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PHYTOCHEMICAL SCREENING, GC-MS ANALYSIS, *IN VITRO* ANTIBACTERIAL ACTIVITY AND ANTIOXIDANT PROPERTY OF *ECLIPTA PROSTRATA L*

Nalini Devi D[1]and Mohamed Saleem Gani A[2]

[1] Research scholar in Biochemistry (category-B), Bharathiar University, Coimbatore-46

[2]Associate Professor in Science & Humanities, M.A.M College of Engineering, Siruganur, Trichy-5.

***Corresponding Author Email: mail2nalinidevi@gmail.com**

ABSTRACT

In the present investigation attempt was taken to explore phyto chemical components from the various solvents such as ethanol, methanol and aqueous extracts of the whole plant of Eclipta prostrata L by Gas chromatography and Mass spectroscopy (GC-MS).100gms of the powdered sample was subjected to the sox let extraction followed by rotary evaporator and investigated using PerkinElmer GC-MS. In which the antibacterial activities were determined by using ethanolic extract of this plant. The GC-MS analysis revealed the presence of various compounds with peak area like 2-cyclopentene-1-tri Dacanoic acid, Octa hydropentalen-1-ol, Tri6,4,0,027)] Dodecane. Dodecanoicacid, tridecanoicacid, Hentriacontane, Heptane 2,2,3,3,5,6,6 Hepta methyl, sulfurous acid Hexy Monyl ester. Extracts and metabolites from this plant have been known to possess pharmacological properties and also exhibit antibacterial activity.

KEY WORDS

Eclipta prostrata L, GC-MS analysis, ethanolic, 2-cyclopentene-1-tri Dacanoic acid, Octahydropentalen-1-ol, Tri6, 4, 0,027)] Dodecane.Dodecanoicacid, tridecanoic acid, Hentriacontane, Heptanes 2,2,3,3,5,6,6 Hepta methyl, sulfurous acid Hexy Monyl ester.

1. INTRODUCTION

The herb *Eclipta prostrata L (*Asteraceae,) commonly known as Bhringraja (Sanskrit), Maka (Marathi) and Bhangra (Hindi) has been reported to show protective effect on experimental liver damage in rats and mice (Kirtikar and Basu,1999). It grows commonly in moist places as a weed in warm temperature to tropical areas worldwide. It is widely distributed throughout the India, China, Thailand and Brazil (saxena, 1993). The plant has been reported for the treatment of liver cirrhosis and infective hepatitis. The plant is known to have some important pharmacological activities such as anti-inflammatory, analgesic, hepatoprotective and also possess antimicrobial activity (Chandra et al.1987). *Eclipta prostrata L* has been used in traditional systems of medicine and also by traditional healers especially in south region of India for the treatment of liver diseases since ancient times. The phytochemical screening is very important in identifying new sources of therapeutically and industrially important compounds like alkaloids, saponins, flavanoids, steroids, phenolic compounds, coumarin, luteolin, wedelolactone, triterpenoids, proteins, amino acids and reducing sugar etc. The present study aimed to investigate the phytochemical constituents present in the ethanol, methanol and aqueous extracts of the whole plant of *Eclipta prostrata L (*kapoor, 2001).

2. MATERIALS & METHODS

2.1 Collection and preparation of plant materials

Eclipta Prostrata (L.) L were collected from Trichy, Tamilnadu, India and confirmed by Dr. S. John Britto, The Rapinat Herbarium, ST. Joseph's college, Tiruchirappalli, (Ref.No: DND 001/2014) The leaves were thoroughly washed thoroughly, and the leaves were shade dried and coarsely powdered in a grinder. Then it is sieved and stored in airtight container for further activities.

2.2. Soxhlet Extraction:

Eclipta Prostrata (L.) powder consisting phytoconstituents were extracted with 70% Ethanol by soxhlet apparatus. The powdered plant sample was packed in a thimble, sealed properly with cotton. Ethanol in the round bottomed flask is boiled up to its boiling point, the vapours of it passes through the packed powder and collected as condensed extract in the RB flask. After repeated extraction of about15-20 cycles the extract was evaporated to expel solvents, which was then used for further process. Finally, crude extract was obtained. The crude extract was stored at 4°C until further use.

