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Screening of Some Selected Medicinal Plants Extracts for *In-vitro* Antimicrobial Activity

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Abstract: Antimicrobial activities of 55 plant extracts were evaluated against twelve microbial strains using macrobroth dilution assay. Twenty one extracts exhibited antimicrobial activity against the tested microorganisms in range of 0.20 to 6.25 mg/ml. Extracts from *Madhuca longifolia, Parkia biglandulosa*, *Pterospermum acerifolium* showed highest antimicrobial potential among the tested plants (MIC 0.20-12.5 mg/ml). Bio-assays showed presence of multiple specifically active compounds at different R_f values in various plant extracts. Acetone and ethanol extract of *M. longifolia, P. biglandulosa; P. acerifolium* shows greater antibacterial activity as compared to their water extracts and could be the potential source to develop new antimicrobial agents.

Key words: Antimicrobial activity · Medicinal plants · Macrobroth dilution assay · Bioautography · Plant Extracts

INTRODUCTION

Ayurveda is ancient health care system and is practiced widely in India, Srilanka and other countries [1]. Ayurveda system of medicine use plants to cure the ailments and diseases. Despite the availability of different approaches for the discovery of therapeuticals, natural products still remain as one of the best reservoir of new structural types. They are used directly as therapeutic agents, as well as starting material for the synthesis of drugs or as models for pharmacologically active compounds [2] . In modern time plants have been sources of analgesics, anti-inflammatory, antineoplastic drugs, medicine for asthma, anti arrhythmic agents and antihypertensive.

In last three decades numbers of new antibiotics have produced, but clinical efficacy of these existing antibiotics is being threatened by the emergence of multi drug-resistant pathogens [3]. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs [4]. According to World Health Organization (WHO) medicinal plants would be the best source to obtain a variety of drugs [5]. About 80 % of individuals from developed countries use traditional medicine, which has compound derived from medicinal plants. Therefore such plants should be investigated to understand their properties, safety and efficacy and for a search of new potent antimicrobial compounds and fractions [6].

The aim of this study was to evaluate the antimicrobial activity of medicinal plant used in Ayurveda and traditional medicinal system for treatment of manifestations caused by microorganisms. Therefore, extracts of the nine plants i.e. *Barleria cuspidate* (Heyne) (Acanthaceae), *Cordia dichotoma* (Forsk) (Boraginaceae), *Cryptolepis buchanani* (R and S) (Asclepiadaceae), *Jasminum grandiflorum* (Oleaceae), *Madhuca longifolia* (Koenig) (Sapotaceae), *Pandanus tectorius (*Soland) (Pandanaceae), *Parkia biglandulosa* (W. and A.) (Mimosaceae), *Phyla nodiflora* (Linn) (Verbenaceae), *Pterospermum acerifolium* Willd (Stericuliaceae) were evaluated for their antimicrobial potentials.

MATERIALS AND METHODS

Plant Materials: The different parts of plants according to their use in Ayurveda and traditional systems of medicine were collected from various regions of Pune, Maharastra during October to February (Table 1). Plants were identified by Dr T. K. Mandal, Research Officer (Ay) at Regional Research Institute (Ay), Nehru Garden, Kothrud, Pune, where the voucher samples were preserved. The plant material was dried in shade.

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Table 1: List of Plant Used and their Ayurvedic Uses

