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Evaluation of the Antibacterial Potential of Some Plants Against Human Pathogenic Bacteria

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Abstract: Plants are rich source of antibacterial agents, which could be exploited in human disease management. Aqueous extracts of leaves of 46 plants selected based on an ethnobotanical survey from Mysore region Karnataka (India) were subjected to *in vitro* antibacterial activity assay against 14 important human pathogenic bacteria employing cup diffusion method. Antibacterial activity of the twelve plants aqueous extracts was compared with antibiotics. MIC was determined for aqueous extracts of the plants that recorded antibacterial activity. It is indicated that only twelve plants (26%) exhibited antibacterial activity against test pathogens and the spectrum of activity was varied among the pathogens. The inhibitory activity was highly significant in the aqueous extracts of *Acacia nilotica*, *Oxalis corniculata* and *Lawsonia inermis*. Most of the plant extracts showed significant antibacterial activity than bacitracin. MIC of aqueous extract of twelve plants varied between 4-50 µl. Results indicate the potential of these plants for further work on isolation and characterization of the active principle responsible for antibacterial activity and its exploitation as therapeutic agent.

Key words: Antibacterial activity · *Acacia nilotica* · *Oxalis corniculata* · *Lawsonia inermis*

INTRODUCTION

Many higher plants accumulate extractable organic substances in quantities sufficient to be economically useful as pharmaceuticals/antibiotics. Species of higher plants are much less surveyed for antibacterial activity [1]. Plants have been a rich source of medicines because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection. It is estimated that only one percent of 2,65,000 flowering plants on earth have been studied exhaustively for their chemical composition and medicinal value [2].

In many developing countries traditional medicine is one of the primary health care systems [3, 4]. India is well known for Ayurveda, which is one of important traditional medicine practiced. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented [5]. Plants grown in this region are not systematically tested for their biological activities in general and antimicrobial activity in particular.

Alternatives to available antibiotics for disease management are increasingly felt due to the increase in the resistance of bacterial isolates. This has necessitated the requirement of second and third line drug [6]. Antibacterial active principles isolated from higher plants is appears to be one of the important alternative approaches to contain antibiotic resistance and the management of disease. It is believed that plant based drugs cause less or no side effect when compared with synthetic antibiotics [7]. Large scale evaluation of the local flora exploited in traditional medicine for various biological activities is a necessary first step in the isolation and characterization of the active principle and further leading to drug development. In view of these forty-six plant species were screened for antibacterial potential against important fourteen human pathogenic bacteria was screened for antibacterial activity against fourteen important human pathogenic bacteria.

MATERIALS AND METHODS

Plant Material: Fresh disease free leaves of forty-six plant species were collected from Mysore, Karnataka, India [Table - 1]. The leaves were washed thoroughly several times with running tap water and once with sterile distilled water. The leaf material was then air-dried on a sterile blotter under shade. A voucher specimen of all the plants has been deposited in the herbarium of Department of Studies in Botany, University of Mysore, Mysore.

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Table 1: List of plant species tested for antibacterial activity

