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Biological screening of various medicinal plant extracts for antibacterial and antitumor activities

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Abstract: Bioassays of 2 types (antibacterial and antitumor) were performed to show the biological activities of 16 different plants grown in Bolu, Turkey: *Clinopodium vulgare* L. subsp. *vulgare* L., *Salvia verticillata* L. subsp. *amasiaca* (Frey & Bornm.) Bornm., *Salvia tomentosa* Mill., *Mentha pulegium* L., *Melilotus officinalis* (L.) Desr., *Melilotus alba* Desr., *Medicago lupulina* L., *Galega officinalis* L., *Xeranthemum annuum* L., *Cichorium intybus* L., *Plantago lanceolata* L., *Plantago major* L. subsp. *major*, *Fumaria officinalis* L., *Galium palustre* L., *Echium vulgare* L., and *Sambucus nigra* L. For each plant, 3 different extracts (aqueous, ethanol, and methanol) were obtained, and a total of 48 extracts were evaluated. Antibacterial activity was evaluated with 10 bacteria, including *Streptococcus pyogenes*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Serratia marcescens*, *Proteus vulgaris*, *Enterobacter cloacae*, and *Klebsiella pneumoniae* by disk diffusion method. All plants except *M. alba*, *M. lupulina*, *X. annuum*, *G. palustre*, and *S. nigra* showed inhibitory activity against both gram-positive and gram-negative bacteria. The best inhibitory activity was observed with aqueous extract of *M. officinalis* (22.5 mm); it performed better than all positive controls (erythromycin, ampicillin, carbenicillin, tetracycline, and chloramphenicol; 7–20 mm) against *P. aeruginosa*. Antitumor activity was evaluated with *Agrobacterium tumefaciens*-induced potato disk tumor assay. The best antitumor activity was obtained with the methanolic extract of *M. alba* and aqueous extract of *F. officinalis* (100% tumor inhibition).

Key words: Bioassays, antibacterial, antitumor, biological activity, medicinal plants

Introduction

For many years, plants have been used as therapeutic resources either as herbal teas or other homemade remedies, as crude extracts, or as standard enriched fractions in pharmaceutical preparations such as tinctures, fluid extracts, powders, pills, and capsules (1). The World Health Organization estimates that 80% of people in developing countries (65% of the world's population) still rely on traditional medicine (2). Plants have been rich sources of medicines because they produce a host of bioactive molecules, most of which probably evolved as chemical defenses against infection (3). Bioassays are adaptable for screening and testing plant extracts (4). Extracts from a broad spectrum of plant species contain substances that possess antitumor activity (1). New drugs from plant secondary metabolites are being developed and studied for their antibacterial and anticancer activities (5). Increasingly, natural products have potential for the treatment of cancer. Cancer incidence and mortality increased by approximately 22% up until 1990. In 2000 there were 10 million new cases and over 6 million deaths worldwide (6). Plant-derived drugs are playing an important role in the upgrowth of cancer therapy (5). Most of the active compounds in these extracts remain unidentified, and their presence is only detected by biological tests.

