The phenomenon of skin lightening among young adults, and the effect of selected plant extracts on tyrosinase and melanogenesis activity

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Keywords

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Tyrosinase

Abstract

The phenomenon of skin lightening among young adults, and the effect of selected plant extracts on tyrosinase and melanogenesis activity

Skin lightening practice refers to the use of skin lightening products to achieve a fairer skin complexion. This is common practice among people of several Asian, North American, South American countries. On the African continent, the practice is popular in countries such as Nigeria, Togo, Senegal, Mali and South Africa. It has been reported that skin lightening products contain dangerous chemicals such as hydroquinone, mercury and steroids. The chronic use of these chemicals can lead to severe adverse effects such as skin irritations, skin infections and skin damage. Despite the health risks associated with this practice, demand and prevalence is on the rise as the global skin lightening industry is estimated to be worth USD 24 billion by 2027 and, thus, arises the need to identify less toxic alternatives.

Africa`s forests are known as the world`s second largest tropical reservoir and holds significant biodiversity resources in which only 10.8% of its flora is known to be applied in traditional medicine. As the use of skin lightening agents are accompanied with the emergence of several complications, several studies have shown the use of natural plant extracts as a potential alternative with limited side effects to current synthesized skin lightening products. Plants are well known as an alternate treatment option for various ailments and the diversity of plants used in traditional medicine globally has been estimated between approximately 10 000 to 53 000 species. Considering the growing demand of skin lighteners which lead to an increase in the emergence of side effects and the alternative prospects presented by plant extracts, the overall aim of this study is to investigate the perceptions, knowledge and practice of skin lightening among young adults at

a university within the Western Cape, South Africa. In addition, the study aims to evaluate the potential effect of plant extracts on melanin and enzyme tyrosinase activity on skin cells. The objectives were as follows: 1) to conduct a cross-sectional survey among male and female young adults aged 18 to 30 to explore their perceptions on skin lightening, 2) to conduct a comprehensive literature search on *in vitro* and *in vivo* studies to identify plant extracts capable of inhibiting melanogenesis and enzyme tyrosinase activity and 3) to assess the *in vitro* bio-activity of selected plant extracts on melanocytes.

Research conducted to investigate influences and perceptions of skin lightening practice revealed a 10.6% prevalence of skin lightening product use among the study population. Furthermore, analysis to understand associations disclosed that engaging with social media platforms such as Instagram and Facebook and the use of skin lightening products by family members were strongly associated with the use of skin lightening by participants. To explore plant extracts which have melanogenesis and tyrosinase inhibitory capabilities that could potentially act as an alternate treatment strategy to current toxic skin lightening chemicals, an in-depth literature search conducted identified 35 plant species distributed across 15 African countries. Of these 35 plants, *Salvia officinalis* and *Harpephyllum caffrum* were selected for further study to investigate their individual and combined effects in exhibiting skin lightening effects on B16 melanoma cells. *S. officinalis* was most effective in reducing the melanin content of the cells in a time and dose dependent manner, while *H. caffrum* increased the melanin content of the cells showing significance at the highest concentrations. In comparison to the negative control, the combined extract showed negligible inhibitory effects on melanin content across most concentrations across all exposure times. However, at 800 µg/mL across all exposure times the extract had a stimulatory effect after 24-hour exposure leading to a significant decrease after longer exposure at 48- and 72-

hours. Most notably, all extracts showed more effective inhibitory responses towards TYR activity than melanin content. This suggest that their mechanism of efficacy could be TYR-specific and none of the extracts were toxic to the cells at the concentrations tested. These results highlight the promising skin lightening potential presented by available extracts used individually and in combination to be used as alternative skin lightening agents.

Keywords: skin lightening, melanin, tyrosinase, *Salvia officinalis*, *Harpephyllum caffrum*, combined extract

Declaration

I declare that "*The phenomenon of skin lightening among young adults, and the effect of selected plant extracts on tyrosinase and melanogenesis activity*" is my own work, that it has not been submitted for any degree or examination in any other university, and that all the sources that I have used or quoted have been indicated and acknowledged by complete references.

Laurentia Joan Opperman……15 November 2023

Acknowledgements

Isaiah 58:11 NKJV

‘The Lord will guide you continually,

And satisfy your soul in drought,

And strengthen your bones;

You shall be like a watered garden,

And like a spring of water, whose waters do not fail.’

Psalm 121: 1 – 4 NKJV

‘I will lift up my eyes to the hills—

From whence comes my help?

My help *comes* from the Lord,

Who made heaven and earth.

He will not allow your foot to be moved;

He who keeps you will not slumber.

Behold, He who keeps Israel

Shall neither slumber nor sleep’

I dedicate this thesis to my father, Johannes Opperman, and brother, Warren Opperman. I hope I have made you proud. And to my mother, Loretta Dulcé Opperman, whom we lost to cancer 25 years ago, I wish I could share this with you.

Thank you Jesus for your mercy, grace and strength through this journey. I am because of you.

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List of Abbreviations

µg/mL	microgram per millilitre
DCT	Dopachrome tautomerase (DCT)
EMU	Epidermal-melanin unit
H&E	Haematoxylin and Eosin
Hg	Mercury
HQ	Hydroquinone
KA	Kojic Acid
L-DOPA	3,4-dihydroxy-l-phenylalanine
mg/mL	milligrams per millilitre
NC	Negative control
PC	Positive control
ROS	Reactive oxygen species
SA	South Africa
SL	Skin lightening
SLP	Skin lightening product
TC	Topical steroids
TRP-1	Tyrosinase-related protein-1

TYR	Tyrosinase
U/mL	Units per millilitre
UV	Ultraviolet
v/v	volume per volume
w/v	weight per volume
WC	Western Cape

Chapter 1

Introduction

1.1 Background of the Study

Skin lightening (SL) refers to the cosmetic practice of applying agents to depigment the skin (Davids et al., 2016). This practice is common among both sexes within certain countries (Africa, Asia, North and South America) where various depigmenting agents are used (Lartey et al., 2017). The desire for fairer skin has become a widespread trend despite its adverse health effects (Ho et al., 2017). According to Benn et al., 2016; Blay, 2011; Jacobs et al., 2016, this practice is driven by a complex range of historical, cultural, sociopolitical and psychological motivations. This was evident in South Africa (SA) during the apartheid regime when skin lightening products (SLPs) were a highly lucrative market and were one of the most common household products after soap, tea and tinned or powdered milk (Dlova et al., 2015a; Thomas, 2012).

SLPs inhibit the synthesis of melanin and alter the chemical structure of the skin. This group of compounds can be categorized by their ability to interfere with melanin synthesis or transport, with the most common target being the inhibition of the tyrosinase (TYR) enzyme (Gillbro and Olsson, 2011; Olumide, 2010). Commonly used SLPs contain chemicals that are toxic and dangerous with mercury-containing compounds, hydroquinone, corticosteroids and retinoids being principle sources of skin damage (Jennifer et al., 2012; Davids et al., 2016).

In light of increasing side effects associated with commercially available chemical-derived lightening agents, several studies have been conducted to find natural agents that reduce melanogenesis with minimal adverse effects (Chang, 2009; Mapunya et al., 2012; Stapelberg et

al., 2019). Botanicals and various naturally occurring ingredients have been traditionally used as topical treatment agents for many generations as they are a rich source of bioactive chemicals and are potentially safe (Leyden et al., 2011; Jennifer et al., 2012). Africa possesses a unique variety of geographic and climatic factors with several of these plants being traditionally used for the treatment of different skin ailments (Máthé et al., 2017; Mapunya et al., 2012). Several African plants such as the South African *Greyia flanaganii* and the Algerian *Garcinia kola* have shown to possess anti-pigmentation capabilities. Although research conducted on such plants provide information pertaining the potential of natural extracts as SLPs, data regarding African natural SL alternatives are still limited (Mapunya et al., 2011; Okunji et al., 2007a).

1.2 Statement of the Problem

The estimated prevalence of SL practice among the African population is 25 - 96%, indicating that this practice is quite popular on the continent (Peltzer et al., 2016). More specifically, a study conducted by Dlova et al., 2015a reported a 32.3% use of SLPs among a study population of 600 women in Durban, with Keakile, 2016 indicating that the practice is also present among South African young adults. The adverse effects associated with skin lightener use such as mercury, corticosteroids and hydroquinone is not uncommon (Etnawati et al., 2019). Regardless of its ban in many countries and associated side effects, mercury-containing products and other SLPs remain readily available from various sources including markets and non-pharmaceutical shop (Olumide, 2010; Robinson, 2011; Uram et al., 2010; Gbetoh and Amyot, 2016). According to the World Health Organization, 2019, further restrictions are needed to protect the public from the illegal sale and its negative consequences such as conducting public health awareness, increasing awareness among health professionals and improving legislation. Thus, it is important to establish the reasons behind SL practices and understand the public`s knowledge pertaining to the negative and positive

aspects of this practice. Furthermore, with the growing demand for SL formulations it is necessary to identify the alternative options available (Kim et al., 2015).

1.3 Motivation of the Study

Epidemiological studies on this topic of research is very limited in SA and even more so in the Western Cape (WC) Province. Thus, this study serves to describe and understand the perceptions and practices influencing this growing phenomenon among young adults. This information can provide important preliminary data that would inform future comparative studies across SA. Despite the serious and life-threatening complications associated with the chronic use of these products, the use of skin lighteners is still a widespread and common practice in several African countries (Dlova et al., 2012; Olumide, 2010). By identifying indigenous African plant extracts that is available in published and unpublished literature and testing these using an *in vitro* skin model, we are able to suggest natural alternatives to current chemical-derived SL formulations.

This study aimed to identify the factors influencing SL among young adults and, identify African indigenous plant extracts which have melanin and enzyme tyrosinase inhibitory capabilities.

1.4 Objectives of the study

This study comprises of 3 objectives:

1. Explore the perceptions, knowledge and practice towards SL at a tertiary institution within the WC, SA with the use of a cross-sectional survey.
2. Review and identify indigenous African plant extracts which have potential SL properties.
3. Assess the *in vitro* TYR and melanin inhibitory activity of selected plant extracts identified in the review (objective 2).

1.5 Research Questions

The following research questions guided this study:

1. Which factors influence the practice and knowledge of SL among young adults of the WC?
2. To what extent are these young adults aware of the negative consequences associated with the use of skin lighteners?
3. Which indigenous African plant extracts have been identified as potential inhibitors of enzyme tyrosinase and melanogenesis?
4. Which indigenous African plant extracts exhibits the potential to be used as an alternative to current chemical skin lighteners?

Chapter 2

Review of Literature

2.1 Structure and Function of the Skin

The human skin is the largest organ of the body and accounts for 15% of an adult`s total body weight. Skin consists of a stratified, cellular epidermis and an underlying dermis of connective tissue (Kolarsick et al., 2011; McGrath et al., 2004). The skin performs various functions including protection against mechanical and chemical damage, as well as protection against microbial factors which may alter the physiological status of the body (Costin and Hearing, 2007). The skin comprises of three distinct layers, namely; (1) the epidermis, which is the most superficial layer, void of blood vessels; (2) the dermis, which provides structural strength, flexibility and contains the blood supply to epidermis and (3) the hypodermis containing subcutaneous fat cells providing thermal insulation (Casey, 2002; D'Orazio et al., 2013; Kolarsick et al., 2011; Kusuma et al., 2010).

The epidermis serves as the point of contact between the body and the environment and is the primary defense against factors such as viral, bacterial and fungal infections (D'Orazio et al., 2013; Kusuma et al., 2010). The epidermis is composed of various cell populations, with keratinocytes and melanocytes residing in the basal layer and are the main constituents which form the epidermal-melanin unit (EMU). In the EMU, a single melanocyte is surrounded by approximately 36 keratinocytes which function in producing crucial structural components of the skin`s barrier. The melanocytes produce melanin and its molecular structure functions in protecting the skin against the harmful effects of ultraviolet (UV) radiation by absorbing UV light (Costin and Hearing, 2007; Davids and Kleemann, 2011; Eckert and Rorke, 1989; Campos et al., 2013; D'Mello et al., 2016).

Melanin is a dark polymer of oxidized tyrosine which is present in humans, plants, fungi and is the determinant of skin colour in humans (Chen et al., 2015; Jawaid et al., 2009; Mapunya et al., 2012). Melanogenesis is the process of melanin biosynthesis, a combination of enzymatic and chemical reactions which eventually leads to the formation of melanin. This process takes place within membrane-bound granules found within a dendritic melanocyte called melanosomes (Hwang and Lee, 2007; Stapelberg et al., 2019). It is initiated upon exposure to UV radiation and the melanin produced can be of two basic types; eumelanin (dark, brown or black) or pheomelanin (light, red or yellow) (Mapunya et al., 2012; Parvez et al., 2006). Melanosomes are then distributed to the surrounding keratinocytes via the melanocyte dendrites and functions to protect the skin against UV radiation by absorption and prevents free radical generation protecting the skin from sun damage (Hwang and Lee, 2007; Kindred and Halder, 2010). This protective effect is executed by melanin`s optical and chemical filtering properties, including its ability to remove reactive oxygen species (ROS) which in excess, could directly lead to oxidative stress within the cells (Ndiaye et al., 2014; Slominski et al., 2004).

Contrary to popular opinion, skin colour is not determined by the number of melanocytes which is approximately the same in all races. However, it is determined by the activity of the melanocytes and the number, size and distribution of the melanosomes (de Mendonça et al., 2013). In terms of pigmentation in light- and dark-skinned individuals, the major structural differences are in the size and groupings of the melanosomes. In light-skinned individuals, melanosomes are smaller and grouped in clumps, while in dark-skinned individuals, the melanosomes are large, single organelles (Mapunya et al., 2012). It has been reported that dark-skinned individuals have a higher total melanin content and higher amount of eumelanin in comparison to light-skinned individuals (Sharma et al., 2013). Furthermore, studies performed on cultured human melanocytes showed

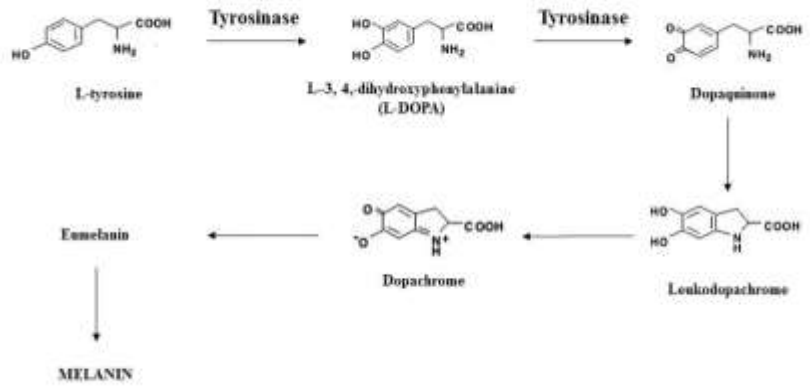
that dark-skin derived melanocytes have a higher ratio of eumelanin to pheomelanin in comparison to the melanocytes derived from light-skin (Sharma et al., 2013; Wakamatsu et al., 2006).

2.2 Tyrosinase (TYR) and Melanogenesis

There are a number of specific enzymatic and structural proteins necessary for a melanosome to become fully developed and equipped enough to produce melanin (D'Mello et al., 2016). These enzymes are translated in the cytoplasm of the melanocyte and transported to the melanosomes (Davids & Kleemann., 2013). Three enzymes are critical for melanogenesis; (1) tyrosinase (TYR), (2) tyrosinase-related protein-1 (TRP-1) and (3) dopachrome tautomerase (DCT) (Campos et al., 2013). While TYR, a copper-containing and rate-limiting enzyme, is responsible for the critical steps of melanogenesis, the latter enzymes (TRP-1 and DCT) are involved in the further modification of melanin into different types (Costin and Hearing, 2007; Jawaid et al., 2009). Mutations in the above-mentioned enzymes dramatically affect the quantity and quality of the melanin produced. This is evident in albinism in which mutations disrupt the function of TYR resulting in this inherited hypopigmentary disorder (Yamaguchi et al., 2007).

When the skin is exposed to UV radiation, melanogenesis is enhanced by the activation of TYR (Gillbro and Olsson, 2011). Melanogenesis is divided into 2 distinct phases. Firstly, the oxidation of the amino acid L-tyrosine which produces the orthoquinone, 3,4-dihydroxy-l-phenylalanine (L-DOPA) and secondly, a polymerisation process leading to the formation of pigment (Jawaid et al., 2009). TYR catalyzes two oxidation steps in melanogenesis. (1) The hydroxylation of L-tyrosine to 3,4-dihydroxy-l-phenylalanine (L-DOPA) and (2) the oxidation of L-DOPA to dopaquinone (John et al., 2005). Furthermore, TYR catalyzes the spontaneous polymerization of DOPAquinone to leukoDOPAchrome and subsequently to DOPAchrome, which undergoes a biochemical chain-reaction ending with the formation of melanin (Jawaid et al., 2009; Slominski and Paus, 1993).

After the formation of the DOPAchrome, the melanin pathway divides into the synthesis of the different types of melanin, eumelanin and pheomelanin, executed by the spontaneous conversion of leukoDOPAchrome and DOPAchrome (Gillbro and Olsson, 2011).



Scheme 1. Illustration of the melanogenesis pathway (Opperman et al., 2020)

2.3 Hyperpigmentation

Hyperpigmentation is represented by an increase in the deposition of melanin within the skin and is caused by a range of physiological phenomena to genetic disorders with UV exposure being its main influence (Bastonini et al., 2016; Speeckaert et al., 2014). Hyperpigmentation is a common dermatological condition found in all skin types. However, it is most commonly found in dark-skinned individuals and requires long-term management (Rigopoulos et al., 2007; Woolery-Lloyd and Kammer, 2011).

A few disorders of hyperpigmentation include; melasma (brown pigmented patches on the face), solar lentigines (also known as age spots - appear as brown patches on skin) and ephelides (also known as freckles – clusters of concentrated melanin) (Stapelberg et al., 2019; Mapunya and Lall,

2011). Damage to the skin such as inflammation or any other injury could almost immediately lead to changes in pigment leading to hyperpigmentation (Woolery-Lloyd and Kammer, 2011). Other forms of the abnormal accumulation of pigment include post-inflammatory hyperpigmentation which is an acquired hypermelanosis which occurs after acne, eczema, contact dermatitis, physical trauma, thermal burns, etc (Campos et al., 2013; Draelos, 2007; Woolery-Lloyd and Kammer, 2011). Hyperpigmentation of the skin is the most common complaint from patients consulting with dermatologists. Regardless of the nature of the problem, patients normally seek means to restore their normal skin colour in which skin lighteners can be prescribed as means of a hyperpigmentory treatment (Dadzie, 2010; Ortonne and Bissett, 2008; Pandya and Guevara, 2000).

2.4 Phenomenon of Skin lightening

SL is defined as the cosmetic practice of applying products to achieve a fairer skin tone and, according to Dlova et al., 2014, SLPs are medically indicated as treatment for hyperpigmentation and are being misused for self-medication purposes in the desire of a fairer skin tone (Masum et al., 2019). This aesthetic practice is especially prevalent worldwide and represents a significant global health problem (Sagoe et al., 2019). It appears that individuals are not deterred from practicing SL despite the associated adverse complications and, it has become a popular means of potentially harmful body modification practice (Julien, 2014). As such, several African governments (Nigerian, South African, Kenyan, Zimbabwean) have placed a prohibition on the sale and import of mercury- and hydroquinone-containing SLPs (Lewis et al., 2011). Despite this, an estimated prevalence of 25 to 96% in Africa indicates that the use of SLPs are quite popular on the continent with existing literature documenting use in many African countries including Cameroon, Kenya, Togo and Senegal. Furthermore, 77% of Nigerian woman, 51.3% of a Ghanaian

study population, and 32.3 % of a study population in Durban, SA have used SLPs (Agyemang-Duah et al., 2019; Ahmed and Hamid, 2016; Dlova et al., 2015a; Ekesiobi and Ude, 2015; Peltzer et al., 2016).

According to Benn et al., 2016; Blay, 2011 and Jacobs et al., 2016, this practice is driven by a complex range of historical, cultural, sociopolitical and psychological motivations. Jacobs et al., 2016 and Olumide, 2010 reported that racial prejudice, together with the global media industry, fueled the notion that an individual with fair skin are seen as more attractive. During the 1960s and 70s, at the peak of South African apartheid law, SLs were a highly lucrative market (Dlova et al., 2015a). During this time, 60% of urban African women reported using SLPs, which made it the 4th most common household product used after soap, tea and tinned or powdered milk. Some consumers would even make their own formulations with a combination of SL and household products, with these dangerous combinations leading to serious side effects (Jacobs et al., 2016; Thomas, 2012).

Benn et al., 2016 reported that SL use is becoming more common among young adults, including teenagers. This is supported by several studies such as that conducted by Peltzer et al., 2016 among undergraduate students at 27 universities in 26 countries across Asia, Africa and the Americas. Results of this study revealed that SL practice is prevalent among young adults at universities in 25 of the 26 countries. This study reported that the highest prevalence were recorded in Thailand (84%), Tunisia (41%), Nigeria (34%), Bangladesh (29%) and Colombia (28%). SL use has been reported to be common practice among women in sub-Saharan Africa with a prevalence ranging between 25% to 77% (Mahe et al., 1993; Teclessou et al., 2018). More recent studies have also investigated SL prevalence among adolescents and young adults of other sub-Saharan African countries including Ghana which reported 66% prevalence among high school students, 12%

among Cameroonian adolescents and 44% among Lesotho women aged 15 – 64 (Geczik et al., 2023; Motlohi et al., 2023; Nkoro et al., 2023).

Various factors are associated with SL prevalence such as age, level of income, relationship status, body image and skin colour dissatisfaction (Raj et al., 2022; Wone et al., 2022). January et al., 2018 reported that 93% of the study population used SL products because they perceived light skin as a symbol of beauty. In addition, 65% thought light skin was a sign of wealth and 23% agreed that light skin symbolized power. Participants enrolled in a study conducted by Atadokpédé et al., 2015 stated that initial motivations of the practice were aesthetic (78%), but continued with the practice to achieve a fairer skin (76%). In KwaZulu Natal of South Africa, Keakile, 2016 conducted a cross sectional study among 147 university students. In this study, participants showed that the factors that influence SL practice included being popular among their peers (41%), to look more trendy and fashionable (39%) and to attain a high social class or rank (37%). Furthermore, 29% and 18% of the participants agreed that factors that influence SL are to acquire a marriage partner and to achieve success in their careers (Keakile, 2016). Additional influences also included familial use in which Osei et al., 2018 showed that 72% of participants stated that they have been approached by family members and friends to make use of SL creams and 66% revealed that they do use it. Although several studies have been conducted to explore reasons for SL practice, additional investigation to further understand these influences can be advantageous. This data could provide valuable insight for the development of targeted educational interventions that could lead to changes in perceptions of beauty and skin colour (Tesfamariam et al., 2023).

2.5 Skin lighteners

Cosmeceuticals are topical cosmetic-pharmaceutical products meant to enhance the user`s beauty and not elicit any negative effects on the structure or function of the skin (Sarkar et al., 2013).

However, SLs inhibit the synthesis of melanin and, in this way alter the chemical structure of the skin (Olumide, 2010). These agents can cause damage by affecting the epidermis which is designed to protect against any injury, thus, damage to the epidermis makes the user susceptible to bacterial, fungal and viral infections (Peltzer et al., 2016). Since TYR plays a significant role within the melanogenesis pathway, it is a common target by SLs to inhibit melanin biosynthesis with differences in their mechanism of inhibition (Lai et al., 2019; Park et al., 2010). These can be categorized by their ability to interfere with melanin synthesis, transport and additional mechanisms necessary for the melanocyte environment (Kim et al., 2012). This is achieved by the use of preparations such as soaps, capsules/pills, injections, creams and even natural SL recipes which are usually applied to large surface areas to maintain a fair skin complexion (Rozen et al., 2012; Darj et al., 2015). Due to the chronic application of these products, side effects which include irritant and contact dermatitis, ochronosis and infection become more prevalent (Egbi and Kasia, 2021). Thus, skin lighteners have become more well-known among dermatologists and clinicians for their negative effects following the emergence of multiple reports on the complications associated with its use caused by the active ingredients (Alrayyes et al., 2019).

