

Full Length Research Paper

Antibacterial and antioxidant activities of root extract of *Onosma dichroanthum* Boiss. in north of Iran

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Onosma dichroanthum Boiss. (Boraginaceae) is locally known as "Hava Chobeh" in north of Iran, which has been used by the rural people as anti-inflammation and antiseptic to treat skin burns and wound healing. In this context, the antimicrobial potential of acetone, chloroform, methanol, ethanol and n-hexane-dichloromethane extracts from root plant against Gram positive and negative bacteria were studied by "agar dilution" and "well diffusion" methods, and the antioxidant properties were obtained by various methods including reducing power (RP), total antioxidant capacity (TAC) and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Results showed that Gram positive bacteria were more sensitive than Gram negative bacteria, especially to acetone extract in the well diffusion method. *Bacillus cereus* with minimum inhibitory concentration (0.078 mg/ml) was the most sensitive bacterium but no significant difference has been seen in antibacterial activity of various solvents. The antioxidant activity with IC₅₀ was measured by TAC and RP methods 1 and 0.72 mg/ml respectively. Therefore, reducing power assay had more antioxidant activity than TAC method. IC₅₀ in DPPH method was 4.08 mg dry weight. These results indicated that the use of *O. dichroanthum* root can be a confirmation for using this plant in traditional medicine, as antiseptic and antioxidant effect.

Key words: *Onosma dichroanthum* Boiss., antibacterial, antioxidant activity.

INTRODUCTION

Reactive oxygen species (ROS) including free radicals such as (O₂⁻, OH[•]) and non free radicals (H₂O₂, ¹O₂) along with different forms of active oxygen are involved in diverse physicochemical processes in the body (Qureshi et al., 2009) which have main role in the pathogenesis of different diseases, such as neurodegenerative disorders, cancer, cardiovascular diseases, arteriosclerosis, cataracts and inflammation (Conforti et al., 2008a). Antioxidants prevent diseases by various mechanisms including, scavenging free radicals, against oxidative stress and inhibiting lipid peroxidation (Miller and Rice-Evans, 1997). More than 80% of the world's population to

prevent and treat of their diseases depends upon traditional medicine (Shanmuga Priya et al., 2002). Therefore, attention to traditional medicine and the use of medicinal plants is being widespread and plants still represent as a largest source of natural antioxidants and antimicrobial components (Conforti et al., 2008a; Sokmen et al., 1999). Currently, widely use of medicinal and aromatic plants, as antioxidants in food and drug industries in the world, is widespread (Kırca and Arslan, 2008), and extravagant with antibiotics, in the last years, this has led to dissemination of resistance genes and emergence of bacterial resistance (Karthy et al., 2009). The Boraginaceae family includes about 100 genera and 2000 species distributed in temperate and tropical zones (Naz et al., 2006a), the main secondary metabolites of this family are alkaloids, naphthoquinones, polyphenols, phytosterols, terpenoids and fatty acids, which show

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antioxidant and antibiotic activities (Li et al., 2010).

Wound healing property of this family attribute to antibacterial, antiviral, antioxidant and anti-inflammatory activities of phenolic components, such as flavonoids, phenolic acids and naphthoquinones such as alkannin and shikonin (Li et al., 2010; Salman et al., 2009). *Onosma* is an important genus of this family with 150 species widespread in the east and the central Asia and in the Mediterranean area; these species have been used as an antioxidant, antibacterial, antiviral and anti-inflammatory agents (Martonfi et al., 2008; Ahmad et al., 2009). The aim of the present study was to evaluate the antibacterial and antioxidant activities of *Onosma dichroanthum*, which has been used by the rural healers of Mazandaran province in healing wounds and burns.

MATERIALS AND METHODS

Plant materials

The roots of *O. dichroanthum* were collected in Kiasar mountainous region in north of Iran during April and May 2010. Kiasar is located in south east of Sari in Mazandaran province, country region with height of (1300 to 2750 m). The climate is semi humid to mountainous cold, soil tissue of this region is sandy clay loam, pH: 7.2, electric conduction: 3.1, the most annual rainfall (49 mm) is in Mar. The voucher specimen was authenticated by botanist M.R. Joharchi (2010) and has been deposited at the Herbarium of Ferdowsi University of Mashhad (FUMH), Khorasan Razavi province. The roots were washed and dried in a hot air oven at 50°C for 8 to 10 h. The dried material was kept in an air-tight container at 4°C.

