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Short Communication

Antibacterial Activity and Preliminary Screening of Phytochemicals of Whole Plant of *Enicostemma littorale*

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ABSTRACT. *Enicostemma littorale is a glabrous perennial herb belonging to the family Gentianaceae. The whole plant is used in traditional medicine for the treatment of various diseases. The decoction is used to treat skin diseases such as kiranthi, itching and scabies. Phytochemical screening was carried out to identify the active functional groups of E. littorale plant which revealed the presence of alkaloids, saponins, steroids, flavonoids, glycosides and triterpenoids. Antibacterial activity of the aqueous extract of the whole plant of E. littorale on eight bacterial isolates was investigated. They were Staphylococcus aureus – NCTC 6571, Escherichia coli – NCTC -10418, Pseudomonas aeruginosa – NCTC – 10662 and five wild strains of Methicillin resistant Staphylococcus aureus (MRSA). Antibacterial activity was performed by the cut well diffusion and agar dilution methods. The aqueous extract of E. littorale showed growth inhibitory action against S. aureus (NCTC 6571 and 5 MRSA strains). Growth inhibitory action against E. coli and P aeruginosa could not be demonstrated using the agar dilution method, although inhibition of E. coli was shown using the cut well method. Further exploration of activity of the aqueous extract against a wider range of skin pathogens would be helpful. The ability of the aqueous extract of E. littorale to inhibit the growth of bacteria is an indication of its antibacterial potential which may be employed in the management of bacterial infections.*

Keywords: *Antibacterial activity; Enicostemma littorale; phytochemical screening*

INTRODUCTION

Considering the significance of traditional medical practices in global health care, the World Health Organization (WHO) has been encouraging and promoting traditional practices for the last few decades, in order to increase worldwide acceptability of herbal drugs throughout the globe and prove the clinical efficacy of these old remedies.

Enicostemma littorale is a glabrous perennial herb belonging to the family Gentianaceae which is found in Sri Lanka. In traditional medicine, the whole plant is used as a decoction and as a paste to treat skin diseases such as kiranthi, itching and scabies. The paste is used for dermal applications (Krishnan, 1949). This plant has been reported to have hypoglycemic, antitumour, hepato protective and antioxidant activities. The antimicrobial activity of aqueous, hydro alcoholic, methanolic, chloroform and ethyl acetate extract of leaves of this

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plant has been tested against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Shigella sonnei, Pseudomonas aeruginosa, Proteus vulgaris, Aspergillus niger* and *Candida albicans*. Five alkaloids, two sterols and volatile oil are reported in this plant (Abirami and Gomathinayakam, 2011).

Since the last decade, the increased failure of chemotherapeutics due to antimicrobial resistance exhibited by pathogenic microbes has led to the screening of several medicinal plants for their potential antimicrobial activity. With the advancement of modern medicinal technology, it is now easier to identify specific botanical constituents and assess their potential for antimicrobial activity.

The objectives of the present study were,

1. Preliminary phytochemical screening of *E. littorale* to enable further investigate the anti-microbiological activity of the individual compounds.

2. To evaluate the antibacterial activity of an aqueous extract of the whole plant of *E. littorale* against bacterial isolates [*Staphylococcus aureus – NCTC 6571, Escherichia coli – NCTC - 10418, Pseudomonas aeruginosa* – NCTC – 10662 and five wild strains of Methicillin resistant *Staphylococcus aureus* (MRSA)] isolated from clinical samples.

METHODOLOGY

Plant collection

The fresh whole plant was collected from its natural habitat (Western part of Jaffna district) during 2011. The plant material was identified and authenticated on the comparison of herbarium sheet prepared for this plant and the documented herbarium in the National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. The whole plant was washed thoroughly, dried under shade at room temperature, ground into coarse powder and packed in polythene bags.

Phytochemical screening

The freshly prepared methanolic extract (5 g plant material refluxed with 50 ml of MeOH) and water extract (1g of plant material boiled in 10 ml of water) were chemically tested qualitatively for the presence of chemical constituents such as alkaloids, saponins, tannins, steroids, flavonoids, glycosides and triterpenoids. They were identified using characteristic colour changes using standard procedures described by Farnsorth, (1996) and Edeoga *et al.* (2005) as given below.

