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GC-MS Determination of Bioactive compounds of *Enicostemma littorale* (Blume)

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ABSTRACT

In this study, the bioactive compounds of Enicostemma littorale have been evaluated using GC-MS. The chemical compositions of the whole plant methanol extract of Enicostemma littorale were investigated using Perkin-Elmer Gas chromatography-mass spectrometry. The mass spectra of the compounds, found in the extract was matched with characterization and measurement of the central Electrochemical Research institute. GC-MS analysis of E.littorale whole plant methanol extract revealed the existence of the ether compound-Laminaribiitol (79.93%), 12-hydroxy-9-octadecenoic acid (9.546%). Myricetin (4.7519%), 3.3-Methylenebis (4-hydroxycoumarin) (2.811%), catechin (2.002%). The results of this study offer a base of using E.littorale as herbal alternative for the synthesis of antifungal agents.

Key words : GC-MS analysis, Bioactive compounds, *Enciostemma littorale,* methanol extract.

INTRODUCTION

Several plant products have been shown to exert a protective role against the formation of free radicals and playing a beneficial role in maintaining disease condition [1]. *Enicostemma littorale* Blume (Family-*Gentianaceae*) a glabrous herb commonly used as a bitter tonic and substitute for *swertia chirayita* (Roxb.ex Flem.) Karst., is also called as chhotachirayta. It is also reported to possess antitumor [2], hypoglycaemic [3] and antimalarial activities [4]. The anticancer activity of methanolic extract of the plant has been evaluated against Dalton's ascetic lymphoma in swiss albino mice [5]. In the present study authors have evaluated the hypolipidemic and antilipid peroxidative activities of the plant against P-DAB induced heptotoxicity in animals.

Plant is man's friend in survival, giving him food and fuel and medicine from the days beyond drawn of civilization [8]. Plant continue to be a major source of medicine, as they have throughout human history [9]. *Coriandrum sativum* L. commonly known as coriander and belonging to the family Apiaceae (Umbelliferae), is cultivated throughout the world for its nutritional value. The fresh leaves of C. *sativum* are a strong smelling, annual herb, is generally

used in three forms; young leaves, dried ripe fruits (commonly termed coriander seeds) and oil. Coriander leaves stimulate appetite and the fresh juice is often recommended for patients suffering from Vitamin A,B and C deficiencies and also for the relief of anxiety and insomnia [10].

Plants are rich source of secondary metabolites with interesting biological activities. In general these secondary metabolites are an important source with a variety of structural arrangements and properties [6]. Natural products from microbial sources have been the primary source of antibiotics but with the increasing recognition of herbal medicine as an alternative form of health care, the screening of medicinal plants for active compounds has become very significant because they may serve as talented source of bulk antibiotic prototypes [7]. The family polygonaceae consists of several important medicinal plants with wide range of biological activities and interesting phytochemical constituents. *Enicostemma littorale* (Blume) was selected for the present study, based on its therapeutic value and the degree of research work which is not done mostly in earlier. In this study, *E.littorale* whole plant methanol extract was used of the screeing of differed phytochemical constituents by GC-MS analysis.

MATERIALS AND METHODS

Plant material

Enicostemma littorale was collected from A.V.V.M. Sri Pushpam College Campus, Thanjavur District, Tamil Nadu, India and with Rapinat Herbarium, St.Josephs College, Trichy.

Preparation of extract

The whole plant material of *E.littorale* was collected from wild, shade dried and pulverized to powder in a mechanical grinder. Required quantity of the whole plant powder of *E.littorale* was weighed, transferred to flask, treated with the methanol until the powder was fully immersed, incubated over night and filtered through a whatmann No.1 Filter paper along with sodium sulphate to remove the sediments and traces of water in the filter paper. Before filtering, the filter paper along with sodium sulphate was wetted with absoluted alcohol. The filtrate is then concentrated to 1ml by bubbling nitrogen gas into the solution. The extract contains both polar and non-polar components of the plant material and 2µl sample of the solutions was employed in GC-MS for analysis of different compounds.

GC-MS analysis

GC-MS analysis of the methanol extract of *E.littorale* was performed using a perkin Elmer GC clarus 500 system comprising AOC-20i auto-sampler and a Gas chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with a Elite-5MS (5% Diphenyl / 95% Dimethyl Poly Siloxane) Fused capillary column (30 x 0.25µm 1D x 0.25µm dF). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization system was operated in electron impact mode with ionization energy of 70ev. Helium gas (99.999%) was used as carrier gas at a constant flow rate of 1ml/min, and an injection volume of 2µl was employed (split ratio of 10:1). The injector temperature was maintained at 250^0C, the ion-source temperature was 200^0C, the oven temperature was programmed at 110^0C (isothermal for 2 min), with an increase of 10^0 c/min to 200^0C, then 5^0C/min to 280^0C, ending with a 9 min isothermal at 280^0C. Mass spectra were taken at 70ev; a scan interval of 0.5 seconds and fragments from 45-450Da. The solvent delay was 0 to 2 min and the total GC-MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass detector used in this analysis was Turbo-Mass Gold-Perkin

Elmer and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver 5.2.

Identification of phytocomponents

Interpretation on mass-spectrum GC-MS was conducted using the database of central electrochemical research institute characterization and measurement laboratory having more than 62,000 patterns. The spectrum of the unknown components was compared with the spectrum of known components stored in the central electrochemical research institute characterization and measurement laboratory. The name, molecular weight and structure of the components of the test materials were ascertained.

