

Research Article – Ethnomedicine

Some endemic medicinal plants of Andamans with antimicrobial potential

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Abstract

The present study was aimed to determine the antimicrobial activity of some endemic plant species used in folkloric medicine by the inhabitants of Andaman Islands, India. The ethanol extracts prepared from the leaves of four plants viz; *Alstonia kurzii*, *Tabernaemontana crispera*, *Mangifera andamanica* and *Vitex diversifolia* were assessed for antibacterial activity against clinically isolated human pathogenic bacteria and antifungal activity against some phytopathogenic fungi. The ethanol extracts showed more inhibition towards Gram positive than Gram negative bacteria and the bacterial strains showed more susceptibility than the fungal strains tested. Among the plants, *Vitex diversifolia* exhibited the highest antibacterial activity and *Mangifera andamanica* showed the highest antifungal activity.

Key words: Endemic plants of Andamans, *Mangifera andamanica*, *Vitex diversifolia*, antibacterial activity, antifungal activity.

Introduction

Andaman and Nicobar Islands also known as Bay Islands represent one of the hotspots of rich biodiversity regions in India and are usually acknowledged as a 'botanical paradise'. Its unique geographical set up and physical isolation, situated away from much human disturbance has resulted in high degree of biological endemism. These islands have been objects of scientific curiosity in terms of their species diversity. More than 86% of the area is under luxuriant forest cover with 2500 angiospermic species, out of which 353 (14%) species are endemic (Montesinos, 2007). A large number of these plants are attributed to possess medicinal values. Among the endemic species about 52 plants are used in medicaments. Since time immemorial the primitive aborigines inhabiting these islands use a host of these medicinal plants for sustaining their livelihood (Kilani, 2006). Some of the plants like *Alstonia kurzii* are used in curing fever and filaria, the leaf decoctions of *Tabernaemontana crispera* have been used in clearing ulcers and in stomachache. The bark of *Mangifera andamanica* is used to cure dysentery. The bark and leaves of *Vitex diversifolia* are used in intestinal troubles (Crafton, 1983). As modern medicine could hardly reach the people,

especially those dwelling in the remote villages amidst deep forests and these plants serve as the mainstay of the population for curing diverse ailments.

The present scenario is an alarming increase in the incidence of human infections due to the rising prevalence of pathogenic microorganisms. Infectious diseases account for about one half of all the deaths in tropical countries (Marjorie, 1999). Concerns have been raised due to the emergence of multiple drug resistance. The indiscriminate use of commercial antibiotics has astronomically decreased the potential of chemical therapeutic agents in clinical use. In addition to human infections, plants are also constantly threatened by a variety of disease causing microorganisms which results in overall losses in crop yield worldwide (Martin, 1995; Palombo and Semple, 2001). Use of synthetic pesticides has also posed problems regarding environmental impact and potential health risk. Therefore, considering the deleterious effects of synthetic products, search for novel antimicrobials is necessary for the management of human as well as crop diseases. Possible alternatives may be the use of medicinal plants and their extracts which are not associated with many side effects, contrary to the synthetic chemicals.

Traditional medicines have always served as man's resort when attacked by infective agents such as bacteria and fungi (Cowan, 1999). Research during the last decades have convincingly shown that "green medicine" is safe and more dependable due to their

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efficacy, safety and fewer side effects. Plant products provide unlimited opportunities for new drug leads because of the matchless availability of chemical diversity. In crude forms they show interesting combination of activities (El-Mahmood *et al.*, 2008). Plants have supplied over 25% of prescription drugs used in human medicine and such pharmacologically active plants have also provided leads to biopesticides (Savary *et al.*, 2006).

The phytochemical research based on ethnopharmacological informations is generally considered as an effective approach in the discovery of new anti-infective agents from higher plants (Kartining *et al.*, 1991). This study for the first time reports the antimicrobial activity of plant species endemic to Andaman Islands. *Alstonia kurzii*, *Tabernaemontana crispa*, *Mangifera andamanica* and *Vitex diversifolia*. The uses of these plants in traditional medicine are also documented.

Materials and Methods

Plant material

The plant materials were collected from different sites located at the tropical rain forests of Port Blair (lat.11°41'13.04"N; long 92°43'30.16E), South Andamans (India). Leaves of *Alstonia kurzii* Hook and *Tabernaemontana crispa* Roxb belonging to the family Apocynaceae were collected from Brichgunj. Leaves of *Mangifera andamanica* King belonging to the family Anacardiaceae were collected from Chidiatapu and leaves of *Vitex diversifolia* kurz belonging to Verbenaceae family were collected from Port Mout region. The voucher specimens were deposited in the herbarium of Department of Botany, Annamalai University.

