Annals of Plant Sciences 8.1 (2019) pp.3484-3488

ANNALS OF PLANT SCIENCES
ISSN: 2287-688X
OPEN ACCESS
www.annalsofplantsciences.com

Research Article

Evaluation of antimicrobial activity of three important medicinal plants of South India

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Abstract: India is found to be a country with rich biodiversity and enormous treasure of herbal plants and consequently called as medicinal garden of the world. Plants are the richest source of natural antimicrobial agents. In recent years drug resistance to human pathogenic bacteria has been commonly reported from all over the world. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases; one approach is to screen local medicinal plants for possible antimicrobial properties. The present study was designed to evaluate the antimicrobial efficacy of hexane, chloroform and methanol crude extracts of the leaves of three important medicinal plants viz., *Biophytum sensitivum* (L.) DC, *Bougainvillea spectabilis* L. and *Caesalpinia bonducella* (L.) Fleming, collected from in and around Visakhapatnam District. The antimicrobial activity of the crude extracts was tested against three Gram Positive bacteria (*Bacillus subtilis* MTCC 441, *Enterococcus faecalis* MTCC 439, *Staphylococcus aureus* MTCC 737), Three Gram Negative bacteria (*Escherichia coli* MTCC 443, *Proteus vulgaris* MTCC 426 and *Pseudomonas aeruginosa* MTCC 1688) and three Fungal strains (*Candida albicans* MTCC 227, *Epidermophyton floccosum* MTCC 613 and *Trichophyton mentagrophytes* MTCC 7687) using agar well diffusion assay. Our results demonstrated that methanol extracts of these plants leaves have concentration dependent antibacterial activity against some of the tested organisms. Further studies should be undertaken to elucidate the exact mechanism of action of antimicrobial effect to identify the active ingredients which can be used for drug development program.

Keywords: antibacterial activity, *Biophytum sensitivum, Bougainvillea spectabilis, Caesalpinia bonducella*, Zone of inhibition.

Introduction

In recent years drug resistance to human pathogenic bacteria has been commonly reported from all over the world. Microorganisms that develop antimicrobial resistance sometimes they referred as "superbugs". The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. The rising prevalence of antibiotics resistant pathogenic microorganisms in the last decades raises the demand for finding new alternative antimicrobial agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases; one approach is to screen local medicinal plants for possible antimicrobial properties.

Plant materials remain an important resourse to combat serious diseases in the world. 80% of the world's population is dependent on the traditional medicine and a major part of the traditional therapies involves the use of plant extracts or their active constituents. Since ancient times, herbs and their essential oils have been known for their varying degrees of antimicrobial activity. India is found to be a country with rich biodiversity and enormous treasure of herbal plants and consequently called as medicinal garden of the world (**Bhutani and Gohil, 2010**).

There are about 17,000 species of higher plants of which approximately 8,000 species are considered medicinal and used by traditional medicinal systems (**Prakasha *et al.,* 2010**) and several thousands have been claimed to possess medicinal properties. The ethnobotanical information reports about 800 plants that may possess antidiabetic potential (**Alarcon-Aguilara *et al.,* 1998**).

Medicinal plants are being used for both prophylactic and therapeutic management of diabetes (**Shukia *et al.,* 2000**). Prophylactic action may be attributed to healthy organs and their cellular tissue especially beta cells of pancreas, hepatic tissue and preventive action on diabetic inducers. Therapeutic action may be due to curative action on affected tissue of pancreas, liver and organs related to diabetes. Some bioactive compounds within the plants are responsible for their medicinal value. The most prominent of these bioactive compounds are alkaloids, tannins, flavonoids and phenolic compounds (**Shihabudeen *et al.,* 2010**). Phytochemical concentrations may vary in different plants which result in unique medicinal properties for a specific plant. Medicinal and aromatic plants contain biologically active chemical substances such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds (**Harborne, 1977; Sofowora, 1993**), which have curative properties. These

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complex chemical substances of different compositions are found as secondary plant metabolites in one or more of these plants. In the investigation is to evaluate the potentiality of plant extracts on standard microorganism strains as well as on the multi-drug resistant bacteria.

