synergistic actions of these compounds contribute to the overall antioxidant capacity of the extracts, enabling them to neutralize ROS, inhibit enzymes involved in inflammation, and mitigate oxidative stress-induced damage.

The findings from this study provide scientific validation for the traditional use of *S. africana* in treating pediatric skin conditions, particularly cradle cap, among indigenous communities in South Africa. The antioxidant-rich extracts hold promise for the development of therapeutic

formulations targeting skin disorders associated with oxidative damage, inflammation, and premature aging.

These promising antioxidant results warrant further research to identify and characterize the specific compounds responsible for this activity in *S. africana* stem-bark. This could lead to developing new natural therapeutic agents or skincare products from this South African plant. While these initial findings are encouraging, further research is needed to fully understand the extract's potential. This should include *in vivo* studies and clinical trials to confirm its safety and effectiveness, particularly for treating oxidative stress-related conditions and improving skin health. Such research is especially relevant for communities with limited healthcare access who could benefit from natural therapeutic alternatives.

CHAPTER FIVE

Exploring the Therapeutic Potential of *Spirostachys africana* Sond. Stem-Bark Extracts for Assessing the Skin Irritation Strength



Local South African woman applying traditional medicine for skin protection.

Source: <https://za.pinterest.com/>

5.1 Introduction

Skin irritation, commonly known as dermatitis, is a common and often distressing condition that can have a undesirable influence on an individual's quality of life and create a psychological burden **(Dalgard et al., 2015; Behera et al., 2022)**. According to **Anderson and Meade (2014)**, the skin is the biggest organ in the body of any human being, accounting for 10% of total body weight and functioning as a protective barrier against external elements. However, its integrity can be jeopardized by a variety of irritants, causing skin irritation and discomfort. Skin irritation, characterized by redness, itching, swelling, or inflammation, can be caused by allergies, chemical exposures, infections, or medical problems **(Novak-Bilić et al., 2018)**.

Skin irritation has far-reaching psychological and social implications in addition to physical suffering. Chronic or severe skin irritation can cause sleep difficulties, anxiety, and a lower quality of life **(Christensen and Jafferany, 2023)**. Furthermore, obvious skin irritation can cause social stigma, negatively impacting an individual's confidence and general well-being. **(Jankowiak et al., 2020)**. Skin irritation pathophysiology is complicated, involving the irritant, skin barrier function, and the immune system's inflammatory response **(Kim et al., 2019)**. Irritants can damage the stratum corneum, the epidermis' outermost layer, impairing its barrier function and allowing irritants to penetrate deeper skin layers **(Lee and Friedman, 2016)**.

The pathophysiology of skin irritation is characterized by a complex interplay between the irritant, the skin barrier function, and the immune system's inflammatory response **(Lee et al., 2020)**. Irritations can damage the epidermis, triggering an inflammatory cascade that releases pro-inflammatory mediators like cytokines and chemokines (such, IL-1 β , TNF, IL-17, and IL-22), these mediators recruit and activate immune cells, causing skin irritation **(Schitteck, 2011; Jiang et al., 2020)**.

The usual methods for managing skin irritation include identifying and avoiding the irritants that cause it, following recommended skincare routines, and, in certain cases, using topical or systemic medications to ease symptoms and reduce inflammation **(Purnamawati et al., 2017)**. The necessity of alternate, secure, and efficient therapeutic approaches is highlighted by the possibility that many traditional medicines may be ineffective or have unfavourable side effects **(Ekor, 2014)**. South Africa has a dermatologist shortage, which is exacerbated by the fact that the majority of dermatologists are found in metropolitan areas and are inaccessible to 70% of the rural population,

with just three dermatologists per million people (**Tiwari et al., 2022**). In this context, investigating natural sources, such as medicinal herbs, for anti-irritant qualities shows promise.

Previous research has proven the antibacterial and antioxidant qualities of *Spirostachys africana* extracts, adding scientific backing to their ethnomedicinal applications (**Mathabe et al., 2006; Amoo et al., 2012; Alliya, 2019**). By combining traditional knowledge with modern scientific techniques, this research endeavours to corroborate the ethnomedicinal utilization of this plant and contribute to the development of effective and accessible therapeutic solutions for skin-related concerns, particularly in resource-limited settings.

5.2 Material and methods

5.2.1 Plant material

The stem-bark extracts of *S. africana* were collected as described in Chapter 3, Section 3.2.1 (**Figure 5.1**).

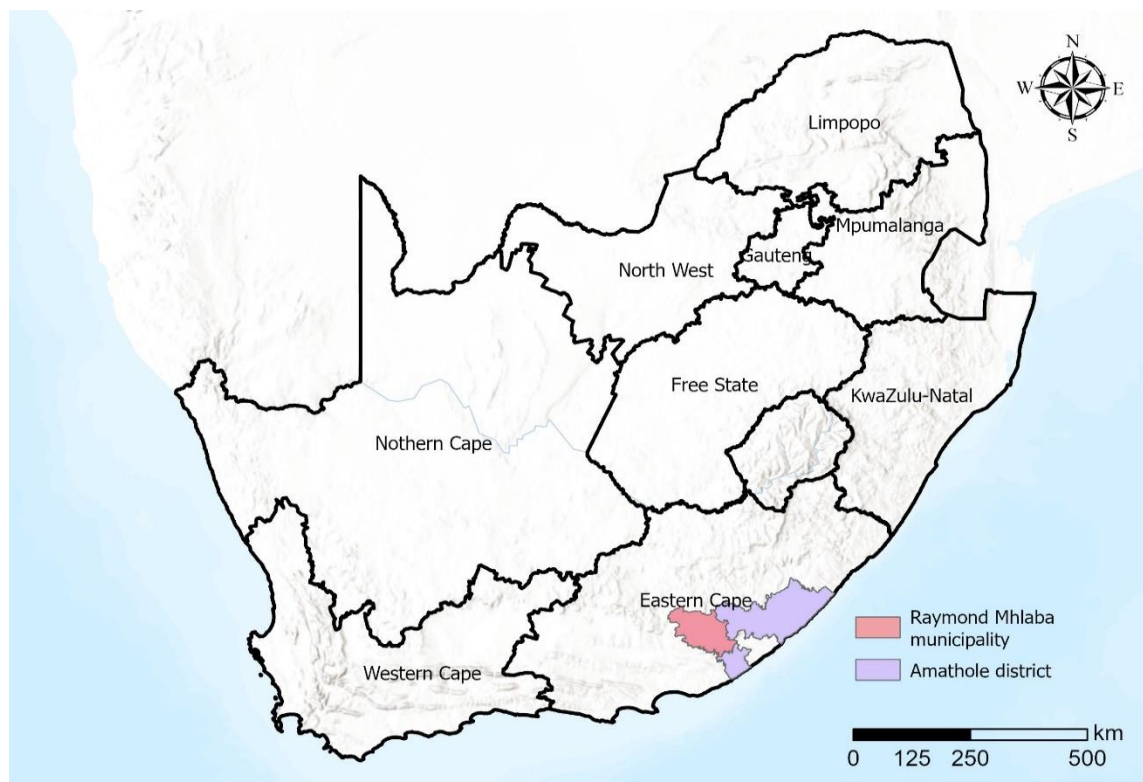


Figure 5.1: The sampling area is located in the Raymond Mhlaba municipality, situated in the Amathole district in South Africa's Eastern Cape Province (**Thibane et al., 2019**).

5.2.2 Sample preparation

The preparation of *S. africana* stem-bark extracts involved several steps. First, the plant material was dried in an oven at 50 °C for 48 h. The dried material was then ground into a fine powder using an MF 10 grinder (Merck Chemical (Pty) Ltd). The powder was extracted using three different solvents – ethanol (EtOH), de-ionized water, and tap water - in an ultrasonic bath for 1 h. After extraction, the mixtures were filtered through Whatman's No. 1 filter paper. Finally, the crude extracts were concentrated under vacuum and dried using a cool air flow. Sodium lauryl sulfate (SLS) at 1.0% concentration was used as a positive control.

5.2.3 Skin irritation tests

The skin irritation test used adult male Wistar rats as model organisms to assess skin irritation properties. To evaluate the possibility for skin irritation, a single application test was performed on the rats in accordance with the requirements mentioned in ISO 10993-10:2021 for medical materials and devices (ISO, 2021). All experimental protocols were approved by the Animal ethics clearance, which was obtained from the University of Limpopo, South Africa with clearance number: **Turfloop Research Ethics Committees/112/2024: Post Graduate (TREC/112/2024: PG)**. The used adult male Wistar rats (310 ± 16 g) were obtained from a local commercial laboratory and used in the Photobiology laboratory at Sefako Makgatho Health Sciences University in South Africa. The rats were kept in a temperature-controlled room (23-25 °C, humidity 30-70%). All the rats needed to acclimatize for one week before the experiment.

A day before the experiment, aqueous solutions of known skin irritants, sodium lauryl sulfate (SLS) at 1.0% concentration (positive control) and EtOH at 10.0% concentration (**Sekizawa et al., 1994; Lachenmeier, 2008**), were prepared. De-ionized water served as a negative control. These solutions were applied topically to the rat skin, exposing a clean skin area measuring roughly 2 cm x 5 cm. Prior to applying the solution, the rat's hair was removed. The hair removal region was gently washed with warm water, and the skin was examined for damage. Each rat was fitted with a single piece of drug-containing paste on its back and secured with medical tape, which provided outstanding protective, absorptive, and therapeutic qualities. After 6 h of continuous exposure to the medical treatment, the paste was removed, and the skin's surface was gently cleansed multiple times with warm water. Finally, excess water was gently brushed away.

The steps for a single application test was as follows: The anesthetized Wistar rat's dorsolateral surfaces were depilated, and four-square zones measuring 4 cm² were defined (zones 1-4). Gauze patches were moistened with aliquots of the test solutions and placed in contact with these zones: 1% SLS (zone 1), 10% EtOH (zone 4), de-ionized water (zone 3), and *S. africana* tap water extract (zone 2) (**Figure 5.2**). The patches were applied to the skin surface using an adhesive polyurethane film (Hydrofilm®, Paul Hartmann AG) and remained on the rats for 4 h.

In the single skin irritation test, the colour, morphology, and texture of subjected skin from the rat was observed using Konica Minolta Chromameter CR-400. Measurements were taken at 24, 48, and 72 h after treatment. Data were processed using moorVMS-PC V3.1 software and reported as means $\pm$ standard deviation.

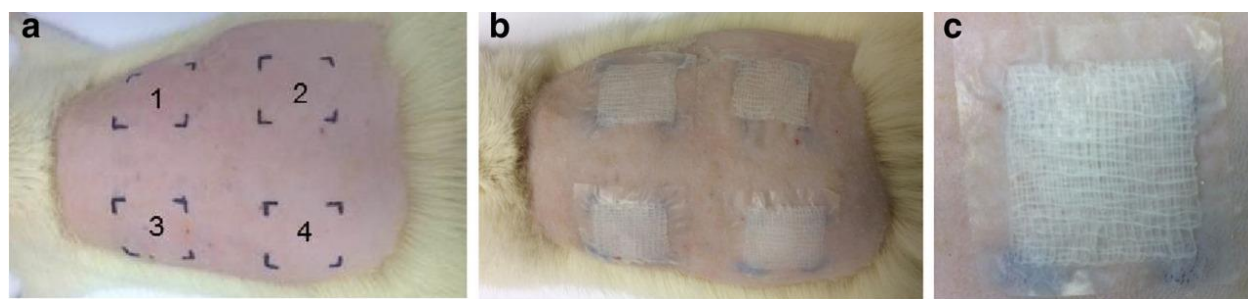


Figure 5.2: Single application test for rat skin irritation determination. In step (a), the dorsolateral side was depilated and zones marked; in step (b), gauze patches containing testing solutions were applied and attached with adhesive film; and in step (c), the gauze patch was fixed. Zone 1 was treated with 1% sodium lauryl sulphate (SLS), zone 4 was treated with 10% ethanol (EtOH) extract, whereas zone 3 was treated with de-ionized water and zone 2 treated with tap water extract. Source as per **Siraeva et al. (2016)**.

5.2.4 Statistical analysis

Statistical analysis was treated with GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA) and data was reported as mean $\pm$ standard deviation (SD). The results were graphically presented to visualize the skin irritation visual scores of *S. africana* stem-bark extracts.

5.3 Results

The positive control (1% SLS) had the highest visual score, around 2.0, indicating a high level of skin irritation at all time points (24, 48, and 72 h) (**Figure 5.3**). In addition, the EtOH extract showed very similar patterns, with visual scores increasing gradually over time, reaching around 1.5-1.6 at 72 h. This shows that the EtOH extracts have moderate skin irritation potential, which is lower than the positive control but higher than the negative control. The negative control (de-ionized water) exhibited the lowest visual scores at 24 and 48 h, indicating minimal skin irritation to non-irritation, as expected. While the water extract had the second lowest maximum visual score at 24 and 48 h, it had a non-irritation score at 72 h.

Overall, **Figure 5.2** reveals that the EtOH extract of *S. africana* stem-bark under investigation had moderate skin irritation potential, while the SLS caused significant irritation, and the negative control (de-ionized water) and water showed non-irritation. The irritation potential appears to increase over time for all tested materials.

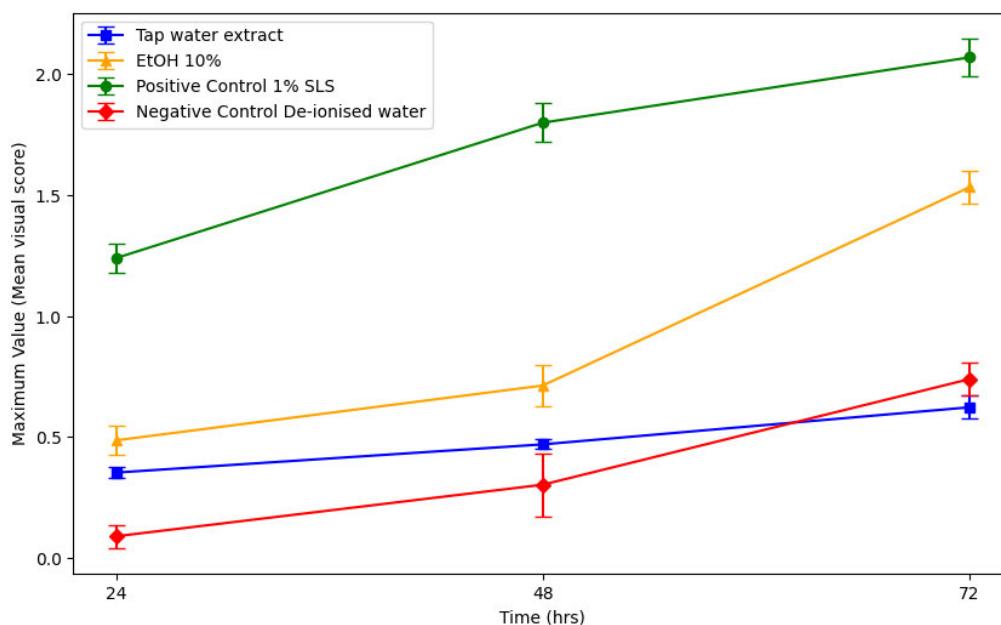


Figure 5.3: Skin irritation potential of *S. africana* stem-bark tap water and 10% EtOH extracts compared to controls (positive control 1% SLS and negative control de-ionized water) over time.

