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Screening of selected aromatic plants belonging to Labiateae and Verbenaceae family for their antimicrobial activity

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ABSTRACT

The present study aimed that 17 plant species belonging to Lamiaceae and Verbenaceae families were screened for their antimicrobial activity. The crude extracts of root, stem, leaf, inflorescence and whole plant were prepared in n-Hexane, ethyl acetate, methanol and D/W and tested against six gram positive and six gram negative bacteria by agar well diffusion method and the zone of inhibition was measured. The MIC value was examined by the twofold serial broth dilution method. The results showed that *Bacillus cereus, Klebsiella pneumoniae, Serratia marcescens* and *Enterococcus faecalis* were found to be most susceptible organism. The n-hexane crude extracts inhibited the growth of *Klebsiella pneumonia* (91.17%), *Bacillus cereus* and *Serratia marcescens* (88.23%), ethyl acetate extract *Enterococcus faecalis* (85.29%), *Bacillus cereus* (82.35%) while methanolic extract found to inhibit the growth of *Bacillus cereus* (76.47%). Least to no activity was found in D/W extract. *Salmonella paratyphi, Escherichia coli, Micrococcus luteus* were found to most resistant organism for all tested crude plant extracts. The MIC values were observed in the range of >8 mg/ml to 0.25 mg/ml of selected crude plant extracts against tested organisms. HPTLC finger printing and TLC-bioautography of certain active extracts demonstrated the presence of common phytochemical compound in plant extracts. The results obtained in present study suggested that these plant extracts can be a source of active principle for antibacterial activity.

KEYWORDS: Antimicrobial activity, MIC, TLC- Bioautography, HPTLC

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INTRODUCTION

Medicinal plants are a major source of drugs for the treatment of various health disorders especially in rural parts of India and different other countries. The plant based local medicines used dates back to more than 5000 B.C.

Nowadays large number of companies used plant based ingredient for preparation of various allopathic medicines. There are more than 10,000 species of plants in the world, as compared to animal's species. A plant contains thousands of chemicals material which act against diseases and infections of humans and animals when properly used. Furthermore, according to assessment of WHO about 80% of world population for their health care depend on plants and 30% of pharmaceutical companies depends on plants for the preparations.[1] Some reports indicated that Pharmaceutical companies are using 90 popular medicinal plants and their extracts for various drugs preparations. Scientists throughout the world are trying to explore the valuable assets of medicinal plants to help the suffering human being.[2] Mostly, the developed countries import raw material from developing countries and prepared medicines after processing export to developing countries back at high priced.[1]

Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of mankind. The search for longevity and eternal health for remedies to relive pain and discomfort drove, early man need to search his immediate natural surrounding and led to the use of many plants, and animal products, etc and the use of a various therapeutic agents increase to cure diseases. Today, there is renewed interest in plant derived drugs is mainly due to the current widespread belief that "green medicine is safe and more dependable than the synthetic drugs, lots of which have harmful side effects.[3]

Nature has very rich botanical wealth and a large number of diverse types of plants grow wild in different regions of India. The use of plant parts to cure specific ailments has been vogue from ancient times.[4]

India is rich in medicinal plant diversity. All known types of agro-climatic, ecologic and edaphic conditions are met within India and is rich in biodiversity, such as species diversity, genetic diversity and habitat diversity.[5]

In recent years, the use of medicinal and aromatic plants (MAPs) has increased greatly in western countries. In Europe, at least 2,000 medicinal and aromatic plant species are traded commercially among which 1,200 to 1,300 plants are native to Europe. The increase in demand for medicinal and aromatic plants is putting pressure on natural

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resources. India is one of the richest countries in the world in regard to genetic resources of medicinal and aromatic plants and exhibits a wide range in climate and topography, which has great impacts on its vegetation and floristic composition. Moreover, agro climate conditions are conducive for introducing and domesticating new exotic plant varieties.[6]

Medicinal and aromatic plants are generally known as "Chemical Goldmines" as they contain natural chemicals, which are good enough suitable to human and animal systems. All these chemicals cannot be synthesized in laboratories. Aromatic plants contain natural antioxidant constituents such as phenolic compounds, which have attracted a great deal of public and scientific interest because of their health-promoting effects as antioxidants.[7] Phytochemicals are present in a variety of plants utilized as important components of both human and animal diets. These include vegetables, herbs, seeds and fruits.[8] Medicinal and aromatic plants are known to produce certain bioactive molecules which inhibit the bacterial or fungal growth (antimicrobial activity) and have little toxicity to host cells are considered the candidates for developing new antimicrobial drugs.[9]

The member of Lamiaceae for their antimicrobial activity was found on antibiotic resistant bacteria[10] and aqueous and alcoholic extract of O*cimum kilimanjaricum* against some S*taphylococcous* species[11] Isolation of potential antibacterial and antioxidant compounds from *Ocimum basilicum*.[12,13] Biochemical composition and antibacterial activity of *Lantana camara* with yellow, lavender, red and white flowers[14] and antifungal activity in member of Verbenaceae.[15]

Aromatic and medicinal plants are an immense sustainable source of natural compounds with various beneficial properties. Therefore, such plant materials have been used since ancient times for various applications, particularly healing of diseases, flavouring of foods and formulation of fragrances. Some of these plants now a days are grown commercially and serve for the production of variety of ingredients. The need of hour is to screen a number of medicinal plants for promising biological activity. In this study, 17 traditionally used medicinal and aromatic plants belonging to two different families were selected to screen the antimicrobial principles present in them. Phytochemical analysis of active plant extracts for their phytoconstituents and the active group of active extracts is reported here.

