Biotechnology & Biotechnological Equipment

ISSN: 1310-2818 (Print) 1314-3530 (Online) Journal homepage: informahealthcare.com/journals/tbeq20

Antibacterial and Antitumor Activities of Some Wild Fruits Grown in Turkey

Arzu Ucar Turker, Arzu Birinci Yildirim & Fatma Pehlivan Karakas

To cite this article: Arzu Ucar Turker, Arzu Birinci Yildirim & Fatma Pehlivan Karakas (2012) Antibacterial and Antitumor Activities of Some Wild Fruits Grown in Turkey, Biotechnology & Biotechnological Equipment, 26:1, 2765-2772, DOI: 10.5504/BBEQ.2011.0156

To link to this article: https://doi.org/10.5504/BBEQ.2011.0156

Published online: 16 Apr 2014.

ARTICLE
DOI: 10.5504/BBEQ.2011.0156 MB
MEDICAL BIOTECHNOLOGY

ANTIBACTERIAL AND ANTITUMOR ACTIVITIES OF SOME WILD FRUITS GROWN IN TURKEY

Arzu Ucar Turker, Arzu Birinci Yildirim, Fatma Pehlivan Karakas
Abant Izzet Baysal University, Department of Biology, Bolu, Turkey
Correspondence to: Arzu Ucar Turker
E-mail: turker_a@ibu.edu.tr

ABSTRACT

Two different bioassays (antibacterial and antitumor) were performed to show the biological activities of eight different wild fruits [Viburnum opulus L. (guelder rose), Viburnum lantana L. (wayfaring tree), Cornus mas L. (cornelian cherry), Pyracantha coccinea Roemer (firethorn), Rubus caesius L. (dewberry), Crataegus tanacetifolia (Lam.) Pers (tansy-leaved thorn), Crataegus monogyna Jacq. (hawthorn) and Rosa canina L. (dog rose)] grown in Turkey. For each fruit, 8 different extracts (aqueous and ethanol extracts prepared from hot and cold treatments of fresh and dried fruits) were obtained and a total of 64 extracts were evaluated. The disc diffusion assay (Kirby-Bauer Method) was used to screen for antibacterial activity. Among the tested fruits, best antibacterial activity was obtained with fresh fruits of wayfaring tree, firethorn and hawthorn. Hot ethanol extracts of these fruits showed strong antibacterial activity against S. aureus, S. epidermidis and S. pyogenes. Antitumor activity was evaluated with potato disc tumor induction assay. Best antitumor activity was obtained with cold water extract of fresh fruits of R. caesius (100% inhibition). Cold or hot ethanol extracts of fresh V. lantana fruits (90.5% and 95.2%, respectively), cold water extract of fresh C. monogyna fruits (85.7%) and hot ethanol extracts of fresh C. tanacetifolia fruits (71.4%) also exhibited strong tumor inhibition.

Biotechnol. & Biotechnol. Eq. 2012, **26**(1), 2765-2772

Keywords: antibacterial, antitumor, *Viburnum opulus*, *Viburnum lantana*, *Cornus mas*, *Pyracantha coccinea*, *Rubus caesius*, *Crataegus tanacetifolia*, *Crataegus monogyna*, *Rosa canina*

Introduction

The medicinal plants have been widely used for the treatment of common animal and human infectious diseases since antiquity (49) and plant products have been used with varying success to cure and prevent diseases throughout history (47). The use of most medicinal plants discovered by traditional societies has not been verified scientifically and bioassays can provide initial screening data about the biological activities of these plants. Kirby-Bauer test (disc diffusion method) is the most widely used standard method currently performed by the National Committee for Clinical Laboratory Standards on disc diffusion susceptibility testing (6). *Agrobacterium tumefaciens* induced potato disc tumor assay is based on antimitotic activity and the validity of this bioassay is predicted on the observation that certain tumorigenic mechanisms are similar in plants and animals (13, 19, 39). It was demonstrated that inhibition of crown gall tumor initiation on potato disc showed an apparent correlation with compounds and plant extracts known to be active in the 3PS (*in vivo*, murine leukemia) antitumor assay (13, 18, 19, 39). Biological screening studies of medicinal plants are important because folkloric usages of these plants gain some scientific justification.

Guelder rose (*Viburnum opulus)* fruits have diuretic, laxative and sedative actions. In folklore medicine, the traditional beverage "gilaburu" prepared from the fruits has been used for the treatment of gallbladder, liver diseases and diabetes in the central Anatolia region of Turkey (2, 7). Antioxidant (2, 50, 52), anticancer (34), antibacterial (34, 52, 62), antinociceptive and anti-inflammatory (1) activities of *V. opulus* have been reported. Yilmaz et al. (63) reported the antinociceptive and anti-inflammatory activity of *V. lantana* leaves. Antioxidant activity of *V. lantana* was evaluated by Altun et al. (2). Yilmaz et al. (62) indicated the antimicrobial activities of the essential oils of *V. opulus* and *V. lantana*. Cornelian cherry (*Cornus mas*) fruits are astringent, febrifuge and nutritive. They have been used traditionally in the treatment of bowel complaints, fevers, cholera and diarrhea (7, 11, 22). Antibacterial (16, 30, 36), cytotoxic (53) and antioxidant (17, 23, 25, 45, 53, 59) activities of cornelian cherry have been recorded. In European countries, firethorn (*Pyracantha coccinea*) fruits have been used as a heart soother (7, 22). In folk medicine in Turkey, decoction of firethorn leaves has been used for the treatment of diarrhea (for humans and animals) (61). Ripe fruits of dewberry (*Rubus caesius*) are diuretic and used in the treatment of constipation. On the other hand, raw fruits have been used in the treatment of diarrhea and excessive consumption may cause constipation (7, 22). Serteser et al. (56) evaluated the antioxidant activity of *R. caesius*. There are many studies about the anticancer activities of *Rubus* spp. (4, 10, 14, 27, 41, 54, 55, 57). Fruits and flowers of many *Crataegus* spp. are well known in folk medicine as a heart tonic (7, 22). They have nervine, antispasmodic, bradycardiac, hypotensive and diuretic activities (7, 11, 22). Antioxidant activities of *C. tanacetifolia* and *C. monogyna* have been documented (5, 9, 46, 56). Moreover, anticancer activities of some *Crataegus* spp.