Figure 5.4 shows the maximum visual score (representing the highest level of skin irritation observed) for the two extracts of *S. africana* stem-bark and the two controls: tap water extract, 10% EtOH extract, positive control 1% SLS, and negative control de-ionized water at 72 h after application. The positive control, 1% SLS, had the highest maximum visual score of 2.07 ± 0.08 , indicating a high level of skin irritating as expected for a positive control irritant. In contrast, the negative control, de-ionized water, had the second lowest maximum visual score around 0.74 ± 0.07 , demonstrating minimal to no skin irritation potential. While the 10% EtOH extract samples showed low irritant maximum visual scores around 1.5, suggesting a moderate level of skin irritation potential. The water extract indicated the lowest amount of irritation, with a maximum visual score of about 0.62 ± 0.05 .

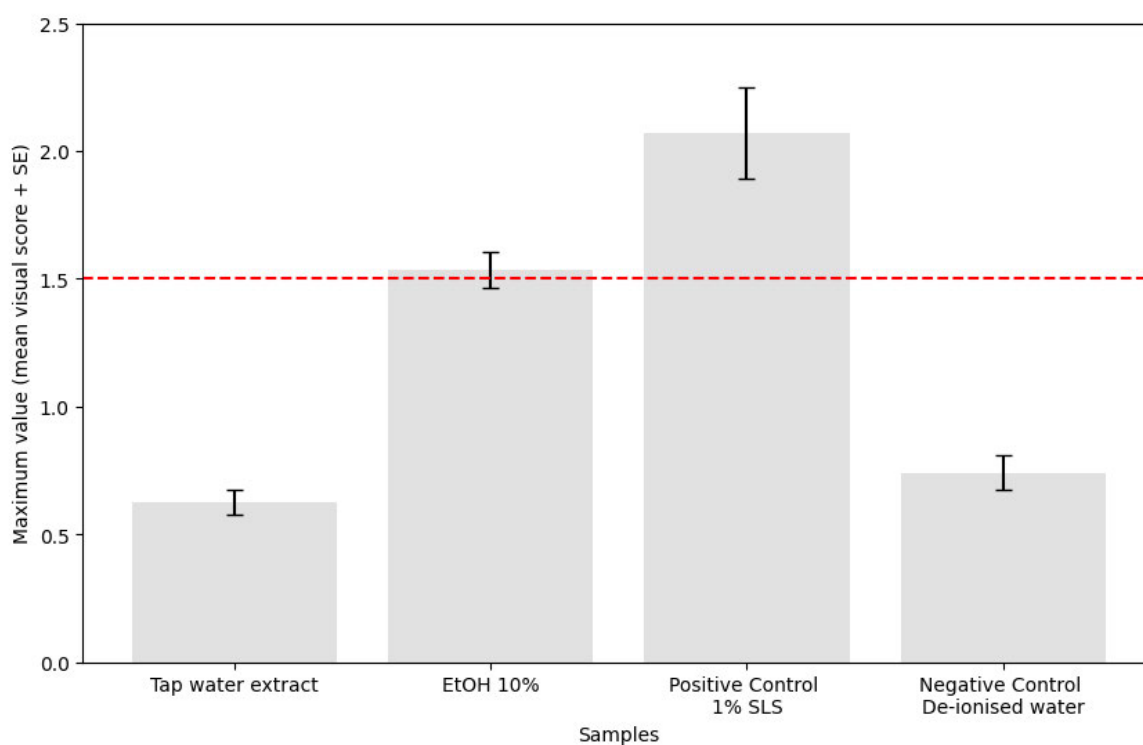


Figure 5.4: Maximum visual skin irritation scores for (tap water and 10% EtOH extracts) and controls (positive control 1% SLS and negative control de-ionized water) at 72 h after application. The red dashed line at 1.5 is the irritancy threshold → any sample above it is considered a high irritant, while those below are non-irritant or mild. The red line: 10% EtOH extract samples showed low irritant maximum visual scores around 1.5.

5.4 Discussion

Skin irritation is defined as reversible damage produced by skin exposure caused by chemicals. In this study, possible skin irritating properties was examined by *in vitro* testing. This chapter looked at skin irritation properties of the plant extract only, assessed by a visual score. Irritating components primarily cause skin irritation by producing an inflammatory reaction. Repeated exposure to skin irritants can cause damage ranging from mild redness (erythema) to severe chemical burns with tissue death (necrosis), which are challenging to treat (Jusoh et al., 2019). When irritants contact the skin, they compromise its protective barrier, directly damaging the epidermal keratinocytes (skin cells). This triggers the body's innate immune response, leading to various inflammatory reactions in the skin (Turner et al., 2014).

The skin irritation tests conducted on rat models revealed important insights into the anti-irritant properties of the water and EtOH extracts of *S. africana*. Single skin irritation test revealed that erythema occurred on the skin within a few days of topical treatment of *S. africana* extracts, with different extracts being mild and reversible.

Sodium lauryl sulfate (SLS) is a well-known cleansing chemical that has been demonstrated to alter the content of lipids in the skin, eradicate lipids from the stratum corneum surface, and impair the functions of the skin barrier (Törmä et al., 2008). The visual scoring results indicated that the positive control (1% SLS) caused significant irritation as expected (Marrakchi and Maibach, 2006; Leoty-Okombi et al., 2021). The positive control, skin irritation response score of 2.07 ± 0.08 at 72 h was the highest, as severe irritation. After single application of 10% EtOH extract of *S. africana*, skin irritation response visual scores were consistently in the range of 1.5-1.6 at 72 h (Figures 5.3 and 5.4), which was moderate irritation. Negative control (de-ionized water) showed mild irritation with the visual score around 0.74 ± 0.07 . The skin irritation response score of the tap water extract indicated the lowest irritation of 0.62 ± 0.05 for the rest of time as shown in Figure 5.4 among all the samples tested.

The traditional use of *S. africana* by the *isiXhosa* people for treating various skin conditions is supported by the observed anti-irritant effects of its extracts, particularly the water extract (Singh et al., 2020). This suggests the plant has potential as a natural therapeutic agent for managing skin

irritation. Firstly, earlier research has demonstrated the antibacterial and antioxidant activities of *S. africana* extracts (**Mathabe et al., 2006; Amoo et al., 2012; Alliya, 2019**), which could contribute to their protective effects against skin irritation. Secondly, *S. africana* extracts may contain compounds that can modulate the inflammatory response, thereby mitigating the irritant effects. For example, phytochemicals have been shown to operate as oxidative enzyme inhibitors, radical chain reaction inhibitors, free radical scavengers, metal chelators, and antioxidant enzyme cofactors (**Michalak, 2023**). According to certain research, plant extracts stimulate endogenous antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH-PX), which protect the skin from increased ROS levels under oxidative stress (**Hoang et al., 2021; Ibrahim et al., 2022; Piao et al., 2023**).

Plant materials can regulate the expression and activation of numerous cytokines, comprising TNF- α , IL-1 β , IL-6, and IL-8 (**Michalak, 2023**). However, it is vital to acknowledge that previous studies have recognized *S. africana* as a plant with potentially poisonous properties that could lead to skin irritation (**Lennox and Bamford, 2015**). This is primarily a result of the existence of toxic compounds in the sap latex of the tree (**Wink and Van Wyk, 2008**). The latex contains certain phytochemicals, which may contribute to its irritant potential. Therefore, future studies should incorporate investigations into the appropriate dosage and concentration of *S. africana* stem-bark extracts to strike a balance between therapeutic efficacy and potential toxicity. Previous research on other medicinal plants has shown that the right concentration or dosage range is crucial for their safe and effective use as therapeutic agents (**George, 2011**). For instance, studies on *Abrus precatorius* and *Gloriosa superba* have demonstrated that while low to moderate concentrations exhibit beneficial effects, excessively high concentrations can be detrimental to a person's health, potentially causing adverse reactions or toxicity (**Fennell et al., 2004; Nasri and Shirzad, 2013; Tamilselvan et al., 2014**). Therefore, it is essential to establish the optimal dosage and formulation of *S. africana* extracts that maximize their anti-irritant properties while minimizing potential side effects or irritation. To establish the safe dosage range and find any possible side effects connected to the use of *S. africana* extracts, toxicological evaluations, including *in vitro* and *in vivo* investigations, ought to be conducted. Furthermore, to guarantee dependable and consistent therapeutic outcomes, phytochemical profiling of the active ingredients and standardisation of the extraction procedure would be essential. Future studies can help produce safe and efficient natural medicinal compounds derived from *S. africana* to treat skin irritation by addressing these factors.

Furthermore, clinical trials are needed to determine the effectiveness and safety of these extracts in human subjects, especially in resource-constrained settings where access to traditional treatments may be the only option available.

5.5 Conclusions

This study included two *S. africana* stem-bark extracts as well as two controls: tap water extract, 10% EtOH extract, positive control 1% SLS, and negative control de-ionised water. EOtH extract showed moderate skin irritation while the positive control caused severe skin irritation. The results support conventional knowledge and emphasize the value of looking into natural sources for secure and efficient medicinal options. To help these extracts become natural therapeutic agents for the treatment of skin irritation, further studies are required to completely comprehend the mechanisms underlying their anti-irritant qualities. The findings align with traditional knowledge and highlight the importance of exploring natural sources for safe and effective therapeutic solutions. Maintaining a delicate balance of both oxidants and antioxidants in a system has cosmetic benefits since oxidants at low levels initiate cell signalling pathways. Further studies are required to understand the processes underlying these extracts' anti-irritant qualities and to promote their use as natural medicinal agents.

CHAPTER SIX

Unravelling the Anti-Tyrosinase Activity of *Spirostachys africana* Stem-Bark Extracts: Towards Natural Solutions for Hyperpigmentation



Isixhosa woman with traditional medicine applied on her face.

Source: <https://www.zkhiphani.co.za/>

6.1 Introduction

Skin pigmentation disorders, such as hyperpigmentation and age spots, are common dermatological concerns that can significantly impact an individual's appearance and self-esteem. These medical conditions are commonly connected with an imbalance in the generation and distribution of melanin, the pigment responsible for the colour of the skin, hair, and eyes. **(Thawabteh et al., 2023)**. Tyrosinase, a multifunctional copper-containing enzyme, plays a crucial role in the melanogenesis process, catalyzing the rate-limiting steps in the biosynthesis of melanin **(dos Santos Videira et al., 2013; Zolghadri et al., 2019)**. As a result, inhibiting tyrosinase activity has emerged as a viable technique for the development of skin lightening agents and the therapy of hyperpigmentation diseases **(Slominski et al., 2004)**.

Tyrosinase is a copper type-3 protein family multifunctional metalloenzyme that comprises two copper ions in the active site and six histidine amino acid residues **(Kanteev et al., 2015)**. This oxidoreductase enzyme plays a critical role in the redox processes within epidermal melanocytes, catalyzing the hydroxylation of L-tyrosine to L-3,4-dihydroxyphenylalanine (L-DOPA) and the subsequent oxidation of L-DOPA to its corresponding quinone **(Pillaiyar et al., 2017)**. These sequential enzymatic reactions are vital for melanogenesis, leading to the production of melanin, an indolic heteropolymer responsible for variations in skin pigmentation **(Djafarou et al., 2023)**.

Melanin is the skin's primary defensive mechanism against UV radiation and photocarcinogens **(Zolghadri et al., 2019)**. However, the uncontrolled alteration of tyrosinase activity can result in a variety of hyperpigmentation-related skin disorders, such as acanthosis nigricans, melasma, lentigines, and an increased risk of skin cancer **(Pillaiyar et al., 2018; Zolghadri et al., 2019)**. Consequently, the development of safe and effective tyrosinase inhibitors has garnered significant interest in the field of cosmeceuticals and dermatological therapeutics. In this context, investigating natural sources, such as medicinal herbs, for anti-tyrosinase action shows a great potential. *Spirostachys africana*, an indigenous South African medicinal plant traditionally used by the *isiXhosa* people for treating various skin conditions, including cradle cap in infants, has gained attention for its therapeutic properties **(Singh et al., 2020)**. Previous studies have demonstrated the antibacterial and antioxidant potential of *S. africana* stem-bark extracts, lending scientific support to its ethnomedicinal applications **(Mathabe et al., 2006; Amoo et al., 2012; Alliya, 2019)**.

However, the plant's potential anti-tyrosinase activity and its implications for skin protection remain largely unexplored.

This part of the study aimed to investigate the anti-tyrosinase activity of *S. africana* stem-bark extracts, providing insights into their potential as natural skin-lightening agents and their role in mitigating hyperpigmentation disorders.

6.2 Material and methods

6.2.1 Plant material

The collection of *S. africana* stem-bark material was previously discussed in Chapter 3, Section 3.2.1 (**Figure 5.1**).

6.2.2 Sample preparation for anti-tyrosinase activity

After being dried for 72 h at 50 °C, the stem-bark material was ground into a fine powder using a grinder (MF 10, Merck Chemical (Pty) Ltd) and extracted for 1 h in an ultrasound bath. The extraction mixtures were filtered and vacuum-dried to near-dryness. The residues were resuspended in extraction solvents (100% ethanol and 100% methanol) to the concentrations required. Kojic acid (200) was used as a positive control. The dried material was weighed, resuspended in water, and filtered through a sterile 0.22 µm filter unit (Millex® GV, Molsheim, France) to obtain a sterile 50 mg/ml starting concentration, which was then diluted to lower concentrations based on the test. The solution was filtered through Whatman No. 1 filter paper, and the filtrate was processed as described for extract preparation. The resuspended extracts were tested for anti-tyrosinase activity.

