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Research Article

INFLUENCE OF TOTAL PHENOLIC CONTENT AND TOTAL FLAVONOID CONTENT ON THE DPPH RADICAL SCAVENGING ACTIVITY OF *ECLIPTA ALBA* (L.) HASSK.

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ABSTRACT

Natural products from plants provide unlimited opportunities for discovery of new drugs. The current study was focused on determination of total phenolic and flavonoid content and their influence on antioxidant activity. The total phenolic content of the plant extracts was determined by the Folin-Ciocalteu method and the total flavonoid content was determined using the Dowd method. The *in vitro* antioxidant activity was measured in terms of free radical scavenging activity of the fractions by 1, 1- diphenyl-2-picrylhydrazyl (DPPH) assay. The total phenolic content of the extracts was in the range of 1.467 ± 0.231 mg GAE/g extract in the aqueous extract to 8.933 ± 0.231 mg GAE/g extract in the ethanol extract. TFC varied from 0.5 mg RE/g extract in the aqueous extract to 30.667 ± 1.155 mg RE/g extract in the ethanol extract. The ethanol extract (IC_{50}= 190.782 ± 31.669 µg/ml) had the highest DPPH radical scavenging activity while the aqueous extract(IC_{50}= 1145.586 ± 35.072 µg/ml) had the lowest DPPH radical scavenging activity. IC_{50} value showed a stronger negative relation with the total flavonoid content (r = -0.850, P = 0.150) as compared to the total phenolic content (r = -0.718, P = 0.282) thereby suggesting a pronounced influence of the flavonoids in the antioxidant activity in terms of DPPH radical scavenging. It is evident from the study that the antioxidant activity in terms of DPPH radical scavenging is influenced by the total flavonoid and phenolic content.

Keywords: *Eclipta alba*, Total phenolic content (TPC), Total flavonoid content (TFC), DPPH radical scavenging activity.

INTRODUCTION

Natural products from plants either as crude extracts or as pure compounds, provide unlimited opportunities for discovery of new drugs because of the unmatched availability of chemical diversity [1]. The medicinal properties of plants have been investigated in the recent scientific developments throughout the world due to their potent biological activities, no side effects and economic viability. Reactive oxygen species (ROS) that are constantly formed in living organisms during the process of cellular respiration, or induced by exogenous sources such as pollution, ionizing radiation and drugs [2], can harm vitally important structures, such as cell membranes, deoxyribonucleic acid (DNA) and other components vital for normal cell function, thus creating preconditions for the emergence of degenerative and malignant diseases. Under normal conditions, the production of ROS is in equilibrium with the antioxidant protection of the organism. Increased production of ROS and / or reduced antioxidant protection of the organism can lead to tissue damage and disease. This situation is called oxidative stress, which is the cause or contributing factor in the pathology of many diseases [3].

Living organisms are protected against oxidative damage with endogenous antioxidant system [4] and exogenous antioxidants, which may be naturally occurring or synthetic. Antioxidants are naturally occurring in plants and help to counter the detrimental effects of reactive oxygen species (ROS) and free radicals which causes degenerative human diseases such as cancer, heart diseases and cerebrovascular diseases [5]. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. The main characteristic of an antioxidant is its ability to trap these free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals and inhibit the oxidative mechanisms that lead to degenerative diseases.

Recently, natural food derived antioxidant such as vitamins and phenol phytochemicals have received growing attention. This is because they are known to function as chemo preventive agents against oxidative damage [6]. Phenolics are ubiquitous secondary metabolites in plants and comprise a large group of biologically active ingredients (above 8000 compounds) - from simple phenol molecules to polymeric structures with molecular mass above 3000 Da [7]. On the basis of the number of phenol subunits, the modern classification forms two basic groups of phenolics-simple phenols and polyphenols. The group of simple phenols contain also the so called "phenolic acids" or phenols with carboxyl group underlying the specificity of their function. Polyphenols contain at least two phenol rings. Flavonoids, a subject of comprehensive studies in recent years belongs to this group. More than 4000 flavonoids have been identified in different higher and lower plant species. Phenolic compounds are well known as radical scavengers, metal chelators, reducing agents, hydrogen donors, and singlet oxygen quenchers[8]. It is reported that phenolic compounds in plants possess strong antioxidant activity and may help to protect cells against the oxidative damage caused by free-radicals [9].

