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Original Article

Qualitative and quantitative phytochemical analysis and *in-vitro* biological activity of *Rheum ribes* L. different parts

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ABSTRACT

The methanol extracts from different parts of *Rheum ribes* were subjected to qualitative and quantitative phytochemical and *in vitro* biological activity (antioxidant, anti-urease, anticholinesterase). Qualitative phytochemical tests were performed using standard analysis methods and these studies revealed the presence of phenolics and tannins. Following this, a quantitative determination of total phenolics and tannins contents was carried out. The antioxidant activity of the extracts were assayed using DPPH, FRAP, TEAC/ABTS and CUPRAC techniques. In addition, the anti-urease and anticholinesterase activity of the extracts were examined using indophenol and Ellman methods, respectively. In this study, it was determined that the macerated flowers extract contained higher total phenolic and tannins contents than the other extracts. According to the results obtained from the antioxidant experiment, the macerated extract of flowers showed the strongest ABTS.+ scavenging and ferric reducing antioxidant power activity. The macerated leaves and Soxhlet radix extracts exhibited the strongest DPPH. scavenging and cupric reducing antioxidant activity, respectively. The young shoots extracts obtained using the Soxhlet methods showed the highest anticholinesterase activity. All extracts obtained from different parts of the plant were found to have very low anti-urease activity when compared to the anti-urease activity of standard compound. Therefore, methanol extracts from plant's flowers, leaves and young shoots can be used as a natural antioxidant and anticholinesterase agent respectively, for the pharmaceutical and food industry in the future.

Keywords: *Rheum ribes*, antioxidant, anti-uresae, anticholinesterase, phytochemical analysis

INTRODUCTION

Free radicals are produced continuously in our bodies (naturally or due to environmental impacts), and play a role in many diseases (cancer, Parkinson's disease, Alzheimer's disease, aging etc). Antioxidants are agents that clear free radicals and prevent them from doing damage. Because of the side effects of synthetic antioxidants, it has been more meaningful to use natural antioxidant sources such as fruits, vegetables, and grain foods (Baskar et al. 2011). It is widely known today that gastric and duodenal ulcers a usually caused by *Helicobacter pylori*. This organism releases urease that converts urea into ammonia and the released ammonia protects it from the acidic environment of the stomach. For this reason, the natural source compounds that inhibit urease activity are very important (Amin et al. 2013).

Rheum ribes L. (Rhubarb) belonging to Polygonaceae family is an annual species that is distributed across the temperate and subtropical regions of the world. This species is grown between 2300 and 2700 altitude in the rocky countryside of Turkey and known as "Işgın, Işkın, Uşgun and Uçgun". The edible parts of the plant are the young shoots and petiols, which were eaten raw or cooked (Davis and Cullen, 1967; Bulut et al. 2016). *Rheum* species are valuable to the pharmaceutical industry due to the presence of phytochemical contents (anthracene derivatives, tannins and phenolic compounds). *R.*

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ribes's young shoots and petioles are used against diarrhea and vomiting. The roots of the plant have been used in the treatment of diabetes, hypertension, ulcer and diarrhea. *Rheum ribes* contains vitamins (A, B1, B2 and C), some elements (potassium, magnesium and calcium) organic acids (citric acid and malic acid), anthraquinones (chrysophanol, physcion and emodin), flavonoid compounds (quercetin, 5- desoxyquercetin, quercetin 3-*0*-rhamnoside, quercetin 3-*0*- galactoside and auercetin 3-0-rutinoside), and tannins (Tosun and Kızılay, 2003; Andiç et al. 2009; Sindhu et al. 2010; Shafaghat et al. 2014; Polat et al. 2015; Shahi et al. 2016).

In recent years, the number of studies on plant extracts showing antioxidant, anticholinesterase and anti-urease activity has increased. In addition, it is known that extraction methods are very important in biological activities. Therefore, the aim of this study was to analyse qualitative and quantitative phytochemicals and to determine the antioxidant, anticholinesterase and anti-urease activities of *R.ribes* extracts obtained using different extraction methods.

