Romanian Biotechnological Letters *Vol. 17, No.6, 2012*

ORIGINAL PAPER

Evaluation of the *In vitro* Bioactivities of Mahaleb Cherry (*Prunus mahaleb* L.)

Received for publication, September 15, 2012
Accepted, October 22, 2012

BERRIN ÖZÇELİK*[1], UFUK KOCA[2], DURMUŞ ALPASLAN KAYA[3], NAZIM ŞEKEROĞLU[4],
[1]*Gazi University, Pharmacy Faculty, Pharmaceutical Microbiology Department, 06330, Ankara- Turkey*
[2]*Gazi University, Pharmacy Faculty, Pharmacognosy Department, 06100, Ankara- Turkey*
[3]*Mustafa Kemal University, Faculty of Agriculture, Department of Field Crops, 31030, Antakya-Turkey*
[4]*Kilis 7 Aralik University, Art and Science Faculty, Biology Department, 79000, Kilis-Turkey*
**Corresponding Author. Mailing address: Gazi University, Pharmacy Faculty, Pharmaceutical Microbiology Department, 06330, Ankara-Turkey;*
E-mail: berrin@gazi.edu.tr, microberr@yahoo.com

Abstract

The mahaleb cherry (Prunus mahaleb L.), a wild member of the Rosaceae family, is an important rootstock for cherry and sour cherry cultivars. Besides horticultural importance, all parts of the plant, specifically the seeds and fruits have been used to give flavor and taste to a variety of its cousins in Mediterranean countries for centuries. The seeds of mahaleb are an important industrial crop in Turkey; moreover, folkloric usage of the other parts of the plant has also been recorded. In the present study, methanol and n-hexane extracts from different parts of the mahaleb cherry including the flower, leaves, branches, fruits, fruit stalk, seeds and seed coat were screened for in-vitro antibacterial, antifungal and radical scavenging activity using the DPPH method. Standard strains of E. coli, P. aeruginosa, P. mirabilis, K. pneumoniae, A. baumannii, S. aureus, E. faecalis and B. subtilis with their pathogens from clinical isolates as well as fungi (C. albicans, C. parapsilosis, C. tropicalis and C. krusei) were used to determine the antimicrobial activity. All extracts showed antibacterial activity against gram-positive standard bacteria with concentrations between 16-64 µg mL^{-1} and against gram-negative bacteria (8-64 µg mL^{-1}). Additionally, the extracts demonstrated antifungal activity at concentrations between 16-64 µg mL^{-1}. The minimal inhibition concentration (MIC) value of the extracts against all gram-positive and gram-negative isolated strains was 250 µg mL^{-1} excluding B. subtilis (MIC: 64 µg mL^{-1}). As for antifungal activity, all methanol and n-hexane extracts were found to inhibit C. krusei, with a MIC value of 64 µg mL^{-1}, which is better than the control fluconazole (64 µg mL^{-1}). Methanol extracts of mahaleb had better antioxidant activity than all n-hexane extracts. The methanol extract of fruit samples (Mfr) displayed better antioxidant activity (86.25±0.12) than the reference BHA solution (83.633±0.22) at all concentrations except for 100 µg mL^{-1}. Antioxidant levels of methanol extracts from mahaleb seed kernel (Msk), leaves (Ml), branches (Mb) and fruit stalks followed the Mfr samples. The strong antimicrobial, antifungal and antioxidant activities of the mahaleb plant indicate that in the future, it may be important for clinical nutrition and the food and pharmaceutical industries.

Key words: Antibacterial, Antifungal, Antioxidant, Mahaleb cherry, *Prunus mahaleb* L., Rosaceae

Introduction

The genus *Prunus* belongs to the subfamily Prunoidae in the *Rosaceae* family and has vital plant species that produce a great deal of industrial raw materials for the horticulture, ornamental, food, and pharmaceutical industries. Crops of the genus, other than potted-fresh cut ornamentals, apricots, plums, almonds, peaches, and cherry laurels have mainly been used as fresh fruits, processed sweets, jams, juices, candies and natural sweeteners for pharmaceutical products. Of these plant species, *Prunus mahaleb* L. (syn. *Cerasus mahaleb*

L. Mill. – Rosaceae) is a large perennial shrub or deciduous small tree that is found throughout Mediterranean countries, as well as central Europe, northern Africa, and western and central Asia. The plant, which is known as the St. Lucie cherry or the Rock cherry in Europe; the mahlab, mahleb, mahaleb cherry in Northern Africa and the Middle east; and Mahlep in Turkey, has pure white blossoms that are small (8-20 mm diameter) and arranged in groups of 3-10 on a 3-4 cm long raceme in early spring. The fruit is a small thin-fleshed cherry-like drupe that is 8–10 mm in diameter; it is green at first and then turns red then dark purple to black when ripe in mid to late summer (C. BRICKELL [1]).

Wild in nature or cultivated as an ornamental in home gardens, the mahaleb cherry and its products are a valuable export item of Turkey (E. SEZIK & AL [2]). According to recent statistical data from the Eagean Exporters Union of Turkey, 951,000 kg of mahaleb products was exported to other countries from Turkey. The total income from this exportation was $13.274. The countries that import the most mahaleb cherry products are Germany, Australia, the USA, Egypt, Israel and Greece (EGEIB [3]).

