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Antibacterial Activities of Some Folk Medicinal Plants of Eastern Ghats

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Antibacterial activity in the crude extracts of the selected parts (roots/ leaves/ fruits/ seeds/ bark) of different traditional folk medicinal plants of North Coastal Andhra Pradesh was examined. Of the 14 plants studied only 10 plants showed various levels of antibacterial activity against Gram positive (*Bacillus subtilis*, *Staphylococcus aureus*) and Gram negative (*Escherichia coli*, *Klesbsiella pneumonia* & *Proteus vulgaris*) bacteria with the diameters of growth inhibition zone in the range of 10–25mm. However, *Abelmoschus moschatus* (Fabaceae), *Thespesia populnea* (Malvaceae), *Abutilon indicum* (Malvaceae) and *Amaranthus spinosis* (Amaranthaceae) have not shown any antimicrobial activity in the extracts with solvents such as methanol, 95% ethyl alcohol and methanol: chloroform: water (MCW – 12:5:3) mixture. *Eclipta prostrata* (Compositae), which is used as folk medicine, showed antibacterial activity only in the ethyl alcohol extract of its leaves. Present studies indicate that the ten plants, which showed antibacterial activity, may be used as good sources for the isolation of the active principle(s) for therapeutic applications.

Key words: Eastern Ghats, Antibacterial activity, fFolk medicinal plant, Gel diffusion.

Indian Ayurvedic system is one of the noteworthy systems of traditional medicine practice that uses mainly certain plants for the treatments of ailments in both man and other animals. Although the popularity of herbal medicine recorded a sharp decline after the introduction of allopathic chemical drugs, herbal medicines are gaining growing interest because of their cost-effective, eco-friendly attributes, and true relief from disease condition. The harmful side effects and high cost of the other forms of treatment and their non-availability to the poor populations, who live in remote areas, are also the reasons for the demand for herbal medicine. The increasing prevalence of multidrug resistant strains of bacteria and the recent emergence of strains with reduced susceptibility to antibiotics raises the specter of

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untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies[1]. Contrary to the synthetic drugs, antimicrobial substances of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases[2]. Traditional medicines are used by about 70 per cent of the world population. Antibacterial properties of certain Indian medicinal plants were reported based on folklore information and only few reports are available on inhibitory activity against certain pathogenic bacteria and fungi[3-4]. Indian folk medicine comprises numerous herbal prescriptions for therapeutic purposes which may be as varied as healing wounds, treating inflammation due to infection, skin lesions, leprosy, diarrhoea, scabies, venereal diseases, snake bite and ulcers etc. Use of plants as a source of medicine has been inherited and is an important component of the health care system in India. In these systems of Indian medicine, most practitioners formulate and dispense their own recipes; hence this requires proper documentation and research[5]. Higher plants produce a large number of diverse chemical compounds with different biological activities[6]. It is believed that these compounds may have important ecological roles. India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world[7]. Biologically active compounds from natural sources have always been of great interest to scientists working on infectious diseases. In recent years there has been a growing interest to evaluate plants possessing antibacterial activity against various diseases[8].

North coastal Andhra Pradesh has wide range of forest area. Especially, Visakhapatnam is one such area where traditional healing systems are still popular among the local people as most of the tribals of north coastal Andhra Pradesh are living in Visakhapatnam, Vizianagaram and Srikakulum districts. But so far, few systematic ethnobotanical surveys have been made in this area, except the documentation of medicinal plants used by the tribes like siddis[9], Gowlis[10] etc. In an effort to fulfill the need of systematic documentation of folklore medicinal plants of these districts, we have focused our efforts primarily on documentation as well as screening of plant species of Visakhapatnam district used by tribals for the treatment of various bacterial diseases. The present study was carried out to investigate the antibacterial activity of extracts from some medicinal plants used in folk medicine.

MATERIAL AND METHODS

Plant material

Plants and some of their parts were collected from Eastern Ghats of Visakhapatnam especially from Kambalkonda area, an eco-tourism forest of Visakhapatnam. After collection the plants were deposited and authenticated at Herbarium of Andhra University. They were also identified with the help of Dr. S. Hara Sriramulu, Plant taxonomist, Dr. V.S.Krishna PG College of Andhra University, Visakhapatnam. The required plant material was thoroughly washed first with tap water and then rinsed with distilled water and shade dried at room temperature. The dried plant material was finely powdered and used for solvent extraction.

