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Extraction of phytochemical constituents from the root of *Eclipta alba* through gas chromatography-mass spectrometry procedure

Satheesh Naik K^{*1}, Gurushanthaiah M², Kavimani M³, Mahesh GM⁴¹Research Scholar, Department of Anatomy, Bharath Institute of Higher Education and Research (Bharath University), Chennai, Tamil Nadu, India²Department of Anatomy, Basaveshwara Medical College, Chitradurga, Karnataka, India³Department of Anatomy, Sree Balaji Medical College and Hospital, Chennai, Tamil Nadu, India⁴Department of Anatomy, Basaveshwara Medical College, Chitradurga, Karnataka, India

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ABSTRACT

The higher herbal values of *Eclipta Alba* have a long history in the treatment of various ailments in traditional and folk medicine in many tropical and subtropical countries. Medicinal plants play a significant role in human culture and civilization. *Eclipta Alba* plant roots were powdered. By using simple soxhlation method, methanol extract was prepared. The powdered extract of *Eclipta alba* and methanol extract was concentrated and analyzed using Gas Chromatography-Mass Spectroscopy to identify the biochemical components of *Eclipta Alba* root extract in the present study. A wide range of active ingredients such as 2- Thiophene carbaldehyde, 5-[5-(thien-2-yl) thien-2-yl]-0.08%, Dodecanoic acid - 0.19, 9-Octadecenamide, (Z)- (CAS) OLEOAMIDE - 0.29, Lolilide - 0.37 and- 44.86. This study supports, methanolic extract of *eclipta alba* has potential antimicrobial, antioxidant and hepatoprotective activities.



* Corresponding Author

Name: Satheesh Naik K
 Phone: +91-9542093368
 Email: nsatheesh43@gmail.com

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INTRODUCTION

Eclipta Alba (Asteraceae) a common weed of agriculture and also widely used medicinal herb in traditional medicine of the East countries. This plant has positive outcomes as antianaphylactic (Patel, M.B. *et al.*, 2010), antihyperglycemic (Ananthi, J. *et al.*, 2003) and hepatoprotective activity reported by previous literature (Veeru.p. *et al.*, 2009). The name *Eclipta Alba* derived from Greek word meaning "Deficient" due to the absence of the bristles and awns on the fruits whereas *Eclipta alba* means

white which refers to the color of the flower. This annual herb is found very commonly in paddy growing areas of India. In Traditional Chinese Medicine, *Eclipta* is said to nourish yin, tonify the kidney and cool the blood. Arabian medicine also uses *Eclipta* known as "Kadim-el-bint" to support liver function. *Eclipta alba* is an important plant of Ayurvedicmatria media (Singh.A.P. *et al.*, 2010). Its pharmacological activities viz., analgesic, antimicrobial, antiviral, antifungal, antinociceptive, anti-inflammatory, antioxidant, anti-hyperglycemic, hepatoprotective, immunomodulatory, hair growth and wound healing activities were summarized (Thorat, R.M. *et al.*, 2010), (Neerja, P.V.*et al.*, 2012) & (Khan, A.V. *et al.*, 2008) reported ethnomedical uses of *Eclipta*.

In many branches of science and technology, Gas chromatography (GC) is one of the widely used technique and has a significant role component determination and their proportions in the mixture. Mass spectrometric detector (MSD) allows obtaining the molecule fingerprint and indicates its mass spectrum. The information on the molecular weight, the composition of elements provided by

Mass spectrum. By using a high-resolution mass spectrometer, the functional groups, geometry and spatial isomerism of the molecule can also be obtained (Elena Stashenko. *et al.*, 2010).

METHODOLOGY



Figure 1: *Eclipta Alba*

Extraction of plant materials: The *Eclipta Alba* is mostly growing in paddy fields during the rainy and summer season in Karnataka state. This herb plant was identified in the Department of Botany, Govt Science College, Davangere University, and Chitradurga. The mature plants (including roots) were collected from the adjoining village area of Chitradurga city and washed thoroughly with running tap water then with deionised water. Roots of the plant were removed separately and shade dried at room temperature for more than 15 days. *Eclipta Alba* dried roots are charged to extractor along with Methanol. It is extracted by heating the mass for 5-6 hours, in a Soxhlet apparatus. This process is repeated. The extract was filtered and centrifuged at 1500 rpm for 20 minutes to remove any plant debris. Supernatants were stored at 22 °C until assayed. It is packed in food grade, virgin, polythene bags. Fig.1

GC-MS Analysis

Sample preparation: About 1 g of the sample was taken in a vial, and 5 ml of methanol added. The sample was sonicated for 15 mins and supernatant layer taken for gc-ms analysis.

Column

Restek Rtx-5 capillary column, length: 30 m, internal diameter: 0.25 mm, film thickness: 0.25 µm.

Column programming

The rate of heating - 10 °C/min, temperature - 60 °C & 330 °C and Hold time 0 min & 10 min.