MATERIAL AND METHODS

Plant material and extract preparation: The *R. ribes* was collected on 20th May 2016 from Van-Gürpınar, Turkey and identified by Dr. Gizem Bulut from Marmara Universty. The voucher specimen was deposited in the Pharmacy Faculty Herbarium (MARE) and the voucher specimen number was MARE 18817. The young shoots, leaves, radix and flowers of the plant were cut into small pieces. The small pieces (10 g) were extracted using the maceration, Soxhlet and ultrasonic bath methods with a methanol solvent. After extraction was complete, the samples were filtered through filter paper, the solvents were evaporated with a rotary evaporator and the crude extracts were stored in a refrigerator at 4 ºC.

Preliminary qualitative phytochemical analysis: Phytochemical analysis of *R. ribes* was carried out using standard procedure to identify the possible bioactive compound(s) (Trease and Evans, 2002; Sharma and Agarwal, 2015). The qualitative results are expressed as (+) for the presence and (-) for the absence of phytochemicals (Table 1).

Table 1. Preliminary qualitative phytochemical analysis of different parts of *R. ribes*

Phytochemicals	Radix	Flower	Leaves	Young shoots
Alkaloids	-	-	-	-
Glycosides	-	-	-	-
Saponins	-	-	-	-
Tannins	+	+	+	-
Cardiac glycosides	-	-	-	-
Phenols	+	+	+	+

+: Presence of the phytochemical, –: absence of the phytochemical from the extract

Quantitative determination of chemical constituents

Extract yield percentage and total phenolic contents: The extraction yield was calculated to determine the effectiveness of the solvents in extracting the active compounds from the plant material. The total phenolic contents of the 12 different plant extracts were determined using the FCR method (Ozsoy et al. 2008). The total phenolic contents in the extracts were given as µg gallic acid equivalents/mg extract.

Determination of tannins content: The amount of tannin contained in the different extracts was determined using the Folin-Ciocalteu method (Vijay and Rajendra, 2014). The tannin contents in the extracts were expressed as µg tannic acid equivalents in microgram per milligram of extract (µgTAE/ mg extract).

***In vitro* evaluation of antioxidant assays**

DPPH radical scavenging activity: The free radical scavenging ability of 12 different extracts was examined with the DPPH· method. The results obtained in the DPPH radical experiment were given as IC_{50} = µg/mL (Wei et al. 2010).

Trolox equivalent antioxidant activity: The $ABTS^{\cdot+}$ scavenging activity of the different extracts from the plant was evaluated using the TEAC/ABTS method. The standard curve was prepared using trolox and the data obtained in the experiment was expressed as mM trolox/mg extract (Re et al. 1999).

Ferric reducing/antioxidant power (FRAP) assay: The ferric reducing/antioxidant power of the different extracts was evaluated using the FRAP method. The standard curve was prepared using $FeSO_4.7H_2O$ and the data obtained in the experiment was expressed as mM Fe^{2+}/mg extract (Benzie and Strain, 1996).

Cupric reducing antioxidant capacity (CUPRAC): The cupric reducing antioxidant capacity of the different extracts was evaluated using the CUPRAC method. The CUPRAC values of the plant extracts were reported as trolox equivalents (mM trolox/mg extract) (Taskin et al. 2017).

In vitro anti-urease activity: In this study, the anti-urease activities of 12 different extracts obtained from the plant were evaluated according to the method of Ghous et al., 2010 and the results were given as a percentage of enzyme inhibition (Ghous et al. 2010).

Anticholinesterase activity of extracts: Inhibition of cholinesterases was evaluated using a 96-well microplate reader based on the method of Ellman et al. with some modifications (Ellman et al. 1961). The experiments were performed in triplicate in each case and the results were given as a percentage of enzyme inhibition. Galantamine was used as a reference.

Statistical analysis

The antioxidant, anticholinesterase and anti-urease experiments were done in triplicate and all the data was shown as mean±SD. The data was analyzed using the Graphpad Prism 5 program. Statistical differences between the experimental groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Multiple Comparison test. Mean values were considered statistically significant when $p<0.05$.