GC-MS Analysis

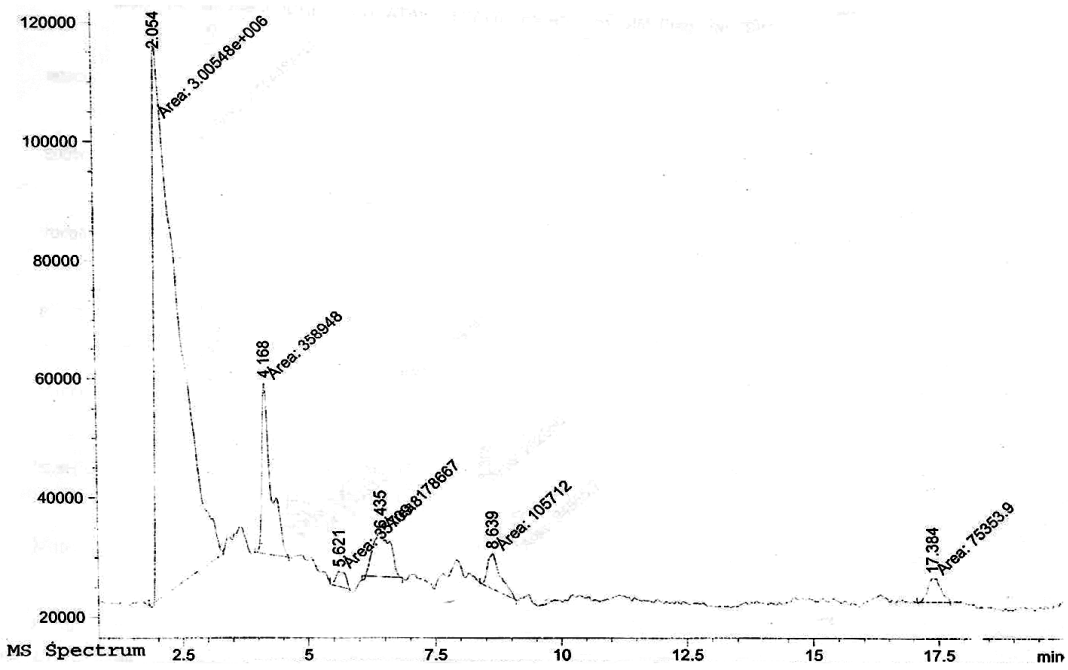

Fig.1 GC-MS chromatogram of *Enicostemma littorale* Methanolic extracts

RESULTS AND DISCUSSION

The studies on the active principles in the *Enicostemma littorale* whole plant methanol extract by GC-MS analysis clearly showed the presence of six compounds (Table-1). The active principles with their retention time (RT), molecular Formula, Molecular weight (MW), and concentration (Peak area%) are presented in Table-1. The GC-MS chromatogram of the seven peaks of the compounds detected was shown in figure-1. The mass spectrum and structure of the compounds identified were presented. The total number of compounds identified in methanol extracts. The results revealed that laminaribiitol (79.93%), 12-hydroxy-9-octadecenoic acid (9.546%), myricetin (4.7519), 3.3-Methylenebis (4-hydroxycoumatrin) (2.811). Cabechin (2.002), unknown (0.961).

Table 1 phto components identified in the *Enicostemma littorale*

S. No.	RT	Peak area in %	M.wt	Name of compounds
1.	2.03	79.93	344.3	Laminaribiitol
2.	4.218	9.546	298.5	12-hydroxy-9-octadecenoic acie
3.	5.671	0.961	345	Unknown
4.	6.403	4.7519	318.2	Myricetin
5.	8.549	2.811	336.2	3,3'-methylenebis (4-hydroxycoumarin)
6.	17.392	2.002	290.3	Catechin

[11] reported the ethanolic extract of *Mussaenda frondosa* has been subjected to GC-MS analysis. Twenty chemical constituents have been identified. The major chemical constituents are (-) – Quinic acid (32.87%), 4-(1E) – 3-HYdroxy-1-propeny1) -2-Methoxyphenol (8.30%), Naphthalene, decahydro-2-methoxy-(7.20%), 1,2,3-Benzenetriol (7.70%). The bioactive compounds of *Polygonum glabrum* have been evaluated using GC-MS. The chemical compositions of the whole plant ethanol extract of *P.glabrum* were investigated using perkin-Elmer Gas chromatography – mass spectrometry while the mass spectra of the compounds found in the extract was matched with the National Institute of Standards and Technology (NIST) library. GC-MS analysis of *P.glabrum* whole plant ethanol extract revealed the existence of the ether compound-propane 1,1-diethoxy-(64-86%), alkane compound-2-Heptane, 5-ethyl-2,4-dimethyl-(13.51%), Sulphur compound – Tiophene – 2 – Caroboxamide, N-(2-Furfuryl) – (8.11%), alcoholic compound – 1,14 – Tetra – decanediol (5.41%), and plasticizer compounds – 1,2 – Benzenedi carboxylic acid, isodecyloctyl ester (5.41%) and 1,2,3 – Benzenetriol (2.79%). The results of this study offer a base of using *P.glabrum* as herbal alternative for the synthesis of antimicrobial agents [12].

CONCLUSION

In the present study twenty chemical constituents have been identified from methanolic extract of the whole plant of *Enicostemma littorale* by Gas chromatogram Mass spectrometry (GC-MS) analysis. The presence of various bioactive compounds justifies the use of whole plant for various ailments by traditional practitioners.

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