6.2.3 Anti-tyrosinase activity assay

The inhibitory effects of kojic acid (200) as positive control and *S. africana* stem-bark extracts prepared using 100% ethanol (EtOH) and 100% methanol (MeOH) on tyrosinase activity were evaluated using the dopachrome method with 3,4-dihydroxy-L-phenylalanine (L-DOPA) as the substrate. Negative controls included all components except the substrate, while kojic acid served

as the positive control (**Chang, 2009**). This protocol was based on the method described by **Momtaz et al. (2008)** with slight modifications.

Initially, *S. africana* stem-bark extracts were solubilized in dimethyl sulfoxide (DMSO) to a concentration of 20 µg /mL. The samples were then diluted to 400 µg/mL in potassium phosphate buffer (0.1 M, pH 6.5) and subjected to two serial dilutions across a 96-well microtiter plate. In each well, 70 µL of the plant extract was combined with 30 µL of mushroom tyrosinase enzyme (333 units/mL in potassium phosphate buffer, 0.1 M, pH 6.5), and the mixture was incubated at room temperature for 10 min.

To initiate the enzymatic reaction, 110 µL of 12 mM L-DOPA was added to each well. The plate was further incubated for 30 min at room temperature, and absorbance was measured at 492 nm using a PerkinElmer VICTOR® Nivo™ Multimode Plate Reader. The percentage inhibition of tyrosinase activity was calculated using the following formula:

$$\% \text{ tyrosinase inhibition} = ((A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}) \times 100$$

Where A_{control} and A_{sample} denote the absorbance of the control and sample, respectively. The % tyrosinase inhibition was plotted versus plant extract concentration using the normalized logarithmic regression curve. To establish reliability, the experiment was conducted in duplicate and performed three times.

6.3 Statistical analysis

Statistical analysis was conducted to determine the significance of the inhibitory effects of kojic acid and *S. africana* stem-bark extracts on tyrosinase activity. All experimental procedures were conducted in duplicate and repeated three times to ensure the reliability of the results. Data were presented as mean ± standard deviation (SD). All statistical analyses were carried out using GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). The results were graphically presented as bar charts or line graphs to visualize the inhibitory effects and compare the effectiveness of different extracts.

6.4 Results

The inhibitory effects of kojic acid (200) and *S. africana* stem-bark extracts prepared using 100% ethanol (EtOH) and 100% methanol (MeOH) on tyrosinase activity were investigated across a range of concentrations ($0.5 - 2.5 \times 10^{-6}$ mol/L). **Figure 6.1** shows the association between the final concentration of each chemical or extract and residual enzyme activity.

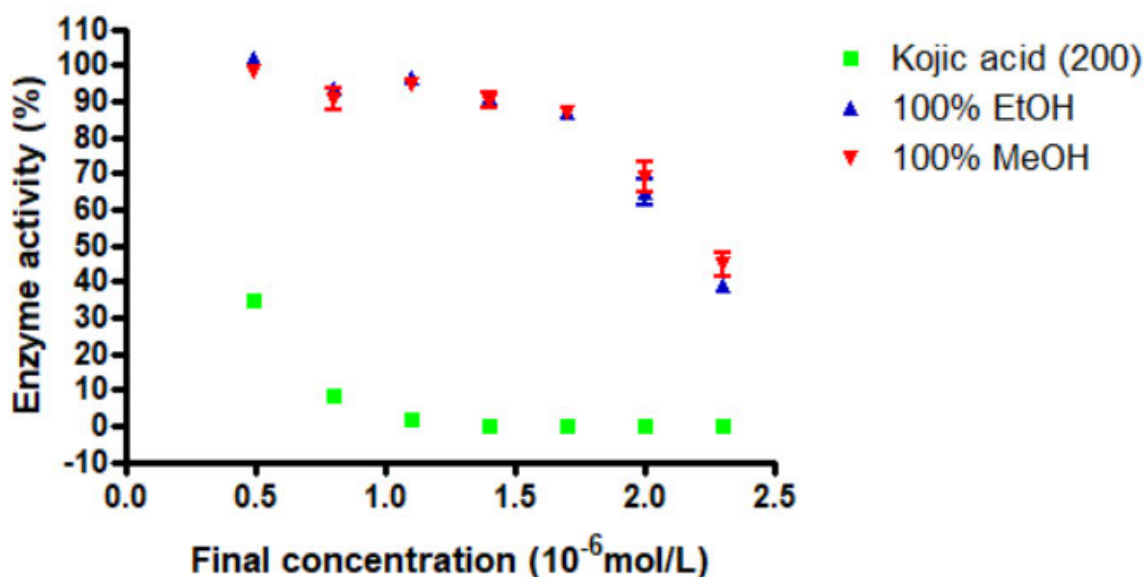


Figure 6.1: Tyrosinase inhibition by kojic acid and *S. africana* stem-bark extracts (100% ethanol (EtOH) and 100% methanol (MeOH)). Kojic acid (KA) served as a positive control, demonstrating potent inhibition.

Kojic acid, a well-known tyrosinase inhibitor, served as a positive control and showed substantial inhibitory effect at all concentrations tested. At the lowest concentration examined (0.5×10^{-6} mol/L), kojic acid reduced enzyme activity to approximately 35%. This inhibitory effect intensified with increasing concentrations, reaching near-complete inhibition ($\leq 5\%$ residual activity) at concentrations of 1.0×10^{-6} mol/L and above. The dose-response curve for kojic acid exhibited a steep decline in the lower concentration range, followed by a plateau at higher concentrations, indicative of its high potency and efficacy as a tyrosinase inhibitor.

In contrast, both *S. africana* stem-bark extracts prepared with 100% EtOH and 100% MeOH displayed markedly different inhibition profiles. At lower concentrations ($0.5 - 1.5 \times 10^{-6}$ mol/L), these extracts exhibited minimal inhibitory effects, with enzyme activity remaining above 90%. As concentrations increased, a gradual decline in enzyme activity was observed for both extracts. At the highest concentration tested (2.5×10^{-6} mol/L), the EtOH and MeOH extracts reduced enzyme activity to approximately 40% and 45%, respectively. The inhibition patterns of the EtOH and MeOH extracts were remarkably similar, with the EtOH extract showing a slightly higher inhibitory effect at concentrations above 1.5×10^{-6} mol/L. However, the differences between these two extracts were minimal as evidenced by the overlapping error bars. It is noteworthy that the dose-response curves for the EtOH and MeOH extracts of *S. africana* stem-bark did not reach a plateau within the concentration range tested, suggesting that higher concentrations might yield further inhibition. This indicates that the active compounds in the extracts may have a lower potency or are present in lower concentrations compared to pure kojic acid.

6.5 Discussion

This study examined the inhibitory effects of kojic acid and *S. africana* stem-bark extracts (made using 100% ethanol and 100% methanol) on the activity of tyrosinase, a crucial enzyme in melanin formation (Tief et al., 1996). The findings revealed notable variations in inhibitory potencies among these substances, with kojic acid outperforming the plant extracts.

Kojic acid, a positive control, effectively inhibited enzymes at all doses tested, even concentrations as low as 1.0×10^{-6} mol/L. This result is consistent with earlier research that have reported IC_{50} values for kojic acid in the micromolar range (Neeley et al., 2009; Wang et al., 2022). The mechanism of kojic acid's inhibition has been attributed to its capacity to chelate copper at the active site of tyrosinase, thereby inactivating the enzyme (Chang, 2009). In contrast, the ethanolic and methanolic extracts of the stem-bark of *S. africana* displayed notably lower inhibitory effects on the activity of tyrosinase. The presence of compounds with a moderate tyrosinase inhibitory action is indicated by the progressive decrease in enzyme activity that occurs as the extract concentration increases. This discovery was consistent with earlier research on a variety of plant extracts, where complex mixtures of compounds often show significant but weaker inhibitory effects (Wang et al., 2023).

The identical inhibitory patterns of ethanolic and methanolic extracts indicate that both solvents were equally successful at extracting active chemicals from *S. africana* stem-bark. This conclusion is helpful for future phytochemical studies since it demonstrates that both solvents might be used for extraction without appreciably changing the tyrosinase inhibitory capability. However, methanol, a highly polar solvent, can produce a better extraction yield than ethanol. For example, one study discovered that methanol had a 33.2% extraction yield, while ethanol had a 12.2% yield (**Suharni et al., 2021**).

The minimal inhibition seen at lower extract concentrations (below 1.5×10^{-6} mol/L) suggests that the active chemicals are present in low concentrations or have lower binding affinities for tyrosinase than kojic acid. However, the increasing inhibitory effect at higher concentrations calls for additional exploration. Fractionation and isolation of active molecules from these extracts might result in the identification of novel tyrosinase inhibitors (**Hwang et al., 2022**). The stark contrast between kojic acid's potency and the moderate inhibitory effects of *S. africana* extracts underscores the challenges in discovering plant-based tyrosinase inhibitors that can match the efficacy of known synthetic inhibitors. Nevertheless, it is crucial to remember that plant extracts often contain multiple compounds that may act synergistically or have additional beneficial properties beyond tyrosinase inhibition, including anti-inflammatory or antioxidant effects (**El-Nashar et al., 2021**).

The current investigation offers insightful information about the possibilities of *S. africana* as a source of tyrosinase inhibitors, but it also has limitations. The *in vitro* nature of the assay may not fully reflect the complexity of tyrosinase inhibition in cellular or tissue environments. Additionally, **Wang et al. (2023)** highlights that the crude extracts may contain compounds that interfere with the assay or have opposing effects on tyrosinase activity.

Future investigations aimed at identifying and isolating the active chemicals present in stem-bark extracts from *S. africana* should use bioassay-guided fractionation. Research on the structure-activity relationship of these substances may help design stronger inhibitors, as demonstrated by (**Vigbedor et al., 2022**). Furthermore, investigating how these extracts affect cellular melanin production and evaluating their safety profiles would be crucial steps towards potential cosmetic or therapeutic applications.

Although less effective than kojic acid, the moderate tyrosinase inhibitory activity of *S. africana* extracts raises the possibility that this plant could be used to create natural skin-lightening

products or as a component of a multi-component strategy to treat hyperpigmentation diseases. Additional benefits that complement the tyrosinase inhibitory effects of these extracts may be found by further investigating their broader biological activities, like their antioxidant or anti-inflammatory qualities.

6.6 Conclusions

This research study offers quantitative data on the tyrosinase inhibitory potential of *S. africana* stem-bark extracts in comparison to kojic acid. While the plant extracts showed moderate inhibition, the results highlight the need for further phytochemical investigation and compound isolation to fully explore the potential of *S. africana* as a source of natural tyrosinase inhibitors.

CHAPTER SEVEN

Anti-inflammatory Action of *Spirostachys africana* Extracts, Specifically Evaluating their Ability to Inhibit Nitric Oxide (NO) Production and 15-Lipoxygenase (15-LOX) Activity



A South African child with inflammatory rash on her face.

Source: <http://archpedi.jamanetwork.com>

7.1 Introduction

Inflammation is a key physiological reaction required to protect the body against detrimental stressors like metabolic stress, damage, and infections (**Torres et al., 2015; Antonelli and Kushner, 2017**). While inflammatory processes are necessary for immunological defence, they are equally critical in creating many chronic illnesses. Current anti-inflammatory drugs often have limited efficacy or cause adverse side effects with long-term use, highlighting the need for safer and more effective alternatives (**Sohail et al., 2023**). In this context, natural products particularly those derived from medicinal plants have emerged as promising sources of novel anti-inflammatory compounds with potential therapeutic applications (**Nyakudya et al., 2020; Aware et al., 2022**).

Spirostachys africana Sond. (Euphorbiaceae), also known as "*tamboti*" in southern Africa, is a vital medicinal herb with a lengthy traditional therapeutic history (**Singh et al., 2020**). Indigenous people have traditionally used several portions of this plant to treat inflammatory disorders, wounds, and microbiological infections (**Singh et al., 2020**). Despite its historical significance, extensive scientific research into its molecular mechanisms of action remains limited.

Lipoxygenases (LOX), specifically the 15-lipoxygenase-1 (15-LOX-1) enzyme, are important biological targets in inflammatory processes. These enzymes catalyse the oxygenation of arachidonic acid, producing bioactive metabolites that are important in inflammatory and immunological responses (**Claesson, 2009**). Inhibiting such enzymatic pathways is a strategic method for controlling inflammatory processes. Early phytochemical studies indicate that *S. africana* has a wide range of bioactive components, including diterpenes, flavonoids, and phenolic compounds, which may contribute to its anti-inflammatory activities (**Mabona and Van Vuuren, 2013**). However, the molecular pathways and enzymatic interactions are not fully understood.

Additionally, inflammatory mediators like nitric oxide (NO) further exemplify the complex nature of immune responses. While essential for cellular signaling and host defense, inappropriate NO production can contribute to various pathological conditions. Understanding the modulation of such mediators is crucial for developing targeted therapeutic interventions.

This research study investigated the anti-inflammatory properties of *S. africana* stem-bark extracts. Various solvents were employed to identify the most effective extraction method for isolating compounds with anti-inflammatory activity. Specifically, the study evaluated the extracts'

ability to inhibit 15-lipoxygenase-1 (15-LOX-1), an enzyme involved in the biosynthesis of pro-inflammatory leukotrienes, as well as their capacity to suppress nitric oxide (NO) production in activated macrophages a crucial contributor to inflammatory responses.

7.2 Material and methods

7.2.1 Plant material

The collection of *S. africana* stem-bark material was previously discussed in Chapter 3, Section 3.2.1 (**Figure 5.1**).

7.2.2 Sample preparation for anti-inflammatory activity

The stem-bark material was oven dried in an oven at 50 °C for 72 h, or until constant dryness was achieved. The dried material were then ground into fine powder using a grinder (MF 10, Merck Chemical (Pty) Ltd). Ten grams of stem-bark powder were extracted separately in 100 mL of dichloromethane, petroleum ether, ethanol, and distilled water, then macerated for 24 h at ambient temperature (25°C). To ensure extensive phytochemical extraction, four mentioned different solvents with various polarities were selected.

Following maceration, Whatman No. 1 filter paper was used to filter mixtures to exclude solid particles and plant debris. The organic solvents were then carefully evaporated to dryness using a rotary evaporator, concentrating the extracted compounds. To ensure reproducibility and minimize potential variations, the extraction process was carried out twice with identical plant material.

In a fume hood at room temperature, a regulated stream of air was used to remove any remaining solvents as the last step. The method facilitated the production of dried crude extracts, preserving the integrity of the extracted phytochemicals and preparing them for subsequent anti-inflammatory activity assessments.

