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Antimicrobial activity and phytochemical screening of crude extract from selected medicinal plants

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Abstract
This work aims to evaluate the antimicrobial activity of n-Hexane, ethyl acetate, methanol and distilled water crude extract of selected medicinal plants. The potential of this extracts was analysed by agar well diffusion and two-fold serial dilution method against selected six gram-positive, six gram-negative bacteria and five fungi using positive control. Ethyl acetate flower extract of *Raphanus sativus L.* showed better antibacterial activity against EN (25mm), ST (24 mm), KP, SM and BC (22 mm). Good antifungal activity was observed in methanol extract of *Raphanus sativus L.* flower against AF(20mm), *Cestrum nocturnum L.* leaf against CA (20mm) whereas ethyl acetate extract of *Amaranthus hybridus L. Var. paniculatus* leaf inhibited AF (17mm), *Ficus krishnae* leaf CA(15mm) and D/W *Indigofera sps* whole plant extract inhibited AF (17mm). MIC and MBC value was observed in the range of 0.125mg/ml to >2mg/ml of selected crude plant extracts against tested organisms. HPTLC fingerprinting and bioautography of selected crude extracts demonstrated the presence of bioactive principle for antimicrobial activity.

Keywords: Antimicrobial activity, MIC, MBC, bioautography, HPTLC &phytochemical

Introduction
Medicinal plants are the gifts of the nature to cure limitless number of diseases among human being and source of modern medicine. The basic molecular and active structures for synthetic fields are provided by rich natural sources. This burgeoning worldwide interest in medicinal plants reflects recognition of the validity of many medicinal claims regarding the value of natural product in health care.

Herbal medicine is still the mainstay of about 75-80% of the whole population, mainly in developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and side effects. However, the last few years have seen a major increase in their use of medicinal plants in the developed world [1].

Plants used in traditional medicine contain a vast array of substances that can be used to treat chronic and infectious diseases. Medicinal herbs practiced in traditional folk medicine in India were screened for the presence of antibacterial activity [2]. The use of plants for therapeutic purposes in medical practice in form of teas, syrups, tinctures, among others have been used as medicines and in many cases come to be the sole therapeutic resource of certain communities and ethnic groups [3, 4]. Thus, knowledge about the therapeutic potential of plants is of great scientific and medical interest, as an effective alternative to the battle against resistant microorganisms [5].

The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines as alternative to synthetic drugs [6]. Despite an increasing frequency and severity of antimicrobial resistance, the future development of new anti-microbial agents is threatened by the cessation of research in this field by many major pharmaceutical companies [7, 8].

A few studies reported by [9-11] indicate that the plant extracts can enhance the *in vitro* activity of certain antibiotics against strains of multi-drug resistant (MDR) *Staphylococcus aureus* and other pathogens. These studies prompted the search for such MDR pump or efflux pump inhibitors from medicinal plants. The phytochemical constituents of each extract were determined and correlated with the antimicrobial action. The active extracts were tested against selected bacteria and fungi. The discovery of antimicrobial phytochemicals, their mechanisms of action, and their inclusion in possible treatments and therapies rapidly increase.

Therefore now a days it necessary to focused on special group of phytochemicals such as flavonoids, terpenoids, phenols etc. or inform of pure compounds and no complex mixtures, or specific type of bacterial resistance [12-15]. The abundance of plants on the earth's surface has led to an increasing interest in the investigation of different extracts obtained from traditional plants as potential sources of new antimicrobial agents. Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components [16].

The demand for more and more drugs from plant sources is continuously increasing. It is therefore, essential to evaluate plants of medicinal value systematically for various ailments that are used in traditional medicine. Hence, there is need to screen medicinal plants for their promising biological activity. Several researchers reported antimicrobial potential of crude plant extracts from different parts of medicinal and aromatic species i.e. twenty-two Indian plants are screened [17]; *Allium sativum*, *Zingiber officinale*, *Caryophyllus aromaticus*, *Cymbopogon citratus*, *Mikania glomerata* and *Psidium guajava* [18]; Bioactive potential of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi* belonging to the family Umbelliferae [19].

In the present investigation, 28 plant species belonging to 21 different families were selected for the screening potential antimicrobial activity against the selected micro-organisms, which can cause severe infectious disease in the community and to detect presence of phytochemical constituent for selected crude extract.

Materials and Methods

Plant materials

Twenty eight plant species belonging to different families were collected in the form of leaf, flower, fruit and whole plant from different localities Vallabh Vidhyanagar, Gujarat (Table-1). All the specimens were identified by referring "Flora of Gujarat state" [20] and confirmed with the help of Dr. A.S. Reddy (Taxonomist) and Dr. Sandip Patel, Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar.

Extract preparation

Plant materials (leaves, flowers, fruits and whole plants) were collected and washed thoroughly with running tap water and dried at room temperature and powdered with grinder (Sumit Mixer-Grinder, India). Extract was prepared by infusion extraction method using ethyl acetate, methanol and distilled water [21].

