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RESEARCH ARTICLE

Determination of some trace and heavy elements, *in vitro* antioxidant activity, total phenolic and Phenethyl isothiocyanate content in Watercress from Turkey

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ABSTRACT

The purpose of this study was to define the content of total phenolic and phenylethyl isothiocyanate, the in vitro antioxidant activity, and the content of Trace and Heavy Elements of watercress (*Nasturtium officinale* R. Br., Brassicaceae). It has ecological and economic importance and has been used by humans for many years both for food and medical purposes. Now it is reported to have a chemopreventive effect. In this study, Watercress samples were collected from Malatya, Turkey. Spectrophotometric methods were used to determine the antioxidant activity and the content of total phenolic and phenylethyl isothiocyanate. To determine heavy metals and trace elements of watercress were used inductively coupled plasma-mass spectrometry (ICP-MS). Our results indicated that the extract of watercress has a high antioxidant capacity. This feature was related to phenolic compounds and phenylethyl isothiocyanate. Our analysis with ICP-MS has revealed that the watercress plant has high iron content and toxic heavy metals did not bioaccumulate in the watercress plant. So it is recommended to consume the watercress plant as food according to these results. However, toxicity studies should be carried out in living beings regarding plant intake.

Keywords: ICP-MS, DPPH, PEITC, Watercress

INTRODUCTION

Watercress (*Nasturtium officinale* R. Br.) is a perennial, rhizome, aquatic plant belonging to the Brassicaceae family (Gill et al., 2007). It grows in clean and continuously flowing freshwater (Rose et al., 2000) (Image 1). Watercress has been used as a food by people for many years and is also considered a medicinal plant. Although its nutritional properties are known, it is a plant with valuable biological properties that have not been discovered and are not widely known. *Nasturtium officinale* R.Br. is used for stomach diseases in Turkey (Demirci and Özhatay, 2012; Bulut et al., 2019). Also, it is involved in the multiherbal recipes with *Malva sylvestris, Foeniculum vulgare*, etc. to be used for reducing abdominal pain in Turkey (Bulut and Tuzlacı, 2013). It is reported that is hypolipidemic and cardioprotective, anti-inflammatory (Bahramikia and Yazdanparast, 2008), antioxidant (Shahani et al., 2017), anticancer (Boyd et al., 2006), and antibacterial (Zafar et al., 2017). *Nasturtium officinale* R.Br. has been shown to have phytoremediation capability in both water and soil reservoirs. Furthermore, their properties can be increased under the effect of various factors such as the adding of chelating agents and planting with plants with similar characteristics (Kara, 2005).

Watercress has a rich glucosinolate content (Bell and Wagstaff, 2014) which is hydrolyzed to isothiocyanates as a result of the activity of myrosinase (β-thioglucoside glucohydrolase; EC 3.2.3.1) enzyme in cases such as chewing, cooking, or chopping. Plants in this group have a bitter taste due to their bioactive components. Gluconasturtiin (2-phenylethyl glucosinolate) in watercress is a glucosinolate component that has an aliphatic and indole structure (Boyd et al., 2006) and metabolizes

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to 2-phenylethyl isothiocyanate which is the main isothiocyanate in the leaves and stems of watercress (Fig. 1). Watercress also contains high conc. of carotenoids such as β-carotene, lutein, and zeaxanthin (Kopsell et al., 2007), and flavanolic components such as quercetin, campherol, and isorhamnetin (Martínez-Sánchez et al., 2008). Watercress is a very important source of vitamins and minerals. This plant contains B1, B2, vitamin A and vitamin C, iron, folic acid, iodine, protein, and calcium and sulfur compounds that affect its characteristic odor, which also increases the nutritional benefits of the plant (Palaniswamy et al., 2003).