Quercetin (Sigma Aldrich, Germany) and indomethacin (Merck, Germany) were evaluated at the same concentrations and served as the positive control, while wells containing LPS-activated but untreated cells served as the negative control.

7.2.3 The 15-lipoxygenase (15-LOX) inhibitory assay

For the 15-lipoxygenase (15-LOX) inhibition test (Pinto del Carmen et al., 2007) a spectrophotometric technique to determine LOX activity was used, with modifications by Dzoyem and Eloff (2015). The assay evaluates the inhibition of soybean 15-lipoxygenase activity by samples in the presence of linoleic acid (Merck, Germany) based on the formation of the complex ferrous sulphate/xylitol orange with absorption at 560 nm. The 15-lipoxidase from *Glycine max* (Sigma Aldrich, USA) was incubated for 5 min with 50 mM Tris-HCl and extracts of varying concentrations. A substrate (140 uM linoleic acid) was added to all the wells except for blanks and incubated for 20 mins at 25 °C, followed by the termination of the assay by adding FOX mixture (100 uM xylitol orange, 100 uM ferrous sulphate (Merck, Germany), methanol and 30 mM sulphuric acid) and incubated for 30 mins at 25 °C. The substrate was added to the blanks and the 15-LOX inhibitory activity was assessed by computing the percentage of the hydroperoxide production inhibition from the variations in absorbance values at 560 nm using the Epoch BioTek Stats (Agilent, USA). The experiment was performed in triplicate and repeated three times. The percentage of 15-LOX inhibition was computed as follows:

$$\% \text{ Inhibition} = \frac{AB-AA}{AA} \times 100,$$

where AB= absorbance of control and AA= absorbance of test sample. The graphs were plotted using linear regression and the IC₅₀ values were calculated to determine the concentration value at which the extract produces 50% inhibition of hydroperoxide-release.

7.2.4 Inhibition of nitric oxide (NO) production in lipopolysaccharide-stimulated RAW macrophage cells

7.2.4.1 Cell culture and treatment with samples

Murine RAW 264.7 macrophage cells were sourced from the American Type Culture Collection (Rockville, MD, USA). The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with L-glutamine, 10% fetal bovine serum (FBS), and 1% penicillin/streptomycin solution (Capricorn Scientific, Germany). Cultures were maintained at 37 °C in a humidified atmosphere with 5% CO₂.

Cells were seeded into 96-well plates at a density of 40,000 cells per well and incubated for 24 h under the same conditions. Following incubation, the media were aspirated, and the cells were activated with lipopolysaccharide (LPS, 1 µg/mL) prior to the addition of varying concentrations of extracts (100, 50, 25, 12.5, 6.25, and 3.125 µg/mL) diluted in serum-free DMEM. The DMSO concentration (Merck, Germany) in all treatments did not exceed 0.1%.

Quercetin (Sigma Aldrich, Germany) and indomethacin (Merck, Germany) were evaluated at the same concentrations and served as the positive control, while wells containing LPS-activated but untreated cells served as the negative control. The plates were incubated for an additional 24 h at 37 °C in a humidified environment with 5% CO₂.

7.2.4.2 Measurement of nitrites and determination of percentage of nitric oxide inhibition

The amount of nitric oxide (NO) produced by treated cells was measured using the Griess reagent method, as described by (Adebayo et al.,2015). Briefly, after 24 h of incubation, 100 µL of the culture supernatant was collected and transferred to new 96-well plates. An equal volume of Griess reagent (Sigma Aldrich, USA) was added to each well in the dark. The plates were covered with aluminum foil and incubated for 15 min in the dark.

The absorbance was measured at 550 nm using a BioTek Synergy™ HT Multi-Detection Microplate Reader (Agilent, USA). Nitrite concentrations were determined through regression analysis using a standard curve generated from serial dilutions of sodium nitrite.

The percentage inhibition of NO production by the extracts was calculated by comparing NO levels in treated wells to those in the control wells (cells incubated with triggering agents and DMSO but without extracts, considered as 0% inhibition). The formula used to calculate the percentage inhibition of NO production was:

$$\% \text{ Inhibition} = 100 - \frac{((\text{Abs Control} - \text{Abs Blank}) - (\text{Abs Extract} - \text{Abs Blank}))}{\text{Abs (Control)} - \text{Abs (Blank)}} \times 100$$

7.2.5 Statistical analysis

The data is represented as means ± standard error (SE). Mean differences were separated using one-way analysis of variance (ANOVA). The Fisher's multiple range tests was used, where $p < 0.05$ indicated statistical significance. Data were analyzed using the Statistical Package for Social Sciences (SPSS) for Windows version 22.2 (SPSS Inc., Chicago, IL, USA).

7.3 Results

7.3.1 The 15-lipoxygenase (15-LOX) inhibitory assay

The anti-inflammatory potential of *S. africana* extracts was assessed using the 15-lipoxygenase (15-LOX) inhibitory assay, with results presented as IC₅₀ values on **Table 7.1**. The distilled water extract demonstrated moderate inhibitory activity (IC₅₀ = 0.447 ± 0.057 µg/mL), while the ethanol extract showed slightly enhanced inhibition (IC₅₀ = 0.421 ± 0.079 µg/mL), suggesting improved extraction of active compounds with ethanol. Conversely, the petroleum ether extract exhibited lower activity (IC₅₀ = 0.746 ± 0.14 µg/mL), and the dichloromethane extract displayed the weakest inhibition (IC₅₀ = 3.56 ± 0.73 µg/mL). Quercetin, a known 15-LOX inhibitor, had an IC₅₀ of 2.02 ± 0.34 µg/mL, indicating that certain *S. africana* extracts were more effective than this standard. These research findings show the potential of the distilled water and ethanol extracts as sources of natural 15-LOX inhibitors for anti-inflammatory applications.

Table 7.1: Anti-inflammatory activities of various extracts of *Spirostachys africana* by determining their IC₅₀ values (the concentration required to inhibit 50% of 15- lipoxygenase [15-LOX] activity).

IC ₅₀ in µg/mL	
Extracts	15-LOX
Distilled water	0.447 ± 0.057
Ethanol	0.421 ± 0.079
Petroleum ether	0.746 ± 0.14
Dichloromethane	3.56 ± 0.73
Quercetin	2.02 ± 0.34

7.3.2 Inhibition of nitric oxide (NO) production in lipopolysaccharide-stimulated RAW macrophage cells

Nitric oxide inhibition by *S. africana* extracts was tested in LPS-stimulated RAW macrophages as shown in **Table 7.2**. Ethanol extracts showed the highest inhibition at lower concentrations, with $17.91\% \pm 0.02$ at $3.125 \mu\text{g/mL}$, while petroleum ether extracts displayed moderate inhibition, peaking at $15.93\% \pm 0.01$ at $6.25 \mu\text{g/mL}$. Dichloromethane and quercetin extracts exhibited weak activity, with quercetin achieving only $4.74\% \pm 0.01$ inhibition at $100 \mu\text{g/mL}$. Indomethacin, a positive control, demonstrated strong inhibition across all concentrations, with a maximum of $41.52\% \pm 0.01$ at $25 \mu\text{g/mL}$. DMSO controls showed negligible inhibition, confirming the reliability of the experimental procedure. These results suggest that ethanol and petroleum ether extracts contain bioactive compounds capable of modulating NO production, warranting further research to isolate and identify these components for potential therapeutic use.

Table 7.2: Percentage of nitric oxide (NO) inhibition in Lipopolysaccharide (LPS) isolated *Spirostachys africana* stem-bark extracts.

Extract	Concentration (µg/mL)	NO Inhibition (% of LPS)
Distilled water	50	11.63 ± 0.03
	25	13.13 ± 0.03
	12.5	9.79 ± 0.03
	6.25	12.90 ± 0.02
	3.125	13.14 ± 0.02
DMSO	0.1%	1.99 ± 0.02
DMSO	0.05%	0.00 ± 0.00
Ethanol	50	9.74 ± 0.02
	25	11.63 ± 0.02
	12.5	5.05 ± 0.02
	6.25	16.38 ± 0.02
	3.125	17.91 ± 0.02
DMSO	0.03%	0.00 ± 0.00
DMSO	0.01%	0.00 ± 0.00
Petroleum ether	50	7.69 ± 0.01
	25	13.46 ± 0.01
	12.5	4.74 ± 0.01
	6.25	15.93 ± 0.01
	3.125	9.66 ± 0.01
Quercetin	100 µg/mL	4.74 ± 0.01
Quercetin	50 µg/mL	0.00 ± 0.00
Dichloromethane	50	0.00 ± 0.00
	25	2.90 ± 0.00
	12.5	4.56 ± 0.00
	6.25	2.33 ± 0.00
	3.125	6.42 ± 0.00
Quercetin	25 µg/mL	0.00 ± 0.00
Quercetin	12.5 µg/mL	0.00 ± 0.00
Indomethacin	50 µg/mL	33.13 ± 0.01
	25 µg/mL	41.52 ± 0.01
	12.5 µg/mL	28.99 ± 0.01
	6.25 µg/mL	17.64 ± 0.01
	3.125 µg/mL	28.39 ± 0.01
Cells without LPS		39.71 0.01

7.4 Discussion

The present study explored the anti-inflammatory qualities of *S. africana* extracts by evaluating their capacity to prevent two key markers of inflammation: 15-lipoxygenase (15-LOX) activity and NO production in LPS-stimulated RAW macrophages. The findings demonstrate promising inhibitory activities, particularly for the ethanol and petroleum ether extracts, which implies the existence of bioactive substances with possible medical uses.

15-LOX inhibitory activity

The 15-LOX enzyme participates in producing pro-inflammatory leukotrienes, which renders it a desirable target for the creation of anti-inflammatory medications (**Dobrian et al., 2011**). The ethanol and distilled water extracts showed the strongest inhibitory activity with IC_{50} values of $0.421 \pm 0.079 \mu\text{g/mL}$ and $0.447 \pm 0.057 \mu\text{g/mL}$, respectively, outperforming the known inhibitor quercetin ($IC_{50} = 2.02 \pm 0.34 \mu\text{g/mL}$). This result highlights the efficiency of these extraction methods in isolating compounds capable of modulating 15-LOX activity. The petroleum ether extract demonstrated moderate activity, with an IC_{50} of $0.746 \pm 0.14 \mu\text{g/mL}$, while the dichloromethane extract displayed the weakest activity ($IC_{50} = 3.56 \pm 0.73 \mu\text{g/mL}$), suggesting a lower yield of active compounds in non-polar solvents. The performance of ethanol and distilled water extracts underscores the significance of choosing suitable solvents to optimize the extraction of active constituents. The results also suggest that the ethanol extract may contain both polar and semi-polar bioactive compounds, enhancing its inhibitory potential.

NO Inhibition in LPS-Stimulated Macrophages

NO is a crucial mediator of inflammation and its overproduction contributes to the pathogenesis of inflammatory diseases (**Abdulkhaleq et al., 2018**). Ethanol extracts of *S. africana* displayed the uppermost NO inhibition, particularly at lower concentrations, with $17.91\% \pm 0.02$ inhibition at $3.125 \mu\text{g/mL}$. This concentration-dependent activity is indicative of potential bioactive compounds that are effective even at low doses. Petroleum ether extracts also displayed moderate NO inhibition, peaking at $15.93\% \pm 0.01$ at $6.25 \mu\text{g/mL}$. These results suggest that ethanol and petroleum ether extracts contain compounds that can modulate NO production, although their differing profiles may reflect variations in the nature or concentration of active constituents extracted by each solvent. In contrast, the dichloromethane extracts showed minimal inhibition, indicating limited extraction of NO-inhibitory compounds. Similarly, quercetin

exhibited weak inhibition ($4.74\% \pm 0.01$ at $100 \mu\text{g/mL}$), which was unexpected given its known anti-inflammatory qualities. This could be credited to solubility issues, or the limited concentration range tested. Positive control indomethacin demonstrated robust inhibition, with a maximum of $41.52\% \pm 0.01$ at $25 \mu\text{g/mL}$, validating the experimental procedure and serving as a benchmark for the test extracts.

Comparative Analysis and Implications

The overall results suggest that ethanol extracts are superior in both 15-LOX and NO inhibition assays, their capacity to provide anti-inflammatory agents. Ethanol's capacity to extract a broad spectrum of bioactive compounds may account for its consistent activity across both assays. Petroleum ether extracts, though less effective than ethanol extracts, also demonstrated moderate activity, suggesting that non-polar compounds may support the anti-inflammatory qualities of *S. africana*.

The dichloromethane extracts' poor performance in both assays suggests that this solvent is less effective in extracting relevant bioactive compounds from *S. africana*. **Mtunzi et al. (2017)** made the same observations, where dichloromethane was found to be less effective in isolating bioactive compounds, possibly due to its non-polar nature and limited ability to solubilize polar or semi-polar compounds. This highlights the importance of solvent selection when targeting specific bioactive extracts in medicinal plants.

Conversely, the strong performance of the distilled water extract in the 15-LOX assay, coupled with its moderate activity in the NO inhibition assay, suggests that water-soluble polar compounds contribute significantly to the plant's anti-inflammatory activity. Similar findings were documented by **Ondua et al. (2019)**, who observed strong 15-LOX inhibitory activity in methanol/water (35%) and hexane extracts, emphasizing the relevance of polar solvents in extracting active constituents. These parallels reinforce the hypothesis that extraction solvent polarity plays a crucial role in isolating anti-inflammatory agents from plant materials.

Future Directions

These results necessitate additional research to separate and pinpoint the precise bioactive compounds causing the observed effects. Advanced approaches, such as bioassay-guided

fractionation and mass spectrometry, can help to identify the molecular structures of active compounds. Furthermore, additional assays, including *in vivo* inflammation models, are required to corroborate these conclusions and determine the therapeutic potential of these extracts. The promising efficacy of ethanol and petroleum ether extracts implies that they have the potential to become natural anti-inflammatory medications for use in the curing inflammatory illnesses. In conclusion, this investigation indicates the anti-inflammatory properties of *S. africana* extracts, notably the ethanol and petroleum ether extracts, in modifying 15-LOX activity and NO generation. These results provide the framework for further investigation geared towards developing plant-based medicines that target.

7.5 Conclusion

The findings align with traditional knowledge and highlight the importance of exploring natural sources for safe and effective therapeutic solutions. These findings highlight the potential of these extracts for further exploration as natural anti-inflammatory agents.

