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Research Paper

In Vitro Antibacterial Potential of Some Medicinal Plants Against Human Enteric Pathogens

Microbiology

KEYWORDS : Medicinal plant, enteric pathogen, antibacterial, disc diffusion.

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ABSTRACT

To investigate in vitro antibacterial potentials of five different medicinal plant leaf extracts against enteric pathogens. Five solvent were used Viz., hexane, chloroform, ethyl acetate, acetone and methanol extracts. Leaf extracts were evaluated against enteric bacterial pathogens by using standard disc diffusion, determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC). Methanol extract of Vitex negundo leaves showed potent antibacterial activity (inhibition zone: 7.8-20.7 mm, MIC: 15.62 – 31.25 µg/mL, MBC: 31.25 -62.5 µg/mL) against all the pathogenic enteric bacteria (Escherichia coli, Salmonella typhi, Vibrio cholera, Vibrio fluvialis, Shigella flexneri, Proteus vulgaris and Proteus mirabilis) tested. For the first time it was observed that methanol extract of V. negundo leaves exhibited strong vibriocidal activity in vitro conditions. Therefore, it will be useful to identify and isolate the active compounds of this extract that could be a good alternative medicine to treat cholera.

INTRODUCTION

The different systems of medicine practised in India, Ayurveda, Siddha, Unani, Amchi and local health traditions, utilize a large number of plants for the treatment of human diseases. Around 400 plants were mentioned having therapeutic values were mentioned by Rigveda, Yajurveda, Atharvana veda (Rajasekarapandiyan *et al.,* 2007). Infectious disease is one of the leading causes of death worldwide, particularly, in developing countries. Infections due to a variety of bacterial etiologic agents, such as pathogenic *Escherichia coli, Vibrio cholerae*, *Shigella* sp*.,* *Salmonella* sp*.,* (Kamruzzaman, *et al*., 2013, Dubey, 2013).

The natural products not only possess significant pharmacological properties but also provide a source of very important lead compounds. The natural products discovered from medicinal plants have provide numerous clinically used medicines (Nagarsekar *et al*., 2010, Kannathasan *et al*., 2011). Multiply resistant organisms render therapy more precarious and costly and sometimes unsuccessful. In developing countries, multidrug resistant enteric disease agents such as *Vibrio cholera, Escherichia coli* and *Shigella* sps. threaten and circumvent public health measures (Islam *et al*., 2011)

In this paper the antimicrobial screening results of selected Indian medicinal plants are presented along with references to their traditional uses.

MATERIALS AND METHODS

Collection of Plant Material

Healthy and well grown leaves of selected plants (*Calotropis gigantea*, *Lantana camara*, *Thevetia peruviana*, *Thespesia populnea* and *Vitex negundo*) were collected from the area of Kolli Hills, Namakkal district, Tamil Nadu, India. The leaves were immediately brought to the laboratory using separate polythene bags. First they were washed with tap water, then surface sterilized in 10 per cent sodium hypochlorite solution to prevent the contamination of any microbes, then rinsed with sterile distilled water and air dried in shade at room temperature. 5 plants voucher specimen were (BSI/SRC/5/23/2013-2014/Tech/492) deposited and authenticated by Dr. G.V.S. Murthy, Scientist 'F' Botanical Survey of India, Coimbatore, Tamilnadu, India.

Preparation of plant extracts

The leaves of the plants were air dried at room temperature for 10 days then powdered using a mixer grinder. The powdered leaves (100 g) were extracted in a Soxhlet apparatus for 72 h with Hexane, chloroform, ethyl acetate, acetone and methanol (Vogel, 1978). The extracts were pooled and the solvent was evaporated using a rotary evaporator under reduced pressure at 400C. The crude extracts thus obtained were kept at 4℃ until further assay.

Antibacterial assay of plant Extract

Microorganisms used

The antimicrobial activity of five plants were tested against seven strains of Gram – negative enteric pathogens viz, *Escherichia coli* (MTCC1677), *Salmonella typhi* (MTCC 8767), *Vibrio cholera* (MTCC 3906), *Vibrio fluvialis* (MTCC 4432), *Shigella flexneri* (MTCC 1457), *Proteus mirabilis* (MTCC 3310) and *Proteus vulgaris* (MTCC 7299). These standard strains were obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India.

