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Antimicrobial activity and phytochemical studies on some Indian medicinal plants against selected human pathogens

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ABSTRACT

The acetone, ethanol, methanol extracts of 10 plants were evaluated for antimicrobial activity against medically important bacteria viz. Streptococcus sp., Staphylococcus sp., Klebsiella sp., E.coli, Salmonella sp., Pseudomonas sp., Candida sp. The invitro antimicrobial activity was performed by agar well diffusion method. The acetone and ethanol extracts were inactive compared to methanol extracts. The methanol extracts showed the maximum antimicrobial activity against the test organisms.Amongst the plant species screened, methanol extract of Indian aloe showed maximum inhibitory activity (44mm) against E. coli. The phyto-chemical analysis revealed the presence of tannins, glycosides, flavonoids, reducing sugars, anthraquinones.

Keywords: Medicinal plant extracts, antimicrobial activity, phytochemical screening.

INTRODUCTION

Plants, which yield medicine or drugs, are called medicinal plants. The branch of science that deals with the medicinal plants, their identification, cultivation, collection, preservation, extraction and the preparation of drug from such medicinal plants is called as pharmacognosy [1].

The practice of using plant as sources of medicine dates back to about 5000 B.C. During 16th century Botanists developed a belief called “Doctrine of Signatures”. According to this belief certain plant organs were modelled upon structural principles similar to those of human organs. These plant organs constituted more or less specific remedies for disease of human organs, which they resembled most [9].

Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments. It is clear that the plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of much intractable disease [10].

Antibiotic resistance has become a global concern. There has been an increasing incidence of multiple resistances in human pathogenic microorganism in recent years, largely due to indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases. This has forced scientist to search for new antimicrobial substances from various source like medicinal plants [11].

According to World Health Organization (WHO), more than 80% of the world's population relies on traditional medicines for their primary health care needs (Andy *et al.*, 2008). The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on human body. The most important bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants [5].

Herbal medicine represents one of the most important fields of traditional medicine. It is estimated that about 75% of the 120 biologically active plant derived compounds, presently in the use worldwide, have been derived through follow up researches to verify the authenticity of the data from folk and ethnomedicinal uses.[6].

Scientific investigations of medicinal plants have been initiated in many countries because of their contributions to health care. The primary benefits of using plant-derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment [4].

MATERIALS AND METHODS

Collection of Plant Samples

To perform this experimental studies ten medicinal plants (*Azadirachta indica, Aristolochia indica, Pyllanthus niruri, Acorus calamus, Aloe vera, Andrographis paniculata, Asparagus racemsous, Eucalyptus globolus, Clitoria ternatea, Acalypha indica)* were collected from Ayurvedic medical shop.

Processing of Samples

Leaves, roots and whole plants were used to extract bioactive fractions. The part of plants which was used to extract, washed to remove soil and dust particles with water. Then they are dried under shaded place. Dried plant materials were blended to form a fine powder and stored in airtight bottles.

Test Organisms

The human pathogens were obtained from Vivek Laboratory at Nagercoil and were maintained in Nutrient agar slant at 4°C. They includes *Staphylococcus sp., Streptococcus sp., Klebsiella sp., Escherichia coli, Salmonella sp., Pseudomonas sp., Candida sp.*

Preparation of Plant Extract

About 10g of each plant powder were dissolved in 50ml of ethanol, methanol and acetone respectively and kept in shaker for two days. Then the extract was filtered and it is dried in oven at 40°C. Then the extract was stored under refrigeration at 4°C for further studies [4].

Assay of Antimicrobial Activity Using Agar Well Diffusion Method
The 20ml of sterilized Mueller Hinton Agar was poured into each Petri plates. The wells were punched over the agar plates using sterile gel puncher. Fresh culture of pathogens were swabbed on the respective plates and 100µl of each extract were poured into the wells. The plates were incubated for 24hrs at 37°C. After incubation, the diameter of inhibition zones formed around each wells were measured and expressed in millimeter (mm).

Each of the extracts was autoclaved to determine the stability of the crude extracts at the temperature of 121°C for 15 minutes [3].

Antimicrobial activity of Commercially available antibiotics
The antimicrobial activity of selected plant extract were compared with the commercially available antibiotics. For this study, sterile Mueller Hinton Agar plates were prepared and the test organisms were swabbed over the surface of agar plates using sterile cotton swab. The selected antibiotic disc such as Gentamycin, Ciprofloxacin, Penicillin, Ampicillin, Kanamycin were placed on the surface of the plates. The plates were incubated at 37°C for 24hrs, after incubation the diameter of inhibition zones were measured in millimeter (mm), [3].