Reactive oxygen species (ROS) have high chemical reactivity that causes destructive and irreversible damage to cellular components including lipids, proteins, and DNA (Riley, 1994). This oxidative stress contributes to the occurrence of different human diseases such as atherosclerosis, arthritis, inflammation, emphysema, diabetes, cirrhosis, and cancer as well as tissue damage (Nordberg and Arner, 2001). Cells have a variety of antioxidant defense mechanisms, and the effectiveness of this antioxidant defense system changes under pathological conditions. Therefore, the inability to remove free radicals and/or their overproduction may play an significant role in causing tissue damage (Halliwell, 1994).

Recently, there has been an increasing interest in natural antioxidants in plants that can be used to reduce the severity of oxidative damage. For this purpose, many medicinal plants have been researched in terms of their scavenger activities of ROS (Prior, 2003). It has been shown in various studies that watercress has an antioxidative effect against lipid peroxidation and DNA damage (Fogarty et al., 2013; Yazdanparast et al., 2008).

Image 1. Photograph of Watercress (*Nasturtium officinale* R.Br.) from Malatya, Turkey.

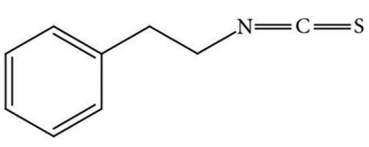

Fig 1. Structure of PEITC.

The determination of minerals in various biological materials is becoming increasingly important due to their role in metabolic processes and their positive effects on human health and the negative effects of elements that cause toxicity with increasing environmental pollution. Studies show that minerals used for both nutrition and treatment are found in different concentrations in different parts of plants such as roots, stems, and leaves. The functions of minerals in the body either come out with the reactions they cause by directly participating in the system, or indirectly by taking part in the structure of hormones and enzymes (Gharibzahedi and Jafari, 2017). In light of all this information, this study, it is aimed to investigate the in vitro antioxidant capacity, PEITC content, and element composition in order to determine the factors affecting the biological activity of watercress grown in Malatya and its surroundings. Although there are some studies that analyze certain elements in watercress, the results to be obtained in terms of the absence of large-scale studies involving trace and heavy elements will shed light on the studies in this field.

MATERIALS AND METHODS

Preparation of plant material

The aerial parts of *Nasturtium officinale* R. Br were collected from the pond in Arguvan country, city of Malatya, Turkey. A voucher specimen (no:1001) was supplied from the Herbarium of the Faculty of Pharmacy, İnönü University, Malatya, Turkey. The freeze-dried watercress was powdered and extracted with 70% methanol by heating at 70°C for 30 minutes and filtered. This procedure was repeated 3 times and then centrifuged at 4000 rpm for 10 minutes. The obtained extract was concentrated with a rotary evaporator (Heidolph Laborata 4011-digital) at 90 rpm and 45°C for 2 hours and dried.

Assay of free radical scavenging activity

The antioxidant activity of *Nasturtium officinale* R. Br was determined using the stable 1,1-diphenyl-2-picryl hydrazyl radical (DPPH) by the Blois method (Blois, 1958). The method is based upon the ability of the extracts to give a proton and electron to decolorize the purple-colored DPPH solution. By taking 3 mL of watercress extract and standard solutions, then 1 mL 0.1 mM DPPH was added. Afterward 30 minutes of incubation in the dark, absorbance at 517 nm was measured and the % inhibition of DPPH radical was calculated. The test was carried out

in triplicate. Butylated hydroxy toluene (BHT) (7.8-500 μg/mL) was used as a standard. Results are evaluated with calculation data by using the equation below. % DPPH radical scavenging activity = ($A_{Control}$-A_{Sample} or $A_{Standard}$)/ $A_{Control}$x100. A Control refers to the absorbance of the control reaction, A Sample refers to the absorbance of the sample or standard if the sample or A is the standard.

