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Research Article

ANTIOXIDANT POTENTIAL OF METHANOLIC EXTRACT OF *ECLIPTA ALBA*, AN INDIAN TRADITIONAL MEDICINAL PLANT

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ABSTRACT

Now a day's demand is for identification of natural antioxidants due to their advantages over the synthetic ones. Therefore the objective of this present investigation is to study the antioxidant potential of the methanolic extract of *Eclipta alba.* The plant *E. alba* is an annual herb, known to address several hair problems and anti-ageing and hepato-protective properties. The free radical scavenging ability of *E. alba* was carried out using the different *in-vitro* models such as DPPH, ABTS, hydrogen peroxide and nitric oxide assays. Screening of phytoconstituents confirms the presence of tannins, phenolics, flavonoids and alkaloids which are the main responsible for the antioxidant property. The obtained results were indicating the strong antioxidant potential of the extract when compared to the standard in almost all the assays studied. The current study provides preliminary evidence to advocate the importance of *E. alba* as an important medicinal plant.

KEYWORDS: *Eclipta Alba,* Free Radical Scavenging Ability, Phyto-Constituents, Medicinal Plant.

INTRODUCTION

During the recent decade there has been significant attention towards the area of free radical chemistry and antioxidant studies[1]. Reactive oxygen species such as hydrogen peroxide, nitric oxide, hydroxyl radical, superoxide and hydroxyl ion, are produced as a byproduct of cellular activity[2]. The accumulation of free radicals in the cells causes adverse effects like damage DNA, proteins and lipids synthase, leading to cell death. FAD, NADPH, etc., plays a vital role in controlling Reactive oxygen species (ROS)[3]. Biological activities of ROS have proven to be toxic to cells. By definition, radicals possess an unpaired electron which makes them highly reactive and thereby able to damage all macromolecules including lipids proteins and nucleic acids [4].

Many herbal plants contain antioxidant compounds which protect the cell against degenerative effects of ROS which is free radicals such as singlet oxygen, superoxide, peroxyl, radicals, hydroxyl radicals[5]. The concept of oxalate stress is the loss of balance between ROS production and antioxidant defenses which results in deregulation of the cellular function leading to various diseases like arthritis, asthma, diabetes etc.[6,7] Antioxidant is the substance that neutralizes free radicals and their actions, by natural antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxides, glutathione reductases, thioredoxin thiols and disulfides bonding which form the bonding system in every cell . Alpha-tocopherol is a chain breaking antioxidant which prevents the propagation of free radicals reaction in all cell membrane in the human body. Other non-enzymatic oxidant includes carotenoid, flavonoid and related polyphenols, alpha lipoic acid and glutathione.

It has been found that oxidative stress is among the major contributing factors in the indication of many chronic and degenerative diseases including atherosclerosis, diabetes mellitus, cancer, immune dysfunctions and is involved in ageing. There is growing attention towards natural antioxidant from herbal source. Epidemiologically and *in vitro* studies on medicinal plants and vegetables strongly have supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in a biological system. Since the beneficial effects of antioxidant are abundant to human health, synthetic antioxidant such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are often added to food. Due to carcinogenicity, synthetic antioxidants are known to prompt liver and kidney dysfunction. Hence, interest in discovering naturally occurring antioxidants for application in food or medicine to replace synthetic antioxidant such as BHA and BHT has intensified remarkably.

Plant-derived secondary metabolites such as phenols, flavonoids, terpenoids, steroids, etc., are found to have antioxidant propriety activity. More than 8000 different structures of phenolic compounds with antioxidant activity are known to be present in secondary metabolites secreted by plants. Stem, leaves, fruits etc., of the plant kingdom, are the major source of phenolic compounds. Phenolic compounds possess one or more aromatic ring and hydroxyl group respectively. These compounds are one among the well-established compounds used in Ayurveda and Homeopathic medicine, for the treatment of heart disease. These compounds diminish the development of atherosclerosis through acting as antioxidants towards the low-density lipoprotein, and phenolic content was also determined to evaluate their probable contribution to the total antioxidant capacity.

The plant *Eclipta alba* is an annual herb belonging to family *Asteraceae*. The leaves of the plant are opposite, pointed and sessile. It is additionally referred to as Bringaraja and

Karisalakanni that is found as a standard weed throughout India ascending to 6000 feet. *Eclipta alba* has been utilized in numerous components of tropical and sub-tropical regions like Africa, South America, Asia etc. The plant is usually utilized in hair tonic everywhere in India for healthy black and long hair. Several *in vitro* studies describe its anti-ageing and anti-hepatotoxic properties.

