

## Antioxidant and antibacterial activities of *Ranunculus marginatus* var. *trachycarpus* and *R. sprunerianus*

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Received: 12.09.2008

**Abstract:** Hexane, ethyl acetate, methanol, and aqueous extracts of *Ranunculus marginatus* d'Urv. var. *trachycarpus* (Fisch. & Mey.) Azn. and *R. sprunerianus* Boiss. were tested in vitro for their antioxidant and antibacterial activities. Antioxidant activity of the extracts was determined by DPPH radical scavenging and Trolox equivalent antioxidant capacity assays. Methanol extracts showed the highest antioxidant activity in both assays. The total phenolics in the extracts were determined colorimetrically by using the Folin-Ciocalteu reagent. The total flavonoid content of the extracts was evaluated by a spectrophotometric method. The results obtained in the antioxidant activity tests were in positive correlation with the total phenolic and flavonoid contents of the extracts. An antibacterial activity analysis was carried out using paper disk diffusion and micro-well dilution techniques. All of the extracts displayed antibacterial activity against the tested bacteria in the disk diffusion method. The minimal inhibitory concentrations (MICs) of all the extracts of both *Ranunculus* species were found to be between 128 and 256 µg/mL.

**Key words:** Antibacterial activity, antioxidant activity, flavonoid, phenol, *Ranunculus marginatus* var. *trachycarpus*, *Ranunculus sprunerianus*

### *Ranunculus marginatus* var. *trachycarpus* ve *R. sprunerianus*'un antioksidan ve antibakteriyel aktiviteleri

**Özet:** *Ranunculus marginatus* d'Urv. var. *trachycarpus* (Fisch. & Mey.) Azn. ve *R. sprunerianus* Boiss. bitkilerinin hekzan, etil asetat, metanol ve su ekstralarının antioksidan ve antibakteriyel aktiviteleri in vitro olarak test edilmiştir. Ekstrelerin antioksidan aktivitesi DPPH radikal süpürücü ve Troloks eşdeğeri antioksidan kapasite yöntemleriyle tayin edilmiştir. Her iki yöntemde de metanol ekstraları en yüksek antioksidan aktiviteyi göstermiştir. Ekstrelerdeki total fenol miktarı Folin-Ciocalteu reaktifi kullanılarak kolorimetrik olarak tayin edilmiştir. Ekstrelerin total flavonoid içeriği spektroskopik bir yöntem kullanılarak değerlendirilmiştir. Antioksidan aktivite deneylerinin sonuçları, ekstraların total fenol ve flavonoid miktarları ile uyumludur. Antibakteriyel aktivite tayininde disk difüzyon ve mikrodilüsyon teknikleri kullanılmıştır. Disk difüzyon yönteminde bütün ekstralar test edilen bakterilere karşı aktivite göstermiştir. Her iki *Ranunculus* türünün ekstralarının minimal inhibitör konsantrasyonlarının (MİK) 128-256 µg/mL arasında olduğu bulunmuştur.

**Anahtar sözcükler:** Antibakteriyel aktivite, antioksidan aktivite, flavonoid, fenol, *Ranunculus marginatus* var. *trachycarpus*, *Ranunculus sprunerianus*

## Introduction

*Ranunculus* (Ranunculaceae) is a widespread and temperate genus, represented by about 84 species in the flora of Turkey (1-3). Various parts of the plants of the *Ranunculus* species, including roots, herbs, and flowers, have been used extensively in traditional medicine in Turkey to treat a variety of illnesses, such as constipation, rheumatism, hemorrhoids, edema, abscesses, and jaundice (4-7). Some of the plants belonging to this genus have also been used in Turkish folk medicine for their emmenagogue, galactagogue, irritant, and wound-healing properties (4). In the northwestern part of the Black Sea region of Turkey, fresh leaves of *Ranunculus ficaria* L. subsp. *bulbifera* (Marsden-Jones) Lawalrée are consumed as a salad after being mixed with yogurt (8).