The active ingredients found in these preparations include highly cogent corticosteroids, mercury (Hg) salts and hydroquinone (HQ), being a major ingredient used for hyperpigmentation treatment (Alanzi et al., 2018; Kim et al., 2012). The ‘big four’, which includes Hg-containing compounds, hydroquinone, corticosteroids and retinoids are still denoted as the principle sources of skin damage, each linked to its own health risks and problems (Davids et al., 2016; López et al., 2012). Sections 2.5.1 to 2.5.4 below elaborates on the consequences of the use of each.

2.5.1 Mercury (Hg)-containing compounds

Hg is a heavy metal applied as a topical application for the treatment of psoriasis and other skin disorders (Pierre, 2008). Hg-containing products, such as mercurous chloride, oxide and ammoniated Hg were first introduced into the market during the first decade of the 20th century. These eventually became popular as skin lighteners and became the oldest known skin lighteners (Olumide et al., 2008).

Hg functions by replacing the copper that is necessary for the tyrosinase pathway and is commonly used in skin lighteners as it is easily absorbed through the skin (Al-Saleh, 2016). The dermal application of Hg absorbs into the bloodstream leading to health complications for the user and the developing fetus in childbearing users. In childbearing or breastfeeding users, this can lead to permanent nephrological and neurological defects in the infant which manifests as cognitive and behavioral deficits including attention-deficit/hyperactivity disorder (ADHD) (Al-Saleh et al., 2011; Al-Saleh et al., 2013; Dickenson et al., 2013; Uram et al., 2010). Chronic application can lead to Hg accumulation in several body tissues, where its aggregation is highest in the kidneys, leading to nephrotoxicity and other complications related to its accumulation such as neurotoxicity (Al-Saleh et al., 2009). For these reasons, Hg-containing preparations have been banned in several African countries, including SA, Zimbabwe, Gambia and Kenya (Maneli et al., 2016). Other adverse complications include paradoxical hyperpigmentation, discolouration and fragility of the nails (Naidoo et al., 2016). Paradoxical hyperpigmentation occurs by either an increase in melanin production, by a mechanism unknown, or via the direct deposition of metallic Hg granules within the dermis (Dadzie and Petit, 2009).

2.5.2 Hydroquinone (HQ)

HQ is considered the 'gold standard' among topical treatments for hyperpigmentation disorders, such as melasma or post-inflammatory hyperpigmentation, and is widely used as a component of many SLPs (Mahe et al., 2003; Westerhof and Kooyers, 2005). It may be used effectively and safely under careful supervision by a dermatologist and should be employed in ranges between 2 to 4% with a maximum limit concentration of 2% in SA (Maneli et al., 2016). Although, higher concentrations (up to 7 %) of HQ can be applied with great efficacy, this has shown to lead to quicker resistance, hyperpigmentation and an elevated risk of ochronosis (Naidoo et al., 2016; Obagi, 2013). The supervised use of prescription topical HQ has low risk of side effects, however, it is the unsupervised use of these formulations that have been reported to be linked to side effects, such as ochronosis (Desai, 2014).

HQ functions as a skin lightener by inhibiting TYR, decreasing the conversion of dopa to melanin. Moreover, it interferes with the cellular metabolism of melanosomes by inhibiting both DNA and RNA synthesis within melanocytes (Katsambas and Stratigos, 2001). Although HQ is sourced from plants and isolated from *Cystoseria jabokae* and *C. adriatica*, HQ has been banned due to its serious safety concerns in African countries and further banned in other countries due to its label as a carcinogen (Amponsah et al., 2014; Maneli et al., 2016; Momtaz et al., 2008a; Smit et al., 2009). HQ can be denoted as a potent cytotoxic agent to melanocytes with relatively high melanocyte-specific toxicity as it only targets cells with high TYR activity, such as epidermal melanocytes (Katsambas and Stratigos, 2001; Parvez et al., 2006). The cytotoxic effect of HQ is not only limited to melanocytes and the dose required to affect cellular metabolism is much higher than that for melanotic cells, contributing to its cytotoxic nature (Oyedeji et al., 2011).

Common side effects related to HQ use are skin irritation and contact dermatitis, both of which can be treated with topical steroids (Rendon and Gaviria, 2005). However, the most dangerous side effect associated with chronic use is exogenous ochronosis which is described as brown pigmentation/discolouration on areas of the body exposed to sunlight followed by loss of skin elasticity (Naidoo et al., 2016; Nduka et al., 2019). Other side effects of HQ use include; loss of skin elasticity, impaired wound healing, cataracts and more (Oyedeji et al., 2011).

2.5.3 Topical Corticosteroids (TC)

First introduced in dermatology in 1952, TCs revolutionized the treatment of skin diseases. It is amongst the most commonly used treatment in dermatological practice, but also popular for its use as a depigmenting agent (Chohan et al., 2016; Gul et al., 2014; Nnoruka and Okoye, 2006). TCs are prescription medication that are representative of anti-inflammatory agents and should not be present within cosmeceuticals. However, these are readily available as over-the-counter preparations with no prescription needed (Nnoruka and Okoye, 2006; Maneli et al., 2016; Park et al., 2010). International drug companies that produce TCs are well aware of the misuse of this product. However, despite this and due to the high profits, these companies proceed with the production and sales of these products (Olumide, 2010).

The pigment-reducing effect occurs due to vasoconstriction in the cutaneous vessels leading to the impression of immediate skin pigment reduction (Gul et al., 2014). Side effects related to its use are more prevalent than systemic reactions with side effects being more localized to the site of TC application. These usually occur with prolonged exposure and depend on the potency of the TC used (Coondoo et al., 2014). These effects include cutaneous atrophy, acne, hypopigmentation, telangiectasia and striae (Chohan et al., 2016). Further complications also include contact eczema and the possibility of bacterial and fungal infections (Glenn, 2008). Systemic side effects

associated with its chronic use include Cushing`s syndrome which is characterized by obesity, thin skin, bone wasting leading to osteoporosis, high blood pressure, gonadal dysfunction and hyperandrogenism (Castinetti et al., 2012; Naidoo et al., 2016).

2.5.4 Retinoids

Most SL agents available include some form of retinoids (Davids et al., 2016). Tretinion is a common topical retinoid that is used for the treatment of inflammatory lesions in mild to moderate acne (Arbab and Eltahir, 2010). As retinoids are lipophilic molecules, they have the ability to diffuse through cellular and other phospholipid membranes and easily penetrate the epidermis (Sorg et al., 2006). It could be for this reason that retinoids acts as penetration enhancers when mixed with other SL agents such as hydroquinone and mequinol (a SL alternative to hydroquinone) (Arbab and Eltahir, 2010; Draelos, 2007). An example of this is The Kligman formula recommended in 1975. It is a combination therapy used for melasma treatment which combines hydroquinone (5%) with a retinoid (tretinoin, 0.1%) and a steroid (dexamethasone, 0.1%) which enhances the efficacy of each individual ingredient (Eshghi and Ashari, 2016; Shankar et al., 2014; Woolery-Lloyd and Kammer, 2011). Since its production, 'The Kligman' formula has been modified in different ways to suit various skin types with the most recent modification becoming quite popular in countries such as India (Majid, 2010). An example of one of these could be found in India where this product has been extensively used in a cream-based combination of HQ, tretinoin and mometasone. One of the modified Kligman formulaes, which is meant to be used for 4-8 weeks, is generally used for 12 weeks or more. Side effects caused by this due to corticosteroids such as mometasone include atrophy, telangiectasias, and/or an acneiform eruption (Shankar et al., 2014).

Retinoids functions as skin lighteners by the following: (1) interfering with the transfer of melanosomes, (2) increase keratinocyte turnover, (3) inhibit the transcription of TYR (Burger et al., 2016). A side effect of retinoid use includes irritant dermatitis which is characterized by erythema, dryness and scaling. These changes induce damage to the skin barrier and allows for an increase in the access of other SL agents, such as those previously mentioned above (Draelos, 2007).

2.6 Plants as potential skin lighteners

The utilization of botanicals was the principle source of cosmetics before the discovery and introduction of methods for the synthetization of chemicals that possess similar properties. These chemicals are generated from natural sources and its derived compounds (Aburjai and Natsheh, 2003; Lahlou, 2013). Indigenous plants hold a long history of being applied in the treatment of ailments by many people of all continents (Mwinga et al., 2019). The diversity of plants used in traditional medicine worldwide has been estimated at approximately 10 000 to 53 000 species with many of these making massive contributions to the discovery of modern medicine, indicating the continued importance of natural sources (Elansary et al., 2015; Lahlou, 2013). In addition, an estimated 80% of the world`s population taps into traditional medicine for skin care treatment (Ryan et al., 2011). Africa holds significant biodiversity resources with its forests being the world`s second largest tropical reservoir. These reservoirs are located within tropical and sub-tropical climates which gives Africa`s plants strong UV rays and exposure to a multitude of pathogens (bacteria, fungi, viruses) proposing that African plants could amass more chemoprotective substances in comparison to plants from the northern hemisphere (Atawodi, 2005; Huang et al., 2009). This large reservoir could also be described by its land mass of approximately 216 634 000

hectares of closed forest with an estimation of over 5 400 medicinal plant taxa with over 16 300 uses listed (Van Wyk, 2011).

Only about 10.8% of Africa`s flora is known to be used for traditional medicine (Van Wyk, 2011). The usage of extracts for skin care has become very popular and has been found to play a major role in the treatment of various skin disorders (De Wet et al., 2013). Additionally, the traditional use of plants against skin diseases is a common practice in the domestic medicine of many cultures. These may provide leads for more effective anti-pigmentation compounds and natural molecules obtained from plant extracts offer an enticing avenue for future research (Aburjai and Natsheh, 2003; Momtaz et al., 2008b).

In the light of increased complications in commercially available chemical- and fungal-derived lightening agents, several studies have been conducted to find natural agents that reduce melanogenesis with limited adverse effects (Mapunya et al., 2011; Chang, 2009; Stapelberg et al., 2019). The readily availability of agents that cause low activity, high cytotoxicity, high mutagenicity and poor skin penetration (such as HQ) found in current lightening agents has contributed to the motivation for the search of herbal alternatives (Nerya et al., 2003). Various cosmetic formulations contain herbal extracts that make up approximately one-third of the product. These herbal extracts possess several actives that work together to improve the efficacy of the product (Kanlayavattanakul and Lourith, 2018b; Schmidt, 2012). Medicinal plants are rich in bioactive chemicals and the common perception that they are limited in adverse effects and are potentially safe, are making them highly desirable (Jennifer et al., 2012; Kanlayavattanakul and Lourith, 2018a). Research has also indicated that botanicals are more effective in their melanogenesis inhibitory effects and that natural ingredients could indeed offer safer alternatives to medical cosmetics (Stapelberg et al., 2019; Otang-Mbeng and Sagbo, 2020). Literature reports

on a variety of tyrosinase inhibitors obtained from natural sources which are applied as depigmentors or for the treatment of skin hyperpigmentory disorders (Ribeiro et al., 2015). Examples of these are mulberry and licorice which are well-known components found in skin lighteners. This also includes lemon extract added into preparations as a potent skin lightener but is added in low concentrations as it can easily induce skin irritation, with other discoveries including flavonoids, polyphenols and yeast derivatives (Smit et al., 2009; Otang-Mbeng and Sagbo, 2020).

There is a plethora of plants used in the traditional treatment of skin ailments and various African plants have been identified as inhibitors of TYR and melanin production. These plants substantiate the contribution the flora of the continent can make towards the exploration of natural products as skin lighteners (Etsassala et al., 2019; Muddathir et al., 2017; Stapelberg et al., 2019; Mapunya et al., 2012). For example, Kamagaju et al., 2013 evaluated the effect of 5 different Rwandan plant extracts on TYR function and melanin content of human melanoma cells. The results from this study showed that some extracts such as an ethyl acetate extract from the leaves of *Dolichopentas longiflora* was a more potent inhibitor of pigment production in comparison to its kojic acid control. In addition, results from the same study on the overall pigmentation effect of *Protea madiensis* on whole cells melanogenesis, which is the most comprehensive as it reflects the complete melanogenesis cycle, showed that the extract quite strongly inhibits melanogenesis (Kamagaju et al., 2013). Okunji et al., 2007a also described that *Garcina kola* from Algeria elicited a significant inhibitory response on TYR activity and further concluded that it could indeed be a potential source of new TYR inhibitors. A recent study investigated the SL effects of 4 Senegal plant extracts on a reconstructed human pigmented epidermis (RHPE), which is described as the closest *in vitro* model to human skin as it is formed in a co-culture of human keratinocytes and

melanocytes. The results showed that none of the extracts tested were toxic to the cells and some of the extracts exhibited depigmenting effects comparable to that of kojic acid positive control. Other plants studied also included those from South African such as *Greyia radlkoferi* in which Lall et al., 2016 showed that the extract inhibited 50% of melanin production at a concentration of 12.5 µg/ml, while maintaining cell viability above 50%. Further analysis needs to be conducted to evaluate the efficacy of already studied extracts in SL formulations. However, the current data still emphasizes the potential presented by Africa`s natural resources to provide alternative agents to current toxic products found on the market.

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Chapter 3

A cross-sectional study investigating the factors influencing skin lightening prevalence among university students in South Africa

3.1 Introduction

Complexion is deemed as one of the most compelling standards of beauty and there is a belief that a fair complexion is synonymous with beauty (Juliano, 2022; Hafeez et al., 2022). These perceptions, along with several historical, cultural and psychological factors, have played an influential role in motivating body modifications practices such as skin lightening (SL) (Thomas, 2012; Swami et al., 2013). SL allows individuals to attain a fairer skin tone through the use of various preparations such as topical creams and soaps which contain chemicals that function by reducing melanin production (Joana et al., 2016; Rozen et al., 2012; Egbi and Kasia, 2021). Skin lightening products (SLPs) are clinically prescribed for the treatment of several skin diseases such as melasma and post-inflammatory hyperpigmentation (Ladizinski et al., 2011). However, some are reported to be commercially available without a prescription and are obtained via street vendors and markets which facilitates the misuse of these products for cosmetic purposes (Amodu et al., 2018).

SLPs are well-known in several countries of the world including South American, Asian and Middle Eastern countries. Sagoe et al., 2019 reported its prevalence in several African countries including Ghana, Kenya, South Africa (SA) and Tanzania, with the African continent revealing an estimated prevalence of 25 to 96% (Li et al., 2008; Peltzer et al., 2016; Rambaran, 2013; Sagoe et al., 2019). Motivations for the desire for fair skin has been reported to be related to perceptions of

beauty, marital prospects, job prospects and earning potential (Li et al., 2008). These reasons were supported by participants in a study conducted by Ahmed and Hamid, 2016 who reported their desire to look appealing and attracting a desired marriage partner. Other reasons cited in this study also included gaining social acceptance as participants perceived lighter skin to be synonymous with such opportunities. Similar results were also obtained from a survey study conducted at a Nigerian tertiary institution, in which 56% of participants agreed that lighter skin tones are a factor of beauty (Amodu et al., 2018). These ideas are further glamorized by cosmetic advertisements in media which feature light-skinned models as symbols of attraction and desire (Olatunji et al., 2019).

The most commonly used SLPs contain agents such as hydroquinone, corticosteroids and mercury with studies showing that these compounds have detrimental effects including skin irritation, contact dermatitis and exogenous ochronosis (Ahmed and Hamid, 2017; Ogunbiyi et al., 2009; Rendon and Gaviria, 2005; Altraide et al., 2021). Despite these side effects, SLPs are promoted as beneficial to the skin which is leading to an increase in demand. Current predictions estimate that the global SL industry will be worth USD 24 billion by 2027, which is more than double its worth of USD 8.3 billion as reported in 2018 (Cristaudo et al., 2013; Abd Wahil et al., 2020; Cheng et al., 2021). A qualitative study conducted in Eritrea found that 59% of the participants were aware of the potential problems related to SLP use, yet there was a 26% usage reported (Amahazion, 2017). In another study of 262 Somaliland participants, 91% and 88% of the participants reported SLPs can cause local and systemic effects respectively, with half of the participants (52%) reporting SL prevalence (Yusuf et al., 2019). These studies confirm that, even though users are aware of the side effects, they remain unperturbed and continue using SLPs (Abd Wahil et al.,

2020). Despite the ban on the sale and use of SLPs, it is clear that consumers are undeterred and access to the products via illegal channels are still prevalent (Motseki, 2019).

SLP usage remains a large public health issue which needs further evaluation in SA (Dlova et al., 2014). A number of studies have been conducted to determine SL prevalence worldwide. However, limited studies have been performed to understand this phenomenon among adults within the SA context such as that of Dlova et al., 2014; Keakile, 2016 and (Rahiman et al., 2021). These same studies have also indicated the emergence of SL use among the young adult population of the country. To gain a comprehensive understanding of this phenomenon, its influences and determine an estimated prevalence of the practice among this specific cohort, a questionnaire-based survey was conducted. Thus, this study intended to explore the factors influencing skin lightening perceptions, knowledge and use among undergraduate students attending a tertiary institution in SA.

Methodology

3.2.1 Study design

This study, which is of a cross-sectional design, was implemented to evaluate the knowledge, perceptions and practice towards skin lightening among young adults at an institution of higher education in Cape Town, SA.

3.2.2 Inclusion and Exclusion Criteria

The target population included undergraduate students enrolled for classes and between the ages of 18 – 30. All prospective participants were provided with an information leaflet, consent form and questionnaire which can be seen in Appendix 2, 3 and 4, respectively. Participants that did not meet the inclusion criteria, as well as those that did not provide signed consent, were excluded from the study.

3.2.3 Ethical considerations

This study was approved by the Biomedical Science Research Ethics Committee of the University of the Western Cape (BM18/3/21) (Appendix 1). Participation in this study was entirely voluntary and participants were free to decline involvement or withdraw at any point without prejudice. Following return of signed informed consent forms, participants were provided with a questionnaire.

3.2.4 Study tool and design

All participants completed a paper-based, structured, self-administered questionnaire (Appendix 4) which was adapted from Keakile (2016). This study tool was piloted among a group of 30 students for reliability and validity. The sections of the survey produced a strong Cronbach Alpha value of above 0.7 respectively thus, finding the questionnaire to be reliable and valid.

The questionnaire comprised of four sections and included questions relating to: 1) Demographic and general details of the participant; 2) skin tone and skin lightening practices; 3) perceptions of skin lightening, and 4) participants` knowledge of the negative consequences of skin lightening. In each section of the questionnaire, participants had to indicate which responses best resonated with them.

3.2.5 Participant confidentiality

Raw data was quantitatively coded, categorized and assigned corresponding numerical values. Data was also protected by being stored onto a password protected computer and questionnaires were secured in a cupboard to be destroyed after a period of 5 years. Access to data was only granted to supervisor and student researcher.

3.2.6 Sampling size and strategy

The undergraduate student population registered for the 2019 academic year was recorded at 18 476, as collected from the administrative staff for each of the 7 faculties of the institution. The sample size was calculated based on the following assumptions: 95% confidence interval, 5% level of significance, a non-response rate of 10%. This yielded a final sample of 740 students. The multistage sampling method was applied to select participants in which 20% of the departments at the institution were selected using simple randomization. From this randomization, the following departments were selected: Department of Academic Development, Department of Geography and Tourism, Department of Biodiversity and Conservation Biology, Department of Information Systems, Department of Language Education, Department of Restorative Dentistry, Department of Dietetics and Nutrition, Department of Public Law and the Department of Biotechnology. The sample size was proportionally allocated to the study years (i.e. year 1, year 2, year 3) in the

selected departments. Convenient 'in class' sampling was conducted which comprised of all students present during lectures or practical sessions.

3.2.7 Data analysis

The data which was compiled using Microsoft excel was analyzed with the assistance of a biostatistician. Descriptive analysis was conducted on data in each section of the questionnaire producing frequency tables. The data was also investigated for possible associations between the use of skin lighteners and categorical variables, namely practice, perceptions, and knowledge. This was conducted using Pearson chi-square analysis. Multiple logistic regression models were fitted to determine the odds ratios with 95% confidence intervals to determine predictors for the use of skin lightening. In addition, the estimation of the confidence interval for a proportion prevalence was determined using the Wilson test. In this study, a p-value <0.05 was considered statistically significant.

3.3 Results

A total of 793 questionnaires were distributed across 9 departments and all 793 students accepted the invitation to participate. This study used convenient sampling which is one of the most commonly used sampling methods in which all individuals in a particular place can be sampled (Acharya et al., 2013, Nulty, 2008). Implementation of this allowed for the participation of all students present in the lecture and/or practical venues, leading to the high participation rate. After exclusion, 765 responses were analyzed leading to a 100% response rate with a final study sample comprising of 383 first year students, 206 second year students and 170 final year students.

The results are presented as follows with Table 1 reflecting all sociodemographic characteristics related to the study participants. Based on the demographics collected, majority of the study participants (97.3%) were aged between 18-25. Most identified as either black (48.1%) or coloured

(43.4%), and more than half (60%) of the respondents were female. Of the 765 participants, 50.1% were in their first year of registration and most were registered for BComm (37.3%). The largest portion of participants (98.2%) stated to be single with 79.7% indicated to live in urban areas.

Table 3.1: Demographic characteristics

Characteristic		**Frequency**	**Percentage**
Age	18 - 25	745	97.3
	26 – 30	20	2.7
Sex	Male	306	40
	Female	458	59.9
Race	Black	368	48.1
	Coloured	331	43.4
	Indian	31	4.1
	White	29	3.8
	Other	1	0.1
Marital Status	Married	12	1.6
	Single	751	98.2
Geographical region	Rural	152	19.9
	Urban	609	79.7
Year of Study	First	383	50.1
	Second	206	26.9
	Third	170	22.2
Registered Degree	Biotechnology	16	2.1

	Medical Biosciences	16	2.1
	Chemical Sciences	3	0.4
	Education	106	13.9
	Biodiversity Conservation Biology	22	2.9
	Environmental Water Sciences	2	0.3
	Restorative Dentistry	77	10
	Sports and Recreation	1	0.1
	BCommerce	285	37.3
	Computer Science	19	2.5
	BAdmin	23	3
	Law	140	18.3
	BArts	50	6.5

Table 3.2 depicted below, as well as Tables A and B found in Appendix 5 and 6, show the frequency response of participants in relation to questions based on factors of practice, perceptions and knowledge. As illustrated in Table 2, 10.6% indicated prevalence of SL usage while 88% reported they have not used SLPs. Of the sample population, 96% of participants indicated satisfaction with their skin colour while 9.3% of participants indicated inclination to change their skin colour given the opportunity. In relation to skin tone preference, 54.4% stated they preferred a medium complexion, in comparison to a fair/light complexion (20.9%) and a dark complexion (14.5%). Of the 765 that participated, 45.5% stated that family and friends was a factor influencing skin colour preference with 25.2% indicating social media, and more specifically Instagram (30.3%), as a strong motivating factor. Participants revealed that SLPs could be obtained from

either the supermarkets (4.6%), pharmacy (3.9%) or from family and friends (2.4%). It was also reported that participants (30.6%) knew family/friends that used SLPs and obtained these products from the places including the supermarket (12.3%), pharmacy (7.1%), skin care clinic (5%) and other family members or friends (3.7%). Products commonly used by family members or friends included creams (24.4%) and soaps (4.2%). Only 19.4% reported to have read the ingredients list of SLPs and were knowledgeable of the common ingredients found in these products which included both chemical and natural ingredients (8.6%) and natural/plant-based ingredients (6.7%). The most frequently mentioned natural/plant-based ingredients were aloe (1.1%), aloe vera (0.8%) and tea tree oil (0.4%).

