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EVALUATION OF ANTIOXIDANT ACTIVITY IN RELATION WITH THEIR PHENOLIC AND FLAVANOID CONTENT OF *Eclipta alba* L LEAF COLLECTED FROM FIVE DIFFERENT GEOGRAPHIC REGIONS OF TAMIL NADU.

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Abstract

The present study examines antioxidant activity, total phenol and flavonoid content of the five different leaf extracts prepared from the *Eclipta alba* leaf collected from five different geographically regions of Tamil Nadu. Butylated Hydroxy Toluene (BHT), Gallic acid (GA) and Quercetin (Q) were taken as a reference standard in case of antioxidant activity, total phenolic and total flavonoid content respectively. The leaf extracts were evaluated for antioxidant activities by DPPH (1, 1 – diphenyl -2- picrylhydrazyl) radical scavenging assay. Among five different regions *E. alba* leaf maximum antioxidant activity was found in S1 chengalpet leaf extract (61.70 %) DPPH radical scavenged with lowest IC50 value of 62.50µg when compared with other sample. Total phenol and flavonoid contents were quantitatively estimated. Total phenolic content measured by Folin-Ciocalteau method varied from 41.15 to 53.70 µg Gallic Acid Equivalents (GAE)/g and the total flavonoid contents as measured by aluminium chloride method varied from 46.53 to 64.30 µg Quercetin Equivalents (QE)/g. The S1 Chengalpet region leaf extract of *Eclipta alba* was found maximum in total phenol and flavonoid contents were 53.70 µg GAE /g and 64.3 µg QE /g respectively.

Keywords *Eclipta alba, Antioxidant, DPPH, Total Phenol & Flavonoid.*

INTRODUCTION

Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years. Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind [1] .Therapeutic benefits can be traced to specific plant compounds; many herbs contain dozens of active constituents that, together, combine to give the plant its therapeutic value [2]. A growing body of evidence indicates that secondary plant metabolites play important roles in human health and may be nutritionally important [3]. Phytochemical screening of various plants has been reported by many workers [4, 5]. These studies have revealed the presence of numerous chemicals including alkaloids, flavonoids, steroids, phenols, glycosides and saponins. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites [6]. A number of studies have focused on the biological activities of phenolic compounds which are antioxidants and free radical scavenger [7- 9].

Free radicals (superoxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and peroxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA [10, 11]. It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others [12, 13].

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The most effective way to eliminate free radicals which causethe oxidative stress is with the help of antioxidants. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promotingtheir decomposition and suppressing such disorders [14-16]. In addition, phenolic compounds and flavonoids are also widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, antiinflammatory, anti-carcinogenic etc. [17].

The crude extracts of herbs, spices and other plant materials, rich in phenolics and flavonoids are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food [18]. *Eclipta alba*, an erect or prostrate much branch, annual herb with rooted nodules; leaf opposite, strigose with oppressed hairs on both sides, flowers white in heads , and ray compressed. The plant is bitter, acrid, thermogenic, alterative, antiinflammatory, antihelminthi, vulnerary, ophthalmic, digestive, carminative, haematinic, diuretic and is useful in hepato spleenomegaly, gastropathy, anorexia, skin diseases, ulcers, jaundice etc. Because of this multiple therapeutic effects the leaf of this magic herbal was selected.

In the present study five different geographic regions *Eclipta alba* leaf samples were screened for phytochemical constituents such as total phenolic and total flavanoid content and antioxidant activity to assess and identify the antioxidant potential of the plant leaf extract.

MATERIALS AND METHODS

Collection and processing of plant material

The leaf of *Eclipta alba* from five different regions of Tamil Nadu namely Chengalpet, Vellingiri Hills, Arani, Padavedu, and Gummidipoondi and the leaf samples from different regions were assigned with marking S_1, S_2, S_3, S_4, S_5 respectively. All the leaf samples were cleansed and shade dried for a week and grounded into powder and preserved for further use.

Preparation of plant extract

Preparation of the plant leaf extract was done according to a combination of the standard methods [19, 20]. About 15g of dried leaf fine powder of *Eclipta alba* plant materials were extracted with following solvent in ethyl acetate then to the 80% methanol for 30 minute using an Ultra Turax mixer (13, 000 rpm) after every step of extraction leaf extract is evaporated to dry and soaked in to the next solvent. Finally the leaf extract sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rotatory evaporator at 40°C to a constant weight. The concentrated leaf extract samples were stored in airtight container in refrigerator below 10º C for further analysis.