The safety, efficacy and quality of some of the bioactive principles have been scientifically validated, and antioxidant activity was also studied for the isolated phytochemical present in it. Therefore the objective of this present investigation is to study the free radical scavenging ability of *E. alba* using the different *in-vitro* model in search of preliminary experiments evidence to advocate the importance to use *E. alba*.

MATERIALS & METHODS

Plant Material Collection and Preparation of the Extract

The whole plant of *Eclipta alba* was collected from Mysore Karnataka during July-August, 2018. The whole fresh plant of *E. alba* was washed with running tap water, cut into small pieces followed by shade drying at room temperature and ground into fine powder using a blender. For extraction, 30 g of fine dry powder was loaded in soxhlet apparatus with 250 ml of methanol and process was continued until a drop of solvent from the siphon tube does not leave residue when evaporated [8,9]. Finally, the solvent was removed from the yield by using rotary evaporator at a temperature of 40° C.

Phytochemical Analysis

The preliminary phytochemical screening of *E. alba* was carried out according to the standard methods. The presence of important phytochemicals like flavonoids, terpenoids, phenols, saponins and tannins was evaluated. Standard protocols were employed for the confirmation of the phytochemicals such as flavonoids, tannins and phenolic compounds with ferric chloride test and gelatin test, terpenoids with Liebermann Burchard`s analysis and saponins with the ability to produce stable foam[10].

Estimation of Total Antioxidant Assay

Determination of Scavenging Effect on DPPH Radicals

The scavenging effects of the samples for DPPH radical were monitored according to the technique described by Brand-Williams[11]. 2.5 ml of the test sample was added to 2.5 ml of 0.18 mM DPPH methanol solution. The mixture was then vortexed for 1 minute and then left to stand at room temperature for 30 minutes in the dark and its absorbance read at 520 nm, the ability to scavenge of DPPH radical was calculated using the following equation:

$$\text{Scavenging (\%)} = \frac{(A0 - A1)}{A0} X\ 100$$

where,

A_0 = absorbance of a standard that was prepared in the same conditions, but without extract,

A_1 = absorbance of plant extract samples.

ABTS Assay

Antioxidant activity of *E. alba* extracts as per ABTS decolourisation assay was measured using the method reported by Shah[12] with some modification[13,14]. The working solution of ABTS radical was made by reacting ABTS (9.5 ml,7mM) with potassium persulfate (245 µl, 100mM), and the volume was made up to 10ml with distilled water. The solution was kept in the dark at room temperature for 18h and then diluted with potassium phosphate buffer (0.1 M pH 7.4) to get an OD of 0.7 at 734 nm. The plant sample was prepared in methanol with dilution 20-100 µg/ml. The sample (10µl) was placed in a test tube and mixed thoroughly with 2.99 ml ABTS radical working solution. The absorbance of the resulting clear mixture was recorded at 734 nm. The per cent antioxidant activity of the sample was determined using the following equation:

$$\text{Scavenging (\%)} = \frac{(A0-A1)}{A0} X\ 100$$

where

A0 = Absorbance of ABTS + radical

A1 = Absorbance of extract or standard

where A0 and A1 is the absorbance of the control and the sample, respectively. 10 µl of the methanol in place of the sample was used as the control.

Hydrogen Peroxide Scavenging Assay

Hydrogen peroxide is the least reactive molecule among reactive oxygen species and is stable under physiological pH and temperature in the absence of metal ions. It can be generated through a dismutation reaction from superoxide anion by superoxide dismutase; it can produce the radical hydroxyl ion in the presence of metal ions[15]. A solution of 40mM H_20_2 was prepared in phosphate buffer (pH 7.4). The different extracts of *E. alba* fruit extract (1µg/ml) were added to the hydrogen peroxide solution (0.6 ml). After 10 minutes of incubation, the absorbance of hydrogen peroxide at 230 nm was determined against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as the standard.