Phytochemical Analysis
The qualitative phytochemical analysis of ten plant extracts were determined as follows:

Tannins
To 0.5ml of plant extracts, 1ml of water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for gallic tannins and green black for catecholic tannins [6].

Glycosides
2ml of plant extracts were treated with 1ml of glacial acetic acid and add few drops of ferric chloride and concentrated sulphuric acid. Reddish brown coloration at the junction of two layers and the bluish green colour in the upper layer indicates the presence of glycosides [6].

Flavonoids
2 ml of plant extracts were treated with few drops of concentrated hydrochloric acid and magnesium ribbon. Pink-tomato red colour indicates the presence of flavonoids [8].

Reducing Sugars
To 0.5ml of plant extracts, 1ml of water and 5-8 drops of Fehling's solution was added and heated over water bath. Brick red precipitate indicates the presence of reducing sugars [6].

Anthraquinones
2ml of plant extracts were treated with 1ml of dilute ammonia and shaken vigorously. Pink-red colour in the ammonical layer indicates the presence of anthraquinones.

RESULT

The antimicrobial activity of acetone, ethanol and methanol extracts of ten Indian medicinal plants were investigated using agar well diffusion method, against the selected human pathogens such as *Staphylococcus sp., Streptococcus sp., Escherichia coli, Klebsiella sp., Salmonella sp., Pseudomonas sp., Candida sp.* All the examined extract showed varying degrees of antimicrobial activities against the pathogens. The Phytochemical test were done to find the presence of active chemical constituents such as glycosides, reducing sugars, tannins, flavonoids, anthraquinones.

Table - 1 showed the antimicrobial activity of acetone extract of Indian Aloe (*Aloe vera*) showed maximum zone of inhibition (32 mm) against *Candida sp.* The Neem (*Azadirachta indica*), Indian Aloe (*Aloe vera*), Green Chiretta (*Andrographis paniculata*), Indian Acalypha (*Acalypha indica*) showed the minimum inhibitory zone (10mm) against *Klebsiella sp*., *Salmonella sp.*, *Escherichia coli* and *Streptococcus sp*. The antimicrobial activity of ethanol extract of Eucalyptus (*Eucalyptus globolus*) showed maximum zone of inhibition (42 mm) against *Streptococcus sp.* and the Sweet Flag (*Acorus calamus*) showed the minimum inhibitory zone (10 mm) against *Klebsiella sp.* The antimicrobial activity of methanol extract of Indian Aloe (*Aloe vera*) exhibited maximum inhibitory zone (44 mm) against *Escherichia coli.* The Neem (*Azadirachta indica*), Indian Birthwort (*Aristolochia indica*), Chanca Piedra (*Pyllanthus niruri*), Green Chiretta (*Andrographis paniculata*) showed minimum inhibitory zone (10 mm) against *Streptococcus sp., Escherichia coli* and *Staphylococcus sp.*

Table - 2 showed the antimicrobial activity of acetone extract of Wild Asparagus (*Asparagus racemosus*) showed maximum zone of inhibition (40 mm) against *Klebsiella sp.* and the Indian Acalypha (*Acalypha indica*) showed the minimum zone of inhibition (8 mm) against *Staphylococcus sp*. The antimicrobial activity of ethanol extract of Green Chiretta (*Andrographis paniculata*) exhibited maximum zone of inhibition (38 mm) against *Pseudomonas sp.* and the minimum inhibitory zone (10 mm) was showed by *Asparagus racemosus* against *Staphylococcus sp*.

The antimicrobial activity of methanol extract of *Eucalyptus (Eucalyptus globolus)* showed the maximum zone of inhibition (40 mm) against *Salmonella sp.* The Neem (*Azadirachta indica*), Green Chiretta (*Andrographis paniculata*) and Indian Acalypha (*Acalypha indica*) showed maximum zone of inhibition (10 mm) against *Streptococcus sp.* and *Salmonella sp.* respectively.