Phytochemical studies carried out on various *Ranunculus* species revealed that they produce compounds belonging to different secondary metabolite groups, including triterpene saponins (9,10), alkaloids (11,12), flavonoids (13-17), fatty acids and organic acids (18-21).

Although several plants belonging to this genus have been shown to possess important biological properties such as antibacterial (19,22), antiviral (23), antimicrobial (24,25), anti-inflammatory (26), antiprotozoal (27), xanthine oxidase inhibitory (28), and nematocidal activities (22), to our knowledge there have been no reports on the activity of *R. marginatus* d'Urv. var. *trachycarpus* (Fisch. & Mey.) Azn. and *R. sprunerianus* Boiss. Therefore, the present study aims to evaluate the antioxidant and antibacterial activities of different extracts prepared from these two *Ranunculus* species.

## Materials and methods

### Plant material

*Ranunculus marginatus* d'Urv. var. *trachycarpus* (Fisch. & Mey.) Azn. and *R. sprunerianus* Boiss. were collected in May 2004 from Akdağ, Karaburun, in İzmir province, Western Anatolia, and identified by Lütfi Bekat, Department of Botany, Faculty of Science, Ege University. Voucher specimens of *R. marginatus* var. *trachycarpus* (No. 1326) and *R. sprunerianus* (No. 1327) are deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Ege University.

### Preparation of extracts

Hexane, ethyl acetate, methanol, and aqueous extracts were separately prepared from 20 g batches of the air-dried and powdered whole plants by percolation at room temperature. Then the extraction solvents were evaporated under reduced pressure to dryness (29). The yields of hexane, ethyl acetate, methanol, and aqueous extracts of *R. marginatus* var. *trachycarpus* were 1.31%, 1.96%, 11.80%, and 19.97% and of *R. sprunerianus* were 0.40%, 0.85%, 14.60%, and 25.71%, respectively. All the extracts were stored at -20 °C.

### Determination of total flavonoid content

Flavonoid contents of the samples were determined spectrophotometrically by measuring the flavonoids in  $AlCl_3$ -complex form from a purified ethyl acetate phase obtained after acid hydrolysis (30,31). The results were expressed as g per 100 g of dry extract (Table 1).

### Determination of total phenol content

The total phenols were determined according to the colorimetric reaction with the Folin-Ciocalteu reagent (32). Dried extracts were diluted in distilled water (1:10 v/v). As a phenolic standard, gallic acid (Sigma-Aldrich Chemie, Steinheim, Germany) was used and prepared in methanol:water (1:1, v/v) at concentrations of 50-250 mg/L. A solution of an extract (0.5 mL) of gallic acid was mixed with 5 mL of the Folin-Ciocalteu reagent (1:10; diluted in distilled water) and 4 mL of sodium carbonate (1 M). The mixtures were heated at about 45 °C in a water bath for 15 min and the total phenols were determined colorimetrically at 765 nm. Total phenol values were expressed as mg of gallic acid equivalents per g of dry extract (Table 1).

### DPPH-radical scavenging activity assay (DPPH-RSC)

The capacity of the plant extracts to scavenge the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was measured as described previously (33). Extracts (0.25 mg) were dissolved in 4 mL of methanol (0.0625 mg/mL). Then it was mixed with 0.5 mL of a methanolic solution of DPPH (1 mM) and allowed to stand at room temperature for 30 min. The optical density of the mixture was measured at 517 nm.

DPPH-RSC values were expressed as a percentage of DPPH radical discolouration. The synthetic antioxidant butylated hydroxytoluene (BHT, Sigma-Aldrich Chemie, Steinheim, Germany) was used as a positive control (1 mg/mL) (Table 1).

