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Fatty acid compositions and antioxidant activities of *Ranunculus isthmicus* subsp. *tenuifolius* and *Ranunculus rumelicus*

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Abstract

The content of fatty acids in plants and especially the ratio of unsaturated to saturated fatty acids is very important. Phenolic compounds and flavonoids with antioxidant properties are useful in scavenging harmful radicals. In this study, fatty acid compositions and antioxidant activities of the aerial parts of *Ranunculus isthmicus* subsp. *tenuifolius* (syn. *Xiphocoma tenuifolia*) and *Ranunculus rumelicus* from Turkey were evaluated. Fatty acid methyl esters (FAMEs) of the oil extracts of two *Ranunculus* species were prepared. The fatty acid compositions of plants were investigated by gas chromatography (GC). Unsaturated fatty acids were detected in higher amounts than saturated fatty acids. The primary unsaturated fatty acids of the *R. isthmicus* subsp. *tenuifolius* oil were linoleic acid (38.42%), oleic acid (18.24%), palmitoleic acid (8.96%), and palmitic acid (8.12%) were determined fatty acid. Linoleic acid (24.09%), palmitoleic acid (16.25%) and palmitic acid (10.32%) were found in *R. rumelicus* oil as the major fatty acids. The antioxidant activities of methanol extracts of two plants were evaluated by DPPH•, ABTS•+, and CUPRAC assays. The extracts' total phenolics and flavonoid contents were determined by Folin-Ciocalteu and aluminium chloride methods, respectively. The methanolic extract of *Ranunculus isthmicus* subsp. *tenuifolius* demonstrated the higher antioxidant activity compared to *R. rumelicus*. The extracted oil from both *Ranunculus* species is a good source of essential fatty acids, especially linoleic acid. According to the antioxidant activity findings, it was determined that the extracts showed a significant correlation with the total phenolic and flavonoid contents.

Key words: Antioxidant activity, Linoleic acid, Oleic acid, Palmitic acid, *Ranunculus*

1. Introduction

Fatty acids are the main component of lipids have many important biological properties such as energetic, metabolic, and structural activities. Moreover, they can be associated with some chronic and degenerative diseases. For example, the consumption of mono/poly unsaturated fatty acids is beneficial in reducing cholesterol and thus the risk of myocardial diseases (Zyriax and Windler, 2000). Polyunsaturated fatty acids especially, linoleic acid act as regulators in cellular functions by participating in the structure of membrane phospholipids and other components. Although many plants are used for their unsaturated fatty acid content, studies on wild plant lipid composition are scarce. For this reason, it is important to carry out studies to identify new sources of high unsaturated fatty acid content.

The genus *Ranunculus* (Ranunculaceae family) is a common type of genus represented by 84 species in Turkey. *Ranunculus isthmicus* subsp*. tenuifolius* (syn. *Xiphocoma tenuifolia*) is an endemic species that is disturbed in the west and northwest Anatolia (Davis, 1965; Davis, 1988). *Ranunculus* species have been used in traditional medicine for wound healing, antihemorrhoidal, antirheumatic, maturation of abscesses and treatment of some skin diseases (Ugurlu and Secmen, 2008; Kaval et al., 2014; Macit and Gokce, 2015). Flavonoids (Erdogan et al., 2012), alkaloids (Zhang et al., 2007), essential oils (Terzioglu et al., 2008), and fatty acids (Chen et al., 2006, Tavakoli et al., 2012; Erdogan et al., 2014) were isolated from some *Ranunculus* species. The fatty acid compositions of this species are poorly studied. The fatty acid composition of *Ranunculus pedatus* subsp. *pedatus* was reported by us (Erdogan et al., 2014). Also, the fatty acid composition of *Ranunculus ternatus* (Chen et al., 2006) and Ficaria kochii from Iran were investigated (Tavakoli et al., 2012). *Ranunculus marginatus* var. *trachycarpus, R. sprunerianus, R. repens, R. arvensis* and *R. pedatus* subsp. *pedatus* were previously studied in antioxidant activities (Mantle et al., 2000; Campos et al., 2003; Kaya et al., 2010; Bhatti et al., 2015). However, there is no report on the fatty acid compositions and antioxidant activities *of Ranunculus isthmicus* and *Ranunculus rumelicus*. This study aims to evaluate the fatty acid compositions of the aerial parts of two *Ranunculus* species from Turkey. In addition, the antioxidant activities with total phenolic and flavonoid contents of *Ranunculus isthmicus* and *Ranunculus rumelicus* were aimed to investigated.