(26, 28, 40, 51) and antibacterial activities of *C. tanacetifolia* leaves (8) and fruits (24) have been determined. Tadic et al. (58) reported the anti-inflammatory, gastroprotective, free-radical-scavenging and antimicrobial activities of ethanolic extracts of hawthorn berries mixture (*C. monogyna* and *C. oxycantha*). Dog rose (*Rosa canina*) has astringent, aperient, stomachic, carminative, diuretic, laxative, ophthalmic, anti-inflammatory and tonic activities (11, 12). Fruits have been used in folk medicine in the treatment of colds, influenza, minor infectious diseases, scurvy, diarrhea and gastritis (7, 11, 22). Antioxidant (29, 32, 42, 44, 56), antibacterial (31, 32), antidiabetic (42) and antitumor (33) activities of dog rose have been recorded.

The objective of this study was to assess the antibacterial and antitumor activities of aqueous and ethanol extracts of eight different fresh or dried wild fruits.

Materials and Methods

Plant material and extraction

Ripe fruits of eight plants were collected from Bolu Mountain, Turkey. Identification of species was done by using *Flora of Turkey and the East Aegean Islands* (15) and voucher specimens were deposited at the Abant Izzet Baysal University (AIBU) Herbarium, Bolu, Turkey. Half of the collected fruits were used fresh and the other half were dried in an oven at 40 °C. Aqueous and ethanol extracts of fruits were prepared as hot and cold treatment.

Aqueous extract preparation

a. 150 ml cold water was added into forty grams of fruit samples and kept at room temperature on a shaker for 12 hours. The extract was then filtered and lyophilized.

b. 150 ml boiled water was added into forty grams of fruit samples and kept at 50 °C in a waterbath for 12 hours. The extract was then filtered and lyophilized.

Ethanol (EtOH) extract preparation

a. 150 ml EtOH was added into forty grams of fruit samples and kept at room temperature on a shaker for 12 hours. The extract was filtered and concentrated *in vacuo*. The residue was then dissolved with 10 ml distilled water and lyophilized.

b. 150 ml EtOH was added into forty grams of fruit samples and kept at 50 °C in a water bath for 12 hours. The residue was then dissolved with 10 ml distilled water and lyophilized.

The botanical names of the studied wild fruits, their family, collection numbers and yield (%) for each extraction are summarized in **Table 1**. All lyophilized extracts were dissolved in water to produce a final concentration of 100 mg/ml.

Antibacterial assay

Sixty-four fruit extracts were tested for their antibacterial activity. The disc diffusion assay (Kirby-Bauer Method) was used to screen for antibiotic activity (3). The microorganisms used were: *Streptococcus pyogenes* (ATCC® 19615), *Staphylococcus aureus* (ATCC® 25923) and *Staphylococcus epidermidis* (ATCC® 12228), which are Gram-positive bacteria, and *Escherichia coli* (ATCC® 25922), *Pseudomonas aeruginosa* (ATCC® 27853), *Salmonella typhimurium* (ATCC® 14028), *Serratia marcescens* (ATCC® 8100), *Proteus vulgaris* (ATCC® 13315), *Enterobacter cloacae* (ATCC® 23355) and *Klebsiella pneumoniae* (ATCC® 13883), which are Gram-negative bacteria.

Each lyophilized bacteria disc (Microtrol Discs, BD®) was transferred to test tubes containing 5 ml of Tryptic Soy Broth (TSB) and incubated overnight at 37 °C. One bacteriological loop from each broth was streaked on Tryptic Soy Agar (TSA) plates and incubated for 2 days at 37 °C. After 2 days, a single colony was removed and streaked on TSA plate and incubated at 37 °C for 2 additional days. The turbidity of each broth culture was adjusted with saline to obtain a turbidity visually comparable to that of an 0.5 McFarland standard and then Mueller Hinton agar plates were inoculated by using cotton swabs.

All extracts were sterilized by filtering through a 0.22 µm filter (Millex®) and sterile filter paper discs (Glass Microfibre filters, Whatman®; 6 mm in diameter) were impregnated with 13 µl of extract. There were five replicates in each plate and two plates for each extract tested for each bacterium. Positive controls consisted of five different antimicrobial susceptibility test discs (Bioanalyse®): Erythromycin (15 µg) (E-15), Ampicillin (10 µg) (AM-10), Carbenicillin (100 µg) (CB-100), Tetracycline (30 µg) (TE-30) and Chloramphenicol (30 µg) (C-30). Four antibiotic discs were used for each plate and run in duplicate. Water was used as a negative control. Inoculated plates with discs were placed in a 37 °C incubator. After 16 to 18 hrs of incubation, inhibition zone diameter (mm) was measured. All experiments were repeated three times.

Antitumor assay

The antitumor activity of all extracts was assessed with the potato disc method as modified by McLaughlin's group (18). *Agrobacterium tumefaciens* (ATCC® 23341) was cultured on Yeast Extract Media (YEM) for 2-3 days at 28 °C. Camptothecin (Sigma®) (tumor suppressant) served as a positive control and water was used as a negative control. Six to seven loops of *A. tumefaciens* were added to 10 ml Phosphate buffered saline (PBS). Suspensions of *A. tumefaciens* in PBS were standardized to 1.0×10^9 Colony Forming Units (CFU) as determined by an absorbance value of 0.96 ± 0.02 at 600 nm (13). All extracts and control solutions were filter sterilized (sterile 0.22 µm filter, Millex®). The test solutions consisted of 600 µl extract or control solution, 150 µl sterile distilled water and 750 µl of the standardized *A. tumefaciens* in PBS.

