Selected South African Plants with Tyrosinase enzyme inhibition and their Effect on Gene Expression

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Highlights

- South African plants were Bioprospected for their anti-tyrosinase activity.
- The plants *O. trichocarpum* and *V. karoo* exhibited tyrosinase enzyme inhibition.
- Mouse melanocyte tyrosinase gene expression levels were down regulated by *K. thyrsiflora*, *M. pillansii* and *V. Karoo*.

Abstract

Hyperpigmentation causes patches of skin to blemish and may lead to serious skin disorders. Prevention of hyperpigmentation would require suppressing the melanogenesis pathway which uses the rate limiting enzyme tyrosinase. South African plant extracts, *Myrsine pillansii, Rapanea melanophloeos, Vachellia karroo, Kalanchoe thyrsiflora, Ormocarpum trichocarpum* and *Myrsine africana* were tested for their tyrosinase inhibiting potential at both the tyrosinase activity and tyrosinase gene expression levels. The plant extracts, *O. trichocarpum* and *V. karroo,* had the most effective inhibition of 50% of the tyrosinase enzyme at concentrations of 2.95 μg/ml and 6.84 μg/ml respectively. The cytotoxicity of the plant extracts were investigated using B16-F10 mouse melanocyte cells. The tyrosinase gene expression levels were examined on the B16-F10 mouse melanocyte cells treated with the South African plant extracts, through real-time reverse transcription polymerase chain reaction (RT-qPCR). Three of the mouse melanocyte samples treated with *K. thyrsiflora, M. pillansii* and *V. karroo* showed significant down regulation of tyrosinase gene expression (p-value < 0.05) at 1.2, 3.7, and 12.7 fold respectively. These plant extracts indicate depigmenting potential through inhibition of tyrosinase directly and at the transcriptional level and therefore, should be investigated further.

Keywords: Tyrosinase, Gene expression, Medicinal plants, Hyperpigmentation, Melanogenesis pathway

Abbreviations: real-time reverse transcription polymerase chain reaction (RT-qPCR); dimethyl sulphoxide (DMSO); 2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT); tyrosinase gene (TYR); carnitine palmitoyltransferase 2 gene (CPT2); analysis of variance (ANOVA); microphthalmia transcription factor (MITF); cyclic adenosine monophosphate (cAMP)

1. Introduction

Hyperpigmentation is a common condition which can cause patches of skin to blemish and become darker. This problem may affect the skin colour of all ethnicities and although it is aesthetically displeasing, the problem may persist to more serious skin disorders. Ailments such as melasma, ephelide, solar lentigines or melanoma skin cancer are just a few of the negative consequences of hyperpigmentation (Ortiz-Ruiz et al. 2016). Hyperpigmentation overproduces melanin, the complex polymer responsible for pigmentation of hair, eyes and skin, within melanocyte cells (Ana Sofia Ribeiro, 2015). Melanocyte cells are distributed in the basal layer of the dermis and they produce and secrete melanin through a physiological process known as melanogenesis. Melanogenesis is a combination of chemical and enzymatic reactions which requires the rate limiting enzyme tyrosinase for the first few steps in the melanin biosynthesis pathway (Slominski et al., 2004). Tyrosinase is a copper containing mono-oxygenase which catalyses both the hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenols to o-quinones. It is these unstable o-quinones which eventually form melanin (Ortiz-Ruiz et al., 2016).

Prevention of hyperpigmentation would require suppressing the melanogenesis pathway and although many synthetic compounds exist in the cosmeceutical industry, they all have adverse damaging effects. One such example is hydroquinone, a synthetically formulated compound that has been used as the skin-depigmenting agent worldwide. It has a cytotoxic effect on melanocyte cells with harmful concentrations as low as 1%. These side effects can include erythema, dermatitis and impaired wound healing with prolonged use (Solano et al., 2006). Arbutin, a derivative of hydroquinone extracted from cranberries, blueberries and wheat is a natural and safer depigmentation alternative (Alexis et al., 2010). Natural ingredients could therefore offer safer alternatives to medical cosmetics.