MATERIAL AND METHODS

(i) Plant material

Seventeen plant samples were collected either in the form of leaves, stem, inflorescence and Root, Whole plant from different localities of Gujarat state. (Table 1) All the plants were identified by Dr A.S.Reddy and Dr Sandip Patel, Department of Biosciences, Sardar Patel University. The collected plant materials were air dried under shade at room temperature and ground with grinder into powder

(ii) Preparation of extracts

Extracts were prepared by cold sequential extraction method in which 100mg of dry powdered material was soaked in n-Hexane at Room temperature for 24 hrs. Extracts was filtered through Whatmann filter paper no.1. The filtrates were centrifuged at 5000rpm for 10 min to remove solid debris. The supernatant was collected and concentrated by solvent recovering assembly. (J-Sil, India) and dried completely at R.T and stored in a refrigerator until further use. The filterate collected on filter paper was completely dried and resuspended in to each of 500ml of ethyl acetate, methanol and D/W at R.T for 24 hrs sequentially. The extract was filtered and filtrate was centrifuged at 5000rpm for 10 min and the supernatant were collected. All fractions were stored in a refrigerator until use.

(iii) Microorganisms

In the current study six gram positive and six gram negative bacteria were tested which were obtained from authentic centre MTCC, ATCC and NCTC. The bacterial cultures were grown in nutrient broth medium (Hi media pH 7.4) and maintained on nutrient agar slants (Table 2)

(iv) Antimicrobial assay[16]

Sensitivity test was performed by agar well diffusion method.[17] An inoculum size by 10^8 CFU/ml of bacterium, compared with 0.5 Mc Farland turbidity standard was used. About 100µl of plant extract (Stock 100mg/ml) was added carefully in a well of 8mm diameter in a nutrient agar plate. Plates were kept in a refrigerator for prediffusion of extracts.

Minimum inhibitory concentration was determined by two-fold serial broth dilution method. The MIC was tested in the concentration range of 0.125-8.0mg/ml. Plates and tubes were incubated for 24hrs at 37°C in an incubator. The zone of inhibition was measured excluding the well diameter to evaluate the antimicrobial activity. Tubes showing no turbidity was recorded as the MIC value.

Antibiotic such as Ciprofloxacin and Doxycyclin 20µg/ml and 100% DMSO, a dissolving solvent were used as positive and negative control respectively. Bioassay was carried out in duplicate and experiment was repeated twice.

(v) HPTLC of plant extract

HPTLC of the sixteen plant extracts with significant antimicrobial activity carried out. Different solvent systems were used for different classes of compounds based on the polarity of organic solvent. Precoated Silica gel $G_{60}F_{254}$ plated (Merck, Germany were used as describe).[18]

About 10 µl of plant extract was applied to the TLC chromatogram. Solvent system used were n-Hexane: Diethylether (4:6), Toluene:Ethylacetate(6:4), Chloroform:Methanol(8:2) was used as mobile phase. Individual Rf for each spot was measured. TLC spots were visualized under U.V light and adequate TLC reagents were used to detect the phytoconstituent.

(vi) TLC-bioautography

For direct bioautography assay agar overlay assay was used with minor modification. BC, SE, EN, ST and KP was used as test strain.[19]

About 10 µl of plant extract was spotted on preparative Merck 10×10 cm chromatographic Silica gel- 60 Plates. Only two solvent system (Chloroform: Methanol; Ethyl acetate: Toluene) was used. One milliliter of (10^8 CFU/ml) broth culture was used for every 10ml of Nutrient agar. The

Table 1 List of the aromatic plants collected from various localities for testing the antimicrobial activity

No.	Plant name	Family	Part Used	Locality
1	*Anisomeles indica* (L).	Labiatae	Leaves,stem, inflorescence	Rajpipla
2	*Anisomeles heyneana* Bth		Leaves,stem, inflorescence, root	Rajpipla
3	*Lavandula bipinnata (Roth)* O.Ktze		Leaves,stem, inflorescence	Rajpipla
4	*Leucas aspera* Spr.		Whole plant	Rajpipla
5	*Leucas stelligera* Wall.		Whole plant	Rajpipla
6	*Leucas martinicensis (Jacob.)* R.Br		Whole plant	Rajpipla
7	*Moschosma polystachyum* (L.)Bth.		Leaves, stem	Rajpipla
8	*Ocimum sanctum* L		Leaves,	Vallabh vidyanagar
9	*Ocimum canum* Sims.		Leaves,stem, inflorescence	Anand Agriculture university campus, Anand
10	*Pogostemon parviflorus* Bth.		Leaves,stem, inflorescence	Saputara
11	*Pogostemon purpurascens* Dalz.		Leaves,stem, inflorescence	Rajpipla
12	*Salvia plebeia* R. Br.		Whole plant	Saputara
13	*Clerodendrum infotunatum* L.	Verbenaceae	Whole plant	Jaunpur (U.P)
14	*Lantana camara* L.		Leaves	SPUUniversity campus,Bakrol
15	*Lantana salvifolia* Jacab (wild)		Leaves, stem	Kabir vad
16	*Lantana salvifolia Jacab.* (cultivated)		Whole plant	Valsad
17	*Phyla nodiflora* (L.) Greene		Whole plant	Vadtal

Table 2 Selected microorganisms

Types of microorganism	Microorganism strains	Causes
Gram positive	***Bacillus cereus*** (ATCC 11778)	Food poisoning, vomiting, Diarrhoea,
	Bacillus subtilis (ATCC 6051)	Food poisoning
	Staphylococcus aureus (Isolated)	Wound infection, Pneumonia
	Staphylococcus epidermidis (ATCC 155)	Infection of prosthetic medical device
	Micrococcus luteus (ATCC 4698)	Septic shock, septic arthritis
	Enterococcus faecalis (Isolated)	Carcinoma, dysplacia, inflammatory bowel disease
Gram negative	***Escherichia coli*** (ATCC 25922)	Bloody diarrhoea, kidney diseases.
	Salmonella typhi (NCTC8394)	Typhoid, enteric fever
	Salmonella paratyphi A (MTCC 735)	Paratyphoid fever and typhoid
	Pseudomonas aeruginosa (ATCC 25668)	Septicemia, pneumonia, dermatitis
	Klebsiella pneumoniae (ATCC 15380)	Pneumonia, flu, chill and cough
	Serratia marcescens (Isolated)	Bacteremia, urinary and respiratory infection

developed chromatogram was placed in sterilized petriplates. Culture was added to 42°C N.A mixed and poured over the chromatograms as a thin layer. The zone of inhibition of bacterial growth could be seen around the active chromatogram spots.