Potatoes (*Solanum tuberosum* L.) were washed and scrubbed with a brush under running water and surface sterilized by immersion in 10% commercial bleach (Domestos®) for 20 min. Tubers were then placed on sterile paper towels and cut along either side revealing the largest surface area available. The trimmed tubers were then immersed in 20% commercial bleach (Domestos®) for 15 min. Cylinders (10 mm diameter) were cut from the center of potato tissue (skin portion was eliminated)

using a cork borer on sterile paper towels and placed in sterile distilled water with lactic acid (pH 4.0). Cylinders were rinsed twice more using sterile distilled water with lactic acid. Each cylinder was cut into 0.5 cm discs after excluding 1 cm end pieces. These discs were transferred to 24-well culture plates containing water-agar (15 g/L). Each disc was overlaid with 50 µl of appropriate inoculum. No more than 30 min elapsed between cutting the potato discs and inoculation (37). Plates were incubated at 28 °C in the dark for 2 weeks. After 2 weeks, discs were stained with Lugol's reagent (I_2KI; 5% I_2 plus 10% KI in distilled water) and tumors on each disc were counted. Lugol's reagent stains the starch in potato tissue to dark blue to dark brown color, but the tumors do not take up the stain and appear creamy to orange. Experiments were repeated three times. Percent inhibition of tumors was calculated using the following formula:

% inhibition = [(solvent control mean - tested extract mean) / / solvent control mean] × 100 (37, 38, 39).

Bacterial viability testing

Standardized bacterial suspension (1×10^9 CFU of *A. tumefaciens* in PBS) was serially diluted with PBS to 1×10^3 CFU. Bacterial viability was determined by incubating 1 ml of each fruit extract with 1 ml of bacterial suspension (1×10^3 CFU of *A. tumefaciens* in PBS) in microcentrifuge tubes (4 tubes per extract) and left for 30 min. Then, 30 min after inoculation, 0.1 ml of inoculum (bacteria + extract) was removed and inoculated on YEM media by the spread plate technique. After 24 h of incubation of inoculated plates at 28 °C, colony counts were done. Also, bacterial growth was evidenced by growth across the plates (13).

Data analysis

All data were analyzed by analysis of variance (ANOVA) and mean values were compared with Duncan's Multiple Range Tests using SPSS vers. 15 (SPSS Inc., Chicago, IL, USA).

Results and Discussion

Screening of antibacterial and antitumor activities of 64 crude extracts obtained from 8 different wild fruits was conducted. Extraction solvents (water and ethanol) were used as cold or hot treatment to show the effect of temperature on extracts prepared from both fresh and dried fruits (**Table 1**).

Among the studied fruit extracts, best antibacterial activity was obtained with *V. lantana*, *P. coccinea* and *C. monogyna* against *S. aureus*, *S. epidermidis* and *S. pyogenes*, which are Gram-positive bacteria (**Table 1**). Generally, Gram-positive bacteria commonly seem to be more susceptible to the inhibitory effects of the plant extracts than the Gram-negative bacteria do. The susceptibility of Gram-positive bacteria may arise from their cell wall structure consisting of a single layer, whereas the Gram-negative cell wall is a multi-layered structure and quite complex (60). Among the Gram-negative bacteria used, *S. marcescens* and *P. aeruginosa* were not vulnerable to any of the tested fruit extracts. The tested fruits extracts showed just a little activity (≤ 8 mm) against the other Gram-negatives used (*S. typhimurium*, *P. vulgaris*, *K. pneumonia*, *E. cloacae* and *E. coli*). Positive controls (reference antibiotics) generally showed antibacterial activity to our test organisms (**Table 1**) and no inhibition was observed with the extraction solvents (water and ethanol).

Although the extracts obtained from dried *V. lantana* fruits did not show any activity, extracts obtained from fresh fruits exhibited antibacterial activities. Especially, the hot ethanolic extract was better than the cold ethanolic extract against *S. aureus*, *S. epidermidis* and *S. pyogenes* (**Table 1**). Among the tested antibiotics, *S. epidermidis* was more sensitive to the hot ethanolic extract of *V. lantana* (11.3 mm) than to tetracycline (10.3 mm). Only the cold ethanol extract of fresh *V. opulus* fruits showed moderate level of antibacterial activity (between 8.8 and 9.4 mm) against *S. aureus*, *S. epidermidis* and *S. pyogenes*. Sagdic et al. (52) reported that 10 and 15% concentrations of methanolic extraction of dried *V. opulus* fruits exhibited inhibition against tested bacteria (*Aeromonas hydrophila*, *Bacillus cereus*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Yersinia enterocolitica*). They concluded that phenolic compounds behaved as antioxidants and antimicrobial agents because of the reactivity of the phenol moiety (52). Yilmaz et al. (62) tested the essential oils of *V. opulus* and *V. lantana* against *E. coli*, *K. pneumoniae*, *P aeruginosa*, *E. faecalis*, *S. aureus* and *B. cereus* and no activity was observed against all the test microorganisms. However, *V. opulus* extracts showed moderate activity against *S. aureus* (8.8 mm), little activity against *K. pneumoniae* (7.0 mm) and *S. typhimurium* (7.5 mm) and no activity against *P. aeruginosa*, *P. vulgaris* and *E. coli* in our study. In addition, *V. lantana* displayed strong inhibition against *S. aureus* (12.8 mm), just little inhibition against *K. pneumoniae* (7.1 mm) and *E. coli* (7.0 mm) and no inhibition against *P aeruginosa*.

The extracts obtained from fresh *P. coccinea* and *C. monogyna* fruits, especially the hot ethanolic extracts, showed strong antibacterial activity against *S. aureus*, *S. epidermidis* and *S. pyogenes*. The hot ethanolic extracts of fresh *P. coccinea* fruits had inhibition zones 11.3 mm, 9.8 mm and 14.9 mm against *S. aureus*, *S. epidermidis* and *S. pyogenes*, respectively. In addition, the hot ethanolic extract of fresh *C. monogyna* fruits also exhibited inhibition zones 12.3 mm, 10.1 mm and 13.6 mm against *S. aureus*, *S. epidermidis* and *S. pyogenes*, respectively. Oskay and Sari (43) evaluated the antimicrobial activity of ethanol extract of raw *P. coccinea* fruits and no inhibition was observed against tested bacteria (*S. aureus*, *E. coli*, *Micrococcus luteus*, *Bacillus subtilis*, *B. cereus*, *S. typhimurium*, *Pseudomonas fluorescens*, *P. vulgaris*, *S. marcescens*, *S. epidermidis*, *Enterococcus faecalis*, *Enterobacter cloaceae*, *E. aerogenes* and *Candida albicans*). Similarly, *S. marcescens*, S. *typhimurium, P. aeruginosa*, *P. vulgaris*, *K. pneumonia*, *E. cloacae* and *E. coli* were not vulnerable to any *P. coccinea* fruit extracts. However, *S. aureus*, *S. epidermidis* and *S. pyogenes* were strongly inhibited