South Africa is considered one of the world's biodiversity hotspots with over 22 000 indigenous species, many often used for medicinal purposes (Forest F., 2014). In this study the Southern African plants, *Myrsine pillansii, Rapanea melanophloeos,, Vachellia karroo, Kalanchoe thyrsiflora, Ormocarpum trichocarpum,* and *Myrsine africana* were investigated for their tyrosinase inhibiting potential. The selection of plants for this study were based on traditional usages for skin related diseases, such as, wound healing, allergies, acne and skin cancer. These plants were also shown to contain polyphenols, tannins and derivatives of vitamin C, chemical constituents associated with skin toning and depigmentation.

The aim of the present study was to investigate Southern African plants*: M. pillansii, R. melanophloeos, V. karroo, K. thyrsiflora, O. trichocarpum,* and *M. africana's* potential to down-regulate melanogenesis, both by inhibiting tyrosinase and down-regulating the tyrosinase gene expression.

2. Methods and Materials

2.1 Plant Extractions

Fresh leaves and stems of *M. pillansii, R. melanophloeos, V. karroo, K. thyrsiflora, O. trichocarpum,* and *M. africana* were collected in January 2016 from the Manie van der Schiff Botanical Garden. The plant species identity was confirmed and a voucher number was then deposited at the H.G.W.J Scheickerdt Herbarium of the University of Pretoria. The plant materials were shade-dried at room temperature for two months, the material was ground and the final fine powder material was weighed. The plant material was then suspended in a 1:10 solvent 99% ethanol (Merck) to dry weight ratio. Ethanol can dissolve both polar and non-polar substances and was therefore used as a solvent in this study since a large range of secondary metabolites would then be extracted and tested (Eloff, 1998).

2.2 Tyrosinase Inhibition Assay

The *M. pillansii, R. melanophloeos, V. karroo, K. thyrsiflora, O. trichocarpum,* and *M. africana* plant extracts were tested for their anti-tyrosinase activity and as a measure of comparison, a known tyrosinase inhibitor, kojic acid, was used as the positive control (Nerya et al. 2003). In triplicate, the plant samples and kojic acid were dissolved in dimethyl sulphoxide (DMSO) to a final concentration of 20 mg/ml. These samples were diluted with potassium phosphate buffer (pH 6.5) (Sigma Aldrich, South Africa), in a 24-well plate to eight concentrations, ranging from 600 µg/ml to 37.5 µg/ml. For each sample, 70 µl of each dilution was aliquoted in triplicate to the wells of a 96-well plate in eight concentrations ranging from 200 µg/ml to 1.563 µg/ml. A negative DMSO control (200 mg/ml), diluted with potassium phosphate buffer, was included in six wells. For all the samples 30 µl of the prepared tyrosinase enzyme (333 units/ml) was then aliquoted into each well. The plate was incubated for 5 minutes at room temperature. After the incubation, 110 µl of the L-tyrosine substrate was added to the wells and the plate was further incubated at room temperature for 30 minutes.

The BIOTEK power XS multi-well plate reader (A.D.P, Weltevreden Park, South Africa) was used to read the optical densities of each well at a wavelength of 492 nm. Using GraphPad prism software (San Diego, California, USA) the IC_{50} values, coefficients of determination (R^2), and statistical significance was calculated for each sample.

2.3 XTT Cytotoxicity Assay

The plant extracts, *M. pillansii, R. melanophloeos, V. karroo, K. thyrsiflora, O. trichocarpum,* and *M. africana* were also tested on mouse melanocyte B16-F10 cells with increasing concentration intervals and the resulting cell viability was measured. Mouse

melanocyte cells were purchased from Highveld Biological (South Africa) and cultured with minimum essential medium cell culture medium containing 10% Foetal Bovine Serum, 1.5 g/l NaHCO3, 2 mM l-glutamine, 10 g/ml penicillin, 10 g/ml streptomycin and 0.25 g/ml fungizone. The cell viability assay was conducted as described by the 2,3-Bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) cell proliferation kit II (Sigma-Aldrich, South Africa) with few modifications. Actinomycin D was used as the positive control since it induces cytotoxicity at a known concentration (Syme et al. 2004). The crude extract and actinomycin D were tested at 400-6.25 μg/ml in triplicate in a 96 well plate. The samples were incubated for 72 hours at 37°C in 5% CO_2. After this incubation period, XTT reagent (1 mg/ml XTT with 0.383 mg/ml PMS) was added to all samples. The samples were then left to incubate for 3 hours and the BIOTEK power XS multi-well plate reader (A.D.P, Weltevreden Park, South Africa) was used to read the optical densities of each well at a wavelength of 405 nm. GraphPad prism software (San Diego, California, USA) was then used to calculate the IC_{50} values for each sample.