#### **Trolox equivalent antioxidant capacity assay (TEAC)**

The 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical cation ( $ABTS^{+}$ ) was produced by reacting ABTS aqueous solution (7 mM) with 2.45 mM of ammonium persulfate; the mixture was allowed to stand in the dark at room temperature for 12-16 h before use. The  $ABTS^{+}$  solution was diluted with 5 mM of phosphate buffered saline (PBS) (pH 7.4) to obtain an absorbance of 0.70 ( $\pm 0.02$ ) at 734 nm. Then 1 mL of the diluted  $ABTS^{+}$  solution was mixed with 10  $\mu$ L of extract (0.1 mg/mL) or Trolox standards (0-15  $\mu$ M) and the absorbance was measured. The percentage inhibition of absorbance at 734 nm was calculated and TEAC values were evaluated from the decreases in absorbance in the Trolox standard curve (34) (Table 1).

#### **Antibacterial activity test**

Antibacterial activity of the extracts of *R. marginatus* var. *trachycarpus* and *R. sprunerianus* was evaluated using the paper disk diffusion technique and by determining the minimal inhibitory concentration (MIC). The bacterial strains used in the antibacterial tests are listed in Tables 2 and 3. Lyophilized bacteria were obtained from the culture collection of the Department of Microbiology, Faculty of Science, Ege University.

#### **Disk diffusion assay**

The antibacterial activity of the crude extracts was tested by the paper disk diffusion technique (35,36). The extracts were dissolved in DMSO and then 20  $\mu$ L of each extract (1024  $\mu$ g/mL) of *R. marginatus* var. *trachycarpus* and *R. sprunerianus* were absorbed onto sterile 6-mm diameter filter paper disks (Schleicher and Schüll, Nr 2668, Dassel, Germany).

The bacterial strains were inoculated on Mueller-Hinton broth (Oxoid) and incubated for 24 h at  $37 \pm 0.1$  °C. Adequate amounts of autoclaved Mueller-Hinton Agar (Oxoid) were dispensed onto sterile

plates and allowed to solidify under aseptic conditions. The counts of bacterial strains were adjusted to yield approximately  $1.0 \times 10^7$ – $1.0 \times 10^8$  mL<sup>-1</sup> using the standard McFarland counting method. Then 0.1 mL of the test organisms were inoculated with a sterile swab on the surface of the appropriate solid medium in the plates.

Agar plates containing the bacteria were incubated for 1 h before placing the extract-impregnated paper disks on the plates. The sterile disks impregnated with different extracts were then placed on the agar plates and incubated at  $37 \pm 0.1$  °C for 24 h. Antibacterial activity was evaluated by measuring the zone of inhibition (in mm) against the test organisms. All experiments were done under sterile conditions in duplicate and repeated three times. Ceftazidime (Fluka) and Sulbactam/Ampicillin (Oxoid) (10 mg/disk) were used as positive controls. DMSO was used as a negative control.

#### **Micro-well dilution assay**

The minimal inhibitory concentration (MIC) values were determined for the bacterial strains that were sensitive to *R. marginatus* var. *trachycarpus* and *R. sprunerianus* by micro-dilution assay according to the procedures developed by the National Committee of Clinical Laboratory Standards (37,38).

The bacterial strains were inoculated on Mueller-Hinton broth (Difco) and incubated for 24 h at  $37 \pm 0.1$  °C. The inocula of the bacterial strains were prepared from 24 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity.

Dilution series of the extracts were prepared in test tubes. Final concentrations of the extracts were 1024 to 0.5  $\mu$ g/mL in the medium. The 96-well plates were prepared by dispensing extract into each well of broth and the inocula to obtain  $1 \times 10^8$  CFU/mL. Extract prepared at the concentration of 1024  $\mu$ g/mL was added into the first wells. Then its serial dilutions (512, 256, 128, 64, 32, 16, 8, 4, 2, 1, and 0.5  $\mu$ g/mL) were transferred into the consecutive wells. The final well, containing Mueller-Hinton broth without extract and the inocula on each strip, was used as a negative control. The plate was covered with a sterile plate sealer. The plates were incubated at 37 °C for 24 h.