TABLE 1

Botanical names of studied wild fruits, their family, collection numbers, yield (%) for each extraction, and antibacterial activity of fruit extracts

Botanical name	Family	Collection #	Yield (%)*	Fruit condition	Treatments	Mean diameter of inhibitory zones (mm ± SE)		
						S.aureus	*S.epidermidis*	*S.pyogenes*
			1.1		Cold water	- -	- -	- -
			1.6	Fresh	Hot water	- -	- -	- -
			1.4		Cold EtOH	- -	- -	7.1 ± 0.1 n
Viburnum opulus L.	Caprifoliaceae	AUT-2029	4.2		Hot EtOH	- -	- -	- -
			5.3		Cold water	- -	- -	- -
			6.4	Dry	Hot water	8.8 ± 0.2 g	8.8 ± 0.2 cde	9.4 ± 0.3 ij
			6.6		Cold EtOH	- -	- -	- -
			18.4		Hot EtOH	- -	- -	8.3 ± 0.3 lm
			0.2		Cold water	- -	- -	- -
			1.8	Fresh	Hot water	- -	8.5 ± 0.7 de	- -
			1.5		Cold EtOH	- -	- -	- -
Viburnum lantana L.	Caprifoliaceae	AUT-2030	3.0		Hot EtOH	9.4 ± 0.2 g	9.4 ± 0.2 cde	10.3 ± 0.2 hi
			11.3		Cold water	- -	- -	- -
			10.7	Dry	Hot water	8.3 ± 0.3 g	- -	10.3 ± 0.2 hi
			0.5		Cold EtOH	- -	- -	- -
			6.0		Hot EtOH	12.8 ± 0.2 e	11.3 ± 0.6 c	13.3 ± 0.2 g
			0.4		Cold water	- -	- -	- -
			0.8	Fresh	Hot water	- -	- -	- -
			3.8		Cold EtOH	- -	- -	- -
Cornus mas L.	Cornaceae	AUT-2031	4.7		Hot EtOH	- -	- -	- -
			1.6		Cold water	- -	- -	- -
			3.2	Dry	Hot water	- -	9.3 ± 0.9 cde	9.3 ± 0.3 jk
			0.9		Cold EtOH	- -	- -	- -
			8.0		Hot EtOH	8.3 ± 0.4 g	- -	7.6 ± 0.2 mn
			0.8		Cold water	- -	- -	- -
			2.9	Fresh	Hot water	- -	- -	- -
			3.2		Cold EtOH	- -	- -	- -
Pyracantha coccinea Roemer	Rosaceae	AUT-2032	4.1		Hot EtOH	- -	- -	9.1 ± 0.1 jkl
			4.4		Cold water	- -	- -	- -
			7.5	Dry	Hot water	- -	- -	9.4 ± 0.2 ij
			4.7		Cold EtOH	- -	- -	- -
			9.0		Hot EtOH	11.3 ± 0.2 ef	9.8 ± 0.3 cde	14.9 ± 0.3 f
			0.8		Cold water	- -	- -	- -
			2.7	Fresh	Hot water	- -	- -	- -
			3.4		Cold EtOH	- -	- -	- -
Rubus caesius L.	Rosaceae	AUT-2033	4.4		Hot EtOH	- -	7.6 ± 0.3 e	7.3 ± 0.2 n
			14.1		Cold water	- -	- -	- -
			16.7	Dry	Hot water	- -	- -	- -
			6.2		Cold EtOH	- -	- -	- -
			14.1		Hot EtOH	- -	- -	7.3 ± 0.2 n
			2.6		Cold water	- -	- -	- -
			4.5	Fresh	Hot water	- -	- -	- -
			2.8		Cold EtOH	- -	- -	- -
Crataegus tanacetifolia (Lam.) Pers	Rosaceae	AUT-2034	4.7		Hot EtOH	- -	- -	- -
(endemic)			1.1		Cold water	- -	- -	- -
			2.7	Dry	Hot water	- -	- -	8.4 ± 0.2 klm
			2.3		Cold EtOH	- -	- -	- -
			5.0		Hot EtOH	- -	10.1 ± 0.4 cde	- -
			0.5		Cold water	- -	- -	- -
			0.6	Fresh	Hot water	- -	- -	- -
			1.7		Cold EtOH	- -	- -	- -
Crataegus monogyna Jacq.	Rosaceae	AUT-2035	4.1		Hot EtOH	- -	- -	- -
			4.4		Cold water	- -	- -	- -
			6.3	Dry	Hot water	11.0 ± 0.2 f	7.6 ± 0.3 e	10.6 ± 0.3 h
			1.3		Cold EtOH	- -	- -	- -
			7.7		Hot EtOH	12.3 ± 0.3 ef	10.1 ± 0.6 cde	13.6 ± 0.3 g
			0.1		Cold water	- -	- -	- -
			5.2	Fresh	Hot water	- -	- -	- -
			1.0		Cold EtOH	- -	- -	- -
Rosa canina L.	Rosaceae	AUT-2036	3.1		Hot EtOH	- -	- -	- -
			1.6		Cold water	- -	- -	- -
			2.5	Dry	Hot water	- -	- -	7.1 ± 1.0 n
			0.1		Cold EtOH	- -	- -	- -
			0.8		Hot EtOH	- -	- -	8.1 ± 0.1 mn
					Ampicillin	50.5 ± 5.8 a	33.8 ± 4.3 b	53.5 ± 0.8 a
					Carbenicillin	48.3 ± 2.6 b	38.5 ± 4.4 a	47.5 ± 0.6 b
					Chloramphenicol	28.3 ± 0.9 d	35.3 ± 1.8 b	36.3 ± 1.4 e
					Erythromycin	28.3 ± 1.0 d	35.8 ± 1.6 b	37.8 ± 0.9 d
					Tetracycline	32.8 ± 0.9 c	10.3 ± 0.7 cd	38.8 ± 0.8 c

*Yield (%) = Weight of extract (g) / 40 g of fruit sample × 100. Means with the same letter within columns are not significantly different at $P > 0.05$.