Materials and Methods

Medicinal plants for the study

Three medicinal plants ***Biophytum sensitivum* (L.) DC**, ***Bougainvillea spectabilis* L.** and ***Caesalpinia bonducella* (L.) Fleming** were selected based on the ethanomedical importance. Which were grown in Visakhapatnam District, Andhra Pradesh.

Preparation of plant extract

The leaves were collected from the selected medicinal plants and were washed with distilled water. The washed leaves were categorized according to their species and age differences and dried at room temperature for seven days under shade. After drying the materials were powdered separately by using electric grinder. 100 grams of ground weighed material of fine coarse powder was successively extracted by different solvents of hexane, chloroform and methanol in a specific sequence based on increasing polarity. The soxhlet hot extraction procedure for each of the above solvents was run for about 6 hours, until a colorless solvent was seen in the siphon tube, which indicated complete extraction. The solvents were removed under reduced pressure and controlled temperature by rotary evaporator. The extracts were dried and stored in a clean glass bottle and kept at 2 to 8 degree centigrade for antimicrobial screenings and further investigation. In each step of the experiment, sterility conditions were maintained for the effectiveness and accuracy in results without contamination.

Test Microorganisms

The antimicrobial activity of the crude extracts was tested against Authentic pure cultures of both Gram positive and Gram-negative bacteria. Three Gram Positive bacteria including *Bacillus subtilis* MTCC 441, *Enterococcus faecalis* MTCC 439, *Staphylococcus aureus* MTCC 737, Three Gram Negative bacteria including *Escherichia coli* MTCC 443, *Proteus vulgaris* MTCC 426 and *Pseudomonas aeruginosa* MTCC 1688 and Three Fungal strains of *Candida albicans* MTCC 227, *Epidermophyton floccosum* MTCC 613 and *Trichophyton mentagrophytes* MTCC 7687 were preocured from MTCC IMTECH, Chandigarh, India.

Antimicrobial Activity

The antimicrobial activity of hexane, chloroform and methanol crude extracts was tested against both three Gram +ve bacteria including *Bacillus subtilis* MTCC 441, *Enterococcus faecalis* MTCC 439, *Staphylococcus aureus* MTCC 737, Three Gram Negative bacteria including *Escherichia coli* MTCC 443, *Proteus vulgaris* MTCC 426 and *Pseudomonas aeruginosa* MTCC 1688 and Three Fungal strains of *Candida albicans* MTCC 227, *Epidermophyton floccosum* MTCC 613 and *Trichophyton mentagrophytes* MTCC 7687.

The antimicrobial activity was carried out by using standard method of **Perez et al., (1990)** employing 24 hours old cultures with given plant extract by using Agar-Well diffusion method. The medium was sterilized by autoclaving at 120°C (15 lb/in2). About 20mL of the Nutrient Agar Medium/Potato Dextrose Agar seeded with the respective strains Bacteria/Fungal were transferred aseptically into each sterilized Petri plate. The Plates were left at room temperature for solidification. Each plate, a single well of 6 mm diameter was made using a sterile borer. The test crude extracts of hexane, chloroform and methanol were freshly reconstituted with suitable solvents (DMSO) and tested at concentrations of 100mg/ml. The samples and the control along with standard antibiotic (Ciprofloxacin/ Fluconazole) were placed in 6 mm diameter well. In Antimicrobial assay for fungal plates were incubated at 28 ± 2°C whereas 37 ± 2°C for bacteria. Activity diameter of the zone of inhibition was measured using Himedia antibiotic zone scale.

Results and Discussion

Antimicrobial activity

The invention and development of antibiotics are most powerful and successful achievements of modern science and technology for the management of infectious diseases. All the extracts were inhibited growth of almost all the selected bacteria in the range of 7-18mm. Among them the methanol extract showed great activity against *E. coli* and moderate activity was reported against *P.vulgaris*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The methanol extract exhibited significant activity against all the studied microorganisms. While the chloroform extract showed moderate activity against all selected pathogens and the Hexane extracts of all the plants extracts showed minimum activity against the selected microorganisms.

