



REGULAR ARTICLE

IN VITRO ANTIMICROBIAL POTENTIALITIES OF DIFFERENT SOLVENT EXTRACTS OF ETHNOMEDICINAL PLANTS AGAINST CLINICALLY ISOLATED HUMAN PATHOGENS

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SUMMARY

Antimicrobial efficiency of 20 ethnomedicinal plants (crude leaf extracts) were examined using water, benzene and acetone as solvents and tested against seven human pathogens like *Escherichia coli* (MDR), *Staphylococcus aureus* (MDR), *Klebsiella pneumoniae*, *Bacillus cereus*, *Vibrio cholerae* and *Candida albicans*. Among the tested plants, *Albizia lebbek* (L.) Willd., *Cleistanthus collinus* (Roxb.) Bth., *Emblica officinalis* (*Phyllanthus emblica* L.), *Eucalyptus deglupta* [*Eucalyptus tereticornis* (Smith)], *Eupatorium odoratum* [*Chromolaena odorata* (L.) King & Robin], *Oxalis corniculata* L., *Hevea brasiliensis* (Willd.ex A. Juss.) Mull. Arg., and *Lantana camara* L. showed profound antimicrobial activity (> 11 mm inhibition zone), MIC (0.35-0.80 mg / ml) and MBC (0.45 – 1.0 mg / ml) values. The organic extracts of these plants could be a possible source to obtain new and effective herbal medicines to treat infections, which may caused by multi-drug resistant (MDR) strains of microorganisms from community as well as hospital settings. The study for the first time justified the ethnic uses of plant parts against infectious diseases.

Key words: Antimicrobial activity, Ethnomedicinal plants, *In vitro* sensitivity, MIC, MBC

S. Maji et al. *In vitro* Antimicrobial Potentialities of Different Solvent Extracts of Ethnomedicinal Plants against Clinically Isolated Human Pathogens. J Phytol 2/4 (2010) 57-64

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1. Introduction

Pathogenic bacteria have developed resistance against existing antibiotics due to indiscriminate use of antimicrobial drugs to treat the infectious diseases [Davis 1994; Qadrie et al., 2009]. The worldwide emergence of *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and many other β -lactamase producers has become a major therapeutic problem. Multi-drug resistant strains are widely distributed in hospitals and are increasingly being isolated from community acquired infections [Davis 1994; Qadrie et al., 2009]. All this has resulted in severe consequences including increased treatment failure and health care cost. This has urged the microbiologists all

over the world to formulate new antimicrobial agents and evaluation of the efficacy of natural plant products as the substitute for chemical antimicrobial agents [Pattnaik and Sharma, 2004; Alviano and Alviano, 2009].

Bioactive compounds are normally accumulated as secondary metabolites in all plant cells but their concentration varies according to the plant parts, season, climate and particular growth phase. Leaf is one of the highest accumulatory plant parts of such compounds and people are generally preferred it for therapeutic purposes. Some of the active compounds inhibit the growth of disease causing microbes either singly or in combination [Cowan 1999]. They can inhibit the growth of

microbes by binding their surface proteins, breaking the peptide bonds, acting as chelating agents, altering their biochemical systematics or by preventing utilization of available nutrients to the microorganisms. Some compounds also cause lyses of microbial cells [Cowan 1999].

India is one of the twelve mega biodiversity centers having more than 45,000 plant species. Its diversity is unmatched due to the presence of sixteen different agro-climatic zones, 10 vegetative zone and 15 biotic provinces. The traditional system of medicine, i.e. Ayurveda, Homoeopathy, Unani, Sridhar, Armchar and others in India based on herbal drugs [Mukherjee and Wahile, 2006]. Finding healing power in plants is also an ancient idea in India [Shahina et al., 2007]. Traditional healers claim that their medicine is cheaper and more effective than modern medicine [Parekh et al., 2005; Mandal et al., 2007]. The rural population in different parts of the world is more disposed to traditional ways of treatment because of the easy availability and cheaper cost [Alviano and Alviano, 2009; Benli et al., 2009; Van Vuuren, 2008]. Medicinal plants are as the source of antimicrobial compounds and for this reason the World Health Organization advocated that both developed and developing countries should interact with the traditional medicine with a view of safe and effective remedies of ailments [WHO 1978]. The selection of crude plant extracts for screening programmes has the potential of being more successful as an initial step than the screening of pure compounds isolated from natural products.

