Pharmacognostic standardization, antioxidant and antimicrobial activities of *Erythrophleum suaveolens* (Guill. & Perr.) Brenan and *Peltophorum pterocarpum* (DC) Backer ex K. Heyne

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ABSTRACT

Background: The plants *Erythrophleum suaveolens* (Guill. & Perr.) Brenan and *Peltophorum pterocarpum* (DC) Backer ex K. Heyne from the family Fabaceae have been used traditionally as emetic and purgative and as medicines for dysentry, insomnia, skin diseases, constipation.

Objectives: The objective of the present study was to establish the pharmacognostic standards of *Erythrophleum suaveolens* and *Peltophorum pterocarpum* and to study the antioxidant and antimicrobial activities of the two plants.

Methods: Microscopic, phytochemical and physicochemical evaluations were performed on the leaf samples of the plants using standard procedures. Leaf extracts were evaluated for total phenolic content using Folin-ciocalteu reagent; *in vitro* antioxidant activity using DPPH assay, while antimicrobial activity was assessed using the agar diffusion method.

Results: Foliar micro-morphological studies on the plants revealed sharp generic variations in sizes and types of stomata, shapes of epidermal cells on the adaxial and abaxial surfaces, sizes and types of trichomes. The physico-chemical results obtained are within the standard limits. Methanol extract of *P. pterocarpum* had the highest phenolic content (62.0±1.23 mg GAE/g) and the highest antioxidant activity as revealed by its low IC_{50} value of 21.57±0.62 µg/mL. The antimicrobial study revealed that the extracts exhibited diverse activities against different microorganisms with zones of inhibition ranging from 2.0 to 15.0 mm. The MIC of the extracts ranged from 0 .78 to 3.125 mg/mL and MBC/MFC from 1.56 to 6.25 mg/mL for sensitive organisms at the tested concentrations.

Conclusion: The results provide the parameters for the standardization of the plants and also justify their ethnomedicinal uses.

Keywords: *Peltophorum pterocarpum, Erythrophleum suaveolens,* standardization, antioxidant, antimicrobial

Standardisation pharmaco-gnostique, antioxydant et activités antimicrobiennes *d'Erythrophleum suaveolens* (Guill. & Perr.) Brennan et *Peltophorum pterocarpum* (DC) Backer ex K. Heyne

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RESUME

Contexte: Les plantes *Erythrophleum suaveolens* (Guill. & Perr.) Brenan et *Peltophorum pterocarpum* (DC) Backer ex K. Heyne de la famille des Fabaceae ont été utilisées traditionnellement comme émétiques et purgatives et comme médicaments pour la dysenterie, l'insomnie, les maladies de la peau, la constipation.

Objectifs: L'objectif de la présente étude était d'établir les normes pharmacognostiques d'*Erythrophleum suaveolens* et *Peltophorum pterocarpum* et d'étudier les activités antioxydantes et antimicrobiennes des deux plantes.

Méthodes: Des évaluations microscopiques, phytochimiques et physicochimiques ont été effectuées sur les échantillons de feuilles des plantes en utilisant des procédures standard. Les extraits de feuilles ont été évalués pour la teneur phénolique totale en utilisant le réactif Folin-ciocalteu; l'activité anti-oxydante *in vitro* en utilisant le dosage DPPH, tandis que l'activité antimicrobienne a été évaluée en utilisant la méthode de diffusion en agar.

Résultats: Des études micro-morphologiques foliaires sur les plantes ont révélé de fortes variations génétiques des tailles et des types de stomates, des formes des cellules épidermiques sur les surfaces adaxiales et abaxiales, des tailles et des types de trichomes. Les résultats physico-chimiques obtenus sont dans les limites du standard. L'extrait méthanol de *P. pterocarpum* a eu la teneur phénolique la plus élevée (62,0 ± 1,23 mg GAE/g) et l'activité anti-oxydante la plus élevée, comme le révèle sa faible valeur IC_{50} de 21,57 ± 0,62 µg/mL. L'étude antimicrobienne a révélé que les extraits présentaient diverses activités contre plusieurs micro-organismes avec des zones d'inhibition allant de 2,0 à 15,0 mm. La CMI des extraits variait de 0,78 à 3,125 mg / mL et de MBC/MFC de 1,56 à 6,25 mg / mL pour les organismes sensibles aux concentrations testées.

Conclusion: Les résultats fournissent les paramètres pour la normalisation des plantes et justifient également leurs utilisations ethno-médicales.

Mots-clés: *Peltophorum pterocarpum*, *Erythrophleum suaveolens*, standardisation, antioxidant, antimicrobien

INTRODUCTION

The importance of plants as sources of traditional medicines cannot be over emphasized. Medicinal plants are the most important source of life saving drugs for the majority of the world's population. Medicinal plants have a long standing history in many indigenous communities and continue to provide useful tools for treating diseases.1 One major obstacle that might impair the potential use of traditional medicine especially in developing countries is the lack of documentation and standardization. Therefore, medicinal plants should be investigated for the better understanding of their properties, safety and efficiency.[2] For phytopharmaceuticals to be regarded as rational drug, they should be standardized and their pharmaceutical quality must be ensured.[3] The process of standardization can be achieved by stepwise pharmacognostic studies. These studies help in identification and authentication of the plant material. Correct identification and quality assurance of the starting materials is an essential prerequisite to ensure reproducible quality of herbal medicine, which will contribute to its safety and efficacy. Simple pharmacognostic techniques used in standardization of plant material include its morphological, anatomical and biochemical characteristics.[4] The present study was aimed at the standardization of two plants: Peltophorum pterocarpum (DC) Backer ex K. Heyne and Erythrophleum suaveolens (Guill. & Perr.) Brenan, belonging to the family Fabaceae.