The MIC was defined as the lowest concentration of an extract or a substance to inhibit the growth of microorganisms after 24 h. Gentamicin and Ampicillin (Sigma Aldrich Chemical Co., St. Louis, MI, USA) were used as standard antibacterial agents. Their dilutions were prepared from 128 to 0.25 µg/mL concentrations in microtiter plates. All of the assays were performed in triplicate.

## Results and Discussion

The antioxidant activity of the extracts prepared from the above-mentioned *Ranunculus* species are reported in Table 1. Similar results were obtained in both of the assays employed for the determination of the antioxidant activity. The strongest activity was detected in the methanol extracts of the tested *Ranunculus* species. A positive correlation between the total phenolic and flavonoid contents of the extracts and the antioxidant activity was observed. It is already well known that polyphenols are the major plant compounds with antioxidant activity and that flavonoids are among the important polyphenolic components detected in plant extracts (39-41). Moreover, in a study by Mantle et al. (42), the antioxidant activity of methanol-water (8:2) extracts prepared from the leaves and flowers of *R. repens* L. were reported as  $0.13 \pm 0.01$  and  $0.12 \pm 0.01$  mM TE/g dry weight, respectively. In another study, it has been reported that flavonoid or phenolic components played a significant role in the free radical scavenging capacity of *R. sardous* Crantz. pollen (43). Since

flavonoids are among the major constituents isolated from the *Ranunculus* species and have also been considered as good taxonomic markers in this genus (14,16), they may very well be the main components that contribute to the antioxidant activity observed in the present study.

The antibacterial activity levels of the extracts of *R. marginatus* var. *trachycarpus* and *R. sprunerianus*, evaluated by the disk diffusion and micro-well dilution techniques, are reported in Tables 2 and 3, respectively. In the disk diffusion assay (Table 2), the maximal inhibition zones ranged between 7 and 12 mm, and, in the micro-well dilution assay (Table 3), the MIC values of the extracts were between 128 and 256 µg/mL, which indicate that all of the extracts of *R. marginatus* var. *trachycarpus* and *R. sprunerianus* showed significant activity against the tested bacterial species. The highest susceptibility to the tested extracts was displayed by the gram-negative bacterium *Enterobacter aerogenes*, with an inhibition zone ranging between 10 and 12 mm and a MIC value of 128 µg/mL. Moreover, all of the plant extracts showed significant activity against another gram-negative bacterium, *Escherichia coli*, with an inhibition zone of 9-10 mm and an MIC value of 128 µg/mL. Interestingly, besides these two gram-negative bacteria, *Pseudomonas aeruginosa*, which is also a gram-negative bacterium, turned out to be the least susceptible among those tested with an inhibition zone of 7-8 mm and a MIC value of 256 µg/mL.

Table 1. Total phenolic, total flavonoid contents, and antioxidant activity of *Ranunculus marginatus* var. *trachycarpus* and *R. sprunerianus*.

Extract	<i>R. marginatus</i> var. <i>trachycarpus</i>				<i>R. sprunerianus</i>			
	Total Phenol (mg/g dry mass)	Total Flavonoid (g %)	DPPH- RSC (%)	TEAC (mM)	Total Phenol (mg/g dry mass)	Total Flavonoid (g %)	DPPH- RSC (%)	TEAC (mM)
hexane	122.80 ± 3.95	0.149 ± 0.01	10.50 ± 0.30	1.84 ± 0.72	131.5 ± 3.07	0.135 ± 0.01	27.60 ± 0.06	4.15 ± 0.56
ethyl acetate	131.72 ± 4.20	0.155 ± 0.01	22.34 ± 0.33	2.42 ± 0.90	140.2 ± 5.25	0.179 ± 0.02	37.20 ± 0.09	5.87 ± 0.71
methanol	694 ± 30.70	0.709 ± 0.03	76.58 ± 0.98	31.24 ± 1.45	368 ± 17.5	0.560 ± 0.04	85.34 ± 0.33	28.45 ± 2.04
aqueous	331.67 ± 3.02	0.405 ± 0.05	45.50 ± 0.50	17.92 ± 1.90	234 ± 15.3	0.335 ± 0.04	61.09 ± 0.29	7.26 ± 1.54

Results are mean ± SD of three replicate analysis.

