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Extraction of Bio-active compounds of *Eclipta Alba* through GC-MS Analysis.

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ABSTRACT

Eclipta alba is one of higher value herbs with long history of traditional and folk medicine for treatment of various ailments in many tropical and sub tropical countries. Medicinal plants have had a crucial role in human culture and civilization. The roots of the plant Eclipta Alba were collected, washed, shade dried and powdered. Methanol extract was prepared by simple soxhalation method. All the extracts were concentrated and analyzed using Gas Chromatography Mass Spectroscopy for the identification of biochemical components present in the root extract of Eclipta alba. A wide range of active ingredients such as 2-Thiophenecarbaldehyde, 5-[5-(thien-2-yl)thien-2-yl]- 0.08%, Dodecanoic acid - 0.19, 9-Octadecenamide, (Z)-(CAS) OLEOAMIDE - 0.29, Loliolide – 0.37 and– 44.86. This study supports, methanolic extract of *eclipta alba* has potential antimicrobial, antioxidant and hepatoprotective activities.

Keywords: Root extract of *Eclipta alba,* GC-MS analysis, 2 – Thiophenecarbaldehyde, 5-[5-(thien-2-yl)thien-2-yl], zizanyl acetate

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INTRODUCTION

Eclipta alba (Asteracea) a common weed of agriculture is one of the important medicinal herbs used in the traditional medicine of the East. This plant is considered rejuvenative Eclipta alba is also reported with antianaphylactic (1), antihyperglycemic (2) and hepatoprotecive (3) The genus name comes from the Greek word meaning “Deficient” with reference to the absence of the bristles and awns on the fruits and the specific Eclipta alba means white which refers to the flower’s colour (4). 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 important plant of Ayurvadic matria media (5). Its pharmacological activities viz., analgesic, antimicrobial, antiviral, antifungal, antinociceptive, anti-inflammatory, antioxidant, anti-hyperglycemic, hepatoprotective, immunomodulatory, hair growth and wound healing activities were summarized (6), (7) & (8) reported ethno medical uses of Eclipta.

Gas chromatography (GC) is a widely applied technique in many branches of science and technology. GC has played a fundamental role in determining how many components and in what proportion they exist in a mixture. The most used, is the mass spectrometric detector (MSD), which allows obtaining the "fingerprint" of the molecule, i.e., its mass spectrum. Mass spectra provide information on the molecular weight, elemental composition, if a high resolution mass spectrometer is used, functional groups present, and, in some cases, the geometry and spatial isomerism of the molecule (9).

MATERIALS AND METHODS

Collection and 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 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. Dried Eclipta Alba roots are charged to extractor along with Methanol. It is extracted by heating the mass for 5-6 hours, in a Soxhalate 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 c until assayed. It is packed in food grade, virgin, polythene bags. Fig;1

Fig 1: Eclipta Alba

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GC-MS Analysis: Sample preparation: About 1 g of sample was taken in 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: 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, Ionisation mode: Electron impact ionization(EI), Ionisation energy: 70 eV, Mass library: NIST 5 and WILEY, at Vittal Mallya Scientific Research Foundation, Bangalore,

RESULTS AND DISCUSSION

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 (Fig.2) of roots of *E. alba* analysis showed the presence of 26 Chemical compounds table:1

Fig 2: GC-MS chromatogram

Table 1: Phytochemical components of Eclipta alba root extract by GC-MS Analysis

Peak	R. Time	I. Time	F.Time	Name of the Compound	Molecular Formula	Peak Area%	Molecular Weight
1	22.599	22.575	22.633	2-Thiophenecarbaldehyde, 5-[5-(thien-2-yl)thien-2-yl]-	C13H8OS3	0.08	276
2	18.706	18.675	18.725	Benzyl .beta.-d-glucoside	C13H18O6	0.09	270
3	17.902	17.875	17.925	OCTADECA-9,12-DIENOIC ACID METHYL ESTER	C19 H34O2	0.10	294
4	15.520	15.492	15.558	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester	C11 H12 O4	0.12	208
5	19.284	19.250	19.317	2,2':5',2''-Terthiophene	C12H8S3	0.12	248
6	21.452	21.425	21.483	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H3O4	0.13	330
7	12.279	12.242	12.317	Dodecanoic acid	C12 H24 O2	0.19	200
8	17.073	17.033	17.117	Benzenepropanoic acid, 2,5-	C11 H14 O4	0.23	210