Assay of total phenolic content

The total phenolic content amount that in the extracts was defined using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965). 200 μL of the samples were placed in test tubes and 1 mL of Folin-Ciocalteu reagent and 0.8 mL of sodium carbonate (7.5%) solution were added. After test tubes were stirred and incubated for 30 minutes. Absorbances were measured at 765 nm. It was carried out in triplicate. Gallic acid (1 mg/mL stock solution in methanol), a phenolic compound, was used as a standard. The total amount of phenolic content of the extracts was calculated according to the standard graph. The amount of phenolic substance was expressed as mg gallic acid equivalent [mg GAE (Gallic Acid Equivalent)/g sample] in 1 g sample.

Assay of phenethyl isothiocyanate content

Determination of phenethyl isothiocyanate (PEITC) content was carried out using the UV spectrophotometric method (Zhang et al., 1992). 90 μL of K_2HPO_4 (pH 8), 90 μL of methanol, 10 μL of sample and 10 μL of benzene-1,2-dithiol dissolved in 80 mM methanol were added to 96 wells and mixed well. The samples were then incubated at 60°C for 90 min and cooled to room temperature. The absorbance of Phenethyl isothiocyanate was measured at 365 nm and quantification was performed using the calibration curve of PEITC (0.312-10 ppm).

Assay of trace and heavy elements in watercress by ICP-MS

Ultra pure water was used to prepare all solutions. Standard stock solutions were prepared in 2% (v/v) HNO_3 with the concentration of 1000 μg/L for all elements. All ICP-MS standarts was obtained Inorganic Ventures. To determination trace elements and heavy metals content of watercress were measured using inductively coupled plasma-mass spectrometry (Thermo Scientific ICAPQC, USA). Firstly 0.1 g iyophilized sample was digested in Teflon vessels with 25 mL suprapure HNO_3 and 25 mL deionized water in microwave oven (Milestone D5, USA). After cooling, the clear supernatant was transferred to polypropylene tubes and diluted to 20 mL with deionized water. After digestion process samples were analyzed with ICP-MS. The operating parameters were showed in Table 1.

The sampler probe was washed between injections by rinsing with ultrapure water for 30 seconds, followed by washing with 2% HNO_3 for 45 seconds, and finally rinsing with ultrapure water for 45 seconds. To ensure the accuracy of the results, each measurement of the samples and standards was repeated three times (Klimek-Szczykutowicz et al., 2019).

Calculation of bioaccumulation of heavy elements in watercress

The BCF (bioconcentration factor, L/kg) was determined with equation as:

$$BCF = Cp/Cw$$

Cw is metal concentration in water (mg/L) and Cp is metal concentration in whole plant tissue (mg/kg, dry).

Statistical analysis

All analyses were repeated in three times and results were given as mean±SD. IC50 values were obtained by linear regression analysis.

RESULTS

DPPH radicals scavenging activity

In this study, the antioxidant activity of Watercress was evaluated by the DPPH method. Results are presented in Table 2. and Fig. 2. Also, the BHT standard calibration curve is given in Fig. 3. The DPPH radical was inhibited to 86.00% and 92.24% by watercress and BHT, respectively. The value IC_{50} values were found 290.7 μg/mL for watercress and 270.9 μg/mL was for BHT.

Total phenolic compounds and PEITC content in watercress

The total phenolic content of watercress was measured by Folin-Ciocalteu reagent. The obtained data is given in Table 3. The standard calibration curve of gallic acid is presented in Fig. 4. The amount of PEITC was determined with UV-Vis spectrophotometer. The maximum wavelength was determined as 760 nm for

Table 1: The operating parameter of ICP-MS.

Radiofrequency power	1550 W
Nebulizer gas	0.96 L/min
Plasma gas	0.88 L/min
Nebulizer pressure	3.01 bar
Dwell time	0.01 ms
Spray chamber temperature	37°C

Table 2: DPPH inhibition (%) and IC_{50}values of Watercress and BHT standart

	DPPH Inhibition (%)	IC_{50}(μg/mL)
BHT Standart (500 μg)	92.24±0.06	270.9±0.18
Watercress (500 μg)	86.00±0.647	290.7±2.18

gallic acid and 365 nm for PEITC. The results were given in Table 3. The standard calibration curve of PEITC is shown in Fig. 5. The contents of total phenolic and PEITC of watercress were 57.2 mg GAE/g extract, and 2.433 µg/mg dry weight, respectively.