In spite of the development of advanced/modern medicine, tribal people reside in a forest area of West Midnapore district are generally preferred to use plants extract to cure different infectious disease (Pal and Jain, 1998a; Pal and Jain, 1998b; Mishra et al., 1996; Maity, 2006). Some ethnobotanical surveys reflected the medicinal use of forest plants in this area [Pal and Jain, 1998, Mishra et al., 1996; Pakrashi and Mukhopadhyay, 2001]. However,

analytical study is essential to establish the effectiveness of the plant products.

In the present work, antimicrobial potentialities of 20 different ethnomedicinal forest plants were examined against locally isolated pathogens and among them two are multi drug resistant bacteria.

2. Materials and Methods

Plant materials

Fresh leaves from 20 forest plants were collected in the month of November from Gurguripal forest, Midnapur, West Bengal, India. This is one of the largest and lateritic forest areas in this district. The identity of the selected plant species was confirmed and voucher specimens were deposited at the Department of Botany in this institute.

The leaves were washed with 70% alcohol and rinsed with sterilized distilled water. Then the leaves were air dried and homogenized to powder and stored in airtight bottles.

Extraction

Dried leaves powder were mixed with extracting solvent like water (W), benzene (B), and acetone (A) in a ratio of 1:10 (w/v) and kept on a rotary shaker for 24 h at 20°C. Then the mixture was filtered and sterilized by using Sintered glass filter (Grade 5, pore size 1-2 μ , Borosil). The filtrate was freeze dried and used as stock sample.

Microorganisms

Pathogenic microorganisms tested in this study were isolated locally from different clinical samples like urine and pus. These were collected from a registered pathological center. Pathogenic bacteria were grown on nutrient broth (HiMedia) at 37°C for 12-14 h and of fungus on Sabouraud dextrose broth (HiMedia) at 28°C for 48 h and were maintained on respective agar slants at 4°C. The bacterial isolates were identified following their morphology and biochemical characteristics by using standard carbohydrate fermentation kit (HiMedia, India) in our laboratory. The fungal isolate was identified on the basis of morphology and reproduction characteristics. These

clinically isolates like *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus cereus*, *Vibrio cholerae*, and *Candida albicans* were preserved and tested their sensitivity against commercial antibiotics. Among the isolates, *Escherichia coli* and *Staphylococcus aureus* were found to be multi drug resistant (MDR) stains against penicillin amoxicillin-clavulanic acid, ampicillin, cefixime, ceftazidime, amikacin, ofloxacin, azithromycin, vancomycin, clindamycin and nitrofurantoin.

Antimicrobial susceptibility testing

The antibacterial potentialities of plant extracts were evaluated by agar well diffusion method [Olutiola et al., 1991] using Mueller Hinton agar medium. The microorganisms were activated by inoculating a loopful of the strain in the nutrient broth (20 ml) in a 100 ml Erlenmeyer flask and incubated at 37°C on a rotary shaker for 24 h. Then 0.1 ml of fresh inoculum (containing around $1-2 \times 10^6$ cfu / ml as per McFarland standards) was spread onto the surface of sterile Mueller Hinton agar using a sterilized glass spreader. Wells were made on the seeded plates with the help of a sterilized cup-borer (4mm). The collected different extracts were further dissolved in DMSO (dimethyl sulfoxide, 4%, v/v). The diluted extracts (50µl) were dispensed into the well and the plates were incubated aerobically at 37°C (for bacteria) or 28°C (for fungi). In the same way a negative and a positive control wells were made with only DMSO and Ciprofloxacin (50µg/ml) respectively. The entire microbial assay was carried out under strict aseptic conditions. The zones of inhibition (mm) of the different extracts were examined after 24 h.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC of the plant extracts were determined by diluting the raw extracts in water to various concentrations. Equal volume of diluted extract and nutrient broth (DS) were mixed in a test tube and 0.1 ml of active inoculum was added to each tube. The

tubes were incubated aerobically at 37°C for 24 hr. Two control tubes were maintained for each test batch that included as antimicrobial control (tube containing extract and the growth medium without inoculum) and organism control (the tube containing the growth medium and the inoculum). The lowest concentration of the extract that completely inhibited bacterial growth (no turbidity) in comparison to control was regarded as MIC.

