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Research Article

ANTIBACTERIAL ACTIVITY OF ETHNOMEDICINALLY USED MEDICINAL PLANTS BY THE TRADITIONAL HEALERS OF THIRUVALLUR DISTRICT, TAMILNADU, INDIA

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ABSTRACT

The objective of this study was to collect information about medicinal plants and their uses, by the knowledge obtained from the traditional healers in Thiruvallur district, Tamilnadu, India. It also determines antimicrobial activity of ten different plants selected based on spearman rank correlation. The traditional healers of Thiruvallur district use different species of medicinal plants belonging to different families for the treatment of various diseases. Commonly maximum number of species has been used for fever followed by skin infection, wound healing and antiseptic. Herbs were found to be the most used plants followed by climbers and shrubs. The spearman rank correlation was used to analayse the knowledge about medicinal plants. Antibacterial activity of ten medicinal plants (Justicia gendarussa, Tephrosia purpurea, Phyllanthus maderaspatensis, Elephantopus scaber, Trichodesma indicum, Rhinacanthus nasatus, Sida cordifolia, Lepidagathis cristata, Evolvulus nummularius and Aerva lanata) was determined by measuring the diameter of zone of inhibition that is the mean of triplicates+standard deviation of three replicates. The traditional healers in Thiruvallur district possess rich ethno-botanical knowledge. This study on medicinal plants will attract ethano botanist, phytochemist and pharmacologist in identifying novel antibacterial compounds.

Keywords: Antibacterial; Medicinal plants; Traditional Medicine

INTRODUCTION

Plants are considered not only as dietary supplement to living organism but also traditionally used for treating many health problems. Medicinal plants and its products are a source of many potent and newer powerful herbal drugs (Srivastava et al., 1996)1. The active principles of many drugs found in plants are secondary metabolites (synthesized during secondary metabolism of the plant) (Ghani, 1990; Dobelis, 1993)2. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. The plant kingdom harbors an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Moreover, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive.

However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (Shariff, 2001)3. The screening of plant extracts and its products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan,2003)4. Numerous studies have identified compounds within herbal plants that are effective antibiotics (Basile et al., 2000)5. Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics (Okpekon et al., 2004)6; some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria (Kone et al., 2004)7. It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity (Ebana et al., 1991; Manna and Abalaka, 2000)8. The result of above studies and literature survey indicates the need for further research into traditional health system.

Table1: Medicinal plants selected and their uses

Medicinal plants	Family	Vernacular name(in Tamil)	Chemical constituents	Uses
Justicia gendarussa	Acanthaceae	Neernotchi	Vasicine,Vasicinone, Vasicinol,Vasicol and Vasmetine	Useful in treating chronic rheumatism,inflammation,bronchitis,vaginal discharge,eye disease and fever
Tephrosia purpurea	Fabaceae	Kolunchi	Glycosides.isoflavones,Tephrosin,sterols and lanceolatin-B	Useful in treating skin infection,asthma,ulcer,urinary disorders,pain and inflammation.
Phyllanthus maderapatensis	Euphorbiaceae	Nila-nelli	Essential oils,maderin and mucilage	Useful in treating ulcers,spleen enlargement,skin infection and fever
Elephantopus scaber	Asteraceae	Aanaikalsuvati	Lupeol,stigmasterol,Elephantopin.scarbetopin and Germacranolide dilactone 11,13 dihydrodeoxvelephantopin	Useful in treating bronchitis,small pox,bladder stones,skin infection and wounds
Trichodesma indicum	Boranginaceae	Kavizhthumbai	Non steroidal compounds hexacosane,oleic acid and linoleic acid	Useful in treating eye diseases,arthritis,anorexia,skin diseases,snake bite poisoning and fever
Rhinacanthus nasatus	Acanthaceae	Nagamalli	Quinol,terpenoids,steroids,benzenoids,anthroquinone,quinone,glycoside and carbohydrate	Useful in treating hepatitis.diabetes,hypertension and skin diseases
Sida cordifolia	Malvaceae	Sitramutti	Alpha-phenethylamines,carboxylated tryptamines and quinazoline alkaloids	Useful in treating asthma, nervous disorders,skin infection and gastric disorders (S. Sankaranarayanan et al 2000)[9]
Lepidagathis cristata	Acanthaceae	karappanpoondu	6-hydroxyluteolin,6-hydroxluteolin-7-apioside,a new tryptophan derived alkaloid cristatin A	Useful in treating eczema.psoriasis and other skin diseases.and fever
Evolvulus nummularius	Convolvulaceae	Aakhukarni	Beta-sitosterols.glucoside,stigma sterol,d-mannitol,urosolic acid and oleanolic acid	Useful in treating bleeding,dysentery,wounds and skin infection
Aerva lanata	Amaranthaceae	Sirupeelai	Flavanoids,Glycosides,aervoside,betulin,campesterol and vanillic acid	Useful in treating urinary calculi.haematemesis.bronchitis,nasal bleeding.cough and fracture

MATERIALS AND METHODS

Plant material

The plants (listed in table 1) were collected from Thiruvallur district, Tamilnadu, India. The plant was identified with the help of available literature and authenticated by Dr. S. Sankaranarayanan, Head of the department, Department of Medicinal Botany, Sri Sairam Siddha Medical College, Tambaram, Chennai. Collected plant material was air-dried under shade at room temperature, ground with an electric grinder into fine powder and stored in airtight containers.