The mahaleb tree and its products have many uses. The mahaleb tree is robust and resistant to disease in nature; therefore, it has been used as the rootstock for the horticultural production of cultivar cherries in most Mediterranean countries (R. GERCEKCIOGLU & al. [4]; M. A. MORENO & al. [5]). Additionally, various parts of the plant, particularly the bitter almond tasting ground seed kernels and every so often, the fruits, have been used as a pleasing spice in special ‘patisseries’ and bakeries in Mediterranean countries. Although the tree is not cultivated, imported fruits and seed kernels are ground and mixed with white flour for their special fragrance, then used in home baking and the candy industry in Egypt (S. R. MARCOS & al. [6]). The plant parts have been used as a tonic to heal various ailments in traditional medicine in Turkey (T. BAYTOP [7]). For instance, the slightly bitter fruits and seeds of the mahaleb tree have been used as a tonic for the heart and a traditional medicine for diabetes and gastrointestinal problems. The resins obtained from the outer surface of the wood have been used for gastritis for centuries. Decoctions prepared from stems, fruit stalks, leaves and flowers have locally been used as an herbal tea to treat colds and asthma in the winter. The oil of the kernels had been exploited in liqueur, varnishes and special wines because of its aromatic taste (E. SEZIK & AL [2]). The seed kernels have also been used to treat diarrhea in children in the Sudan and as sedatives and vasodilators in Arabic countries (M. S. AL-SAID & al. [8], A.A. MARIOD & al. [9]).

Due to the special fragrance, previous studies mostly focused on the seeds. The seed kernels have a high protein content and fixed oil (27-40%), which is valuable for industrial usage. Besides proteins, sucrose and fatty acids, previous studies found that the seeds contain coumarins, tannins, and traces of hydrocyanic acid, which was confirmed after hydrolysis (E. SEZIK & AL [2]). Moreover, although coumarin derivatives (coumarin, dihydrocoumarin, herniarin) have been primarily found in the seed kernels (M. S. AL-SAID & al. [8]), others have been isolated from the dried bark (J. MASTELIC & al. [10]). The appealing properties and the healing ability of the mahaleb cherry may be a result of the coumarin derivatives. The importance of industrial usage, the scientific literature and the traditional knowledge on medicinal uses of the mahaleb cherry lead us to investigate its biological activities. Thus, in the present study, the antimicrobial, antifungal and antioxidant potentials of different plant parts were investigated, which have not reported for the different components of the mahaleb. In order to evaluate the usage of the different parts of the plant, the flowers, leaves, fruits, fruit stalk, branches and seed kernels were extracted with lipophilic *n*-hexane and hydrophilic methanol, sequentially. These extracts were analyzed for their antimicrobial and antioxidant activities together with chromatographical analysis in comparison with each other.

Materials and methods

Plant Materials. In the present study, various parts of the mahaleb tree, including the flowers, leaves, and whole fruits were handpicked from wild plants in the districts of Mardin, located in the southeast part of Turkey. The prepared herbarium sample was confirmed by biologist Kamil Aydin, and a voucher sample was deposited in the herbarium of Kilis 7 Aralık University, Department of Biology. All plant parts except fruits were collected in early spring 2009. The fruit ripens in late summer and was therefore collected in the late summer of 2009. Following collection, all plant parts were air dried in the shade at room temperature. Dried samples were separated individually and classified as follows: mahaleb flowers (MF), mahaleb leaves (ML), seed kernels (MSK), and mahaleb seed coats (MFR, the fleshy fruit part), mahaleb fruit stalks (MFS), mahaleb branches (MB), and mahaleb resins (MR).

Preparation of the extracts. All the solvents used were HPLC grade. Methanol and *n*-hexane were obtained from Merck (Merck, Darmstadt, Germany). Dried aerial parts were ground to powder using a mortar and pestle. The ground materials (up to 20 g) were extracted with 3x 300 ml of *n*-hexane at room temperature. Following filtration, the combined extracts were evaporated to dryness *in vacuo* to yield 2.16% crude *n*-hexane extracts for mahaleb flowers (MF-H), 2.19% for mahaleb leaves (ML-H), 27.20% for mahaleb seed kernels (MSK-H), for 12.1 % for mahaleb fleshy seed coats (MFR-H), 3.52% for mahaleb fruit stalks (MFS-H), 1.02% for mahaleb branches (MB-H), and 1.04% for mahaleb resins (MR-H). The remaining ground materials were air dried again to evaporate the remaining *n*-hexane, then re-extracted with 3x 300 ml of methanol, which produced 12.04% methanolic extracts for mahaleb flowers (MF-M), 12.07% for mahaleb leaves (ML-M), 9.42% for mahaleb seed kernels (MSK-M), 40.14% for mahaleb fleshy seed coats (MFR-M), 19.80% for mahaleb fruit stalks (MFS-M), and 9.80% for mahaleb branches (MB-M).

Microbiological studies

Test materials. The extracts were dissolved in dimethylsulphoxide (80%) and EtOH (20%) to a final concentration of 256 μg mL^{-1} and used as the stock solutions. Ampicillin, gentamicin, levofloxacin, ketoconazole and fluconazole were used as the standard antibacterial and antifungal drugs. Reference antibacterial agents of ampicillin (AMP), gentamicin (GM) and levofloxacin (LFX) and the reference antifungal agents ketoconazole (KET) and fluconazole (FLU) were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and were dissolved in phosphate buffer solution (ampicillin**,** pH: 8.0; 0.1 mol mL^{-1}), dimethylsulphoxide (ketoconazole) or in water (gentamicin, levofloxacin and fluconazole). The stock solutions were prepared in medium according to the Clinical and Laboratory Standards Institute (CLSI [11]).

