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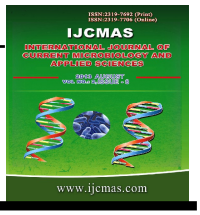

Original Research Article

Screening of eight plant extracts for their antimicrobial properties

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Keywords

Antimicrobial; Plant extracts; *Azadirachta indica Ficus benghalensis Vinca rosea Mangifera indica Emblica officinalis*

A B S T R A C T

Eight plants were screened for their potential antimicrobial activity. The plants screened were *Azadirachta indica (Meliaceae), Emblica officinalis (Phyllanthaceae), Terminalia arjuna (Combretaceae), Calendula officinalis (Asteraceae), Ficus benghalensis (Moraceae), Vinca rosea (Apocynaceae), Mangifera indica (Anacardiaceae)* and *Mentha piperita (Lamiaceae)*. The activity was tested using agar well diffusion method against 13 microbes that includes six bacterial strains and seven fungal strains. Among the three solvents used the extractive yield was maximum with methanol. *Proteus vulgaris*, among bacteria was observed to be the most resistant one, slightly inhibited by n-hexane extract of *Calendula officinalis* (leaf), methanolic extract of *Emblica officinalis* (leaf) and methanolic extracts of *Calendula officinalis*. Antifungal activity was found to be less in case of all the extracts except in case of methanolic extracts of *Emblica officinalis* (fruit) and n-hexane extract of *Azadirachta indica* which considerably inhibited *Aspergillus fumigatus* and *Cladosporium herbarum* respectively.

Introduction

Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants growing in different regions of the word. Plants have provided men with all his needs in terms of shelter, clothing, food, flavour and fragrance and not the least, medicines. Plants also have been the focus of religious life for many people around the world, being a major source of stimulation to the mythic imagination. For centuries, plants have been used throughout the word as drugs and remedies for various diseases since they have greater potential for producing newer drugs of great benefit to mankind. India is one of the largest users of medicinal plants using more than 7000 plant species for cure and has an abundance of plants, used in the traditional treatments of various disease on an empirical basis (Jain 1994; Ali *et al*., 2008).

Plants have formed the basis of sophisticated traditional medicine systems

among which are Ayurveda, Unani and Chinese etc. The first mention of medicinal use of plants has been documented in "Rig-Veda" (3000 2500 BC), which mentioned 67 plants having therapeutic effects. Scientific experiments on the antimicrobial properties of plant components were first documented in the late 19th century (Cowan, 1999). The properties of traditionally used drugs have been documented in detail in "Ayurveda", one of the sacred texts of the Hindu philosophy. It is the foundation stone of ancient medical science of India followed by "Susruta Samhita" and "Charaka Samhita".

Plant derived medicines are still the mainstay of about 80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with human body and fewer side effects (De Smet, 1997; Ullah and Khan, 2008). The quest for plants with antimicrobial properties continue to receive attention due to development of multiple drug resistance, an increased awareness of the limited ability of synthetic pharmaceutical products to control major diseases and adverse effects of synthetic products on host such as hypersensitivity, immunosuppression and allergic reactions. The relatively lower incidence of adverse reaction to the plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is also encouraging both the consuming public and national health care institutions to consider plant medicines as alternative to synthetic drugs (Itokawa *et al*., 2008).

In recent years, much attention has been paid to extract and isolated biologically active compounds from various plant species in light of their usefulness in medicine. So far, more than one million biologically active plant derived components have been isolated from higher plants (Efferth and Greten, 2012). These are mostly the secondary metabolites. Plant based antimicrobial represents a vast untapped source of medicines and needed to be explored further. They have enormous therapeutic potential and are effective in treatment of infectious disease (Harborne *et al*., 1998). In this context, Medicinal plants are rightly said to be "Tradition of yesterday and drugs of tomorrow".

Materials and Methods

Plant collection

All the 8 plants used in this study were collected from different locations in Sahibabad (Ghaziabad) during the months of January to April. Fresh plants / plant parts, collected from different locations, were washed thoroughly under running tap water and then oven dried at 50°C overnight. The dried material was grinded into fine powder and stored in dark bottles.

Microorganisms

All the microbial culture used in present study were obtained in and freeze-dried form from a Microbial Type Culture Collection, IMTEC Chandigarh. The bacterial strains used were *Bacillus subtilis* MTCC 121, *Staphylococcus aureus* MTCC 1430, *Proteus vulgaris* MTCC 1771, *Escherichia coli* MTCC 1610, *Klebsiella pneumoniae* MTCC 661 and *Salmonella typhimurium* MTCC 98. The fungal cultures used in the present study were obtained from characterized microbial culture stock of Inderprashta Engineering College, Ghaziabad, Sahibabad. The fungal strains used were

Aspergillus niger, *Aspergillus flavus*, *Aspergillus fumigatus*, *Trichoderma viride*, *Cladosporium herbarum*, *Fusarium graminearum* and *Fusarium oxysporum*.

