Tyrosinase inhibition by extracts and constituents of *Sideroxylon inerme* L. stem bark, used in South Africa for skin lightening

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Objective: To investigate the stem bark of *Sideroxylon inerme* L. and its compounds for tyrosinase-inhibition activity and to evaluate the mechanism involved of the most potent compounds in tyrosinase inhibition.

Materials and methods: Three different extracts (acetone, methanol and dichloromethane) of *Sideroxylon inerme* L. were evaluated for their inhibitory effect *in vitro* on the monophenolase and diphenolase activated forms of tyrosinase, using a colorimetric procedure. This test was used for bioactivity-guided isolation of two active compounds using column chromatography and TLC. Active extracts were also investigated for their inhibitory effect on melanogenesis in cultured B16 melanoma cells. Antioxidant activities of the methanolic extract of *Sideroxylon inerme* and purified compounds were investigated using the 1,2-diphenyl-2-picrylhydrazyl (DPPH) antioxidant assay. The inhibition of tyrosinase activity relative to the inhibition of its activity at the transcripational level was also studied by determination of the degree of expression of mRNAs for this gene by using extract of *Sideroxylon inerme*-treated cells (B16F10) and semi-quantitative RT-PCR.

Results: Methanolic and acetic extracts of the stem bark of *Sideroxylon inerme* showed significant inhibition of monophenolase activity (IC50 values of 63 μg/ml and 82 μg/ml, respectively). The methanolic extract also exhibited 37% reduction of melanin content at 6.2 μg/ml in melanocytes without being significantly toxic to the cells. Examination for inhibition of monophenoloxidase *in situ* on TLC, followed by column chromatographic purification of the stem bark extract of *Sideroxylon inerme*, resulted in the isolation of two active compounds, epigallocatechin gallate and procyanidin B1, with IC50 values against monophenolase of 30 μg/ml and >200 μg/ml, respectively. Epigallocatechin gallate exhibited a greater anti-tyrosinase activity than arbutin. *Sideroxylon inerme* bark extracts, epigallocatechin gallate and procyanidin B1 exhibited antioxidant DPPH radical scavenging activities with EC50 values of 1.54 μg/ml, 1.33 μg/ml and 1.68 μg/ml, respectively and were not particularly cytotoxic. During mechanism studies it was evident that at the transcriptional level, *Sideroxylon inerme* (25 μg/ml) was acting as a potent tyrosinase inhibitor compared to controls (untreated cells and kojic acid).

Conclusion: The bark extract of *Sideroxylon inerme* and the two isolated compounds warrant further investigation in clinical studies to be considered as skin-depigmenting agents.

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1. Introduction

The dominant component of normal skin colour is provided by melanin (Launey and Land, 1984), although there are actually four chromophores that contribute to skin colour; haemoglobin, oxyhaemoglobin, carotenoids and melanin(s) (Summers, 2006).

Melanin plays the main role in skin colour and pigmentation and up to 10% of skin cells in the innermost layer of the epidermis produce melanin. Upon exposure of the skin to UV radiation, melanogenesis is initiated through an enzyme called tyrosinase (Parvez et al., 2006; Vamos-Vigyazo, 1981). Tyrosinase is a multifunctional copper-containing enzyme and in fungi and vertebrates, it catalyzes the initial step in the formation of the pigment melanin from tyrosine (Kim and Uyama, 2005). Use of tyrosinase inhibitors such as kojic acid and hydroquinone is becoming increasingly important in the cosmetic industry due to their anti-pigmenting effects. Such synthetic agents often result in inflammation of the skin so alternatives to them are being sought, including naturally occurring...
compounds. The traditional use of plants against skin disease, and especially for cosmetecutical purposes, is a common practice in the domestic medicine of many cultures, and may provide leads for better anti-pigmentation compounds (Pieroni et al., 2004).

One such traditionally used plant for skin-lightening purposes is Sideroxylon inerme L. (Sapotaceae), a large, evergreen tree, commonly known as white milkwood in South Africa (Van Wyk et al., 1997). It occurs across a wide range in southern Africa. Zulus and Xhosas (South African tribes) use the bark for several medicinal purposes (Watt and Breyer-Brandwijk, 1962). In the form of a paste, the bark is widely used as a skin lightener, particularly in KwaZulu—Natal province of South Africa (Van Wyk and Gerick, 2000).