The MBC was determined by sub-culturing the test dilution (used in MIC) on to a fresh solid medium and incubated further for 24 h. The concentration of plant extract that completely killed the organisms was taken as MBC.

3. Results and Discussion

The Midnapore district (in between 21°36'35"N - 22°57'10"N and 86°35'50"E - 88°12'40"E) is one of tribal rich (33% of total population) district in India. The groups like *Lodha*, *Kheria (Sabar)*, *Munda*, *Santal*, *Kohl*, *Oraon*, *Mahali* and *Bhumij* are primitive tribes in the Gurguripal forest area of this district. Tribal people in this district are generally used plant extract for curing different systemic and superficial infection; and their medicinal system extensively studied and reported by Pal and Jain [1998]; Mishra et al.[1996]; Maity [2002]. The results from this screening justified the folklore use of some of the plants used by tribal people.

The present study describes the effectiveness of the 20 plant extracts against different locally isolated pathogenic organisms. Among them, 19 plant extracts were exhibited significant antimicrobial activity against at least one of the tested pathogenic organisms (Tab. 1). The extracts of *Albizia lebeck*, *Cleistanthus collinus*, *Embllica officinalis*, *Eucalyptus deglupta*, *Eupatorium odoratum*, *Oxalis corniculata* and *Hevea brasiliensis* were exhibited highest zone of inhibition (>11mm) against *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Bacillus cereus*, *Vibrio cholerae* and *Candida albicans*. The zone of inhibition of 11-13mm was showed by *Lantana camara* against

Klebsiella pneumoniae, *Staphylococcus aureus*, *Bacillus cereus*, *Vibrio cholerae* and *Candida albicans*. The extract of *Butea frondosais*, *Melastoma malabathricum*, *Terminalia Arjuna*, and *Lycopodium japonicum* showed moderate activity (8 to 11mm) against all the tested microorganisms. The plants like *Adina cordifolia*, *Asparagus racemosus*, *Aegle marmelos*, *Cassia Tora*, *Dillenia pentagyna*, *Valeriana wallichii* showed little activity (5 to 8mm) against all pathogenic microorganisms. *Ocimum basilicum* showed moderate antibacterial activity (05mm–08mm) but it exhibited higher antifungal activity (12 mm). It was also been observed that the antimicrobial substance are more extracted from plant cell in organic solvent like in acetone and benzene than in water.

Our preliminary investigation showed that all aqueous, benzene and acetonic extracts of ethnomedicinal plants (leaves) were active against the locally isolated human pathogens like *Escherichia coli* (MDR), *Klebsiella pneumoniae*, *Staphylococcus aureus* (MDR), *Bacillus cereus*, *Vibrio cholerae*, and *Candida albicans*. The extracts of eight plants like *Albizia lebbbeck*, *Cleistanthus collinus*, *Emblica officinalis*, *Eucalyptus deglupta*, *Eupatorium odoratum*, *Oxalis corniculata*, *Hevea brasiliensis*, and *Lantana camara* showed significant antimicrobial activity against multi-drug resistant clinically isolated organisms in comparison to other twelve plants. Though, the mechanism of the action of these plant constituents is not yet fully

known it is clear that the effectiveness of the extracts largely depends on the type of solvent used. The organic extracts provided more powerful antimicrobial activity as compared to aqueous extracts. This observation clearly indicates that the existence of non-polar residues in the extracts which have higher both bactericidal and bacteristatic abilities. Cowan [1999] mentioned that most of the antibiotic compounds already identified in plants are reportedly aromatic or saturated organic molecules which can easily solubilized in organic solvents.

The MIC values of plant extracts were exhibited significant at 0.35- 0.80 mg / ml and represented in Table II. Among the tested plants, *Albizia lebbbeck*, *Cleistanthus collinus*, *Emblica officinalis*, *Eucalyptus deglupta*, *Eupatorium odoratum*, *Oxalis corniculata* and *Hevea brasiliensis* showed the low MIC values (0.35mg/ml-0.60 mg/ml). The acetonic extracts of *Emblica officinalis*, *Eucalyptus deglupta*, *Oxalis corniculata* and *Hevea brasiliensis* showed the best activity (lowest MIC values). The MBC values (Table III) varied from plant to plant and it was significant at 0.45 - 1.00 mg / ml. The lowest MBC values of 0.40 mg/ml and 0.50 mg/ml were found in acetone and benzene extracts of *Emblica officinalis* against *Staphylococcus aureus* (MDR) and *Klebsiella pneumoniae* respectively. Among the aqueous extracts, *Oxalis corniculata* showed the lowest MBC value of 0.50 mg/ml.