Crude extraction

Twenty grams of dried plant material was extracted sequentially with 50 ml. n-hexane, chloroform, and methanol at room temperature. Each solvent was allowed to remain in contact with the plant material for 48 HRS in each occasion. The extracts were filtered through Whatmann filter paper No. 1 and solvent was allowed to evaporate from the extract. The residue was dried over anhydrous sodium sulphate to remove traces alcohol. Then the extract were kept in the refrigerator at 4°C for further use.

Yield of Extract

Three organic solvents, in series of their increasing polarity were used for the extraction purpose i.e. n-hexane, chloroform and methanol. The yield was calculated in terms of percentage (%) i.e. grams of extract obtained per 100 gm of initial material used.

Preparation of inoculums

The bacterial culture were grown in nutrient broth at 37°C until the optical density reaches the absorbance of Mc Farland No. 5 standard i.e. approximately 0.132 at 600 nm. At this absorbance, a concentration of 10^8 cells/ml was obtained. This suspension was used as inoculum for the antimicrobial susceptibility testing. In case of fungi, the spores were suspended in a sterile water blanks to obtain the required concentration i.e. 10^8 cells/ml.

Antimicrobial assay

To access the antimicrobial activity of different plant extracts Agar well diffusion method was used. In this method, the plant extract was dissolved in appropriate solvent. Agar plates were prepared and inoculated with the microbial suspension and then agar was punched to form well of 7 mm diameter. 100 µl of the test extract (100 µg/ml of DMSO) was suspended in the wells. A control well was loaded with equal amount of the solvent (DMSO). The plates were then incubated at 37°C for 24 to 48 HRS. After incubation, all plates were analysed for the appearance of inhibition zone around the extract loaded well and the clear zone of growth inhibition was measured in terms of diameter (mm).

Results and Discussion

Traditional healers used primarily water as solvent, but it has been documented that plant extracts extracted in organic solvents showed profoundly distinct antimicrobial activity from aqueous extract (Parekh *et al*., 2005). Hence, in the present work two organic solvents namely n-hexane, and methanol were used for the purpose of extraction. The extractive yield (%) in n-hexane ranges from 0.3% to 3.7%, in chloroform from 0.5% to 6.9% and in methanol it ranges from 0.8% to 10%. Among the three solvents, the yield percentage was observed to be maximum with methanol. The result justifies the claimed use of methanol as a better solvent for consistent extraction of antimicrobial substances from medicinal plants (Parekh *et al*., 2005). The observations can be rationalized in terms of the ability of plant metabolites to dissolve in each solvent.

Table.1 Antimicrobial activities of different plant extracts

Plant name	Part		BS	SA	PV	EC	KP	ST	AN	AFL	AFU	TV	CH	FG	FO
Azadirachta indica (Meliaceae)	Leaf	H (0.9)	--	12	--	--	13	14	--	--	5	--	12	--	2
		C (1.7)	--	17	--	--	--	--	--	--	--	--	--	--	--
		M (4.3)	11	10	--	--	13	--	5	--	2	4	--	--	--
	Fruit	H (0.4)	--	--	--	7	--	--	3	--	--	--	--	--	--
		C (1.8)	--	--	--	--	--	--	--	--	--	--	--	--	--
		M (1.3)	--	--	--	--	2	--	--	--	--	--	--	--	--
Emblica officinalis (Phyllanthaceae)	Leaf	H (2.5)	--	--	--	--	--	2	--	--	--	--	--	3	--
		C (1.4)	--	--	--	--	--	--	--	--	10	--	--	--	--
		M (5.2)	2	--	3	--	--	--	--	9	--	--	--	--	--
	Fruit	H (0.6)	2	--	--	5	--	--	--	--	--	--	--	--	--
		C (1.0)	--	--	--	--	--	--	--	--	--	--	--	--	--
		M (3.1)	--	--	--	--	--	7	--	--	16	--	--	--	--
Terminalia arjuna (Combretaceae)	Leaf	H (1.1)	--	12	--	--	--	--	--	--	--	--	--	--	--
		C (1.4)	--	15	--	--	14	14	--	--	--	--	--	--	5
		M (2.0)	7	15	--	--	16	11	--	--	2	--	--	3	--
	Stem	H (0.3)	--	--	--	--	--	--	--	--	--	--	--	--	--
		C (0.9)	--	2	--	--	--	--	--	--	--	--	--	--	--
		M (0.8)	--	--	--	--	--	--	--	--	--	--	--	--	--
Calendula officinalis (Asteraceae)	Leaf	H (2.5)	--	--	3	--	--	--	--	--	--	--	--	--	--
		C (1.3)	--	--	--	--	12	--	--	--	--	--	--	--	--
		M (5.7)	2	12	--	--	14	10	--	--	--	2	--	--	--
	Flower	H (1.2)	--	--	--	--	--	--	--	--	--	--	--	--	--
		C (2.8)	--	--	--	--	--	--	5	--	--	3	--	--	--
		M (4.5)	--	10	--	--	13	5	--	7	--	--	3	--	--
Ficus benghalensis (Moraceae)	Leaf	H (2.3)	--	--	--	--	--	--	--	--	--	--	--	--	--
		C (6.9)	--	--	--	4	--	--	--	--	--	--	--	--	--
		M (4.2)	11	--	--	12	--	--	10	--	--	--	--	8	--
Vinca rosea (Apocynaceae)	Leaf	H (1.2)	--	12	--	--	--	--	--	--	--	--	--	--	--
		C (2.5)	--	12	--	--	11	12	--	--	--	--	--	--	--
		M (5.0)	--	--	2	--	--	--	--	--	--	--	--	--	--
	Flower	H (0.4)	--	--	--	--	--	--	--	--	--	--	--	--	--
		C (0.5)	--	--	--	--	--	--	--	--	--	--	--	--	--
		M (1.5)	--	--	--	--	--	--	--	--	--	2	--	--	--
Mangifera indica (Anacardiaceae)	Leaf	H (3.7)	--	--	--	--	--	--	--	--	--	--	--	--	--
		C (6.7)	--	11	--	--	9	10	--	--	--	--	--	--	--
		M (1.0)	--	12	--	--	15	12	--	--	--	--	--	--	--
	Stem	H (1.2)	--	--	--	--	--	--	--	--	--	--	2	--	--
		C (2.0)	--	--	--	--	--	--	--	--	--	--	--	--	--
		M (3.5)	--	--	--	--	--	--	--	--	--	--	--	--	--
Mentha piperita (Lamiaceae)	Leaf	H (0.8)	--	15	--	--	6	--	--	--	10	--	--	--	--
		C (1.6)	--	13	--	--	10	8	--	--	--	--	--	--	--
		M (1.4)	--	13	--	--	14	10	--	--	--	--	--	--	--