Preparation of microorganisms and inoculums. Antibacterial activity tests were carried out against the standard (ATCC; American type culture collection, RSKK; Culture collection of Refik Saydam Central Hygiene Institute) and isolated strains (clinical isolates were obtained from the Department of Microbiology, Faculty of Medicine, Gazi University). The following gram-negative strains were used as standards to determine antibacterial activity: *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 10145, *Proteus mirabilis* ATCC 7002, *Klebsiella pneumoniae* RSKK 574, and *Acinetobacer baumanii* RSKK 02026. The following strains were used as gram-positive controls to determine antibacterial activity: *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6633. Isolated strains of *E. coli*, *P. mirabilis* and *K. pneumoniae* have an extended spectrum β-lactamase (ESβL) enzyme, the *P. aeruginosa* isolate is resistant to gentamicin, and the *A. baumannii* isolate is resistant to cephalosporin. The gram-positive strains of *S. aureus* are resistant to methicillin (MRSA), the *E. faecalis* isolation is resist to

cephalosporin, and the *B. subtilis* isolate is resist to ceftriaxon. *C. albicans* ATCC 10231, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 13803, and *C. krusei* ATCC 6258 were used for the determination of antifungal activity. Mueller Hinton Broth (MHB; Difco) and Mueller Hinton Agar (MHA; Oxoid) were used to grow and dilute the bacterial suspensions (B. OZCELIK & al. [12]). The synthetic medium RPMI-1640 with L-glutamine was buffered to pH 7 with 3-[N-morpholino]-propansulfonic acid, and culture suspensions were prepared as described previously (B. OZCELIK & al. [13]). The microorganism suspensions used for inoculation were prepared at 10^5 cfu (colony forming unite mL^{-1}) by diluting fresh cultures in McFarland to a density of 0.5 (10^8 cfu/ml). Suspensions of bacteria and fungi were added to each well of the diluted extracts at a density of 10^5 cfu mL^{-1} for fungi and bacteria. The bacterial suspensions used for inoculation were prepared at 10^5 cfu mL^{-1} by diluting fresh cultures in McFarland to a density of 0.5 (10^8 cfu mL^{-1}). The fungi suspension was prepared using the spectrophotometric method of inoculation (CLSI [11], I. ORHAN & al. [14]).

Antibacterial and antifungal tests. The micro dilution method was used to test for antibacterial and antifungal activity. Media was placed into each well of 96-well micro plates. The compounds (256 μg mL^{-1}) were added to the first rows of micro plates, and two fold dilutions of the compounds (128-0.125 μg mL^{-1}) were made by dispensing the solutions to the remaining wells. Ten microliters of culture suspension was inoculated into each well. DMSO (80%) and EtOH (20%); pure microorganisms; and pure media were used as controls. All organisms were tested in duplicate in each experiment. The sealed micro plates were incubated at 35ºC for 24 h and 48 h in a humid chamber. The lowest concentration of the extracts that completely inhibited macroscopic growth was determined, and the minimum inhibitory concentrations (MICs) were reported as described in a previous study (U. KOCA & al. [15], OZCELIK & al. [13]).

Radical Scavenging Assay. The antioxidant activity of the extracts was determined by the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH; Sigma-Aldrich D9132-1G Steinheim, Germany) radical. The assay is based on the electron-donation ability of the extracts that is measured by bleaching the purple-colored ethanol solution of the DPPH. The *n*-hexane extracts were dissolved in methanol, and the others were dissolved in ethanol (75%). Four dilutions (100, 500, 1000 and 2000 μg L^{-1}) were made containing 77 μl of the corresponding extract and 3 ml of the DPPH solution dissolved in either methanol or ethanol according to the type of the extract while keeping the concentration at 6X 10-5 mol/L. A synthetic antioxidant butylated hydroxyanisole (BHA) (Sigma B-1378 Steinheim, Germany) was used as a reference. After incubation in the dark for 15 min, the absorbance was measured at 515 nm using a spectrophotometer (PG Instruments GB). The radical scavenging effect was calculated as follows: antiradical effect (%) = $(A_{blank}-A_{extract})/A_{blank}X100$. The blank is the absorbance of the control, which contains all reagents except the extract; $A_{extract}$ is the absorbance of the test extract or the reference.

Results

The results of antibacterial (Table 1, Table 2) and antifungal activities (Table 3) of the tested extracts obtained from the different parts of *P. mahaleb* L. are shown in Table 1, Table 2, and Table 3. The radical scavenging activity of the extracts was assayed using the DPPH radical scavenging activities of the different extracts at concentrations of 100, 500, 1000 and 2000 μg L^{-1}; they are presented in Table 4. Moreover, preliminary phytochemical analysis of the extracts was also analyzed using the TLC method.

The results obtained from the antibacterial and antifungal tests are shown in Table 1, Table 2, and Table 3, including the results from the different components (flowers, leaves,

seed kernels, fleshy seed coats, fruit stalks, branches, and resins) of *P. mahaleb* L. in *n*-hexane (*Hx*.) or methanolic (MeOH) extracts.