DPPH-RSC of synthetic antioxidant BHT (1 mg/mL) was determined as  $86.54 \pm 2.4\%$ .

Table 2. Antibacterial activity of *Ranunculus marginatus* var. *trachycarpus* and *R. sprunerianus* by the disk diffusion method.

Microorganism		<i>R. marginatus</i> var. <i>trachycarpus</i>				<i>R. sprunerianus</i>				Standard	
		hexane extract	EtOAc extract	MeOH extract	aqueous extract	hexane extract	EtOAc extract	MeOH extract	aqueous extract	CF20	SAM20
<i>Streptococcus faecalis</i>											
ATCC 8043	G(+)	11	12	8	10	9	8	10	9	17	19
<i>Staphylococcus aureus</i>											
ATCC 6538/P	G(+)	8	8	7	7	8	7	9	9	24	23
<i>Staphylococcus epidermidis</i>											
ATCC 12228	G(+)	11	12	8	8	12	10	10	8	12	19
<i>Bacillus subtilis</i>											
ATCC 6633	G(+)	10	8	7	7	9	7	9	9	24	13
<i>Salmonella typhimurium</i>											
CCM 5445	G(-)	10	10	9	7	9	9	8	8	20	15
<i>Pseudomonas aeruginosa</i>											
ATCC 27853	G(-)	8	8	8	7	8	8	7	8	28	-
<i>Enterobacter aerogenes</i>											
ATCC 13048	G(-)	12	12	10	10	11	10	12	10	18	12
<i>Escherichia coli</i>											
ATCC 29998	G(-)	9	10	10	9	9	10	10	9	22	12

CF20: Cefazidime (20 mg); SAM20: Sulbactam (10 mg)/Ampicillin (10 mg).

Values (mean of three replicates) indicate zone of inhibition in mm and include filter paper disk diameter (6 mm); G: gram reaction; "-": no inhibition

Table 3. The MIC values ( $\mu\text{g/mL}$ ) of *Ranunculus marginatus* var. *trachycarpus* and *R. sprunerianus* against bacteria tested in the micro-well dilution assay.

Concentration range (1024-0.5 $\mu\text{g/mL}$ )											
Microorganism		<i>R. marginatus</i> var. <i>trachycarpus</i>				<i>R. sprunerianus</i>				Standard	
		hexane extract	EtOAc extract	MeOH extract	aqueous extract	hexane extract	EtOAc extract	MeOH extract	aqueous extract	GN	AMP
<i>Streptococcus faecalis</i>											
ATCC 8043	G(+)	128	128	256	128	128	256	128	128	16	2.0
<i>Staphylococcus aureus</i>											
ATCC 6538/P	G(+)	256	256	256	256	256	256	128	128	1.0	0.5
<i>Staphylococcus epidermidis</i>											
ATCC 12228	G(+)	128	128	256	256	128	128	128	256	1.0	0.5
<i>Bacillus subtilis</i>											
ATCC 6633	G(+)	128	256	256	256	128	256	128	128	4.0	0.5
<i>Salmonella typhimurium</i>											
CCM 5445	G(-)	128	128	128	256	128	128	256	256	1.0	1.0
<i>Pseudomonas aeruginosa</i>											
ATCC 27853	G(-)	256	256	256	256	256	256	256	256	2.0	16.0
<i>Enterobacter aerogenes</i>											
ATCC 13048	G(-)	128	128	128	128	128	128	128	128	2.0	4.0
<i>Escherichia coli</i>											
ATCC 29998	G(-)	128	128	128	128	128	128	128	128	1.0	8.0

G: gram reaction; GN: Gentamicin; AMP: Ampicillin.