CHAPTER EIGHT

Conclusions and Future Perspectives

8.1 Overview of the study

This current study concentrated on evaluating the cosmeceutical potential of extracts derived from *Spirostachys africana*, a versatile plant species traditionally used for child skincare by *isiXhosa* communities in South Africa's Eastern Cape Province. The goal of the study was to close the gap between accepted practices and present scientific methods, harnessing the rich biodiversity and cultural heritage of the region. **Figure 8.1** shows the graphical representation of the thesis.

Chapter 1 provided a comprehensive introduction, highlighting the significance of medicinal plants in healthcare systems worldwide, particularly in developing nations. It emphasized the frequency of skin ailments in Africa and the need to explore natural, culturally rooted remedies to address these conditions.

Chapter 2 offered an in-depth review of *S. africana*'s phytochemistry, medicinal properties, and potential for skincare applications. This chapter delved into the plant's rich phytochemical profile, including alkaloids, flavonoids, saponins, terpenoids, and tannins, each with distinct biological activities and therapeutic potential. While acknowledging the potential toxicity associated with certain compounds, this chapter underscored the importance of scientific validation and standardization to ensure the safe and effective utilization of *S. africana* in modern healthcare practices.

Chapter 3 presented experimental findings on the antibacterial potential of *S. africana* extracts against common dermatological pathogens such as *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus*. The results demonstrated potent antibacterial activity, particularly in dichloromethane (DCM) and ethanol (EtOH) extracts, with least inhibitory concentrations (MICs) ranging from 0.19 to 1.56 µg/mL. These findings aligned with the plant's ethnomedicinal applications and highlights its potential as a cradle of natural antimicrobial agents for topical applications.

Chapter 4 evaluated the *S. africana* stem-bark extracts' antioxidant potential. The antioxidant ability of the extracts was evaluated using numerous *in vitro* assays, including the DPPH radical scavenging and CLAMS assay. The results demonstrated significant free radical scavenging and lipid peroxidation inhibition capabilities, attributed to the presence of phytochemicals like saponins, tannins, and flavonoids with anti-inflammatory, and antioxidant qualities.

Chapter 5 investigated the anti-irritant properties of *S. africana* stem-bark extracts for side effects caused by the skin irritation. Skin irritation tests using a rat model revealed that the water extract demonstrated the least overall irritation potential, with a maximum visual score of 0.62 ± 0.05 at 72 h, which indicated its suitability for topical applications and promise as a natural remedy for managing the side effect caused by skin irritation.

Chapter 6 examined the anti-tyrosinase activity of the extracts, exploring their potential for skin lightening and pigmentation regulation, which could be beneficial in addressing hyperpigmentation disorders. However, the study found minimal evidence of anti-tyrosinase activity from the *S. africana* stem-bark extracts.

Chapter 7 focused on the anti-inflammatory potential of *S. africana* extracts, specifically evaluating their ability to inhibit nitric oxide (NO) production and 15-lipoxygenase (15-LOX) activity. These assays aimed to identify bioactive extracts capable of modulating inflammatory pathways, a critical feature in managing inflammatory-related disorders. The study revealed that ethanol and distilled water extracts demonstrated notable activity in the 15-LOX assay, with IC_{50} values comparable to or better than the positive control, quercetin and indomethacin. Similarly, the ethanol and petroleum ether extracts showed moderate NO inhibition, indicating potential anti-inflammatory properties. Conversely, dichloromethane extracts exhibited poor performance in both assays, highlighting the significance of solvent selection in isolating bioactive mixtures. The results underscored the potential of *S. africana* extracts as natural anti-inflammatory agents, warranting further exploration to characterize their active constituents.

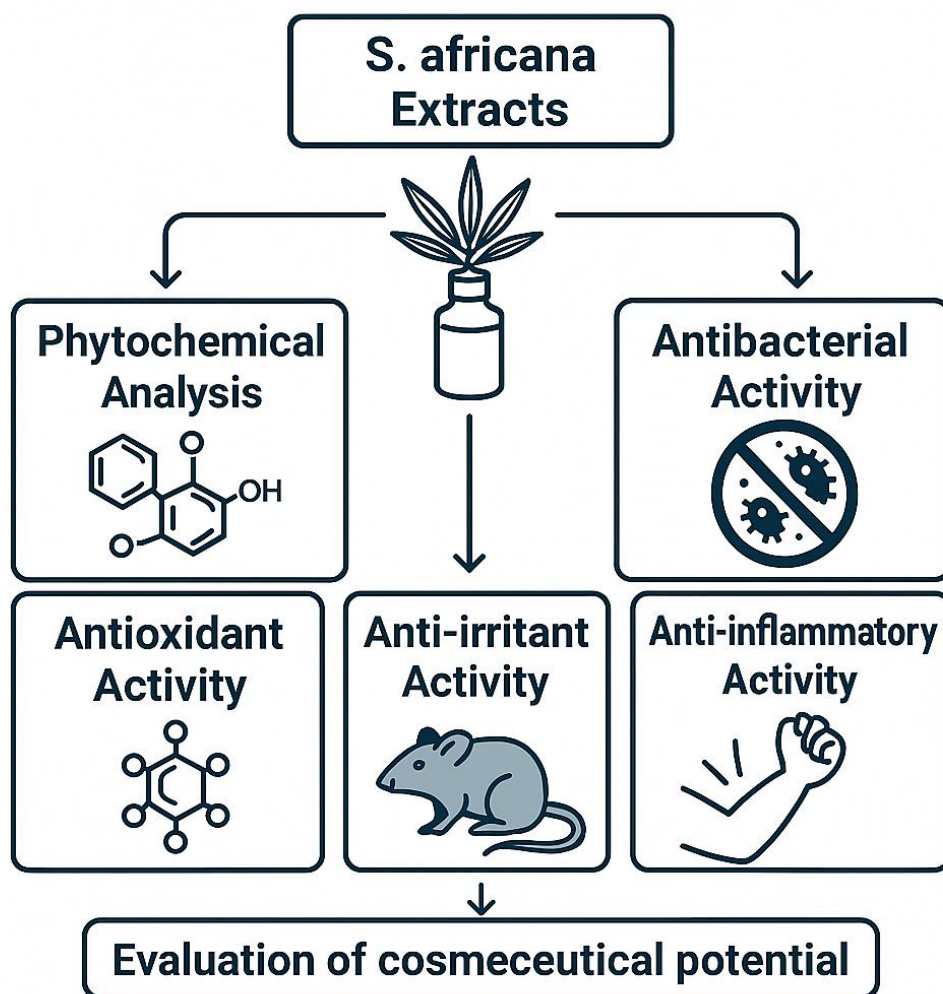


Figure 8.1: Graphical representation of the current study.

8.2 Significance and implications

This investigation has important ramifications for how traditional knowledge and contemporary scientific methods might be combined to create natural, plant-based cosmeceutical solutions. By investigating the therapeutic potential of *S. africana*, an indigenous medicinal plant traditionally utilized by the *isiXhosa* communities of South Africa, this research contributes to the preservation of cultural heritage and the conservation of biodiversity.

The findings validate the traditional use of *S. africana* in treating paediatric skin conditions, particularly cradle cap, by demonstrating its antibacterial, anti-inflammatory, antioxidant, anti-irritant, and anti-tyrosinase properties. The observed bioactivities of the plant extracts can be

accredited to the existence of several phytochemicals, including saponins, tannins, flavonoids, and terpenoids, which possess antimicrobial, antioxidant, anti-inflammatory, and qualities beneficial for skin condition.

The study has substantial implications for the development of novel, plant-based cosmeceutical products targeting various skin concerns, such as infections, oxidative stress, inflammation, and hyperpigmentation. The antibacterial action of *S. africana* extracts on skin-relevant bacterial strains suggests their potential as natural anti-infective agents for topical applications. The antioxidant aptitude of the extracts and their ability to inhibit lipid peroxidation indicate their potential in alleviating skin disorders related to oxidative stress. Additionally, the anti-irritant properties of the water extract, particularly its low skin irritation potential, make it a potential candidate for the advancement of safe and non-irritant topical formulations.

Furthermore, the study adds to the growing body of knowledge on the therapeutic potential of indigenous medicinal plants and emphasizes the importance of further investigating natural sources of bioactive compounds to develop effective and accessible treatments, particularly for vulnerable populations in resource-limited settings.

The implications extend beyond the cosmeceutical industry, as the results may open the door to the creation of new medicinal substances for various skin conditions, addressing the shortage of accessible and affordable dermatological care in many regions of the world.

Overall, the current study demonstrates the significance of integrating traditional medicinal knowledge with modern scientific techniques, fostering collaboration between researchers, healthcare professionals, and indigenous communities. By combining these approaches, the study contributes to the responsible utilization of natural resources, the preservation of cultural heritage, and the development of sustainable and culturally relevant solutions for addressing skin-related concerns.

8.3 Recommendations for future research

While this current study has contributed important insights into the cosmeceutical potential of *S. africana* extracts, further research is recommended to address the following aspects:

- i. Isolate and identify the specific compounds responsible for its antibacterial, anti-inflammatory, antioxidant, anti-irritant, and anti-tyrosinase activities. Techniques such as: HPLC-MS/GC-MS for compound separation and identification. This is critical for quality assurance and standardising the formulation for product development.
- ii. Conduct *in vivo* and clinical research to evaluate the efficacy, safety, and potential adverse effects of *S. africana* stem-bark extracts or their extracted bioactive components in animal models and human subjects, respectively. This should involve isolating bioactive compounds via HPLC and clarify their antimicrobial and anti-tyrosinase activity.
- iii. Formulation development: Explore the incorporation of *S. africana* stem-bark extracts or their bioactive compounds into cosmeceutical formulations, such as creams, lotions, or other topical products, specifically tailored for skincare applications. This may involve investigating factors such as stability, penetration and compatibility with other ingredients.
- iv. Sustainable sourcing and conservation: Implement strategies for the sustainable sourcing and conservation of *S. africana*, as well as explore alternative methods such as cultivation and domestication to ensure the long-term availability of this valuable medicinal plant resource.
- v. Collaboration and knowledge exchange: Encourage collaboration among healthcare professional's researchers, traditional healers, and communities to facilitate knowledge exchange and promote the ethical incorporation of traditional plant-based medicines into mainstream healthcare practices.

REFERENCES

- Abdulkhaleq L., Assi M., Abdullah R., Zamri-Saad M., Taufiq-Yap Y., Hezmee M., 2018.** The crucial roles of inflammatory mediators in inflammation: A review. *Veterinary World* 11, 627.
- Abdullahi A.A., 2011.** Trends and challenges of traditional medicine in Africa. *African Journal of Traditional, Complementary and Alternative Medicines* 8, 115–123.
- Afolayan A.J., Grierson D.S., Mbeng W.O., 2014.** Ethnobotanical survey of medicinal plants used in the management of skin disorders among the *Xhosa* communities of the Amathole District, Eastern Cape, South Africa. *Journal of Ethnopharmacology* 153, 220-232.
- Akhalwaya S., Van Vuuren S., Patel M., 2018.** An *in vitro* investigation of indigenous South African medicinal plants used to treat oral infections. *Journal of Ethnopharmacology* 210, 359-371.
- Al-Khayri J.M., Sahana G.R., Nagella P., Joseph B.V., Alessa F.M., Al-Mssallem M.Q., 2022.** Flavonoids as potential anti-inflammatory molecules: A review. *Molecules* 27, 2901.
- Allemann I.B., Baumann L., 2009.** Botanicals in skin care products. *International Journal of Dermatology* 48, 923-934.
- Alliya A.A., 2019.** Anti-bacterial activity of the crude extract and fractions of *Spirostachys africana* against multi-drug resistant bacteria. MSc thesis, University of Venda.
- Alnuqaydan A.M., 2024.** The dark side of beauty: an in-depth analysis of the health hazards and toxicological impact of synthetic cosmetics and personal care products. *Frontiers in Public Health* 12, 1439027.
- Aly R., 2011.** Microbial infections of skin and nails, in Baron, S. (Eds). *Medical Microbiology*, Vol. 4th Galveston (TX): University of Texas Medical Branch at Galveston.
- Amarowicz R., Pegg R., Rahimi-Moghaddam P., Barl B., Weil J., 2004.** Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry* 84, 551-562.
- Amoo S.O., Aremu A.O., Moyo M., Van Staden J., 2012.** Antioxidant and acetylcholinesterase-inhibitory properties of long-term stored medicinal plants. *BMC Complementary and Alternative Medicine* 12, 1-9.

- Anderson S.E., Meade B.J., 2014.** Potential health effects associated with dermal exposure to occupational chemicals. *Environmental Health Insights* 8, 51-62.
- Arabski M., Węgierek-Ciuk A., Czerwonka G., Lankoff A., Kaca W., 2012.** Effects of saponins against clinical *E. coli* strains and eukaryotic cell line. *BioMed Research International* 2012, 286216.
- Ärnlöv J., Larsson A., 2015.** Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990-2013: A systematic analysis for the Global Burden of Disease Study 2013. *The Lancet* 386, 743-800.
- Arnold T.H., Prentice C., Hawker L., Snyman E., Tomalin M., Crouch N., Pottas-Bircher C., 2002.** Medicinal and magical plants of southern Africa: An annotated checklist. National Botanical Institute.
- Aware C.B., Patil D.N., Suryawanshi S.S., Mali P.R., Rane M.R., Gurav R.G., Jadhav, J.P., 2022.** Natural bioactive products as promising therapeutics: A review of natural product-based drug development. *South African Journal of Botany* 151, 512-528.
- Baikar S., Malpathak N., 2010.** Secondary metabolites as DNA topoisomerase inhibitors: a new era towards designing of anticancer drugs. *Pharmacognosy Reviews* 4, 12.
- Barbieri R., Coppo E., Marchese A., Daglia M., Sobarzo-Sánchez E., Nabavi S.F., Nabavi S.M., 2017.** Phytochemicals for human disease: An update on plant-derived compounds antibacterial activity. *Microbiological Research* 196, 44-68.
- Beach R., Gantsho N., Flesche J., Scott C., Khumalo N., 2010.** Possible drug reaction, eosinophilia and systemic symptoms (DRESS) syndrome in an infant from ingestion of *Spirostachys africana* complicated by measles co-infection. *South African Journal of Child Health* 4, 112-113.
- Behera P.R., Palepu S., Sirka C.S., Ranjan R., Pradhan S., Singh A.K., Behera P.R., Singh A., 2022.** Psychosocial distress and quality of life among patients with a chronic skin disorder at a tertiary care hospital in Eastern India: A hospital-based case-control study. *Cureus* 14.
- Besharati M., Maggolino A., Palangi V., Kaya A., Jabbar M., Eseceli H., De Palo P., Lorenzo J.M., 2022.** Tannin in ruminant nutrition. *Molecules* 27, 8273.