Peltophorum pterocarpum is a decidous tree growing up to 15-25 m. The leaves are bipinnate, 30-60 cm long with 16-20 pinnae, each pinna with 20-40 oval leaflets 8-25 cm long and 4-10 mm broad.[5] The flower extract is known to be a good sleep inducer and used in insomnia treatment.[6] The bark is used as medicine for dysentry, as eye lotion, embrocation for pains and sores.[7] Flowers are used as astringent to cure or relieve intestinal disorders after pain at childbirth, sprains, bruises and swelling, or as eye lotion, muscular pain and sores.[7] The ethanol extract and petroleum ether extract of Peltophorum pterocarpum was investigated to elicit cardiotonic effect.[8] The acetyl and butyrl cholinesterase effects of Peltophorum pterocarpum had been reported, providing support for its folkloric claim as a memory enhancer.[9] The flowers and bark are also reported to have antimicrobial activity.[10-11] The leaf and bark of this plant are reported to contain phenolic compounds that showed antibacterial, antioxidant and hypoglycemic activity.[6-7, 12-13]

Erythrophleum is mostly found in the tropical rain forest region of Africa. The species of E. suaveolens are 25-30 m in height and have a rough and blackish bark. Leaves are alternate and have [2-3] pairs of pinnate, which carry [7-13] leaflets. The leaflets are 5 by 2.5 cm, green coloured and ovate.14 The tree when wounded yields a red juice, which like the bark is used by natives as an ordeal poison for their arrow.[15] Sassy bark is used as emetic and purgative. In the Republic of Congo, the dried powdered bark is taken as a snuff to cure headache. In Kenya, a diluted decoction of the wood is used as an antihelminthic especially against tapeworm. The stem bark of Erythrophleum suaveolens had been reported to compose of alkaloids, saponins, cardiac glycosides and tannins.[16] The chloroform extract of the stem bark of E. suaveolens was found to contain the amide, norcassaide and a new diterpenoid alkaloid: norerythrosuaveolide (characterized as 7b-hydroxy-7-deoxo-6-oxonorcassaide), which possess antifungal and antibacterial properties.[17] Aqueous and chloroform extracts of E. suaveolens containing saponins, tannins, steroids and alkaloid were reported to be responsible for its bioactivity.[18] To the best of our knowledge, there had been no report on the pharmacognostic studies of these two plants. Also, there is scarce information on the antioxidant and antimicrobial activities of the leaf fractions of the two plants. This study determines various identification characters for the two plants: Peltophorum pterocarpum and Erythrophleum suaveolens, their antioxidant and antimicrobial activities were also evaluated.

MATERIALS AND METHOD

Plant materials

The plants-Peltophorum pterocarpum and Erythrophleum suaveolens were collected from the Botanical Garden, University of Ibadan and Sasha area of Ibadan, Ibadan between August and September, 2014. Plants were identified and authenticated at the Department of Pharmacognosy Herbarium, University of Ibadan (DPHUI), Ibadan, Nigeria where voucher specimens (DPHUI 1450 and DPHUI 1384) were deposited. The leaves of the two plants were air dried separately for about two weeks after which they were oven dried at 40 ^{o}C for about 2 h and then grounded using an electric blender. The powdered samples were used for phytochemical analysis, while fresh leaves of the two plants were used for microscopic studies.

Plant extraction

The powdered leaves of Erythrophleum suaveolens and Peltophorum pterocarpum (250 g each) were

successively macerated individually with n- hexane, chloroform or dichloromethane, ethyl acetate and methanol for a period of 72 h, with stirring using a glass rod and then filtered. This extraction procedure was repeated until solvents obtained were clear in each case. Filtrate was concentrated using a rotary evaporator and then stored at 4 °C until used for other analyses.

Pharmacognostic studies

Microscopic analysis

Leaf epidermises were obtained by scrapping and chemical treatment. For scrapping, fresh leaves of E. suaveolens and P. pterocarpum were placed on a white tile and a sharp blade was used to scrape the upper surface of the leaf in order to obtain the abaxial surface, while the lower portion of the leaf was scraped to obtain the adaxial surface. Also, epidermal peels were obtained by chemical method by irrigating fresh leaves in concentrated Trioxonitrate (V) acid (HNO3) in covered petri dish and warmed on a water bath (60ºC) for 3-5 min. When tissue disintegration was indicated by bubbles, the epidermis was peeled using camel hair brush and carefully transferred into petri dishes containing distilled water for cleansing (X4). The adaxial and abaxial surfaces obtained were cleared in 40% NaOCl solution for 10-15 min. The decolourized epidermal peels were then rinsed thoroughly in distilled water (X5), dehydrated through ethanol series (30%-95%) and stained with Safranin O for 2 min. Specimens were mounted in glycerol, covered with cover slip and ringed with nail vanish to prevent dehydration. Observations and measurements were made with a light microscope using the micrometer eye piece. Qualitative and quantitative features of epidermal cells, stomata and trichomes were assessed and photomicrographs of the specimens were taken.[19]

Physico-chemical analysis

Physicochemical analyses were carried out on powdered samples following standard procedures.[20-22] Moisture content, total ash value, acid insoluble ash value, methanol-soluble extractive value, alcohol extractive value and water-soluble extractive values were evaluated.

Phytochemical screening

The powdered leaves of the two plants were screened phytochemically for the presence of anthraquinones, alkaloids, tannins, cardiac glycoside, saponins, alkaloids and flavonoids using previously described standard procedures. 20-21

Determination of total phenolic content (TPC)

The total phenolic content of the different solvent extracts of leaves of Peltophorum pterocarpum and Erythrophleum suaveolens were determined using the Folin-Ciocalteau reagent.[23] Calibration curve was prepared by mixing different concentrations of 0.5 mL Gallic acid (6.25-200 µg/mL) with 2.5 mL of Folin-Ciocalteau reagent (5: 50 mL of water) and 2 mL of Sodium bicarbonate. The mixture was left at room temperature for about 30 min. The absorbance was measured at 760 nm. Results were expressed as Gallic acid equivalents/g (GAE/g) of extract. All determinations were carried out in triplicate.

