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REGULAR ARTICLE

SCREENING OF ANTIMICROBIAL PROPERTIES OF CERTAIN INDIAN MEDICINAL PLANTS

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SUMMARY

Crude extracts were prepared from the leaves of five medicinal plants viz., *Aegle marmelos, Chloris virgata, Collinsonia anisata, Feronia limonia* and *Cassia auriculata* using methanol as solvent and screened for their antibacterial activity against four bacterial pathogens. The tested bacterial strains were *Escherichia coli, Salmonella typhi, Proteus mirabilis* and *Klebsiella pneumoniae*. Among the five plants tested, the methanol extracts of *Aegle marmelos* and *Cassia auriculata* exhibited higher antibacterial activity. The antibacterial potential of the extracts were found to be dose dependent. The phytochemical analysis of the plants were carried out. The antibacterial activities of the leaves were due to the presence of various secondary metabolites.

Keywords: Antibacterial activity, Bacterial pathogens, Secondary metabolites.

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1. Introduction

Herbal medicines have been known to man for centuries. Therapeutic efficacy of many indigenous plant for several disorders has been described by practitioners of traditional medicine [1].

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 [2].

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 [3].

Cultivation of medicinal plants offers considerable scope for rural employment and export for foreign exchange earnings. India is already a major exporter of medicinal plants. Infectious diseases are one of the important health hazard all over the world, both in

developing and developed countries. Several synthetic antibiotics are employed in the treatment of infections and communicable diseases [4].

The harmful microorganisms can be controlled with drugs and this result in the emergence of multiple drug resistant bacteria and it has created alarming clinical situations in the treatment of infections. The pharmacological industries have produced a number of new antibiotics, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to synthetic drugs which are utilized as therapeutic agents [5].

Therefore, actions must be taken to reduce this problem, such as to minimize the use of antibiotics, develop research of resistance, among microorganism and to continue studies to develop new drugs, either synthetic or natural to control pathogenic microorganism. Certain natural products such as plants and different parts of plant-leaves, roots, flowers, fruits, barks, seeds and oils are widely used for some chronic and acute diseases.

2. Materials and methods

Collection of plant materials

The fresh and healthy leaves of plants viz., *Aegle marmelos, Chloris virgata, Collinsonia anisata, Feronia limonia* and *Cassia auriculata* were collected from Cuddalore, Tamil Nadu, India. The leaves were washed thoroughly with tap water followed with sterilized distilled water for the removal of dust and sand particles. The leaves were shade dried for few days and then powered. This was used as new material for the extraction of antimicrobial compounds against the microbes.

Culture used

Microorganisms chosen were isolated from the clinical specimens that came for culture and sensitivity testing done in the laboratory of department of microbiology, Annamalai University, Annamalainagar. The cultures identified were *E. coli, Salmonella typhi, Proteus mirabilis* and *Kliebsella pneumoniae*.

Preparation of plant extracts

The preparations of different plant extracts were done by the following the modified method described by Perumal Samy et al. [6].

Solvent extraction methods

The shade dried and powdered leaf materials were used for the solvent methanol extraction. About 50 gm of leaf powder was weighed and mixed with solvent (1:3 w/v), which was incubated for two days, after the incubation period the slurry was filtered through whateman no.1 filter paper in a beaker and allowed it for evaporation. The residence was dissolved with dimethyl sulfoxide (DMSO) with different concentrations and checked it for antimicrobial activity.

Antimicrobial susceptibility test

Disc diffusion method was adopted for evaluation of antimicrobial activity of different medicinal plant leaves. Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45°C. The cooled media was poured on to sterile petriplates and allowed for solidfication. The plates with media were seeded with the respective microbial suspension using sterile swab. The disc impregnated with respective leaf extract at different concentration (100, 200 and 300 mg/ml) individually were placed on the four corners of each petridishes, control disc was also placed. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm [7].

Phytochemical analysis

The leaf extract of *Aegle marmelos, Chloris virgata, Collinsonia anisata, Feronia limonia, Cassia auriculta* were analyzed for the presence of Saponins, Tennis, Alkaloids, Flavonoids, Triterpenoids, Steroids, Anthraquinones, Glycosides, Coumarin, Gum, Starch and Protein [8].

Test for saponins

The extract 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.

Test for Tannins

Five ml of the extract and a few drops of 1% lead acetate were added yellow precipitate was formed, which indicates the presence of tannins.