Bacterial strains

Microorganisms used for the determination of antibacterial activities of isolated compounds were Gram positive; Staphylococcus aureus MTCC 29213, Gram negative; Vibrio cholerae MTCC 12657. Both bacterial strains were obtained from Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology Sector 39-A, Chandigarh – 160036, India. Different bacterial strains were maintained on nutrient agar and subcultures were freshly prepared before use. Bacterial cultures were prepared by transferring two to three colonies into a tube containing 20 ml nutrient broth and grown overnight at 37 °C.

Preparation of Methanolic leaf extracts

Dried leaf powders from the plants (listed in table 1) (500 g) individually was extracted with 1litre of methanol (at room temperature) after 24 hrs of soaking. The extracts were collected, filtered, centrifuged at 4000 rpm for 10 minutes and the supernatant was collected, concentrated in a vacuum rotary evaporator and used for analysis.

Agar disc diffusion assay

The antibacterial activity was studied using the disc-diffusion method10. Bacteria were grown overnight on Muller Hinton agar plates. Five young colonies were suspended with 5ml of sterile saline (0.9%) and the density of the suspension adjusted to approximately 3×108 colony forming units (CFU). The swab was used to inoculate the dried surface of MH agar plate by streaking four times over the surface of the agar, rotating the plate approximately by 90 ° to ensure an even distribution of the inoculums. The medium was allowed to dry for about 3 min before adding a sterile paper disc of 5 mm diameter. Each disc was tapped gently down onto the agar to provide uniform contact.

5, 10, 15 and 20 µl of the methanolic leaf extract of each plant individually were introduced on each disc (five replicates) and 7% methanol alone served as a negative control. The plates were incubated at 37 °C for 24 h; inhibition zones were measured and calculated.

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration of the isolated compounds was determined by dilution method11. The strains were grown in Mueller Hinton broth to exponential phase with an A560 of 0.8, representing 3×108 CFU/ml. Different dilutions of the methanolic leaf extract of each plant individually were prepared to give concentrations at 5, 10, 15 and 20 µg/ml respectively. 0.5 ml of each concentration was added into separate test tubes containing 4ml of MH broth inoculated with 0.5 ml bacterial suspension at a final concentration of 108 CFU/ml. Each MIC was determined from five independent experiments performed in duplicate. The tubes containing 4.5 ml of bacterial inoculates and 0.5 ml of 7% methanol were used as bacterial controls, 4.5 ml of uninoculated Mueller Hinton broth and 0.5 ml PBS served as a blank. The tubes were incubated at 37 °C for 18 h; inhibition of bacterial growth was determined by measuring the absorbance at A560 nm.

ANTIBACTERIAL ACTIVITY OF 10 MEDICINAL PLANTS BY DISC DIFFUSION METHOD

Table2: Disc diffusion method

Medicinal plants	S.aureus			
	5µg/ml	10µg/ml	15µg/ml	20µg/ml
Aerva lanata	2.33±0.58	3.33±0.58	4.33±0.58	5.33±0.58
Elephantopus scaber	10.5±0.5	12.4±0.1	13.93±0.60	15.5±0.5
Evolvulus nummularis	8.1±0.5	8.5±0.1	10.5±0.5	12.4±0.1
Justicia gendarussa	17.5±0.5	20.33±0.58	22±1	24.33±0.58
Lepidagathis cristata	8.1±0.5	8.5±0.1	10.5±0.5	11.33±0.58
Phyllanthus maderapatensis	6.5±0.5	8.5±0.1	9.17±0.29	10.5±0.5
Rhinacanthus nasatus	4.33±0.58	5.33±0.58	5.83±0.58	6.17±0.29
Sida cordifolia	6.5±0.5	8.5±0.1	9.17±0.29	10.5±0.5
Tephrosia purpurea	6.17±0.29	9.17±0.29	10.5±0.5	12.4±0.1
Trichodesma indicum	10.5±0.5	12.4±0.1	13.93±0.60	15.5±0.5

The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates ± SD of three replicates.