Sl.No.	Name of the Plant	Family
1	*Acacia nilotica* (L.) Del.	Mimosaceae
2	*Achras zapota* L.	Sapotaceae
3	*Aegle marmelos* Corr.	Rutaceae
4	*Aloe vera* Linn.	Liliaceae
5	*Anacardium occidentale* L.	Anacardiaceae
6	*Artocarpus heterophyllus* Lamb.	Moraceae
7	*Azadirachta indica* A. Juss.	Meliaceae
8	*Boerhaavia rependa* Willd.	Nyctanginaceae
9	*Calotropis gigantea* R. Br.	Asclepidaceae
10	*Catharanthus roseus* (L.) G. Don.	Apocyanaceae
11	*Clerodendron inerme* Gaertn.	Verbenaceae
12	*Coleus aromaticus* Benth.	Lamiaceae
13	*Cuscuta chinensis* Lam.	Cuscutaceae
14	*Datura stramonium* L.	Solanaceae
15	*Delonix regia* Raf.	Caesalpinaceae
16	*Derris indica* (Lawk.) Bennet	Fabaceae
17	*Dolichos lablab* L.	Fabaceae
18	*Emblica officinalis* Gaertn.	Euphorbiaceae
19	*Eucalyptus globulis* Labill.	Myrtaceae
20	*Euphorbia pulcherrima* Willd.	Euphorbiaceae
21	*Hibiscus vitifolius* L.	Malvaceae
22	*Jacaranda acutifolia* Humb and Bonpl.	Bignoniaceae
23	*Lantana camara* L.	Verbenaceae
24	*Lawsonia inermis* L.	Lythraceae
25	*Leucas aspera* L.	Myrtaceae
26	*Macroslen parasiticus* (L.) Danser.	Loranthaceae
27	*Mimosa pudica* L.	Mimosaceae
28	*Mimusops elengi* L.	Rubiaceae
29	*Morinda tinctoria* Roxb.	Sapotaceae
30	*Moringa oleifera* Lam.	Moringaceae
31	*Murraya koenigii* (L.) Spreng.	Rutaceae
32	*Oxalis corniculata* L.	Oxalidaceae
33	*Peltophorum pterocarpum*(DC.) Baker ex Heyne.	Caesalpinaceae
34	*Phyllanthus acidus* Linn.	Euphorbiaceae
35	*Plumbago zeylanica* L.	Plumbaginaceae
36	*Polyanthia longifolia* HK. F & T.	Annonaceae
37	*Psidium guajuva* L.	Myrtaceae
38	*Punica granatum* L.	Punicaceae
39	*Salvia offinalis* L.	Lamiaceae
40	*Samanea saman* Prain.	Mimosaceae
41	*Sapindus laurifolius* Vahl.	Sapindaceae
42	*Spathodea campanulata* Beaur.	Bignoniaceae
43	*Syzygium cumini* (L.) Skeels	Myrtaceae
44	*Tabebuia argentia* Britt.	Bignoniaceae
45	*Tamarindus indica* L.	Caesalpinaceae
46	*Viscum orientale* Willd.	Viscaceae

Extraction: Leaf samples (50g) of the plants were thoroughly washed, blot dried and macerated with 100ml sterile distilled water in a waring blender (Waring international, New Hartford, CT, USA) for 10 min. The macerate was first filtered through double layered muslin cloth and then centrifuged at 4000*g* for 30 min. The supernatant was filtered through Whatmann No. 1 filter paper and heat sterilised at 120°C for 30 min. This served as a mother extract. The extracts were preserved aseptically in sterile brown bottles at 5°C until further use.

Bacterial Cultures: Clinical isolates of *Citrobacter* sp., *Escherichia coli*, *Klebsiella* sp., *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Salmonella typhimurium*, *Salmonella paratyphi* A, *Salmonella paratyphi* B, *Shigella boydii*, *Shigella flexneri*, *Shigella sonnei, Staphylococcus aureus* and *Streptococcus faecalis* were obtained from the Department of Microbiology, Government Medical College, Mysore, India. All the test strains were maintained on nutrient agar slopes (Hi-Media) and were subcultured once in every two-week. These bacteria served as test pathogens for antibacterial activity assay.

Antibacterial Activity Assay: Antibacterial activity of the aqueous extracts was determined by cup diffusion method on nutrient agar medium [8]. Cups are made in nutrient agar plate using cork borer (5 mm) and inoculum containing 10^6 CFU/ml of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then 50 µl of the aqueous extract was placed in the cups made in inoculated plates, the treatments also included 50 µl of sterilized distilled water, which served as control. All the plates were incubated for 24 h at 37°C and zone of inhibition if any around the wells was measured in mm (millimeter). For each treatment 12 replicates were maintained. Antibiotics (10mcg) Bacitracin and Ciprofloxacin were used as reference to determine the sensitivity of each bacterial species tested.