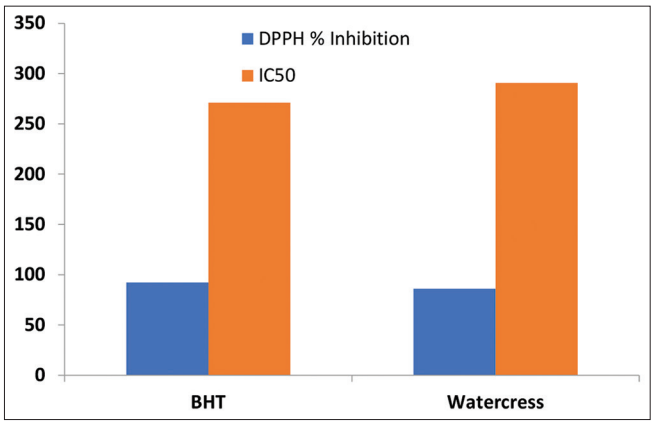

Fig 2. The IC50 values and DPPH inhibition (%) of BHT and Watercress.

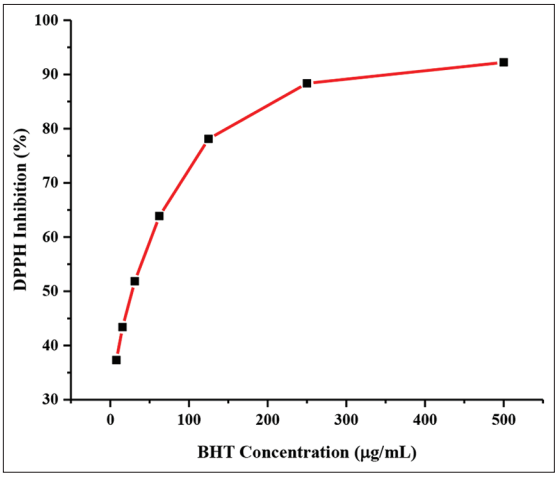

Fig 3. Standart calibration curve of BHT.

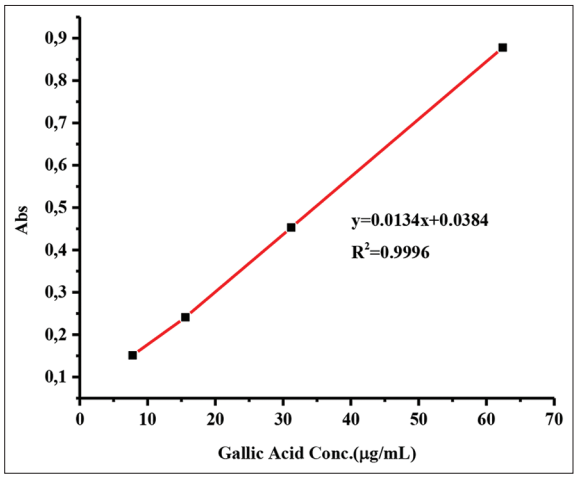

Fig 4. The standart calibration curve of gallic acid.

The trace and heavy element concentrations and BCF values in the watercress

The trace and heavy element concentrations and BCF values in the watercress can be ranged as follows: Fe>Mn>Zn>Al>Cu>Se>As>Pb>Mo>Ni>Co>Hg>Sb>Sn>Au>Be>Cd>Cr (Table 4). When BCF<1 or BCF=1, it shows that heavy metals are not bioaccumulating in the plant, if BCF is greater than 1, it shows that the plants are accumulative (Liu et al., 2005). The BCF values of the all elements are examined, it is seen that all values are less than 1 (Table 5). This results means that the watercress plant is not bioaccumulative.

