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ANTIMICROBIAL ACTIVITY OF SOME INDIAN MEDICINAL PLANTS

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Abstract

The antimicrobial potential of seventy-seven extracts from twenty-four plants was screened against eight bacteria and four pathogenic fungi, using microbroth dilution assay. Lowest concentration of the extract, which inhibits any visual microbial growth after treatment with p-iodonitrotetrazolium violet, was considered to be minimum inhibitory concentration (MIC). Water extracts of *Acacia nilotica, Justicia zelanica, Lantana camara* and *Saraca asoca* exhibited good activity against all the bacteria tested and the MIC was recorded in range of 9.375-37.5 µg/ml and 75.0-300.0 µg/ml against the bacterial and fungal pathogens, respectively. The other extracts of *Phyllanthus urinaria, Thevetia nerifolia, Jatropha gossypifolia Saraca asoca, Tamarindus indica, Aegle marmelos, Acacia nilotica, Chlorophytum borivilianum, Mangifera indica, Woodfordia fruticosa and Phyllanthus emblica* showed antimicrobial activity in a range of 75-1200 µg/ml.

Key Words: Antibacterial, Antifungal, Medicinal Plants, Microbroth dilution assay, folkloric medicine

Introduction

The number of multi-drug resistant microbial strains and the appearance of strains with reduced susceptibility to antibiotics are continuously increasing. This increase has been attributed to indiscriminate use of broad-spectrum antibiotics, immunosuppressive agent, intravenous catheters, organ transplantation and ongoing epidemics of HIV infection (Graybill, 1988; Ng, 1994; Dean and Burchard, 1996; Gonzalez et al, 1996). In addition, in developing countries, synthetic drugs are not only expensive and inadequate for the treatment of diseases but also often with adulterations and side effects. Therefore, there is need to search new infection-fighting strategies to control microbial infections (Sieradzki et al, 1999).

The aim of this study was to evaluate the antimicrobial activity of some medicinal plant used in Ayurveda and traditional medicinal system for treatment of manifestations caused by microorganisms. Therefore, extracts of the following twenty-four plants from different families were tested for their potential activity against microbial pathogens: *Justicia zelanica, Phyllanthus urinaria, Thevetia nerifolia, Acacia leucophloea, Solanum surattense, Tephrosia purpurea, Jatropha gossypifolia, Pithecolobium dulce, Holoptelea integrifolia, Lantana camara, Saraca asoca, Tamarindus indica, Aegle marmelos, Acacia nilotica, Woodfordia fruticosa, Mangifera indica, Phyllanthus emblica, Chlorophytum borivilianum, Chlorophytum laxum, Chlorophytum tuberosum, Abutilon indicum, Bombax ceiba, Calotropis procera and Bacopa monnieri*.

Materials and Methods

Plant materials

The different parts of plants used in Ayurveda and traditional systems of medicine were collected from various regions during October to February (Table 1). Plants were identified by Dr A.M. Gurav (Botanist) at Regional Research Institute (Ay), Nehru Garden, Kothrud, Pune, where the voucher samples were preserved. The plant material was dried in shade.

Preparation of plant extracts

The powdered plant materials were extracted successively with n-hexane, chloroform, acetone, methanol and water to afford corresponding fractions (Dabur et al, 2004). Solvents were evaporated under reduced pressure and stored at °C for use.

Micro-organisms

Clinical isolates of the microorganisms were used along with the standard strains. Quality control strains of *Aspergillus fumigatus* ITCC 4517, *A. flavus* ITCC 5192, *A. niger* ITCC 5405, *Candida albicans* ITCC 4718 obtained from Indian Type Culture Collection, IARI, Delhi. *Salmonella typhi* MTCCB 733, *Escherichia coli* MTCCB 82, *Pseudomonas aeruginosa MTCCB 741, Staphylococcus aureus* MTCCB *737, Bacillus cereus* MTCCB 1272, *K. aerogenes* 99/209, *P. vulgaris* 99/345, and *Sh. boydis* 01/21 were included in each test as recommended by the National Committee for Clinical Laboratories Standards (NCCLS), purchased from Institute of Microbial Technology, Chandigarh, India.

