The Evaluation of Antioxidant Activities and Phenolic Compounds in Leaves and Inflorescence of *Artemisia dracunculus* L. by HPLC

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Abstract

Background: ***Artemisia dracunculus*** **L. belongs to the Anthemideae tribe of Asteraceae family. This plant has been used in traditional folk medicine. Also, it's anti-bacterial, anti-fungal, antioxidant, anti-inflammatory and hepatoprotective activity has been proved.**

Objective: The purpose of this study was to evaluate the antioxidant activity of leaves and inflorescence of *A. dracunculus* and also to identify its phenolic compounds.

Methods: ***A. dracunculus*** **was collected in inflorescenceing season from Urmia. In this study, phenolic and flavonoid content, and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity for methanol extracts of leaves and inflorescences of *A. dracunculus* were investigated. Also, phenolic compounds were determined and measured by high performance liquid chromatography (HPLC).**

Results: Phenolic content of methanolic extracts of inflorescence of the *A. dracunculus* showed higher values than leaves. The highest flavonoid content was determined in leaves. There was a positive correlation between total phenolic content and the percentage of DPPH radical scavenging. Based on the results of the HPLC, seven phenolic compounds were detected in leaves and inflorescences (gallic acid, p-hydroxy benzoic acid, vanillic acid, p-coumaric acid, syrinjic acid, ferolic acid, sinapic acid).

Conclusion: ***A. dracunculus*** **contained phenolic compounds and showed significant antioxidant activity. High performance liquid chromatography results indicated significant differences ($p<0.05$) between different parts in case of phenolic compounds.**

Keywords: ***Artemisia dracunculus*** **L., Antioxidant activity, HPLC, Phenoic compounds**

Introduction

Artemisia dracunculus L. or tarragon belongs to the Anthemideae tribe of Asteraceae family. *A. dracunculus* is a woody, perennial subshrub with stem heights ranging from 40 to 150 cm. Aerial stems arise from thick, horizontal rhizomes growing in clusters and singly. Leaves are alternate, 1.2_8.0 cm long and 1_6 mm wide. Basal leaves are cleft with one to three lobes. The inflorescence is a panicle with numerous flowers [1, 2]. Its main source is alluvial valleys and various parts of Russia and Siberia. But nowadays it has become a native to the western regions of North America. Also, it is grown in the most areas of Iran and has dispersed everywhere [3]. The fresh and dried leaves are commonly used in salads and soups. This plant has been used in traditional folk medicine as appetizer, gastric tonic, diuretic, anti-scurvy and anti-worm [3].

The important groups of the *A. dracunculus* bioactive secondary metabolites, are essential oil, coumarins, flavonoids and phenolic acids [4] and also reported that hydroxycinnamates such as 1-caffeyolquinic acid, chlorogenic acid, caffeic acid, caffeoyltaric acid, 5-feruloylquinic acid, 1-4-Dicaffeoylquinic acid are main phenolic components of tarragon leaves [5]. This herb has antifungal and antioxidant [6], anti-bacterial, anti-inflammatory, and hepatoprotective [7] as well as Antihyperglycemic activities [8].

Free radicals are unstable, highly reactive molecules including reactive species of oxygen and nitrogen. Production of free radicals in living systems is associated with the cellular and metabolic damage, rapid aging, cancer, cardiovascular diseases, degenerative diseases of the nervous system and inflammation [9].

Polyphenols are secondary metabolites in plants. Phenolics can be defined as substances which possess an aromatic ring and have one or more hydroxyl groups. Plants contain a large variety of phenolic derivatives, including benzoic acids, cinnamic acid derivatives, flavonoids, isoflavonoids, lignans, and tannins [10].There are about 8000 naturally occurring plant phenolics and about half of them are flavonoids. These flavonoids are closely related structures, based on the C15 heterocyclic nucleus of flavones and varying chiefly in the number of phenols, such as phenolics acid, phenyl propanoids, and phenolics quinones [11]. The compounds such as flavonoids, which contain hydroxyl functional groups, are responsible for antioxidant effect in plants [12]. Antioxidant properties of phenolic compounds are because of their reduction capacity which allows them to act as an iron chelation and hydrogen denaturation agents [13].