Evaluation of antibacterial activity

The test solution was prepared with 10mg/mL of crude extracts dissolved in 5% Dimethyl sulphoxide (DMSO). A Sterile Disc (HiMedia) 6mm were impregnated with different concentration 10µl, 20µl and 30µl of the extract to obtain 100mg, 200mg and 300mg/disc and allowed to dry at room temperature. Ciprofloxacin (5µg/disc) was used as the positive control and 5% DMSO was used as blind (negative) control (Bauer *et al*, 1966). Petri plates were prepared by pouring 20 mL of Mueller Hinton agar and allowed to solidify. Plates were dried and 0.1 of standardized inoculum suspension was poured and uniformly spread. After drying the disc impregnated the extract disc were placed on the surface of the plate. The inoculated plates were incubated at 37 °C for 24 h and the zone of inhibition was observed and measured in millimeters.

Minimum Inhibitory Concentration (MIC)

500µl of various concentrations (500, 250, 125, 62.50, 31.25, 16.12, 8.06 and 4.03µg/mL) of extract stock solutions were mixed with 500µl of Mueller Hinton broth and 50 µl of bacterial pathogens suspensions individually. Mueller Hinton broth alone served as negative control. Whole setup in duplicate was incubated at 37°C for 24 h. The MIC was the lowest

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concentration of the synthetic compounds that did not permit any visible growth of bacteria during 24 h of incubation after inoculation examined on the basis of turbidity (Ravikumar *et al*., 2010a).

Minimum Bactericidal Concentration (MBC)

To avoid the possibility of misinterpretations due to the turbidity of insoluble compounds if any the MBC was determined by sub-culturing the above (MIC) serial dilutions after 24 h in nutrient agar plates using 0.01 mL culture inoculate and incubated at 370C for 24 h. MBC was regarded as the lowest concentration that prevents the growth of bacterial colony on this solid media (Ravikumar *et al*., 2010b).

Statistical analysis

All the data antibacterial activities were examined as mean ±SD. One – sample t test was carried out to determine the significant differences (P<0.05) between the means, the analysis was carried out using Statistical Package of Social science (SPSS package software, version 11.5, Chicago, IL, USA).

RESULTS AND DISCUSSION

The antimicrobial activity of crude leaf extracts of Calotropis gigantea, Lantana camara, Thevetia peruviana, Thespesia populnea and *Vitex negundo* and the solvent hexane, chloroform, ethyl acetate, acetone and methanol. Antibacterial potential of leaf extract was assessed in terms of zone of inhibition of bacterial growth. The results of the antibacterial activities carried out in 100, 200 and 300 mg/mL of each leaves was used for antimicrobial screening. The antibacterial activity of the extract increased linearly with increase in volume of extract (mg/mL). The methanol extract have shown more sensitive in all plant compared to other solvent.

Antibacterial activity of methanol extract of Thevetia peruviana extracted highest activity in Shigella flexneri (7mm - 11mm) followed by Vibrio cholera (7.7mm - 10mm) and Salmonella typhi (7.5mm - 9.8mm) (Table-1). Highest antibacterial activity of Thespesia populnea was observed in Shigella flexneri (7.5mm – 11mm) and Vibrio cholera (7.2mm – 10mm) and Salmonella typhi (7.5mm – 9.8mm) (Table -2).

Table: 1 Antibacterial activity of methanol extract of Thevetia peruviana

Microorganism	Zone of inhibition (mg/mL)			MIC	MBC	Ciprofloxacin (5 µg/disc)
	100	200	300			
Escherichia coli	7.0± 0.6	8.2± 0.6	9.0± 0.5	125	250	31.6 ± 3.51
Salmonella typhi	7.5± 0.6	8.0± 0.6	9.8± 0.9	125	250	30.8 ± 1.60
Vibrio cholera	7.2± 0.6	8.8± 0.7	10± 1.0	62	125	33.1 ± 2.64
Vibrio fluvialis	7.7± 0.6	8.2± 0.6	9.2± 0.7	125	250	32.1 ± 2.61
Shigella flexneri	7.0± 0.6	7.8± 0.5	11 ± 1.0	62	125	28.8 ± 1.50
Proteus vulgaris	6.7± 0.6	7.5± 0.5	8.0± 0.5	250	500	29.3 ± 1.95
Proteus mirabilis	7.0 ± 0.5	7.8± 0.5	9.0± 0.5	250	500	30.3 ± 2.08

a – Diameter of zone of inhibition including disc diameter of 6 mm; mean ± SD; MIC – Minimum Inhibitory Concentration; MBC – Minimum Bactericidal Concentration.