Antibacterial screening

Antibacterial activities of the extracts were determined by the microbroth dilution assay as described by Buwa and Staden (2006). The water and ethanol plant extracts were dissolved in corresponding extracting solvents at a concentration of 2400 µg/ml. Acetone extracts were also dissolved in ethanol while the other extracts were dissolved in DMSO. Proper controls were kept for each experiment. The bacterial strains used as inocula were grown at 37 ^{o}C to get OD 0.6 at 600 nm and used for susceptibility testing. Lowest concentration, which inhibited any visual growth, was considered to be minimum inhibitory concentration (MIC).

Antifungal screening

All the extracts were dissolved in DMSO to achieve a concentration of 2400 µg/ml. Microbroth dilution assay for *Candida albicans* was performed as described by Espinel- Fromtling et al (1993). *Aspergillus* species cultures were grown on Sabouraud dextrose agar at 37 ^{o}C until sporulation occurs, typically for 5 days. The spores were harvested in Abouraud dextrose broth and the numbers of colony forming units (CFU) per milliliter were determined by plating serial dilutions on Sabouraud dextrose agar plates. For susceptibility tests, serial two fold dilution of extracts were made in Sabouraud dextrose in 100 µl volumes and were inoculated with 100 µl of the spore suspensions having 2 x 10^4 to 2 x 10^5 CFU/ml in Sabouraud dextrose broth. The cultures were incubated for 48 h at 37 ^{o}C (Dabur et al, 2004). MICs were determined at the lowest concentration that inhibited visible fungal growth.

Bioassay for antibacterial activity of *Acacia nilotica*

The bioassay described by Begue and Kline (1972) was used. The TLC chromatogram of methanol extracts of *A. nilotica* was developed by n-butanol: acetic acid: water (5:1:4) and dried overnight to remove residual solvent. One plate was sprayed with vanillin reagent and the others with all the six bacteria used in this study. Ten milliliter of highly dense fresh bacterial culture was centrifuged at 5300-x g for 20 min to concentrate