Phenolics have been shown to possess a wide spectrum of biochemical activities such as antioxidant, antimutagenic, anticarcinogenic as well as ability to modify the gene expression. Numerous epidemiological studies confirm significant relationship between high dietary intake of flavonoids and the reduction of cardiovascular and carcinogenic risk [7]. The formulation of preventive and healthy nutrition requires information about phenolic and flavonoid composition in plant foods and their influence on various biological properties.

Based on numerous evidence on the strong biological activity of phenolic compounds and on the scarcity of confirmatory data for their influence on the antioxidant activity, the current study was focused on determination of total phenolic and flavonoid content of the four solvent extracts of *Eclipta alba* and their influence on antioxidant activity of the plant.

E. alba is small branched annual herbaceous plant with a long history of traditional medicinal uses in many countries especially in tropical and subtropical regions. The herb has been known for its curative properties and traditionally has been utilized to cure various ailments. Considering its wide array of medicinal properties and use in traditional medicine, *E. alba* was selected for the present study.

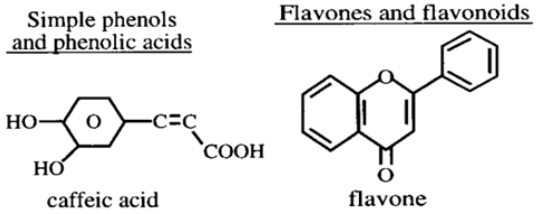

Fig. 1: Simple phenols and flavonoids

MATERIALS AND METHODS

Preparation of plant extracts

The plant samples were collected locally and processed. The cleaned and shade dried plant material was ground into fine powder using electric blender. Plant extracts in the different organic solvents were prepared by successive cold maceration method. 50 grams of dried powder was extracted successively by soaking in 500 ml petroleum ether, ethyl acetate and ethanol for 48 hours with intermittent shaking. The extracts were filtered through Whatman No. 1 filter paper into pre-weighed beakers. The filtrates were dried in a rotatory vacuum evaporator until a constant dry weight of each extract was obtained. The residues were stored aseptically at 5°C for further use. The aqueous extract was prepared by soaking 50 g of dried plant material in 500 ml of sterile distilled water containing 1% chloroform for 48 hours with intermittent shaking. The extract was filtered through a double layer muslin cloth and then centrifuged at 3500 rpm for 20 minutes. The supernatant was then filtered through Whatman No. 1 filter paper and then by 0.2 µ membrane filter. The extract was dried and preserved aseptically at 5^0C for further use.

Determination of Total Phenolic Content

The total phenolic content of the plant extracts was determined by the Folin-Ciocalteau method [10]. The extract diluted to 0.25 mg/ml was used in the analysis. A 0.5 ml aliquot of the diluted extract was mixed with 2.5 ml of 10% Folin-Ciocalteu's reagent in water. The mixture was vortexed and after 2 minutes, 2 ml of 7.5% Na_2CO_3 was added. The mixture was incubated for one hour at room temperature and absorbance was measured at 765 nm against blank. Blank was concomitantly prepared, containing 0.5 ml distilled water, 2.5 ml 10% Folin-Ciocalteu's reagent and 2 ml of 7.5% of Na_2CO_3 dissolved in water. Gallic acid was used as standard for preparing the calibration curve. The total phenolic content in extracts was expressed in terms of Gallic acid equivalent (mg of GA/g of extract).

Determination of Total Flavonoid Content

The total flavonoid content was determined using the Dowd method [11]. 5 mL of 2 % aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (0.05 mg/mL). Absorption readings at 415 nm using spectrophotometer were taken after 10 minutes against a blank sample consisting of a 5 mL extract solution with 5 mL methanol without $AlCl_3$. The total flavonoid content was determined using a standard curve with rutin as the standard. Total flavonoid content is expressed as rutin equivalent (mg of RE/ g of extract).