- Bhambhani S., Kondhare K.R., Giri A.P., 2021.** Diversity in chemical structures and biological properties of plant alkaloids. *Molecules* 26, 3374.
- Bhat R., 2013.** Plants of *Xhosa* people in the Transkei region of Eastern Cape (South Africa) with major pharmacological and therapeutic properties. *Journal of Medicinal Plants Research* 7, 1474-1480.
- Bhat R., 2014.** Medicinal plants and traditional practices of *Xhosa* people in the Transkei region of Eastern Cape, South Africa. *Indian Journal of Traditional Knowledge* 13, 292-298.
- Bittner Fialová S., Rendecková K., Mučaji P., Nagy M., Slobodníková L., 2021.** Antibacterial activity of medicinal plants and their constituents in the context of skin and wound infections, considering European legislation and folk medicine. A review. *International Journal of Molecular Sciences* 22, 10746.
- Bowler P., Duerden B., Armstrong D.G., 2001.** Wound microbiology and associated approaches to wound management. *Clinical Microbiology Reviews* 14, 244-269.
- Bredenkamp C.L., 2019.** A Flora of the Eastern Cape Province. *Strelitzia* 41. Pretoria. South African National Biodiversity Institute.
- Brueton V., Yogeswaran P., Chandia J., Mfenyana K., Modell B., Modell M., Nazareth I., 2010.** Primary care morbidity in Eastern Cape province. *South African Medical Journal* 100, 309-312.
- Cavalcante G., da Silva Cabral A., Silva C., 2018.** Leishmanicidal Activity of Flavonoids Natural and Synthetic: A Minireview. *Mintage Journal of Pharmaceutical and Medical Sciences* 7, 25-34.
- Chang T.-S., 2009.** An updated review of tyrosinase inhibitors. *International Journal of Molecular Sciences* 10, 2440-2475.
- Chen Y.F., Yang C.H., Chang M.S., Ciou Y.P., Huang Y.C., 2010.** Foam properties and detergent abilities of the saponins from *Camellia oleifera*. *International Journal of Molecular Sciences* 11, 4417-4425.
- Chintamunnee V., Mahomoodally M.F., 2012.** Herbal medicine commonly used against non-communicable diseases in the tropical island of Mauritius. *Journal of Herbal Medicine* 2, 113-125.
- Christensen R.E., Jafferany M., 2023.** Psychiatric and psychologic aspects of chronic skin diseases. *Clinics in Dermatology* 41, 75-81.

- Coates Palgrave K., 2002.** Trees of southern Africa. Veld & Flora 63, 8.
- Cocks M., Dold A., 2000.** The role of ‘African Chemists’ in the health care system of the Eastern Cape province of South Africa. Social Science & Medicine 51, 1505-1515.
- Comino-Sanz I.M., López-Franco M.D., Castro B., Pancorbo-Hidalgo P.L., 2021.** The role of antioxidants on wound healing: A review of the current evidence. Journal of Clinical Medicine 10, 3558.
- Dai J., Mumper R.J., 2010.** Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules 15, 7313-7352.
- Dalgard F.J., Gielier U., Tomas-Aragones L., Lien L., Poot F., Jemec G.B., Misery L., Szabo C., Linder D., Sampogna F., 2015.** The psychological burden of skin diseases: A cross-sectional multicenter study among dermatological out-patients in 13 European countries. Journal of Investigative Dermatology 135, 984-991.
- Das A.K., Islam M.N., Faruk M.O., Ashaduzzaman M., Dungani R., 2020.** Review on tannins: Extraction processes, applications and possibilities. South African Journal of Botany 135, 58-70.
- De Wet H., Nciki S., van Vuuren S.F., 2013.** Medicinal plants used for the treatment of various skin disorders by a rural community in northern Maputaland, South Africa. Journal of Ethnobiology and Ethnomedicine 9, 1-10.
- Del Giudice P., 2020.** Skin infections caused by *Staphylococcus aureus*. Acta Dermatovenereologica 100.
- Department: Science and Technology, 2008.** Indigenous Knowledge Systems. Available at: https://www.dst.gov.za/images/pdfs/IKS_Policy%20PDF.pdf.
- Desai S.D., Desai D.G., Kaur H., 2009.** Saponins and their biological activities. Pharma Times 41, 13-16.
- Devi N., Kumar A., Garg A., Hussain A., Khathuriya R., 2018.** A review on herbal cosmetics. World Journal of Pharmaceutical Research 7, 298-310.
- Djafarou S., Mermer A., Barut B., Yilmaz G.T., Boulebd H., 2023.** Synthesis and evaluation of the antioxidant and anti-tyrosinase activities of thiazolyl hydrazone derivatives and their application in the anti-browning of fresh-cut potato. Food Chemistry 414, 135745.

- Dlova N.C., Hamed S.H., Tsoka-Gwegweni J., Grobler A., 2015a.** Skin lightening practices: An epidemiological study of South African women of African and Indian ancestries. *British Journal of Dermatology* 173, 2-9.
- Dlova N.C., Mankahla A., Madala N., Grobler A., Tsoka-Gwegweni J., Hift R.J., 2015b.** The spectrum of skin diseases in a black population in Durban, KwaZulu-Natal, South Africa. *International Journal of Dermatology* 54, 279-285.
- Dobrian A.D., Lieb D.C., Cole B.K., Taylor-Fishwick D.A., Chakrabarti S.K., Nadler J.L., 2011.** Functional and pathological roles of the 12-and 15-lipoxygenases. *Progress in Lipid Research* 50, 115-131.
- Dold T., Cocks M., 2005.** Imbhola yesiXhosa: traditional Xhosa cosmetics. *Veld & Flora* 91, 123-125.
- dos Santos Videira I.F., Moura D.F.L., Magina S., 2013.** Mechanisms regulating melanogenesis. *Anais Brasileiros de Dermatologia* 88, 76-83.
- Draelos Z.D., 2008.** The cosmeceutical realm. *Clinics in Dermatology* 26, 627-632.
- Dua V.K., Verma G., Singh B., Rajan A., Bagai U., Agarwal D.D., Gupta N., Kumar S., Rastogi A., 2013.** Anti-malarial property of steroidal alkaloid conessine isolated from the bark of *Holarrhena antidysenterica*. *Malaria Journal* 12, 1-6.
- Ekor M., 2014.** The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in Pharmacology* 4, 177.
- El-Nashar H.A., El-Din M.I.G., Hritcu L., Eldahshan O.A., 2021.** Insights on the inhibitory power of flavonoids on tyrosinase activity: A survey from 2016 to 2021. *Molecules* 26, 7546.
- Eloff J.N., 1998.** A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica* 64, 711-713.
- Fennell C., Light M., Sparg S., Stafford G., Van Staden J., 2004.** Assessing African medicinal plants for efficacy and safety: Agricultural and storage practices. *Journal of Ethnopharmacology* 95, 113-121.
- Fisher F., Africa C., Klaasen J., Fisher R., 2025.** South African medicinal plants traditionally used for wound treatment: An ethnobotanical systematic review. *Plants* 14, 818.
- Flohr C., Hay R., 2021.** Putting the burden of skin diseases on the global map. *British Journal of Dermatology* 184, 189-190.

- García E., Halpert E., Borrero E., Ibañez M., Chaparro P., Molina J., Torres M., 2020.** Prevalence of skin diseases in children 1 to 6 years old in the city of Bogota, Colombia. *World Allergy Organization Journal* 13, 100484.
- George P., 2011.** Concerns regarding the safety and toxicity of medicinal plants. An overview. *Journal of Applied Pharmaceutical Science*, 40-44.
- Górniak I., Bartoszewski R., Króliczewski J., 2019.** Comprehensive review of antimicrobial activities of plant flavonoids. *Phytochemistry Reviews* 18, 241-272.
- Grace O., Prendergast H., Jäger A., Van Staden J., Van Wyk A., 2003.** Bark medicines used in traditional healthcare in KwaZulu-Natal, South Africa: An inventory. *South African Journal of Botany* 69, 301-363.
- Grierson D., Afolayan A., 1999.** Antibacterial activity of some indigenous plants used for the treatment of wounds in the Eastern Cape, South Africa. *Journal of Ethnopharmacology* 66, 103-106.
- Groner V.P., Nicholas O., Mabhaudhi T., Slotow R., Akçakaya H.R., Mace G.M., Pearson R.G., 2022.** Climate change, land cover change, and overharvesting threaten a widely used medicinal plant in South Africa. *Ecological Applications* 32, e2545.
- Gulcin İ., Alwasel S.H., 2023.** DPPH radical scavenging assay. *Processes* 11, 2248.
- Gurunathan S., Han J.W., Kwon D.-N., Kim J.-H., 2014.** Enhanced antibacterial and anti-biofilm activities of silver nanoparticles against Gram-negative and Gram-positive bacteria. *Nanoscale Research Letters* 9, 1-17.
- Hartshorne S., 2003.** Dermatological disorders in Johannesburg, South Africa. *Clinical and Experimental Dermatology* 28, 661-665.
- Hay R., Bendeck S.E., Chen S., Estrada R., Haddix A., McLeod T., Mahé A., 2006.** Skin diseases. *Disease Control Priorities in Developing Countries*. 2nd edition.
- Hekmatpou D., Mehrabi F., Rahzani K., Aminiyan A., 2019.** The effect of aloe vera clinical trials on prevention and healing of skin wound: A systematic review. *Iranian Journal of Medical Sciences* 44, 1-9.
- Hoang H.T., Moon J.-Y., Lee Y.-C., 2021.** Natural antioxidants from plant extracts in skincare cosmetics: Recent applications, challenges and perspectives. *Cosmetics* 8, 106.
- Hutchings A., Scott A.H., 1996.** Zulu medicinal plants: An inventory. University of Natal Press.

- Hwang Y., Lee J., Jung H.J., Ullah S., Ko J., Jeong Y., Park Y.J., Kang M.K., Yun H., Kim M.-S., 2022.** A novel class of potent anti-tyrosinase compounds with antioxidant activity, 2-(substituted phenyl)-5-(trifluoromethyl) benzo [d] thiazoles: *in vitro* and *in silico* insights. *Antioxidants* 11, 1375.
- Ibrahim A.M., El-Karim R.M.G., Ali R.E., Nasr S.M., 2023.** Toxicological effects of Saponin on the free larval stages of *Schistosoma mansoni*, infection rate, some biochemical and molecular parameters of *Biomphalaria alexandrina* snails. *Pesticide Biochemistry and Physiology* 191, 105357.
- Ibrahim N., Abbas H., El-Sayed N.S., Gad H.A., 2022.** *Rosmarinus officinalis* L. hexane extract: Phytochemical analysis, nanoencapsulation, and *in silico*, *in vitro*, and *in vivo* anti-photoaging potential evaluation. *Scientific Reports* 12, 13102.
- Iqbal E., Salim K.A., Lim L.B., 2015.** Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniiothalamus velutinus* (Airy Shaw) from Brunei Darussalam. *Journal of King Saud University-Science* 27, 224-232.
- Jadid N., Rachman R.Y., Hartanti S.R., Abdulgani N., Wikanta W., Muslihatin W., 2016.** Methanol extract of *Piper retrofractum* Vahl: Potentially mediates mast cell stabilization. *International Journal of Pharma and Bio Sciences* 7, 379-383.
- Jankowiak B., Kowalewska B., Krajewska-Kulak E., Khvorik D.F., Niczyporuk W., 2020.** Relationship between self-esteem and stigmatization in psoriasis patients. *Advances in Dermatology and Allergology/Postępy Dermatologii Alergologii* 37, 597-602.
- Jiang Y., Tsoi L.C., Billi A.C., Ward N.L., Harms P.W., Zeng C., Maverakis E., Kahlenberg J.M., Gudjonsson J.E., 2020.** Cytokinocytes: the diverse contribution of keratinocytes to immune responses in skin. *JCI insight* 5.
- Joshi H., 2012.** Potentials of traditional medicinal chemistry in cosmetology industry; prospectives and perspectives. *Anaplastology an Open Access Journal* 1, 43.
- Kadam V.S., Chintale A.G., Deshmukh K.P., Nalwad D.N., 2013.** Cosmeceuticals an emerging concept: A comprehensive review. *International Journal of Research in Pharmacy and Chemistry* 3, 308-316.
- Kannathasan K., Senthilkumar A., Venkatesalu V., 2019.** Crystal structure and antibacterial evaluation of epifriedelinol isolated from *Vitex peduncularis* Wall. ex Schauer. *Arabian Journal of Chemistry* 12, 2289-2292.

- Kanteev M., Goldfeder M., Fishman A., 2015.** Structure-function correlations in tyrosinases. *Protein Science* 24, 1360-1369.
- Karimkhani C., Dellavalle R.P., Coffeng L.E., Flohr C., Hay R.J., Langan S.M., Nsoesie E.O., Ferrari A.J., Erskine H.E., Silverberg J.I., 2017.** Global skin disease morbidity and mortality: An update from the global burden of disease study 2013. *JAMA Dermatology* 153, 406-412.
- Kasote D.M., Katyare S.S., Hegde M.V., Bae H., 2015.** Significance of antioxidant potential of plants and its relevance to therapeutic applications. *International Journal of Biological Sciences* 11, 982.
- Katibi O.S., Dlova N.C., Chateau A.V., Mosam A., 2016.** The prevalence of paediatric skin conditions at a dermatology clinic in KwaZulu-Natal Province over a 3-month period. *South African Journal of Child Health* 10, 121-125.
- Kawser Hossain M., Abdal Dayem A., Han J., Yin Y., Kim K., Kumar Saha S., Yang G.-M., Choi H.Y., Cho S.-G., 2016.** Molecular mechanisms of the anti-obesity and anti-diabetic properties of flavonoids. *International Journal of Molecular Sciences* 17, 569.
- Khan M.I., Ahhmed A., Shin J.H., Baek J.S., Kim M.Y., Kim J.D., 2018.** Green tea seed isolated saponins exerts antibacterial effects against various strains of gram positive and gram negative bacteria, a comprehensive study *in vitro* and *in vivo*. *Evidence-Based Complementary and Alternative Medicine*.
- Kim J., Kim B.E., Leung D.Y., 2019.** Pathophysiology of atopic dermatitis: Clinical implications. In *allergy and asthma proceedings*. Ocean Side Publications 40, 84-92.
- Kozłowska A., Szostak-Wegierek D., 2014.** Flavonoids-food sources and health benefits. *Roczniki Państwowego Zakładu Higieny* 65, 79-85.
- Kregiel D., Berłowska J., Witonska I., Antolak H., Proestos C., Babic M., Babic L., Zhang B., 2017.** Saponin-based, biological-active surfactants from plants. *Application and Characterization of Surfactants* 6, 184-205.
- Kumar S., Korra T., Thakur R., Arutselvan R., Kashyap A.S., Nehela Y., Chaplygin V., Minkina T., Keswani C., 2023.** Role of plant secondary metabolites in defence and transcriptional regulation in response to biotic stress. *Plant Stress* 8, 100154.
- Lachenmeier D.W., 2008.** Safety evaluation of topical applications of ethanol on the skin and inside the oral cavity. *Journal of Occupational Medicine and Toxicology* 3, 1-16.