All extracts exhibited antibacterial activity against the standard strains of *A. baumannii* (8-16 μg mL^{-1}), *P. mirabilis* (16 μg mL^{-1}), *P. aeruginosa, and E. coli* (32-64 μg mL^{-1}). The same degree of inhibition has been observed against isolated gram-negative strains at concentrations of 128 μg mL^{-1}.

Moreover, all of the extracts exerted an inhibitory effect on standard stains of *S. aureus* at concentration of 32 μg mL^{-1}, except methanol extracts from the flowers (Mf-MeOH), *n*-hexane extracts of the fruit (Mfr-*Hx*.) and branches (Mb-*Hx*.) (64 μg mL^{-1}). Antibacterial activity was observed at these same concentrations against *E. faecalis* except with the methanol extracts of mahaleb flowers (MIC; 64 μg mL^{-1}), where the activity was found at 16 μg mL^{-1} against *B. subtilis* and from Mf-MeOH, Mfr-*Hx*., and Mb-*Hx* (MIC; 64 μg mL^{-1}).

As for antifungal activity, both methanol and *n*-hexane extracts were found to inhibition *C. krusei* with a MIC value of 64 μg $mL^{-}1$ as compared with the control fluconazole (64 μg mL^{-1}).

The antioxidant activities of the extracts were determined using the DPPH radical scavenging capacity assay. Hydrophilic extracts displayed better antioxidant activity than the lipophilic extracts (Table 4). The Mfr sample had the greatest antioxidant activity (86.25%) compared to a reference BHA solution (83.63%) at 2000 μg mL^{-1}, 1000 μg mL^{-1}, and 500 μg mL^{-1} , which was followed by the seed kernels (Msk), leaves (Ml), branches (Mb) and fruit stalks (Mfs) respectively. The lipophilic extracts did not display significant antioxidant activity with the selected method. Although the results were not as exciting with the *n*-hexane extracts, Mb (14.10±0.15) displayed the highest antioxidant activity followed by Mfs-*Hx* (8.95±0.25), Mf- *Hx* (6.81±0.40), Msk- *Hx* (6.75±0.12) and Ml- *Hx* (4.78±0.01). The *n*-hexane extract of the Mfr did not show any activity, which may be due to the high hydrophilic content of the fruits.

Table 1. Antibacterial activity of the extracts from *P. mahaleb* against gram-negative bacteria represented by MIC (μg mL^{-1}) values.

Extracts	*Gram-negative bacteria*									
	E. coli		*P. aeruginosa*		*P. mirabilis*		*K. pneumonae*		*A. baumannii*	
	ATCC 35218	Isolated strain	ATCC 10145	Isolated strain	ATCC 7002	Isolated Strain	RSKK 574	Isolated strain	RSKK 02026	Isolated strain
Mf-MeOH[a]	32	128	32	64	16	128	64	128	8	128
Ml-MeOH[b]	32	128	32	64	16	128	32	128	8	128
Msk-MeOH[c]	32	128	32	64	16	128	32	128	8	128
Mfr-MeOH[d]	32	128	32	64	16	128	32	128	8	128
Mfs-MeOH[e]	32	128	32	64	16	128	32	128	8	128
Mb-MeOH[f]	32	128	32	64	16	128	32	128	8	128
Mr-MeOH[g]	32	128	32	64	16	128	32	128	8	128
Mf-*Hx*[h]	32	128	32	64	16	128	32	128	8	128
Ml-*Hx*[i]	32	128	32	64	16	128	32	128	8	128
Msk-*Hx*[k]	32	128	32	64	16	128	64	128	8	128
Mfr-*Hx*[m]	64	128	32	64	16	128	32	128	8	128
Mb-*Hx*[n]	64	128	32	64	16	128	32	128	16	128
AMP[p]	2	>128	-	-	2	>128	2	>128	2	>128
LFX[r]	0.12	0.5	1	64	<0.12	1	0.12	1	0.12	64
GM[s]	-	-	0.5	2	-	-	-	-	-	-

a: Methanol extracts of mahaleb flowers; **b:** Methanol extracts of mahaleb leaves; **c:** Methanol extracts of mahaleb seed kernels; **d:** Methanol extracts of mahaleb fleshy seed coats; **e:** Methanol extracts of mahaleb fruit stalks; **f:** Methanol extracts of mahaleb branches; **g:** Methanol extracts of mahaleb resins; **h:** *n*-hexane extracts of mahaleb flowers; **i:** *n*-hexane extracts of mahaleb leaves; **k:** *n*-hexane extracts of mahaleb seed kernels; **m:** *n*-hexane extracts of mahaleb fleshy seed coats; **n:** *n*-hexane extracts of mahaleb branches; **p:** Ampicilline, r: Gentamicine, s: Levofloxacin;

Table 2. Antibacterial activity of the extracts from *P. mahaleb* L. against gram-positive bacteria represented by MIC (µg mL^{-1}) values.

