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## RESEARCH ARTICLE

### IN VITRO ANTIMICROBIAL ACTIVITY STUDIES ON *Enicostema littorale* (Lam), Raynal. WHOLE PLANTS

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#### ABSTRACT

*Enicostema axillare* (Lam.). Raynal, syn. *E.littorale* Blume (Family) *Gentianaceae* is a perennial herb found throughout the greater part of India. Locally it is known as *Chota chirayita* and used in indigenous medicines in the treatment of fevers and as bitter tonic and forms one ingredients of many hypoglycemic marketed formulations. In the present study in vitro antimicrobial activity (Minimum Inhibitory Concentration by Micro-titre plate method of Chloroform, Ethyl acetate, Methanol, Petroleum ether extract of whole plant has been evaluated. Four Bacterial Species and two fungal strains used for study are: *Staph aureus*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella sonnei*, and two fungal strains are: *Aeromonas hydrophila*, *Candida albicans*. It was observed that Ethyl acetate, Methanolic extract showed prominent antimicrobial activity against all micro organisms.

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#### INTRODUCTION

*Enicostema axillare* (*E.littorale*) Lam Raynal (synonym *Enicostema littorale* Blume, *Gentianaceae*) is a perennial herb found throughout India. And common in coastal areas. The plant is used in Folk medicine to treat diabetes mellitus, rheumatism abdominal ulcers. Hernia, swelling itching hypoglycemic (Raghu Bir S Rawat, 2006) and anticancer (Rai, 1946) activities have been reported. These reported activities and many of the ethnomedical Uses of the plant are related to its antioxidant activity. Swertiamarin, alkaloids, steroids, triterpenoids, saponins flavonoids, xanthone.<sup>4</sup>) Many such compounds has protective effects due to their antioxidant properties (Maroo *et al.*, 2003). The methanolic extract showed the prominent antiviral activity the preliminary test to evaluate the anti viral activity and hence the antimicrobial activity shows the broad spectrum of antibiotics. The various phytochemical tests are methanolic extract showed the presence of tannins, flavanoid, alkaloid, betacyanin, quinone, glycosides, phenol.

#### MATERIALS AND METHODS

##### Plant material and preparation of extracts

The aerial plant parts of *Enicostema littorale* at flowering stage were collected from the Tirunelveli District, Alangulam, and March 2010. The plant species was identified (by Botanist Dr.P.Jayaraman at Plant Anatomy Research Centre (PARC). The collected material was dried under the dry shade and powdered.

The powdered plant material was extracted using solvents of increasing polarity chloroform, ethyl acetate, methanol, petroleum ether, in a soxhlet extraction apparatus.

##### Microbial Strains and Standard drugs

*Staphylococcus aureus*, *Shigella sonnei*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Aeromonas hydrophila*, *Candida albicans* was used as microbial and fungal strains for the study were Ciproflaxin. The Minimum Inhibitory Concentration (MIC) of aqueous and ethyl acetate, against *S. aureus*, *A. hydrophila*, *K. pneumoniae*, *V. fischeri*, *B. subtilis*, *S. paratyphi*, *S. pyogenes* and *E. coli* were determined by broth micro dilution method as per the standard National Committee for Clinical Laboratory Standards (NCCLS). Overnight MH broth cultures were used to prepare inocula of 10<sup>6</sup> CFU/ml. The MIC was defined as the lowest concentration of antimicrobial agent that prevented turbidity after 24 h of incubation at 37°C.

##### Minimal Bactericidal Concentration (MBC)

MBC is the smaller concentration of the drug necessary for elimination of 99.9% of the microorganisms tested. The MBC was determined after the MIC assays. Tubes where the MIC results showed no bacterial growth, The MIC<sub>99</sub> was considered as MBC. Bacterial growth was evaluated for the MBC determination. After 24 h, at 35°C, if MIC = MBC or if MBC is one, two or three dilutions above of MIC, the drug is considered bactericide.

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Table 1. MBC of different extract

extract	<i>Aeromonas hydrophila</i>	<i>Candida albicans</i>	<i>Pseudomonas aeruginosa</i>	<i>Salmonella paratyphi</i>	<i>Staphylococcus aureus</i>
Chloroform	12.5	6.25	0.360	12.5	3.10
Ethyl acetate	6.25	0.360	<0.360	12.5	6.25
Petroleum ether	6.25	12.5	6.25	25	12.5

Values recorded in mg/ml, test was done in triplicates

Table 2. MIC of different strains (Methanolic extract)

S.No	Organisms	1mg	5m	10mg	25mg	50mg	Control	MBC
1.	<i>S.aureus</i>	NG	NG	NG	NG	NG	G	<1mg.
2.	<i>Ps.aeruginosa</i>	G	NG	NG	NG	NG	G	5mg
3.	<i>S.typhi</i>	NG	NG	NG	NG	NG	G	5mg
4.	<i>Shi.sonnei</i>	NG	NG	NG	NG	NG	G	5mg

*S.aureus* inhibits <1mg/ml concentration where as 5mg/ml inhibits *P.aeruginosa*, *S.typhi*, and *S.sonnei*.

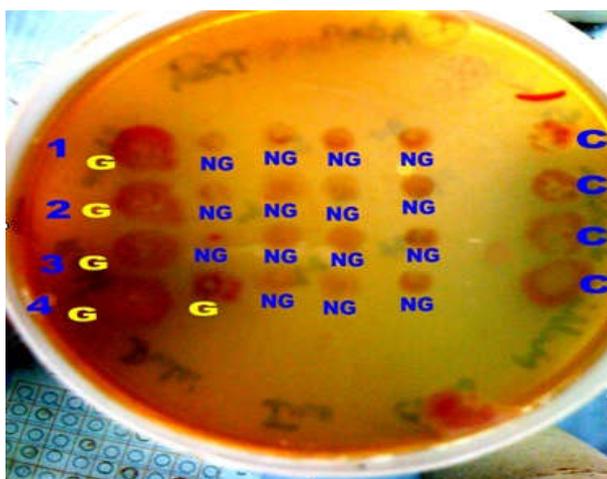


Fig. 1. Anti microbial activity of Methanolic extract of *Enicostema littorale*

## RESULTS AND DISCUSSION

Results of phytochemical investigation of *E. axillare* showed the presence of tannins, flavanoid, alkaloid, betacyanin, quinone, glycosides, and phenol. According to ethanobotanical claim this plant is used in typhoid fever, dropsy, malaria and skin diseases. A plant of *Enicostema littorale* contains phenolic and terpenoids compounds hence present study has undertaken to evaluate antimicrobial activity. Antimicrobial activity on Gram positive, Gram negative bacteria and some fungal strains have been performed.

The results of antimicrobial activity are shown in Table 1 and 2. All 4 extracts exhibited prominent antimicrobial activity against all micro-organisms used in the study. It is observed that methanol, ethyl acetate extracts showing prominent antimicrobial activity against all microorganisms as compare to petroleum ether, chloroform extracts. Minimum Inhibitory concentration of methanolic extract treated in a different strain, *S. aureus* inhibits 1mg/ml concentration where as 5mg/ml inhibits *P. aeruginosa*, *S. typhi*, and *S. sonnei* (Fig. 1.).

## Conclusion

The prominent antimicrobial activity may be due to presence of higher content of tannins, phenolic acid, flavanoid, terpenoids, glycoside. Further scope involves isolation and identification of different constituents responsible for these activities.

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