The structure and mechanism of action in others have been elucidated (1), and some compounds such as rubomycin, vinblastine, vincristine, colchamine, and camptothecin have been investigated for their activities in chemotherapy (5). Most of the identified compounds are products of plant secondary metabolism and belong to the classes of alkaloids, polyphenols, triterpenes, or steroid glycosides (1). *Clinopodium vulgare* is one of the curative plants used in folk medicine for wound healing and treatment of warts due to viral infection (7). It also has cytotoxic and antitumor effects (1). *Salvia* species are widespread plants in many countries. They were reportedly used for memory-enhancing purposes in European folk medicine (8). *S. tomentosa* has been used in traditional medicine for the treatment of flatulent dyspepsia, laryngitis, pharyngitis, stomatitis, gingivitis, glossitis, hyperhidrosis, and galactorrhea (9). Antimicrobial and antioxidant activities of the various extracts of *S. tomentosa* and *S. verticillata* were studied (10). *Mentha pulegium* is one of the *Mentha* species commonly known as pennyroyal. The flowering aerial parts of *M. pulegium* have been used traditionally for the treatment of cold, sinusitis, cholera, food poisoning, bronchitis, and tuberculosis (11). They are also used for antiseptic, antiflatulent, carminative, expectorant, diuretic, and antitussive purposes (12). The abortifacient effect of *M. pulegium* essential oil in rat myometrium, cytotoxic activity against different human cell lines, and antioxidant activity (11) were also reported. For centuries, *Melilotus* species have been used to reduce spasm, in liver diseases, and as diuretics (12). For inflammation-related therapy *M. officinalis* (sweet clover) has antiinflammatory and antioxidant activities (12). The plant contains coumarin, coumaric acid, dicoumarol, melilitin, essential oil, and slime (13). *Medicago lupulina* (black medic) contains saponins (a large group of triterpenes) and steroid glycosides (14). *Galega officinalis* is widely used in folk medicine as an antidiabetic or for increasing lactation (15). Recent experimental investigations indicate that crude aqueous extracts and gel filtered fractions of the plant suppress platelet aggregation (15). This plant has been used for treatment of the plague, malignant fevers, and parasitic infection and was also widely cultivated as cattle feed in ancient times (16). The ethanolic (60%) extract of *G. officinalis* was tested against both gram-positive and gram-negative bacteria as the plant was suggested to hasten skin healing after surgery, and the antibacterial effect was shown (15). It is believed that galegine is the major compound in *G. officinalis* that causes injury (15). *Cichorium intybus* (chicory) is widely used in India as a traditional treatment for diabetes mellitus (17). It has been shown to affect cholesterol uptake and tumor development in mice and has antiinflammatory properties (18). *Plantago* is the most important genus of the family Plantaginaceae and is used in traditional medicine around the world for different purposes. *P. lanceolata* has been used for inflammation, cellular regeneration, and ulcers and possesses antimicrobial and nematicidal activities (19). The aerial parts of *P. lanceolata* are also used to treat bronchial catarrh and inflammation of the mucous membrane of the pharynx (20). *P. major* has been used for the treatment of skin diseases, infectious diseases, tumors, high fever, pains, and problems concerning the digestive organs, respiratory organs, reproduction, and circulation (21). *P. major* is also used as a remedy for colds and viral hepatitis (21). *Fumaria* species have been used for the treatment of hepatobiliary system diseases (22). *F. officinalis* is a useful plant for the eyes and is used to remove skin blemishes. Today, herbalists use it to treat skin diseases, conjunctivitis, rheumatism, hypertension, and infections as well as to cleanse the kidneys (22). It is also used for dermatological indications (e.g., milk crust, eczema, and scabies) or as a diuretic or laxative (22). Extracts of *Galium* species have long been used in folk medicine for a variety of purposes, mainly as diuretics, astringents, and choleretics and in the treatment of some stomach diseases, gout, and epilepsy (23). *Echium vulgare* (24) has been used as an expectorant and laxative. *Sambucus nigra* (elderflower) is recommended by the German Commission E for upper respiratory tract infections (25). In the meantime, however, promising elderberry properties such as antioxidative, antibacterial (26), antiviral (27), and antiinflammatory (28) actions have been detected.

The objective of this study was to assess the antibacterial and antitumor activities of aqueous, methanol, and ethanol extracts of 16 different plants grown in Bolu, Turkey.