RESULTS AND DISCUSSION

Emergency of multi-drug resistance in human and animal pathogenic bacteria as well as undesirable side effect of certain antibiotics has triggered immense interest in the search for new antimicrobial drugs of plant origin. In present study n-Hexane, ethyl acetate, methanolic and D/W extract of 17 Aromatic and Medicinal plants have been tested against six gram positive and six gram negative bacteria.

The antimicrobial activity of extracts and their potency was quantitatively assessed by the zone diameter and presence or absence of inhibition zone respectively in given (Table 3 to 5) and the plants which have not showed effective antimicrobial activity are excluded. The minimum inhibition zone was in the range of 0.25mg to >8mg/ml is given (Table 6 to 8).

Only the n-hexane, ethyl acetate and methanol extract was tested as better solvent compare to water. The results of screening are encouraging as out of 17 plants, 34 extracts showed antibacterial

Table 3 Antibacterial activity of the crude n-Hexane extracts of selected plant species

			Zone of inhibition (mm) against											
			Gram positive						Gram negative					
No	Plant name	Part used	BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	KP	SM
1	*Anisomeles indica.*	L	9	0	4	0	5	0	0	6	0	7	7	8
		INF	7	0	5	10	0	5	0	7	0	0	7	7
		S	5	0	0	0	0	7	0	0	0	7	5	6
2	*Anisomeles heyneana*	L	7	7	6	6	4	9	0	7	0	7	7	6
		INF	9	8	8	9	6	8	0	9	0	0	8	11
		S	7	9	7	0	4	0	0	7	0	0	8	6
		R	14	6	13	10	4	8	0	13	0	9	17	13
3	*Lantana camara*	L	12	9	10	13	7	9	15	14	0	13	12	13
4	*Lantana salvifolia*	L	7	0	0	0	0	6	0	7	0	0	5	3
		S	5	0	0	0	0	3	0	0	0	0	0	6
5	*Lavandula bipinnata*	L	9	9	10	9	6	8	8	10	0	13	10	12
		INF	26	16	17	17	19	18	18	14	7	30	16	14
		S	6	6	0	0	0	0	0	7	0	7	5	7
6	*Leucas aspera*	WP	3	0	4	0	4	5	0	5	0	0	4	7
7	*Leucas stelligera*	WP	7	4	0	5	0	6	0	4	0	11	6	7
8	*Leucas martinenssis*	WP	7	0	0	0	0	0	0	0	0	0	7	0
9	*Moschosma polystachyum*	L	9	5	9	8	5	10	0	0	5	11	9	10
		S	9	9	8	6	9	4	5	10	0	8	7	8
10	*Ocimum sanctum*	L	18	9	12	8	7	12	0	14	0	0	17	14
11	*Ocimum canum*	L	6	4	0	0	0	3	0	5	0	0	6	8
		INF	7	6	6	0	0	0	0	6	0	7	8	6
		S	6	5	0	4	0	5	0	7	0	7	7	8
12	*Pogostemon parviflorus*	L	5	5	4	2	2	7	0	5	0	0	6	8
		INF	6	0	0	0	0	9	0	4	0	0	7	2
		S	6	6	3	6	0	6	0	7	0	0	8	7
13	*Pogostemon purpurascens*	L	9	0	6	6	0	8	0	4	0	6	6	8
		INF	11	7	9	11	8	8	9	10	0	7	9	9
		S	6	0	0	0	0	0	0	6	0	7	7	14
14	*Phyla nodiflora*	WP	15	13	21	16	4	14	0	15	0	6	15	16

Table 3 ***Continue***

No	Plant name	Part used	Zone of inhibition (mm) against											
			Gram positive						Gram negative					
			BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	KP	SM
15	*Salvia plebeian*	WP	8	6	5	5	0	7	0	7	0	0	9	8
16	Ciprofloxacin (20µg/ml)		11	10	14	11	9	12	7	14	8	9	10	22
17	Doxycline (20µg/ml)		14	12	11	5	8	9	15	19	11	4	13	20

BC-*Bacillus cereus;* BS-*Bacillus subtilis;* SA- *Staphylococcus aureus ;* SE-*Staphylococcus epidermidis;* ML-*Micrococcus luteus;* EN-*Enterococcus faecalis* EC-*Escherichia coli;* ST-*Salmonella typhi;* SP-*Salmonella paratyphi ;* PS-*Pseudomonas aeruginosa ;*
KP-*Klebsiella pneumoniae ;* SM-*Serratia marcescens* R- Root, S-Stem, L- Leaf, INF- Inflorescences, WP- Whole plant

Table 4 Antibacterial activity of the crude ethyl acetate extracts of selected plant species

