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Full Length Research Paper

Antimicrobial activity of leaf extracts of Indian medicinal plants against clinical and phytopathogenic bacteria

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The ethnobotanical efficacy of Indian medicinal plants; *Achyranthes aspera, Artemisia parviflora, Azadirachta indica, Calotropis gigantean, Lawsonia inermis, Mimosa pudica, Ixora coccinea, Parthenium hysterophorus* and *Chromolaena odorata* were examined using agar disc diffusion method against clinical bacteria (*Escherichia coli* and *Staphylococcus aureus*) and phytopathogenic bacteria (*Xanthomonas vesicatoria* and *Ralstonia solanacearum*). Leaves were extracted using different solvents such as methanol, ethanol, ethyl acetate and chloroform. Among treatments, maximum in vitro inhibition was scored in methanol extracts of *C. odorata* which offered inhibition zone of 10, 9, 12 and 12 mm against *E. coli, S. aureus, X. vesicatoria and R. solanaccearum*, respectively, followed by chloroform extract of the same plant leaf with inhibition zone of 8, 4, 4 and 4 mm, respectively. A significant inhibition of *E. coli* was found in aqueous and in all tested solvent extracts of *A. indica*. In case of *S. aureus*, maximum inhibition of 8 mm was obtained in aqueous extracts of A. indica and 6 mm from methanol extract of *L. inermis*. The minimum inhibitory concentration (MIC) value for the clinical bacteria ranged between 0.35 to 4.0 mg/ml and 0.25 to 4.0 mg/ml for phytopathogenic bacteria when tested with all four solvents extracts of *C. odorata*. Whereas, extracts of *A. aspera, A. parviflora, C. gigantean, L. inermis, M. pudica* and *I. coccinea* were found to be ineffective or showed poor inhibition on tested human and phytopathogenic bacteria.

Key words: Indian medicinal plants, solvents, leaves extracts, clinical and phytopathogenic bacteria, antimicrobial assay.

INTRODUCTION

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine. Thus it is a logical approach in drug discovery to screen traditional natural products.

Approximately 20% of the plants found in the world have been submitted to pharmaceutical or biological test and a sustainable number of new antibiotics introduced on the market are obtained from natural or semi synthetic resources. It has been reported that between the years 1983 and 1994 (Cragg et al., 1999), the systematic screening of antibacterial plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant bacteria. Several workers throughout the world have carried out anti-microbial studies on some medicinal plants including *Betula pendula* (Mukhtar et al., 2002) and *Ageratum houstonianum* (Bowers et al., 1976). According to World Health Organization (Santos et al., 1995) medicinal plants would be the best source to obtain a variety of drugs.

Current advancements in drug discovery technology and search for novel chemical diversity have intensified

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the efforts for exploring leads from Ayurveda the traditional system of medicine in India. Ayurvedic system of medicine has its long history of therapeutic potential.

The use of plant extracts and phytochemicals both with known antimicrobial properties is of great significance, in the past few years a number of investigations have been conducted world wide to prove antimicrobial activities from medicinal plants (Alonso-Paz et al., 1995; Nascimento et al., 1990). For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. According to World Health Organization (Santos et al., 1995) medicinal plants would be the best source to obtain a variety of drugs. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are a part of the essential oils (Jansen et al., 1987) as well as tannin (Saxena et al., 1994).

There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases (Rojas et al., 2003). Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections (Benkeblia, 2004). Green plants represent a reservoir of effective chemotherapeutants and can provide valuable sources of natural pesticides (Mahajan and Das, 2003). Biopesticides has been suggested as an effecttive substitute for chemicals (Kapoor, 2001). Reports are available on the use of several plant by-products, which posses antimicrobial properties, on several pathogenic bacteria and fungi (Bylka et al., 2004; Shimpi and Bendre, 2005; Kilani, 2006). Here, we evaluate the potential of several plant extracts for antibacterial activity against important human pathogenic and phytopathogenic bacteria.