Table 1. Antimicrobial activity (zone of inhibition, mm) of various plant extracts [Acetone (A), water (W) and benzene (B)] against clinical pathogens.

Botanical name of the Plants (voucher specimen number)	Name of the Organism																	
	<i>E. coli</i> (MDR)			<i>K. pneumoniae</i>			<i>S. aureus</i> (MDR)			<i>V. cholerae</i>			<i>C. albicans</i>			<i>B. cereus</i>		
	A	W	B	A	W	B	A	W	B	A	W	B	A	W	B	A	W	B
<i>Adina cordifolia</i> (Roxb.) Hk. f ex Bran. (VUM033)	08	05	06	09	04	05	07	05	05	06	04	04	07	05	06	04	04	04
<i>Aegle marmelos</i> (L.) Corr. (VUM028)	04	04	04	05	04	04	5.5	04	04	04	04	04	04	04	04	04	04	04
<i>Albizia lebbbeck</i> (L.) Willd (VUM010)	13	10	11	11	07	10	11	04	09	12	09	09	13	07	06	09	09	08
<i>Asparagus racemosus</i> Willd. (VUM111)	04	04	04	07	04	05	05	04	04	04	04	04	06	04	05	04	04	04
<i>Butea frondosa</i> Koenig ex. Roxb. (VUM009)	08	05	07	08	06	06	08	04	05	09	04	05	07	04	04	09	04	05
<i>Cassia Tora</i> L. (VUM013)	07	05	05	07	04	04	06	04	04	07	04	04	06	04	05	04	04	05
<i>Chromolaena odorata</i> (L.) King & Robin (VUM091)	10	04	08	12	04	09	12	04	12	09	04	13	10	08	13	07	12	09
<i>Cleistanthus collinus</i> (Roxb.) Bth. (VUM087)	13	05	07	15	04	11	14	09	11	14	15	11	13	08	10	11	09	09
<i>Curculigo orchoides</i> Gaertn. (VUM034)	04	04	04	04	04	04	04	04	04	04	04	04	04	04	04	04	04	04
<i>Dillenia pentagyna</i> Roxb. (VUM022)	07	05	06	04	04	05	07	05	05	07	04	04	5.5	05	04	06	05	04
<i>Eucalyptus tereticornis</i> (Smith) (VUM051)	12	04	07	13	04	10	16	04	07	12	04	11	10	04	09	09	06	07
<i>Hevea brasiliensis</i> (Willd. ex A. Juss.) Mull. Arg (VUM041)	15	09	09	17	11	13	07	05	06	09	05	05	12	08	09	12	07	10
<i>Lantana camara</i> L. (VUM045)	04	04	05	11	04	08	12	04	07	11	08	08	13	10	06	12	04	05
<i>Lygodium pinnatifidum</i> (L.) Sw. (VUM015)	08	06	08	08	04	05	08	05	05	09	05	07	07	04	04	09	05	07
<i>Melastoma malabathricum</i> L. (VUM077)	09	04	04	08	04	05	09	04	07	13	08	08	08	04	07	09	04	05
<i>Ocimum basilicum</i> L. (VUM063)	06	04	05	06	04	04	08	04	05	07	05	05	12	04	04	04	04	04
<i>Oxalis corniculata</i> L. (VUM101)	13	09	10	15	10	12	10	07	09	09	05	07	04	06	07	15	11	08
<i>Phyllanthus emblica</i> L. (VUM044)	13	11	13	13	13	12	17	12	14	12	09	12	12	09	10	10	12	10
<i>Terminalia arjuna</i> (Roxb.) Wt. & Arn. (VUM029)	07	05	07	07	04	04	11	06	09	11	07	09	07	05	05	09	04	04
<i>Valeriana jatamansii</i> Jones (VUM025)	04	04	04	07	06	09	06	04	04	07	04	04	04	04	04	05	04	05
Ciprofloxacin	16			20			14			22			18			27		
Solvent (DMSO)	0			0			0			0			0			0		

