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Research Article

Tyrosinase Inhibition, Antioxidant Activity, and Bioactive Compound Profiling in Acetone Extracts of *Erythrophleum suaveolens* Stem Bark

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ABSTRACT

This study investigates the inhibitory activity of *Erythrophleum suaveolens* bark extracts on mushroom tyrosinase, a key enzyme in melanin synthesis, as well as ascertaining the antioxidant properties and elucidating the phyto-constituents in the most potent extract. Extracts were obtained using five solvents: n-hexane, dichloromethane, acetone, methanol, and distilled water. Among these, the acetone extract demonstrated the best tyrosinase inhibitory activity compared to the enzyme standard inhibitor, kojic acid, at the highest concentration (400 µg/ml). Specifically, the acetone extract demonstrated an inhibitory activity value at 50% inhibition (IA50) of 39.2 ± 0.20 µg/ml for mushroom tyrosinase diphenolase activity, surpassing other extracts, including hexane (65.8 ± 1.06 µg/ml) and kojic acid (45.1 ± 0.10 µg/ml). Furthermore, the acetone extract showed dose-dependent antioxidant properties, with ferric reducing antioxidant power of 57.16 ± 0.11 mg vitamin C/g extract, DPPH radical scavenging activity of 118.7 ± 0.16%, and iron chelating activity of 26.4 ± 0.07% at the highest concentration (100 µg/ml), suggesting potential de-pigmenting effects. High-performance liquid Chromatography (HPLC) analysis, which separates, identifies and quantifies extract components via an acetonitrile/water (80:20, v/v) mobile phase and a Phenyl stationary phase, revealed a rich flavonoid content in the acetone extract, with rutin (1.418 ppm) and quercetin (4.758 ppm) being the most prominent compounds. This bioactive compound profile reveals the inhibitory properties of the *E. suaveolens* acetone extract and underscores its potential as a natural tyrosinase inhibitor with promising implications for improving skin appearance and health.

Keywords: *Tyrosinase, Secondary metabolites, High-Performance Liquid Chromatography, Erythrophleum suaveolens*

INTRODUCTION

Erythrophleum suaveolens, a tropical tree native to West and Central Africa, is recognised for its significant stature in forest ecosystems. First described by Procter in 1851, it is closely related to the Coffee-nut tree (*Gymnocladus canadensis*) of the United States (Brunken *et al.*, 2008). Commonly known as "African rosewood" or "scented rosewood," this species is referred to by various names across cultures, including "Igi obo" (Yoruba) and "Inyi" (Igbo). Belonging to the Fabaceae family, *E. suaveolens* has a rich history of traditional medicinal use among indigenous populations (Maroyi, 2023). The therapeutic versatility of *E. suaveolens* is attributed to its secondary metabolites, such as alkaloids, flavonoids, tannins, and terpenoids (Akanji and Adewumi, 2020). These phytochemicals have been linked to diverse biological activities, including anti-inflammatory, antimicrobial, antidiabetic, and anticancer effects (Son, 2019), suggesting their potential for pharmaceutical and cosmetic applications. Tyrosinase is a copper-containing enzyme essential in melanin biosynthesis (Momtaz et al.,

2008; Omotoyinbo et al., 2020), significantly influences skin and hair pigmentation. Imbalances in melanin production can lead to hyperpigmentation disorders, which pose concerns in dermatology and the cosmetics industry (Cestari et al., 2014; Pillaiyar et al., 2017). Although synthetic tyrosinase inhibitors have been utilised, safety and side effect concerns have driven interest in natural alternatives (Chang, 2009). The traditional use of E. suaveolens and emerging scientific evidence position it as a promising candidate for developing safe and effective tyrosinase inhibitors.

Numerous plant extracts, including oats, walnuts, and chamomile, have been investigated for their effects on hyper- or hypopigmentation disorders (Momtaz et al., 2008). Tyrosinase is critical in pigment formation, alongside other components like haemoglobin and carotenoids; however, melanin remains the dominant contributor (Summers, 2006; Momtaz *et al.*, 2008). Tyrosinase, also a polyphenol oxidase exhibits two enzymatic activities: cresolase activity (hydroxylation of L-tyrosine) and catechol oxidase activity (oxidation of diphenols), both crucial for melanin production. Overproduction of melanin can result in dermatological conditions such as dark spots and freckles, often exacerbated by reactive oxygen species (ROS) generated during biosynthesis (Koga *et al.*, 1992; Simon *et al.*, 2009). Therefore, controlling melanisation through tyrosinase inhibition could have broad applications in dermatology and cosmetic products.

