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Phytochemical Screening of Solvent Extracts from *Eclipta prostrata* L.: A Preliminary Investigation

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Abstract

Eclipta prostrata L., classified within the Asteraceae family, is a well-acknowledged medicinal plant with ethnomedical significance, used in Ayurveda, Unani, and Siddha formulations. Traditionally used in Ayurveda and Chinese medicine due to its hair growth potential and other significant pharmacological properties. Different solvent extracts, viz., aqueous, ethanol, and methanol of *Eclipta prostrata*, were screened for the existence or non-existence of diverse phytochemical compounds in order to identify the right solvent for further studies. Ethanol, followed by methanol, was found to be the best solvent. Also, the total alkaloid, phenol, and saponin contents of *Eclipta prostrata* leaves were determined to be 2.11 per cent, 41.35 mg g^{-1}, and 2.41 per cent, respectively. The phytochemical profiling of methanolic extracts of the dried whole plant by GC-MS analysis revealed a diverse spectrum of phytochemicals, prominently featuring n-Hexadecanoic Linolenic acid, 10E,12Z-Octadecadienoic acid, and Phytol.

***Key words*:** *Bhringaraj, Eclipta,* Solvent, Phytochemicals, Screening, Chemo profiling

Medicinal plants remain the most readily accessible therapeutic resource within the conventional primary healthcare system for humans. The existence of diverse phytochemical components, pharmacological properties, economic feasibility, and the absence of side effects renders them the core component of the conventional healthcare system. Although certain herbs are often classified as weeds, they have been used in traditional medicine [1]. *Eclipta prostrata* L. is the prime example of such a weed with ethnomedical significance, belonging to the Asteraceae family used in Ayurveda, Unani, and Siddha formulations. *Bhringaraj* or false daisy (*Eclipta prostrata* L.) is an herbaceous annual plant with diminutive branches and clusters of white axillary or terminal inflorescences that flourish in moist habitats. The plant is native to Asia and exhibits extensive distribution across tropical, subtropical, and warm temperate climatic zones globally [2]. The cosmopolitan distribution of this species, considering it as indigenous to the regions of North, Central, and South America and subsequently disseminated throughout Europe, Africa, the Middle East, Asia, and Australasia, was described [3].

It is an annual herb acknowledged as the "king of hairs" because of its ability to promote hair growth. It has been conventionally employed in the realms of Ayurveda and traditional Chinese herbal pharmacology. The leaf extracts of *E. prostrata* exhibited significant antimicrobial activity against *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*. The plant is further esteemed for its characteristics of notable pharmacological relevance, such as hepatoprotective, antidiabetic, analgesic, anti-inflammatory, neuroprotective, antioxidant, antimalarial, antimicrobial, immunomodulatory, and anticancer [4]. Furthermore, the extracts sourced from *E. prostrata* function as a naturally occurring environmentally sustainable hair colour [5], plant dye for cotton fabric [6], and for the biosynthesis of silver, gold, copper, palladium, and zinc oxide nanoparticles [7]. *Eclipta prostrata* has gained attention for its applications in sustainable practices, particularly as a source of natural dyes and in nanotechnology. The phytochemical composition of the species is attributed to these properties.

In light of this context, the current research endeavors comparative evaluation of different solvents, viz., aqueous, ethanol, and methanol, for finding the various phytochemical compounds of the *E. prostrata*, thereby identifying the best solvent for extraction so that maximum phytochemical compounds are obtained. Furthermore, the study evaluated the total alkaloid content, total phenol content, and saponin content of *Eclipta prostrata* leaves.

MATERIALS AND METHODS

Fresh *bhringaraj* plants were collected from the germplasm maintained at the Department of Plantation, Spices, Medicinal, and Aromatic Crops, College of Agriculture, Kerala Agricultural University, Thrissur (latitude: 10.547726° N and longitude: 76.282487° E). The plant specimens were

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thoroughly washed to eliminate any attached soil and particulate matter, followed by drying in a shaded location, and were further used for extraction. The botanical specimen was pulverized utilizing an electrical grinder. Ten grams of pulverized whole plant material, which included the root, stem, and leaf components, were used for extraction using different solvents employing a Soxhlet apparatus. The resultant extracts were filtered and subsequently desiccated at a temperature of 40ºC utilizing a rotary evaporator. The extracts were preserved at a temperature of 4°C until their eventual utilization.