Microorganisms

The microorganisms used for the antibacterial activity screening were eight bacteria (3 Gram positive and 5 Gram negative). These bacteria are *Staphylococcus aureus* ATCC (1885), *Bacillus cereus* ATCC (6633), *Micrococcus luteus* ATCC (79840), *Escherichia coli* ATCC (ESBL+), *Citrobacter freundii* ATCC (24580), *Yersinia enterocolitica* PTCC (1151), *Salmonella typhimurium* PTCC (1639) and *Shigella dysenteriae* PTCC (1188). These bacteria were procured from Pasteur Institute of Iran.

Phytochemical tests

2,2-Diphenyl-1-picryl hydrazyl (DPPH), Butylated hydroxyanisole (BHA), methanol, ethanol, acetone, chloroform, n-hexane-dichloromethane and Dimethyl Sulfoxide (DMSO) were purchased from Sigma and Merck Co (German).

Extraction

Powdered root (25 g) of *O. dichroanthum* Boiss. with 250 ml of various solvent acetone, chloroform, methanol (100, 80%), ethanol were separately extracted by maceration method, and n-hexane-dichloromethane solvent (1:1) by reflex method. Extracts were filtered with Whatman No. 1 filter paper. The filtrates obtained from extracts were evaporated into dry rotary evaporator at 40°C and were stored at 4°C (Pourmorad et al., 2006; Ozgen et al., 2003).

Antimicrobial activity tests

Well diffusion method

Antibacterial activity was measured by using a well diffusion method (Hassan et al., 2009). Dilutions of each extract were prepared in 1 ml volume of DMSO to give a fraction from 1/2 to 1/512 (100 mg/ml to 0.39 mg/ml final concentration). Mueller Hinton Agar (MHA) was inoculated by 1.5×10^8 CFU/ml of suspension of each tested bacteria. Then, 50 μ l of each concentration of extracts poured into each well on the MHA media. The plates were incubated at 37°C for 24 h, and activity was then determined by measuring the diameter of zones showing complete inhibition (mm), DMSO was used for negative control, Gentamicin and Erythromycin as positive control.

Agar dilution method

Minimum inhibitory concentration (MIC) was determined by the agar dilution method. In this method, serial dilutions of different extracts were prepared in DMSO. 2 ml of different dilutions poured into plates containing 18 ml sterile MHA medium at temperature about 50°C, the mixture was homogenized immediately before solidification to obtain final dilutions from 10 mg/ml to 0.078 mg/ml. Then 30 μ l of suspension (0.5 McFarland) of tested bacteria were spread on the surface of media. The plates were incubated at 37°C for 24 h. MIC was defined as the lowest concentration of each extract, in which no growth of the tested bacteria was observed.

Antioxidant activity tests

Reducing power assay

The reducing power assay was determined according to Arabshahi-Deloue and Urooj (2007) method. At first, the dried extract (12.5 to 1000 μ g) 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.

Total antioxidant capacity

This experimental procedure was adapted from Arabshahi-Delouee 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 μ g 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 cold the samples and measured their 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.

2,2-Diphenyl-1-picrylhydrazyl radical scavenging capacity assay

The ability of the extract for free radical scavenging was assessed

Table 1. The means of inhibition zones (mm) from standard antibiotics and most concentration (½) of various extracts *Onosma dichroanthum* Boiss.

Microorganisms	Inhibition zones (mm)							
	Plant extracts					c	Antibiotic	
	Acetone	Ethanol	Methanol	Chloroform	n-hexan-dichloromethan	Dimethyl Sulfoxide	Erythromycin	Gentamicin
<i>S. a</i>	16	15	15	13	14	-	29	24
<i>B. c</i>	17	15	14	14	12	-	38	31
<i>M. l</i>	14	11	14	13	13	-	38	31
<i>E. c</i>	-	-	-	-	-	-	14	21
<i>C. f</i>	-	-	-	-	-	-	13	26
<i>Y. e</i>	-	-	-	-	-	-	10	22
<i>S. t</i>	-	-	-	-	-	-	12	24
<i>S. d</i>	-	-	-	-	-	-	10	20

-: No inhibition c: Negative control ;*S. a*: *Staphylococcus aureus*, *B. c*: *Bacillus cereus*, *M. l*: *Micrococcus luteus*, *E. c*: *Escherichia coli*, *C. f*: *Citrobacter freundii*, *Y. e*: *Yersinia enterocolitica*, *S. t*: *Salmonella typhimurium*, *S. d*: *Shigella dysenteriae*.

