Investigation of the Phytochemical and Anti microbial Activities of *Senna Alata* And *Psidium Guajava* Leaves Extract As Possible Excipients In Topical Formulations

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Abstract

The leaves of Senna alata and Psidium guajava were collected, dried, pulverized and extracted using methanol and ethanol while the leaf extracts were screened for phytochemical, elemental, proximate compositions and antifungal and antibacteria activities. From the phytochemical test such chemical constituents as alkaloid, carbohydrate, cyonogenic glycoside, anthraquinone, saponin, tannin and flavonoids were found to be present in both extracts. Study on the qualitative analysis test reveals the moisture, ash and acid insoluble ash contents of both the S.alata and P.guajava extracts respectively as: (12.5,3.67)(6.0,3.29) and (1.5,0.25). The pH of both extracts were 6.6 and 6.2 for S.alata and P.guajava respectively while in consideration of the proximate principles, the carbon hydrate, proteins, fat and crude fibre contents of S.alata and P.guajava leaf extracts were found to be present at percentage composition of (53.7, 68.0), (5.1,18.6), (5.3, 0.5) and (25,8.7) respectively.Elemental analysis of the extracts showed presence of such metals in ppm as: Magnesium (10.663,22.482), calcium (0.700,0.945), potassium (27.266,187.444), lead (nil) and zinc (0.093, 0.031) respectively for S.alata and P.guajava leaf extracts. The extracts were seen to possess anti-microbial /antifungal properties hence from the study, combination of both extracts could be of greater value upon inclusion in Pharmaceutical topical formulations.

Key words: Senna alata, Psidium guajava, Extracts, phytochemical, anti-microbial, Excipients

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I. Introduction

Infectious diseases are being of daily increase due to various factors including, climate change, antibiotic resistance and microbial adaptation. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella typhi*, *Mycobacterium tuberculosis*, *Vibrio cholerae*, *Escherichia coli*, *Candida albicans*, *Mycosporum canis*, *Trichophyton rubrum* and others, have been investigated and discovered to be the most common causative agents of infectious diseases like pneumonia, endocarditis, septic arthritis, cholera, typhoid, tuberculosis, cellulitis, ringworm, impetigo and psoriasis. To treat these infections both synthetic and topical herbal formulations have been used for a long time [1]. In recent time, herbal medicines are gaining importance and are used as an alternative to synthetic drugs, as they seem to be associated with little or no side effects [2]. Medicinal plants like Neem, Turmeric, Garlic, Aloe vera have been used to treat infectious diseases for a long time and they have also been found useful in treatment of skin infections. Due to the indiscriminate use of the antimicrobial drugs, developments of drug-resistance by microbes hasbeen frequently reported [3].

As a result of the development of bacterial resistance to antibiotics and other synthetic antimicrobials, investigations on thousands of phytochemicals from plants which inhibit different types of microorganisms with different mechanisms and which are found to be relatively safe and have broad spectrum antimicrobial activity in the treatment of resistant microbial strains are being carried out [4].

Senna alata



This is an important medicinal tree, as well as an ornamental flowering plant in the subfamily Caesalpinioideae and family Fabiaecea. It is also known as emperor's candlesticks, candle bush, candelabra bush, Christmas candles, empress candle plant, ringworm shrub, or candle tree. It is native to most of the Neotropics (from Mexico and the West Indies to Paraguay), and can be found in diverse habitats [5].

Medicinal uses

Senna alata (also known as *Cassia alata*) is often called the ringworm bush because of its very effective fungicidal properties, for treating ringworm and other fungal infections of the skin. The leaves are ground in a mortar to obtain a material which appears like "green cotton wool". This material often is mixed with same amount of vegetable oil and rubbed on the affected area twice or thrice a day with fresh preparation being made every day. Its active ingredients include the yellow chrysophanic acid which is proven to have laxative effect probably due to its anthraquinone content [6].