Undoubtedly, there is growing interest in natural sources of nutrients and health–promoting compounds. Within these compounds, polyphenols and antioxidants have aroused special attention, which is understandable because of their role as potential protective and preventive molecules against chronic ailments,such as atherosclerosis and cardiovascular diseases, ischemic heart disease, Alzheimer's disease, cancer and in the entire aging process [14, 15, 16]. The aim of this study was to determine phenolic compounds, their content and antioxidant activity in leaves and inflorescence of Iranian tarragon. Due to many factors including climate, soil and height, extraction methods and methods for measuring antioxidants are involved in the amount of plant secondary metabolites, such as phenolic

content and antioxidant properties, necessity of this research is justified [17].

Materials and Methods

Plant materials

A. dracunculus was collected from a vegetable farm in Urmia in inflorescenceing season in August 2012. The plant divided into two sections of inflorescences and leaves, then were dried at room temperature and darkness. After ensuring the dryness, it was grinded with small grinding mill.

Extraction

3 g of each sample was extracted with 50 ml of methanol as solvent for 2 h on a magnetic Shaker and then centrifuged at 3000 rpm for 30 min, the supernatant was stored in a refrigerator at 4° C [18].

Determination of total phenol content

Total phenolic content of the extracts was determined using the Folin-Ciocalteau reagent [19]. Folin-Ciocalteau reagent was diluted 10 times with distilled water. *A. dracunculus* extract solution (20 µL) was mixed with 1 ml diluted Folin- Ciocalteau reagent, 1 ml sodium bicarbonate solution (7.5%), and 1 ml distilled water. The mixture was incubated at room temperature for 15 min. The absorbance of the solution was determined at 730 nm using a spectrophotometer (Biowave, S2100, UK) and compared with agallic acid equivalents (GAE) calibration curve. The total phenolic content was expressed as mg gallic acid equivalents of gram dry weight.

Determination of total flavonoid content

Flavonoid content was determined, as described by Bonvehi et al. (2001), with some modifications [20]. An appropriate dilution (0.1 ml extract, 0.8 ml distilled water) of the extract was mixed with the same volume (1 ml) of 2% AlCl3 in methanol solution (5% acetic acid in methanol). The mixture was allowed to react for 10 min and the absorbance was read at 430 nm against a sample blank without reactants. Quercetine was used as standard for the calibration curve. Total flavonoid content of the extracts was expressed as mg quercetine equivalents (QE) of gram dry weight.

Determination of DPPH radical scavenging activity

The free radical scavenging capacity of extracts was determined using DPPH [21]. Two ml of freshly prepared methanol solution of DPPH (0.004%) was added to 20 µL of extracts and allowed to stand at room temperature for 30 min. The absorbance of sample solution was measured at 517 nm, compared with that of control solution (maximum absorbance). Control solution was prepared containing the same volume without any extract. Scavenging percentage of the DPPH free radical was measured using the following equation: DPPH radical scavenging percentage= [(AControl –ASample)/AControl] ×100.

Extraction for the analysis of phenolic compounds by high performance liquid chromatography (HPLC)

For this purpose, 70 mL of HPLC-specific methanol was mixed with 20 ml of HPLC-specific water, 9.96 ml of HCl and 0.080 g ascorbic acid. Then 15 ml of the prepared solution was mixed with 0.5 g of *A. dracunculus* powder, and then placed under reflux conditions for an hour at 80 °C. After heating, the solution was centrifuged for 20 min at 6000 rpm, the filtrate was used for HPLC analysis [22].

Specifications of HPLC machine

HPLC model Knuer was used for analysis. Flow rate was 0.8 ml per minute and mobile phase composition used was water/acetic acid 2% (A), methanol (B) and Percentage of mobile phase transmittal in different times are shown in Table 1. Type of the filling material was C_{18} reversed Phase and column length was 25 cm and particle size, 5 mm. UV wavelength of 280 nm and injection volume of 20 ml was used. To perform the test, room temperature was considered 25 °C. Software Chrom Gate was also used for analyzing.

Methodology

Used standards in this project were purchased from Sigma Company. First, for quantitative measurement, 0.1 mg of each of the standards was dissolved in one ml of HPLC – specific methanol and various concentrations were prepared and each was loaded three times each to the device. The under curve area against plotted injection, the line equation and standards correlation coefficient was calculated. Then prepared extracts were loaded on the device to identify and determine phenolic compounds. And for quantitative analysis, obtained chromatograms of each loaded sample was compared to obtained chromatogram from relative loaded standards, finally the concentration of these compounds was calculated in mg per gram of extract.