Pillaiyar *et al.* (2017) highlighted the importance of tyrosinase inhibitors in addressing hyperpigmentation. Various plant extracts have shown potential, with notable inhibitors including vitamin C and liquorice (Pieroni *et al.*, 2004; Zaveri and Patel, 2012). Furthermore, the imbalance between the body's free radical production and its antioxidant defences, known as oxidative stress, underlies many chronic diseases, including cancer and cardiovascular disorders (Sharifi-Rad *et al.*, 2020). Antioxidants like those in *E. suaveolens* can neutralise harmful free radicals and mitigate oxidative damage (Martemucci *et al.*, 2022).

With recent studies exploring the tyrosinase inhibitory and antioxidant properties of *E. suaveolens* extracts (Jacques *et al.*, 2019), a comprehensive assessment of this plant's therapeutic and cosmetic potential is necessary. This research aims to evaluate the tyrosinase inhibition of *Erythrophleum suaveolens* stem bark extracts, assess their antioxidant activities, and employ High-Performance Liquid Chromatography (HPLC) to identify and quantify bioactive compounds in the most effective extract.

MATERIALS AND METHODS

Plant material

Erythrophleum suaveolens (Guill & Perr.) was collected from the medicinal garden of Olusegun Agagu University of Technology, Okitipupa. The stem bark of the plant was identified and verified at the Botany Department, with voucher specimens deposited in their Herbarium under specimen snumber: BSH 294.

Processing and preparation of extracts

Stem bark was dried for 20 days. The drying area was monitored using a hygrometer to maintain consistent ambient humidity levels. The drying process was also conducted in a well-ventilated space to facilitate air circulation, further stabilising the moisture content at an ambient temperature of 25°C. The dried materials were pounded with mortar and pestle to produce coarse particles before being pulverised using Kanchan International Blender (China).

Exactly 50 g of pulverised stem bark was dispensed into five conical flasks, after which 250 ml of extracting solvent: n-hexane, dichloromethane, acetone, methanol and distilled water was added and shaken vigorously to obtain a mixture. Extraction was done by decoction at 50°C with constant stirring for 5 hours in an oscillating water bath (WHY-2, China), after which the extract was then allowed to stand overnight. The mixtures were initially filtered using muslin cloth and subsequently with Whatman No.1 filter paper. The residue underwent the same procedure twice for exhaustive extraction of phyto-constituents while the filtrates obtained in each process were evaporated using a Rotary evaporator (OEM, India). Finally, the extracts were air-dried at ambient temperature to obtain dry extract.

$$\text{Yield (\%)} = \frac{\text{Dry wt. of recovered extract}}{\text{Initial wt. of part extracted}} \times 100 \quad (1)$$

Tyrosinase inhibition assay

Tyrosinase inhibitory activity assays were measured spectrophotometrically following the method described by Liu *et al.* (2012). Enzyme - Mushroom tyrosinase (MERCK) 50 U/ml was prepared using 50 mM phosphate buffer, pH 6.5, 0.35 ml of various concentrations of extract: 3.1, 6.2, 12.5, 25, 50, 100, 200 and 400 µg/ml (Krasavin *et al.*, 2015) were added to 0.15 ml of tyrosinase, respectively and then incubated for 10 mins at room temperature (25°C). After, 0.55 ml of 12 mM L-DOPA was added to each mixture and further incubated at room temperature for 20 mins. The reaction mixture without plant extract served as a control, and the change in absorbance was measured using U.V./Visible Spectrophotometer (BOSCH, Germany) at 492 nm against the blank. Kojic acid (standard inhibitor) was used as a positive control at all concentrations considered while tests were performed in triplicate determination.

$$\text{Inhibition (\%)} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \times 100 \quad (2)$$

Inhibitory activity of extracts at 50% (IA_{50})

The extract inhibitory activity at 50% was extrapolated from the tyrosinase inhibition data graph. The values represent the extract concentration at which 50% of enzyme diphenolase activity was inhibited.