DISCUSSION

Plant-based foods contain substantial amounts of bioactive compounds that provide desirable health benefits beyond basic nutrition. Over the last decade, special attention has been paid to edible plants, particularly those that are rich in secondary metabolites, also called phytochemicals, and there is an increasing interest in the antioxidant activity of such phytochemicals in the diet today (Podsedek, 2007). The pharmacological potential of watercress is specified by its rich chemical composition whereas the most important group of secondary metabolites in the plant are glucosinolates, in addition to other compounds such as isothiocyanates, polyphenols, vitamins and carotenoids. Isothiocyanates are the metabolites of glucosinolates, and the main isothiocyanate available in watercress leaves and stems is 2-phenylethyl isothiocyanate (Jeon et al., 2017). Owing to these valuable chemical components, watercress acts as a good source of natural antioxidants, and strong epidemiological evidence indicates that these compounds can help protect the human body against the damage induced by reactive oxygen species (Shahani et al., 2017).

PEITC is a versatile agent that aims at more than one process responsible for cancer growth and development (Gupta et

Table 3: Total phenolic compounds and PEITC content in watercress

	Total Phenolic Content (mg GAE/g Extract)	PEITC (µg/mg Dry Weight)
Watercress	57.20±0.325	2.433±0.0475

Table 4: Mean concentrations of trace and heavy metals, n=3.

Element	BCF value×10^{-3}	Element	BCF values×10^{-3}	Element	BCF value×10^{-3}
Al	16.32	Cr	0.0001	Ni	1.01
As	1.97	Cu	6.55	Pb	1.09
Au	0.17	Fe	184.9	Sb	0.19
Be	0.15	Hg	0.24	Se	3.18
Cd	0.14	Mn	39.1	Sn	0.17
Co	0.41	Mo	1.09	Zn	22.4

Table 5: Bioconcentration factor (BCF) for heavy elements in the watercress plant.

Element	Conc.(µg/kg)± sd	RSD	Element	Conc.(µg/kg) ± sd	RSD	Element	Conc.(µg/kg) ± sd	RSD
Al	32636.09±2225.3	6.82	Cr	0.02±0.01	2.41	Ni	2027.43±191.7	9.46
As	3948.84±218.45	5.53	Cu	13108.55±938.04	7.16	Pb	2190.46±97.05	4.43
Au	338.12±4.68	1.38	Fe	369915.57±33157.0	8.96	Sb	378.11±9.78	2.59
Be	307.47±3.05	0.99	Hg	474.32±71.84	15.15	Se	6361.79±2136.93	33.59
Cd	298.95±2.03	0.68	Mn	78226.11±6201.88	7.93	Sn	342.02±1.40	0.41
Co	812.61±64.79	7.97	Mo	2176.66±139.58	6.41	Zn	44857.10±4108.87	9.16

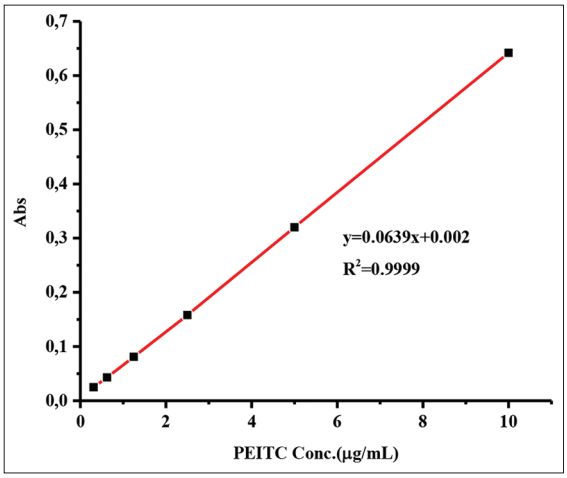

Fig 5. The standart calibration curve of PEITC.

al., 2014). In our study, we used the spectrophotometric method to designate the amount of PEITC and determined 2.433 µg of PEITC in our watercress sample. In other study, 0.233-0.688µmol/100g of PEITC amount was determined in watercress (Palaniswamy et al., 2003). It is reported that the amounts of glucosinolates or isothiocyanates in different plants or vegetables in the cruciferous family differ according to their environmental and genetic characteristics (Tang et al., 2014).