Preparation of extracts

The method of Alade and Irobi (1993)[11] was adopted for preparation of plant extracts with little modifications[12–13]. 20 gms of shade dried, powdered plant material were soaked separately in 100 ml of methanol: chloroform: water (MCW–12:5:3) mixture, Methanol and ethanol for 72 hrs. Each mixture was stirred periodically using a sterile glass rod and/or orbital shaker. At the end of 72 hrs of extraction, each extract was filtered through cheesecloth for the required processes.

Microbiological studies

The following test organisms were used in this study: *Escherichia coli* (ATCC 11775), *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 12600), *Klebseilla pneumonia* (ATTC 13883) and *Proteus vulgaris* (ATTC 13315). The bacterial strains were obtained from Andhra University, Visakhapatnam, India. The bacteria were grown in nutrient broth (Himedia Pvt, Ltd., Bombay; India) at 37°C and maintained on nutrient agar slants at 4°C and stock at-20°C. Antibacterial activities of the extracts of different plants and their parts were determined by agar well diffusion method[12-13]. Nutrient agar plates were prepared by pour plate method using 20 ml of nutrient medium. The molten sterile nutrient agar medium was cooled to 45°C and mixed

thoroughly with 1.0 ml of growth culture of concerned test organism (1×10^8 cells) and then poured into sterile flat-bottomed petridish (9.0 × 1.5 cm diameter) and allowed to solidify. Wells of 4 mm size were made with sterile cork borer and 20 µl of extract of powdered plant material (2 mg /ml) was added to each well aseptically. Then the plates were incubated at 37°C for 24 hours and the zones of inhibition were measured in millimeters (mm) using Himedia zone of inhibition scale. Results presented are the average values of triplicates. Respective pure solvents served as negative controls; Penicillin and streptomycin have been used as positive controls.

Antibacterial susceptibility Testing

The minimum inhibitory concentration (MIC) was determined for extracts, which showed high antibacterial activity with the agar diffusion method[12-13]. Extracts were serially diluted two fold with corresponding organic solvents up to a final concentration of 0.0625 mg/ml. 20 µl of each diluted extract was introduced in triplicate to all corresponding wells made in petriplates that were already seeded with the standardized inoculum (1×10^8) of the test bacterial cells. All test plates were incubated at 37°C for 24 h. The lowest concentration of each extract, which showed a clear zone of inhibition, was taken as the MIC.

RESULTS AND DISCUSSION

Antibacterial activity in the extracts of 14 plants belonging to different botanical families has been evaluated *in vitro* against five bacterial species, which are known to cause dermal and mucosal infections, besides other infections in humans especially in the tribal people (Tables 1&2). Three different organic solvents i.e., ethyl alcohol, methanol and methanol: chloroform: water mixture (12: 5: 3 volume ratio) were used for preparing plant extracts. In these studies, only 10 plants showed antibacterial activity against some of the test organisms. Most putative and active plants identified are *Punica granatum*, *Cliotoria ternata*, *Lantana camara*, *Euonymous glaber* and *Randia dumetorum* whereas the plants, *phyllanthus niruri*, *Eclipta prostrata, Tribulus terrestris* and *Thespesia populnea* did not show significant antibacterial activity (Table 2).

Punica granatum (pomegranate) fruit is used in folk medicine for antiviral, anti-helminthic, anti-fungal and antibacterial against methicillin resistant *Staphylococcus aureus*. Fruit extract also found to show anti-proliferative, anti-invasive, anti-icosanoid, pro-apoptotic actions and promotes tissue healing. It is effective against respiratory diseases and modulates immune response. In our studies both MCW and ethanol extracts of *P.granatum* fruit rind showed very significant antibacterial activity against *B.subtilis* and *P.vulgaris*, whereas MCW extract exhibited similar action only on both *E.coli* and *K.pneumonia*. These two organisms are the most frequent etiological agents of urinary and respiratory infections respectively[14]. Therefore, MCW and methanol extracts of fruit rind of this plant are most active against both gram-negative *E. coli* and *K. pneumonia*. Methanol extract exclusively showed significant activity against all four gram-negative bacteria tested. We believe that these results are the first reports on the fruit rind of *P.grantum*. MIC values are very significant against all bacteria tested. This might be the reason for the people to use the fruit rind of this plant as folklore medicine for curing the dysentery and diarrhoea in tribal areas of North Coastal Andhra Pradesh.

The tropical forage legume *Clitoria ternatea* (L.) has important agronomic traits such as adaptation to a wide range of soil conditions and resistance to drought. It is resistant to a number of pathogens and pests. Aqueous extract of its roots enhances memory by increasing the functional growth of neurons of the amygdala[15]. A small and highly basic protein was purified from seeds of *C. ternatea*, which is found to show antifungal, insecticidal, antipyritic, Anti-inflammatory and analgesic activities. The extract was found to possess nootropic, anxiolytic, anti-depressant, anti-convulsant and anti-stress activity[16]. Ethanolic and MCW extracts of root of this plant showed very significant broad spectrum of antibacterial activity against all bacterial strains tested. MIC values of the extracts ranged from 0.0625 to 0.152 mg/ml.