2.4 Mouse Melanocyte Cell Treatments with Plant Extracts, RNA Extractions and cDNA Synthesis

The tyrosinase and cytotoxicity assay results provided a selectivity index and concentrations of plant extracts below the toxicity level and above the anti-tyrosinase activity were selected for each plant species as seen in Table 2. Mouse melanocyte cell treatments with the plant extracts were undertaken in a 24-well plate with minimum essential medium, B16-F10 mouse melanocyte cells were cultured to 1 x 10^6 cells per well. Controls included a solvent control of DMSO (2%) and a negative control of untreated cells. Each well was then treated with the selectivity index concentration of *M. pillansii, R. melanophloeos, V. karroo, K. thyrsiflora, O. trichocarpum,* or *M. africana* in triplicate and incubated for 72 hours at 37°C at a 5% CO_2 level. After incubation total RNA was extracted from B16-F10 mouse melanocyte cells using the Qiagen RNeasy minikit (Qiagen, Hilden, Germany). The additional on-column DNAse digestion using the RNase free DNase purification kit (Qiagen, Hilden, Germany) was included to eliminate genomic DNA contamination. The quality of the RNA samples were assessed on the NanodropTM 2000 spectrophotometer (Thermo Scientific, Minnesota, USA) by obtaining the $OD_{260/280}$ and $OD_{260/230}$ ratios. Further evaluation of the RNA quality included a Seakem® LE Agarose (Lonza Rockland, USA) gel electrophoresis of all the RNA samples. Extracted RNA (500 ng) was used to prepare cDNA using the cDNA synthesis kit (Thermo Scientific, South Africa). Lipoic acid, a known transcription inhibitor was used as a positive control.

2.5 PCR Primers

Specific primers for the two genes *M. musculus* tyrosinase (P11344) and *M. musculus* carnitine palmitoyltransferase 2 (CPT2) (NC_000070) were designed with Primer3 Software (version 0.4.0). Selected primers for each gene were as follows: Tyrosinase Forward Primer: 5' CCAGTGCCTTGTATATGC 3', Tyrosinase Reverse Primer: CCTTGAACCGCTAGAGAA 3', CPT2 Forward Primer: 5'

CCTGCCAAGAAGTGACACAGA 3', CPT2 Reverse Primer: ATCCAGGGGATATGCATTGA 3'.

2.6 Real Time quantitative PCR Relative Expression Analysis of Tyrosinase cDNA in Mouse Melanocyte Cells

The real-time quantitative PCR (RT-qPCR) analysis was conducted with the Biorad CFX96 TouchTM Real-Time PCR detection system (Bio-rad Labratories, Johannesburg, South Africa) and the SYBR® Green Master Mix (Life Technologies, Johannesburg, South Africa). The expression of the tyrosinase gene (TYR) across all samples was quantified by assessing its stability against the housekeeping reference gene, CPT2, with three technical replicates for each sample. Concisely each 10 µl reaction comprised of 1x SYBR® Green Master Mix, 0.2 µM forward, 0.2 µM reverse primer, 1.25 ng/µl cDNA template and millipore water. The samples were run for 10 min/95°C then forty cycles of amplification were carried out with the following thermal profile: 10 sec/95°C, 30 sec/ 59°C, 10 sec/72°C. To verify the primer specificity, a melting point analysis was included where the samples were heated from 65°C to 95°C with 5 second increments. The Biorad CFX ManagerTM software was used to generate crossing point values as well as the melting curves, standard curves and amplification plots for the TYR and CPT2 genes. The TYR gene relative expression values were normalized by the reference gene CPT2 cDNA (Vandesompele et al. 2002). Statistical analysis of the relative expression data was done with GraphPad Prism 5.04 software using log-transformed normal distributed relative quantification data. A one-way Analysis of variance (ANOVA), measured at a $p \leq 0.05$ level of statistical significance, was used to detect significant differences between the DMSO and plant extract mouse melanocyte treated samples.

3. Results

Table 1 summarizes the botanical name, authority name, family, voucher specimen and plant parts utilized of the six Southern African plants used in this study.

