Research Article

Phytochemical Analysis of Leaf Extract of *Eclipta alba* (L.) Hassk by GC-MS Method

*Anand.D[1], John Wyson.W[1], Saravanan.P[1], Rajarajan.S[2]

[1]*P.G & Research Dept of Botany, Ramakrishna Mission Vivekananda College (Autonomous), Mylapore, Chennai – 600 004. India*
[2]*Former Head & Founder, P.G & Research Dept. of Microbiology and Biotechnology, Presidency College (Autonomous), Chennai – 600 005. India*

ABSTRACT

Plants have been an important source of medicine since ancient era. *Eclipta alba* has an important role in the traditional Ayurvedic, Siddha and Unani systems of Medicine. The aim of the present study is to determine the potential bioactive components of leaves of *Eclipta alba* using Gas Chromatography – Mass Spectrometry analysis (GC-MS). The chemical compositions of methanol extract of plant leaves was investigated using Perkin – Elmer GC-MS while the mass spectra of the compounds found in the leaf extract was matched with the National Institute of Standards and Technology library. The GC-MS analysis provided peaks of eight different phytochemical compounds namely 10-Octadecenoic acid, methyl ester (25.89%), c-Sitosterol(18.94%), 9,19- Cyclocholestan-3-ol-7-one,4a-dimethly-[20R] (12.14%), Dodecanoic acid, 10 methyl, methyl ester (11.61%), Tridecanol, 2-ethyl-2-methyl (10.20%), 1,2 Benzenedicarboxylic acid, butyl octy ester (10.13%), 1-Heptatriacotanol (7.46%), Oleic acid, eicosyl ester (3.58%). This will be further considered for pharmacological activities and isolation of individual components would however, help to find new drugs.

Keywords: *Eclipta alba*, GC-MS analysis, Methanolic, Bioactive components, Oleic acid, eicosyl ester.

INTRODUCTION

Medicinal plants have been used by human being since ages in traditional medicine due to their therapeutic potential and the search on medicinal plants have led to the discovery of novel drug candidates used against diverse diseases. According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs[1]. *Eclipta alba* is a medicinal herb, belonging to family Asteraceae which is widely distributed throughout Asia. It is common in marshy lands, hedges and roadsides. The branches are hairy, reddish brown, and can grow up to 40cm high. The roots are found growing at the thickened nodal points[2]. *E.alba* arranged in small clusters and the dry fruit formed by fusion of two carpels which do not break open and each has just one seed. *Eclipta alba* is widely used in India as a cholagogue and deobstruent in hepatic enlargement[3]. The decoction of the whole plant is effective for fever[4]. *E.alba* is a source of coumestans-type compounds used in phytopharmaceutical formulations of medicines prescribed for treatment of cirrhosis of the liver and infectious hepatitis[5]. *Eclipta* is traditionally used for blackening, promoting hair growth and strengthening the hair. It is used as anti venom against snake bite in China and Brazil. Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents are playing a significant role in the identification of crude drugs[6]. Therefore, proper scientific knowledge is required to investigate and explore the exact standardization of such medicinally important plant.