Table 3.2: Prevalence and Influences of skin lightening practice

Characteristic		Frequency	Percentage
Satisfaction with skin colour	Yes	734	95.9
	No	30	3.9
Inclination to change skin colour	Yes	71	9.3
	No	690	90.2
Skin tone preference	Fair/light complexion	160	20.9
	Medium complexion	416	54.4
	Dark complexion	11	14.5
	More than one complexion	52	6.8
Factors influencing preference	Social media	193	25.2
	Advertisements	20	2.6
	Television	41	5.4

	Family and friends	384	45.5
	More than one	93	12.2
If social media, specific social media platforms	Facebook	48	6.3
	Instagram	232	30.3
	Tinder	4	0.5
	Whatsapp	126	16.5
	More than one	86	11.2
Usage of products to lighten skin	Yes	81	10.6
	No	672	88
Supplier skin lightening products	Family or friend	18	2.4
	Pharmacy	30	4
	Skin care clinic	11	1.4
	Supermarket	35	4.6
	More than one	7	1
	Cosmetic store	3	0.4
	Korea	1	0.1
Familial skin lightening usage	Yes	234	30.6
	No	512	67
If yes, familial means of access to skin lightening	Family, other members or friends	28	3.7
	Pharmacy	54	7.1
	Skin care clinic	38	5
	Supermarket	94	12.3

	More than one	9	1.2
	Vendors	10	1.3
	Indian store	1	0.1
	Hawkers	1	0.1
	South Korea	1	0.1
If yes, familial method of skin	Soap	32	4.2
lightening	Creams	187	24.4
	Home remedies	10	1.3
	More than one	39	5.1
	Street corners	3	0.4
Read ingredient list	Yes	148	19.4
	No	442	57.8
If yes, type of ingredient in	Chemical (synthetic)	29	3.8
product	Natural (plant based)	51	6.7
	Both (chemical & natural)	66	8.6
	Organic (synthetic- and toxin-free)	16	2.1
	More than one	10	1.3
Natural/organic or specify	Healing	1	0.1
	Herbs	2	0.3
	Aloe vera/coconut oil	3	0.4
	Aloe	8	1.1
	Aloe vera	6	0.8

	Tea tree oil	3	0.4
	Dawn moisturizer	1	0.1
	Lemon	3	0.4
	Coconut oil	1	0.1
	Tumeric	2	0.3
	Marula extracts	1	0.1
	Tumeric, mayonnaise, oats, eggs	1	0.1
	Mercury	1	0.1
	Benzoyl peroxide	1	0.1
	Sunlight	1	0.1
	Aloe, honey	1	0.1
	Types of vitamin C creams	1	0.1
	Cocoa	1	0.1
	Imbola	1	0.1
	Sunflower	1	0.1
	Rooibos, Vitamin E	1	0.1
	Cocoa nut	1	0.1
	Honey, sugar	1	0.1
	Tea tree oil/green tea	1	0.1
	Jojoba oil	1	0.1
	Organic baobab oil	1	0.1

According to Table A in Appendix 5, 71.9% agreed that individuals lighten their skin to look fashionable/trendy. Although majority of respondents (43%) rejected the notion that people used SLs to become more successful in their careers, 27.3% still agreed with this statement. Many also acknowledged that these products are used to seem more appealing to the opposite sex (65.2%), to obtain a 'higher social class/ranking' (64%), and also to impress their partners (52.3%). In addition to this, 82.5% of study population recognized that the want to appear more beautiful also had influence. Just below half of the total respondents (47.6%) reported that SL was also motivated by the need for treatment of medical conditions.

In terms of SL knowledge, Table B in Appendix 6 showed that approximately 61.7% reported that SL use can cause skin damage in the form of rashes/irritations. Several participants showed to be knowledgeable about the practice leading to skin infections (49.5%) and skin tearing or breaking (53.1%). However, participants still showed a lack of knowledge in terms of the systemic effects related to SL use. Only 31% agreed that SLP use could lead to mercury poisoning and intake of other harmful substances which could lead to organ failure, and approximately 60% of respondents revealed to be unsure if SL use leads to a prevention in immune system responses.

To investigate the association between SL usage and factors such as demographics, practice, perceptions and knowledge, the Pearson chi-square analysis (Table 3) was conducted. The results showed that sociodemographic variables such as sex, race, marital status, geographical region, study year and registered degree were not significantly associated with SLP usage. Further analysis conducted on participants` responses to questions related to SL practice revealed that inclination to change skin colour ($p = 0.008$) and social media ($p = 0.022$) were significantly associated with SLP usage. No statistical significance was found between SLP use and familial usage, as well as SLP use and perceptions such as the need to impress their partners or to appear appealing to the

opposite sex. However, results showed a significant association between SL usage and being knowledgeable of SL consequences such as skin damage ($p = 0.014$) and skin infections ($p = 0.025$).

Table 3.3: Association between usage of skin lighteners with demographics, practice, perceptions, and knowledge

Questionnaire section		**Usage of skin lighteners**	
Demographics		Yes (Count %)	*p*-value
Age group	18 - 25	47.2	0.065
	26 - 30	8.9	
Sex	Male	9.2	0.513
	Female	11.8	
Race	Black	12.4	0.190
	Coloured	8.9	
	Indian	16.1	
	White	3.5	
Marital status	Married	25	0.104
	Single	10.5	
Geographical region	Rural	11.4	0.789
	Urban	10.7	
Year of Study	1	10.9	0.997

	2	10.7	
	3	10.9	
Degree	Biotechnology	18.8	0.693
	Medical Biosciences	0	
	Chemical Sciences	33.3	
	Education	11.3	
	Biodiversity Conservation Biology	13.6	
	Environmental Water Sciences	0	
	Restorative Dentistry	10.4	
	Sports and Recreation	0	
	BCommerce	9.9	
	Computer Science	22.2	
	BAdmin	14.3	
	Law	8.9	
	BArts	14	

Practice			
Satisfaction with skin colour	Yes	10.4	0.095
	No	20	
Inclination to change skin colour	Yes	20.3	0.008*
	No	9.8	
Skin tone preference	Fair/light complexion	13.9	0.300
	Medium complexion	10.9	
	Dark complexion	8.2	
	More than one	5.9	
Factors influencing preference	Social media	11.6	0.959
	Advertisements	15	
	Televisions	12.5	
	Family and friends	10.4	
	More than one	10.9	
Social media platforms	Facebook	17	0.022*
	Instagram	12	

	Tinder	25	
	Whatsapp	2.4	
	More than one	10.8	
	Other	0	
Supplier of products	Family or friend	68.8	0.352
	Pharmacy	72.7	
	Skin care clinic	90.9	
	Supermarket	91.4	
	More than one	85.7	
	Cosmetic store	66.7	
	Korea	100	
Familial skin lightening usage	Yes	18.5	0
	No	7.5	
POI supplier of product	Family, other members or friends	22.2	0.194
	Pharmacy	17.3	
	Skin care clinic	13.2	

	Supermarket	18.3	
	More than one	33.3	
	Vendors	20	
	Indian store	100	
	Hawkers	0	
	South Korea	100	
POI method of skin lightening	Soap	23.3	0.607
	Creams	21.2	
	Home remedies	40	
	More than one	28.2	
	Street corners	33.3	
Read ingredient list	Yes	17.7	0.062
	No	11.7	
Type of ingredient in product	Chemical (synthetic)	24	0.758
	Natural (plant based)	21.6	
	Both (chemical & natural)	16.9	

	Organic (synthetic- and toxin-free)	12.50	
	More than one	10	
Specify ingredient	Healing	0	0.386
	Herbs	0	
	Aloe vera/coconut oil	50	
	Aloe	0	
	Aloe vera	25	
	Tea tree oil	0	
	Dawn moisturizer	33.33	
	Lemon	0	
	Coconut oil	33.33	
	Tumeric	0	
	Marula extracts	0	
	Tumeric, mayonnaise, oats, eggs	0	
	Mercury	100	
	Benzoyl peroxide	0	

	Sunlight	0	
	Aloe, honey	100	
	Types of vitamin C creams	100	
	Cocoa	0	
	Imbola	0	
	Sunflower	100	
	Rooibos, Vitamin E	0	
	Cocoa nut	0	
	Honey, sugar	0	
	Tea tree oil/green tea	100	
	Jojoba oil	0	
	Organic baobab oil	0	
Perceptions			
To look fashionable/trendy	Agree	11.2	0.442
	Disagree	12.7	
	Don`t know	7.8	

Be more successful in their careers	Agree	9.8	0.789
	Disagree	11.6	
	Don`t know	10.6	
Obtain a ‘higher social ranking/class’	Agree	11.5	0.671
	Disagree	9.3	
	Don`t know	9.6	
Impress their partners	Agree	12.9	0.149
	Disagree	7.8	
	Don`t know	9	
Seem more appealing to opposite sex	Agree	12.4	0.150
	Disagree	7.9	
	Don`t know	7.6	
To appear more beautiful	Agree	11.9	0.067
	Disagree	8.5	
	Don`t know	3.7	
Treatment for medical conditions	Agree	11.1	0.675

	Disagree	12.6	
	Don`t know	9.7	
Knowledge			
Skin damage in the form of	Agree	9.3	0.014*
rashes/irritations	Disagree	21.2	
	Don`t know	10.8	
Skin infections (bacterial or fungal)	Agree	8.9	0.025*
	Disagree	19.1	
	Don`t know	10.9	
Skin tearing or breaking	Agree	9.3	0.331
	Disagree	12.9	
	Don`t know	12.6	
Mercury poisoning	Agree	9.4	0.592
	Disagree	13.5	
	Don`t know	11.1	
Prevention of immune system responses	Agree	12.90	0.534

	Disagree	11.72
	Don`t know	9.85

*Indicates significance at $p < 0.05$

According to Ahmed and Hamid, 2017 and Bamerdah et al., 2023, certain factors such as age, sex, and familial usage have been associated with SLP usage. Therefore, multiple logistic regression analysis (Table 3.4) was performed on these influential factors to determine their potential as predictors of SL usage within this cohort.

As previously described, models to determine odds ratios were fitted to measure any associations between an exposure and an outcome. In this study, it was used to determine predictors for use of skin lighteners. According to Szumilas, 2010, an odds ratio (OR) less than 1 in comparison to the reference category is considered to have a lower odds of outcome and an odds ratio of more than 1 compared to the reference category is considered to have a higher odds of outcome. Therefore, the results indicated that participants in age groups 20 and 21 had a higher odds of usage ($p = 0.03$ and 0.001 respectively) than participants aged 18, with those aged 21 being almost 4 times more likely to use SLPs. Further evaluation also indicated that females are 1.6 more likely to participate in the practice in comparison to males ($p = 0.041$). In addition, it was observed that overall, participants that reported no familial SLP usage had a statistically significant ($p = 0.001$) lower odds (OR = 0.3) of SL use. However, analysis of reported familial use among population groups showed that odds of SLP usage is 6.1 times higher ($p = 0.001$) among those of the non-African study population group.

Table 3.4: Multiple logistic regression analysis for predictors for use of skin lighteners

Variable	OR	*p*	95% CI for OR
Age group			
18	Ref	Ref	Ref
20	2	0.03*	1.1 – 3.6
21	4	0.001*	2.1 – 7.2
Familial skin lightening usage			
Yes	Ref	Ref	Ref
No	0.3	0.001*	0.1 – 0.5
Non-african study population			
No	Ref	Ref	Ref
Yes	6.1	0.001*	2.6 – 14.3
African study population			
No	Ref	Ref	Ref
Yes	1.7	0.106	0.9 – 3.4
Sex			
Male	Ref	Ref	Ref
Female	1.6	0.041*	1 – 2.4

OR: odds ratio; Ref: reference category; * Indicates significance at $p < 0.05$; CI: confidence interval

3.4 Discussion

Of the 765 respondents, 95.9% of the study population expressed their satisfaction with their skin colour. However, 10.6% of respondents still revealed to have used SLPs in the past. This reported prevalence is supported by research conducted among a similar cohort of students registered for classes at a health sciences faculty of a tertiary institution within the same province in which 12% of the sample indicated SL usage (Rahiman et al., 2021). Adebimpe et al., 2020 and Asumah et al., 2022 also conducted studies within other African countries among undergraduate students in Southwestern Nigeria and Ghana, which indicated a higher SL use of 38% and 26.3% respectively (Adebimpe et al., 2020; Asumah et al., 2022). The high statistic reported in Nigeria could be due to the popularity of the practice in west African countries as reported by the World Health Organization (WHO) in which 77% of the female population stated to have used SLPs (Egbi and Kasia, 2021). Although SLP use is widely common among women, our study demonstrated growing interest in usage among males. Despite logistic regression analysis showing that females are more likely to practice (odds ratio: 1.6), 9.2% of males revealed to use SLs. This evidence reveals the increased popularity among men as they are becoming more interested in grooming and the maintenance of their appearance (Cheong and Kaur, 2019).

In comparison to data available from other African countries, evidence within the WC suggests that SL prevalence is low. Nonetheless, it remains pertinent to investigate the motivating factors associated with this practice. A recent review listed several factors which included sociocultural background, skin conditions and other psychological factors such as self-esteem and body image to be associated with SL usage (Al-Sarraf, 2022). However, upon statistical examination of some of these factors (race, sex, degree) in this study, there showed to be no significant association between sociodemographic variables and SL usage. Ibekwe et al., 2020 reported similar results among 200 university students which also showed no significant association between these

sociodemographic variables and SLP usage. Considering both of these studies were conducted among tertiary educated individuals, the lack of association between sociodemographic variables and usage could be suggestive of the level of education (Al-Sarraf, 2022; Ibekwe et al., 2020). Data collected in the current study also showed that participants were aware of the adverse effects associated with SLP. These results support studies as those conducted by Alrayyes et al., 2019 and Keakile, 2016 in which it was proposed that a higher level of education leads to an increased likelihood of awareness of social issues and the negative effects associated with such practices. A similar conclusion can also be made regarding this cohort`s awareness of SLP side effects as majority of participants agreed it can lead to skin damage, infection and tearing/breaking. This corroborates research conducted at other institutions of higher education as that of Apak, 2018, in which participants also showed to be knowledgeable of the consequences of SLP use.

Overall, approximately 25% of participants in the current study indicated social media as an influence and elaborated by identifying Instagram to be of the most common platforms. Instagram is used by several companies as a means of encouraging product consumerism and many turn to it for information regarding skin care and beauty products (Sommerlad, 2021; Hassan et al., 2021). Other platforms that showed a significant association with SLP usage was Tinder, a popular dating app that uses the swipe-concept in which first impressions are made on users` profile pictures (Ward, 2017; Nair and Padmakumar, 2020). Since its inception in 2012, the app has gained more than 50 million users in almost 200 countries with an average of approximately 10 million users daily, all possibly '…looking for love…', '…want to start dating…' or just looking to 'keep it casual,' as described by the app`s website. (Barrada and Castro, 2020). This desire to find a partner could also be supported by more than 60% of participants in the current study agreeing that individuals practice SL to appear more beautiful and to seem more appealing to the opposite sex. Additionally, individuals` reported SLP use is motivated by the desire to look fashionable/trendy

and to obtain a ‘higher social ranking/class’. Similar findings were reported by Kamagaju et al., 2016 and Ahmed and Hamid, 2016 in which participants expressed the reasons for SLP use to be driven by their desire to be attractive and to ‘look pretty/fashionable’. Despite no associations found between SL perceptions and usage, responses from participants still highlight the influence of perceptions on SL use.

According to the sociodemographic data reported, majority of the study participants identified as black and just over half of this population indicated their preference for a medium complexion in comparison to a fair/light or a darker complexion. This evidence suggests that there still remains a preference for a lighter skin complexion and this could be indicative of longstanding ideals that skin color influences perceptions of beauty (Regencia et al., 2023). Bamerdah et al., 2023 and Fakorede, 2022 described similar outcomes in which their study participants stated fairer skin increases chances of marriage, employability and socioeconomic outcomes. Only a minority of the participants in the current study agreed that SL is practiced to be more successful in their careers. This is similar with results reported by Hossain, 2020 in which only 34% of the 385 university students that participated in the study agreed that fairer skin has influence on employment opportunities. Although the perception that employment opportunities is influenced by fairer skin was less commonly reported in the current study, the results show that the idea of fairer-skinned individuals having an advantage in these areas over darker-skinned individuals still remains.

Several social factors such as family, culture and social roles can determine consumer behaviours, with family and friends stated to be one of the most influential of the social factors. It was also stated that individuals place a high value on ‘word-of-mouth’ communication from a trusted source or someone known personally (Hafeez et al., 2022). Results obtained from this study showed that some participants were aware of family or friends making use of SLPs. Although there was no

significant association reported between familial and individual usage, multiple regression analysis revealed that familial exposure could act as a predictor of SLP usage, with it being less likely for those who have no exposure to familial SLP usage to engage in this practice. Additional analysis conducted to determine other influential factors which could be predictors of SL usage showed that there was a higher likelihood of SLP use among females, as well as those aged 20 and 21 in which they are 2 and 4 times more likely to use SLPs, respectively. These results validate research conducted by Askari et al., 2013 which showed the popularity of SL practice amongst those aged between 20 – 30, and further mentioned that those within this age group are more inclined towards use of such products (Hamed et al., 2010; Ravichandran, 2013; Rusmadi et al., 2015). Possible reasons for this could be due to body image dissatisfaction which is particularly common among adolescents due to the physiological, psychological and social changes they are experiencing and can still continue into the young adult years (Clay et al., 2005; Quick et al., 2013).

SL use has been stated to be common among those of African descent and Nyoni-Kachambwa et al., 2021 showed that dark-skinned women were more likely to use SLPs than light-skinned women (Benn et al., 2019). However, the results obtained in this study showed the inverse as those of the non-African population group showed to be 6.1 times more likely to use SLPs if familial SL use is reported in comparison to those of African population groups which showed to be only 1.7 times likely. The common assumption is that SL use is exclusive to people of colour, but the results obtained for the current study indicate its popularity among the non-African population (Shrestha, 2013). This could be due to individuals of this population group desiring to tone their skin to achieve a more uniform complexion, remove blemishes and/or treat other skin diseases and reduce any visible signs of aging (Blay, 2011; Mendoza, 2014). However, Shrestha, 2013 also further

explained that its use is also motivated by marketing strategies targeting fair-skinned individuals wishing to sustain their fair complexion and perceived privileges accompanied with it.

Data with current evidence on SL practice across several international countries is available, however, evidence within SA remains scarce. Thus, this study served to address a gap in research by showing the prevalence of SL use among the SA young adult population. The factors that were identified as influence of SL practice which were showed to be familial SL use and social media as the most common. Despite results from this study and other studies indicating that people are aware of the negative side effects, usage remains prevalent and on the rise.

3.5 Conclusion

The high response rate achieved through the use of convenient 'in class' sampling provided results more likely to be representative of the target population and contributed imperative insight into the phenomenon of SLP use among the young adult population of SA. In addition to this, as this study provides evidence on influential factors on SLP usage among young adults such as social media, specific intervention strategies targeted for these specific platforms can be devised to increase awareness of such dangerous practices.

3.6 Limitations

The research tool selected for this study, which was a questionnaire, was both cost-effective and efficient. However, social desirability bias, in which participants tend to answer questions in a way that could be viewed favorably by others, is a potential contributor of bias in studies of this nature. Participant responses may be exaggerated as well as participants could feel too embarrassed to fill out the questionnaire honestly. In an attempt to minimize these limitations, participants` responses were completely anonymous, allowing them to be more honest without the fear of judgement.

Conducting additional studies of this nature could lead to recommendations for possible interventions necessary to reduce usage and harm to the public. In addition, data collection could include other tertiary institutions across other faculties, expanding the sample size to allow for generalizability and provide data that is better representative of the population and the overall prevalence. This could also be made possible with the use of an electronic questionnaire accessed by following a link shared via electronic devices and participants would then click the option/s that suit best applies to them. This system also negates the need for capturing of responses manually, minimizing the risk of human error.

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Chapter 4

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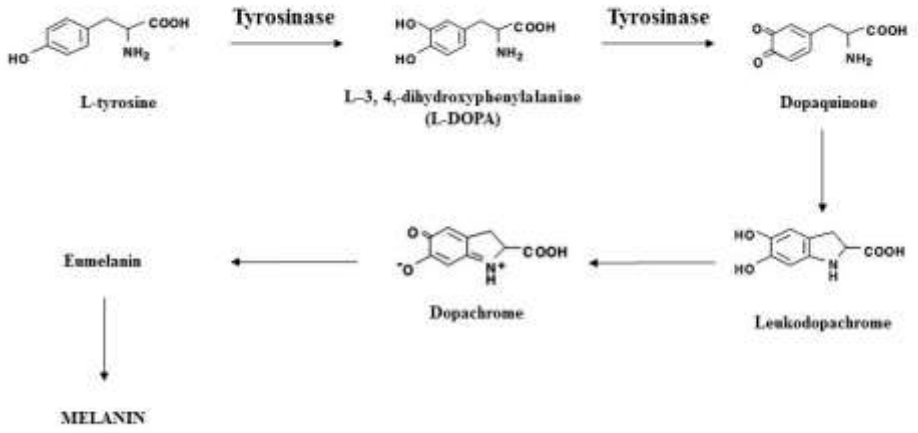
Tyrosinase and Melanogenesis Inhibition by Indigenous African Plants: A Review

4.1. Introduction

Melanin is a widespread natural pigment that is responsible for color in hair, skin, and eyes. It provides protection against the deleterious effects of ultraviolet (UV) irradiation (Masum et al., 2019). Melanogenesis is the physiological process of melanin formation in which TYR, a copper-dependent enzyme, initiates the first step. Tyrosinase catalyzes the rate-limiting step where L-tyrosine is converted to L-3,4,-dihydroxyphenylalanine (L-DOPA), leading to the eventual formation of the pigment [Illustrated by Scheme 1] (Chang, 2009; Jawaid et al., 2009; Mapunya and Lall, 2011; Videira et al., 2013). Abnormal TYR activity leads to pigmentary disorders, such as the abnormal accumulation of melanin (hyperpigmentation) that accounts for most dermatology visits (Campos et al., 2013; Chiocchio et al., 2018; Hollinger et al., 2018). Skin lighteners can be divided by their mechanisms of action, such as inhibition of tyrosinase transcription, inhibition of melanosome transfer, and accelerated epidermal turnover, with the most common target being tyrosinase (TYR) inhibition (Couteau and Coiffard, 2016; Gillbro and Olsson, 2011). By decreasing the activity and/or expression of TYR, melanogenesis can be inhibited, leading to reduced melanin production (Sari et al., 2019).



Scheme 1. Illustration of the melanogenesis pathway (Opperman et al., 2020)

The skin lightening industry is one of the fastest-growing segments of the global beauty industry. Global industry analysts (GIA) have predicted that by 2020, the universal skin lightening market will reach $23 billion (Mohiuddin, 2019). A recent meta-analysis provided evidence of the global prevalence of skin lightening use by reporting an estimate of 27.7%, with Africa at a current estimated prevalence of 27.1% (Sagoe et al., 2019). Previous epidemiological studies have also reported a high prevalence of skin lightener use among African populations. This is evident among South African, Senegalese, and Nigerian study populations that revealed between 32 to 75% skin lightener use (Adebajo, 2002; Dlova et al., 2015b; Wone et al., 2000).

This practice is motivated by a long-standing history of social divisions, including societal pressures and stigmas, leading to the demand for lighter skin tones (Charles, 2003; Charles, 2009). Creams, lotions, soaps, and injections indicated as a treatment for hyperpigmentation disorders are exceedingly abused as self-medication to achieve a lighter skin complexion (Arbab and Eltahir, 2010; Dlova et al., 2014). In many African countries, a variety of these skin lightening preparations are easily obtained over-the-counter without a medical prescription, despite this being a

requirement by law (Dlova et al., 2015b; Nnoruka and Okoye, 2006). The most frequently used ingredients include steroids, mercury, hydroquinone (HQ) (considered the gold standard), and its derivatives (Nnoruka and Okoye, 2006). Health concerns associated with the long-term use of these skin lightener ingredients include exogenous ochronosis and infectious dermatosis (Mulholland et al., 2013; Villareal et al., 2013). Furthermore, heavy metal exposure can lead to damage to the circulatory and urinary systems (Michalek et al., 2019). Due to their toxicity, these compounds have been prohibited as skin lightening compounds in several African countries, including South Africa, Nigeria, Kenya, and the Ivory Coast (Chan, 2011; Davids et al., 2016). Despite this ban, these damaging chemicals are often illegally introduced into cosmetic formulations and, the public continues to gain access via informal channels such as street vendors, markets, and non-pharmaceutical shops (Gbetoh and Amyot, 2016; Kamagaju et al., 2016). In contrast, botanicals and natural ingredients offer safer alternatives as they may not exhibit the same kind of toxicity as synthetic compounds and could exhibit much less harmful side effects (Di Petrillo et al., 2016). Despite this, consumers are not generally aware that natural products are composed of a variety of chemical compounds that could lead to the development of some adverse reactions. These potential effects could be overcome by researchers chemically characterizing extracts with respect to its composition (Ribeiro et al., 2015).