One per cent weight by volume of stock solution of each extract was prepared by mixing one gram of bhringaraj extract with 100 ml of each respective solvent (water, ethanol, and methanol) in isolation. The extracts mentioned above underwent preliminary phytochemical screening in accordance with the established protocols [8].

Mayer's test for alkaloids: Alkaloids were indicated by a pale yellow or white precipitate when two millilitres of the test solution were treated with Meyer's reagent.

Lead acetate test for tannins and phenolic compounds: The addition of lead acetate solution (10%) to each test solution resulted in the formation of a white precipitate, validating the existence of tannins and phenolic compounds.

Salkowski test for terpenoids and steroids: An aliquot of one millilitre of concentrated sulphuric acid was introduced to two millilitres of each test solution. The red hue in the lower stratum signified steroid presence, whereas a yellow hue denoted triterpenoid presence.

Ammonia test for flavonoids: A two-millilitre aliquot of the test solution was blended with two millilitres of dilute ammonia solution (10%) and several drops of concentrated sulfuric acid, whereby the detection of flavonoids is indicated by the emergence of a yellow-orange hue, which subsequently diminishes over time.

Keller Killiani test for cardiac glycosides: To ascertain the existence of cardiac glycosides, two milliliters of each test solution were treated with glacial acetic acid, concentrated sulfuric acid, and a minimal quantity of ferric chloride, resulting in a greenish hue in the upper stratum and a reddish-brown coloration at the junction as markers of existence.

Foam test for saponins: The consistent generation of stable foam upon vigorous agitation of five millilitres of each test solution validated the existence of saponins.

Benedict's test for carbohydrates: A red precipitate serves as an indicator of carbohydrate availability achieved by adding one millilitre of Benedict's reagent to two millilitres of each test solution, which were subsequently heated in a water bath for two minutes.

Ninhydrin test for amino acids: The development of a blue or violet shade signifies the presence of amino acids in the solution, achieved through the integration of Ninhydrin solution (0.5%) into two milliliters of each test solution, thereafter subjected to thermal exposure in a water bath for a duration of two minutes.

Hydroxyanthraquinone test for anthraquinone glycosides: The red tint in the solution reflects the existence of hydroxyanthraquinone, accomplished by the combination of one millilitre of potassium hydroxide (10%) solution and two millilitres from each test solution.

Determination of total alkaloid content

The alkaloid concentration in *Eclipta prostrata* leaf samples was assessed [8]. The leaf sample (5g) was combined with 200 ml of ethanol containing 10% acetic acid and allowed to macerate for four hours. The filtered extract was concentrated to 25 per cent of its primary volume in a water bath, followed by precipitation through the introduction of concentrated ammonium hydroxide. Subsequently, the precipitate was subjected to washing with dilute ammonium hydroxide, followed by filtration to isolate the residue, which was dried and assessed to determine the total alkaloid concentration.

$$\text{Total alkaloid content (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

Determination of total phenol content

For the quantification of the total phenol content of leaf samples, 0.1 gram of the leaf sample was homogenized with 80 per cent ethanol (10 ml), subsequently subjected to centrifugation at 10,000 rpm for 20 minutes, followed by re-extraction of the residue, culminating in the pooling and evaporation of both supernatants to dryness. Five millilitres of distilled water was used to dissolve the residue. Consequently, one millilitre of the extract was measured and subsequently mixed with three milliliters of distilled water in a test tube, to which Folin-Ciocalteu reagent (0.5 ml) and 20 per cent sodium carbonate (2 ml) solution were incorporated and mixed rigorously for three minutes. Subsequently, the test tube underwent a one-minute immersion in boiling water followed by an hour incubation in darkness, after which the absorbance was quantified at 650 nm relative to a reagent blank. The phenolic concentration in samples was quantified through the standard gallic acid calibration curve at varying concentrations, articulated in mg GAE g^{-1} [9].