Extracts of the bark of Sideroxylon inerme were tested for tyrosinase inhibition to see if this could explain the traditional use as a skin lightener. In order to more quickly identify the compounds responsible for the effect, an in situ TLC spray reagent procedure was used similar to that described recently (Wangthong et al., 2007).

2. Materials and methods

2.1. Materials and chemicals

The bark of Sideroxylon inerme was collected during July 2006 in Venda. The plant was identified by Prof. B. van Wyk, at the H.G.W.J. Schwelckerdt Herbarium of the University of Pretoria where a voucher specimen (PRU 96216) was deposited. The bark was dried outside, shielded from the sun, at ambient temperatures. L-Tyrosine, L-DOPA, tyrosinase, arbutin and kojic acid were obtained from Sigma–Aldrich (Kempton Park, South Africa). Cell culture reagents and equipment were purchased from Highveld Biological (Sandringham, South Africa), LASEC (Randburg, South Africa) and The Scientific Group (Midrand, South Africa). The B16F10 mouse melanocyte cell line was obtained from Highveld Biological (Sandringham, South Africa).

2.2. Extraction

The dried bark of Sideroxylon inerme was ground to a fine powder. Forty gram portions of the bark powder were each extracted with 200 ml of acetone, dichloromethane or methanol separately, each with stirring at room temperature, to give three extracts. This process was repeated three times and the solvent was then removed under vacuum to yield dry extracts. The percentage yields of each extract, acetone, dichloromethane and methanol were 6%, 5.2% and 8% w/w, respectively.

2.3. Colorimetric tyrosinase inhibition assay

Extracts/purified compounds were dissolved in dimethyl sulfoxide (DMSO) to a final concentration of 20 mg/ml. This extract stock solution was then diluted to 600 μg/ml in 50 mM potassium phosphate buffer (pH 6.5). Arbutin and kojic acid were used as positive controls. 70 μl of each sample solution of different concentrations (3.1–400 μg/ml) were combined with 30 μl of tyrosinase (333 Units/ml in phosphate buffer, pH 6.5) in triplicate in a 96-well microtitre plates. After incubation at room temperature, to give three extracts. This extract stock solution was then diluted to 600 μg/ml in 50 mM potassium phosphate buffer (pH 6.5). Arbutin and kojic acid were used as positive controls. 70 μl of each sample solution of different concentrations (3.1–400 μg/ml) were combined with 30 μl of tyrosinase (333 Units/ml in phosphate buffer, pH 6.5) in triplicate in a 96-well microtitre plates. After incubation at room temperature for 5 min, 110 μl of substrate (2 mM L-tyrosine or 12 mM L-DOPA) were added to each well. Final concentrations of the extract were 3.1, 6.2, 12.5, 25, 50, 100, 200 and 400 μg/ml. Final concentrations of pure compounds and positive controls were 1.5, 3.1, 6.2, 12.5, 25, 50, 100 and 200 μg/ml. Microtitre plates were incubated for 30 min at room temperature. Optical densities of the wells were then determined at 492 nm with the BIO-TEK PowerWave XS multi-well plate reader (A.D.P., Weltevreden Park, South Africa). The IC50 value was determined by the use of MSTATC software (East Lansing, MI, US).

2.4. Tyrosinase inhibition bioautography assay

The procedure was similar to that reported recently (Wangthong et al., 2007). Tyrosinase solution was prepared by dissolving 1 ml of 3333 units enzyme in 10 ml phosphate buffer. The substrate was prepared by dissolving 0.0036 g of L-tyrosine in 10 ml phosphate buffer. Methanol extract (10 μL) of Sideroxylon inerme (20 mg/ml) and positive control (arbutin) were dissolved in acetone and subjected to TLC (silica gel/acetone: methanol 1:49) using two plates. After removal of the mobile phase, one plate was sprayed with acidic vanillin, a detecting agent for terpenoids and related compounds, while the other plate was sprayed with L-tyrosine as substrate (2 mM) and incubated at room temperature for 10 min. After 10 min, the plate was sprayed with the tyrosinase solution and left at room temperature for 30 min. The background colour of the plate assumed a purlish-grey colour and clear zones of tyrosinase-enzyme inhibition were observed on the plates, indicating the active compounds present in the extract.