Botanicals and natural ingredients provide abundant sources of treatment for various diseases such as cancer, diabetes, and dermatological conditions (Kumari et al., 2018; Mapunya et al., 2012). The use of plants is a common practice in traditional medicines of many cultures using several plant extracts as cosmetics to improve skin health (Momtaz et al., 2008b; Twilley and Lall, 2014). This could be attributed to plant extracts being a rich source of vitamins, antioxidants, oils/essential oils, and other bioactive compounds, which provide the body with nutrients necessary for healthy

skin (Ribeiro et al., 2015). Plants also constitute a variety of chemical compounds that elicit various pharmacological activities with the possibility that these compounds act synergistically to produce a net pharmacological effect (Abdillahi et al., 2011). Examples of such compounds include polyphenols and flavonoids. Polyphenols are widely distributed in plants, and several polyphenol types have been reported as being responsible for melanogenesis inhibition (Muddathir et al., 2017; Zhu and Gao, 2008). Flavonoids and chalcones are a group of polyphenols with flavonoids being one of the most explored and most numerous groups of polyphenols (Mapunya et al., 2011). Flavonoids are found within the leaves, seeds, bark, and flowers of most plants, and have been studied for its oxidation of L-DOPA and have shown good antityrosinase. Furthermore, chalcones exhibit a wide array of biological activities with a number of chalcones eliciting antityrosinase activity (Lee et al., 2016; Momtaz et al., 2008a; Ali, 2017).

The significant advancement of research using plant extracts in cosmetics demonstrates the growing interest of researchers and pharmaceutical companies in developing natural skin lightening products (Huang et al., 2009). The objective of this review was to examine existing literature to identify and document indigenous African plant species capable of inhibiting the enzyme TYR and melanogenesis for possible use as alternatives to current skin lightening formulations.

4.2. Methods

A computerized literature search was performed using the following databases: MEDLINE, SCOPUS, GOOGLE SCHOLAR, MEDLINE EBSCOHOST, and SCIENCE DIRECT databases.

In addition, the South African National Electronic Thesis Database (ETD) was searched for grey literature, which included Masters and Doctoral theses. The following key terms were used for the retrieval of articles in the databases: “skin lightening”, “tyrosinase”, “melanin”,

"antimelanogenesis", "antityrosinase", "melanogenesis", "tyrosinase inhibition", "melanin inhibition". For an article to be considered eligible, the following criteria needed to be met: (1) The use of indigenous African plant extracts (alone or in combination with other African plants); (2) performed *in vivo* and *in vitro* studies only; (3) investigated tyrosinase and melanogenesis inhibition. This literature search also had no restrictions on the following: Language; date of publication, and publication status (inclusive of published, unpublished, in the press and in progress). Studies that reported on both non-African (not indigenous to Africa and imported plants) and African plants were isolated, and only the African plants were included in this study. In addition, studies that included tests other than tyrosinase and melanin assays were isolated, and only the tyrosinase and melanin assays were reported on. All qualitative studies were excluded. Three independent reviewers completed the above-described methods independently. Any disagreements between the reviewers were discussed and resolved.

The articles that had been retrieved through the computerized literature searches were combined, giving a total of 128 articles. A preliminary analysis of the titles and abstracts of each article was performed, and all duplicates were excluded. After the screening of abstracts by at least 2 authors, the articles classified as ineligible based on the previously described criteria were excluded, and a total of 49 articles underwent a full-text review. After further application of the exclusion and inclusion criteria, a total of 36 articles were classified as eligible for discussion in this review.

4.3 Results

Tables 4.1 and 4.2 summarizes the plant species identified along with their melanin and tyrosinase results, respectively. In both tables, the plant names are arranged according to their family, along with the region the plants are found in Africa and plant part used.

In this study, 35 plant species distributed across 31 genera and 21 families were identified as being effective as TYR and melanogenesis inhibitors. In addition, the plants identified in this study were distributed among 15 African countries and 9 South African provinces. 17 (47.2%) were found in South Africa, with 19 (52.7%) found within other African countries. The most represented families were Fabaceae (5 plant species), Melianthaceae (3 plant species), Sapotaceae (3 plant species), Chenopodiaceae (2 plant species), Proteaceae (2 plant species), Clusiaceae (2 plant species), Rhizophoraceae (2 plant species), and Lamiaceae (2 plant species). The rest of the families were represented with only 1 plant species—Anacardiaceae, Apiaceae, Asteraceae, Brassiaceae, Capparaceae, Euphorbiaceae, Myrsinaceae, Pedaliaceae, Picrodendraceae, Poaceae, Podocarpaceae, Rubiaceae, and Thymelaeaceae.

Table 4.1. Summary of the plant species identified and their melanin results.

Family	Plant Name	Region	Part Used	Results	Reference
Anacardiaceae	*Harpephyllum caffrum*	SA (EC)	Leaves Bark	26% melanin inhibition at 6.25 µg/mL	(Mapunya et al., 2012)
Chenopodiaceae	*Arthrophytum scoparium*	TUN	Stems	52% melanin inhibition	(Chao et al., 2013)
Clusiaceae	*Garcinia livingstonei*	SA (KZN)	Bark	isolated compounds, <0.25 MC at 25 µg/mL	(Mulholland et al., 2013)
Lamiaceae	*Salvia officinalis*	EGY	Aerial parts	MC at 27% at 10, 20 and 40 µg/mL	(Sallam et al., 2016)
Melianthaceae	*Greyia flanaganii*	SA (EC)	Leaves	20% melanin inhibition at 6.25 µg/mL	(Mapunya et al., 2011)
Melianthaceae	*Greyia radlkoferi*	SA (MP)	Leaves	isolated compound,	(Lall et al., 2016)

				50% melanin inhibition at 12.5 µg/mL	
Myrsinaceae	*Myrsine africana*	SA (EC, FS, GAU, KZN, LP, MP, NW, WC)	Shoots	50% melanin inhibition at 50 µg/mL	(Kishore et al., 2018)
Myrsinaceae	*Myrsine africana*	SA (EC, FS, GAU, KZN, LP, MP, NW, WC)	Shoots	18% melanin inhibition at 12.50 µg/mL	(Momtaz et al., 2008a)
Pedaliaceae	*Sesamum angolense*	RWA	Leaves	cell pellets indicate no significant inhibition	(Kamagaju et al., 2013)
Picrodendraceae	*Hyaenanche globosa*	SA (WC)	Leaves Roots	–	(Momtaz et al., 2008a)

			Stems		
Proteaceae	*Protea madiensis*	NIG, ETH	Root bark	cell pellets indicates strong inhibition	(Kamagaju et al., 2013)
Proteaceae	*Serruria furcellata*	SA (WC)	Aerial parts	94.3% melanin inhibition at 50 µg/mL	(Sonka, 2018)
Rhizophoracea	*Cassipourea congoensis*	SEN, NIG, DRC, UGA, TZA, MLI	Roots	isolated compounds, <0.2 pg/mL MC at 10 µg/mL and 100 µg/mL	(Takou et al., 2019)
Rubiaceae	*Dolichopentas longiflora*	RWA	Leaves Roots	cell pellet indicate increase	(Kamagaju et al., 2013)
Sapotaceae	*Argania spinosa*	MAR	Fruits	55% melanin inhibition at 50 µg/mL	(Bourhim et al., 2018)

Sapotaceae	*Argania spinosa*	MAR	Fruits	>50% melanin inhibition at 1/100	(Villareal et al., 2013)
Sapotaceae	*Sideroxylon inerme*	SA (KZN)	Stem-bark	37% melanin inhibition at 6.2 µg/mL	(Momtaz et al., 2008b; Momtaz, 2007)
Sapotaceae	*Vitellaria paradoxa*	MLI, ETH, UGA	Fruit	Cameroon = 10.1% MC at 100 µg/mL Chad = 10.2% MC at 100 µg/mL Sudan = 10.9% MC at 100 µg/mL	(Zhang et al., 2018)
Thymelaeaceae	*Thymelaea hirsuta*	TUN	Leaves	> 50% melanin inhibition of melanin	(Kawano et al., 2007)
Thymelaeaceae	*Thymelaea hirsuta*	TUN	Leaves	isolated compound, 37% melanin inhibition at 0.1 µg/mL	(Miyamae et al., 2009)
Thymelaeaceae	*Thymelaea hirsuta*	TUN	Leaves	isolated compound,	(Villareal et al., 2010)

		50% melanin inhibition at 1 µg/mL isolated compound, 33% melanin inhibition at 0.1 µg/mL

This table indicates the melanin inhibition or MC (melanin content) at various concentrations of plant extracts (µg/mL). Provinces in South Africa (SA) - EC: Eastern Cape; FS: Free State; GAU: Gauteng; KZN: KwaZulu-Natal; LP: Limpopo; MP: Mpumalanga; NW: NorthWest; WC:Western Cape; Other African countries - ALG: Algeria; DRC: Democratic Republic of Congo; EGY: Egypt; ETH: Ethiopia; GHA: Ghana; IC: Ivory Coast; MAR: Morocco; MLI: Malawi; NIG: Nigeria; RWA: Rwanda; SEN: Senegal; SUD: Sudan; TUN: Tunisia; TZA: Tanzania; UGA: Uganda; - : not significant.

Table 4.2. Summary of the plant species identified and their tyrosinase results.

Family	Plant Name	Region	Part Used	Results (IC_{50} or Other Values)	Reference
Anacardiaceae	*Harpephyllum caffrum*	SA (EC)	Leaves Bark	92% inhibition of L-tyrosine at 500 µg/mL 60% inhibition of L-DOPA at 500 µg/mL IC_{50} = 40 ± 0.035 µg/mL	(Mapunya et al., 2012)
Anacardiaceae	*Hyaenanche globosa*	SA (EC)	Leaves Bark	L-DOPA at 500 µg/mL = 42% inhibition L-tyrosine at 500 µg/mL = 92% inhibition IC_{50} 27.1 ± 042 µg/mL	(Momtaz et al., 2008a)
Anacardiaceae	*Hyaenanche globosa*	SA (EC)	Leaves Bark	90.4% TYR inhibition at 200 µg/mL	(Momtaz et al., 2010)
Apiaceae	*Pituranthos scoparius*	TUN	Aerial parts	IC_{50} 125.01 ± 0.72 µg/mL using L-tyrosine IC_{50} 270.51 ± 0.76 µg/mL using L-DOPA	(Jdey et al., 2017)

Asteraceae	*Helichrysum niveum*	SA (WC)	Aerial parts	Isolated compound, 35.63 ± 4.67 µg/mL	(Popoola et al., 2015)
Brassiaceae	*Rorippa nasturtium-aquaticum*	SA (EC)	Leaves	IC_{50} 22.24 µg/mL	(Thibane et al., 2019b)
Brassiaceae	*Rorippa nasturtium-aquaticum*	SA (EC)	Leaves	IC_{50} 1.513 µg/mL	(Thibane et al., 2019a)
Capparaceae	*Cleome arabica*	TUN	Aerial parts	IC_{50} 124.4 ± 0.69 µg/mL L-tyrosine IC_{50} 243.43 ± 2.71 µg/mL using L-DOPA	(Jdey et al., 2017)
Chenopodiaceae	*Haloxylon articulatum*	ALG, MAR, TUN	Shoot	IC_{50} = 160 µg/mL using L-DOPA as substrate IC_{50} using L- tyrosine not significant	(Jdey et al., 2017)
Clusiaceae	*Garcinia kola*	ALG	Seed	79% TYR inhibition at 500 µg/mL	(Okunji et al., 2007b)

Euphorbiaceae	*Macaranga hurifolia*	NIG, GHA	Leaves Stem bark	Leaf extracts = 159.42 mg KAE/g Bark extracts = 160.42 mg KAE/g*	(Sadeer et al., 2019)
Fabaceae	*Ceratonia siliqua*	ALG	Leaves	crude extract, 50% TYR inhibition at 200 µg/mL isolate compounds, 90% TYR inhibition at 200 µg/mL	(Momtaz, 2007)
Fabaceae	*Ormocarpum trichocarpum*	SA (KZN, LP, MP)	Leaves Stems	IC_{50} 2.95 ± 1.76 µg/mL using L-tyrosine	(Stapelberg et al., 2019)
Fabaceae	*Rhynchosia villosa*	SA (EC, KZN, MP)	Root	56.40% TYR inhibition at 100 µg/mL	(Rondo, 2017)
Fabaceae	*Vachellia karroo*	SA	Roots	IC_{50} 6.84 µg/mL	(Stapelberg et al., 2019)

		(EC, FS, GAU, KZN, MP, NC, NW, WC)			
Fabaceae	*Acacia nilotica*	SUD SA (GAU, KZN, LP, MP, NW)	Pods	pod extract, IC_{50} 8.61 ± 0.94 µg/mL using L-tyrosine	(Muddathir et al., 2017)
			Bark	pod extract, 98.3% TYR inhibition at 500 µg/mL	
Fabaceae	*Acacia nilotica*	SUD SA (GAU, KZN, LP, MP, NW)	Pods	IC_{50} 12.97 ± 1.07 µg/mL	(Lall et al., 2019)
			Bark		
Lamiaceae	*Plectranthus ecklonii*	SA (EC, KZN, MP)	Aerial parts	IC_{50} 61.73 ± 2.69 µg/mL	(Nyila, 2011)

				>70% at 100 µg/mL	
Lamiaceae	*Plectranthus ecklonii*	SA (EC, KZN, MP)	Aerial parts	IC_{50} 21.58 µg/mL	(Ronauld, 2016)
Lamiaceae	*Salvia barrelieri*	ALG	Aerial parts	27% TYR inhibition at 1.5 mg/mL	(Lehbili et al., 2018)
Melianthaceae	*Greyia flanaganii*	SA (EC)	Leaves	95% TYR inhibition at 200 µg/mL Isolated compound, IC_{50} 17.86 µg/mL	(Mapunya and Lall, 2011)
Melianthaceae	*Bersama abyssinica*	IC	Leaves	148.94 mg KAE/g	(Sinan et al., 2021)
Melianthaceae	*Greyia radlkoferi*	SA (MP)	Leaves	IC_{50} = 17.96 µg/mL using L-tyrosine IC_{50} using L- DOPA not significant	(Lall et al., 2016)

Myrsinaceae	*Myrsine africana*	SA (EC, FS, GAU, KZN, LP, MP, NW, WC)	Shoots	IC_{50} 0.12 ± 0.001 mg/mL	(Kishore et al., 2018)
Myrsinaceae	*Myrsine africana*	SA (EC, FS, GAU, KZN, LP, MP, NW, WC)	Shoots	L-DOPA at 500 µg/mL = 62% inhibition L-tyrosine at 500 µg/mL = 83% inhibition IC_{50} 22.51 ± 0.42 µg/mL	(Momtaz et al., 2008a)
Myrsinaceae	*Myrsine africana*	SA (EC, FS, GAU, KZN,	Shoots	IC_{50} 27.4 µg/mL using L-tyrosine	(Stapelberg et al., 2019)

		LP, MP, NW, WC)			
Pedaliaceae	*Sesamum angolense*	RWA	Leaves	IC_{50} 24 µg/mL	(Kamagaju et al., 2013)
Poaceae	*Sorghum bicolor*	TUN	Stalk	40% TYR inhibition (in comparison to untreated control)	(Lee et al., 2017)
Podocarpaceae	*Podocarpus elongates*	SA (KZN)	Stems	74% TYR inhibition at 1 mg/mL EC_{50} = 0.14mg/mL	(Abdillahi et al., 2011)
Proteaceae	*Protea madiensis*	NIG, ETH	Root bark Leaves	31 ± 4 µg/mL	(Kamagaju et al., 2013)
Proteaceae	*Serruria furcellata*	SA (WC)	Aerial parts	95.49% TYR inhibition at 200 µg/mL 80.84% TYR inhibition at 50 µg/mL	(Sonka, 2018)

Rhizophoraceae	*Cassipourea congoensis*	SEN, NIG, DRC, UGA, TZA, MLI	Roots	crude extract, >80% TYR inhibition at 10 µg/mL and 100 µg/mL	(Takou et al., 2019)
Rhizophoraceae	*Cassipourea flanaganii*	SA (EC, KZN)	Bark	IC_{50} 22.24 ± 1.32 µg/mL	(Popoola et al., 2015)
Rhizophoraceae	*Cassipourea flanaganii*	SA (EC, KZN)	Bark	IC_{50} 1.425 µg/mL	(Thibane et al., 2019a)
Rubiaceae	*Dolichopentas longiflora*	RWA	Leaves Roots	IC_{50} 26 ± 2 µg/mL	(Kamagaju et al., 2013)
Sapotaceae	*Sideroxylon inerme*	SA (KZN)	Stem-bark	70% TYR inhibition at 200 µg/mL	(Momtaz et al., 2008b; Momtaz, 2007)

This table indicates TYR inhibition, EC_{50} (concentration at which the plant extract exhibits 50% of its maximum response) and IC_{50} (concentration at which half the original TYR activity is inhibited) values of plant extracts (µg/mL or KAE/g; KA equivalent per grams or mg/mL). Provinces in South Africa (SA) - EC: Eastern Cape; FS: Free State; GAU: Gauteng; KZN: KwaZulu-Natal; LP: Limpopo;

MP: Mpumalanga; NW: North West; WC: Western Cape; Other African countries - ALG: Algeria; DRC: Democratic Republic of Congo; EGY: Egypt; ETH: Ethiopia; GHA: Ghana; IC: Ivory Coast; MAR: Morocco; MLI: Malawi; NIG: Nigeria; RWA: Rwanda; SEN: Senegal; SUD: Sudan; TUN: Tunisia; TZA: Tanzania; UGA: Uganda

4.4 Discussion

African forests are the world's second-largest tropical reservoir holding very promising plant materials with various biological activities, which has attracted considerable research interest (Bourhim et al., 2018). Up to 90% of Africa's human population depends directly on traditional medicine. Plants form a central component of the African traditional healthcare system and is probably the oldest of all therapeutic systems (Bene et al., 2019; Elansary et al., 2015). The importance of this resource can be illustrated by the comprehensive list of African medicinal plants in which more than 5400 plant taxa and over 16,300 medicinal uses for the plants have been identified. The use of plant extracts as topical treatments has been practiced for many generations with extracts being used for the treatment of various skin ailments, including wounds, skin infections, and inflammation (Van Wyk, 2011; Leyden et al., 2011). The demand for cosmetic skin-lightening products is growing, with predictions particular to Asia and Africa forecasting the beauty industry to be worth an estimated $US 31.2 billion by 2024 (Kim et al., 2015; Mohiuddin, 2019). This significant increase can also be accounted for by the pleasant aromatics and the general consensus that plant extracts are safer than synthetic products available. Thus, there is an ever-growing endeavour to explore plant-based melanogenesis inhibitors (Jennifer et al., 2012; Parvez et al., 2007)

Various plant extracts and compounds have been investigated for their anti-tyrosinase and antimelanogenic effects (Lee et al., 2015). Three methods are extensively used to study tyrosinase activity, which includes 2 radiometric assays (tyrosinase hydroxylase and melanin formation activities) and one spectrophotometric assay (dopa oxidase activity). Tyrosinase hydroxylase assay estimates the tyrosinase hydroxylase activity of tyrosinase by measuring tritiated water released from L-[3,5-^{3}H]-tyrosine. The melanin formation activity assay estimates the radioactive melanin

synthesized from L-[U-^{14}C]-tyrosine while the dopa oxidase activity measures the rate of dopachrome formation, of which all three are in vitro assays (Jara et al., 1988). These assays also include the use of positive controls whose potencies are well-known, such as kojic acid (KA), to which the substance of interest can be compared (Faig et al., 2017). Results obtained from these assays are often presented in IC_{50} values, which refers to the concentration of plant extract at which half the original TYR activity is inhibited (Momtaz et al., 2008a).

As shown in the results described in Table 2, plants reported from the Fabaceae family were only tested for their ability as TYR inhibitors, and all proved to be strong inhibitors. Further results obtained by Lall et al., 2019, supported the findings for *Ormocarpum* and *Acacia nilotica*, which demonstrated the lowest IC_{50} value of 2.95 µg/mL and showed to have the highest TYR inhibition of 98.3% (IC_{50} 8.61 µg/mL), respectively (Muddathir et al., 2017; Stapelberg et al., 2019). *Cassipourea congoensis* demonstrated significant effects of both crude extract and isolated compounds on melanin and TYR activity, respectively (Takou et al., 2019). *Rorippa nasturtium-aquaticum* (Brassiaceae) showed in studies conducted by both Thibane et al., 2019b and Thibane et al., 2019a that the extract is an effective TYR inhibitor (IC_{50} values of 22.24 and 1.513 µg/mL respectively) when compared to the kojic acid (KA) control (19.38 and 1.421 µg/mL, respectively). It is also noted that KA is the most prominent (91.7%, 33 articles) positive control used in the studies identified, due to its well-established potency in literature (Faig et al., 2017). Arbutin, a HQ derivative, was the second most common (35.3%, 12 articles) used positive control as it is generally used in cosmetics as a hypopigmenting agent (Chang, 2012).

Studies on *Thymelaea hirsuta* (Thymelaeaceae) reported that this extract inhibited more than 50% of melanin at 1 µg/mL (Kawano et al., 2007). Furthermore, isolated compounds of this extract indicated that melanin production was reduced by 37% at 0.1 µg/mL, in comparison to its arbutin

control, which only inhibited 33% of melanin at a higher concentration—100 µg/mL (Miyamae et al., 2009). These results are further supported by Villareal et al. (Villareal et al., 2010), who concluded that isolated compounds of *Thymelaea hirsuta* at 0.1 µg/mL (33% reduction of MC) is as effective as arbutin, a common depigmenting agent, at 100 µg/mL. *Rhizophoraceae extracts* (*Cassipourea flanaganii and Cassipourea congoensi*s) exhibited compelling skin lightening properties with IC_{50} values obtained from studies conducted on *Cassipourea flanaganii*, which indicated values (1.425 µg/mL and 22.24 µg/mL, respectively) comparable to their KA controls (1.421 µg/mL and 19.38 µg/mL, respectively) (Sonka, 2018; Takou et al., 2019). *Argania spinosa* (Sapotaceae) effectively inhibited melanogenesis at 55% after 72 hours' exposure (Bourhim et al., 2018). These findings are supported by Villareal et al. [23], showing that there is a greater than 50% reduction in melanin content after 72 hours of exposure. Although significant results were obtained from separate studies, the difference in the result could be attributed to the researchers investigating different parts of the same plant (Table 1) as well as differences in plant preparation and assay protocols.

The plants in this study were distributed among 15 African countries with studies, including data from 9 South African provinces. Twenty plants species were investigated using aerial parts/leaves with these plants being collected in different regions of their respective countries and/or provinces. Thus, in natural ecosystems, factors affecting the plant's performance include climate, soil, and geographic locations yielding various molecular complexes, thus, emphasizing the environment's crucial role in the metabolism of plants (Zargoosh et al., 2019; Correia et al., 2008).

The results obtained from the TYR and melanin assays of *Harpephyllum caffrum* showed the bark extract to have the highest inhibitory effect on TYR and melanin production in comparison to the leaf extract of the same species (Mapunya et al., 2012). These results are further corroborated by

a review conducted by Lall and Kishore, 2014, where it was noted that *Harpephyllum caffrum* and *Greyia flanaganii*, among other listed plants, showed promising pharmacological activities, a finding that warrants further scientific investigation. Similar comparisons can be seen with *Ceratonia siliqua* concerning TYR activity where its isolated compounds (90% inhibition) were shown to be a more potent TYR inhibitor than its crude extract (50% inhibition) at the same concentrations (200 µg/mL).