Extracts	*Gram-positive bacteria*					
	S. aureus		*E. faecalis*		*B. subtilis*	
	ATCC 25923	Isolated strain	ATCC 29212	Isolated Strain	ATCC 6633	Isolated strain
Mf-MeOH[a]	64	128	64	128	32	64
Ml-MeOH[b]	32	128	32	128	16	64
Msk-MeOH[c]	32	128	32	128	16	64
Mfr-MeOH[d]	32	128	32	128	16	64
Mfs-MeOH[e]	32	128	32	128	16	64
Mb-MeOH[f]	32	128	32	128	16	64
Mr-MeOH[g]	32	128	32	128	16	64
Mf-*Hx*[h]	32	128	32	128	16	64
Ml-*Hx*[i]	32	128	32	128	16	64
Msk-*Hx*$_k$	32	128	32	128	16	64
Mfr-*Hx*[m]	64	128	32	128	32	64
Mb-*Hx*[n]	64	128	32	128	32	64
AMP[p]	<0.12	>128	0.5	>128	0.12	0.5
LFX[s]	0.25	128	0.5	32	-	-

a: Methanol extracts of mahaleb flowers; **b:** Methanol extracts of mahaleb leaves; **c:** Methanol extracts of mahaleb seed kernels; **d:** Methanol extracts of mahaleb fleshy seed coats; **e:** Methanol extracts of mahaleb fruit stalks; **f:** Methanol extracts of mahaleb branches; **g:** Methanol extracts of mahaleb resins; **h:** *n*-hexane extracts of mahaleb flowers **i:** *n*-hexane extracts of mahaleb leaves; **k:** *n*-hexane extracts of mahaleb seed kernels; **m:** *n*-hexane extracts of mahaleb fleshy seed coats; **n:** *n*-hexane extracts of mahaleb branches; **p:** Ampicilline, **s:** Levofloxacin

Table 3. Anti-fungal activity of the extracts of *P. mahaleb* L. represented as MIC (µg mL^{-1}) values.

Extracts	*Fungi*			
	C. albicans ATCC 10231	*C. parapsilosis* ATCC 90028	*C. tropicalis* ATCC 13803	*C. krusei* ATCC 6258
Mf-MeOH[a]	32	32	64	64
Ml-MeOH[b]	16	32	32	64
Msk-MeOH[c]	32	32	32	64
Mfr-MeOH[d]	32	32	32	64
Mfs-MeOH[e]	32	32	32	64
Mb-MeOH[f]	32	32	32	64
Mr-MeOH[g]	32	32	32	64
Mf-*Hx*[h]	32	32	32	64
Ml-*Hx*[i]	32	32	32	64
Msk-*Hx*[k]	32	32	32	64
Mfr-*Hx*[m]	32	32	32	64
Mb-*Hx*[n]	32	32	32	64
KET[t]	0.5	1	2	4
FLU[v]	2	4	4	64

a: Methanol extracts of mahaleb flowers; **b**: Methanol extracts of mahaleb leaves; **c:** Methanol extracts of mahaleb seed kernels; **d:** Methanol extracts of mahaleb fleshy seed coats; **e:** Methanol extracts of mahaleb fruit stalks; **f:** Methanol extracts of mahaleb branches; **g:** Methanol extracts of mahaleb resins; **h:** n-hexane extracts of mahaleb flowers; **i:** n-hexane extracts of mahaleb leaves; **k:** n-hexane extracts of mahaleb seed kernels; **m:** n-hexane extracts of mahaleb fleshy seed coats; **n:** n-hexane extracts of mahaleb branches; **t:** Ketoconazole; **v:** Fluconazole.

Table 4. Antioxidant activity of the extracts from *P. mahaleb* L. at various concentrations.

Extracts	**Antioxidant Activity % at Different Concentrations**			
	2000 µg mL^{-1}	**1000 µg mL^{-1}**	**500 µg mL^{-1}**	**100 µg mL^{-1}**
Mf-MeOH[a]	68.68±0.32	62.12±0.57	43.53±0.53	46.22±0.95
Ml-MeOH[b]	78.71±0.54	76.02±0.52	61.034±0.61	30.28±0.55
Msk-MeOH[c]	76.34±0.18	78.71±0.19	62.07±0.61	42.13±0.61
Mfr-MeOH[d]	86.25±0.12	81.43±0.34	78.46±0.40	29.86±0.18
Mfs-MeOH[e]	73.07±0.26	67.99±0.28	58.41±0.34	24.72±0.81

Mb-MeOH[f]	75.24±0.18	78.29±0.19	65.07±0.61	52.53±0.61
Mf-*Hx*[h]	6.92±0.40	6.62±0.10	6.20±0.15	6.15±0.08
Ml-*Hx*[i]	4.78±0.01	4.20±0.15	3.70±0.15	2.85±0.44
Msk-*Hx*[k]	6.75±0.12	6.56±0.05	6.20±0.14	5.27±0.20
Mfr-*Hx*[m]	NA	NA	NA	NA
Mfs- *Hx*[n]	8.95±0.25	5.9±0.05	4.069±0.18	2.78±0.26
Mb-*Hx*[o]	14.10±0.15	11.19±0.27	8.67±0.22	6.64±0.26
BHA[z]	83.63±0.22	78.85±0.85	67.32±0.27	40.48±0.22

a: Methanol extracts of mahaleb flowers; **b:** Methanol extracts of mahaleb leaves; **c:** Methanol extracts of mahaleb seed kernels; **d:** Methanol extracts of mahaleb fleshy seed coats; **e:** Methanol extracts of mahaleb fruit stalks; **f:** Methanol extracts of mahaleb branches; **h:** *n*-hexane extracts of mahaleb flowers; **i:** *n*-hexane extracts of mahaleb leaves; **k:** *n*-hexane extracts of mahaleb seed kernels; **m:** *n*-hexane extracts of mahaleb fleshy seed coats; **n:** *n*-hexane extracts of mahaleb branches; **o:** *n*-hexane extracts of mahaleb fruit stalks; **z:** Butylated hydroxyanisole; **NA:** No data available