Table 1: Selected Indian medicinal used to treat various kinds of human diseases

S.N.	Plant (Voucher number)	Plant part used	Ayurvedic or Traditional Uses
1.	*Abutilon indicum* (245)	Whole plant	The plant is used to treat impotency, rheumatism, menorrhoea, polyuria, gout and hemorrhagic diseases
2.	*Acacia leucophloea* (3218)	Bark	Bark of plant is used as antimicrobial, anthelmentic, expectorant and blood purifier. It is also used to treat skin diseases (leprosy), ulcer, gum bleeding, mouth ulcer, dry cough, dysentery, diabetes and fever
3.	*Acacia nilotica,* (591)	Bark	Bark is used to treat cough, acute gonorrhoea dysentery, diarrhoea, cancers, syphilitic affections and genitourinary affections
4.	*Aegle marmelos* (346)	Fruit	Fruits are used in diarrhoea and dysentery
5.	*Bacopa monnieri* (371)	Leaves	Leaves of plant are used to treat epilepsy, insanity and other nervous disorders
6.	*Bombax ceiba* (579)	Bark	Bark of plant is demulcent, tonic and expectorant and used to treat ulcer
7.	*Calotropis procera* (97)	Whole plant	Plant is used to treat leprosy
8.	*Chlorophytum borivilianum* (577)	Root	Roots are used to treat diarrhoea and dysentery and also used as demulcent and galactogogue
9.	*Chlorophytum laxum* (574)	Root	Roots are used to treat diarrhoea and dysentery and also used as demulcent and galactogogue
10.	*Chlorophytum tuberosum* (372)	Root	Roots are used to treat diarrhoea and dysentery and also used as demulcent and galactogogue
11.	*Holoptelea integrifolia* (146)	Stem Bark	Stem bark is externally used in inflammation and internally used to treat piles, skin disease anthelmentic and obesity
12.	*Jatropha gossypifolia* (3325)	Latex and Leaf	Root is used in diarrhoea and dysentery. Oil used as purgative and locally applies in skin disease and arthritis. Latex and leaf juice are used to treat ulcer, skin disease (leprosy) and gum infections
13.	*Justicia zeylanica* (3128)	Leaf	Leaf of the plants are used in microbial infections, bronchitis, asthma, fever and arthritis
14.	*Lantana camara* (507)	Leaf flower	Leaf juice is used as antimicrobial in skin diseases
15.	*Mangifera indica* (122)	Root	Roots are used in menorrhoea, leucorrhoea and scabies
16.	*Phyllanthus emblica* (37)	Fruit and Seed	Fruits and seeds are used to treat asthma, bronchitis and biliousness
17.	*Phyllanthus urinaria* (342)	Whole plant	Plants is used to treat cough, bronchitis, skin disease, enlarged spleen and liver, jaundice, and fever
18.	*Pithecolobium dulce* (3306)	Root	Root and bark decoctions are taken orally to treat diarrhoea; fruit pulp is taken orally to stop blood flow in case of heamoptysis. The seed juice is inhaled into the nostrils against chest congestion.
19.	*Saraca asoca,* (119)	Bark	Bark is used to treat menorrhoea, bowel, pimple, weakness, hemorrhage, dropsy and uterine sedative
20.	*Solanum* surattense (99)	Whole plant	Plant is used to treat skin disease, cough, cold, bronchitis and asthma
21.	*Tamarindus indica* (369)	Whole Plant	Plant is used to treat diarrhoea, lotions and pustules, sores, boils, asthma and amenorrhea
22.	*Tephrosia purpurea* (219)	Whole plant Root	Plant is recommended in ulcers, spleenomegaly, liver dysfunction, anthelmentic, cough, cold, Skin disease (antimicrobial) and fever
23.	*Thevetia nerifolia* (1470)	Root	Paste of root is recommended to apply externally to treat Leprosy (Skin disease), Syphilis, internally heart disease (action like digitalis) and fever
24.	*Woodfordia fruticosa* (96)	Flower	Flowers are used to treat ulcer, wounds, cough and small pox

Table 2: MIC of plant extracts against the microorganisms by micro dilution broth assay.