Table 1: List of medicinal plants collected from various localities of Gujarat

No.	Plant name	Family	Plant part	Location
1.	*Alangium salviifolium* (L). Wang.	Alangiaceae	Leaf	Lambhvel road
2.	*Amaranthus sps*.	Amaranthaceae	Flower	Santokpura
3.	*Amaranthus hybridus L. Var. p*aniculatus.	Amaranthaceae	Leaf	Vadtal road
4.	*Bauhinia variegata* Linn.	Caesalpiniaceae	Flower	B.R.D Campus
5.	*Cestrum nocturnum* L.	Solanaceae	Leaf	Valasan
6.	*Chenopodium album* L.	Chenopodiceae	Whole plant	Vadtal road
7.	*Cichorium intybus* L.	Asteraceae	Whole plant	Vadtal road
8.	*Citrus medica* Linn. Var. medica	Rutaceae	Leaf, Flower	Vadtal road
9.	*Clitoria ternatea* L.	Fabaceae	Leaf	Valasan
10.	*Coccinia grandis* (L.) Voigt	Cucurbitaceae	Whole plant	Botanical garden
11.	*Digera muricata (L.) Mart.*	Amaranthaceae	Whole plant	Karamsad
12.	*Dombia natalensis*	Sterculliaceae	Flower	Botanical garden
13.	*Euphorbia tirucalli* L.	Euphorbiaceae	Whole plant	Santokpura
14.	*Ficus racemosa*	Moraceae	Leaf	Botanical garden
15.	*Ficus krishnae*	Moraceae	Leaf	Botanical garden
16.	*Hibiscus rosa-sinensis* L	Malvaceae	Flower	Botanical garden
17.	*Hygrophila auriculata* Heine	Acanthaceae	Whole plant	Santokpura
18.	*Indigofera sps*.	Fabaceae	Whole plant	Vadtal road
19.	*Jatropha gossypifolia* L.	Euphorbiaceae	Leaf	Lambhvel road
20.	*Millingtonia hortensis* L. f	Bignoniaceae	Leaf	Vadtal road
No.	**Plant name**	**Family**	**Plant part**	**Location**
21.	*Momordica charantia* L.	Cucurbitaceae	Leaf	Vadtal road
22.	*Moringo oleifera* Lam.	Moringaceae	Flower	Botanical garden
23.	*Mukia maderaspatana (*L.)M.Roem	Cucurbitaceae	Whole plant	Vadtal road
24.	*Parkia biglobosa* Weight. & Arn.	Mimosaceae	Flower	B.R.D. campus
25.	*Pithecellobium dulce* C.E. P. Mart.	Mimosaceae	Flower	Vadtal road
26.	*Raphanus sativus* L	Brassicaceae	Flower, Fruit	Santokpura
27.	*Thunbergia grandiflora* Roxb.	*Thunbergiaceae*	Flower	Botanical garden
28.	*Vitex negundo* L	Verbenaceae	Leaf	Botanical garden

Selected microorganisms

12 bacterial strains and 5 fungal strains used in the study, among these were six Gram-positive namely *Bacillus cereus* (ATCC 11778), *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (Isolated), *Staphylococcus epidermidis* (ATCC 155), *Micrococcus luteus* (ATCC 4698), *Enterococcus faecalis* (Isolated) and six Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Salmonella typhi* (NCTC8394), *Salmonella paratyphi* (MTCC 735), *Pseudomonas aeruginosa* (ATCC 25668), *Klebsiella pneumoniae* (ATCC 15380), *Serratia marcescens* (Isolated) and fungal strains is *Aspergillus niger* (MTCC40211), *Candida albicans* (MTCC 183), *Trichoderma harzianum* (Isolated), *Fusarium oxysporum* (Isolated), *Aspergillus flavus* (MTCC4613). All the tested strains are reference strains, and were collected from MTCC (Microbial type culture

collection, Chandigarh), ATCC (American type culture collection, Manassas, Virginia) and NCTC (National collection of type culture). The bacterial and fungal cultures were grown on nutrient agar medium (Hi Media, pH 7.4) at 37 ºC and potato dextrose agar medium (Hi Media, pH 5.6) at 27 ºC respectively. Both the cultures were maintained at 4 °C.

Antibacterial assay

In the present study, the antibacterial activities of leaf, stem, flower and fruit crude extracts prepared in different solvents were screened by agar well diffusion method [22]. An inoculum size of 1×10 CFU/ml of bacteria which compared with 0.5 McFarland turbidity in a refrigerator for 30 minutes for pre-diffusion of plant extract and turbidity standards was used [23]. Each extract of 100 µl (stock solution 100 mg/ml) was added in a previously marked sterile nutrient agar petriplates and the wells were punched with sterile cork borer and filled with each plant extract. Plates were placed then incubated at 37 ºC for 24 hours. After incubation all the plates were examined and zone of inhibition (excluding well diameter in mm) was measured as a property of antimicrobial activity. Antibiotic such as ciprofloxacin and doxycycline (20µg/ml) as a positive control and 100% DMSO and solvents i.e. hexane, ethyl acetate and methanol as a negative controls.

Minimum inhibitory concentration (MIC)

In the present study, minimum inhibitory concentration (MIC) was evaluated by serial broth dilution method [24] for the plant extracts showing more than 7mm to 30mm of inhibition. Density of bacterial suspension was maintained uniformly throughout the experiment at $1x10^8$ CFU/ml by comparing with 0.5 Mc Farland turbidity standards. 40µl of plant extract from stock solution (100 mg/ml) was taken into the first dilution tube and added 960µl of nutrient broth and mixed well. 500µl of solution from first dilution tube was taken and added 500µl of nutrient broth into second tube, this step was repeated 5times and from last tube 500µl solution was discarded. Final volume was made up to 1ml by adding 500µl of test organism in each tube. The MIC was tested in the concentration range between 8mg/ml to 0.250 mg/ml. Tubes were incubated at 37 ºC for 24 hours in an incubator. 100µl (0.1%) 2,3,5 – triphenyl tetrazolium chloride solution as a growth indicator was incorporated in each tube to find out the bacterial inhibition and tubes were further incubated for 30 minutes at 37 ºC. Bacterial growth was visualized when colorless 2, 3, 5-triphenyl tetrazolium chloride was converted into red color formazon in the presence of live bacteria. MIC assay was repeated thrice by using DMSO and nutrient broth as controls.

Minimum bactericidal count (MBC): To determine the MBC, for each set of test tubes in the MIC determination for selected crude extract, l00µl of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient agar plate by spreading for bacteria. Plates inoculated with bacteria were then incubated at 37 ºC for 24 hours. After incubation the concentration at which no visible growth was seen was noted as the minimum bactericidal count. [25].