Antioxidant assay using DPPH

The free radical scavenging activity of the extracts and Gallic acid as positive control was measured using the stable DPPH radical (2,2-diphenyl-1-picrylhydrazyl) method previously reported with slight modifications.24 About 2 mL of each extract and control at various concentrations (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.625, and 0.812 µg/mL) were added to 3 mL of freshly prepared DPPH solution (0.004%) in methanol. The reaction was incubated for 30 min at room temperature and absorbance was measured at 515 nm using a UV spectrophotometer. All experiments were repeated three times independently. The degree of decolourisation of DPPH from purple to yellow indicated the scavenging efficiency of the extract. The percentage inhibition of DPPH free radical scavenging activity was calculated using the following equation:

Percent inhibition = 1- Asample /ADPPH × 100

Where: ADPPH = Absorbance of DPPH

Asample = Absorbance of sample (extract/Gallic acid)

The % inhibition data was then plotted against concentration fitted in a graph and IC^{50} (half-maximal inhibitory concentration) value was calculated by linear regression analysis.

Antimicrobial screening of plant extracts

Microorganisms used: Two gram positive (Staphylococcus aureus (NCTC 6571) and Bacillus subtilis) and five gram negative bacteria (Escherichia coli (NCTC 10), Acinetobacter baumannii (NCTC 6373), Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis) and two fungi (Aspergillus niger, and Candida albicans) were collected from the Department of Medical Microbiology, University College Hospital (UCH), Ibadan. The organisms were selected due to their medical relevance. The organisms were sub-cultured in nutrient broth and nutrient agar for use.

Culture media: About 40 g of Mueller-Hinton Agar

(MHA) and 68 g of Sabouraud Dextrose Agar (SDA) were dispensed each into 1000 mL of distilled water, which was allowed to soak for 10 min. It was swirled to mix and sterilized by autoclaving for 15 min at 121 ^{o}C, allowed to cool at 47 ^{o}C, mixed well and poured into plates.

Brain Heart infusion broth preparation

About 40 g of Brain heart infusion was dissolved in 1 litre of distilled water, mixed well and distributed into final containers. It was sterilized by autoclaving at 121^{o}C for 15 min.

Determination of antibacterial and antifungal activities

Antibacterial and antifungal activities of leaf extracts of the two plants were determined by using agar well diffusion method proposed by the clinical and Laboratory Standards institute.[25] The concentration of bacterial and fungal cell suspension used were equilibrated to a 0.5 McFarland standard. For antibacterial, briefly, all samples were dissolved in dimethylsulfoxide (DMSO 10%, v/v) at 10 mg/mL. Inoculum of the bacterial strains was spread using sterile swabs into Petri dishes with 15 mL of Mueller-Hinton Agar (MHA) and incubated at room temperature for 5 min after which sterile Cork borer of 6 mm diameter was used to make five ditches (wells) on each inoculated plate and filled with 100 µL of the extract, DMSO was used as a negative control. The experiment was carried out in triplicate for each bacterium. The plates were left on the bench for 30 min to ensure adequate diffusion of the extracts and thereafter were incubated at 37 °C for 24 h after which the diameter of all the resulting zones of inhibition were measured to the nearest millimetre along two axes and the mean of the two measurements was calculated.

Antibiotic susceptibility test was carried out on the test bacteria as control. Multi-sensitivity disc bearing different antibiotics of GBMTS-NEG (Lot: NH05/P) (Abtec Biological Ltd. Liverpool L9 7AR, UK) with their concentrations; Cefixime (5 µg), Ofloxacin (5 µg), Ceftazidine (30 µg), Gentamicin (10 µg), Ciprofloxacin (30 µg), Erythromycin (5 µg), and Nitrofurantoin (300 µg) were used against each of the test bacteria inoculated on Mueller Hinton agar plates, plates were incubated at 37 °C for 24 h.

The antifungal activity was evaluated by dissolving samples in dimethylsulfoxide (DMSO 10%, v/v) at 10 mg/mL. Inoculum of the fungal strains was spread using sterile swabs into Petri dishes with 15 mL of Sabouraud Dextrose Agar (SDA) and incubated at room temperature for 5 min after which sterile Cork borer of 6 mm diameter was used to make five wells on each inoculated plate and filled with 100 µL of the extracts, DMSO was used as a negative control and Fluconazole (10 mg/mL) was included in the assay as positive control. The experiment was carried out in triplicate for each fungus. The plates were left on the bench for 30 min to ensure adequate diffusion of the extracts and thereafter were incubated at 37°C for 24 h after which the diameter of all the resulting zones of inhibition were measured to the nearest millimeter along two axes and the mean of the two measurements were calculated.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

To determine MIC, two fold serial dilutions of the plants extract and their fractions were prepared in sterile BHI broth to achieve a decreasing concentration ranging from 100-0.78 mg/mL in eight labeled test tubes. Each was seeded with 100 µL of the standardized inoculums. The inoculated culture tubes were incubated at 37 °C for 18-24 h. A group of tubes containing only seeded broth (without plant extract) was kept as control. The lowest concentration that did not permit any visible growth when compared with control was considered as the MIC.

Minimum Bactericidal Concentration is defined as the lowest concentration of antimicrobial agent that will prevent the growth of an organism after subculture on to antibiotic- free media.[26-28] One hundred micro litre aliquot from the tube showing MIC was placed on MHA plate antibiotic– free and was spread over the plate. After incubation at 37 °C for 24 h, the plates were examined for the growth of bacteria to determine the concentration of extract at which 99.9% killing of bacterial isolates was achieved.