S. No.	Botanical name	Family	VN	Common name	Plant part used	Ayurvedic or Traditional uses
1	*Barleria cuspidate* (Heyne)	Acanthaceae	642	Sahacharabhed	Leaves	Toothaches, Healing of Cuts and wounds, Cough, Inflammation
2	*Cordia dichotoma* (Forsk)	Boraginaceae	31	Sebesten plum	Leaves and Stem bark	Dyspepsia, Fever, Diarrhoea, Leprosy, Gonorrhoea, Burning Sensation
3	*Cryptolepis buchanani* (R and S)	Asclepiadaceae	351	Jambu Patra	Root	Diarrhoea, Fever, Cough, Dyspepsia, Etching, Vata rakta (gout)
4	*Jasminum grandiflorum* (Linn)	Oleaceae	666	Svarnajuthica	Leaves	Nasal Haemorrhage, Dermatosis, Leprosy, Malignant ulcer.
5	*Madhuca longifolia* (Koenig)	Sapotaceae	461	Mahua	Leaves and Stem bark	Astringent, Stimulant, Emollient, Demulcent, Rheumatism, Piles and Nutritive.
6	*Pandanus tectorius* Soland	Pandanaceae	692	Screw pine, Ketki	Leaves	Purgative, Leprosy, Smallpox, Scabies, Syphillis, Rheumatism, Headache, Blood disorder.
7	*Parkia biglandulosa* (W.andA.)	Mimosaceae	845	Chenduphala	Stem bark	Haemagglutinating, Ulcer, Nutritive, for tanning.
8	*Phyla nodiflora* (Linn)	Verbenaceae	656	Wildsage, Jalpippali	Whole plant	Cardiac tonic, Promotes eyesight and Spermatogenesis, Piles, Fever, Diuretics.
9	*Pterospermum acerifolium* Willd	Stericuliaceae	671	Karnikara, Hathipaila	Stem bark	Inflammation, For bleeding piles, Ulcers, Smallpox, Tumors and Leprosy.

VN- Voucher No of the Plant

Preparation of Plant Extracts: The powdered plant materials were extracted successively with n-hexane, chloroform, acetone, methanol and water to afford corresponding fractions [7]. Solvents were evaporated under reduced pressure and dry fractions were stored at 4°C for use.

Micro-Organisms: Clinical isolates of the microorganisms were used along with the standard strains. Quality control strains of *Aspergillus flavus* NCIM 549*, Aspergillus fumigatus* NCIM 902, *Aspergillus nigar* NCIM 620*, Candida albicans* NCIM 3471 *and Saccnaromyces creveaceae* NCIM 3284 obtained from Indian Type Culture Collection, National Chemical Laboratory, Pune. Seven bacterial stains *Escherichia coli* ATCC 11775*, Enterobacter aerogenes* ATCC13048*, Klebsiella pneumoniae* ATCC 15380*, Pseudomonas aeruginosa* ATCC10145*, Proteus vulgaris* ATCC 33420*, Staphylococcus aureus* ATCC 12600*, Salmonella typhi* ATCCB 23564 were included in each test as recommended by the Clinical Laboratories Standards Institute (CLSI), purchased from Bangalore Genei, India.

Antibacterial Screening: The *in vitro* antibacterial activity of plant extracts was determined by macrobroth dilution method [8]. Stock solutions (200 mg/ml) of plant extracts were prepared in suitable solvents viz. DMSO, acetone and water and filtered using 0.45 um sterile filters. Nutrient broth media was used for serial dilution. Nine serial dilutions were prepared, ranging from concentration of 25 mg/ml to 0.10 mg/ml of plant extract. The tubes were inoculated with 100 µl-of bacterial strain inoculums with a concentration of 10^6cell/ml. Ampicilln was used as a standard drug for comparison as a positive control. Nutrient broth was used as negative control. The tubes were incubated aerobically at 37°C for 18-24 h and the MIC of each sample was determined by using tetrazolium salts to indicate bacterial growth [6].

Antifungal Screening: Stock solutions (200 mg/ml) of plant extracts were prepared as discussed above. Macrobroth dilution assay was performed as described by Fromtling [9]. Seven serial dilutions in Sabourated Dextrose broth were prepared; ranging from concentration 25 mg/ml to 0.39 mg/ml. The tubes were inoculated with 100 µl inoculum of approximately 10^5 spores/ml. Tubes were incubated at 35°C for 48 hours. MICs were determined as per described by [10].

