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Phytochemical Analysis of *Eclipta prostrata* L. (L.) Leaves

K. Priya[1*], Preethy John[1], P.T.A. Usha[1], B.J. Kariyil[1], R. Uma[2] and M.S. Hogale[1]

[1]Department of Veterinary Pharmacology and Toxicology,
[2]Department of Veterinary Biochemistry, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India

**Corresponding author*

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A B S T R A C T

Eclipta prostrata (L.) belonging to Asteraceae plant family possesses antihepatotoxic, antipyretic, anti-inflammatory, antibacterial, anthelmintic, insecticidal, antihaemorrhagic, antihyperglycaemic, antimytotoxic, antioxidant and immunomodulatory properties. In the present study the phytochemical screening of *Eclipta prostrata* (L.) leaves through qualitative phytochemical test, Fourier- transform infrared spectroscopy (FTIR) analysis and Gas chromatography- mass spectrometry (GC-MS) analysis was done. The methanolic extract of leaves of *Eclipta prostrata* (L.) was used for GC- MS analysis. The leaf powder prepared was used for qualitative phytochemical test and FTIR analysis. The qualitative phytochemical test revealed the presence of steroids, tannins, saponins, flavonoids, diterpenes and triterpenes. The FTIR analysis showed the presence of structurally similar compounds like L (-)-glyceraldehyde unnatural form, heptyl-beta-d-glucopyranoside, tomatine, b-cyclodextrin, chitin, octyl-beta-d-glucopyranoside, streptomycin sulphate, digitonin, pectin ex apples, a-cyclodextrin. The GC-MS analysis exposed the compounds Propanedinitrile dimethyl (0.59%), Pentadecane (2.09%), Heptadecane (9.84%), Neophytadiene (20.80%), 1, 3- Propanediol 2- hydroxymethyl (1.15%), Citronellyl butyrate (1.94%), Citronellyl propionate (5.12%), Heptadecanoic acid, methyl ester(2.31%), 1-Allyloxyl- octa- 2,7- diene (0.44%), 6(E), 9(Z), 13(E)- Pendectriene (1.59 %), Phytol (28.72 %), Pentanoic acid, 4- Methyl (0.07 %), Ethanamine, 2, 2'- Oxybsin [N, N- Dimethyl (1.85%), 2 Methoxysulpholane (1.82 %), Cyclopropane, Methoxymethylene(0.64%), Ethyl- 2- Oxo- 4- Methyl- 3- Pentenoate (0.68%), Squalene (19.18%), Butanoic acid, 4-(Ethoxyhydroxyphosphinyl) (0.35%) and dl- alpha- Tocopherol (1.03%).

Introduction

The phytochemical constituents are mainly secondary metabolites secreted by all plants in small quantities. These compounds play a significant role in survival of the plants under harmful conditions and also in the protection from microbes. Research around the globe has proved that the phytochemicals from the plants possess various medicinal properties. Hence the phytochemical screening is necessary to find out the bioactive profile of plants having therapeutic significance.

The Asteraceae is the largest flowering plant family in the world with 24000 - 30000 accepted species and 1600 - 1700 genera, cosmopolitan in distribution except in Antarctica (Funk *et al.,* 2005). *Eclipta prostrata* L. (L.) commonly known as false daisy and bhringaraj belonging to this family is well known for its hair growth promoting capacity. This herb is also reported to have antihepatotoxic, antihyperglycaemic, immunomodulatory antipyretic, antioxidant, antibacterial, anthelmintic, antimytotoxic, anti-inflammatory, antihaemorrhagic properties (Mithun *et al.,* 2011). Literatures mentioning bioactive profile of this plant of Kerala origin were few. Hence this study was conducted to find out the active principles present in the *E. prostrata* leaves collected from Kerala.

Materials and Methods

Collection of plants and authentication

Eclipta prostrata L. whole plant was procured from Thrissur and authenticated at Botanical Survey of India, Coimbatore. The leaves were collected; shade dried and pulverized using an electrical pulveriser. About 50 g powder was taken and extracted with methanol using accelerated solvent extractor (Dionex ASE 150, Thermoscientific). The methanolic extract was then concentrated using a rotary vacuum evaporator under reduced pressure and temperature and stored under refrigeration (4ºC) until further use.