Statistical analysis

All the assays were carried out in triplicate. The results are expressed as mean values and standard error (SE) of the mean. The differences were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's tests. For all analyses, p-values<0.05 were considered statistically significant. Data were analyzed using SPSS version 16 software.

Results

The total phenolic and flavonoid content as well as DPPH radical scavenging activities of methanolic extracts of leaves and inflorescences of the *A. dracunculus* are shown in Table 2.

Identified phenolic compounds of various organs of *A. dracunculus* by HPLC are given in Table 3. HPLC results indicate significant differences between the leaves and inflorescence in case of phenolic compounds. Compounds identified in the leaves and inflorescences include galic acid, p-hydroxy benzoic acid, vanillic acid, p-coumaric acid, syrinjic acid, ferolic acid and sinapic acid. The amount of ascorbic acid, rutin and caffeic acid was below the detection limit. HPLC chromatograms are shown in Figures 1 and 2.

Table 1- Percentage of mobile phase transmittal in different times

Time	solution (A) water/acetic acid 2%	solution (B) methanol
0	100	0
5	95	5
15	90	10
26	0	100

Table 2- Total phenolic content, total flavonoid content and DPPH radical scavenging activity in leaves and inflorescence of *Artemisia dracunculus*

Plant organs	Leaves	Inflorescence
TPC (mgGAE/g)	58.03 ± 2.85	81.46 ± 2.47
TFC (mg QE/g)	7.51 ± 0.18	6.75 ± 0.18
DPPH (%)	86.43 ± 0.15	92.03 ± 0.11

Data are represented as mean values and standard error (SE) of the mean.

Table 3 - Phenolic compounds (mg /100g dry sample) in leaves and inflorescence of *A. dracunculus*

Phenolic compound \ Plant organ	Leaves	Inflorescence
Ascorbic acid	–	–
Gallic acid	2.022 ± 0.10	2.03 ± 0.05
Rutin	–	–
Caffeic acid	–	–
P-hydroxy benzoic acid	3.21 ± 0.03	3.08 ± 0.00
Vanillic acid	0.30 ± 0.02	0.27 ± 0.01
P-coumaric acid	3.84 ± 0.05	27.90 ± 0.06
Syrinjic acid	14.15 ± 0.04	81.54 ± 0.07
Ferolic acid	4.54 ± 0.03	4.60 ± 0.03
Sinapic acid	124.18 ± 0.02	141.44 ± 0.12

Data are represented as mean values and standard error (SE) of the mean. Symbol (-) represents the composition being below the detection limit.

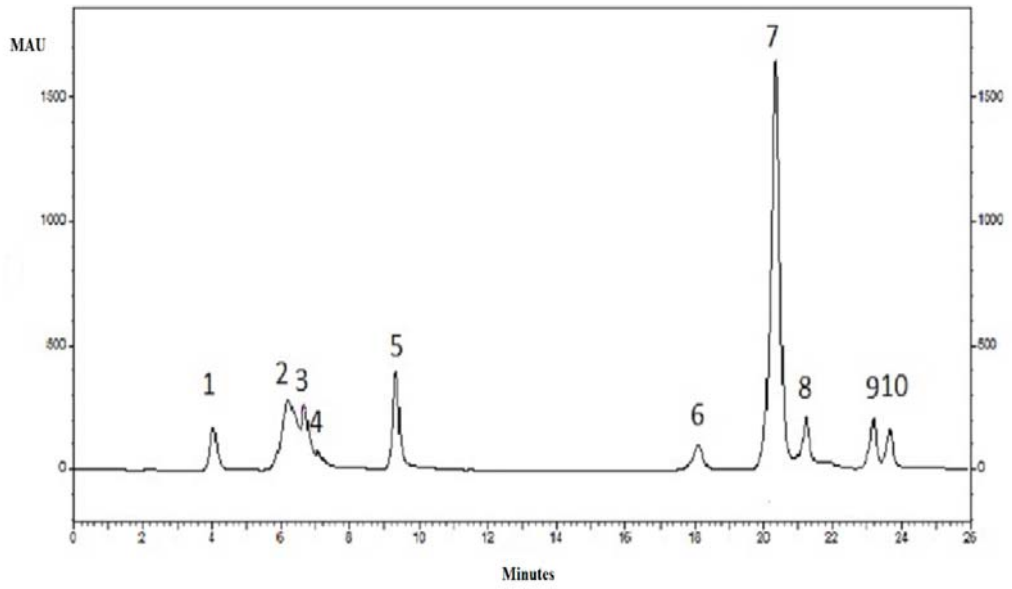

Figure 1- HPLC chromatogram of phenolic standards. Peaks: 1- Ascorbic acid, 2- Gallic acid, 3- Rutin, 4- caffeic acid, 5- P-hydroxy benzoic acid, 6- Vanillic acid, 7- P-coumaric acid, 8- Syrinjic acid, 9- Ferolic acid, 10- Sinapic acid.