No	Plant name	Part used	Zone of inhibition(mm) against											
			Gram positive						Gram negative					
			BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	KP	SM
1	*Anisomeles indica*	L	10	6	0	0	0	13	0	8	0	0	8	7
		INF	9	0	0	10	0	17	0	11	0	0	8	10
		S	7	0	0	0	0	8	0	0	0	7	5	0
2	*Anisomeles heyneana*	L	6	5	6	9	0	8	0	8	7	10	7	7
		INF	9	0	7	5	0	7	0	8	0	0	8	6
		S	7	9	0	0	0	0	0	4	0	0	4	5
		R	11	0	11	0	0	10	0	11	0	0	10	9
3	*Lantana camara*	L	16	0	11	16	7	11	16	12	0	12	16	15
4	*Lantana salvifolia*	L	7	7	0	8	0	11	0	8	0	0	7	8
		S	6	0	0	0	0	6	0	0	0	0	6	3
5	*Lavandula bipinnata*	L	0	0	0	5	0	0	0	4	0	0	0	5
		INF	9	7	6	8	7	9	5	7	0	9	8	8
		S	0	0	0	0	0	0	0	7	0	4	0	0
6	*Leucas aspera*	WP	0	0	0	0	1	5	0	7	0	0	0	0
7	*Leucas stelligera*	WP	8	0	0	7	0	8	0	7	0	0	8	6
8	*Leucas martinenssis*	WP	5	0	0	8	0	0	0	0	0	0	7	3
9	*Moschosma polystachyum*	L	7	0	5	7	0	8	7	6	0	7	7	7
		S	5	7	5	6	13	5	0	6	6	6	7	8
10	*Ocimum sanctum*	L	9	0	0	0	0	7	0	14	0	0	3	10
11	*Ocimum canum*	L	11	12	0	0	0	6	0	9	0	0	1	0
		INF	8	7	6	0	0	0	0	8	0	7	8	7
		S	6	6	0	0	0	7	0	7	0	5	7	8
12	*Pogostemon parviflorus*	L	6	6	0	0	0	7	0	6	0	0	6	7
		INF	8	0	0	0	0	8	0	0	0	0	8	3
		S	0	5	0	3	0	8	0	8	0	0	10	7
13	*Pogostemon purpurascens*	L	13	0	13	13	0	14	0	9	0	0	14	12
		INF	14	12	12	7	6	11	0	13	0	0	13	12
		S	0	0	0	0	0	0	0	0	0	0	0	0
14	*Phyla nodiflora*	WP	17	17	25	15	0	23	0	16	0	12	17	17
15	*Salvia plebeian*	WP	10	11	11	9	10	11	0	9	0	0	11	6

Table 4 ***Continue***

No	Plant name	Part used	Zone of inhibition(mm) against											
			Gram positive						Gram negative					
			BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	KP	SM
16	Ciprofloxacin(20µg/ml)		11	10	14	11	9	12	7	14	8	9	10	22
17	Doxycline (2µg/ml)		14	12	11	5	8	9	15	19	11	4	13	20

BC-*Bacillus cereus;* BS-*Bacillus subtilis;* SA- *Staphylococcus aureus;* SE-*Staphylococcus epidermidis;* ML-*Micrococcus luteus;* EN-*Enterococcus faecalis* EC-*Escherichia coli;* ST-*Salmonella typhi;* SP-*Salmonella paratyphi ;* PS-*Pseudomonas aeruginos;*KP-*Klebsiella pneumoniae ;* SM-*Serratia marcescens*
R- Root, S-Stem, L- Leaf, INF- Inflorescences, WP- Whole plant

Table 5 Antibacterial activity of the crude methanol extracts of selected plant species

No	Plant name	Part used	Zone of inhibition (mm) against											
			Gram positive						Gram negative					
			BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	KP	SM
1	*Anisomeles indica*	L	8	0	0	0	0	12	0	3	0	0	8	7
		INF	8	0	0	11	0	12	0	8	0	0	7	8
		S	7	0	0	0	0	9	0	0	0	0	0	0
2	*Anisomeles heyneana*	L	5	8	4	8	0	7	0	5	7	5	6	4
		INF	2	0	0	6	0	5	0	5	0	0	0	4
		S	4	7	0	0	0	0	0	4	0	0	4	5
		R	7	0	7	0	0	6	0	8	0	0	7	7
3	*Lantana camara*	L	8	0	5	13	5	5	9	7	0	5	12	12
4	*Lantana salvifolia*	L	6	9	0	7	0	4	0	5	0	0	6	4
		S	5	0	0	0	0	0	0	0	0	0	6	0
5	*Lavandula bipinnata*	L	0	7	0	0	0	0	0	0	0	0	4	0
		INF	8	0	0	4	0	0	0	0	0	7	6	0
		S	7	0	0	0	0	0	0	0	0	8	0	5
6	*Leucas aspera*	WP	2	0	7	0	0	0	0	0	0	0	0	5
7	*Leucas stelligera*	WP	5	0	0	8	0	7	0	6	0	0	6	6
8	*Leucas martinenssis*	WP	7	13	0	12	0	7	0	7	0	7	8	3
9	*Moschosma polystachyum*	L	6	3	5	0	0	9	6	0	4	6	7	5
		S	4	5	0	5	13	0	5	5	0	5	4	6
10	*Ocimum sanctum*	L	0	1	0	7	0	0	0	0	0	0	6	0
11	*Ocimum canum*	L	8	9	0	0	0	0	0	7	0	0	8	8
		INF	6	0	0	0	0	0	0	6	0	0	5	0
		S	7	7	0	6	0	3	0	5	0	5	6	7
12	*Pogostemon parviflorus*	L	6	3	0	0	0	11	0	6	0	6	7	6
		INF	0	0	0	0	0	8	0	4	0	0	0	2
		S	7	5	0	0	0	5	0	3	0	0	7	8
13	*Pogostemon purpurascens*	L	10	0	0	0	0	12	0	8	9	0	0	0
		INF	6	0	0	0	0	8	7	11	6	6	0	0
		S	6	6	0	0	0	0	0	7	0	6	7	7
14	*Phyla nodiflora*	WP	11	9	12	11	0	14	0	11	0	0	11	13
15	*Salvia plebeian*	WP	9	6	5	5	6	5	0	7	0	0	13	6