Table 2. The MIC values (mg/ml) from various extracts of *O. dichroanthum* Boiss. by Pour plate method.

Minimum inhibitory concentrations Extracts (MIC)			
Extracts	<i>S. a</i>	<i>B. c</i>	<i>M. l</i>
Acetone	0.312	0.078	0.312
Ethanol	0.312	0.156	0.312
Methanol	0.156	0.156	0.312
Chloroform	0.156	0.156	0.312
n-hexane-dichloromethane	0.156	0.156	0.156

S. a: *Staphylococcus aureus*, *B. c*: *Bacillus cereus*, *M. l*: *Micrococcus luteus*

by the method of Kirca and Arslan (2008). The aliquots of plant extract (20 to 40 to 60 to 80 to 100 µl) were mixed with a methanolic solution of DPPH- (1 mm, 600 µl) and brought to 6 ml with solvent. After incubating in dark and room temperature that absorbance was measured at 517 nm. A DPPH- blank sample (containing 5.4 ml of methanol and 600 µl of DPPH-solution) was prepared. The percent decrease in absorbance was recorded for each concentration and percentage inhibition was calculated according to the following formula:

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

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 (IC_{50}).

RESULTS AND DISCUSSION

Antibacterial results

Antibacterial activity of dried extracts (acetone,

chloroform, methanol, ethanol and n-hexane-dichloromethane extracts) has been shown in Table 1. The results showed that diameter of inhibitory zones for Gram positive bacteria in well diffusion method were between 11 to 17 mm. All Gram positive bacteria were sensitive to various extract of root plant. Acetone extract was the most effective plant extracts and *B. cereus* with 17 mm inhibition zone in acetone extract was the most susceptible bacterial strain. None of Gram negative bacteria showed sensitivity to different extracts of roots *O. dichroanthum*. Although we were not able to demonstrate the therapeutic effects of this extract on wound lesion in an animal model (Zarghami Moghaddam et al., 2011).

Table 2 shows the MIC value of different kind of extract of root *O. dichroanthum* against tested Gram positive bacteria. The lowest MIC was seen against *Bacillus cereus* which was between 0.078 mg/ml to 0.156 mg/ml and acetone extraction had the best inhibitory effect and lowest MIC.

The reason for higher sensitivity of Gram positive bacteria in comparison with negative bacteria could be ascribed to the differences between their cell wall compositions. Negi et al. (2005) stated Gram positive bacteria contain an outer peptidoglycone layer, which is an ineffective permeability barrier, but higher resistance of Gram-negative bacteria due to external agents attributed to the presence of LPS in their outer membranes and the presence of different digestive enzymes in periplasmic space, which make them resistant to antibiotics, detergent and hydrophilic dyes. No studies have yet been carried out about this subject, and this study is the first evaluation of antibacterial and antioxidant activities of *O. dichroanthum*, in north of Iran. But different studies were carried out on another species of *Onosma* (Benli et al., 2007; Ahmad et al., 2009). Our results were in agreement with the findings of Khalil et al.

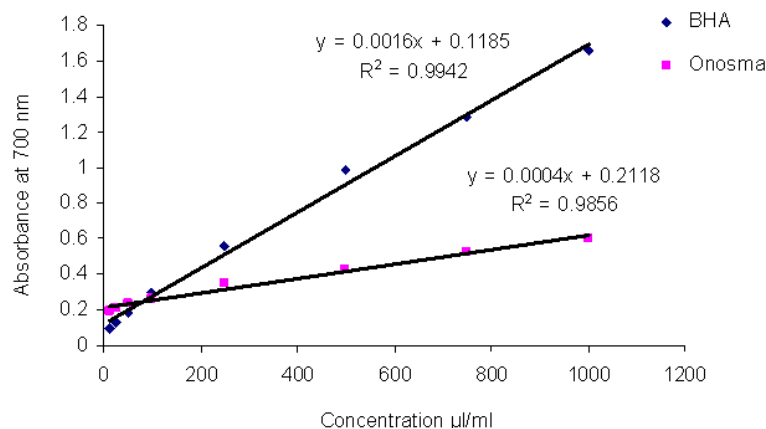


Figure 1. Reducing power of root extract of *O. dichroanthum* Boiss. BHA was used as positive. control.