Test for Alkaloids

Five ml of the extract was added to 2 ml of HCl. To this acidic medium, 1 ml of Drangendroff's reagent was added. An orange or red precipitate produced immediately indicates the presence of alkaloids.

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.

Test for Triterpenoids

Ten mg of the extract was dissolved in 1 ml of chloroform to which 1 ml of acetic anhydride was added following the addition of 2 ml of concentrated sulphuric acid formation of reddish violet colour indicates the presence of triterpenoids.

Test for Steroids

About 100 mg of the extract was dissolved in 2 ml of chloroform. Sulphuric acid was carefully added to form a low layer. A reddish brown colour at the interface is indicate for the presence of steroids.

Test for Anthraquinones

Five ml of the extract solution was hydrolysed with diluted concentrated sulphuric acid to which1 ml of dilute ammonia was added. Rose pink colouration suggest the positive response for anthraquinones.

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 indicate the presence of glycosides.

Test for Coumarin

To about 100 mg of the extract a few drops of 10% sodium hydroxide was added yellow colour formation indicate the presence of coumarin.

Test for Gum

To the seed powder drops of water was added swelling of seeds / forms adhesive indicate the presence of gum.

Test for Starch

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

Test for Protein

To the extract drops of picric acid was added formation of yellow colour indicate the presence of starch.

3. Results

The antimicrobial activity of crude leaf extract of *Aegle marmelos, Chloris virgata, Collinsonia anisata, Feronia limonia* and *Cassia auriculata* were studied in different concentrations (100mg/ml, 200 mg/ml, 300mg/ml) against four pathogenic bacterial strains.

Antibacterial potential of leaf extract was assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial activities are presented in Table 1. Different

concentrations viz., 100, 200, and 300mg/ml of each leaves were used for antimicrobial screening. The antibacterial activity of the extract increased linearly with increase in concentration of extract (mg/ml). The methanol extracts have shown significant antibacterial activity. The results revealed that *Proteus mirabilis* and *Klebsilla pneumoniae* were more sensitive as compared to *Escherichia coli* and *Salmonella typhi*, the growth inhibition zone measured ranged from 5-19mm for all the sensitive bacteria.

The inhibitory effect of *Aegle marmelos* extract showed at 100, 200, 300mg/ml) were (15, 16, 17mm) for *Proteus mirabilis*, (18, 19, 19mm) for *Klebsilla pneumoniae*, (10, 11, 13) for *E. coli* and (6, 7 and 9mm) for *Salmonella typhi* respectively. In *Chloris virgata* the diameter of the inhibition zone were (10, 12 and 13mm) aganist *Proteus mirabilis*, (13, 14, and 16mm) for *Klebsilla pneumoniae*, (9, 9 and 9mm) for E. coli and (6, 7 and 8mm) for Salmonella typhi at 100, 200 mg/ml conc. respectively.

Collinsonia anisata showed inhibitory effect in terms of diameter of the inhibition zone ranged from 10 to 13mm for Proteus mirabilis (12 to 18mm) for *Klebsiella pneumoniae* (8 to 10mm) for E.coli and (5 to 6mm) for *Salmonella typhi*. The antimicrobial activity of *Feronia limonia* in terms of utilization zone were 12, 13 and 15mm for Proteus mirabilis (12, 13 and 14mm) for *Klebsiella pneumoniae* (6, 7 and 8 mm) for E. coli and (10, 10 and 11 mm) for *Salmonella typhi*. Whereas *Cassia auriculata* recorded zone of inhibition of 10, 10 and 12 mm for Proteus mirabilis, (12, 16 and 18 mm) for *Klebsiella pneumoniae*, (8, 10 and 10 mm) for *E. coli* and (9, 10 and 11 mm) for *Salmonella typhi* at 100, 200 and 300 mg/ml concentration.