MATERIALS AND METHODS

Plant material

Fresh leaves of *Achyranthes aspera*, *Artemisia parviflora, Azadirachta indica, Calotropis gigantean, Lawsonia inermis, Mimosa pudica, Ixora coccinea, Parthenium hysterophorus* and *Chromolaena odorata* were collected from different locations of Mysore (12.18 N – 76.42 E, 770 m above sea level (ASL)), Karnataka (India), during 2007 - 2008. The plants were identified taxonomically and authenticated at the Herbarium, Department of Botany, Mysore. Fresh leaves were washed thoroughly 2 - 3 times with running tap water and then with sterile water followed by shade-dried, powdered and used for extraction.

Test microorganisms

Human pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus* were collected from JSS medical college Mysore. India. Plant pathogenic bacteria such as Xanthomonas vesicatoria and Ralstonia solanacearum were collected from the culture collection of Department of Applied Botany and Biotechnology, University of Mysore, India. All the test bacterial species were maintained on nutrient agar media.

Preparation of aqueous plant extracts

25 g of shade dried, powder of plant materials were macerated separately with 50 ml of sterile distilled water using pestle and mortar. The macerate was first filtered through four layer of muslin cloth and then filtrate was centrifuged at 8,000 rpm for 15 min at room temperature. Supernatant was filtered through Whatman No. 1 filter paper and heat sterilized at 120ºC for 30 min. The extract was preserved aseptically in a brown bottle at 4oC until further use.

Preparation of solvent extractions

25 g of shade dried, powder of plant materials were filled separately in the thimble and extracted successively with 150 ml each of methanol, ethanol, ethyl acetate and chloroform using a Soxhlet extractor for 48 h. All the extracts were concentrated using rotary flash evaporator. After complete solvent evaporation, each of these solvent extract was weighed and preserved at 4oC in airtight bottles until further use. 1 g of each solvent residue was dissolved in 10 ml of respective solvents were used as the test extracts for antimicrobial activity assay.

Anti-bacterial activity assay

Antibacterial activity of aqueous extract and solvent extracts; methanol, ethanol, ethyl acetate and chloroform was determined by disc diffusion method on nutrient agar medium (Anonymous, 1996). Sterile Whatmann filter discs (6 mm diameter) were made in nutrient agar plate using sterile cork borer (5 mm) and inoculum containing 106 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50 µl each of all aqueous and solvent extracts were placed in the discs made in inoculated plates. The treatments also included 50 µl of solvents served as control and chloromphenicol as a standard control. The plates were incubated for 24 h at 37ºC and zone of inhibition if any around the wells were measured in mm (millimeter). Each treatment consists of three replicates and repeated at least twice. Minimum inhibitory concentration (MIC), which was determined as the lowest concentration of *C. odorata* plant extracts inhibiting the growth of the organism, was determined based on the readings.

RESULTS

The ethnobotanical efficacy of various solvent extracts of *A. aspera, A. parviflora, A. indica, C. gigantean, L. inermis, M. pudica, I. coccinea, P. hysterophorus* and *C. odorata* against both human and plant pathogenic bacteria showed varied level of inhibition (Table 1). Among treatments, maximum in vitro inhibition of tested bacteria *E. coli, S. aureus, X. vesicatoria* and *R. solanaccearum* was scored in methanol extracts of *C. odorata* which offered inhibition zone of 10, 9, 12 and 12 mm respectively. Further, chloroform extract of *C. odorata* was effective against all four tested bacteria which re-

Table 1. Zone of inhibitory activity (in millimeter) of different plant extracts against clinical and phytopathogenic bacteria.