Several studies have been carried out to determine the antimicrobial activity of extracts, essential oils, and compounds isolated from various *Ranunculus* species. Previously, Barbour et al. (24) investigated the antimicrobial activity of methanol and aqueous extracts of the flowers of *R. cuneatus* Boiss. and the whole plants of *R. myosuroides* Boiss. & Kotschy, together with several other plant species collected from Lebanon, by the disk diffusion and minimal inhibitory concentration methods. They found that aqueous extracts of these *Ranunculus* plant parts did not result in the inhibition of the test organisms, namely, *Escherichia coli*, *Proteus* sp., *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella enteridis*, *Salmonella typhi*, *Staphylococcus faecalis*, and *Candida albicans*. They also reported that the methanol extracts of whole plants of *R. myosuroides* and flowers of *R. cuneatus* showed an efficacious inhibitory effect against 88.8% and 77.7% of the tested microorganisms, respectively. The MIC value for the methanol extract of *R. myosuroides* was also determined and found consistent at 1/2.5 (herbal weight/methanol volume ratio) for all test microorganisms, with the exception of *Streptococcus faecalis*. They detected no efficacy against this bacterium at any serial dilution of the extract.

Quave et al. (44) tested the effect of the ethanol extract of the leaves, stems, and flowers of *R. acris* L. on the planktonic growth and biofilm formation of methicillin-resistant *Staphylococcus aureus* (ATCC 33593), a common cause of skin and soft tissue infection, together with some other plant species. There was no growth detected within the MIC range of 8-512 µg/mL, and no IC<sub>50</sub> was identified for biofilm formation within the concentration range of 4-128 µg/mL.

In a recent study, antimicrobial and essential oil compositions of *Ranunculus constantinopolitanus* (DC.) d'Urv. and *R. arvensis* L. were reported. The essential oils of these plants were tested against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*, and *Candida albicans* using the micro-well dilution method. The essential oil of *R. constantinopolitanus* showed activity against *Enterococcus faecalis*, *Staphylococcus aureus*, and *Candida albicans*, whereas the essential oil of *R.*

*arvensis* displayed activity against *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Candida albicans*. It was indicated that the tested extracts showed stronger antimicrobial activity against the gram-positive bacteria when compared to the gram-negative bacteria (25).

In another study, by Noor et al. (19), six compounds, including R(+)-dalbergiphenol, R(+)-4-methoxydalbergione, methyl-3,4,5-trihydroxybenzoate, 4-hydroxy-2-benzoic acid, rho-hydroxy cinnamic acid, and beta-sitosterol, were isolated and their antibacterial activity was evaluated.

It has been documented in the literature that major groups of antimicrobial metabolites from plants include phenolics and polyphenols (simple phenols and phenolic acids, quinones, flavones, flavonoids, flavonols, tannins, and coumarins), terpenoids, essential oils, alkaloids, lectins, and polypeptides (45). Some of this group of metabolites, such as flavonoids (14-16), phenolic acids (19), alkaloids (11), and essential oils (25), have been found to be synthesized by several *Ranunculus* species. In conclusion, the data obtained in previous studies indicate that *Ranunculus* species possess antioxidant (42,43) and antimicrobial (19,22,24,25) properties. The findings in our study also demonstrate that *R. marginatus* var. *trachycarpus* and *R. sprunerianus* have significant antioxidant and antibacterial activities. Further investigations are necessary to identify the compounds responsible for the activity of the screened efficacious extracts.

## Acknowledgements

The authors would like to thank Dr. Lütfi Bekat for the identification of plant material.