Antifungal Activity

The fungal spores were harvested in sterile distilled water from seven days old culture for determination of antifungal activity. The fungal spores count was counted using haemocytometer under aseptic condition, in laminar air flow the potato dextrose agar medium pour into presterilized petriplate and inoculated by fungal strain respectively and kept for 10-15 minutes for solidifing. Each extract of 100 µl (stock solution 100 mg/ml) was added in a previously marked sterile Potato dextrose agar petriplates and the wells were punched with sterile cork borer and filled with each plant extract. Plates were placed then incubated at 27 ºC for 48 hours. After incubation all the plates were examined and zone of inhibition (excluding well diameter in mm) was measured as a property of antifungal activity. Antibiotic such as Fluconazole and Ketacozole (20µg/ml) as a positive control and 100% DMSO and solvents i.e. hexane, ethyl acetate and methanol as a negative controls.

HPTLC fingerprinting

Sixteen different plant extracts(Table 2) which showed significant antimicrobial activity were selected for HPTLC finger printing based on the results obtained. Separation of the compounds present in the particular plant extract were carried out by High Performance Thin Layer Chromatography (HPTLC) (Camag, Switzerland). 20 µl of each selected extract from stock of plant extracts (100 mg/ml) prepared in DMSO was applied on a 0.25 mm thick 10x10 cm precoated silica gel G60 F254 plate (Merck, Germany) using a Camag Linomat 5 sample applicator (Camag, Switzerland).For different extract different solvent system *viz*. n-Toluene: Hexane: Diethylether (7:2:1), Toluene: Ethylacetate (8:2), Chloroform: Methanol (9:1)s) was used as mobile phase(Table-2). The plates were dried by air drier to remove the solvent vapour. The plates were then visualized in UV chamber and then scanned with a CAMAG TLC Scanner 3 (Camag, Switzerland) at 254 and 366 nm to record Rf value. Chromatogram and absorption spectra were examined and recorded. The chromatograms were developed by using the 10% Antimony trichloride for presence of terpenoids. The colour, Rf value and spectra of the resolved bands were recorded. The plates were photographed at 254 nm and 366 nm with the help of Camag Reprostar, Switzerland.

Table 2: Selected plant extracts for HPTLC finger printing with selected mobile phase

Organic solvent	Plant parts	Plant name	Mobile phase
n- Hexane	Flower	*P. biglandulosa*	Toluene: Hexane: Diethylether (7:2:1)
	Leaves	*A. salviifolium*	
		J. gossypifolia	
	Whole plant	*C. album*	
		Indigofera sps.	
Ethyl acetate	Flower	*Dombia natalensis*	Toluene: Ethylacetate (8:2)
		M. oliefera	
		R. sativus	
		F. krishnae	
		J. gossypifolia	

	Leaves	*M. charantia*	
		M. hortensis	
		V. negundo	
	Whole plant	*D. muricata*	
Methanol	Flower	*P. dulce*	Chloroform: Methanol (9:1)
	Leaves	*A. savifolium*	
		F. racemose	
		M. charantia	
		M. hortensis	
	Whole plant	*E. tirucalli*	

Bio autography

Twenty one plant extracts in (n-hexane, ethyl acetate, methanol) were selected (Table 3) for bio autography to checked the antimicrobial activity by using semiautomatic Camag Linomate 6 sample applicator (Camag, Switzerland). 20µl of each sample from 100mg/ml stock solution was spotted on 0.25 mm thick 10×10 cm precoated silica gel 60 F254 plate (Merck, Germany). The band length was 2 mm with 17.5 mm distance between two track horizontally and 20 mm vertically. The sample was loaded at dosages speed of 70nl/sec and then dried with hot air blower. Nutrient agar medium seeded with selected bacterial strains (Table 3) was overlaid with the sample loaded silica gel plate and incubated at 37^0C for 24 hrs. The plate was flooded with 0.1% 2,3 5 tetraphenly tetrazolium chloride to visualize inhibition area which appear as yellow in colour against pink red background (Lawn of living organism) [25].

Table 3: List of selected plant extracts for bio autography

Organic solvents	Plant parts	Plant name	Bacterial strains
n- Hexane	Flower	*P. biglandulosa*	BC, PS, ST, BS, EN
	Leaves	*A. salviifolium*	
		J. gossypifolia	
	Whole plant	*C. album*	
		Indigofera sps.	
Ethyl acetate	Flower	*Dombia natalensis*	BC, ST, EN, BS, EC
		M. oliefera	
		R. sativus	
	Leaves	*F. krishnae*	
		J. gossypifolia	
		M. charantia	
		M. hortensis	
		V. negundo	
	Whole plant	*D. muricata*	
Methanol	Flower	*P. dulce*	SM, SE, EN, BS, KP
	Leaves	*A. savifolium*	
		F. racemosa	
		M. charantia	
		M. hortensis	
	Whole plant	*E. tirucalli*	

Bacillus cereus (BC) *Salmonella typhi (*ST)
*Staphylococcus epidermidis (*SE) *Klebsiella pneumoniae (*KP)
*Enterococcus faecalis (*EN*) Bacillus subtilis (BS)*
Pseudomonas aeruginosa (PS) *Serratia marcescens* (SM)

Table 4: Antimicrobial activity of crude n-Hexane extract of selected plant species.

