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Full Length Research Paper

Effects of solvent type on phenolics and flavonoids content and antioxidant activities in *Onosma dichroanthum* Boiss.

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In our research, 8 kinds of solvents extracts from roots of *Onosma dichroanthum* Boiss. were used to examine the effects of extraction solvent on total phenolics (TP), total flavonoids (TF) , total anthocyanin (TA) content and antioxidant activity by 1,1-diphenyl-2-picryl hydrazyl radical scavenging (DPPH), total antioxidant capacity (TAC), reducing power (RP) and antioxidant activity (AA) were determined spectrophotometrically. Results showed that extraction solvent had significant effects on TP, TF, TA content and antioxidant activity of acetone extract. The highest content of TP, TF and TA were found in acetone extracts. The TP varied from 4.5 ± 0.7 to 125.6 ± 3.01 mgGAE g^{-1} dry weight, TF contents were between 9.8 ± 3 to 41 ± 2.3 mgQUE $g^{-1}$and TA were 11.5± 3.4 to 47.8± 6.8 mgECGgr^{-1}. Effective concentration (EC_{50}) (antioxidant activity) in TAC, RP and 2,2- diphenyl-1-picrylhydrazyl (DPPH) methods were measured at 0.495, 0.844 mg/ml and 4.21 mg dw, respectively and amount of antioxidant activity (AA%) was reported at 38.02%. The greater amount of phenolic compounds which leads to more potent radical scavenging effect was shown by acetone extract. Additionally, amount of phenolic compounds and antioxidant activities increased in acetone extract. Thus, a positive correlation existed between antioxidant activity and their total phenolic content. Acetone solvent showed the greatest capability in extracting antioxidants and inhibiting the free radicals produced.

Key words: Solvents, total phenol, flavonoid, anthocyanin, 1,1-diphenyl-2-picryl hydrazyl radical scavenging (DPPH), *Onosma dichroanthum* Boiss.

INTRODUCTION

Free radicals induced tissue injury contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia, central nervous system injury, gastritis, cancer and AIDS (Pourmorad et al., 2006).

Therefore currently, there is a world-wide trend in the therapeutic potentials of medicinal plants as antioxidants in reducing such free radical induced tissue injury and use of them as antioxidants in foods and drugs (Yanishlieva et al., 2006). Epidemiological studies have shown that many of these antioxidant compounds possess anti-inflammatory, anti-atherosclerotic, anti-tumor, anti-mutagenic, anti carcinogenic, antibacterial or antiviral activities (Owen et al., 2000; Sala et al., 2002). Medicinal value of plants is related in their phytochemical components and their secondary metabolites such as: Phenolic compounds, flavonoids, alkaloids and tannins (Mohammedi et al., 2011) and some evidence suggests that the biological actions of these compounds are related to their antioxidant activity (Ghasemzadeh et al.,

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2011). Flavonoids are a group of polyphenolic components synthesized by plants with known properties which include free radical scavenging, inhibition of hydrolytic and oxidative enzymes, anti-inflammatory action, reduce blood-lipid and glucose and to enhance human immunity (Atoui et al., 2005). Today's plant secondary metabolites are the interest subject of research, but their extraction as part of phytochemical or biological investigations presents specific challenges that must be addressed throughout the solvent extraction process.

Technique of secondary metabolites isolation such as phenol and flavonoid components from a plant material depends on the type of compounds and solvent extraction (Goli et al., 2004; Ghasemzadeh et al., 2011). Previous studies showed that solvent polarity had important effect on polyphenol contents and their antioxidant activity. (Turkmen et al., 2006; Lapornik et al., 2005; Ghasemzadeh et al., 2011; Siddhuraju et al., 2003; Sultana et al., 2007).