The MIC and MBC of the two plants extracts were determined by macro dilution broth method.[26-27]

RESULTS

Microscopic analysis

Epidermal peel of Erythrophleum suaveolens had no trichomes on the adaxial and abaxial surfaces, while simple unicellular trichomes were observed on both epidermal surfaces of Peltophorum pterocarpum (Figures 1 and 2). Stomata were not found on the adaxial surfaces of the two plants, while their abaxial surfaces had anomocytic and paracytic stomata. Stomata and few trichomes were seen on the abaxial surface of P. pterocarpum compared to its adaxial surface, which had numerous trichomes. Adaxial and abaxial epidermal cells were slightly sinuous in E. suaveolens, while they were polygonal in P. pterocarpum. Epidermal cells were more in number on the adaxial surfaces of the

two plants than on the abaxial surfaces. Adaxial epidermis of E. suaveolens obtained by scrapping had druse crystals (Figure 1e). Also, E. suaveolens had larger epidermal cells [(31.3±1.8) x (14.9±0.8) µm, (33.1±1.6) x (13.8±0.5) µm] on the adaxial and abaxial surfaces, respectively compared to the cells [(24.0±2.0) x (14.8±0.6), (27.6±1.5) x (16.1±0.6) µm] observed on the adaxial and abaxial surfaces of P. pterocarpus. Stomata of P. pterocarpum were of higher dimension [(22.4±0.8) x (5.8±0.3) µm] than that of E. suaveolens [(13.8±0.5) x (6.1±0.3) µm] (Table 1). Other diagnostic features observed on the epidermis of E. suaveolens include: coastal cell, contiguous cell, and secretory cells.

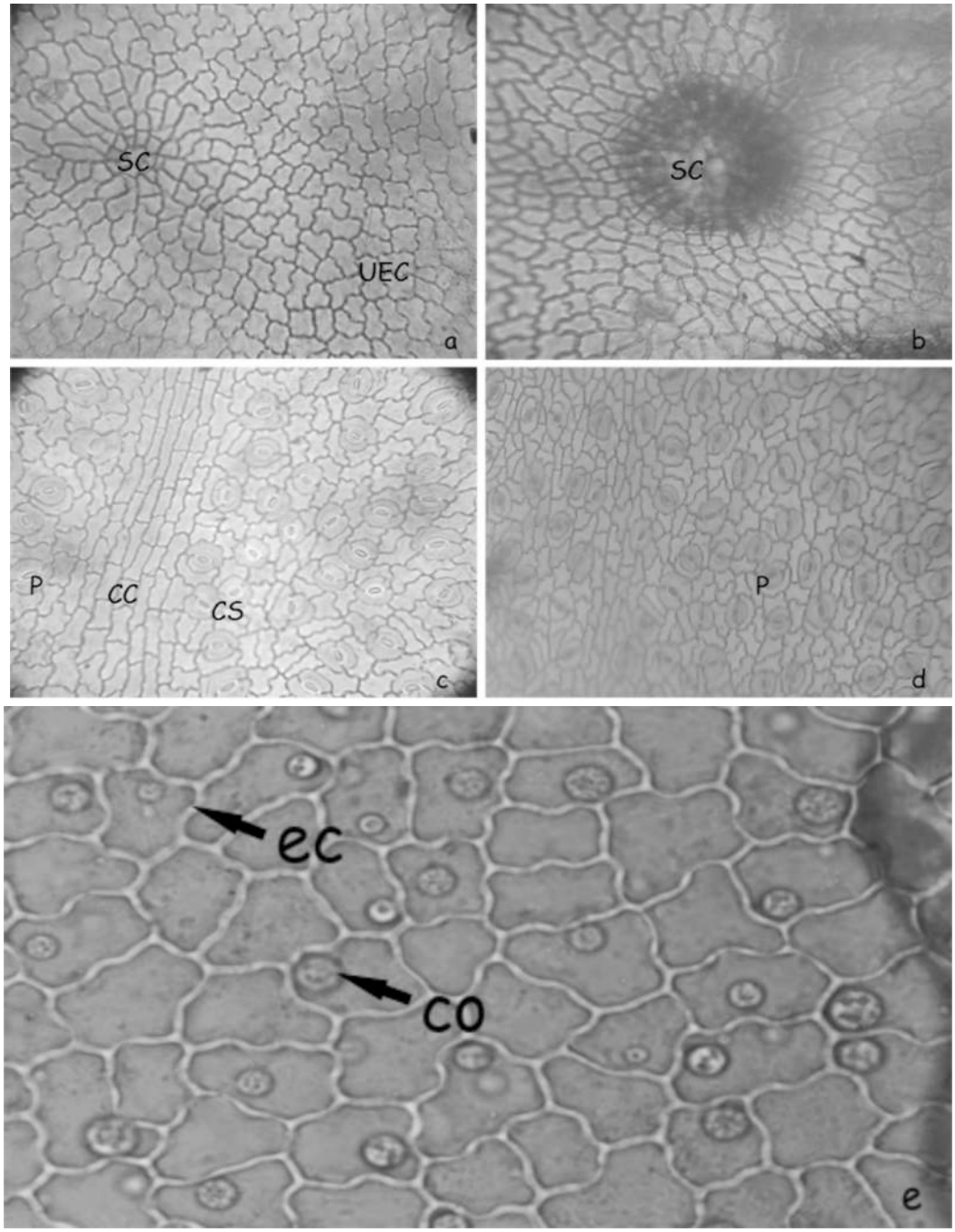

Plate 1: Epidermal morphology of *Erythrophleum suaveolens* (photo taken at x400): (a & b) Adaxial surface; (c & d) Abaxial surface; e. Adaxial surface showing epidermal cells and Druse crystals (epidermis obtained by scrapping). CC- Coastal cell, CS- Contiguous cell, P- Paracytic stomata, UEC- Undulating Epidermal Cell, SC- Secretory cell