Nitric Oxide Scavenging Assay

Nitric oxide radical scavenging activity was measured spectrophotometrically[16]. 1.0 ml of sodium nitroprusside in phosphate buffer was mixed with the different concentration of extract mg/ml in phosphate buffer the tubes were then incubated at 25 °C for two hours. At the end of the second hour, 1.5 ml of the reaction mixture was removed and diluted with 1.5 ml Griess reagent the absorbance was immediately measured at 546 nm. The tube without extract was taken as control.

$$\text{Scavenging (\%)} = \frac{(A0 - A1)}{A0} X\ 100$$

Where

A0 = Absorbance of control reaction; A1 = Absorbance of test

Statistical Analysis

The radical scavenging activity data is analyzed by mean ± SE subjected to univariate analysis. $P < 0.0001$ by 2 way ANOVA test using the trial version of graph pad prism.

RESULTS

Phytochemical Analyses

Preliminary biochemical screening of *Eclipta alba* extract has indicated in Table 1. The extract shows the presence of various phytoconstituents such as tannins, phenolics, flavonoids and alkaloids which are responsible for the rich antioxidant property of the extract.

DPPH Radical Scavenging Assay

DPPH radical scavenging activity was found to increase with an increase in concentrations of *Eclipta alba* extract (Table.2). Decrease in absorbance as a result of the antioxidative effect of soluble solids of *E. alba.* The highest % scavenging activity was found was 81.31% at 500 µg/ml concentration which is almost equal to the standard (control) at given concentration.

ABTS Radical Scavenging Activity of *Eclipta alba*

The presence of antioxidants in the tested extract of *Eclipta alba* shows the reduction in ferricyanide complex to ferrous state, it shows that rising concentration of the extract resulted in a simultaneous increase of reducing power. The highest reducing

ability was found with *Eclipta alba* extract at 57.69% at 500µg/ml (table 3). It was found that the reducing ability of the extract was increased with increase in the concentration.

Hydrogen Peroxide Assay

The antioxidant potential of *Eclipta alba* extracts was investigated by *in vitro* hydrogen peroxide scavenging experiment, and the obtained results are shown in Table .4. In this study, extracts were subjected to evaluate the ability of different solvent fractions to scavenge the hydrogen peroxide radicals. From the result, it was found that the % scavenging activity of methanolic fractions of *E. alba* was appreciable (60.17%) at higher concentration studied (500 µg/ml); which is compared with standard ascorbic acid (60.87%) in the process of scavenging hydrogen peroxide radicals.

Nitric Oxide Radical Scavenging Activity of *Eclipta alba*

Nitrous oxide radicals generated from sodium nitroprusside at physiological pH were significantly inhibited by *Eclipta alba* extract. Per cent inhibition was concentration dependent and maximum at 500 µg/ml concentration of the extract. The highest scavenging effect found with *Eclipta alba* extract was 52.7% as shown in table 5.

TABLE 1: PHYTOCHEMICAL ANALYSIS

Phytochemical Test	Result
Tannin	+
Phenolic	+
Flavonoid	+
Alkaloids	+
Steroids	-
Saponins	-

+ Presence; - Absent

TABLE 2: PER CENT SCAVENGING ACTIVITY OF DPPH BY ASCORBIC ACID

Sample concentration (µg/ml)	Scavenging activity (%)	
	Eclipta alba	Ascorbic acid (control)
100	30.75±1.15	34.43
200	46.30±0.62	48.19
100	64.1±0.78	66.41
400	71.37±0.78	77.13
500	81.31±0.60	80.31

TABLE 3: PER CENT SCAVENGING ACTIVITY OF ABTS BY ASCORBIC ACID

Sample concentration (µg/ml)	Scavenging activity (%)	
	Eclipta alba	Ascorbic acid (control)
100	31.43±0.46	32.61
200	40.44±0.43	41.07
300	46.66±0.55	53.44
400	51.21±0.63	56.44
500	57.69±0.81	65.44

TABLE 4: PER CENT SCAVENGING ACTIVITY OF HYDROGEN PEROXIDE ASSAY

Sample concentration (µg/ml)	Scavenging activity (%)	
	Eclipta alba	Ascorbic acid(control)
100	25.79±0.33	29.86
200	30.67±036	35.80
300	45.04±0.36	45.83
400	52.64±1.52	56.07
500	60.17±0.39	60.87

TABLE 5: PER CENT SCAVENGING ACTIVITY OF NITRIC OXIDE RADICAL SCAVENGING ASSAY

Sample concentration (µg/ml)	Scavenging activity (%)	
	Eclipta alba	Ascorbic acid (control)
100	21.02±0.28	24.14
200	26.03±0.14	29.46
300	37.62±0.86	36.23
400	48.13±0.39	46.38
500	52.17±0.49	50.99