Estimation of saponin content

The saponin content of leaf samples of *Eclipta prostrata* was determined [10]. Grounded leaf samples (20 g) were immersed in 20 per cent ethanol (200 ml) and subjected to four hours of heating process in a hot water bath at around 55°C with constant stirring, followed by filtration to isolate the residue and subsequent re-extraction. The combined filtrates were subsequently concentrated to 40 ml at 90°C in a hot water bath. The concentrate was introduced into a separating funnel (250 ml), augmented with 20 ml of diethyl ether, and subjected to vigorous agitation resulting in the formation of two distinct layers; the ethyl layer was eliminated, preserving the aqueous layer, to which 60 ml of n-butanol was introduced. The extract underwent two sequential washes with five per cent sodium chloride solution (10 ml) followed by evaporation over a water bath and subsequent drying to achieve a stable weight. The saponin percentage was quantified.

$$\text{Saponin content (\%)} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

Phytochemical profiling

The phytochemical profile of methanolic extracts of dried whole plant of *E. prostrata* was quantified using the gas chromatography-mass spectrometry analysis at Centre for Analytical Instrumentation – Kerala (CAI-K), KSCSTE-Kerala Forest Research Institute (KFRI), Peechi, Thrissur, using the Shimadzu Nexis GC- 2030 instrument, comprising an AOC-

30/20i auto-sampler. The chemical constituents were identified by comparing the mass spectra and time indices with the NIST 20 library, and their peak area was expressed as percentage.

RESULTS AND DISCUSSION

The findings of the preliminary comparative phytochemical evaluation of various solvent extracts *viz.*, aqueous, methanol, and ethanol of dried *Eclipta prostrata* sample are presented in (Table 1). Tannins, phenolic compounds, terpenoids, steroids, and amino acids were tested positive in all three extracts examined. Alkaloids, flavonoids, cardiac glycosides, carbohydrates, and amino acids were detected in both ethanol and methanol extracts yet were absent in aqueous extracts. Conversely, saponins were identified in aqueous and ethanol extracts, while they were not found in methanol extract. Ethanol, followed by methanol, was found to be the best solvent for the extraction of phytochemicals, which might be attributed to its proficiency in solubilizing a greater spectrum of phytochemicals.

The existence of a varied assortment of phytochemicals may facilitate the multitude of pharmacological properties linked to the plant. The phenolic compounds are associated with antioxidant, hepatoprotective, and anticancer properties, whereas coumestan derivatives such as wedelolactone, along with alkaloids and saponins, exhibit antibacterial effects. Additionally, triterpenoids and specific fractions contribute to hepatoprotective activities, while luteolin and flavonoids demonstrate anticonvulsant properties; wedelolactone's efficacy in diabetic treatment and its role, alongside β-sitosterol, in promoting hair growth, as well as triterpenes' effectiveness against osteoporosis, is noteworthy [11].

Table 1 Preliminary phytochemical screening of *Eclipta prostrata* L. using different solvents

Phytochemicals	Aqueous extract	Ethanol extract	Methanol extract
Alkaloids	-	+	+
Tannins and phenolic compounds	+	+	+
Terpenoids and steroids	+	+	+
Flavonoids	-	+	+
Cardiac glycosides	-	+	+
Saponins	+	+	-
Carbohydrates	-	+	+
Amino acids	+	+	+
Anthraquinone glycosides	-	+	+

The existence of steroids, tannins, flavonoids, diterpenes, triterpenes, and saponins within the methanolic extract derived from desiccated *Eclipta prostrata* foliage was supported by the earlier findings [12]. The identification of alkaloids, tannins, phenolic substances, terpenoids, and steroids in the aqueous and ethanol extracts derived from the desiccated whole plant of *Eclipta prostrata* was substantiated [13].

Table 2 Estimated values of different phytochemical compounds

S. No.	Phytochemical compound	Estimated value
1.	Total alkaloid content	2.11 %
2.	Total phenol content	41.35 mg GAE g^{-1}
3.	Total saponin content	2.41 %

The total alkaloid, phenol, and saponin content of *Eclipta prostrata* leaves were determined following the standard procedures mentioned, employing ethanol as the solvent for the extraction process, and are specified in (Table 2). The concentration of alkaloids in the *Eclipta prostrata* leaves was confirmed to be 2.11 per cent. Alkaloids represent a substantial group of phytochemical compounds that are responsible for a variety of pharmacological effects observed in *Eclipta prostrata*. The alkaloid concentration present in the leaves of *Eclipta prostrata* was found to be approximately 0.4 mg g^{-1}, and the isolated alkaloids exhibit considerable antibacterial efficacy against human pathogenic microorganisms, particularly *Staphylococcus aureus* and *Escherichia coli* [14].