DPPH radical scavenging assay

The free radical scavenging activity of the fractions was measured *in vitro* by 1, 1- diphenyl-2-picrylhydrazyl (DPPH) assay [12]. About 0.25 mM solution of DPPH in DMSO was prepared and 1 ml of this solution was added to 3.0 ml of the fraction dissolved in DMSO at different concentrations. The mixture was mixed and incubated at room temperature for 30 min and the absorbance was measured at 517nm against blank. A reaction mixture without test sample served as control. The percentage scavenging was determined and was compared with that of ascorbic acid which was used as the standard. The percentage scavenging was calculated by the following equation:

$$\% \text{ scavenging activity} = \frac{\text{Control Abs} - \text{Test Abs}}{\text{Control Abs}} \times 100$$

The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of radicals by 50%.

Statistical analysis

Experiments were performed in triplicate and results obtained were presented as mean ± standard deviation (sd). Statistical analysis was performed using SigmaPlot 10 and SigmaStat 3.5. All pairwise multiple comparison procedures were performed by the Holm-Sidak method at an overall significance level = 0.05.

RESULTS AND DISCUSSION

The total phenolic content (TPC) of the four extracts of *E. alba* is shown in table 1. The total phenolic content was determined using Gallic acid as standard. A calibration curve was prepared (figure 2) and the total phenolic content of the extract was expressed as milligram of Gallic Acid Equivalent per gram of extract i.e. mg GAE/g extract. TPC increased from petroleum ether extract to the ethanol extract but decreased in the aqueous extract. This may be due to the action of enzymes that remain active in the aqueous medium [13] but are inactivated in the organic solvents. The total phenolic content of the extracts was in the range of 1.467 ± 0.231 mg GAE/g extract in the aqueous extract to 8.933 ± 0.231 mg GAE/g extract in the ethanol extract.

Table 1: TPC of *E. alba* extracts (in mg GAE/g extract)

Extract	Mean± sd
Petroleum ether	2 ± 0
Ethyl acetate	4.267 ± 0.231
Ethanol	8.933 ± 0.231
Aqueous	1.467 ± 0.231

* The results show a statistically significant difference at P = <0.001

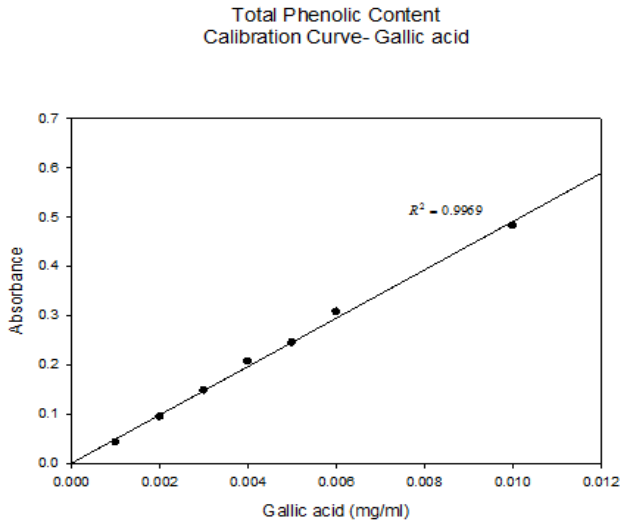

Fig. 2: Total phenolic content calibration curve of Gallic acid

Phenolic compounds are known to contribute to the quality and nutritional values of foods in terms of color, aroma and flavor and also in providing health-beneficial effects [14, 15]. They also serve in plant defence mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and damage by microorganisms, insects and herbivores [16]. The phenolic compounds have been recognised as antioxidant agents which act as free radical terminators [14]. The significant differences in the phenolic content of the extracts may be attributed to different solvents used. The study shows that extraction by cold maceration using ethanol yielded maximum phenolic content.

The total flavonoid content (TFC) was determined from the calibration curve of Rutin (figure 3) and expressed as milligram of Rutin Equivalent per gram of extract i.e. mg RE/g extract (Table 2). TFC varied from 0.5 mg RE/g extract in the aqueous extract to 30.667 ± 1.155 mg RE/g extract in the ethanol extract and increased in a trend similar to the total phenolic content. Flavonoids are well-known antioxidant constituents of plants and are known to possess a broad spectrum of biological and chemical activities including radical scavenging activity [17]. Flavonoids have been reported to be responsible for antioxidant activities of plants through their scavenging or chelating activity [18, 19, 20].