Materials and methods

Plant material and extraction

The 16 plant species studied were collected from the campus of Abant İzzet Baysal University, Bolu, Turkey. Identification of the species was made using *Flora of Turkey and the East Aegean Islands* (29), and voucher specimens were deposited at the Abant İzzet Baysal University (AIBU) Herbarium, Bolu, Turkey. All plant samples and voucher numbers are presented in Table 1. Collected plants were dried in an oven at 40 °C and then ground into a powder. For extraction, 3 different solvents [water, methanol (MeOH), and ethanol (EtOH)] were used. For aqueous extraction, 50 g from each plant sample were extracted with 400 mL water at 80 °C in a water bath for 18 h. The extract was then filtered and lyophilized. For alcoholic extractions, 50 g of plant sample were Soxhlet-extracted with 500 mL MeOH or EtOH at 55 °C for 18 h. The extract was then vacuum-evaporated. For antibacterial and antitumor assays, each residue was dissolved in sterile distilled water in order to obtain a final concentration of 100 mg/mL. Plant materials, designation of treatments, and yield (%) for each extraction are summarized in Table 1.

Antibacterial bioassay

The disk diffusion assay (Kirby–Bauer method) was used to screen for antibacterial activity (30). The gram-negative bacteria *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) and the gram-positive bacteria *Streptococcus pyogenes* (ATCC® 19615), *Staphylococcus aureus* (ATTC® 25923), and *Staphylococcus epidermidis* (ATCC® 12228) were the microorganisms used. Each lyophilized bacteria disk (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, streaked on a TSA plate, and incubated at 37 °C for 2 additional days. The turbidity of each broth culture was then adjusted with saline to obtain turbidity visually comparable to that of a 0.5 McFarland standard.

All extracts were sterilized by filtering through a 0.22-μm filter (Millex®), and sterile filter paper disks (glass microfiber filters, Whatman®; 6 mm in diameter) were impregnated with 13 μL of extract. There were 5 replicates in each plate and 2 plates for each extract tested for each bacterium. Positive controls consisted of 5 different antimicrobial susceptibility test disks (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). For each plate, 4 antibiotic disks were used and run in duplicate. Water was used as a negative control. Inoculated plates with disks were placed in a 37 °C incubator. After 16 to 18 h of incubation, the inhibition zone diameter (mm) was measured. All experiments were repeated 3 times.

Potato disk tumor induction assay

The antitumor activity of extracts was assessed by potato disk method as modified by McLaughlin's group (4,31). *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. Suspensions of *A. tumefaciens* in phosphate buffered saline (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 (32). 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 immersed in 20% commercial bleach (Domestos®) for 15 min. Cylinders (10 mm in diameter) were cut from the center of the 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 disks after excluding 1-cm end pieces. These disks were transferred to 24-well culture plates containing

Table 1. Family and plant species names of studied plants, collection times, collection numbers, part used, extract, and yield (%) for each extraction.

Family and plants species	Collection time	Collection number	Part used	Extract	Yield (%)*
LAMIACEAE					
Clinopodium vulgare L.	July 2010	AUT-1901	Aerial	Water	20.0
subsp. *vulgare* L.				EtOH	9.0
				MeOH	9.0
Salvia verticillata L. subsp.	July 2010	AUT-1903	Leaves	Water	12.0
amasiaca (Freyn & Bornm.) Bornm.				EtOH	5.0
				MeOH	9.0
Salvia tomentosa Mill.	July 2010	AUT-1904	Leaves	Water	3.8
				EtOH	6.3
				MeOH	12.5
Mentha pulegium L.	August 2010	AUT-1918	Aerial	Water	11.9
				EtOH	15.0
				MeOH	20.0
FABACEAE					
Melilotus officinalis (L.) Desr.	May 2010	AUT-1911	Aerial	Water	33.8
				EtOH	5.0
				MeOH	20.0
Melilotus alba Desr.	May 2010	AUT-1915	Aerial	Water	18.9
				EtOH	2.5
				MeOH	15.0
Medicago lupulina L.	May 2010	AUT-1920	Aerial	Water	12.5
				EtOH	2.5
				MeOH	12.5
Galega officinalis L.	September 2010	AUT-1912	Aerial	Water	25.8
				EtOH	5.8
				MeOH	18.5
ASTERACEAE					
Xeranthemum annuum L.	July 2010	AUT-1902	Aerial	Water	30.0
				EtOH	9.0
				MeOH	12.0
Cichorium intybus L.	July 2010	AUT-1908	Aerial	Water	6.5
				EtOH	4.0
				MeOH	7.5
PLANTAGINACEAE					
Plantago lanceolata L.	July 2010	AUT-1905	Leaves	Water	12.5
				EtOH	5.0
				MeOH	4.0
Plantago major L. subsp. *major.*	July 2010	AUT-1933	Leaves	Water	22.3
				EtOH	27.2
				MeOH	16.4