Injector

300 °C, Flow mode: Linear velocity, Split: 1:10, Sample injection: 1 µl, Interface: 330 °C, Ion source:

200 °C, Detector voltage: 1.5 kV, Mass scan range: 40-600 m/z, Ionization mode: Electron impact ionization (EI), Ionization energy: 70 eV, Mass library: NIST 5 and WILEY, at VittalMallya Scientific Research Foundation, Bangalore.

RESULTS AND DISCUSSION

Figure 2: GC-MS chromatogram

The *Eclipta Alba* roots showed the presence of 26 phytochemical chemical compounds by using based on their time of retention (RT) formula and weight of molecules, chemical structure and concentration (peak area %). (Fig.2) by using GC-MS chromatogram analysis were recorded and tabulated [Table1].

DISCUSSION

The role of phytochemical compounds increased significantly due to their nutritional incidence in health and disease (Steinmetz K. *et al.*, 1991). In recent years, the study of the organic compounds from plants and their activity was widely increased. The present study aimed to develop a useful method for the phytochemicals present in the plant extracts and determination of organic compounds by a quantitative method in the plant. The diversity of medicinal plants and herbs containing various phytochemicals with biological activity can be of the valuable therapeutic key (Liu RH. *et al.*, 2003). Phytochemical constituents are responsible for the medicinal activity of plant species (Raaman.N.2006). GC-MS analysis of *Eclipta alba* by using methanol divulged showed eight possible bioactive compounds Tridecanol, 2-ethyl-2-methyl, 1- Heptatriacotanol, c-Sitosterol, Oleic acid, eicosyl ester, 9, 19- Cyclocholestan-3-ol-7-one, 4a-dimethyl-[20R], 10- Octadecenoic acid, methyl ester, 1, 2 Benzenedicarboxylic acid, butyl octy ester, Dodecanoic acid, 10 methyl, methyl ester.11, whereas the current study showed seven compounds c-Sitosterol, Glycine, N[(3a, 5a, 12a]- 3, 12-dihydroxy 24-oxocholan-24-yl]-, Oleic acid, eicosyl ester, Ethanol, 2-(9, 12-octadecadienyloxy), (ZZ), 10-Octadecanoic acid, methyl ester, Pentadecanoic acid, 14methyl, methyl ester, Diethyl Phthalate which are divergent. They have many biological properties. Oleic acid, eicosyl ester reported containing anti-inflammatory, cancer preventive, dermatitogenic Hypocholesterolemic and anemiagenic Insectifuge (Sheela D. *et al.*, 2013). *E. prostrata* chemical constituents namely Two oleanane-type glycosides eclalbasaponin I and eclalbasaponin II along with the ubiquitous steroid, stigmasterol were isolated from an n-hexane extract of the stem bark of the plant. The water extract of *E. prostrata* (whole plant) exhibited the potent inhibitory activity against antiviral activity HIV-1 integrase (HIV-1

Table 1: Gas chromatography-mass spectrometry Analysis - Phytochemical components of Eclipta alba root extract

Peak	R. Time	I. Time	F.Time	Name of the Compound
5	19.284	19.250	19.317	2, 2':5', 2''-Terthiophene
6	21.452	21.425	21.483	Hexadecanoic acid, 2-hydroxy-1- (hydroxymethyl) ethyl ester
7	12.279	12.242	12.317	Dodecanoic acid
8	17.073	17.033	17.117	Benzenepropanoic acid, 2, 5 - dimethoxy
15	15.044	14.900	15.083	2-Cyclohexen-1-One, 4- Hydroxy-3, 5, 5-Trimethyl -4-(3- Oxo
16	25.628	25.592	25.650	Retinol, acetate (CAS) Vitamin an acetate
17	25.679	25.650	25.717	Cholesta-6, 22, 24-triene, 4, 4- dimethyl
18	25.536	25.508	25.592	(E)-2-Methyl-4(2', 4', 4'- trimethylbicyclo[4.1.0] hept-2'-en3'-yl)-1, 3-butadi
9	23.486	23.458	23.525	9-Octadecenamide, (Z)- (CAS) Oleoamide
10	16.248	16.208	16.300	Pentadecanoic acid, 14-methyl-, methyl ester(CAS) Methyl14- Meth
11	14.458	14.417	14.500	4-((1E)-3-Hydroxy-1-propenyl)-2- methoxyphenol
12	14.869	14.833	14.900	(-)-Loliolide
13	23.792	23.758	23.833	2, 6, 10, 14, 18, 22- Tetracosahexaene, 2, 6, 10, 15, 19, 23hexamethyl-, (allE)-
14	25.918	25.892	25.942	-3Oxatricyclo[20.8.0.0E7, 16]Triconta1 (22), 7(16), 9, 13, 23, 29-
1	22.599	22.575	22.633	2-Thiophenecarbaldehyde, 5-[5- (thien-2-yl)thien-2-yl]-
2	18.706	18.675	18.725	Benzyl .beta.-d-glucoside
3	17.902	17.875	17.925	Octadeca-9, 12-Dienoic Acid Methyl Ester
4	15.520	15.492	15.558	2-Propenoic acid, 3-(4-hydroxy-3- methoxyphenyl)-, methyl ester
19	14.539	14.500	14.600	2-Propenoic acid, 3-(4- hydroxyphenyl)-, methyl ester (CAS) Methyl p-hy
23	16.627	16.575	16.683	Hexadecanoic acid
24	13.175	13.050	13.300	Quinic acid
25	27.759	27.700	27.833	3-(1, 5-Dimethyl-Hexyl)- 3A, 10, 10, 12B-Tetramethyl1, 2, 3, 3A
26	27.618	27.533	27.700	Zizanyl acetate
20	31.208	31.150	31.283	Periplogenin
21	27.410	27.367	27.450	-Ethyanyl-3, 5-dimethyladamantane
22	25.474	25.433	25.508	1-Ethyanyl-3, 5- dimethyladamantane