3.1 Tyrosinase Inhibition Assay

The substrate, L-tyrosine was used to determine the monophelase and diphenolase activity of the mushroom enzyme tyrosinase. The tyrosinase activity was measured by the IC_{50} value, which is the concentration of the plant extract required to inhibit 50% of the tyrosinase activity. Table 2 below presents the tyrosinase inhibition potential of the selected plant extracts. Some selected plants showed good anti-tyrosinase activities with low IC_{50} values. The plant extract *V. karroo (*6.84 µg/ml) inhibited the tyrosinase enzyme activity at a concentration close to that of the control, kojic acid (6.45 µg/ml) and *O. trichocarpum's* (2.95 µg/ml) activity was even better than that of kojic acid. Certain plant constituents are known to be common only in specific plant families. Both *V. karroo* and *O. trichocarpum* belong to the Fabaceae, a plant family well-known for containing high levels of polyphenolic compounds, such as flavanoids (Musabayane, 2012).

Table 1. Botanical name, authority name, family, voucher specimen and plant parts utilized of selected South African medicinal plants

Plant extract	Common Name	Family	Voucher Specimen Number	Plant Parts Utilized	Medicinal uses	References
Myrsine pillansii (L.) Adamson	African boxwood	Myrsinaceae	Q.R.17	Leaves and stems	Blood purifier, wound healing, skin treatment	(Watt & Breyer-Brandwijk, 1962)
Rapanea melanophloeos (L.) Mez	Cape beech	Myrsinaceae	Q.R.18	Leaves and stems	Treat stomach, respiratory and nervous system disorders, wound healing	(Gwala, 2011)
Myrsine africana (L.)	Large cape myrtle	Myrsinaceae	S.M.95503	Leaves	Anthelmintic and anti-acne.	(Chopra et al., 1956)
Vachellia karroo Hayne	Sweet thorn tree	Fabaceae	BC77	Roots	Treatment of colds and acne.	(Madureira et al., 2012)
Ormocarpum trichocarpum (Taub.) Engl.	Hairy caterpillar-pod	Fabaceae	BC19	Leaves	Treatment of stomach and skin ailments.	(Chukwujekwu et al., 2012)
Kalanchoe thyrsiflora Harv.	Paddle plant	Crassulaceae	BC16	Leaves	Treatment of earaches, acne and colds.	(Pooley, 1998)

Table 2. The tyrosinase inhibition potential and cytotoxicity of the selected Southern African plant extracts.

Plant Sample	Extract Yield (%)	Tyrosinase IC_{50} (µg/ml)	± SD[a]	Cytotoxicity (µg/ml)	± SD[a]
M. pillansii	15.79	231.10	1.67	169.79	1.93
R. melanophloeos	13.25	102.80	1.32	79.23	2.64
V. karroo	11.36	6.84	2.11	153.4	1.74
K. thyrisflora	9.08	14.3	1.21	131.4	2.34
O. trichocarpum	5.69	2.95	1.76	254.9	1.10
M. africana	7.69	27.4	2.05	155.4	1.83
Kojic acid*	-	6.45	0.99	-	-
Lipoic acid**	-	452.50	1.01	-	-
Actinomycin D***	-	-	-	0.00915	-

*Positive control for anti-tyrosinase assay

**Positive control for tyrosinase gene expression

***Positive control for cytotoxicity assay

[a]SD indicates standard deviation

The plants *K. thyrsiflora (*14.3 µg/ml) and *M. africana* (27.4 µg/ml) showed slightly higher IC_{50} values than Kojic acid but still had tyrosinase inhibiting activity potential. The plants *M. pillansii (*231,10 µg/ml) and *R. melanophloeos (*102.8 µg/ml) had high IC_{50} values, but

through this assay only tyrosinase inhibitors which directly affect the activity of the tyrosinase enzyme, are quantified. However, the inhibition of melanogenesis may include other mechanisms of action. It is crucial therefore to study the plant extracts at other inhibiting levels. For this study, the *in vitro* effect of the plant extracts on the genetic expression of tyrosinase within mouse melanocyte cells, was examined further.