Qualitative Antibacterial Activity Assay by Bioautography: Bio-autography was done with agar overlay method. TLC plates were prepared using CAMAG HPTLC applicator (Model- Linomat 5) and developed in different solvent systems, dried for overnight under a stream of air to remove residual solvents. Inoculum was prepared by suspending the microorganism in nutrient

broth media with an approximate concentration of 10^6 cell/ml just before applying to the overlay. The TLC plates were placed in a sterile petri dish and covered with 4.5 ml of inoculum. It was kept at low temperature for some time, once the media has solidified; the plates incubated for 15 h at 36°C. Plates were sprayed with an aqueous solution of phenyl tetrazolium chloride 2.0 mg/ml. After incubating for about 1 h at 36°C; clear zones on chromatograms indicates inhibition of growth [11].

Solvent-solvent Fractionation: Solvent-solvent fractionation was used as a preliminary separation to simplify complex extracts with promising activity by fractionating chemical compounds into broad groups based on their solubility. The residue was dissolved in 1:1 mixture of chloroform and water and two phases was separated by separating funnel. Water fraction was mixed with equal volume of n-butanol in a separating funnel to yield water (W) and butanol (BT) fraction. The chloroform fraction was taken to dryness and after complete drying was extracted with equal volume of hexane and 10% water in methanol. This yielded Hexane (HE) fraction and 10% water/methanol was further diluted to 20% water/methanol by addition of water. This was mixed with equal volume of carbon tetrachloride in a separating funnel; yielded carbon tetrachloride fraction (CT) and 20% water /methanol was further diluted to 35% water/methanol with water by addition of water and was mixed with equal volume of chloroform in separating funnel, yielded chloroform (CH) and 35% water/methanol fraction(W/M) [6]. In all cases, equal volumes of solvents were used and extraction process repeated with a small volume three times. TLC and bioassay of six fractions obtained was carried out as described above. R_f values were also determined for active components. Zone of inhibitions were compared with that of standard antibiotic Kanamycin.

RESULTS

Plant Extracts: Properties of the plant used in the study are shown in the Table 1. Plants parts are selected as per use in Ayureveda and traditional system of medicines. Percent yield of plants extracts varies from 0.51 to 27.65% (Table 2). In most of the cases the amount of residue extracted with water and ethanol is high as compared to that of other solvents. Percent yield in case of hexane, chloroform and acetone extract was recorded to be variable and lower which varies from 0.51 to 10.59% where as in ethanol and water extract there variability was found to 0.85 to 27.65%.

Antibacterial Activity: Various parts of nine plants were evaluated for their antimicrobial potential against twelve microorganisms microbroth dilution assay. Table 3 summarizes the results obtained for the plant species presented some activity against at least one microorganism. Ampicillin, the positive control used in this study shows MICs in the range 0.05-0.20 mg/ml against different bacterial strains. Fifty five extracts of nine plants tested for their antimicrobial potential and seven plants were found to be active. MIC values for different plant extracts are shown in Table 3. *P. nodiflora* and *C. dichotoma* plant extract were found to be inactive. *B cuspidate* and *P. tectorius* extracts showed variable weak activity in the range of 6.25 to 25.00 mg/ml. Highest antimicrobial potential was observed with ethanol extract of *P. acerifolium* (MIC 0.20 mg/ml) against *P. aeruginosa*,*P. vulgaris, S. aureus;* acetone extract of *P. biglandulosa* (MIC 0.20 mg/ml) against *K. pneumoniae*, *P. aeruginosa*, *P. vulgaris* and *S. aureus* (Table 3) and ethanol extract of *M. longifolia* (Stem bark) (MIC 0.20 mg/ml) against *P. aeruginosa*, *P. vulgaris, S. typhi.* In *C. buchanani.* Acetone extracts of these plants were observed to be active against all the strains in

