

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *ECLIPTA ALBA* L.

THENMOZHI M*, JAYANTHI M

Department of Biotechnology, Vels Institute of Science, Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu, India.
Email: drmmthenmozhi@gmail.com

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ABSTRACT

Objective: Earlier various studies on pharmacological and therapeutic properties of *Eclipta alba* L. have been reported. The present investigation is on phytochemical screening and antioxidant activity of *E. alba* L. using different solvent extracts.

Method: The shade-dried and grounded *E. alba* L. plant materials were treated with petroleum ether, chloroform, ethyl acetate, ethanol, methanol, and water (aqueous) solvents. The extracted residue was then dissolved in dimethylsulfoxide and further used for phytochemical screening and antioxidant studies.

Results: *E. alba* L. on different solvent extract, the extractive yield was higher in methanol (14.22±0.99% w/w). The phytochemical screening analysis showed high number of secondary metabolites in the aqueous extract compared to other solvent studied. The metabolite flavonoids, phenols, and tannins are likely to be antioxidant potential compound. Hence, further, the total flavonoid content determination showed high of 0.875±0.020 µg/g in methanol extract. Further antioxidant property of *E. alba* L. was studied by method such as 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity, nitric oxide scavenging activity, metal chelating activity, and superoxide dismutase activity which showed high antioxidant potency with IC₅₀ values of 62.76±0.63 µg/ml, 96.24±0.34 µg/ml, 72.29±0.61 µg/ml, and 108.40±0.52 µg/ml, respectively, in the petroleum ether extract.

Conclusion: From the present study, the substantiate result of antioxidant studies has been obtained from petroleum ether extract. Furthermore the extractive value and flavonoid content was observed to be high in methanol extract. The antioxidant potency of *E. alba* L. was found to be high in petroleum ether extract compared to other solvent studied.

Keywords: *Eclipta alba* L., Methanol, Antioxidant, Flavonoid, 1,1-diphenyl-2-picrylhydrazyl, Radical scavenging.

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INTRODUCTION

Eclipta alba L. is small branched annual herbaceous plant with a long history of traditional medicines uses in many countries, especially in tropical and subtropical regions. *E. Alba* is a source of coumestan-type compounds used in pharmaceutical formulations of medicines prescribed for the treatment of cirrhosis of the liver and infectious hepatitis [1].

E. alba is traditionally used for blackening, promoting hair growth, and strengthening the hair. It is used as antivenom against snake bite in China and Brazil. The herb has been known for its curative properties and has been utilized as antimutagenic, analgesic, antibacterial, antihepatotoxic, antihemorrhagic, antihyperglycemic, antioxidant, and immunomodulatory properties, and it is considered as a good rejuvenator too.

Phytochemical constituents are the basic source for the establishment of several pharmaceutical industries. The constituents are playing a significant role in the identification of crude drugs. Therefore, proper scientific knowledge is required to investigate and explore the exact standardization of such medicinally important plant.

Moreover, plant-based medicines also have an enormous potential to provide low cost, easily accessible, and safe method of treatment. Herbal medicines are widely accepted in complementary and alternative medicine, especially in cancer patients with poor socioeconomic condition. Hundreds of plants possessing anticancer properties have been identified, and they are the source of alternative medicine for cancer therapy in various regions of the globe [2].

The medicinal properties of plants have been investigated in the recent scientific developments throughout the world, due to their potent antioxidant activities, no side effects, and economic viability [3].

METHODS

Plant collection and identification of plants

The plants used in this study *E. alba* L. Hassk No. BSI/SRC/5/23/10-11/Tech-706 (Asteraceae) was collected from Namakkal District, Tamil Nadu, and it was authenticated at the Botanical Survey of India, South Circle, Coimbatore, Tamil Nadu.

E. alba (L.) Hassk
Kingdom: Plantae
Order: Asterales
Family: Asteraceae
Genus: *Eclipta*
Species: *E. alba*

Preparation of powder

The *E. alba* L. was collected and dried under shade without any contamination [4]. These dried materials were mechanically powdered, sieved using 80 mm mesh, and stored in airtight containers. These powdered materials were used for further extraction analysis.

Extraction for analysis

The dried plants of *E. alba* L. were powdered and treated with different solvents, namely petroleum ether, chloroform, ethyl acetate, ethanol, methanol, and water (aqueous). The solvents were evaporated and residues were dissolved in dimethylsulfoxide (DMSO). It was used for the phytochemical and antioxidant activity.