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2. Material and Methods

2.1. Plant materials

The aerial parts of *Ranunculus isthmicus* Boiss. subsp*. tenuifolius* (syn. *Xiphocoma tenuifolia* Steven) P.H. Davis and *Ranunculus rumelicus* Griseb. were collected during flowering (May 2016) from Izmir (village of Odemis, Bozdag mountain an altitude of 1130 m). They were identified by M. Ali Onur (Ege University). Voucher specimens (nos. 1345, and 1364, respectively) were deposited in the Herbarium of the Faculty of Pharmacy, Department of Pharmacognosy, Ege University, Izmir, Turkey. The plants were analyzed 6 months later collected.

2.2. Fatty acid analysis

40 grams of overground sample were extracted for oil using petroleum ether at 60 °C for 24 hours in a Soxhlet system (Erdogan et al., 2014). The solvent was evaporated by a rotary evaporator. The obtained oil was

transesterified to determine fatty acid composition (Yıldırım et al., 2009). The method described by Ichihara et al. was followed to prepare fatty acid methyl esters (FAMEs) using transmethylation (Ichihara et al., 1996). FAMEs were analyzed on an HP (Hewlett Packard) Agilent 6890N model gas chromatograph (GC), equipped with a flame ionization detector (FID) and fitted to an HP-88 capillary column (100 m length, 0.25 mm and 0.2 μm thickness). Injector and detector temperatures were set at 240 and 250 ºC, respectively. The oven was held at 160 ºC for 2 min. Thereafter the temperature was increased up to 140 ºC at the rate of 4 ºC / min, then increased up to 200 ºC at the rate of 2 ºC / min and held at 200 ºC for 46.75 min. The total run time was 70 min. Helium was used as carrier gas (1 mL/min). Identification of fatty acids was made by comparison of spectra and retention times with standard Supelco 37 Component FAME Mix. The methyl esters of fatty acids were quantified using the standard internal mass and relative percentage of GC in relation to each component. Each reported result was given in the average value of three GC analyses. The results were offered as means ±SD.

2.3. Indexes of lipid quality

The atherogenicity (IA) and thrombogenicity (TI) indices were calculated according to the following equations (Turchini et al., 2003).

Index of atherogenicity (IA): IA = [(4 × C14:0) + C16:0 + C18:0] / [ΣMUFA + ΣPUFA-ω6 + ΣPUFA-ω3].

Index of thrombogenicity (IT): IT = (C14:0 + C16:0 + C18:0) / (0.5MUFA + 0.5PUFA-ω6 + 3PUFA-ω3 + PUFA-ω3/PUFA-ω6)

2.4. Extraction

Methanol extracts were prepared from 40 grams of aerial plant parts, which were air-dried and powdered before extracting with 400 ml methanol in a Soxhlet apparatus for 12 hours. In the rotary evaporator (Buchi-R200) (40 °C), the solvents were evaporated to dryness. For two days, the extracts were put away at +4 °C in the dark.

2.5. Antioxidant activity assays

2.5.1. DPPH• radical scavenging assay

DPPH• (2,2'-diphenyl-1-picrylhydrazyl) scavenging action measurement of methanol extracts was assessed by the method described by (Ceylan et al., 2016). 4 mL of 0.004 percent DPPH• in methanol is added to 1 mL of 1 mg/mL methanol extracts. After 30 minutes, the absorbance was measured at 517 nm. The percentage of free radical inhibition was determined as follows: % Inhibition = [(Ab– As)/Ab] × (100)

(Ab: the absorbance of the control, As: the absorbance of the test sample). The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage against extract concentration. Trolox was used as standard.

