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Original Article

PHYTOCHEMICAL SCREENING AND ANTIOXIDANT POTENTIAL OF *ECLIPTA PROSTRATA* (L) L-A VALUABLE HERB

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ABSTRACT

Objective: In this study, phytochemical screening, antioxidant activity, total phenolic, flavonoid contents and reducing potential of *Eclipta prostrata* (L.) L plant prepared in different solvents (methanolic, ethanolic and double distilled water) was evaluated by various assays.

Methods: Polyphenolic contents, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity, Hydrogen peroxide (H_2O_2) radical scavenging activity and reducing power of the plant was estimated by modified protocol.

Results: In DPPH free radical, methanolic extract of plant was most potent in activity with 50% inhibition at 333.34 µg/ml and 398.12 µg/ml concentration respectively. Total phenolic content was maximum in the methanolic extract of plant (52.5±0.64 mg/g of gallic acid equivalent) and total flavonoid was maximum in aqueous extract of plant (193.12±1.65 mg/g of rutin equivalent). High reducing capacity of plant was observed in case of methanolic extract. A significant correlation was found between antioxidant activity and polyphenolic content (total phenols and total flavonoids). Moreover, a significant correlation was found between antioxidant activity and reducing potential of plant extract, denoting that reducers are important contributors to antioxidant.

Conclusion: The study shows *E. prostrata* as an important natural source of antioxidants.

Keywords: *Eclipta prostrata*, Plant extract, Antioxidant activity, Polyphenolic content, DPPH activity, H_2O_2 scavenging activity, Reducing potential

INTRODUCTION

Medicinal plants have been used in folk medicine since date back in different part of the world against a variety of diseases. The interest in medicinal plants for their phytochemical constituents, biological activities and antioxidant activity have been studied since long back due to the absence of side effects and its economic viability [1]. The practice of using plants as a source of food and medicine has recently showed revival of interest globally. Oxidative stress produces free radicals. Free radicals like reactive oxygen species (ROS) and reactive nitrogen species (RNS) are constantly formed in an organism during the process of cellular respiration and from sources like pollution, ionizing radiation and drugs. These radicals contribute in the development of many diseases like Alzheimer, cancer, heart disease and rheumatoid arthritis [2]. Thus, when the endogenous antioxidant becomes inadequate for free radicals balancing, a demand for exogenous antioxidants is increased in the body to prevent oxidative damage of cells and tissues [3]. The plant contains numerous free radical trapping molecules like phenols, flavonoids, carotenoids and vitamins which are medicinally important [4]. Phenolic groups quench free radicals by hydrogen donation [5].

Antioxidants are naturally occurring molecule which inhibits the oxidizing chain reactions by delaying or inhibiting the oxidation of molecules [6]. These antioxidants are used to maintain food quality by arresting the oxidative deterioration of lipids. They slow down the chronic disease by enhancing the body's natural antioxidant defences by adding with dietary antioxidants [7]. Natural antioxidants are preferred over synthetic antioxidants for not only as an eco-friendly approach but also for its low manufacturing than artificial antioxidant having multifarious benefits [8, 9]. Enormous research opportunities exist in the field of plants antioxidant and their effects on human health. Antioxidant activities are present due to biologically active polyphenol components such as flavonoids and phenolic acids [10].

The extracts of various plant species show preventive action against anti-inflammation, cancer, cardiovascular, and neurodegenerative disease [11]. The plant based natural antioxidants like alkaloids, flavonoids, phenols, tocopherols in foods is gaining much attention [12]. These natural biologically active agents exhibit anti-carcinogenic potential and offer diverse health-contributing effects because of their antioxidative and antimicrobial attributes [13]. *Eclipta prostrata* is a medicinal herb widely distributed in tropical and sub-tropical region of Asia. It is a valuable medicinal herb reported for its various pharmacological and biological activities like hair growth promotion [14], hepatoprotective [15], antidiabetic [16], antimicrobial property [17] and anticancerous activity [18]. Phytochemical study and reducing potential was not earlier reported from the whole plant. The objective of the present work was to study phytochemical screening, polyphenolic content and antioxidant activity of the plant. Reducing potential of *E. prostrata* was evaluated for the first time in methanolic, ethanolic and aqueous extracts derived from the plant.

