Research Article

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

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ABSTRACT

Since ancient times aromatic plants had not only been used to impart flavour and aroma to food but also for their medicinal & preservative properties. *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 roots of *Eclipta alba* using Gas Chromatography – Mass Spectrometry analysis (GC-MS). The phytochemical compositions of methanol extract of plant root were investigated. Using Perkin – Elmer GC-MS while the mass spectra of the compounds found in the root extract was matched with the National Institute of Standards and Technology library and WILEY. The Methonolic extract of plant root was revealed the presence of 51 medicinally important bioactive compounds among those 49, 25, 50, 16, 18, 27, 34, 43, 2 &7 - zizanyl acetate –showed highest peak of 44.86%, followed by D-Allose - 10.11%, 3-(1,5-DIMETHYL-HEXYL)-3A,10,10,12B-TETRAMETHYL-1,2,3,3A-7.22%,1,2-Benzenediol(CAS) Pyrocatechol- 2.93%, 2-Furancarboxaldehyde, 5-(hydroxymethyl)-2.86 %, QUINIC ACID - 2.64%, Hexadecanoic acid - 2.49%, 2-Cyclopenten-1-one, 2-hydroxy - 2.09%, 1-Ethynyl-3,5-dimethyladamantane - 2.07%. Pentanoic acid, 4-oxo- (CAS) Levulinicacid - 2.00% identified during analysis. 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, - zizanyl acetate, 4-oxo- (CAS) Levulinic acid.

INTRODUCTION

The Indian traditional medicine like Ayurveda, Siddha which is largely therapeutic nature has a rich heritage and history.[1] 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[2]. In the health of an individual and the communities medicinal plants are playing major role. The medicinal value of these plants lies in the Phytochemicals, which produce a physiological action on the human body. Phytochemicals are naturally occurring biochemical compounds inplants for colour, flavour, smell and texture for pollination and define mechanism[3].

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[4]. *Eclipta alba* is widely used in India as a cholagogue and deobstruent in hepatic enlargement[5]. *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[6]. *Eclipta* is traditionally used for blackening, promoting hair growth and strengthening the hair. The constituents are playing a significant role in the identification of crude drugs[7]. Therefore, proper scientific knowledge is required to investigate and explore the exact standardization of such medicinally important plant.

Gas chromatographic-mass spectrometry (GC-MS) is a very powerful and ubiquitous analytical technique used for direct analysis of components existing in medicinal plants[8]. Not only can a GC-MS separate the volatile components of complex mixtures, but it can also record a mass spectrum of each component. Methanolic solvent is used to procure the phytocomponents due to high polarity (Molecules with permanent dipoles). Hence, the objective of the present study is to identify the phytochemical constituents of methonolic root extracts of *E.alba* with the aid of GC-MS technique.[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 Fig;1. This process is repeated. The extracts are combined and filtered. Then concentrated this is charged to drier unit to dry and separated the product in a powder form. It is packed in food grade, virgin, polythene bags.

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	temperature	Hold time
	60 °C	0 min
10 °C/min	330 °C	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, India.

Figure 1: *Eclipta alba*

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 of roots of *E. alba* analysis showed the presence of 51 Chemical compounds table: 1

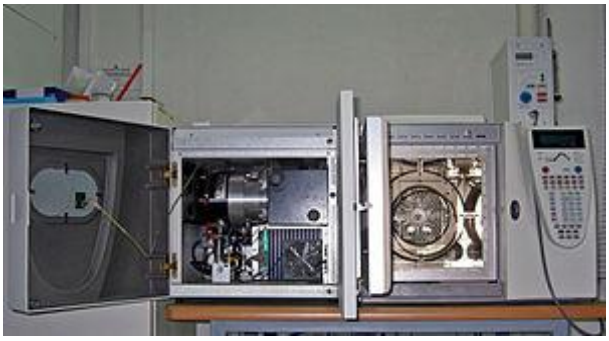

Figure 2: GC-MS chromatogram

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-(Trimethyl silyl) Propargyl)-1,4-Naphthoquinone,L Alanine, Ethyl ester-,Formamide,N-[(dibutylamino)methyl]-N-methyl, Trans-2-((phenylthio)methyl)-1-(2-propenyl)-1,2,3,4 tetra hydronaphthalene, 2-Acetonyl-3-cyano-2,3-dimethyl cyclobutane-1-carboxylic acid,5,5'-dicarboxy-3'-(2-chloro ethyl) -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[14-16]. 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

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.