				dimethoxy			
9	23.486	23.458	23.525	9-Octadecenamide, (Z)- (CAS) OLEOAMIDE	C18 H35 N O	0.29	281
10	16.248	16.208	16.300	Pentadecanoic acid, 14-methyl-, methyl ester (CAS) METHYL 14-METH	C17 H34 O2	0.31	270
11	14.458	14.417	14.500	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	C10 H12 O3	0.36	180
12	14.869	14.833	14.900	(-)-Loliolide	C11 H16 O3	0.37	196
13	23.792	23.758	23.833	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	C30H50	0.41	410
14	25.918	25.892	25.942	3-OXATRICYCLO[20.8.0.0E7,16]TRICONTA-1(22),7(16),9,13,23,29-H	C29 H42 O	0.45	406
15	15.044	14.900	15.083	2-CYCLOHEXEN-1-ONE, 4-HYDROXY-3,5,5-TRIMETHYL-4-(3-OXO	C13 H18 O3	0.46	222
16	25.628	25.592	25.650	Retinol, acetate (CAS) Vitamin a acetate	C22 H32 O2	0.66	328
17	25.679	25.650	25.717	Cholesta-6,22,24-triene, 4,4-dimethyl-	C29H46	0.69	394
18	25.536	25.508	25.592	(E)-2-Methyl-4(2',4',4'-trimethylbicyclo[4.1.0]hept-2'-en-3'-yl)-1,3-butadi	C15 H22	0.71	202
19	14.539	14.500	14.600	2-Propenoic acid, 3-(4-hydroxyphenyl)-, methyl ester (CAS) Methyl p-hy	C10 H10 O3	0.76	178
20	31.208	31.150	31.283	Periplogenin	C23H34O5	1.30	390
21	27.410	27.367	27.450	-Ethynyl-3,5-dimethyladamantane	C14H20	1.41	188
22	25.474	25.433	25.508	1-Ethynyl-3,5-dimethyladamantane	C14H20	2.07	188
23	16.627	16.575	16.683	Hexadecanoic acid	C16 H32 O2	2.49	256
24	13.175	13.050	13.300	QUINIC ACID	C7 H12 O6	2.64	192
25	27.759	27.700	27.833	3-(1,5-DIMETHYL-HEXYL)-3A,10,10,12B-TETRAMETHYL-1,2,3,3A	C30 H50	7.22	410
26	27.618	27.533	27.700	zizanyl acetate	C17 H26 O2	44.86	262

DISCUSSION

There is an increasing interest in the phytochemical compounds, which could be relevant to their nutritional incidence and their role in health and disease 10. In recent years, the interest for the study of the organic compounds from plants and their activity has increased. The aim of the present study was to develop a rapid method for the phytochemicals present in the plant extracts and quantitative determination of organic compounds in plant. Diversity of medicinal plants and herbs containing various phytochemicals with biological activity can be of valuable therapeutic key 11. Phytochemical constituents are responsible for medicinal activity of plant species12. The Previous studies reported that the GC-MS analysis of *Eclipta alba* using methanol divulged the presence of eight possible bio active compounds 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.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-Octadeconic acid, methyl ester, Pentadecanic acid,14methyl,methyl ester, Diethyl Phthalate which are divergent. 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 13. *E. prostrata* reported to contain many 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 and earlier authors claimed that the water extract of *E. prostrata* (whole plant) exhibited the most potent inhibitory activity against antiviral activity HIV-1 integrase (HIV-1 IN) 14. Dasyscyphin-C (saponins) a newer isolated compound from *E. prostrata* reported to have anticancer-cytotoxic activity 15. It was tested under *in vitro* conditions in HeLa (Human cervical carcinoma) & vero cell lines. At the concentration of 50g/ml it showed a good anticancer-cytotoxic activity on HeLa cells 16. A rat hepatic stellate cell line (HSCs) was used as *in vitro* assay system, the methanolic extract of aerial parts of *Eclipta prostrata* showed significant inhibitory activity on HSCs proliferation. It has been reported that the importance of free carboxylic acid at C-28 position *Bacillus subtilis*, and *Staphylococcus aureus* 17.

In the present study we observed the presence of 26 componants 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)ethyl ester, 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-,(all-E)-,3OXATRICYCLO[20.8.0.0E7,16]TRICONTA-1(22),7(16),9,13,23,29-H, 2-CYCLOHEXEN-1-ONE, 4-HYDROXY-3,5,5-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, -Ethynyl-3,5-dimethyladamantane, 1-Ethynyl-3,5-dimethyladamantane and Hexadecanoic acid, our study not in agreement with the previous literature.

In conclusion the presence of various bioactive compounds justifies the use of the root 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

CONCLUSION

The presence of various secondary metabolites such as glycosides, phytosterols, alkaloids, oils, Saponins, phenols and Flavanoids were believed to exhibit the antibiotic, antiobeisty and Hepatoprotective properties of *Eclipta Alba roots*. On the basis of the results obtained, the present work conclude that the roots of Eclipta Alba are rich in phytochemical constituents even though the phytochemical screening of the root extracts of samples had shown variation in their phytochemical constituents with the presence and or absence of some components. Most components were present in aqueous and methanolic extracts of Roots. The present work highlights the possible use of *Eclipta Alba* root extracts as a source of Hepatoprotective activity and Antiobeisty properties.

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