Albizzia libbeck is commonly called Indian Siris or East Indian Walnut tree is used in folk remedies for abdominal tumors, boils, cough, eye ailments, flu and lung ailments. Seed oil used

Table 1. Antibacterial activities of selected medicinal plants of North Coastal A.P

S. No	Botanical Name, Family	Part used	Formulations	Antibacterial activity				
				SA	BS	EC	KP	PV
1.	*Albezzia lebbeck*, Mimosaceae	Bark	Ethanol	1+	1+	1+	1+	1+
			Methanol	2+	2+	3+	3+	2+
			MCW	2+	1+	1+	2+	2+
2.	*Abelmoschus moschatus*, Fabaceae	Seeds	Ethanol, Methanol & MCW	–	–	–	–	–
3.	*Punica granatum*, Myrtaceae	Fruit rind	Ethanol	2+	3+	2+	2+	3+
			Methanol	2+	2+	3+	3+	3+
			MCW	2+	3+	3+	3+	3+
4.	*Clitoria ternata*, Fabaceae	Roots	Ethanol	2+	1+	2+	2+	1+
			Methanol	2+	3+	3+	2+	2+
			MCW	2+	2+	2+	3+	2+
5.	*Lantana camara*, Verbenaceae	Leaves	Ethanol	2+	2+	2+	2+	2+
			Methanol	2+	1+	2+	1+	2+
			MCW	2+	1+	2+	1+	1+
6.	*Thespesia populnea*, Malvaceae	Seeds	Ethanol, Methanol & MCW	–	–	–	–	–
7.	*Tribulus Terrestris*, Zygophyllaceae	Fruit	Ethanol	–	2+	–	–	–
			Methanol	2+	2+	–	–	–
			MCW	2+	1+	–	–	–
		Leaves	Ethanol	–	2+	–	–	1+
			Methanol & MCW	–	–	–	–	–
8.	*Abutilon indicum*, Malvaceae	Leaves	Ethanol, Methanol & MCW	–	–	–	–	–
9.	*Eclipta prostrata*, Compositae	Leaves	Ethanol	3+	2+	1+	–	–
			Methanol & MCW	–	–	–	–	–
10.	*Euonymous glaber*, Myrtaceae	Leaves	Ethanol	3+	3+	3+	3+	3+
			Methanol	3+	2+	3+	3+	3+
			MCW	3+	3+	3+	3+	3+
11.	*Randia dumetorum*, Rubiaceae	Leaves	Ethanol	2+	2+	1+	1+	2+
			Methanol	1+	2+	1+	1+	2+
			MCW	1+	1+	1+	1+	1+
12.	*Phyllanthus niruri*, Ephorbiaceae	Whole plant	Ethanol	–	–	–	–	–
			Methanol	2+	1+	2+	1+	2+
			MCW	1+	1+	1+	1+	1+
13.	Calophyllum inophyllum, Clusiaceae	Fruits	Ethanol	1+	1+	1+	1+	1+
			Methanol	2+	3+	3+	3+	3+
			MCW	3+	3+	3+	3+	3+
14.	*Amaranthus spinosis*, Amaranthaceae	Whole plant	Ethanol, Methanol & MCW	–	–	–	–	–

Bacteria tested: SA – *Staphylococcus aures*, BS– *Bacillus subtilis*, EC– *Escherichia coli*, KP– *Klebseilla pneumonia*, PV– *Proteus vulgaris*. Antibacterial activity key: – No Inhibition, 1+ : <10mm Inhibition, 2+: 10-15 mm Inhibition, 3+: 15-25 mm Inhibition

for leprosy. Bark saponins are identified as anti-fertility factors[17]. In the present study we observed a broad spectrum of activity against all organisms tested with little bit of variation.

Lantana camara L. is regarded as a notorious weed and a popular ornamental garden plant, which is used in folk medicine in many parts of the world. It has a broad spectrum of anti-fungal, antibacterial and anti-mutagenic properties[18]. It is also used as an antipyretic and mosquito repellants[19]. *L.camara* is one of the ingredients in an ointment made with ethanolic extracts of leaves of Senna alata, *L.camara* and *Madicarpus scaber* for the treatment of dermatophilosis. In our studies we found that the broad spectrum of antibacterial activity in MCW and ethanol extracts of Leaves of *L.camara* against all tested organisms. This shows that there is a correlation between its popularity as folk medicinal plant with the present experimental observation on its antibacterial activity.