Preliminary phytochemical screening

Phytochemical screening was performed for the analysis of different phytoconstituents such as carbohydrates, proteins, steroids, amino acids, glycosides, alkaloids, flavonoids, vitamins, saponins, tannins and

phenol compounds, quinones, and fixed oils and fats. The screening was performed with some modifications of the method [5].

Determination of antioxidant activity

Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce free radicals, thereby leading to chain reactions that may damage the cells of organisms. Some of the mechanisms are by donating a hydrogen atom or electron to the radical, scavenging free radical, chelating metal ions, inhibiting β -carotene bleaching, and quenching singlet oxygen. Each assay specifically detects any one of the abilities of the antioxidant. Therefore, it is necessary to use more than one type of antioxidant assay [6].

Determination of total flavonoids

The amount of flavonoids was determined spectrophotometrically [7]. 1 ml of plant extract was mixed with 1 ml of 2% aluminum trichloride in ethanol. The mixture was diluted with ethanol to 25 ml and allowed to stand for 40 min at 20°C, and the absorbance was measured at 415 nm against the sample blank. The results were expressed as quercetin equivalent per gram of dried sample.

1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay

The radical scavenging activity of plant extracts and ascorbic acid was measured by the DPPH method [8]. 1 ml of 0.135 mM DPPH solution in methanol was mixed with 1 ml of extract. The reaction mixture was vortexed and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm. A reaction mixture without test sample served as control. The ability to scavenge DPPH radical was calculated by the following equation:

$$\text{DPPH radical scavenging activity (\%)} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]}{(\text{Abs}_{\text{control}})} \times 100$$

Nitric oxide (NO) radical scavenging activity

NO radical scavenging activity was measured by spectrophotometric method [9]. Test samples at different concentrations (1000–9000 μg) were dissolved in DMSO. With 1 ml of the test solution, 1 ml of sodium nitroprusside (5 mm) in phosphate buffer saline was mixed and incubated for 30 min at 25°C. An assay medium without test solution served as control. After 30 min, 1 ml of incubated solution was taken out and an equal amount of Griess reagent was added. The absorbance of the chromophore formed during the diazotization of the nitrile with sulfanilamide and the subsequent coupling with naphthyl ethylenediamine dihydrochloride was measured at 546 nm. Catechin was used for comparison. The percentage scavenging activity was calculated as follows:

Nitric oxideradicalscavenging activity (%) =

$$\frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]}{(\text{Abs}_{\text{control}})} \times 100$$

Fe²⁺ chelating activity

Ferrous ion chelating activity assay was based on the principle of the Fe²⁺ chelating ability of the test samples by measuring the ferrous iron-ferrozine complex formed at 562 nm [10]. Two different concentrations of sample extracts were added to 0.1 ml of 2 mM ferrous chloride, 0.2 ml of 5 mM ferrozine, and 3.7 ml of methanol. The solution was allowed to react for 10 min. The absorbance at 562 nm was measured against blank. The chelating activity of the extracts on Fe²⁺ was compared with that of ethylenediaminetetraacetic acid (EDTA) (0.01 mM) and citric acid (0.025 M). The percentage of ferrous ion chelating activity was calculated as follows:

$$\text{Fe}^{2+} \text{ Chelation activity (\%)} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]}{(\text{Abs}_{\text{control}})} \times 100$$

Superoxide dismutase (SOD)

Superoxide scavenging activity was based on the inhibitory action of SOD on the rate of base-catalyzed autooxidation of pyrogallol [11]. The assay medium contained 1 ml of different concentrations of the test sample, 2 ml of water, 3 ml of 0.05 M Tris buffer, and pH 8.2, and the reaction was initiated by the addition of 0.02 ml of pyrogallol (60 mm) and the reading was recorded every 1 min at 420 NM. The chelating activity of the extracts on SOD was compared with that of quercetin. The percentage of superoxide scavenging activity was calculated as follows:

$$\text{Superoxide scavenging activity (\%)} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]}{(\text{Abs}_{\text{control}})} \times 100$$

RESULTS AND DISCUSSION

Extractive values for different solvents

The extractive values are successive with petroleum ether, chloroform, ethyl acetate, ethanol, methanol, and aqueous extract, and their percentage yield was calculated (Table 1). The increased amount of extract value indicates more solubility of the phytoconstituents. In the present study, methanol extract has shown a higher amount of value compared to all other extracts, followed by ethanol, chloroform, aqueous, and petroleum ether. Very poor extract value was observed in ethyl acetate. Based on the solvent polarity and extraction method, the

Table 1: Solvent extraction methods and yield from powder of *Eclipta alba* L.