Table 5 ***Continue***

No	Plant name	Part used	Zone of inhibition (mm) against											
			Gram positive						Gram negative					
			BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	KP	SM
16	Ciprofloxacin(20µg/ml)		11	10	14	11	9	12	7	14	8	9	10	22
17	Doxycline (20µg/ml)		14	12	11	5	8	9	15	19	11	4	13	20

BC-*Bacillus cereus;* BS-*Bacillus subtilis;* SA- *Staphylococcus aureus;* SE-*Staphylococcus epidermidis;*
ML-*Micrococcus luteus;* EN-*Enterococcus faecalis* EC-*Escherichia coli;*
ST-*Salmonella typhi;* SP-*Salmonella paratyphi ;* PS-*Pseudomonas aeruginosa;*
KP-*Klebsiella pneumoniae ;* SM-*Serratia marcescens*
R- Root, S-Stem, L- Leaf, INF- Inflorescences, WP- Whole plant

Table 6 Minimum inhibitory concentration of effective n-Hexane plant extracts

Plant name	Part used	MIC (mg/ml) n –Hexane											
		Gram positive						Gram negative					
		BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	KP	SM
Anisomeles indica	L	2	-	-	-	-	-	-	-	-	0.5	4	2
	INF	2			8	-	4	-	2	-	-	0.5	1
	S	-	-	-	-	-	>8	-	-	-	4	-	-
Anisomeles heyneana	L	2	0.25	-	-	-	-	-	8	-	2	1	-
	INF	0.5	2	2	2	-	4	-	0.5	-	-	0.5	1
	S	8	1	4	-	-	-	-	1	-	-	0.5	-
	R	2	-	8	4	-	8	-	4	-	4	2	4
Lantana camara	L	2	2	8	8	>8	4	>8	>8	-	2	1	1
Lantana salvifolia	L	>8	-	-	-	-	-	-	8	-	-	-	-
	S	-	-	-	-	-	-	-	-	-	-	-	
Lavandula bipinnata	L	2	>8	1	>8	-	>8	8	4	-	8	1	0.5
	INF	2	>8	4	2	4	>8	4	8	1	4	1	4
	S	-	-	-	-	-	-	-	4	-	8	-	>8
Leucas aspera	WP	-	-	-	-	-	-	-	-	-	-	-	4
Leucas stelligera	WP	4	-	-	-	-	-	-	-	-	0.5	-	8
Leucas martinenssis	WP	8	-	-	-	-	-	-	-	-	-	>8	-
Moschosma polystachyum	L	4	-	4	8	-	4	-	-	-	4	2	4
	S	4	2	>8	-	>8	-	-	8	-	4	8	1
Ocimum sanctum	L	2	2	1	2	>8	2	-	2	-	-	0.5	0.5
Ocimum canum	L	-	-	-	-	-	-	-	-	-	-	-	>8
	INF	1	-	-	-	-	-	-	-	-	8	8	-
	S	-	-	-	-	-	-	-	0.25	-	4	2	0.5
Pogostemon parviflorus	L	-	-	-	-	-	8	-	-	-	-	-	4
	INF	-	-	-	-	-	>8	-	-	-	-	1	-
	S	-	-	-	-	-	-	-	>8	-	-	8	4
Pogostemon purpurascens	L	0.5	-	-	--	-	>8	-	-	-	-	-	1
	INF	8	0.5	4	4	1	1	2	1	-	0.5	2	4
	S	-	-	-	-	-	-	-	-	-	2	2	1

Table 6 ***Continue***

		MIC (mg/ml) n –Hexane											
		Gram positive						Gram negative					
Plant name	**Part used**	**BC**	**BS**	**SA**	**SE**	**ML**	**EN**	**EC**	**ST**	**SP**	**PS**	**KP**	**SM**
Phyla nodiflora	WP	4	1	1	8	-	>8	-	>8	-	-	4	2
Salvia plebeian	WP	0.5	-	-	-	-	0.5	-	0.25	-	-	0.25	8

BC-*Bacillus cereus;* BS-*Bacillus subtilis;* SA- *Staphylococcus aureus;* SE-*Staphylococcus epidermidis;* ML-*Micrococcus luteus;* EN-*Enterococcus faecalis* EC-*Escherichia coli;* ST-*Salmonella typhi;* SP-*Salmonella paratyphi ;* PS-*Pseudomonas aeruginosa;*
KP-*Klebsiella pneumoniae ;* SM-*Serratia marcescens*
R- Root, S-Stem, L- Leaf, INF- Inflorescences, WP- Whole plant

Table 7 **Minimum inhibitory concentration of effective ethyl acetate plant extracts**