Onosma dichroanthum Boiss. (Boraginaceae), with red root extract and locally known as" Hava Chobeh", is one of the most important medicinal plants, which has been used in traditional medicine in North of Iran as single use or combination with another medicinal herbs as antiseptic and anti-inflammatory to healing wounds and burns. The *Onosma* L. genus with 150 species widespread in the East and the Central Asia and in the Mediterranean area (Martonfi et al., 2008), which has been used as anti-inflamation to treat of hemorrhoids, stomach ulcers, as antioxidant and stimulant to treat of heart palpitation, burn wound healing, rheumatism, bladder pain and kidney irritation (Salman et al., 2009; Ahmad et al., 2009) According to some studies, polyphenols (flavonoids, phenolic acids), rosmarinic acid and caffeic acid, phytosterols, alkaloids, naphthoquinones (alkannin, shikonin), terpenoids, fatty acids were the main secondary metabolites of the roots of species in this family, with have antioxidant activity (Li et al., 2010; Salman et al., 2009; Petersen and Simmonds, 2003). It is not clear correlating, which the type of solvents and their effecting to total phenolics and flavonoids in O. *dichroanthum* and evaluating the antioxidant activity of them. On the other hand, little is known about phenolic and flavonoid contents and antioxidant activity of *O. dichroanthum* extracted by different solvents. Therefore, the objectives of this research were to investigate the effect of different extracting solvents on total phenolics (TP), total flavonoids (TF), total anthocyanin (TA) and antioxidant activity in 1,1-diphenyl-2-picryl-hydrazyl (DPPH), total antioxidant capacity (TAC), reducing power (RP) and antioxidant activity (AA) methods.

MATERIALS AND METHODS

Plant materials

O. dichroanthum roots were collected in Kiasar Mountainous region (1800 m) of Mazandaran province in North of Iran during April and May 2010. This voucher of specimen was identified by a botanist (Joharchi, M.R.) and has been deposited at the Herbarium of Ferdowsi University of Mashhad (FUMH), Khorasan Razavi provience.

The plant raw materials were washed and dried in a hot air oven at 50°C for 8 to 10 h. Roots were ground to a fine powder using a laboratory mill and the powdered materials were maintained at room temperature (22 to 24°C), and protected from light.

Extract preparation

Extract preparation for flavonoids, phenol and anthocyanin tests

Powdered roots (5 g) of *O. dichroanthum* 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 24 h in a mechanical shaker at room temperature. Extracts were filtered with a filter paper (Whatman No. 1) and was stored at 4°C.

Extract preparation for antioxidant activity

The dried roots (45 g) were extracted overnight with 300 ml acetone, in a mechanical shaker at room temperature. Extract was filtered with Whatman No. 1 filter paper. The filtrate obtained from acetone was evaporated to dryness at 40°C in a rotary evaporator and stored at 4°C (Arabshahi-Deloue and Urooj, 2007).

Total phenols determination

Total phenolic content was estimated by the Folin Ciocalteu method, based on the procedure of (Pourmorad et al., 2006). A 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 colorimetry at 765 nm. Gallic acid was used as a standard for calibration curve. Total phenol values are 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. It remained at room temperature for 30 min; the absorbance of the reaction mixture was measured at 415 nm with a spectrophotometer, and quercetin was used as a standard for calibration curve. Total flavonoids values are 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 (Giusti and Wrolstad, 2001). Two dilutions of berry extracts were prepared, one with potassium chloride buffer (pH 1.0) (1.86 g KCl in 1 L of distilled water, pH value adjusted to 1.0 with concentrated HCl), and the other with sodium acetate buffer (pH 4.5) (54.43 g $CH_3CO_2Na \cdot 3H_2O$ in 1 L of distilled water, pH value adjusted to 4.5 with concentrated HCl). Absorbance was measured simultaneously at 510 and 700 nm after 15 min incubation at room

temperature. The content of total anthocyanins was expressed in mg of cyanidin-3-glucoside equivalents per dry weight.