Preparation of the extracts

Healthy and well grown leaves were collected and washed with tap water. Surface sterilization was done using 10 per cent sodium hypochlorite solution to prevent contamination of any microbes. After rinsing with sterile distilled water, the leaves were air dried at room temperature. These samples were ground into a fine powder.

Ethanol extract

Thirty grams of powdered leaf samples were soaked in 100 ml of ethanol for seven days accompanied with continuous stirring. The solution was then filtered using Whatmann filter paper and concentrated in a rotary evaporator at 40°C.

Preparation of test solution and disc

The test solution was prepared with known weights of ethanol extracts, dissolved in 5% dimethyl sulfoxide (DMSO). Whatmann No.1

sterile filter paper discs (6mm) were impregnated with 10µl of the extract and allowed to dry at room temperature.

In vitro Studies

Microorganisms used

Five strains of human pathogenic bacteria were used viz; *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. These clinically isolated strains were obtained from Department of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalai nagar. The stock cultures were maintained on nutrient agar medium at 4°C. Four strains of plant pathogenic fungi namely *Fusarium oxysporum*, *Pythium aphanidermatum*, *Sclerotium rolfsii* and *Macrophomina phaseolina* used in the study were obtained from the Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai nagar. The pure fungal cultures were maintained on Potato Dextrose Agar medium at 28±2°C.

Preparation of inocula

Twenty four hours old culture of selected bacteria were mixed with physiological saline and turbidity was adjusted by adding sterile physiological saline until a Mac Farland turbidity standard of 0.5 (10⁶ colony forming units (CFU) per ml) was obtained. The fungi were sub cultured on Potato Dextrose Agar and incubated at 28± 2°C for seven days. The growth was scraped aseptically, crushed and macerated thoroughly in sterile saline and fungal suspension was standardized spectrophotometrically to an absorbance of 0.4 optical density at 500 nm.

Antibacterial and antifungal assays

Disc Diffusion Method

The agar diffusion method (Bauer *et al.*, 1966) was followed for antibacterial susceptibility test. Petri plates were prepared by pouring 20 ml of Mueller Hinton Agar and allowed to solidify for the use in susceptibility test against bacteria. Plates were dried and 0.1 ml of standard inoculum suspension was poured and uniformly spread. The excess inoculum was drained and the plates were allowed to dry for five minutes. After drying, the sterile discs with extracts were placed on the surface of the plate. Positive control Ciprofloxacin (5µg/disc) was used as a reference standard. The inoculated plates were incubated at 37°C for 24 hours.

For antifungal assays, petriplates were prepared by pouring 20 ml of Potato Dextrose Agar and allowed to solidify. The plates were dried and using a sterile loop, a plug of inoculum from the actively

Table 1. Traditional uses of some endemic medicinal plants of Andaman

Sl. No.	Botanical name	Local name	Habit	Family	Folk medicinal uses	Parts used	Preparations
1	<i>Alstonia kurzii</i> Hook	Chattiyan	Tree	Apocynaceae	Used in chest pain, Epilepsy, fever, filaria.	Bark Leaf	Paste prepared is applied for chest pain. An Ayurvedic drug (Saptachada) extracted and used for epilepsy.
2	<i>Tabernaemontana crispa</i> Roxb.	Tawzala	Small tree	Apocynaceae	Used for ulcers, Borers, Stomachache. Alcoholic drink preparation.	Leaf decoctions Fruits Young shoots	Leaf decoction and fruit ground with little amount of water. Alcoholic drink (handia) prepared from young shoots.
3	<i>Mangifera andamanica</i> king	Jungli Am	Tree	Anacardiaceae	Used mainly to cure dysentery.	Bark Fruit	Bark and fruits used to cure dysentery. Fruits are eaten by tribals.
4	<i>Vitex diversifolia</i> Kurz	Jungli Pyinma	Small tree	Verbenaceae	To cure worm troubles, Tooth diseases, Skin diseases, Intestinal troubles, amoebiasis. Buds used to clean teeth.	Bark Fruit Leaves	Source of an Ayurvedic drug, Nirgundi. Bark, fruits and leaves are commonly used.

