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Phytochemical Evaluation and *in Vitro* Antibacterial Activity of *Sphaeranthus indicus* (L.)—An Important Antijaundice Medicinal Plant

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Abstract

An investigation was carried out to study the antibacterial activity of *Sphaeranthus indicus* from leaf, stem and root extracts by the sequential cold maceration method against selected laboratory bacterial pathogens such as *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Pseudomonas putida* by the agar well diffusion method. Zone of inhibition measured (mm) was compared with standard antibiotics such as Tetracycline, Erythromycin and Ampicillin. The organic solvents such as ethanol, methanol, petroleum ether, chloroform as well as distilled water extracts were employed. Among all the extracts, tested ethanolic leaf extracts have showed more antibacterial activity against *Klebsiella pneumoniae*. Phytochemical screening methods were also done to identify the major secondary metabolites in the present species such as alkaloids, flavonoids, phenols, steroids and tannins. This study concluded that *Sphaeranthus indicus* had the sufficient antibacterial activity due to the presence of various secondary metabolites.

Keywords

Sphaeranthus indicus, Antibacterial Activity, Agar Well Diffusion, Phytochemical Screening

1. Introduction

Sphaeranthus indicus (Linn.) is one of the important herbaceous medicinal plants belonging to the family Asteraceae. It is commonly known as "Boddasoram" in Telugu and "East Indian globe thistle" in English [1]. *S. indicus* has long been used in the indigenous medicine. The herb is bitter and hot with a sharp sweet

taste and the juice of the plant is styptic and said to be useful in treating jaundice, diseases of the spleen, elephantiasis, anaemia, pain in the uterus and vagina, epileptic convulsions, leukoderma, dysentery and hemicranias (Ayurveda) [2]. The bark powder mixed with whey is a valuable remedy for piles. Flowers are credited with alternative, depurative and tonic properties. The oil obtained from the root is aphrodisiac, used in prolapsus ani (Unani) [2]. The whole herb is used in ayurvedic preparations to treat epilepsy and mental disorders [3] and hepatitis [4].

Phytochemical investigation of plants is an interesting area of research, leading to the isolation of several novel compounds. The therapeutic value and pharmacological action of a drug was due to the presence of certain chemical constituents such as various forms of glycosides, tannins, phenolic compounds, lipids, fixed oils and volatile oils, resins, various kinds of alkaloids gums, mucilages, pectin etc. These phytochemicals are of immense importance to mankind [5]. Medicinal plants are the only important natural source to the development of drugs without any adverse effects, in which the secondary metabolites are responsible for the activity against harmful bacteria pathogens. The first step towards this goal is the biological and phytochemical screening of plant extracts and extracts from traditional preparations used in popular medicine [6] [7]. Successful strategies for investigation of these preparations involve the selection of test crude extracts based on a combination of ethnopharmacology and daily healer's practices. The presence of secondary metabolites was very important for the medicinal activity. It was confirmed by preliminary phytochemical analysis. There is a constant and crucial need to innovate novel antimicrobial compounds with diverse chemical structures and unique mechanisms of action because there has been an alarming increase in the prevalence of new and re-emerging infectious diseases. Another massive concern is the development of resistance to the antibiotics in contemporary medical use. The present study focused to reveal the presence of secondary metabolites and antibacterial activity of asteraceae member *S. indicus* plant extracts against laboratory bacterial strains.

2. Materials and Methods

2.1. Collection of Plant Material

The plant material of *S. indicus* (Linn.) for this experiment was collected from the Herbal garden of Dravidian University, Kuppam, Andhra Pradesh, India, and also from the fields in the surroundings of the Dravidian University campus.

2.2. Preparation of Plant Extracts

The plants were collected from the field and initially rinsed with distilled water to free from soil particles and dried on paper towel under shade for one week and stored in airtight containers at room temperature. The dried leaves were coarsely powdered in a blender before subjecting for extraction.