Antioxidant activity tests

Antioxidant activity

Total antioxidant activity was determined by the 2,2- diphenyl-1-picrylhydrazyl (DPPH) method described by Ayhan et al. (2009). A 0.1 ml of sample was mixed with 0.9 ml of 100 mM Tris–HCl buffer (pH 7.4) to which 1 ml of DPPH (500 μM in ethanol) was added. The mixture was shaken vigorously and left to stand for 30 min. Absorbance of the resulting solution was measured at 517 nm by a UV–Visible spectrophotometer. The antioxidant activity was calculated using the following equation:

$$\text{Antioxidant activity (AA\%)} = 1 - (A_{\text{Sample (517 nm)}} / A_{\text{Control (517 nm)}}) \times 100$$

Reducing power assay

Dried extract (12.5 to 1000 µg) in 1 ml of the corresponding solvent was combined with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide ($K_3Fe(CN)6$; 10 g L^{-1}), after the mixture was incubated at 50°C for 30 min. Then, 2.5 ml of trichloroacetic acid (100 g L^{-1}) were added and the mixture centrifuged at 1650 g for 10 min. Then, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (1 g L^{-1}), and the samples absorbance was measured at 700 nm (Arabshahi-Deloue and Urooj, 2007).

1,1-diphenyl-2-picryl hydrazyl radical scavenging capacity assay

The ability of the extract for free radical scavenging was assessed by the method of Kırca and Arslan (2008). The aliquots of 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 methanol. Then for 15 min incubation in the dark and at room temperature, after that 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:

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

A_{DPPH} is the absorbance value of the $DPPH^-$ blank sample and $A_{Extract}$ is the absorbance value of the test solution. The plots of the 'percentage inhibitions amounts of dried plants (mg) in the extract' were used to find the concentration at which 50% radical scavenging occurred (EC_{50}), (Kirca and Arslan, 2008).

Total antioxidant capacity

This experimental procedure was adapted from Arabshahi-Deloue and Urooj (2007) method, which is based on the reduction of Mo (VI) to Mo (V) by the sample and observation of a green phosphate/Mo (V) complex at acidic pH. An aliquot of 0.1 ml of sample solution, containing 12.5 to 1000 µg of dried extract in corresponding solvent, was combined in a tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). They were incubated in a thermal block at 95°C for 90 min. Then we got the samples cold and measured the absorbance at 695 nm. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent was used for the sample, and was incubated under the same conditions as the rest of the samples (Arabshahi-Deloue and Urooj, 2007).

Statistical analysis

For all assays, data were expressed as means ± S.E. and significance differences for multiple comparisons were determined using analysis of variance (ANOVA). Differences at $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Total phenolics, flavonoids and anthocyanin

The level of phenol, flavonoids and anthocyanin compounds in different solvent extracts of the roots of *O. dichroanthum* are shown in Table 1. The results indicated that the TP content of various extract had significant variation, ranging from 4.5 to 125.6 mg $GAEgr^{-1}$ dry weight for those of solvents extracts, TF content 9.8 to 41 mg $QUEgr^{-1}$ and TA content from 11.5 to 47.8 mg $CGEgr^{-1}$. In all of solvents, High content of TF (41 $mgEQUgr^{-1}$), TP (125.6 $mgEGAgr^{-1}$) and TA (47.8 $mgECGgr^{-1}$) obtained from acetone extract ($p \le 0.05$). While Ethyl acetate and methanol solvents had the lowest content of phenol and flavonoid compounds, when compared with the other solvent (Table 1, Figures 1, 2 and 3).