Thespesia populnea bark was found to show considerable antioxidant and anticarcinogenic activities against K562 cell lines. Aqueous extract of its fruit is being used to treat a variety of skin ailments including wounds[20]. MCW and ethanol extract of seeds of this plant did not show any activity against all organisms tested in the present studies. This indicates that the pulp of the fruit may be responsible for showing antibacterial activity and not the seeds.

Tribulus terrestris (Puncturevine) is a natural herb used as folk medicine for treatment of hypertension, diuresis, colic pains, hypercholesterolemia, antifungal, anticancer, anti-helminthic, anti-hyperglycemic and broad spectrum of antibacterial activity[21]. This plant seeds are recommended for enhanced physical performance regardless of scientific evidence of effect in China, Korea, India and other countries, to provide a popular alternative for men and women seeking to improve their sex performance and also as energizer and vitalizer in the indigenous system of medicine[22]. Nevertheless of the previous reports on fruits and leaves of *T.terrestris,* it was observed to show antibacterial activity on tested gram-positive than gram-negative bacteria. Alcohol extract of leaves showed only mild activity against gram-negative *P.vulgaris*.

Table 2. Traditional uses of some folk medicinal plants selected for antibacterial investigations

S. No	Botanical Name, Family	Telugu local Name/ Hindi Name	Part used	Traditional uses
1.	*Albezzia lebbeck*, Mimosaceae	Dirisana/ Bansa	Bark	Skin diseases, Leprosy, Asthma, Leucoderma
2.	*Abelmoschus moschatus*, Fabaceae	Kasturibenda vittulu/ Mushkdana/	Seeds	Urinary troubles, Gonorrhoea,
3.	*Punica granatum*, Myrtaceae	Danimma chettu/ Anar	Fruit rind	Diarrhoea, Dysentery
4.	*Clitoria ternata*, Fabaceae	Shanku, ushpi/Aparajita	Roots	Leprosy, Leucoderma, Bronchitis, Asthma
5.	*Lantana camara*, Verbenaceae	Talambrala poola chettu/ Ghaneri	Leaves	Decotion used in Malaria, Atoxy, Rheumatism
6.	*Thespesia populnea*, Malvaceae	Ganga Ravi/ Parsipu	Seeds	Scabies, Psoriasis, Leprosy
7.	*Tribulus terrestris*, Zygophyllaceae	Palleru/ Hussuk	Fruits & Leaves	Gonorrhoea, Skin diseases
8.	*Abutilon indicum*, Malvaceae	Tuttura benda/ Kanghi	Leaves	Syphillis, Nervous weakness
9.	*Eclipta prostrata*, Compositae	Guntakalagara/ Mochkand	Leaves	Gonorrhoea, Jaundice
10.	*Euonymous glaber*, Myrtaceae	Spindle tree	Leaves	Skin diseases
11.	*Randia dumetorum*, Rubiaceae	Manga chettu	Leaves	Respiratory diseases.
12.	*Calophyllum inophyllum*, Clusiaceae	Ponna chettu/Surpan	Fruits	Skin diseases, Leprosy, Scabies
13.	*Phyllanthus niruri*, Ephorbiaceae	Nela vusiri/Jungli amla	Whole plant	Jaundice, Skin diseases, Urinary diseases
14.	*Amaranthus spinosis*, Amaranthaceae	Mulla thotakura	Whole plant	Leprosy, Bronchitis

Table 3. Minimum inhibitory concentrations (MIC) of medicinal plants of North Coastal Andhra Pradesh

S. No.	Name of the Plant Formulations	Microbial species	*S. aureus*	*B. subtilis*	*E.coli*	*K.pneumonia*	*P.vulgaris*
			MIC values in mg/ml				
1.	*Albezzia lebbeck*	Ethyl alcohol	+	+	+	++	++
		Methanol	+	+	+	+	+
		MCW	+	+	+	++	+
2.	*Punica granatum*	Ethyl alcohol	+++	++++	++	++	+++
		Methanol	+++	++++	++++	++++	++++
		MCW	+++	++++	++	+++	+++
3.	*Clitoria ternata*	Ethyl alcohol	++	++++	++	++	++
		Methanol	+++	+++	++	++	++
		MCW	++	++	++	++	++
4.	*Lantana camara*	Ethyl alcohol	++	++	++	++	++
		Methanol	++	++	++	++	++
		MCW	+	+	+	+	+
5.	*Tribulus Terrestris*	Ethyl alcohol	–	++	–	–	–
		Methanol	++	++	++	–	–
		MCW	++	++	–	–	–
6.	*Tribulus Terrestris*	Ethyl alcohol	–	++	–	–	–
7.	*Eclipta prostrata*	Ethyl alcohol	++++	+++	++	–	–
8.	*Euonymous glaber*	Ethyl alcohol	++++	++++	+++	+++	++++
		Methanol	++++	++++	++++	++++	++++
		MCW	++++	++++	++++	++	++++
9.	*Randia dumetorum*	Ethyl alcohol	++	+++	+	++	+++
		Methanol	+++	++	+	++	+++
		MCW	+++	++	++	++	+++
10.	*Phyllanthus niruri*	Methanol	++	+	++	+	++
		MCW	++	+	+	+	++
11.	ControlsAntibiotics	Ampicillin	+++	+++	++	++	++
	Streptomycin		+++	+++	++++	+++	++++