Minimum levels of detection for known inhibitors of tyrosinase were determined by spotting 10 μl of serial dilutions of their stock solutions onto the plate and then spraying, using the enzyme procedure detailed above. The concentration below which no white zone could be observed was taken to calculate the minimum detectable amount.

2.5. Isolation of active constituents

Acetone and methanolic extracts of the bark of Sideroxylon inerme exhibited similar tyrosinase inhibitory activity (Table 1). The methanolic extract was selected for the isolation and identification of active principle(s). One kilogram of air-dried bark of the plant was milled into a fine powder using a commercial grinder. The powder was extracted twice, each time with 41 of methanol at 50°C for 24 h. The combined methanol extract was filtered and the filtrate was concentrated to dryness under reduced pressure in a rotary evaporator.

The dried methanolic extract of the bark of Sideroxylon inerme (30.05 g) was re-dissolved in 80% ethanol (ethanol/distilled water; 80:20) and partitioned with n-hexane, ethyl acetate and n-butanol. The organic layers were evaporated to dryness at 40°C to give 8 g, 10.5 g, 7 g and 5 g of n-hexane, ethyl acetate, n-butanol and aqueous fractions, respectively. Bioassay of these fractions of Sideroxylon inerme showed that the ethyl acetate fraction demonstrated the highest inhibition (70% at 200 μg/ml) of monophenolase activity (Fig. 2). It was therefore, subjected to fractionation on a Sephadex column LH-20 (7 × 50 cm) using a gradient of H2O:ethanol of decreasing polarity (0% to 100% Ethanol). Forty-four fractions were collected and those with similar TLC profiles (TLC plates were developed using ethyl acetate/formic acid:acetic acid:distilled water: 13.5:10:1:1.5, as eluent. Acidic vanillin was used as a detecting agent. Fractions exhibiting similar TLC profile were combined together to provide fifteen major fractions (1B to 15B). Fraction 6B (100 mg) and 12B (120 mg) were chromatographed separately using Sephadex columns LH-20 (Sigma–Aldrich, South Africa) using 100% ethanol as eluent, to obtain pure epigallocatechin gallate 1 from 6B (pale orange powder, yield: 0.035%) and pure procyanidin B1 from 12B (pale orange powder, yield: 0.084%) (Fig. 1). Compounds were identified by mass spectrometric and NMR data which were identical to those in the literature for these two compounds (Thompson et al., 1972; Porter, 1988).
2.6. Cell culture

The mouse melanocyte cell line, B16F10, was cultured in complete Basal Medium containing 10% foetal bovine serum, 1.5 g/l NaHCO₃, 2 mM l-glutamine, 10 μg/ml penicillin, 10 μg/ml streptomycin and 0.25 μg/ml fungi zone at 37 °C with 5% CO₂ in a humidified atmosphere. Cells were sub-cultured in a ratio of 1:3 on every third or fourth day. For in vitro experiments, B16F10 cells were resuspended in complete DMEM medium containing 10% foetal bovine serum, 1.5 g/l NaHCO₃, 2 mM l-glutamine, 10 μg/ml penicillin, 10 μg/ml streptomycin and 0.25 μg/ml fungizone.

2.7. Determination of extract toxicity and melanin content in melanocytes

*Sideroxylon inerme* (methanol extract) and epigallocatechin gallate exhibited significant anti-tyrosinase activity (Table 1), so were selected for further investigation of their ability to inhibit the production of melanin in melanocyte cells. B16F10 cells (mouse melanocytes) in complete DMEM medium were added into the wells of a 96-well plate (10⁴ cells per well) and 24-well plate (10⁵ cells per well). After an overnight incubation at 37 °C in 5% CO₂ and a humidified atmosphere, extract samples and positive controls; arbutin and kojic acid were added to the cells to final concentrations of 1.5, 3.1, 6.2, 12.5, 25, 50 and 100 μg/ml. Plates were incubated at 37 °C in 5% CO₂, humidified atmosphere for 3 days. The toxicity of the extracts on the B16F10 cells was assayed using XTT (sodium 1-(phenyl amino-carbonyl)-3,4-tetrazolium)-bis[4-methoxy-6-nitrobenzene sulfonylic acid hydrate] cytotoxicity assay. Fifty microlitres of XTT reagent (1 mg/ml XTT with 0.383 mg/ml 2-(4-methoxy-6-nitrobenzene sulfonylic acid hydrate) cytotoxicity assay) were added to the wells and incubated for 1 h. The optical densities of the wells were then measured at 450 nm (690 nm reference wavelength). By referring to the control (medium with DMSO), cell survival was assessed. The effect of the extracts/compounds on melanin synthesis was determined by washing the cells in the 24-well plate with PBS and lysing with 200 μl of sterile distilled water. Optical densities were determined at a wavelength of 405 nm. The effect on melanin production was determined by referring to the control sample (medium with DMSO). The absorbances of a series of known concentrations of pure melanin were used to construct a calibration curve to determine the amount of melanin produced by the cells.