The total phenol content of *Eclipta prostrata* leaves was 41.35 mg GAE g^{-1}. Phenolic compounds form a vital group of phytochemicals that primarily serve as antioxidants, safeguarding humans from oxidative stress triggered by reactive oxygen species (ROS) and free radicals. The total phenolic concentration of both aqueous and ethanolic extracts obtained from *Eclipta prostrata* was found to be 29.34 ± 0.96 and 36.73 ± 0.49 mg gallic acid equivalent (GAE)/g extract, respectively [15]. the total phenolic content of the hydro-alcoholic extract derived from the desiccated whole plant of *Eclipta prostrata* was measured to be 98.39 mg GAE/g of dry mass [16].

The concentration of saponins present in the foliage was ascertained to be 2.41 per cent. A systematic investigation illustrated that the saponin fractions sourced from *Eclipta prostrata* are pivotal in their antimicrobial efficacy [17]. The leaves of *Tridax procumbens*, a member of the Asteraceae family, were reported with a saponin content of 10.30 mg/100g and 103.52mg/100g on a wet and dry weight basis, respectively [18]. The saponin content of dried leaves of *Eclipta prostrata* was determined to be 2.10 % [19], employing the ultrasonic-assisted extraction (UAE) technique as a fast extraction method.

The GC-MS analysis of methanolic extracts from the dried whole plant of *Eclipta prostrata* identified 23 distinct compounds, as detailed in the GC-MS chromatogram (Fig. 1) and accompanying Table 3. The principal compounds detected included n-Hexadecanoic acid (21.36 %), Linolenic acid (12.77%), 10E,12Z-Octadecadienoic acid (10.08%), Phytol (9.02%), Propenenitrile, 2-(2-benzothiazolyl)-3-(2-methoxyphenyl)- (6.47%), Methyl hexadecanoate (6.46%), and 2-Hydroxy-3,5,6-trimethylbenzo-1,4-quinone (3.37%). Further compounds such as Octadecanoic acid (2.1%), Squalene (1.98%), Methyl DL-pyroglutamate (1.87%), Loliolide (1.85%), Pyranone (1.79%), Heptadecane (1.45%), 2-Amino-3-hydroxypyridine (1.2%), Neophytadiene (1.2%), 3-Cyclopentylpropionic acid, 2-dimethylamino ethyl ester (1.16%), Cyclohex-2-enone, 3-(N', N'-dimethylhydrazino)- (1.02%), Octanoic acid, 2-dimethylamino ethyl ester (0.74%), indole (0.68%0, 6,10,14-Trimethyl-2-pentadecanone (0.66%), and Tetradecanoic acid (0.5%) were detected in minimal quantities, collectively indicating a diverse biochemical profile.

n-Hexadecanoic acid may significantly contribute to anti-inflammatory responses and possess therapeutic potential for rheumatic conditions [20], while Linolenic acid has been

associated with protective effects against cardiovascular diseases, cancer, neurodegeneration, osteoporosis, inflammation, and oxidative stress [21]. Recent studies have revealed that Phytol, a diterpene within long-chain unsaturated acyclic alcohols exhibited various effects, including anxiolytic, metabolism modulation, cytotoxicity, antioxidant activity, induction of autophagy and apoptosis, antinociceptive, anti-inflammatory, immune modulation, and antimicrobial properties [22]. Additionally, quinones demonstrate cytoprotective capabilities against oxidative stress, thereby underscoring their significance in neurodegenerative disease treatment [23], while further investigations highlighted their anticancer potential [24] and anticoagulant properties [25].

Squalene, recognized primarily as a cholesterol synthesis intermediate, exhibits various pharmacological effects, including hypolipidemic, hepatoprotective, cardioprotective, antioxidant, and antitoxicant properties, proving beneficial in managing type 2 diabetes mellitus and enhancing the efficacy of certain antitumor agents while mitigating their adverse effects [26]. Further, the other compounds present in trace amounts may also contribute to significant medicinal properties associated with the plant.