Table 2: Percentage Yields of Plants extracts

			Percentage Yield of Extracts				
S. No	Name of Plant	Part Used	Hexane	Chloroform	Acetone	Ethanol	Water
1.	*B. cuspidate*	Leaf	4.40	5.00	2.56	12.3	4.06
	C .dichotoma	Leaf	1.48	1.65	0.76	1.09	14.51
	C. dichotoma	Stem bark	0.97	1.78	0.32	1.15	7.19
	C. buchanani	Root	2.0	0.51	1.02	0.85	6.74
	J. grandiflorum	Leaf	1.50	10.59	7.71	9.50	19.0
	M. longifolia	Leaf	1.23	1.30	2.60	5.0	13.08
	M. longifolia	Stem Bark	2.30	0.55	2.25	5.0	13.0
	P. tectorius	Leaf	2.05	1.36	1.14	8.41	2.83
	P. biglandulosa	Stem bark	0.82	1.35	1.49	2.70	24.32
	P. nodiflora	Whole Plant	1.88	1.40	2.05	4.0	27.65
	P. acerifolium	Stem bark	0.93	0.52	1.21	3.26	15.31

Table 3: MIC of plant extracts against the microorganisms by Macro Dilution broth assay

	MIC in mg/ml											
Plants	A	B	C	D	E	F	G	H	I	J	K	L
B. cuspidate (Leaves)												
Hexane	12.5	12.5	-	-	-	-	-	-	-	-	-	-
Chloroform	25.0	25.0	-	-	-	-	-	-	-	-	-	25.0
Acetone	12.5	25.0	12.5	-	-	25.0	-	-	25.0	-	-	-
Ethanol	-	-	-	-	-	-	-	12.5	25.0	25.0	-	25.0
Water	-	-	-	-	-	-	-	-	-	-	-	-
C. dichotoma (Leaves)												
Hexane	-	-	-	-	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	--	-	-	-	-
Acetone	-	-	-	-	-	-	-	-	-	-	-	-
Ethanol	-	-	-	-	-	-	-	-	-	-	--	-
Water	-	-	-	-	-	-	-	-	-	-	-	-
C. dichotoma (Stem bark)												
Hexane	-	-	-	-	-	--	-	--	-	-	-	3.125
Chloroform	-	-	-	-	-	-	-	-	-	-	-	-
Acetone	-	-	-	-	-	-	-	-	-	-	-	-
Ethanol	-	-	-	-	-	-	-	-	25.0	-	-	3.125
Water	-	-	-	-	-	-	-	-	-	-	-	-
C. buchanani (Root)												
Hexane	-	-	-	-	--	-	-	-	-	-	-	-
Chloroform	-	-	-	3.125	3.125	-	-	-	-	-	-	25.0
Acetone	6.25	6.25	6.25	3.125	1.56	3.125	6.25	-	25.0	-	-	-
Ethanol	-	-	-	-	-	-	-	-	25.0	-	-	-
Water	-	-	-	-	-	-	-	-	-	-	-	12.5
J. grandiflorum (Leaves)												
Hexane	-	-	-	3.125	-	-	12.5	-	-	--	-	-
Chloroform	1.56	3.125	3.125	3.125	3.125	6.25	6.25	-	-	-	-	25.0
Acetone	-	6.25	6.25	6.25	6.25	-	-	-	-	-	-	-
Ethanol	12.5	-	-	12.5	-	12.5	-	-	-	-	-	-
Water	12.5	12.5	-	12.5	12.5	12.5	-	-	25.0	-	-	25.0
M. longifolia (Leaves)												
Hexane	-	-	-	-	-	-	-	-	-	-	25.0	12.5
Chloroform	6.25	6.25	6.25	-	-	12.5	-	-	-	-	-	--
Acetone	3.125	3.125	1.56	1.56	3.125	1.56	3.125	-	12.5	-	25.0	6.25
Ethanol	1.56	3.125	3.125	3.125	3.125	1.56	6.25	-	25.0	-	25.0	3.125
Water	3.125	1.56	1.56	1.56	1.56	1.56	3.125	-	12.5	-	-	12.5
M. longifolia(Stem bark)												
Hexane	-	-	-	-	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-	-	-	-	-
Acetone	6.25	6.25	3.125	12.5	6.25	3.125	6.25	-	-	-	-	-
Ethanol	-	-	25.0	0.20	0.20	0.39	0.20	-	-	-	-	25.0
Water	3.125	3.125	6.25	0.78	0.78	0.78	6.25	-	-	-	-	-
P. tectorius (Leaves)												
Hexane	-	-	-	-	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-	-	-	-	25.0
Acetone	12.5	12.5	25.0	25.0	12.5	25.0	12.5	-	12.5	-	-	12.5
Ethanol	12.5	12.5	12.5	6.25	6.25	12.5	12.5	12.5	6.25	12.5	-	6.25
Water	-	-	-	-	-	-	-	-	-	-	-	-