Determination of Minimal Inhibitory Concentration (MIC): MIC was determined by both agar and broth dilution methods. For broth dilution tests, 0.1ml of standardized suspension of bacteria (10^6 CFU/ml) was added to each tube containing different concentrations of the aqueous extracts (02-50 µl/ml) and incubated for 24h. at 37°C. In agar plating method dilutions having 02-50 µl of aqueous extracts was placed in the cups on the inoculated plate and incubated as mentioned above. The lowest concentration of the tube or plate that did not show any visible growth by macroscopic evaluation was considered as the MIC.

The data was subjected to stastical analysis of. SPSS for windows.

RESULTS

Among the forty-six plant species tested only twelve species recorded different degrees of antibacterial activity as evidenced by the zone of inhibition [Table 2], whereas the other plant species did not show any inhibitory activity.

Table 2: Antibacterial activity measured as a zone of inhibition (mm) of aqueous extracts of twelve plant species on fourteen human pathogenic bacteria

Sl. No.	Plants	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	*Acacia nilotica*	13.33±1.11	15.75±0.13	11.00±0.90	15.92±1.84	09.17±0.17	11.42±0.47	11.92±0.47	12.42±0.78	08.83±0.11	15.00±0.60	14.33±1.11	13.25±0.28	38.50±3.61	12.83±0.43
2	*Anacardium occidentale*	00.00±0.00	09.17±0.11	08.92±0.02	09.92±0.15	09.17±0.11	12.08±0.15	10.17±0.17	09.00±0.00	08.33±0.14	07.00±0.00	10.08±0.23	00.00±0.00	09.83±0.11	00.00±0.00
3	*Emblica officinalis*	00.00±0.00	14.25±0.13	09.58±0.15	13.08±0.23	11.08±0.29	11.83±0.17	10.58±0.15	12.25±0.13	00.00±0.00	00.00±0.00	14.75±0.22	00.00±0.00	14.92±1.06	15.42±0.87
4	*Lawsonia inermis*	10.17±0.11	12.17±0.58	10.00±0.17	08.92±0.15	10.17±0.11	11.42±0.26	10.67±0.56	16.92±0.68	11.83±0.11	13.17±1.46	10.75±0.91	13.50±1.36	17.50±1.37	16.83±0.92
5	*Macroslen parasiticus*	08.83±0.11	00.00±0.00	00.00±0.00	09.00±0.21	08.83±0.11	09.17±0.11	08.58±0.34	09.92±0.15	08.50±0.15	0.00±0.00	0.00±0.00	0.00±0.00	09.42±0.15	00.00±0.00
6	*Manilkara zapota*	16.33±0.15	15.75±0.35	00.00±0.00	16.00±1.81	08.75±0.18	11.83±0.37	11.92±0.47	14.92±0.15	08.67±0.14	15.50±0.58	14.33±1.11	13.58±0.19	24.25±2.83	12.17±0.64
7	*Oxalis corniculata*	16.58±0.61	17.50±0.38	12.23±0.19	00.00±0.00	17.00±0.37	14.08±1.09	19.08±0.88	16.17±0.34	20.25±0.64	10.17±0.47	15.25±1.44	00.00±0.00	15.50±1.96	20.00±1.82
8	*Punica granatum*	09.83±0.11	12.33±0.14	09.08±0.19	12.17±0.21	10.67±0.14	10.58±0.19	08.00±0.00	10.42±0.15	09.92±0.19	08.00±0.30	14.25±0.28	0.00±0.00	15.08±1.40	13.50±0.19
9	*Samanea saman*	14.83±0.27	15.83±0.10	15.92±0.50	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	12.83±0.21	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	21.08±1.28	0.00±0.00
10	*Syzygium cumini*	09.50±0.15	0.00±0.00	09.50±0.15	11.33±0.22	10.00±0.00	09.83±0.21	09.25±0.13	08.50±0.15	10.75±0.25	08.00±0.00	13.83±0.52	07.33±0.14	09.00±0.00	00.00±0.00
11	*Tamarindus indica*	00.00±0.00	00.00±0.00	10.00±0.25	10.50±0.06	13.17±0.21	09.58±1.16	09.33±0.56	00.00±0.00	10.33±0.14	00.00±0.00	09.83±0.17	0.00±0.00	10.63±0.81	22.00±0.00
12	*Viscum orientale*	09.42±0.23	0.00±0.00	09.67±0.14	10.50±1.06	00.00±0.00	12.75±0.25	12.17±0.68	11.08±0.15	12.00±0.00	12.17±0.78	13.00±0.90	11.67±0.50	11.42±0.69	00.00±0.00
13	Bacitracin	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	26.75±0.84	0.00±0.00
14	Ciprofloxacin	19.62±0.18	0.00±0.00	20.25±0.16	18.25±0.16	34.25±0.16	27.75±0.16	27.63±0.18	20.25±0.16	18.75±0.31	17.75±0.16	27.63±0.18	21.75±0.16	18.13±0.48	0.00±0.00