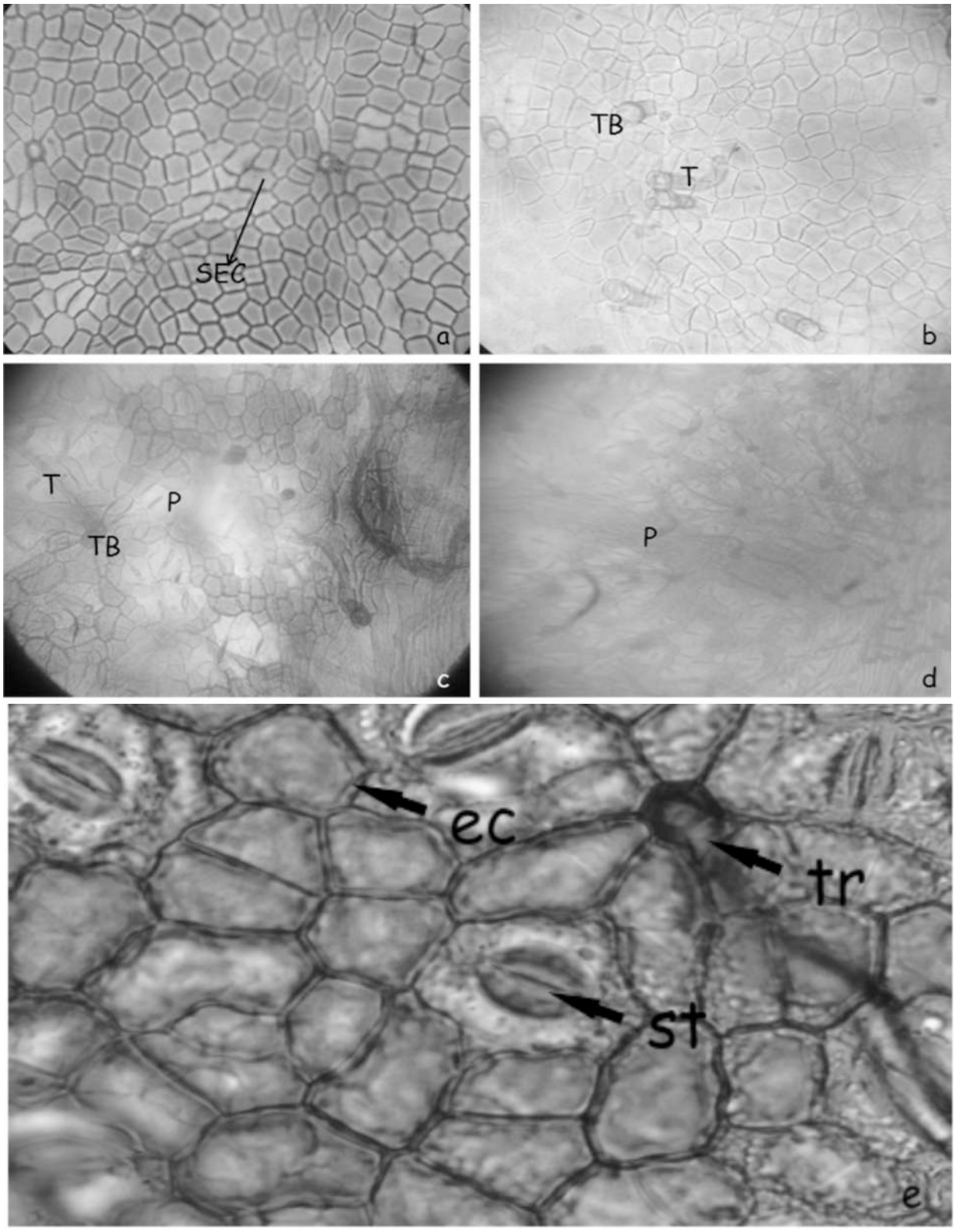

Plate 2: Epidermal morphology of *Peltophorun pterocarpum* (photo taken at x400): (a & b) Adaxial surface; (c & d) Abaxial surface; e. Adaxial surface showing epidermal cells, stomata and trichomes (epidermis obtained by scrapping). P- Paracytic, T- Simple Unicellular Trichome, TB- Trichome Base, SEC- Straight Epidermal Cell

Table 1: Combined and quantitative features of the epidermal micro-morphology of Erythrophleum suaveolens and Peltophorum pterocarpum

Taxa	*Erythrophleum suaveolens*		*Peltophorum pterocarpum*	
	Adaxial	Abaxial	Adaxial	Abaxial
Trichome type	No trichomes	No trichomes	Simple unicellular	Simple unicellular
Trichome length (µm)	No trichomes	No trichomes	111.2±4.86	114.3±10.11
Trichome width (µm)	No trichomes	No trichomes	11.8±0.75	12.5±1.05
Stomata type	No stomata	Anomocytic and paracytic	No stomata	Anomocytic and paracytic
Stomata Number/mm^2	No stomata	23.0 (33.6±1) 40.0	No stomata	10.0 (13.4±0.6) 19.0
Stomata length (µm)	No stomata	10.0 (13.8±0.5) 17.5	No stomata	15.0 (22.4±0.8) 25.0
Stomata width (µm)	No stomata	5.0 (6.1±0.3) 7.5	No stomata	5.0 (5.8±0.3) 10.0
Stomata index (SI)	No stomata	12.4 (20.8±0.7) 23.9	No stomata	9.9 (11.9±0.5) 16.2
Epidermal cell shape	Slightly sinuous	Slightly sinuous	Polygonal	Polygonal
Epidermal Number/mm^2	128.0 (151.9±3.4) 184.0	110(129±3.27)162	121(162.8±4.9)190	84.0 (98.5±1.9) 116.0
Epidermal cell length (µm)	12.5 (31.3±1.8) 45.0	20.0(33.0±1.6) 47.5	10.0 (24±2) 40.0	15.0 (27.6±1.49) 47.5
Epidermal cell Width (µm)	10.0 (14.9±0.8) 22.5	10.0 (13.8±0.5) 17.5	10.0 (14.8±0.6) 17.5	12.5 (16.1±0.6) 22.5

