Antioxidant activity, phenol, flavonoid and anthocyanin contents in various extracts of *Onosma dichroanthum* Boiss. in north of Iran

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Abstract

In the present study we carried out a record of the relative antioxidant activity to secondary metabolites content (phenol, flavonoid and anthocyanin) of *Onosma dichroanthum* Boiss. in various solvent extracts of plant root. The roots were collected in Kiasar Mountain (1800 m) in Mazandaran province, then dried and different extracts were obtained by acetone, ethanol and methanol (absolute and hydro alcoholic), ethyl acetate, chloroform and n-hexane-dichloromethane.The total phenol varied from 4.5 ± 0.7 to 125.6 ± 3.01 mgGAE g^{-1} in the extracts. Flavonoid contents were between 9.8 ± 3 to 41 ± 2.3 mgQUE g^{-1}, while anthocyanin contents were 11.5± 3.4 to 47.8± 6.8 mgECGgr^{-1}. DPPH (1,1-diphenyl-2-picryl hydrazyl) radical scavenging effect of the extracts was determined spectrophotometrically. The highest radical scavenging effect was observed in acetone extract of plant with EC50 4.06 mg DW. There was a positive correlation between antioxidant activity and total phenolic content for acetone extract. Thus, it was concluded that phenolic compounds were the predominant antioxidant components in the investigated plant species. Therefore, analysis of these results showed acetone was the best solvent to release most secondary metabolites of plant roots, these results will be confirmed the uses of this plant as anti-inflammatory to treat of burn and wounds in their traditional medicine.

Keywords: Onosma dichroanthum Boiss.; antioxidant; flavonoids; phenols; anthocyanin; 1,1-diphenyl-2-picryl hydrazyl

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Introduction

Free radicals due to increases of technology, radiation, chemical pollutants, toxins, preservative drugs, deep fried fast foods as well as physical stress, including atherosclerosis,

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arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS (Pourmorad et al., 2006). Currently, there is a worldwide trend and interest towards the use of medicinal and aromatic plants as antioxidants in foods (Yanishlieva et al., 2006). Besides, they are well known and have been traditionally used as natural drugs as antioxidants, anti inflammation and antiseptics to treat many ranges of illness. Therefore consuming antioxidants as free radical scavengers may be necessary (Cai et al., 2004; Katalinic et al., 2006; Wong et al., 2006; Yanishlieva et al., 2006). In vascular plants, more than 4000 phenolic and polyphenolic compounds were the most antioxidants which have been identified, namely, phenolic acids, tannins, coumarins, anthraquinones, flavonoids, phenolic diterpens and anthocyanin (Middleton and Kandaswami, 1993; Trease and Evans, 1989), which have the ability to scavenge free radicals, donate hydrogen atoms or electrons, or chelate metal cations (Pietta et al., 1998). High correlation was reported between the antioxidant capacity and total phenol and flavonoides contents of plants (Silva et al., 2007; Tawaha et al., 2007). Besides antioxidant capacity, phenolic compounds exhibit a wide range of biological activities, including anti-carcinogenic, anti-inflammatory, anti-viral, anti-allergic, estrogenic, immune-stimulating agents, antiallergenic, anti-artherogenic, anti-inflammatory, anti-microbial, antithrombotic, anti stress, anti hyperglycemia, cardioprotective and vasodilatory effects (Balasundram et al.,2006).Therefore, supplementing a food product with antioxidant plant phenols may provide a health benefit as well. Over the past few years, medicinal and aromatic plants have been extensively studied for their antioxidant capacities in the different regions of the world (Koleva et al., 2002; Mantle et al., 2000; Oke and Hamburger, 2002; Pourmorad et al., 2006). Flavonoids the major groups of 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).

Onosma dichroanthum Boiss., belongs to Boraginaceae family with red root extract. Locally known as" Hava Chobeh", *Onosma dichroanthum* Boiss. is one of the most important medicinal plants in North of Iran where it has been used in traditional medicine in isolation or combined with other medicinal herbs as antiseptic, wound healing and anti-inflammatory to treat inflammation of ulcer, sore, burn and wounds. *Onosma* is an important genus of Boraginaceae family with 150 species widespread in the East and Central Asia as well as in the Mediterranean area (Martonfi et al., 2008) ,which traditionally are used as stimulant in rheumatism, bladder pain, kidney irritation, palpitation of heart, burn wound healing (Ahmad et al., 2009), hemorrhoids and stomach ulcers (Salman et al., 2009). The main secondary metabolites of the roots of this family are alkaloids, naphthoquinones (alkannin, shikonin), polyphenols (flavonoids, phenolic acids), phytosterols, terpenoids, fatty acids, rosmarinic acid and caffeic acid (Li et al., 2010; Salman et al., 2009). High correlation was reported between total flavonoids and phenolic compounds of plants with free radical scavenging activity, inhibition of hydrolytic and oxidative enzymes (Pourmorad et al., 2006; Kirca and Arslan., 2008).

