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ANTIMICROBIAL ACTIVITY IN SELECTED INDIAN MEDICINAL PLANT EXTRACTS USING DISC DIFFUSION METHOD

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ABSTRACT

The use of medicinal plants in the treatment of respiratory illness, dermatophytes and infectious diseases is common in traditional medicine. t. Therefore, in the present investigation the antimicrobial activity was evaluated in different parts of seven selected medicinal plant extracts such as seeds of *Citrullus colocynthis, Mucuna prurita, Strychno potatorum*, *Strychnosnux-vomica,* Roots of *Glycyrrhiza glabra*, *Plumbago* zeylanica and *Salacia oblonga.* The extracts were obtained using methanol, ethylacetate and chloroform. The antimicrobial potential of 21 extracts collected using three different solvents were subjected to screening against five strains of the bacteria species, *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus* and *Proteus mirabilis* using disc diffusion method. Broad-spectrum antibiotics, Ampicillin, Cephalotaxine and Gentamicin were used as control drugs. The plant extracts showed more activity that is significant against Gram-positive bacteria than Gram-negative bacteria. Among the three solvent extracts, methanol extract have higher activity against bacteria. Ethyl acetate and chloroform extracts indicates moderate inhibition zone in medicinal plant extracts. This result showed that the plant extracts inhibited bacterial growth but their effectiveness is varied.

KEY WORDS: Antimicrobial activity, disc diffusion method, inhibition zone

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INTRODUCTION

Medicinal plants and their parts represent a rich source of antibacterial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. They have been widely used as traditional treatments for numerous human diseases. In less developed countries low income people such as farmers, people of small isolate villages and native communities use herbal medicine for the treatment of common infections. It is necessary to evaluate, in a scientific base, the potential use of herbal medicine for the treatment of infectious diseases produced by common pathogens[1]. In India, the use of different parts of several medicinal plants to cure specific ailments has been in vague from ancient times[2]. The present trend in modern medicine is towards a change from the use of cellulose coated medicinal pills to extracts of plant supplied either in pure forms or in synthetic versions for curing many human ailments. Thus, plants have provided the blue prints for the modern medicine[3]. In recent years, many possible sources of natural antibiotics have been in use for several infectious diseases, mostly bacterial and fungal. In view of this, the searches for new anti-microbial agents from medicinal plants are even more urgent in the countries like India where infectious diseases of bacterial origin are not only rampant, but the causative agents are also developing an increasing resistance against many of the commonly used antibiotics. The medicinal plants and of different parts, which are rich in a wide variety of secondary metabolites belonging to chemical classes such as steroids, alkaloids, glycosides, saponins, flavonoids, tannins, and carbohydrates, are generally superior in their anti-microbial activities. Seeds and Roots of experimental plants have been used for treating many diseases in traditional medicines[4]. In addition, the increasing failures of chemotherapeutics and antibiotic resistance exhibited pathogenic microbial infectious agents have led to the screening of several medicinal plants for their potential antimicrobial activity. From over 3, 00,000 species of higher plants to occur in nature, only about 2 percent have been screened so for. Extract of plants from 157 families have been reported to be active against microorganisms[5]. Compound extracted from different parts of the plants and their actions against diseases [6] were shown in Table 1. In the present investigation, the antimicrobial susceptibility of different parts of seven-selected medicinal plant extracts such as seeds of *Citrullus colocynthis, Mucuna prurita, Strychno potatorum, Strychnosnux-vomica,* Roots of *Glycyrrhiza glabra*, *Plumbago* zeylanica and *Salacia oblonga* have been evaluated against five strains of bacteria species.

