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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 10 (4): 637-640, 2007
ISSN 1028-8880
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In vitro Antioxidant Activity of *Polygonium hyrcanicum*, *Centaurea depressa*, *Sambucus ebulus*, *Mentha spicata* and *Phytolacca americana*

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Abstract: Extracts of five plants were investigated for their total flavonoids, phenol contents and their radical scavenging activity using DPPH assays: *Polygonium hyrcanicum*, *Centaurea depressa*, *Sambucus ebulus*, *Mentha spicata and Phytolacca americana.* Quercetin and butylated hydroxy toluene were used as standard reference with well-documented antioxidant activity. Total flavonoid content in these plants ranged from 31.6 to 109.5 mg g^{-1} and the amount of free phenolic compounds was between 32 and 287.5 mg g^{-1} extract powder. Free phenolic compounds content were in the order: *P. hyrcanium*> *M. spicata*> *S. ebulus*> *C. depressa*> *P. americana.* It was also observed that all methanolic extract samples of studied plants showed free radical scavenging activity. The highest antioxidant activity was found in *P. hyrcanium* with an IC 50 equal to 0.036 mg mL^{-1} that is higher than BHT (IC 50 = 0.054). A correlation between radical scavenging capacities of extracts with total phenolic compounds content was observed. This result indicates that *P. hyrcanium contains* high levels of phenolic compounds that may contribute to higher free radical scavenging activity compared to the other extracts of the plants in this study.

Key words: Antioxidant, DPPH, flavonoids, phenols, Iranian plants

INTRODUCTION

Reactive Oxygen Species (ROS), including hydroxyl radicals, superoxide anion radical and hydrogen peroxide are continuously formed during life as a result of the metabolism of oxygen. These ROS are of major significance in human physiology as well as the food industry. In the former, oxidative stress has been linked to some diseases such as cancer and tissue damage in rheumatoid arthritis. Most living species have efficient defense systems to protect themselves against the oxidative stress induced by ROS (Cerutti, 1991). Recent investigations have shown that the antioxidant properties of plants could be correlated with oxidative stress defense and different human diseases (Tiwari, 2001). Antioxidants, as oxygen scavengers, can interfere with the oxidation process by reacting with free radicals. Phenolic compounds are a class of antioxidant agents to act as free radical terminators (Shahidi *et al*., 1992).

Antioxidant defense systems can reduce side effects induced by ROS in living cells. Synthetic antioxidants, such as butylated hydroxy anisole (BHA) and butylated hydroxy toluene (BHT) are widely used in the food and cosmetic industry, administration of high dose of BHT induced liver and lung damage (Lanigan and Yamarik, 2002; Devi *et al*., 2003) and long-term administration of BHT is capable to induce oxidative and metabolic alterations in heart similarly to some pathological disorders (Faine *et al*., 2006). Herbal medicine are natural origins to have many biological properties, therefore, development and utilization of more effective antioxidants of natural origins are desired.

The objective of the present research, is to carry out a survey for the relative levels of antioxidant activity in selected Iranian plants species which are used in traditional medicine. Also this study compared the antioxidant effects of the plants with BHT, in order to find an easily accessible source of natural antioxidant, which will have been used in food and pharmaceutical industry.

MATERIALS AND METHODS

Chemicals: 1,1-Diphenyl-2-picryl hydrazyl (DPPH) and quercetin were purchased from Sigma Chemical Co.

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(St., Louis, USA). Gallic acid, tert-butyl-4-hydroxy toluene (BHT), Folin Ciocalteu reagentand methanol were purchased from Merck Co. (Germany).

Plant materials: Whole parts of *Polygonium hyrcanicum* (Polygonaceae) (PH), *Centaurea depressa* (Compositae) (CD), *Sambocus ebulus* (Carprifoliaceae) (SE), *Mentha spicata* (Labiatae) (MS) and *Phytolacca americana* (Phytolaccaceae) (PA) were collected from northern provinces of Iran (Gilan and Mazandaran) on 2004.

Plants materials were dried at room temperature and powdered in a grinder. Fifty grams of each plant powder was extracted with 500 mL of methanol for 48 h. The extracts were freeze-dried after evaporating the solvent under vacuum at temperature below 50°C. This study was done in mazandarn university of medical sciences.