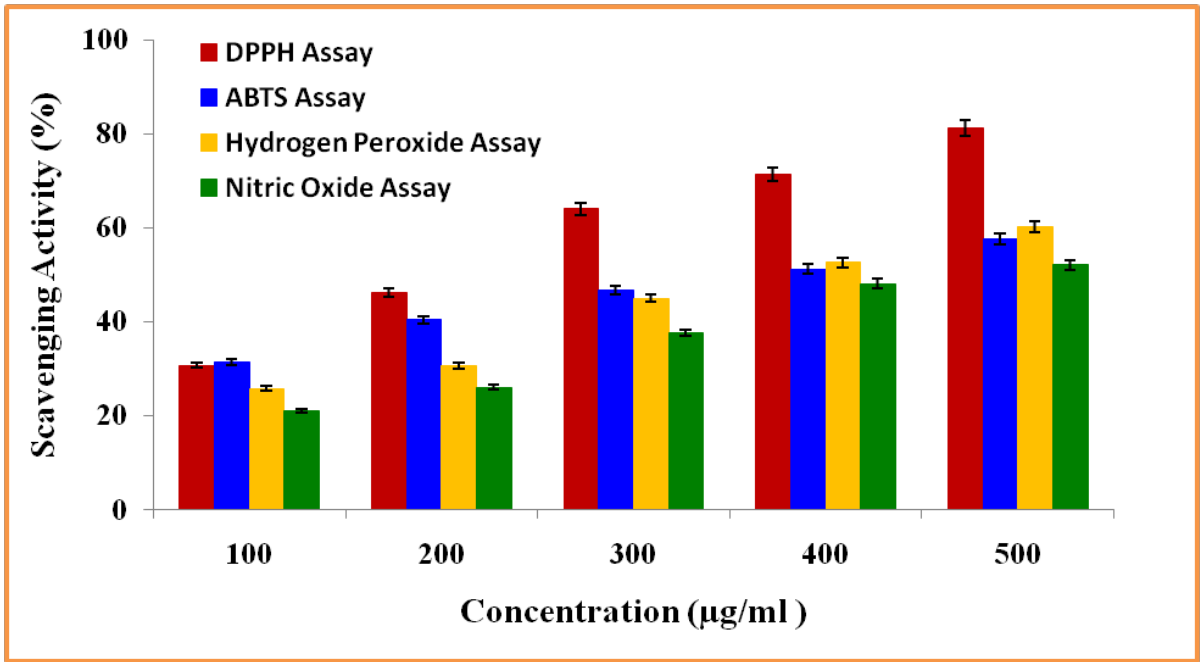

Figure 1: Per cent Radical Scavenging Activities

DISCUSSION

The medicinal plant considers as a rich resource of ingredients which can be used in drug development and synthesis. Plants play a critical role in the development of human culture around the whole world. The increased interest in the plant-derived drug is mainly because of the widespread belief that herbal medicine is safer than costly synthetic medicines which possess side effects. Hence, there is a need to screen medicinal plants for promising biological activity. Further, there is a continuous development of resistant strains which pose them need for search and development of a new drug to cure diseases.

The present study was carried out for the phytochemical screening of principle bioactive compounds and to evaluate antioxidant activities in methanolic extract of *Eclipta alba.* The extract was screened for the presence of phytochemical constituents, which reviled the presence of the secondary metabolites like tannin, phenolic, flavonoid and alkaloids and absence of steroids and saponins.

Excessive generation of reactive oxygen species leads to a variety of pathological conditions such as inflammation, diabetes, hepatic damage, cancer much other physiological disorder. Flavonoids from different plant source have been reported to have anti-inflammatory, antiarthritic and antioxidant activity. The phenolic compounds have been known to act as an antioxidant due to their capability to donate electrons as well as the effectiveness of stabilizing radical intermediates in the prevention of oxidation all the cellular and physiological level. Potential of the antioxidant property was evaluated through various biological activity studies (Figure 1). When compared to the different methods studied, the scavenging activities were found to increase with an increase in concentrations. The percentage scavenging activity found to be high at DPPH scavenging assay when compared to the other methods studied.

CONCLUSION

Current investigation reports on preliminary screening of phytochemicals and total phenolic content followed by the antioxidant efficiency of the *Eclipta alba* extract by various assays. The result concludes the efficacy of methanolic extract in scavenging the free radicals using different *in vitro* experimental models which confirms the presence of phytoconstituents and total phenolic compounds may be responsible for the antioxidant potential of the methanolic *E. alba* extract.

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