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Findings of Gas chromatography - Mass Spectrometry (GCMA) Analysis of Eclipta Alba Plant Root

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Abstract :
Background*: Eclipta alba L. Hassk. (Asteraceae), commonly known as Bhringaraja (Sanskrit), Maka (Marathi) and Bhangra (Hindi) has been reported to show protective effect on experimental liver damage in rats and mice. This study was designed to investigate the phytochemical compositions of methanol extract of Eclipta Alba root through GCMS analysis.*
Material and methods*: 5 grams of plant material was extracted with 250 ml 99% methanol. For methonolic extract dried powder samples were extracted in a Soxhlet apparatus with methanol until becoming colourless. 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.*
Results*: In the Methonolic extract of Eclipta alba plant root, we observed the presence of 25 medicinally important bioactive compounds among those 13,21,19,12,17,1,16 & 25 - showed lowest peak of - 2(3H)-Furanone, 5-acetyldihydro-**0.09%,** Pentenoic acid, 4-methyl-3-4methylene-, isopropyl ester - **0.14%,** Phenol, 4-ethenyl-2-methoxy-**0.27%,** 4H-Pyran-4-one, 3-hydroxy-2-methyl- (CAS) Maltol - **0.36,** 2,3-DIHYDRO-BENZOFURAN - **0.92%,** 1H-Imidazole, 4,5-dihydro-2-methyl- **1.53%,** 1,2-Benzenediol (CAS) Pyrocatechol - **2.93%** and D-Allose - **10.11** identified during analysis.*
Conclusions*: The presence of various secondary metabolites such as glycosides, alkaloids, Saponins, and Flavanoids are believed to exhibit the, antiobeisty and Hepatoprotective properties of Eclipta Alba roots.*
Keywords: *Bhringraj (Eclipta Alba), GC-MS analysis, 2(3H)-Furanone, 5-acetyldihydro and D-Allose*

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I. Introduction

Eclipta alba L. Hassk. (Asteraceae), commonly known as Bhringaraja (Sanskrit), Maka (Marathi) and Bhangra (Hindi) has been reported to show protective effect on experimental liver damage in rats and mice [1]. The plant will be used for the treatment of liver cirrhosis and infective hepatitis [2]. The medicinal properties of plants have been investigated in the recent scientific studies throughout the world, because of their potent antioxidant activities, there will be no side effects and economic viability [3]. In Ayurveda, extract of the root powder is used for treating hepatitis, and enlarged spleen disorders. In most of the parts of India, it grows commercially as a medicinal crop. It is an annual, erect or prostate entirely pubescent herb, often rooting at nodes with opposite, sessile, usually oblong, 2.5–7.5 cm long leaves with appressed hairs. Floral heads 6–8 mm in diameter, solitary, white; achene compressed and narrowly winged. The aerial parts of the plant are used in medicine like Ayurveda, Siddha.

In another study, the areal parts and root of alcoholic extraction exhibited significant Hepatoprotective activity against CCl4-induced liver injury in rats. In yet another study, treatment with 50% ethanol extract of E.alba (100 & 250mg/100g body weight) was found to protect the mice from hepatotoxic action of paracetamol as evidenced by significant reduction in the elevated serum transaminase levels [4].

The total alcoholic extract of E. prostrata exhibited a dose-dependent activity in albino rats when compared to standard drugs. The activity was assessed by studying the lipid profiles of serum, liver and heart of the control and drug-treated animals Two studies reported efficacy of E.alba in the treatment of infective hepatitis in adults and jaundice in children, respectively [5-7]. 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 [8]. Phytochemicals are naturally occurring biochemical compounds in plants for colour, flavour, smell and texture for pollination and define mechanism [9]. The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950s by Roland Gohlke and Fred. The development of affordable and miniaturized computers has helped in the simplification of the use of this instrument, as well as allowed great improvements in the amount of time it takes to analyze a sample. In 1996 the top-of-the-line high-speed GC-MS units completed analysis of fire accelerants in less than 90 seconds, whereas first-generation GC-MS would have required at least 16 minutes. This has led to their widespread adoption in a number of fields. In this context, our study was aimed to investigate the phytochemical compositions of methanol extract of Eclipta Alba root through GCMS analysis.

II. Material And Methods

Collection and extraction of plant materials

Fresh plant parts (Including roots) collected from adjoining village area of Chitradurga city and taxonomic identity of the plant was confirmed in the Department of Botany, Govt Science College, Davangere University, and Chitradurga. The mature plants were washed thoroughly with running tap water, then with deionised water (Fig 1). Plant material (Roots) was extracted with 250 ml (1:50, w/v) of solvent; 99% methanol. For methonolic extract dried powder samples were extracted in a Soxhlet apparatus for 5 – 6 hrs, until becoming colourless. The extract was filtered. The filtrate was 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.

GC-MS Analysis:

Sample preparation: About 1 g of Eclipta alba root extract 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 (Fig 2).

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.

III. 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 roots of E. alba analysis showed the presence of 25 Chemical compounds Table 1.

IV. Discussion

The use of a mass spectrometer as the detector in gas chromatography was developed during the 1950s by Roland Gohlke and Fred. 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 the phytochemical analysis of the different alcohol and aqueous plant extract of Eclipta alba mostly contained higher quantities of glycosides, followed by flavonoids and alkaloids. Alkaloids and tannins were entirely absent in most of the tested aqueous extracts. The Phytochemical screening of the alcohol and aqueous extracts of Tylophora indica revealed the presence of glycosides, alkaloids, flavanoids and tannins in all the plant extracts studied. [11].

The metabolites are of various pharmacological im-portance. The presence of tannin in most of plant extract could be responsible for possible antitumor and anti oxidant activities [12]. Thenmozhi et al., (2011) identified the phytochemicals present in methanol extracts of Eclipta alba.

