

## Antibacterial activity of selected medicinal plants used by the tribal people

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### Abstract

**Background:** In traditional medical systems, plants have a major role in the treatment of various ailments and this has made the search for new medicines from plants which are used by the traditional practitioners. Still there is a growing interest to utilize plants or plant derived molecules to cure microbial infections. The present study is attempted to collect the selected ethnomedicinal plants from forest areas of Tirunelveli district in Western Ghats of Tamil Nadu, India and to evaluate their antibacterial activity against five bacterial strains.

**Methods:** The ethnomedicinal information was collected through questionnaire among the Kanikar and Paliyar tribal who are practicing traditional medicine in Tirunelveli district, Western Ghats region of Tamil Nadu. Crude n-Hexane and methanolic extract prepared from selected medicinal plants were tested against two gram positive bacteria (*Staphylococcus aureus* MTCC 7443 and *Bacillus subtilis* MTCC 7415) and three gram negative bacteria (*Escherichia coli* MTCC 7436, *Klebsiella pneumoniae* MTCC 7407 and *Vibrio cholerae* MTCC 3906) at 100µg and 500µg/ml concentration by disc diffusion method. MIC of crude methanol extract prepared from selected plants was also tested by adopting broth dilution method.

**Results:** There are 32 plant species belonging to 24 families which are used to cure fever, cold, cough and other respiratory problems were collected with the help of 25 tribal practitioners. The methanolic extract of 19 plants showed inhibitory activity against one or more bacterial strains only at 500 µg/disc concentration. N-hexane extract of all the plants showed no activity. In particular, the methanol extract of seven plants namely *Ruta graveolens*, *Mukia maderaspatana*, *Terminalia chebula*, *Curculigo orchoides*, *Camellia sinensis*, *Cleome viscosa* and *Rauvolfia serpentina* were showed promising antibacterial activity against most of the tested strains. The MIC test results showed that the extracts from seven plants exhibited activity against atleast one bacterial strain between 125 µg/ml to 175 µg/ml concentration.

**Conclusion:** Most of the plants used in traditional medicine are used as either single or mixed with other ingredients and taken as maceration, infusion or decoction. The present study ascertains the value of plants used in herbal treatment, which could be of considerable interest to the potential for discovery of antibacterial principles for the development of new drugs.

**Key words:** Antibacterial activity, Ethnomedicine, Herbal drugs, plant extracts.

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### Background

Since the time of civilization plants have been an essential part of human society. The medicinal plants have been used by humans from the pre-historical times to treat various infectious and non-infectious

diseases. The ethnomedicinal information plays a vital role in the treatment of human diseases as well as in novel drug discovery. According to the world health organization (WHO) as much as 80% of world's population depends on traditional medicine for their primary health care needs (1).

Medicinal plants are one of the most sensitive commodity areas of research in the world today. Therefore, the demand for plant based therapeutics has increased many folds in both developing and developed countries due to the growing recognition that they are natural products, being non-narcotic, having no side effects, easily available at affordable prices (2). Many countries would like to keep their information and knowledge about medicinal plants to themselves for fear of being marginalized in the race to exploit the commercial values of medicinal plants. People who live in rural areas of the India are familiar with the medicinal properties of plants. Such details were usually passed on in the past from parent to offspring in the family and uses of plants and the various combinations or mixes made were kept as a family secret, along with the development of knowledge at family level. A large number of the country's rural population depends on medicinal plants for treating various illnesses. Studies have pointed out that many drugs that are used in commerce have come from folk-use and use of plants by indigenous cultures (3). Ethnomedicinal information plays a vital role in the treatment of human diseases as well as in novel drug discovery (3). Medicinal plants play a vital role in natural resource management (4). The detection of antifungal activity in *Portulaca oleracea* by a single cell bio assay system and bioactive compounds isolated from plant species used in herbal medicines (5). The World Health Organization (WHO) indicates that more than half of the world's population does not have access to adequate health care services. The present work is attempted to study the antibacterial activity of selected ethnomedicinal plants used by the tribal people in Tirunelveli district of Tamil Nadu, India.