Allopathic medicines which are commercially available was used against the selected human pathogens. Table - 3 showed the maximum inhibitory zone was observed in Ciprofloxacin (34 mm) against *Streptococcus sp.* and the minimum inhibitory zone was exhibited in Ampicillin (6 mm) against *Streptococcus sp.*

The phytochemical analysis of plant extracts using acetone, ethanol and methanol was showed in Table - 4. From the phytochemical analysis catecholic tannins were found in Chanca Piedra (*Pyllanthus niruri)* and Eucalyptus (*Eucalyptus globolus*) in the solvents such as acetone, ethanol and methanol. The methanol extract of Indian Aloe (*Aloe vera*) and ethanol extract of Neem (*Azadirachta indica*) showed the presence of tannins. Glycosides were found in ethanol extract of Sweet Flag (*Acorus calamus*), Green Chiretta (*Andrographis paniculata*), Indian Acalypha (*Acalypha indica*), Indian Aloe (*Aloe vera*). The methanol extract of Indian Birthwort (*Aristolochia indica*) and the acetone extract of Neem (*Azadirachta indica*), Sweet Flag (*Acorus calamus*) showed the presence of glycosides. Flavonoids were observed only in methanol extract of Chanca Piedra (*Pyllanthus niruri).* Reducing sugars were found in all the plant extracts except in acetone extract of Indian Aloe (*Aloe vera*). Anthraquinones were observed in the acetone and methanol extract of Wild Asparagus (*Asparagus racemosus*).

Table - 1. Antimicrobial activity of acetone, ethanol, methanol extract of medicinal plants against human pathogens - before autoclaving

Plants	Extracts	Zone of inhibition (mm)						
		Staphylococcus sp	*Streptococcus sp*	*Klebsiella sp*	*E.coli sp*	*Salmonella sp*	*Pseudomonas sp*	*Candida sp*
Azadirachta indica	A	16	18	10	18	16	14	22
	E	20	18	18	22	34	18	12
	M	14	10	20	18	20	18	30
Aristolochia indica	A	20	14	22	16	20	12	24
	E	24	12	16	12	24	18	14
	M	20	14	18	10	12	16	14
Pyllanthus niruri	A	20	22	16	22	30	18	20
	E	32	20	20	24	18	20	24
	M	18	10	18	16	24	20	32
Acorus calamus	A	18	12	14	18	20	18	18
	E	22	24	10	38	18	14	20
	M	16	14	20	22	16	18	24
Aloe vera	A	20	30	16	14	10	12	32
	E	14	40	18	24	14	16	14
	M	18	16	16	44	12	20	12
Andrographis paniculata	A	18	18	12	10	14	16	16
	E	14	14	16	12	16	16	16
	M	10	14	20	22	12	14	20
Asparagus racemosus	A	24	30	16	12	34	18	20
	E	20	12	12	40	18	14	22
	M	26	16	22	24	14	16	14
Eucatyptus globolus	A	22	16	18	16	20	20	26
	E	20	42	40	30	14	16	26
	M	18	20	20	22	18	16	24
Clitoria ternatea	A	28	18	12	32	22	24	14
	E	18	30	20	22	14	18	20
	M	24	36	16	32	16	14	16
Acalypha indica	A	12	10	14	18	22	16	24
	E	20	18	18	36	16	14	14
	M	14	12	24	18	16	16	22

"A" - Acetone *"E" - Ethanol* *"M" – Methanol*

Table - 2. Antimicrobial activity of acetone, ethanol, methanol extract of medicinal plants against human pathogens - after autoclaving

Plants	Extracts	Zone of inhibition (mm)						
		Staphylococcus sp	*Streptococcus sp*	*Klebsiella sp*	*E.coli sp*	*Salmonella sp*	*Pseudomonas sp*	*Candida sp*
Azadirachta indica	A	14	14	16	18	12	12	18
	E	16	16	14	20	16	16	20
	M	12	10	16	20	24	18	22
Aristolochia indica	A	12	20	16	16	28	12	22
	E	26	16	16	16	26	20	24
	M	16	14	20	14	22	18	38
Pyllanthus niruri	A	12	18	28	34	20	20	22
	E	12	14	16	18	22	28	28
	M	14	18	20	22	18	30	24
Acorus calamus	A	18	30	18	22	18	24	22
	E	26	30	12	18	24	30	20
	M	20	12	12	24	20	16	22
Aloe vera	A	12	30	20	18	22	20	16
	E	20	14	24	12	18	16	28
	M	18	30	12	16	16	22	18
Andrographis paniculata	A	18	12	18	16	12	22	16
	E	14	10	12	18	28	38	24
	M	24	10	8	14	10	20	20
Asparagus racemosus	A	16	16	40	12	12	16	32
	E	8	16	26	20	20	20	22
	M	16	16	18	18	18	16	20
Eucatyptus globolus	A	20	20	28	36	22	20	20
	E	22	24	32	32	36	22	16

