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REGULAR ARTICLE

PRELIMINARY SCREENING OF ANTIMICROBIAL PROPERTIES OF FEW MEDICINAL PLANTS

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SUMMARY

Crude extracts were prepared from the leaves of ten medicinal plants viz., *Alpinia galanga, Artabotrys uncinatus, Commelina benghalensis, Costus igneus, Euphorbia cyathopora, Justicia gendarussa, Kalanchoe pinnata, Panicum antidotale, Sauropus androgynous* and *Hibiscus* using methanol as solvent and screened for their antibacterial activity against ten bacterial pathogens. The tested gram positive bacterial strains were *Bacillus cerus, Bacillus megaterium, Micrococcus leuteus, Staphylococcus aureus, Streptococcus lactis*, and gram negative strains were *Pseudomonas aeruginosa, Escherichia coli, Enterobacter aerogenes, Klebsiella pneumoniae* and *Salmonella typhimurium*. Among the ten plants tested, the methanol extracts of Alpinia galanga, *Artabotrys uncinatus, Costus igneus* and Yellow *Hibiscus* exhibited higher antibacterial activity when compared to the other plant extracts. These four plant extracts were further used for the phytochemical analysis. Results of the phytochemical analysis indicated the presence of alkaloids, phenolic compounds and flavanoids. The antibacterial activities of the leaves were due to the presence of various secondary metabolites

Key words: Antibacterial activity, Medicinal plants, Methanol extract, Secondary metabolites

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1. Introduction

Innumerable biologically active compounds that are found in plants [1-3] possess antibacterial properties [4, 5]. Plant produced compounds are of interest as sources of safer or more effective substitutes for synthetically produced antimicrobial agents [6]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The world health organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [7].

India has about 2000 species of medicinal plants and a vast geographical area with high production potential and varied agro-climatic conditions. For a long period of time, plants have been a valuable source of natural products for maintaining human health, last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purpose has gradually increased. According to the world health organization, medicinal plants would be the source to obtain a variety of drugs [8].

In recent years, secondary plant metabolites (phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents [9]. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections [6]. Since time immemorial, man has used various parts of plants in the treatment and prevention of various ailments [10].

The aim of this study was to evaluate the activity of extracts from 10 plants against several Gram-positive and Gram-negative bacterial strains in vitro.

2. Materials and Methods

2.1. Collection of plants

The fresh and healthy leaves of plants viz. *Alpinia galanga, Artabotrys uncinatus,*

Commelina benghalensis, Costus igneus, Euphorbia cyathopora, Justicia gendarussa, Kalanchoe pinnata, Panicum antidotale, Sauropus androgynous and *Yellow hibiscus* were collected from Vellore, Tamil Nadu, India. The vernacular name and their family names were given in Table 1. The collected leaves were washed thoroughly with tap water followed with sterile distilled water for the removal of dust and soil particles. The leaves were shade dried for few days and then powdered.

Table 1. Ethnobotanical information of some medicinal plants species selected for antibacterial activity

Plant Name	Common name	Family
Alpinia galanga	Galanga	Zingiberaceae
Artabotrys uncinatus	Bhandari Vine, Manorangini	Annonaceae
Commelina benghalensis	Benghal dayflower	Commelinaceae
Costus igneus	Spiral Flag, Fiery Costus	Costaceae
Euphorbia cyathophora	Painted Leaf Poinsettia	Euphorbiaceae
Hibiscus (Yellow)	Yellow Hibiscus	Malvaceae
Jusiticia gendarussa	Gandarusa, Karunochi	Acanthaceae
Kalanchoe pinnata	Life plant, Air plant	Crassulaceae
Panicum antidotale	Blue Panicgrass	Poaceae
Sauropus androgynus	Star-Gooseberry, Sweet leaf	Phyllanthaceae

2.2. Cultures used

Bacterial strains were obtained from the National Chemical Laboratory (NCL), Pune, India. Among the ten organisms investigated, five were gram-positive organisms and five were gram-negative organisms. The list of the organisms were given in Table 2.