2.8. RNA extraction and reverse transcription

It was considered beneficial to determine whether the inhibition of tyrosinase activity was related to the inhibition of its activity at the transcriptional level. Therefore, the degree of expression of mRNAs for this gene was determined by using cells (B16F10) treated with *Sideroxylon inerme* bark extract and semi-quantitative RT-PCR. Total RNA was extracted from B16F10 cultured cells using the RNeasy Plant Mini Kit (Qiagen, EU). The quality of the total RNA sample was evaluated by determination of the A260/A280 ratio. To prepare a cDNA pool from each RNA sample, total RNA (1 μg) was reverse transcribed using DNasey Plant Mini Kit (Qiagen, EU). Each cDNA pool was stored at −20 °C until RT-PCR analysis was performed.

2.8.1. PCR primers

Specific oligonucleotide primer pairs to be used for RT-PCR were purchased from Inqaba Biotechnical Industries (South Africa). As internal control, mouse GADPH mRNA was also amplified using mouse GADPH amplimers. The sequences of the primers used for reverse transcription polymerase chain reaction (RT-PCR) were as follows: Tyrosinase forward primer: 5′ CGAGCCCTGCTGCTCCCTCCTAAG3′, Tyrosinase reverse primer: 5′ CACGACTACGCTGCTCCCTCCTAAG3′, GAPDH forward primer: 5′ CCAATGTCCTGCCTGCATCAAG3′ and GAPDH reverse primer: 5′ GCTGTTGAAGTCGCAGGAGA3′.

2.8.2. Semi-quantitative RT-PCR analysis of tyrosinase mRNA

Two micrograms of total DNaseI-treated and column-purified RNA extracted from mouse cells treated with methanolic extract
Sideroxylon inerme (25 µg/ml) and from untreated control mouse cells were reverse transcribed into first strand cDNA using Promerl Reverse Transcriptase (Promega, Madison, WI) according to manufacturer’s instructions. Each PCR contained the following components: 1 µl of cDNA, 1 X buffer, 2.5 mM MgCl2, 0.2 mM dNTPs, 0.2 µM each primer and 1 unit of Taq polymerase in a total reaction volume of 20 µl. DNA amplification was performed using a GeneAmp PCR System 2 400 (PerkinElmer, California, USA). The cycling conditions were 95 °C for 5 min, 35 cycles of 95 °C for 30 s, 58 °C for 30 s and 72 °C for 6 min. Products were removed after 23, 25, 28, 30 and 32 cycles and run on a 2% (w/v) agarose gel alongside a standard. The gel was photographed under UV light and the amount of DNA represented in each DNA band was calculated using the VersaDocTM 4000 (Bio-Rad Laboratories, Inc. Life Science Research Group, California, USA). The data was entered in Microsoft Excel and the expression of TYR was standardised to the expression of the internal control gene, GAPDH. A bar graph representing the percentage inhibition of the expression of TYR in the different treatments compared to that in the untreated control sample was derived from this data (Fig. 2).

2.9. Antioxidant activity of Sideroxylon inerme

Since melanin formation beneath the skin proceeds through a free radical mechanism, it could be disrupted by selective use of antioxidants, potent enough to poison this reaction. Antioxidant activities of the methanolic extract of Sideroxylon inerme and purified compounds were, therefore, investigated using the 1,2-diphenyl-2-picrylhydrazyl (DPPH) (Sigma–Aldrich, South Africa).


diphenyl-2-picrylhydrazyl (DPPH) (Sigma–Aldrich, South Africa)

According to Toit et al. (2001) for each sample, a dilution series (8 dilutions) was prepared in a 96 well ELISA plate by adding distilled water (100 µl) as a dilution medium. Final concentrations of the methanolic extract of Sideroxylon inerme ranged from 7.8 to 1000 µg/ml and of purified compounds ranged from 1.9 to 250 µg/ml. Each concentration was tested in triplicate. Ascorbic acid (AA) was used as a positive control and was tested at concentrations ranging from 7.8 to 1000 µg/ml. Ninety microlitres (90 µM) of methanolic DPPH was added to each well. The plates were covered with aluminium foil and incubated at room temperature for 1 h before being read by a BIO-TEK PowerWave XS multi-well plate reader (A.D.P., Weltevreden Park, South Africa).