- Lall N., 2017.** Indigenous knowledge-based cosmeceuticals for natural and green cosmetics. Available at: https://www.up.ac.za/research-matters/news/post_2991402-indigenous-knowledge-based-cosmeceuticals-for-natural-and-green-cosmetics. [Accessed on 08 February 2024].
- Lall N., Kishore N., 2014.** Are plants used for skin care in South Africa fully explored? *Journal of Ethnopharmacology* 153, 61-84.
- Lee T., Friedman A., 2016.** Skin barrier health: Regulation and repair of the stratum corneum and the role of over-the-counter skin care. *Journal of Drugs in Dermatology* 15, 1047-1051.
- Lemmens R., Louppe D., Oteng-Amoako A., 2012.** *Timbers 2. Plant Resource of Tropical Africa.*
- Lennox S.J., Bamford M., 2015.** Use of wood anatomy to identify poisonous plants: Charcoal of *Spirostachys africana*. *South African Journal of Science* 111, 1-9.
- Leoty-Okombi S., Gillaizeau F., Leuillet S., Douillard B., Le Fresne-Languille S., Carton T., De Martino A., Moussou P., Bonnaud-Rosaye C., André V., 2021.** Effect of sodium lauryl sulfate (SLS) applied as a patch on human skin physiology and its microbiota. *Cosmetics* 8, 6.
- Lim T.Y., Lim Y.Y., Yule C.M., 2009.** Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four *Macaranga* species. *Food Chemistry* 114, 594-599.
- Liu H.-M., Cheng M.-Y., Xun M.-H., Zhao Z.-W., Zhang Y., Tang W., Cheng J., Ni J., Wang W., 2023.** Possible mechanisms of oxidative stress-induced skin cellular senescence, inflammation, and cancer and the therapeutic potential of plant polyphenols. *International Journal of Molecular Sciences* 24, 3755.
- Lobo V., Patil A., Phatak A., Chandra N., 2010.** Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacognosy Reviews* 4, 118.
- Lu Y., Knoo T.-J., Wiart C., 2014.** Phytochemical analysis and antioxidant activity determination on crude extracts of *Melodinus eugeniifolus* barks and leaves from Malaysia. *Pharmacology and Pharmacy* 5, 773-780.
- Ma E.Z., Khachemoune A., 2023.** Flavonoids and their therapeutic applications in skin diseases. *Archives of Dermatological Research* 315, 321-331.
- Mabogo D.E.N., 1990.** The ethnobotany of the Vhavenda. MSc Thesis: University of Pretoria.
- Mabona U., Van Vuuren S., 2013.** Southern African medicinal plants used to treat skin diseases. *South African Journal of Botany* 87, 175-193.

- Macáková K., Kolečkář V., Cahlíková L., Chlebek J., Hošťálková A., Kuča K., Jun D., Opletal, L., 2014.** Tannins and their influence on health. In *Recent Advances in Medicinal Chemistry* 1, 159-208. Elsevier Publisher.
- Mahomoodally M.F., 2013.** Traditional medicines in Africa: an appraisal of ten potent African medicinal plants. *Evidence-Based Complementary and Alternative Medicine* 2013, 617459.
- Mancuso G., Midiri A., Gerace E., Biondo C., 2021.** Bacterial antibiotic resistance: The most critical pathogens. *Pathogens* 10, 1310.
- Mandrioli D., Silbergeld E.K., 2016.** Evidence from toxicology: The most essential science for prevention. *Environmental Health Perspectives* 124, 6-11.
- Mapunya M.B., Nikolova R.V., Lall N., 2012.** Melanogenesis and antityrosinase activity of selected South African plants. *Evidence-Based Complementary and Alternative Medicine* 2012, 374017.
- Marrakchi S., Maibach H.I., 2006.** Sodium lauryl sulfate-induced irritation in the human face: Regional and age-related differences. *Skin Pharmacology and Physiology* 19, 177-180.
- Martin K.I., Glaser D.A., 2011.** Cosmeceuticals: The new medicine of beauty. *Missouri Medicine* 108, 60.
- Marutescu L., Popa M., Saviuc C., Lazar V., Chifiriuc M.C., 2017.** Botanical pesticides with virucidal, bactericidal, and fungicidal activity, in (Eds). *New Pesticides and Soil Sensors*, 311-335: Elsevier Publisher.
- Mathabe M., Nikolova R., Lall N., Nyazema N., 2006.** Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa. *Journal of Ethnopharmacology* 105, 286-293.
- Mathabe M.C., Hussein A.A., Nikolova R.V., Basson A.E., Meyer J.M., Lall N., 2008.** Antibacterial activities and cytotoxicity of terpenoids isolated from *Spirostachys africana*. *Journal of Ethnopharmacology* 116, 194-197.
- Maury G., Rodríguez D., Hendrix S., Arranz J., Boix Y., Pacheco A., Díaz J., Morris-Quevedo H., Dubois A., Aleman E., 2020.** Antioxidants in Plants: A valorization potential emphasizing the need for the conservation of plant biodiversity in Cuba. *Antioxidants* (Basel, Switzerland) 9, 1048.

- McGaw L., Jäger A., Van Staden J., 2000.** Antibacterial, anthelmintic and anti-amoebic activity in South African medicinal plants. *Journal of Ethnopharmacology* 72, 247-263.
- McMullen R.L., Dell'Acqua G., 2023.** History of natural ingredients in cosmetics. *Cosmetics* 10, 71.
- Michalak M., 2022.** Plant-derived antioxidants: Significance in skin health and the ageing process. *International Journal of Molecular Sciences* 23, 585.
- Michalak M., 2023.** Plant extracts as skin care and therapeutic agents. *International Journal of Molecular Sciences* 24, 15444.
- Mikail H.G., Mohammed M., Umar H.D., Suleiman M.M., 2022.** Secondary metabolites: The natural remedies. IntechOpen. ISBN: 9781803552095.
- Mohan V., Tresina P., Daffodil E., 2016.** Antinutritional factors in legume seeds: characteristics and determination. *Encyclopedia of Food and Health*, 211-220. Elsevier Publisher.
- Molino S., Francino M.P., Henares J.A.R., 2023.** Why is it important to understand the nature and chemistry of tannins to exploit their potential as nutraceuticals? *Food Research International* 173, 113329.
- Momtaz S., Mapunya B., Houghton P., Edgerly C., Hussein A., Naidoo S., Lall N., 2008.** Tyrosinase inhibition by extracts and constituents of *Sideroxylon inerme* L. stem-bark, used in South Africa for skin lightening. *Journal of Ethnopharmacology* 119, 507-512.
- Mothibe M.E., Sibanda M., 2019.** African traditional medicine: South African perspective. *Traditional and Complementary Medicine* 2019, 1-27.
- Mtunzi F.M., Ejidike I.P., Ledwaba I., Ahmed A., Pakade V.E., Klink M.J., Modise S.J., 2017.** Solvent–solvent fractionations of *Combretum erythrophyllum* (Burch.) leaf extract: Studies of their antibacterial, antifungal, antioxidant and cytotoxicity potentials. *Asian Pacific Journal of Tropical Medicine* 10, 670-679.
- Mudau R.I., Ledwaba M.B., 2023.** *Bulbine angustifolia* Poelln. Available at: <https://pza.sanbi.org/bulbine-angustifolia>. [Accessed on 20 January 2024].
- Mukandiwa L., Naidoo V., Eloff J.N., 2012.** *In vitro* antibacterial activity of seven plants used traditionally to treat wound myiasis in animals in Southern Africa. *Journal of Medicinal Plants Research* 6, 4379-4388.
- Mukherjee A., Sasikala W.D., 2013.** Drug–DNA intercalation: From discovery to the molecular mechanism. *Advances in Protein Chemistry and Structural Biology* 92, 1-62.

- Mulaudzi F., 2001.** Synergy between indigenous knowledge systems, modern health care system and scientific research. A vision for the 21st century. *Health SA Gesondheid : Journal of Interdisciplinary Health Sciences* 6.
- Mulaudzi R.B., Ndhhlala A.R., Kulkarni M.G., Van Staden J., 2012.** Pharmacological properties and protein binding capacity of phenolic extracts of some Venda medicinal plants used against cough and fever. *Journal of Ethnopharmacology* 143, 185-193.
- Mwinga, J.L., Makhaga, N.S., Aremu, A.O., Otang-Mbeng, W., 2019.** Botanicals used for cosmetic purposes by *Xhosa* women in the Eastern Cape, South Africa. *South African Journal of Botany* 126, 4-10.
- Naidoo K., Coopposamy R., 2011.** A comparative analysis of two medicinal plants used to treat common skin conditions in South Africa. *African Journal of Pharmacy and Pharmacology* 5, 393-397.
- Nasri H., Shirzad H., 2013.** Toxicity and safety of medicinal plants. *Journal of Herbmmed Pharmacology* 2, 21-22.
- Nawas Z.Y., Tong Y., Kollipara R., Peranteau A.J., Woc-Colburn L., Yan A.C., Lupi O., Tying S.K., 2016.** Emerging infectious diseases with cutaneous manifestations: Viral and bacterial infections. *Journal of the American Academy of Dermatology* 75, 1-16.
- Ncube B., Finnie J.F., Van Staden J., 2012.** Quality from the field: The impact of environmental factors as quality determinants in medicinal plants. *South African Journal of Botany* 82, 11-20.
- Neeley E., Fritch G., Fuller A., Wolfe J., Wright J., Flurkey W., 2009.** Variations in IC₅₀ values with purity of mushroom tyrosinase. *International Journal of Molecular Sciences* 10, 3811-3823.
- Ndhlovu P. T., Mooki O., Otang Mbeng W., Aremu A.O., 2019.** "Plant species used for cosmetic and cosmeceutical purposes by the Vhavenda women in Vhembe District Municipality, Limpopo, South Africa." *South African Journal of Botany* 122, 422-431.
- Nie Y., Stürzenbaum S.R., 2019.** Proanthocyanidins of natural origin: Molecular mechanisms and implications for lipid disorder and aging-associated diseases. *Advances in Nutrition* 10, 464-478.
- Nobles T., Harberger S., Krishnamurthy K., 2023** Cradle Cap. Publishing; 2024 Jan. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK531463/>. [Accessed on 27 March 2024].

- Novak-Bilić G., Vučić M., Japundžić I., Meštrović-Štefekov J., Stanić-Duktaj S., Lugović-Mihić L., 2018.** Irritant and allergic contact dermatitis—skin lesion characteristics. *Acta Clinica Croatica* 57, 713-719.
- Nwobodo D.C., Ugwu M.C., Anie C., O., Al-Ouqaili M.T.S., Ikem J.C., Chigozie U.V., Saki M., 2022.** Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. *Journal of Clinical Laboratory Analysis* 36, e24655.
- Nyahangare E.T., Mvumi B.M., Mutibvu T., 2015.** Ethnoveterinary plants and practices used for ecto-parasite control in semi-arid smallholder farming areas of Zimbabwe. *Journal of Ethnobiology and Ethnomedicine* 11, 1-16.
- Nyakudya T.T., Tshabalala T., Dangarembizi R., Erlwanger K.H., Ndhlala A.R., 2020.** The potential therapeutic value of medicinal plants in the management of metabolic disorders. *Molecules* 25, 2669.
- Okello D., Lee J., Kang Y., 2020.** Ethnopharmacological potential of *Aspilia africana* for the treatment of inflammatory diseases. *Evidence-Based Complementary and Alternative Medicine* 2020, 8091047.
- Ondua M., Njoya E.M., Abdalla M.A., McGaw L.J., 2019.** Anti-inflammatory and antioxidant properties of leaf extracts of eleven South African medicinal plants used traditionally to treat inflammation. *Journal of Ethnopharmacology* 234, 27-35.
- Oninla O., Oninla S., Otiike-Odibi B., Oripelaye M., Olanrewaju F., Mohammed T., 2019.** African Skin: Different types, needs and diseases. *International Journal of Tropical Disease and Health*, 1-13.
- Oyebode O., Kandala N.-B., Chilton P.J., Lilford R.J., 2016.** Use of traditional medicine in middle-income countries: a WHO-SAGE study. *Health Policy and Planning* 31, 984-991.
- Ozioma E.-O.J., Chinwe O.A.N., 2019.** Herbal medicines in African traditional medicine. *Herbal Medicine* 10, 191-214.
- Paczosa M.K., Mecsas J., 2016.** *Klebsiella pneumoniae*: Going on the offense with a strong defense. *Microbiology and Molecular Biology Reviews* 80, 629-661.
- Palgrave K.C., 1990.** *Trees of Southern Africa*. Cape Town: Struik Publishers.
- Panche A.N., Diwan A.D., Chandra S.R., 2016.** Flavonoids: An overview. *Journal of Nutritional Science* 5, e47.