In other findings, the effect of different solvents on TP content are very considerable and indicate the highest content of phenolic and antioxidant compounds release of barley flour with mixtures of ethanol and acetone solvents (Bonoli et al., 2004; Chatha et al., 2006; Siddhuraju et al., 2003). Our results showed that the acetone and ethanol were better solvents for flavonoid, phenol and anthocyanin extraction compared to another solvent. It is similar to results reported by another research. However, ethanol and acetone were better solvents compared to water solvent (Kallithraka et al., 1996; Yilmaz et al., 2006). Turkmen et al. (2006) report that solvents with different polarity (ethanol and acetone) have significant effect on polyphenol content and antioxidant activity in higher content in more polar solvents (Turkmen et al., 2006). But Jayaprakasha et al. (2001) in another study showed that the ineffectiveness of acetone, methanol and water for the extraction of total phenols of grapes seeds (*Vitis vinifera*). Similar to our finding, the free radical scavenging power of antioxidant components is very much associated with their TP and TF content (Ghasemzadeh et al., 2010). The plant extracts with higher levels of total phenolics and flavonoids also exhibit greater free radical scavenging (Yingming et al., 2004; Ghasemzadeh et al., 2010). According to the previous studies on *Zingiber officinale* Roscoe (ginger) DPPH activities varieties with high level of TP and TF have high activity of free radical scavenging (Hasna et al., 2009; Praven et al., 2007; Ghasemzadeh

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

Solvent	Secondary metabolites		
	Phenol (mgGAE g^{-1})	Flavonoid (mgQUE g^{-1})	Anthocyanin (mgECGgr^{-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
Ethanol80% (Aq/ethanole)	17.7±5.2	19.3±4.5	30.6±1.9
Methanol	8.8±0.5	19.5±12	11.5±3.4
Methanol80% (Aq/methanole)	7.9 ±1.2	9.8±3	13.1±1.9
Chloroform	18.2 ±1.1	-	-
Ethyl acetate	4.5 ±0.7	20.9±4.1	-
N-hexan dichloromethane	5.6 ±0.3	40.8±1.1	-

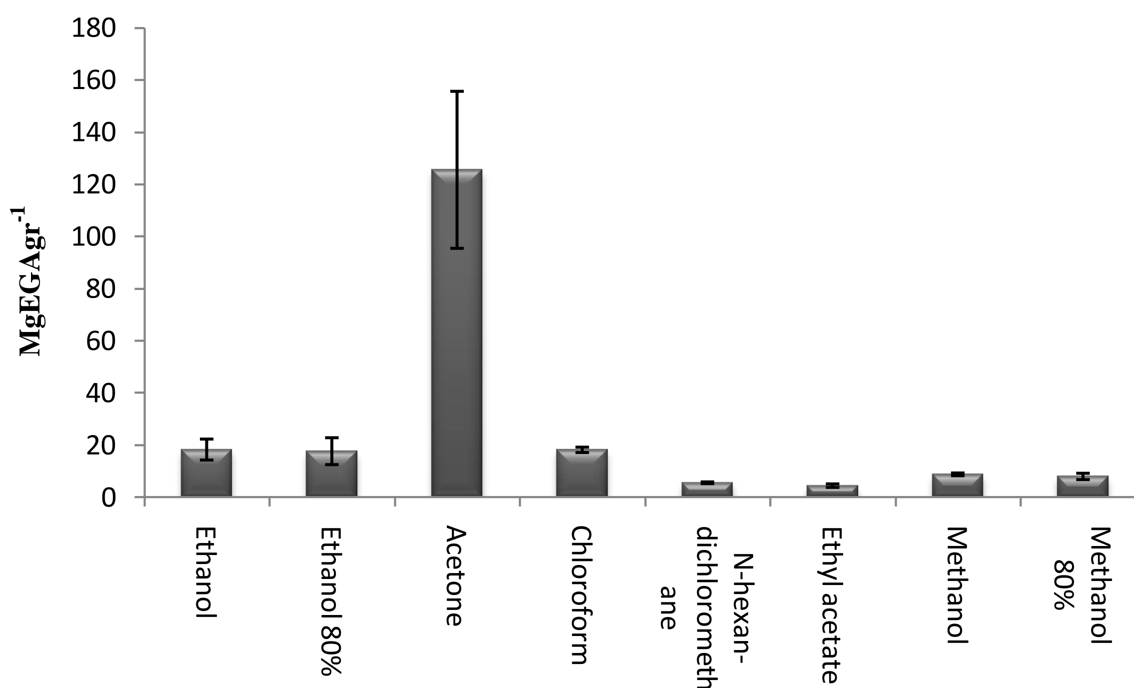

Figure 1. The quantity of total phenol of various extracts of *Onosma dichroanthum* Boiss. roots.

et al., 2011). The various studies show that, with change in solvent polarity, its ability to dissolve especial group of antioxidant compounds alters and influences the antioxidant activity estimation (Zhou et al., 2004).