The results of present investigation showed broad spectrum antimicrobial activity against the tested bacteria. *Biophytum sensitivum* (L) DC showed broad spectrum of antibacterial activity against the tested bacteria than antifungal activity. All the extracts of *Biophytum sensitivum* inhibition growth of almost all the selected microorganisms in the range from 7 to 14mm. Among them the methanol extract of *B. sensitivum* showed great activity against *Staphylococcus aureus* (14mm) Table 1.

Bougainvillea spectabilis L. showed promising activity against different bacterial and fungal strains. Antimicrobial potency was assessed by the presence or absence of inhibition zones and zone diameters (mm). The methanol extract showed very good activity against *E.coli* (15mm) Table 2.

Caesalpinia bonducella (L.) Fleming methanol extract showed the inhibition activity against *Staphylococcus aureus* (15mm) and the least activity was noted in *Staphylococcus aureus* (7mm) in hexane extract (Table 3).

Table 1: Antimicrobial activity of *Biophytum sensitivum* (L.) DC

Organism	Zone of Inhibition (mm)			
	Control	Hexane Extract	Chloroform Extract	Methanol Extract
Bacillus subtilis	23±0.4	8±0.3	11±0.5	14±0.2
Enterococcus faecalis	24±0.4	7±0.5	10±0.7	11±0.5
Staphylococcus aureus	24±0.8	9±0.4	8±0.6	14±0.4
Escherichia coli	26±0.1	9±0.6	9±0.4	12±0.6
Proteus vulgaris	25±0.6	10±0.7	11±0.7	9±0.7
Pseudomonas aeruginosa	24±0.3	11±0.4	11±0.5	12±0.8
Candida albicans	24±0.5	10±0.3	12±0.3	13±0.4
Epidermophyton floccosum	23±0.3	10±0.4	11±0.2	10±0.4
Trichophyton mentagrophytes	24±0.5	11±0.5	9±0.5	13±0.4

Values are Mean±Standard Error; Control 10µg; Crude extract 100mg/ml concentration

Table 2: Antimicrobial activity of *Bougainvillea spectabilis* L.

Organism	Zone of Inhibition (mm)			
	Control (10µg)	Hexane Extract	Chloroform Extract	Methanol Extract
Bacillus subtilis	23±0.4	8±0.7	9±0.2	12±0.7
Enterococcus faecalis	24±0.4	9±0.4	10±0.5	11±0.6
Staphylococcus aureus	24±0.8	8±0.6	9±0.3	13±0.4
Escherichia coli	26±0.1	10±0.8	8±0.3	15±0.8
Proteus vulgaris	25±0.6	8±0.2	12±0.6	13±0.5
Pseudomonas aeruginosa	24±0.3	11±0.1	10±0.8	13±0.3
Candida albicans	24±0.5	8±0.5	9±0.4	12±0.2
Epidermophyton floccosum	23±0.3	9±0.1	8±0.5	10±0.7
Trichophyton mentagrophytes	24±0.5	8±0.7	10±0.2	11±0.7

Values are Mean±Standard Error; Control 10µg; Crude extract 100mg/ml concentration

Table 3: Antimicrobial activity of *Caesalpinia bonducella* (L.) Fleming

Organism	Zone of Inhibition (mm)			
	Control (10µg)	Hexane Extract	Chloroform Extract	Methanol Extract
Bacillus subtilis	23±0.4	11±0.3	10±0.8	14±0.4
Enterococcus faecalis	24±0.4	9±0.5	12±0.8	12±0.3
Staphylococcus aureus	24±0.8	7±0.6	14±0.6	15±0.6
Escherichia coli	26±0.1	9±0.3	10±0.7	11±0.2
Proteus vulgaris	25±0.6	12±0.4	13±0.3	13±0.7
Pseudomonas aeruginosa	24±0.3	13±0.5	10±0.6	10±0.8
Candida albicans	24±0.5	9±0.7	13±0.5	14±0.4
Epidermophyton floccosum	23±0.3	10±0.5	14±0.3	10±0.6
Trichophyton mentagrophytes	24±0.5	8±0.3	10±0.8	11±0.4

Values are Mean±Standard Error; Control 10µg; Crude extract 100mg/ml concentration

Determination of the minimum inhibitory concentration (MIC)