GC-MS analysis

20g of powdered sample is soaked with 60ml ethanol overnight and filtered through ash less filter paper with sodium sulphate. The extract is concentrated to 1ml by bubbling nitrogen into the solution. The extract contains both polar and non-polar phyto-components. The GC-MS was performed by using column Elite-5MS (5% diphenyl/95% dimethyl polysiloxane), 30× 0.25 mm × 0.25 µm df, equipment: GC-clarus 500 perkin Elmer carrier gas:1ml per minute, split:10.1 detector: Mass detector Turbo mass gold perkin software: turbomass 5.2. 2µl of ethanolic extract of the whole plant of *Eclipta prostrata L* was employed for GC-MS analysis. The 2µl sample extract injected into the instrument was detected by the Turbo mass software. The GC-MS extraction process was maintained at a temperature of 110 ^{0}C with 30minutes.the injector temperature was set at 250 ^{0}C [mass analyzer]. The different parameters were involved in the operation of the clarus500MS, were standardized. The helium gas was used as the carrier gas at a constant flow rate of 1.0mL/min.MS program: Library used NIST version year 2005 (Inlet line temperature: 200 ^{0}C; source temperature: 200 ^{0}C). Mass spectra were taken at 70 eV; a scan interval 0f 0.5s and fragments from 45 to 450 Da. The MS detection was completed in 36 minutes. (Woo et al., 2012)

Phytochemical screening

Phytochemical analysis of ethanol extract of *Eclipta Prostrata (L.) L* was carried out qualitatively to test for the presence of phenols, alkaloids, proteins, amino acids, tannins, carbohydrates, flavonoids, Phytosterols, saponins etc.

3.1. Detection of alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test for the presence of alkaloids.

(a) Mayer's test: Filtrates were treated with Mayer's reagent (Potassium mercuric iodide). Formation of a yellow color precipitate indicates the presence of alkaloids.

(b) Hager's test: Filtrates were treated with Hager's reagent (saturated picric acid solution). Formation of a yellow colored precipitate indicates the presence of alkaloids.

3.2. Detection of saponins

(a) Foam test: Small amount of extract was shaken with little quantity of water. If foam produced persists for ten minutes, it indicates the presence of saponins.

3.3. Detection of carbohydrates

Extracts were dissolved individually in 5ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

(a) Benedict's test: Filtrates were treated with Benedict's reagent and heated on a water bath. Formation of an orange red precipitate indicates the presence of reducing sugars.

(b) Molisch's test: Filtrates were treated with 2 drops of alcoholic α-napthol solution in a test tube and 2ml conc. sulphuric acid was added carefully along the sides of the test tube. Formation of violet ring at the junction indicates the presence of carbohydrates.

3.4. Detection of Phytosterols

(a) Liebermann bur chard's test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride. Boiled and cooled. Conc. Sulphuric acid was added carefully along the sides of the test tube. Formation of brown ring at the junction indicates the presence of Phytosterols.

3.5. Detection of flavonoids:

(a) Zinc hydrochloric acid reduction test: To the alcoholic solution of extracts, a pinch of Zinc dust and conc. HCl was added. Appearance of magenta color after few minutes indicates the presence of flavonoids.

(b) Alkaline reagent test: Extracts were treated with few drops of sodium hydroxide solution. Formation of intensive yellow color, which becomes colorless of dilute acid, indicates the presence of flavonoids.

3.6. Detection of proteins and amino acids:

(a) Xanthoproteic acid test: The extracts were treated with few drops of conc. nitric acid solution. Formation of yellow color indicates the presence of proteins.

(b) Ninhydrin test: To the extracts were treated with 0. 25% Ninhydrin reagent was added and boiled for few minutes. Formation of blue color indicates the presence of amino acids.