Mass spectrophotometry coupled with chromatographic separations such as Gas chromatography (GC-MS) is normally used for direct analysis of components existing in traditional medicines and medicinal plants[7]. The unknown organic compounds in a complex mixture can be determined by interpretation and also by matching the spectra with reference spectra[8]. Methanolic solvent is used to procure the phytocomponents due to high polarity [9](Molecules with permanent dipoles). Hence, the objective of the present study is to identify the phytochemical constituents of methonolic leaf extracts of *E.alba* with the aid of GC-MS technique.
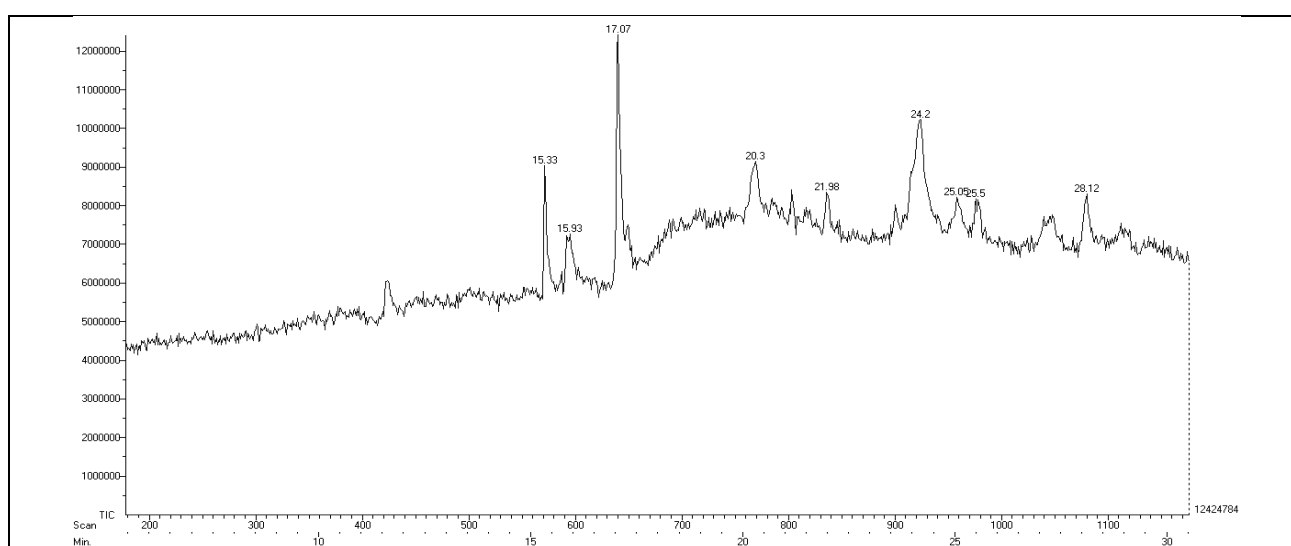
MATERIALS AND METHODS

Collection of Plant Material: The leaves of *E.alba* were collected during the month of December from the natural habitats of Thiruvalluvar district, Tamil nadu, India. The plant material was identified and authenticated by Department of Botany, Ramakrishna Mission Vivekananda College, Chennai India. The leaves were washed with running tap water and finally washed with distilled water to remove the dirt and dried under shade for two weeks.

Preparation of Leaf Extracts: The leaf was powdered in electric grinder and 100 gm was extracted in soxhlet

Author for Correspondence: E-mail: anandesingh@yahoo.co.in

Table 1: Phytocomponents identified in the methanolic leaf extract of *Eclipta alba* by GC-MS

NO	Retention Time	Name of the compound	Molecular Formula	Peak area (%)	Molecular Weight
1.	15.33	Dodecanoic acid,10 methyl, methyl ester	$C_{14}H_{28}O_2$	11.61	228.37
2.	15.93	1,2 Benzenedicarboxylic acid, butyl octyl ester	$C_{20}H_{30}O_4$	10.13	334.44
3.	17.07	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	25.89	296.48
4.	21.98	Oleic acid, eicosyl ester	$C_{38}H_{74}O_2$	3.58	562.99
5.	25.05	1-Heptatriacotanol	$C_{37}H_{76}O$	7.46	536.99
6.	28.12	Tridecanol, 2-ethyl-2-methyl-	$C_{16}H_{34}O$	10.20	242.44
7.	20.3	9,19- Cyclocholestan-3-ol-7-one,4a-dimethly-[20R]	$C_{29}H_{48}O_2$	12.14	428.69
8.	24.2	c-Sitosterol	$C_{29}H_{50}O$	18.94	414.70

Fig. 1: GC-MS Chromatogram of methanolic extract of Eclipta alba

apparatus using methanol for 6 hours. The methanolic extracts were dried under reduced pressure using rotary evaporator to get the crude and were stored at 4°C until further used. The extract contains polar components of the plant material, and 2 µl of the sample of the solutions was employed in GC-MS analysis of different compounds.

GC-MS Analysis: GC-MS analysis of the methanol extract of *E.alba* was performed in a Perkin–Elmer GC Clarus 500 system comprising an 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 a capillary column (30 × 0.25 µm ID × 0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization energy of 70 eV. Helium gas (99.999%) was used as a carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2µl was employed (a split ratio of 10:1). The injector temperature was maintained at 250°C, the ion-source temperature was 200°C, the oven temperature was programmed from 110°C (isothermal for 2 min), with an increase of 10°C/min to 200°C, then 5 °C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a

scan interval of 0.5 s and fragments from 45 to 450 Da. 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 compounds: Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the known component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained.