Antioxidant activity

Antioxidant capacity of the acetone extracts in TAC, RP, AA and DPPH ranged from 0.238 to 0.844 mg ml^{-1}. Results in this study show that considerable different had been seen between reducing power of butylated hydroxyanisole (BHA) and acetone extract, which extract with the highest amount of EC_{50} (0.495 mg/ml) had the weakest activity whereas, BHA had the least amount of EC_{50} (0.238 mg/ml) and the most potent reducing agent (Figure 4).

The most appropriate and fastest way of evaluating the antioxidant activity of plant extracts is DPPH stable free radical method. Recently, DPPH scavenging method has been widely used in antioxidant activity studies of herb extracts (Chatha et al., 2006; Canadanovic-Brunet et al., 2005; Pinelo et al., 2004). In fact, free radical scavenging method (DPPH) showed the reduction of alcoholic DPPH solutions in the presence of a hydrogen donating antioxidant (Koleva et al., 2002).

The results showed that the plant extract had the capability of scavenging the $DPPH^-$ radicals and the inhibition activity in this extract is increased at high concentration and EC_{50} were 0.787 and 4.21 mg dw for BHA and extract respectively (Figure 5).

In TAC method, total antioxidant capacity is increased

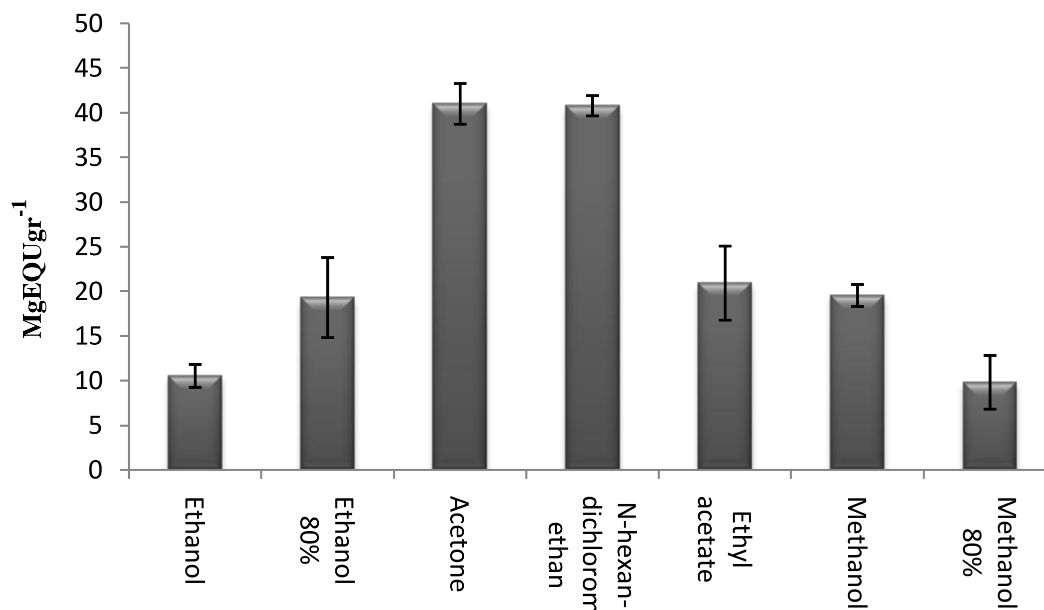

Figure 2. The quantity of total flavonoid of various extracts of *Onosma dichroanthum* Boiss. roots.