Determination of total flavonoid concentration: Flavonoid content was determined with aluminum chloride colorimetric method from the procedure reported by Chang *et al.* (2002). Briefly plant extracts in methanol were separately mixed with 10% aluminum chloride, 1 M potassium acetate. The absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). Quercetin at concentrations 12.5 to 100 μg mL^{-1} was used to make the calibration curve.

Determination of total phenols: Folin Ciocalteu reagent was used for the determination of total phenols adapted from McDonald *et al.* (2001). A dilute extract of each plant or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent and aqueous Na_2CO_3. The mixtures were remained for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using gallic acid. Total phenol values are expressed as a gallic acid equivalent (mg g^{-1} dry mass) which is a common reference compound.

Measurement of free radical scavenging activity: The free radical-scavenging capacity of methanolic extracts were determined as bleaching of the stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) (Koleva *et al.*, 2002; Orhan *et al.*, 2003; Poli *et al.*, 2003). The experiment was performed for triple times. BHT and quercetin were used as positive controls. IC50 values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals.

Statistical analysis: The statistical significance between antioxidant activity values of the extracts was evaluated with a Mann-Whitney U-test. p-values less than 0.05 was considered to be statistically significant.

RESULTS AND DISCUSSION

Flavonoid and phenol content of plant extracts: Antioxidants may play a major role in the prevention of number of diseases, including cardiovascular and some forms of cancer. Multiple substances contribute to the total antioxidant capacity of plants. Flavonoids and phenolic compounds are good sources of natural antioxidants. Flavonoids are known to act as antioxidants, both as radical scavengers and as metal chelators (Kessler *et al.*, 2003; Lebeau *et al.*, 2000). Hence, in this study the total flavonoid contents of five herb extracts were measured by using 10% $AlCl_3$ reagent. Table 1 shows the total flavonoid contents of samples tested as quercetin equivalent by reference to a standard curve ($y = 0.0067x + 0.0132$, $r^2 = 0.999$). The flavonoid contents in MS and PH were obtained 109.4±2.57 and 76.6±7.7 mg g^{-1} of extract powder respectively. In fact, MS and PH showed higher antioxidant capacity than CD, SE and PA. Total phenols, as determined by the Folin Ciocalteu method, are reported (Table 1) as gallic acid equivalents by reference to a standard curve ($y = 0.05x + 0.0545$, $r^2 = 0.9873$). The amounts of total phenolic compounds in the samples were ranged between 32±2.5 and 287.5±0.15 mg g^{-1} of extract powder (Table 1). The amount of total phenolic compounds in PH was 287.5±0.15 mg g^{-1} that was higher than other plant extracts. Phenols are one of the most important plant constituents because of their scavenging ability due to their hydroxyl groups (Shahidi *et al.*, 1992). Therefore phenolic compounds may contribute directly to antioxidant action in these plants.

Antioxidant activity: Free radical scavenging is one of the most important accepted mechanisms for antioxidant activity. DPPH stable free radicals scavenging method can be used to evaluate the antioxidant activity of a specific compound or extract in a short time (Tiwari, 2001). DPPH scavenging capacity of the extracts was compared with BHT and quercetin as standards. The values of the 50% inhibition concentration (IC 50) of PH, MS, CD, SE, PA,

Table 1: The amount of flavonoids and phenols in five methanolic plant extracts

Sample	Flavonoids (mg g^{-1})	Phenol (mg g^{-1})
PH	76.6±7.7	287.5±0.15
MS	109.4±2.6	84±7
CD	42.25±1.7	44.5±3.5
SE	49.6±5.5	68±1
PA	31.6±1.5	32±2.5

Extracts: PH = *Polygonium hyrcanium*, MS = *Mentha spicata*, CD = *Centaurea depressa*, SE = *Sambucus ebulus*, PA = *Phytolacca americana*. Each value in the table was obtained by calculating the average of three experiments±standard deviation