3.7. Detection of phenols

Ferric chloride test: Extracts were treated with few drops of ferric chloride solution. Formation of bluish black color indicates the presence of phenols.

3.8. Detection of Tannins

Gelatin test: To the extract, 1% gelatin solution containing sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

TABLE-I: Phytochemical analysis of *Eclipta Prostrata (L.)* with aqueous and ethanol solvents

SL NO.	Test name	Leaf extract		Stem extract		Root extract	
		Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
1.	Alkaloids	+	+	+	+	+	+
2.	carbohydrates	+	+	+	+	+	+
3.	Steroids	-	+	+	+	-	+
4.	Glycosides	+	+	-	+	+	+
5.	Saponins	+	+	+	+	+	+
6.	Tannins	-	+	-	+	-	+
7.	Phenolic	+	+	+	+	+	+
8.	Flavonoids	+	+	+	+	+	+
9.	Amino acids	+	+	+	+	+	+
10.	Terpenoids	+	+	+	+	+	+

TABLE-II: GC-MS report for ethanolic extract of *Eclipta Prostrata (L.)*

SL. NO	Chemical constituents	Molecular formulae	Molecular weight	Peak area	Retention time
1.	2-cyclopentene-1-tridecanoic acid	C18H32O2	280	2.86	0.20
2.	Tetra tetracontane	C44H90	618	17.61	17.40
3.	N-(3-methylButyl) acetamide	C7H15ON	129	19.20	19.60
4.	Dodecane,2,6,11-tetramethyl	C15H32	212	20.58	21.00
5.	2-propyn-1-amine, N, N Dimethyl	C5H9N	83	20.75	21.20
6.	Fumaric acid,2-methylallyl Penta decyl ester	C23H42O4	380	21.30	21.40
7.	Sulfurous acid, Hexyl Nonyl ester	C15H32O3S	292	22.05	22.20
8.	Phthalic acid,2-methyl Butyl Octyl ester	C21H32O4	348	23.38	23.00
9.	Heptanes 2,2,3,3,5,6,6 Hepta methyl	C14H3O	198	23.64	23.20
10.	Sulfurous acid, Hexyl Penta Decyl ester	C23H36O4	376	24.38	23.40
11.	Hexatriacontane	C36H74	506	25,23	24.20
12.	Octatriacontane,1,38-dibromo	C38H76Br2	690	25,79	25.40
13.	Dotriacontane	C42H86	590	27.29	25.60
14.	Hexane,1-(hexyloxy)-5-Methyl	C13H28O	200	27.98	27.40
15.	Hentriacontane	C31H64	436	29.15	28.00
16.	Phthalic acid, Bis (2-Pentyl) ester	C18H26O4	306	29.87	29.20

Antimicrobial assay

The pathogenic bacterial species were collected, and it was determined. Bacterial strains consisted of *Shigellaboydii, E.coli, Klebsilla pneumonia, Pseudomonas Sp*.and *Salmonella paratyphi* A. The antifungal effect of *Eclipta prostrata*L. Was determined against fungal strains such as Aspergiller niger and candida albicans. The strains were Sub cultured bimonthly and the cultured strains were allowed to grow for one week and stored at 5 ^{0}C for further analysis. Muller Hinton agar (MHA) was used as the media for culturing of bacterial strains. The stock cultures were maintained in sabouraud dextrose broth and two different strains of fungal pathogens were maintained in sabouraud dextrose broth for 24 hours until used for antifungal activity. The discs were immersed in different concentrations like 50µg to 250µg/ml allowed evaporating. After that the plates were incubated at room temperature (27 ^{0}C ± 2) for 24hours.After incubation, plates were observed for zones of inhibition and recorded in millimeters.

Evaluation of Anti-microbial activity

The antimicrobial activity of the methanol extracts of various parts and *in vitro* grown plant of *Eclipta prostrata*L. was evaluated through disc-diffusion method.