The plant extracts were prepared by sequential cold maceration method using ethanol, methanol, petroleum ether, chloroform and distilled water as a solvent

[8]. 50 g of dried powder of plant material was soaked in 250 ml ethanol for 24 hr at room temperature under shaking condition at 120 rpm. This solution was filtered with the help of whatman® No. 1 filter paper. The filtrate was collected in petri dishes and allowed solvent to be evaporated at room temperature. The dried extract was stored in 2 ml eppendorf tube and further used for antimicrobial assay after dilution. The filter cake was dried at room temperature and stored separately. The dried powder of filter cake was sequentially resuspended in 250 ml methanol, petroleum ether, chloroform and distilled water to prepare dried extract in each solvent. After extraction in each solvent, remaining filter cake was dried and further used with next solvent for extraction. All the dried extracts were stored at 4˚C.

2.3. Phytochemical Screening

Phytochemical analysis of all the evaporated solvent extracts was conducted following the procedure of Indian pharmacopoeia [9].

2.3.1. Test for Alkaloids

200 mg of plant material was dissolved in 10 ml of methanol and filtered. For 2 ml filtrate and 1% HCl + steam, 1 ml filtrate + 6 drops Mayer's reagent/Wagner's reagent/Dragendorff's reagent was added. Creamish precipitate/brownish red precipitate/orange precipitate indicated the presence of respective alkaloids.

2.3.2. Test for Tannins

200 mg of plant material was dissolved in 10 ml of distilled water and filtered. For 2 ml filtrate + 2 ml $FeCl_3$ was added. Blue/black precipitate indicated the presence of tannins.

2.3.3. Test for Flavonoids

200 mg of plant material was dissolved in 10 ml of ethanol and filtered. For 2 ml filtrate + conc. HCl + magnesium was added. Ribbon pink/tomato red color indicated the presence of flavonoids.

2.3.4. Test for Steroids

(Liebermann-Burchard reaction)

200 mg of plant material was dissolved 10 ml of chloroform and filtered. For 2 ml of filtrate + 2 ml of acetic anhydride + conc. H_2SO_4 was added. Blue/green ring indicated the presence of steroids.

2.3.5. Test for Phenols

1 ml of each solvent extracts dissolved in alcohol/water was separately treated with 1 ml of neutral $FeCl_3$. The change in colour indicated the presence of phenols.

2.4. Antimicrobial Screening

2.4.1. Laboratory Test Organisms and Their Cultural Conditions

The test bacterial strains used for the study were *B. subtilis*, *S. aereus*, *P. putida*, *E. coli* and *K. pnemoniae.* Specific growth conditions were maintained. The bac-

teria used for the study were obtained from Department of Microbiology, Sri Venkaterwara University, Tirupati. All the cultures were maintained at 4˚C in nutrient agar slants.

2.4.2. Preparation of Inoculums

A loop full of the test culture was taken from respective strains of agar slants and sub cultured in to fresh tubes containing nutrient broth and incubated for overnight at 37˚C. The obtained cultures was centrifuged at 5000 rpm for 15 min. Bacterial suspension was added to fresh media which gives final concentration of 107 cfu /ml [10].

2.4.3. Antibacterial Activity Assay

The antibacterial activity was tested by agar well diffusion method [11] as adopted earlier and with little modifications was used [12]. Seeded agar was made using nutrient agar medium. After the medium preparation it was sterilized and allowed to cool, so that the medium gets solidified. Just before solidification 0.1 ml of diluted inoculum (10^5 cfu/ ml) of test organism was added to the medium and then it was poured into the sterilized petri dishes under aseptic conditions. Under sterile conditions, wells of 4 mm diameter were punched into the agar medium with the help of sterile cork borer. These wells were filled with 50 µl of plant extract of 500 µg/ml concentration and solvent Dimethyl sulphoxalate (DMSO) as control. The plates were incubated at 37˚C for 24 hrs. The antibacterial activity was evaluated by measuring the zone of inhibition (in mm) against test organisms. The antibiotics ampicilline, tetracycline and erythromycin at 500 µg/ml concentration each were used as positive controls.

2.4.4. Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined by macro broth dilution method [13]. The reconstituted extract was serially diluted two-fold in nutrient broth medium. Duplicate tubes of each dilution were inoculated with 5 × 10^5 cells (cfu) of the bacterial strain and cultures incubated at 37˚C for 18 hours. MIC was taken as the highest dilution (least concentration) of controls. MIC was taken as the highest dilution (least concentration) of extract showing no detectable growth in the macro-broth assay.

