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ANTIOXIDANT ACTIVITY OF *GALIUM APARINE* L. FROM PUNJAB, PAKISTAN

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Abstract

The petroleum ether, chloroform, methanol and aqueous extracts of *Galium aparine* were screened for their antioxidant activity. DPPH radical scavenging ability and total phenolic contents of the plant were determined. The percentage yield, colour and texture of the plant extracts were also determined. Methanol extract of the selected plant had the highest antioxidant for reducing DPPH (84.33%) while its water extract exhibited least antioxidant activity for reducing DPPH (8.77%). These results were compared with the standards i.e., α-Tocopherol and BHT. Methanol extract of the plant had highest amount of total phenols (124.8 µg of Gallic acid equivalent to one gm of extract) which correlates with the DPPH radical scavenging assay. The study exposed that the utilization of these plants would exert several valuable effects by virtue of their antioxidant activity.

Introduction

Interest in herbal medicines has increased in a number of countries, especially in Pakistan, over the last few decades. Now the medicinal plants have become the aim for the hunt by cosmopolitan drug companies and study institutes for new drugs (Akerele, 1993).

Medicinal plants have become the heart of passionate study in terms of corroboration of their traditional uses through the determination of their definite pharmacological effects. Synthetic drugs are not only expensive and deficient for the cure of diseases but also often with adulterations and side effects. Therefore, there is needed to look for new strategies to manage a variety of diseases.

Antioxidants are nutrients in our foods which can avert or sluggish the oxidative damage to our body. When our body cells use oxygen, they obviously produce free radicals (by-products) which can be harmful. Free radical damage may direct to cancer. Antioxidants act as free radical scavengers and hence check and fix damage done by these free radicals. Antioxidants may also augment immune defense and therefore, poorer the risk of cancer and infection. Examples of antioxidants comprise beta-carotene, lycopene, vitamins C, E, A and other substances (Miller *et al*., 2000). Antioxidants are broadly applied as food stabilizer to afford assurance next to oxidative ruin of foodstuff by free radicals (Gülçin *et al*., 2002).

Masuda *et al*., (1999) examined the antioxidant actions on the methanolic extracts of leaves of 39 plant species growing on subtropical seashores, by three kinds of assay methods, which included DPPH radical scavenging assay, linoleic acid oxidation assay. Two extracts from *Excoecaria agallocha* and *Terminalia catappa* exhibited extraordinarily effective antioxidant action in the entire test schemes. Javanmardi *et al*., (2003) determined total antioxidant activity and total phenolic contents *Ocimum basilicum*. A direct +ve connection was present among antioxidant activity and total phenolic content of the experienced plant accessions. *Ocimum basilicum* acquire important antioxidant possessions. Sun & Ho (2005) evaluated antioxidant behavior of *Fagopyrum esculentum* extracts. The extract of acetone demonstrated the uppermost total phenolics content and maximum DPPH scavenging activity.

Chin-Yuan (2006) extracted dried *Polygonum aviculare* L., by ethanol for antioxidant activity. The results indicated that *Polygonum aviculare* L., extract clearly had antioxidant effects.

All extracts *Rosa nutkana*, *R. pisocarpa* and *R. woodsii* exhibited strong antioxidant activity as reported by Ocksook *et al*., (2007). Pericarp extracts of *R. nutkana* had elevated phenolic content and exhibited better antioxidant activity than extracts of seeds. Seed extracts of *R. woodsii* had a superior phenolic concentration as well as better antioxidant activity than extracts of pericarp. Nooman *et al*., (2008) evaluated free radical scavenging activity of some common therapeutic plants using DPPH free radical using ascorbic acid as standard antioxidant. On the whole antioxidant activity of *Camellia sinensis* was the distinct, *Eugenia caryophyllus*, *Piper cubeba*, *Zingiber officinale* and *Piper nigrum* were also active in a descending order.

Material and Methods

Collection and preparation of samples: *Galium aparine* Linn. (Rubiacea) were obtained from diverse regions of Punjab, Pakistan. The collected plant materials were authenticated by Dr. Zaheer-ud-din Khan, Professor and Chairperson Department of Botany, GC University Lahore. These plant samples were submitted to GC University Herbarium and Voucher Number was obtained. The plant was dried in shady places at room temperature and crushed to make excellent powder, in addition, preserved in glass bottles to make further use.

Two hundred fifty grams of each dried plant material was soaked in 250 ± 50ml of non polar and polar solvents for instance petroleum ether, chloroform, methanol and Water. The crude extracts were obtained by using masceration method for a week in every solvent.

The physical appearance of plant extracts was noted. The colour, texture and chemical nature of plant extracts was noted. Percentage yield was also noted (Tables 1 and 2).

Table 1. Physical appearance of the plant.

Plant	Solvent	Colour	Texture	Chem. Nature
Galium aparine	Pet. ether	Dark green	Granular	Non crystalline
	Chloroform	Dark green	Granular	Non crystalline
	Methanol	Dark green	Granular	Non crystalline
	Water	Brown	Sticky	Non crystalline

Table 2. Percentage yield of the plant.