TABLE 2

Botanical and common names of studied wild fruits, and antitumor activity of fruit extracts

Botanical name	Common names (English and Turkish)	Fruit condition	Treatments	Mean # of tumors (±SE)				% Tumor inhibition
			Cold water	20.7	±	2.9	rstu	71.4
		Fresh	Hot water	21.2	±	5.1	qrstu	71.4
			Cold EtOH	27.8	±	4.2	opqrst	61.9
V. opulus	Guelder Rose		Hot EtOH	26.7	±	2.9	opqrst	66.7
	Gilaburu		Cold water	0.0	±	0.0	-	100
		Dry	Hot water	0.0	±	0.0	-	100
			Cold EtOH	20.8	±	5.2	rstu	71.4
			Hot EtOH	10.8	±	1.6	uvw	85.7
			Cold water	51.2	±	5.9	cdefgh	30.3
		Fresh	Hot water	42.9	±	4.3	ghijklmn	42.9
			Cold EtOH	61.2	±	4.0	bcde	19.7
V. lantana	Wayfaring tree		Hot EtOH	14.7	±	2.7	tuv	81
	Germişek		Cold water	63.1	±	6.5	abc	17.1
		Dry	Hot water	5.7	±	1.3	vw	90.5
			Cold EtOH	37.7	±	5.2	hijklmno	48.7
			Hot EtOH	4.1	±	0.8	vw	95.2
			Cold water	59.4	±	3.9	bcdef	22.4
		Fresh	Hot water	32.9	±	4.2	lmnopqr	57.1
			Cold EtOH	53.6	±	4.5	cdefg	28.9
C. mas	Cornelian cherry		Hot EtOH	37.1	±	3.3	ijklmno	52.4
	Kızılcık		Cold water	32.5	±	4.6	lmnopqr	57.9
		Dry	Hot water	16.7	±	2.3	stuv	76.2
			Cold EtOH	45.8	±	4.0	fghijkl	39.5
			Hot EtOH	29.6	±	4.1	nopqrs	61.9
			Cold water	61.8	±	5.7	bcd	18.4
		Fresh	Hot water	37.6	±	4.6	hijklmno	47.6
			Cold EtOH	71.4	±	4.4	ab	6.6
P. coccinea	Firethorn		Hot EtOH	30.0	±	3.3	nopqrs	61.9
	Ateş dikeni		Cold water	54.4	±	4.3	cdefg	28.9
		Dry	Hot water	49.6	±	2.9	defghi	33.3
			Cold EtOH	47.6	±	4.1	efghijk	36.8
			Hot EtOH	32.2	±	2.7	lmnopqr	57.1
			Cold water	52.4	±	4.4	cdefg	31.6
		Fresh	Hot water	0.0	±	0.0	-	100
			Cold EtOH	30.7	±	3.0	mnopqr	59.2
R. caesius	Dewberry		Hot EtOH	21.9	±	3.2	pqrstu	71.4
	Böğürtlen		Cold water	30.6	±	3.1	mnopqr	59.2
		Dry	Hot water	34.6	±	4.1	klmnopq	57.1
			Cold EtOH	47.4	±	3.3	fghijk	38.2
			Hot EtOH	15.2	±	2.3	tuv	81
			Cold water	50.8	±	4.8	cdefghi	3.9
		Fresh	Hot water	49.3	±	3.9	defghi	33.3
			Cold EtOH	48.7	±	4.3	defghij	35.5
C. tanacetifolia	Tansy-leaved thorn		Hot EtOH	43.9	±	4.6	ghijklm	42.9
	Sarı alıç		Cold water	51.1	±	3.9	cdefgh	32.9
		Dry	Hot water	29.9	±	3.5	nopqrs	61.9
			Cold EtOH	47.7	±	3.9	efghijk	36.8
			Hot EtOH	21.3	±	2.5	qrstu	71.4
			Cold water	53.2	±	4.1	cdefg	30.3
		Fresh	Hot water	11.6	±	1.7	uvw	85.7
			Cold EtOH	52.3	±	4.2	cdefg	31.6
C. monogyna	Hawthorn		Hot EtOH	14.6	±	1.6	tuv	81
	Kırmızı alıç		Cold water	54.8	±	4.2	cdefg	27.6
		Dry	Hot water	15.8	±	2.1	tuv	81
			Cold EtOH	45.7	±	5.4	fghijkl	39.5
			Hot EtOH	26.7	±	3.4	opqrst	61.9
			Cold water	35.3	±	3.6	jklmnop	55.3
		Fresh	Hot water	45.3	±	4.1	ghijkl	38.1
			Cold EtOH	44.3	±	5.0	ghijkl	42.1
R. canina	Dog rose		Hot EtOH	73.9	±	5.9	a	0
	Kuşburnu		Cold water	42.0	±	4.7	ghijklmn	44.7
		Dry	Hot water	50.6	±	4.5	cdefghi	33.3
			Cold EtOH	45.0	±	3.2	ghijkl	40.8
			Hot EtOH	37.5	±	6.3	hijklmno	47.6
			Water	74.3	±	5.6	a	-
			Camptothecin	0.0	±	0.0		100

Means with the same letter within columns are not significantly different at $P > 0.05$

by the hot ethanol extract of fresh and ripe *P. coccinea* fruits in our study (**Table 1**).