The aim of the present study was to evaluate and screen some solvents with respect to their total phenolic, flavonoid , anthocyanin content and antioxidant activity, as potential sources of natural antioxidants.

Materials and Methods

Plant materials

The roots of *Onosma dichroanthum* Boiss. were collected in Kiasar Mountainous region (1800m) of Mazandaran province in North of Iran during April and May 2010. A voucher specimen was identified and has been deposited at the Herbarium Museum of the Ferdowsi University of Khorasan Razavi province.The plant raw materials were cleaned and air-dried at room temperature. Roots were ground to a fine powder using a laboratory mill, passed through a 24 mesh sieve, to provide homogeneous powder for the analysis. Powdered materials were maintained at room temperature (22–23 °C), and protected from light until required for analyzes.

Preparation of the extracts

Powdered roots (5g) of *Onosma dichroanthum* Boiss. with 250 ml of various solvent acetone, methanol and ethanol (absolute and 80%), ethyl acetate, chloroform and n-hexane-dichloromethane were extracted by maceration method for 24h in a mechanical shaker at room temperature. Extracts were filtered with a piece of filter paper (Whatman No. 1) and were stored at 4 °C (Tawaha et al., 2007).

Solvents

Folin Ciocalteu reagent, aluminum chloride, ethanol, methanol, acetone, chloroform, ethyl acetate and n-hexan dichloromethane were purchased from Merck Co. (Germany).

Total phenols determination

Total phenolic content was estimated by the Folin Ciocalteu method, based on the procedure suggested by Pourmorad et al. (2006). Then 0.5 ml of plant extracts or Gallic acid (standard phenolic compound) was mixed with Folin Ciocalteu reagent (5 ml) 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 colorimeter at 765 nm. Gallic acid was used as a standard for calibration curve. Total phenol values were expressed in terms of mg equal Gallic acid in 1 g powder dry plant.

Total flavonoids determination

Total flavonoids content of each extract was determined by aluminum chloride method (Pourmorad et al., 2006). Plant extracts (0.5 ml) were separately mixed with 1.5 ml of solvent, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. They were kept at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer. Quercetin was used as a standard for calibration curve. Total flavonoid values were expressed in terms of mg equal Quercetin in 1 g powder dry roots plant.

Total anthocyanin determination

The total anthocyanin content was measured by the pH-differential method described by Giusti and Wrolstad (2001), using 2 buffer systems: potassium chloride buffer, pH 1, and sodium acetate buffer, pH 4.5. The sample diluted with corresponding buffer and they were kept at room temperature for 15 min, the absorbance was measured at 510 and 700 nm. Total anthocyanins were calculated as cyanidin-3-glucoside according to the following equation:

$$A = [(A_{510}-A_{700})_{\text{pH 1.0}}-(A_{510}-A_{700})_{\text{pH 4.5}}]$$

$$\text{TAC} = (A \times \text{DF} \times \text{MW} \times 100)/\text{MA}$$

where MW, DF and MA are molecular weight, dilution factor and molar absorptivity respectively**.**

Antioxidant activity test

1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging capacity Assay

The ability of the extract for free radical scavenging was assessed by the method suggested by Kirca and Arslan (2008). The aliquots of plant extract (20–40–60–80–100 µL) were mixed with a methanolic solution of DPPH (1 mm, 600 µL) and brought to 6 mL with solvent. After incubation in the dark at room temperature for 15 min absorbance was measured at 517 nm. The percent decrease in absorbance was recorded for each concentration and percentage inhibition was calculated according to the following formula:

$$\text{inhibition\%} = [(A_{DPPH} - A_{\text{Extract}}) / A_{DPPH}] \times 100$$

where A_{DPPH} is the absorbance value of the $DPPH^{\cdot}$ blank sample and A_{Extract} is the absorbance value of the test solution. The plots of the 'percentage inhibition amounts of dried plants (mg) in the extract' were used to find the concentration at which 50% radical scavenging occurred (EC50). The EC50 values were reported as 'mg Dry Weight (DW)' (Kirca and Arslan, 2008).