Table 2. List of microorganisms used in this study

Sl No	Name	NCIM No.
	Gram-positive organism	
1	*Bacillus cereus*	2458
2	*Bacillus megaterium*	2032
3	*Micrococcus leuteus*	2704
4	*Staphylococcus aureus*	2672
5	*Streptococcus lactis*	2606
	Gram-negative organism	
6	*Enterobacter aerogenes*	5139
7	*Escherichia coli*	2810
8	*Klebsiella pneumoniae*	2707
9	*Pseudomonas aeruginosa*	2200
10	*Salmonella typhimurium*	2501

2.3. Preparation of plant extracts

The shade dried and powdered leaf materials were used for the solvent methanol extraction. About 50 grams of the leaf samples was extracted with 500 ml of methanol using soxhlet apparatus at 80^0 C. Further, the solvent was evaporated using a rotary vacuum evaporator. The residence was dissolved with dimethyl sulfoxide (DMSO) and used for antimicrobial activity.

2.4. Preparation of inoculum

Stock cultures were maintained at 4°C on slopes of nutrient agar. Active cultures for experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of Mueller-Hinton broth (MHB), that were incubated without agitation for 24 hrs at 37°C. The cultures were diluted with fresh Mueller- Hinton to achieve optical densities to 2.0 x 10^6 colony forming units (CFU/ml).

2.5. Antimicrobial activity

The disc diffusion method was used to screen the antimicrobial activity. In vitro antimicrobial activity was screened by using Mueller Hinton Agar (MHA) obtained from Himedia (Mumbai). The MHA plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1 % inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. The 50 mg concentrations of extracts were loaded on 6 mm sterile disc. The loaded disc was placed on the surface of medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs. For each bacterial strain, pure solvent is used as control. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. These studies were performed in triplicate and mean values were presented.

2.6. Phytochemical analysis

The leaf extract of *Alpinia galanga, Artabotrys uncinatus, Costus igneus and Hibiscus* were analyzed for the presence of Saponins, Phenolic compounds, Alkaloids, Flavonoids, Glycosides and Starch [11].

2.7. Test for saponins

The extract (50mg) was diluted with 20 ml of distilled water and it was agitated in a graudated cylinder for 15 minutes. The formation of 1 cm layer of foam showed the presence of saponins.

2.8. Test for Phenolic compounds

The extract is dissolved in distilled water and to this few drops of 1% lead acetate were added a bulky white precipitate was formed, which indicates the presence of phenolic compounds

2.9. Test for Alkaloids

50mg of extract is mixed with few ml of dilute HCl and then it is filtered. To this acidic medium, 1 ml of Drangendroff's reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.

2.10. Test for Flavonoids

To one ml of the extract, a few drops of dilute sodium hydroxide was added. An intense yellow colour was produced in the plant extract, which become colourless on addition of a few drops of dilute acid indicates the presence of flavonoids.

2.11. Test for Glycosides

About 100 mg of extract was dissolved in 1 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1 ml of concentrated sulphuric acid. A brown ring obtained indicated the presence of glycosides.

2.12. Test for Starch

To the extract drops of iodine solution was added formation of blue colour indicated the presence of starch.

3. Results

The antibacterial activity of 10 plant species extract was assayed *in vitro* by disc diffusion method against 10 bacterial species. Table 3 summarizes the microbial growth inhibition of methanol extract of the screened plant species.

Table 3. Antimicrobial activity of crude leaf extract of some Indian medicinal plants

	Zone of inhibition in millimeter									
	1	2	3	4	5	6	7	8	9	10
Gram-positive organism										
Bacillus cereus	-	-	-	16	1	7	-	12	12	13
Bacillus megaterium	11	10	-	14	2	-	-	-	-	-
Micrococcus leuteus	8	11	-	12	1	8	-	-	-	-
Staphylococcus aureus	7	15	-	13	-	9	1	12	-	-
Streptococcus lactis	12	16	1	17	1	8	3	-	13	-
Gram-negative organism										
Enterobacter aerogenes	7	15	1	12	12	2	-	-	-	12
Escherichia coli	6	7	-	9	-	4	-	-	-	-
Klebsiella pneumoniae	9	-	-	10	1	9	2	-	-	-
Pseudomonas aeruginosa	9	11	-	15	1	11	2	-	13	-
Salmonella typhimurium	10	15	-	19	12	-	-	-	-	13