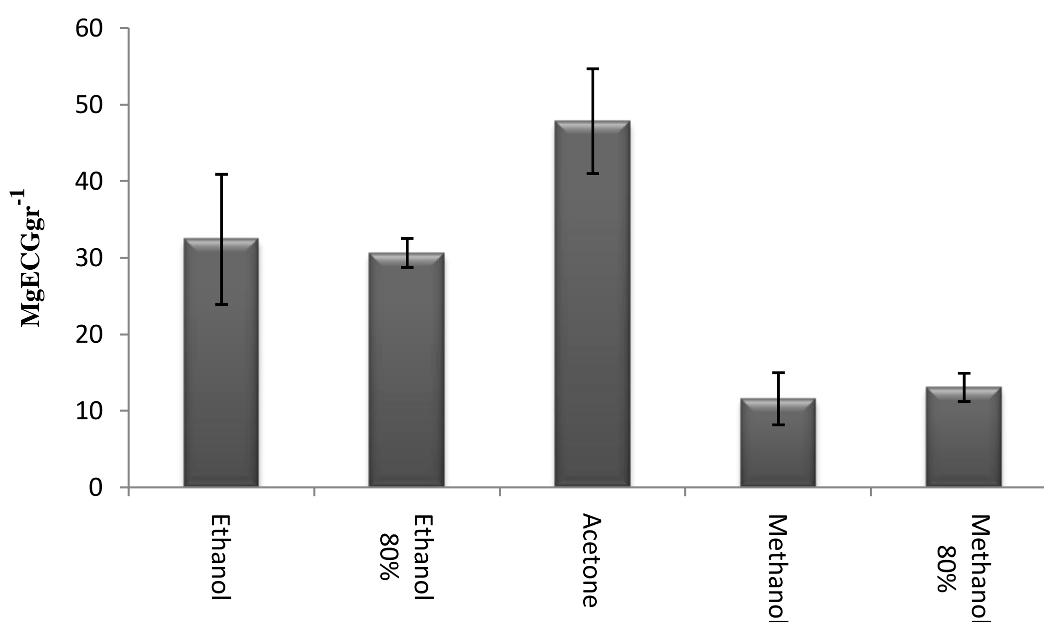

Figure 3. The total anthocyanin content of various extracts of *Onosma dichroanthum* Boiss. roots.

at high concentrations for BHA and plant extract and the the ability of BHA is approximately double, and EC_{50} is reported 0.844 mg/ml for extract and 0.456 mg/ml for BHA (Figure 6), and in Antioxidant Activity method, the amount of AA% reported 38.02%. According to our study, the high contents of these phytochemicals in *O. dichroanthum* Boiss. can explain its high radical scavenging activity.

In similar studies about variety of species belongs to Boraginaceae family (*Lithospermum erythrorhizon*, *Cordia multispicata* and *Tournefortia bicolor, Ehretia laevis, C. myxa* and *Borago officinalis*), reporters show that the high quantities of total phenole and flavonoids, which important correlation between their antioxidant activity was observed (Cai et al., 2004; Conforti et al., 2008). By Correia et al. (2010) the total phenolic content and antioxidant activity from leaves of two Boraginaceae species; *C. multispicata* and *T. bicolor* Sw. measured. Their results