In this study, the antioxidant capacity of watercress was assessed by the DPPH radical scavenging method. The percentage of scavenging of the DPPH radical by watercress exhibited a higher antiradical activity (86.00%) compared to the positive control (92.24%). Other studies, where standards such as ascorbic acid and trolox were used, have also revealed that watercress has a similar DPPH radical scavenging effect (Bahramikia and Yazdanparast, 2010; Aires et al., 2013). These results indicate that watercress extract has strong free radical scavenging and antioxidant activities compared to standard compounds. The antioxidant activities of plant extracts largely result from the presence of phytochemicals, such as polyphenols, which are known to have the capacity to absorb, neutralize and remove reactive oxygen species. Redox properties, conjugated ring structures and the presence of carboxylic groups are held responsible for this activity (Vuolo et al., 2019).

The elemental content of the watercress was examined by the ICP-MS method. Our analysis results have revealed that the watercress plant has a high iron content. Our study findings were similar to the study results of Kawashima and Soares (2003) and de Souza et al. (2011). It can be said that watercress is rich in mineral content and its consumption as food can be beneficial.

In our study, toxic and heavy metal levels in watercress were also analyzed. It was Hutchinson, (1975) who first reported that macrophytes could collect elements from aquatic environments. Hutchinson stated that toxic elements such as cadmium, lead and mercury were at much higher levels in plants than in aquatic environments. Heavy metal terms are used for elements such as Cd, Cr, Cu, Hg, Ni, Pb and Zn, which are usually associated with pollution and toxicity problems. Biochemically, the negative effects caused by excessive concentrations of these metals are the reactions of ATP and ADP with phosphate groups, damage on cell membranes, reactions with -SH groups, replacement of main ions and competition with main metabolites. Organisms can tolerate these irregularities, which appear upon the intake of most elements, with their homeostatic mechanisms (Alloway and Ayres, 1993).

In metal accumulation research on the tissues of vascular aquatic plants, these plants were found to have 10 and 10^6 times higher metal accumulation than the aquatic environment near them (Kovacs et al., 1984). For this reason, macrophytes, directly and indirectly, play a significant role in heavy metal cycling in aquatic ecosystems (St-Cyr et al., 1994). Metal accumulation depends on the plant species, plant organs, metal ions in the water, the passage of metal-contaminated particles, and abiotic factors such as pH and temperature (Lewis, 1995; Lewander et al., 1996).

According to the findings of our study, toxic heavy metals did not bioaccumulate in the watercress plant. When all the results are considered, it is recommended to consume the watercress plant as food for health. However, toxicity studies should be carried out in living beings regarding plant intake. Moreover, there is a need for advanced studies researching the effects of watercress in respect of remediation, which is important for the ecosystem.

CONCLUSION

Watercress is a plant consumed as food by the local people. The results showed that this plant has high iron content and at the same time, the level of toxic elements is low. In addition, it has been shown that the antioxidant capacity of watercress was good and this activity was associated with total phenolic and PEITC levels. According to the results, watercress is suggested to consume limited doses as daily food that may be beneficial for health. However, dose and toxicity studies are needed for treatment efficacy.

Conflict of interest statement

The authors have no conflicts of interest to declare.

Author contributions

Uyumlu and Çağlar Yılmaz conceived and planned the experiment. Uyumlu carried out the biochemical experiments of watercress. Çağlar Yılmaz contributed to the ICP-MS analysis. Uyumlu and Çağlar Yılmaz wrote the manuscript.

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