Table 1 continued.

PAPAVERACEAE					
Fumaria officinalis L.	May 2010	AUT-1906	Aerial	Water	14.8
				EtOH	8.0
				MeOH	20.0
RUBIACEAE					
Galium palustre L.	July 2010	AUT-1921	Aerial	Water	13.5
				EtOH	6.3
				MeOH	15.0
BORAGINACEAE					
Echium vulgare L.	May 2010	AUT-1907	Aerial	Water	5.5
				EtOH	2.5
				MeOH	5.0
CAPRIFOLIACEAE					
Sambucus nigra L.	June 2010	AUT-1917	Leaves and fruits	Water	16.0
				EtOH	5.0
				MeOH	10.0

*Yield (%) = weight of extract (g) / 50 g of plant sample × 100.

water–agar (15 g/L). Each disk was overlaid with 50 µL of the appropriate inoculum. No more than 30 min elapsed between the cutting of the potato disks and inoculation (4). Plates were incubated at 28 °C in the dark for 2 weeks. After 2 weeks, disks were stained with Lugol's reagent (I_2KI; 5% I_2 + 10% KI in distilled water) and the tumors on each disk were counted. Lugol's reagent stains the starch in potato tissue dark blue to dark brown in color, but the tumors do not take up the stain and appear creamy to orange. Experiments were repeated 3 times. Percent inhibition of tumors was calculated using the following formula:

% inhibition = [(solvent control mean – tested extract mean) / solvent control mean] × 100 (4).

Data analysis

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

Results and discussion

Forty-eight different extracts prepared with 3 kinds of solvents (water, methanol, and ethanol) of 16 different plant species were tested in order to screen and show their potential as antibacterial (Table 2) and antitumor (Table 3) agents. The Kirby–Bauer test (disk diffusion method) is the most widely used standard method for antibacterial bioassay. It is currently performed by the National Committee for Clinical Laboratory Standards for disk diffusion susceptibility testing (30). Bacterial growth was generally sensitive to the reference antibiotics tested (Table 2). Since final concentrations of all extracts were adjusted with distilled water, it was used as a negative control, and there was no inhibition with this control solvent.

Although tested extracts showed especially high antibacterial activity against gram-negative bacteria and no significant or weak activities (except *G. officinalis*) against gram-positive bacteria, gram-positive bacteria seem more susceptible to the inhibitory effects of the plant extracts in general. This may come from their cell wall structure, consisting of a single layer. The gram-negative cell wall is a quite complex, multilayered structure and more resistant to the inhibitory effects of the plant extracts (33,34).

The best antibacterial activity was obtained with aqueous extract of *M. officinalis* (22.5 mm), which was stronger than the positive control antibiotics against *P. aeruginosa* (Table 2). The antibacterial

Table 2. Antibacterial activity of plant extracts, positive controls [ampicillin (10 µg), carbenicillin (100 µg), chloramphenicol (30 µg), erythromycin (15 µg), and tetracycline (30 µg)], and negative control (water). Means with the same letter within columns are not significantly different at P > 0.05.