Minimum inhibitory concentration (MIC)

The ethanolic extract of *Eclipta prostrata*L. antimicrobial activity were further tested against all the organisms for the evaluation of its antibacterial and antifungal efficiency at different concentrations (50µg to 250µg/ml) by using filter paper disc diffusion method. The zone of inhibition was calculated in millimeters. Activity index was calculated by comparing the zone of inhibition by plant extract with that of standard drug.

$$\text{Activity index} = \frac{\text{Inhibition zone of test sample (extract)}}{\text{Inhibition zone of standard drug}}$$

Figure I : GC-MS report for ethanol extract of *Eclipta Prostrata (L.)*

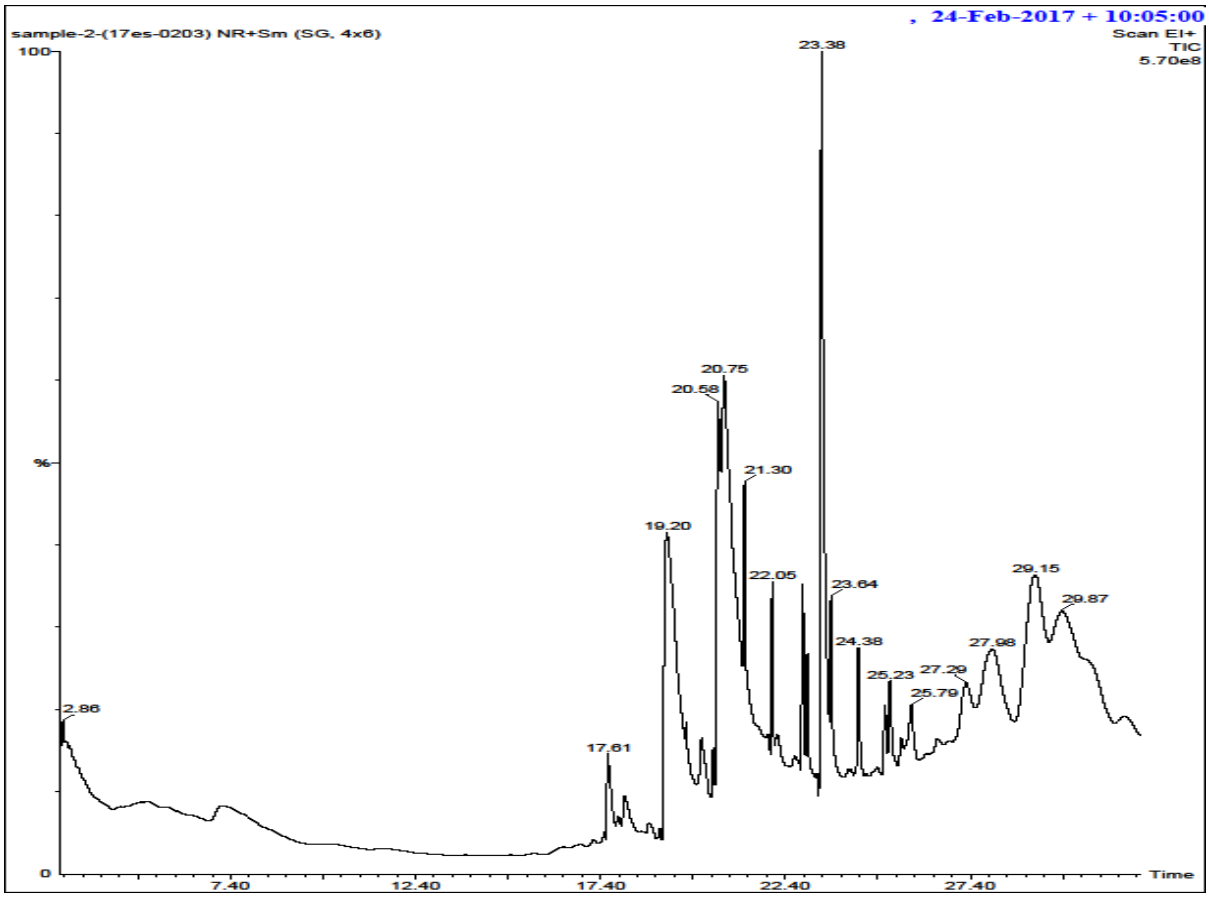

Table –III represents antimicrobial activity of *Eclipta prostrata L*.