RESULTS
The identification of phytochemical compounds is based on their retention time (RT), molecular formula, molecular weight (MW), chemical structure and concentration (peak area %). GC-MS chromatogram of leaves of *E. alba*
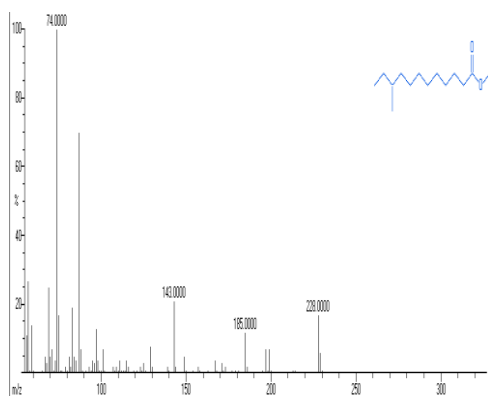
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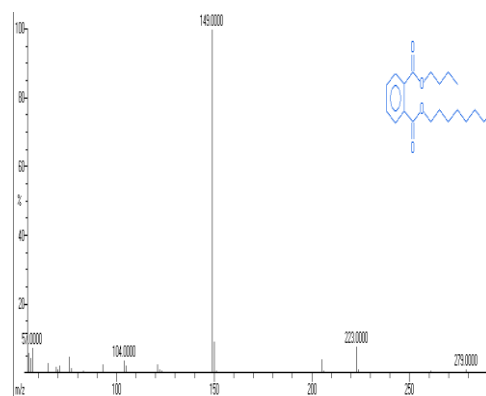*Figure 2 A. Dodecanoic acid,10 methyl, methyl ester*

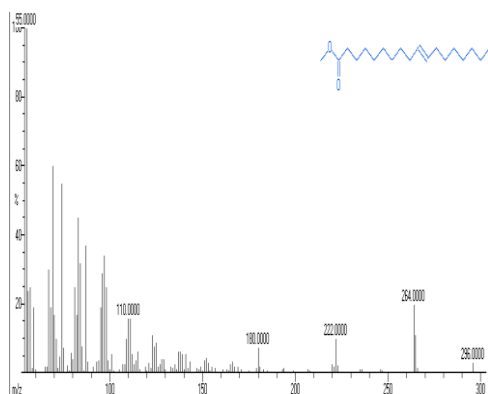*Figure 2 B. 1,2 Benzenedicarboxylic acid, butyl octyl ester*
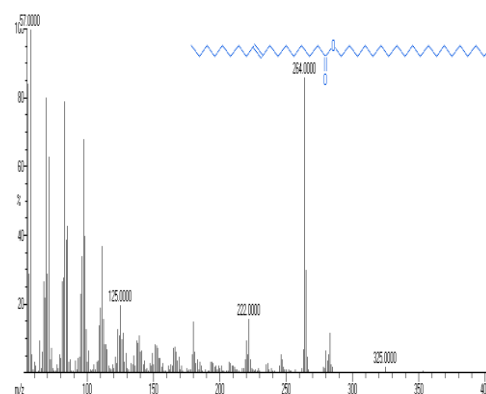

Figure 2 C.10-Octadecenoic acid, methyl ester
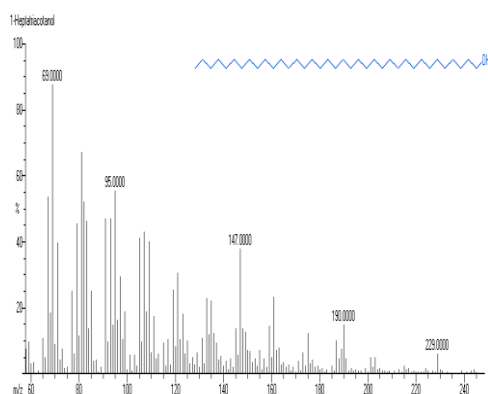