2.5.2. ABTS•+ radical cation decolorization activity

ABTS•+ [2,2'- azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diamonium salt] solution was mixed with potassium persulfate ($K_2S_2O_8$), and the mixture was allowed to react for 15-16 h in the dark at room

temperature (Machado et al., 2013). The radical solution was diluted with acetic acid (20 mM, pH 4.5) to obtain a solution with an absorbance of 0.70±0.02 at a wavelength of 734 nm, ABTS•+ solution (2 mL) was added to 200 µL of the diluted sample (1 mg/mL). Absorbance was scanned for 15 minutes in 734 nm. Trolox was utilized as a standard solution. The percentage decrease of absorbance against a blank sample (distilled water) was calculated using the equation: % Inhibiton = $[(Abs_1 – Abs_2) / Abs_1] x (100)$
(Abs_1 : the initial absorbance, Abs_2 : the absorbance at 15 min).

2.5.3. Cupric ion reducing antioxidant capacity (CUPRAC assay)

As indicated by Apak et al., with certain alterations Cu (II) reducing force was analyzed (Apak et al., 2004). 1 mL $CuCl_2$ (10 mM), 1 mL neocuproine (7.5 mM), and 1 mL NH_4Ac buffer (1 M, pH 7.0) solutions were added into a tube. Then, 0.5 mL of different concentrations of extract were mixed, and the total volume was brought up to 4.1 mL with deionized water. The mixture absorbance was recorded against a blank at 450 nm after 30 min incubation at room temperature. The results of the assay were evaluated by using EC_{50} values and were expressed as mg of TE (Trolox equivalents) /mL.

2.6. Determination of total phenolic and flavonoid contents

The amount of total phenolic content in the extract was determined according to the Folin-Ciocalteu method (Ozay and Mammadov, 2016). The total phenolic content was expressed as gallic acid equivalents (GAE) in milligram per gram of extract (mg GAE/g extract).

The total flavonoid content of the extract was determined according to the reported method in the literature (Chang et al., 2002). 0.5 mL of sample solutions (1 mg/mL) was mixed with 2 mL of distilled water and subsequently with 0.15 mL 5% of $NaNO_2$ solution. After 6 min incubation, 0.15 mL of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, followed by adding 2 mL of 4% NaOH solution to the mixture. The mixture was made up to 5 mL with methanol and mixed well. The absorbance was measured at 510 nm after incubation for 15 min. The total flavonoid content was expressed in milligrams of rutin equivalents (RE) per gram of extract.

2.7. Statistical analysis

All examinations of each example were completed three times, and obtained results have appeared as means±SD. One-way analysis of variance (ANOVA) ($p<0.05$) was applied, and Person's correlation was utilized to decide the correlation coefficient of total phenolic substance and antioxidant activity.

3. Results and Discussion

The yields of petroleum extracts of *Ranunculus isthmicus* subsp. *tenuifolius* and *Ranunculus rumelicus* (syn. *Xiphocoma tenuifolia*) were 2.89% and 2.21%, respectively. The fatty acid compositions of *Ranunculus isthmicus* subsp. *tenuifolius* and *Ranunculus rumelicus* are given in Table 1. The composition of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) of the taxa were calculated. The major unsaturated fatty acid of the taxa was linoleic acid (C18:2ω6), followed by palmitoleic acid (C16:1ω7), and palmitic acid (C16:0) as

