Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Preliminary study on the antimicrobial activity of *Enicostemma littorale* using different solvents

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ARTICLE INFO

Article history:
Received 10 March 2012
Received in revised form 15 May 2012
Accepted 15 July 2012
Available online 20 July 2012

Keywords:
Antimicrobial activity
Enicostemma littorale
Extracts
Medicinal plants

ABSTRACT

Objective: To study the antimicrobial activity of *Enicostemma littorale (E. littorale)* using different solvents. **Methods:** Chloroform, methanol and acetone extracts of different parts of *E. littorale* (leaf, stem and root) were evaluated for antimicrobial activity using disc diffusion method against some gram-negative species such as *Escherichia coli, Klebsiella pnemoniae, Pseudomonas aeruginosa, Salmonella typhi* and gram-positive species *Staphylococcus aureus, Bacillus* cereus, *Bacillus subtilis* and two fugal species *viz., Aspergillus fumigates* and *Aspergillus flavus*. **Results:** The chloroform extracts showed the highest antibacterial activity. Among leaf, stem and root extracts, the stem extracts showed maximum antibacterial activity. All of the used extracts had no significant antifungal activity against *Aspergillus fumigates* and *Aspergillus flavus*. The chloroform stem extract showed highest activity (about 20 mm inhibition zone) against *Bacillus subtilis* (at 500 mg/mL) followed by the methanolic stem extract which showed highest activity against the same organism. The lowest antibacterial activity was observed by the acetone leaf extract (about 8 mm inhibition zone) against *Escherichia coli*. **Conclusions:** The findings of the study indicate littorale could also be a new source for antibiotics discovery.

1. Introduction

Enicostemma littorale Blume (White Head) (*E. littorale*) is a perennial glabrous medicinal herb (Gentianaceae). It is found distributed throughout the greater part of India and common in coastal areas. The plant is pungent and very bitter, antihelmintic, cures fever and vata diseases. It is also used as stomachic, laxative, antidiabetic, and crushed plant material is applied to snake-bites[1]. *E. littorale* is rich source of alkaloids, catechins, saponins, sterols, triterpinoids, phenolic acids, flavonoids and xanthones. It also contains minerals like iron, potassium, calcium, silica, phosphate, chloride sulphate and carbonate[2]. In recent years, pharmaceutical companies have spent considerable time and money in developing therapeutics based upon natural products extracted from plants[3,4]. The rising incidence of multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources[5,6]. Because of its abundant and widespread availability, this study set out to investigate the antimicrobial activity of the different parts of (leaf, stem and root) Enicostemma littorale using different solvent systems.

2. Materials and methods

2.1. Plant material

The plants were collected from Erode district of Tamilnadu, India in August to September at the end of flowering season. The taxonomic identification of the plant was done comparing with exist herbarium in the botany department of Annamalai University.

2.2. Extract preparation

The plant materials *viz*., leaf, stem, roots were collected. One hundred grams of each powdered plant material were extracted with chloroform, methanol and acetone by soxhlet apparatus. The organic solvent was removed by evaporation using rota vapor at not more than 40 ℃. The residue was then placed in an oven at 40 ℃ for about 48 h to remove the water. The resulting dried mass was then powdered,

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Foundation Project: This study was finacially supported by Department of Science and Technology.

packed into a glass vial and stored in a desiccator over silica gel until use. 500 mg/mL extract were used against all the microorganisms.

2.3. Bacterial and fungal species

The seven bacterial species which was used in this study were, the gram-negative species: *Escherichia coli, Klebsiella pnemoniae, Pseudomonas aeruginosa, Salmonella typhi* and gram-positive species: *Staphylococcus aureus, Bacillus cereus, Bacillus subtilis* and two fugal species *viz.*, *Aspergillus fumigates* and *Aspergillus flavus*. They were identified according to standard phenotype tests.

2.4. Determination of antimicrobial activity

Antibacterial activity of each extract of the organic extract of plant samples (500 mg/mL) were evaluated by the paper disc diffusion method[7]. Stock culture of tested test bacteria were grown in nutrient broth medium at 37 for 24 hours. Final bacterial numbers were adjusted to 0.5 Mc Farland turbidometry. A lawn culture then prepared on Muller-Hinton agar using sterile cotton swab. All the fungal cultures were inoculated onto Sabouraud Dextrose Agar plates. Sterile filter paper discs (6 mm for bacteria and fungi) were placed on these cultures and impregnated with reconstituted extract in minimum amount of solvent at concentrations of 500 mg/mL were placed on the culture plates previously seeded with the 0.5 McFarland and 106 cfu/mL cultures of bacteria and fungi, respectively. Paper discs impregnated with 20 μL of a solution of 10 mg/mL of chloramphenicol (for bacteria) and streptomycin (for fungi) as standard antimicrobials were used for comparison. Antimicrobial activity was determined by measurement of inhibition zone around each paper disc. For each extract three replicate trials were conducted against each organism.