3.2 XTT Cytotoxicity Assay

Potential tyrosinase inhibiting compounds could be restricted according to their cytotoxicity levels. If the compound is too cytotoxic, then it would not be regarded as safe and would be an unlikely candidate for future cosmetic applications. To ensure the plant extracts were not toxic or damaging to the melanocyte cells, a cytotoxicity assay was completed on B16-F10 mouse melanocyte cells. Based on the metabolic activity of the cells, the cell number and viability of the cells were calculated. The IC_{50} values obtained from this assay represented the concentrations at which 50% of the cells were inhibited for each plant extract. As seen in Table 2, all the plant extracts, except *R. melanophloeos* had an IC_{50} value greater than 100 µg/ml, and were therefore regarded as safe. The toxicity of *R. melanophloeos* is moderate, with the correct concentrations and careful consideration its usage can be easily regulated in cosmetic applications.

3.3 Real Time quantitative PCR Relative Expression Analysis of Tyrosinase cDNA in Mouse Melanocyte Cells

Relative quantification provides a means to compare the potential changes in gene expression with the fold difference indicating whether the change in treatment is significant or not (Fraga et al., 2008). RT-qPCR analysis of the treated and untreated mouse melanocyte samples for both the TYR and CPT2 genes were performed with three technical replicates each. The CPT2 gene is expressed in mouse melanocyte cells at levels similar to the expression of tyrosinase (Fraga et al. 2008). Therefore we could obtain an indication of whether the tyrosinase expression was increased or decreased for each sample when compared to CPT2 levels. Besides normalising the tyrosinase gene expression levels by relative quantification, the reference gene CPT2, also ensured that the comparison made was between equivalent amounts of starting sample.

The relative expression of tyrosinase, normalised against the CPT2 reference gene, can be seen in Figure 1. The untreated mouse melanocyte cells were used as a control and indicated the second highest expression of the tyrosinase gene. Surprisingly the extract, *R. melanophloeos*, upregulated the expression of the tyrosinase gene whilst all the other treatments down-regulated the tyrosinase mRNA compared to the untreated control. Since the plant extracts were dissolved in DMSO prior to treatment, and DMSO clearly had an effect on tyrosinase expression, it was used as a measure of comparison. The relative expression of the plant treated samples were compared against the DMSO control and a fold-change was calculated.