Table: 2 Antibacterial activity of methanol extract of Thespesia populnea

Microorganism	Zone of inhibition (mg/mL)			MIC	MBC	Ciprofloxacin (5 µg/disc)
	100	200	300			
Escherichia coli	6.5± 0.6	8.3 ± 0.6	9.5 ± 0.5	125	250	31.6 ± 3.51
Salmonella typhi	7.5± 0.6	8.0 ± 0.6	9.8 ± 0.9	125	250	30.8 ± 1.60
Vibrio cholera	7.2± 0.6	8.8 ± 0.7	10 ± 1.0	62. 50	125	33.1 ± 2.64
Vibrio fluvialis	7.7± 0.6	8.5± 0.6	9.2 ± 0.7	125	250	32.1 ± 2.61
Shigella flexneri	7.5± 0.6	7.8 ± 0.5	11± 1.0	62. 50	125	28.8 ± 1.50
Proteus vulgaris	6.7± 0.6	8.5 ± 0.5	8.0 ± 0.5	250	500	29.3 ± 1.95
Proteus mirabilis	7.0 ± 0.5	7.8 ± 0.5	9.0 ± 0.5	125	250	30.3 ± 2.08

a – Diameter of zone of inhibition including disc diameter of 6 mm; mean ± SD; MIC – Minimum Inhibitory Concentration; MBC – Minimum Bactericidal Concentration.

Antibacterial activity of methanol extract of *Calotropis gigantea* exerted highest activity in *Proteus mirabilis* (7.2mm – 10mm) followed by *Proteus vulgaris* (7.5mm- 9.8mm) and *Vibrio fluvialis* (7.5mm – 9.5mm) (Table-3). Highest antibacterial activity of *Lantana camara* observed in *Shigella flexneri* (7.9mm -11mm) followed by *Vibrio cholera* (7.4mm-10mm) and *Escherichia coli* (7.5mm-9.9mm) (Table-4).

Table: 3 Antibacterial activity of Methanol extract of Calotropis gigantea

Microorganism	Zone of inhibition (mg/mL)			MIC	MBC	Ciprofloxacin (5 µg/disc)
	100	200	300			
Escherichia coli	7.5 ± 0.6	8.0 ± 0.8	9.1 ± 0.9	62	125	31.6 ± 3.51
Salmonella typhi	7.0 ± 0.7	8.5 ± 0.5	9.0 ± 0.8	125	250	30.8 ± 1.60
Vibrio cholera	7.8 ± 0.7	8.2 ± 0.7	9.1 ± 0.8	62	125	33.1 ± 2.64
Vibrio fluvialis	7.5 ± 0.7	8.2 ± 0.7	9.5 ± 0.9	62	125	32.1 ± 2.61
Shigella flexneri	6.5± 0.6	8.3 ± 0.6	9.5 ± 0.5	62	125	28.8 ± 1.50
Proteus vulgaris	7.5± 0.6	8.0 ± 0.6	9.8 ± 0.9	125	250	29.3 ± 1.95
Proteus mirabilis	7.2± 0.6	8.8 ± 0.7	10 ± 1.0	31	62	30.3 ± 2.08

a – Diameter of zone of inhibition including disc diameter of 6 mm; mean ± SD; MIC – Minimum Inhibitory Concentration; MBC – Minimum Bactericidal Concentration;

Table: 4 Antibacterial activity of Methanol extract of Lantana camara

Microorganism	Zone of inhibition (mg/mL)			MIC	MBC	Ciprofloxacin (5 µg/disc)
	100	200	300			
Escherichia coli	7.5 ± 0.6	8.7 ± 0.7	9.9 ± 1.0	62.5	125	31.6 ± 3.51
Salmonella typhi	7.0 ± 0.6	7.9 ± 0.6	9.0 ± 0.8	62.5	125	30.8 ± 1.60
Vibrio cholera	7.4 ± 0.6	8.7 ± 0.7	10 ± 1.0	31.25	62.5	33.1 ± 2.64