Further comparisons can also be observed by the contrast in results obtained for TYR assays from the use of substrates L-DOPA and L-tyrosine. Here, several plant extracts have proven to be more effective in targeting the inhibition of the oxidation of either L-DOPA or L-tyrosine. This is illustrated by the TYR assay results obtained for *Haloxylon articulatum*, *Greyia radlkoferi*, *Pituranthos scoparius*, *Myrsine africana*, *Hyaenanche globose*, and *Cleome Arabica* (Jdey et al., 2017; Momtaz et al., 2008a; Lall et al., 2016). Additional studies also included extracts of *Dolichopentas longiflora*, where preparations exhibited a stimulatory response on melanogenesis, whereas the IC50 value for TYR activity (26 ± 2 µg/mL) showed contrasting results. This included *Sesamum angolense* of the same study, where pellets of the cells that were treated with the extract indicated no significant inhibition. However, the IC_{50} value (24 µg/mL) obtained indicated that the plant extract can illicit an inhibitory response (Rondo, 2017). Due to the complexity of pigment production, melanogenesis regulation takes place at different levels and various means of interference are possible—providing a possible explanation for the above-described contrasting results (Brenner and Hearing, 2008; Smit et al., 2009). Mechanisms of depigmenting include; (1) tyrosinase inhibition, (2) decrease in DOPA polymerase, (3) induction of anti-inflammatory, and (4) anti-oxidant effects (Fisk et al., 2014).

Extracts from the Lamiaceae family also proved to be effective inhibitors with *Plectranthus ecklonii* showing an IC_{50} value of 21.58 µg/mLwith more than 70% TYR inhibition and *Salvia offcinalis* decreasing MC to 27% at various concentrations (Sallam et al., 2016; Nyila, 2011; Ronauld, 2016). In addition, other plant extracts elicited a significant inhibitory response on both melanin and TYR activities. These include *Garcinia livingstonei* and *Garcinia kola* (Clustiaceae), *Myrsine africana* (Myrsinaceae), *Protea madiensis*, and *Serruria furcellata*—both from the Proteaceae family and *Sideroxylon inerme* (Sapotaceae). Species from other families such as Clustiaceae (*Garcinia livingstonei* and *Garcinia kola*) exhibited significant activities with *G. livingstonei* exhibiting a large decrease of melanin concentration at 25 µg/mL and the seeds of *G. kola* inhibiting 79% of tyrosinase at 500 µg/mL (Mulholland et al., 2013; Okunji et al., 2007a).

4.5 Conclusion

Several studies have been conducted to identify inhibitors from both natural and synthetic sources, and a number of research papers have been published and regularly updated in this aspect. This study was conducted as a means of identifying plant-based skin lightening alternatives to the current toxic substances. Despite the serious and life-threatening complications associated with the chronic use of these products, the use of skin lighteners is still a widespread and common practice in several African countries (Dlova et al., 2012; Olumide, 2010).

All plants identified in this study showed competent antimelanogenesis and antityrosinase capabilities, with the most effective of the extracts being the following: *Acacia nilotica*, *Cassipourea congoensis*, *Cassipourea flanaganii*, *Garcinia kola*, *Greyia flanaganii*, *Greyia radlkoferi*, *Hyaenanche globosa*, *Myrsine africana*, *Ormocarpum trichocarpum*, *Plectranthus ecklonii*, *Protea madiensis*, *Rorippa nasturtium-aquaticum*, *Serruria furcellata*, *Sesamum angolense*, and *Vachellia karroo*. The reproducibility of the identified studies and interpretation of

the results is limited by the inconsistencies in methodologies and means of plant extraction in these studies. Other variables also include geographical location and varied climate regions.

This review shows that plants of the African continent have the potential to act as melanin and TYR inhibitors and can be used to replace synthetic and other derived chemicals. Although many of these plants have been effective in their pigment reduction properties, plants are still known to cause allergic reactions and elicit phototoxic effects (Fisk et al., 2014). This is due to natural products being a complex mixture of chemical compounds, a fact often unknown to consumers. To combat this, extracts should be chemically characterized with respect to the product composition (Ribeiro et al., 2015). In addition, it is imperative that toxicity studies be conducted to establish a safe dose range. These findings could aid in the production and commercialization of these plants in natural-based remedies for cosmetic and skincare product industries.

4.6 References

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Chapter 5

The *in vitro* effect of *Harpephyllum caffrum* and *Salvia officinalis* on tyrosinase and melanin function

5.1 Introduction

Melanin is the primary determinant of skin colour and is produced via melanogenesis, a process that takes place within melanosomes located in melanocytes (Damodaran and Nair, 2023, Li et al., 2022). External factors such as UV light and inflammatory stimuli influence melanin production (Ding et al., 2020). However, the most crucial of all melanogenesis-associated factors is the enzyme TYR that initiates melanogenesis via the conversion of L-tyrosine to dopaquinone (Roulier et al., 2020). Irregularities during this process can lead to either a decrease in melanin production (hypopigmentation) causing disorders such as albinism and vitiligo, or an increase in melanin synthesis (hyperpigmentation) leading to disorders of like melasma, age spots or post-inflammatory inflammation (Lambert et al., 2019, Thawabteh et al., 2023).

Several options are available to treat pigmentation conditions which are primarily administered through topical creams and/or oral medications (Thawabteh et al., 2023). Hyperpigmentation conditions treated with topical creams comprise chemicals such as hydroquinone, arbutin, kojic acid (KA), niacinamide and retinoids. These treatments can be directly applied to the affected area but are often misused for the cosmetic purpose of SL and practiced without the supervision of a medical professional (Lambert et al., 2019, Tesfamariam et al., 2023, Yasir et al., 2018, Yélamos et al., 2021, Lee et al., 2023). Several of these SL products are also available from many sources worldwide including informal markets and online stores (Jain et al., 2012, Rosen and Givens, 2022). Chandorkar et al. (2021) reports that many of these non-registered products are advertised

under the pretense of ‘skin brighteners’ or ‘skin toners’ and have not been subjected to the appropriate safety and efficacy studies. They contain toxic chemicals that, with chronic use, lead to severe adverse events which include topical damage to the skin, damage to the kidneys and the nervous system to highlight a few, emphasizing the need for safer alternatives (Eagle et al., 2014, Rosen and Givens, 2022, Ricketts et al., 2020). As such, there is an increase in the demand for natural ingredients to be incorporated into dermatological-cosmetic products as they offer fewer side effects and can be cost effective (Omar et al., 2021, Hanif et al., 2020).

Africa is a large biodiversity resource that holds an estimated 40 000 to 45 000 plant species of which 5 000 species are utilized medicinally (Mahomoodally, 2013). The use of African plants for aromatic and medicinal purpose is widespread and has been practiced for many generations. A large variety of plants have been applied in the treatment of various skin ailments as they comprise various bioactive compounds that possess several properties including antifungal and antibacterial (Mapunya et al., 2011, Okigbo et al., 2008, Sitarek et al., 2020). Studies conducted by Abd El-Ghani (2016), Agyare et al. (2016) and Cock and van Vuuren (2020) documented African plants and their various medicinal uses. Similar evidence was also collected based on an in-depth review of literature previously conducted (Chapter 4) in which several plant extracts were documented to have exhibited melanin and enzyme TYR inhibitory properties (Opperman et al., 2020). Thirty-five African plants species where identified and *H. caffrum* and *S. officinalis* and, as such, were then selected for further investigation in this study based on their commercial availability within the country.

H. caffrum is a plant from the family Anacardiaceae, commonly known as ‘wild plum’ or ‘umgwenya’ (Moodley et al., 2013). Its natural distribution is restricted to southern Africa ranging from the Eastern Cape and is a large evergreen tree that grows up to 15 meters tall with the tree

bark being popularly used in traditional medicine (Dlamini, 2004, Gericke, 2018, Moodley et al., 2014). Cosmeceutical utilization of this plant includes topical application for the treatment of acne and eczema and plant leaves prepared for use as a face mask. In addition, other studies conducted to evaluate the plant`s pharmacological properties show that bark extracts hold antifungal and antibacterial properties (Sagbo and Mbeng, 2018, Buwa and Van Staden, 2007, Mapunya et al., 2012). To our knowledge, only one study has reported on the effect of *H. caffrum* on melanin and TYR activity. In this study, Mapunya et al. (2012) found that both its leaf and bark extracts are potential inhibitors. In addition, the leaf extract showed to be more effective in inhibiting tyrosinase but also proveds to be cytotoxic to mouse melanomas. In contrast, the bark extract showed was more effective in reducing melanin production without being toxic to the cells and was therefore recommended as an alternative SL agent.

S. officinalis, also known as common or true sage, is known as a culinary spice (Nair, 2023). Its origins are reportedly from the Mediterranean and Northern African zones including Egypt, but it`s growth has been globally naturalized (Said-Al Ahl et al., 2015, Mazarie et al., 2019, Ghorbani and Esmaeilizadeh, 2017). Its cosmeceutical uses include lotions to improve the condition of hair and skin and its medical purposes include treatment of body wounds (Mapunya and Lall, 2011, Paun et al., 2017). Garcia et al. (2012) also signified that *S. officinalis* has antiseptic, anti-inflammatory and antioxidant properties. Data related to its SL properties are limited. However, studies by Juee (2022), Lianza et al. (2020), Oliveira et al. (2013) and Sallam et al. (2016) have shown that *S. officinalis* does indeed inhibit melanin and TYR function. Juee (2022) showed that the methanolic extract of *S. officinalis* had the same tyrosinase inhibitory efficacy when compared to the kojic acid control. Furthermore, Oliveira et al. (2013) showed that *S. officinalis* increased melanin production in a concentration-dependent manner without affecting TYR activity.

Although none of these studies tested the cytotoxic effects of the extract, these results still provide valuable insights to its potential as an SL alternative.

Although studies have been conducted to determine the skin lightening properties of *H. caffrum* and *S. officinalis*, there remains little to no anecdotal evidence reporting their potential combined effects (Mapunya et al., 2012, Momtaz et al., 2008, Sallam et al., 2016). Kanthraj (2010) has stated that therapies used in combination could result in increased efficacy in comparison to therapies used individually. Jeong et al. (2023) further described that plants used in combination could lead to increased pharmacological action by simultaneously acting on several targets. This combination could cause the use of a lower overall dose of the extracts and potentially minimize the side effects.

Studies have been conducted to emphasize the efficacy of combination plant extracts such as that of Otieno et al. (2008) and García-Muñoz et al. (2023) which emphasized the benefits of combination extracts against infectious diseases and metabolic diseases, respectively. In relation to the use of combination extracts on skin, Bhagavathula et al. (2009) showed that the joint use of curcumin and ginger extract on abrasion wounds in rat skin facilitated an accelerated healing process in comparison to rats treated with a topical corticosteroid. Furthermore, Ha and Le (2023) showed that the ethanolic extracts of *Psidium guajava* L. and *Syzygium samarangense* produced optimal anti-tyrosinase activity when combined in a 1:1 ratio.

Although available evidence highlight the benefits associated with the use of combined extracts, research related to the SL effects of combined plant extracts is also limited. Thus, the aim of this study was to determine whether the individual and combined effects of *H. caffrum* and *S. officinalis* holds potential in decreasing melanogenesis and TYR function without being cytotoxic to skin cells.

5.2 Methodology

5.2.1 Plant extracts

The commercially prepared hydro-ethanolic bark extract *Harpephyllum caffrum* (80% w/v) was purchased from Zuplex Botanicals (South Africa) and hydro-ethanolic leaf extract *Salvia officinalis* (62% w/v) was gifted from Parceval Pharmaceuticals (South Africa). For the cell culture experiments, the hydro-ethanolic extracts were first tested individually using a 200 mg/mL stock solution which was generated by diluting each extract in RPMI 1640 medium (Cat no. 21875034, ThermoFisher Scientific, South Africa) supplemented with 10% heat-inactivated foetal bovine serum (HI-FBS) (Cat no. 12389962, Hyclone, Little Chalfont, UK), antibiotic (penicillin (100 U/mL), streptomycin (100 mg/mL) (GIBCO, NY, USA) and stored at 4˚C. Following this, extracts were combined in a 1:1 ratio as shown by evidence provided by Diwakar et al. (2012) and Diwakar et al. (2014) which reported that plant extracts combined in this ratio was most effective in inhibiting melanin production in comparison to extracts tested individually. Thus, to also ensure the combined extract was tested at the same stock concentration as the individual extract stock solutions in this study, equal volumes (1:1 v/v) of *Harpephyllum caffrum* and *Salvia officinalis* extracts were combined and further diluted in supplemented RPMI 1640 medium to generate a 200mg/mL stock solution.

5.2.2 Cell line

The murine tumor cell line, B16 melanoma, was a gift from the Radiation Biophysics Division of iThemba LABS (Cape Town, South Africa). This cell line was selected due to their common use for this purpose as they produce melanin and contain melanogenesis-associated tyrosinase, similar to that of the melanogenic mechanisms of normal human melanocytes (Takahashi et al., 2012, Oyekunle, 2019). In addition, they are relatively easy to culture *in vitro* (Yan et al., 2014).

The cells were seeded and propagated in complete RPMI 1640 medium supplemented with 10% heat-inactivated foetal bovine serum (HI-FBS) (Cat no. 12389962, Hyclone, Little Chalfont, UK), antibiotic (penicillin (100 U/mL) and streptomycin (100 mg/mL) (GIBCO, NY, USA) and maintained in a humidified incubator with 5% CO_2 at 37 °C. Treatments were carried out 24 h after plating to allow for attachment and cells were subsequently treated with optimized concentrations of 100, 200, 400, 600 and 800 µg/mL of the plant extract for 24, 48 and 72 h.

5.2.3 Determination of cell proliferation and viability

The crystal violet (CV) assay was used to determine the effects of the ethanolic extracts on cell proliferation and viability as previously described (Saotome et al., 1989). Cell proliferation is determined via the triarylmethane dye that binds to ribose molecules such as DNA in nuclei. The B16 cells were seeded into 96-well tissue culture plates (Greiner Bio-one, Kremsmunster, Austria) at a density of 1×10^4 cells/well and allowed to adhere overnight. The cells were treated with the various concentrations of the plant extracts for 24, 48 and 72 h. Cells without the extract treatments served as a negative control. A similar study conducted by Oyekunle (2019) on B16 cells used 6% Dimethylsulphoxide (DMSO) (Sigma Aldrich, Germany) as a positive control which showed to be effective in reducing cell viability. Thus, in the current study, cells treated with 6% DMSO served as a positive control. At the end of each period, the cell proliferation levels were assessed via CV assay (stock 0.1%: PBS) with 0.2% Triton-X 100 as the solubilizing agent and detected using a microplate spectrophotometer reader (Biochrom EZ Read 400 Microplate Reader, Cambridge, United Kingdom) at 570 nm.

5.2.4 Determination of cytotoxicity

To evaluate the cellular cytotoxicity, the lactate dehydrogenase (LDH) assay was used. This assay determines the release of the LDH enzyme which is released into the cell culture when the plasma

membrane is damaged (Kumar et al., 2018). LDH activity was measured using an LDH-cytotoxicity kit (Cat no. 11644793001, Roche Diagnostics, Mannheim, Germany) and was performed as per the manufacturer's instructions. Briefly, the B16 cells were seeded in 96-well plates at a density of 0.5×10^4 cells/well. After 24 h, various concentrations of the plant extracts were added, and the cells were incubated for 24, 48 and 72 h. At the end of each time point, the LDH activity was measured, and the absorbance was read at 490 nm using a microplate reader (Biochrom EZ Read 400 Microplate Reader, Cambridge, United Kingdom).

5.2.5 Melanin content assay

The experiment was performed according to the technique previously described by Yan et al. (2022) with slight modifications. The B16 cells were seeded into 96-well tissue culture plates (Greiner Bio-one, Kremsmunster, Austria) at a density of 0.25 x 10^4 cells/well and allowed to adhere overnight. The cells were treated with the various concentrations of the three plant extracts for 24, 48 and 72 h. Cells without extract treatments served as a negative control. A similar study conducted by Oyekunle (2019) on B16 cells used Kojic Acid (KA) as a positive control. In this study, KA tested at 300 µg/mL was most effective in reducing melanin content without being cytotoxic to the cells. Thus, in the current study, cells treated with 300 µg/mL of KA (Sigma Aldrich, Germany) served as a positive control. At the end of each time period, cells were washed with phosphate buffered solution (PBS) and solubilized with 1mol/L NaOH and incubated at 37˚C for 60 min. The melanin content was evaluated using a microplate spectrophotometer reader (Biochrom EZ Read 400 Microplate Reader, Cambridge, United Kingdom) at 492 nm.

5.2.6 Measurement of TYR activity

Tyrosinase activity was measured as previously described by Yan et al. (2022) with slight modifications. The B16 cells were seeded into 96-well tissue culture plates (Greiner Bio-one,

Kremsmunster, Austria) at a density of 0.25 x 10^4 cells/well and allowed to adhere overnight. The cells were treated with the various concentrations of the three plant extracts for 24, 48 and 72 h. Cells without extract treatments served as a negative control and cells treated with 300 µg/mL of KA was used as a positive control. At the end of each time period, cells were washed with phosphate buffered solution (PBS) and lysed with 1% Triton X-100. Plates were then shaken for 15 min after which 1% 3, 4-Dihydroxy-L-phenylalanine (L-DOPA) (Sigma Aldrich, Germany) solution was added to each well and incubated at 37˚C for 120 min. The tyrosinase activity was evaluated using a microplate spectrophotometer reader (Biochrom EZ Read 400 Microplate Reader, Cambridge, United Kingdom) at 492 nm.

5.2.7 Cellular morphological changes

To evaluate the cellular morphological changes occurring after the B16 cells were treated with the extracts, a haematoxylin and eosin (H&E) cell stain was conducted as previously described (Vorster et al., 2012). Coverslips were soaked in ethanol, flamed then placed in 6-well plates. Cells were seeded onto coverslips at a density of 25×10^4 cells per well and allowed to attach overnight. Cells were subsequently exposed to the different concentrations of the plant extracts and appropriate controls respectively for 24, 48 and 72 h, after which coverslips were fixed with Bouin's fixative (30 min) and 70% ethanol (20 min). Coverslips were rinsed with water, stained with haematoxylin (20 min), rinsed with water and 70% ethanol (20 min) after which they were stained with eosin (2 min). Coverslips were then dehydrated stepwise with ethanol (70%, 90%, 100%) and xylene, mounted on microscope slides with Dibutylphthalate Polystyrene Xylene (DPX) mountant (Cat no. 44581, Sigma-Aldrich, St. Louis, USA) and dried overnight. Qualitative evaluation (40 × magnification) was conducted using the 360 4i Nikon (Tokyo, Japan) microscope.

5.3 Statistical analysis

Statistical data processing was performed using GraphPad Prism software version 10.0.0 for Windows (Boston, Massachusetts, USA). The data was presented as mean and standard error of mean from triplicate measurements. One-way analysis of variance (ANOVA) was used to evaluate statistical significance of differences among groups. Statistical significance was determined in comparison to either the negative or positive control and was accepted at the level of $p < 0.05$.

5.3 Results

5.3.1 Effect of the plant extracts on cell viability and cell proliferation

Figure 5.1A shows that *S. officinalis* displayed a significant ($p < 0.05$) decrease in cell viability in comparison to the negative control across all concentrations at most of the exposure times, except after 24-hour treatment at concentrations 200, 400, 600, 800 µg/mL. The extract elicited a response that decreased the cell viability in a time- and dose-dependent manner. This trend can be observed specifically across concentrations 100 and 400 µg/mL, where the extract caused outspoken decreases in cell viability at 400 µg/mL with a 9.6% and 10% decrease after longer exposure at 48- and 72-hour exposure. *H. caffrum* and the combined extract (Figure 5.1B and C, respectively) displayed similar trends as that of *S. officinalis* in which the cell viability decreased in a time- and dose-dependent manner in comparison to the negative control. Results obtained after treatment with *H. caffrum* displayed a statistically significant decrease at 100 and 400 µg/mL in which the largest decrease (4.4%) in cell viability is observed after 48-hour treatment at the lowest concentration (100 µg/mL). The combined extract did not have any significant effects on the cell viability of the B16 cells.

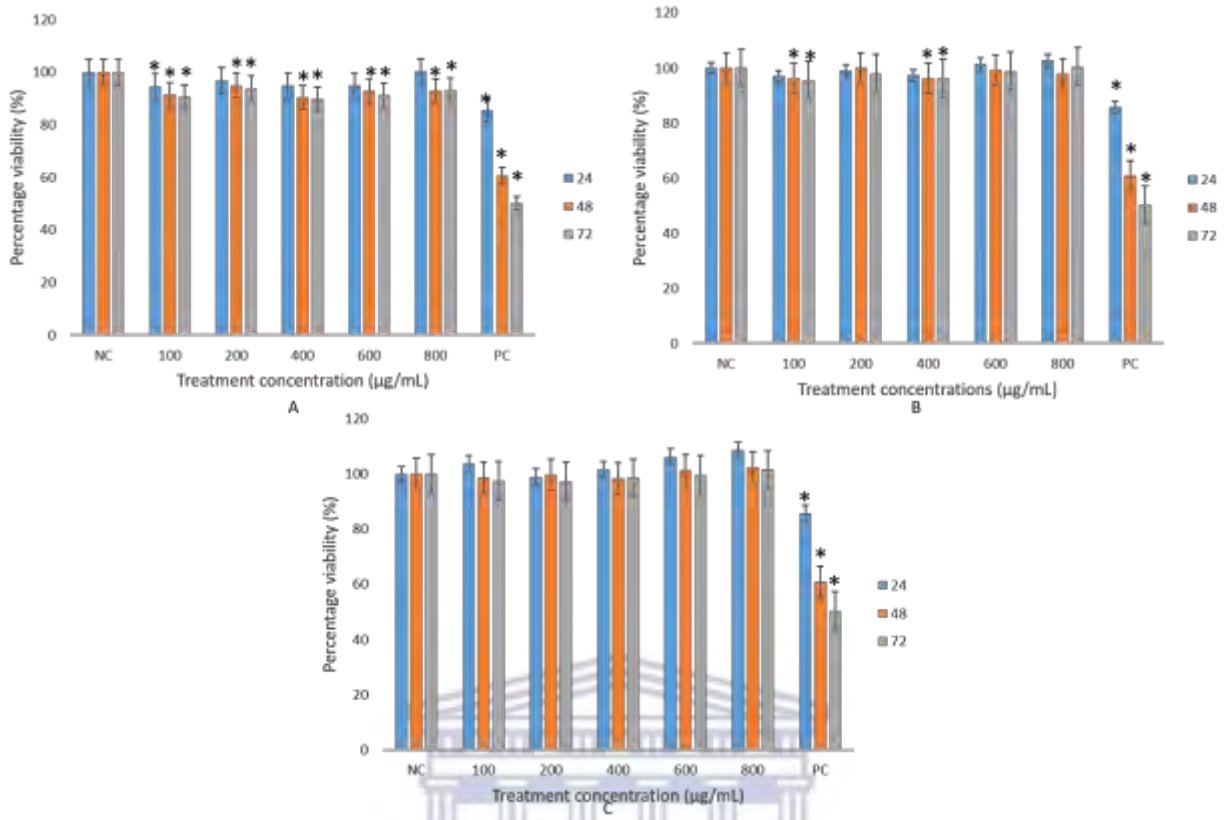

Figure 5.1. The cell viability of murine B16 melanoma cells treated with 100, 200, 400, 600 and 800 µg/mL concentrations of *Salvia officinalis* (A), *Harpephyllum caffrum* (B) and the combined extract (C) for 24, 48 and 72 hours determined using a crystal violet assay. NC: negative control - untreated cells with media; PC: positive control - cells treated with 6% DMSO. Data represent the mean ± SEM, n = 3, where * indicates statistical significance at level $p < 0.05$ compared to the NC.

5.3.2 Effect of the plant extracts on cell cytotoxicity

The results from the LDH assay on the B16 cells are displayed in Figure 5.2 (A, B and C). These results confirmed that all three selected extracts exhibited negligible cytotoxicity towards the B16 cells after exposure to the various dosages at all times compared to the negative control. The only significant ($p < 0.05$) increase in cytotoxicity was observed in the positive control at all exposure times.

