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## Estimation of terpenoid content and its antimicrobial property in *Encostemma littorale*

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**Abstract:** *Encostemma littorale* is traditionally used in India as a stomachic, bitter tonic, carminative to reduce fever and as a tonic for appetite loss. In Indian ayurvedic medicine, *E. littorale* is taken in combination with other herbs, especially for diabetes. This study aims to determine the presence of terpenoid content in *E. littorale* both qualitatively and quantitatively. The plant sample is extracted with 5 different solvent viz. ethanol, petroleum ether, chloroform, water and acetone. The presence of terpenoid was estimated by phytochemical analysis and quantitative study using linalool reagent. Antimicrobial activity of the terpenoid extract was tested against *E. coli* and *B. Subtilis*. Formation of reddish brown color indicated the presence of terpenoid. The total amount of terpenoid present in the *E. littorale* sample was found to be 0.7%. Considerable diameter of zone of inhibition was observed against *E. coli* and *B. Subtilis*.

**Keywords:** *E. littorale*, terpenoids, linalool, antimicrobial, *E. coli*, *B. Subtilis*.

### Introduction

Medicinal plants act as an alternative source for treating several ailments since their usage increasing day by day <sup>1</sup>. Medicinal plants have been used as sources of medicine in virtually all cultures <sup>2</sup>. During the last decade, the exploration of traditional medicine and its medicinal properties has expanded globally. *Encostemma littorale* (*E. littorale*) is a perennial herb with lanceolate leaves and is found throughout India. It is called as Vellarugu in Tamil <sup>3</sup>. In Indian ayurvedic medicine, *E. littorale* is taken in combination with other herbs, especially for diabetes <sup>4</sup>. The plant possesses valuable medicinal properties but most of the advantages are still confined to tribal areas because of raw knowledge and absence of proper scientific standardization. It is believed that the plant have many bioactive compounds like alkaloids, saponins, flavonoids, steroids, tannins, proteins and reducing sugar <sup>5</sup>. The quantitative analysis of the secondary metabolite terpenoids also referred to as terpenes is less explored and these phytoconstituents are the largest group of natural compounds. Many terpenes have biological activities and are used for the treatment of human diseases. The anticancer drug Taxol® and the antimalarial drug Artemisinin are two of the most renowned terpene-based drugs <sup>6</sup>. Hence, this study focuses on determining the terpenoid content in *E. littorale* and consecutively its antibacterial activity.

### Materials and Methods

#### Materials Required

Methanol, Chloroform, petroleum ether, H<sub>2</sub>SO<sub>4</sub>, Linalool (Sigma, Cat. No. L 2602), *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus cereus*, *Bacillus subtilis*, agar, nutrient broth,

antibiotic discs, test tubes, test tube stand, mortar and pestle, beaker, pipette, weighing machine, separating funnel, UV Spectrophotometer.

### Sample collection and processing

The *Enicostemma littorale* plants were collected from Thirunelveli Accession located at South India. It was identified by experts in Vel Tech High Tech Dr. Rangarajan Dr. Sakunthala Engineering College. The collected leaves of the plant were cleansed. It was shade dried for 10 days. The dried leaves of the plant were pulverized by using mortar and pestle and the powdered leaves of *Enicostemma littorale* was packed in a air tight sterilized bottle. Then this *Enicostemma littorale* plant powder was used for further research analysis.

### Qualitative analysis

The qualitative phytochemical analysis for the presence of terpenoid was determined by the methods described elsewhere<sup>7,8</sup>. 0.8 g of plant sample was taken in a test tube and 10 ml of methanol was poured in it. The mixture was shaken well and filtered to take 5 ml extract of plant sample. Then 2 ml of chloroform were mixed in extract of selected plant sample and 3 ml of sulphuric acid were added in selected sample extract. Formation of reddish brown color indicates the presence of terpenoids in the selected plants.

### Quantitative analysis

The previously prepared sample for qualitative analysis was transferred from assay tube to Colorimetric cuvette [95% (v/v) Methanol will be used as blank] to read the absorbance at 538 nm. For the standard curve 200 $\mu$ l of previously prepared Linalool solution in methanol<sup>9</sup> will be added to 1.5 ml Chloroform & serial dilution must be done [dilution level-100mg/200 $\mu$ l to 1mg/200 $\mu$ l Linalool Conc.] In case of serial dilution total volume of 200 $\mu$ l will be made up by addition of 95% (v/v) Methanol.

Alternatively, the total terpenoid content was determined by the method described elsewhere<sup>10</sup>. 100 mg of plant powder were taken and soaked in ethanol for 24 hour. The extract was filtered and the filtrate was extracted with petroleum ether using separating funnel. The ether extract was treated as total terpenoids.