Discussion

The present study covered the *in vitro* antibacterial, antifungal, and antioxidant activities of extracts obtained from the different parts of *P. mahaleb* L. (Table 1, Table 2, Table 3, and Table 4). Moreover, preliminary phytochemical analysis was performed on the extracts using the thin layer chromatography method, which has not been previously performed for any plant part but the seeds. This study shows that the different plant materials have valuable antimicrobial activity against the isolated and standard strains that were used, as well as antifungal activity against common micro fungi. According to our results, the antioxidant activity of the methanol extracts was significantly superior to the *n*-hexane extracts. Preliminary phytochemical screening showed that phenolic and terpenic compounds were the best predictors of high antibacterial and microbial activity.

Resistance has emerged with the usage of antibiotics. Although the extent and the speed with which bacteria develop resistance to antimicrobial drugs vary, resistance has developed to all antimicrobial drugs. Moreover, there is an increase in the number of reported clinical problems caused by bacterial resistance to multiple antimicrobial drugs. The use of medicinal plants, possibly in the diet, is a feasible alternative to antimicrobial drugs because most are safe, have few side effects, cost less and are effective against a wide range of antibiotic-resistant microorganism. Additionally, medicinal plants have commonly been utilized as a source of therapeutic agents worldwide. Recently, herbal medicines have increasingly been used to treat diseases, including several infections, and are known to produce chemicals that are naturally toxic to bacteria (R. D. LIN & al. [16]).

In this study we evaluate the antimicrobial properties of *P. mahaleb* L. using the micro dilution technique, against five standard gram-negative bacteria: *E. coli* (ATCC 35218), *P. aeruginosa* (ATCC 10145), *P. mirabilis* (ATCC 7002), *K. pneumoniae* (RSKK 574), *A. baumannii* (RSKK 02026) and their human pathogens. *Escherichia coli* and *Acinetobacter baumannii* are opportunistic nosocomial pathogens that are associated with significant morbidity and mortality. *Pseudomonas aeruginosa* is a highly prevalent opportunistic pathogen that has low antibiotic susceptibility due to the multidrug efflux pumps with chromosomally-encoded antibiotic resistance genes and the low permeability of the bacterial cellular envelopes. Additionally, plasmid-mediated extended spectrum β-lactamases (ESβLs) are common in the family Enterobacteriaceae. *Klebsiella pneumoniae* is the most common species in which ESβL enzymes have been reported worldwide (T. KOHLER & al. [17]). The minimum inhibitory concentrations (MICs) were determined for extracts as well as for the reference compounds (ampicillin, levofloxacin, gentamicin) under identical conditions (Table 1). The same degree of inhibition was observed against *E. coli*, *P. aeruginosa*, and *K.*

pneumoniae at concentrations of 32-64 μg mL^{-1}. Additionally, all extracts showed the most potent effect against *A. baumannii* (MICs; 8-16 μg mL^{-1}) followed by *P. mirabilis* (MICs; 16 μg mL^{-1}). All extracts screened exerted more inhibition of the quality control strains (MICs; 8-64 μg mL^{-1}) than the isolates (MICs ≥128 μg mL^{-1}) (Table 1, Table 2). A previous study that was conducted with ethanol extracts of the seeds from *Prunus mahalep* using the disc diffusion method to determine antimicrobial activity against various species of gram-negative and gram-positive bacteria showed that *P. mirabilis* was the most susceptible to extract and could be inhibited with 0.4 g mL^{-1}. Additionally, an inhibitory effect was also observed against the gram-positive bacteria *B. anthracis* and *S. aureus* using the same concentration. This report is consistent with ours, in that *P. mirabilis* was more susceptible to the plant extracts than the other gram-negative bacteria (*E. coli*, *P. aeruginosa*, *K. pneumoniae*) (Table 1). In our study, *P. mahaleb* L. extracts exerted antibacterial activity against *S. aureus*, *E. faecalis*, *and B. subtilis* with MIC values between 16-64 μg mL^{-1} (Table 2). Less antibacterial activity was observed against the drug resistant strains (64- 128 μg mL^{-1}). The bacterium *S. aureus* was used because of its importance as a human pathogen and because it rapidly develops resistance to many antimicrobial agents, causing therapeutic problems.

Candida species are the most widespread and threatening fungal pathogens today and are responsible for the majority of invasive and non-invasive fungal infections. Currently, only four classes of antifungal drugs (polyene macrolides, azoles, flucytosine, and ecinocandins) are available for treatment of systemic mycoses. Unfortunately, none are ideal with regard to efficacy, the antifungal spectrum or safety. *n*-Hexane extracts and methanol extracts of mahaleb flowers, mahaleb leaves, mahaleb seed kernels, mahaleb fleshy seed coats, mahaleb fruit stalks and mahaleb branches demonstrated antifungal activity against *C. krusei*, but not *C. albicans*, *C. parapsilosis*, or *C. tropicalis* (Table 3).