Qualitative analysis of Antioxidant activity

The qualitative antioxidant activity of leaf extracts of *Eclipta alba* was determined by following the standard color spot method [21]. 50µL of leaf extracts of *Eclipta alba* were taken in the microtiter plate. 100µL of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

DPPH Free radical scavenging activity

The antioxidant potential of different leaf extracts of the plant were determined using DPPH free radical scavenging assay by the standard method [22]. Leaf extract of 100µl were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample measured

containing the same amount of methanol and DPPH solution was prepared and measured as a control. Subsequently, at every 5 min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicate. Free radical scavenging activity was calculated by the following formula:

% DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample/ (Absorbance Of control)] x 100

IC50 for reference standard and leaf sample was calculated. IC50 is the concentration of reference standard or leaf sample is required to inhibit or scavenge 50% of DPPH radicals.

Estimation of Total phenol content

Total phenolic content of the leaf extracts sample was determined by the standard Folin Ciocalteau spectrophotometric method [23]. For the analysis, 0.5 ml of dry powdered ethanolic leaf extracts were added to 0.1 ml of Folin-Ciocalteau reagent (0.5N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of Sodium carbonate (Na_2CO_3) was added and the mixture was allowed to stand for 30 minutes after mixing. The absorbance was measured at 760 nm in a UV-Visible Spectrophotometer. The total phenolic contents were expressed as mg gallic acid equivalents (GAE)/g leaf extract. The experiment was carried out in triplicate.

Estimation of Total Flavonoid Content

Total flavonoids content of different region *E. alba* leaf extracts sample was determined by the standard Aluminium chloride spectrophotometric method [24]. In brief, 0.5 ml of leaf extracts of *Eclipta alba* at a concentration of 1mg/ml were taken and the volume was made up to 3ml with methanol. Then 0.1ml AlCl3 (10%), 0.1ml of potassium acetate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance was recorded at 415 nm after 30 minutes of incubation. A standard calibration plot was generated at 415nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of leaf extract.

RESULTS AND DISCUSSION

Qualitative analysis of Antioxidant activity

Results for qualitative analysis of antioxidant activity of leaf extract were shown in Table 1. The samples observed for its bleaching from purple to yellow and pale pink were considered as strong positive and weak positive respectively. Sample S1 (Chengalpet region *E.alba*) leaf sample shows strong positive result shown yellow color spot indicates the strong antioxidant activity followed by the S3 & S4 samples shows moderate antioxidant activity whereas S2 & S5 sample shows mild antioxidant activity.

Table.1 Qualitative analysis of antioxidant activity of *Eclipta alba*

SAMPLE/STANDARD	RESULT
BHT	+++
S_1	+++
S_2	+
S_3	++
S_4	++
S_5	+

+++ = Strong positive ++ = moderate positive + = mild positive - = Negative

Antioxidant Activity by DPPH Radical Scavenging Assay

Different region *Eclipta alba* leaf samples were analyzed for antioxidant activity by DPPH radical scavenging assay and the results were shown in Table 2 & Figure 1. Maximum percentage (81.44%) of DPPH radical scavenging activity was recorded at 100 µg/ml of reference standard sample.Highest antioxidant activity with lowest IC50 (51.25µg) value was recorded in reference standard. Highest antioxidant activity with lowest IC50 value was recorded in S1 (62.50 µg) Chengalpet sample followed by the sample S4 (80.75µg),S3 (81.20 µg) then S5 (94.40µg) & S2 (97.25µg) samples.

Table 2: DPPH Free Radical Scavenging Assay of *Eclipta alba*

Reference Standard & Plant Sample	DPPH % Inhibition (Concentration µg/ml)					IC50
	20	40	60	80	100	
BHT	36.44±0.74	48.23±1.32	54.37±0.90	66.84±1.39	81.44±2.23	51.25
S_1	24.69±0.29	32.29±1.45	44.12±2.48	53.88±2.16	61.70±0.12	62.50
S_2	21.82±0.24	29.95±1.66	36.81±0.21	43.20±1.76	51.24±1.47	97.25
S_3	23.46±1.25	30.37±1.28	38.37±1.15	46.69±1.31	54.37±2.34	81.20
S_4	25.22±0.41	31.36±0.24	39.41±1.17	49.47±1.43	56.16±1.21	80.75
S_5	19.79±1.62	27.73±1.23	33.17±0.89	41.26±2.14	52.22±1.37	94.40