Source	Extract	*E. Coli*	*S. aureus*	*X. vesicatoria*	*R. solanacearum*
Achyranthes aspera	Aqueous	3 mm	-	-	3 mm
	Methanol	-	-	-	-
	Ethanol	-	*	-	-
	Ethyl acetate	2mm	-	5mm	-
	Chloroform	*	-	*	4mm
Artemisia	Aqueous	-			
	Methanol	-	-	1mm	1mm
	Ethanol	-	2mm	2mm	-
	Ethyl acetate	*	*	*	-
	Chloroform	-	*	-	2mm
Azadirachta indica	Aqueous	10mm	8mm	-	-
	Methanol	9mm	5mm	1mm	4mm
	Ethyl acetate	10mm	-	-	-
	Chloroform	6mm	-	*	*
Calotropis gigantean	Aqueous	-	-	1.5mm	-
	Ethanol	3mm	-	-	*
	Ethyl acetate	-	-	4mm	-
	Chloroform	-	-	-	2.5mm
Lawsonia inemis	Aqueous	-	*	-	-
	Methanol	-	6mm	1mm	-
	Ethanol	-	-	-	*
	Ethyl acetate	-	*	-	-
	Chloroform	-	-	-	*
Mimosa pudica	Aqueous	5mm	1mm	-	-
	Methanol	-	-	-	-
	Ethanol	-	-	-	*
	Ethyl acetate		-	2mm	-
	Chloroform	-	*	-	*
Ixora coccinee	Aqueous	-	-	*	-
	Methanol	-	2mm	-	-
	Ethanol	-	3.5mm	-	0.5mm
	Ethyl acetate	-	-	-	-
	Chloroform	*	*	1mm	*
Parthenium hysterophorus	Aqueous	-	-	-	-
	Methanol	9mm	-	-	-
	Ethanol	-	*	*	4mm
	Ethyl acetate	5mm	-	-	-
	Chloroform	7mm	-	-	4mm
Chromolaena odorata	Aqueous	-	-	-	-
	Methanol	10mm	9mm	12mm	12mm
	Ethanol	-	-	-	-
	Ethyl acetate	-	*	-	-
	Chloroform	12mm	11mm	7mm	9mm

Values are the average of at least three determinations.
-: Not active; *: shows poor inhibition of bacterial growth.

corded significant inhibition zone of 12, 11, 7 and 9 mm respectively. A significant inhibition zone of clinical bacteria *E. coli* was found in aqueous and all tested solvent extracts of *A. indica* showing 10, 9, 12, 10 and 6mm inhibition, which was followed by 9, 5 and 7 mm of *E. coli* inhibition from methanol, ethyl acetate and chloroform

Table 2. Minimum inhibitory concentration (MIC) of *Chromolaena odorata* for antibacterial activity.

Source	Extracts	MIC (mg/ml)			
		E. coli	***S. aureus***	***X. vesicatoria***	***R. solanacearum***
Chromolaena odorata	Aqueous extract	4.00	1.00	4.00	2.00
	Methanol	4.00	4.00	2.00	2.00
	Ethanol	2.00	4.00	2.00	4.00
	Ethyl acetate	4.00	1.00	4.00	4.00
	Chloroform	4.00	4.00	4.00	4.00
	Chloromphenicol	8.00	10.00	9.00	8.00

Values are the average of at least three determinations.

extract of *P. hysterophorus*. In case of human pathogenic bacteria *S. aureus* maximum inhibition of 8 mm was obtained in aqueous extracts of *A. indica* and 6 mm from methanol extract of *L. inermis*. Whereas, extracts of *A. aspera, A. parviflora, C. gigantean, L. inermis, M. pudica* and *I. coccinea* were found to be ineffective or showed poor inhibition on tested human and phytopathogenic bacteria.

Plant extract of *C. odorata* in methanol showed MIC of 2.0 mg/ml against phytopathogenic bacteria *X. vesicatoria* and *R. solanaccearum,* whereas 4.0 mg/ml for clinical bacteria *E. coli* and *S. aureus*. Chloroform leaf extract showed MIC of 4.0 mg/ml against all tested bacteria. Aqueous extract showed MIC of 4.0 mg/ml against *E. coli* and *X. vesicatoria*, whereas MIC of 1.0 and 2.0 mg/ml were found against *S. aureus* and *R. solanaccearum*, respectively (Table 2). The MIC of 2.0 and 4.0 mg/ml was found against all the tested bacteria when ethanol extracts of *C. odorata* were used. The MIC of 4.0 mg/ml was found against *E. coli, X. vesicatoria* and *R. solanaccearum* when ethyl acetate extract was used, whereas MIC of 1.0 mg/ml was found against *S. aureus*.