S.NO	R. Time	I. Time	F.Time	Name of the Compound	Molecular Formula	Peak Area	Molecular Weight
1	3.760	3.692	3.833	1H-Imidazole, 4,5-dihydro-2-methyl-	C4H8N2	1.53	84
2	3.875	3.833	3.958	2-Cyclopenten-1-one, 2-hydroxy	C5H8O	2.09	98
3	4.530	4.483	4.575	Phenol (CAS) Izal	C6H6O	1.02	94
4	4.700	4.575	4.733	2-Hydroxy-gamma-butyrolactone	C4H6O3	1.01	102
5	5.381	5.342	5.417	N,N'-Dimethylpiperazine	C6H14N2	0.40	114
6	5.450	5.417	5.483	Acetic acid, pentyl ester (CAS) n-Amyl acetate	C7H14O2	0.32	130
7	5.582	5.483	5.608	Pentanoic acid, 4-oxo- (CAS) Levulinic acid	C5H8O3	2.00	116
8	5.633	5.608	5.683	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	C6H8O3	0.35	128
9	5.926	5.883	5.958	Cyclopentane, 1-acetyl-1,2-epoxy-	C7H10 O2	0.77	126
10	6.067	5.958	6.108	Phenol, 2-methoxy- (CAS) Guaiacol	C7H8O2	0.30	124
11	6.139	6.108	6.192	Cyclopropylcarbinol	C4H8O	0.87	72
12	6.411	6.375	6.458	4H-Pyran-4-one, 3-hydroxy-2-methyl- (CAS) Maltol	C6H6O3	0.36	126
13	6.564	6.542	6.592	2(3H)-Furanone, 5-acetyldihydro-	C6H8O3	0.09	128
14	6.711	6.675	6.758	Pentanoic acid, 4-oxo- (CAS) Levulinic acid	C5H8O3	0.33	116
15	6.851	6.808	6.892	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	C6H8O4	1.15	144
16	7.638	7.583	7.725	1,2-Benzenediol (CAS) Pyrocatechol	C6H6O2	2.93	110
17	7.858	7.725	7.908	2,3-DIHYDRO-BENZOFURAN	C8H8O	0.92	120
18	8.000	7.908	8.083	2-Furancarboxaldehyde, 5-(hydroxymethyl	C6 H6 O3	2.86	126
19	9.233	9.192	9.283	Phenol, 4-ethenyl-2-methoxy-	C9 H10 O2	0.27	150
20	9.710	9.675	9.750	Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol	C8 H10 O3	0.34	154
21	10.291	10.250	10.325	-Pentenoic acid, 4-methyl-3-4methylene-, isopropyl ester	C10 H16 O2	0.14	168
22	10.919	10.883	11.000	Phenol, 2,3,5-trimethyl- (CAS) 2,3,5-Trimethylphenol	C9 H12 O	0.65	136
23	11.037	11.008	11.058	2-Hexene, 2-methyl- (CAS) 2-Methyl-2-hexene	C7H14	0.24	98
24	11.449	11.417	11.483	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	C13 H18 O	0.42	190
25	11.737	11.492	11.950	D-Allose	C6 H12 O6	10.11	180
26	12.279	12.242	12.317	Dodecanoic acid	C12 H24 O2	0.19	200
27	13.175	13.050	13.300	QUINIC ACID	C7 H12 O6	2.64	192
28	14.458	14.417	14.500	4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol	C10 H12 O3	0.36	180
29	14.539	14.500	14.600	2-Propenoic acid, 3-(4-hydroxyphenyl)-, methyl ester (CAS) Methyl p-hy	C10 H10 O3	0.76	178
30	14.869	14.833	14.900	(-)-Loliolide	C11 H16 O3	0.37	196
31	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

32	15.520	15.492	15.558	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester	C11 H12 O4	0.12	208
33	16.248	16.208	16.300	Pentadecanoic acid, 14-methyl-, methyl ester (CAS) METHYL 14-METH	C17 H34 O2	0.31	270
34	16.627	16.575	16.683	Hexadecanoic acid	C16 H32 O2	2.49	256
35	17.073	17.033	17.117	Benzenepropanoic acid, 2,5-dimethoxy	C11 H14 O4	0.23	210
36	17.902	17.875	17.925	OCTADECA-9,12-DIENOIC ACID METHYL ESTER	C19 H34O2	0.10	294
37	18.706	18.675	18.725	Benzyl .beta.-d-glucoside	C13H18O6	0.09	270
38	19.284	19.250	19.317	2,2':5',2''-Terthiophene	C12H8S3	0.12	248
39	21.452	21.425	21.483	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	C19H3O4	0.13	330
40	22.599	22.575	22.633	2-Thiophenecarbaldehyde, 5-[5-(thien-2-yl)thien-2-yl]-	C13H8OS3	0.08	276
41	23.486	23.458	23.525	9-Octadecenamide, (Z)- (CAS) OLEOAMIDE	C18 H35 N O	0.29	281
42	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
43	25.474	25.433	25.508	1-Ethynyl-3,5-dimethyladamantane	C14H20	2.07	188
44	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
45	25.628	25.592	25.650	Retinol, acetate (CAS) Vitamin a acetate	C22 H32 O2	0.66	328
46	25.679	25.650	25.717	Cholesta-6,22,24-triene, 4,4-dimethyl-	C29H46	0.69	394
47	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
48	27.410	27.367	27.450	-Ethynyl-3,5-dimethyladamantane	C14H20	1.41	188
49	27.618	27.533	27.700	zizanyl acetate	C17 H26 O2	44.86	262
50	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
51	31.208	31.150	31.283	Periplogenin	C23H34O5	1.30	390
						100.00	

CONCLUSION

Medicinal plants were the potent source of human health due to the presence of active phytochemical compounds that are responsible for its various pharmacological Activities. 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 presence of various secondary metabolites such as glycosides, phytosterols, alkaloids, oils, Saponins, phenols and Flavonoids were believed to exhibit the antibiotic, anti-obesity and Hepatoprotective properties of *Eclipta alba roots*. The present work highlights the possible use of *Eclipta Alba* root extracts as a source of Hepato-protective activity and Anti-obesity properties.

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