Table 2. Extract yield percentage, total phenolic and total tannins contents of different parts of *R.ribes*

Samples	Total phenolic (µgGAE/mg extract)			Extract yield (%)			Tannins (µgTAE/mg extract		
	Ultrasonic bath	Soxhlet	Maceration	Ultrasonic bath	Soxhlet	Maceration	Ultrasonic bath	Soxhlet	Maceration
Radix	135.00±0.007^{a}	122.00±0.003^{a}	83.00±0.008^{a}	5.21^{a}	20.46^{a}	18.44^{a}	123.00±0.003^{a}	109.00±0.005^{a}	61.00±0.010^{a}
Flowers	105.00±0.003^{b}	140.00±0.001^{b}	167.00±0.002^{b}	10.72^{b}	17.44^{b}	11.82^{b}	184.00±0.005^{b}	140.00±0.018^{b}	229.00±0.005^{b}
Leaves	163.00±0.007^{c}	158.00±0.004^{c}	139.00±0.005^{c}	12.62^{c}	65.03^{c}	55.80^{c}	210.00±0.007^{c}	141.00±0.006$^{c.b}$	178.00±0.014^{c}
Young shoots	58.00±0.002^{d}	67.00±0.001^{d}	53.00±0.004^{d}	8.74^{d}	30.79^{d}	19.62^{d}	ND	ND	ND

Values are mean of triplicate determination (n=3) ± standard deviation
Means with different superscripts (a-d) are significantly different, p<0.05
GAE-Gallic acid equivalents.
TAE-Tannic acid equivalents
ND: Not determined

RESULTS AND DISCUSSION

Qualitative phytochemical analysis of *R. ribes*

The phytochemical screening of different parts of a plant exhibited negative test for alkaloids, glycosides, saponins, cardiac glycosides (Table 1). Although all the plant's different parts showed positive test for phenols, and tannins, only young shoots showed negative test for tannins. It was known that the phytochemical compounds (phenols, tannins) that were qualitatively analyzed in *R. ribes* were medically important. Tannin-containing drugs have traditionally been used to protect inflamed surfaces of the mouth and throat. In addition, recent studies have shown that tannins are effective as antitumor and anti-HIV agents. Phenols are important compounds of some medicinal plants, and are used as coloring agents, flavored aromatizators and antioxidants (Trease and Evans, 2002; Buzzini et al. 2008). The phenols and tannins compounds identified in the methanol extract from *R.ribes'* different parts may be responsible for the biological activities. It is known that *R. ribes* contain tannins and phenol compounds (Amiri et al. 2015). The results of our study are consistent with the literature.

Quantitative phytochemical analysis of *R. ribes*

Extract yield percentage, total tannins and total phenolic contents: The total phenolic, tannins contents and yield percentage of methanol extracts from different parts of plant were analysed and are presented in Table 2. Leaf extracts obtained using ultrasonic bath and Soxhlet (163.00 µgGAE/mg extract, 158.00 µgGAE/mg extract) showed higher total phenolic contents than the macerated leaves extract (139.00 µgGAE/mg extract), respectively. In addition, it was found that the flowers extract (167 µgGAE/mg extract) obtained using the maceration method exhibited the highest total phenolic contents. When compared to all the data obtained in this study, the young shoots extracts obtained from the three methods were found to exhibit the lowest total phenolic contents. When the yields percentage of the different extracts were compared, the leaf extract obtained using the Soxhlet method was found to have a higher recovery over the other extracts. The total phenolic contents of the chloroform and methanol extracts from the roots and stems of *R. ribes* have been reported before (Öztürk et al. 2007). In this study, it was found that the roots's chloroform extract (48.66±1.23 µg pyrocatechol equivalent/mg extract) had higher phenolic contents than the others, while the one containing least phenolics was the stems's chloroform extract (22.68 ±1.10 µg pyrocatechol equivalent/mg extract). When we compared our study with data in literature, we found that methanol extract of radix (83.00 µgGAE/mg extract) showed higher total phenolic contents than radix's chloroform extract (48.66 µg pyrocatechol equivalent/ mg extract).