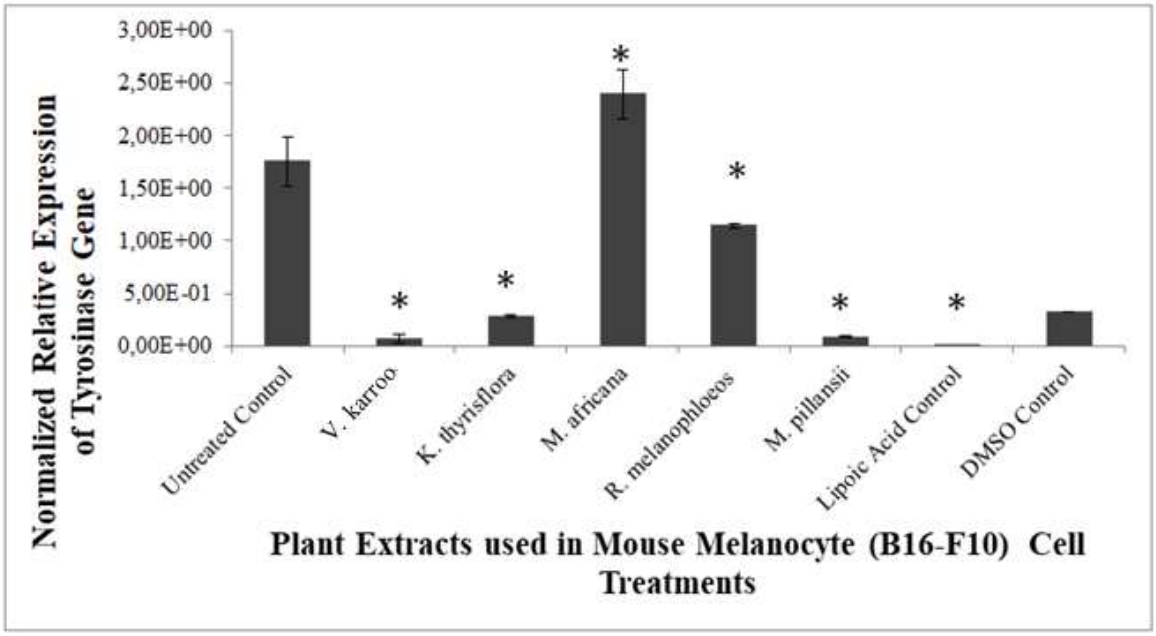

Fig 1. Normalised RT-qPCR expression profiles of the tyrosinase mRNA in B16-F10 mouse melanocyte cells. The melanocyte cells were treated with *M. pillansii, R. melanophloeos, V. karroo, K. thyrsiflora* and *M. africana* as well as lipoic acid, DMSO and untreated controls. The treatments were performed in triplicate and the average tyrosinase gene relative expression values were normalized by dividing the amount of input tyrosinase cDNA (extrapolated from the respective standard curves) by the input cDNA of the reference gene CPT2. Standard error of the average relative expression is indicated by the error bars in the figure. Statistical analysis of the relative expression data was done by one-way ANOVA analysis. * Indicates a statistical significant change in mean expression value when compared to the DMSO control. The extracts *M. pillasnii* and *V. karroo* significantly inhibited tyrosinase gene expression (p-value < 0.05).

The one way ANOVA test indicated that all the samples were significantly different to the DMSO control at a calculated probability equal or smaller than 0.05. The plant extract, *M. africana* up-regulated the tyrosinase mRNA expression at the tested concentrations by 2.7 fold respectively, when compared to the untreated control. Whilst the plant extract *R. melanoploeos* up-regulated tyrosinase expression by 3.5 fold respectively when compared to the DMSO control. These plant extracts are therefore possibly decreasing melanin production through other mechanisms besides down-regulating the expression of tyrosinase gene.

Levels of tyrosinase mRNA were down-regulated by *K. thyrsiflora, M. pillansii* and *V. karroo* by 1.2, 3.7, and 12.7 fold when compared to the DMSO control. The positive control, lipoic acid is known to inhibit tyrosinase gene expression with the lowest 0.004 relative expression to the tyrosinase gene expression. Lipoic acid down-regulated the expression of the tyrosinase gene by 81.3 fold compared to the DMSO control.

4. Discussion

The greatest anti-tyrosinase activity was observed by the plant extracts, *V. karroo* and *O. trichocarpum*, equal or better than that of kojic acid. The positive control, kojic acid chelates the copper ion in the tyrosinase enzyme to induce inhibition on catecholase and monophenolase activities of tyrosinase (Nerya et al., 2003). Kojic acid binding to the

tyrosinase active centre is a form of competitive inhibition preventing the catalysed oxidation of L-Dopa. Kojic acid has been used in skin depigmenting products at concentrations of 1 to 4% (Nerya et al. 2003) but its instability and potential side effects such as erythema, sensitization and irritant contact dermatitis reduces its choice for use in cosmetics (Monteiro et al., 2013).

When observing our plant extracts, *V. karroo* and *O. trichocarpum*, used in the present study, their inhibition of tyrosinase could have been due to competitive inhibitors present. Competitive inhibitors compete against melanin substrates, such as L-Dopa, for the same active centres of the enzyme. Therefore the inhibitor and substrate are mutually exclusive and may be copper chelators, non-metabolizable analogues or substrate derivatives of the true substrate (Chang, 2009). Since copper is an essential part of the enzymatic activity, tyrosinase can be inhibited by compounds which bind to the copper (Chang, 2009). The *V. karroo* and *O. trichocarpum* extracts most likely act through competitive inhibition since the Fabaceae family are known to contain many phenolic flavonoid components (Musabayane, 2012). In a previous study, the Asian plant, *Sophora japonica* (Fabaceae), has been proven to be a potent tyrosinase inhibitory agent which contains the anti-tyrosinase activity of several important flavonoids, such as rutin and quercetin (Lai et al., 2014). Flavonoids contain a phenol structural group which may be a structural analogue to the L-tyrosine substrate (Chang, 2009). This phenolic structure would compete with the substrate for the active site of tyrosinase, inhibiting its activity.