Table 2. MIC (mg / ml) performance of different extracts of leaves against pathogenic organisms

Name of the Plants	Name of the Organism																	
	<i>E. coli (MDR)</i>			<i>K. pneumonias</i>			<i>S. aureus (MDR)</i>			<i>V. cholerae</i>			<i>Candida sp</i>			<i>B. cereus</i>		
	A	W	B	A	W	B	A	W	B	A	W	B	A	W	B	A	W	B
<i>Adina cordifolia</i> (Roxb.) Hk.f ex Bran.	0.76	-	0.78	0.79	-	-	0.75	-	-	0.75	-	-	0.80	-	-	-	-	-
<i>Aegle marmelos</i> (L.) Corr.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Albizia lebbek</i> (L.) Willd	0.40	0.50	0.45	0.50	0.50	0.45	0.40	-	0.56	0.50	0.60	0.59	0.41	0.60	0.50	0.59	0.60	0.60
<i>Asparagus racemosus</i> Willd.	-	-	-	0.80	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Butea frondosa</i> Koenigex. Roxb.	0.65	-	0.76	0.76	-	-	0.79	-	-	0.78	-	-	0.78	-	-	0.80	-	-
<i>Cassia Tora</i> L.	0.76	-	-	0.80	-	-	0.80	-	-	-	-	-	-	-	-	-	-	-
<i>Chromolaena odorata</i> (L.) King & Robin	0.53	-	0.50	0.51	-	0.52	0.50	-	0.55	0.60	-	0.40	0.50	0.60	0.45	0.60	0.53	0.60
<i>Cleistanthus collinus</i> (Roxb.) Bth.	0.45	0.50	0.50	0.40	-	0.45	0.40	0.54	0.46	0.40	0.40	0.45	0.39	0.60	0.53	0.54	0.60	0.60
<i>Curculigo orchioides</i> Gaertn.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dillenia pentagyna</i> Roxb.	0.80	-	-	-	-	-	0.76	-	-	-	-	-	-	-	-	-	-	-
<i>Eucalyptus tereticornis</i> (Smith)	0.48	-	0.50	0.45	-	0.50	0.35	-	0.60	0.46	-	0.45	0.47	-	0.60	0.60	0.60	0.35
<i>Hevea brasiliensis</i> (Willd. ex A. Juss.) Mull. Arg	0.40	0.57	0.50	0.35	0.50	0.45	0.58	-	0.60	0.60	-	-	0.58	0.58	0.60	0.54	0.60	0.59
<i>Lantana camara</i> L.	-	-	-	0.45	-	0.55	0.53	-	0.60	0.55	0.59	0.60	0.53	0.59	0.60	0.51	-	-
<i>Lygodium pinnatifidum</i> (L.) Sw.	0.70	-	0.75	0.80	-	-	0.70	-	-	0.75	-	-	0.80	-	-	-	-	-
<i>Melastoma malabathricum</i> L.	0.62	-	-	0.79	-	-	0.80	-	0.79	0.65	0.80	0.80	0.79	-	-	0.80	-	-
<i>Ocimum basilicum</i> L.	-	-	-	-	-	-	0.78	-	-	-	-	-	0.60	-	-	-	-	-
<i>Oxalis corniculata</i> L.	0.45	0.56	0.50	0.35	0.45	0.53	0.56	0.58	0.59	0.60	-	-	-	-	0.60	0.50	0.57	0.62
<i>Phyllanthus emblica</i> L.	0.45	0.50	0.45	0.45	0.50	0.45	0.35	0.42	0.42	0.46	0.60	0.46	0.48	0.59	0.53	0.56	0.54	0.58
<i>Terminalia arjuna</i> (Roxb.) Wt. & Arn.	0.65	-	0.75	0.77	-	-	0.68	0.80	0.79	0.70	0.80	0.79	0.76	-	-	-	-	-
<i>Valeriana jatamansii</i> Jones	-	-	-	-	-	0.80	-	-	-	-	-	-	-	-	-	-	-	-

Table 3. Determination of MBC (mg) value of different ethnomedicinal plants against pathogenic organisms