DISCUSSION

Plant based antimicrobial compounds have enormous therapeutical potential as they can serve the purpose without any side effects that are often associated with synthetic antimicrobials. The methanol, ethanol, ethyl acetate and chloroform and aqueous extracts of the leaves of *A. aspera, A. parviflora, A. indica, C. gigantean, L. inermis, M. pudica, I. coccinea, P. hysterophorus* and *C. odorata* were subjected to a preliminary screening for antimicrobial activity against two human pathogenic bacteria *E. coli* and *S. aureus* and two phytopathogenic bacteria *X. vesicatoria* and *R. solanacearum*. It was clear from the present results, that both methanol and chloroform leaves extracts of *C. odorata* exhibited pronounced activity against all the tested four bacteria. The results show that the methanol extract of *C. odorata* showed more inhibitory effect than the other plant extracts. This tends to show that the active ingredients of the plant parts are better extracted with methanol than other solvents. The methanol extracts contain alkaloids, coumarins and tannins (Okemo, 1996). Coumarins and tannins have antibacterial and antihelminthic properties (Hedberg et al., 1983), also Eloff (1998) and Cowan (1999) found that methanol was more efficient than acetone in extracting phytochemicals from plant materials. The absence of antibacterial activity of chloroform, ethyl acetate and ethanolic extracts of *C. odorata* indicates the insolubility of the active ingredients in these solvents. In general the activities against test bacterial culture used have shown good activity when compared with standard antibiotics. In another research, dichloromethane and aqueous extracts from the leaves as well as ethyl acetate extracts from the flowers have shown antibacterial activity against *Staphylococcus aureus* (Kameda et al., 1987). The minimum inhibitory concentration (MIC) for clinical bacteria was ranged between 0.35 to 4.0 mg/ml and 0.25 to 4.0 mg/ml for phytopathogenic bacteria when tested with all four solvents extracts of *C. Odorata*. Various investigators demonstrated that the extract of the leaves of *C. odorata* at low concentrations (from 0.1 to 5 mg/ml) inhibits the growth of *Pseudomonas aeruginosa, E. coli, S. aureus* and *Neisseria gonorrhoea* (Irobi, 1992; Bamba et al., 1993; Caceres et al., 1995). *Chromolaena* species (Asteraceae) have been chemically investigated; flavornoids and terpenoids are extensively distributed in this genus (Amaro-Luis and Delgado, 1993; Biller et al., 1994). The presence of these flavonoids in *C. moritziana* could contribute to the observed antibacterial activity (Baez et al., 1998). In the present investigations the antibacterial activity of *C. odorata* against phytopatho-genic bacteria such as *Ralstonia solancearum* and *Xanthomonas vesicatoria* has been demonstrated for the first time.

High activity against the Gram-positive organism *E. coli* was found in aqueous and all tested solvent extracts of *A. indica.* In case of human pathogenic *S. aureus*, maximum inhibition of 8 mm was obtained in aqueous extracts of *A. indica.* Similar observations were reported from nimbolide isolated from neem seed oil showing antibacterial activity against *S. aureus* and Staphylococcus coagulase (Nazma and Rao, 1977). Also antimicrobial effects of neem extract have been demonstrated against *Streptoco-*

ccus mutans and *S. faecalis* (Almas, 1999).

These might be due to presence of triterpenoids, phenolic compounds, carotenoids, steroids, valavinoids, ketones and tetratriterpenoids azadirachtin (Kraus, 1995). Shariff et al., 2006 reported that *Rauvolfia tetraphylla* and *Physalis minima* leaf and callus extracted in chloroform were found to inhibit *E. coli* and *X. vesicatoria* at minimum inhibitory concentration (MIC) ranged between 0.25 to 6 mg/ml. Though, *Parthenium hysterophorus* is well known for its antimalarial (Hopper et al., 1990) and antiamoebic (Sharma and Bhutani, 1998) and allelopathic properties (Kanchan, 1975), it failed to inhibit the tested bacteria except for *E. coli* where a fair to good inhibition was obtained.

C. odorata methanolic extracts posses a broad spectrum of activity against a panel bacteria responsible for the most common bacterial diseases. These primary extracts open the possibility of finding new clinically effective antibacterial compounds. *C. odorata* providing active extracts are found in different locations of Mysore and are well known plants as most of them are used for various medical purposes (Phan et al., 2001).

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 antibacterial evaluation of some plants forms a primary platform for further phytochemical and pharmacological studies to discover new antibiotic drugs.

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