(2009), who reported that the extracts of *Onosma roussaiei* affected on tested Gram positive, but no effect on Gram negative bacteria. Also Ozgen et al. (2003) showed that the root extract of *Onosma argentatum* has antioxidant and antimicrobial effects agent against *S. aureus*, *Bacillus subtilis* and *E. coli*. Benli et al. (2007) reported that the methanol extracts of leaf and flower *Onosma bornmuelleri* were inactive on Gram positive and negative bacteria and fungi such as *Enterococcus gallinarum*, *B. subtilis*, *E. coli*, *Shigella*, *Streptococcus pyogenes*, *S. aureus*, *Listeria monocytogenes*, *Candida* (*albicans*, *crusei*), *Saccharomyces cerevisiae*, while, the seed extract of *Onosma hispidum* and *Onosma bulbotrichum* have antibacterial activity (Naz et al., 2006b; Fazly Bazzaz and Haririzadeh, 2003). Ahmad et al. (2009) demonstrated that the aqueous fraction of *Onosma griffithii* Vatke had moderate antibacterial activity (42.3%) against *Staphylococcus aureus*, also against *B. subtilis* n-hexane and chloroform fractions showed 29.72 and 33.42% antibacterial activity respectively.

Researches has showed, polyphenols (phenolic acids and flavonoids) existed in the plants of this family have various pharmaceutical activities such as antioxidant, anti-inflammatory, antiviral, anti-bacterial and hepato-protecting activity (Li et al., 2010). These secondary metabolites produced by plants are effective against pathogenic microorganisms in plant and human (Rojas et al., 2003).

Antioxidant activity

Reducing power assay

Various studies have indicated that the electron donation capacity of bioactive compounds is associated with antioxidant activity (Siddhuraju et al., 2002; Arabshahi-Deloue, 2007). In reducing power assay, the antioxidant ability of extract to reduce iron (III) to iron (II) was

examined. Results showed that at high concentrations, significant difference were seen between reducing power of BHA and plant extract. Reducing power of them was increased at high concentration, but at low concentrations (12.5 to 25 to 50 µl/ml) the amount of reducing power for extract was more than that the BHA, and the amount of IC₅₀ was 0.238 mg/ml for BHA and 0.720 mg/ml for plant extract. BHA contains the least amount of IC₅₀ and the most potent reducing agent, whereas extract had the highest amount of IC₅₀ and the weakest activity (Figure 1). Ebrahimzadeh et al. (2010) reported that aerial parts extract of *O. demawendicum* exhibited a high reducing power at concentrations 25 to 400 µl/ml that was significantly better than vitamin C as positive control.

Total antioxidant capacity

Antioxidants through their scavenging power are useful for the management of diseases. Silva et al. (2007) and Tawaha et al. (2007) reported high correlation between the antioxidant capacity and secondary metabolites contents of plants, based on our results, total antioxidant capacity was increased at high concentrations for BHA and plant extract but at low concentrations were found to be rather similar to each others. In high concentrations, the ability of BHA was approximately double, and IC₅₀ is reported 0.456 mg/ml for BHA and 1 mg/ml for extract. The extract had the highest amount of IC₅₀ and the least in the activity, and BHA contained the least amount of IC₅₀ and the most potentiality (Figure 2). Different studies were carried out about antioxidant activity on another species of Boraginaceae family (Ozen, 2010).

2,2-Diphenyl-1-picrylhydrazyl radical scavenging capacity assay

DPPH is a stable free radical generally used to determine

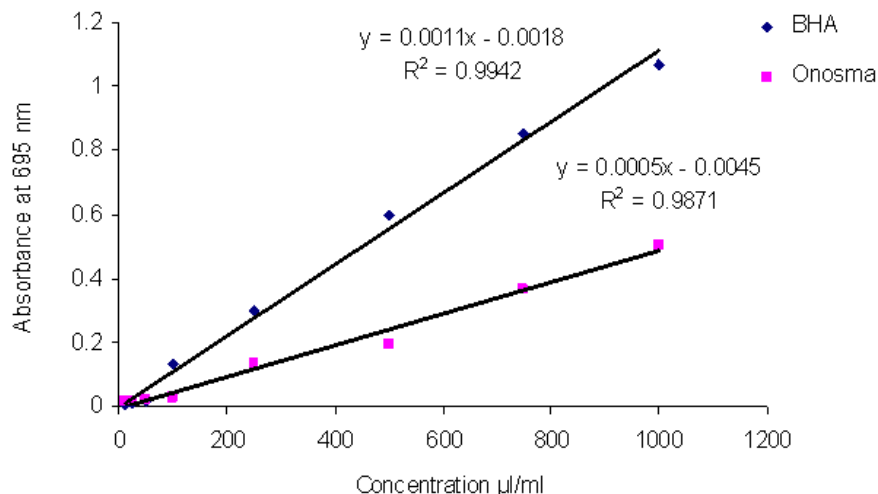


Figure 2. Total antioxidant activities of ethanol extract of *O. dichroanthum* Boiss., BHA was used as positive control.