Psidium guajava



This plant is popularly known as guava, a small tree belonging to the myrtle family (Myrtaceae). It is native to tropical areas from North to South America, although the tree has been grown by many other countries having tropical and subtropical climates, thus deriving production around the world [7]. Traditionally, preparations of the leaves have been used in folk medicine in several countries, mainly as anti-diarrheal remedy, other several uses have been described elsewhere. Depending upon the illness, the application of the remedy is either oral or topical. The consumption of decoction, infusion, and boiled preparations is the most common way to overcome several disorders, such as rheumatism, diarrhea, diabetes mellitus, and cough., in India and China. In Southeast Asia the decoction is used as gargle for mouth ulcers and as anti-bactericidal in Nigeria. For skin and wound applications, poultice from the plant is externally used in Mexico, Philippines, and Nigeria where it is also used as a chewing stick for oral care [8].

This study is aimed atextraction of the leaf component from *Senna alata* and *Psidium guajava* using methanol and ethanol and screening the leaf extract for phytochemical composition, antifungal and antibacterial activities and for further consideration as excipient in Pharmaceutical topical formulations.

METHOD

II. Materials:

Leave extracts of *Senna alata* and *Psidum Guajava* (University of Port Harcourt), glycerine (E, Merck, Darustadt), All the chemicals used where of analytical grades and includes: Ethanol, methanol, Disinfectants, Wiji's solution, n hexane, acetone, Potassium iodide solution, Sodium thiosulphate solution, Glacial Acetic acid, Phenolpthalein Solution, 0.5M Hydrochloric acid, Concentrated Sulphuric acid (SIGMA-ALORICH 2.5L, LOT NO. 83280, GERMANY), Standard Glucose and Boric acid, Mueller Hinton agar (MHA), Nutrient agar and Potato dextrose agar (PDA), Bacterial isolates of *Escherichia coli, Proteus mirabilis, Salmonella typhi* and, *Staphylococcus aureus* and the fungal isolates of *Aspergillus niger* and *Candida albicans*((Divic Medical laboratories, Port Harcourt).

Apparatus:

Soxhlet apparatus (Borosilicate Glass, England), Heating mantle (PEC Medicals, USA), Rotary evaporator, light microscope, pH meter (Helmreasinn, PHS-25), Brookfield viscometer (DV2T), Round bottom flask (1000 ml, Borosilicate Glass, Unicon, India), glass rod (Pyrex), Thermostat USA), Milling machine (Corona Landers), (Labscience, England), Analytical Weighing balance (Aculab Sartorius Group), Borosilicate Glass, Unicon, India), thermometer, crucibles, Weighing scale (Avery), distillation flask, porcelain evaporating dish, dessicator, hallow cathode lamp, autoclave, incubator, petri dishes (disposable),universal bottles, MacCarteney bottles,

Collection and identification of plant materials:

The leaves of the *Senna alata* and *psidum guajava* plants were collected in the morning hours from the plant garden of Department of Pharmacognosy and phytotherapy, University of PortHarcourt.

Preparation of leaf powder

The *Senna alata* and *psidum guajava* leaves were collected, washed, cut into small pieces and left to dry in the open at atmospheric temperature and the dried leavespulverized to obtain a coarse powder using an electric blender then the powder stored in a closed vessel for analysis.

Extraction of plant material

The coarse powder material was subjected to soxhlet extraction separately and successively with methanol and ethanol. These extracts were concentrated to dryness in flask evaporator under reduced pressure and controlled temperature ($40-50^{\circ}$ C). The extracts from methanol and ethanol were separately and appropriately stored in air tight containers.

Percentage yield determination

The weight of dried powdered sample was initially noted then the weight of extract determined. Therefore,

Percentage Yield = <u>Weight of extract in grams X 100</u> Weight of powder material 1----- eq1

Physicochemical Properties of the Extract Solubility of the extract

Solubility analysis of the powder samples was carried out according to conventional methods using both polar (distilled water) and non-polar solvents

The solubility of 1% w/v of the extracts were tested in various solvents as: water, acetone, ethanol and chloroform, to determine extent of dispersibility.

Organoleptic properties of the extract

The physical characteristics (colour, odours, texture and appearance) of the extracts obtained were observed and recorded.

Rheology of the extracts

A 1% w/v of the extracts prepared was withdrawn with 2ml pipette up to certain volume then the rate of flow per unit time (seconds) was noted.

pH of the extract

A dispersion of 0.1% w/v of the extracts were separately made with water in a beaker and pH electrodeinserted. The test was conducted in triplicates and readingstaken when stable.