Name of the Plants	Name of the Organism																	
	<i>E. coli (MDR)</i>			<i>K. pneumonias</i>			<i>S. aureus (MDR)</i>			<i>V. cholerae</i>			<i>Candida sp</i>			<i>B. cereus</i>		
	A	W	B	A	W	B	A	W	B	A	W	B	A	W	B	A	W	B
<i>Adina cordifolia</i> (Roxb.) Hk.f ex Bran.	0.85	-	0.90	0.85	-	-	0.90	-	-	0.80	-	-	0.90	-	-	-	-	-
<i>Aegle marmelos</i> (L.) Corr.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Albizia lebbek</i> (L.) Willd	0.50	0.60	0.55	0.60	0.60	0.55	0.50	-	0.65	0.65	0.75	0.80	0.55	0.70	0.65	0.75	0.75	0.80
<i>Asparagus racemosus</i> Willd.	-	-	-	0.90	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Butea frondosa</i> Koenigex. Roxb.	0.75	-	0.85	0.90	-	-	0.95	-	-	0.90	-	-	01	-	-	0.95	-	-
<i>Cassia tora</i> L.	0.85	-	-	0.90	-	-	01	-	-	-	-	-	-	-	-	-	-	-
<i>Chromolaena odorata</i> (L.) King & Robin	0.65	-	0.65	0.60	-	0.65	0.45	-	0.80	0.65	-	0.60	0.55	-	0.85	0.80	0.75	0.75
<i>Cleistanthus collinus</i> (Roxb.) Bth.	0.60	0.65	0.60	0.55	-	0.50	0.50	0.65	0.65	0.60	0.60	0.55	0.45	0.75	0.70	0.65	0.80	0.80
<i>Curculigo orchioides</i> Gaertn.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Dillenia pentagyna</i> Roxb.	0.90	-	-	-	-	-	0.95	-	-	-	-	-	-	-	-	-	-	-
<i>Eucalyptus tereticornis</i> (Smith)	0.60	-	0.65	0.60	-	0.65	0.45	-	0.80	0.65	-	0.60	0.55	-	0.85	0.80	0.75	0.75
<i>Hevea brasiliensis</i> (Willd. ex A. Juss.) Mull. Arg	0.60	0.65	0.70	0.50	0.60	0.70	0.70	-	0.85	0.85	-	-	0.70	0.80	0.80	0.60	0.70	0.65
<i>Lantana camara</i> L.	-	-	-	0.60	-	0.60	0.70	-	0.85	0.70	0.80	0.75	0.60	0.90	0.90	0.60	-	-
<i>Lygodium pinnatifidum</i> (L.) Sw.	0.80	-	0.85	0.90	-	-	0.90	-	-	0.65	-	-	01	-	-	-	-	-
<i>Melastoma malabathricum</i> L.	0.70	-	-	0.95	-	-	01	-	01	0.90	0.90	0.90	0.90	-	-	0.90	-	-
<i>Ocimum basilicum</i> L.	-	-	-	-	-	-	0.90	-	-	-	-	-	0.85	-	-	-	-	-
<i>Oxalis corniculata</i> L.	0.50	0.60	0.70	0.50	0.50	0.65	0.65	0.70	0.80	0.85	-	-	-	-	0.65	0.65	0.65	0.85
<i>Phyllanthus emblica</i> L.	0.55	0.65	0.60	0.55	0.60	0.50	0.40	0.65	0.50	0.70	0.60	0.55	0.55	0.65	0.75	0.65	0.75	0.75
<i>Terminalia arjuna</i> (Roxb.) Wt. & Arn.	0.80	-	0.90	0.95	-	-	0.75	01	0.95	0.65	01	0.95	01	-	-	-	-	-
<i>Valeriana jatamansii</i> Jones	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

The MIC value of the active plant extracts obtained in this study were lower than the MBC values (Table 2 & 3) suggesting that the plant extracts were bacteriostatic at lower concentration but bactericidal at higher concentration.