Figure 2 D. Oleic acid, eicosyl ester
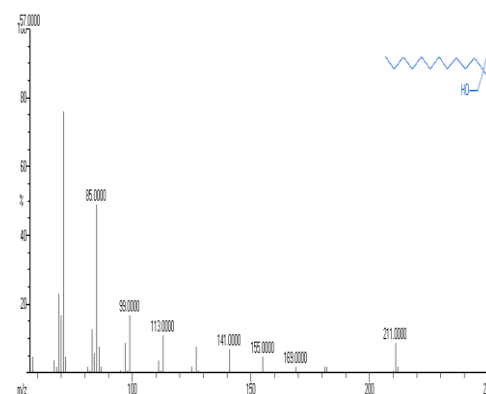

Figure 2 E. 1-Heptatriacotanol

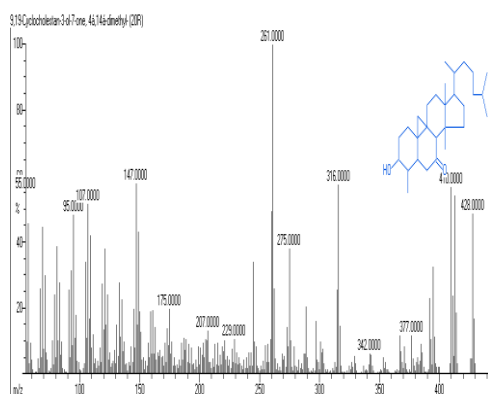*Figure 2 F. Tridecanol, 2-ethyl-2-methyl-*

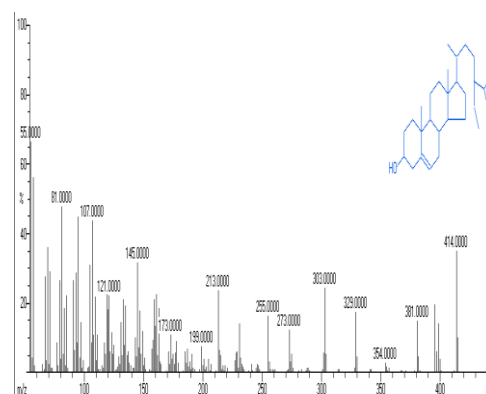*Figure 2 G. 9,19- Cyclocholestan-3-ol-7-one,4a- dimethly-[20R]*

Figure 2 H. c-Sitosterol

analysis showed the presence of eight different compounds namely Tridecanol, 2-ethyl-2-methyl (retention time 28.12), 1-Heptatriacotanol (retention time 25.05), c-Sitosterol (retention time 24.2) Oleic acid, eicosyl ester (retention time 21.98), 9,19- Cyclocholestan-3-ol-7-

one,4a-dimethly-[20R] (retention time 20.3), 10-Octadecenoic acid, methyl ester (retention time 17.07), 1,2 Benzenedicarboxylic acid, butyl octy ester (retention time 15.93), Dodecanoic acid,10 methyl, methyl ester (retention time 15.33) (Table-1) and (Figure-1). The individual fragmentations of the components are illustrated in Figures 2A-2H.

DISCUSSION

Traditional medicine also known as indigenous or folk medicine comprises medical knowledge systems that developed over generations within various societies before the era of modern medicine. Traditional medicines are prepared from a single plant or combination of more than one plant. Indian contribution to herbal market and emphasis on novel research is continuously increasing. Phytochemical constituents are responsible for medicinal activity of plant species[10]. Hence, in the present study phytochemical screening of *E.alba* was carried out, qualitative phytochemical analysis of this plant confirms the presence of bio active compounds.

The results of methonolic extracts of *E. alba* leaves clearly implies that the strength of active principle depends upon the use of solvent besides the type of plant species to achieve the positive results. The identified phytochemical compounds have many biological properties. For instance, Oleic acid, eicosyl ester reported to contain anti-inflammatory, cancer preventive, dermatitigenic Hypocholesterolemic and anemiagenic Insectifuge[11]. 1-Heptatriacotanol is an alcoholic compound which showed antimicrobial activity[12].

Previous, studies reported that the phytochemical studies of *E. alba* using methanol solvent yielded eleven bio active compounds[13] which are N-(3,4,4-Trimethyl-1,2-Dioxethane-3-yl-MethoxyCarbonyl)Glycine, Silane, Acetamide, 1H-Pyrimido[4,5,6-IJ][2,7]Naphthyridine-6-Carbonitrile,2-Ethyl-5,8-Dimethoxy-, Acetonitrile- D3, 3-Methoxy-5-(Methoxymethoxy)-7-Methyl-6-(3-(Trimethylsilyl)Propargyl)-1,4-Naphthoquinone, L-Alanine, Ethylester-, Formamide,N-[(dibutylamino)methyl]-N-methyl-, Trans-2-((phenylthio)methyl)-1-(2-propenyl)-1,2,3,4-tetrahydronaphthalene, 2-Acetonyl-3-cyano-2,3-dimethylcyclobutane-1-carboxylic acid, 5,5'-dicarboxy-3'-(2-chloroethyl)-4-(2-acetoxyethyl)-3,4'-dimethylpyrromethane, whereas eight dissimilar compounds were observed in the present study namely Tridecanol , 2-ethyl-2-methyl, 1-Heptatriacotanol , c-Sitosterol,Oleic acid, eicosyl ester, 9,19- Cyclocholestan-3-ol-7-one,4a-dimethly-[20R], 10-Octadecenoic acid, methyl ester, 1,2 Benzenedicarboxylic acid, butyl octy ester, Dodecanoic acid,10 methyl, methyl ester. Hence, the differences in plant components might arise from several environmental (climatical, seasonal, and geographical) and genetic differences, which were the important factors influencing the quality of medicinal herbs.