Detection of alkaloids

A few drops of 2N hydrochloric acid was added to 2 ml of the methanolic extract and heated in a water bath (50 °C). The solution was filtered and Wagner's reagent added to the filtrate. The formation of a red colour precipitate indicates the presence of alkaloids.

Detection of tannins

Sodium chloride solution (0.9 %) was added to 2 ml of the methanolic extract and heated in a water bath (50 °C). The solution was filtered and 1% $FeCl_3$ solution added to the filtrate. The appearance of blackish green colour indicates the presence of tannins.

Detection of saponins

Aqueous extract (1 ml) was mixed with 9 ml of distilled water and shook vigorously for 15 seconds. The extract was allowed to stand for 10 min. Formation of stable foam (1 mm) indicates the presence of saponins.

Detection of steroids

10 ml of chloroform was added to 2 ml of the methanolic extract. 1 ml of acetic anhydride was added, followed by the addition of 2 ml of concentrated sulphuric acid along the sides of the test tube. The appearance of blue-green colour formation at the junction indicates the presence of steroids.

Detection of triterpenoids

The test for triterpenoids was same as that for steroids. The appearance of red or brown colour at the junction indicates the presence of triterpenoids.

Detection of cardiac glycosides

A few drops of glacial acetic acid, ferric chloride and 3-4 drops of concentrated sulphuric acid were added to 1 ml of the methanolic extract. The appearance of blue-green colour indicates the presence of glycosides.

Detection of flavonoids

The metal magnesium (0.5 g) and 10 drops of conc. hydrochloride acid were added to 1 ml of the methanolic extract. The formation of tomato red colour indicates the presence of flavonoids.

Preparation of aqueous extract for antibacterial assay

Forty grams of *E. littorale* were added to 480 ml distilled water (12 times), boiled until the volume was reduced to 60 ml (1/8) and further concentrated to obtain 30 ml using a reduced flame.

Test microorganisms

The plant extract was assayed for antibacterial activity against eight bacterial isolates which were obtained from the Department of Microbiology, Faculty of Medicine, University of Peradeniya. The bacteria included, *S. aureus*–NCTC 6571, *E. coli*–NCTC-10418, *P. aeruginosa*–NCTC–10662 and five wild strains of methicillin resistant *S. aureus* (MRSA).

Antibacterial assay

The antibacterial activity of the whole plant aqueous extract was evaluated using the cut well diffusion and agar dilution methods. All the experiments were conducted using standard aseptic techniques.

The bacterial isolates were maintained on nutrient agar slopes at room temperature. Each isolate was sub-cultured on blood agar and checked for purity before use.

Preparation of bacterial inoculums.

Isolated bacterial colony of each test strain was picked, using a sterile cotton wool swab, inoculated in to 2 ml of sterile normal saline and vortexed for five seconds to disperse the bacteria evenly. An inoculums density of 10^5/ml was obtained by comparing with MacFarland standard 0.5 (Derek *et al.,* 2001).

Cut well method

Mueller–Hinton Agar (MHA) was used for this bioassay. The MHA plate was inoculated with 1 ml of the liquid bacterial culture. The petridish was rotated to spread the liquid bacterial culture equally and excess culture was removed from the plate and allowed to dry at 37 °C for 15 minutes. The wells with 12 mm in diameter and 4 mm in depth were bored into the MHA using a sterile cork borer and the well completely filled with the test extract. The plates were left on the bench for 30 minutes for absorption of extract and then incubated at 37 °C for 24 h. The plates were examined for inhibition of growth around the well and diameters of the zones of inhibition were measured.

Agar dilution method

Table 1. Dilution series of agar and *E. littorale* aqueous extract

Dilutions	Volume of MHA (ml)	Volume of aqueous extract (ml)	Dilution
Plate 1	20	-	control
Plate 2	16	4	1/5
Plate 3	18	2	1/10
Plate 4	19	1	1/20

Dilutions were prepared in sterile universal bottles as shown in Table-1. After capping and mixing the solutions, the medium was poured into sterile petridishes, marked into eight partitions and labeled. A dilution of 1/10 in sterile distilled water was prepared. 10 µL of a 1/10 dilution of each test strain (prepared as given above) was inoculated its corresponding areas. Three replicates were carried out for the entire procedure.