2.5. Determination of minimum inhibitory concentration

To determine the MBC, a loopful of broth was collected from those tubes which did not show any growth and inoculated on sterile nutrient agar (for bacteria) and sabouraud dextrose agar (for fungi) by streaking. Nutrient agar and sabouraud agar only were streaked with the test organisms, respectively, to serve as control. Plates inoculated with bacteria were then incubated at 37 for 24 h, while those inoculated with fungi were incubated at room temperature (28 ℃) for 48 h. After incubation, the lowest concentration at which no visible growth was noted as the minimum bacterial concentration.

2.6. Determination of minimum bactericidal concentration

To determine the minimum inhibitory concentration (MBC), a loopful of broth from those tubes which did not exhibit any visible growth in the MIC assay was cultured on freshly prepared sterile Muller-Hinton agar and then incubated at 37 ℃ for 18 - 24 h. After incubation the highest dilution (least concentration) that inhibited colony formation on a solid medium was considered as MBC.

3. Results

The results of the different solvent extracts of Enicostemma littorale was presented in the Table 1. The antimicrobial activity was determined by the presence or absence of inhibition zone around the discs. The results exhibited that *Aspergillus fumigates* and *Aspergillus flavus* were the most resistant strains. All the used extracts showed significant antibacterial activity against bacterial strains. Among the plant extracts chloroform extracts showed maximum antibacterial activity than methanol and acetone extracts. Among these leaf, stem and root extracts the stem extracts showed maximum antibacterial activity. All of the used extracts had no significant antifungal activity against *Aspergillus fumigates* and *Aspergillus flavus*. The chloroform stem extract showed highest activity (about 20 mm inhibition zone) against Bacillus subtilis (at 500 mg/mL) followed by the methanolic stem extract showed highest activity against the same organism. The lowest antibacterial activity was observed by the acetone leaf extract (about 8 mm inhibition zone) against *Escherichia coli*. Results of minimum inhibitory concentration (MIC) and minimum

Table 1.
Antimicrobial activity of different parts of Enicostemma littorale using different solvents (mm).

No	Organism	Diameter of zone of inhibition										
		Leaf extracts (500 mg/mL)			Stem extracts (500 mg/mL)			Root extracts (500 mg/mL)			Antibiotics	
		CL	ME	AC	CL	ME	AC	CL	ME	AC	Ch	St
1	*Escherichia coli*	10	9	8	14	15	10	12	14	9	15	15
2.	*Klebsiella pneumoniae*	10	11	11	12	16	11	12	13	13	17	17
3.	*Pseudomonas aeruginosa*	16	14	14	17	17	16	18	16	11	18	17
4.	*Salmonella typhi*	10	13	9	12	18	16	12	16	11	15	15
5.	*Staphylococcus aureus*	11	14	11	12	15	12	9	11	12	4	8
6.	*Bacillus cereus*	16	13	16	18	18	17	13	11	10	20	18
7.	*Bacillus subtilis*	18	18	9	20	19	11	18	15	10	22	24

CL: Chloroform extract, ME: Methanol extract, AC: Acetone extract, Ch: chloramphenicol, St: streptomycin

Table 2

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of different parts of *E. littorale* using different solvents (mg/mL).

No	Organism	Leaf extracts						Stem extracts						Root extracts					
		MIC			MBC			MIC			MBC			MIC			MBC		
		CL	ME	AC	CL	ME	AC	CL	ME	AC	CL	ME	AC	CL	ME	AC	CL	ME	AC
1	*Escherichia coli*	18	18	18	18	18	18	15	15	15	15	15	18	18	20	20	20	22	22
2.	*Klebsiella pneumoniae*	15	15	18	18	18	20	15	15	15	18	18	18	18	18	20	20	20	20
3.	*Pseudomonas aeruginosa*	8.5	8.5	10	8.5	8.5	10	8.5	8.5	8.5	10	10	10	15	15	15	15	15	18
4.	*Salmonella typhi*	10	10	10	15	15	18	8.5	8.5	8.5	15	15	18	10	10	10	18	18	18
5.	*Staphylococcus aureus*	18	18	18	20	20	20	15	15	15	20	20	20	18	18	18	18	18	18
6.	*Bacillus cereus*	15	15	15	15	15	18	8.5	8.5	8.5	8.5	8.5	10	15	15	15	15	15	18
7.	*Bacillus subtilis*	8.5	8.5	8.5	10	10	10	8	8	8	8	8	10	15	15	15	15	15	18

MIC: Minimum Inhibitory Concentration, MBC: Minimum Bactericidal Concentration, CL: Chloroform extract, ME: Methanol extract, AC: Acetone extract.

bacterial concentration (MBC) for general extracts are shown in Table 2. The results showed that Escherichia coli had the highest MIC (20 mg/mL) and MBC (22 mg/mL) for root extract while the lowest value of MIC and MBC (8 mg/mL) was shown by Bacillus subtilis for stem extract. The MIC and MBC values were generally higher for the root extracts against the test organisms compared to those of the leaf and stem extracts.