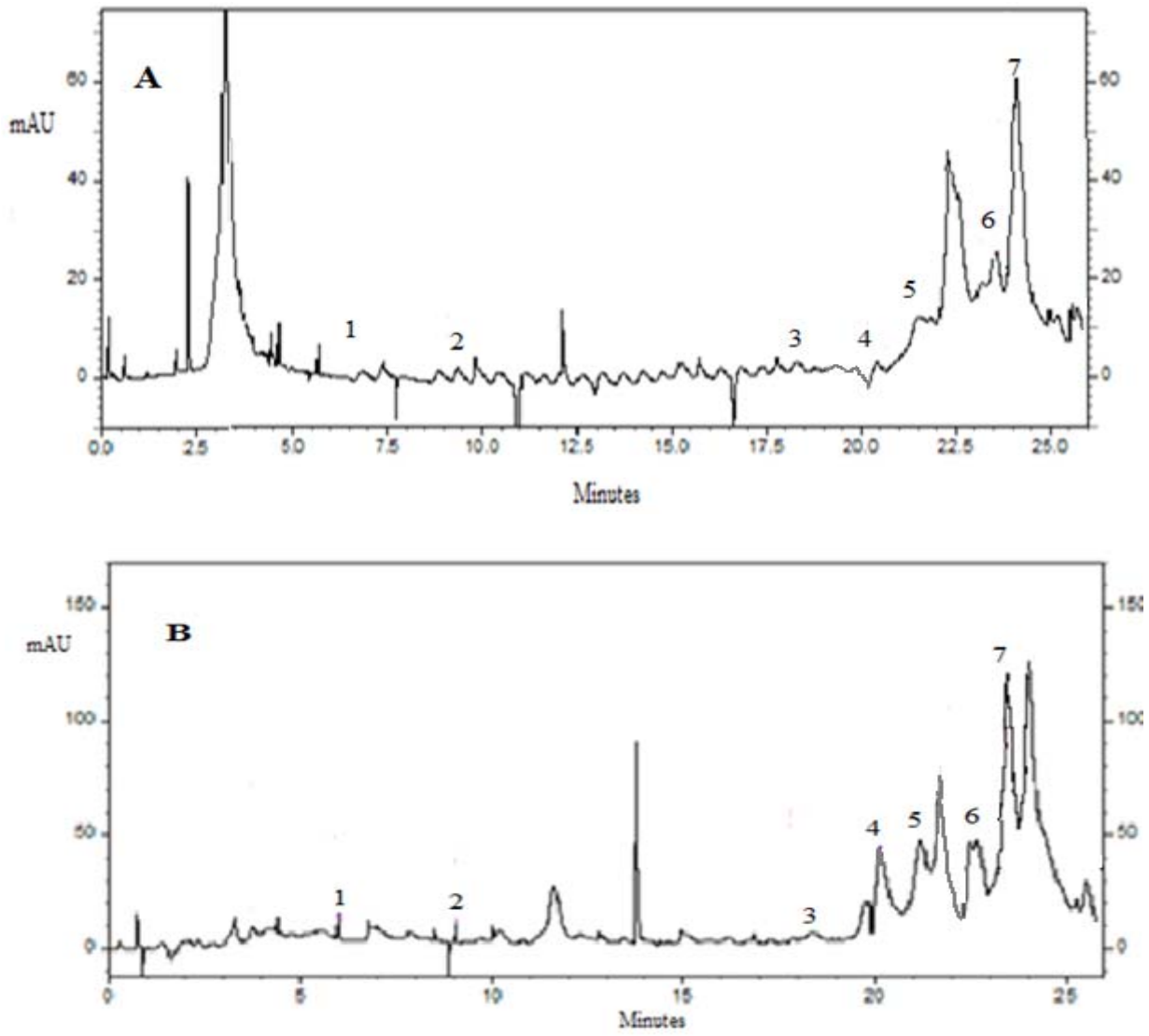

Figure 2- HPLC chromatograms of leaves (A) and inflorescence (B) of *A. dracunculus*. Peaks: 1- Gallic acid, 2- P-hydroxy benzoic acid, 3- Vanillic acid, 4- P-coumaric acid, 5- Syrinjic acid, 6- Ferolic acid, 7- Sinapic acid.