	M	24	34	30	24	40	24	26
Clitoria ternatea	A	18	14	18	30	12	36	20
	E	12	16	22	22	10	32	18
	M	18	16	18	20	14	16	18
Acalypha indica	A	8	14	10	18	12	16	10
	E	16	12	20	14	12	14	20
	M	14	14	16	20	10	20	16

"A" - Acetone *"E" - Ethanol* *"M" - Methanol*

Table - 3. Antimicrobial activities of commercially available antibiotics against human pathogens

Microorganisms/Antibiotic	**Gentamycin**	**Ciprofloxacin**	**Penicillin**	**Ampicillin**	Kanamycin
	Zone of inhibition (mm)				
Staphylococcus sp.	24	32	22	20	**28**
Streptococcus sp.	28	34	-	6	**24**
Pseudomonas sp.	18	-	-	-	-
Klebsiella sp.	22	30	-	8	**18**
Escherichia coli	12	24	-	8	**12**
Salmonella sp.	12	8	36	-	**14**
Candida sp.	**20**	**26**	**-**	**14**	**28**

Table – 4. Phytochemical analysis of plant extracts

Medicinal Plants	**Tannins**			**Glycosides**			**Flavonoids**			**Reducing sugars**			**Anthraquinones**		
	Solvents														
	E	**M**	**A**	**E**	**M**	**A**	**E**	**M**	**A**	**E**	**M**	**A**	**E**	**M**	**A**
Azadirachta indica	+	–	–	–	–	+	–	–	–	+	+	+	–	–	–
Aristolochia indica	–	–	–	–	+	-	–	–	–	+	+	+	–	–	–
Pyllanthus niruri	+	+	+	–	–	–	–	+	–	+	+	+	–	–	–
Acorus calamus	–	–	–	+	–	+	–	–	–	+	+	+	–	–	–
Aloe vera	–	+	–	+	–	–	–	–	–	+	+	-	–	–	–
Andrographis paniculata	–	–	–	+	–	–	–	–	–	+	+	+	–	–	–
Asparagus racemosus	–	–	–	–	–	–	–	–	–	+	+	+	–	+	+
Eucalyptus globolus	+	+	+	–	–	–	–	–	–	+	+	+	–	–	–
Clitoria ternatea	–	–	–	–	–	–	–	–	–	+	+	+	–	–	–
Acalypha indica	–	-	–	+	–	–	–	–	–	+	+	+	–	–	–

"E" - Ethanol, *"M" - Methanol, "A" - Acetone*
"+" - Present, *"–" - Absent*

DISSCUSION

Infectious disease are a major cause of morbidity and mortality worldwide. The number of multi-drug resistant microbial strains and the appearance of strains which reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agents, intravenous catheters, organ transplantation and ongoing epidermidis of human immunodeficiency virus (HIV) infections. This situation provided the impetus to the search for new antimicrobial substances from various source like medicinal plants.

This research aimed at assaying the efficiency of the ten different plants using various solvent extraction procedure against the selected human pathogens.

In this present study, preliminary screening for antimicrobial activity showed, that the methanol extract of Indian Aloe (*Aloe vera*) exhibited maximum inhibitory zone (44mm) against *Escherichia coli* while the acetone extract of Sweet Flag (*Acorus calamus*) showed least inhibitory activity against *Klebsiella sp*.

The results of the effect of temperature (121^{o}c for 20 minutes) on the activity of plant extracts against the human pathogens showed significiant changes. For instance, the untreated (non-autoclaved) methanol extract of Indian Aloe (*Aloe vera*) showed 44mm zone of inhibition where as the autoclaved methanol extract of Indian Aloe showed 16mm zone of inhibition. The non-autoclaved acetone extract of wild Asparagus (*Asparagus racemosus)* showed 16mm zone of inhibition where as the autoclaved acetone extract showed 40mm of inhibitory zone.

Results obtained from this study, indicate that the plant extracts showed the strongest antimicrobial activity than the commercially available antibiotics. For example, Ciprofloxacin showed the maximum zone of inhibition (34mm) against *Streptococcus sp*. but the methanol extract of Eucalyptus (*Eucalyptus globolus*) and the methanol extract of Butterfly Pea (*Clitoria ternatea*) showed the maximum zone of inhibition (42mm and 36mm) against *Streptococcus sp*.

The phytochemical analysis showed the presence of tannins, glycosides, flavonoids, reducing sugars, anthraquinones were present in some of the plant extracts.

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