No	Plant name	Plant part	Zone of inhibition (mm)											
			Gram positive						Gram negative					
			BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	KP	SM
1	*Alangium salviifolium*	Leaf	5	4	5	8	-	4	5	3	-	-	5	5
2	*Chenopodium album*	Whole plant	7	8	-	4	-	3	6	6	-	-	5	5
3	*Indigofera sps.*	Whole plant	8	4	-	3	-	5	5	5	-	12	4	4
4	*Parkia biglobosa*	Flower	3	3	-	-	-	-	-	-	-	8	-	-
5	*Raphanus sativus*	Flower	5	-	-	-	-	1	-	8	-	-	4	4
		Fruit	-	-	-	-	-	4	-	-	-	-	-	-
6	Ciprofloxacin (20 µg/ml)		11	10	14	11	9	12	7	14	8	9	10	22
7	Doxycycline (20 µg/ml)		14	12	11	5	8	9	15	19	11	4	13	20

BC-*Bacillus cereus*; EC-*Escherichia coli*; BS-*Bacillus subtilis*; ST-*Salmonella typhi*
SA-*Staphylococcus aureus*; SP-*Salmonella paratyphi*; SE-*Staphylococcus epidermidis* PS-*Pseudomonas aeruginosa*; ML-*Micrococcus luteus*; KP-*Klebsiella pneumoniae* EN-*Enterococcus faecalis*; SM-*Serratia marcescens*

Table 5: Antimicrobial activity of crude Ethyl acetate extract of selected plant species.

No	Plant name	Plant part	Zone of inhibition (mm)											
			Gram positive						Gram negative					
			BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	KP	SM
1.	*Clitoria ternatea*	Leaf	5	4	7	-	5	-	7	8	-	-	6	5
2.	*Digera muricata*	Whole plant	9	7	4	5	-	3	6	6	-	-	9	8
3.	*Dombeya natalensis*	Flower	10	3	2	4	10	5	5	8	-	-	8	6
4.	*Ficus krishnae*	Leaf	7	12	6	10	3	5	2	6	-	-	4	4
5.	*Hibicus rosa-sinensis*	Flower	5	-	4	-	-	4	6	-	-	-	8	6
6.	*Indigofera sps.*	Whole plant	4	-	-	-	-	8	4	-	-	-	-	-
7.	*Jatropha gossypifolia*	Leaf	4	5	-	3	4	9	7	5	-	-	5	6
8.	*Millingtonia hortensis*	Leaf	7	-	-	4	-	-	11	8	-	2	7	7
9.	*Momordica charantia*	Leaf	11	5	7	8	9	10	6	8	-	-	8	4
10.	*Moringo oleifera*	Flower	1	9	-	-	-	1	6	12	-	-	6	11
11.	*Mukia maderaspatana*	Whole plant	4	-	3	2	-	-	8	5	-	-	5	-
12.	*Raphanus sativus*	Flower	22	14	15	17	9	25	19	24	-	17	22	22
		Fruit	-	-	-	-	-	6	-	-	-	-	-	-
13.	*Vitex negundo*	Leaf	14	7	-	9	2	9	4	10	9	11	14	-
14.	Ciprofloxacin (20 µg/ml)		11	10	14	11	9	12	7	14	8	9	10	22
15.	Doxycycline (20 µg/ml)		14	12	11	5	8	9	15	19	11	4	13	20

BC-*Bacillus cereus*; EC-*Escherichia coli*; BS-*Bacillus subtilis*; ST-*Salmonella typhi*
SA-*Staphylococcus aureus*; SP-*Salmonella paratyphi*; SE-*Staphylococcus epidermidis* PS-*Pseudomonas aeruginosa*; ML-*Micrococcus luteus*; KP-*Klebsiella pneumoniae* EN-*Enterococcus faecalis*; SM-*Serratia marcescens*

Table 6: Antimicrobial activity of crude Methanol extract of selected plant species.

No	Plant name	Plant part	Zone of inhibition (mm)											
			Gram positive						Gram negative					
			BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	KP	SM
1.	*Alangium salviifolium*	Leaf	3	8	5	9	-	10	5	5	-	10	4	4
2.	*Cichorium intybus*	Whole plant	7	-	-	-	-	-	-	-	-	-	8	6
3.	*Digera muricata*	Whole plant	8	5	3	1	5	-	6	7	7	-	5	5
4.	*Euphorbia tirucalli*	Whole plant	6	7	12	4	7	10	7	5	7	5	7	8
5.	*Ficus racemosa*	Leaf	7	6	-	9	4	4	7	7	-	4	7	8
6.	*Jatropha gossypifolia*	Leaf	3	4	6	-	-	8	3	4	-	-	5	-
7.	*Millingtonia hortensis*	Leaf	8	13	11	12	-	10	13	13	-	3	8	12
8.	*Momordica charantia*	Leaf	10	4	9	10	8	11	9	10	-	-	8	8
9.	*Mukia maderaspatana*	Whole plant	3	-	-	-	-	-	8	1	-	-	6	5
10.	*Parkia biglobosa*	Flower	7	5	-	5	-	7	8	8	-	-	8	7
11.	*Pithecellobium dulce*	Flower	7	-	-	11	6	-	4	8	-	-	-	5
12.	*Raphanus sativus*	Flower	3	-	-	-	5	-	-	6	-	-	1	1
		Fruit	-	-	-	-	-	6	4	-	-	-	5	-
13.	Ciprofloxacin (20 µg/ml)		11	10	14	11	9	12	7	14	8	9	10	22
14	Doxycycline (20 µg/ml)		14	12	11	5	8	9	15	19	11	4	13	20

BC-*Bacillus cereus*; EC-*Escherichia coli*; BS-*Bacillus subtilis*; ST-*Salmonella typhi*
SA-*Staphylococcus aureus*; SP-*Salmonella paratyphi*; SE-*Staphylococcus epidermidis*; PS-*Pseudomonas aeruginosa*; ML-*Micrococcus luteus*; KP-*Klebsiella pneumoniae*; EN-*Enterococcus faecalis*; SM-*Serratia marcescens*

Table 7: Antimicrobial activity of crude Distilled water extract of selected plant species

No	Plant name	Part used	Zone of inhibition (mm)											
			Gram positive						Gram negative					
			BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	KP	SM
1.	*Alangium salviifolium*	Leaf	-	7	-	3	-	5	-	-	-	-	-	2
2.	*Ficus krishnae*	Leaf	-	7	-	-	-	-	-	-	-	-	-	-
3.	*Moringo oleifera*	Flower	12	-	-	4	6	13	1	3	-	5	-	-
4.	*Raphanus sativus*	Flower	2	1	-	-	8	-	-	9	-	-	2	1
		Fruit	-	3	-	-	-	1	-	-	-	-	-	-
5.	Ciprofloxacin (20 µg/ml)		11	10	14	11	9	12	7	14	8	9	10	22
6.	Doxycycline (20 µg/ml)		14	12	11	5	8	9	15	19	11	4	13	20