Although ethanol extracts of *C. tanacetifolia* fruits showed moderate activity against *S. epidermidis* (10.1 mm) and *S. pyogenes* (8.4 mm) and little activity against *P. vulgaris* (7.1 mm), other tested bacteria were not inhibited by this extract in our study (**Table 1**). Similarly, Guven et al. (24) reported that ethyl acetate extract of *C. tanacetifolia* did not show activity against S. *typhimurium*. However, they found inhibitory activity of ethyl acetate extracts against *E. coli* (9 mm), *P. aeroginosa* (12 mm), *S. aureus* (9 mm), *K. pneumoniae* (12 mm) and *P. vulgaris* (9 mm). Benli et al. (8) showed that methanol extract of *C. tanacetifolia* leaves had antibacterial effects on *Bacillus subtilis*, *S. aureus* and *Listeria monocytogenes*. In our study, the hot ethanolic extracts of fresh *C. monogyna* fruits exhibited strong inhibition against *S. aureus*, *S. epidermidis* and *S. pyogenes*. In addition, *E. coli* has just little sensitivity (7.4 mm) to the aqueous extract of *C. monogyna* (data not shown). Similarly, Proestos et al. (46) showed that *C. monogyna* extract has a slight inhibitory activity against *E. coli* but no activity against *S. aureus*. Tadic et al. (58) evaluated the antibacterial activity of ethanol extract of hawthorn berries mixture (*C. monogyna* and *C. oxycantha*). Although *S. typhimurium*, *E. faecalis*, *Pseudomonas talaasii*, *Proteus mirabilis* and *Sarcinaa lutea* were not vulnerable, *E. coli*, *S. aureus*, *S. epidermidis*, *B. subtilis*, *M. luteus*, *M. flavus*, *P. aeruginosa* and *Lysteria monocytogenes* showed sensitivity to this extract.

The hot or cold ethanol extract of fresh *C. mas* fruits had little or moderate activity (7-9.3 mm) against *S. aureus*, *S. epidermidis*, *S. pyogenes* and *E. cloacae*. In addition, the hot or cold aqueous extract of fresh *C. mas* fruits had little inhibition against *E. coli* (7.1 mm and 7.4 mm) (**Table 1**). Krisch et al. (30) reported that methanol extract of *C. mas* fruits showed strong inhibition but aqueous extracts displayed very little inhibition against *E. coli* and *S. marcescens* (data not shown). Similarly, *E. coli* showed very little sensitivity and *S. marcescens* was not sensitive to aqueous *C. mas* fruit extracts in our study. Mamedov and Craker (36) reported that the fatty oil from drupes of *C. mas* exhibited significant activity against *S. aureus* and *E. coli*. Ethanolic extract of *C. mas* bark showed moderate inhibition (9-10 mm) against *S. aureus*, *P. aeruginosa*, *P. vulgaris* and *Micrococcus luteus* (16).To our knowledge, there is no study about the antibacterial activity of *R. caesius* fruits up to now. In our study, *R. caesius* fruit extracts showed very little inhibition against *S. epidermidis* and *S. pyogenes* (**Table 1**). However, according to Rauha et al. (48), *E. coli*. *S. aureus*, *S. epidermidis*, *B. subtilis* and *M. luteus* were vulnerable to 70% aqueous acetone extracts of dried *Rubus chamaemorus* L. and *R. idaeus* L. fruits. Krisch et al. (30) reported that *B. subtilis*, *B. cereus*, *E. coli* and *S. marcescens* were not sensitive to aqueous extract of *R. idaeus* fruits but methanol extracts strongly inhibited *E. coli* and *S. marcescens*. In addition, aqueous extract of *R. fruticosus* fruits showed strong inhibition capacity against *B. subtilis* and *B. cereus* but little activity against *E. coli* and *S. marcescens*. On the other hand, all tested bacteria were inhibited by methanol extracts of *R. fruticosus* fruits (30).

S. pyogenes was the only bacterial pathogen that was slightly inhibited by the hot or cold ethanol extract of fresh *R. canina* extract (**Table 1**). Similarly, Kumarasamy et al. (31) reported that *S. aureus*, *S. epidermidis*, *P. aeroginosa* and *S. marcescens* were not sensitive but only *E. coli* was inhibited by methanol extract of *R. canina* seeds.

A prerequisite for the potato disc tumor induction assay is that the extract or substance being tested does not show antibacterial activity toward *A. tumefaciens* (20). Inhibition of crown gall formation on potato discs is caused by two effects: by anti-tumorogenesis or by decreasing the viability of the *A. tumefaciens*. Viability tests were carried out with all extracts to distinguish between these possibilities. Bacterial viability was determined by incubating fruit extracts with 1×10^3 CFU of *A. tumefaciens* bacterial suspension and left for 30 min. As the attachment of the bacterium to a tumor-binding site is complete within 15 min following inoculation (21, 35), 30 min exposure was chosen in the experiment. There was no difference in bacterial growth across the plates between control (only *A. tumefaciens*) and tested extracts (*A. tumefaciens* + fruit extracts) in terms of colony counts (ranged from 9.2×10^3 to 13×10^3 CFU), except for *V. opulus* extracts. All tested extracts other than *V. opulus* did not affect the viability of the bacterium. Thus, the observed inhibition of tumor formation for these extracts was due to inhibition of tumor formation and not to reduction of bacterial viability. On the other hand, *V. opulus* extracts affected the viability of the bacterium and *A. tumefaciens* bacterial growth was not observed across the plates. Therefore, it could be concluded that the inhibition of crown gall formation on potato disc is caused by decreasing the viability of the *A. tumefaciens* for *V. opulus* extracts. It was not possible to evaluate the antitumor activity of *V. opulus* extracts by the potato disc bioassay because they have very strong antibacterial activity against *A. tumefaciens*. Although the results herein did not prove an anti-tumor effect for the extracts of *V. opulus*, antioxidant activity of this fruit was recorded (2, 52). Moreover, Laux et al. (34) reported that lipid aldehydes isolated from *V. opulus* fruits inhibited the growth of *Helicobacter pylori* and induced apoptosis in a gastric cancer cell line *in vitro*. In the future, the anticancer activity of this plant should be studied using different cancer cell lines.

Since the final concentrations of all extracts were adjusted with distilled water, it was used as a negative control and no inhibition was observed with water. No tumor formation was observed with camptothecin (100% inhibition).