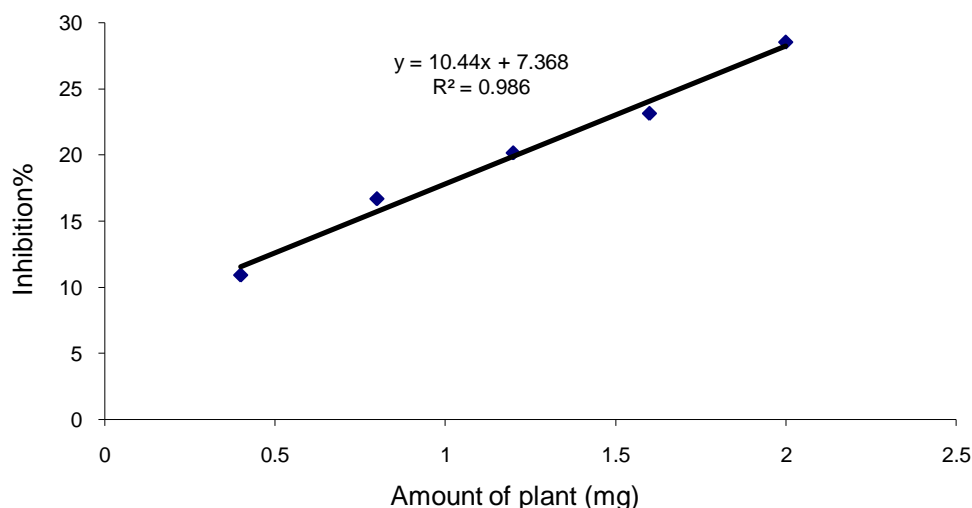


Figure 3. Inhibition of the DPPH solution in various amounts of dried roots of *O. dichroanthum* Boiss.

the ability of compounds to scavenge free radicals. Pourmorad et al. (2006) stated that free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. The most appropriate and fastest way of evaluating the antioxidant activity of plant extracts is DPPH stable free radical method. Figure 3 shows the amount inhibition of the DPPH solution in various amounts of dried roots of *O. dichroanthum*. The results showed that the plant extract has the capability of scavenging the DPPH- radicals. The inhibition activity in this extract was increased at high concentration and IC_{50} in DPPH radical scavenging capacity assay was 4.08 mg dry weight.

In various studies about variety of species belongs to

Boraginaceae family was observed the high correlation between quantity of total phenol and antioxidant activity (Cai et al., 2004; Conforti et al., 2008b). Some flavonoids agents have been found in this family which, through scavenging or chelating process, showed antioxidant activity (Pourmorad et al., 2006). Torane et al. (2011) reported the amount of secondary metabolites and antioxidant activity of ethanol extract of leaves and stem of *Ehretia laevis*. Their findings showed that quantity of IC_{50} of leaves and stem were respectively 2.44 and 29.88 µg/ml in DPPH free radical method.

In the other research by Conforti et al. (2008a), the EtOH (70%) extract of *Borago officinalis* leaves (Boraginaceae) was used for survey of antioxidant

activity and the amount of IC₅₀ report in DPPH method 58 µg/ml and β-Carotene method 4 µg/ml. Ozgen et al. (2003) stated that *Onosma argentatum* extract has 98% antioxidant activity at 0.1% concentration and the IC₅₀ was reported 0.0076% w/v. Also Ebrahimzadeh et al. (2010) showed that the amount of IC₅₀ for *O. demawendicum* aerial parts was 221 µg/ml in DPPH method.

Conclusion

The data presented in this study demonstrate that *O. dichroanthum* possess antibacterial and also antioxidant activities against free radical produced in different condition. We introduce the acetone extract as a good solvent in extracting (secondary metabolites) for future research. Our results confirm the use of this plant by the rural healers, in the traditional medicine, in single use or combination with other herbs especially with animal fat as antiseptic, anti-inflammatory and for healing of wound and burn, although we could not demonstrate the therapeutic effects of root extract of *O. dichroanthum* on wound lesion in an animal model. We offer the effects of these extracts to be investigated via *in vivo* and clinical models.

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