Viscosity of the extract

The sample to be evaluated was introduced into a clean 600ml beaker. A temperature probe was attached to the spindle guard leg and the viscometer lowered into the beaker until the spindle is fully immersed in the sample. The procedure was repeated at different temperature (10°C, 25°C and 45°C while the Brookfield viscometers, DV 2T was turned on, to obtain a stable % Torque between 10% and 1000%. The displayed viscosity, was recorded in Centipose (Cp) RP and %Torque on the viscosity data sheet.

Density of the extract

The weight of an empty measuring cylinder was recorded, a known amount of the extract was transferred into the measuring cylinder and weighed. The procedure was repeated for the other extract. The density was calculated using the formula:

 $M_2 = M_1 - M_0$

V₁-V₀ ----- Eq 2

Mass of extract (M_2) = weight of cylinder and extract (M_1) – weight of empty cylinder (M_0) Volume of extract (V_2) = volume of cylinder and extract (V_1) – volume of empty cylinder (V_0)

Conductivity of the extracts

The electrical conductivity of the extracts were obtained using a conductivity probe meter(Prob-Conductometer). The electrode was inserted into the beaker containing 0.1% w/v of the extract and the readings recorded when it was stable. The tests were conducted in triplicates and the mean triplicate readings was calculated.

Proximate analysis

Carbohydrate content was determined using Cleg- Anthrone method Absorbance of the standard glucose was read and the value of the carbohydrate as glucose calculated as: % CHO as glucose = 25 x absorbance of sample Absorbance of standard glucose ----- eq3

Protein Content was determined following Kjeldahl method involving: digestion, distillation, and titration. % Organic Nitrogen = Titre value x 1.4 x 100 x 100 1 1

000 x 20 x 0.1	eq
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Where, Titre value = the volume of HCl used in titrating the ammonium distillate. 1.4= nitrogen equivalent to the normality of HCl used in the titration 100 = the total volume of digestion, 100 = percentage factor, 20 = conversion factor from gram to milligram, 0.1= the weight (g) of sample digested.

Moisture Content: This was, carried out using the Air Oven Method % moisture = Weight of fresh sample – Weight of dried sample x 100

Weight of sample used

----- eq5

Lipid content: This was determined using Soxhlet extraction technique and the percentage lipid conted calculated using the formular:

% lipid = Weight of flask and extract – Weight of empty flask x 100 Weight of simple extracted ----- ea6

Fibre content: This was obtained by difference using sum of other parameters from 100 hence Fibre content = 100 - \sum (other parameters) ----- eq7

Elemental Analysis

Sample was ashed in a muffle furnace at a temperature of 63°C for 3 hours. The ashed sample was dissolved in 10ml concentrated hydrochloric acid and was heated on an electro-thermal heater hotplate. The solution of the ash was diluted to 50ml distilled water. The solution was analyzed for metal ion by Atomic Absorption Spectrophotometer[9].

TheLead (Pb) content was analyzed at 283.3mm wavelength,Magnesium at285.2nm wavelength, Sodium content at 589nm wavelength while forPotassium content samples were digested and the solution made up to 50 ml with distilled water and the Potassium content analyzed at 766 nm wavelength

Determination of Antimicrobial properties

Test Organism

Bacterial isolates of *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhi* and, *Staphylococcus aureus* and the fungal isolates of *Aspergillus niger* and *Candida albicans* were used.

All the pure cultures were suspended in nutrient broth and incubated at 37°C for 18 h. Mueller Hinton agar (MHA) and Nutrient agar were used in the test for antibacterial activity while potato dextrose agar (PDA) was used for determining antifungal activity.

Evaluation

All the extracts from methanol and ethanol (*Senna alata* and *Psidum guajava*) were evaluated by the agar disk diffusion method and screened for their antibacterial and antifungal activities against the *Escherichia coli, Proteus spp, Salmonella typhi, Staphylococcus aureus* and the fungi *Candida albicans and Aspergillous niger*. Mueller-Hinton sterile agar plates were seeded with indicator bacterial strains and allowed to stay at 37°C for 3 hours. The zones of growth inhibition around the disks were measured after 18 to 24 hours of incubation at 37°C for bacteria and 48 to 96 hours for fungi at 28°C. The sensitivities of the microorganism species to the plant extracts were determined by measuring the sizes of inhibitory zones (including the diameter of disk) on the agar surface around the disks, and values <8 mm were considered as not active against microorganisms.