Table -1
List of Medicinal Plant Parts & Uses

Botanical Name & *Family*	Parts used	Medicinal uses/Actions
Citrullus colocynthis Cucurbitaceae	Seeds	The pulp is used for varicose veins and piles. A paste of root is applied to various inflammations and swellings. The cataplasm of leaves is applied in migraine and neuralgia. Seeds exhibits antibacterial activity against a number of Gram-positive and Gram-negative bacteria.
Mucuna prurita Papilionaceae Fabaceae	Seeds	Astringent, nervine tonic, local stimulant, used in impotence, spermatorrhoea, urinary troubles, leucorrhoea, traditionally used for male virility. Also used in depressive neurosis. Hair on fruit—vermifuge, mild vesicant; used for diseases of liver and gallbladder.
Strychnos potatorum Loganiaceae; Strychnaceae	Seeds	Antidiabetic, antidysenteric, emetic. Mannogalactan from seeds reduces cholesterol and triglycerides) Seeds are also applied to abscesses, and venereal sores (internally in gonorrhoea).
Strychnos nux-vomica *Loganiaceae; Strychnaceae*	Seeds	Used in emotional disorders, insomnia, hysteria, epilepsy, paralytic and neurological affections, retention or nocturnal incontinence of urine, spermatorrhoea, sexual debility and impotence, general exhaustion; as antidote to alcoholism; GIT disorders. In Chinese medicine a paste made of Nux vomica seeds is applied topically for treating facial paralysis.
Glycyrrhiza glabra Papilionaceae;	Root	Demulcent, expectorant, antiallergic, anti-inflammatory, spasmolytic, mild laxative, antistress, antidepressive, antiulcer, liver protective, estrogenic,

Fabaceae		emmenagogue,antidiabetic. Used in bronchitis, dry cough, respiratory infections, catarrh, tuberculosis; genitourinary diseases, urinary tract infections; abdominal pain, gastric and duodenal ulcers, inflamed stomach, mouth ulcer. Also used for adrenocorticoid insufficiency.
Plumbago zeylanica Plumbaginaceae	Root	Root—intestinal flora normalizer, stimulates digestive processes; used for dyspepsia. Root paste is applied in order to open abscesses; a paste prepared with milk, vinegar or salt and water, is used externally in leprosy and other obstinate skin diseases. A cold infusion is used for influenza and black-water fever
Salacia Oblonga Hippocrateaceae; Celastraceae	Root	Root bark—used for the treatment rheumatism; also for gonorrhoea, swellings and skin diseases.

COLLECTION OF PLANT MATERIALS

Fresh seeds and roots of seven different medicinal plants viz., Seeds of Citrullus colocynthis, Mucuna prurita, Strychnos potatorum, Strychnosnux-vomica, Roots of Glycyrrhiza glabra, Plumbago *zeylanica* and Salacia oblonga were collected from mazhilam herbal garden in Salem District and Kolli Hills area in Namakkal District of Tamil Nadu. Samples were washed in fresh running water to eliminate dust, dirt and possible parasites, and then treated with deionized water. The plant parts were dried in shade and powdered using a mechanical grinder.

SOLVENT EXTRACTION OF PLANT MATERIALS

A known quantity of medicinal plants powder was filled in extraction thimble and soxhlet extracted successively for 48 hrs with different solvents like, Ethyl acetate, Methanol and Chloroform with constant temperature not exceeding the boiling point of the solvent. The extract was filtered with whatman filter paper (No.1) and concentrated in vacuum and dried at 45ºC for solvent removal. The extracts were stored in sterile bottles under refrigerated conditions until use. The dry weight of the plant extracts were obtained by solvent evaporation were weighed and dissolved in dimethyl sulfoxide (DMSO) to obtain a final concentration (mg/ml). The extract thus obtained was directly used in the assay of antimicrobial activity.

SELECTION OF BACTERIAL STRAINS

Microorganisms Bacillus cereus (MTCC NO: 1272), Escherichia coli (MTCC NO: 1687), Klebsiella pneumoniae (MTCC NO: 7028), Staphylococcus aureus (MTCC NO: 96) and Proteus mirabilis (MTCC NO: 425) were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology, Sector 39-A, Chandigarh-160036, India.

ANTIMICROBIAL ACTIVITY

The antimicrobial activity was performed by agar disc diffusion method. The Mueller Hinton agar (Hi-Media-M173) was inoculated and poured into the sterile Petri plates (Hi-media). The disc (0.6 cm) was saturated with various concentrations of solvents extract. The plates were incubated overnight at 37 ºC. The antimicrobial efficiency of ethyl acetate, chloroform and methanol extracts of seven medicinal plants of different parts were quantitatively assessed on the basis of zone of inhibition[7]. The results were compared with the standard antibiotics of Ampicillin, Cephalotaxine and Gentamycin .