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## References

- Davis PH. *Ranunculus*. In: Davis PH ed. Flora of Turkey and the East Aegean Islands, Vol 1. Edinburgh University Press, 1965: pp. 146-197.
- Davis PH. *Ranunculus* L. In: Davis PH, Mill RR, Tan K eds., Flora of Turkey and the East Aegean Islands Supplement, Vol 10. Edinburgh University Press, 1988: pp. 18-22, 231.
- Güner A. *Ranunculus* L. In: Güner A, Özhatay N, Ekim T, Baser KHC eds., Flora of Turkey and the East Aegean Islands Supplement 2, Vol 11., Edinburgh University Press, 2000: p. 14.
- Baytop T. Therapy with Medicinal Plants in Turkey (Past and Present), 2nd ed. Nobel Tıp Kitabevleri Ltd. Sti, 1999: pp.165, 375.
- Ezer N, Avcı K. Folk medicines of Çerkeş (Çankırı) in Turkey. Hacettepe University Journal of the Faculty of Pharmacy 24: 67-80, 2004.
- Gurhan G, Ezer N. Plants used for hemorrhoid treatment in folk medicine I. Hacettepe University Journal of the Faculty of Pharmacy 24: 37-55, 2004.
- Sezik E, Yeşilada E, Honda G et al. Traditional medicine in Turkey X. Folk medicine in Central Anatolia. J Ethnopharmacol 75: 95-115, 2001.
- Sadıkoglu N, Alpınar K. Bartin: from an ethnobotanical point of view. In: Gurkan E, Tuzlacı E eds. XIII th Meeting on Plant Originated Crude Drugs Proceeding Book, Marmara University press, 2000 pp. 87-100.
- Marston A, Cabo M, Lubrano C et al. Clarification of the saponin composition of *Ranunculus ficaria* tubers. Nat Prod Commun 1: 27-32, 2006.
- Wegner C, Hamburger M, Kunert O et al. Tensioactive compounds from the aquatic plant *Ranunculus fluitans* L. (Ranunculaceae). Helv Chim Acta 83: 1454-1464, 2000.
- Bonora A, Tosi B, Dall'Olio G et al. Quaternary alkaloids in rhizomes of *Ranunculus serbicus*. Phytochemistry 29: 2389-2390, 1990.
- Zhang L, Yang Z, Tian JK. Two new indolopyridoquinazoline alkaloidal glycosides from *Ranunculus ternatus*. Chem Pharm Bull 55: 1267-1269, 2007.
- Gluchoff-Fiasson K, Fiasson JL, Waton H. Quercetin glycosides from European aquatic *Ranunculus* species of subgenus *Batrachium*. Phytochemistry 45: 1063-1067, 1997.
- Fiasson JL, Gluchoff-Fiasson K, Dahlgren G. Flavonoid patterns in European *Ranunculus* L. subgenus *Batrachium* (Ranunculaceae). Biochem Syst Ecol 25: 327-333, 1997.
- Markham KR, Mitchell KA, Campos M. An unusually lipophilic flavanol glycoside from *Ranunculus sardous* pollen. Phytochemistry 45: 203-204, 1997.
- Prieto JM, Braca A, Morelli I et al. A new acylated quercetin glycoside from *Ranunculus lanuginosus*. Fitoterapia 75: 533-538, 2004.
- Liang Y, Chen Z, Liu L. Studies on chemical constituents of *Ranunculus japonicus*. Zhongguo Zhongyao Zazhi 33: 2201-2203, 2008.
- Chen J, Yao C, Xia LM et al. Determination of fatty acids and organic acids in *Ranunculus ternatus* Thunb using GC-MS. Spectros Spect Anal 26: 1550-1552, 2006.
- Noor W, Gul R, Ali I et al. Isolation and antibacterial activity of the compounds from *Ranunculus repens* L. J Chem Soc Pak 28: 271-274, 2006.
- Tian JK, Sun F, Cheng YY. Chemical constituents from the roots of *Ranunculus ternatus*. J Asian Nat Prod Res 8: 35-39, 2006.
- Chi Y, Yang Y, Yu S. Effect and composition of organic acid of *Radix ranunculus ternati*. Nanjing Zhongyiyao Daxue Xuebao 23: 365-367, 2007.
- Sener B, Bingöl F, Erdogan I et al. Biological activities of some Turkish medicinal plants. Pure & Appl Chem 70: 403-406, 1998.
- Li H, Zhou C, Pan Y et al. Evaluation of antiviral activity of compounds isolated from *Ranunculus sieboldii* and *Ranunculus sceleratus*. Planta Med 71: 1128-1133, 2005.
- Barbour EK, Sharif MA, Sagherian VK et al. Screening of selected indigenous plants of Lebanon for antimicrobial activity. J Ethnopharmacol 93: 1-7, 2004.
- Terzioğlu S, Yaşar A, Yaylı N et al. Antimicrobial activity and essential oil compositions of two *Ranunculus* species from Turkey: *R. constantinopolitanus* and *R. arvensis*. Asian Journal of Chemistry 20: 3277-3283, 2008.
- Prieto JM, Recio MC, Giner RM et al. Pharmacological approach to the pro- and anti-inflammatory effects of *Ranunculus sceleratus* L. J Ethnopharmacol 89: 131-137, 2003.
- Orhan I, Sener B, Atıcı T et al. Turkish freshwater and marine macrophyte extracts show *in vitro* antiprotozoal activity and inhibit Fab I, a key enzyme of *Plasmodium falciparum* fatty acid biosynthesis. Phytomedicine 13: 388-393, 2006.
- Khan WN, Ali I, Gul R et al. Xanthine oxidase inhibiting compounds from *Ranunculus repens*. Chem Nat Comp 44: 95-97, 2008.
- Unver N, Kaya GI, Ozturk HT. Antimicrobial activity of *Sternbergia sicula* and *Sternbergia lutea*. Fitoterapia 76: 226-229, 2005.
- Deutsches Arzneibuch (DAB 10) Amtliche Ausgabe. Deutscher Apotheker Verlag, Stuttgart; 1996.
- Miliauskas G, Venskutonis PR, van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem 85: 231-237, 2004.
- McDonald S, Prenzler PD, Antolovich M et al. Phenolic content and antioxidant activity of olive extracts. Food Chem 73: 73-84, 2001.