Phytochemical analysis

The freshly prepared extracts were subjected to standard phytochemical analysis for different constituents such as tannins, alkaloids, flavonoids, glycosides, saponins and phenols as described [10].

Glycosides: To 1ml of the extract was added 2ml of acetic acid and then cooled in an ice bath at 4°C. To this mixture 1ml of concentrated tetraoxosulphate (VI) acid (H_2SO_4) was added dropwise. The formation of an oil

layer on top of solution indicated the presence of glycosides

Alkaloids: The dry powder samples (1 gm) were placed in a test tube and ammonia solution (3 ml) was added to it. They were allowed to stand for few minutes. Then chloroform (10 ml) was added to the test tube samples which was shaken and then filtered to remove the powder samples. The chloroform was evaporated using a water bath and Mayer's reagent (2 ml) was added. A cream coloured precipitate was immediately produced which indicates the presence of alkaloids [11].

Saponins: Five drops of olive oil wereadded to 2ml of theplant extract andthe mixture shaken vigorously. The formation of a stable emulsion indicated the presence of saponins

Tannins: Two drops of 5% FeCl_3 was added to 1ml of the plant extract. The appearance of a dirty-green precipitate indicated the presence of tannins

Flavonoids: To 1ml of the extract was added 3 drops of ammonia solution (NH_3) followed by 0.5ml of concentrated HCl. The resultant pale brown coloration of the entire mixture indicated the presence of flavonoids

Steroids: To 1ml of the plant extract was added 1ml of concentrated tetraoxosulphate (VI) acid (H_2SO_4) . A red coloration confirmed the presence of steroids

Test for Resins: To 5ml of the extract was added 5ml of copper acetate solution. The mixture was shaken vigorously and allowed to separate. The appearance of a reddish-brown precipitate indicated the presence of resins

Qualitative Evaluation of S.alata and P.guajava leaf extract

The ash value, acid insoluble ash, water-soluble ash and were determined as described adopting the AOAC method of analysis. The moisture content of the powdered leaves was determined by loss on drying method [12].

Results

ANTIMICROBIAL ANALYSIS



III.

Plate 1: Culture of Salmonella spp



Plate 2: Culture of Staphlococus aureus



Plate 3: Culture of E.coli

Results of Quantitative Evaluation of the Leaf extracts of S. alata and P.guajava

Samples	Evaluation parameters values				
(leaf extracts)	(%w/w)				
	Moisture content	Ash content	Acid insoluble ash		
S.alata	12.5	6.0	1.5		
P. guajava	3.67	3.29	0.25		



Fig3: Effect of test/sample extracts on inhibition of selected micro organisms

PHYTOCHEMICAL ANALYSIS

Chemical constituents	Test	Psidum guajava	Senna alata
Alkaloid	Hager's test	+	+
Carbohydrate		+	+
Saponin	Foams test	+	+
Cardenolide		+	+
Cyanogenic glycoside	Bontragers tests	+	+
Triterpenoids	Legals tests	+	+
Tannin	Tannin tests	+	+
Free anthraquinone	Anthraquinone tests	+	+
Combined anthraquinone		+	+
Flavonoid	Sodium hydroxide tests	+	+
Fixed oil		+	+

PHYSICOCHEMICAL PROPERTIES OF EXTRACT IN VARIOUS SOLVENTS

			-					
Properties	$S_1 + H_2O$	S_1	$S_1 +$	$S_1 +$	$S_2 + H_2O$	$S_2 + CHCl_3$	$S_2 +$	$S_2 +$
		+CHCl ₃	Ethanol	Acetone			Ethanol	Acetone
pH	6.8±0.23	4.0±0.65	5.5 ± 0.18	5.5±017	6.2±0.14	5.1±0.32	4.5±	4.4±
Viscosity (c ^p)	2.5	2.5	2.5	2.5	3.00	2.00	3.00	2.00
Temp. (⁰ C)	31.0±0.01	260±0.23	29.0±0.23	28.0±0.17	31.0±0.25.m/S	28.0±0.03	29.0±0.067	29.0±
Conductivity(m/S)	0.01 ± 0.00	0.00 ± 0.01	0.03±0.14	0.00±0.13	0.01±0.24	0.00 ± 0.00	0.01±0.86	0.03±
Rheology (v/s)	0.026±0.01				0.024±0.13			