RESULTS AND DISCUSSION

The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones. Phytochemicals derived from plant products serve as a prototype to develop less toxic and more effective medicines in controlling the growth of microorganism [8,9] . These compounds have significant therapeutic application against human pathogens including bacteria, fungi or virus. Numerous studies have been conducted with the extracts of various plants, screening antimicrobial activity as well as for the discovery of new antimicrobial compounds [10,11]. Therefore medicinal plants are finding their way into pharmaceuticals, Neutralacetials and Food supplements. In the present study, different parts of seven medicinal plants like Seeds and Roots with different solvent extracts

viz., methanol, ethyl acetate and chloroform. They were screened to determine the inhibition level against the bacterial species, which are reported to cure different diseases and ailments. It was confirmed that all tested plant extracts were able to exhibit inhibitory effect on both gram positive and gram negative bacteria[12].The percentage yield of ethyl acetate, chloroform, methanol extracts were given in Table 2 and 3. Susceptibility of each plant extracts were tested by serial micro dilution and Disc Diffusion method was determined. The extract yield was considerably more in methanol than ethyl acetate and chloroform. The extract yield in methanol ranged from 0.8 to 15.46% while in ethyl acetate and chloroform the ranges were from 0.47% to 8.80% and 0.53% to 4.21% respectively. Methanolic extract yield was maximum in Citrullus colocynthis (15.46%) followed by Glycyrrhiza glabra (8.70%) and the minimum yield was in Strychnos nux-vomica (0.80%). Maximum ethyl acetate extract yield was in Citrullus colocynthis (8.80%), while minimum yield was in Strychnos nux-vomica (0.47%) Maximum Chloroform extractive yield was in Mucuna prurita (4.21%), while minimum yield was in Plumbago zeylanica (0.53%).

Table 2
Antimicrobial activity of Methanol, Ethyl acetate and Chloroform extracts of the Medicinal plant parts (SEEDS) against different microbial strains

Zone of inhibition (mm)							
Gram-positive bacteria							
Plants	**Extracts (µl) (yield in %)**		**Bacillus cereus**	**Staphylococcus aureus**	**Escherichia coli**	**Klebsiella pneumoniae**	**Proteus mirabilis**
Citrullus colocynthis	Methanol (15.46%)	20µl	8	8	-	14	15
		40µl	11	10	7	16	18
		60µl	13	14	10	19	22
		80µl	17	18	14	24	25
	Ethyl acetate (8.80%)	20µl	9	8	7	-	7
		40µl	12	8	9	8	8
		60µl	14	12	14	9	10
		80µl	15	17	21	12	14
	Chloroform (1.75%)	20µl	9	7	-	7	8
		40µl	13	7	7	8	8
		60µl	15	10	11	11	10
		80µl	20	13	14	11	13
Mucuna prurita	Methanol (4 .70%)	20µl	10	9	7	8	-
		40µl	14	10	7	9	8
		60µl	11	16	10	11	10
		80µl	25	26	12	13	9
	Ethyl acetate (4.50%)	20µl	7	9	9	8	7
		40µl	8	13	12	12	7
		60µl	11	19	12	16	9
		80µl	12	22	15	20	12
	Chloroform (4.21%)	20µl	7	11	-	-	8
		40µl	9	15	8	-	8
		60µl	9	22	10	8	11
		80µl	11	26	14	8	15
Strychnos potatorum	Methanol (2.30%)	20µl	7	9	13	10	9
		40µl	10	12	17	13	9
		60µl	14	17	21	15	11
		80µl	19	20	26	21	14
	Ethyl acetate (2.50%)	20µl	10	8	-	8	-
		40µl	11	9	7	8	9
		60µl	17	14	10	14	12
		80µl	21	23	11	15	13
	Chloroform (2.23%)	20µl	8	9	7	-	-
		40µl	9	14	9	8	-
		60µl	10	18	10	8	9
		80µl	13	25	18	9	11
	Methanol	20µl	13	10	8	7	9