3. Results and Discussion

3.1. Qualitative Phytochemical Analysis

Preliminary investigation of phytochemical analysis of selected crude extracts revealed the presence of various compounds such as alkaloids, flavonoids, phenols, tannins and steroids. Alkaloids, flavonoids detected in the extracts are compounds that have been documented to possess a variety of medicinal properties and health promoting effects. Phenolics are the largest group of phytochemicals and have been said to account for most of the antioxidant activity of plant extract. These classes (such as alkaloids, phenols, tannins, steroids and flavonoids) of compounds are known to have curative activity against several

pathogens and therefore could suggest the use traditionally for the treatment of various illnesses [14]. From the results depicted in Table 1 higher concentration of alkaloids were present in ethanol leaf extract and methanol stem extract than the root extracts. Maximum concentrations of flavonoids were present in ethanol, methanol leaf and root extracts compared to stem extracts. Higher concentration of phenols was recorded in ethanol leaf and stem extracts when compared to root extracts. Ethanol stem extract, methanol root extracts shows higher concentrations of steroids. Lower concentrations of steroids were present in methanol and petroleum ether leaf extracts, chloroform extracts of stem and chloroform and petroleum ether extracts of root. Higher concentrations of tannins were present in ethanol and petroleum ether leaf and ethanol stem extracts. A moderate concentration of tannins was present in methanol and chloroform leaf extract compared to stem and root extracts.

The most of the phytochemicals classified as secondary metabolites are produce mainly by the shoot part of the plant, often their function in the plant is unknown, but certain phytochemiclas have structural, functional and general defence against plant pathogens so the preliminary phytochemical studies received pronounced importance, because the crude drugs posses varied composi-

Table 1. Phytochemical analysis of secondary metabolites such as alkaloids, flavonoids, phenols, steroids and tannins in different plant parts of *S. indicus*. + Less; + + Moderate; + + + High; + + + + Very high.

Type of extract	Alkaloids	Flavonoids	Phenols	Steroids	Tannins
LEAF					
Ethanol	++++	++	+++	++	+++
Methanol	+++	++	++	+	++
Chloroform	+	+	++	-	++
Petroleum ether	+++	-	+++	+	+++
Aqueous	++	-	++	-	-
STEM					
Ethanol	+++	+	++	-	+++
Methanol	++++	++	+	++	++
Chloroform	++	-	++	+	+
Petroleum ether	+++	+	++	++	-
Aqueous	+	-	-	-	-
ROOT					
Ethanol	+++	++	++	++	++
Methanol	++	++	+	++	++
Chloroform	-	+	-	+	-
Petroleum ether	+	-	+	+	++
Aqueous	-	-	+	-	-

tion of secondary metabolites [15] [16]. Considering the high economical and pharmacological importance of secondary plant metabolites, industries are deeply interested in utilizing plant tissue culture technique for large scale production of these substances [17].

3.2. Antimicrobial Activity Studies

In India, 70% of its population resides in villages. In spite of the accessibility to western medicine, people in these villages still continue to depend on herbal remedies, for treatment of their health problems. Plant species have long been the principal ingredients of traditional medicine and their use dates back to the beginning of human civilization. As compared to synthetic antimicrobial agents, plant based antimicrobials are cost effective, affordable and exhibit lesser side effects. As microbes are rapidly evolving their defence mechanism, so does the resistance develops against many of the antibiotics which were once effective. The search for new antimicrobial compounds has always been a need.

Zone of inhibition was measured (mm) compared with standard antibiotics such as ampicillin, erythromycin and tetracycline. The organic solvents such as ethanol, methanol, petroleum ether, chloroform and aqueous extracts were employed. Among the solvent extracts used ethanol leaf extract shows very high activity (3.0 - 11.0 mm one of inhibition) against all the tested organisms. All the stem extracts (ethanol, methanol and petroleum ether) were also shows high activity (3.5 - 9.0 mm of inhibition) when compared to root extracts. Aqueous extracts which showed less activity or else poor activity. Ethanol root extracts shows a moderate to less activity (2.0 - 7.0 mm of zone of inhibition) against all the tested organisms. Aqueous leaf extract showed antimicrobial activity (4.5 mm of zone of inhibition) against *K. pneumoniae* and it does not have any effect on other organisms tested. Methanol extract showed (9.0 mm of zone of inhibition) against *K. pneumoniae.* The obtained results of the crude extracts were compared with the standard antibiotics such as Tetracycline, Erythromycin and Ampicillin.