the major saturated fatty acid. 14 fatty acids were identified in *Ranunculus isthmicus* subsp. *tenuifolius* oil. Linoleic acid (38.42%), oleic acid (18.24%), and palmitoleic acid (8.96%) were determined as the major fatty acids, and palmitic acid (8.12%) was found as saturated fatty acid in *Ranunculus isthmicus* subsp. *tenuifolius* oil. We also identified 15 fatty acids in the oil of *Ranunculus rumelicus*, the major fatty acid was linoleic acid (24.09%). Other fatty acids with a high percentage were palmitoleic acid (16.25%) and palmitic acid (10.32%). Our findings are in good agreement with those in the previous research. The reason for the difference between the two plants in terms of fatty acid content may be the different growing places, altitude, climatic conditions, and genetic diversity. The indexes of atherogenicity (IA) and thrombogenicity (IT) for *Ranunculus isthmicus* subsp. *tenuifolius* and *Ranunculus rumeli* are presented in Table 2. The IA of the oils of *R. isthmicus* subsp. *tenuifolius* and *R. rumelicus* were determined as 0.106 and 0.164. The IT of the oil of *R. isthmicus* subsp. *tenuifolius* and *R. rumelicus* were calculated as 0.187 and 0.223, respectively. The highest ω3/ω6 ratio of 0.127 was found to be in *R. rumelicus.*

The yields of methanol extracts of *Ranunculus isthmicus* subsp. *tenuifolius* and *Ranunculus rumelicus* were 28.04% and 21.89%, respectively. Table 3 shows the antioxidant activities of *Ranunculus isthmicus* subsp. *tenuifolius* and *R. rumelicus* extracts. The total phenolic content of *Ranunculus isthmicus* subsp. *tenuifolius* extract was higher than *R. rumelicus* extract ($p < 0.05$). For the antioxidant activities "DPPH•, ABTS•+, and CUPRAC assays were determined for" methanol extracts *Ranunculus isthmicus* subsp. *tenuifolius,* with Trolox equivalent values of 8.246 mg/mL (DPPH•), 3.402 mg/mL (ABTS•+) and 3.001 mg/mL(CUPRAC) was found to have higher antioxidant activity than *R. rumelicus* with 6.308 (DPPH•), 2.051 (ABTS•+) and 2.632 (CUPRAC) TEAC mg/mL. The TPC of the extracts of *R. isthmicus* subsp. *tenuifolius* and *R. rumelicus* were determined as 854.06 and 672.08 mg gallic acid equivalents of the dry matter (mgGAE/g). TFC of MeOH extracts of *R. isthmicus* subsp. *tenuifolius* and *R. rumelicus* were calculated as 7.63, and 6.02 mgRE/g, respectively.

Some previous reports have also concerned fatty acid compositions of the aerial parts from *Ranunculus* species (Chen et al., 2006; Tavakoli et al., 2012; Erdogan et al., 2014). Similarly, linoleic, oleic, palmitoleic, and palmitic acids were the dominant fatty acids among the species. For example, Tavakoli et al. reported that the major components of the aerial parts of *Ficaria kochii* oil from Iran were palmitic acid (25.9%), linolenic acid (25.3%), and linoleic acid (17.5%) (Tavakoli et al., 2012). Similarly, we detected linoleic acid (24.47%) and palmitoleic acid (18.94%) as dominant fatty acids in the aerial parts of *Ranunculus pedatus* subsp. *pedatus* oil (Erdogan et al., 2014). In another work, the unsaturated fatty acids and oleic acid account for 58.19% and 35.68% of the aerial parts of *Ranunculus ternatus* from China (Chen et al., 2006). Monounsaturated fatty acids such as linoleic acid intake lead to a decrease in low-density lipoprotein (LDL) cholesterol and increased high-density lipoprotein (HDL) cholesterol (DiNicolantonio and O'Keefe, 2018). Therefore, it is important to have a sufficient amount of linoleic acid in the diet because it is an essential fatty acid. Lack of essential fatty acids such as linoleic acid in the body, mostly causes cardiovascular (and many other) diseases and its progression. Linoleic acid has a protective effect against heart diseases and is important for the development of the brain and retina. It is anti-inflammatory and acne-reducing when applied topically to the skin (Parikh et al., 2005). The role of linoleic acid in infant growth is important and in the developing brain, and it plays a role as a precursor to arachidonic

acid required for normal neural development linoleic acid and arachidonic acid represent the main PUFA in muscles (Marangoni et al., 2020).

Table 1. Fatty acid compositions (%) of investigated *Ranunculus* species (n=3).