	(0.80%)	40µl	16	15	13	9	11
		60µl	24	21	16	15	13
		80µl	26	29	21	19	15
	Ethyl acetate (0.47%)	20µl	8	8	-	-	-
		40µl	11	10	7	8	-
		60µl	13	14	10	11	8
		80µl	17	18	14	15	10
	Chloroform (0.75%)	20µl	12	9	8	7	-
		40µl	17	11	8	9	-
		60µl	23	15	11	9	-
		80µl	28	21	13	10	-

Table 3

Antimicrobial activity of Methanol, Ethyl acetate and Chloroform extracts of the Medicinal plant parts (ROOTS) against different microbial strains

Zone of inhibition (mm)							
Gram-positive bacteria							
Plants	**Extracts (µl) (yield in %)**		**Bacillus cereus**	**Staphylococcus aureus**	**Escherichia coli**	**Klebsiella pneumoniae**	**Proteus mirabilis**
Glycyrrhiza glabra	Methanol (8.70%)	20µl	12	14	9	14	16
		40µl	16	17	12	17	19
		60µl	21	19	14	21	26
		80µl	32	24	21	25	28
	Ethyl acetate (3.26%)	20µl	7	9	9	8	-
		40µl	11	10	7	8	9
		60µl	14	12	13	10	15
		80µl	17	14	17	12	17
	Chloroform (1.80%)	20µl	10	9	8	-	-
		40µl	11	10	7	7	-
		60µl	17	17	12	8	-
		80µl	21	23	15	8	-
Plumbago zeylancia	Methanol (3.45%)	20µl	12	10	-	7	8
		40µl	16	16	8	7	10
		60µl	20	19	10	10	12
		80µl	25	23	13	13	15
	Ethyl acetate (1.45%)	20µl	8	8	7	11	12
		40µl	11	10	10	14	15
		60µl	16	13	14	24	20
		80µl	17	15	16	29	26
	Chloroform (0.53%)	20µl	8	8	-	14	15
		40µl	11	10	7	16	18
		60µl	13	14	10	19	22
		80µl	17	18	14	24	25
Salacia Oblonga	Methanol (4.15%)	20µl	12	9	8	9	7
		40µl	16	10	8	12	10
		60µl	19	14	14	15	11
		80µl	25	15	17	19	15
	Ethyl acetate (2.25%)	20µl	11	12	8	9	8
		40µl	14	15	12	14	8
		60µl	19	21	13	16	12
		80µl	23	26	16	20	15
	Chloroform (1.50%)	20µl	9	8	-	-	9
		40µl	11	10	7	11	11
		60µl	18	15	13	12	12
		80µl	24	22	15	14	14

(-) – No activity

The antibacterial activity of all the extracts is shown in Table 2 and 3. The chloroform extract of Strychnosnux-vomica and the extract of Glycyrrhiza glabra in Methanol did not show any activity against Gram-negative bacteria. While the methanol extract shows significant antibacterial activity against bacterial strains.The methanol extracts of the studied plants were more potent than the ethyl acetate extracts.The present study investigated that methanol is a better solvent for consistent extraction of antimicrobial substances from

medicinal plants. The most susceptible bacterium was Klebsiella pneumonia followed by Proteus mirabilis amongst the Gram-negative bacteria, while amongst Gram-positive strains, the most susceptible was Bacillus cereus followed by Staphylococcus aureus. The most resistant Gram-negative bacteria were Klebsiella pneumonia followed by Proteus mirabilis showed moderate activity. The most resistant Gram positive bacterium was Staphylococcus aureus followed by Bacillus cereus. The maximum antibacterial activity was shown by Methanol extract of Glycyrrhiza glabra against Bacillus cereus followed by Strychnosnux-vomic against Staphylococcus aureus.

CONCLUSION

The present investigation concluded that the antimicrobial activity of medicinal plants of different parts which are extracted using the solvents such as methanol, ethyl acetate and chloroform were having strong activity against gram positive and gram negative bacteria. The medicinal plant extracted using methanol shows very strong activity among three solvents.

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