Plant samples	Pet. Ether	Chloroform	Methanol	Water
Galium aparine	0.24	1.43	0.63	0.35

DPPH free radical scavenging activity: DPPH radical scavenging activity of plant extracts was determined by comparison with that of known antioxidant, Butylated hydroxytoluene (BHT) according to Erasto *et al*., (2004).One ml of 500 µM (0.2mg/ml) DPPH in methanol was fluxed with equal volume of test compounds at various concentrations, fluxed well and kept in dark for half an hour. The absorbance at 517 nm was observed in the presence of different concentrations of plant samples. A blank sample was also run to determine the absorbance of DPPH before interacting with the compounds. The sample was done in triplicate and mean value was calculated.

The percent of DPPH decoloration of the samples was calculated according to the formula:

$$\text{Antiradical activity} = A_{control} - A_{sample} / A_{control} \times 100$$

Quantification of total phenolic content: Total Phenolics of crude extracts were determined by the method of Makkar *et al*., (1993) which includes 0.1ml of sample (0.5mg/ml) combined with 2.8 ml of 10% Na_2CO_3 and 0.1 ml of 2 N Foloin-Ciocalteu reagent. After 40 min absorbance at 725 nm was noted by UV-visible spectrophotometer. Total phenols were calculated as milligrams of gallic acid equivalents (GAE) per gram of extracts by computing with the standard calibration curve constructed for different concentrations of Gallic acid.

Results

DPPH free radical scavenging activity: A flat concentration assay was carried out with extracts of *Galium aparine* and the results are presented in Fig 1. The results had shown a direct comparison of the plant's activity with the standard antioxidants i.e., BHT and α-Tocopherol. All the extracts possessed good antioxidant activity on the DPH radical.

Quantification of total phenolic content: Total phenolic content of plant samples was determined. Great variation in total phenolic content was found in plant extracts as in Fig. 2.

Discussion

Galium aparine in methanol had shown substantial value of 84.33% DPPH scavenging activity while least value was given by aqueous extract i.e., 8.77%. Lakić *et al*., (2010) evaluated the antioxidant property of methanol extracts of *Galium verum* measuring the scavenging activity of extracts on 2, 2-diphenyl-1-pycrylhydrazil (DPPH). The extracts expressed very strong scavenger activity, reducing the DPPH (Fig. 3).

Yang *et al*., (2009) reported six different phenolic compounds of *Galium aparine* and the present study confirms the presence of high phenolic compounds in the plant. The present study also provides useful information for ethnopharmacological studies.

The data of the plant can be useful for the pharmacological industry for the production of novel antidote commencing the plant against various diseases.

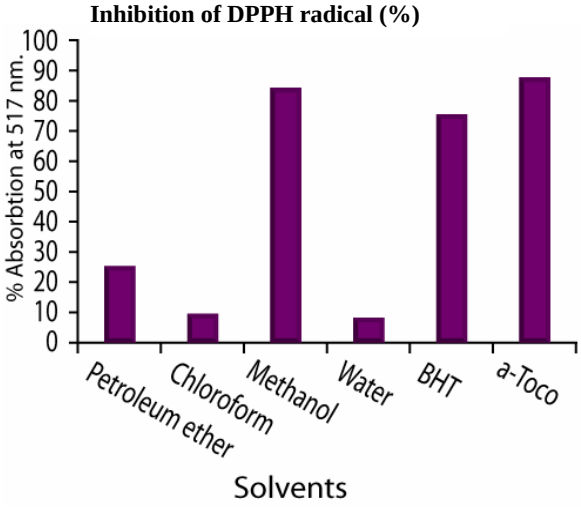

Fig. 1. DPPH free radical scavenging activity of Extracts and standard antioxidant BHT and α-Tocopherol.

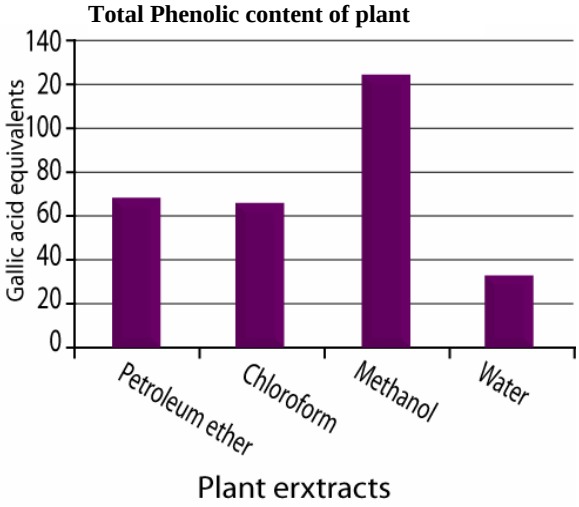

Fig. 2. Total Phenolic content of plant extracts.

Fig. 3. *Galium aparine* in its natural habitat.

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