Mounting evidence indicates that consumption of natural compounds may lower the risk of serious health disorders because of their antioxidant activities (M. L. HERTOG & al. [18], SURH [19]). Antioxidants in food lower rancidity and the formation of oxidation products, maintain clinical nutritional quality, and increase shelf life (S. J. JADHAV [20]). Tocopherol, tertiary-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) are the most commonly used synthetic antioxidants. However, due to the reported adverse effects of synthetic antioxidants, such as toxicity and carcinogenicity, the use of natural antioxidants obtained from edibles and edible by-products has become a viable alternative (G. M. WILLIAMS & [21]). This study shows that the antioxidant activity of hydrophilic extracts is superior to that of lipophilic extracts, which is in agreement with (A.A. MARIOD & al. [9]), who also showed that the hydrophilic, methanol, ethyl acetate, water and *n*-hexane extracts of mahaleb seedcakes had higher antioxidant activities than *n*-hexane extracts. It is also possible that phenolic compounds that are present in hydrophilic extracts may be responsible for the observed antioxidant capacity of the extracts. The same researchers used the β-Carotene Bleaching assay to estimate the effect of mahaleb seedcake extracts on the oxidation of β-Carotene by hyperoxides. The antioxidants from the hydrophilic methanol mahaleb extracts reduced the oxidation of β-Carotene the most, followed by water, *n*-hexane and ethylacetate extracts. The seed kernels have high protein content and fixed oil (27-40 %), which is valuable for industrial usage. Besides proteins and fatty acids, the studies revealed that the seeds contain coumarins, catechin tannins, traces of hydrocyanic acid (which was confirmed after hydrolysis) and sucrose (E. SEZIK & AL [2]). Moreover, although coumarin derivatives (coumarin, dihydrocoumarin, herniarin) are mainly found in the seed kernels (M. S. AL-SAID & al. [8]), they have also been isolated from the dried bark (J. MACTELIC [10]). Our preliminary phytochemical analysis of the methanolic and hexane extracts revealed that mahaleb branch extracts are also very rich in these compounds as compared to other parts. Studies on the volatile constituents

of the air-dried flowers, leaves, stem-bark and wood found that coumarins are a major component of the bark (34.1%) (S. J. JADHAV [20]). Fatty acids and esters were also identified in the seed kernels of the plant. Palmitic, (4.60%), stearic (1.80%) and palmitoleic acid (0.30%) were reported as the major fatty acids by OZGUL-YUCEL [22]. Additionally, unusual fatty acids, including conjugated linolenic acid (CLNA) were also reported. a-Eleostearic (18:3 9*c,*11*t,*13*t*) acid was the dominant conjugated linolenic acid followed by b-eleostearic (18:3 9*t,*11*t,*13*t*) and catalpic (9*t,*11*t,*13*c*) acid. CLNA has a strong cytotoxic effect on human leukemia cells (R. SUZUKI & al. [23]). Moreover, the anticarcinogenic effect and antioxidant properties of purified a-eleostearic acid were previously shown (P. DHAR & al. [24], M. L. HERTOG & al. [25], H. KOHNO & al. [26]). In addition to the hydophilic compounds, volatiles and fatty acids also contribute to the antioxidant activity, which increases the nutritional value of the plant.

Conclusions

The results of the present study suggest that *P. mahaleb* L extracts possess compound(s) with antimicrobial properties against bacteria, some bacterial pathogens and fungi as well as antioxidant activity. To our knowledge, our study is the first report the detailed antimicrobial and antioxidant activities of the extracts obtained from *P. mahaleb* L components. Further studies are being conducted to elucidate the compounds responsible for the antimicrobial activity, as well as any pharmacological or toxicological properties that the extracts may have. Furthermore, phytochemical analysis will likely reveal the individual phytochemical responsible for the observed biological effects.

Studies on flora and medicinal plants are important approaches for the future discovery of novel drugs and food additives. In the search for plant-derived antimicrobial and antiviral agents, the screening of medicinal plants has lead to the discovery of a remarkably high number of compounds that are currently applied in several therapeutic areas.