Bacillus subtilis (BS), Staphylococcus aureus (SA), Proteus vulgaris (PV), Escherichia coli (EC), Klebsiella pneumoniae (KP), Salmonella typhimurium (ST), Aspergillus niger (AN), Aspergillus flavus (AFL), Aspergillus fumigatus (AFU), Trichoderma viride (TV), Cladosporium herbarum (CH), Fusarium graminearum (FG), Fusarium oxysporum (FO); (H: n-hexane, C: Chloroform, M: methanol)

n-Hexane extract of *Terminalia arjuna* (stem), *Calendula officinalis* (flowers), *Ficus benghalensis* (leaf), *Vinca rosea* (flower) and *Mangifera indica* do not exhibit any activity against all the 13 test of organisms. The n-hexane extract of *Azadirachta indica* (leaf) was found to have considerable inhibitory effect against *Staphylococcus aureus* (Ahmad and Beg, 2001), *Klebsiella pneumoniae* (Koul *et al*., 1990), *Salmonella typhimurium* (Baswa *et al*. , 2001) and *Cladosporium herbarum* (Verma *et al*., 2008). Chloroform extract of *Azadirachta indica* (leaf) and *Emblica officinalis* (fruits) was inactive against all the organisms while other were found to have a profound inhibitory activity against few of the test organisms. (Table.1). *Terminalia arjuna* (Stem) were found to have no inhibitory activity, whereas the methanolic extracts of *Terminalia arjuna* (leaf) and *Azadirachta indica* (leaf) exhibited less to profound antimicrobial activity against 6 of the test organisms.

Staphylococcus aureus was observed to be the most susceptible bacterial, inhibited by sixteen of the test extracts, followed by *Klebsiella pneumoniae*. The most resistant one were *Proteus vulgaris* and *Escherichia coli* inhibited only by 3 and 4 of the test extracts respectively. Less susceptibility of Gram-negative bacteria can be explained in reference to their outer membrane which is known to present a barrier to the penetration of numerous antibiotic molecules and also the periplasmic space contains enzymes that are able to break down the foreign molecules introduced from outside and the efflux pumps which reduces the cellular level of antibiotics (Ash *et al*., 2002). The activity of plant extracts against both Gram positive and Gram-negative bacteria may be an indicator of the presence of broader spectrum antibiotic compounds in the plant (Ahmad *et al*., 1998; Ahmad and Beg 2001).

The antifungal activity was found to be less in case of all the extract except in case of methanolic extracts of *Emblica officinalis* (fruit) and n-hexane extract of *Azadirachta indica* which considerably inhibited *Aspergillus fumigatus* and *Cladosporium herbarum* respectively. The variation in the susceptibility of bacteria and fungi for the test plant extracts may be possibly due to the differences in the chemical nature of cell wall and cell membranes of both the organisms (Cowan, 1999).

The broad spectrum of antibacterial activity found in the study may be attributed to the presence of secondary metabolites of various chemical nature present in plants. Different plants possess different constituents and in different concentrations, which accounts for differential antimicrobial activity, as also suggested earlier by Wallace (2004). The study of these constituents may help in the discovery of new chemical classes of antibiotics that could serve as selective agents for the maintenance of human health and may provide biochemical tools for the study of infectious disease.

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