Phytochemical screening

The leaves were tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins (Harborne, 1991).

Fourier transform infrared (FTIR) spectroscopy

Functional groups present in *E. prostrata* leaves were identified using Fourier transform infrared (FTIR) spectroscopy (Perkin Elmer, FTIR spectrophotometer) as described by Swapna *et al.,* (2012). About 2.0 mg of *E. prostrata* leaves powder and 298 mg of dry fine powder of potassium bromide (KBr) were mixed well using mortar and pestle. The KBr- sample mixture was transferred to an evacuable die that has a barrel diameter of 13 mm and the die was pressed at around 8 to 10 tons for 1-2 min in a hydraulic hand press. Re-crystallization of the KBr results in a clear transparent disk about one millimetre thick and the infrared spectrum was recorded in the scan range from 4000 cm^{-1} to 400 cm^{-1} on FTIR spectrophotometer with a resolution of 0.5 cm^{-1}. The structurally related compounds were identified through Fluka library supplied by Perkin-Elmer.

GC-MS analysis

The GC-MS analysis of the extract was carried out at Kerala Forest Research Institute, Peechi, Thrissur using Shimadzu GCMS Model Number: QP2010S. It was performed as per the protocol described by Anand *et al.,* (2014) with minor modifications.

The compounds were separated on Rxi-5Sil MS capillary column (30 m × 0.25 mm; i.d., 0.25 µm film). The sample dissolved in methanol, filtered in 0.22-micron syringe filter, was used for analysis. The column oven temperature was programmed from an initial temperature of 80ºC (4 min), then temperature raised to 280ºC at the rate of 5ºC min-1, finally 280ºC was maintained isothermally with a final time of 6 min. The injection temperature and ion source temperature were 260ºC and 200ºC, respectively. Helium (99.999%) was used as the carrier gas with a

flow rate of 1 mL min-1. The ionizing energy was 70 eV. All data were obtained by collecting the full-scan mass spectra within the scan range 50–500 amu. Compounds were identified using the National Institute of Standards and Technology (NIST 11) and WILEY 8 library.

Results and Discussion

On phytochemical screening of *E. prostrata* leaves, the presence of steroids, tannins, saponins, flavonoids, diterpenes and triterpenes were detected. This result was in accordance with the findings of Arunachalam *et al.,* (2009). But a few researchers (Dhandapani, *et al.,* 2007; Kumari *et al.,* 2006) reported the presence of steroids, phenols, reducing sugars and carbohydrates in ethanolic and aqueous extract. The differences may be due to solvent capacity to extract the active principles, difference in the extraction methods and collection time.

Fourier transform infrared spectroscopy analysis showed the presence of structurally similar compounds in the leaf powder of *E. prostrata*. The compounds identified were L (-)-glyceraldehyde unnatural form, Heptyl-beta-d-glucopyranoside, Tomatine, b-Cyclodextrin, Chitin, Octyl-beta-d-glucopyranoside, Streptomycin sulphate, Digitonin, Pectin ex apples, a-Cyclodextrin.
There were about 19 active principles found in the methanolic extract of *E. prostrata* L. (L.) leaves through GC- MS analysis. Wide range of alcohols, fatty acids, esters, diterpenoids, triterpenoids and many other acyclic alkanes were found in the extract like propanedinitrile dimethyl (0.59%), pentadecane (2.09%), heptadecane (9.84%), neophytadiene (20.80%), 1, 3- propanediol 2-hydroxymethyl (1.15%), citr1llyl butyrate (1.94%), citr1llyl propionate (5.12%), heptadecanoic acid, methyl ester (2.31%), 1-allyloxyl- octa- 2,7- diene (0.44%), 6(E), 9(Z), 13(E)- pendectriene (1.59 %), phytol (28.72%), pentanoic acid, 4- methyl (0.07 %), ethanamine, 2, 2'- oxybsin [N, N- dimethyl (1.85%), 2 methoxysulpholane (1.82%), cyclopropane, methoxymethylen e(0.64%), ethyl- 2- Oxo- 4- methyl- 3- pentenoate (0.68%), squalene (19.18%), butanoic acid, 4-(Ethoxyhydroxyphosphinyl) (0.35%) and dl-alpha- tocopherol (1.03%). This result was varied from the results of Wyson *et al.,* (2016) who reported the presence of 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,14 methyl, methyl ester, diethyl Phthalate. Lin *et al.,* (2010) reported that heptadecane, pentadecane, phytol was presented in the aerial parts of *E. prostrate* (Table 1–3; Fig. 1 and 2).