Table 2: TFC of *E.alba* extracts (in mg RE/g extract)

Extract	Mean±sd
Petroleum ether	8±0
Ethyl acetate	27.333±1.155
Ethanol	30.667±1.155
Aqueous	0.5±0

* The results show a statistically significant difference at P = <0.001

The DPPH radical scavenging activity was determined as percent inhibition of DPPH radical and reported as IC_{50} value of the extract. (Table 3). IC_{50} was recorded highest for aqueous extract (1145.586 ± 35.072 µg/ml) and lowest for the ethanol extract (190.782 ± 31.669 µg/ml). Ascorbic acid was used as standard. A lower IC_{50} value implied a better antioxidant activity of the extract. Thus, the ethanol extract had the highest DPPH radical scavenging activity followed by ethyl acetate extract and petroleum ether extract. The aqueous extract had the lowest DPPH radical scavenging activity.

The correlations studies revealed a negative correlation between the IC_{50} value and the TPC and TFC (Table 4). IC_{50} value showed a stronger negative relation with the total flavonoid content as compared to the total phenolic content there by suggesting a pronounced influence of the flavonoids in the antioxidant activity in terms of DPPH radical scavenging.

Our findings are in agreement with the results of various other workers [21, 22, 23, 24, 25] suggesting correlation between antioxidant activity and phenolics and flavonoid content. But there are also reports of no such correlation [26, 27]. Hence it may be said that the phenolics and flavonoids are not the only metabolites influencing the antioxidant activities of the plant extracts. Wide variety of other phytochemicals may also have an influence on the antioxidant potentials of different plants.

Table 3: IC_{50} of *E.alba* extracts (in µg/ml)

Extract	Mean±sd
Petroleum ether	436.643±12.911
Ethyl acetate	350.775±25.378
Ethanol	190.782±31.669
Aqueous	1145.586±35.072
Ascorbic acid	4.689±0.556

* The results show a statistically significant difference at P = <0.001

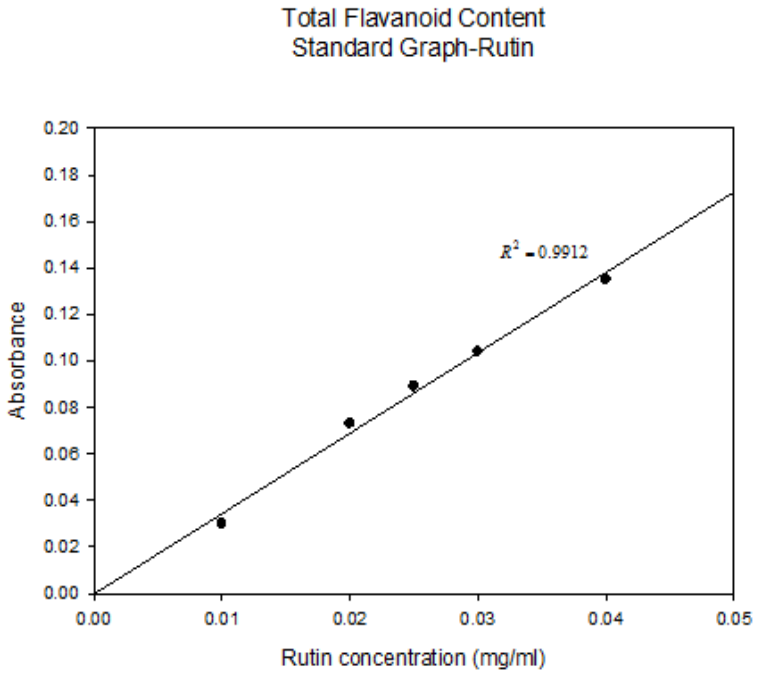

Fig. 3: Total Flavonoid Content calibration graph of Rutin

Table 4: Correlation between IC_{50}, TPC and TFC

		TFC	TPC
IC50	Correlation Coefficient (r)	-0.85	-0.718
	P Value	0.15	0.282

CONCLUSIONS

It is evident from the study that the antioxidant activity in terms of DPPH radical scavenging is influenced by the total flavonoid and phenolic content. But detailed studies on the role of different phytoconstituents in influencing the antioxidant activities are required for proper understanding of the mechanisms and for their use as functional foods and in pharmaceutical industry.

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