4. Discussion

Medicinal plants have long history of use and their uses are wide spread in both developed and developing countries. On the other hand, in modern medicine due to indiscriminate and irrational use of antimicrobial drugs the infectious microorganisms have developed resistance. Hence new alternative antimicrobial drug regimens are required to combat the existing infectious diseases.

The potential of higher plants as source for new drugs is still largely unexplored. Medicinal plants are widely used by all sections of people either directly as folk remedies or in different indigenous systems of medicine or indirectly in the pharmaceutical preparations of modern medicines. The use of plant extract or plant-derived chemicals to treat disease has stood the test of time. In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries, because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs[8].

Numerous plants used in traditional medicine are effective in treating various ailments caused by bacterial and viral infections. Research has shown that medicinal plants exhibit antimicrobial activity[9]. Because of their antibacterial properties, herbs are used as new source for antibiotics discovery[10, 11]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. These phytochemicals are the active constituents that exhibit some biological activities concerning antioxidant, antimicrobial, anti-inflammatory and anticancer activities. Exploration of the chemical constituents of the plants and pharmacological screening is of great importance which leads for the development of novel agents[12]. Plants are the important raw materials for pharmacological research and drug development[13]. The plants represent an unlimited source of phytochemicals. The photochemicals present in plants consist of primary and secondary metabolites. The higher plants collectively accumulate as many as 1 00 000 secondary metabolites that can be mainly classified into alkaloids, tannins, flavonoids *etc*.[14].

In the present study an attempt has been made to screening of different solvent extracts for their antimicrobial activity against several pathogenic bacteria and fungi like *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, *Aspergillus fumigates* and *Aspergillus flavus* by using disc diffusion method. The chloroform extract of *E. littorale* gave good and excellent activity against all the tested bacteria except fungi. Results showed that chloroform extract has antimicrobial activity higher than methanol and acetone extracts. The results showed that the stem extracts showed good antimicrobial activity than the leaf and root extracts. The mature stem may contain other secondary metabolities and bitter principles of the plant[15]. The chloroform extract of *Capparis zeylanica* exhibited *in vitro* antibacterial activity against gram negative and gram positive bacteria[16]. Extracts of leaves and stems of *Gynandropsis gynandra* and *Buchholzia coriaceae* were screened phytochemically for the presence of secondary metabolites and for *in vitro* antibacterial and antifungal properties[17-22]. Different solvents have been reported to have the capacity to extract different phytoconstituents depending on their solubility or polarity in the solvent[23]. Chloroform extracts obtained in this study might have higher solubility for more of active antimicrobial phytoconstituents, consequently displaying the highest relative antimicrobial activity. The antibacterial activity was more pronounced on the gram-positive bacteria (*Bacillus subtilis*) than the gram-negative bacteria (*Escherichia coli*). The reason for the difference in sensitivity between gram-positive and gram-negative bacteria might be ascribed to the differences in morphological constitutions between these microorganisms, gram-negative bacteria having an outer phospholipidic membrane carrying the structural lipo polysaccharide components. This makes the cell wall impermeable to antimicrobial chemical substances. The gram-positive bacteria on the other hand are more susceptible having

only an outer peptidoglycan layer which is not an effective permeability barrier. Therefore, the cell walls of gram-negative organisms which are more complex than the gram-positive ones act as a diffusional barrier and making them less susceptible to the antimicrobial agents than are Gram-positive bacteria[24-27]. In spite of this permeability differences, however, some of the extracts have still exerted some degree of inhibition against gram-negative organisms as well. Antimicrobial agents are considered "miracle drugs" that are our leading weapons in the treatment of infectious diseases. The ability of certain microorganisms to withstand attack by antimicrobials and the uncontrolled rise in resistant pathogens threatens lives. Development of drug resistance in human pathogens against commonly used antibiotics has demanded for the search of new antimicrobial substances, chemotherapeutic agents, and agrochemicals that combine antimicrobial efficacy with low toxicity, and minor environmental impact. The result of this study leads to the discovery of new biologically-active molecules by the pharmaceutical industry and the adoption of crude extracts of plants.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors acknowledged the Department of Science and Technology (DST) for financial support. The work was carried out as part of project work under "Innovation in Scientific Pursuit for Inspired Research programme" (INSPIRE), by Department of Science and Technology (DST), Government of India, New Delhi awarded to the first author.

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