



## Evaluation of phytochemical and antioxidant activities from different parts of *Nasturtium officinale* R. Br. in Mazandaran

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### Abstract

The relationship between secondary metabolites content and antioxidant activity was studied in aerial parts of *Nasturtium officinale* R. Br., at various altitudes and periods of growth in north of Iran. Total phenolics (TP), and total flavonoids (TF) contents in aerial parts of the plant were investigated spectrophotometrically and their antioxidant activities were obtained by Total Antioxidant Capacity (TAC) method and then compared with standards such as butylated hydroxyanisole, butylated hydroxytoluene. Findings demonstrated that the TP content of aerial parts of *Nasturtium officinale* were 8.03 to 9.35 mgGAEg<sup>-1</sup> in vegetative period and 6.5 to 7.65 mgGAEg<sup>-1</sup> in generative period. Also, TF contents were 26.5 to 31.11 mgQuEg<sup>-1</sup> in vegetative period and 36.89 to 42.65 mgQuEg<sup>-1</sup> in generative period. The highest secondary metabolites content of TP were found in vegetative period. Aerial parts in high altitudes (1400 m) had also high amount of phenolic and flavonoid compounds. IC<sub>50</sub> contents in aerial parts of the plant were 932 to 1494 µg/ml in TAC method. The highest antioxidant activity and radical scavenging effects were observed in vegetative period with the lowest IC<sub>50</sub>, 932 to 1227.5 µg/ml, whereas generative period with the highest amount of IC<sub>50</sub> had the weakest antioxidant activity. Findings showed a positive correlation between antioxidant activity and the most important secondary metabolites, which explains why this plant is used by the rural healers in north of Iran for nutrition and anti-inflammation.

**Keywords:** *Nasturtium officinale*; secondary metabolites; antioxidant activity; Mazandaran province

### Abbreviations:

BHA: butylated hydroxyanisole; BHT: butylated hydroxytoluene; TAC: total antioxidant capacity; TF: total flavonoid; TP: total phenol; Mo: molybdenum

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### Introduction

Reactive oxygen species including free radicals such as (O<sub>2</sub><sup>-•</sup>, OH<sup>•</sup>) and non-free radicals

(H<sub>2</sub>O<sub>2</sub>, <sup>1</sup>O<sub>2</sub>) along with different forms of active oxygen are involved in various physicochemical processes in the body (Qureshi et al., 2009; Ozen, 2009). Application of many synthetic antioxidants (BHA, BHT) in the food industries shows toxic properties for human and animal health,

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therefore scientists are forced to find new substances from various sources such as medicinal plants (Wangensteen et al., 2004; Tepe et al., 2006; Nickavar and Abolhasani, 2009).

Herbs have been used for medical treatment since ancient times (Dragland et al., 2003). Recent investigations have shown that the positive treatment effects and antioxidant properties of medicinal plants could be correlated with bioactive components such as alkaloids, bitters, flavonoids, bioflavonoids, glycosides, mucilage, saponins, tannins, phenols, phenolic acids, guinones, coumarins, terpenoids, essential oils, lectins and polypeptides (Cicek Rathert et al., 2010; Ozen, 2009). The use of these compounds as natural antioxidants plays an important role in protecting and prevention of DNA damage (Ozen, 2009) cancer, atherosclerosis diseases, and the aging processes (Sanchez-Moreno et al., 1999). Also these compounds have been effective in protecting the plant against microorganisms, fungi, insects, and herbivores (Cicek Rathert et al., 2010).

*Nasturtium officinale* R.Br. (Watercress) belonging to Brassicaceae family with local name of "Outare" is one of the most important mountainous medicinal herb in most parts in north of Iran that grows on the wet and around water habitats and has been used by the rural healers as nutritive, anti-inflammatory and antioxidant agent. This plant is a vegetable, consumed raw or cooked in salads, soups and other recipes in European and Turkish cuisine and also used to cure abdominal pain in traditional medicine (Ozen, 2009) and for treatment of diseases such as diabetes and bronchitis (Bahramikia et al., 2009).

*Nasturtium officinale* R. Br. is a main source of essential vitamins and minerals phytochemicals, such as lutein and zeaxanthin (Anonymous, 2003). Vegetables are an important source of ascorbic acid. Vitamin is valuable for its antioxidant effect, stimulating the immune system and other health benefits (Cruz et al., 2006). It is considered an excellent functional food for the prevention of cancer and related diseases which is reported to decrease breast cancer risk (Cruz et al., 2006, Ozen, 2009), treat lung and other

cancers in humans (Kelloff et al., 1996), diabetes, bronchitis, and diuresis (Miraldi et al., 2001), scurvy, tuberculosis, influenza, asthma, and as antimicrobial agent (Ozen, 2009), antiulcerogenic (Alkofahi and Atta, 1999), antiestrogenic and anticarcinogenic (Tamayo et al., 2000) and the spreading of pancreas, gastric (Jiao et al., 1998; Ozen, 2009) and finally used as nutritional supplement and digestive aid (Sezik et al., 2001).