*Concentrations: Plant extracts made @ 2 mg/ ml; antibiotics @ 2 mg/ml

Minimum inhibitory concentration key: +: 1.0 – 2.0 mg/ml; ++: 0.10 – 0.90 mg/ml; +++: 0.01 – 0.090 mg/ml; ++++: 0.001– 0.0090 mg/ml; –: No significant results

Eclipta prostrata is an important Chinese medicinal plant with a reputation for its action against snakebite and its butanolic extract has been shown to exhibit anti-venom potental[23]. Methanol extracts obtained from the leaves, bark, and roots of this plant have been screened for antibacterial and antifungal activities and found to display a broadest spectrum of activity[24]. This plant is also used in the traditional medicine of Perak, Peninsular Malaysia. Unlike previous reports, we found only a narrow range of antibacterial activity especially on *S.aureus, B.subtilis,* and *E.coli* in which *S.aureus* was highly inhibited by alcohol extract but not MCW extract of leaves.

The antibacterial activity of different plant extracts with respect to each organism tested is depicted in Table 1. *Punica granatum* (Fruit rind), *Clitoria ternate* (Root), *Randia dumetorium* (Leaves), *Lantana camara* (Leaves), *Euonymous glaber* (Leaves) and *Calophyllum inophyllum* (fruits) exhibited broad spectrum of antibacterial activity. The remaining plants were found to have moderate antibacterial activity. In many reviews it was noticed that MCW extracts exhibited the highest degree of antibacterial activity as compared to alcoholic extracts from different medicinal plants. Therefore, it is clear from Table-2, specific antibacterial activity found in some of the plants studied in the present investigations appear to have a fairly good degree of correlation with their traditional therapeutic use. Medicinal herb is considered to be a chemical factory as it contains a multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresins, sesquiterpene lactones and oils (essential and fixed). The antibacterial activity of the root of the *Clitoria ternata* is being reported for first time and it may be an important source for many antibacterial compounds. The results obtained in the present studies authenticate and support the use of these plants in folklore medicine for treatment of different skin and other diseases caused by the bacterial pathogens. Investigations on the antibacterial activity of two plants such as *Euonymous glaber* and *Randia dumetorium* are not well explored. These two plants including *P. grantum* showed significant antibacterial activity than the remaining plants. Four plants i.e., *Abelmoschus moschatus*, *Thespesia populnea*, *Abutilon indicum* and *Amaranthus spinosis* did not show any activity against organisms tested. Srinivasan *et al*[25] reported that the water extract of *A. indicum* showed significant antibacterial activity against *Bacillus subtilis* and not against any other bacteria used in our studies. Water extract of *Tribulus terrestris* also showed activity against *E.coli*, *K.pneumonia*. Out of 14 plants studied against *K. pneumonia* only eight plants (S.No. 1, 3-5, and 10-13 in Table -2) showed considerable antibacterial activity. Ethanolic and MCW extracts of *Euonymous glaber* showed significant activity against *K.Pneumonia*. Almost similar responses were observed against *P.vulgaris* and *E.coli* with the above eight plants.

Significant zones of inhibition were also observed against both gram positive and gram-negative bacteria. These results marked the beginning of what will most certainly be a large leap forward in understanding how medicinal plants function in regulating response to infections with microbial pathogens. To protect, preserve and exploit our vast biodiversity, we should focus our studies on medicinal plants to exploit and make use of the folklore knowledge of the tribals. Further work is needed to isolate the active principle from the various extracts and to characterize their phyto-pharmaceutical properties. Research into the antibacterial effects of some local medicinal plants will boost the use of these plants in the treatment of diseases caused by the test bacterial species. The results of this present investigations indicated that a large number of medicinal plants of North Coastal Andhra Pradesh have high potential of antibacterial activity.

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