Data are presented as the mean ± SD values of triplicate determinations

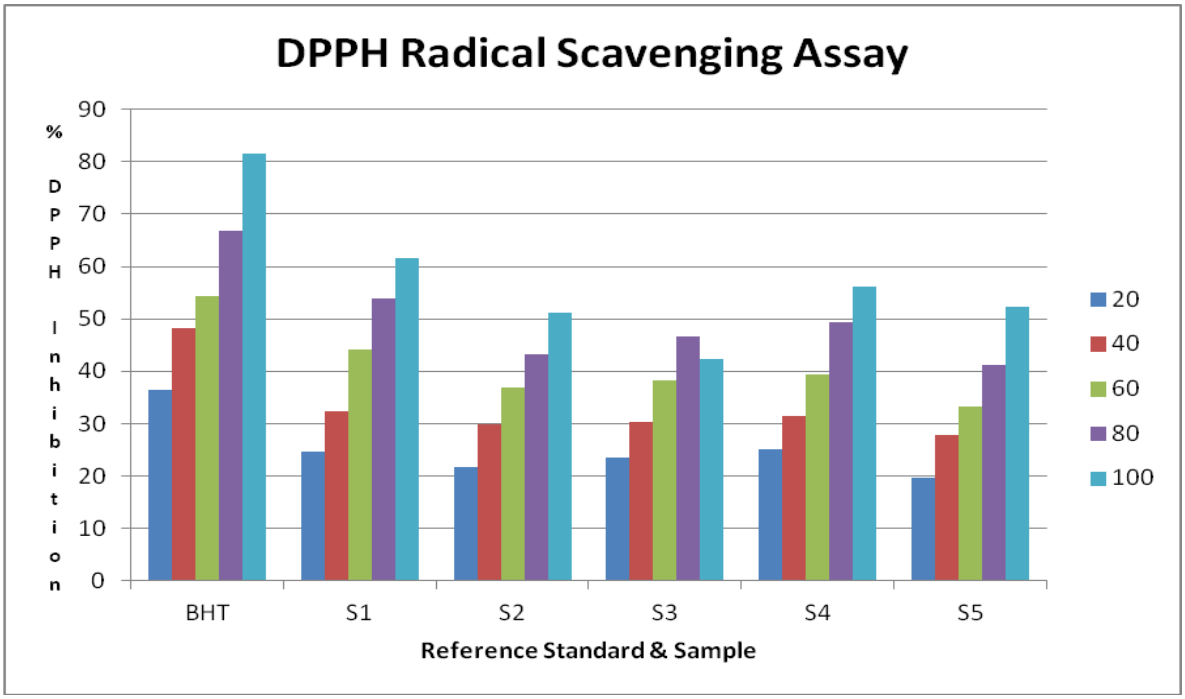

Figure 1: DPPH Radical Scavenging Assay of Eclipta alba; BHT: Reference standard, Eclipta alba S1-Chengalpet, S2- Vellingiri Hills, S3 –Arni, S4 - Padavedu and S5 -Gummidipoondi..

Estimation of Total phenolic content

Results for total phenolic contents of the leaf extracts in terms of Gallic acid equivalent per gram of leaf extract were shown in Table 3 and Figure 2. The total phenolic content of leaf extract lie between 41.15 µg GAE/ g to 53.70 µg GAE/g. Total phenolic content of *Eclipta alba* leaf extract was found to be maximum in S1 Chengalpet (53.70 µg GAE/g) followed by S4 Padavedu (45.50 µg GAE/g),S5 Gummidipoondi (41.15 µg GAE /g), S3 Arani (39.90 µg GAE/g), S2 Vellingiri Hills (37.40 µg GAE/g).

Phenolics are the most widespread secondary metabolite in plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as radical scavengers. Phenolic compounds are a class of antioxidant agents which act as free radical terminators [28]. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers [29]. Phenolic compounds are important plant antioxidants which exhibited considerable

scavenging activity against radicals. Thus, antioxidant capacity of a sample can be attributed mainly to its phenolic compounds [30-32]. Phenolic compounds are effective hydrogen donors, making them good antioxidants. Similarly, Shahidi and Naczk reported that naturally occurring phenolics exhibit antioxidant activity [33]. Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants. The values of flavonoid content varied from plants. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable.