Physico-chemical analysis and phytochemical screening

Physico-chemical parameter is a valuable analytical tool in the identification of plant samples and crude drugs. The result of the physico-chemical parameters is shown in Table 2. Moisture content, Ash values and Extractive values of studied samples were all recorded. Moisture contents according to British Herbal Pharmacopoeia should not be more than 14% in medicinal plants. The results obtained from this study indicates that the moisture contents is within the limits as *Erythrophleum suaveolens* and *Peltophorum pterocarpum* have moisture content values of 4.57±0.01% and 6.25±0.37% , respectively. The total ash values of the leaves of *Erythrophleum suaveolens* and *Peltophorum pterocarpum* were 4.8% and 5.13%, respectively, while the acid insoluble ash values were 1.57% and 1.76% for the leaves of *Erythrophleum suaveolens* and *Peltophorum pterocarpum,* respectively. According to the British herbal pharmacopoeia, the limit for total ash and acid insoluble ash should not be more than 12% and 2.5%, respectively.

Alkaloids, saponins, tannins, flavonoids and cardiac glycosides were present in both plants at varying degrees. Anthraquinone was absent in the two samples. Table 2 gives the result of the phytochemical screening of the powdered samples of the two plants.

Table 2: Physicochemical parameters and phytochemical constituents of *Erythrophleum suaveolens* and *Peltophorum pterocarpum* leaves

Parameters	*Erythrophleum suaveolens*	*Peltophorum pterocarpum*
Physical	%	%
Total ash	4.80±0.04	5.13±0.01
Acid insoluble	1.57±0.01	1.76±0.05
Alcohol soluble extractive	40.00±11.5	29.30±4.37
Water soluble extractive	25.30±0.67	23.30±3.84
Moisture content	4.57±0.01	6.25±0.37
Phytochemical constituents	Present/absent	Present/absent
Alkaloids	+	+
Anthraquinones	-	-
Saponins	+	+
Tannins	+	+
Flavonoids	+	+
Cardiac glycosides	+	+

+: present, -: absent

Total phenolic content (TPC) and DPPH antioxidant activity

Total phenolic content was estimated using the Folin-Ciocalteu reagent and expressed as milligrams of gallic acid equivalent (GAE). *Peltophorum pterocarpum* extracts had higher TPC than extracts of *Erythrophleum suaveolens.* The methanol extract of both plants contained higher phenolics compared to other extracts. Methanol extract of *P. pterocarpum* had TPC of 62.0±1.23 mg GAE/g of extract, while that of *E. suaveolens* had TPC of 8.17±0.12 mg GAE/g of extract (Table 3). The DPPH radical scavenging-based antioxidant potential of the extracts was evaluated by their IC_{50}. Here, IC_{50} means the concentration of antioxidant required for 50% scavenging of DPPH radicals in the specified time. *In vitro* antioxidant activities of all the extracts were measured with the standard antioxidant, gallic acid. The extracts had a dose-dependent activity where scavenging activity increased proportionate to the increase in concentration of the extracts (Table 3). The methanol extract of both plants also possess higher antioxidant activity. The methanol extract of *P. pterocarpum* had the highest antioxidant activity with the IC_{50} value of 21.57±0.62 µg/mL (Table 3).

Table 3: Total Phenolic content (TPC) and DPPH antioxidant activity of Erythrophleum suaveolens and Peltophorum pterocarpum

	Total phenolic content (mg GAE/g)		DPPH antioxidant activity IC_{50} (µg/mL)	
Extract	*Erythrophleum suaveolens*	*Peltophorum pterocarpum*	*Erythrophleum suaveolens*	*Peltophorum pterocarpum*
n-hexane	1.32±0.03	-	200.68±0.01	268.79±8.60
chloroform/DCM	6.64±3.32	3.86±1.34	259.66±6.44	139.83±2.08
ethylacetate	0.714±0.04	4.50±1.06	191.68± 1.77	176.24±4.53
methanol	8.17±0.12	62.0±1.23	134.89±0.01	21.57±0.62

Data are presented as mean ± S.E.M (n=3). DCM= Dichloromethane

Antimicrobial activity

The agar-well diffusion method for the determination of antibacterial and antifungal activity showed the various zones of inhibition of the plant extracts against the micro-organisms as shown in Table 4. The result obtained showed that the ethyl acetate extract of *Erythrophleum suaveolens* had the highest zone of inhibition of 13.79 mm against *Staph. aureus* and 12.30 mm against *Bacillus subtilis*. The zones of inhibition of 10.64 mm, 12.30 mm and 10.0 mm were recorded for *E. coli, Candida albicans* and *Aspergillus niger*, respectively. Hexane extract had the highest activity against *Acinetobacter baumannii, Pseudomonas aeruginosa, Proteus mirabilis and Aspergillus niger* with zones of inhibition of 13.0 mm, 9.67 mm, 11.33 mm and 10.0 mm, respectively. However, the methanol extract of *E. suaveolens* had the highest zone of inhibition of 11.70 mm against *Klebsiella pneumonia*e. For *Peltophorum pterocarpum,* the highest zones of inhibition of 10.70 mm, 9.33 mm, 11.33 mm, 8.33 mm and 12.30 mm were obtained from the ethyl acetate extract for *Staphylococcus aureus, Bacillus subtilis, Acinetobacter baumannii, Pseudomonas aeruginosa and Candida albican*s, respectively while methanol extract had the highest activity against *E. coli, K. pneumonia and C. albican*s with zones of inhibitions of 10.70 mm, 11.00 mm and 15.0 mm, respectively.