 $S_1 = S.alata S_2 = P.guajava$

DISPERSIBILITY OF EXTRACTS IN VARIOUS SOLVENTS

Extracts	Water	Acetone	Chloroform	Ethanol
S.alata	Slightly dispersible	Dispersible	Dispersible	Dispersible
P.guajava	Dispersible with	Dispersible with	Dispersible with	Dispersible with
	little sediment	sediment	sediment	sediment

ELEMENTAL ANALYSIS



Fig.4: Elemental composition of S.alata and P.guajava leaf extracts



Proximate analysis

Fig 5: Proximate analysis of Senna alata and P. guajava leaf extracts

IV. Discussion

The therapeutic assessment of medicinal herbs, needs involve an indispensable study on their phytochemical, pharmacological, toxicological, and nutritional implications. In consideration of the nutritional constituents of *S. alata* and P.guajava leaves, several components assessed include moisture content, carbohydrate, crude lipid, crude fibre and ash as in Fig.5. Various studies reveals that storage capacity, easeof food absorption and antimicrobial properties are connected to the nutritional composition of herbs [13]. Minerals such as potassium, iron, magnesium, calcium, zinc, copper, and chromium have been assessed in the leaf extractsand has often helped in formulation of herbal medicines, dietary supplements and topical medicines [14].

The total ash value (%) obtained, was 6.0 and 3.29 in *S. alata* and *P. guajava* leaf extracts respectively and this implies that the plants have normal contents of inorganic and organic compounds [12].

Proximate analysis of a food is the nutritional composition of that food and it is the estimate of the nutritive value of human food in its chemical form. The proximate analysis as shown in Figure 5, reveals that the protein content is relatively low in*S. alata* (5.1%) than in *P.guajava* (18.6%) but protein contributes to the formation of hormones which controls varieties of body functions such as growth, tissue repairs and

maintenance of body function [14]. The fat content of S. alata (5.3%) was higher than that of P.guajava (0.5%) though thebeneficial effect of moderate fat content can be useful for storage and transport forms of metabolic fuel. Also, high fat content can be exploited for nutritional advantage in health and improved emmolliencyin topical formulations [15]. The crude fibre content of S. alata (25%) was higher than that of *P.guajava* (8.7%). Carbohydrate content was lower in S. alata (53.7%) than in P. guajava (68%) and the relatively appreciable carbohydrate content can be used as energy sources and also it is necessary in the digestion and assimilation of other food. However, this study reveals that S. alata and P.guajava contains essential nutrients for sustenance of human and animal health. Phytochemical screening of the leave extracts reveals the presence of various bioactive compounds such as saponins, tannins, flavonoids, and cardiac glycosides, which are the basis of therapeutic potentials of medicinal plants. The presence of tannins as reported, is capable of lowering available protein by antagonistic competition and can therefore elicit protein deficiency which may lead to "Kwashiokor" [16]. Saponin is responsible for anti-yeast, anti-fungal, antidote, antimicrobial and anti-inflammatory activities and protection against potential invading organisms (17). Flavonoids, also known as vitamin p or plan modifier, could enhance activities as antihypertensive, anti-rheumatism as well as antimicrobial. It has been reported that many plant containing flavonoids could improve diuretic and the antioxidants properties, hence the leaves of these plants can be of use in such cases as depicted by Essiett et al. (2010),. Cardiac glycosides were detected in the extracts and this compound could be useful in the treatment of terminal illnesses as asthma [19].

Quantitative evaluation is an important parameter in setting standard for crude drugs. However, the values of solvent extractives can be a means of providing preliminary information on the quality of the excipient. The results of the moisture content in *S. alata* and *P.guajava*are: 12.5 and 3.67% respectively. This was lower than maximum value of 14% and hence indicates less chances of microbial degradation of the drug formulated with it during storage because excess moisture can result in the breakdown of important constituents by enzymatic activity and as a result may encourage the growth of yeast and fungi[20]. Glycosides were detected in the leaf of *S. alata* and *P.guajava*, and reference to its long implication as stimulant in cases of cardiac failure and diseases, justifies an established function of the plants in the treatment and management of hypertension [21]. Alkaloids have been found to have micro biocidal effect and their antidiarrheal effect is probably due to the action on small intestine. In addition, they have been found to be effective as antihypertensive, antifungal, anti-inflammatory, and anti fibrogenic [22].