MATERIALS AND METHODS

Chemicals

2,2-Diphenyl,1-picryl hydrazyl (DPPH), ascorbic acid, gallic acid, rutin, trichloroacetic acid (TCA), potassium ferricyanide ($K_3Fe(CN)_6$), ferric chloride ($FeCl_3$), Folin-Ciocalteu phenol reagent, aluminium chloride ($AlCl_3$), rutin, sodium carbonate (Na_2CO_3), sodium potassium tartarate (Na-K tartarate) were purchased from Hi-Media Ltd and Merck.

Plant collection

Plant of *E. prostrata* was collected from the campus of Banaras Hindu University, Varanasi, during the months of June-July. Plant material was collected from a different location to make a composite sample. The plant was authenticated at Botanical Survey of India (BSI), Allahabad. Collection number BHU-171 and voucher number-91926 was given by BSI to the plant flora.

Percentage yield

Plants were washed thoroughly for dust removal under running tap water. The plant material was oven dried at 40-45 °C for 24 h. The

plant was crushed in the electrical grinder. Extraction was done from 20 g of whole plant powder consisting of root, stem and leaf in 250 ml of the solvent by using a Soxhlet apparatus for 12 h. Extracts was then filtered and evaporated to dryness at 40 ºC in the rotatory evaporator. Extracts were stored at 4 °C till use. Percentage yield (w/w) of crude extract was calculated with the original amount of coarse powder used for extraction [19].

Preparation of sample extract

One gram of plant extract was dissolved in 10 ml of respective solvent to make a stock solution of 100 mg/ml. Extracts were further diluted as per requirement in different experiments.

Phytochemical screening

The initial phyto-compound testing of the various solvent extract was performed using standard protocol [20-21].

DPPH free radical scavenging activity

The free radical scavenging activity of methanolic, ethanolic and aqueous extracts of the plants was measured by 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) assay [22]. Different concentration of plant extracts (100-600 µg/ml) were added to 3 ml of DPPH solution in methanol (0.25 mM). The mixture was incubated for 10 min at room temperature in the dark, after which the absorbance was measured at 517 nm by using spectrophotometer (Thermo Scientific UV 1). Ascorbic acid was used as a standard and methanol acted as blank.

Hydrogen peroxide radical scavenging activity

The hydrogen peroxide (H_2O_2), the radical scavenging activity of methanolic, ethanolic and aqueous extracts of the plants, was measured according to Ruch *et al.*, [23]. The plant extracts of different concentration (100-600 µg/ml) in the volume of 1 ml were added to 2 ml of H_2O_2 (40 mM) prepared in (50 mM, pH-7.4) phosphate buffer. The plant samples were incubated for 10 min at room temperature. The absorbance was determined at 230 nm against a blank solution containing phosphate buffer only (Thermo Scientific UV 1). Ascorbic acid was used as a standard.

Estimation of total phenolic content and flavonoid content

Total phenolic content (TPC) was measured by Folin–Ciocalteu assay [24]. In brief, 0.5 ml of plant extract (200 µg/ml) prepared with different solvent (methanol, ethanol and water) was mixed with 0.5 ml Folin-Ciocalteu reagent and 3 ml of 20% Na_2CO_3. Absorbance was taken at 650 nm. The standard graph was prepared with gallic acid as a reference compound and expressed as mg/g of gallic acid equivalent (GAE).

Total flavonoid content (TFC) was determined by$AlCl_3$ colorimetric method [25]. The plant extract (1 ml, 200 µg/ml) prepared with different solvent (methanol, ethanol and water) was taken in which 100 µl $AlCl_3$ (10% w/v), 100 µl Na–K tartrate and 2.8 ml distilled water were added and kept for 30 min. Absorbance was taken at 415 nm. The standard graph was prepared with rutin as a reference compound and expressed as mg/g of rutin equivalents (RE).

Reducing potential capacity (RPC)

The reducing capacity assessment of methanolic, ethanolic and aqueous plant extract was determined according to the method of Athukorala *et al.*, [26] with minor modifications. The extracts were used at different concentrations (50-300 µg/ml) prepared in 1 ml of different solvents were mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide [$K_3Fe(CN)_6$] (30 mM). The mixture was incubated at 50 °C for 20 min. Then, 2.5 ml TCA (0.6 M) was added to the mixture which was then centrifuged for 10 min at 3000 rpm. The upper organic layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 1 mM) and the absorbance was measured at 700 nm by a spectrophotometer. Ascorbic acid was used as a standard.