The main purpose of this study was to evaluate total phenolic and flavonoid contents, and antioxidant activity in aerial parts of *Nasturtium officinale* R.Br which has been used by the rural healers in north of Iran to prevent and treat nutrition and inflammation problems.

## Materials and Methods

### Plant materials

The aerial parts of *Nasturtium officinale* R. Br. were collected in localities of Mazandaran province (200m-1400m) in different periods of plant growth, (Mar- Apr, 2011). This voucher of specimen was identified and has been deposited at the Herbarium Museum of the Golestan University. The plant materials were washed, then dried and ground to a fine powder using a laboratory mill and the materials were maintained at room temperature (21–23 °C), protected from light.

### Extraction of plant materials for phenol and flavonoid tests

The aerial parts of *Nasturtium officinale* R. Br. (5g) were extracted overnight with 100 ml of methanol solvent, in a mechanical shaker at room temperature. Each extract of plant was filtered with Whatman No. 1 filter paper and stored at 4 °C.

### Extract preparation for antioxidant activity

The obtained extracts were filtered with Whatman No. 1 filter paper. The filtrates were evaporated into dry at 40°C in a rotary with evaporator and stored at 4 °C (Arabshahi-Deloue and Urooj, 2007).

Table 1

Comparison of secondary metabolites of aerial parts of *Nasturtium officinale* in various regions and at different periods of growing.

Test	Vegetative period		Generative period	
	Nosrat Abad (200m)	Touska Cheshme (1400m)	Nosrat Abad(200m)	Touska Cheshmeh (1400m)
Total Phenol mgGAEg <sup>-1</sup>	8.03±1.01	9.35±1.14	6.5± 0.3	7.65± 0.39
	ab	a	b	b
Total FlavonoidmgQuEg <sup>-1</sup>	26.57±1.16	31.11±1.45	36.89± 2.23	42.65± 1.09
	d	c	b	a

Significance differences: a, b, c, d

## Phytochemical tests

### Total phenols determination

Total phenolic contents were determined by FolinCiocalteu method (Pourmorad et al., 2006). 0.5 ml of sample organic acid (standard phenolic compound) was mixed with FolinCiocalteu reagent (5 ml) and aqueous Na<sub>2</sub>CO<sub>3</sub> (4 ml, 1 M). The mixture was allowed to stand for 15 min and the total phenols were determined by colorimeters 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 was estimated by the Aluminum chloride method, based on the procedure described by 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, and quercetin was used as a standard for calibration curve. Total flavonoids values are expressed in terms of mg equal quercetin in 1 g dry plant powder.

### Antioxidant activity test

#### Total antioxidant capacity

This experimental procedure was adapted from Arabshahi-Delouee 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 12.5-1000 µg of dried extract in corresponding solvent, was combined in a tube with 1 ml of reagent solution (0.6 M sulphoric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). They were incubated in a thermal block at 95 °C for 90 min. Samples were then cooled and their absorbance was measured at 695 nm. A typical blank solution containing 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-Delouee and Urooj, 2007).

### Statistical analysis

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

## Results

### Total phenolics and flavonoids

Phytochemical compounds of aerial parts of *Nasturtium officinale* in various regions are shown in Table 1. Comparison of the results indicated that the TP content of aerial parts of plant were within 8.03 to 9.35 mgGAEg<sup>-1</sup> in vegetative period and 6.5 to 7.65 mgGAEg<sup>-1</sup> in generative period. In addition, TF contents were between 26.5 and 31.11 mgQuEg<sup>-1</sup> in vegetative period and between 36.89 and 42.65 mgQuEg<sup>-1</sup>

Table 2  
Comparison of IC<sub>50</sub> in various parts of *Nasturtium officinale* in TAC method

Test	Vegetative period		Generative period		BHA	BHT
	Nosrat Abad (200m)	TouskaCheshmeh (1400m)	Nosrat Abad (200m)	TouskaCheshmeh (1400m)		
TAC (μgml <sup>-1</sup> )	1227.5	932	1494	1356	122.979	184.695

in generative period. The highest contents of secondary metabolites of TP were found in vegetative period compared with the other period. The generative period had high amount of flavonoid compounds and aerial parts in high altitudes (1400m) had high amount of phenolic and flavonoid compounds as compared with low altitudes (Table 1 and Figs. I and II).