		MIC (mg/ml) ethyl acetate											
		Gram positive						Gram negative					
Plant name	**Part used**	**BC**	**BS**	**SA**	**SE**	**ML**	**EN**	**EC**	**ST**	**SP**	**PS**	**KP**	**SM**
Anisomeles indica	L	2	-	-	-	-	4	-	2	-	-	0.25	8
	INF	2	-	8	-	-	2	-	4	-	-	8	-
	S	1	-	-	-	-	4	-	-	-	0.5	-	-
Anisomeles heyneana	L	-	-	-	8	-	8	-	0.5	8	8	0.5	0.25
	INF	4	-	8	-	-	8	-	1	-	-	1	-
	S	4	4	-	-	-	-	-	-	-	-	-	-
	R	4	-	8	-	-	8	-	1	-	-	8	8
Lantana camara	L	2	-	>8	8	4	>8	>8	8	-	4	4	8
Lantana salvifolia	L	1	4	-	>8	-	4	-	8	-	-	4	>8
	S	-	-	-	-	-	-	-	-	-	-	-	-
Lavandula bipinnata	L	-	-	-	-	-	-	-	-	-	-	-	-
	INF	4	2	-	8	>8	>8	-	2	-	4	0.5	8
	S	-	-	-	-	-	-	-	2	-	-	-	-
Leucas aspera	WP	-	-	-	-	-	-	-	2	-	-	-	-
Leucas stelligera	WP	8	-	-	4	-	>8	-	8	-	-	4	-
Leucas martinenssis	WP	-	-	-	>8	-	-	-	-	-	-	4	-
Moschosma polystachyum	L	1	-	-	1	-	4	8	-	-	0.5	4	1
	S	-	4	-	-	8	-	-	-	-	-	8	8
Ocimum sanctum	L	1	-	-	-	-	8		1	-	-	-	4
Ocimum canum	L	8	4	-	-	-	-	-	2	-	-	-	-
	INF	4	8	-	-	-	-	-	8	-	2	8	4
	S	-	-	-	-	-	8	-	2	-	-	0.5	8
Pogostemon parviflorus	L	-	-	-	-	-	4	-	-	-	-	-	0.25
	INF	4	-	-	-	-	>8	-	-	-	-	1	-
	S	-	-	-	-	-	>8	-	0.25	-	-	8	0.5
Pogostemon purpurascens	L	0.5	-	4	4	-	4	-	4	-	-	4	2
	INF	2	8	2	-	-	4	-	2	-	-	2	8
	S	-	-	-	-	-	-	-	-	-	-	-	-
Phyla nodiflora	WP	8	4	2	8	-	>8	-	0.5	-	2	8	>8
Salvia plebeian	WP	2	4	4	2	2	8	-	1	-	-	4	-

BC-*Bacillus cereus;* BS-*Bacillus subtilis;* SA- *Staphylococcus aureus;* SE-*Staphylococcus epidermidis;* ML-*Micrococcus luteus;* EN-*Enterococcus faecalis* EC-*Escherichia coli;* ST-*Salmonella typhi;* SP-*Salmonella paratyphi;* PS-*Pseudomonas aeruginos;*
KP-*Klebsiella pneumonia;* SM-*Serratia marcescens*
R- Root, S-Stem, L- Leaf, INF- Inflorescences, WP- Whole plant

Table 8 Minimum inhibitory concentration of effective methanol plant extracts

		MIC (mg/ml) methanol											
		Gram positive						Gram negative					
Plant name	**Part used**	**BC**	**BS**	**SA**	**SE**	**ML**	**EN**	**EC**	**ST**	**SP**	**PS**	**KP**	**SM**
Anisomeles indica	L	8	-	-	-	-	>8	-	-	-	-	8	0.5
	INF	4	-	-	2	-	8	-	2	-	-	8	2
	S	1	-	-	-	-	>8	-	-	-	-	-	-
Anisomeles heyneana	L	-	4	-	2	-	8	-	-	8	-	-	-
	INF	-	-	-	-	-	-	-	-	-	-	-	-
	S	-	2	-	-	-	-	-	-	-	-	-	-
	R	1	-	0.5	-	-	-	-	0.5	-	-	8	2
Lantana camara	L	4	-	-	>8	-	-	8	2	-	-	4	8
Lantana salvifolia	L	-	>8	-	2	-	-	-	-	-	-	-	-
	S	-	-	-	-	-	-	-	-	-	-	-	-
Lavandula bipinnata	L	-	4	-	-	-	-	-	-	-	-	-	-
	INF	4	-	-	-	-	-	-	-	-	4	-	-
	S	-	-	-	-	-	-	-	-	-	1	-	-
Leucas aspera	WP	-	-	4	-	-	-	-	-	-	-	-	-
Leucas stelligera l	WP	-	-	-	>8	-	>8	-	-	-	-	-	-
Leucas martinenssis	WP	4	4	-	2	-	>8	-	8	-	4	8	-
Moschosma polystachyum	L	-	-	-	-	-	>8	-	-	-	-	4	-
	S	-	-	-	-	2	-	-	-	-	-	-	-
Ocimum sanctum.L	L	-	-	-	1	-	-	-	-	-	-	-	-
Ocimum canum	L	2	1	-	-	-	-	-	2	-	-	4	4
	INF	-	-	-	-	-	-	-	-	-	-	-	-
	S	8	4	-	-	-	-	-	-	-	-	-	8
Pogostemon parviflorus Bth	L	-	-	-	-	-	8	-	-	-	-	4	-
	INF	-	-	-	-	-	8	-	-	-	-	-	-
	S	1	-	-	-	-	-	-	-	-	-	2	>8
Pogostemon purpurascens	L	2	-	-	-	-	>8	-	>8	>8	-	-	-
	INF	-	-	-	-	-	>8	>8	8	-	-		-
	S	-	-	-	-	-	-	-	8	-	-	8	8
Phyla nodiflora	WP	2	0.5	8	4	-	8	-	2	-	-	0.5	0.5
Salvia plebeian	WP	0.5	-	-	-	-	-	-	0.5	-	-	0.5	-

BC-*Bacillus cereus;* BS-*Bacillus subtilis;* SA- *Staphylococcus aureus;* SE-*Staphylococcus epidermidis;* ML-*Micrococcus luteus;* EN-*Enterococcus faecalis* EC-*Escherichia coli;* ST-*Salmonella typhi;* SP-*Salmonella paratyphi;* PS-*Pseudomonas aeruginos;* KP-*Klebsiella pneumonia;* SM-*Serratia marcescens*
R- Root, S-Stem, L- Leaf, INF- Inflorescences, WP- Whole plant

activity against one or more tested bacteria. Seven plants namely *Phyla nodiflora, Lavendula bipinnata, Pogostemone purpurences, Pogostemone parviflorous, Ocimum sanctum, Lantana camara, Anisomeles indica* demonstrated broad spectrum antibacterial activity (Figure 1).