S. No	Extracts	Extractive values in % (W/W)
1	Petroleum ether	8.01±0.18
2	Chloroform	12.58±0.24
3	Ethyl acetate	7.87±0.15
4	Ethanol	12.97±0.12
5	Methanol	14.22±0.19
6	Aqueous	11.35±0.10

Data represented a mean±SE. SE: Standard error

Table 2: Preliminary phytochemical screening of *Eclipta alba* L.

S.No	Constituents	Extracts					
		PE	CH	EA	ET	ME	AQ
1	Carbohydrates	+	+	+	+	+	+
2	Proteins	+	+	+	+	+	+
3	Steroids	+	-	+	-	-	+
4	Amino acids	+	+	+	+	+	+
5	Glycosides	+	+	+	+	+	+
6	Alkaloids	-	+	-	-	-	+
7	Flavonoids	+	+	+	+	+	+
8	Saponins	-	+	-	+	-	+
9	Tannins and phenol compounds	+	+	+	+	+	+
10	Quinones	+	-	+	-	+	+
11	Fixed oils and fats	+	+	+	+	+	+
12	Terpenes	-	-	-	-	-	-

+: Presence, -: Absence of phytochemicals. PE: Petroleum ether, CH: Chloroform, EA: Ethyl acetate, ET: Ethanol, ME: Methanol, AQ: Aqueous

Table 3: Flavonoid content of *Eclipta alba* L.

S.NO	Extracts	Content mg/g dry weight
1	Petroleum ether	0.420±0.014
2	Chloroform	0.793±0.009
3	Ethyl acetate	0.666±0.014
4	Ethanol	0.714±0.009
5	Methanol	0.875±0.020
6	Aqueous	0.608±0.011

Data are represented as mean±SE. SE: Standard error

Table 4: Antioxidant property of *Eclipta alba* L.

S.No	Extracts	IC ₅₀ (µg/ml)			
		DPPH Activity	NO scavenging activity	Fe ²⁺ Chelating activity	SOD activity
1.	Petroleum ether	65.76±0.63	96.24±0.34	72.29±0.61	108.40±0.52
2.	Chloroform	39.23±0.71	52.68±0.58	44.18±0.46	68.76±0.56
3.	Ethyl acetate	50.68±0.55	63.82±0.43	52.24±0.49	83.89±0.76
4.	Ethanol	43.88±0.76	59.67±0.56	48.51±0.39	77.34±0.56
5.	Methanol	33.10±0.38	44.10±0.45	32.74±0.55	55.74±0.63
6.	Aqueous	54.21±0.61	72.76±0.69	62.16±0.71	92.93±0.59

Mean±SE. SE: Standard error; DPPH: 1,1-diphenyl-2-picrylhydrazyl, SOD: Superoxide dismutase

yield of phytoconstituents/extract differs significantly [12,13]. Similar to our present study, extract yield was found to be high in methanol in *Eclipta prostrata* L. [14]. It has been reported that extract yield was found to be high in methanol [14].

Phytochemical screening

The present study revealed that the carbohydrates, proteins, amino acids, glycosides, flavonoids, tannin and phenol compound, and fixed oils and fats present in all extracts (Table 2). However, the steroids were present only in petroleum ether, ethyl acetate, and aqueous extracts. Alkaloids were present in chloroform, methanol, and aqueous extracts. Saponins were present in chloroform, ethanol, and aqueous extracts. Quinones were present in petroleum ether, ethyl acetate, methanol, and aqueous extracts. Terpenes were determined to be absent in all the extracts. Compared to all other solvent extracts, aqueous extracts were shown high values of secondary metabolites. Phytochemical screening using different solvents has been reported on this plant [15,16]. The phytoconstituents such as flavonoids, tannins, and phenolic compound extracts were liable for its antioxidant property.