All the tested organisms are highly sensitive to the ethanol leaf extract (6.5 - 11.0 mm) than the standard antibiotics such as tetracycycline, erythromycin and ampicillin, which showed more or less activity (5.0 - 12.5 mm) on the tested organisms compared with ethanol leaf extract. In present investigation, the overall leaf extracts show high inhibitory activity against all the tested bacterial strains followed by stem and root extracts. Medicinal plants are one of the most important sources of drugs. The use of different parts of several medicinal plants to cure specific human ailments has been in vogue from ancient times. Natural products drug discovery will be more holistic, personalized and involve wise use of ancient and modern therapeutic skills in a complementary manner so that maximum benefits can be occurred to the patients and community [18]. The medicinal plants occupy significant place in modern medicine as a raw material of some important drugs. However world health organization (WHO) also has been recognized the importance of traditional medicine and has been active in

creating strategies, guidelines and standards for botanical medicine. Among the bacterial strains *B. subtilis*, gram positive bacteria contaminates wounds, *S. aureus* is one of the causative bacterium in community acquired pneumonia. *Klebsiella* species causes urinary and respiratory tract infections, opportunistic infection and pneumonia [19] [20]. Antibacterial properties of leaf and stem extracts can be effectively used for wound healing, septicemia and other common infectious diseases. Similar results were reported in *Adhatoda vasica* as a wound healing agent [21] [22] (**Figure 1** & **Figure 2**, **Table 2**).

The present study reveals that the active principles present in the aerial parts of plant are very active against all the tested bacterial strains compare to roots. Based on earlier reports, among the great variety of secondary compounds found in plants, phenolics and terpenoids represent the main antimicrobial agents. Plants based antimicrobial drugs has enormous therapeutic potential as they can serve the purpose with lesser side effects.