The MIC and MBC results are shown in Table 5. In gram positive bacteria, the lowest MIC of 0.78 and MBC of 1.56 µg/mL were obtained in the ethyl acetate extract of *P. pterocarpum* against *Staph. aureus,* while in gram negative bacteria they were obtained in the methanol extract against *A. baumannii* and *P. mirabilis*. The ethyl acetate extract gave the lowest inhibitory and fungicidal concentrations in *A. niger* and *Candida albicans.* For *Erythrophleum suaveolens,* the ethyl acetate fraction had the lowest MIC and MBC. Also the ethyl acetate fraction had the lowest minimum inhibitory and fungicidal concentration against fungi at 0.78 µg/mL and 1.56 µg/mL, respectively (Table 6).

Table 4: Zone of inhibition (mm) MEAN ± S.E.M of *Erythrophleum suaveolens* and *Peltophorum pterocarpum* extracts

Orgs	Gram positive bacteria			Gram Negative Bacteria			Fungi		
Erythrophleum suaveolens									
	S. aureus	*B. subtilis*	*E. coli*	*A. baumannii*	*K. pneumoniae*	*P. aeruginosa*	*P. mirabilis*	*C. albicans*	*A. niger*
NHE	12.33±0.88	8.67±1.20	9.33±1.76	13.00±1.15	7.67±0.88	9.67±1.20	11.33±0.88	5.67±0.88	10.00±1.15
DCM	4.00±0.58	5.33±0.88	0.00	4.00±0.58	5.00±0.58	0.00	4.67±0.88	2.00±1.00	3.00±0.58
EAE	13.70±1.20	12.33±0.88	10.67±0.88	7.30±0.88	9.00±0.58	8.33±0.88	11.00±0.58	12.30±1.20	10.00±1.15
ME	12.00±1.15	10.33±1.45	9.67±1.20	11.30±0.88	11.70±0.88	9.00±0.58	11.00±0.58	9.00±0.58	9.00±1.15
Peltophorum pterocarpum									
NHE	5.67±0.33	8.00±1.15	10.00±1.15	10.33±0.33	9.33±0.33	10.0±1.15	9.67±1.20	8.67±0.33	8.67±0.88
CE	5.33±0.67	7.00±1.15	7.00±1.15	9.00±0.58	6.00±0.58	9.00±0.58	5.00±1.00	11.00±1.00	5.00±0.58
EAE	10.70±0.88	9.33±1.76	8.67±0.33	11.33±1.20	10.33±0.67	8.33±0.88	11.00±0.58	12.30±1.15	10.00±1.15
ME	8.33±0.88	7.67±1.45	10.70±0.33	11.00±0.88	11.00±1.00	9.00±0.58	8.00±0.58	15±0.58	8.00±1.00
DMSO	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Keys: ME-Methanol extract; NHE- *n*-Hexane extract; DCM- Dichloromethane extract;
CE- chloroform extract; EAE- Ethylacetate extract.

Table 5: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of Peltophorum pterocarpum extracts

Organisms/ Extract	NHE		CE		EAE		ME	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Gram positive								
S. aureus	3.125	6.25	3.125	6.25	1.56	3.125	0.78	1.56
B. subtilis	1.56	3.125	6.25	12.5	3.125	6.25	1.56	3.125
Gram negative								
E.coli	1.56	3.125	6.25	12.5	3.125	6.25	3.125	6.25
A. baumannii	1.56	3.125	3.125	6.25	0.78	1.56	3.125	6.25
K. pneumonia	0.78	1.56	6.25	12.5	3.125	6.25	0.78	1.56
P. aeruginosa	1.56	3.125	3.125	6.25	3.125	3.125	0.78	1.56
P. mirabilis	0.78	1.56	12.5	25	1.56	3.125	0.78	1.56
FUNGI								
A. niger	0.78	1.56	3.125	6.25	3.125	6.25	3.125	6.25
C. albicans	3.125	6.25	3.125	6.25	3.125	6.25	12.50	25.00

ME-Methanol extract, CE- Chloroform extract, NHE- N-Hexane extract, EAE-Ethylacetate extract

Table 6: Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of Erythrophleum suaveolens extracts

Organisms/ Extract	NHE		DCM		EAE		ME	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
Gram positive								
Staph. aureus	3.125	6.25	12.5	25	0.78	1.56	1.56	3.125
B. subtilis	6.25	12.5	12.5	25	1.56	3.125	3.125	6.25
Gram negative								
E.coli	1.56	3.125	ND	ND	1.56	3.125	1.56	3.125
A. baumannii	1.56	3.125	25	50	1.56	3.125	0.78	1.56
K. pneumonia	3.125	6.25	12.5	25	1.56	3.125	1.56	3.125
P. aeruginosa	1.56	3.125	ND	ND	1.56	3.125	3.125	6.25
P. mirabilis	3.125	6.25	6.25	12.5	1.56	3.125	0.78	1.56
FUNGI								
A .niger	6.25	12.5	12.5	25.00	0.78	1.56	1.56	3.125
C. albicans	3.125	6.25	12.5	25.00	0.78	1.56	3.125	6.25

ME-Methanol extract, CE- Chloroform extract, NHE- N-Hexane extract, EAE-Ethylacetate extract MIC- Minimum Inhibitory Concentration, MBC- Minimum Bactericidal Concentration.