The radical scavenging capacities of the samples were determined by using a BIOTEK plate reader to measure the disappearance of DPPH at 550 nm. The radical scavenging activity was measured in terms of the amount of antioxidants necessary to decrease the initial DPPH absorbance by 50% (EC50) (Toit et al., 2001). The EC50 value for each sample was determined graphically by plotting the absorbance of DPPH as a function of the sample concentration in µg/ml for the standard and samples (Table 2). The EC50 is the amount of antioxidant necessary to decrease the initial DPPH absorbance by 50%. The results are expressed as the mg AA equivalents/g dry weight and are calculated as follows: EC50 [mg/ml]/EC50 sample (g/ml) × mg AA equivalents/g dry weight = Zero mg/ml was taken as 100%.

2.10. Statistical analysis

The final results were expressed as the mean (standard deviation, ±S.E.M.). The group means were compared using the ANOVA test (MSTATC software, East Lansing, MI, USA) and the Duncan’s Multiple Range Test was applied to compare the means. Values were determined to be significant when P was less than 0.01 (P<0.01).

3. Results

3.1. Development of a TLC autobiographic assay for tyrosinase inhibitory activity

During the anti-tyrosinase bioautographic assay, clear zones indicating inhibition of monophenolase were observed when TLC plates spotted with extract was sprayed with l-tyrosine substrate and tyrosinase enzyme. No further darkening of plates sprayed with tyrosine was observed 10 min after the second (enzyme) spray was applied, so this was considered to be a sufficient time for maximal enzyme activity. Minimal detectable limits for known inhibitors of tyrosinase were found to be 0.7 µg for kojic acid and 1.6 µg for arbutin. A similar experiment using L-DOPA was used to detect diphenoilase inhibition.

Table 2

<table>
<thead>
<tr>
<th>Samples</th>
<th>EC50 (µg/ml)</th>
<th>mg AA equivalents/g dry weight (EC50 value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sideroxylon inerme</td>
<td>1.54</td>
<td>2168.1</td>
</tr>
<tr>
<td>Epigallocatechin gallate</td>
<td>1.33 (2.9)</td>
<td>2510.6</td>
</tr>
<tr>
<td>Procyanidin B1</td>
<td>1.68 (4.9)</td>
<td>1987.6</td>
</tr>
<tr>
<td>Ascorbic acid (AA)</td>
<td>3.34 (18.9)</td>
<td></td>
</tr>
</tbody>
</table>
3.2. Tyrosinase inhibitory activity

Results of tyrosinase inhibitory activity by the methanol and acetone extracts of Sideroxylon inerme were similar (Table 1) showing significant inhibition \( (P<0.01) \) (70%) of tyrosinase activity at 200 \( \mu g/ml \). No significant reduction \( (P<0.01) \) of tyrosinase activity was obtained for the dichloromethane extract at any concentration tested.

The methanolic extract was subjected to phase partitioning. Of the hexane, butanol and ethyl acetate fractions, the ethyl acetate fraction exhibited the highest anti-tyrosinase activity and the highest inhibition of melanin production in melanocyte cells (40% at 25 and 50 \( \mu g/ml \)) \( (P<0.01) \). The purified compounds, epigallocatechin gallate 1 and procyanidin B1 2, isolated from this fraction, exhibited IC50 values of 30 \( \mu g/ml \) (65.5 \( \mu M \)) and >200 \( \mu g/ml \) (>346 \( \mu M \)) against monophenolase activity (58% and 55%, respectively at 200 \( \mu g/ml \)) while kojic acid and arbutin demonstrated IC50 values of 1.1 \( \mu g/ml \) and 149 \( \mu g/ml \). These compounds did not show any inhibition of DOPA auto-oxidation at the concentrations tested (except kojic acid which gave IC50 50.5 \( \mu g/ml \)). Table 1 depicts the IC50 values of crude extracts, isolated compounds from the methanolic extract of Sideroxylon inerme and the positive controls.