1. *Citrobacter* sp. 2. *Escherichia coli* 3. *Klebsiella* sp. 4. *Proteus mirabilis* 5. *Pseudomonas aeruginosa* 6. *Salmonella paratyphi* A
7. *Salmonella paratyphi* B 8. *Salmonella typhi* 9. *Salmonella typhimurium* 10. *Shigella boydii* 11. *Shigella flexneri* 12. *Shigella sonnei*Values are expressed as mean ± S.D. of 12 replicates 13. *Staphylococcus aureus* 14. *Streptococcus faecalis*

Table 3: MIC of aqueous extracts of twelve plant species on fourteen human pathogenic bacteria

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Acacia nilotica	10	10	50	08	40	10	10	10	40	10	10	10	04	10
Anacardium occidentale	nd	50	40	30	40	10	30	50	50	50	30	nd	30	nd
Emblica officinalis	nd	10	20	10	30	10	30	10	nd	nd	10	nd	06	08
Lawsonia inermis	30	20	30	40	40	10	30	10	30	10	30	10	04	08
Macroslen parasiticus	40	nd	nd	40	40	40	50	40	50	nd	nd	nd	20	nd
Manilkara zapota	10	10	nd	10	40	10	20	10	40	10	10	10	06	10
Oxalis corniculata	08	08	04	nd	10	08	06	06	04	10	08	nd	06	08
Punica granatum	40	30	40	10	20	20	50	40	40	40	10	nd	10	06
Samanea saman	10	20	10	nd	nd	nd	nd	nd	nd	nd	nd	nd	04	nd
Syzygium cumini	30	nd	40	10	30	20	40	20	30	40	10	50	50	nd
Tamarindus indica	nd	nd	40	50	10	20	40	40	40	nd	40	nd	30	10
Viscum orientale	30	nd	40	50	nd	30	10	20	10	40	10	20	10	nd

1. *Citrobacter* sp. 2. *Escherichia coli* 3. *Klebsiella* sp. 4. *Proteus mirabilis* 5. *Pseudomonas aeruginosa*
6. *Samonella paratyphi* A 7. *Salmonella paratyphi* B 8. *Salmonella typhi* 9. *Salmonella typhimurium* 10. *Shigella boydii*
11. *Shigella flexneri* 12. *Shigella sonnei* 13. *Staphylococcus aureus* 14. *Streptococcus faecalis* nd = not determined
Values are expressed as mean ± S.D. of 12 replicates