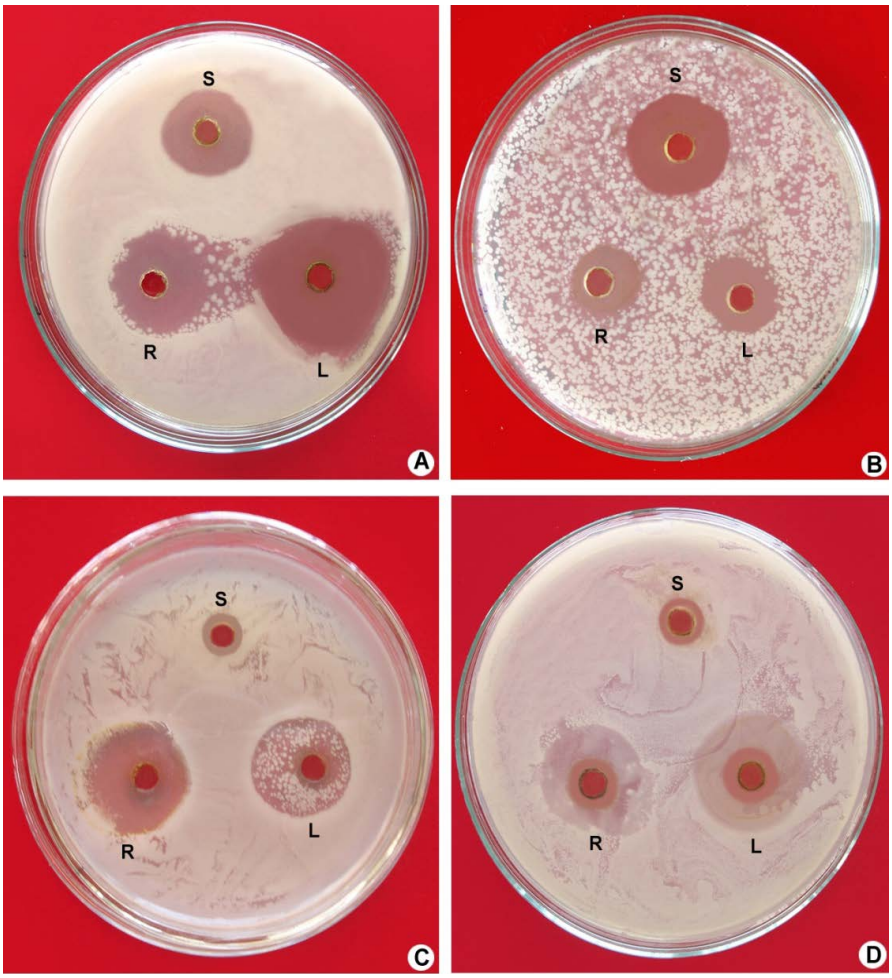

Figure 1. Antibacterial activity of leaf (L), stem (S) and root (R) solvent extracts of field grown *S. indicus* plants. (A) Plate tested with ethanol leaf, stem and root extract against *Klebsiella pneumoniae*, (B) Plate tested with methanol leaf, stem and root extract against *Klebsiella pneumoniae*, (C) Plate tested with ethanol leaf, stem and root extract against *Bacillus subtilis*, (D) Plate tested with ethanol leaf and stem extract against *Pseudomonas putida*.

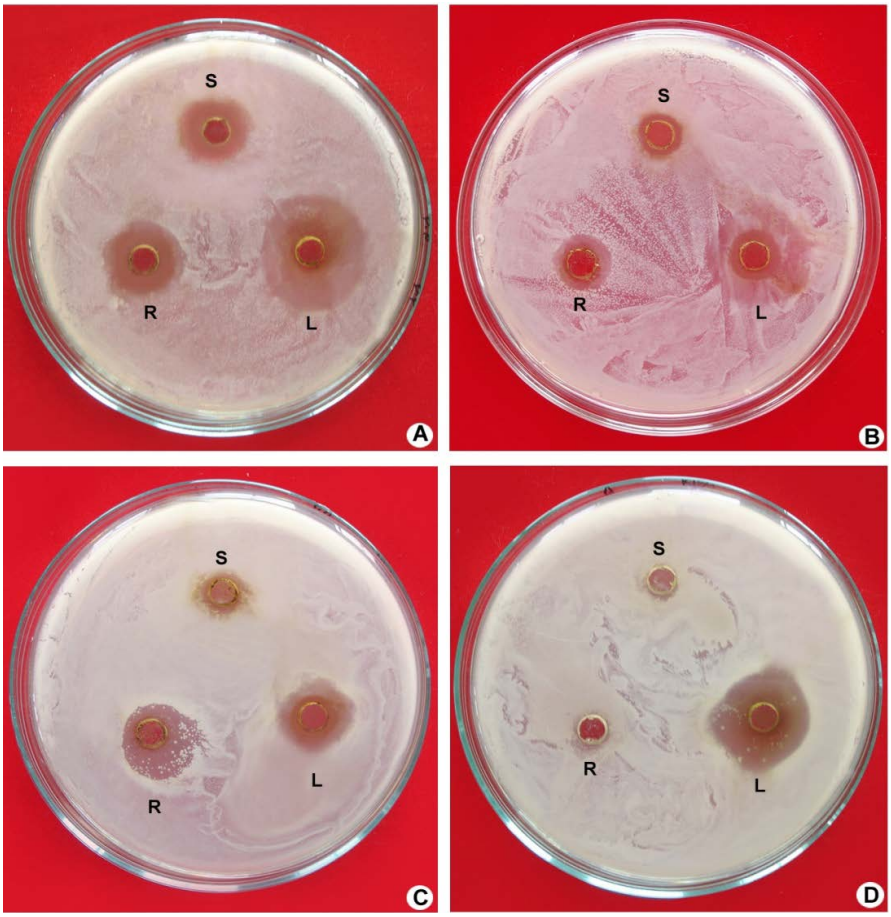

Figure 2. Antibacterial activity of leaf (L), stem (S) and root (R) solvent extracts of field grown *S. indicus* plants. (A) Plate tested with methanol leaf, stem and root extract against *Staphylococcus aureus*, (B) Plate tested with chloroform leaf, stem and root extract against *Escherichia coli*, (C) Plate tested with petroleum ether leaf and stem extract against *Staphylococcus aureus*, (D) Plate tested with aqueous leaf extract against *Klebsiella pneumoniae*.