The hot ethanolic extracts of fresh *V. lantana* fruits showed strong antitumor activity (95.2%). On the other hand, the cold water extract of fresh *R. caesius* and *C. monogyna* fruits exhibited strong antitumor action (100% and 85.7%, respectively). The hot ethanolic extracts of fresh *C. tanacetifolia* fruits also showed 71.4% tumor inhibition (**Table 2**). Generally, fresh fruit extracts gave more tumor inhibition

for *V. lantana*, *R. caesius*, *C. monogyna* and *C. tanacetifolia*. Altun et al. (2) reported that maceration of dried *V. lantana* fruits in cold distilled water showed strong 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Some studies about anticancer activities of some *Rubus* spp. have been recorded (4, 10, 14, 27, 41, 54, 55, 57). Saenz et al. (51) reported that hexanoic extract of *C. monogyna* demonstrated significant cytotoxic activity against larynx cancer cells. Anticancer activities of other *Crataegus* spp. have also been reported (26, 28, 40, 51).

The antitumor activity of the ethanol extracts of *C. mas* was better than that of the aqueous extracts. Among *C. mas* fruit extracts, best tumor inhibition was obtained with the cold ethanol extract from fresh fruits (76.2%) (**Table 2**). Studies about the antioxidant activities of *C mas* fruits were documented (23, 45, 59). Furthermore, Savikin et al. (53) demonstrated the cytotoxic and antioxidant properties of the methanol extracts of leaves and flowers of *C. mas*. All extracts of *C. mas* possessed potential cytotoxic activity towards HeLa and LS174 human cancer cell lines *in vitro*, with stronger inhibition against the growth of HeLa cells than against LS174 cell growth (53). Among *R. canina* fruit extracts, tumor inhibition activity was less than 55% (**Table 2**). Larsen and Christensen (33) found moderate level of DLGG (1,2-Di-O-a-linolenoyl-3-O-b-D-galactopyranosyl-sn-glycerol) that can be an indicator of antitumor activity in *R. canina* fruits.

Conclusions

The antibacterial and antitumor activities of 64 different extracts obtained from 8 different wild fruits grown in Turkey were evaluated. Best antibacterial activity was obtained with *V. lantana*, *P. coccinea* and *C. monogyna* against *S. aureus*, *S. epidermidis* and *S. pyogenes*. Furthermore, best antitumor activity was obtained with *R. caesius, V. lantana and C. monogyna*. Generally, the extracts obtained from dried fruits did not exhibit any antibacterial activity or just a little activity ($\leq$ 8 mm) and the hot ethanolic extracts exhibited better inhibition against the tested bacteria. Similarly, best antitumor activity was observed with the extracts obtained from fresh fruits. With these results, the tested fruits have some scientific justification as medicinal plants. In the future, identification of active components can be attempted for fruits having strong bioactivity.

Acknowledgements

The authors are grateful to The Scientific and Technological Research Council of Turkey (TUBITAK) for financial support [107T280(TBAG/HD-305)]. We are grateful to Dr. Hakan Turker for his technical support.

REFERENCES

1. **Altun M.L., Citoglu G.S., Yilmaz B.S., Ozbek H.** (2009) Pharm. Biol., **47**, 653-658.
2. **Altun M.L., Citoğlu G.S., Yilmaz B.S., Coban T.** (2008) Int. J. Food Sci. Nutr., **59**, 175-80.
3. **Andrews J.M.** (2004) J. Antimicrob. Chemother., **53**, 713-728.
4. **Bagchi D., Sen C.K., Bagchi M., Atalay M.** (2004) Biochem.-Moscow, **69**, 75.
5. **Barros L., Carvalho A.M., Ferreira I.C.F.R.** (2011) Phytochem. Analysis, **22**, 181-188.
6. **Barry A.L., Thornsberry C.** (1985) In: Manual of Clinical Microbiology (E.H. Lennette, Ed.), American Association for Microbiology, Washington D.C., 978-987.
7. **Baytop T.** (1999) Türkiye'de Bitkiler ile Tedavi, Nobel Tıp Kitabevleri.
8. **Benli M., Yigit N., Geven F., Guney K., Bingol U.** (2008) Cell Biochem., **26**, 844-851.
9. **Bernatoniene J., Masteikova R., Majiene D., Savickas A., Kevelaitis E., Bernatoniene R., Dvorackova K., Civinskiene G., Lekas R., Vitkevicius K., Peciura R.** (2008) Med. Lit., **44**, 706-712.
10. **Cekic C., Ozgen M.** (2010) J. Food Comp. Anal., **23**, 540-544.
11. **Chevallier A.** (1996) The Encyclopedia of Medicinal Plants, Darling Kindersley Limited, London.
12. **Chrubasik C., Roufogalis B.D., Muller-Ladner U., Chrubasik S.** (2008) Phytother. Res., **22**, 725-733.
13. **Coker P.S., Rodecke J., Guy C., Camper N.D.** (2003) Phytomed., **10**, 133-138.
14. **Dai J., Patel J.D., Mumper R.J.** (2007) J. Med. Food, **10**, 258-265.
15. **Davis P.H.** (1972) Flora of Turkey and the East Aegean Islands, Vol. 4, Edinburgh Univ. Press, Edinburgh.
16. **Dulger B., Gonuz A.** (2004) Pakistan J. Biol. Sci., **7**, 1559-1562.
17. **Ersoy N., Bagci Y., Gok V.** (2011) Sci. Res. Essays, **6**, 98-102.
18. **Ferrigini N.R., Putnam J.E., Anderson B., Jacobsen L.B., Nichols D.E., Moore D.S., McLaughlin J.L.** (1982) J. Nat. Prod., **45**, 679-686.
19. **Galsky A.G., Wilsey T.P., Powell R.G.** (1980) Plant Physiol., **65**, 184-185.
20. **Ghisalberti E.L.** (1993) In: Bioactive Natural Products, Detection, Isolation and Structural Determination (S.M. Colegate, R.J. Molyneux, Eds.), CRC Press, 9-49.
21. **Glogowski W., Galsky A.G.** (1978) Plant Physiol., **61**, 1031-1033.
22. **Grieve M.** (1982) A Modern Herbal, Vol I and II, Dover Publications, Inc., New York.
23. **Gulcin I., Beydemir S., Sat I.G., Kufrevioglu O.I.** (2005) Acta Aliment., **34**, 193-202.
24. **Guven K., Yucel E., Cetintas F.** (2006) Pharm. Biol., **44**, 79-83.

