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

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## Abstract

The relationshipbetween 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) contentsin aerial parts of the plantwere investigated spectrophotometrically and their antioxidant activitieswere obtained byTotal Antioxidant Capacity (TAC) method and then compared with standards such as butylatedhydroxyanisole, butylatedhydroxytoluene. Findings demonstrated that the TP content of aerial parts of *Nasturtium officinale*were 8.03 to 9.35 mgGAE$g^{-1}$ in vegetative period and 6.5 to 7.65 mgGAE$g^{-1}$ in generative period. Also, TF contents were 26.5 to 31.11 mgQuE$g^{-1}$in vegetative period and 36.89 to 42.65 mgQuE$g^{-1}$ in generative period. The highest secondary metabolitescontentsof TP were found in vegetative period. Aerial parts in high altitudes (1400 m) hadalso high amount of phenolic and flavonoid compounds. $IC_{50}$ contents in aerial parts of the plant were 932 to 1494 µg/mlin TAC method.The highest antioxidant activity and radical scavenging effects were observed in vegetative period with the lowest $IC_{50}$, 932 to 1227.5 µg/ml, whereas generative period with the highest amount of $IC_{50}$ had the weakest antioxidant activity. Findings showeda 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 nutritionand anti-inflammation.

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

Abbreviations:
BHA: butylatedhydroxyanisole; BHT: butylatedhydroxytoluene; TAC: total antioxidant capacity; TF: total flavonoid; TP: total phenol; Mo: molybdenum

**Mazandarani, M., A. Momeji, P. Zarghami Moghaddam.**2013. 'Evaluation of phytochemical and antioxidant activities from different parts of Nasturtium officinale R. Br.in Mazandaran'*Iranian Journal of Plant Physiology* 3 (2), 659-664.

## Introduction

Reactive oxygen species including free radicals such as ($O_2^{-\bullet}$, $OH^{\bullet}$) and non-free radicals ($H_2O_2$, $^1O_2$) 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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Received: June, 2012
Accepted: January, 2012

therefore scientists are forcedto find new substances from various sources suchas 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 (CicekRathert 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 (CicekRathert et al., 2010).

*Nasturtium officinale* R.Br. (Watercress) belonging toBrasicacea 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 habitatsand 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 Turkishcuisine 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 antioxidanteffect, stimulating the immune system and other healthbenefits (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 (Kellof et al., 1996), diabetes, bronchitis, and diuresis (Miraldiet 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 asnutritional 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 *Nasturtiumofficinale* 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 materialsfor phenol and flavonoid tests

The aerial partsof *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 ℃.

### Extract preparation for antioxidant activity

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

Table 1

Comparison ofsecondary 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 mgGAE$g^{-1}$ | 8.03±1.01 ab | 9.35±1.14 a | 6.5± 0.3 b | 7.65± 0.39 b |
| Total FlavonoidmgQuE$g^{-1}$ | 26.57±1.16 d | 31.11±1.45 c | 36.89± 2.23 b | 42.65± 1.09 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 samplesorgallic acid (standard phenolic compound) was mixed with FolinCiocalteu reagent (5 ml) and aqueous $Na_2CO_3$ (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. Theywere 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 1gdry plantpowder.

### 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 12.5-1000µg 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 absorbancewas 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-Deloue 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*Nasturtiumofficinale*in various regions are shownin Table 1. Comparison ofthe results indicated that the TP content of aerial parts of plant werewithin 8.03 to 9.35 mgGAE$g^{-1}$ in vegetative period and 6.5 to 7.65 mgGAE$g^{-1}$ in generative period. In addition, TF contents were between 26.5 and 31.11 mgQuE$g^{-1}$in vegetative period and between 36.89 and 42.65 mgQuE$g^{-1}$

Table 2
Comparison of IC50 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 ($\mu gml^{-1}$) | 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. $IC_{50}$ contents in aerial parts of *Nasturtium officinale* werebetween 932 and 1494 µg/ml) in TAC method.The highest antioxidant activity and radical scavenging effect were observed in vegetative period with the lowest $IC_{50}$ (932 to 1227.5 µg/ml), whereas generative period with the highest amount of $IC_{50}$ 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 constituentsin 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)whichhave a main role in the pathogenesis of different diseases such as

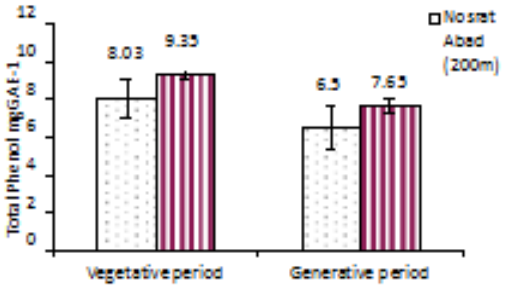


Fig. I. Total phenol contents of aerial parts of *Nasturtium officinale*

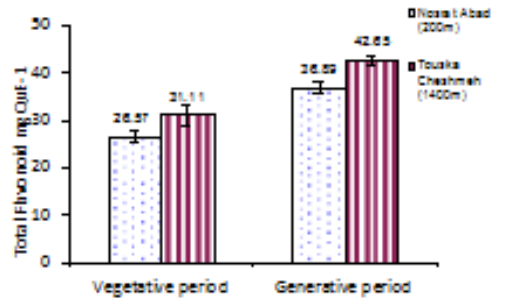


Fig. II. Total flavonoid contents of aerial parts of *Nasturtium officinale*

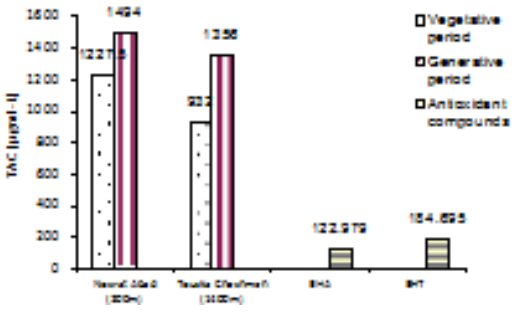


Fig. III. Amount of IC50 in aerial parts of *Nasturtium officinale*, TAC method

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 toAugustiBoligon (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 speciesincluding *Ehretialaevis, Cordiamyxa, Sylibummarrianum*, *Lithospermumerythrorhizon*, *Cordiamultispicata*, *C. multispicata*, *Tournefortia bicolor, Heracleumgorganicum, Artemisia anuua, Onosmadichloraanthum*and *Boragoofficinalis* (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; MokarramShah 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 periodcontainthe 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.

## Acknowledgements

The authors are grateful to technical help of laboratory officer of RCMP(Research Center of Medicinal Plants) in Islamic Azad University, Gorgan Branch.

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