References

1. C. BRICKELL, The Royal Horticultural Society. A-Z Encyclopedia of Garden Plants. Dorling Kindersley London. New York. Stutttgart. Moscow. p. 581-583. (1981).
2. E.SEZIK, A. Başaran, Turkish Mahalep and its oil. *J. Fac. Pharm. Gazi Univ.* 21, 121-127, (1985).
3. EGEIB, Exportation Statistics of Aegean Exporters Union. http://www.egeliihracatcilar.com/StandartRaporlar/12_20101231.asp. (2011).
4. R. GERÇEKÇIOĞLU, Ç. ÇEKIÇ, The Effects of Some Treatments on Germination of mahaleb *(Prunus mahaleb* L.) Seeds. *Turk. J. Agric. For.* 23, 145-150, (1999).
5. M. A. MORENO, L. MONTANES, M.C. TABUENCA, R. CAMBRA, The performance of adara as a cherry. *Hort. Sci.* 65, 85–91, (1996).
6. S.R. MARCOS, M. EL-DAKHAKHANY, The sugar and amino acid pattern of mahaleb kernels. *Planta Med.* 10, 341- 344, (1962).
7. T. BAYTOP, Curing with Plants in Turkey (In the past and today). Istanbul University Press, Nr.3255-Faculty of Pharmacy Nr:40, Sanal Publishing. Istanbul, 271-272, (1984).
8. M.S. AL-SAID, M.S. HIFNAWY, Dihydrocoumarin and Certain Other Coumarins from *Prunus mahaleb* Seeds. *J. Nat. Prod.* 49(4), 721-721, (1986).
9. A.A. MARIOD, R.M. IBRAHIM, M. ISMAIL, N. ISMAIL, Antioxidant activities of phenolic rich fractions (PRFs) obtained from black mahlab (*Monechma ciliatum*) and white mahlab (*Prunus mahaleb*) seedcakes. *Food Chem.* 118, 120-127, (2010).
10. J. MASTELIC, I. JERKOVIC, M. MESIC, Volatile constituents from flowers, leaves, bark and wood of *Prunus mahaleb* L. *Flavour and Fragrance J.* 21(2), 306-313, (2005).
11. CLSI, Clinical and Laboratory Standards Institute (CLSI). Reference method for broth dilution antifungal susceptibility testing of yeast; approved standard- third edition. CLSI document M27-A3, Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania

USA, (2008).
12. B. ÖZÇELIK, M. KARTAL, I. ORHAN, Cytotoxicity, antiviral antimicrobial flavonoids and phenolic acids. *Parm. Biol.* 1–7, (2011).
13. B. ÖZÇELIK, I. GÜRBÜZ, T. KARAOGLU, E. YEŞILADA, Antiviral and antimicrobial activities of three sesquiterpene lactones from *Centaurea solstitialis* L. ssp. solstitialis extract. *Microbiol. Res.* 164, 545-552, (2009).
14. İ. ORHAN, B. OZCELİK, B. ŞENER, Evaluation of antibacterial, antifungal, antiviral, and antioxidant potentials of some edible oils and their fatty acid profiles. *Turk. J. Biol*. 35, 251-258, (2011).
15. U. KOCA, B. ÖZÇELIK, Antiviral, Antibacterial, and Antifungal Activities of *Centaurea tchihatcheffii* Extracts. *Tur. J. Pharm. Sci.* 6(2), 125-134, (2009).
16. R.D. LIN, Y.P. CHIN, M.H. LEE, Antimicrobial Activity of Antibiotics in Combination with Natural Flavonoids against Clinical Extended-Spectrum–Lactamase (ESβL)-producing *K. pneumonia. Phytoteraphy Res.* 19, 612–617, (2005).
17. T. KOHLER, J.C. PECHERE, P. PLESIAT, Bacterial antibiotic efflux systems of medical importance. *Cell. Mol. Life Sci.* 56, 771-778, (1999).
18. M.L. HERTOG, E.M. FESKENS, P.H. HOLLMAN, M.B. KATAN, D. KROMHOUT, Dietary antioxidant flavonoids and risk of coronary heart-diseases the Zutphen elderly study. *Lancet* 342, 1007–1011, (1993).
19. Y.J. SURH, Anti-tumor promoting potential of selected spice ingredients with antioxidative and anti-inflammatory activities: A short review. *Food Chem. Toxicol.* 40, 1091–1097, (2002).
20. S.J. JADHAV, S.S. NIMBALKAR, A.D. KULKARNI, D.L. MADHAVI, Lipid oxidation in biological and food systems. In: D.L. Madhavi, S.S. Deshpande and D.K. Salunkhe, Editors, *Food Antioxid.* 5–63, (1996).
21. G.M. WILLIAMS, M.J. IATROPOULOS, J. WHYSNER, Safety assessment to butylated hydroxyanisol and butylated hydroxytoluene as antioxidant food additives. *Food Chem. Toxicol.* 37, 1027–1038. (1999).
22. S. ÖZGÜL-YÜCEL, Determination of conjugated linolenic acid content of selected oil seeds grown in Turkey. *J. Am. Oil Chem. Soc.* 82, 893-897, (2005).
23. R. SUZUKI, R. NOGUSHI, T. OTA, A. MASAYUKI, K. MIYASHITA, T. KAWADA, Cytotoxic effect of conjugated trienoic fatty acids on mouse tumor and human monocytic leukemia cells. *Lipids* 36(5), 477-482, (2001).
24. P. DHAR, S. GHOSH, D.K. BHATTACHARYYA, Dietary effects of conjugated octadecatrienoic fatty acid (9 cis, 11 trans, 13 trans) levels on blood lipids and nonenzymatic in vitro lipid peroxidation in rats. *Lipids* 34(2), 109-114, (1999).
25. M. IGARASHI, T. MIYAZAWA, Preparation and fractionation of conjugated trienes from α-linolenic acid and their growth-inhibitory effects on human tumor cells and fibroblasts. *Lipids* 40(1), 109-113, (2005).
26. H. KOHNO, Y. YASUI, R. SUZUKI, M. HOSOKAWA, K. MIYASHITA, T. TANAKA, Dietary seed oil rich in conjugated linolenic acid from bitter melon inhibits azoxymethane-induced rat colon carcinogenesis through elevation of colonic PPARgamma expression and alteration of lipid composition. *Int. J. Cancer* 110, 896–901 (2004).