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BIOCHEMICAL COMPOSITION OF *ENICOSTEMMA LITTORALE* BLUME EXTRACTS

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Abstract

Analysis of organic solvent extracts of *Enicostemma littorale* reveals the presence of total phenols, vitamin C and E and carbohydrates. The organic solvent extracts of *E. littorale* exhibited antibacterial activities.

Enicostemma littorale Blume is a perennial glabrous medicinal herb. It is distributed in India and in tropical Africa, Southeast Asia and Malaysia (Ali *et al.* 2008). The plant has a number of antioxidant phytochemicals including alkaloids, catechins, saponins, sterols, triterpinoids, phenolic acid flavonoids and xanthones. The plant is used in folk medicine to treat diabetes mellitus, rheumatism, ulcers, cancer and inflammation (Murali *et al.* 2002). This paper deals with the quantitative determination of phytoconstituents and the antimicrobial activity of *E. littorale.*

Fresh *E. littorale* plant samples were collected from Erode district, Tamilnadu, India at the end of the flowering season. The plant materials were separated into root, stem and leaf and air dried in a shade at room temperature. The samples were ground to a fine powder. From it 100 g samples was extracted with chloroform, methanol and acetone by using a soxhlet apparatus. The organic solvent was removed by evaporation using rota evaporator. The residue was then placed in an oven at 40°C for about 48 h to remove the residual water. The resulting dried mass was then powdered, packed into a glass vial and stored in a desiccator. These extracts were used for phytochemical and antibacterial screening. The separated parts into leaves, flowers, stems and roots were cut into small pieces and then used for estimation of protein, carbohydrate and vitamins.

Total phenolic content was estimated after Malick and Singh (1980) and vitamin E after Backer *et al.* (1980). Vitamin C was extracted and estimated following Omaye *et al.* (1979). The total protein content was determined after Bradford (1976) and the total carbohydrate content was determined following Dubois *et al*. (1974).

The bacterial cultures used in this study were obtained from the Department of Medical Microbiology, Raja Muthiah Medical College, Annamalai University, India. The culture consists of three from each of Gram-positive and Gram-negative strains (Table 1). Antibacterial activity of each extract of the plant samples (500 mg/ml) were evaluated by the paper disc diffusion method (Brantner and Grein 1994). Stock culture of test bacteria was grown in nutrient broth medium at 37ºC for 24 h. Final bacterial numbers were adjusted to 0.5 Mc Farland Turbidometry. A lawn culture then prepared on Muller-Hinton agar using sterile cotton swabs. Sterile filter paper discs (6 mm) impregnated with reconstituted extract at the concentration of 500 mg/ml were placed on the culture plates previously seeded with the 0.5 McFarland and 10^6 cfu/ml cultures of bacteria. Paper discs impregnated with 20 µl of a solution of 10 mg/ml of chloramphenicol and streptomycin as standard antimicrobials were used for comparison. Antimicrobial activity was determined by measurement of zone of inhibition around each paper disc. For each extract three replicates were used against each organism.

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Data were subjected to statistical analysis using statistical software package SPSS version 14 (SPSS Inc, Chicago, USA). One way analysis of variance (ANOVA) followed by Duncan multiple range test were employed and the differences between individual means were deemed to be significant at $p < 0.05$.

Vitamin C content in root, stem, leaves and flowers of *E. littorale* ranged from 0.37 - 4.42 mg/g FW (Table 1). The flowers contained more vitamin C than leaves, stem and root. The vitamin E content in leaves, stem, root and flowers of *E. littorale* ranged from 1.51 to 2.66 mg/g FW (Table 2). The highest vitamin E content was observed in leaves of *Moringa oleifera* (Sanchez-Machado *et al.* 2006). The total phenolic content of root, stem, leaves and flowers in the different parts of *E. littorale* ranged from 4.69 to 7.90 GAE/g DW and showing the highest amount in flowers (Table 1).

Table 1. Biochemical components in different parts of *Enicostemma littorale*.

Biochemical components	Leaf	Stem	Root	Flower
Vitamin C (mg/g FW)	4.11 ± 0.05	3.61 ± 0.04	0.37 ± 0.26	4.42 ± 0.34
Vitamin E (mg/g FW)	2.66 ± 0.07	2.48 ± 0.02	1.51 ± 0.05	2.51 ± 0.11
Total phenols (GAE/g DW)	5.59 ± 0.06	5.89 ± 0.01	4.69 ± 0.11	7.90 ± 0.02
Protein (mg/100g)	6.08 ± 0.04	6.04 ± 0.02	5.85 ± 0.01	4.01 ± 0.02
Carbohydrate (mg/100g)	10.21 ±0.07	9.18 ± 0.03	8.21 ±0.05	6.01 ± 0.01

Each value is the mean of three replicates ± standard deviation.

Table 2. Antibacterial activity of different extracts of *Enicostemma littorale* using different solvents.

	Diameter of zone of inhibition (mm)										
Bacteria	Leaf extract (500 mg/ml)			Stem extract (500 mg/ml)			Root extract (500 mg/ml)			Antibiotic	
	CL	ME	AC	CL	ME	AC	CL	ME	AC	Ch	St
Escherichia coli	10	9	8	14	15	10	12	14	9	15	15
Klebsiella pneumoniae	10	11	11	12	16	11	12	13	13	17	17
Pseudomonas aeruginosa	16	14	14	17	17	16	18	16	11	18	17
Staphylococcus aureus	11	14	11	12	15	12	9	11	12	4	8
Bacillus cereus	16	13	16	18	18	17	13	11	10	20	18
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

Among the plant extracts, chloroform extract showed maximum antibacterial activity in comparison to methanol and acetone extracts. Among the leaf, stem and root extracts, the stem extract showed maximum antibacterial activity. The chloroform extract of stem showed the highest activity (about 20 mm inhibitory zone) against *Bacillus subtilis* (at 500 mg/ml) followed by the methanolic stem extract (Table 2). The chloroform extract of *E. littorale* showed better activity against all the tested bacteria. Results showed that the chloroform extract has higher antibacterial activity than that of methanol and acetone extracts. The stem extracts displayed higher antibacterial activity than the leaf and root extracts. This might be due the maturity of the stem which contain other secondary metabolites and bitter principles of the plant. Earlier results suggested that antimicrobial

compounds from natural leaf and stem extracts might benefit oral health as plaque-control agents for the prevention of dental caries and periodontal disease (Yim *et al.* 2010). Antimicrobial assessment of the crude extracts of *Gunnera perpensa* indicated that the highest sensitivity was obtained from the stem extracts, and the least activity was noted for the root extracts (Drewes *et al.* 2005).

From the present research it is evident that *E. littorale* is rich in vitamin C, vitamin E and it has good antibacterial activity. Therefore, the plant may contribute significantly to the nutrient requirements of human being and may act as antibiotics.

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