Table.1 Qualitative test of *E.prostrata* leaves

Active principle	Result
Steroids	Present
Alkaloids	Absent
Phenolic compounds	Absent
Tannins	Present
Flavonoids	Present
Glycosides	Absent
Diterpenes	Present
Triterpenes	Present
Saponins	Present

Table.2 FTIR analysis of *E. prostrata* leaves powder

Search Score	Structurally related compounds
0.629568	l(-)-glyceraldehyde unnatural form
0.607445	heptyl-beta-d-glucopyranoside
0.604807	Tomatine
0.598818	b-cyclodextrin
0.591086	Chitin
0.574454	octyl-beta-d-glucopyranoside
0.543647	streptomycin sulphate
0.516638	Digitonin
0.51234	pectin ex apples
0.506237	a-cyclodextrin

Table.3 GC-MS analysis of methanolic extract of *E. prostrata* leaves

Peak	**Retention time**	**Area %**	**Name**
1	17.974	0.59	Propanedinitrile, dimethyl
2	19.047	2.09	Pentadecane
3	23.780	9.84	Heptadecane
4	26.716	20.80	Neophytadiene
5	26.806	1.15	1,3- Propanediol, 2- hydroxymethyl
6	27.196	1.94	Citr1llyl butyrate
7	27.578	5.12	Citr1llyl propionate
8	28.496	2.31	Heptadecanoic acid, methyl ester
9	31.702	0.44	1- Allyloxyl- octa- 2,7- diene
10	31.815	1.59	6(E), 9(Z), 13(E)- Pendectriene
11	32.093	28.72	Phytol
12	32.340	0.07	Pentanoic acid, 4- Methyl
13	35.128	1.85	Ethanamine, 2,2'- Oxybsin[N, N- Dimethyl
14	38.004	1.82	2- Methoxysulpholane
15	38.221	0.64	Cyclopropane, Methoxymethylene
16	39.032	0.68	Ethyl- 2- Oxo- 4- Methyl- 3- Pentenoate
17	43.285	19.18	Squalene
18	46.958	0.35	Butanoic acid, 4-(Ethoxyhydroxyphosphinyl)
19	48.514	1.03	dl- alpha- Tocopherol