Plant	**MIC (µg/ml)**											
	A	B	C	D	E	F	G	H	I	J	K	L
A. leucophloea												
Hexane	-	-	-	-	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-	-	-	-	-
Acetone	-	-	-	600	-	-	-	-	-	-	-	-
Methanol	300	-	-	-	-	-	-	-	-	-	-	-
Water	300	-	-	-	300	300	-	ND	-	-	-	-
A. *marmelos*												
Chloroform	ND	-	-	-	ND	-	-	-	-	-	-	-
Acetone	ND	ND	-	ND	ND	ND	ND	ND	-	-	-	-
Methanol	1200	1200	1200	1200	1200	1200	1200	1200	-	-	-	-
Water	ND	ND	-	ND	ND	ND	ND	ND	-	-	-	-
A. nilotica												
Chloroform	-	-	-	-	ND	-	-	-	-	-	-	-
Acetone	ND	300	300	300	300	300	300	300	-	-	-	-
Methanol	75	75	75	75	75	75	75	75	-	-	-	-
Water	18.75	37.5	37.5	37.5	37.5	37.5	37.5	37.5	-	-	-	-
C. borivilianum												
Acetone	ND	-	-	1200	-	-	-	-	-	-	-	-
Methanol	600	-	600	600	-	-	-	-	-	-	-	-
C. laxum												
Acetone	ND	-	1200	-	-	-	-	-	-	-	-	-
Methanol	ND	ND	-	ND	-	ND	ND	ND	-	-	-	-
J. gossypifolia												
Hexane	600	1200	600	ND	600	1200	ND	-	-	-	-	600
Chloroform	-	1200	1200	-	600	ND	ND	-	-	-	-	1200
Acetone	1200	-	1200	1200	ND	1200	1200	ND	-	-	-	1200
Methanol	1200	1200	600	1200	600	ND	ND	ND	-	-	-	1200
Water	1200	1200	600	1200	1200	1200	1200	-	-	-	-	1200
J. zeylanica												
Chloroform	150	75	300	300	600	ND	ND	ND	300	ND	ND	600
Acetone	75	ND	150	300	ND	300	ND	-	300	ND	150	300
Methanol	18.75	18.75	75	37.5	37.5	37.5	37.5	75	75	150	150	75
Water	18.75	18.75	18.75	18.75	9.375	37.5	18.75	37.5	18.75	75	75	150
L. camara												
Chloroform	600	600	600	300	ND	ND	ND	ND	300	ND	ND	600
Acetone	75	ND	300	300	ND	ND	ND	ND	300	ND	150	300
Methanol	300	75	150	300	300	300	300	150	300	-	75	150
Water	75	150	150	37.5	150	75	150	75	150	300	300	300
M. indica												
Acetone	ND	-	1200	-	ND	-	-	-	-	-	-	-
Methanol	ND	600	600	600	ND	600	600	600	-	-	-	-
Water	ND	150	150	150	150	150	150	150	-	-	-	-
P. *emblica*												
Pet ether	ND	ND	-	-	ND	ND	ND	ND	-	-	-	-
Acetone	ND	-	300	300	ND	600	-	-	-	-	-	-
Methanol	ND	-	300	300	ND	300	300	300	-	-	-	-
Water	ND	1200	1200	1200	1200	1200	1200	1200	-	-	-	-
P. urinaria												
Hexane	-	-	-	-	-	-	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-	-	-	-	-
Acetone	300	300	300	300	300	300	300	300	-	-	-	-
Methanol	300	300	300	300	150	300	-	300	-	-	-	-
Water	-	-	-	-	-	-	-	-	-	-	-	-

S. asoca												
Pet ether	ND	1200	-	600	ND	600	600	600				
Chloroform	ND	-	-	-	ND	-	-	-	-	-	-	-
Methanol	ND	600	300	300	ND	300	300	300	600	600	600	600
Water	18.75	18.75	18.75	18.75	18.75	18.75	37.5	37.5	1200	600	600	600
S. xanthocarpum												
Hexane	ND	300	300	300	300	300	300	300	-	-	-	-
Chloroform	300	300	300	-	-	ND	ND	ND	-	-	-	-
Acetone	-	300	300	300	600	ND	ND	ND	-	-	-	-
Methanol	-	300	-	300	600	ND	600	ND	300	300	300	150
Water	600	300	300	300	300	600	-	300	-	-	-	-
T. indica												
Pet ether	ND	-	-	600	ND	600	300	-	-	-	-	-
Chloroform	ND	ND	-	-	ND	-	-	ND	-	-	-	-
Acetone	ND	ND	1200	ND	ND	ND	ND	ND	-	-	-	-
Methanol	ND	300	600	300	ND	150	150	150	-	-	-	-
Water	300	300	150	600	ND	1200	150	600	-	-	-	-
T. purpurea												
Hexane	-	150	-	-	-	ND	ND	-	-	150	-	-
Chloroform	-	-	-	-	-	-	ND	-	-	-	-	-
Acetone	-	-	-	-	-	-	ND	-	-	-		
Methanol	-	-	600	-	-	ND	-	ND	-	-	-	-
Water	300	-	-	-	-	ND	-	ND	-	-	-	-
T. nerifolia												
Hexane	-	-	-	-	-	ND	-	-	-	-	-	-
Chloroform	-	-	-	-	-	-	-	-	-	-	-	-
Acetone	75	150	300	150	300	300	150	300	-	-	-	-
Methanol	600	150	300	300	150	300	300	300	-	-	-	-
Water	150	75	300	75	150	150	300	150	-	-	-	-
W. fruticosa												
Acetone	300	300	300	300	ND	300	600	600				
Methanol	ND	300	300	300	ND	300	600	600				
Water	ND	300	300	300	ND	300	600	600				