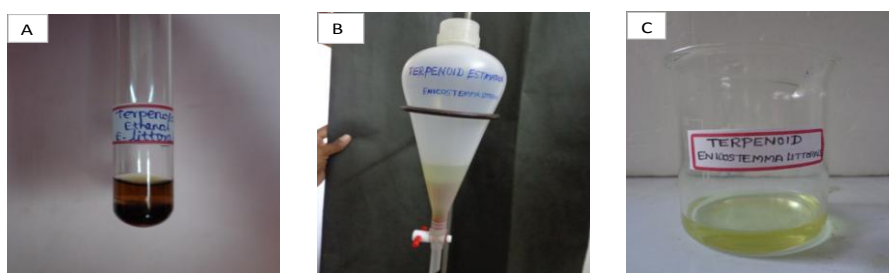
### Antimicrobial activity

Antimicrobial activity of the plant extract (500 mg/mL) was evaluated by disc diffusion method<sup>11</sup>. Stock culture of the test bacteria were grown in nutrient broth at 37C for 24 hr and were swabbed in MH agar to prepare a lawn culture. Sterile filter paper discs were placed and 500 mg/mL of the plant extract was added to the discs. Chloramphenicol (10 mg/mL) paper discs were used as control. Antimicrobial activity was determined by measurement of inhibition zone around paper discs. The experiment was conducted as a triplicate.

### Results and Discussion

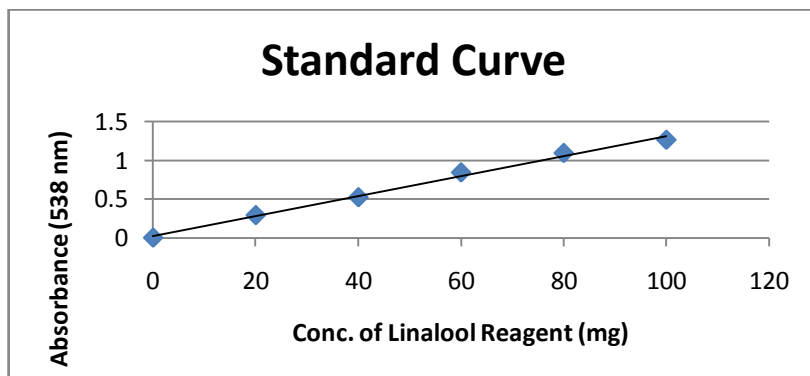
This study has revealed the presence of terpenoids by the formation of reddish brown color (Figure-1A). Terpenes are a large and diverse class of organic compounds, produced by a variety of plants, particularly conifers<sup>8</sup>. The terpenoids are primary constituents of most essential oils. They are widely used in traditional medicines for aromatherapy<sup>12</sup>.

**Figure 1: Estimation of terpenoids. (A) Result of qualitative analysis – Formation of reddish brown color. (B) Petroleum ether extract for quantitative determination of terpenoid content. (C) Isolated terpenoid extract.**



Linalool is a monoterpene which produces geraniol on reaction with chloroform<sup>8</sup>. The reaction gives a characteristic reddish brown color the concentration of which shall be detected by absorbance at 538 nm. The interpretation of the results from the standard curve (Figure- 2) showed that the amount of terpenoid present in the sample was 0.7%. The alternate method for the determination terpenoid revealed the amount of terpenoid to be 70 mg/g sample.

**Figure 2 Standard curve of Linalool reagent to estimate the amount of terpenoid content in the *E. littorale* methanolic extract.**



Terpenoids are reported to have anti-inflammatory, anti-viral, anti-malarial, inhibition of cholesterol synthesis and anti-bacterial activity<sup>6,13</sup>. The antimicrobial activity was determined by the presence and absence of inhibition zone. The extract showed significant antibacterial activity against all the species. The methanolic extract showed maximum inhibitory activity against *Bacillus subtilis* followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Table- 1).

**Table 1 Antibacterial activity of methanolic extract of *E. littorale***

| S No | Organisms              | Diameter of zone of inhibition (mm) |
|------|------------------------|-------------------------------------|
| 1    | Escherichia coli       | 8                                   |
| 2    | Pseudomonas aeruginosa | 13                                  |
| 3    | Staphylococcus aureus  | 13                                  |
| 4    | Bacillus cereus        | 12                                  |
| 5    | Bacillus subtilis      | 17                                  |

This study shall be proceeded to isolate the purest form of terpenoids by chromatographic techniques and shall be observed for the antioxidant potential. On presence of antioxidant property, the research shall be taken forward to determine the cytotoxic activity against any specific cell lines which shall be used for anticancerous therapy.

## Conclusion

The terpenoids were believed to contribute to various medicinal properties. Hence the confirmation on presence of terpenoids and its antimicrobial activity shall drive the research towards determining the cytotoxic effects of the *E. littorale* extract.

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