IN) (Tewtrakul S. *et al.*, 2007). Dasyscyphin-C (saponins) isolated compound from *E. prostrata* have anticancer-cytotoxic activity (Khanna. *et al.*, 2008) and was tested the same under in vitro conditions in HeLa (Human cervical carcinoma) & vero cell lines with concentration of 50g/ml showed a good anticancer-cytotoxic activity on HeLa cells (Mithun NM. *et al.*, 2011). The methanolic extract of *Eclipta prostrata* showed significant inhibitory activity on HSCs proliferation and reported the importance of free carboxylic acid at C-28 position *Bacillus subtilis*, and *Staphylococcus aureus* (Karthikumar. S. *et al.*, 2007).

We observed the presence of 26 components like 2-Thiophenecarbaldehyde, 5-[5- (thien-2-yl)thien-2-yl]-, Octadeca-9, 12-Dienoic Acid Methyl Ester, Benzyl .beta.-d-glucoside, 2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester, 2, 2': 5', 2''-Terthiophene, Hexadecanoic acid, 2-hydroxy-1-

(hydroxymethyl) ethylester, Dodecanoic acid, Benzenepropanoic acid, 2, 5-dimethoxy, 9-Octadecenamide, (Z)- (CAS) Oleoamide, Pentadecanoic acid, 14-methyl-, methyl ester (CAS) Methyl 14-Meth4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, (-)Loliolide, 2, 6, 10, 14, 18, 22-Tetracosahexaene, 2, 6, 10, 15, 19, 23-hexamethyl-, (allE), 3 Oxatricyclo [20.8.0.0E7, 16] Triconta-1(22), 7(16), 9, 13, 23, 29-H, 2-Cyclohexen-1-One, 4-Hydroxy3, 5, 5-Trimethyl-4-(3-OXO, Retinol, acetate (CAS) Vitamin a acetate, Cholesta-6, 22, 24-triene, 4, 4-dimethyl-(E)-2-Methyl-4 (2', 4', 4'-trimethylbicyclo [4.1.0]hept-2'-en-3'-yl)-1, 3-butadi, 2-Propenoic acid, 3-(4-hydroxyphenyl)-, methyl ester (CAS) Methyl p-hy, Periplogenin, -Ethyanyl-3, 5-dimethyladamantane, 1-Ethyanyl-3, 5- dimethyladamantane and Hexadecanoic acid, the components observed by GC-MS method in our study are in agreement with the previous literature.

Table 2: Gas chromatography-mass spectrometry Analysis - Phytochemical components of Eclipta alba root extract (Contd.....)

Molecular Formula	Peak Area%	Molecular Weight
C12H8S3	0.12	248
C19H304	0.13	330
C12 H24 O2	0.19	200
C11 H14 O4	0.23	210
C13 H18 O3	0.46	222
C22 H32 O2	0.66	328
C29H46	0.69	394
C15 H22	0.71	202
C18 H35 N O	0.29	281
C17 H34 O2	0.31	270
C10 H12 O3	0.36	180
C11 H16 O3	0.37	196
C30H50	0.41	410
H C29 H42 O	0.45	406
C13H8OS3	0.08	276
C13H18O6	0.09	270
C19 H34O2	0.10	294
C11 H12 O4	0.12	208
C10 H10 O3	0.76	178
C16 H32 O2	2.49	256
C7 H12 O6	2.64	192
C30 H50	7.22	410
C17 H26 O2	44.86	262
C23H34O5	1.30	390
C14H20	1.41	188
C14H20	2.07	188

CONCLUSION

The Eclipta Alba roots believed to exhibit the anti-biotic, antiobeisty and Hepatoprotective properties due to the presence of various secondary metabolites such as glycosides, phytosterols, alkaloids, oils, Saponins, phenols and Flavonoids. The roots of Eclipta Alba are rich in phytochemical constituents, and most of the components were present in aqueous and methanolic extracts of Roots. Our study highlights the positive role of Eclipta Alba root extracts with their Hepatoprotective and Antiobesity properties for fourth coming studies.

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