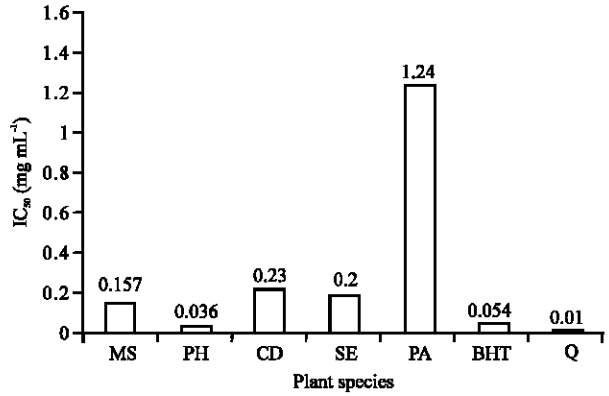

Fig. 1: IC50 values of studied plant extracts for free radical scavenging activity by 1,1-dipheny-2-picrylhydrazyl radicals. Lower IC50 value indicates higher antioxidant activity. Extracts: PH = *Polygonium hyrcanium*, MS = *M. spicata*, CD = *C. depressa*, SE = *Sambucus ebulus*, PA= *Phytolacca americana*, Reference compounds: Butylated hydroxy toluene (BHT) and quercetin (Q)

BHT and quercetin for the DPPH radical were 0.036, 0.157, 0.23, 0.2, 1.24, 0.054 and 0.01 mg mL^{-1}, respectively (Fig. 1). As it is shown in Fig. 1, PH demonstrated the highest antioxidant activity compare to other herbs in this work. BHT showed IC50 equal to 0.054 mg mL^{-1}, while PH extract with IC 50 equal to 0.036 mg mL^{-1}. So it shows higher antioxidant activity than BHT ($p<0.05$). The rank of the scavenging effects of the five herbal extracts decreased as PH> MS> SE> CD> PA. The percentage of antioxidant activity of five plants, BHT and quercetin on DPPH radical has shown in Table 2. In this study, all plants at different concentrations exhibited more than 80% scavenging activity. BHT at a concentration of 0.4 mg mL^{-1} exhibited an antioxidant activity similar to PH at 0.2 mg mL^{-1}, SE and CD at 0.8 mg mL^{-1} and PA at a concentration higher than 4 mg ml^{-1}. In this study, it has been shown the amount of total phenol in PH extract is higher than MS, CD, SE and PA. This result indicates the herbal extracts containing higher phenolic compounds may contribute to scavenge free radicals. The main characteristic of antioxidants is an ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety sources. The free radicals may oxidize nucleic acids, proteins, lipid and DNA and can initiate degenerative diseases (Tiwari, 2001). Antioxidant compounds like phenolic acids, poly phenols and flavanoids scavenge free radicals such as peroxide, lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases. Many flavonoids have shown strong antioxidant properties and quercetin has been established as a strong antioxidant principle (Kessler *et al*., 2003; Lebeau *et al*., 2000). This experiment shows that quercetin has strong antioxidant activity compared to BHT as a synthetic antioxidant.

Table 2: Antioxidant activity of methanolic extracts of the plants on scavenging DPPH stable radical.

Sample	Concentration (mg mL^{-1})	(%) Inhibition
PH	0.2	93±0.5
MS	0.4	88.7±1.5
CD	0.8	92±0.1
SE	0.8	91.3±0.29
PA	4	82.3±1.15
BHT	0.4	93.6±0.47
Quercetin	0.025	95.6±0.4

Extracts: PH = *P. hyrcanium*, MS = *M. spicata*, CD = *Centaurea depressa*, SE = *S. ebulus*, PA = *P. americana*. Reference compounds: Butylated Hydroxy Toluene (BHT) and quercetin. Each value in the table was obtained by calculating the average of three experiments ± standard deviation

The result of this study demonstrates that methanolic extracts of the studied herbs of Iran possess DPPH radical-scavenging effects, which varies between plants. Among the herbs tested, extract of PH exhibits the highest DPPH radicals scavenging activity even more than that BHT. In conclusion, present study shows PH contains the highest amount of total phenols compared to other plants. This result indicates that PH with high levels of total phenolic compounds may contribute to scavenge the free radicals.

ACKNOWLEDGMENT

We are grateful to thank for the financial support granted by the research part of Mazandaran University of Medical Sciences.

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