Similar reports on antimicrobial activities of certain Indian aromatic medicinal plants such as *Pogostemon patchouli, Ocimum basilicum, Lantana camara, Lavendula stoochas, Leuca lavandulefolia, Ocimum sanctum and Pogostemon cabin,* were also reported by other worker.[13;20;21;22;23;24;25.]

However antimicrobial activity of some Indian plants namely *Anisomeles hyneana, Leucas stelligera, Leucas martinensis, Moschoma polystachum, Pogostemone purpurea and lantana salvifolia* reported here for very first time and similarly *Anisomeles indica, Lavandula bipinnata* and *Leucas aspera* also found to be reported first time on activity of crude drug but reports is there on oils.[20]

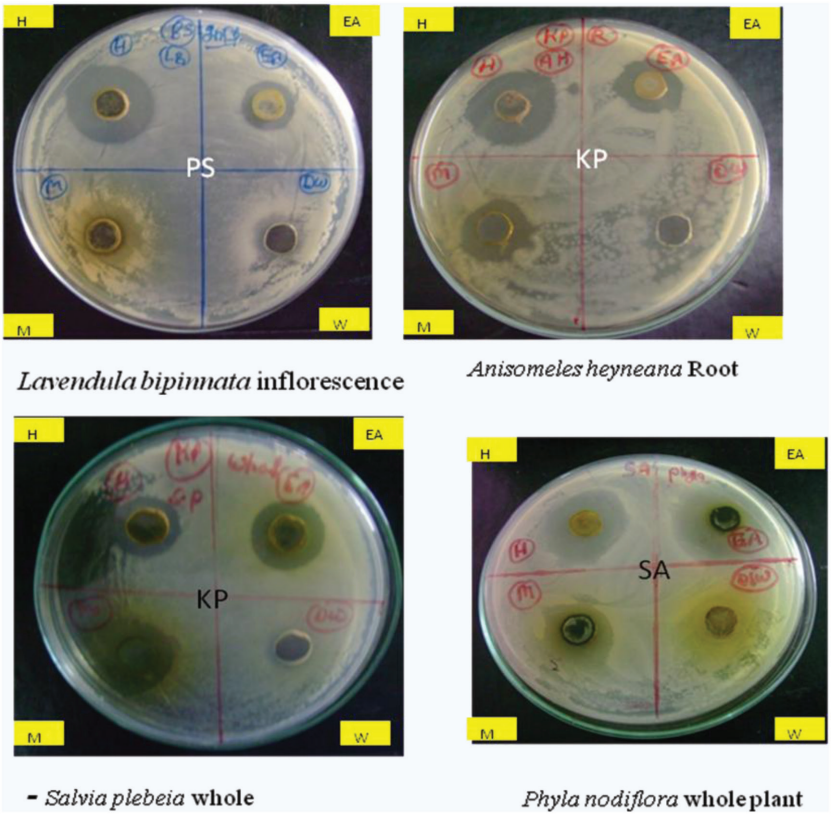

Figure 1 Antimicrobial activity of n-Hexane, Ethyl acetate, Methanol and Distilled water extract against selected microorganisms

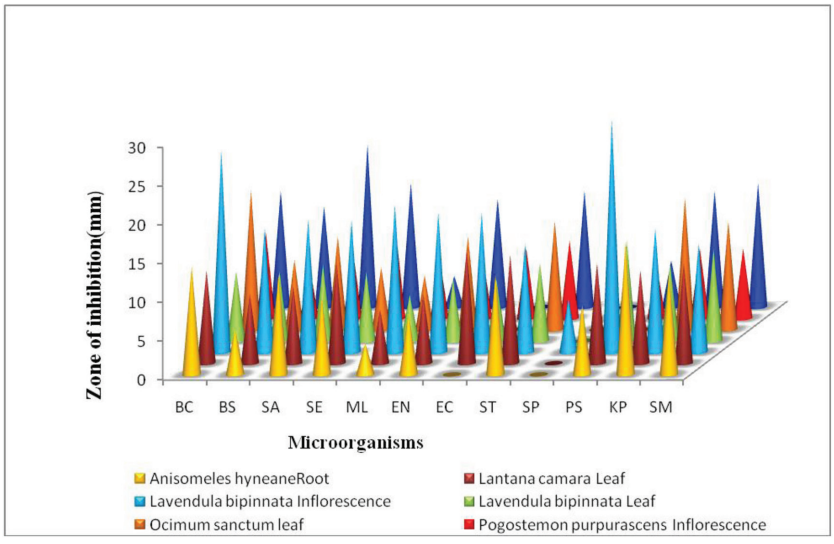

Figure 2 Antimicrobial activity of the crude n-hexane extracts of selected plant species

Overall present inhibition of active plant extract against test bacteria among different solvent solvents. Sensitivity of test strains was is decreasing order of n-hexane extracts BC=KP=SM>ST>EN>BS>SA=SE>ML>EC>SP (Figure 2) while ethyl acetate extract showed BC=KP>SM>EN=ST>SE>BS>SA>PS>ML>EC>SP (Figure 3) while methanolic extract showed BC>KP>ST=SM>EN>BS>SE>PS>SA>EC>ML=SP (Figure 4).

In the case of test bacteria the basis for their differences in susceptibility might be due to difference in cell wall composition by gram positive and gram negative bacteria ST, SP, ML was least sensitive compared to other test bacteria. This results clearly indicated that there is no obstruct with antimicrobial action of plant extracts and their different mode of action on test organisms with that antibiotic resistance. Two plant in this study were also screened previously against other test strains and showed similar results to this study with varying degree of potency.[12,13,14] The difference in potency may be due to the, different sensitivity of test strains, stage of collection of plant sample and method of extraction. Drug resists strains of bacteria were found to be sensitive to tested plant extacts. This study revealed that antimicrobial action of extract and these extract might have different mode of action on test organisms.