BC-*Bacillus cereus*; EC-*Escherichia coli*; BS-*Bacillus subtilis*; ST-*Salmonella typhi*
SA-*Staphylococcus aureus*; SP-*Salmonella paratyphi*; SE-*Staphylococcus epidermidis*; PS-*Pseudomonas aeruginosa*; ML-*Micrococcus luteus*; KP-*Klebsiella pneumoniae*; EN-*Enterococcus faecalis*; SM-*Serratia marcescens*

Table 8: Minimum inhibitory concentration of effective n-Hexane plant extracts.

No. s	Plant name	Plant part	MIC (mg/ml)				
			Gram positive			Gram negative	
			BC	BS	EC	ST	PS
1.	*Chenopodium album*	Whole plant	2	>2	-	-	-
2.	*Indigofera sps.*	Whole plant	>2	-	-	-	2
3.	*Parkia biglobosa*	Flower	-	-	-	-	>2
4.	*Raphanus sativus*	Flower	-	-	-	2	-

BC-*Bacillus cereus*; BS-*Bacillus subtilis*; EC-*Escherichia coli*; ST-*Salmonella typhi* PS-*Pseudomonas aeruginosa*

Table 9: Minimum inhibitory concentration of effective ethyl acetate plant extracts.

Plant name	Plant part	MIC (mg/ml)											
		Gram positive						Gram negative					
		BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	KP	SM
Chenopodium album	Whole plant	-	-	-	-	-	-	2	1	-	-	-	-
Clitoria ternatea	Leaf	-	-	>2	-	-	-	2	2	-	-	-	-
Digera muricata	Whole plant	>2	2	-	-	-	-	-	-	-	-	2	>2
Dombeya natelensis	Flower	2	-	-	-	>2	-	-	2	-	-	2	-
Ficus krishnae	Leaf	1	0.5	-	0.5	-	-	-	-	-	-		-
Hibicus rosa-sinensis	Flower	-	-	-	-	-	-	-	-	-	-	>2	-
Hygrophila auriculata	Whole plant	-	-	-	-	-	-	-	-	-	-	>2	-
Indigofera	Whole plant	-	-	-	-	-	>2	-	-	-	-		-
Jatropha gossypifolia	Leaf	1	-	2	-	>2	1	0.5	1	-	-	1	-
Millingtonia hortensis	Leaf	1	-	-	-	-	-	0.5	0.25	-	-	0.25	0.5
Momordica charantia	Leaf	0.125	-	0.25	0.25	0.25	0.5	-	2	-	-	0.25	-
Moringa oleifera	flower	-	>2	-	-	-	-	-	>2	-	-	-	>2
Mukia maderaspatana	Whole plant	-	-	-	-	-	-	1	-	-	-	-	-
Pithecellobium dulce	Flower	-	-	-	2	-	-	-	-	-	-	-	-
Raphanus sativus	Flower	2	1	0.5	2	>2	1	1	1	-	2	1	2
Vitex negundo	Leaf	0.5	0.25	-	0.5	-	0.5	-	>2	>2	0.5	0.125	-

BC-*Bacillus cereus*; EC-*Escherichia coli*; BS-*Bacillus subtilis*; ST-*Salmonella typhi*
SA-*Staphylococcus aureus*; SP-*Salmonella paratyphi*; SE-*Staphylococcus epidermidis*; PS-*Pseudomonas aeruginosa*; ML-*Micrococcus luteus*; KP-*Klebsiella pneumoniae*; EN-*Enterococcus faecalis*; SM-*Serratia marcescens*

Table 10: Minimum inhibitory concentration of effective Methanol plant extracts.

Plant name	Plant part	MIC (mg/ml)											
		Gram positive						Gram negative					
		BC	BS	SA	SE	ML	EN	EC	ST	SP	PS	KP	SM
Alangium salviifolium	Leaf	-	0.125	-	>2	-	2	-	-	-	>2	-	-
Cichorium intybus	Whole plant	>2	-	-	-	-	-	-	-	-	-	>2	-
Digera muricata	Whole plant	>2	-	-	-	-	-	-	>2	>2	-	-	-
Euphorbia tirucalli	Whole plant	-	>2	>2	-	>2	>2	>2	-	>2	-	>2	>2
Ficus racemosa	Leaf	2	-	-	0.5	-	-	1	1	-	-	0.5	0.5
Jatropha gossypifolia	Leaf	-	-	-	-	-	>2	-	-	-	-	-	-
Millingtonia hortensis	Leaf	1	0.5	1	0.5	-	1	0.5	0.5	-	-	1	1
Momordica charantia	Leaf	0.5	-	0.5	0.25	0.5	0.5	0.25	1	-	-	0.5	0.25
Mukia maderaspatana	Whole plant	-	-	-	-	-	-	2	-	-	-	-	-
Parkia biglobosa	Flower	1	-	-	-	-	2	>2	>2	-	-	2	2
Pithecellobium dulce	Flower	-	-	-	>2	-	-	-	>2	-	-	-	-
Raphanus sativus	Flower	-	-	-	-	-	>2	-	-	-	-	-	-

BC-*Bacillus cereus*; EC-*Escherichia coli*; BS-*Bacillus subtilis*; ST-*Salmonella typhi*
SA-*Staphylococcus aureus*; SP-*Salmonella paratyphi*; SE-*Staphylococcus epidermidis*; PS-*Pseudomonas aeruginosa*; ML-*Micrococcus luteus*; KP-*Klebsiella pneumoniae*; EN-*Enterococcus faecalis*; SM-*Serratia marcescens*

Table 11: Minimum inhibitory concentration of effective Distilled water plant extracts.