Treatments	Extract	Mean diameter (± SE) of inhibitory zones (mm)																											
		Gram +								Gram –																			
		S. pyogenes		*S. aureus*		*S. epidermidis*		*S. marcescens*		*S. typhimurium*		*P. aeruginosa*		*P. vulgaris*		*K. pneumonia*		*E. cloacae*		*E. coli*							
C. vulgare	Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.0 ± 0.0	e	-	-	-	-						
	EtOH	-	-	-	-	-	-	-	-	-	-	-	-	9.0 ± 0.2	f,g,h	9.0 ± 0.0	d	-	-	-	-						
	MeOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
S. verticillata	Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	11.0 ± 0.0	c	10.0 ± 0.2	e	-	-						
	EtOH	-	-	-	-	-	-	-	-	-	-	-	-	9.0 ± 0.1	f,g,h	-	-	-	-	-	-						
	MeOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
S. tomentosa	Water	-	-	-	-	-	-	-	-	8.0 ± 0.0	e	-	-	-	-	-	-	-	-	-	-						
	EtOH	-	-	-	-	-	-	-	-	-	-	-	-	10.0 ± 0.0	e,f	-	-	10.0 ± 0.3	e	-	-						
	MeOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10.17 ± 0.5	e	-	-						
M. pulegium	Water	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
	EtOH	-	-	-	-	-	-	10.0 ± 0.4	e	-	-	-	-	-	-	-	-	-	-	-	-						
	MeOH	8.5 ± 0.43	f	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.0 ± 0.4	f	-	-						
M. officinalis	Water	-	-	-	-	-	-	-	-	-	-	22.5 ± 1.1	a	8.5 ± 0.2	g,h,i	-	-	8.33 ± 0.2	f	9.33 ± 0.2	e,f						
	EtOH	-	-	-	-	-	-	8.2 ± 0.6	f	8.17 ± 0.6	e	9.0 ± 0.0	e	7.5 ± 0.2	i,j	-	-	-	-	9.0 ± 0.4	f						
	MeOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
G. officinalis	Water	-	-	13.33 ± 0.2	f	16.8 ± 0.8	e	8.2 ± 0.2	f	8.5 ± 0.2	d,e	8.5 ± *0.22*	e,f	8 ± 0.4	h,i,j	-	-	8.0 ± 0.1	f	7.7 ± 0.2	g						
	EtOH	-	-	7.7 ± 0.2	i	7.7 ± 0.2	f,g	-	-	9.0 ± 0.4	c,d,e	7.83 ± *0.48*	f	8.3 ± 0.3	g,h,i,j	-	-	-	-	9.0 ± 0.3	f						
	MeOH	11.2 ± 0.5	e	10.0 ± 0.4	g	-	-	i	-	-	-	-	-	-	-	-	-	-	-	-	-						
C. intybus	Water	-	-	-	-	-	-	i	7.7 ± 0.2	f,g	-	-	-	-	-	-	-	8.0 ± 0.3	f	8.0 ± 0.0	g						
	EtOH	-	-	-	-	7.5 ± 0.2	g,h	7.0 ± 0.0	g	-	-	-	-	8.3 ± 0.3	g,h,i,j	-	-	7.67 ± 0.2	f	-	-						
	MeOH	-	-	-	-	-	-	i	-	-	h	-	-	-	-	-	-	-	-	-	-						
P. lanceolata	Water	-	-	8.0 ± 0.2	h	7.5 ± 0.2	g,h	7.7 ± 0.2	f,g	-	-	-	-	9.3 ± 0.9	f,g	-	-	-	-	-	-						
	EtOH	-	-	-	-	7.0 ± 0.3	h	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
	MeOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
P. major	Water	-	-	-	-	-	-	-	-	-	-	-	-	10.7 ± 0.2	e	-	-	-	-	-	-						
	EtOH	-	-	-	-	-	-	-	-	-	-	-	-	7.33 ± 0.2	j	-	-	-	-	-	-						
	MeOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
F. officinalis	Water	-	-	-	-	-	-	-	-	9.5 ± 1.0	c,d	-	-	-	-	-	-	-	-	-	-						
	EtOH	-	-	-	-	-	-	7.8 ± 0.2	f,g	8.0 ± 0.4	e	-	-	-	-	-	-	-	-	-	-						
	MeOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
E. vulgare	Water	-	-	-	-	-	-	8.0 ± 0.4	f	-	-	-	-	-	-	-	-	8.2 ± 0.2	f	-	-						
	EtOH	-	-	-	-	7.5 ± 0.2	g,h	7.0 ± 0.2	g	-	-	-	-	-	-	-	-	-	-	-	-						
	MeOH	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						
Ampicillin		48.3 ± 1.05	a	40.67 ± 0.4	b	22.0 ± 0.6	d	17.7 ± 0.9	d	26.0 ± 0.7	a	8.0 ± 0.5	f	27 ± 0.5	c	9.0 ± 0.4	d	25.0 ± 0.7	d	19.0 ± 0.4	d						
Carbenicillin		46.0 ± 0.2	b	41.5 ± 0.2	a	25.0 ± 0.7	c	20.0 ± 0.5	c	23.3 ± 1.3	b	18.3 ± 0.4	b	33.33 ± 1.7	b	7.3 ± 0.2	f	33.5 ± 0.2	a	20.5 ± 0.2	c						
Chloramphenicol		31.0 ± 0.45	d	23.67 ± 0.2	e	31.0 ± 0.7	a	29.0 ± 0.6	a	26.5 ± 1.4	a	12.7 ± 0.2	d	20 ± 0.7	d	26.7 ± 1.1	b	27.7 ± 0.8	c	27.0 ± 0.6	b						
Erythromycin		38.3 ± 1.05	c	24.17 ± 0.6	d	30.0 ± 0.3	b	7.3 ± 0.5	f,g	10.0 ± 0.3	c	6.3 ± 2.0	g	10 ± 0.1	e,f	10.7 ± 0.2	c	7.5 ± 0.6	f	9.7 ± 0.2	e						
Tetracycline		38.3 ± 1.05	c	30.33 ± 0.2	c	8.2 ± 0.2	f	24.5 ± 0.2	b	24.2 ± 1.1	b	16.7 ± 0.2	c	35 ± 1.0	a	27.7 ± 0.4	a	28.7 ± 0.6	b	29.0 ± 0.6	a						
Water		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-						