Phytochemical analysis of 31 plant extracts demonstrated the presence of common phytoconstituent like phenols tannins, alkaloid, flavonoids, and carotene. The presence of these compounds was detected by thin layer chromatography. Our phytochemical analysis are in agreement with report of other worker.[18] To locate the major active constituents responsible for antimicrobial activity against the most sensitive test strains (ST, SE, BC and KP) TLC bioautography (Figure 5) was performed against six higher active plants extract *Phyla nodiflora, Lantana camara, Anisomeles indica, Selvia plebia.* In the majority of the plants tested by HPTLC fingerprinting showed presence of active compound which was confirmed by spray reagent 10% Antimony trichloride flavonoids of most common active extract and TLC bioautography help us to confirm antimicrobial activity of active compound presence in selected extracts.

It is expected that more active compound might have been detected by TLC bioautography in different solvent system, microbial strain and more plant extracts were used. Thus, our antimicrobial screening results also justify the traditional uses of these plants in various ailments including infectious diseases. Further active phytochemical of these plants against multidrug resistant bacteria and *Candida albicans, Aspergillus niger,* has be characterized and the efficacy of non-toxic extracts has to be evaluated *in vivo*. Study of the synergistic interaction is required to exploit these potential plant extracts in combination therapy of infectious disease caused by multidrug resistant organism.

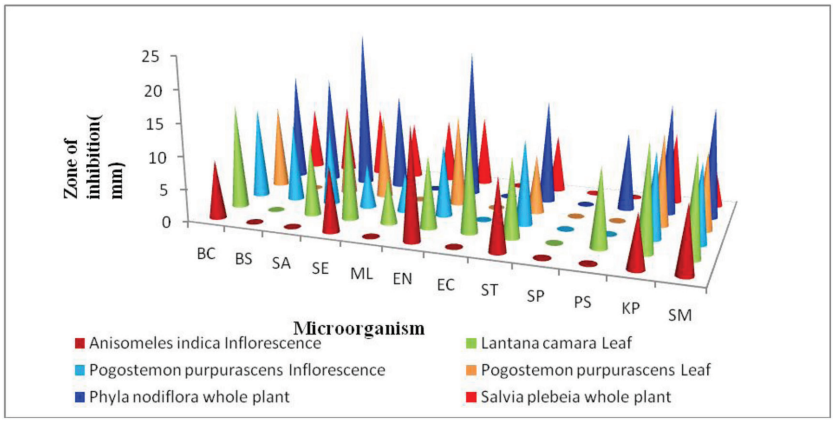

Figure 3 Antimicrobial activity of the crude ethyl acetate extracts of selected plant species

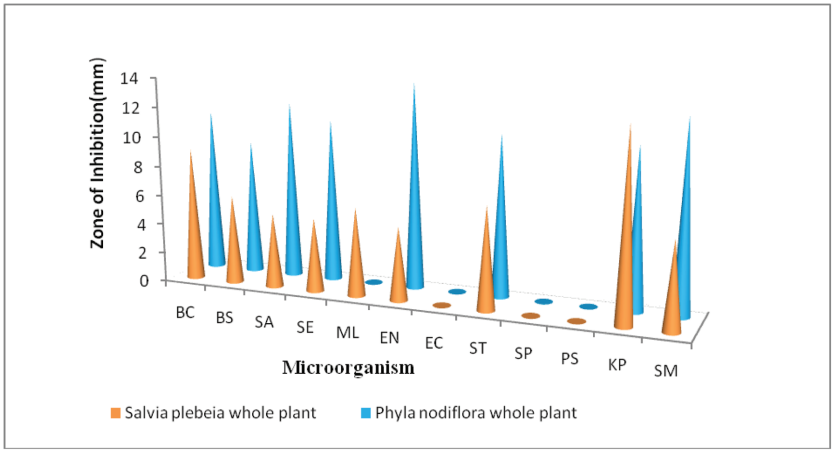

Figure 4 Antimicrobial activity of the crude methanol extracts of selected plant species

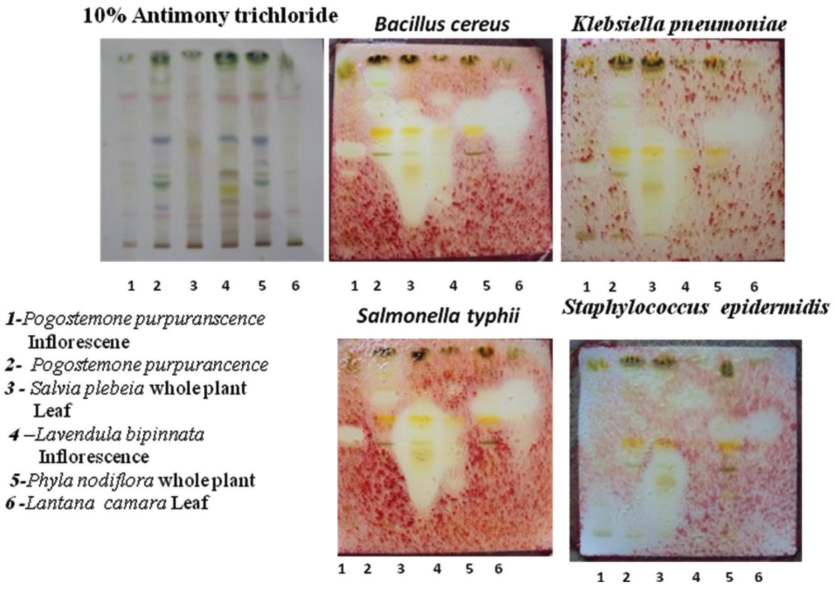

Figure 5 Zone of inhibition by bioautography method of ethyl acetate plant extracts compare with HPTLC profiling

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