Table 3: Estimation of Total phenolic content of *Eclipta alba*

Plant Sample	Total phenol content (µg GAE/g)
S_1	53.70±1.29
S_2	37.40±0.82
S_3	39.90±0.19
S_4	45.50±1.58
S_5	41.15±0.49

Data are presented as the mean ± SD values of triplicate determinations

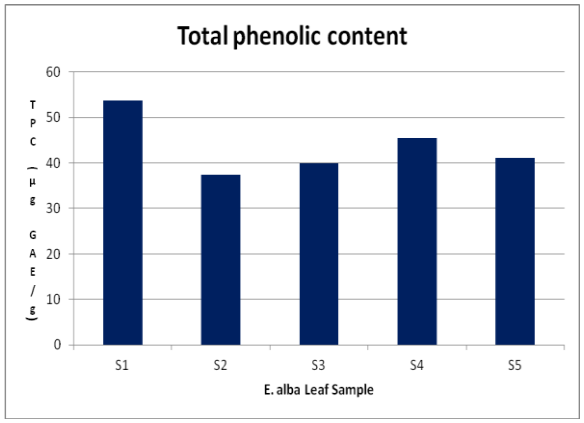

Figure 2: Total Phenolic Content of *Eclipta alba* collected from different regions; S1- Chengalpet, S2- Vellingiri Hills, S3 –Arni, S4 - Padavedu and S5 –Gummidipoondi

Estimation of Total Flavonoid Content

Total flavanoid content results of leaf extracts were shown in Table 4 and Figure 3 in terms of Quercetin equivalent per gram of leaf extract.

Table 4: Estimation of Total Flavonoid content of *Eclipta alba*

Plant Sample	Total Flavanoid content (µg QE/g)
S_1	64.30±1.12
S_2	46.53±0.76
S_3	54.82±1.15
S_4	51.16±0.82
S_5	48.32±1.27

Data are presented as the mean ± SD values of triplicate determinations

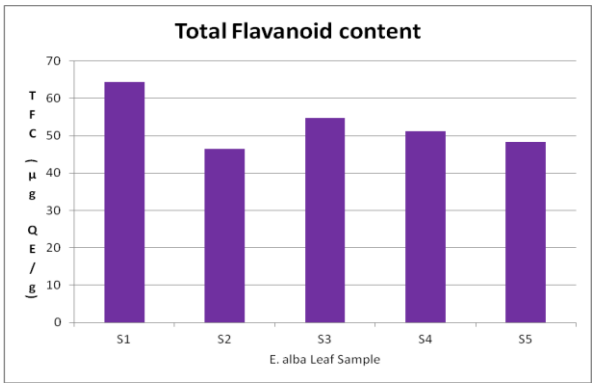

Figure 3: Total Flavanoid Content of *Eclipta alba* collected from different regions ;S1- Chengalpet, S2- Vellingiri Hills, S3 –Arni, S4 - Padavedu and S5 -Gummidipoondi.

The total flavanoid content of leaf extract lie between 46.53 µg QE/ g to 64.30 µg QE/g. Total phenolic content of *Eclipta alba* leaf extract was found to be maximum in S1 Chengalpet (64.30 µg QE/g) followed by S3 Arani (54.82 µg QE/g), S4 Padavedu (51.16 µg QE/g), S5 Gummidipoondi (48.32 µg QE /g), S3 Arani S2 Vellingiri Hills (46.53 µg QE/g). The mechanisms of action of flavonoids are through scavenging or chelating process. Antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups [34, 35].

CONCLUSION

In the conclusions, *Eclipta alba* leaf sample collected from different geographic regions show remarkable differences in antioxidant activity, total phenol and total flavonoid content parameters. The plant leaf sample is acts as a very important sources of therapeutically and industrially important compounds to treat various disease conditions. The results indicate that the plant

Eclipta alba leaf material may become an important source of compounds with health protective potential to use in nutraceutical products of commercial importance. Further studies of the plant *Eclipta alba* should be directed to carry out in vivo studies of its medicinal active components in order to prepare natural pharmaceutical products of high value.

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