Table 1. Antimicrobial activity of crude leaf extract of *Aegle marmelos*, *Chloris virgata*, *Collinsonia anisata*, *Feronia limonia* and *Cassia auriculata* in comparision to methanol extract

Serial No	Plants	Microorganism	Zone of inhibition in millimeter			
			Concentration in mg/ml			
			Control	100	200	300
1.	*Aegle marmelos*	*Escherichia coli*	-	10	11	13
		Salmonella typhi	-	6	7	9
		Proteus mirabilis	-	15	16	17
		Klebsiella pneumoniae	-	18	19	19
2.	*Chloris virgata*	*Escherichia coli*	-	9	9	10
		Salmonella typhi	-	6	7	8
		Proteus mirabilis	-	10	12	13
		Klebsiella pneumoniae	-	13	14	16
3.	*Collinsonia anisata*	*Escherichia coli*	-	8	10	10
		Salmonella typhi	-	5	6	6
		Proteus mirabilis	-	10	11	13
		Klebsiella pneumoniae	-	12	16	18
4.	*Feronia limonia*	*Escherichia coli*	-	6	7	8
		Salmonella typhi	-	10	10	11
		Proteus mirabilis	-	12	13	15
		Klebsiella pneumoniae	-	12	10	14
5.	*Cassia auriculata*	*Escherichia coli*	-	8	10	10
		Salmonella typhi	-	9	10	11
		Proteus mirabilis	-	10	10	12
		Klebsiella pneumoniae	-	12	16	18

Graph 1: Antimicrobial activity of crude leaf extract of *Aegle marmelos*, *Chloris virgata*, *Collinsonia anisata*, *Feronia limonia* and *Cassia auriculata* at 300 mg/ml

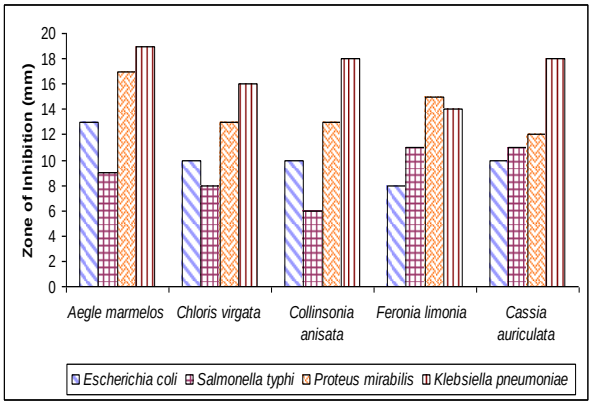

Table 2: Phytochemical composition of seed

Serial No	Secondary metabolite	*Aegle marmelos*	*Cassia auriculata*
	Saponins	+	+
	Tannins	+	-
	Alkaloids	-	-
	Falvonoid	-	-
	Triterpenoids	+	+
	Steroids	+	+
	Glycosides	+	+
	Anthraquinones	-	+
	Coumarin	-	-
	Gum	+	+
	Starch	-	-
	Protein	+	+

(-) – Negative (+) - Positive

The results show that among the leaves tested *Cassia auriculata* and *Aegle marmelos* 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 2. It was observed that, the secondary metabolites such as saponins, triterpenoids, steroids, glycosides, gum and protein were presented in both the plant extracts and alkaloids, falvonoid starch coumarin were found to be absent in both the plant extracts. Tannins were present in *Aegle marmelos* and absent in *Cassia auriculata* but in the case of Anthraquinones is vice versa.

4. Discussion

Recently much attention has been directed towards plant extracts and biologically active compounds isolated from popular plant species. The use of medicinal plants play a vital role in covering the basic health needs in developing countries and the plants may offer a new source of antibacterial, antifungal and antiviral agents with significant activity against infective microorganisms [9, 10].

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 [11,12].

In the present work methanolic extract of *Aegle marmelos* and *Cassia auriculata* extract showed higher activity to the test bacteria such as *Klebseilla pneumoniae*, *Proteus mirabilis*, Escherichia coli and *Salmonella typhi*. The antibacterial activity of *Chloris virgata*, *Collinsonia anisata* and *Feronia limonia* showed more or less equal zone of inhibition or slightly greater against that pathogens when compared to each other.

This study also shows the presence of different phytochemicals with biological activity that can be of valuable therapeutic index. The result of phytochemicals in the present investigation showed that both the plant leaves contain more or less same components like

Saponin, Triterpenoids, Steriods, Glycosides, Gum and Protein (Table 2).

The present observations suggests that the organic solvent extraction was suitable to verify the antimicrobial properties of medicinal plants and they support many investigators. The present study justifies the claimed uses of leaves in the traditional system of medicine to treat various infectious disease caused by the microbes. This study also encourages cultivation of the highly valuable plant in large scale to increase the economic status of the cultivaters in the country [13, 14].

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