Table 3: Continued

P. biglandulosa (Stem bark)												
Hexane	-	-	-	-	-	-	-	-	-	-	-	-
Chloroform	-	-	12.5	-	-	-	-	-	-	-	-	-
Acetone	1.56	1.56	0.20	0.20	0.20	0.20	0.39	12.5	6.25	12.5	-	6.25
Ethanol	1.56	1.56	0.39	0.39	0.39	0.39	0.39	12.5	12.5	6.25	-	3.125
Water	1.56	1.56	0.78	0.39	0.39	1.56	0.39	12.5	25.0	12.5	-	6.25
P. nodiflora (Whole plant)												
Hexane	-	-	-	-	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-	-	-	-	-
Acetone	-	-	-	-	-	-	-	-	-	-	-	-
Ethanol	-	-	-	-	-	-	-	--	-	-	-	-
Water	-	-	-	-	-	-	-	-	-	-	-	-
P. acerifolium (Stem bark)												
Hexane	-	-	-	-	-	-	-	-	-	-	-	-
Chloroform	-	-	-	12.5	-	-	-	-	-	-	-	-
Acetone	3.125	3.125	-	0.39	0.39	0.39	0.39	-	6.25	-	-	25.0
Ethanol	6.25	6.25	6.25	0.20	0.20	0.20	0.78	-	12.5	12.5	-	12.5
Water	12.5	6.25	6.25	0.78	0.78	0.78	0.78	-	-	-	-	-
Ampicilln	0.15	0.20	0.05	0.125	0.175	0.075	0.10	ND	ND	ND	ND	ND
Gentamicin	ND	ND	ND	ND	ND	ND	ND	0.02	0.03	0.02	0.03	0.05

Note: A= *E. coli;* B= *E. aerogenes;* C= *K. pneumoniae;* D= *P. aeruginosa;* E= *P. vulgaris;* F= *S. aureus;* G= *S. typhi;* H= *A.*

the range of 1.56-6.25 mg/ml. Chloroform extract showed growth inhibition at 3.125 mg/ml against *P. aeruginosa* and *P. vulgaris*. n-Hexane, ethanol and water extracts did not show activity against any bacterial species. In *J. grandiflorum*, chloroform extract was found to be active against all seven bacterial strains in the range of 1.56-6.25 mg/ml; while n-hexane, acetone, ethanol and water extract shows activity against one or more strains. *M.* l*ongifolia* (Stem bark) ethanol extract exhibited activity against *P. aeruginosa, P. vulgaris* and *S. typhi* (MIC 0.20 mg/ml). Acetone extract prepared from leaves of *M.* l*ongifolia* found to be active against all tested bacterial strains in the range 1.56-3.125 mg/ml. Acetone, ethanol and water extracts prepared of *P. biglandulosa* were found to be active against all bacteria in a range of 0.20 -1.56 mg/ml. *P. acerifolium* ethanol extract was observed to be most active having MIC value of 0.20 mg/ml for *P. aeruginosa, P. vulgaris* and *S. aureus*. Acetone and water extract were found to be active for bacterial strains in the range of 0.39- 12.5 mg/ml.