Table3: Disc diffusion method

Medicinal plants	V.chlorea			
	5µg/ml	10µg/ml	15µg/ml	20µg/ml
Aerva lanata	3.33±0.58	4.33±0.58	5.33±0.58	8.5±0.1
Elephantopus scaber	5.33±0.58	5.83±0.58	6.17±0.29	9.17±0.29
Evolvulus nummularis	4.33±0.58	5.33±0.58	5.83±0.58	9.17±0.29
Justicia gendarussa	**11.5±0.5**	**12.4±0.1**	**15±1**	**16.93±0.55**
Lepidagathis cristata	10.5±0.5	12.4±0.1	13.93±0.60	15.5±0.5
Phyllanthus maderapatensis	6.5±0.5	8.5±0.1	9.17±0.29	10.5±0.5
Rhinacanthus nasatus	2.33±0.58	3.33±0.58	4.33±0.58	5.33±0.58
Sida cordifolia	9.17±0.29	10.5±0.5	12.4±0.1	13.93±0.60
Tephrosia purpurea	6.5±0.5	8.5±0.1	9.17±0.29	10.5±0.5
Trichodesma indicum	4.33±0.58	5.33±0.58	5.83±0.58	6.17±0.29

The antimicrobial activity was determined by measuring the diameter of zone of inhibition that is the mean of triplicates ± SD of three replicates

ANTIBACTERIAL ACTIVITY OF 10 MEDICINAL PLANTS BY MINIMUM INHIBITORY CONCENTRATION METHOD

Table 4 MIC

Medicinal plants	S.aureus			
	5µg/ml	10µg/ml	15µg/ml	20µg/ml
Aerva lanata	0.719±0.02	0.579±0.05	0.544±0.02	0.459±0.03
Elephantopus scaber	0.610±0.01	0.579±0.05	0.544±0.02	0.559±0.03
Evolvulus nummularis	0.579±0.05	0.544±0.02	0.162±0.02	0.135±0.02
Justicia gendarussa	0.312±0.01	0.263±0.01	0.160±0.01	0.112±0.01
Lepidagathis cristata	0.579±0.05	0.459±0.03	0.367±0.02	0.263±0.01
Phyllanthus maderapatensis	0.629±0.04	0.459±0.03	0.367±0.02	0.263±0.01
Rhinacanthus nasatus	0.719±0.02	0.579±0.05	0.459±0.03	0.312±0.01
Sida cordifolia	0.459±0.03	0.367±0.02	0.263±0.01	0.112±0.01
Tephrosia purpurea	0.719±0.02	0.579±0.05	0.544±0.02	0.367±0.02
Trichodesma indicum	0.752±0.01	0.641±0.01	0.629±0.04	0.544±0.02

The minimum inhibitory concentration was determined by optical density of inhibition that is the mean of triplicates ± SD of three replicates.

Table 5 MIC

Medicinal plants	V.chlorea			
	5µg/ml	10µg/ml	15µg/ml	20µg/ml
Aerva lanata				
Elephantopus scaber	0.579±0.05	0.459±0.03	0.367±0.02	0.263±0.01
Evolvulus nummularis	0.629±0.04	0.459±0.03	0.367±0.02	0.263±0.01
Justicia gendarussa	0.367±0.02	0.263±0.01	0.212±0.01	0.112±0.01
Lepidagathis cristata	0.719±0.02	0.579±0.05	0.459±0.03	0.312±0.01
Phyllanthus maderapatensis	0.459±0.03	0.367±0.02	0.263±0.01	0.112±0.01
Rhinacanthus nasatus	0.719±0.02	0.579±0.05	0.544±0.02	0.367±0.02
Sida cordifolia	0.719±0.02	0.629±0.04	0.459±0.03	0.263±0.01
Tephrosia purpurea	0.729±0.02	0.629±0.04	0.544±0.02	0.367±0.02
Trichodesma indicum	0.579±0.05	0.459±0.03	0.367±0.02	0.263±0.01

The minimum inhibitory concentration was determined by optical density of inhibition that is the mean of triplicates ± SD of three replicates.

RESULTS AND DISCUSSION

The antibacterial activity of 10 different medicinal plants at different concentration was screened by disc diffusion technique and the zone of inhibition was measured in mm diameter (Table 2,3). The medicinal plant Justicia gendarussa was more effective against the Gram positive strain S.aureus compared with Gram (-) V.chlorea with a zone of inhibition percentage of 24.1 and 16.5 respectively at the concentration of 20µl/ml. Furthermore the MIC also support the present findings (table 3,4) respectively. The plant showed a broad spectrum of antimicrobial activity suggesting that the plant possess certain constituents with antibacterial properties that can be used as antimicrobial agents in designing drugs for infectious diseases caused by pathogens. The medicinal properties and pharmacological actions of the plant is well used in traditional medicine. The plant contains many therapeutic uses and has certain biological activity against number of infectious diseases.

CONCLUSION

The results indicated that comparing to all 10 medicinal plants, the plant JUSTICIA GENDARUSSA showing high inhibitory activity against the pathogens. The presence of phytochemicals in the plant is responsible for the inhibitory activity. Hence, there is a need for further research into the medicinal plant JUSTICIA GENDARUSSA and might be useful in treating the bacterial disease.

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