- Passos F.R.S., Araújo-Filho H.G., Monteiro B.S., Shanmugam S., de Souza Araújo A.A., da Silva Almeida J.R.G., Thangaraj P., Júnior L.J.Q., Quintans J.d.S.S., 2022.** Anti-inflammatory and modulatory effects of steroidal saponins and sapogenins on cytokines: A review of pre-clinical research. *Phytomedicine* 96, 153842.
- Patel C., Tyagi S., Kumar U., Pinkesh P., Chaudhari B., Patel S., Mangukia D., 2013.** Importance of different herbal plants in field of cosmetics: A recent review. *Journal of Drug Delivery and Therapeutics* 1, 19-27.
- Patel J., Parmar A., Patel N., 2012.** Traditional herbal cosmetics used by local women communities in district Banaskantha of north Gujarat, India. *Life Sciences Leaflets* 27, 1-6.
- Perveen S., 2018.** Introductory Chapter: Terpenes and Terpenoids. ISBN: 9781838815295.
- Petersen L.M., Charman A.J., Moll E.J., Collins R.J., Hockings M.T., 2014.** “Bush doctors and wild medicine”: The scale of trade in Cape Town's informal economy of wild-harvested medicine and traditional healing. *Society and Natural Resources* 27, 315-336.
- Piao M., Tu Y., Zhang N., Diao Q., Bi Y., 2023.** Advances in the application of phytogetic extracts as antioxidants and their potential mechanisms in ruminants. *Antioxidants* 12, 879.
- Pillaiyar T., Manickam M., Namasivayam V., 2017.** Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry* 32, 403-425.
- Pillaiyar T., Namasivayam V., Manickam M., Jung S.-H., 2018.** Inhibitors of melanogenesis: an updated review. *Journal of Medicinal Chemistry* 61, 7395-7418.
- Pisoschi A.M., Pop A., 2015.** The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry* 97, 55-74.
- Pullar J.M., Carr A.C., Vissers M., 2017.** The roles of vitamin C in skin health. *Nutrients* 9, 866.
- Purnamawati S., Indrastuti N., Danarti R., Saefudin T., 2017.** The role of moisturizers in addressing various kinds of dermatitis: A review. *Clinical Medicine and Research* 15, 75-87.
- Random Harvest, 2024.** *Spirostachys africana*. Available at: <https://www.randomharvest.co.za/South-African-Indigenous-Plants/Show-Plant/PlantId/782/Plant/Spirostachys-africana>. [Accessed on 19 February 2024].

- Rinnerthaler M., Bischof J., Streubel M.K., Trost A., Richter K., 2015.** Oxidative stress in aging human skin. *Biomolecules* 5, 545-589.
- Salatino A., Salatino M.L.F., Negri G., 2007.** Traditional uses, chemistry and pharmacology of *Croton* species (Euphorbiaceae). *Journal of the Brazilian Chemical Society* 18, 11-33.
- Samie A., Obi C., Bessong P., Namrita L., 2005.** Activity profiles of fourteen selected medicinal plants from Rural Venda communities in South Africa against fifteen clinical bacterial species. *African Journal of Biotechnology* 4, 1443-1451.
- Saxena M., Saxena J., Nema R., Singh D., Gupta A., 2013.** Phytochemistry of medicinal plants. *Journal of Pharmacognosy and Phytochemistry* 1, 168-182.
- Schitteck B., 2011.** The antimicrobial skin barrier in patients with atopic dermatitis. *Pathogenesis and Management of Atopic Dermatitis* 41, 54-67.
- Schroeter H., Boyd C., Spencer J.P., Williams R.J., Cadenas E., Rice-Evans C., 2002.** MAPK signaling in neurodegeneration: influences of flavonoids and of nitric oxide. *Neurobiology of Aging* 23, 861-880.
- Sekizawa J., Yasuhara K., Suyama Y., Yamanaka S., Tobe M., Nishimura M., 1994.** A simple method for screening assessment of skin and eye irritation. *The Journal of Toxicological Sciences* 19, 25-35.
- Shackleton S., Steenkamp C., 2004.** The woodcraft industry in the Mpumlanga-Limpopo Province lowveld. *Indigenous forests and woodlands in South Africa: Policy, People and Practice*. University of KwaZulu-Natal Press, Pietermaritzburg, 399-430.
- Shankar R., Lavekar G., Deb S., Sharma B., 2012.** Traditional healing practice and folk medicines used by Mishing community of North East India. *Journal of Ayurveda and Integrative Medicine* 3, 124.
- Sharma M., Kumar A., 2013.** Ethnobotanical uses of medicinal plants: A review. *Life* 50, 52
- Shenefelt P.D. (Ed)^(Eds) 2011.** *Herbal Treatment for Dermatologic Disorders*. Boca Raton (FL): CRC Press/Taylor & Francis. ISBN: 9780429130946.
- Shivanna K.R., 2022.** Climate change and its impact on biodiversity and human welfare. *Proceedings of the Indian National Science Academy* 88, 160-171.
- Sieniawska E., Baj T., 2017.** Chapter 10 - Tannins. Amsterdam, Netherland: Elsevier Science.
- Silver L.L., 2011.** Challenges of antibacterial discovery. *Clinical Microbiology Reviews* 24, 71-109.

- Simpson M.G., 2010.** 8 - Diversity and Classification of Flowering Plants: Eudicots, in (Eds). Plant Systematics (Second Edition), 275-448. Springer.
- Singh K., Baijnath H., Street R., 2020.** *Spirostachys africana*: A review of phytochemistry, traditional and biological uses and toxicity. Indilinga African Journal of Indigenous Knowledge Systems 19, 176-188.
- Siraeva Z., Đặng T., Yergeshov A., Salikhova T., Lopukhov L., Abdullin T., 2016.** Evaluation of skin irritation in rats using simultaneous laser Doppler flowmetry and oxygenation monitoring. BioNanoScience 6, 384-387.
- Slominski A., Tobin D.J., Shibahara S., Wortsman J., 2004.** Melanin pigmentation in mammalian skin and its hormonal regulation. Physiological Reviews 84, 1155-1228.
- Smith C.A., 1996.** Common names of South African plants. South African National Botanical Institute.
- Sofowora A., Ogunbodede E., Onayade A., 2013.** The role and place of medicinal plants in the strategies for disease prevention. African Journal of Traditional, Complementary and Alternative Medicines 10, 210-229.
- Sohail R., Mathew M., Patel K.K., Reddy S.A., Haider Z., Naria M., Habib A., Abdin Z.U., Chaudhry W.R., Akbar A., Patel K.K., 2023.** Effects of non-steroidal anti-inflammatory drugs (NSAIDs) and gastroprotective NSAIDs on the gastrointestinal tract: A narrative review. Cureus 15, e37080.
- Souto A.L., Tavares J.F., Da Silva M.S., Diniz M.d.F.F.M., de Athayde-Filho P.F., Filho J.M.B., 2011.** Anti-inflammatory activity of alkaloids: an update from 2000 to 2010. Molecules 16, 8515-8534.
- Sparg S., Light M., Van Staden J., 2004.** Biological activities and distribution of plant saponins. Journal of Ethnopharmacology 94, 219-243.
- Street R.A., Prinsloo G., 2013.** Commercially important medicinal plants of South Africa: A review. Journal of Chemistry 1, 205048.
- Suharni S., Indarto D., Cilmiaty R., 2021.** Comparison of ethanol and methanol extracts of bay leaves (*Syzygium polyanthum*) in terms of vitamin c, iron and phytochemical levels. In Proceedings of the 1st International Seminar on Teacher Training and Education, ISTED 2021, 17-18 July 2021, Purwokerto, Indonesia.

- Sun L.-c., LI S.-y., Wang F.-z., Xin F.-j., 2017.** Research progresses in the synthetic biology of terpenoids. *Biotechnology Bulletin* 33, 64.
- Suroowan S., Mahomoodally F., 2013.** Complementary and alternative medicine use among Mauritian women. *Complementary Therapies in Clinical Practice* 19, 36-43.
- Tamilselvan N., Thirumalai T., Shyamala P., David E., 2014.** A review on some poisonous plants and their medicinal values. *Journal of Acute Disease* 3, 85-89.
- Thawabteh A., Juma S., Bader M., Karaman D., Scrano L., Bufo S.A., Karaman R., 2019.** The biological activity of natural alkaloids against herbivores, cancerous cells and pathogens. *Toxins* 11, 656.
- Thawabteh A.M., Jibreen A., Karaman D., Thawabteh A., Karaman R., 2023.** Skin pigmentation types, causes and treatment. A review. *Molecules* 28, 4839.
- Thibane V., Ndhlala A., Finnie J., Van Staden J., 2019.** Cosmeceutical efficiency by some plant extracts used traditionally for skin care in inhibiting tyrosinase activity in a human epidermal melanocyte (HEM) cell line. *South African Journal of Botany* 126, 256-260.
- Thirumaran R., Prendergast G.C., Gilman P.B., 2007.** Cytotoxic chemotherapy in clinical treatment of cancer, in (Eds). *Cancer Immunotherapy*, 101-116: Elsevier Publisher.
- Thothela S.N.S., Kola E., Dalu M.T., Ndhlovu P.T., 2025.** Indigenous knowledge and utilisation of *Strychnos spinosa* Lam. in Sub-Saharan Africa: A systematic review of its medicinal, nutritional, and cultural significance. *Diversity* 17, 228.
- Tief K., Hahne M., Schmidt A., Beermann F., 1996.** Tyrosinase, the key enzyme in melanin synthesis, is expressed in murine brain. *European Journal of Biochemistry* 241, 12-16.
- Timilsena Y.P., Phosanam A., Stockmann R., 2023.** Perspectives on saponins: Food functionality and applications. *International Journal of Molecular Sciences* 24, 13538.
- Tiwari R., Amien A., Visser W.I., Chikte U., 2022.** Counting dermatologists in South Africa: number, distribution and requirement. *The British Journal of Dermatology* 187, 248.
- Törmä H., Lindberg M., Berne B., 2008.** Skin barrier disruption by sodium lauryl sulfate-exposure alters the expressions of involucrin, transglutaminase 1, profilaggrin, and kallikreins during the repair phase in human skin *in vivo*. *Journal of Investigative Dermatology* 128, 1212-1219.
- TreeSA, 2024.** *Spirostachys africana*. Available at: <https://treesa.org/spirostachys-africana/>. [Accessed on 18 March 2024].

- Tshabalala T., Mutanga O., Abdel-Rahman E., 2022.** Predicting the geographical distribution shift of medicinal plants in South Africa due to climate change. *Conservation* 2, 694–708.
- Twilley D., Rademan S., Lall N., 2020.** A review on traditionally used South African medicinal plants, their secondary metabolites and their potential development into anticancer agents. *Journal of Ethnopharmacology* 261, 113101.
- Tyavambiza C., Dube P., Goboza M., Meyer S., Madiehe A.M., Meyer M., 2021.** Wound healing activities and potential of selected african medicinal plants and their synthesized biogenic nanoparticles. *Plants* 10, 2635.
- Ullah A., Munir S., Badshah S.L., Khan N., Ghani L., Poulson B.G., Emwas A.-H., Jaremko M., 2020.** Important flavonoids and their role as a therapeutic agent. *Molecules* 25, 5243.
- van Cooten M.H., Bilal S.M., Gebremedhin S., Spigt M., 2019.** The association between acute malnutrition and water, sanitation, and hygiene among children aged 6–59 months in rural Ethiopia. *Maternal & Child Nutrition* 15, e12631.
- Van Wyk A., Prinsloo G., 2018.** Medicinal plant harvesting, sustainability and cultivation in South Africa. *Biological Conservation* 227, 335-342.
- Van Wyk B.-E., Gericke N., 2000.** People's plants: A guide to useful plants of Southern Africa. Briza publications.
- Van Wyk B., 2013.** Field guide to trees of southern Africa. Penguin Random House South Africa.
- Vaou N., Stavropoulou E., Voidarou C., Tsigalou C., Bezirtzoglou E., 2021.** Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. *Microorganisms* 9, 2041.
- Vashi N.A., Patzelt N., Wirya S., Maymone M.B.C., Kundu R.V., 2018.** Dermatoses caused by cultural practices: Cosmetic cultural practices. *Journal of American Academy of Dermatology* 79, 19-30.
- Verschaeve L., Kestens V., Taylor J., Elgorashi E., Maes A., Van Puyvelde L., De Kimpe N., Van Staden J., 2004.** Investigation of the antimutagenic effects of selected South African medicinal plant extracts. *Toxicology in vitro* 18, 29-35.
- Vigbedor B.Y., Osei-Owusu J., Kwakye R., Neglo D., 2022.** Bioassay-Guided Fractionation, ESI-MS Scan, Phytochemical Screening, and Antiplasmodial Activity of *Afzelia africana*. *Biochemistry Research International* 2022, 6895560.

- Vincken J.-P., Heng L., de Groot A., Gruppen H., 2007.** Saponins, classification and occurrence in the plant kingdom. *Phytochemistry* 68, 275-297.
- Vuolo M.M., Lima V.S., Junior M.R.M., 2019.** Phenolic compounds: Structure, classification, and antioxidant power, in (Eds). *Bioactive Compounds*, 33-50: Elsevier Publisher.
- Wang W., Gao Y., Wang W., Zhang J., Yin J., Le T., Xue J., Engelhardt U.H., Jiang H., 2022.** Kojic acid showed consistent inhibitory activity on tyrosinase from mushroom and in cultured B16F10 cells compared with arbutins. *Antioxidants* 11, 502.
- Wang W., Yang L., Wang W., Zhang J., Engelhardt U.H., Jiang H., 2023.** Inhibitory activities of samples on tyrosinases were affected by enzyme species and sample addition methods. *International Journal of Molecular Sciences* 24, 6013.
- Wenk C., Itin P.H., 2003.** Epidemiology of pediatric dermatology and allergology in the region of Aargau, Switzerland. *Pediatric Dermatology* 20, 482-487.
- Widyawati P.S., Budianta T.D.W., Kusuma F.A., Wijaya E.L., 2014.** Difference of solvent polarity to phytochemical content and antioxidant activity of *Pluchea indica* leaves extracts. *International Journal of Pharmacognosy and Phytochemical Research* 6, 850-855.
- Williams V., Victor J., Crouch N., 2013.** Red listed medicinal plants of South Africa: status, trends, and assessment challenges. *South African Journal of Botany* 86, 23-35.
- Wink M., 2016.** Alkaloids: properties and determination. In *Encyclopedia of Food and Health* (Eds) Caballero, B., Finglas, P. M., Toldrá, F. Academic Press.
- Wink M., Van Wyk B.-E., 2008.** Mind-altering and poisonous plants of the world.
- Xaba N.N., Nkomo S.L., Harrypersad K., 2022.** Whose Knowledge? Examining the Relationship between the Traditional Medicine Sector and Environmental Conservation Using a Stakeholder Analysis: Perceptions on Warwick Herb Market Durban South Africa. *International Journal of Environmental Research and Public Health* 19, 11900.
- Yang W., Chen X., Li Y., Guo S., Wang Z., Yu X., 2020.** Advances in pharmacological activities of terpenoids. *Natural Product Communications* 15, 1-13.
- Zandavar H., Babazad M.A., 2023.** Secondary metabolites: Alkaloids and flavonoids in medicinal plants. In *Herbs and Spices-New Advances*. ISBN: 9781837684762.

Zolghadri S., Bahrami A., Hassan Khan M.T., Munoz-Munoz J., Garcia-Molina F., Garcia-Canovas F., Saboury A.A., 2019. A comprehensive review on tyrosinase inhibitors. *Journal of Enzyme Inhibition and Medicinal Chemistry* 34, 279-309.