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. 1-Heptatriacotanol is an alcoholic compound which showed antimicrobial activity.

Previous studies reported that the phytochemical studies of E. alba using methanol solvent yielded eleven bio active compounds, which are The active compounds shows 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 IS reported to contain anti-inflammatory, hepatoprotecive, cancer preventive, dermatitigenic, hypocholesterolemic properties [13].

In our Research we foud the presence of 25 phytochemical compounds, which are 2(3H)-Furanone, 5-acetyldihydro-, -Pentenoic acid, 4-methyl-3-4methylene-, isopropyl ester, 2-Hexene, 2-methyl- (CAS) 2-Methyl-2-hexene, Phenol, 4-ethenyl-2-methoxy-, Phenol, 2-methoxy- (CAS) Guaiacol, Acetic acid, pentyl ester (CAS) n-Amyl acetate, Pentanoic acid, 4-oxo- (CAS) Levulinic acid, Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol, 2,5-Dimethyl-4-hydroxy-3(2H)-furanone, 4H-Pyran-4-one, 3-hydroxy-2-methyl- (CAS) Maltol, N,N'-Dimethylpiperazine, 4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one, Phenol, 2,3,5-trimethyl- (CAS) 2,3,5-Trimethylphenol, Cyclopentane, 1-acetyl-1,2-epoxy-, Cyclopropyl carbinol, 2,3-DIHYDRO-BENZOFURAN, 2-Hydroxy-gamma-butyrolactone, Phenol (CAS) Izal, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, 1H-Imidazole, 4,5-dihydro-2-methyl-, Pentanoic acid, 4-oxo- (CAS) Levulinic acid, 2-Cyclopenten-1-one, 2-hydroxy, 2-Furancarboxaldehyde, 5-(hydroxymethyl, 1,2-Benzenediol (CAS) Pyrocatechol and D-Allose.

Through our study, the presence of various phytochemical compounds justifies the use of the E. alba root 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.

V. Conclusions

The presence of various bioactive compounds justifies the use of the plant leaves for various ailments by traditional practitioners. On the basis of the results obtained, the present work conclude that the roots of Eclipta Alba are also 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 flavanoids are believed to exhibit the antibiotic, antiobeisty and hepatoprotective properties in Eclipta Alba roots. The present work highlights the potential use of Eclipta Alba root extracts as a medicine with hepatoprotective and antiobesity properties.

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Figure 1: Eclipta alba plant

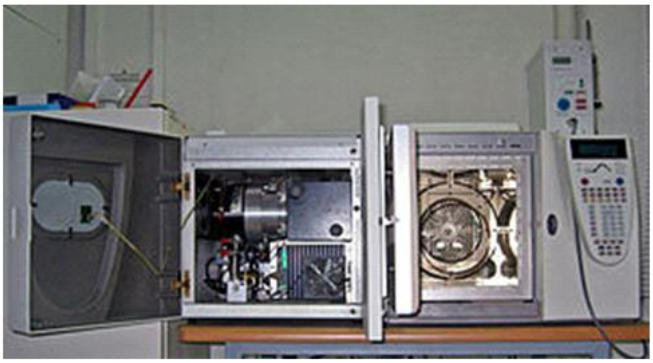

Figure 2: GCMS analysis equipment

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

Peak	R. Time	I. Time	F.Time	Name of the Compound	Peak Area%	Molecular Weight
13	6.564	6.542	6.592	2(3H)-Furanone, 5-acetyldihydro- trioxide	0.09	128
21	10.291	10.250	10.325	-Pentenoic acid, 4-methyl-3-4methylene-, isopropyl ester	0.14	168
23	11.037	11.008	11.058	2-Hexene, 2-methyl- (CAS) 2-Methyl-2-hexene	0.24	98
19	9.233	9.192	9.283	Phenol, 4-ethenyl-2-methoxy-	0.27	150
10	6.067	5.958	6.108	Phenol, 2-methoxy- (CAS) Guaiacol	0.30	124
6	5.450	5.417	5.483	Acetic acid, pentyl ester (CAS) n-Amyl acetate	0.32	130
14	6.711	6.675	6.758	Pentanoic acid, 4-oxo- (CAS) Levulinic acid	0.33	116
20	9.710	9.675	9.750	Phenol, 2,6-dimethoxy- (CAS) 2,6-Dimethoxyphenol	0.34	154
8	5.633	5.608	5.683	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	0.35	128
12	6.411	6.375	6.458	4H-Pyran-4-one, 3-hydroxy-2-methyl- (CAS) Maltol	0.36	126
5	5.381	5.342	5.417	N,N'-Dimethylpiperazine	0.40	114
24	11.449	11.417	11.483	4-(2,6,6-Trimethylcyclohexa-1,3-dienyl)but-3-en-2-one	0.42	190
22	10.919	10.883	11.000	Phenol, 2,3,5-trimethyl- (CAS) 2,3,5-Trimethylphenol	0.65	136
9	5.926	5.883	5.958	Cyclopentane, 1-acetyl-1,2-epoxy-	0.77	126
11	6.139	6.108	6.192	Cyclopropyl carbinol	0.87	72
17	7.858	7.725	7.908	2,3-DIHYDRO-BENZOFURAN	0.92	120
4	4.700	4.575	4.733	2-Hydroxy-gamma-butyrolactone	1.01	102
3	4.530	4.483	4.575	Phenol (CAS) Izal	1.02	94
15	6.851	6.808	6.892	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	1.15	144
1	3.760	3.692	3.833	1H-Imidazole, 4,5-dihydro-2-methyl-	1.53	84

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