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Full Length Research Paper

Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants

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In present study, we carried out a systematic record of the relative antioxidant activity in selected Iranian medicinal plant species' extracts. The total phenol varied from 24.1 ± 1 to 289.5 ± 5 mg g $^{-1}$ in the extracts. Flavonoid contents were between 25.15 ± 0.8 and 78.3 ± 4.5 mg g^{-1}. 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging effect of the extracts was determined spectrophotometrically. The highest radical scavenging effect was observed in *Mellilotus officinalis* with IC_{50} = 0.018 mg ml $^{-1}$. The potency of radical scavenging effect of *M. officinalis* extract was about 4 times greater than synthetic antioxidant butylated hydroxy toluene (BHT). The greater amount of phenolic compounds leads to more potent radical scavenging effect as shown by *M. officinalis* extract.

Key words: Antioxidant, radical scavenger, flavonoids, phenols, 1,1-diphenyl-2-picryl hydrazyl.

INTRODUCTION

Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Kumpulainen and Salonen, 1999; Cook and Samman, 1996). Free radicals due to environmental pollutants, radiation, chemicals, toxins, deep fried and spicy foods as well as physical stress, cause depletion of immune system antioxidants, change in gene expression and induce abnormal proteins. Oxidation process is one of the most important routs for producing free radicals in food, drugs and even living systems. Catalase and hydroperoxidase enzymes convert hydrogen peroxide and hydroperoxides to nonradical forms and function as natural antioxidants in human body. Due to depletion of immune system natural antioxidants in different maladies, consuming antioxidants as free radical scavengers may be necessary (Halliwell, 1994; Kuhnan, 1976; Kumpulainen and Salonen, 1999; Younes, 1981).

Currently available synthetic antioxidants like butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT), tertiary butylated hydroquinon and gallic acid esters, have been suspected to cause or prompt negative health effects. Hence, strong restrictions have been placed on their application and there is a trend to substitute them with naturally occurring antioxidants. Moreover, these synthetic antioxidants also show low solubility and moderate antioxidant activity (Barlow, 1990; Branen, 1975).

Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury. Besides well known and traditionally used natural antioxidants from tea, wine, fruits, vegetables and spices, some natural antioxidant (e.g. rosemary and sage) are already exploited commercially either as antioxidant additives or a nutritional supplements (Schuler, 1990). Also many other plant species have been investigated in the search for novel antioxidants (Chu, 2000; Koleva et al., 2002; Mantle et al., 2000; Oke and Hamburger, 2002) but generally there is still a demand to find more information concerning the antioxidant potential of plant species. It has been mentioned the antioxidant activity of plants might be due to their phenolic compounds (Cook and Samman, 1996). Flavonoids are a group of

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polyphenolic compounds with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action (Frankel, 1995). Some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Gryglewski et al., 1987). An easy, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay using 1,1-diphenyl-2-picryl hydrazyl (DPPH) stable radical spectrophotometrically. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases (Koleva et al., 2002).

In particular, despite widespread use of wild plants as medicines in Iran, the literature contains few reports of antioxidant activity and chemical composition of these plants. In present study, we carried out a systematic record of the relative free radical scavenging activity in selected Iranian medicinal plant species, which are being used traditionally: *Mellilotus officinalis* (Fabaceae), *Equisetum maximum* (Equisetaceae), *Plantago major* (plantaginaceae), *Adiantum capillus-veneris* (Adiantaceae) *and Urtica dioica* (Urticaceae). We have also found the relationship of total flavonoid and phenol contents with antioxidant activity. In the longer term, plant species (or their active constituents) identified as having high levels of antioxidant activity *in vitro* may be of value in the design of further studies to unravel novel treatment strategies for disorders associated with free radicals induced tissue damage.

MATERIALS AND METHODS

Chemicals

1,1-Diphenyl-2-picryl hydrazyl (DPPH) and quercetin were purchased from Sigma Chemical Co. (St., Louis, USA). Gallic acid, tert-butyl-4-hydroxy toluene (BHT), Folin Ciocalteu reagent, and methanol were purchased from Merck Co. (Germany).

Plant materials

Whole parts of the plants *Mellilotus officinalis* (Fabaceae), *Equisetum maximum* (Equisetaceae), *Plantago major* (plantaginaceae), *Adiantum capillus-veneris* (Adiantaceae) and *Urtica dioica* (Urticaceae) were collected from Northern provinces of Iran (Gilan and Mazandaran). Plants' materials were dried at room temperature and ground in a mortar. Fifty grams of each plant powder was extracted in 500 ml of methanol by maceration (48 h). The solvent was removed under the vacuum at temperature below 50 ^{o}C and the extracts were freeze-dried.

Total flavonoids determination

Aluminum chloride colorimetric method was used for flavonoids determination (Chang et al., 2002). Each plant extracts (0.5 ml of 1:10 g ml^{-1}) in methanol were separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 µg ml^{-1} in methanol.

Total phenols determination

Total phenols were determined by Folin Ciocalteu reagent (McDonald et al., 2001). A dilute extract of each plant extract (0.5 ml of 1:10 g ml^{-1}) or gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1 M). The mixtures were allowed to stand for 15 min and the total phenols were determined by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg L^{-1} solutions of gallic acid in methanol : water (50:50, v/v). Total phenol values are expressed in terms of gallic acid equivalent (mg g $^{-1}$ of dry mass), which is a common reference compound.

Free radical scavenging activity determination

The stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) was used for determination of free radical-scavenging activity of the extracts (Koleva et al., 2002). Different concentrations of each herbal extract were added, at an equal volume, to methanolic solution of DPPH (100 µM). After 15 min at room temperature, the absorbance was recorded at 517 nm. The experiment was repeated for three times. BHT and quercetin were used as standard controls. IC_{50} 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 were considered to be statistically significant.