Determination of percentage and statistical analysis

Percentage scavenging activity (%) was calculated by the formula.

$$\text{Inhibition } \% = \frac{C - T}{C} \text{ X } 100$$

Where, C is the absorbance of control, T is absorbance of the test samples/standard. The antioxidant activity of the extract was expressed as IC_{50}. IC_{50} was calculated through linear regression analysis. IC_{50} value is the concentration (in µg/ml) of extract that inhibits the formation of radical by 50 %. The above experiments were performed four times in independent manner and repeated for thrice. Data was represented as mean±standard error (SE) by applying one-way Analysis of Variance (ANOVA). Tukey's multiple range tests were used for separation of means when ANOVA was significant ($p \leq 0.001$) (SPSS 16.0; Chicago, IL, USA). The graphs were prepared in Sigma plot 11.0.

RESULTS AND DISCUSSION

Percentage yield

The maximum percentage yield of extract was 42.8%, 39.2% and 36% for aqueous, methanolic and ethanolic extract respectively. The percentage yield of plant extract varied significantly in different extraction solvents. Variation in % yield in the different solvent may be due to various degree of solubility of crude extract in different polarity solvents. Almost similar 33.66 % yield in methanolic extract of aerial part of the same plant was reported [27]. Percentage yield of various plant extracts depends upon the extraction conditions such as solvent polarity for the production of a fraction with adequate yield [19].

Phytochemical screening in various solvents

In the present study, the phytochemical testing showed the presence of various bioactive compounds like carbohydrate, alkaloids, tannins, steroids and flavonoids (table 1). There are other reports of screening of metabolites from the leaf, stem and roots of this plant in other solvents [28]. The distribution of bioactive compounds varies in plant and extraction solvents play an important role in screening. Medicinal herbs are rich in phenolic compounds, flavonoids, tannins; lignin and other compounds [29] which may be responsible for the antioxidant activities of the extract [30].

Table 1: Phytochemical screening of plant in different solvents

Phytochemicals	Test performed	Methanolic extract	Ethanolic extract	Aqueous extract
Carbohydrate	Fehling test	+	-	+
Phenols	Ferric chloride test	+	+	+
Flavonoids	Ammonia test	+	+	+
Alkaloids	Wagner's test	+	+	-
Steroids	Salkowski test	+	+	+
Tannins	Lead acetate test	+	+	-
Saponins	Frothing test	+	+	+
Glycosides	Nitroprusside test	+	+	+
Amino acids	Ninhydrin test	+	+	+

Note: += Presence; -= Absence of phytochemicals

Table 2: Antioxidant activity of *E. prostrata* by DPPH free radical scavenging method in different solvents

Concentration(µg/ml)	Percentage inhibition (mean±SE)			
	Methanolic	Ethanolic	Aqueous	Ascorbic Acid
100	24.11±0.39f	18.15±0.56f	15.99±0.42f	25.12±0.29f
200	36.69±0.59e	22.68±0.64e	23.91±0.37e	39.34±0.20e
300	48.86±0.32d	33.61±0.46d	36.09±0.42d	56.25±0.22d
400	58.41±0.24c	55.75±0.82c	47.42±0.24c	65.15±0.14c
500	68.65±0.37b	61.49±0.49b	54.80±0.55b	86.47±0.38b
600	73.40±0.53a	69.34±0.46a	63.92±0.37a	95.22±0.32a
IC_{50}	333.34	366.46	448.16	271.47

Data represented as mean±SE (n=4). One way ANOVA followed by Tukey's test. All data is significant at p<0.001; a,b,c = different letter shows significant difference between means.