Antifungal Activity: Extracts prepared from stem bark of *M.* l*ongifolia, P. nodiflora* and *C. dichotoma* were found to be ineffective against fungal strains. *P. biglandulosa* was observed to be active against fungal strains in a range of 3.125- 12.5 mg/ml. *C. dichotoma (s*tem bark) hexane and water extracts showed limited antifungal activity against *S. creveaceae* only (Table 3). Ethanol and acetone extract prepared from leaves of *M. longifolia* were found to be active against *A. fumigatus A. nigar* and *S. creveaceae* in the range of 3.125- 25.0 mg/ml. In *P. tectorius* only acetone and ethanol extract showed antifungal activity in the range of 6.25-25.0 mg/ml. Acetone and ethanol extracts of *P. acerifolium* showed antifungal activity at 6.25- 12.5 mg/ml against *A. fumigatus, C. albicans* and *S. creveaceae.*

Agar Over-lay Assay: Some selected plants extracts were subjected to further fractionation. TLC- Bioautography assays were performed for extracts as well for most active fractions. Bioautography showed presence of one or more active compounds in extracts and fractions at different R_f values. In *C. buchanani* (CB) (acetone extract) two compounds CB1 and CB2 at R_f values 0.47 and 0.93 respectively were found to be active. Compound CB1 was found to be active against *S. aureus* and *K. pneumoniae* while CB2 showed zone of inhibition for *E. coli* only (Fig 1). Chloroform extract of *J. grandiflorum* revealed presence of four active compounds; JG1, JG2, JG3 and JG4 at R_f values 0.24, 0.36, 0.86 and 0.97 respectively. Compound JG1 found to be active against *K. pneumoniae* and *S. aureus;* compound JG2 and JG3 against *S. typhi* and JG4 against *E. coli*. Multiple active compounds active against *S. typhi* in JH fraction were observed (Fig 1). *P. biglandulosa* (PB) acetone extract formed zones of inhibitions against *S. aureus* at R_f values 0.44 (PB1),

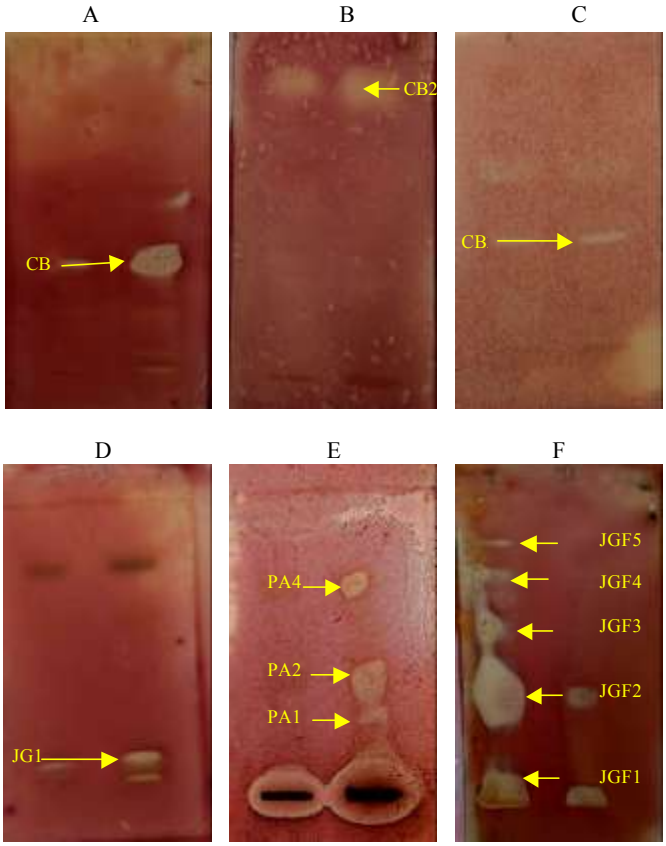

Fig. 1: Bio-assays of acetone extract of *C. buchanani* (Root) with *S. aureus* (A), *E. coli* (B) and *K. pneumoniae* (C); Chloroform extract of *J. grandiflorum* (Leaf) with *S. aureus* (D) and Ethanol extracts of *P. acerifolium* (Stem Bark) with *S. aureus* (E). F: - Bioassay profile of *J. grandiflorum* (CH fraction) from chloroform extract with *S. aureus*.