All the test bacteria were inhibited by *Acacia nilotica* and *Lawsonia inermis* demonstrating broad spectrum of activity. *Manilkara zapota* showed inhibitory activity against all bacteria except *Klebsiella* sp., where as *Oxalis corniculata* was effective against all the tested bacteria except *Shigella sonnei* and *Proteus mirabilis. Viscum orientale* was effective against all test bacteria except *E. coli*, *Pseudomonas a eruginosa Proteus mirabilis* and *Streptococcus faecalis*. *Emblica officinalis* was not effective against *Citrobacter* sp., *Salmonella typhimurium*, *Shigella boydii* and *Shigella sonnei.* The antibiotic bacitracin was not effective against any of the Gram-negative test bacteria where as the activity was observed only against *Staphylococcus aureus* which is Gram-positive. Ciprofloxacin recorded broad spectrum of activity and was not effective against *E. coli* and *Streptococcus faecalis*.

Highly significant degree of activity was observed against all the test bacteria in case of *Acacia nilotica* and *Lawsonia inermis*. The zone of inhibition in case of *Acacia nilotica* varied between 9mm to 35.5mm. Where as in case of *Lawsonia inermis* it varied between 9 to 17.5mm. Highest degree of zone of inhibition was observed against *Staphylococcus aureus* by both the plants. More than 20mm of zone of inhibition was recorded by *Acacia nilotica, Lawsonia inermis, Manilkara zapota, Samanea saman* and bacitracin against *Staphylococcus aureus*. The zone of inhibition in case of *Oxalis corniculata* was 19mm against *Salm.* paratyphi B and more than 20mm against *Salm. typhimurium.*

Minimal Inhibitory Concentration [MIC] of the twelve different plants extract varied against different test pathogens. Some plants extract did not show any activity

even at 50 µl concentration. The MIC of the plant extract required for the test pathogens is presented in Table 3. Lowest MIC of 4 µl against *Staph. aureus* was observed in case of *Acacia nilotica*, *Emblica officinalis* and *Samanea saman*. Where as highest MIC of 50 µl was needed to inhibit *Klebsiella* sp. by *Acacia nilotica.*

DISCUSSION

Ethnobotanical approach is one of the common methods that are employed in choosing the plants for pharmacological study [2]. India is one of the twelve mega biodiversity centers having more than 45,000 plant species. Its diversity is unmatched due to the presence of sixteen different agroclimatic zones, 10 vegetative zone and 15 biotic provinces [9]. Use of plants as a source of medicine has been inherited and is an important component of the health care system. Approximately 20% of the plants found in the world have been submitted to pharmacological or biological tests [10]. The systemic screening of plant extracts for antibacterial activity is a continuous effort to find new antibacterial compounds. Considering the rich diversity of plants in Karnataka, it is necessary to screen plants for their antibacterial activity.

A special feature of higher angiospermic plants is their capacity to produce a large number of organic chemicals of high structural diversity. Hence more than 46 plant species of the local flora have been screened for their antibacterial potential for the first time and among these 12 plant species have been identified d to posses antibacterial activity against human pathogenic bacteria. *Acacia nilotica*, *Oxalis corniculata* and *Lawsonia inermis* possess broad spectrum of activity and a high degree of activity against the test pathogens. The results of the present investigations suggest that these three plants are important candidate plants for further investigations on isolation and characterization of the bioactive principle responsible for antibacterial activity.

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* antibacterial activity assay [11] and in the recent years several reports available on the antibacterial activity of plant extracts on human pathogenic bacteria [12-18] Evaluation of parasitic angiosperm plant for antibacterial potential has not been attempted by any of the earlier workers. In the present investigation two angiosperm parasites *Macroslen parasiticus* and *Viscum orientale* were screened and it is interesting to note that *V. orientale* was active against eleven test bacteria and *Macroslen parasiticus* was active against eight test bacteria. The degree of activity observed was less than 12mm in both the plants. The antibacterial potential of three plants has been demonstrated for the first time and further investigation is in progress to isolate and characterize the active principles.

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