Antioxidant DPPH activity

Antioxidant capacity is one of the most important parameters for quality check of medicinal bioactive and functional components in the plant. In the present study, different solvents i.e. water; methanol and ethanol were used for extraction. All extracts of *E. prostrata* plant prepared in different solvent showed significant free radical scavenging activity (table 2). The methanolic extract (IC_{50}=333.34 µg/ml) of the plant showed highest scavenging activity followed by ethanolic (IC_{50}=366.46 µg/ml) and aqueous (IC_{50}=448.16 µg/ml**)** extract**.**

DPPH radical scavenging activity is a simple and fast method to screen the scavenging potentials of plant sample [31]. DPPH is neutralized by antioxidants present in extract due to their ability to donate electrons. The degree of DPPH scavenging can be used to signify the hydrogen-donating capacity of antioxidants. The reduction in absorbance is the indicator of the increased antioxidant capacity of the extract [32]. The bioactive compounds present in plant extracts were able to discolour DPPH solution turning its purplish colour to yellowish coloured diphenyl-picryl hydrazine at low concentration. Ascorbic acid has also been used as standard antioxidant to determine the IC_{50} of extracts in other plants [33]. Similar pattern of antioxidant activity was observed in methanolic leaf extract of *E. prostrata* [34].

Antioxidant H_2O_2 scavenging activity

All the extracts of this plant have shown significant scavenging activity (table 3). The scavenging activity of the methanolic extract was found highest (IC_{50}=398.12 µg/ml) followed by ethanolic (IC_{50}=449.12 µg/ml) and aqueous (IC_{50}=473.96 µg/ml) extract**.**

Table 3: Antioxidant activity of *E. prostrata* by H_2O_2 radical scavenging in different solvents

Concentration(µg/ml)	Percentage inhibition (mean±SE)			
	Methanolic	Ethanolic	Aqueous	Ascorbic Acid
100	23.17±0.20f	20.16±0.45f	19.36±0.24f	25.86±0.38f
200	31.00±0.23e	28.39±0.32e	24.10±0.42e	36.80±0.30e
300	48.81±0.38d	40.10±0.41d	35.89±0.40d	48.33±0.31d
400	50.64±0.42c	47.87±0.35c	45.69±0.23c	57.07±0.29c
500	57.49±0.44b	56.02±0.47b	52.90±0.23b	61.87±0.39b
600	60.95±0.37a	58.89±0.43a	59.30±0.20a	74.16±0.38a
IC_{50}	398.12	449.12	473.96	342.56

Data represented as mean±SE (n=4). One way ANOVA followed by Tukey's test. All data is significant at p<0.001. a,b,c = different letters shows significant difference between means.

The plant extracts scavenge H_2O_2 by donating an electron to hydrogen peroxidase, thereby converting it into the water. In this assay, methanolic extract of *E. prostrata* showed greater inhibition in comparison to aqueous extract. It may be due to the presence of higher phenolics in methanolic extract of the plant. This suggests that aqueous extract required significantly high concentration to show its potency. H_2O_2 radical scavenging activity was also reported from different extracts of *Crataegus monogyna* [35].

Total phenolic and flavonoid content

Total phenolic content was reported as mg/g of GAE in reference to a standard curve (y=0.001x+0.05, R^2=0.997). TPC was found higher in methanolic (52.5±0.64) followed by ethanolic (44.25±1.75) and aqueous (13.25±0.85) extract of *E. prostrata*. All TPC value was represented as mg/g of GAE.

Total flavonoid content was calculated by a standard curve (y=0.0008x+0.198, R^2=0.994) and reported as mg/g of RE. Total flavonoids were found higher in aqueous (193.12±1.65 mg/g) extract followed by ethanolic (162.5±1.73 mg/g) and then methanolic extract (144.62±1.62 mg/g) (table 4).

The phenolic and flavonoids present in the plants are secondary metabolites which act as antioxidants and help in free radical scavenging, metal ions chelation [36] and protects against human disease like cardiac-disorder, thrombosis, hepatotoxicity, anti-carcinogenic, anti-mutagenic, etc [37]. The phenolic content was found to be maximum in the methanol extract of the plant. Similarly, higher phenolic content in an organic solvent has also been reported [38]. The presence of secondary metabolites like phenol and flavonoid contents in plant extract depend on the solvent used [39, 40].

In this study, flavonoids content showed the highest activity in aqueous extract, it might be due absence of tannin and alkaloid in an aqueous solvent as observed in screening experiment. Flavonoids reported to be responsible for antioxidant activities of the plant through their scavenging activity [41]. Flavonoids might show higher antioxidant activity in aqueous extract due to structure and substitution pattern of hydroxyl group.