1. Alpinia galanga; 2. Artabotrys uncinatus; 3. Commelina benghalensis; 4. Costus igneus; 5. Euphorbia cyathophora; 6. Hibiscus (Yellow); 7. Jusiticia gendarussa; 8. Kalanchoe pinnata; 9. Panicum antidotale; 10. Sauropus androgynus

Among the ten plants the maximum antibacterial activity was shown by *Costus igneus,* followed by *Alpiniga galanga, Artabotrys uncinatus and Hibiscus,* respectively. Moderate activity was observed in *Euphorbia cyathophora, Panicum antidotale* and *Sauropus androgynus*, where as the other three plants namely *Commelina benghalensis, Jusiticia gendarussa* and *Kalanchoe pinnata*, methanol extracts showed very minimum activity.

The antibacterial results indicated that among the ten plants *Costus igneus, Alpiniga galangal, Artabotrys uncinatus and Hibiscus* were found to be more effective against all the microbes tested. Based on these results, the preliminary screening of their secondary metabolites were done and presented in Table 4. *Costus igneus*, *Alpiniga galanga Hibiscus* and *Artabotrys uncinatus* showed the presence of Alkaloids, whereas *Alpiniga galanga* and *Hibiscus* showed the presence of Flavonoids. *Costus igneus, Artabotrys uncinatus* revealed the presence of glycosides. *Costus igneus* showed the presence of Saponins. *Hibiscus, Artabotrys uncinatus* and *Costus igneus*, showed the presence of phenolic compounds. The phytochemical analysis result indicated that the presence of alkaloids, flavanoids, phenolic compounds and glycosides in the plant extracts, these compounds might be responsible for antibacterial activity against micro-organisms.

Table 4. Phytochemical composition of some plant species

	Alpinia galanga	*Artabotrys uncinatus*	*Costus igneus*	*Hibiscus*
Alkaloids	+	+	+	+
Saponins	-	-	+	-
Carbohydrates	-	-	-	-
Phenolic compounds	-	+	+	+
Glycosides	-	+	+	-
Flavanoids	+	-	-	+

4. Discussion

Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The world health organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the words population. There are about 45,000 plant species in India with capacity to produce a large number or organic chemicals concentrated hotspot in the region of Eastern Himalayas, of high structural diversity [12, 13]. In the present work methanolic extract of *Costus igneus, Alpiniga galangal, Artabotrys uncinatus and Hibiscus* showed higher activity to the majority of the organism tested. Some of the plants were ineffective in this study do not possess antibiotic properties, or the plant extracts may have contained antibacterial constituents, just not in sufficient concentrations so as to be effective. It is also possible that the active chemical constituents were not soluble in methanol, or the drying process may have caused conformational changes to occur in some of the chemical constituents found in these plants (14, 15). The result of phytochemicals in the present investigation showed that all the four plant leaves contain components like alkaloids, phenolic compounds and flavanoids. This study also shows the presence of different phytochemicals with biological activity that can be of valuable therapeutic index [15, 16].

The potential for developing antimicrobials from higher plants appears rewarding as it will lead to the development of a phytomedicine to act against microbes. Plant-based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials [17]. Continued further exploration of plant-derived antimicrobials is needed today. Further research is necessary to determine the identity of the antibacterial compounds from within these plants and also to determine their full spectrum of efficacy. However, the present study of in vitro antimicrobial evaluation of some plants forms a primary platform for further phytochemical and pharmacological studies.

In the present study, we have found that the biologically active phytochemicals were present in the methanol extracts of few medicinal plants. The antibacterial properties of these extracts may be due to the presence of above mentioned phytochemicals. Further studies are in progress in our laboratory to isolate the active components.

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