The anti-tyrosinase assay only quantifies tyrosinase inhibitors, which directly affect the activity of the tyrosinase enzyme. However, the inhibition of melanogenesis may include down-regulating the genetic expression of tyrosinase within mouse melanocyte cells. The positive control for tyrosinase gene expression studies was lipoic acid, an anti-oxidant dithiol-containing cofactor for many mitochondrial enzymes (Gorąca et al., 2011). Lipoic acid contains both antioxidant and dopaquinone activity and has been proven to block the expression of the microphthalmia transcription factor (MITF) which subsequently inhibits the expression of tyrosinase (Lu et al., 2011). Although lipoic acid is very effective in depigmentation, it is unfeasible as a cosmetic since it is toxic to cells and might interrupt the expression of off-target genes, such as ribosomes or CPT2 (Kim et al., 2008).

The natural plant extracts *K. thyrsiflora*, *M. pillansii* and *V. karroo*, exhibited down-regulation of the tyrosinase gene expression. Similar to lipoic acid, these plant extracts most likely down-regulated tyrosinase expression through the suppression of MITF expression, which is the most influential in modulating transcriptions of melanogenic enzymes (Park et al., 2009). MITF influences tyrosinase gene expression through the cyclic adenosine monophosphate (cAMP) dependant signal pathway (Ebanks et al., 2009).

Anti-inflammatory agents are known as good cAMP inhibitors (Gallardo and Johnson, 2004; Nanda et al., 2006). Aromatic-turmerone, a major bioactive compound of the curcumin herb *Curcuma longa*, has been well known for its anti-inflammatory properties (Hucklenbroich et al., 2014). In 2011 this compound was also discovered to down-regulate tyrosinase gene

expression through suppression of the cAMP pathway (Park et al., 2011). Resveratrol, an aromatic hydroxstillbene, with anti-oxidant and anti-inflammatory activity, exhibited down regulation of tyrosinase gene expression by reduction of MITF and tyrosinase promoter activities (Ebanks et al. 2009). This mechanism of action is most likely present in *V. karroo* which has been reported to possess anti-inflammatory activities (Adedapo et al., 2008).

Other reports have shown MITF activity inhibition by phenolic compounds, especially flavonoids (Chan et al., 2014) which are also capable of modulating the activity of enzymes (Abbhi et al., 2011). One such example is the compound, luteolin a flavone flavonoid, which inhibited both tyrosinase activity and melanin production in MSH stimulated B16-F10 melanoma cells. The flavonoid inhibited the intracellular cAMP levels thereby reducing the production of melanin in the melanoma cells (Choi et al., 2008). From phytochemical analysis flavonoids were abundantly present in *M. africana*, and we can assume their presence in *M. pillansii* (Abbhi et al., 2011).The *M. pillansii* plant might therefore have down regulated the tyrosinase gene through reduction of the intracellular cAMP levels.

Another known set of inhibitors of the cAMP/ MITF pathway includes terpenoids (Muhammad et al., 2016). From a previous study, a terpenoid momilactione B, isolated from rice bran, showed tyrosinase inhibitory mechanisms by affecting the MITF activity (Lee and Choi, 1999). Momilactione B also suppressed the activity of MITF in B16-F10 mouse melanocytes through the cAMP pathway and as a result, decreased the expression of the tyrosinase gene (Lee and Choi, 1999). The plant extract, *K. thyrsiflora,* most likely functions in a similar manner to downregulate tyrosinase since the *Kalanchoe* genus has been reported to contain many terpenoid compounds (Dasgupta et al., 2013).

The South African plants tested in this study contain various secondary metabolites which could convey different bioactivities. Transcriptional inhibition of tyrosinase expression is probably mostly due to terpenoid and flavonoid groups of compounds and compounds containing anti-inflammatory activities. Although regulation of tyrosinase by the plants is seen at transcription level, further analyses on other regulation mechanisms remain essential to confirm the mode of activity of the studied plant extracts.

5. Conclusion

The plant extracts, *M. pillansii, R. melanophloeos, V. karroo, K. thyrsiflora, O. trichocarpum,* and *M. africana* were studied for their melanogenesis inhibiting potential before and during melanin synthesis. This study demonstrated direct inhibition of tyrosinase by *V. karroo* and *O. trichocarpum* and down-regulation of tyrosinase gene expression by *K. thyrsiflora, M. pillansii* and *V. karroo*. In both cases the melanogenesis pathway was suppressed and less melanin was formed. These indigenous South African plants have great potential to be developed into a safe pharmaceutical or cosmeceutical products which would promote even skin tone whilst preventing undesirable hyperpigmentation and its associated ailments. These plants should therefore warrant further investigation in the field of skin-depigmentation.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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