The amounts of tannins contained in different parts of the plant were ascertained in the following order: macerated flower extract (229.00±0.005 µgTAE/mg extract)>ultrasonic bath leaves extract (210.00±0.007 µgTAE/mg extract)>Soxhlet leaves extract (141.00±0.006 µgTAE/mg extract). The results from the total tannins assay showed that the macerated flowers extract had the highest amount of tannins. According to

Table 3. Effects of extracting solvents/methods on the antioxidant activity of *R. ribes* extracts

Samples	DPPH (IC_{50}: µg/mL)			ABTS (mM trolox/mg extract)			FRAP assay (mM Fe^{2+}/mg extract)			CUPRAC assay (mM trolox/mg extract)		
	Ultrasonic bath	Maceration	Soxhlet	Ultrasonic bath	Maceration	Soxhlet	Ultrasonic bath	Maceration	Soxhlet	Ultrasonic bath	Maceration	Soxhlet
Radix	28.00±0.01^{a}	75.00±0.02^{a}	36.00±0.02^{a}	51.70±0.01^{a}	48.80±0.2^{a}	51.60±0.02^{a}	0.13±0.08^{a}	0.18±0.1^{a}	0.15±0.03^{a}	0.78±0.11a	0.62±0.06^{a}	0.91±0.02^{a}
Flowers	5.00±0.02^{b}	4.80±0.05^{b}	23.00±0.02^{b}	51.10±0.02^{b,a}	51.90±0.1^{b}	51.40±0.01^{b,a}	0.15±0.04^{b,a}	0.29±0.6^{b}	0.17±0.02^{b,a}	0.62±0.05b,a	0.61±0.02^{b,a}	0.62±0.07^{b}
Leaves	7.00±0.02^{c,b}	3.00±0.01^{c,b}	20.00±0.02^{c,b}	51.40±0.03^{c,a,b}	49.10±0.1^{c,a}	50.80±0.03^{c,a,b}	0.15±0.02^{c,a,b}	0.21±0.2^{c,a}	0.22±0.04^{c,a,b}	0.67±0.02c,a,b	0.71±0.02^{c,a,b}	0.72±0.05^{c,b}
Young shoots	144.00±0.09^{d}	98.00±0.03^{d}	85.00±0.05^{d}	13.10±0.01^{d}	12.80±0.4^{d}	12.70±0.03^{d}	0.28±0.08^{d}	0.14±0.3^{d,a,c}	0.16±0.01d,a,b,c	0.58±0.17^{d,a,b,c}	0.27±0.07^{d}	0.74±0.01^{d,c}
Ascorbic acid	6.00±0.01^{e,b,c}	6.00±0.01^{e,b,c}	6.00±0.01^{e}									
BHT				1.10±0.12^{e}								
BHA		52.63±0.01^{e,a}	52.63±0.01^{e,a,b}					1.62±0.12^{e}				
		52.63±0.01^{e,b}										

Values are mean of triplicate determination (n=3) ± standard deviation
Means with different superscripts (a-e) are significantly different, p<0.05

the obtained results, it was determined that young shoots obtained using all extraction methods did not contain tannin compounds and also the radix extracts obtained using all extraction methods contained the lowest tannin compounds. To the best of our knowledge, there have been no reports in literature on the total tannins contents of methanol extract from a plant's different parts. Therefore, for the first time in this study, the amount of tannins contained in the different parts of plant was determined and the effects of this compound on biological activity were examined.

In vitro antioxidant activity assays: The DPPH· scavenging activity of different extracts from *R. ribes* different parts are shown in Table 3. Ascorbic acid was used as a positive control. According to the results obtained from the DPPH experiment, the flowers and leaves extracts obtained using the three extraction methods were very close to each other and had a stronger DPPH scavenging activity than the other extracts. Ultrasonic bath (IC_{50} 5.00 µg/mL), maceration (IC_{50} 4.80 µg/mL) flowers and maceration leaves (IC_{50} 3.00 µg/mL) extracts exhibited a stronger DPPH radical scavenging activity than ascorbic acid (IC_{50} 6.00 µg/mL). When comparing extractions methods, it was found that the maceration and ultrasonic bath methods were more suitable methods for the DPPH activity of *R.ribes*. In addition, the young shoots extracts obtained using the three extraction methods exhibited the lowest free radical scavenging activity. The DPPH method was usually applied to measure the activity of polar compounds. The obtained results showed that flowers and leaves extracts were rich in polar compounds. Since these extracts exhibited the highest phenolic contents and DPPH radical scavenging activity, it was found that there was a linear relationship between phenolic compounds and free radical scavenging activity.