Antioxidant assays

Antioxident reducing power

Ferric Reducing Antioxidant Power Assay (FRAP) was determined according to the method described by Pulido *et al*. (2000) and Nam *et al*. (2017). In this method, the antioxidant compound forms a coloured complex with potassium ferricyanide, trichloroacetic acid and ferric chloride, read at 700 nm with the increase in absorbance of the reaction mixture, indicating the reducing power of the samples. Precisely 0.7 mL of extract solution was added to 0.7 mL of 50 mM phosphate buffer (pH 7.0). Afterwards, 0.5 mL of potassium ferricyanide (1% w/v $K_3Fe(CN)_6$) was added, and the mixture was incubated at 50°C for 20 mins. After that, 0.5 mL TCA (10% w/v) was added before centrifuging at 3000 rpm for 10 mins. Aliquot (0.5 mL) from the upper layer of the solution was then mixed with 0.5 ml of distilled water and 0.1 mL of $FeCl_3$ (0.1% w/v). The absorbance was then read at 700 nm against blank. Vitamin C was used as standard.

FRAP value (mg Vit. C/g sample) =

$$\frac{\text{Abs of sample x FRAP value of Standard}}{\text{Abs of Standard}} \text{ x } 100 \qquad (3)$$

Free radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging ability of the extract was determined using the method of Sun and Wang (2010). The DPPH assay is mainly based on electron transfer reaction, and hydrogen-atom abstraction is a marginal reaction pathway (Huang *et al.*, 2008). DPPH is a stable organic nitrogen radical. On accepting an electron from a corresponding donor, its solution loses the characteristic deep purple colour. Precisely 1.8 mL of 0.11 mM DPPH (in 80% ethanol) was added to 0.2 mL of varying concentrations (0, 25, 50, 75, 100 µg/mL) of the extract. The mixture was mixed and incubated at room temperature in the dark for 30 minutes, and absorbance (A sample) was read at 517 nm. Quercetin was used as reference/standard and solution of 0.11 mM DPPH in ethanol served as control (Acontrol). Compared to the reaction mixture's standard absorbance, a low absorbance value indicates a higher free radical-scavenging activity. To calculate the DPPH radical scavenging activity, the following equation was used:

(4)

% Inhibition of DPPH radical=

$$\frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \text{ x } 100$$

Iron Chelation Activity

The extracts' Iron (Fe^{2+}) chelating ability was determined using a modified method by Puntel *et al*. (2005). Freshly prepared 0.5 mM $FeSO_4$ (0.30 mL) was added to a reaction mixture containing 0.34 mL of 0.1 M Tris-HCl (pH 7.4), 0.45 mL saline and 0.05 mL extracts of varying concentrations: 0, 25, 50, 75 and 100 µg/ml. The mixture was allowed to stand for 5 mins before adding 0.02 mL of 0.25% 1,10-phenanthroline (w/v). The absorbance was subsequently measured with a UV-visible spectrophotometer at 510 nm. The Fe^{2+} chelating ability was subsequently calculated with the formula: (5)

$$Fe^{2+} \text{ chelating ability (\%)} = \frac{\text{Abs of control} - \text{Abs of sample}}{\text{Abs of control}} \text{ x } 100$$

HPLC extract analysis

HPLC analysis of phyto-compounds was performed using an Agilent HPLC 1200 system with Chem Station software equipped with an AGILENT 1260 detector and a CHROMSPHERE 5 C18 column (5 µm, 3 mm x 250 mm). A Hamilton microliter syringe was used for a 100 µL sample injection. The column temperature was maintained at 40°C, with a 0.7 mL/min flow rate and column pressure at 180 x 10 Pa. The mobile phase consisted of acetonitrile and water (80:20, v/v), with a modification to include 2% acetic acid in a water-methanol mixture (82:18, v/v) for isocratic elution. Phenolic compounds were quantified at 320 nm, with identification based on retention times and UV-vis spectra. Calibration curves from standard compounds were used for quantification; structurally related substances were used when unavailable, accounting for molecular weight correction factors.

Statistical analysis

Relevant data obtained from this study were analysed using analysis of variance (ANOVA), followed by the appropraite post hoc test. Values of $P < 0.05$ was considered statistically significant. The analysis was performed using GraphPad Prism 6 software.