Statistical analysis

For all assays, data were expressed as means ± S.E. and analysis was carried out using Microsoft Office Excel 2007. The student t-test was applied to test for significant differences.

Results

Flavonoid and total phenol contents of the extracts

As shown in Table 1, the results indicated that the total phenolic content of various extracts had significant variation, ranging from 4.5 to 125.6 mg GAEg^{-1}, dry weight for those of solvents extracts, total flavonoide content 9.8 to 41 mg QUEgr^{-1} and quantity of anthocyanin 11.5 to 47.8 mg CGEg^{-1}. Many solvents such as acetone showed the highest secondary metabolites (Table 1). The findings showed that the acetone extract had the highest quantity of total phenol (125.6 mgEGAg^{-1}), total flavonoid (41 mgEQUg^{-1}) and anthocyanin (47.8 mgECGg^{-1}), while ethyl acetate and methanol 80% extracts had the lowest content of phenol and flavonoid compounds compared with the other solvents (Figs. I, II, III). Therefore analysis of these results shows that the acetonic extract possessd significant activity in release of most secondary metabolites of plant roots and also total phenol had the highest content in acetonic extract compared with the other secondary methabolites (Table 1).

Antioxidant activity

Figure IV shows inhibition of the DPPH solution in various quantities of dried roots of *Onosma dichroanthum* Boiss., The highest radical scavenging effect was observed in acetone extract of plant with EC_{50} 4.06 mg DW. The inhibition activity in this extract is increased at high concentration and the highest radical scavenging activity was observed at 30 minute and 2mg (Figure IV).

Table 1
Comparison of secondary metabolites of various extracts of *Onosma dichroanthum* Boiss. root

Solvents	Phenol (mg g^{-1})	Flavonoid (mg g^{-1})	Anthocyanin (mgg^{-1})
Acetone	125.6±3.01	41±2.3	47.8±6.8
Ethanol	18.3±4	10.5±1.3	32.4±8.5
Ethanol (80%)	17.7±5.2	19.3±4.5	30.6±1.9
Methanol	8.8±0.5	19.5±12	11.5±3.4
Methanol 80%	7.9 ±1.2	9.8±3	13.1±1.9
Chloroform	18.2 ±1.1	31.6±8.2	-
Ethyl acatate	4.5 ±0.7	20.9±4.1	-
N-hexan dichloromethane	5.6 ±0.3	40.8±1.1	-

Each value in the table is obtained by calculating the average of three experiments ± Standard Error.

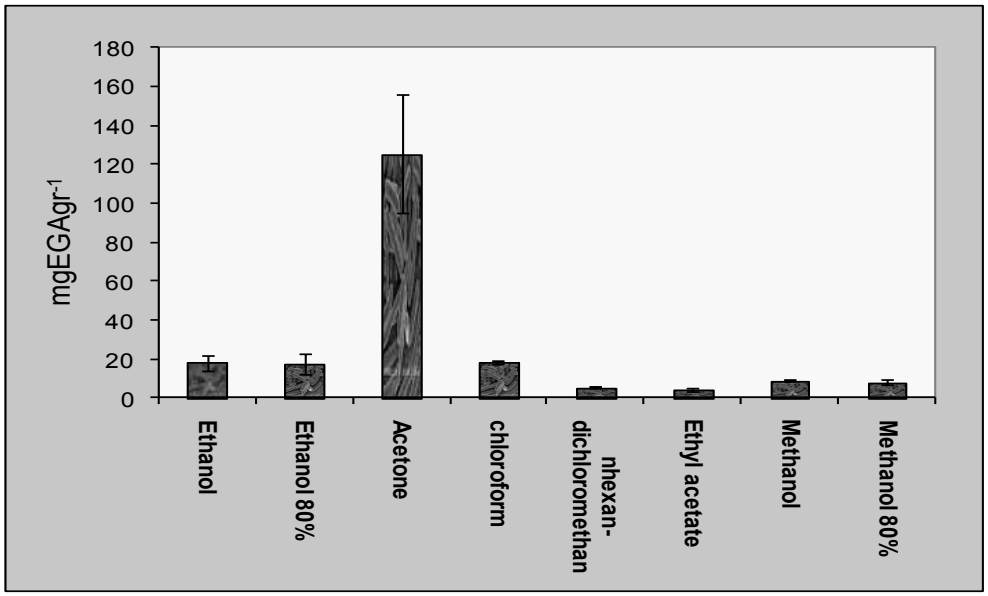

Fig. I. The quantity of total phenol of various extracts of *Onosma dichroanthum* Boiss. roots