No. s	Plant name	Plant part	MIC (mg/ml)				
			Gram positive			Gram negative	
			BC	ML	EN	EC	ST
1.	*Alangium salviifolium*	Leaf	>2	-	-	-	-
2.	*Ficus krishnae*	Leaf	>2	-	-	-	-
3.	*Moringo oleifera*	Flower	>2	-	>2	-	-
4.	*Raphanus sativus*	Flower	-	>2	-	-	>2

BC-*Bacillus cereus*; ML-*Micrococcus luteus*; EN-*Enterococcus faecalis*; EC-*Escherichia coli* ST-*Salmonella typhi*

Table 12: Minimum bacteriocidal count of ethyl acetate extract from selected plant species.

Plant name	Plant Part	MBC (mg/ml)										
		Gram positive						Gram negative				
		BC	BS	SA	SE	ML	EN	EC	ST	PS	KP	SM
Alangium salviifolium	Leaf	-	-	-	-	R	-	-	-	-	-	-
Chenopodium album	Whole plant	-	-	-	-	-	-	-	R		-	-
Citrus medica	Leaf	-	-	-	-	-	-	-		R	-	-
Ficus krishnae	Leaf	R	R		R	-	-	-	-	-	-	-
Jatropha gossypifolia	Leaf	R	-	-	-	-	R	R	R	-	R	-
Millingtonia hortensis	Leaf	R	-	-	-	-	-	-	R	-	R	R
Momordica charantia	Leaf	R	-	R	R	-	R	-	-	-	R	-
Raphanus sativus	Flower	-	R	R	-	-	R	R	R	-	R	-
Vitex negundo	Leaf	R	R	-	R	-	R	-	-	R	R	-

"R" indicates resistance to that particular plant extract.

BC-*Bacillus cereus*; EC-*Escherichia coli*; BS-*Bacillus subtilis*; ST-*Salmonella typhi*

SA-*Staphylococcus aureus*; SP-*Salmonella paratyphi*; SE-*Staphylococcus epidermidis*; PS-*Pseudomonas aeruginosa*; ML-*Micrococcus luteus*; KP-*Klebsiella pneumoniae*; EN-*Enterococcus faecalis*; SM-*Serratia marcescens*

Table 13: Minimum bacteriocidal count of crude methanol extract from selected plant species.

Plant name	Plant Part	MBC (mg/ml) Methanol									
		Gram positive						Gram negative			
		BC	BS	SA	SE	ML	EN	EC	ST	KP	SM
Alangium salviifolium	Leaf	-	R	-	-	-	-	-	-	-	-
Clitoria ternatea	Leaf	-	-	-	R	-	-	-	R	R	-
Ficus racemosa	Leaf	-	-	-	R	-	-	R	R	R	R
Millingtonia hortensis	Leaf	-	R	R	R	-	R	R	R	R	R
Momordica charantia	Leaf	R	-	R	R	R	R	R	R	R	R

"R" indicates resistance to that particular plant extract.

BC-*Bacillus cereus*; EC-*Escherichia coli*; BS-*Bacillus subtilis*; ST-*Salmonella typhi*

SA-*Staphylococcus aureus*; SP-*Salmonella paratyphi*; SE-*Staphylococcus epidermidis*; PS-*Pseudomonas aeruginosa*; ML-*Micrococcus luteus*; KP-*Klebsiella pneumoniae*; EN-*Enterococcus faecalis*; SM-*Serratia marcescens*

Table 14: Antifungal activity in the crude extracts of selected plant species

Extract	Plant name	Plant part	Zone of inhibition (mm)				
			AF	AN	CA	FO	TH
n-Hexane	*Alangium salviifolium*	Leaf	-	-	-	-	9
	Cestrum nocturnum	Leaf	-	-	-	-	10
Ethyl acetate	*Amaranthus hybridus subsp. Cruentus* L.	Leaf	17	-	-	-	-
	Ficus krishnae	Leaf	-	-	15	-	-
	Mukia maderaspatana	Whole plant	-	-	7	-	13
Methanol	*Cestrum nocturnum*	Leaf	-	-	20	-	7
	Ficus krishnae	Leaf	-	-	13	-	-
	Pithecellobium dulce	Flower	5	-	-	-	8
	Raphanus sativus	Fruit	20	-	-	-	-
	Thunbergia grandiflora	Flower	-	-	-	-	9
Distilled water	*Cestrum nocturnum*	Leaf	-	-	10	-	-
	Indigofera sps.	Whole plant	17	-	-	-	6
	Millingtonia hortensis	Leaf	-	-	-	8	-
	Pithecellobium dulce	Flower	10	-	-	-	-
	Raphanus sativus	Fruit	13	-	-	-	5
Ketacozole (20 µg/ml)			10	9	20	12	10
Fluconazole (20 µg/ml)			19	20	21	18	16

AF- *Aspergillus flavus* CA- *Candida albicans* FO- *Fusarium oxysporum* AN- *Aspergiluus niger* TH- *Trichoderma harzianum*

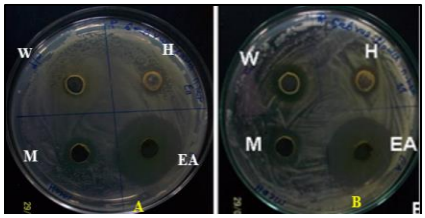

Fig 1: Antibacterial activity of *Raphanus sativus flower extract against Enterococcus faecalis* (A) & *Salmonella typhi* (B)

Fig 2: Antifungal activity of *Indigofera sps* whole plant *extract against Aspergillus flavus*

Fig 3: Antifungal activity of *Cestrum nocturnum* leaf extract against *Candida albicans*

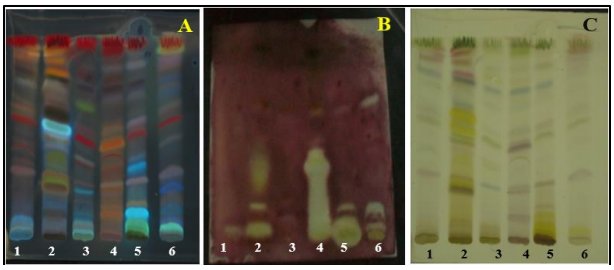

Fig 4: A: Phytochemical fingerprinting of crude methanol extract scanned at 366nm B- Appearance of zone of inhibition in bioautography of crude methanol extract against *Enterococcus faecalis C*- Phytochemical fingerprinting of methanol extract at 546nm after spraying 10% antimony chloride to detect the presence of terpenoids.