RESULTS AND DISCUSSION

Flavonoid and total phenol contents of the extracts

It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler et al., 2003, Cook and Samman, 1996). Phenolic compounds are a class of antioxidant agents which act as free radical terminators (Shahidi and Wanasundara, 1992). The flavonoid contents of the extracts in terms of quercetin equivalent (the standard curve equation: y = 0.0067x + 0.0132, r^2 = 0.999) were between 25.15 ± 0.18 and 78.3 ± 4.5 (Table 1). The flavonoid contents in the extracts of *M. officinalis* (57 ± 5.4 mg g^{-1}) and *A. capillus-veneris* (78.3 ± 4.5 mg g^{-1}) were higher than that in the extracts of *U. dioica, E. maximum* and *P. major.* Table 1 also show the contents of total phenols that were measured by Folin Ciocalteu reagent in terms of gallic acid equivalent (standard curve equation: y = 0.05x + 0.0545, r^2= 0.9873). The total phenol varied from 22.3 ± 3 to 289.5 ± 5 mg g^{-1} in the extract powder. *M. officinalis*

Table 1. Flavonoid and phenol contents in the studied plant extracts.

Plant species	Flavonoid (mg/g)	Phenol (mg/g)
Mellilotus officinalis	57±5.4[1]	289.5±5[1]
Adiantum capillus-veneris	78.3±4.5	22.3±3
Plant ago major	25.15±0.18	31±4
Equisetum maximum	36.2±1.2	54.5±7
Urtica dioica	43.3±0.37	24.1±1

[1]Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

Table 2. Comparison of DPPH radical scavenging activity of the plant extracts and those of BHT and quercetin.

Plant species	Concentration (mg/ml)	Scavenging (%)
Mellilotus officinalis	0.1	94.3±0.6[1]
Adiantum capillus-veneris	4	44±1
Plantago major	0.8	89.3±1.5
Equisetum maximum	0.8	89.6±0.6
Urtica dioica	4	70.8±1
BHT	0.4	93±0.5
Quercetin	0.025	95.6±0.4

[1]Each value in the table was obtained by calculating the average of three experiments ± standard deviation.

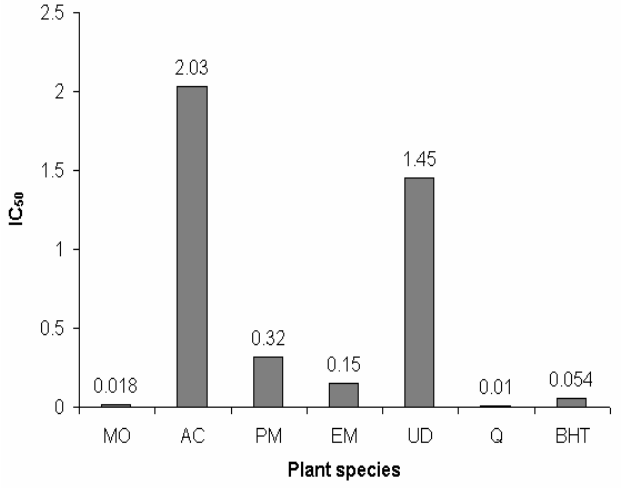

Figure 1. IC50 (mgml-1) values of plant extracts for free radical scavenging activity by DPPH radical. Lower IC50 value indicates higher antioxidant activity. Extracts: MO = Mellilotus officinalis, AC = Adiantum capillus-veneris, PM = Plantago major, EM = Equisetum maximum, UD = Urtica dioica, BHT = Butylated hydroxy toluene and Q = quercetin.

with total phenol contents of 289.5±5 mg g^{-1} had the highest amount among the plants in this study. The compounds such as flavonoids, which contain hydroxyls, are responsible for the radical scavenging effect in the plants (Das and Pereira, 1990; Younes, 1981). According to our study, the high contents of these phytochemicals in *M.* officinalis can explain its high radical scavenging activity.

Antioxidant activity

Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants through their scavenging power are useful for the management of those diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts (Koleva et al., 2002). Figure 1 shows the amount of each extract needed for 50% inhibition (IC_{50}). IC_{50} of the standard compounds, BHT and quercetin were 0.054 and 0.01 mg ml^{-1}, respectively. The highest radical scavenging activity was showed by *M. officinalis* with IC_{50}=0.018 mg ml^{-1} which is higher than that of BHT ($P<0.05$). The radical scavenging activity in the plant extracts decreased in the following order: *Me. Officinalis* > *E. maximum* > *P. major* > *U. dioica* > *A. capillus-veneris*. Most of the plants' extracts at different concentrations exhibited more than 70% scavenging activity (Table 2). The radical scavenging effect of *M. officinalis* at 0.1 mg ml^{-1} was similar to BHT at 0.4 mg ml^{-1}. Therefore, the antioxidant effect of *M. officinalis* was 4 times greater than that of the synthetic antioxidant, BHT.

Conclusion

The result of the present study showed that the extract of *M. officinalis*, which contain highest amount of flavonoid and phenolic compounds, exhibited the greatest antioxidant activity. The high scavenging property of *M. officinalis* may be due to hydroxyl groups existing in the phenolic compounds' chemical structure that can provide the necessary component as a radical scavenger.

Free radicals are often generated as by products of biological reactions or from exogenous factors. The involvements of free radicals in the pathogenesis of a large number of diseases are well documented. A potent scavenger of free radicals may serve as a possible preventative intervention for the diseases (Gyamfi et al., 1999). All of the extracts in this research exhibited different extent of antioxidant activity. *M. officinalis* extract showed a higher potency than BHT in scavenging of DPPH free radical. This may be related to the high amount of flavonoid and phenolic compounds in this plant extract.

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