Reducing potential capacity

The reducing potential capacity of the plant extracts increased as per their concentration in all the three solvents. The methanolic extract showed maximum reducing power followed by ethanolic extract and the minimum was found in aqueous extract. Increased absorbance of the reaction mixture indicated an increase in reducing power capacity (fig. 1).

In this assay, the colour of plant extracts solution changes from yellow to a range of green and blue colour. The reducing capacity of plant extract acts as an indicator of its antioxidant activity [42]. Similar to antioxidant activity, the reducing potential also increases in a concentration-dependent manner [43].

Correlation between antioxidant activity and polyphenolic compounds

The positive, significant and linear correlation was found between total antioxidant activity and polyphenolic contents (TPC & TFC) of various extracts (fig. 2, A-C). Correlation coefficient (R^2) values of different extract showed very high correlation between antioxidant activities and TPC and TFC content (R^2 ranges from 0.965 to 0.999). Thus, it can be said that there found good correlation with antioxidant activity and total phenolic and flavonoid content of *E. prostrata* in this study.

A significant correlation between the polyphenolics and total antioxidant capacity has been reported in another plant system [44].

Table 4: Total phenolic and flavonoid content in *E. prostrata* in different solvent

Total polyphenolic content		
Plant extract	**TPC (mg/g GAE)**	**TFC (mg/g RE)**
Methanol	52.5±0.64a	144.62±1.62c
Ethanol	44.25±1.75b	162.5±1.73b
Aqueous	13.25±0.853c	193.12±1.65a

Data represented as mean±SE (n=4); One-way ANOVA followed by Tukey's test. All data is significant at $p<0.001$; a,b,c = different letters shows significant difference between means.

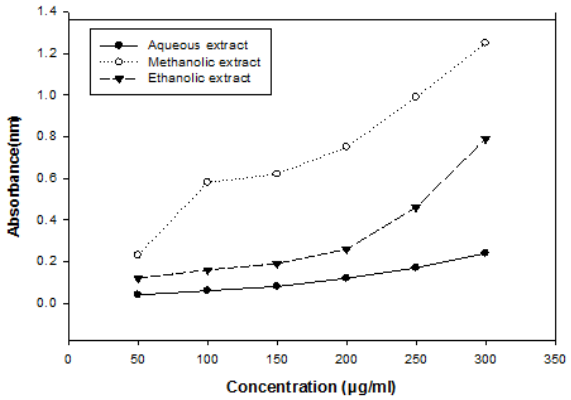
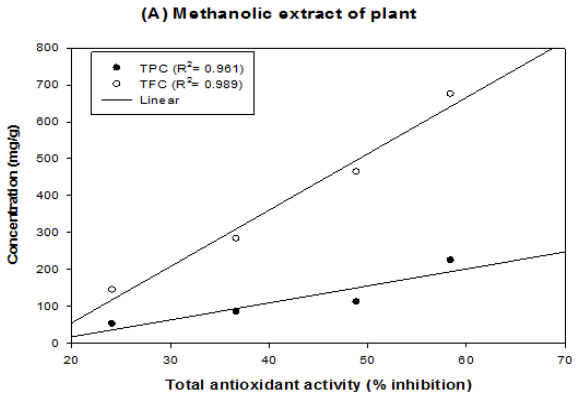
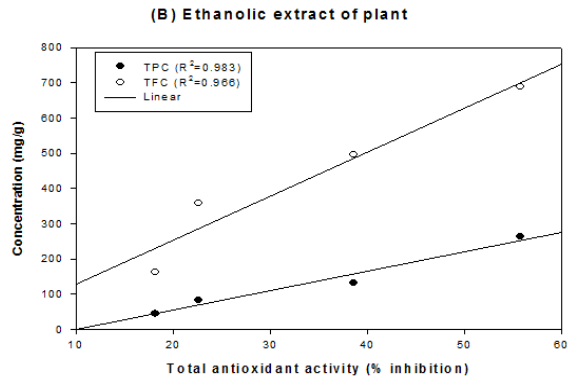