The TEAC/ABTS was a widely used method for measuring the activity of polar and nonpolar compounds in plants. The maceration extract of flowers (51.90 mM trolox/mg extract) exhibited the strongest $ABTS^{\cdot+}$ scavenging activity. In addition, the radix, flowers and leaves extracts obtained using all the extraction methods showed antioxidant activity close to each other and BHA, but the young plant shoots exhibited lower antioxidant activity than BHA and other extracts. When comparing extractions methods, it was found that the all extraction methods were a suitable method for the TEAC/ABTS activity of this species.

In the ultrasonic bath method, the young shoots extract (0.28 mM Fe^{2+}/mg extract) showed a stronger ferric reducing/antioxidant power activity than the other extracts. In the maceration method, the flowers extract (0.29 mM Fe^{2+}/mg) had the highest FRAP values. In addittion, the leaves extract (0.22 mM Fe^{2+}/mg extract) obtained using the Soxhlet method showed a stronger ferric reducing activity than the other extracts. According to the obtained results, the macerated flowers and ultrasonic bath young shoots extracts were found to have stronger ferric reducing activity than the other extracts. The radix extract (0.13 mM Fe^{2+}/mg extract) obtained using the ultrasonic bath method exhibited the lowest ferric reducing/antioxidant power activity. All the extracts from the plant's dif-

Table 4. The anti-urease inhibitory activity of different parts of *R. ribes*

	Urease inhibition (%) (12.5 µg/mL)		
Samples	Ultrasonic bath	Maceration	Soxhlet
Radix	2.33±0.1[a]	12.46±1.06[a]	7.57±0.13[a]
Flowers	6.61±2.4[b]	5.76±0.9[b]	4.33±0.4[b]
Leaves	17.90±0.5[c]	10.79±0.07[c]	16.83±0.4[c]
Young shoots	NA	9.26±0.7[d]	6.12±1.5[d]
Thiourea	78.54±0.60[d]	78.54±0.60[e]	78.54±0.60[e]

Values are mean of triplicate determination (n=3) ± standard deviation
NA: not activity
Means with different superscripts (a-e) are significantly different, $p<0.05$

Table 5. The anticholinesterase activity of different parts of *R. ribes*

	Acetylcholinesterase inhibition (%)		
Samples	Ultrasonic bath	Maceration	Soxhlet
Radix (500 µg/mL)	45.97±1.3[a]	36.05±0.83[a]	37.43±1.53[a]
Flowers (500 µg/mL)	71.90±1.14[b]	65.39±0.25[b]	61.13±0.76[b]
Leaves (500 µg/mL)	64.90±0.35[c]	14.95±2.33[c]	55.32±1.09[c]
Young shoots (200 µg/mL)	84.19±1.82[d]	63.95±0.5[d]	87.98±1.01[d]
Galantamine (500 µg/mL)	93.35±0.06[e]		

Values are mean of triplicate determination (n=3) ± standard deviation
Means with different superscripts (a-e) are significantly different, $p<0.05$

ferent parts had lower FRAP values than BHT compound (1.10 mM Fe^{2+}/mg). The results obtained from this study showed that both maceration and Soxhlet extraction techniques (excluding ultrasonic bath young shoots extract) was the most suitable method to get the most powerful ferric reducing/antioxidant activity.

In the ultrasonic bath method, radix (0.78 mM trolox/mg extract) and leaves (0.67 mM trolox/mg extract) extracts showed a stronger cupric reducing antioxidant activity than other extracts, respectively. In the maceration method, the leaves (0.71 mM trolox/mg extract) and radix (0.62 mM trolox/mg extract) extracts had higher CUPRAC values than the other extracts, respectively. In the Soxhlet method, the radix extract (0.91 mM trolox/mg extract) exhibited the strongest cupric reducing antioxidant activity. It was also found that young shoots (0.74 mM trolox/mg extract) and leaves (0.72 mM trolox/mg extract) extracts showed very close cupric reducing antioxidant results to each other. According to the results obtained from CUPRAC experimental, the radix extracts obtained using Soxhlet and ultrasonic bath methods showed the highest cupric reducing antioxidant activity. When the antioxidant activity of all the extracts was compared to the standard compound, all extracts were found to have lower activity than the BHA (1.62 mM trolox/mg) .