There are several evidences on the presence of antimicrobial metabolites like tannins, flavonoid, glycosides, essential oils, furostanol, spirostanol, saponins, phytosterols, amides, alkaloids, etc in the studied plant species [Olutiola et al., 1991; Une et al., 2001; Govindachari et al., 1969; Bhattacharya et al., 1999; Ganjewala et al., 2009]. Une et al (2001) reported that the leaf extract of *Oxalis corniculata* (3 g/5ml water) showed significant zone of inhibition (60 mm) against ampicillin and mecillinam resistant *E. coli*. *A. lebeck* contains higher quantity of phenolics (41.9 mg/g) and flavanoid (3.0 mg/g) that showed MIC and MBC within the range of 16 – 24 mg/ml against multi-drug resistant *V. cholerae* [Acharyya et al., 2009], which were far higher level than our experimental results (Table. 2). *E. officinalis* is a member of Triphala, a traditional ayurvedic herbal formulation, has higher quantity of phenolics (about 8000 µg/g) and showed potent antibacterial action against the wide spectrum of bacterial isolates (MIC 100µg/ml aqueous extract and 0.1 µg/ml ethanolic extract) [Srikumar et al., 2007]. Ethyl acetate extract of *L. camara* was found to be the most effective against pathogens, antibacterial activities with zone of inhibition value ranging from 10-21 mm [Ganjewala et al., 2009]. The methanol extracts of the roots of *Asparagus racemosus* Willd. showed considerable *in vitro* antibacterial efficacy at 150 µg/ml against many Gram positive and negative bacteria [Mandal et al., 2000]. Khan et al. [Khan et al., 2009] reported that the ethanolic extract of

Eucalyptus globules was not effective against multi-drug resistant clinically isolated bacteria, but Warnke et al [2009] revealed that eucalyptus oil was considerably effective against methicillin-resistant *Staphylococcus aureus*. The ethanolic extract of *Ocimum basilicum* showed a stronger and broader spectrum of antibacterial activity, which inhibited 6% of the 146 bacterial strains with a MIC value of 125 -250 µg/ml [Adiguzo et al., 2005]. But, Durga et al. [2009] mentioned a higher MIC value of its ethanolic extract (200 -600 µg/ml) against pathogenic bacteria.

These evidences have clearly suggested the antibacterial activity of plants at varying degrees with the activity being both strain and dose dependent; and geographical location, developmental stage, and ontogeny influence the accumulation of metabolites and or biochemical compositions of plants.

4. Conclusion

From our preliminary investigation, it has been observed that most of the tested forest plants exhibited antimicrobial activity and among them, *Albizia lebeck*, *Cleistanthus collinus*, *Emblica officinalis*, *Eucalyptus deglupta*, *Eupatorium odoratum*, *Oxalis corniculata*, *Hevea brasiliensis*, and *Lantana camara* showed profound antimicrobial activity and even active against multi-drug resistant *Escherichia coli* and *Staphylococcus aureus*. The results of the present study support the folkloric usage (Table 4) of these plants and suggested that the plant extracts can be effectively used against multidrug resistant microbes causing nosocomial and community acquired infections. This probably first time scientifically explains the use of these plants by the indigenous people in the studied area against a number of infections.

Table 4. Ethnomedicinal uses of selected plants against infectious diseases (Maity, 2002).

Plants	Anti-infections uses
<i>Albizia lebbek</i>	As poultice on ulcer, control leprosy, gum inflammation, skin disease, ring worm etc.
<i>Cleistanthus collinus</i>	Washing agent for clearing septic wound, cure fungal diseases
<i>Emblica officinalis</i>	Against inflammations, ulcerations, G.I. disorders, UTI, hepatitis, convalescence from fever, cough, etc.
<i>Eucalyptus deglupta</i>	To treat tuberculosis, chronic cough, pneumonosis, burn's skin diseases, etc.
<i>Eupatorium odoratum</i>	To control sore throat and gonorrhoeal infection.
<i>Oxalis corniculata</i>	To treat whooping cough, blood dysentery, ulcers, any disease of the eye, and uses as antiseptic and antibacterial agent.
<i>Hevea brasiliensis</i>	To control dad (ring worm) and candidiasis
<i>Lantana camara</i>	As an antiseptic for wounds and externally to contro leprosy and scabies; for treatment of diarrhoea.

Furthermore, active plant extracts are being subjected to various pharmacological evaluations by several methods for the isolation of the therapeutic antimicrobials.

Acknowledgements

The authors are grateful to the University Grants Commission, Govt. of India for financial support [F. No. 32-593 / 2006 (SR) dt.13.7.2007].

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