25. **Hashempour A., Ghazvini R.F., Bakhshi D., Ghasemnezhad M., Sharafti M., Ahmadian H.** (2010) Hort. Environ. Biotechnol., **51**, 83-88.
26. **Hosseinimehr S.J., Azadbakht M., Abadi A.J.** (2008) Environ. Toxicol. Phar., **25**, 51-56.
27. **Johnson J.L, Boniser J.A., Scheerens J.C., Giusti M.M.** (2011) J. Agr. Food Chem., **59**, 1638-1645.
28. **Kao E.S., Wang C.J., Lin W.L., Chu C.Y., Tseng T.H.** (2007) Food Chem. Toxicol., **45**, 1795-1804.
29. **Kilicgun H., Altiner D.** (2010) Pharmacogn. Mag., **6**, 238-241.
30. **Krisch J., Galgoczy L., Tolgyesi M., Papp T., Vagvolgyi C.** (2008) Acta Biol. Szegediensis, **52**, 267-270.
31. **Kumarasamy Y., Cox P.J., Jaspars M., Nahar L., Sarker S.D.** (2002) J. Ethnopharmacol., **83**, 73-77.
32. **Kumarasamy Y., Cox P.J., Jaspars M., Rashid M.A., Sarker S.D.** (2003) Pharm. Biol., **41**, 237-242.
33. **Larsen E., Christensen L.P.** (2007) J. Food Lipids, **14**, 272-279.
34. **Laux M.T., Aregullin M., Rodriguez E.** (2007) Nat. Prod. Com., **2**, 1015-1018.
35. **Lippincott B.B., Lippincott J.A.** (1969) J. Bacteriol., **2**, 620-628.
36. **Mamedov N., Craker L.E.** (2004) Acta Hortic., **629**, 83-86.
37. **McLaughlin J.L.** (1991) In: Methods in Plant Biochemistry (K. Hostetsman, Ed.), Academic Press, London, 1-31.
38. **McLaughlin J.L., Chang C.J., Smith D.L.** (1993) In: Human Medicinal Agents from Plants (A.D. Kinghorn, M.F. Baladrin, Eds.), ACS Symposium Series 534, Washington DC, 112-137.
39. **McLaughlin J.L., Rogers L.L., Anderson J.E.** (1998) Drug Inf. J., **32**, 513-524.
40. **Min B.S., Kim Y.H., Lee S.M., Jung H.J., Lee J.S., Na M.K., Lee C.O., Lee J.P., Bae K.** (2000) Arch. Pharm. Res., **23**, 155-158.
41. **Milivojevic J., Maksimovic V., Nikolic M., Bogdanovic J., Maletic R., Milatovic D.** (2011) J. Food Quality, **34**, 1-9.
42. **Orhan N., Aslan M., Hosbas S., Orhan D.D.** (2009) Pharmacogn. Mag., **5**, 309-315.
43. **Oskay M., Sari D.** (2007) Pharm. Biol., **45**, 176-181.
44. **Ozcan M.** (2000) Acta Aliment, **29**, 377-384.
45. **Pantelidis G.E., Vasilakakis M., Manganaris G.A., Diamantidis G.** (2007) Food Chem., **102**, 777-783.
46. **Proestos C., Boziaris I.S., Kapsokefalou M., Komaitis M.** (2008) Food Technol. Biotechnol., **46**, 151-156.
47. **Raskin I., Ribnicky D.M., Komarnytsky S., Ilic N., Poulev A., Borisjuk N., Brinker A., Moreno D.A., Ripoll C., Yakoby N., O'Neal J.M., Cornwell T., Pastor I., Fridlender B.** (2002) Trends Biotechnol., **20**, 522-531.
48. **Rauha J.P., Remes S., Heinonen M., Hopia A., Kahkonen M., Kujala T., Pihlaja K., Vuorela H., Vuorela P.** (2000) Int. J. Food Microbiol., **56**, 3-12.
49. **Rios J.L., Recio M.C.** (2005) J. Ethnopharmacol., **100**, 80-84.
50. **Rop O., Reznicek V., Valsikova M., Jurikova T., Mlcek J., Kramarova D.** (2010) Molecules, **15**, 4467-4477.
51. **Saenz M.T., Ahumada M.C., Garcia M.D.** (1997) Z. Naturforsch. C., **52**, 42-44.
52. **Sagdic O., Aksoy A., Ozkan G.** (2006) Acta Aliment., **35**, 487-492.
53. **Savikin K., Zdunic G., Jankovic T., Stanojkovic T., Juranic Z., Menkovic N.** (2009) Nat. Prod. Res., **23**, 1731-1739.
54. **Seeram N.P., Adams L.S., Zhang Y.J., Lee R., Sand D., Scheuller H.S., Heber D.** (2006) J. Agr. Food Chem., **54**, 9329-9339.
55. **Seeram N.P.** (2008) J. Agr. Food Chem., **56**, 630-635.
56. **Serteser A., Kargioglu M., Gok V., Baci Y., Ozcan M.M., Arslan D.** (2008) Int. J. Food Sci. Nutr., **59**, 643-651.
57. **Stoner G.D., Chen T., Kresty L.A., Aziz R.M., Reinemann T., Nines R.** (2006) Nutr. Cancer, **54**, 33-46,
58. **Tadic V.M., Dobric S., Markovic G.M., Orevic S.M., Arsic I.A., Menkovic N.R., Stevic T.** (2008) J. Agr. Food Chem., **56**, 7700-7709.
59. **Tural S., Koca I.** (2008) Sci. Hortic., **116**, 362-366.
60. **Turker A., Usta C.** (2006) Biotechnol. Biotech. Eq., **20**, 105-113.
61. **Tuzlaci E., Aymaz P.E.** (2001) Fitoterapia, **72**, 323-343.
62. **Yilmaz N., Yayli N., Misir G., Coskuncelebi K., Karaoglu S., Yayli N.** (2008) Asian J. Chem., **20**, 3324-3330.
63. **Yilmaz B. S, Citoglu G. S, Altun M. L., Ozbek H.** (2007) Pharm. Biol, **45**, 241-245.