Discussion

Various studies results have shown that consumption of foods containing phenolic compounds may have a role in human health. In a prospective study, it was reported that fruits and vegetables rich in antioxidants are preferred to using supplements in order to cope with oxidative damage [23]. Phenolic compounds are a class of antioxidant compounds which act as free radical terminators [24]. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging process [4]. In this study, phenolic content was observed in the inflorescences 81.46 (mg GAE/g) and in the leaves 58.03 (mg GAE/g). The highest flavonoid content was determined in leaves

(7.51 mg QE/g) and the lowest was in inflorescences (6.75 mg QE/g).

Bahramikia et al. (2008) reported the phenolic content of ethanolic extracts of *A. dracunculus* leaves about 27 (mg GAE/g) [25], also Gawlik-Dziki (2012) have reported the total phenolic content in aqueous extract of *A. dracunculus* 26.2 (mg GAE/ml) and total flavonoid content 1.32 (mg QE/ml) [26], which in our study, phenolic and flavonoid content of methanolic extracts of leaves and inflorescence of *A. dracunculus* showed higher values than the two mentioned investigations.

The DPPH radical is long-lived organic nitrogen radical and has a deep purple colour. It iscommercially available and does not have to be generated before assay. In this assay, the purple chromogen radical is reduced by antioxidant/reducing compounds to the corresponding pale yellow hydrazine. The reducing ability of antioxidants towards DPPH can be evaluated by electron spin resonance or by monitoring the absorbance decrease at 515-528 nm until the absorbance remains stable in organic media. This widely used method was first reported by Brand-Williams et al. (1995) [27].

In this experiment, there was a direct correlation between total phenolic content and the percentage of DPPH radical scavenging. The correlation between total phenolic contents and antioxidant activity has been widely studied in different foodstuffs such as fruit and vegetables [28 - 31]. antioxidant activity of fruits and vegetables significantly increases with the presence of high concentration of total polyphenolic content.

The phenolic compounds, due to having hydroxyl groups, have the ability to neutralize free radicals and can act as an electron or hydrogen donor [32]. Phenolic compounds structure is a determining factor in their free radical scavenging and metal chelating activity which it called as the structure-dependent activity. For example, In case of phenolic acids, antioxidant activity is associated to the number and position of hydroxyl groups related to 2-carboxyl functional groups [33]. In the leaves and inflorescences of tarragon identified compounds were gallic acid, p-hydroxy benzoic acid, vanillic acid, p-coumaric acid, syrinjic acid, ferolic acid and sinapic acid. Which the most abundant phenolic acids of inflorescence were sinapic acid (141.44 mg/100g), syrinjic acid (81.54 mg/100g), p-coumaric acid (27.90 mg/100g) and for leaves were sinapic acid (124.18 mg/100g) and syrinjic acid (14.15 mg/100g).

Lin and Harnly, (2012) have identified phenolic compounds in chamomile (*Matricaria chamomilla* L.) and tarragon (*Artemisia dracunculus* L.). The main phenolic components of Chamomile flowers were the glycosides of flavones, while hydroxycinnamates were the main phenolic components of tarragon leaves [5]. Miron et al., (2010) have identified phenolic compounds such as syrinjic acid, vanillic acid, p-hydroxy benzoic acid and p-coumaric acid in Romanian tarragon [34]. These compounds were also detected in our study. Several factors affect the amount of phenolic compounds in plant tissues, some of them including genetic factors, amount of sunlight, soil conditions, degree of maturity at harvest time, environmental and climatic conditions, the post-harvest operations, and quantity and storing conditions can be noted [35].

Conclusion

In this study, it was observed that two parts of *A. dracunculus* contained phenolic compounds which the highest amount was

found in the inflorescence, also leaves and inflorescence of this plant showed significant antioxidant activity. High performance liquid chromatography results indicated significant differences ($p<0.05$) between different parts in case of phenolic compounds.

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