S.NO.	Number of the organisms	Zone of inhibition(mm)
1.	*Pseudomonas Sp*	1.8
2.	*Shigella boydii*	10.6
3.	*Klebsilla pneumonia*	7.1
4.	*Salmonella paratyphi* A	5.1
5.	*E.coli*	6.5
6.	*Aspergiller niger*	5.7
7.	*candida albicans*	4.6

Antioxidant assay

DPPH Radical scavenging activity:

The scavenging effects of samples for DPPH radical were monitored according to the method described by Yen and Chen.Briefly, 2.5ml of the test sample was added to 2.5ml of 0.18mmDPPH ethanol solution.The mixture was then vortexes for 1 minute and then left to stand at room temperature for 30 minutes in the dark and its absorbance was read at520nm.The ability to scavenge the DPPH radical was calculated using the formulae given by Duan et al. Synthetic antioxidants ascorbic acid used as positive controls.

Percentage of inhibition (%) = [(A_o-A1)/A_o] X100

TABLE-IV shows scavenging effects of *Eclipta prostrata L*. vs. standard ascorbic acid

S.NO.	SAMPLE CONCENTRATION (µg/ml)	TEST	CONTROL
1.	**20**	**31.63±2.00Z**	**36.43±1.01Z**
2.	**40**	**48.04±0.03Z**	**51.19±0.04Z**
3.	**60**	**67.19±0.06Z**	**70.41±0.06Z**
4.	**80**	**74.12±1.46Z**	**74.13±1.61Z**
5.	**100**	**80.13±2.00Z**	**80.41±0.03Z**

Test sample- *Eclipta prostrata* L.
Control- Ascorbic acid

3. RESULTS AND DISCUSSION

The active compounds with their peak number concentration (peak area%) and retention time (RT) are present in Table- I and Fig -I shows the presence of 16 bioactive phytochemical compounds in the ethanol extract of Eclipta prostrata L. The individual fragmentations of the components with molecular structure were illustrated in the antimicrobial activity was determined by measuring the diameter of Zone of inhibition. The whole plant extracts (Leaf stem, flower& root) of Eclipta prostrata L. were found to have antimicrobial activity by using solvent like ethanol as well as aqueous. From these results of the various extracts against some pathogenic organisms like Shigella boydii, E.coli, Klebsilla pneumonia, Pseudomonas Sp.and Salmonella paratyphi A Aspergiller niger and candida albicans. Species etc.

From the qualitative of *Eclipta prostrata L*. analysis having various chemical constituents by using aqueous and ethanol extracts showed some medicinal properties. In this analysis was carried out to understand the presence of various chemical constituents such as alkaloids, Tannins, glycosides, flavonoids, Terpenoids and steroids. These compounds are shows the plants having antimicrobial activity and also exhibit the pharmacological activity. The different parts of the plants exhibited activity against the pathogenic organisms such as, *Shigellaboydii, E.coli, Klebsilla pneumonia, Pseudomonas Sp*.and *Salmonella paratyphi* A *Aspergiller niger* and *candida albicans*. Species etc.

From the Disc diffusion method ethanolic extract of *Eclipta prostrata L*. shows zone of inhibition in mm represents in Table –III.

5. CONCLUSION

The various bioactive constituents revealed from the different parts of an *Eclipta prostrata L*. Plant by using ethanol extract suggests that the components having pharmacological activity, antimicrobial activity and also possesses antioxidant properties. This medicinal herb indicates that one of the disease curable medicine like liver cancer, Jaundice, hair stimulator, hepatoprotectant and also skin diseases.

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***Corresponding Author:**
Nalini Devi D*
Email: mail2nalinidevi@gmail.com