(Lane 1- *Ficus racemose leaf*; Lane 2- *Momordica charantia leaf*; Lane 3- *Alangium salviifolium leaf*; Lane 4:- *Millingtonia hortensis leaf*; Lane 5-*Pithocelobium dulce flower*; Lane 6- *Euphorbia tirucalli* whole plant)

Results and Discussion

Now a days the world is facing a growing number of multidrug-resistant microorganisms, numerous studies have been conducted in order to select new antimicrobial compounds, such as those from natural resources which are of extremely importance. This has caused an urgent need for search of new and innovative ways to control bacterial invasions especially by multi resistant pathogens. So, this study has evaluated the antimicrobial activity of selected different extracts in n-Hexane, ethyl acetate, methanol and distilled water extract for their potential antimicrobial activity of 28 selected plant species belonging to different families.

The data pertaining to the antibacterial (Table 4-7) and antifungal (Table 14) potential of the plant extracts and the inhibition zone formed by extracts and minimum inhibitory concentration values MIC was tested between 2 mg/ml and 0.125 mg/ml (Table 8-11) and Minimum bacteriocidal concentration (Table 12-13) of selected crude extract found that all bacterial strains are resistant.

Bacillus cereus, Klebsiella pneumoniae, Salmonella typhi, Escherichia coli were found to be the most susceptible organisms among the tested organisms against all the four extracts. The overall inhibitory activity of n-hexane plant extracts on tested organisms was in decreasing order i.e. BC>ST>KP>EC>PS=SM>EN>BS>SE>SA=ML =SP. The overall inhibitory activity of ethyl acetate plant extracts on tested organisms were i.e. EC>BC>KP>ST>SM>BS=EN>SE>SA>ML>PS>SP.

Escherichia coli was found to be the most susceptible organism and SP most resistant organism for ethyl acetate leaf and flower extracts e.g. *Raphanus sativus* flower extract exhibited better zone of inhibition against EN (25mm) & ST (24mm) (Fig.1). Methanolic extract of selected plant species was most effective against *Klebsiella pneumonia*. The overall inhibitory activity of methanolic plant extract was to be: KP>BC=ST=SM>EN>EC>BS=SA=SE>ML>PS=SP.

The overall inhibitory activity in distilled water was BS>ML=PS>BC=SE=EN=ST>SA=EC=SP=KP=SM. The inhibitory activity in distilled water extracts was not very significant. *Bacillus subtilis* found to be more susceptible while *Salmonella paratyphii* was more resistant against all the four extracts.

Similarly antifungal activity was observed in selected plants extract n-Hexane, ethyl acetate, methanol and D/W (Table.14). Amongst this methanol extract found to be more effective against CA (20mm) in *Cestrum nocturnum* (Fig.3), *Raphanus sativus* (20mm) against AF whereas D/W whole plant extract against AF (17 mm) in *Indigofera sps* (Fig. 2). AN and FO are found to be resistant in all selected extract similarly reported that ethanolic: water (50:50) shows antifungal activity against *Aspergillus flavus, Aspergillus Niger* and *Candida albicans* [26]. A decrease in antifungal activity could imply that the fungi either became resistant to the extracts or that the active compound(s) became unstable during the incubation period.

The MBC values were higher in most cases then the MIC values suggesting that the crude plant extracts were bacteriocidal at high concentration and bacteriostatic at low concentration. In the present study it can be deduced that *Millingtonia hortensis* ethyl acetate leaf extract was strong antimicrobial agent against *Escherichia coli* when compared to other plant extract. Among five different fungi, *Trichoderma harzianum, Candida albicans* was found to be more susceptible while *Aspergillus flavus, Aspergillus Niger* and *Fusarium oxysporum* was found to be more resistant against all plants extracts.

In classifying the antimicrobial activity of gram-positive, gram-negative bacteria and fungi, it would generally be expected that a much greater number of antimicrobial agents shows susceptibility against gram-positive, gram-negative bacteria and fungi due to differences in the cell wall composition. The gram negative bacteria having an outer phospholipidic membrane carrying the structural lipo-polysaccharide components, this makes the cell wall impermeable to lipophilic solutes, while protein constitutes a selective barrier to the hydrophilic solutes [27]. Gram positive bacteria should be more susceptible having only an outer permeability barrier. Additional contribution to intrinsic resistance in Gram-negative bacteria is provided by efflux pumps (Eps) which actively pump out a broad spectrum of compounds (such as antibiotics, toxins, β-lactamase inhibitors, dyes, detergents, lipids, and molecules involved in quorum sensing) from the periplasm to the outside of the cell. The overexpression of EPs (such as Resistance-Nodulation-Division type efflux pumps) is recognized as a major component in the development of the multidrug resistance phenotype in Gram negative bacteria [28, 29]. The ineffectiveness of plant compounds toward Gram negative pathogens has been proposed to be strongly related to EPs as the combination of plant antimicrobials with EPs inhibitors leads to a striking increase in antimicrobial activity [30] similarly the selected plant extract act on primary site of action of chitosan is H^+-Atpase that leads to inhibition of selected fungi. Although the action mechanisms of natural products are distinct, the cytoplasmic membrane ranks as the most common site of action for phytoconstituent. They usually act through cell lysis, triggering the leakage of cellular contents and consequently cell death.