Shahi et al. (2016) investigated the antioxidant activity of maceration methanol extract from *R. ribes* flowers. According to the results obtained, flowers extracted with the concentration of 200 ppm and 300 ppm showed a higher inhibitory activity of free radicals than the BHT compound (Shahi et al. 2016). When we compared our study with this study, it was found that parallel to this study, maceration methanol extract from plant's flowers (IC_{50} 4.80 µg/mL) exhibited stronger DPPH· scavenging activity than ascorbic acid (IC_{50} 6.00 µg/mL). Shafaghat et al. (2016) investigated the free radical scavenging of Soxhlet hexane extract and essential oils from plant and plant's hexane extract (IC_{50} 325.00 µg/mL) and essential oils (IC_{50} 565.00 µg/mL) showed lower DPPH radical scavenging activity compared to the synthetic antioxidant of vitamin C (IC_{50} 26.00 µg/mL). In addition, the plant's essential oils and hexan extract composition were analyzed using GC-GC/MS. The main components of the hexane extract were 9-octadecenoic acid(ω-9), 9, 12- octadecadienoic acid (linoleic acid or ω- 6), hexadecanoic acid, (palmitic acid) , 1,2-benzenedicarboxylic acid diisooctyl, dodecane and γ- linolenic acid. The germacrene-d, α-pinene, terpinolene, p-cymene, bicyclogermane and limonene compounds were analysed as major components in the essential oils of the plant (Shafaghat et al. 2014). The antioxidant activities of chloroform and methanol extracts of the roots and stems of *R.ribes* have been reported before (Öztürk et al. 2007). This study reported that both methanol extracts obtained using the maceration method showed stronger free radical scavenging capacity than the corresponding chloroform extracts, moreover, the stems's methanol extract exhibited better activity than BHT. In addition, both roots extracts exhibited more potent superoxide anion radical scavenging activity than BHT. Except for the roots's extract, the other three extracts showed better metal chelating activity than quercetin. Unlike this study, the antioxidant activity of methanol extracts from different parts of the plant were examined with DPPH, FRAP, ABTS/TEAC and CUPRAC methods and it was determined that the maceration radix extract showed lower DPPH scavenging activity than ascorbic acid.

Anti-urease inhibitory activity: The results for the assessment of urease inhibitory activity of *R. ribes m*ethanol extracts (12.50 µg/mL) obtained using the three extraction methods are shown in Table 4. In the ultrasonic bath method, the leaves extract (17.90%) showed stronger ureae inhibitory activity than the other extracts. It was also found that the radix extract (2.33%) showed the lowest anti-urease activity. In addtion, the young shoots extract didn't show any anti-urease activity. In the maceration method, the radix (12.46%), leaves (10.79%) and young shoots (9.26%) extracts exhibited a stronger anti-urease activity than the flowers extract (5.76%). In the Soxhlet method, the leaves extract (16.83%) showed the strongest anti-urease activity. It was also found that the radix (7.57%) and

young shoots (6.12%) extracts showed close anti-urease activity and that extracts exhibited stronger activity than the flowers extract (4.33%). In this study, among the extracts obtained from three different extraction methods, the leaves extracts obtained using the ultrasonic bath and Soxhlet method exhibited the strongest anti-urease activity. When the anti-urease activities of the extract and standard were compared, it was found that all the extracts from the plant had lower anti-urease activity than standard thiourea (78.54%). The anti-urease activity of the 50% methanol extract of *R.ribes* roots has been previously reported (Nabati et al. 2012). This study showed that the 50% methanol extract had a 98.93% anti-urease activity at a concentration of 10 mg/mL. In our study, the anti-urease activity of methanol extracts from the radix at a concentration of 12.5 µg/mL was investigated and found that maceration radix extract had 12.46% anti-urease activity.