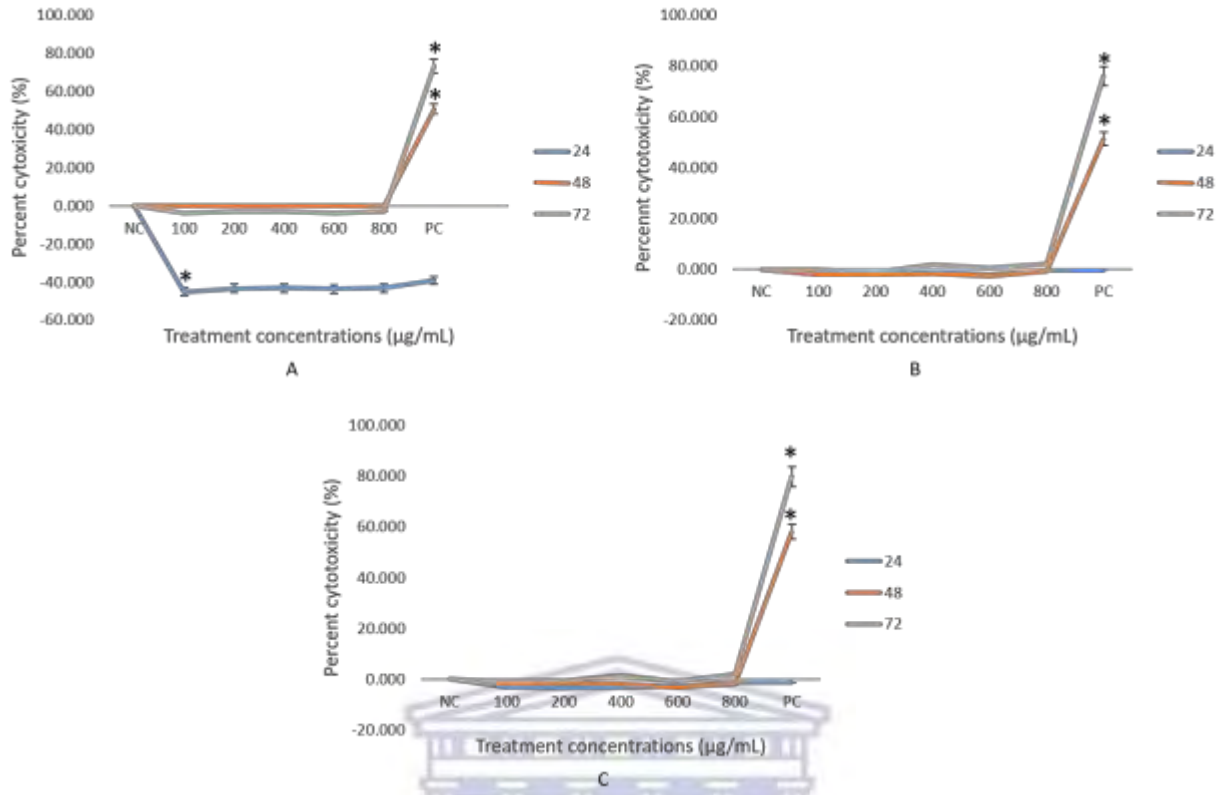

Figure 5.2. The cell cytotoxicity of murine B16 melanoma cells treated with 100, 200, 400, 600 and 800 µg/mL concentrations of *Salvia officinalis* (A), *Harpephyllum caffrum* (B) and the combined extract (C) for 24, 48 and 72 hours determined using a lactate dehydrogenase (LDH). NC: negative control - untreated cells with media; PC: positive control - cells treated with 6% DMSO. Data represent the mean ± SEM, n = 3, where * indicates statistical significance at level $p < 0.05$ compared to the NC.

5.3.3 Effect the plant extracts on melanin content

S. officinalis inhibited the melanin content of the cells in time- and dose-dependent manner (Fig 5.3A). A significant decrease ($p < 0.05$) was observed at the lowest concentration (100 µg/mL) after 48- and 72-hour exposure with the melanin content at 92% and 90.2%, respectively compared to the negative control. In addition, when compared to the negative control, significant ($p < 0.05$) decreases in melanin content can be observed after 48-hour exposure at concentrations 400 µg/mL and the largest decrease (10.7%) after 72-hour exposure at 800 µg/mL. Results show that, in

comparison to the negative control, the positive control (KA at 300 µg/mL) displayed a significant ($p < 0.05$) time-dependent response in which the melanin content decreased with 13%, 15% and 25% after 24, 48 and 72-hour exposure, respectively. In contrast to the trend observed with KA exposure, *H. caffrum* (Figure 5.3B) displayed a stimulatory effect across all concentrations after 24-hour exposure in which there was a 5.7%, 7.9%, 7.6%, 14.31% and 15.7% increase in melanin content, respectively. Statistically significant increases were observed ($p < 0.05$) only after treatment with the higher concentrations (400, 600 and 800 µg/mL) after 24- and 48-hour treatment. The combined extract (Figure 5.3C) elicited similar effects as that of *H. caffrum* in which a stimulatory effect was observed in comparison to the effects of the positive control after 24-hour exposure across all treatment concentrations in which there was also an outspoken significant increase at 800 µg/mL (12.78%). The combined extract then displayed statistically significant inhibitory effects after the longer exposure time (72-hours) at the highest concentration (800 µg/mL). Although significant effects of the extracts were observed in comparison to the untreated negative control, comparisons to the known efficacy of the positive control show that *S. officinalis* have potential melanin inhibitory capabilities.

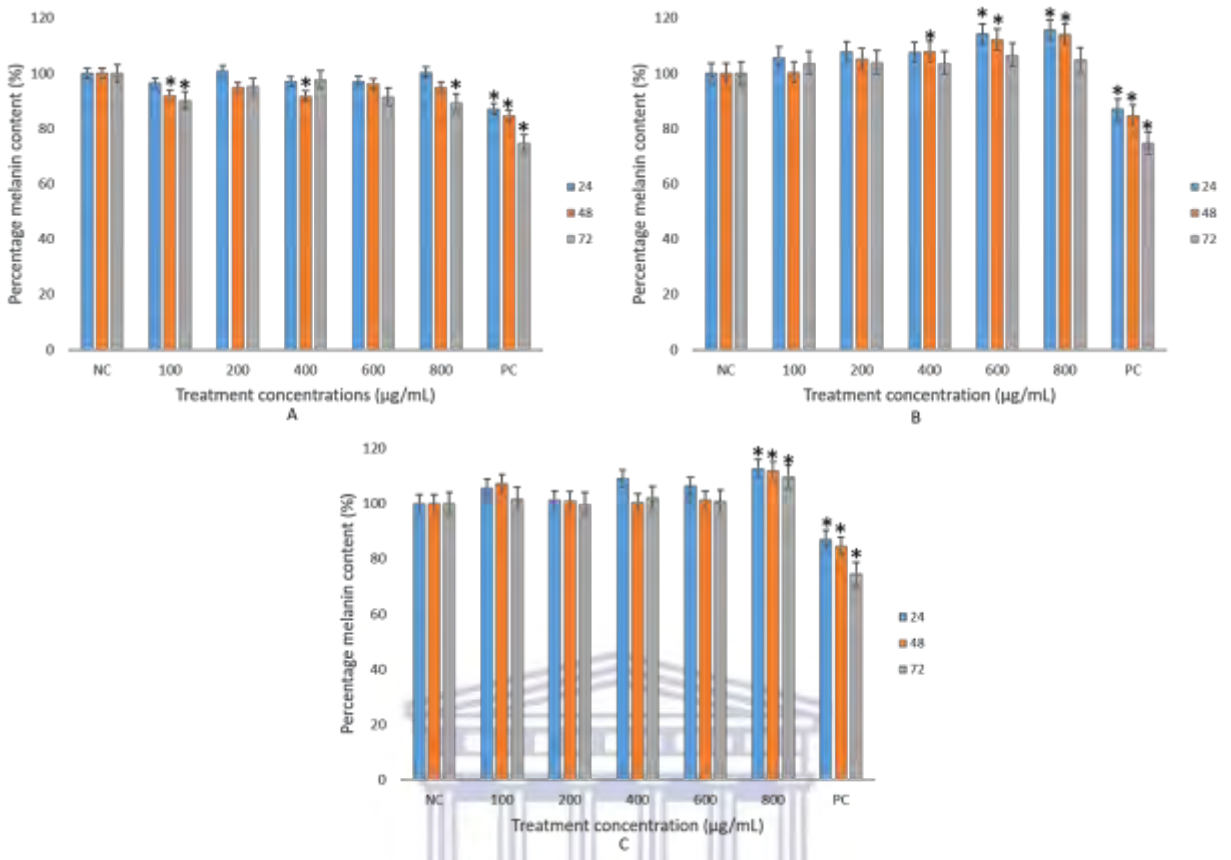

Figure 5.3. The melanin content of murine B16 melanoma cells treated with 100, 200, 400, 600 and 800 µg/mL concentrations of *Salvia officinalis* (A), *Harpephyllum caffrum* (B) and the combined extract (C) for 24, 48 and 72 hours determined using a melanin content assay. NC: negative control - untreated cells with media; PC: positive control - cells treated with 300 µg/mL KA. Data represent the mean ± SEM, n = 3, where * indicates statistical significance at level $p < 0.05$.

5.3.4 Effect of the plant extracts on TYR activity

S. officinalis had no significant effect on TYR activity after 24-hour treatment at the lowest concentration (100 µg/mL). However, a significant ($p < 0.05$) inhibitory effect was observed after longer exposure of 72 hours at 200 (15.3% reduction) and 400 µg/mL (7% reduction), with the largest reduction observed at 600 µg/mL showing a 15.5% decrease in TYR activity. In comparison to the negative control, *H. caffrum* significantly stimulated TYR activity at the higher

concentrations (600 and 800 µg/mL) after 24-hour exposure in which there was a 7.9% and 12.4% increase. However, the extract displayed significant inhibitory effects after the longer exposure with the largest decrease in TYR activity observed after 72-hour treatment with 600 µg/mL of *H. caffrum*. In addition, the combined extract (Figure 5.4C) stimulated TYR activity at 100 µg/mL after 24-hour treatment where significant ($p < 0.05$) increases were observed at 200, 400 and 800 µg/mL, respectively. The combination extract significantly ($p < 0.05$) decreased TYR activity after longer exposure times (48- and 72 hours) at concentrations 200, 400, 600 and 800 µg/mL. The largest inhibition of TYR activity (12.9%) after treatment with the combined extract was observed after 72-hour treatment at 800 µg/mL. Compared to the negative control, KA tested at 300 µg/mL had a time-dependent inhibitory response in which there was a decrease of 10%, 12% ($p < 0.05$) and the largest decrease of 26% ($p < 0.05$) across 24, 48 and 72-hours, respectively. Although significant inhibitory effects of the extracts were observed in comparison to the negative control, all extracts exhibited inhibitory effects comparable to that of the known positive control used in this study, KA.

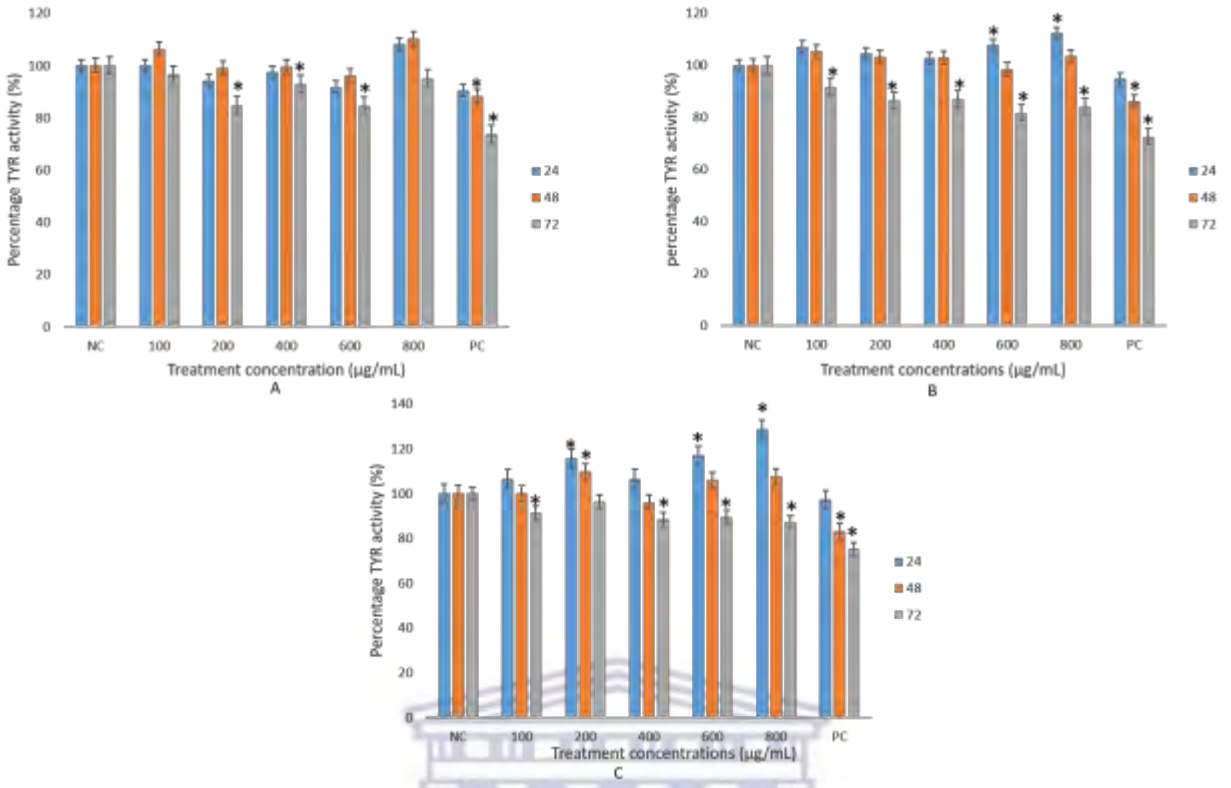

Figure 5.4. The TYR activity of murine B16 melanoma cells treated with 100, 200, 400, 600 and 800 µg/mL concentrations of *Salvia officinalis* (A), *Harpephyllum caffrum* (B) and the combined extract (C) for 24, 48 and 72 hours. NC: negative control - untreated cells with media: positive control - cells treated with 300 µg/mL KA. Data represent the mean ± SEM, n = 3, where * indicates statistical significance at level $p < 0.05$.

5.3.5 Effects of the plant extracts on cell morphology

Qualitative analysis by H&E staining revealed little to no morphological changes in response to the three extracts (*S. officinalis*, *H. caffrum* and the combined extract) after exposure to the B16 cell line at the various concentrations (Figure 5.6). The results of the H&E staining further validated the data obtained from the CV and LDH assays in that the figures displayed mitotically active cells with intact nuclei and cytoplasmic properties across these concentrations and exposure

times. Apoptotic characteristics (hypercondensed chromatin, membrane blebbing) were only observed in samples of the positive control after 24, 48 and 72 h.

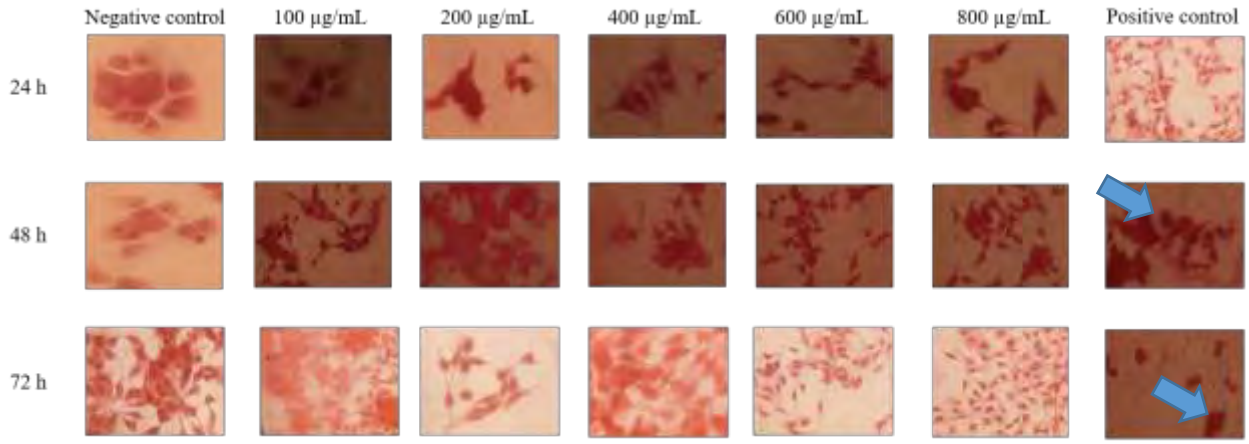

Figure 5.5. Cellular morphology of the B16 melanoma cells after treatment with 100, 200, 400, 600 and 800 µg/mL concentrations of *S. officinalis*. Negative control - untreated cells with media; Positive control: cells treated with 6% DMSO. After treatment, the cells were stained with H&E and the photographs taken at 40x. Arrows show irregularly shaped cells.

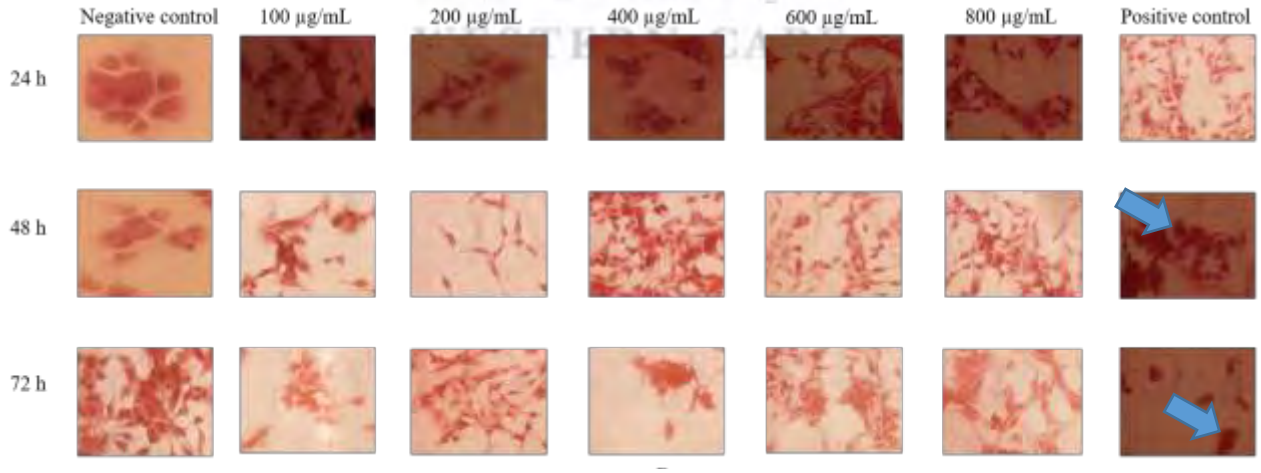

Figure 5.6. Cellular morphology of the B16 melanoma cells after treatment with 100, 200, 400, 600 and 800 µg/mL concentrations of *H. caffrum*. Negative control - untreated cells with media; Positive control:

cells treated with 6% DMSO. After treatment, the cells were stained with H&E and the photographs taken at 40x. Arrows show irregularly shaped cells.

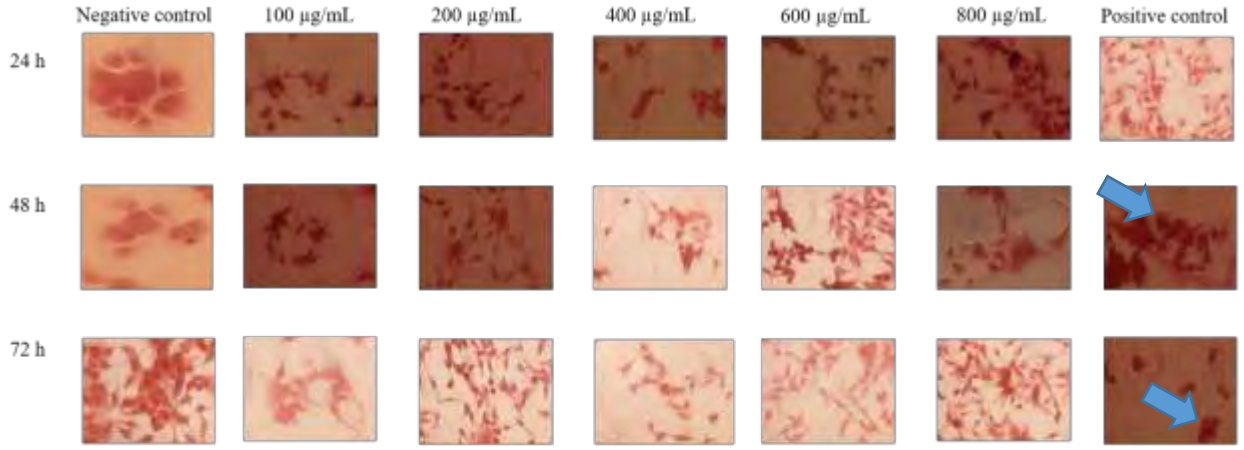

Figure 5.7. Cellular morphology of the B16 melanoma cells after treatment with 100, 200, 400, 600 and 800 µg/mL concentrations of the combined extract. Negative control - untreated cells with media; Positive control: cells treated with 6% DMSO. After treatment, the cells were stained with H&E and the photographs taken at 40x. Arrows show irregularly shaped cells.

5.4 Discussion

The cosmetic industry is a constantly changing market that has a large consumer base with several skin care needs that continuously motivates an industry and its associated companies to provide innovative and new products (Dubey et al., 2022). These products serve various purposes including cleansing, moisturizing, anti-aging as well as skin lightening (Dey and Dubey, 2023). SLs exhibit their efficacy on the skin via several mechanisms including tyrosinase inhibition, melanocyte loss and interference with melanosomal maturation and transfer (Kamakshi, 2012). However, these products are related to various safety concerns associated with the chronic use of their ingredients and thus, several studies have explored alternative options using plant extracts such as *S. officinalis* and *H. caffrum* (Kamakshi, 2012, Mapunya et al., 2012, Sallam et al., 2016). Previous reports, such as that of Madhogaria and Ahmed (2010) has stated that SLPs are often combined to increase their efficacy by more than one mechanism, therefore, achieving a maximum SL effect. Thus, the present study reports on the concentrations of combined extracts on melanin production and TYR activity while also indicating the concentrations that were non-cytotoxic to a skin cell model using B16 cells.

In this study, *S. officinalis* showed to be most effective in reducing the melanin content of B16s at highest concentrations (800 µg/mL) after longer exposure (72 hours) in comparison to the negative control. Similar evidence was reported by Oliveira et al. (2013) in which the extract displayed a concentration-dependant inhibitory response to the melanin production of B16s, in which the highest decrease was observed at the highest concentration tested. *H. caffrum* displayed stimulatory effects across all concentrations and exposure times but only displayed significance after longer exposure times (48- and 72-hour) at the higher concentrations. These results are contrary to that reported by Mapunya et al. (2012) in which melanin production decreased after

treatment with higher dosages. In comparison to the combined extract, the effect showed to be negligible across most concentrations, except at 800 µg/mL across all exposure times. At these concentrations, the combined extract elicited an increase in melanin content after short exposure (24 hour) after which there was significant decrease after 48 and 72-hour exposure. Although none of the extracts had an inhibitory effect stronger than that of the positive control, the results still suggest that *S. officinalis* and *H. caffrum* elicit optimum melanin inhibitory effects at higher dosages after longer exposure times.

The results obtained to assess TYR activity after treatment with the combined extract showed similar trends as that obtained for the melanin content assay. An increase in TYR activity was observed after the shortest exposure time (24-hour) at all treatment concentrations and a subsequent decrease in TYR activity after longer exposure times (48- and 72-hour). In contrast to the other extracts tested, TYR activity after treatment with *S. officinalis* and *H.caffrum* showed a different trend in comparison to the results obtained for the melanin content assay after treatment with the same extract. For the evaluation of TYR activity, *S. officinalis* showed an inhibitory time-dependent response across all treatment concentrations in which the largest decrease was observed after 72-hour exposure at one of the lowest concentrations (200 µg/mL). *H.caffrum* displayed an increase in TYR activity after the shortest exposure time (24-hour) across all treatment concentrations and a subsequent decrease in TYR activity after longer exposure times (48- and 72-hour). In addition, the overall results obtained for the extracts tested on the TYR activity of the B16s suggest that they could be more effective in their lightening effects by directly targeting the enzyme, the same as common lightening agents such as hydroquinone, KA and arbutin (Shivakumar and Jafferany, 2020). This could be due to these extracts possessing secondary plant metabolites such as flavonoids, chalcones, stilbenes, tannins and polyphenols which have been

reported to exhibit effective anti-tyrosinase activity (Mukherjee et al., 2018, Riaz et al., 2021). This can also be observed in *S. officinalis* in which one its polyphenols, rosmarinic acid, displayed effective tyrosinase inhibition at high concentrations (Oliveira et al., 2013). Additional examples can be seen in reports by (Saidi et al., 2020) in which isolated metabolites from Tunisian *Citharexylum spinosum* L. showed the highest anti-tyrosinase effect when compared to its other metabolites tested. The same can be said for several other plants including *Moringa oleifera* and *Adansonia digitata*, which are also in Africa. These plants were phytochemically evaluated and showed to possess metabolites such as tannins, flavonoids and phenols which were reported to be responsible for these plants` efficacy to reduce tyrosinase activity (Zeitoun et al., 2016). Thus, it can be suggested that the extracts in the current study showed a more effective response in inhibiting TYR activity than melanin content due to the presence of these secondary metabolites targeting TYR. In addition, these metabolites could have combined their effects leading to an effective synergistic inhibitory response.