### Materials and Methods

The entire area of Tirunelveli district lies between 77°3' E and 77°9' E longitude and 9°1' N and 9°8' N latitude in the state of Tamil Nadu. The district is spread over an area of about 11,433 km<sup>2</sup>. Tribal people were distributed southern slopes of the Western Ghats in Tamil Nadu. The ethnobotanical

data were collected according to the methodology suggested by Jain (6). Ethnobotanical informations were collected during the months of December 2008 to November 2009. The ethnobotanical data were collected using questionnaire, interviews and discussions with the local tribal people and their uses in traditional medicinal preparation among the Kanikar and Paliyar tribal practicing traditional medicine in Tirunelveli District from different places at Papanasam, Courtallam and Shencottai area of Western Ghats.

### Collection of plant materials

Based on the ethnobotanical information, thirty two medicinal plant species were collected; these plants were used for human herbal medicines. Totally more than 25 respondents were interviewed including males and females that depended on plant as sources of medicines either for self-medication or for treating others for fever, cold, cough and other respiratory problems were collected. All these collected plants were identified with the help of plant taxonomist. Their Latin name, local name, family, parts used, mode of preparation and medicinal uses were documented. Further, the plants will also be screened for anti-TB activity leading to novel drug discovery. The identified medicinal plant species with their medicinal uses and phytochemical preparations were listed out in Table- 1.

### Preparation of extracts

The collected medicinal parts selected plants of different were cleaned and shade dried. Then the plant parts were chopped into small pieces and made into fine powder using blender. About 100 grams of coarsely powdered plant material was exhaustively extracted for 2 hours with methanol (60-70°C) in soxhlet apparatus. The plants materials of different plant species were successively extracted with organic solvents like hexane and methanol basing on order of their polarity using soxhlet's apparatus and the collected extract was filtered and evaporated under reduced pressure using Rota-vapor and these extracts were stored in a refrigerator at 4°C.

### Stock extracts preparation

Stock extracts of all the medicinal plant parts were prepared at 20mg/ml concentration using DMSO (final concentration of 1%v/v). Two working concentration (100 and 500µg/disc) were prepared with distilled water (Milli Q) and sterilized by filtration through 0.45µ membrane filter and stored in sterile dark bottles for subsequent experiment.

### Microorganisms tested

Strains of MTCC bacterial culture was used in this study as follow: *Staphylococcus aureus* MTCC 7443, *Bacillus subtilis* MTCC 7415, *Escherichia coli* MTCC 7436, *Klebsiella pneumoniae* MTCC 7407 and *Vibrio cholerae* MTCC 3906. All these strains were obtained from the Department of Microbiology, Sri Sankara Arts and Science College, Kanchipuram, Tamil Nadu, India and its growth was maintained on nutrient agar slants by periodical sub culturing.

### Antibacterial activity testing

Antibacterial activity of crude extracts was tested by disc diffusion method. Twenty four hours old bacterial cultures was taken and suspended in sterile saline. Turbidity of the bacterial suspension was adjusted to 0.1 OD. 100µl of the diluted suspension of bacterial inoculum was transferred into Muller Hinton Agar (MHA) plates and spreaded using sterile cotton swab. The crude extract impregnated discs were placed over the MHA plates seeded with test organisms. Streptomycin disc (25µg) and filter paper disc charged with DMSO was also tested as positive control and solvent control, respectively. Soon after all the plates were kept at 10°C for 30 minutes followed by incubation at 37°C for 24 hours. All tests were performed in duplicates and antibacterial activity was expressed as zone of inhibition (in mm).

**Minimum inhibitory concentration (MIC) assay:** Crude extracts showing maximum zone of inhibition against all the test organisms were further

tested at lower concentrations (125 µg/ml to 200 µg/ml) by broth dilution method to determine the minimum inhibitory concentration (MIC) (6)

### Results and Discussion

**Ethnomedicinal study:** The ethnobotanical information about the 32 plants collected from 25 local tribal peoples revealed that they are traditionally used for fever, cold, coughs and other respiratory problems. The utility lies through their roots, bark, latex, leaves, fruits and seeds (Table 1). The plant parts used in this study include roots from 5 plants, flowers from 2 plants, rhizome of one plant, leaves from 13 plants, fruits from 2 plants, spikes (dried fruits) of one plant, seeds of 7 plants, tuber of one plant and one whole plant. The 32 plants include 7 trees, 6 climbers, 18 herbs and 1 perini. These are taken internally or applied externally in the form of infusion, decoction, paste or powder (7). The ethanomedicinal information of the collected plants evidenced that they are the rich resource for the isolation of anti-infective molecules. Through this research information gives to as some phytomedicines and the source available in the Western Ghats region for medicinal plant conservation and utilization, there is a need to fully document the medicinal plant species, where they are located, their existing population, place(s) of conservation and their known traditional uses.