activity of *M. officinalis* may explain why *Melilotus* species are used in folk medicine in inflammation-related therapy (caused by *P. aeruginosa*). Generally, ethanolic extract of *M. officinalis* was better than aqueous and methanolic extracts of this plant against *S. marcescens* and *S. typhimurium*. Although *P. vulgaris*, *E. cloacae*, and *E. coli* were susceptible to aqueous extract of *M. officinalis*, no tested bacteria were susceptible to methanolic extracts (Table 2). It is known that the coumarin found in *M. officinalis* has antiinflammatory effects (12); the strong antibacterial activity of *M. officinalis* may be due to coumarin.

C. vulgare extracts had no strong antibacterial activity against the bacteria used. Aqueous or ethanolic extracts of this plant showed just a little activity (8–9 mm) only against *K. pneumonia* and *P. vulgaris* in our study (Table 2). On the other hand, Opalchenova and Obreshkova (7) found a very strong antibacterial activity of 5% propylene glycol and ethanol extracts of *C. vulgare*. Stefanovic et al. (35) also indicated the antibacterial activity of ethanol, ethyl acetate, and acetone extract of *C. vulgare* by determining minimum inhibitory concentrations (MICs) using the microdilution method. The most sensitive bacteria were the gram-positive bacteria *S. aureus* and *Bacillus subtilis*.