A= *E. coli*; B= *S. typhi*, C= *P. aeruginosa*; D= *S. aureus*; E= *B. cereus*; F=*K. aerogenes*, G=*P. vulgaris*, H= *Sh. boydis*, I=*A. fumigatus*; J=*A. flavus*; K=*A. niger*; L=*C. albicans*.

(-) Means No Activity, ND Means Not determined.

the bacteria. The supernatant was discarded and the pellet re-suspended in 4.0 ml of fresh nutrient broth. The plates were sprayed with the concentrated suspension until they were just wet and incubated overnight at 37 °C in 100 % relative humidity. After incubation, the plates were sprayed with 2.0 mg/ml solution of p-iodonitrotetrazolium violet. Clear zones on chromatogram indicated the zone of inhibition of growth of bacteria after incubating for one hour.

Results and Discussion

Various parts of twenty four plants were evaluated for their antimicrobial potential against twelve microorganisms in this study using microbroth dilution assay (Table 1). Table 2 summarizes the results obtained and listed the only plant species that presented some activity against at least one microorganism. Antimicrobial activity of plant extract was considered to be good if its MIC was less than 100.0 µg/ml, moderate if MIC was from 100.0 to 500.0 µg/ml and poor over 500.0 µg/ml.

The water extracts of *A. nilotica*, *J. zeylanica*, *L. camera* and *S. asoca*, were found to be the most active against bacteria as well as fungal pathogens. The wells containing a concentration of 9.375-150.0 µg/ml extracts of water and methanol inhibited the visible growth of all the bacterial species (Table 2). Methanol extracts of *A. nilotica* and *J. zeylanica* exhibited good activity in the range of 18.75-75.0 µg/ml. The chloroform and acetone fractions were found to be less active. The MICs of water and methanol fractions against all the fungi were observed to be in a range of 75.0-300.0 µg/ml. However, *A. nilotica* was observed to be inactive against fungal

pathogens. Water soluble fraction of the flowers and bud of *S. asoca* were reported to have significant inhibitory effect against *Sh. boydis* (Narang et al, 1962) and the 50% ethanolic extract of the whole plant was reported to be inactive. In this study, methanolic extract of stem bark of *S. asoca* exhibited significant inhibitory activity against bacteria used.

T. nerifolia, P. urinaria, W. fruticosa, M. indica, P embilica and *T. indica* showed activity against all the bacterial species used in the present study in a range of 150.0-600.0 µg/ml. *S. xanthocarpum, T. purpurea* and *A. leucophloea* also exhibited low activity against some bacterial species. Antifungal activity of *S. surattense* against *A. fumigatus* had been reported by Dabur et al (2004), additionally, chloroform and hexane extracts (300.0 µg/ml) of the same were found to be active against *S. typhi, E. coli, P. aeruginosa, S. aureus*, while methanol extract inhibited the growth of *A. flavus, A. niger* and *C. albicans.*

The bioassay was performed to characterize the active constituent of *A. nilotica* that was found to be most active plant in the present study. The TLC chromatogram of methanol extracts of *A. nilotica*, showed inhibition of growth of bacteria at Rf 0.564. The duplicate plate exposed to vanillin showed four spots of Rf value 0.600, 0.564, 0.164 and 0.117.

The screenings of these medicinal plants showed that some of the screened plants are potential source of antibacterial agents. This *in vitro* study corroborated the antimicrobial activity of *T. nerifolia, A. nilotica, S. asoca, L. camera, J. zeylanica, P. urinaria, T. nerifolia* and *S. surattense* in Ayurveda.

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