**Antibacterial activity:** The results of the disc diffusion methods are extensively used to investigate the antibacterial activity of natural substances and plant extracts. These assays are based on the use of discs as reservoirs containing solutions of substances to be examined (8). Among the 32 plant extracts tested, nineteen extracts were found to be active against one or more number of test organisms at the concentration of 500µg/disc. Moreover, the methanol extract of seven plants showed most potent antibacterial activity against all the tested strains. Similarly, methanol extract of *Ruta graveolens* showed significant in vitro anti inflammatory activity (9). The study also provides an evidence for the leaves *Ruta* in folk treatment

**Table1: The list of identified species with their medicinal uses of the tribal people and traditional medical practitioners.**

Sl.No.	Botanical Name (Local name and Family)	Parts used and Ethnomedicinal preparation
1	<i>Ruta graveolens</i> L. Seerpatchilai (Rutaceae )	Seerpatchilai leaves powder was used to encourage the onset of menstruation cycle, and it kill the intestinal worms, these leaves infusion is used as an eye wash and eye problems.
2	<i>Phyllanthus emblica</i> L. Kattunelli (Euphorbiaceae )	Kattunelli fruits are astringent and it is useful in ulcerative stomatitis and gastrohelcosis. This seed powdered to be used for gonorrhea, jaundice, cough and diarrhea.
3	<i>Piper longum</i> L Thippili (Piperaceae )	Thippili roots and fruiting spikes are used in treating diarrhea, indigestion, jaundice, abdominal disorder, hoarseness of voice, asthma, cough, malarial fever and wheezing.
4	<i>Piper nigrum</i> L.Kurumilagu (Piperaceae )	Kurumilagu, after drying the pericarp may be removed, the berries well known for their stomachache, antibacterial purpose, treating dyspepsia, vomiting diarrhea and common cold. The powder berries applied topically to cure toothache.
5	<i>Murraya koenigii</i> (L.)Spreng. Kattukarivepilai (Rutaceae )	Kattukarivepilai leaves are extensively used for flavoring curries; they are also useful for the treatment of rheumatism.
6	<i>Hemidesmus indicus</i> (L) R.Br. Nannari (Asclepiadaceae )	Nannari roots paste are used for leprosy, skin disease, asthma, bronchitis, fever and root extract is used for blood purification and general debility.
7	<i>Hibiscus sigittifolius</i> Kurz. Var. Kattusembaruthi (Malvaceae )	Kattusembaruthi roots decoction of 1-2 spoon was taken once to get instant relief from stomach pain, menstrual disorders and body pain.
8	<i>Mukiamaderaspatana</i> (L) M.Roe Musumuzhukai (Cucurbitaceae )	Musumuzhukai –leaves extract is taken internally to cure piles; it is applied to the hair of the head to blacken gray hair and decoction to cure vomiting and intermittent fever.
9	<i>Syzygium cumini</i> L. Navval (Myrtaceae)	Navval seeds and leaves are antibacterial and are used for strengthening the teeth and gums. The fruits and seeds are in diabetes, diarrhea, and ringworm.
10	<i>Terminalia chebula</i> Retz. Kadukkai (Combretaceae)	Kadukkai powder paste used skin diseases, leprosy and wound ulcer and its decoction used for intermittent fever.
11	<i>Gymnema sylvestra</i> (Retz.) R.Br. Shirukuringa (Asclepiadaceae )	Shirukuringa leaves used for inflammation, jaundice, helminthiasis, asthma, bronchitis and intermittent fever. The fresh leaves when chewed have the remarkable property of paralyzing.
12	<i>Elettaria cardamomum</i> Maten Elakkai (Zingiberaceae )	Elakkai seed tonic used for asthma, bronchitis and anorexia.
13	<i>Curculigo orchoides</i> Gaertn. Nilapana kizhangu (Amaryllidaceae)	Nilapana kizhangu –powder used for skin disease, asthma, bronchitis, jaundice and diarrhea.
14	<i>Ocimum sanctum</i> L. Tulasi (Lamiaceae )	Tulasi leaves mixed with water; drink its keeps the blood pressure. A decoction of the plants is used for cough, fever and mouthwash for relieving toothache.
15	<i>Solanum trilobatum</i> Burn. f. Thuthuvalai (Solanaceae)	Thuthuvalai leaves juice is used to treat cough and cold.