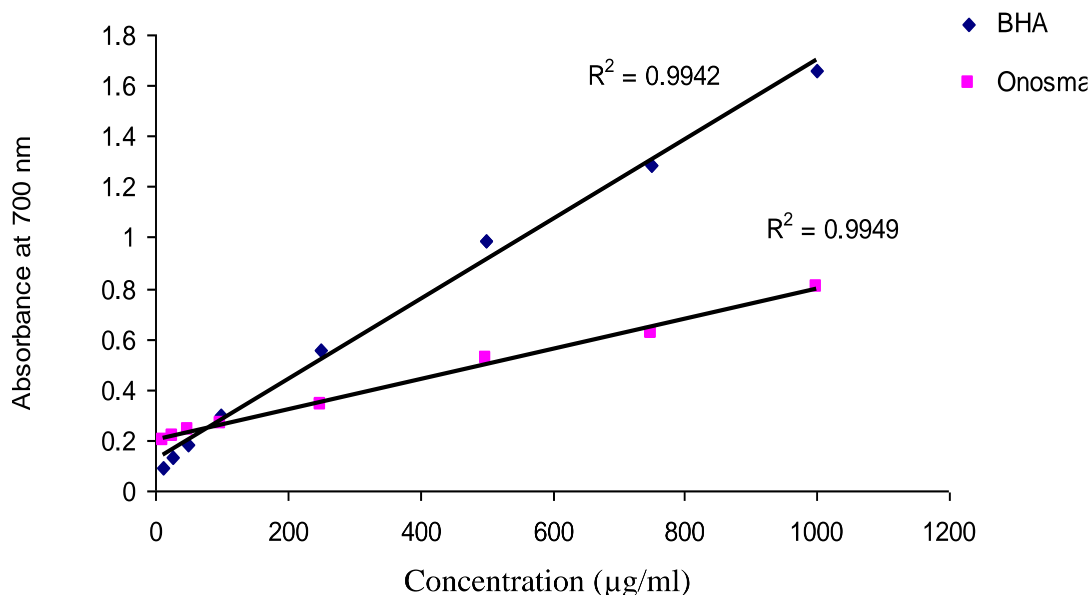

Figure 4. Reducing power of root extract of *Onosma dichroanthum* Boiss.

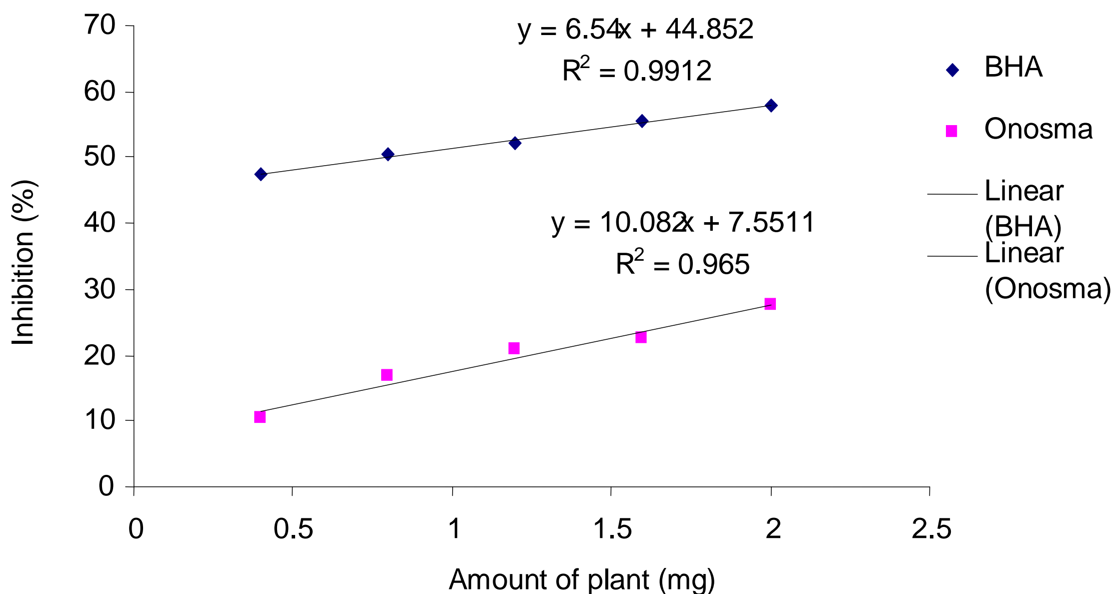

Figure 5. Inhibition of the DPPH solution in various amount of *Onosma dichroanthum* Boiss. Root.

show *T. bicolor* has higher phenol content (68.8 to 1000 mg/g) than *C multispicata* (66.1 to 231 mg/g) and scavenging radicals (IC_{50}) were 12.8 to 437 mg/l which important correlation between quantity of total phenol and antioxidant activity was observed. High correlations were found between the total antioxidant capacity assay and phenolic content of the many herbs, vegetables, fruits and Indian and Chines medicinal plants (Surveswaran et al., 2007; Cai et al., 2004; Dorman et al., 2004).