0.68 (PB2) and 0.88 (PB3). Compound PB1 was found to active against *S. aureus* while compounds PB2 and PB3, both were observed to be active against all the stains tested. In acetone extract of plant *M. longifolia* (ML) (leaves) compound ML1 showed activity for *S. aureus* at R_f value 0.74. In *P. acerifolium* (PA) five compounds PA1, PA2, PA3, PA4 and PA5 founds to be active at R_f value 0.38, 0.46, 0.50, 0.69 and 0.94 respectively. Compounds PA1, PA2 and PA4 were active against *S. aureus* whereas compounds PA3 and PA5 showed activity against *K. pneumonia* and *E. coli*. The chloroform (CH) and W/M fractions of *J. grandiflorum* (JG) were tested for antimicrobial activity using bioassay for *S. aureus* and *K. pneumoniae*. CH fraction showed presence of five active compounds JGF1, JGF2, JGF3, JGF4 and JGF5 at R_f values 0.21, 0.50, 0.63, 0.79 and 0.87 respectively. Compounds JGF1, JGF2 and JGF5 were observed to be active against *S. aureus* and JGF2, JGF3, JGF4 and JGF5 against *K. pneumoniae*. Diameters of zone of inhibition for CH fraction were compared with that of Kanamycin. Diameter of zone of inhibition (±7.5 mm at a concentration of 300 µg of CH fraction) at R_f 0.50 was less than diameter of zone of inhibition developed by kanamycin (± 16.5 mm at a concentration of 300 µg). The CH fraction shows growth inhibition in bio-assay at lowest concentration of 75 µg on TLC plate.

DISCUSSION AND CONCLUSION

Crude plant extracts are generally a mixture of active and non-active compounds. A number of medicinal plant described in Ayurveda still need to be testify according to the modern parameters to ensure their activity and efficacy. Medicinal properties of plants studies are enlisted in Table 1. Drugs used in Ayurveda are mostly prepared by extraction with water. Therefore healers may not be able to extract all the active compound(s). In this study the acetone and ethanol extracts of all plants studied showed greater antibacterial activity as compared to their water extract. Same observations have been

reported earlier by various authors [6, 12, 13]. Precent yields were observed to be at more in case of ethanol and water extracts (Table 2).

With more refined and solitary compound MICs of less than 0.1 mg/ml may be suggestive of good anti-microbial activity. Seven out of nine plants used in this study were found to be endowed with antimicrobial activity in a range of 0.20-25.0 mg/ml. *M. longifolia, P. biglandulosa; P. acerifolium* showed reasonable activity. *B. cuspidate, C. buchanani, J. grandiflorum* and *P. tectorius* were found to be active in the range 1.56-25.0 mg/ml (Table 3). Extract from stem bark of *M. longifolia* were observed to have better activity than leaves. Acetone and water extracts of the plant were found to have broad range antibacterial activity (MIC 0.78 to 12.5 mg/ml). Ethanol extract was observed to have selective but better activity in range of 0.20 to 0.39 mg/ml (Table 3). Methanol extract of flowers, leaves, stem and stem bark of *M. longifolia* had been reported earlier to have antimicrobial activity [14].

P. acerifolium, ethanol and water extracts were found to be active against all bacterial strains tested in the range 0.20-12.5 mg/ml. For *A. fumigatus*, *A. nigar and S. creveaceae* MIC values were observed to be in higher range of 6.25- 12.5 mg/ml. Water extract from leaves of P. *acerifolium* had been reported earlier for prominent antimicrobial activity with 20 mm zone of inhibition against several gram positive and gram negative human pathogenic bacteria [15]. *P. biglandulosa* had been previously reported for anti-inflammatory and antiulcer activity [16] although its antimicrobial potential has not been reported. *P. biglandulosa,* acetone, ethanol, water extracts were found to have broad range activity (MIC 0.20- 1.56 mg/ml) (Table 3).

The agar overlay technique is a hybrid of the two other methods and works successfully with a range of microorganism; including *E. coli*, *P. aeruginosa, S. aureus* and *S. typhi* [17]. Bio-assay of acetone extract of *C. buchanan* showed two active compounds CB1 and CB2 having selective activity. Acetone extract of *C. buchanan* showed antibacterial activity with MIC 1.56- 6.25 mg/ml (Table 4). Same observation has been recorded earlier with water extract of *C. buchanan* leaves [18].