Table 2. Antibacterial activity of the some medicinal plants of Andaman

S. No.	Name of the Bacteria	Mean zone of inhibition(mm) Ethanol exteact (100µg/disc)				MIC (µg/ml)				MBC (µg/ml)				Ciprofloxacin (5µg/disc)
		1	2	3	4	1	2	3	4	1	2	3	4	
1	<i>Bacillus subtilis</i>	14.0±0.8	18.2±1.0	16.2±1.2	20.1±1.1	250	125	125	62.5	500	250	250	125	28.7±1.9
2	<i>Staphylococcus aureus</i>	16.1±0.9	15.8±1.0	14.5±0.9	19.0±1.2	125	250	250	125	250	500	500	250	29.1±2.0
3	<i>Escherichia coli</i>	9.7±0.8	11.9±0.7	10.2±0.7	16.6±1.3	2000	1000	1000	125	4000	2000	2000	250	28.7±1.6
4	<i>Klebsiella pneumoniae</i>	12.2±0.8	NA	NA	13.0±1.0	500	NT	NT	500	1000	NT	NT	1000	23.5±1.5
5	<i>Pseudomonas aeruginosa</i>	11.0±8	8.8±07	9.3±07	11.9±0	1000	200	2000	1000	2000	4000	4000	2000	27.3±1.5

a- Mean zone of three assays; MIC – Minimum inhibitory concentration; MBC- Minimum bactericidal concentration ; NA- No activity; NT- Not tested; 1 - *A. kurzii*; 2 - *T. crispa*; 3 - *M. andamanica*; 4 - *V. diversifolia*

Table 3. Antifungal activity of the some endemic plants of Andamans

S. No.	Name of the Fungus	Mean zone of inhibition(mm) Ethanol extract (100µg/disc)				MIC (µg/ml)				MFC (µg/ml)				Carbendazim (0.25%)
		1	2	3	4	1	2	3	4	1	2	3	4	
1	<i>Fusarium oxysporum</i>	10.7±0.7	12.0±0.3	12.8±0.8	NA	1000	500	500	NT	2000	1000	1000	NT	15.0±1.0
2	<i>Pythium aphanidermatum</i>	9.8±0.8	10.7±0.5	14.7±0.9	11.5±0.8	2000	1000	250	1000	4000	2000	500	2000	15.0±1.0
3	<i>Sclerotium rolfsii</i>	9.4±0.5	NA	13.0±0.9	11.9±0.7	2000	NA	500	1000	4000	NT	1000	2000	18.0±1.0
4	<i>Macrophomina phaseolina</i>	NA	NA	11.8±0.8	9.8±0.8	NT	NA	1000	2000	NT	NT	2000	4000	17.2±1.3

a-Mean zone of three assays; MIC – Minimum inhibitory concentration; MFC- Minimum Fungicidal concentration; ; NA- No activity; NT- Not tested; 1 - *A. kurzii*; 2 - *T. crispa*; 3 - *M. andamanica*; 4 - *V. diversifolia*

growing margin of the Petri culture of each fungal isolate was placed in the centre of Petri dish with the mycelium face down. Discs were placed equidistantly on the surface of the plate with sterile forceps. Here, standard fungicide Carbendazim (0.25%) was used as positive control for comparison. Further the plates were incubated at 28± 2°C for 48 hours (Al-Mughrabi, 2003). For both antibacterial and antifungal assays the zone of inhibition observed was measured in millimeters and recorded. Each assay was performed in triplicate.

Minimum inhibitory Concentration (MIC)

Minimum inhibitory Concentration of the ethanol extracts were tested in Mueller Hinton Broth for bacteria and, following the Broth macro dilution technique (Ericsson and Sherris, 1971). The plant extracts were dissolved in 5% DMSO to obtain 32 mg/ml of stock solution was incorporated into 0.5 ml of Mueller Hinton Broth to obtain a concentration of 16000, 8000, 4000, 2000, 1000, 500, 250 and 62.5 µg/ml. 50 µl of standardized suspension of bacteria was transferred into each tube. The lowest concentration which did not show any bacterial growth after macroscopic evaluation was determined as MIC.

Determination of minimum inhibitory concentration for fungi was done following broth dilution method using serially diluted plant extracts (24). Fungal cultures prepared in Potato Dextrose Broth were incubated at 25°C for 48 hours. The cultures were adjusted with sterilized saline to obtain absorbance of 0.4 optical density at 500 nm. The plant extracts were diluted in 5% DMSO and concentrations of 16000, 8000, 4000, 2000, 1000, 500, 250 and 62.5 µg/ml were prepared. 100 µl of diluted fungal culture, 0.6 ml of Potato Dextrose Broth and plant extracts were mixed well in a test tube. The mixture was incubated at 28°C for 72 hours and lowest concentration at which growth of fungal cells were fully inhibited were determined as MIC. Each antibacterial and antifungal assay was repeated thrice.

Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

The MBC and MFC of the plant extracts were determined by the method of Kartinig *et al.* (1991) by plating 100µl of sample from each MIC assay tube with growth inhibition into freshly prepared Mueller Hinton Agar (for bacteria) and Potato Dextrose Agar (for fungi) and the plates were incubated at 37°C for 24 hours (bacteria) and 28°C for 72 hours (fungi). The MBC and MFC were recorded as the lowest concentration of the extracts that did not permit any visible bacterial and fungal colony growth on the appropriate agar plate during the period of incubation.

Results

Table 1 provides the uses of the selected endemic plant species in traditional medicine by the inhabitants of Andaman Islands. The results of the antimicrobial screening of the ethanol extracts of all the four plant species are shown in Tables 2 and 3. Our results indicated that ethanol extracts of the plants exhibited higher antimicrobial activity against the microorganisms tested. The antibacterial activities of the ethanol extracts were more pronounced on Gram positive than Gram negative bacteria. In addition, bacterial strains showed more susceptibility than the fungal strains tested. The mean zone of inhibition obtained for the ethanol extracts tested against the bacterial strains were between 8.8 and 20.1 mm. The MIC values recorded ranged between 62.5-2000 µg/ml while MBC values obtained were between 125-4000 µg/ml.

The mean zones of inhibition of the ethanol extracts against the fungal strains were recorded between 9.4 and 14.7 mm. The MIC values ranged between 250-2000 µg/ml and the MFC values were between 500-4000 µg/ml. The mean zones of inhibition of the ethanol extracts against the fungal strains were recorded between 9.4 and 14.7 mm. The

MIC values ranged between 250-2000 µg/ml and the MFC values were between 500-4000 µg/ml.

Our results also revealed that, among the four plant species tested, highest antibacterial activity was exhibited by the ethanol extracts of *Vitex diversifolia* with mean zone of inhibition 20.1 mm and lowest MIC value (62.5 µg/ml) and MBC value (250 µg/ml) against *Bacillus subtilis*. While for the fungal strains, ethanol extracts of *Mangifera andamanica* exhibited highest mean zone of inhibition (14.7 mm) with MIC value of 250 µg/ml and MFC value of 500µg/ml against *Pythium aphanidermatum*.

Discussion

Plant based antimicrobials are seen as valuable sources for the design and rational planning of new drug leads which can be used both in medicine and agriculture. The present study showed that the ethanol extract of *Vitex diversifolia* showed potential antibacterial activity against all the bacterial strains tested. The biological activities of *Vitex* species have been well documented by many researchers. The extracts of the leaves and twigs of *V.negundo* were reported to show antibacterial activity against *Escherichia coli* and *Micrococcus pyogenes* (Bratner *et al.*, 1960; Crafton, 1983). Methanolic extracts of stem bark of *V.doniana* exhibited good antimicrobial activity against *Shigella dysenteriae* and potent activity against clinically isolated *Salmonella typhi* and *E.coli* (Gilani and Rahman, 2005). Gram positive bacteria were observed more susceptible than Gram negative bacteria in the study. Similar results were obtained from the ethanol extracts of *Nauclea latifolia* and *Daniella oliveri* when tested for antibacterial activity (Dagar and Singh, 1999). The higher resistance of gram negative bacteria to plant extracts is known to be related to the thick murein layer in their outer membrane, which prevent the entry of inhibitory substances to the cells (Marassas, 1991; Reddy *et al.*, 2004). Moreover, the different sensitivities between gram positive and negative bacteria could be ascribed to the morphological differences between these microorganisms (Al-Mughrabi, 2003).

The present study revealed that the fungal strains showed sensitivity towards the ethanol extracts of *Mangifera andamanica*. A similar strategy of using crude plant extracts against plant pathogens for broad commodity uses has been reported (Kloucek *et al.*, 2005). Preliminary phytochemical analysis of other *Mangifera* species such as *M.indica* had revealed the presence of tannins, glycosides, phenols and saponins, which have been well known to inhibit bacteria, viruses, fungi and pests (Mandal *et al.*, 2000). The presence of such phytoconstituents in the leaf extracts might be probably responsible for its activity. Moreover results of the present study indicate that the

stronger extraction capacity of ethanol could have produced greater number of active constituents responsible for antimicrobial activity of the plants. This confirms many of the previous studies that ethanol is a better solvent for more consistent extraction of antimicrobial substances from medicinal plants (Chawla *et al.*, 1992; Ahmad *et al.*, 1998).

In conclusion, the findings of the study strongly recommends further investigations in the nature of chemical constituents of *Vitex diversifolia* and *Mangifera andamanica* for elucidating the active components responsible for their antimicrobial activity. By the interest that has been generated across the globe into medicinal products, there is now more than even a golden opportunity to continue making worthwhile contributions to health care as well as crop management.

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