RESULTS

Results

The extraction yield obtained from *Erythrophleum suaveolens* stem bark extracts using the different solvent systems (Table 1) revealed that methanol gave the highest yield among all the extracts.

Table 1. Extraction Yield from *Erythrophleum Suaveolens* Stem Bark Extracts

Plant Extracts	Sample Quantity (g)	Extract Yield (g)	Extract Yield (%)
E.s. water	50	5.34	10.68
E.s. methanol	50	9.27	18.50
E.s. acetone	50	4.25	8.50
E.s. dichlorom ethane	50	1.90	3.80
E.s. hexane	50	1.33	2.66

N.B: E.s. = *Erythrophleum suaveolens* stem bark

Inhibitory activity of *Erythrophleum suaveolens* stem bark extracts obtained from the different plants using five solvent systems: water, methanol, acetone, dichloromethane and n-hexane on Mushroom tyrosinase (Figure 1) showed the inhibition by the acetone extract was comparable to that of kojic acid (positive control); 79.73% and 80.11% respectively at highest concentration considered (400 µg/ml) and in almost all the inhibitor concentrations considered. On the contrary, Dichloromethane extract showed the lowest inhibitory activity of 54.8% at 400 µg/ml. Also, the inhibitory activity values of all the extracts at 50% (IA50) for Mushroom tyrosinase diphenolase activity, as reported in Table 2, revealed that *Erythrophleum suaveolens* acetone extract had the best IA50 value; 39.2 µg/ml, comparing favourably to that of kojic acid; 48.3 µg/ml.

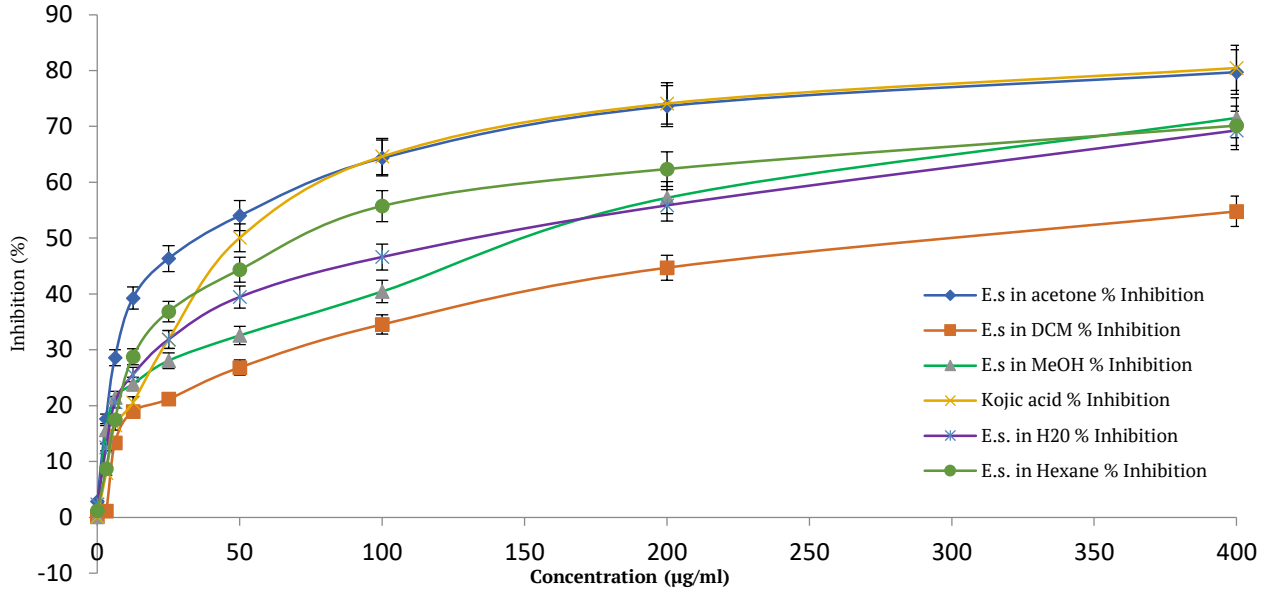


Figure 1. Tyrosinase Inhibition of *Erythrophleum suaveolens* (E.s.) Stem-bark Extracts and kojic acid.