3.3. Minimum Inhibitory Concentrations (MIC) of the Crude Extracts of *S. indicus* against Laboratory Bacterial Strains

MIC was determined by macro broth dilution method [13]. The lowest concentration (highest dilution) of the extract showing no detectable bacterial growth in the macro broth assay (Table 3) when compared with the control tubes was considered as minimum inhibitory concentration (MIC). Among different types of extracts tested; ethanol leaf extract showed lowest MIC values (2.0 - 5.50 µg/ml) against all the bacterial isolates tested. A lowest MIC value was recorded against *S. aureus* and *K. pneumoniae*. Ethanol stem extracts showed the MIC values in the range of (2.50 - 4.0 µg/ml), whereas petroleum ether stem extracts showed MIC values in the range of (2.00 - 5.50 µg/ml). Among the leaf extracts petroleum ether leaf extracts showed MIC values in the range of (4.00 - 7.00 µg/ml). Methanolic stem extract showed highest MIC values (25.50 µg/ml) and ethanolic leaf extract showed highest MIC values (17.50 µg/ml) were recorded. From the data obtained it is evident that lowest MIC values were recorded in leaf

Table 2. Susceptibility of test bacterial strains to leaf, stem and root extracts of *S. indicus* and standard antibiotics. P. ether = Petroleum ether.

Type of extract/ antibiotic used	Zone of inhibition (mm)				
	Bacillus subtilis	*Staphylococcus aureus*	*Escherichia coli*	*Pseudomonas putida*	*Klebsiella pneumoniae*
LEAF					
Ethanol	7.5	8.0	6.5	7.0	11.0
Methanol	6.0	9.0	8.0	5.5	9.0
P. ether	4.0	6.0	3.0	4.0	7.5
Chloroform	5.0	4.5	4.0	3.0	7.0
Aqueous	-	-	-	-	4.5
STEM					
Ethanol	3.5	5.0	5.0	7.0	7.5
Methanol	5.0	6.5	4.0	6.0	9.0
P. ether	4.0	-	3.5	-	5.0
Chloroform	-	3.5	2.5	1.5	4.0
Aqueous	-	-	-	-	-
ROOT					
Ethanol	7.0	3.5	3.0	5.5	5.0
Methanol	2.5	4.0	4.5	1.5	3.0
P. ether	-	4.0	-	2.0	3.5
Chloroform	4.5	2.5	3.0	3.5	-
Aqueous	-	-	-	-	-
STANDARD ANTIBIOTICS					
Tetracycline	12.5	9.0	9.5	10.5	9.5
Erythromycin	9.0	8.5	8.0	7.5	5.0
Ampicillin	11.0	10.5	8.5	5.0	8.0

Table 3. Minimum inhibitory concentration (MIC) of the crude extract of *S. indicus* against the bacterial strains.

Type of extract	MIC (µg/ml)				
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Pseudomonas putida*	*Klebsiella pneumoniae*
LEAF					
Ethanol	3.50	6.00	7.50	3.50	9.50
Methanol	17.50	13.50	9.00	12.50	11.00
Chloroform	-	5.50	7.00	6.50	7.00
Petroleum ether	7.00	12.50	6.50	4.00	5.50
Aqueous	2.00	3.50	7.50	6.50	3.00
STEM					
Ethanol	4.00	13.50	12.50	7.00	2.50
Methanol	8.00	11.00	9.50	25.50	11.50
Chloroform	-	10.00	8.50	12.00	7.00
Petroleum ether	3.50	-	2.0	5.50	4.00
Aqueous	3.50	3.00	-	2.00	3.00
ROOT					
Ethanol	8.50	17.50	8.50	6.00	7.50
Methanol	14.00	15.00	20.00	-	11.50
Chloroform	9.50	-	9.50	25.00	-
Petroleum ether	-	10.50	-	-	8.50
Aqueous	-	-	-	-	-

extracts followed by stem and root extracts.

4. Conclusion

In conclusion, the ethanolic extracts of *S. indicus* showed the abroad spectrum of activity against all the tested organisms which were responsible for some common bacterial infections. However, the results were encouraging enough to practice fractionation of this extracts and to find out the functional properties of phytochemical compounds in order to ascertain useful potential antimicrobial compounds.

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