Bioassay with acetone extract of P. *biglandulosa* exhibited three zones of inhibitions at different R_f values for *S. aureus* (Table 4). Acetone extract of *P. biglandulosa* and chloroform extract of *J. grandiflorum* were subjected to solvent-solvent fractionation. W/M fraction of P. *biglandulosa* was found to be active for K. *pneumoniae* (MIC 0.0625 mg/ml). W/M fraction of plant *P. biglandulosa* showed presence of five active principles (Table 4).

Table 4: Bioautography of active plant Extracts

S. No	Plant	Solvent for Extraction	Solvent System	Compound	Rf value	Strain
1	C. buchanan (Root)	Acetone	Toluene: Ethyl formate:	CB1	0.47	S. aureus, K. pneumoniae
			Formic acid (6:3:1)	CB2	0.93	E .coli
2	J. grandiflorum(Leaves)	chloroform	Cyclohexane:	JG1	0.24	K. pneumoniae, S. aureus
			Ethyl Acetate (7:3)	JG2	0.36	S. typhi
				JG3	0.86	S. typhi
				JG4	0.97	E. coli
3	M.longifolia (Leaves)	Acetone	Toluene: Ethyl formate:	ML1	0.74	S. aureus
			Formic acid (5:4:1)			
4	P. biglandulosa (Stem bark)	Acetone	Toluene: Ethyl formate:	PB1	0.44	S. aureus
			Formic acid (6:3:1)	PB2	0.68	S. aureus, K. pneumoniae
				PB3	0.88	S. aureus, K. pneumoniae
5	P. acrifolium(Stem bark)	Acetone	Toluene: Ethyl formate:	PA1	0.38	S. aureus
			Formic acid (6:3:1)	PA2	0.46	S. aureus
				PA4	0.69	S. aureus
		Ethanol	Toluene: Ethyl formate:	PA3	0.50	K. pneumoniae
			Formic acid (2:7:1)	PA5	0.94	E. coli
6	J. grandiflorum (chloroform extract)	CH fraction	Cyclohexane: ethyl acetate (4:6)	JGF1	0.21	S. aureus
				JGF2	0.50	S. aureus, K. pneumoniae
				JGF3	0.63	K. pneumoniae
				JGF4	0.79	K. pneumoniae
				JGF5	0.87	S. aureus, K. pneumoniae
7	P. biglandulosa(Acetone extract)	W/M fraction	Toluene: Ethyl formate:	PBF1	0.38	E. aerogenes, K. pneumoniae
			Formic acid (4:5:1)	PBF2	0.41	P. vulgaris
				PBF3	0.54	K. pneumoniae
				PBF4	0.60	K. pneumoniae
				PBF5	0.72	S. aureus, S. typhi

The chloroform extract of *J. grandiflorum* had shown the presence of five zones of inhibitions at different R_f values from CH fraction (Table 4). CH fraction showed MIC at 1.56 mg/ml for *K. pneumoniae.* In comparative studies with Kanamycin, utilizing agar over lay assay, zone of inhibition in case of JGF1 and JGF2 were observed 8 mm as compared to 16.5 mm in case of Kanamycin at a concentration of 300 µg. The CH fraction which contains at least five compounds was less active than Kanamycin but isolated compounds may have comparable activity to the Kanamycin (Data not shown).

Bio-assay revealed the presence of specific and selective antimicrobial compounds in the fractions and extracts which may or may not have broad range activities. Broad range activity of plant extracts as per observations in this study was due to presence of multiple antimicrobial compounds or synergic effects of these compounds. Therefore, standardization of active fractions and study for toxicity and *in vivo* efficacy may result in development of better antimicrobial drugs. It may provide nature friendly and cheap drugs accessible to all the people of world. Furthermore, the antimicrobial compounds from *C. buchanani*, *J. grandiflorum, P. biglandulosa, P. acerifolium* have not been reported till date. Therefore further exploration of these plants for isolation of active compounds may be considered.

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