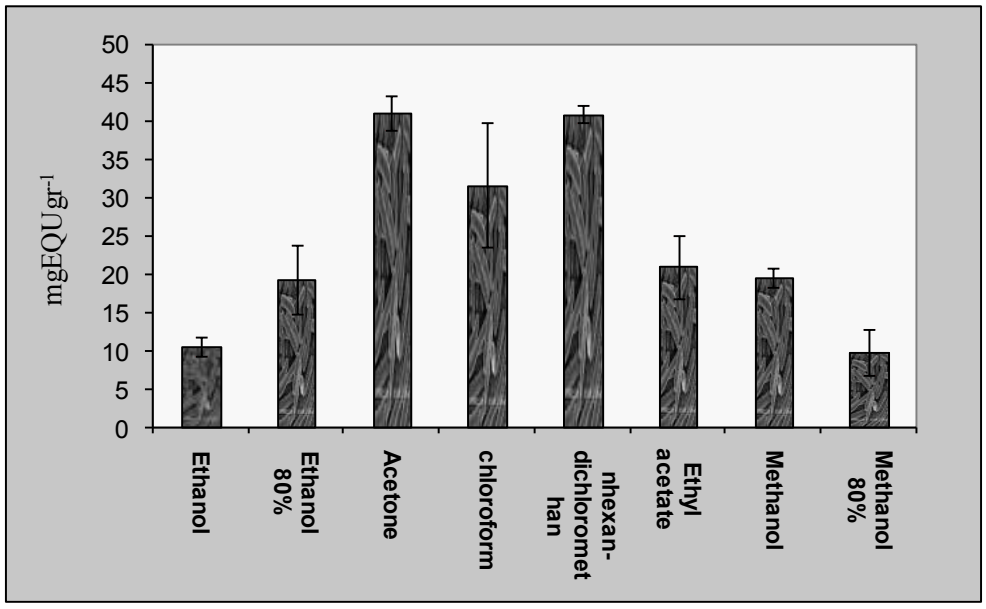

Fig. II. The quantity of total flavonoid of various extracts of *Onosma dichroanthum* Boiss. roots

Discussion

It has been recognized that phenols and 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). High levels of total phenolic content in acetone extract of root (125.6 mgg^{-1}DW) is known to be rich in chlorogenic acid, quercetin, Quercetin and Rosmarinic acids and its glycosides has been shown to possess anti-inflammatory, antioxidative, antiviral as well as antibacterial activity in *in vitro* and *in vivo* models (Petersen and Simmonds, 2003;Youn et al., 2003). There

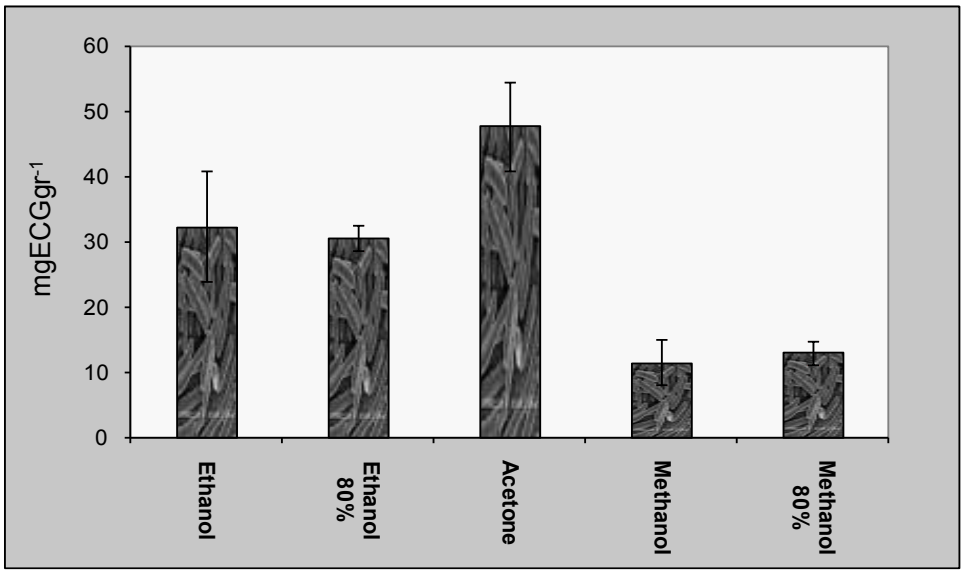

Fig. III. The total anthocyanin content of various extracts of *Onosma dichroanthum* Boiss. roots