Anticholinesterase activity: The results for the assessment of cholinesterase inhibitory activity of plant's different extracts are shown in Table 5. In the ultrasonic bath method, young shoots (84.19%) and flowers (71.9%) extracts exhibited stronger cholinesterase inhibitory activity than other extracts. In the maceration method, the young shoots (63.95%) and flowers (65.39%) extracts exhibited stronger cholinesterase inhibitory activity than other extracts. It was also found that the leaves extract (14.95%) had the lowest anticholinesterase activity. In the Soxhlet method, the young shoots (87.98%) and flowers (61.13%) extracts exhibited the strongest anticholinesterase activity. According to the results obtained from activity assay, the radix extract (37.43%) showed lower anticholinesterase activity than the other extracts. As a result of this experiment, it was found that the young shoots extracts obtained using the three extraction methods exhibited the strongest anticholinesterase activity. It was also found that the young shoots extracts obtained using the ultrasonic bath (84.19%) and Soxhlet (87.98%) methods showed close anticholinesterase activity to the galantamine compound (93.35%). In the present study, the Soxhlet and ultrasonic bath methods were the most extraction methods to get the strongest anticholinesterase activity. In Gholamhoseiniant et al., the *in vitro* anticholinesterase activity of the methanol extract from the rhizomes of the plant was investigated and it was found that this extract showed 72.4% activity at a concentration of 8 mg /mL (Gholamhoseiniant et al. 2009). In another study, it was clearly demonstrated that the treatment with 50% methanol extract from *R.ribes* roots and rhizomes could significantly recover the spatial and passive avoidance memory disorders caused by the destruction of the NBM nucleus in male-wistars rats (Zahedi et al. 2015). In our study, anticholinesterase activities of different parts (radix, flowers, leaves and young shoots) of the plant were investigated and it was found that these parts (especially the young shoots) showed significant activity in accordance with the literature.

CONCLUSION

Rheum ribes is mainly used in medicines and foods in Turkey. Therefore, it was very important to examine the biological activities (antioxidant, anti-urease, and anticholinesterase) of this plant. In this study, the biological activities and chemical contents of different parts of the plant were qualitatively and quantitatively determined. In this study, it was determined that the macerated extract of flowers contained higher total phenolic and tannins contents than other extracts. According to the results obtained, the macerated flowers extract showed the strongest ABTS radical scavenging and ferric reducing activity. The macerated leaves and Soxhlet radix extracts showed the highest DPPH radical scavenging and cupric reducing antioxidant activity, respectively. The young shoots extracts obtained using the Soxhlet methods showed the highest anticholinesterase activity. All extracts obtained from different parts of the plant were found to have very low anti-urease activity when compared to the anti-urease activity of standard compound. Therefore, the methanol extract from the plant's flowers, leaves and young shoots can be used as a natural antioxidant and anticholinesterase agent respectively for the pharmaceutical and food industry in the future.

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REFERENCES

- Amin M, Anwar F, Naz F, Mehmood T, Saari N (2013). Anti-*Helicobacter pylori* and urease inhibition activities of some traditional medicinal plants. *Molecules* **18:** 2135-2149. **[CrossRef]**
- Amiri N, Shafaghat A, Salimi F (2015). Screening of the essential oil, hexane extract, chemical composition, antioxidant activity, and antimicrobial activity of the flower *Rheum ribes* L. from Iran. *J Essent Oil Bear Pl* **18:** 1108-1115. **[CrossRef]**
- Andiç S, Tunçtürk Y, Ocak E, Köse S (2009). Some chemical characteristics of edible wild rhubarb species (*Rheum ribes* L.) Res. *J Agric Biol Sci* **5:** 973-977.
- Baskar R, Shrisakthi S, Sathyapriya B, Shyampriya R, Nithya R, Poongodi P (2011). Antioxidant potential of peel extracts of banana varieties (*Musa sapientum*). *Food Nutr Sci* **2:** 1128-1133. **[CrossRef]**
- Benzie IF, Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal Biochem* **239:** 70-76. **[CrossRef]**
- Bulut G, Biçer M, Tuzlacı E (2016). The folk medicinal plants of Yüksekova (Hakkâri-Turkey). *J Fac Pharm Istanbul* **46:** 115-124.
- Buzzini P, Arapitsas P, Goretti M, Branda E, Turchetti B, Pinelli P, Leri F, Romani A (2008). Antimicrobial and antiviral activity of hydrolysable tannins. *Mini Rev Med Chem* **8:** 1179-1187. **[CrossRef]**
- Davis PH, Cullen J (1967). The Flora of Turkey, Vol 2, Edinburgh university press, Edinburgh, pp. 268-269.
- Ellman GL, Courtney KD, Andress V, Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol* **7:** 88-95. **[CrossRef]**
- Gholamhoseinian A, Moradi MN, Sharifi-far F (2009). Screening the methanol extracts of some Iranian plants for acetylcholinesterase inhibitory activity. *Res Pharm Sci* **4:** 105-112.