RESULTS AND DISCUSSION

Phytochemicals screening

The preliminary phytochemicals analyses revealed the presence of alkaloids, saponins, steroids, flavonoids, glycosides and triterpenoids in the whole plant of *E. littorale* . The tannin was not detected in the whole plant of *E. littorale*.

Antibacterial activity

Aqueous extract of *E. littorale* showed inhibition zone (diameter 14–16 mm) against *S. aureus* (1MSSA, 5 MRSA) and *E. coli*. This extract did not show any activity against *P. aeruginosa*. Activity against *S. aureus* (MSSA and all 5 MRSA) was seen at 1/5 dilution.

Table 2. Inhibition zone (mm) of aqueous extract of *E. littorale* using the Cut well method

Organisms	1st time	2nd time	3rd time
1. *Staphylococcus aureus*–NCTC 6571	15	16	16
2. *E. coli*–NCTC-10418	15	no zone	15
3. *P. aeruginosa*–NCTC–10662	no zone	no zone	no zone
4. MRSA – wild strain 1	15	15	16
5. MRSA – wild strain 2	14	16	16
6. MRSA – wild strain 3	16	no zone	15
7. MRSA – wild strain 4	15	14	16
8. MRSA – wild strain 5	14	16	15.5

In cut well method the aqueous extract has shown activity to all the tested organisms except *P. aeruginosa.*

Table 3. Antibacterial activity of aqueous extract of *E. littorale* using Agar dilution method

Organisms	1st Time			2nd Time			3rd Time		
	1/5	1/10	1/20	1/5	1/10	1/20	1/5	1/10	1/20
1.*Staphylococcus aureus*–NCTC 6571	NG	G	G	NG	G	G	NG	G	G
2. E. coli – NCTC - 10418	G	G	G	G	G	G	G	G	G
3.*P. auruginosa* - NCTC-10662	G	G	G	G	G	G	G	G	G
4. MRSA–wild strain1	NG	G	G	NG	G	G	NG	G	G
5. MRSA–wild strain2	NG	G	G	NG	G	G	NG	G	G
6. MRSA–wild strain3	NG	G	G	NG	G	G	NG	G	G
7. MRSA–wild strain4	NG	G	G	NG	G	G	NG	G	G
8. MRSA–wild strain5	NG	G	G	NG	G	G	NG	G	G

NG - No Growth; G – Growth

In control plate all the organisms have grown. In Agar dilution method this aqueous extract has shown activity against organisms 1, 4, 5, 6, 7, & 8 in 1/5 dilutions. This extract has not shown activity on *E. coli* and *P. aeruginosa.*

In Traditional medicine, *E. littorale* is used for treatment of infections of skin as a paste and a decoction (Murukesamuthaliyar, 1936). *S. aureus* is the commonest organism which causes skin infections, although Gram negative Bacilli such as *E. coli* and *Pseudomonas* sp. as known to contribute in some clinical situations.

Using the cut well method, growth inhibitory action of the aqueous extract of *E. littorale* could be demonstrated against *S. aureus* (MSSA and MRSA). This extract also showed similar inhibitory activity against *E. coli.* However, at a dilution of 1/5, growth inhibitory activity was retained against *S. aureus*, but was not demonstrable against *E. coli*.

Plants produce organic compounds. These compounds are usually secondary metabolites. They are alkaloids, tannins, flavonoids, steroids, glycosides and triterpenoids. These phytochemicals exert antimicrobial activity through different mechanisms. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms (Kam and Liew, 2002). Plant alkaloids inhibit release of autocods and prostaglandins of cell. Likewise flavonoid has antimicrobial activity it forms a complex with cell wall, and binds to adhesions. Terpenoids also disrupt the cell membrane (Prashant *et al,* 2011). Hence, the presence of these compounds in this plant could explain the antimicrobial activities observed.

CONCLUSIONS

Aqueous extract of *E. littorale* shows growth inhibition activity against *S. aureus* (MSSA and MRSA). Although activity against *E. coli* was seen in the cut well method, it could not be demonstrated using the agar dilution method. There was no activity against *P. aeruginosa.* Further exploration of activity of the aqueous extract against a wider range of skin pathogens would be helpful.

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