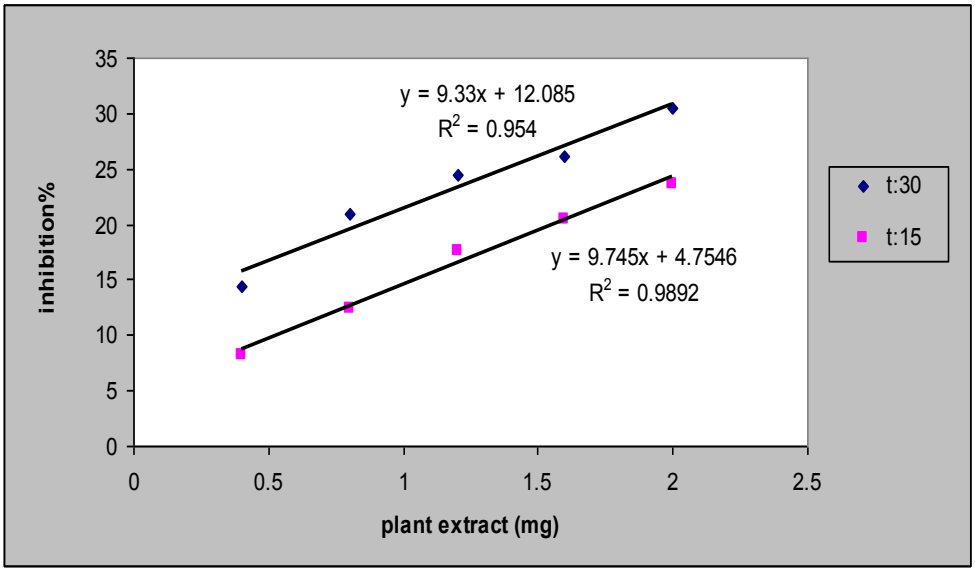

Fig. IV. Inhibition of the DPPH solution in various amounts of dried roots of *Onosma dichroanthum* Boiss. at various times

was a positive correlation between antioxidant activity and total phenolic content for acetone extracts. These results suggested that the phenolic compounds contributed significantly to the antioxidant capacity of the investigated plant species. These results were consistent with the findings of many research groups who reported such positive correlation between total phenolic content and antioxidant activity (Cai et al., 2004; Zheng & Wang, 2001; Pourmorad et al., 2006; Tawaha et al., 2007; Kirca and Arslan, 2008). 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 *Onosma dichroanthum* Boiss. can explain its high radical scavenging activity. In many similar studies about variety of species belonging to Boraginaceae family (*Onosma dichroanthum* Boiss, *Lithospermum erythrorhizon* , *Cordia multispicata* , *C. multispicata* and *Tournefortia bicolor, Ehretia laevis, Cordia myxa* and *Borago officinalis),* reporters were observed the correlation between quantity of total phenol and their antioxidant activity (Cai et al., 2004; Conforti et al., 2008).

According to our study, the high contents of these phytochemicals in *Onosma dichroanthum* Boiss. can explain its high radical scavenging activity. Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS (Pourmorad et al., 2006). Antioxidants through their scavenging power are useful for the management of these diseases. DPPH stable free radical method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extract (Koleva et al., 2002).

The result of the present study showed that the acetone extract of *O. dichloroanthum Boiss.* which contains highest amount of anthocyanin, flavonoid and phenolic compounds, exhibited the greatest antioxidant activity. Acetone extract showed a high potency in scavenging of DPPH free radical which may be related to the high amount of flavonoid and phenolic compounds in this plant extract.In this research project, we showed relationship between total flavonoid and phenol contents with their high antioxidant activity, which could provide potential natural sources of antioxidant compounds. So these *in vitro* studies may be of value in the design of further studies (via *in vivo* and clinical models), to unravel novel treatment strategies for disorders associated with free radicals induced tissue damage. These data demonstrate the acetone extract as the best solvent to release most secondary metabolites of plant roots for future research and also confirmed the uses of this plant in traditional medicine by rural healers of this region as antiseptic and anti-inflammatory to treat ulcer, burn and healing of various wounds.

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