Each value represents means ± S.D. (n=3)

Table 2. Erythrophleum Suaveolens Extracts Inhibitory Activity at 50% (IA_{50})

Extracts	*Erythrophleum suaveolens* IA_{50}(µg/ml)
Water extract	148.6 ± 2.24[a]
Methanol extract	150.5 ± 0.25[a]
acetone extract	39.2 ± 0.20[b]
Dichloromethane extract	294.2 ± 3.41[c]
Hexane extract	65.8 ± 1.06[d]
Kojic acid	48.3 ± 0.74[de]

Values are presented as mean ± S.D. (n = 3); IA_{50}: inhibitory activity at 50%. Values with different superscript(s) in a column are significantly different (P<0.05)

The ferric reducing antioxidant power (FRAP), DPPH radical scavenging activity, and Iron chelating activity of the *Erythrophleum suaveolens* acetone extract revealed that the extracts exhibited a dose-dependent activity in its antioxidant property assays as represented in Table 3.

Table 3. Antioxidant Activity of *Erythrophleum Suaveolens* Acetone extracts

Conc. (µg/ml)	FRAP (mg vit./g ext.)	DPPH scavenging (%)	Iron chelation (%)
25	14.11 ± 0.10[a]	22.70 ± 0.20[a]	3.30 ± 0.10[a]
50	31.80 ± 0.18[b]	35.80 ± 0.20[b]	11.65 ± 0.08[b]
75	51.86 ± 0.14[c]	89.70 ± 0.20[c]	18.75 ± 0.11[c]
100	57.16 ± 0.11[cd]	118.70 ± 0.16[d]	26.40 ± 0.07[d]

Values are expressed as mean ± standard deviation (n=3). Values with different superscript (s) in a column are significantly different (P<0.05)

The spectra and summary of the bioactive compounds resolved in the acetone extract of *Erythrophleum suaveolens* (Table 4) revealed several phenolics and flavonoids, which include gallic acid, catechin, rutin, ferulic acid, quercetin, kaempferol and luteolin also two phenolics: p-coumarin and apigenin.

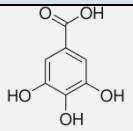
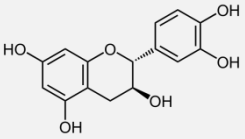
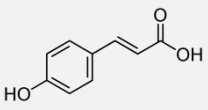
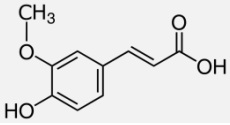
DISCUSSION

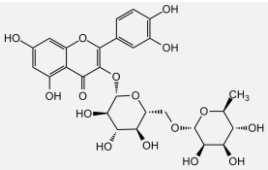
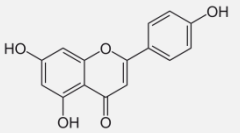
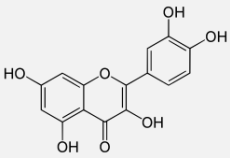
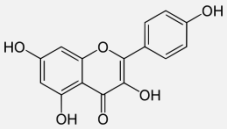
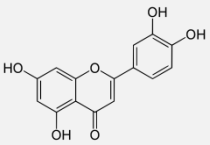
The inhibitory activity of the extracts obtained from *E. suaveolens* bark on mushroom tyrosinase was most pronounced for the acetone extract, which demonstrated an inhibition rate of 79.73% compared to 80.11% for kojic acid

at the highest concentration (400 µg/ml). The extract's inhibitory activity values at 50% (IA50) for mushroom tyrosinase diphenolase activity were notably lower (39.2±0.20 µg/ml) than those of kojic acid (45.1±0.10 µg/ml), suggesting a more potent inhibition. This efficacy can be attributed to the presence of various phytochemicals including glycosides, alkaloids, tannins, terpenoids, steroids, cardiac glycosides, phlobatannins, anthraquinones, and flavonoids, which collectively enhance the tyrosinase inhibitory activity (Loizzo *et al.*, 2012).