Further evaluation was also conducted with a crystal violet assay and an LDH to determine whether the extracts were cytotoxic to the cells. Sallam et al. (2016) explained that a good candidate for use as a SL agent is one that that can lower melanin and/or TYR activity without causing toxicity to cells. In addition, a treatment can be considered as non-cytotoxic at a cell viability reported above 80%. In addition, a result within 80 – 60% is considered as weak, 60 – 40% moderate and below 40% represents strong cytotoxicity (Standardization, 2009). Thus, the results showed that none of the hydro-ethanolic extracts at the concentrations tested exhibited cytotoxicity. The results reported from the current study are consistent with those reported for *H. caffrum* by Mapunya et al. (2012) in which the cytotoxic effect of both leaf and bark extract were tested on B16s. It was concluded that although the leaf extract exhibited SL properties, due to its cytotoxic effects on

cells tested, it should not be considered as an anti-TYR alternative. Another study also conducted on ethanolic extracts generated with the leaves of *H. caffrum* reported similar cytotoxic findings, emphasizing the safety concerns associated with the leaves of this extract (Mapunya et al., 2012, Shabana et al., 2011). Other studies conducted on *S. officinalis* also reported similar findings in which essential oils isolated from the plant`s leaves exhibited low cytotoxicity on human keratinoocytes, demonstrating its viability for incorporation into skin care and cosmetic formulations (Abu-Darwish et al., 2013). Thus, as the individual extracts displayed low cytotoxicity to the cells, it was likely that the combined extract would show similar effects. This suggests that neither of the extracts are harmful to the cells at any of the tested concentrations.

These results show that the combined extract did have significant inhibitory effects on the TYR activity of the cells without being toxic to the cells. Furthermore, the results obtained also suggests that the extracts` individual mode of action could be enzyme TYR-specific and this is also more efficacious in a combined extract. As previously suggested, plant metabolites could be associated with exhibiting SL effects on the cells tested in this study. Further analysis would need to be conducted to identify these specific metabolites and test their skin lightening efficacy on skin cells. Overall, the study proposes the potential melanin and TYR inhibiting benefits of combined plant extracts and its use as alternative ingredients to current SL products.

5.5 Conclusion

To our knowledge, this is the first study to investigate the SL effect of these plant extracts in combination. The results obtained support current evidence available for the respective extracts but also provides evidence on the potential combined effects of these plant extracts on melanin and TYR activities. Furthermore, this research provides valuable insight into the SL possibilities associated with combined extracts that have already shown SL efficacies and supports their

possible investigation. Although the results suggest these extracts are potentially safe, further *in vitro* and *in vivo* evaluation is necessary.

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Chapter 6

Final Discussion and Conclusion

In Chapters 1 and 2, literature revealed that there is a high prevalence of skin lightening practice worldwide, and especially so within the African continent. This practice is associated with a variety of adverse effects that is linked to the chronic use of skin lightening agents that contain dangerous chemicals such as hydroquinone, lead and mercury. This evidence motivating the need to identify less toxic alternatives to current available products. In addition, studies conducted within South Africa has indicated the prevalence of skin lightening practice and even so among the young adult population. Thus, it was worth exploring the factors associated with the emergence skin lightening practice among the South African young adult population and identifying plant extracts that hold potential as effective skin lightening agents.

In Chapter 3, to investigate the motivating factors associated with skin lightening prevalence among young adults, a cross-sectional study was conducted among students attending a tertiary institution within South Africa. The results collected indicated a low prevalence of skin lightening practice. Analysis showed that social media platforms, specifically Instagram, was the most common influence of skin lightening practice. Other influences identified also included familial skin lightening usage. Furthermore, social media was more significantly associated with skin lightening usage. Females, including participants aged 20 and 21, had higher odds of usage and participants of the non-african population group that reported familial skin lightening usage were also more likely to practice skin lightening in comparison to those of the African population group that revealed familial usage. Blay, 2011 and Mendoza, 2014 reported that this increased

likelikehood be due to their desire to tone their skin for various reasons including to achieve a more uniformed skin complexion and to reduce potential visible signs of ageing. This data shows the necessity for specific intervention strategies to increase awareness related to the deleterious side effects of skin lightening.

In Chapter 4, a literature review conducted to identify African medical plants that have been investigated for their SL capabilities found a list of 35 plant species that have melanin and tyrosinase inhibitory efficacy. Various studies have been conducted to identify plant extracts as skin lighteners, however studies providing a comprehensive list of African plants with these properties are limited. As such, this work was able to identify *Salvia officinalis* and *Harpephyllum caffrum* as some of the plant extracts with potential to serve as alternatives to current skin lightening agents. In addition, they could be safer in comparison to synthetic agents used especially since they could be less toxic and readily available for incorporation into skin care formulations.

In Chapter 5, hydroethanolic extracts of *S. officinalis* and *H. caffrum* were selected for further analysis *in vitro*. The study investigated the individual and combined effects of the extracts on the melanin and TYR function, as well as their cytotoxic and proliferative effects on the cells. *S. officinalis* was most effective in reducing melanin content at the 800 µg/mL after 72-hour treatment. *H. caffrum* increased the melanin content across all concentration after 24-hours but only displayed statistical significance across the higher concentrations after 24- and 48-hour exposure. Although the combined extract had negligible inhibitory effects on the melanin content of the cells across most concentrations, the individual and combined extracts showed to be more effective in reducing TYR function. Furthermore, the extracts exhibited no cytotoxic or anti-proliferative effects on the cells at any concentrations or exposure times tested. These results suggesting that the extracts could be TYR-specific in their mechanism of action while being non-

toxic to the cells. This study provides valuable leads for the development of individual and combination extracts that could be less deleterious in their effects. Future studies could include the detection of apoptotic markers to confirm whether the extracts do not induce cell apoptosis.

Limitations in this study included the direct mechanism of potential cell death (apoptosis) not being fully explored in the *in vitro* study. Additionally, the determination of the effects of *S. officinalis*, *H, caffrum* and the combined extract on human skin cells *in vivo* did not fall within the scope of the main objectives of this study.

Despite this, the results found in this study could provide valuable leads for future research. This study has provided more information on the motivating factors associated with skin lightening practice among young adults. Furthermore, similar studies can be conducted to explore the same factors among a larger target population by including other tertiary institutions allowing for generalizability and data that is better representative of the population and the overall prevalence. It would also be beneficial to identify additional plant extracts as potential skin lightening alternatives which were described in the literature search (Chapter 4) and further investigate their prospective efficacies in combination as described in the *in vitro* study (Chapter 5).

Appendix 1

Ethical clearance

UNIVERSITY of the WESTERN CAPE

OFFICE OF THE DIRECTOR: RESEARCH
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09 July 2019

Dr F Rahiman
Medical Biosciences
Faculty of Sciences

Ethics Reference Number: BM18/3/21

Project Title: A cross-sectional study investigating the prevalence, knowledge and attitude concerning the practice of skin lightening.

Approval Period: 14 June 2019 – 14 June 2020

I hereby certify that the Biomedical Science Research Ethics Committee of the University of the Western Cape approved the scientific methodology and ethics of the above mentioned research project.

Any amendments, extension or other modifications to the protocol must be submitted to the Ethics Committee for approval.

Please remember to submit a progress report in good time for annual renewal.

The Committee must be informed of any serious adverse event and/or termination of the study.

Ms Patricia Josias
Research Ethics Committee Officer
University of the Western Cape

BMREC REGISTRATION NUMBER -130416-050

FROM HOPE TO ACTION THROUGH KNOWLEDGE

Appendix 2

Participant Information leaflet

FACULTY OF NATURAL SCIENCES
DEPARTMENT OF MEDICAL BIOSCIENCES

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PARTICIPANT INFORMATION LEAFLET

TITLE OF THE RESEARCH PROJECT: A cross-sectional study investigating the knowledge, perceptions and practices toward skin lightening among young adults

PRINCIPAL INVESTIGATOR: Ms. Laurentia Opperman

ADDRESS: University of the Western Cape, Modderdam Road, Bellville

CONTACT NUMBER: 021 959 3581

You are being invited to take part in a research project entitled, 'A cross-sectional study investigating the knowledge, perceptions and practices toward skin lightening among young adults'. Please take some time to read the information presented here, which will explain the details of this project. Please ask the principal investigator and/or research assistants any questions about any part of this project that you do not fully understand. It is very important that you are fully satisfied that you clearly understand what this research entails and how you could be involved. Also, your participation is entirely voluntary and you are free to decline to participate. If you say no, this will not affect you negatively in any way whatsoever. You are also free to withdraw from the study at any point, even if you do agree to take part.

This study has been approved by the Biomedical Research Ethics Committee at the University of the Western Cape (can be contacted using the following details: Email: research-ethics@uwc.ac.za; Tel: 021 959 2988) and the Unit of Research Integrity (URI) at the Cape Peninsula University of Technology (CPUT) (can be contacted using the following details: Email: Kamaldl@cput.ac.za; Tel: 021 460 3843).

What is this research study all about?
The aim of the study is to investigate how frequently young adults use skin lightening products and also to understand the knowledge and perceptions concerning the practice of skin lightening. This study will be used for research purposes only.

Why have you been invited to participate?
You have been asked to participate as you meet the inclusion criteria for our study. We believe you can make a significant contribution to this study. Should you decide to participate, you will need to sign a consent form. Information regarding the study will be explained to you before you sign the consent form so that you will be able to make an informed decision.

What will your responsibilities be in this study?
Should you decide to participate in this study you will be required to complete a questionnaire at only one point in time. You will be provided with a hard copy of the questionnaire and questions will pertain to the knowledge, perceptions and practices toward skin lightening.

What are the risks, should I decide to participate in this study?
There are no risks anticipated for this study

Anonymity and confidentiality
You will be assured of your confidentiality as you are not required to write down your name on the questionnaire. The questionnaire you complete will be assigned an identifying number and this number will be used for all data capturing purposes. No personal information about you will be recorded or shared with others.

Right and Freedom to withdraw
As a participant you reserve the right to withdraw at any time from the project and they may stop participating at any time without prejudice.

Costs and compensation
There will be no costs involved for the participation in this study. As a participant, you will not be paid for taking part in this study. There will also be no direct benefits to the participants from this study.

Is there anything else that I should know or do?
Should you feel uncomfortable in answering any of the questions and require support please feel free to contact Ms Opperman at Cel: 073 941 7658 or email: 3339588@myuwc.ac.za or Dr. Farzana Rahiman at Tel: 021 959 3581 or email: frahiman@uwc.ac.za. Dr Rahiman and Ms. Opperman will also address any further queries or problems concerning the questionnaire and will refer you to the Health Clinic (CPUT, Bellville campus located on ground floor of the New Library Extension. To be contacted at 021 959 6403; UWC, located on the first floor of the Community and Health Sciences Building (next to 'B' Block). To be contacted at 021 959 2876/5) should that be required.

Appendix 3

Participant informed consent

FACULTY OF NATURAL SCIENCES
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PARTICIPANT CONSENT FORM

Declaration by participant

By signing below, I ..... agree to take part in a research study entitled, 'A cross-sectional study investigating the knowledge, perceptions and practices toward skin lightening among young adults.'

I declare that:

- I have read or had read to me this information and consent form and it is written in a language with which I am fluent and comfortable.
- I have had a chance to ask questions and all my questions have been adequately answered.
- I understand that taking part in this study is **voluntary** and I have not been pressurised to take part.
- I may choose to leave the study at any time and will not be penalised or prejudiced in any way.

Signed at (*place*) ..... on (*date*) .....

....

Signature of participant Signature of witness

Declaration by investigator/researcher

I *(name)* ..... declare that:

- I explained the information in this document to the participant
- I encouraged him/her to ask questions and took adequate time to answer them.
- I am satisfied that he/she adequately understands all aspects of the research, as discussed above.

Signed at (*place*) ..... on (*date*) .....

....

Signature of investigator Signature of witness

Appendix 4

Questionnaire

FACULTY OF NATURAL SCIENCES
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For official purposes only
Date collected:
Questionnaire no:
Informed consent: Yes / No

PART 1: Demographic and General details

Please answer the following questions by either ticking the relevant box or filling in an answer in a box

1. Age: _____
2. Gender: Male ☐ Female ☐
3. What is your race (according to government classification)?:
   ☐ Black ☐ Coloured ☐ Indian ☐ White
   Other: Please specify _____
4. What is your marital status? Married ☐ Single ☐
5. How would you describe the geographical region you are from?
   Rural ☐ Urban ☐
6. What is your current year of study? 1st ☐ 2nd ☐ 3rd ☐
7. What degree are you currently registered for?
   Please specify _____

PART A

Questions relating to skin tone and practice

8. Are you satisfied with the colour of your skin? Yes ☐ No ☐
9. If you were able to change the colour of your skin, would you? Yes ☐ No ☐
10. Which skin tone(s) do you prefer? Fair/light complexion ☐

    Medium complexion ☐ Dark complexion ☐
11. Which factor(s) do you think influenced your preference?

    Social Media ☐ Advertisements ☐ Television ☐ Family and friends ☐
12. If you chose social media, which platforms specifically?

    Facebook ☐ Instagram ☐ Tinder ☐ WhatsApp ☐
13. Have you ever used any products to lighten your skin? Yes ☐ No ☐
14. If yes, where did you get the skin lightening product(s)? Family or friend ☐

    Pharmacy ☐ Skin care clinic ☐ Supermarket ☐

    Other: Please specify_____
15. Have any of your friends or family members lightened their skin? Yes ☐ No ☐
16. If yes, where did they get the product previously mentioned?

    Family, other members or friends ☐ Pharmacy ☐ Skin care clinic ☐

    Supermarket ☐

    Other: Please specify _____
17. If yes, which methods of skin lightening did you or your family member/friend use?

    Soap ☐ Creams ☐ Home remedies ☐

    Other: Please specify _____
18. Do you read the ingredients list of the skin lightening product(s)? Yes ☐ No ☐
19. If yes, what type of ingredient(s) is found in these products?

    Chemical (synthetic) ☐ Natural (plant based) ☐ Both (chemical & natural) ☐

    Organic (synthetic- and toxin-free) ☐
20. If natural/organic, please specify the ingredient(s)

    _____

PART B

According to your perceptions of skin lightening, what makes people lighten their skin? Please circle the statement which resonates with you most.

To look fashionable/trendy	a) Agree b) Disagree c) Don't know
Be more successful in their careers	a) Agree b) Disagree c) Don't know
Obtain a "higher social ranking/class"	a) Agree b) Disagree c) Don't know
To impress their partners	a) Agree b) Disagree c) Don't know
Seem more appealing to the opposite sex	a) Agree b) Disagree c) Don't know
To appear more beautiful	a) Agree b) Disagree c) Don't know
Treatment for medical conditions such as hyperpigmentation or scarring	a) Agree b) Disagree c) Don't know

PART C

What is your knowledge about skin lightening? Please circle the statement which resonates with you most. The effects of skin lightening include:

Skin damage in the form of skin rashes/ irritations	a) Agree b) Disagree c) Don't know
Skin infections (bacterial or fungal)	a) Agree b) Disagree c) Don't know
Skin tearing or breaking	a) Agree b) Disagree c) Don't know
Mercury poisoning and intake of other harmful substances which could lead to organ failure	a) Agree b) Disagree c) Don't know
Prevention of immune system responses	a) Agree b) Disagree c) Don't know

Appendix 5

Table A: Perceptions of skin lightening

Characteristic		Frequency	Percentage
To look fashionable/trendy	Agree	550	71.9
	Disagree	81	10.6
	Don`t know	131	17.1
Be more successful in their careers	Agree	209	27.3
	Disagree	330	43.1
	Don`t know	222	29.1
Obtain a 'higher social ranking/class'	Agree	490	64.1
	Disagree	132	17.3
	Don`t know	139	18.2
Impress their partners	Agree	400	52.3
	Disagree	158	20.6
	Don`t know	20	26.5
Seem more appealing to opposite sex	Agree	499	65.2
	Disagree	102	13.3
	Don`t know	160	20.9
To appear more beautiful	Agree	631	82.5
	Disagree	47	6.1
	Don`t know	83	10.9
Treatment for medical conditions	Agree	364	47.6

	Disagree	113	14.8
	Don`t know	283	36.9

Appendix 6

Table B: Knowledge of skin lightening

Characteristic		**Frequency**	**Percentage**
Skin damage in the form of	Agree	472	61.7
rashes/irritations	Disagree	67	8.8
	Don`t know	213	27.8
	Agree	379	49.5
Skin infections (bacterial or fungal)	Disagree	84	10.9
	Don`t know	288	37.7
	Agree	406	53.0
Skin tearing or breaking	Disagree	87	11.4
	Don`t know	258	33.7
	Agree	237	30.9
Mercury poisoning	Disagree	77	10.1
	Don`t know	437	57.1
	Agree	159	20.8
Prevention of immune system	Disagree	131	17.1
responses	Don`t know	461	60.3

Appendix 7

Journal article

cosmetics

MDPI

Review

Tyrosinase and Melanogenesis Inhibition by Indigenous African Plants: A Review

Laurentia Opperman [1], Maryna De Kock [1], Jeremy Klaasen [1] and Farzana Rahiman [1,2,*]

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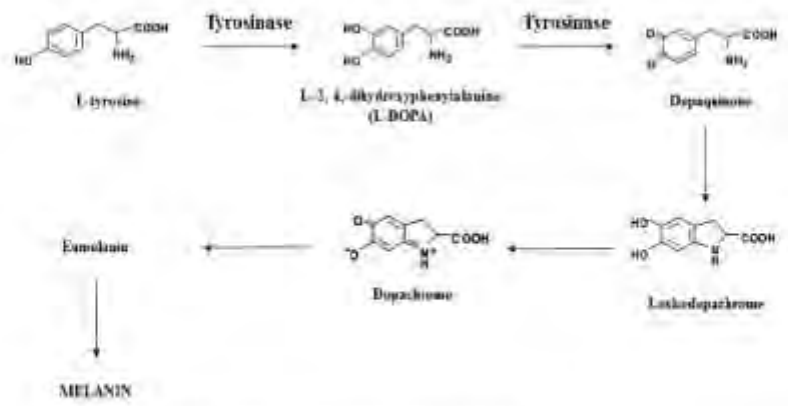
check for updates

Abstract: The indiscriminate use of non-regulated skin lighteners among African populations has raised health concerns due to the negative effects associated with skin lightener toxicity. For this reason, there is a growing interest in the cosmetic development of plants and their metabolites as alternatives to available chemical-derived skin lightening formulations. Approximately 90% of Africa's population depends on traditional medicine, and the continent's biodiversity holds plant material with various biological activities, thus attracting considerable research interest. This study aimed to review existing evidence and document indigenous African plant species capable of inhibiting the enzyme tyrosinase and melanogenesis for potential incorporation into skin lightening products. Literature search on melanin biosynthesis, skin lightening, and tyrosinase inhibitors resulted in the identification of 35 plant species were distributed among 31 genera and 21 families across 15 African countries and 9 South African provinces. All plants identified in this study showed competent tyrosinase and melanogenesis inhibitory capabilities. These results indicate that African plants have the potential to serve as alternatives to current chemically-derived skin lighteners.

Keywords: skin lightening; cosmetics; indigenous plant extracts; tyrosinase; melanogenesis

1. Introduction

Melanin is a widespread natural pigment that is responsible for color in hair, skin, and eyes. It provides protection against the deleterious effects of ultraviolet (UV) irradiation [1]. Melanogenesis is the physiological process of melanin formation in which TYR, a copper-dependent enzyme, initiates the first step. Tyrosinase catalyzes the rate-limiting step where L-tyrosine is converted to L-3,4,-dihydroxyphenylalanine (L-DOPA), leading to the eventual formation of the pigment (Illustrated by Scheme 1) [2–5]. Abnormal TYR activity leads to pigmentary disorders, such as the abnormal accumulation of melanin (hyperpigmentation) that accounts for most dermatology visits [6–8]. Skin lighteners can be divided by their mechanisms of action, such as inhibition of tyrosinase transcription, inhibition of melanosome transfer, and accelerated epidermal turnover, with the most common target being tyrosinase (TYR) inhibition [9,10]. By decreasing the activity and/or expression of TYR, melanogenesis can be inhibited, leading to reduced melanin production [11].



Scheme 1. Illustration of the melanogenesis pathway.

The skin lightening industry is one of the fastest-growing segments of the global beauty industry. Global industry analysts (GIA) have predicted that by 2020, the universal skin lightening market will reach $23 billion [12]. A recent meta-analysis provided evidence of the global prevalence of skin lightening use by reporting an estimate of 27.7%, with Africa at a current estimated prevalence of 27.1% [13]. Previous epidemiological studies have also reported a high prevalence of skin lightener use among African populations. This is evident among South African, Senegalese, and Nigerian study populations that revealed between 32 to 75% skin lightener use [14–16].

This practice is motivated by a long-standing history of social divisions, including societal pressures and stigmas, leading to the demand for lighter skin tones [17,18]. Creams, lotions, soaps, and injections indicated as a treatment for hyperpigmentation disorders are exceedingly abused as self-medication to achieve a lighter skin complexion [19,20]. In many African countries, a variety of these skin lightening preparations are easily obtained over-the-counter without a medical prescription, despite this being a requirement by law [15,21]. The most frequently used ingredients include steroids, mercury, hydroquinone (HQ) (considered the gold standard), and its derivatives [7]. Health concerns associated with the long-term use of these skin lightener ingredients include exogenous ochronosis and infectious dermatosis [22,23]. Furthermore, heavy metal exposure can lead to damage to the circulatory and urinary systems [24]. Due to their toxicity, these compounds have been prohibited as skin lightening compounds in several African countries, including South Africa, Nigeria, Kenya, and the Ivory Coast [25,26]. Despite this ban, these damaging chemicals are often illegally introduced into cosmetic formulations and, the public continues to gain access via informal channels such as street vendors, markets, and non-pharmaceutical shops [27,28]. In contrast, botanicals and natural ingredients offer safer alternatives as they may not exhibit the same kind of toxicity as synthetic compounds and could exhibit much less harmful side effects [29]. Despite this, consumers are not generally aware that natural products are composed of a variety of chemical compounds that could lead to the development of some adverse reactions. These potential effects could be overcome by researchers chemically characterizing extracts with respect to its composition [30].

Botanicals and natural ingredients provide abundant sources of treatment for various diseases such as cancer, diabetes, and dermatological conditions [31,32]. The use of plants is a common practice in traditional medicines of many cultures using several plant extracts as cosmetics to improve skin health [33,34]. This could be attributed to plant extracts being a rich source of vitamins, antioxidants, oils/essential oils, and other bioactive compounds, which provide the body with nutrients necessary for healthy skin [30]. Plants also constitute a variety of chemical compounds that elicit various pharmacological activities with the possibility that these compounds act synergistically to produce a

net pharmacological effect [35]. Examples of such compounds include polyphenols and flavonoids. Polyphenols are widely distributed in plants, and several polyphenol types have been reported as being responsible for melanogenesis inhibition [36,37]. Flavonoids and chalcones are a group of polyphenols with flavonoids being one of the most explored and most numerous groups of polyphenols [38]. Flavonoids are found within the leaves, seeds, bark, and flowers of most plants, and have been studied for its oxidation of L-DOPA and have shown good antityrosinase. Furthermore, chalcones exhibit a wide array of biological activities with a number of chalcones eliciting antityrosinase activity [39–41].