### Antioxidant activity

The total antioxidant activity of sample of plant was compared to BHT and BHT in Table2 and Fig.III. IC<sub>50</sub> contents in aerial parts of *Nasturtium officinale* were between 932 and 1494 μg/ml) in TAC method. The highest antioxidant activity and radical scavenging effect were observed in vegetative period with the lowest IC<sub>50</sub> (932 to 1227.5 μg/ml), whereas generative period with the highest amount of IC<sub>50</sub> had the weakest antioxidant activity.

### Discussion

Evaluation of our results showed that a positive correlation existed between TP contents and their antioxidant activity of aerial parts extract as free radical inhibitor. Phenolic compounds and TF are the most important constituents in scavenging of free radicals and also stabilizing lipid peroxidation (Ozen, 2009).

Quercetin and kaempferol were the most flavonoids found in *Nasturtium officinale* (Cartea et al., 2011). Ozen (2009) found that aerial parts of *Nasturtium officinale* had 88.60 ±2.41 and 74.18 ±1.72 μg pyrocatechol equivalent of phenolic compounds in 1000 mg of water and ethanol extracts, respectively.

Reactive oxygen species are involved in diverse physicochemical processes in the human body (Qureshi et al., 2009) which have a main role in the pathogenesis of different diseases such as

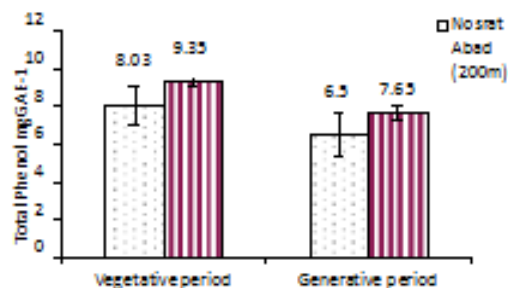


Fig. I. Total phenol contents of aerial parts of *Nasturtium officinale*

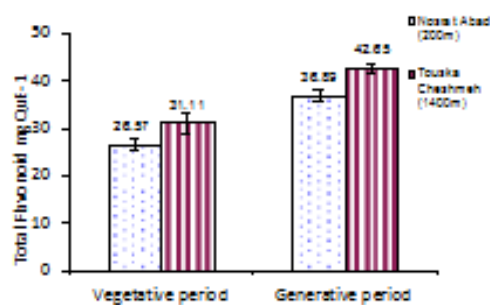


Fig. II. Total flavonoid contents of aerial parts of *Nasturtium officinale*

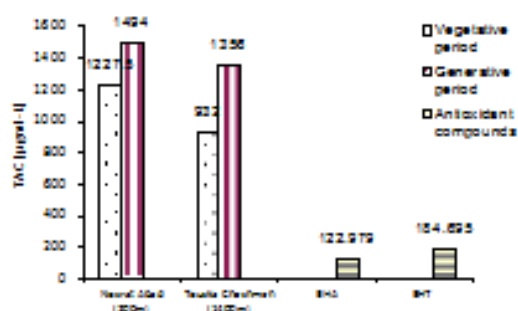


Fig. III. Amount of IC<sub>50</sub> in aerial parts of *Nasturtium officinale*, TAC method

neurodegenerative disorders, cancer, cardiovascular diseases, arthrosclerosis, cataracts and inflammation (Conforti et al., 2008). Ozen

(2009) reported that in *N. officinale*, the antioxidant activity or reduction power of ethanolic extract was more than that in aqueous extract especially in DPPH method. According to Augusti Boligon (2011), the antioxidant activities of organic solvents such as ethanol, dichloromethane and ethyl acetate were more than those from crude extracts in DPPH and TBARS methods. The antioxidant and radical scavenging activity of the crude extract and fractions were found in the following decreasing order, butanolic fraction > ethyl acetate fraction > dichloromethane fraction >.

Our results were in agreements to those of many research studies that reported direct relationships between TP content and their antioxidant activity in a variety of species including *Ehretia laevis*, *Cordiamyxa*, *Sylibum marrianum*, *Lithospermum erythrorhizon*, *Cordiamultispicata*, *C. multispicata*, *Tournefortia bicolor*, *Heracleum gorganicum*, *Artemisia annua*, *Onosmadichloranthum* and *Borago officinalis* (Dorman et al., 2004; Cai et al., 2004; Pourmorad et al., 2006; Surveswaran et al., 2007; Tawaha et al., 2007; Kirca and Arslan, 2008; Conforti et al., 2008; Mokarram Shah et al., 2011; Hadaruga, 2009; Mazandarani et al., 2011; Mazandarani et al., 2012). In sum, the study showed that the aerial parts of *Nasturtium officinale* R. Br. in vegetative period contain the highest amount of TP compounds with antioxidant activity which could be a valuable natural antioxidant source confirming traditional application of this plant by the rural healers as nutritive, diuretic and anti-inflammation.

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