The methanolic extracts of *P.guajava* leaf have been found to have remarkable antimicrobial activity hence species of *Staph aureus*, *Bacillus* and *Salmonella* can be controlled. It also has anti-plaque activity due to the presence of active flavonoids compounds and their derivatives [23]. As a result of the bacteriostatic effects on pathogenic bacteria the *P.guajava* leaf extract could also be useful as antitussive, anti-diarrhea, oral ulcers and insome swollen gums wound [24]. From observation on results obtained, ethanol extracts show low antimicrobial activity (MIC) than that of methanol and due to this, methanolextract appears to be most effective. The antibacterial activity of *P.guajava* appeared high against gram positive bacteria but moderate on gram negative bacterial strains [25]. *P.guajava* leaves has been foundtohave many compounds effective both as fungistatic, bacteriostatic and anti-viral, hence can control viral infections like influenza virus thereby cushioning and curtailing viral resistance which could be enhanced by the protein degradation ability of theextract. The anti oxidant activities of *P.guajava* extract, involve linkage to presence of alkaloids(nitrogencontaining naturally occurring compounds), found to have antimicrobial properties due to the ability to intercalate with DNA [26]. The proximate principles of the *P. guajava* extract showed high percentage carbohydrate content, moderate levels of protein, moisture and crude fibre with low levels of ash and fat (Fig. 5). The low amount of fat showed that it is not a good source of lipids [26].

Leaf extracts with different solvent systems showed antimicrobial properties against different Gram positive and Gram negative bacteria including different fungus. These diverse activities as shown by the leaf extracts of Cassia alata L. is mainly because of different types of phytocomponents along with various minerals present in it. The leaves also contain vitamins like ascorbic acid, riboflavin and niacin [27].

Crude methanolic extract of senna alata has previously been found to inhibit the growth of all the organisms tested except *Candida albicans* and *Saccharomyces* [29] and this has been supported by the out come of the present study. Although, the extracts could demonstrate a concentration-dependent antibacterialactivity The efficacy of this has also been linked to the presence of a flavonoid glycoside.Similarly, *Cassia alata* has been reported to contain anthraquinone, the principal laxative constituent of many plants used as purgatives [30]. Recent studies has revealed that the methanolic fraction of the *S. alata* leaves has activity against *Trichophyton mentagrophytes* [31].

Study of the metallic composition of the extracts, reveals abundance of Fe, Ca, Na, Mg, Zn and Al. Fe is important in immune function, cognitive development, temperature regulation and energy metabolism, it is also required for the synthesis of haemoglobin and myoglobin while its deficiency causes anaemia [32]. Ca along with P is required for formation and maintenance of bones and teeth, blood clothing and muscle contraction. Mg is needed in varieties of enzymes that utilize adenosine triphosphate and it is useful in DNA and RNA synthesis during cell proliferation, important for nerve and heart function as well as ultimate insulin

action on cells and it decreases blood pressure by dilating arteries and preventing abnormal heart rhythm, irritability, convulsion and even death [33]. Na is the major element of the extracellular fluid and is a key factor in retaining body fluid. In conjunction with K, creation of electrical potential, nerve impulses are conducted and the contraction of muscles is enabled. It participates in facilitating the absorption of nutrients such as glucose and amino acids in the small intestine although high levels could be associated with hypertension.

Based on the study therefore, presence of Ca, Mg and K, in the leaf extracts, could collectively give a positive implication to the nutritional importance of *P.guajava* and *S.alata* plant.

V. Conclusion

Extracts of S.alata and P.guajava leaves were successfully obtained adopting the Soxhlet extraction method with the use of solvents as methanol and ethanol. The extracts were rich in phytochemical and proximate and metallic components and exerted appreciable anti-microbial and anti fungal activities. Following the results and the activities as observed therefore, the extracts could be usefulmedicinally, if used as components in pharmaceutical topical formulations. Hence further study is suggested for the incorporation of such excipient especially the methanol extract, in suchtopical formulations. This will help to encourage utilization of the wasting and abundant natural materials and helpimprove the economy of the developing countries.

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