In this study, the higher total phenolic content of the extracts resulted in higher total antioxidant capacity. In previous study, phenolic compounds possessed potent antioxidant activity and also had anticancer, anti-carcinogenic, antimutagenic activities, such as phenolic acids (for example, chlorogenic acid, caffeic acid, ferulic acid), flavonoids (for example, vitexin, quercetin, wogonin, genistein, catechins, isoflavones), quinones (for example. emodin, rhein, aloe-emodin), coumarins (for example, 7-hydroxy- coumarin), stilbenes (for example, resveratrol), curcuminoids (for example curcumin), lignans, etc. (Ho et al., 1994; Gao et al., 2000; Owen et al., 2000; Xiao et al., 2000; Yang et al., 2001; Tapiero et

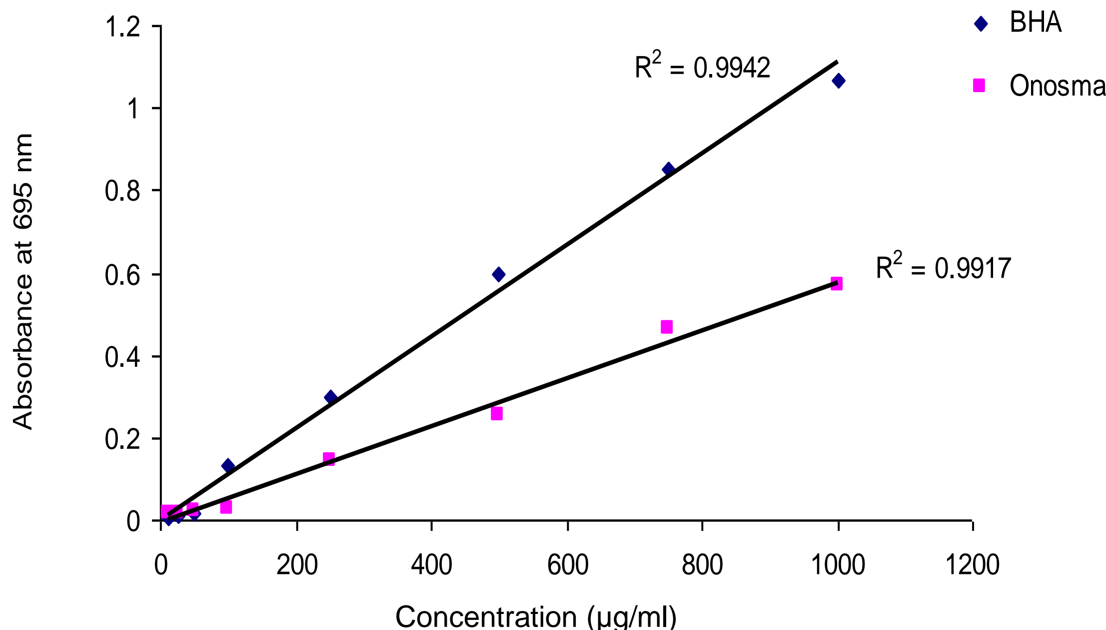

Figure 6. Total antioxidant activities of root extract of *Onosma dichroanthum* Boiss.

al., 2002)

Conclusion

We introduce the acetone extract with highest amount of TP, TF and TC compounds as a good solvent in extracting with highest antioxidant activity and positive correlation existed between antioxidant activity and total phenolic and flavonoid content. These data demonstrate the acetone extract as the best solvent to release of most secondary metabolites of *O. dichroanthum* Boiss. roots for future research, which could provide potential natural sources of antioxidant compounds.

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