3.3. Cytotoxicity

Fig. 3 shows the effect of Sideroxylon inerme (methanolic extract), epigallocatechin gallate, arbutin and kojic acid on cell viability/proliferation and melanin production in B16F10 cells. Sideroxylon inerme showed significant inhibition (37%) of melanin production at 6.2 \( \mu g/ml \) while 80% of cells were viable, thus indicating low levels of cytotoxicity vs inhibition of melanin synthesis. The sample showed some toxicity to the cells when exposed to concentrations greater than 50 \( \mu g/ml \). Epigallocatechin gallate 1 did not show any significant \( (P<0.01) \) inhibition of melanogenesis at the concentrations tested and therefore probably plays a minor role in spite of its known strong antioxidant effects. Kojic acid (a positive control) showed no significant toxicity to B16-F10 cells at the highest concentration tested and exhibited similar reduction in melanin content at 3.1 and 25 \( \mu g/ml \) (60%).

4. Discussion

Plant extracts and their bioactive constituents have been explored previously for tyrosinase inhibitory activity. According to Hara et al. (1997), epigallocatechin gallate, epigallocatechin and epigallocatechin isolated from tea leaves exhibited 95%, 17% and 11% inhibition of monophenolase activity at 150 \( \mu g/ml \), so it is not surprising that the compounds isolated in this instance showed similar effects. No et al. (1999) reported that \((+)-epicatechin (EC), (-)-epigallocatechin (EGC) and (+)-catechin, purified from the methanolic extract of green tea, showed <10%, 40% and <10% inhibitory effects on mushroom tyrosinase at 40 \( \mu M \), respectively. They also demonstrated that galloatechingallate (GCC), isolated from the same extract, exhibited 40% anti-DOPA activity at 50 \( \mu M \).

According to Yamakoshi et al. (2006), little is known of the lightening effect of proanthocyanidin on UV-induced pigmentation of the skin but they showed that grape seed extract, rich in proanthocyanidins, inhibited the activity of mushroom tyrosinase and inhibited melanogenesis without inhibiting the growth of cultured B16 mouse melanoma cells. ‘Quebracho’, an extract rich in proanthocyanidins, isolated from the heartwood of Schinopsis lorentzii with 70% aqueous acetone, showed 47% tyrosinase inhibition (Takagi and Mitsunaga, 2003). Compared with the positive controls (arbutin and kojic acid), epigallocatechin gallate 1 exhibited more anti-tyrosinase activity than arbutin but less than kojic acid. This compound also showed stronger tyrosinase inhibitory activity than procyanidin B1 2.

It was considered beneficial to determine whether the inhibition of tyrosinase activity was related to the inhibition of its activity at the transcriptional level. Therefore, the degree of expression of mRNAs for this gene was determined by using Sideroxylon inerme treated cells (B16F10) and semi-quantitative RT-PCR. The gene for GAPDH served as the housekeeping gene. It was evident that at the transcriptional level, Sideroxylon inerme (25 \( \mu g/ml \)) was acting as a potent tyrosinase inhibitor compared to controls (untreated...
cells and kojic acid) (Fig. 2). A more accurate way to determine the inhibition at the transcript level would be the use of qRT-PCR, which determines the presence of SYBR gene in DNA sample (SYBR is a more sensitive fluorescent dye compared to ethidium bromide which is being used in semi-quantitative RT-PCR).

All the compounds showed strong antioxidant activities (Table 2). The DPPH-scavenging activities of *Sideroxylon inerme* (methanol extract) and its isolated compounds were two times better than those of the antioxidant activity of ascorbic acid. *Sideroxylon inerme*, epigallocatechin gallate and procyanidin B1 exhibited EC50 values of 1.54 μg/ml, 1.33 μg/ml (2.9 μM) and 1.68 μg/ml (4.9 μM) for DPPH antioxidant activity, respectively. Epigallocatechin gallate 1 and procyanidin B1 2 thus have DPPH radical scavenging activities higher than that observed for the standard (ascorbic acid). These compounds are therefore likely to be responsible for the antioxidant activity of the *Sideroxylon inerme* extract. The present findings warrant further investigation in the field of skin-depigmentation agents in clinical studies.

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