Vibrio fluvialis	7.5 ± 0.7	8.6 ± 0.7	9.5 ± 0.8	31.25	62.5	32.1 ± 2.61
Shigella flexneri	7.9 ± 0.5	8.5 ± 0.7	11 ± 1.2	31.25	62.5	28.8 ± 1.50
Proteus vulgaris	7.7 ± 0.4	8.4 ± 0.6	9.8 ± 1.0	125	250	29.3 ± 1.95
Proteus mirabilis	7.0 ± 0.5	8.5 ± 0.6	9.9 ± 1.0	62.5	125	30.3 ± 2.08

a – Diameter of zone of inhibition including disc diameter of 6 mm; mean ± SD; MIC – Minimum Inhibitory Concentration; MBC – Minimum Bactericidal Concentration;

Antibacterial activity of methanol extract of *Vitex negundo* presented in (Table 5). Highest activity was observed for *Vibrio cholera* (8.4mm-20.7mm) followed by *Escherichia coli* (8.9mm-19mm) and *Vibrio fluvialis* (8.4mm-18.3mm).

Table: 5 Antibacterial activity of Methanol extract of Vitex negundo

Microorganism	Zone of inhibition (mg/mL)			MIC	MBC	Ciprofloxacin (5 µg/disc)
	100	200	300			
Escherichia coli	8.9 ± 0.7	12.9 ± 1.5	19.0 ± 2.0	62.5	125	31.6 ± 3.51
Salmonella typhi	8.4 ± 0.5	11.8 ± 1.0	17.0 ± 1.8	62.5	125	30.8 ± 1.60
Vibrio cholera	8.4 ± 0.8	13.8 ± 1.0	20.7 ± 1.9	31.25	62.5	33.1 ± 2.64
Vibrio fluvialis	8.4 ± 0.5	12.8 ± 1.3	18.3 ± 1.9	31.25	62.5	32.1 ± 2.61
Shigella flexneri	9.0± 0.9	12.7 ± 1.5	17.3 ± 1.8	31.25	62.5	28.8 ± 1.50
Proteus vulgaris	8.1 ± 0.5	11.7 ± 1.0	16.3 ± 1.6	125	250	29.3 ± 1.95
Proteus mirabilis	7.8 ± 0.8	11.6 ± 1.0	16.7 ± 1.6	62.5	125	30.3 ± 2.08

a – Diameter of zone of inhibition including disc diameter of 6 mm; mean ± SD; MIC – Minimum Inhibitory Concentration; MBC – Minimum Bactericidal Concentration;

During the second half of the 20th century, the acceptance of traditional medicine as an alternative form of health care and the development of microbial resistance to the available antibiotics led researchers to investigate the antimicrobial activity of medicinal plants (Das *et al*, 2010). *Escherichia coli, Salmonella typhi*, *Vibrio cholera, Vibrio fluvialis Proteus mirabilis* been documented as the most important pathogen associated with dysentery in more countries **(**Arokiyaraj *et al*, 2012)

Since disc diffusion assay has some limitations and this assay only indicate the growth inhibition ability of the extracts against certain microorganisms, it could not tell whether the inhibition zone is for bactericidal or bacteria static activity of the certain extracts (Kumar et al, 2010). Therefore to confirm the nature of activity and specific dose against certain microorganisms, we determined the MIC and MBC, the values ranged between 31.25 to 500 µg/mL for all enteric bacteria. Vitex negundo leaf could kill all types of enteric bacteria tested a range of 31.25 to 62.5 µg/mL of the extract was sufficient to kill the V. cholera.

In vitro bactericidal activity of Vitex negundo leaf methanol extract (500 µg/mL) at different time interval suggested that it has very strong killing activity against all the Vibrio species. These results suggested that the compounds of Vitex negundo leaf responsible for antibacterial activity in vitro are effective to exert its activity in vivo also. This result convinced us to examine the potentiality of this extract whether it can be able to protect mice from the pathogenic V. cholera infection.

Conclusion

In this study, we have shown the antibacterial activity of five different plants and five solvent leaf extract on a wide range of enteric pathogens. Vitex negundo leaf methanol extract is high activity equally in vitro conditions to kill the cholera causing bacteria. If we identified and isolate the active compounds from Vitex negundo leaf. Therefore, further studies should be undertaken to study the active compounds residing in Vitex negundo leaves.

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