DISCUSSION

Evaluation of plant materials is an important aspect of the discovery of phytopharmaceuticals.[29] Identification of the starting material is a critical step in assuring reproducible quality of herbal medicines. Micro-morphological determination of medicinal plants is important in the identification and characterization of medicinal plants. The variations observed in the epidermal morphology of *E. suaveolens* and *P. pterocarpum* from the family Fabaceae could be used to distinguish the species. Anatomical studies on the two plants revealed sharp generic variations in sizes and types of stomata, shapes of epidermal cells on the adaxial and abaxial surfaces, sizes and types of trichomes, all of which could be employed in species identification. No trichomes were observed on both adaxial and abaxial surfaces of *E. suaveolens*, whereas simple unicellular trichomes were observed on the two surfaces of *P. peltophorum*. Also, *E. suaveolens* displayed some diagnostic characters such as: coastal cell and secretory cells that were not observed in *P. pterocarpum*. Contiguous cells were observed on the abaxial epidermises of both species . To the best of our knowledge this is the first report on the standardization of these two plants.

Physico-chemical evaluation of plant drugs is an important parameter in detecting adulteration or improper handling of drugs (African pharmacopoeia). In the present study physical constant parameters such as moisture content, total ash value, acid insoluble ash, water and alcohol extractive values, which form part of the quantitative parameters evaluated in standardizing

herbal medicine, were determined. The total ash is particularly important in the evaluation of the purity of drugs, the presence or absence of foreign organic matter such as silica or metallic salts.[30] According to the British herbal pharmacopoeia, the limit for total ash and acid insoluble ash should not be more than 12% and 2.5%, respectively. The results obtained from this study indicate that the ash values were within limits hence there is absence of impurities. Moisture content according to British Herbal Pharmacopoeia should not be more than 14% in medicinal plants. The moisture content of the two plants was within the stated limit. Determination of moisture content helps to reduce errors in the estimation of the actual weight of the plant material as high moisture content is indicative of susceptibility to microbial degradation and hydrolysis by hydrolytic enzymes. The extractive values are indicative weights of the extractable chemical constituents of crude drugs under different solvent conditions.[31]

Phytochemical screening showed that both species contain the same classes of secondary metabolites. This might possibly be because they belong to the same family. Anthraquinone was absent in both plants. The presence of these secondary metabolites might be responsible for some of the pharmacological activities reported in the plants. Flavonoids have been shown to be highly effective scavengers of most types of oxidizing molecules including singlet oxygen and various free radicals, which are possibly involved in DNA damage and tumour promotion. Tannins are a major group of compounds that act as primary antioxidant or free radical scavengers.[32] Tannins play an important role in promoting wound healing hence plants containing tannins are widely used in treating various diseases. Alkaloids are very important in medicine and they constitute most of the valuable drugs. They have physiological effect on animals.[33] Saponins are a class of glycosides which possess antifungal activities.

Phenolic compounds contain one or more hydroxyl groups attached to an aromatic ring. Compounds containing the phenolic hydroxyl substituents are often referred to as polyphenols. Polyphenolic compounds are widely known for their antioxidant activity. Plant materials rich in phenolics are increasingly being used in food industry because they retard oxidative degradation of lipids and improve the quality and nutritional value of food.[34] *Peltophorum pterocarpum* had higher phenolic content than *E. suaveolens.* Thus, the high phenolic content in the former compared to the latter is indicative of the higher antioxidant activity of *Peltophorum pterocarpum* than that of *E. suaveolens.* A high antioxidant activity (73.29±0.81%) had also been reported in methanol extract of *P. pterocarpum* pod.[35] In the present study, leaf methanol extracts of both plants gave the highest phenolic content compared to the ethylacetate, choloform and *n*-hexane extracts, respectively. Antioxidants prevent free radicals from harming DNA, proteins and cells by donating electrons to stabilize and neutralize the harmful effects of the free radicals. This action helps in protecting the body from degenerative diseases and slows down the aging process.[36]

The agar-well diffusion method for the determination of antibacterial and antifungal activity allowed the observation of zones of inhibition of the plant extracts against the micro-organisms. This therefore showed that the extracts contain substance(s) that inhibited the growth of some of the micro-organisms tested. This result is similar to a previously reported work on the antimicrobial activity of *Erythrophleum africanum*.[37] In line with the result of the present study, the hexane extract of *Peltophorum pterocarpum* had shown the highest zone of inhibition against tested microorganisms in literature.[38] The observed antimicrobial effects could be attributed to the presence of saponins, glycosides, flavonoids, tannins, alkaloids and terpenoids, which have been shown to possess antimicrobial activities.[39] The exhibition of large zone of inhibition by the plants extract against *Staph aureus*, *P. aeruginosa*, *E. coli* and *P. vulgaris* justifies its use by the traditional medical practitioners in the treatment of sores, boils, open wounds, and in the treatment of dysentery. *Staphylococcus aureus* and *P. aureginosa* have been implicated in cases of boils, sores and wounds.[40] The two plants demonstrated good antimicrobial activities worthy of further investigation.

CONCLUSION

The study provides the basis for the identification and evaluation of medicinal plants using accurate and standardized methods. The morphological and anatomical studies carried out on *Peltophorum pterocarpum* and *Erythrophleum suaveolens* aided the characterization of the plant materials qualitatively and quantitatively, which could form part of the standardization process that will help in correct identification and quality assurance of the plant materials. The evaluation of antioxidant, antibacterial and antifungal activities of both plants showed that the plants are promising sources of natural antioxidant and antimicrobial agents.

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