The broth micro-dilution method was used to determine the MIC according to the European Committee on Antimicrobial Susceptibility Testing (**EUCAST**). The tested extracts were dissolved in 10% DMSO and diluted to the higher concentration. Then a serial ½ dilutions of extracts were prepared directly in a microtiter plate containing Mueller Hinton broth to obtain concentrations from 0.0125 to 12.8 mg/mL. The bacterial inoculum was added to give a final concentration of 5×10^5 CFU/mL in each well. Gentamycin used as a positive control at final concentrations from 0.125 to 128 µg/mL. The plate was covered with a sterile sealer and incubated for 24h at 37°C. The MIC was considered as the lowest concentration of the extract that completely inhibits the bacterial growth. The lower the MIC the higher the activity of the extract. The effectiveness of the extracts on tested microbial strains was determined by measuring the minimum inhibitory concentration (MIC) (Table 4). The MICs of hexane, chloroform and mehanolic extracts of the three plant extracts against different tested strains. MIC values obtained from the methanolic extract of *Biophytum sensitivum (L.) DC* showed MIC of 0.8mg/mL against *Escherichia coli* and *E. floccosum*. *Bougainvillea spectabilis* L. showed MIC of 0.8mg/mL against *T.mentagrophytes*.

Table 4: MICs of hexane, chloroform and mehanolic extracts of three plant extracts against different microbial strains

Plant Name	Solvent	*B. subtilis*	*E. faecalis*	*S. aureus*	*E. coli*	*P. vulgaris*	*P. aeruginosa*	*C. albicans*	*E. floccosum*	*T.mentagrophytes*
	Hexane	12.8	12.8	12.8	3.2	6.4	6.4	12.8	12.8	12.8
Biophytum sensitivum (L) DC	Chloroform	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
	Methanol	3.2	6.4	3.2	0.8	1.6	3.2	3.2	0.8	1.6
	Hexane	12.8	12.8	12.8	12.8	12.8	6.4	12.8	12.8	12.8
Bougainvillea spectabilis L.	Chloroform	12.8	6.4	6.4	6.4	6.4	6.4	6.4	6.4	6.4
	Methanol	6.4	3.2	12.8	3.2	3.2	3.2	1.6	3.2	0.8
	Hexane	12.8	12.8	12.8	12.8	12.8	3.2	12.8	12.8	12.8
Caesalpinia bonducella (L) Fleming	Chloroform	1.6	6.4	6.4	6.4	6.4	6.4	3.3	6.4	6.4
	Methanol	3.2	3.2	1.6	3.2	3.2	3.2	6.4	3.2	12.8

In vitro antimicrobial activities of the methanolic leaves extract of three medicinal plants were screened individually by the presence or absence of zone of inhibition. The antibacterial activity of methanol extracts of selected plants leaf extracts against human pathogenic microorganisms are presented in Table (1-3). After analysing the above results, it is clear that gram positive bacteria *S. aureus* is more susceptible than the other microorganisms. The methanol extracts of *Biophytum sensitivum* exhibited excellent antibacterial activity as compared to the standard. These results are agreed with the previous claim of **Deshwal and Vig, [2012]**. This implied that the gram-positive bacteria were more susceptible to the extract than the other microorganism. This is due to the presence of outer membrane that serves as an effective barrier [**Nikaido, 1999; Adesokan et al., 2007**].

Conclusion

In the present the study it is concluded that among the leaf extracts of three medicinal plants *Biophytum sensitivum* possess greater antibacterial activity against tested human pathogenic. *Bougainvillea spectabilis* and *Caesalpinia bonducella* showed moderate antimicrobial activity. Our results demonstrated that methanol extracts of selected plants leaves have age and concentration dependent antimicrobial activity against some of the tested organisms. The results obtained by this study cannot be directly extrapolated to human; further studies should be undertaken to elucidate the exact mechanism of action by which extracts exert their antimicrobial effect to identify the active ingredients which can be used in drug development program for safe health care services.

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Cite this article as:

Gopalakrishnaiah B. and O. Aniel Kumar. Evaluation of antimicrobial activity of three important medicinal plants of South India. *Annals of Plant Sciences 8.1 (2019) pp.3484-3488*.

http://dx.doi.org/10.21746/aps.2018.8.1.1

Source of support: Nil
Conflict of interest: Nil.