There is growing awareness in correlating the phytochemical components and their biological activities[14, 15, 16]. In conclusion the presence of various bioactive compounds justifies the use of the leaf of *E. alba*

for various ailments by traditional practitioners. Isolation of these compounds was supportive to identify new drugs to treat various diseases. Therefore, it is recommended as a plant of phytopharmaceutical importance. Further investigation of the plant with various solvents can increase the isolation of the newer molecules which will be helpful for the study of the pharmacological activities and in discovering drugs from the plant which may prevent the human and the economic losses in the environment.

REFERENCE

1. Pierangeli G, Vital G, Rivera W. Antimicrobial activity and cytotoxicity of Chromolaena odorata (L. f) king and robinson and Uncaria perrottetii (A. Rich) Merr Extracts. Journal of Medicinal Plants Research 2009; 3(7): 511-518.
2. Manik Sharma, Muhammad Yusuf, Showkat Hussain, Abrar Hussain. Phytochemical constituents and pharmacological activities of Eclipta alba Linn (Asteraceae). A Review,International Research Journal of Pharmacy 2012; 3(12).
3. Prakash. M.M.S. Kinthada, Naidu P.V.S, Muralidhar P. Biologically estimation of heavy / toxic metals present in traditional medicinal plant – Eclipta alba. International Journal of Pharmaceutical and Biomedical Science 2011; 2(4): 99-102.
4. Chevallier and Andrew. The encyclopedia of medicinal plants. Edn 1, DK Publishing Inc., New York, USA, 1996: 55-281.
5. Wagner H. Coumestans as the main active principles of the liver drugs Eclipta alba and Wedelia Calendulaceae. Planta Medica 1986; 5: 370-74.
6. Getahun Tadesse, Reneela P, Aman Dekebo. Isolation and characterization of natural products from Helinus mystachnus (Rhamnaceae). Journal of Chemical and Pharmaceutical Research 2012; 4(3):1756-1762.
7. Sermakkani M, Thangapandian V. GC-MS Analysis of Cassia italica leaf methanol extract. Asian Journal of Pharmaceutical and Clinical Research 2012; 5 (2), 90-94.
8. Ronald Hites A. Gas chromatography mass spectroscopy, Handbook of instrumental techniques for analytical chemistry, 1997: 609-611.
9. Israelachvili, Jacob N. Intermolecular and surface forces. Edn 3. San Diego, CA, Academic press/Elsevier, 2011.
10. Kalimuthu K, Prabakaran R. Preliminary phytochemical screening and GC-MS analysis of methanol extract of Ceropegia pusilla, International Journal of Research in Applied Natural and Social Science 2013; 1(3): 49-58.

11. Sheela D , Uthayakumari F. GC-MS Analysis of bioactive constituents from coastal sand dune taxon – Sesuvium portulacastrum (L.), Bioscience Discovery 2013; 4(1): 47-53.

12. Kalaiarasan A, Kumar P, Ahmed John S. GC-MS determination of Bioactive Components of Bulbophyllum kaitense. Reichib Leaves Estern Ghats in India, New York Science Journal 2011; 4(10).

13. Neha Chauhan, Dolly Singh, Painuli R.M. Screening of bioprotective properties and phytochemical analysis of various extracts of Eclipta alba whole plant. International Journal of Pharmacy and Pharmaceutical Science 2012; 4(2): 554-560.

14. Fernie AR, Trethewey RN, Krotzky AJ, Willmitzer L. Innovation - Metabolite profiling: from diagnostics to system biology. Nature Reviews Molecular Cell Biology 2004; 5: 763 - 769.

15. Sumner LW, Mendes P, Dixon RA. Plant metabolomics. largescale phytochemistry in the functional genomics era. Phytochemistry 2003; 62(6): 817 - 836.

16. Robertson DG. Metabonomics in toxicology. A review. Toxicological Sciences 2005;85:809 – 822.