The HPTLC fingerprinting was carried out for sixteen different plant extracts, which showed the presence of terpenoids, phenolic compounds and other active principles. HPTLC fingerprinting was performed for twenty one highly active plant extracts extracted in n-Hexane, ethyl acetate and methanol to find out the active compounds. The plates were developed in chloroform: methanol (8:2), toluene: ethyl acetate (8:2) and Toluene: n-Hexane: Diethyl ether (7:2:1) and the plates were visualized and documented the Rf values at 254nm, 366nm and 546nm in a scanner.

Different bands were observed at different Rf values in all the selected plant extracts (Table-2). Presence of terpenoids,

compounds was confirmed by spray reagents i.e. 10% antimony trichloride. The Rf value was noted between 0.10 and 0.96 for the ethyl acetate extracts of *Dombeya natalensis* flower, *Moringa oliefera* flower, *Raphanus sativus* flower, *Ficus krishnae* leaf, *Jatropha gossypifolia* leaf, *Millingtonia hortensis* leaf, *Momordica charantia* leaf, *Vitex negundo* leaf and *Digera muricata* whole plant. The Rf value was noted between 0.07 and 0.99 for the methanolic extracts of *Pithecellobium dulce* flower, *Alangium salviifolium* leaf, *Ficus racemesa* leaf, *Millingtonia hortensis* leaf, *Momordica charantia* leaf and *Euphorbia tirucalli* whole plant (Fig.4 A-C).

HPTLC fingerprinting of nineteen highly active plant extracts was performed against selected microorganisms for bio autography (Table-3). The plates were developed in chloroform: methanol; ethylacetate: Toluene; Toluene: n-Hexane: diethylether and visualized at 254nm and 366nm and 546nm. Total number of bands counted and number of bands showing zone of inhibition were recorded by using bio autography TLC fingerprinting at different Rf value. Most of the bands detected at 546nm after spraying 10% antimony trichloride (Fig.4 C) produced inhibition bands at almost same Rf values between 0.05-0.81 having probably similar chemical profile. Ethyl acetate extract of *Dombeya natalensis* showed inhibition against all selected extracts between Rf values 0.10 and 0.40. Same inhibition bands were localized at 254nm and 366nm. Ethyl acetate extract of *Moringa oliefera, Raphanus sativus, Vitex negundo, Momordica charantia and Ficus krishnae* showed inhibition between Rf values 0.11 and 0.64. *Ficus racemosa* methanolic extract showed inhibition at Rf value 0.07 against *Bacillus subtilis and Serrtaia marcescens. Momordica charantia* showed inhibition between Rf values 0.24 and 0.61 against *Bacillus cereus* and *Serratia marcescens* while against *Staphylococcus epidermis and Kebsiella pneumonia*, inhibition was observed between Rf values 0.79 and 0.94. *Alangium salviifolium and Millingtonia hortensis* and *Pithocellobium dulce* showed inhibition between Rf values 0.22 and 0.99 against all selected bacterial strains except *Klebsiella pneumoniae*. Methanolic plant extracts showing inhibition bands between Rf values 0.07-0.99 was localized at 254nm and 366nm (Fig.4 B). Bioautography is based on the biological effect of substance to be detected and is extremely useful method, which help in making the position of the detected substance visible on chromatogram [31, 32]. In our study selected extract i.e. n-Hexane plant extracts exhibited inhibition area against BC, ST, BS and EN used as reference organisms. Ethyl acetate plant extracts demonstrated inhibition area against BC, ST, EN, BS and EC. Methanol plant extracts showed inhibition against SM, SE, EN, BS and KP. The selected plant extracts for phytochemical screening revealed the presence of terpenoids showed the inhibitory activity against selected bacteria. The phenolic compounds (polyphenols, tannins, and flavonoid) can act at two different levels: the cell membrane and cell wall of the microorganisms [33]. They can also penetrate into bacterial cells and coagulate cell content [34]. Tannins are one of the phytochemicals which prevent microbial growth, the phenolic and flavonoid are involved in reactions with microbial cell membrane proteins which will stimulate protein precipitation-mediated cell death and enzymes inhibition. The interaction of polyphenols with bacterial plasma membranes can trigger a myriad of effects that contribute to their antibacterial activity. There are numerous evidence suggesting that plant extracts and polyphenols have the ability to disrupt the structure of the bacterial plasma membrane, causing the formation of pores, leakage, altering electrical charge, altering polarity, increasing permeability, modifying fluidity, delocalizing membrane proteins, and other phenomena responsible for antimicrobial activity. Among different solvents, the most significant scavenging effect was exhibited in ethyl acetate & methanolic extract in all the plant parts. This is due to difference in polar and non-polar group found in selected solvent. In our study all selected extract having presence of phytoconstituents i.e. terpenoids which shows presences of higher radical scavenging affinity.

The present study concludes that among different plant extracts *Raphanus sativus* (ethyl acetate flower extract), *Vitex negundo* (ethyl acetate leaf), *Millingtonia hortensis* (ethyl acetate and methanol leaf extract) and *Momordica charantia* (methanol leaf extract) possessed active compounds with antibacterial property. *Cestrum nocturnum* (n-hexane, methanol and distilled leaf extract) and *Ficus krishnae* against *Candida albicans* and *Amaranthus hybridus subsp. cruentus* against *Aspergillus flavus* possessed antifungal activity. These plant extracts possessed active compounds with antimicrobial property, which can be used as a substitute either alone or in combination to combat infectious disease caused by some of the resistant organisms.

Conclusion

Present study revealed that phyto constituent of biological active compounds synergistically act against drug resistant microorganism. These active compounds may be tested for their safety and efficacy to uncover their therapeutic potential in modern medicine against infectious diseases. Further work may be focused on isolation and characterization of active principles.

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