Determination of total flavonoid content

Medicinal plants are an important source of antioxidants [17]. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of certain diseases [18]. Flavonoids are phenolic acids, which serve as an important source of antioxidants found in different medicinal plants and related phytomedicines [19]. The antioxidant activity of flavonoids is due to their ability to reduce free radical formation and scavenge free radicals. The total flavonoid content analysis of the present study was found higher in the methanolic extract (0.875±0.020 mg/g) followed by chloroform (0.793±0.009 mg/g) and ethanol (0.714±0.009 mg/g) (Table 3).

Flavonoids are one of the secondary metabolites of medicinal herbs and it is also known as Vitamin P [20]. It is also found to have anti-inflammatory, and anticancer activity [21]. The total flavonoid content was determined and expressed as mg against the standard equivalents quercetin.

DPPH radical scavenging activity

The antioxidant potential of different solvent extracts of *E. alba* was determined using DPPH radical scavenging activity. DPPH is nitrogen-centered, stable free radical which accepts an electron or hydrogen when reacts with a suitable reducing agent, The sample gets decolorized when reacts its DPPH, which represents the reducing activity [22,23]. The antioxidant potency of the sample along with ascorbic acid revealed the radical scavenging activity with IC₅₀ values of 13.58±0.38 µg/ml (Table 4). The DPPH radical scavenging activity of crude drug showed the highest percentage of antioxidant activity with IC₅₀ values of 65.76±0.63 µg/ml, 54.21±0.61 µg/ml, and 50.68±0.55 µg/ml in petroleum ether, aqueous, and ethyl acetate extract, respectively. From the overall solvent, a comparison study reveals that maximum radical scavenging activity was shown in the petroleum ether extract followed by aqueous extract and minimum activity was shown in methanol extract.

NO scavenging activity

The NO scavenging activity of the present study was performed against standard catechin. Maximal percentage of scavenging activity

along with catechin standard was observed in petroleum ether extract with IC₅₀ value of 96.24±0.34 µg/ml, followed by aqueous and ethyl acetate extract with IC₅₀ value of 72.76±0.69 and 63.82±0.43 µg/ml, respectively (Table 4). The structure and function of cells may be altered due to excess generation of NO in tissues [24]. The concentration of NO depends on the disease affection [25]. Flavonoid and phenol phytoconstituents are the cause for the observed NO scavenging activity of our study.

Fe²⁺ chelating activity

In the present study, iron chelating activity of petroleum ether, chloroform, ethyl acetate, ethanol, methanol, and aqueous extracts of *E. alba* L was determined against standard EDTA. Maximum IC₅₀ value of 72.29±0.61 µg/ml was observed in the petroleum ether extract, and the minimum chelating activity was observed in the methanol extract with IC₅₀ values of 32.74±0.55 µg/ml (Table 4).

The present study illustrates that antioxidant property and metal chelating properties of plant extract are may be due to flavonoid content. Fe²⁺, a transition metal ion, transfers a single electron during the propagation of radical reaction with relatively non-reactive radicals [26]. Chelating agents functions as a secondary antioxidant, by reducing redox potential and stabilizing metal ion oxidation, while metal bonding [27].

SOD activity

Cellular enzyme SOD was studied in the present study to determine radical scavenging activity property. Table 4 depicts the scavenging activity of *E. alba* different solvent extracts against standard rutin, which showed IC₅₀ of 32±0.69 µg/ml. The highest percentage of activity was observed in the petroleum ether extract (IC₅₀=108.40±0.52 µg/ml). The least percentage radical scavenging activity was observed in the methanol extract (IC₅₀=55.74±0.63 µg/ml). Reactive oxygen species protect the living system by antioxidant enzymes such as SOD, catalase, lipid peroxide, and other endogenous antioxidant sources [15]. SOD is a potent enzymatic antioxidant defense system [28]. Very high and significant correlation was observed between reducing power and antioxidant activity. A similar relationship was also found between antioxidant potential and reducing the power of different plant extract [29].

CONCLUSION

It can be concluded from the study that the petroleum ether extract showed significant antioxidant potential compared to other solvent extract studied. However, the extracted value was high in methanol extract. Thus, the potency may be due to the presence of polyphenolic compound (flavonoid). Further studies may reveal the potential of *E.alba* L.

AUTHORS' CONTRIBUTIONS

Thenmozhi M: Corresponding author, research work, and typing, Jayanthi M: Suggestion and correction.

CONFLICTS OF INTEREST

The authors have no conflicts of interest.

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