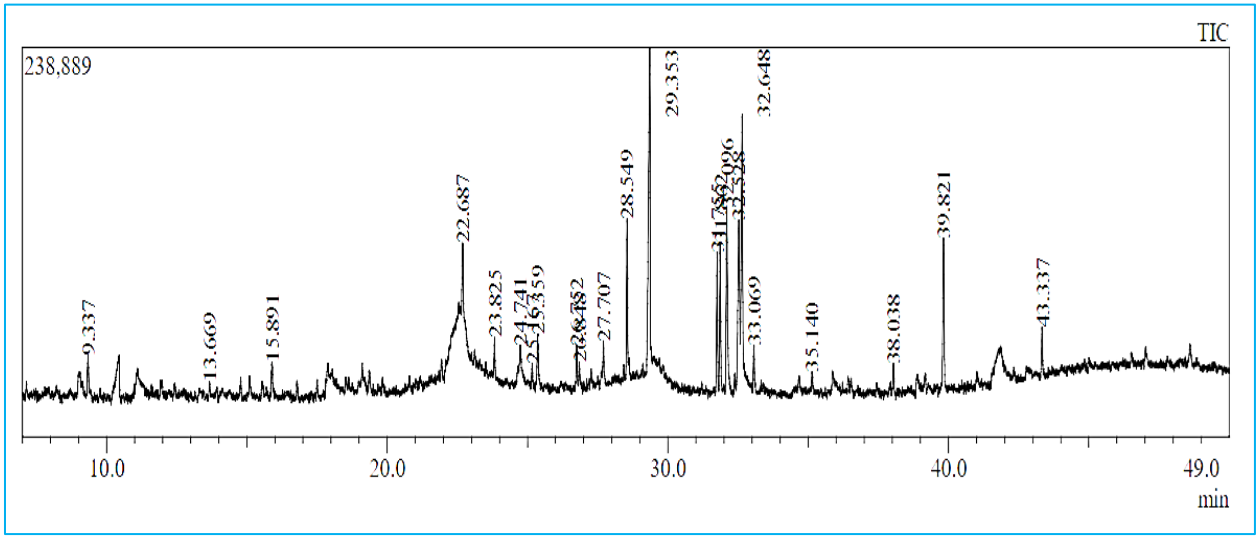

Fig 1 GC-MS chromatogram of methanolic extract of dried whole plant of *Eclipta prostrata*

Table 3 Phytochemical profile of methanolic extract of dried whole plant of *Eclipta prostrata*

Peak	Retention time	Compound	Peak area %
1.	9.337	Pyranone	1.79
2.	13.669	Indole	0.68
3.	15.891	Methyl DL-pyroglutamate	1.87
4.	22.687	2-Hydroxy-3,5,6-trimethylbenzo-1,4-quinone	3.37
5.	23.825	Heptadecane	1.45
6.	24.741	2-Amino-3-hydroxypyridine	1.25
7.	25.167	Tetradecanoic acid	0.50
8.	25.359	Loliolide	1.85
9.	26.752	Neophytadiene	1.20
10.	26.848	6,10,14-Trimethyl-2-pentadecanone	0.66
11.	27.707	Cyclohex-2-enone, 3-(N',N'-dimethylhydrazino)-	1.02
12.	28.549	Methyl hexadecanoate	6.46
13.	29.353	n-Hexadecanoic acid	21.36
14.	31.755	Methyl linoleate	5.50
15.	31.862	Methyl alpha.-linolenate	6.25
16.	32.096	Phytol	9.50
17.	32.528	10E,12Z-Octadecadienoic acid	10.08
18.	32.648	Linolenic acid	12.77
19.	33.069	Octadecanoic acid	2.10
20.	35.140	Octanoic acid, 2-dimethylaminoethyl ester	0.74
21.	38.038	3-Cyclopentylpropionic acid, 2-dimethylaminoethyl ester	1.16
22.	39.821	Propenenitrile, 2-(2-benzothiazolyl)-3-(2-methoxyphenyl)-	6.47
23.	43.337	Squalene	1.98

CONCLUSION

Eclipta prostrata is a rich reservoir of diverse phytochemical compounds, contributing to its pharmacological properties. The current investigation elucidated the effectiveness of various solvents in the extraction of phytochemical compounds from *Eclipta prostrata*. Ethanol, followed by methanol, has been identified as the most effective solvent for the extraction of the highest quantity of phytochemicals and may be employed in subsequent research endeavors. A range of compounds, including alkaloids, tannins, phenolic compounds, terpenoids, steroids, flavonoids, cardiac glycosides, saponins, carbohydrates, amino acids, and anthraquinone glycosides, were identified in the extracts,

potentially contributing to the antioxidative, antimicrobial, hepatoprotective, anticancer, and hair growth-promoting properties. Furthermore, the total alkaloid, phenol, and saponin content of *Eclipta prostrata* leaves were determined by employing ethanol as the solvent. The GC-MS analysis of methanolic extracts revealed a variety of phytochemical compounds that may be critically linked to the plant's pharmacological attributes.

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