Due to its intermediate polarity, acetone is recognised for effectively extracting a broad spectrum of polar and non-polar bioactive compounds. This solvent's capacity to solubilise hydrophilic and lipophilic compounds facilitates the extraction of phenolic compounds and flavonoids essential for bioactivity. Previous studies have demonstrated that acetone can enhance the extraction yield of flavonoids, which are vital for the anti-tyrosinase and antioxidant activities observed in plant extracts (Khatib *et al.*, 2007). The predominance of flavonoids, such as rutin and quercetin, in the acetone extract likely contributes to the observed antioxidant and anti-tyrosinase activities. Flavonoids possess structural features that allow them to scavenge free radicals and chelate metal ions (Abdelsalam *et al.*, 2023), thereby mitigating oxidative stress and inhibiting melanogenesis (Harbone and Williams, 2000). The potent antioxidant properties of flavonoids like rutin and quercetin can be linked to their ability to neutralise reactive oxygen species (ROS), which are known to stimulate tyrosinase activity and subsequent melanin production in the skin (Sarangarajan and Apte, 2006). Their structural characteristics, which include multiple hydroxyl groups, enable these compounds to donate electrons and stabilise free radicals effectively. Consequently, this interaction may explain the dose-dependent antioxidant activity of the acetone extract and its ability to inhibit tyrosinase activity. The interactions between polyphenols and flavonoids with the tyrosinase enzyme at the molecular level are critical in understanding their inhibitory mechanisms. Flavonoids can act as competitive inhibitors by binding to the active site of tyrosinase, preventing the substrate (tyrosine) from accessing the catalytic site (Lee *et al.*, 2015). The nature of these interactions can be reversible or irreversible, depending on the flavonoid's specific chemical structure and the reaction conditions. Therefore, the acetone extract's observed antioxidant and anti-tyrosinase activities can be explained by the dual role of flavonoids: potent radical scavengers and competitive inhibitors of the enzyme involved in melanogenesis. These results highlight the potential for the acetone extract of *E. suaveolens* to serve as a natural alternative for skin treatments targeting hyperpigmentation. Although this study primarily investigated the inhibitory effects of extracts and resolved compounds on mushroom tyrosinase, the relevance of these findings to human tyrosinase remains to be determined due to structural and functional differences between the enzymes. Notably, mushroom tyrosinase is commonly used for initial screenings of hyperpigmenting agents (Momtaz *et al.*, 2008). While the *in vitro assays* provided valuable insights, they do not reflect the complex biological environment of human skin or address potential cytotoxicity concerns. Despite these limitations, analysing chemical constituents through HPLC revealed a correlation with the observed inhibitory activities. Hence, future studies should focus on isolating and characterising the specific compounds responsible for these effects. Such investigations could lead to the development of more targeted and effective tyrosinase inhibitors, enhancing our understanding of the extract's mechanisms and potential applications in human skin treatments.

Table 4. Some Phytochemicals Detected in of *Erythrophleum Suaveolens* Acetone Extract

S/N	Ret. Time (min)	Flavonoid dimer	Area Amount (ppm)	Name of Phenolics	Structure
1	4.688	BB	0.801	Gallic acid	
2	12.235	MM	0.309	Catechin	
3	18.796	-	-	P-coumaric acid	
4	20.440	MM	0.271	Ferrulic acid	

5	23.495	MM	1.418	Rutin	
6	30.190	-	-	Apigenin	
7	37.800	BB	4.758	Quercetin	
8	38.671	BV	0.338	Kampferol	
9	39.069	VB	0.663	Luteolin	

N.B: BB = Biflavonoids, MM = Homodimers, BV = Heterodimers of two different monmers, VB = Heterodimers where the order of the monomers is reversed; all refers to different flavonoid dime.

CONCLUSION

The findings of this study highlight the remarkable inhibitory activity of the acetone extract obtained from *Erythrophleum suaveolens* bark against Mushroom tyrosinase. The extract's inhibitory efficacy and significant redox properties suggest a plausible mechanism for depigmentation, and this dual functionality underscores the versatility of *Erythrophleum suaveolens* bark acetone extract in cosmetic and dermatological applications. Therefore, exploring potential synergistic effects between the various bioactive compounds, optimising formulations for topical application, and comparing the efficacy of these extracts with other established tyrosinase inhibitors would also be valuable avenues for future investigation.

AUTHORS' CONTRIBUTIONS

OVO and DMS worked on the tyrosinase inhibitory assay as well as the antioxidant potential of the most potent stem extract. BIO assisted in the resolution of the bioactive compounds in the choice extract. OVO majorly did the write-up while other co-authors reviewed to make it ready for submission. All authors have read and agreed to the published version of the manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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