Fatty acids Systematic name	**RI**	**RR**
C 6:0	2.08±0.07[a]	3.59±0.02
C 11:0	1.59±0.01	0.09±0.02
C 13:0	1.86±0.06	1.32±0.01
C 16:0	8.12±0.02	10.32±0.01
C 20:0	2.87±0.22	4.25±0.61
C 22:0	0.38±0.46	1.96±0.12
∑SFA[b]	**16.90±1.68**	**21.53±1.34**
C 16:1ω7	8.96±2.42	16.25±1.02
C 18:1ω9	18.24±0.02	8.46±0.08
C 20:1	2.91±0.04	1.14±0.01
C 24:1ω9	-	0.68±0.01
∑MUFA[b]	**30.11±1.06**	**26.53±0.24**
C 18:2ω6	38.42±1.09	24.09±0.01
C 20:2ω6	4.42±0.68	6.27±0.62
C 18:3ω3	2.09±0.02	4.74±0.03
C 20:3ω6	1.28±0.02	6.89±0.03
Total identified	**95.11±0.08**	**94.78±0.04**

[a]Values reported are means ±S.D.; RI: *Ranunculus isthmicus* subsp. *tenuifolium*, RR: *Ranunculus rumelicus*,
[b]SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids

Table 2. IA and IT indexes of *Ranunculus isthmicus* subsp. *tenuifolium* and *R. rumelicus.*

Plant	Indexes of atherogenicity	Indexes of thrombogenicity	ω3/ ω6
R.isthmicus subsp. *tenuifolium*	0.106	0.187	0.047
R. rumelicus	0.164	0.223	0.127

Table 3. Results of antioxidant activities, total phenolic and flavonoid contents of *Ranunculus isthmicus* subsp. *tenuifolium* and *R. rumelicus* (average ± stardard deviation)

	Yields (%)	DPPH• (mgTEAC/mL)	ABTS•+ (mgTEAC/mL)	CUPRAC (mgTEAC/mL)	TPC (mgGAE/g)[a]	TFC (mgRE/g)[b]
R.isthmicus	28.04	8.246±0.86[c]	3.402±0.62	3.041±1.86	854.06 ± 1.89	7.63 ± 0.96
R.rumelicus	21.89	6.308±2.24	2.051±1.86	2.632±0.54	672.08±0.94	6.02±2.02
Trolox	-	0.041±0.002	-	0.045±0.012	-	-
Acetic acid	-	-	0.920±0.011		-	-

[a] Total phenolic content expressed as gallic acid equivalent (mg GAE/g extract)
[b] Total flavonoid content expressed as rutin equivalent (mg RE/g extract)
[c] Results are mean ±SD of three replicate analysis

4. Conclusion

Antioxidants, the human body, cancer, inflammation, diabetes, rheumatoid arthritis, and reactive oxygen species (ROS) with biological molecules cardiovascular reaction can protect from problems (Comlekcioglu, 2020). Similarly, considerable variations in antioxidant capacity values TPC and TFC concentrations *Ranunculus* species were reported in previous studies (Mantle et al., 2000; Campos et al., 2003; Kaya et al., 2010). According to previous studies, it has been determined that the antioxidant activity of the extracts is related to being rich in flavonoid components (Prior and Cao, 2000). Many flavonoids such as vitexin, and isovitexin were isolated from different *Ranunculus* species (Erdogan et al., 2012). Epidemiologic studies have shown an inverse correlation between foods with high antioxidant content and the rate of death from diseases of degenerative origin such as cancer and cardiovascular diseases (Pandey and Rizvi, 2009). Therefore *R. isthmicus* subsp. *tenuifolius* and *R. rumelicus* and their phenolic compounds are important as natural sources of antioxidants and can be used in nutrition and for many pharmacological applications.

The fatty acid compositions of *Ranunculus isthmicus* subsp. *tenuifolius* and *Ranunculus rumelicus* were investigated for the first time. This work showed that these species are a good natural source of linoleic acid.

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Conflicts of Interests

Authors declare that there is no conflict of interests

Statement contribution of the authors

This study's experimentation, analysis and writing, etc. all steps were made by the authors.

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