16	<i>Curcuma aromatic</i> Salisb. asthurimanjal(Zingiberaceae)	Kasthurimanjal rhizomes powder is combination with cow milk and taken to control hiccough, bronchitis, and cough, paste used for leucoderma and skin eruption.
17	<i>Eclipta prostrate</i> L. Kari- salanganni (Asteraceae )	Karisalanganni leaves powder used for anti-inflammatory, eruption, leucorrhoea and enterhaemorrhage.
18	<i>Solanam surattense</i> Burn.f. Kantangathiri (Solanaceae )	Kantangathiri dried fruits powder pasted to leprosy, skin disease, rheumatoid fever, cough, asthma and bronchitis.
19	<i>Camellia sinensis</i> (L.) O. Kuntze Thyilai (Theaceae )	Thyilai leaves infusion used in inflammations, abdominal disorders,
20	<i>Coffea Arabica</i> L. Kappi (Rubiaceae )	Kappi seed powder infusion good for fever, the infusion of ripe roasted seed is good for diarrhea, asthma, whooping cough. A strong coffee is a remedy for poisoning by opium.
21	<i>Piper belle</i> L. Karuppuvet- tilai (Piperaceae )	Karuppuvettilai leaves decoction was used in treating wounds, burns and eczema. The leaves, if topically applied to the chest cure, cough and asthma. Fraction of the spinal column with leaves in recommended for treating cold. The roots are used in treating rheumatism.
22	<i>Mimusops elengi</i> L. Mag- ilam (Sapotaceae )	Magilam dried flower powder prepared lotion for wounds and ulcers, and the powder of dried flowers is a brain tonic, diarrhea and dysentery.
23	<i>Cleome viscosa</i> L. Nayikka- dugu (Capparideaceae )	Nayikkadugu powder of dried seeds are anthelmintic, Carminative and useful in fever, diarrhea, worm infestations and cardiac disorder.
24	<i>Tephrosia purpurea</i> (L.)Pers. Kolangi (Fabaceae)	Kolangi leaves are useful in dyspepsia, pectoral diseases, hemorrhoids, and syphilis, gonorrhea and skin diseases.
25	<i>Vitex leucoxylin</i> Linn. f. Nirnuchi (Verbenaceae )	Nirnuchi inhale boiled leaves extract vapors to relive headache and control for respiratory disease and cold.
26	<i>Ipomoea batatas</i> (L.)Lam. Chakkaravallikkilanku (Convolvulaceae )	Chakkaravallikkilanku leaves decoction is used to control hyperdipsia, Constipation, renal and vesicle calculi, diabetes and fever.
27	<i>Trianthema portulacastrum</i> L. Saranai (Aizoaceae)	Saranai roots can be used as plaster to allay pain even from venereal sores.
28	<i>Nephrolepis biserrata</i> (Sw.) Ferns (Oleandraceae)	Ferns leaves paste is applied to cure eczema.
29	<i>Rauwolfia serpentina</i> (L.) enth. Sarpaganthi (Apocy- naceae )	Sarpaganthi roots reducing blood pressure, its decoction is given during labor pain to increase uterine contraction. Juice of the leaves is used for the cure of corneal opacity of the eyes. The dried roots along with bark intact are used in diseases like bowels and fever.
30	<i>Ocimum gratissimum</i> L. Kattuthulasi (Lamiaceae )	Kattuthulasi seed powder mixed with honey is taken internally to improve digestion and to relieve giddiness and the whole plants is used in treating sunstroke, fever, headache and influenza.
31	<i>Maranta arundinacea</i> L. Koovaikilanku (Maranta- ceae )	Koovaikilanku dried root powder mixed with cow milk and taken early morning three days to control cough and respiratory problems.
32	<i>Gardenia resinifera</i> Roth. Tikkamalli (Rubiaceae )	Tikkamalli dry flowers powder mixed with palm sweet to take twice a day, first thing in the morning and before going to bed at night to control all respiratory diseases. A decoction of the flowers is used for hiccough and bronchitis.