33. Amarowicz R, Pegg RB, Rahimi-Moghaddam P et al. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem* 84: 551-562, 2004.
34. Re R, Pellegrini N, Proteggente A et al. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic Biol Med* 26: 1231-1237, 1999.
35. Bradshaw LJ. *Laboratory Microbiology*, 4th ed. New York USA, Emeritus California State University, Saunders College Publishing, Fullerton, 1992.
36. Collins CM, Lyne PM. *Microbiological Methods*. Butterworths and Co. Ltd., London, 1987.
37. Atlas RM, Parks LC, Brown AE. *Laboratory Manual of Experimental Microbiology*, Mosby-Year Book Inc., St. Louis, Missouri, 1995.
38. National Committee for Clinical Laboratory Standards Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically. Approved Standard- 8th ed. NCCLS Wayne NCCLS document, Pennsylvania USA, M7-A6, 2003.
39. Bouaziz M, Grayer RJ, Simmonds MSJ et al. Identification and antioxidant potential of flavonoids and low molecular weight phenols in olive cultivar chemlali growing in Tunisia. *J Agric Food Chem* 53: 236-241, 2005.
40. Moure A, Cruz JM, Franco D et al. Natural antioxidants from residual sources. *Food Chem* 72: 145-171, 2001.
41. Rice-Evans CA, Miller NJ, Paganga G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic Biol Med* 20: 933-956, 1996.
42. Mantle D, Eddeb F, Pickering AT. Comparison of relative antioxidant activities of British medicinal plant species in vitro. *J Ethnopharmacol* 72: 47-51, 2000.
43. Campos MG, Webby RF, Markham KR et al. Age-induced diminution of free radical scavenging capacity in bee pollens and the contribution of constituent flavonoids. *J Agric Food Chem* 51: 742-745, 2003.
44. Quave CL, Plano LRW, Pantuso T et al. Effects of extracts from Italian medicinal plants on planktonic growth, biofilm formation and adherence of methicillin-resistant *Staphylococcus aureus*. *J Ethnopharm* 118: 418-428, 2008.
45. Cowan MM. Plant products as antimicrobial agents. *Clinical Microbiol Rev* 12: 564-582, 1999.