Fig.1 Peak obtained at FTIR analysis

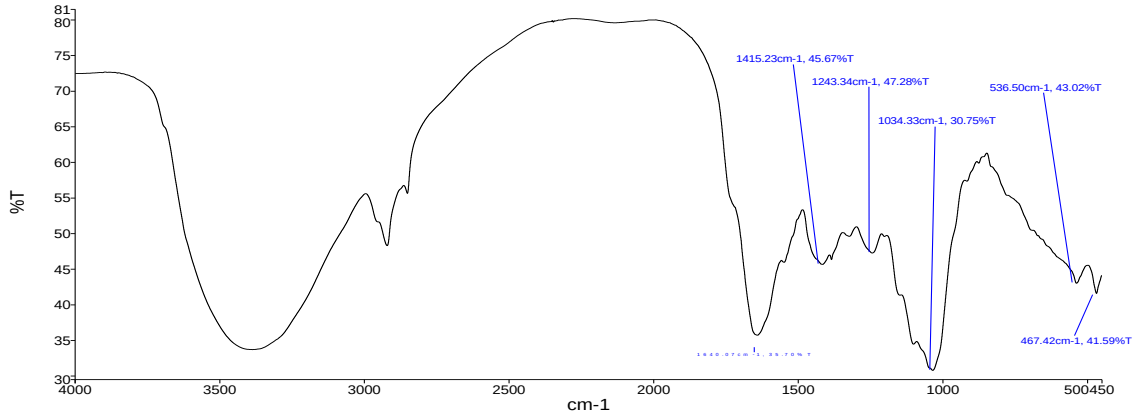
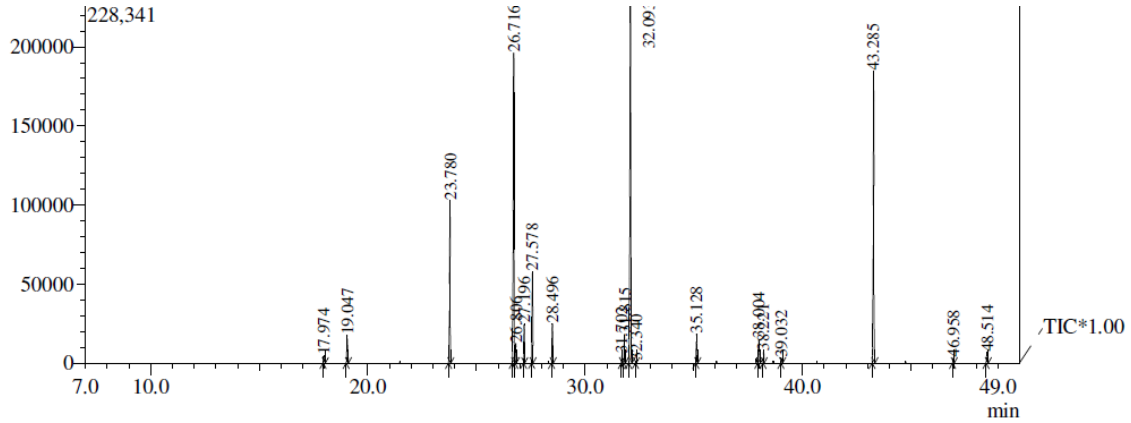


Fig.2 Chromatogram obtained at the GC-MS analysis of methanolic extract of *E. prostrata* leaves



Zubair *et al.,* (2017) reported the presence of phytol and citronellyl butyrate. The differences in the compounds reported in this study and other researchers might be due to difference in the geographical locations of plants which influence the growth and chemical composition of the plants.

The phytol belonging to diterpene group was the principle phyto constituent obtained from the present GC- MS study. phytol is said to have many medicinal properties such as antimicrobial, anti-inflammatory, anticancerous, antidiuretic, antinociceptive, antioxidant activities etc. It also acts as precursor for Vitamin K and E. The squalene belonging to triterpene group was reported to have many medicinal properties such as antibacterial, antioxidant, insecticidal, antitumor, cancer preventive, immunostimulant, chemo preventive and lipoxygenase-inhibitor activities (Seramakkani and Thangapandian, 2012). Neophytadiene possesses antibacterial, antipyretic, analgesic and antioxidant properties and also used for the treatment of head ache, rheumatism and some other skin problems (Singh *et al.,* 2012). D- alpha-tocopherol showed more antioxidant than γ-tocopherol (Seppanen *et al.,* 2010). Moderate supplementation of Vitamin E improved the semen characteristics in cocks and egg qualities in layers by reducing the oxidative stress. It is also said that dl- alpha- tocopherol

in diet of animal improved the resistance against infectious diseases (Rengaraj and Hong, 2015). Butyric acid exposed the anticoccidial effect, anti-inflammatory effect and also reduced the effect of necrotic enteritis (Timbermont *et al.,* 2010). Heptadecanoic acid which is a saturated fatty acid can be used as biomarkers of dietary food intake assessments, risk of coronary heart disease, type II diabetes mellitus as well as used for quantitative internal standards for lipidomic analyses (Jenkins *et al.,* 2015). Citronellyl butyrate and propionate belonged to fatty alcohol esters group used as surfactant, emulsifier and flavouring agents.

In conclusion, this study reveals that methanolic extract of *E. prostrata* leaves contained many important compounds such as phytol, squalene, neophytadiene and dl- alpha tocopherol which might be responsible for medicinal activity of this plant.

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