The significant advancement of research using plant extracts in cosmetics demonstrates the growing interest of researchers and pharmaceutical companies in developing natural skin lightening products [42]. The objective of this review was to examine existing literature to identify and document indigenous African plant species capable of inhibiting the enzyme TYR and melanogenesis for possible use as alternatives to current skin lightening formulations.

2. Materials and Methods

A computerized literature search was performed using the following databases: MEDLINE, SCOPUS, GOOGLE SCHOLAR, MEDLINE EBSCOHOST, and SCIENCE DIRECT databases. In addition, the South African National Electronic Thesis Database (ETD) was searched for grey literature, which included Masters and Doctoral theses. The following key terms were used for the retrieval of articles in the databases: "skin lightening", "tyrosinase", "melanin", "antimelanogenesis", "antityrosinase", "melanogenesis", "tyrosinase inhibition", "melanin inhibition". For an article to be considered eligible, the following criteria needed to be met: (1) The use of indigenous African plant extracts (alone or in combination with other African plants); (2) performed in vivo and in vitro studies only; (3) investigated tyrosinase and melanogenesis inhibition. This literature search also had no restrictions on the following: Language; date of publication, and publication status (inclusive of published, unpublished, in the press and in progress). Studies that reported on both non-African (not indigenous to Africa and imported plants) and African plants were isolated, and only the African plants were included in this study. In addition, studies that included tests other than tyrosinase and melanin assays were isolated, and only the tyrosinase and melanin assays were reported on. All qualitative studies were excluded. Three independent reviewers completed the above-described methods independently. Any disagreements between the reviewers were discussed and resolved.

The articles that had been retrieved through the computerized literature searches were combined, giving a total of 128 articles. A preliminary analysis of the titles and abstracts of each article was performed, and all duplicates were excluded. After the screening of abstracts by at least 2 authors, the articles classified as ineligible based on the previously described criteria were excluded, and a total of 49 articles underwent a full-text review.

After further application of the exclusion and inclusion criteria, a total of 36 articles were classified as eligible for discussion in this review. Tables 1 and 2 summarizes the plant species identified along with their melanin and tyrosinase results, respectively. In both tables, the plant names are arranged according to their family, along with the region the plants are found in Africa and plant part used.

Table 1. Summary of the plant species identified and their melanin results.

Family	Plant Name	Region	Part Used	Results	Reference
Anacardiaceae	*Harpephyllum caffrum*	SA (EC)	Leaves Bark	26% melanin inhibition at 0.25 µg/mL	[22]
Cleomaceae	*Arthropodium capense*	TUN	Stems	32% melanin inhibition	[47]
Crassulaceae	*Cotyledon orbiculata*	Cameroon	Bark	Isolated compounds: ~0.25 MIC at 25 µg/mL	[23]
Lamiaceae	*Salvia officinalis*	EGY	Aerial parts	MC at 25°C at 10, 20 and 40 µg/mL	[41]
Malvaceae	*Grewia flava*	SA (EC)	Leaves	20% melanin inhibition at 6.25 µg/mL	[4]
Malvaceae	*Grewia occidentalis*	SA (WC)	Leaves	Isolated compound: 50% melanin inhibition at 12.5 µg/mL	[47]
Myrtaceae	*Myrtus africana*	SA (EC, PS, GAU, KZN, LP, MP, NW, WC)	Shoots	50% melanin inhibition at 50 µg/mL	[48]
Myrtaceae	*Myrtus africana*	SA (EC, PS, GAU, KZN, LP, MP, NW, WC)	Shoots	18% melanin inhibition at 12.50 µg/mL	[48]
Polygonaceae	*Persicaria senegalensis*	BWA	Leaves	cell pellets indicate no significant inhibition	[47]
Pteridophyta	*Dryopteris globosa*	SA (WC)	Leaves Roots Stems	-	[46]
Rutaceae	*Peltostigma sphaerocarpum*	NIG, GHA	Root bark	cell pellets indicate strong inhibition	[47]
Rutaceae	*Spathelia bicolor*	SA (WC)	Aerial parts	94.3% melanin inhibition at 50 µg/mL	[49]
Sapindaceae	*Cardiospermum corindum*	BEN, NIG, DRC, UGA, TZA, MLI	Roots	isolated compounds: ~0.2 µg/mL MC at 50 ng/mL and 100 µg/mL	[49]
Sapotaceae	*Chrysophyllum magalismontanum*	BWA	Leaves Roots	cell pellet indicate inactive	[47]
Sapotaceae	*Argania spinosa*	MAR	Drum	25% melanin inhibition at 50 µg/mL	[50]
Sapotaceae	*Argania spinosa*	MAR	Fruits	>90% melanin inhibition at 1/100	[51]
Sapotaceae	*Sideroxylon inerme*	SA (KZN)	Stem bark	27% melanin inhibition at 6.2 µg/mL	[52,53]

Table 1. *Cont.*

Family	Plant Name	Region	Part Used	Results	Reference
Sapotaceae	*Vitellaria paradoxa*	MLI, ETH, UGA	Fruit	Cameroon = 10.1% MC at 100 µg/ml Chad = 16.2% MC at 100 µg/ml Sudan = 10.9% MC at 100 µg/ml	[51]
Thymelaeaceae	*Thomasia foliosa*	TUN	Leaves	>50% melanin inhibition of melanin	[54]
Thymelaeaceae	*Thymelaea hirsuta*	TUN	Leaves	isolated compound: 10% melanin inhibition at 0.1 µg/mL	[54]
Thymelaeaceae	*Thymelaea hirsuta*	TUN	Leaves	isolated compound: 90% melanin inhibition at 1 µg/mL isolated compound: 33% melanin inhibition at 0.1 µg/mL	[54]

The table indicates the melanin inhibition or MC (melanin content) at various concentrations of plant extracts (µg/mL). The cases in South Africa (SA)—EC: Eastern Cape; FS: Free State; GAU: Gauteng; KZN: KwaZulu-Natal; LP: Limpopo; MP: Mpumalanga; NW: North West; WC: Western Cape; Other African countries—ALG: Algeria; DRC: Democratic Republic of Congo; EGY: Egypt; ETH: Ethiopia; GHA: Ghana; IC: Ivory Coast; MAR: Morocco; MLI: Malawi; NIG: Nigeria; BWA: Botswana; SEN: Senegal; SUD: Sudan; TUN: Tunisia; TZA: Tanzania; UGA: Uganda; ↑: not significant.

Table 2. Summary of the plant species identified and their tyrosinase results.

Family	Plant Name	Region	Part Used	Results IC_{50} or Other Values	Reference
Anacardiaceae	*Harpephyllum caffrum*	SA (EC)	Leaves Bark	92% inhibition of L-tyrosine at 500 µg/mL 60% inhibition of L-DOPA at 500 µg/mL IC_{50} 49 ± 0.000 µg/mL	[22]
Asteraceae	*Gymnosporia glauca*	SA (EC)	Leaves Bark	L-DOPA at 500 µg/mL = 42% inhibition L-tyrosine at 500 µg/mL = 92% inhibition IC_{50} 27.1 ± 062 µg/mL	[46]
Asteraceae	*Gymnosporia glauca*	SA (EC)	Leaves Bark	91.8% TYR inhibition at 200 µg/mL	[55]
Apiaceae	*Centella asiatica*	TUN	Aerial parts	IC_{50} 125.01 ± 0.72 µg/mL, using L-tyrosine IC_{50} 255.51 ± 0.56 µg/mL using L-DOPA	[52]
Asteraceae	*Helichrysum cymosum*	SA (WC)	Aerial parts	Related compound, 35.61 ± 4.67 µg/mL.	[56]
Brunoniaceae	*Lampranthus amoenus*	SA (EC)	Leaves	IC_{50} 22.24 µg/mL	[56]

Table 2. Cont.

Family	Plant Name	Region	Part Used	Results (IC50 or Other Values)	Reference
Brassicaceae	*Rorippa nasturtium-aquaticum*	SA (EC)	Leaves	IC50 1.517 µg/mL	[illegible]
Capparaceae	*Cleome arabica*	TUN	Aerial parts	IC50 124.4 ± 0.69 µg/mL L-tyrosine IC50 243.43 ± 2.71 µg/mL using L-DOPA	[illegible]
Chenopodiaceae	*Haloxylon articulatum*	ALG, MAR, TUN	Shoot	IC50 180 µg/mL using L-DOPA as substrate IC50 using L-tyrosine not significant	[illegible]
Clusiaceae	*Garcinia kola*	ALG	Seed	29% TYR inhibition at 500 µg/mL	[illegible]
Euphorbiaceae	*Alchornea cordifolia*	NIG, GHA	Leaves Stem bark	Leaf extracts = 139.42 mg KAE/g Bark extracts = 160.42 mg KAE/g	[illegible]
Fabaceae	*Ceratonia siliqua*	ALG	Leaves	crude extract, 50% TYR inhibition at 200 µg/mL isolate compounds, 90% TYR inhibition at 200 µg/mL	[illegible]
Fabaceae	[illegible]	SA (KZN, LP, MP)	Leaves Stems	IC50 2.95 ± 1.20 µg/mL using L-tyrosine	[illegible]
Fabaceae	*Rhynchosia villosa*	SA (EC, KZN, MP)	Root	56.40% TYR inhibition at 500 µg/mL	[illegible]
Fabaceae	*Vachellia karroo*	SA (EC, FS, GAU, KZN, MP, NC, NW, WC)	Roots	IC50 6.84 µg/mL	[illegible]
Fabaceae	*Acacia nilotica*	SUD SA (GAU, KZN, LP, MP, NW)	Pods Bark	pod extract: IC50 8.01 ± 0.94 µg/mL using L-tyrosine pod extract: 69.3% TYR inhibition at 500 µg/mL	[illegible]
Fabaceae	*Acacia nilotica*	SUD SA (GAU, KZN, LP, MP, NW)	Pods Bark	IC50 12.97 ± 1.07 µg/mL	[illegible]
Lamiaceae	*Plectranthus ecklonii*	SA (EC, KZN, MP)	Aerial parts	IC50 81.73 ± 2.89 µg/mL >50% at 100 µg/mL	[illegible]
Lamiaceae	*Plectranthus ecklonii*	SA (EC, KZN, MP)	Aerial parts	IC50 21.58 µg/mL	[illegible]
Lamiaceae	[illegible]	ALG	Aerial parts	27% TYR inhibition at 1.5 mg/mL	[illegible]
Melianthaceae	*Greyia flanaganii*	SA (EC)	Leaves	95% TYR inhibition at 200 µg/mL Isolated compound, IC50 17.86 µg/mL	[illegible]
Melianthaceae	*Bersama abyssinica*	IC	Leaves	148.94 mg KAE/g	[illegible]

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Table 2. Cont.

Family	Plant Name	Region	Part Used	Results (IC50 or Other Values)	Reference
Melianthaceae	*Greyia radlkoferi*	SA (MP)	Leaves	IC50 17.99 µg/mL using L-tyrosine IC50 using L-DOPA not significant	[illegible]
Myrsinaceae	*Myrsine africana*	SA (EC, FS, GAU, KZN, LP, MP, NW, WC)	Shoots	IC50 0.12 ± 0.005 mg/mL	[illegible]
Myrsinaceae	*Myrsine africana*	SA (EC, FS, GAU, KZN, LP, MP, NW, WC)	Shoots	L-DOPA at 500 µg/mL – 82% inhibition L-tyrosine at 500 µg/mL < 93% inhibition IC50 22.91 ± 0.43 µg/mL	[illegible]
Myrsinaceae	*Myrsine africana*	SA (EC, FS, GAU, KZN, LP, MP, NW, WC)	Shoots	IC50 27.3 µg/mL using L-tyrosine	[illegible]
Pedaliaceae	*Sesamum angolense*	RWA	Leaves	IC50 24 µg/mL	[illegible]
Poaceae	*Sorghum bicolor*	TUN	Stalk	40% TYR inhibition (in comparison to untreated control)	[illegible]
Podocarpaceae	*Podocarpus elongatus*	SA (KZN)	Stems	73% TYR inhibition at 1 mg/mL IC50 = 0.14 mg/mL	[illegible]
Proteaceae	*Protea madiensis*	NIG, ETH	Root bark Leaves	31 ± 4 µg/mL	[illegible]
Proteaceae	*Serruria furcellata*	SA (WC)	Aerial parts	95.49% TYR inhibition at 200 µg/mL 80.86% TYR inhibition at 50 µg/mL	[illegible]
Rhizophoraceae	*Cassipourea congoensis*	SEN, NIG, DRC, UGA, TZA, MLI	Bark	crude extract, >80% TYR inhibition at 10 µg/mL and 100 µg/mL	[illegible]
Rhizophoraceae	*Cassipourea flanaganii*	SA (EC, KZN)	Bark	IC50 22.24 ± 1.32 µg/mL	[illegible]
Rhizophoraceae	*Cassipourea flanaganii*	SA (EC, KZN)	Bark	IC50 1.429 µg/mL	[illegible]
Rubiaceae	*Dolichopentas longiflora*	RWA	Leaves Roots	IC50 20 ± 2 µg/mL	[illegible]
Sapotaceae	*Sideroxylon inerme*	SA (KZN)	Stem bark	59% TYR inhibition at 200 µg/mL	[illegible]

The table measures TYR inhibition. EC50 (concentration at which the plant extract exhibits 50% of its maximum response) and IC50 (concentration at which half the original TYR activity is inhibited) values of plant extracts (µg/mL or KAE/g; KA equivalent per gram or mg/mL). Provinces in South Africa (SA): EC: Eastern Cape; FS: Free State; GAU: Gauteng; KZN: KwaZulu-Natal; LP: Limpopo; MP: Mpumalanga; NW: North West; WC: Western Cape; Other African countries: ALG: Algeria; DRC: Democratic Republic of Congo; EGY: Egypt; ETH: Ethiopia; GHA: Ghana; IC: Ivory Coast; MAR: Morocco; MLI: Malawi; NIG: Nigeria; RWA: Rwanda; SEN: Senegal; SUD: Sudan; TUN: Tunisia; TZA: Tanzania; UGA: Uganda.

3. Results and Discussion

In this study, 35 plant species distributed across 31 genera and 21 families were identified as being effective as TYR and melanogenesis inhibitors. In addition, the plants identified in this study were distributed among 15 African countries and 9 South African provinces. 17 (47.2%) were found in South Africa, with 19 (52.7%) found within other African countries. The most represented families were Fabaceae (5 plant species), Melianthaceae (3 plant species), Sapotaceae (3 plant species), Chenopodiaceae (2 plant species), Proteaceae (2 plant species), Clusiaceae (2 plant species), Rhizophoraceae (2 plant species), and Lamiaceae (2 plant species). The rest of the families were represented with only 1 plant species—Anacardiaceae, Apiaceae, Asteraceae, Brassicaceae, Capparaceae, Euphorbiaceae, Myrsinaceae, Pedaliaceae, Picrodendraceae, Poaceae, Podocarpaceae, Rubiaceae, and Thymelaeaceae.

African forests are the world's second-largest tropical reservoir holding very promising plant materials with various biological activities, which has attracted considerable research interest [70]. Up to 90% of Africa's human population depends directly on traditional medicine. Plants form a central component of the African traditional healthcare system and is probably the oldest of all therapeutic systems [71,72]. The importance of this resource can be illustrated by the comprehensive list of African medicinal plants in which more than 5400 plant taxa and over 16,300 medicinal uses for the plants have been identified. The use of plant extracts as topical treatments has been practiced for many generations with extracts being used for the treatment of various skin ailments, including wounds, skin infections, and inflammation [73,74]. The demand for cosmetic skin-lightening products is growing, with predictions particular to Asia and Africa forecasting the beauty industry to be worth an estimated $US 31.2 billion by 2024 [12,75]. This significant increase can also be accounted for by the pleasant aromatics and the general consensus that plant extracts are safer than synthetic products available. Thus, there is an ever-growing endeavour to explore plant-based melanogenesis inhibitors [76,77].

Various plant extracts and compounds have been investigated for their anti-tyrosinase and antimelanogenic effects [78]. Three methods are extensively used to study tyrosinase activity, which includes 2 radiometric assays (tyrosinase hydroxylase and melanin formation activities) and one spectrophotometric assay (dopa oxidase activity). Tyrosinase hydroxylase assay estimates the tyrosinase hydroxylase activity of tyrosinase by measuring tritiated water released from L-[3,5-^{3}H]-tyrosine. The melanin formation activity assay estimates the radioactive melanin synthesized from L-[U-^{14}C]-tyrosine while the dopa oxidase activity measures the rate of dopachrome formation, of which all three are in vitro assays [79]. These assays also include the use of positive controls whose potencies are well-known, such as kojic acid (KA), to which the substance of interest can be compared [80]. Results obtained from these assays are often presented in IC_{50} values, which refers to the concentration of plant extract at which half the original TYR activity is inhibited [40].

As shown in the results described in Table 2, plants reported from the Fabaceae family were only tested for their ability as TYR inhibitors, and all proved to be strong inhibitors. Further results obtained by Lall et al. [65], supported the findings for *Ormocarpum* and *Acacia nilotica*, which demonstrated the lowest IC_{50} value of 2.95 µg/mL and showed to have the highest TYR inhibition of 98.3% (IC_{50} 8.61 µg/mL), respectively [36,65]. *Cassipourea congoensis* demonstrated significant effects of both crude extract and isolated compounds on melanin and TYR activity, respectively [39]. *Rorippa nasturtium-aquaticum* (Brassicaceae) showed in studies conducted by both Thibane et al. [59,60], that the extract is an effective TYR inhibitor (IC_{50} values of 22.24 and 1.513 µg/mL respectively) when compared to the kojic acid (KA) control (19.38 and 1.421 µg/mL, respectively). It is also noted that KA is the most prominent (91.7%, 33 articles) positive control used in the studies identified, due to its well-established potency in literature [80]. Arbutin, a HQ derivative, was the second most common (35.3%, 12 articles) used positive control as it is generally used in cosmetics as a hypopigmenting agent [81].

Studies on *Thymelaea hirsuta* (Thymelaeaceae) reported that this extract inhibited more than 50% of melanin at 1 µg/mL [53]. Furthermore, isolated compounds of this extract indicated that melanin production was reduced by 37% at 0.1 µg/mL, in comparison to its arbutin control, which only inhibited

33% of melanin at a higher concentration—100 µg/mL [54]. These results are further supported by Villareal et al. [55], who concluded that isolated compounds of *Thymelaea hirsuta* at 0.1 µg/mL (33% reduction of MC) is as effective as arbutin, a common depigmenting agent, at 100 µg/mL. Rhizophoraceae extracts (*Cassipourea flanaganii* and *Cassipourea congoensis*) exhibited compelling skin lightening properties with IC_{50} values obtained from studies conducted on *Cassipourea flanaganii*, which indicated values (1.425 µg/mL and 22.24 µg/mL, respectively) comparable to their KA controls (1.421 µg/mL and 19.38 µg/mL, respectively) [48,49]. *Argania spinosa* (Sapotaceae) effectively inhibited melanogenesis at 55% after 72 hours' exposure [50]. These findings are supported by Villareal et al. [23], showing that there is a greater than 50% reduction in melanin content after 72 hours of exposure. Although significant results were obtained from separate studies, the difference in the result could be attributed to the researchers investigating different parts of the same plant (Table 1) as well as differences in plant preparation and assay protocols.

The plants in this study were distributed among 15 African countries with studies, including data from 9 South African provinces. Twenty plants species were investigated using aerial parts/leaves with these plants being collected in different regions of their respective countries and/or provinces. Thus, in natural ecosystems, factors affecting the plant's performance include climate, soil, and geographic locations yielding various molecular complexes, thus, emphasizing the environment's crucial role in the metabolism of plants [82,83].

The results obtained from the TYR and melanin assays of *Harpephyllum caffrum* showed the bark extract to have the highest inhibitory effect on TYR and melanin production in comparison to the leaf extract of the same species [32]. These results are further corroborated by a review conducted by Lall and Kishore. [84], where it was noted that *Harpephyllum caffrum* and *Greyia flanaganii*, among other listed plants, showed promising pharmacological activities, a finding that warrants further scientific investigation. Similar comparisons can be seen with *Ceratonia siliqua* concerning TYR activity where its isolated compounds (90% inhibition) were shown to be a more potent TYR inhibitor than its crude extract (50% inhibition) at the same concentrations (200 µg/mL).

Further comparisons can also be observed by the contrast in results obtained for TYR assays from the use of substrates L-DOPA and L-tyrosine. Here, several plant extracts have proven to be more effective in targeting the inhibition of the oxidation of either L-DOPA or L-tyrosine. This is illustrated by the TYR assay results obtained for *Haloxylon articulatum*, *Greyia radlkoferi*, *Pituranthos scoparius*, *Myrsine africana*, *Hyaenanche globose*, and *Cleome Arabica* [40,45,57]. Additional studies also included extracts of *Dolichopentas longiflora*, where preparations exhibited a stimulatory response on melanogenesis, whereas the IC_{50} value for TYR activity (26 ± µg/mL) showed contrasting results. This included *Sesamum angolense* of the same study, where pellets of the cells that were treated with the extract indicated no significant inhibition. However, the IC_{50} value (24 µg/mL) obtained indicated that the plant extract can illicit an inhibitory response [64]. Due to the complexity of pigment production, melanogenesis regulation takes place at different levels and various means of interference are possible—providing a possible explanation for the above-described contrasting results [85,86]. Mechanisms of depigmenting include: (1) tyrosinase inhibition, (2) decrease in DOPA polymerase, (3) induction of anti-inflammatory, and (4) anti-oxidant effects [87].

Extracts from the Lamiaceae family also proved to be effective inhibitors with *Plectranthus ecklonii* showing an IC_{50} value of 21.38 µg/mL with more than 70% TYR inhibition and *Salvia officinalis* decreasing MC to 27% at various concentrations [44,66,67]. In addition, other plant extracts elicited a significant inhibitory response on both melanin and TYR activities. These include *Garcinia livingstonei* and *Garcinia kola* (Clustiaceae), *Myrsine africana* (Myrsinaceae), *Protea madiensis*, and *Serruria furcellata*—both from the Proteaceae family and *Sideroxylon inerme* (Sapotaceae). Species from other families such as Clustiaceae (*Garcinia livingstonei* and *Garcinia kola*) exhibited significant activities with *G. livingstonei* exhibiting a large decrease of melanin concentration at 25 µg/mL and the seeds of *G. kola* inhibiting 79% of tyrosinase at 500 µg/mL [22,61].

4. Conclusions

Several studies have been conducted to identify inhibitors from both natural and synthetic sources, and a number of research papers have been published and regularly updated in this aspect. This study was conducted as a means of identifying plant-based skin lightening alternatives to the current toxic substances. Despite the serious and life-threatening complications associated with the chronic use of these products, the use of skin lighteners is still a widespread and common practice in several African countries [88,89].

All plants identified in this study showed competent antimelanogenesis and antityrosinase capabilities, with the most effective of the extracts being the following: *Acacia nilotica*, *Cassipourea congoensis*, *Cassipourea flanaganii*, *Garcinia kola*, *Greyia flanaganii*, *Greyia radlkoferi*, *Hyaenanche globosa*, *Myrsine africana*, *Ormocarpum trichocarpum*, *Plectranthus yuklonii*, *Protea madiensis*, *Rorippa nasturtium-aquaticum*, *Serruria furcellata*, *Sesamum angolense*, and *Vachellia karroo*. The reproducibility of the identified studies and interpretation of the results is limited by the inconsistencies in methodologies and means of plant extraction in these studies. Other variables also include geographical location and varied climate regions.

This review shows that plants of the African continent have the potential to act as melanin and TYR inhibitors and can be used to replace synthetic and other derived chemicals. Although many of these plants have been effective in their pigment reduction properties, plants are still known to cause allergic reactions and elicit phototoxic effects [87]. This is due to natural products being a complex mixture of chemical compounds, a fact often unknown to consumers. To combat this, extracts should be chemically characterized with respect to the product composition [90]. In addition, it is imperative that toxicity studies be conducted to establish a safe dose range. These findings could aid in the production and commercialization of these plants in natural-based remedies for cosmetic and skincare product industries.

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