Fig. 1: Reducing power capacity of plant extract of *E. prostrate*





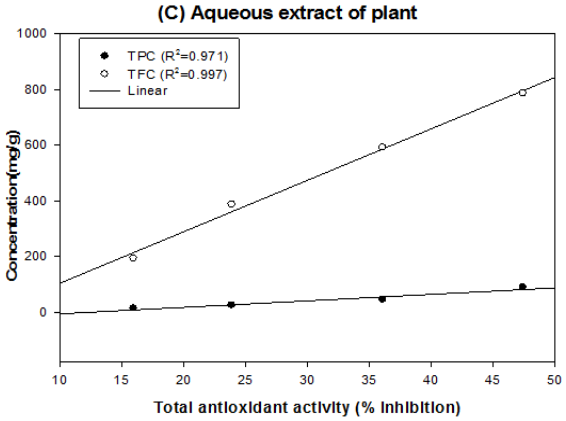
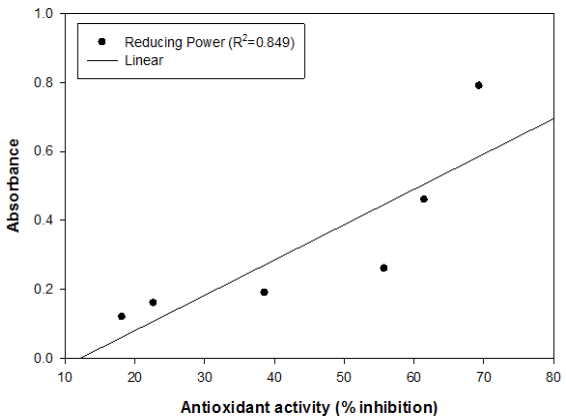


Fig. 2: Correlation between antioxidant activity and polyphenols (TPC and TFC) of *E. prostrata* (A) Methanolic extract of plant (B) Ethanolic extract of plant and (C) Aqueous extract of plant

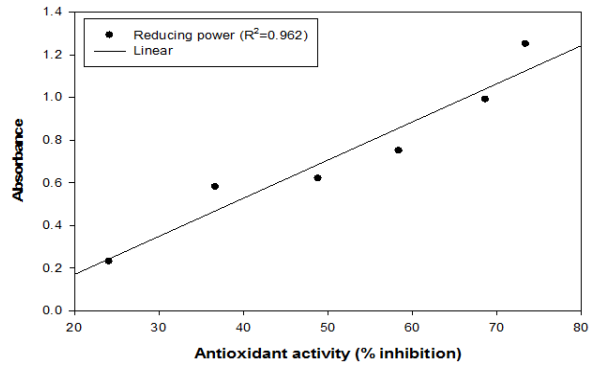
Correlation between antioxidant activity and reducing power

A significant correlation was obtained between total antioxidant activity and reducing the potential of plant extract.

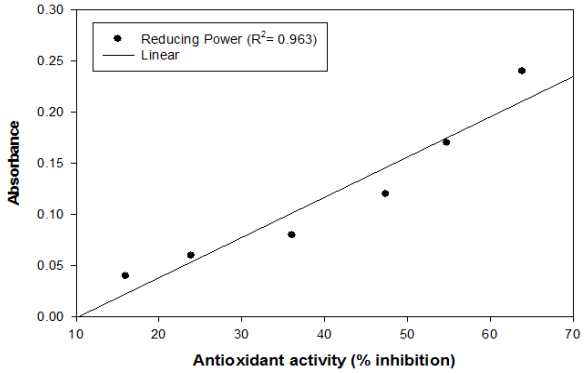
The correlation coefficient (R^2) between antioxidant activity and reducing potential was found (R^2=0.849), (R^2=0.962) and (R^2=0.963) for methanolic, ethanolic and aqueous extract respectively (fig. 3A-C).



(A) Methanolic extract



(B) Ethanolic extract



(C)Aqueous extract

Fig. 3: Correlation between antioxidant activity and reducing power of *E. prostrata* (A) Methanolic extract of plant (B) Ethanolic extract of plant and (C) Aqueous extract of plant

In this study reducing potential and antioxidant activity of extract increased with increase in extract concentration. So, 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 [45].

CONCLUSION

Our study report that whole plant extract of *E. prostrata* plant is a rich source of antioxidant compounds. The antioxidant property, reducing power and polyphenolic content varied significantly in the different extraction solvent. For most of the activity, organic solvents gave more promising results than aqueous one. Thus, extracts of this plant can be used for relieving the oxidative damage caused by stress and may help in preventing aging. Therefore, this plant can be recommended to be used for the production of nutraceuticals and pharmaceuticals.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

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