- Ghous T, Akhtar K, Nasim FUH, Choudhry MA (2010). Screening of selected medicinal plants for urease inhibitory activity. *Biol Med* **2:** 64-69.
- Nabati F, Mojab F, Habibi-Rezaei M, Bagherzadeh K, Amanlou M, Yousefi B (2012). Large scale screening of commonly used Iranian traditional medicinal plants against urease activity. *Daru* **20:** 72. **[CrossRef]**
- Ozsoy N, Can A, Yanardağ R, Akev N (2008). Antioxidant activity of *Smilax excels* L. leaf extracts. *Food Chem* **110:** 571-583. **[CrossRef]**
- Öztürk M, Öztürk FA, Duru ME, Topçu G (2007). Antioxidant activity of stem and root extracts of Rhubarb (*Rheum ribes*): An edible medicinal plant. *Food Chem* **103:** 623-630. **[CrossRef]**
- Polat R, Cakilcioglu U, Ulusan MD, Paksoy MY (2015). Survey of wild food plants for human consumption in Elazığ (Turkey). *IJTK* **1:** 69-75. **[CrossRef]**
- Re R, Pellegrını N, Proteggente A, Pannala A, Yang M, Evans CR (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Bio Med* **26:** 1231-1237. **[CrossRef]**
- Shafaghat A, Amiri N, Salimi F (2014). Screening of flowers essential oil and hexane extract of *Rheum ribes* L. from Iran- Chemical composition, antioxidant and antimicrobial activities. *JPHS* **2:** 115-123.
- Shahi MMN, Rad AHE, Shahi NN, Amin MRB (2016). Study of antioxidant activity and free radical scavengıng power of *Rheum ribes* flower extract. *J Fundam Appl Sci* **8:** 1164-1174. **[CrossRef]**
- Sharma V, Agarwal A (2015). Physicochemical and antioxidant assays of methanol and hydromethanol extract of ariel parts of *Indigofera tinctoria* Linn. *Indian J Pharm Sci* **77:** 729-734. **[CrossRef]**
- Sindhu R, Kumar P, Kumar J, Kumar A, Arora S (2010). Investıgatıons into the anti-ulcer activity of *Rheum rıbes* Linn leaves extracts. *Int J Pharm Pharm Sci* **I2:** 90-93.
- Taskin D, Alkaya BD, Dölen E (2017). HPLC-DAD/ESI-Q-TOF LC/MS Analysis of major phenolic compounds in flowers, leaves and stems of *Achillea grandifolia* and evaluation of their individual antioxidant properties. *Chiang Mai J Sci* **44:** 1-12.
- Tosun F, Kızıla, AÇ (2003). Anthraquinones and flavonoids from *Rheum ribes*. *J Fac Pharm* **32:** 31-35.
- Trease GE, Evans WC (2002). Pharmacognosy. 15th Ed. London: Saunders Publishers; pp. 391-393.
- Vijay DT, Rajendra SB (2014). Estimation of total phenol, tannin, alkaloid and flavonoid in *Hibiscus tiliaceus* L. wood extracts. research and review. *J. Pharmacog Phytochem* **2:** 41-47.
- Zahedi M, Hojjati MR, Fathpour H, Rabiei Z, Alibabaei Z, Basim A (2015). Effect of *Rheum ribes* hydro-alcoholic extract on memory impairments in rat model of Alzheimer's disease. *Iran J Pharm Res* **14:** 1197-1206.
- Wei F, Jinglou C, Yaling C, Yongfang L, Liming C, Lei P (2010). Antioxidant, free radical scavenging, anti-inflammatoryand hepatoprotective potential of the extract from *Parathelypteris nipponica* (Franch.et Sav.) Ching. *J Ethnopharmacol* **130:** 521-528. **[CrossRef]**