Table 2: Antibacterial activity of methanolic extract of selected ethnomedicinal plants from Tirunelveli district of Tamil Nadu, India

Sl. No.	Botanical Name	Parts used	Zone of inhibition (mm)											
			S.aureus	B.subtilis	E. coli				K. pneumoniae				V.cholerae	
					Concentration (µg/disc)									
			100	500	100	500	100	500	100	500	100	500	100	500
1	Ruta graveolens L.	Leaves	-	15.4	-	15.8	-	17.8	-	15.4	-	15.4	-	13.6
2	Phyllanthus emblica L.	Fruits	-	-	-	-	-	09.3	-	-	-	-	-	-
3	Piper longum L.	Spikes	-	-	-	12.4	-	12.6	-	11.8	-	-	-	-
4	Piper nigrum L.	Seeds	-	-	-	-	-	-	-	-	-	-	-	-
5	Murraya koenigii (L.)Spreng.	Leaves	-	-	-	-	-	-	-	-	-	-	-	-
6	Hemidesmus indicus (L.)R.Br.	Roots	-	08.6	-	08.4	-	10.6	-	10.8	-	10.8	-	12.4
7	Hibiscus sigittifolius Kurz. Var.	Roots	-	-	-	-	-	-	-	15.4	-	15.4	-	10.6
8	Mukia maderaspatha (L.)M.Roe	Leaves	-	10.3	-	18.4	-	08.2	-	15.6	-	15.6	-	12.9
9	Syzygium cumini L.	seeds	-	-	-	11.2	-	-	-	-	-	-	-	-
10	Terminalia chebula Retz.	Seeds	-	16.6	-	-	-	15.8	-	-	-	-	-	-
11	Gynmema sylvestra (Retz.)R.Br.	Leaves	-	-	-	10.2	-	-	-	-	-	-	-	-
12	Elettaria cardamomum Maten	Seeds	-	-	-	12.4	-	08.2	-	08.4	-	-	-	-
13	Curculigo orchoides Gaertn.	Tubers	-	-	-	-	-	18.9	-	-	-	-	-	-
14	Ocimum sanctam L.	Leaves	-	-	-	-	-	15.5	-	15.5	-	-	-	-
15	Solan trilobatum Burn. f.	Leaves	-	-	-	-	-	-	-	15.4	-	15.4	-	-
16	Curcuma aromatic Salisb.	Rhizome	-	-	-	-	-	-	-	-	-	-	-	-
17	Eclipta prostrate L.	Leaves	-	-	-	-	-	-	-	-	-	-	-	-
18	Solanam surattense Burn.f.	Fruits	-	-	-	10.2	-	-	-	-	-	-	-	-
19	Camellia sinensis (L.)O.Kuntze	Leaves	-	15.6	06.3	15.4	-	15.6	-	18.9	-	18.9	-	20.2
20	Coffea Arabica L.	Seeds	-	-	-	-	-	-	-	-	-	-	-	-
21	Piper betle L.	Leaves	-	-	-	-	-	-	-	-	-	-	-	-
22	Mimusops elengi L.	Flowers	-	-	-	-	-	-	-	12.4	-	12.4	-	-
23	Cleome viscosa L.	Seeds	-	-	-	-	-	-	-	17.2	-	17.2	-	-
24	Tephrosia purpurea(L.)Pres.	Leaves	-	-	-	-	-	-	-	-	-	-	-	-



## Conclusion

Findings of the present study revealed that the medicinal plants distributed in the Western Ghats region are widely used in the traditional medicine to treat various respiratory infections. Further the results of *in-vitro* antibacterial study evidenced that the seven potential plants identified in this study will be a good source for the isolation of active molecule to fight against various dreadful pathogens.

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