Aqueous extract of *S. verticillata* was more effective than carbenicillin and erythromycin against *K. pneumoniae* in our study (Table 2). Ethanolic and methanolic extracts of *S. tomentosa* also had stronger antimicrobial activity than erythromycin (positive control) against *P. vulgaris* and *E. cloacae* (Table 2). Haznedaroglu et al. (36) showed that essential oil obtained from aerial parts of *S. tomentosa* had weak antibacterial activity against limited microorganisms including *E. coli*, *S. aureus*, and *P. aeruginosa*. In contrast, Tepe et al. (37) reported that the essential oil of *S. tomentosa* exhibited strong antimicrobial activity against *S. aureus*, *S. pneumoniae*, *Clostridium perfringens*, and *Mycobacterium smegmatis*.

Although ethanolic extract of *M. pulegium* showed moderate activity against *S. marcescens* and methanolic extract showed weak activity against *S. pyogenes* and *E. cloacae*, other tested bacteria were not inhibited by *M. pulegium* extracts in our study (Table 2). Mahboubi and Haghi (11) reported antimicrobial activities of the essential oils (major components: piperitone, piperitenone, terpineol, and pulegone) of *M. pulegium*. They also observed significant antibacterial activity with the gram-positive bacteria *S. aureus* and *S. epidermidis*, with inhibition zones and MIC values in the range of 8–21 mm and 0.25–4 µL/mL, respectively, whereas gram-negative bacteria were least susceptible, especially *E. coli*.

Tested extracts of *G. officinalis* exhibited broad-spectrum activity against both gram-positive and gram-negative bacteria (Table 2). This activity against both types of bacteria may be indicative of the presence of broad-spectrum antibiotic compounds or simply general metabolic toxins (34). In particular, aqueous and ethanolic extracts of *G. officinalis* showed antibacterial activity against the gram-positive bacteria *S. aureus* and *S. epidermidis* and the gram-negative bacteria *S. marcescens*, *S. typhimurium*, *P. aeruginosa*, *P. vulgaris*, and *E. coli* (Table 2). Antibacterial activity of the aqueous extract of *G. officinalis* was greater than that of the reference antibiotic tetracycline against *S. epidermidis* (Table 2). Only the methanolic extract of *G. officinalis* showed inhibition against *S. pyogenes* (Table 2). Similarly, Özbucak et al. (38) reported that alcoholic extracts of *G. officinalis* were active against the bacteria species used (*P. aeruginosa* and *E. coli*). Pundarikakshudu et al. (39) also indicated that ethanolic extract of *G. officinalis* was active against the gram-positive bacterium *S. aureus* and the gram-negative bacterium *S. marcescens*.

All extracts of *C. intybus* failed to show any antibacterial activity against *S. aureus*. Aqueous and ethanolic extracts of *C. intybus* were more active than erythromycin against *S. epidermidis* and *E. cloacae* (Table 2). Conversely, Aqil and Ahmad (40) reported that ethanolic extract of *C. intybus* showed a synergistic interaction with tetracycline, chloramphenicol, and ciprofloxacin against *S. aureus* and/or *E. coli*.

P. vulgaris was the only bacterium that was inhibited by aqueous or ethanolic extracts of *P. major* (10.7 mm and 10 mm, respectively) (Table 2). Methanolic extract of *P. lanceolata* did not show any significant antibacterial activity against gram- positive or gram-negative bacteria, and aqueous extract exhibited weak or moderate antimicrobial activity against *S. aureus*, *S. epidermidis*, *S. marcescens*, and *P. vulgaris* in our study (Table 2). Similarly, Bazzaz and Haririzadeh (41) indicated that *P. lanceolata* extracts

were not active against the bacteria species used (*K. pneumonia*, *P. aeruginosa*, *P. vulgaris*, *Shigella sonnei*, *Salmonella paratyphi*, *Vibrio cholerae*, *E. coli*, *S. aureus*, and *Bacillus anthracis*). Moreover, it was reported that pressed juice of fresh *P. lanceolata* had a bactericidal effect (41).

Only aqueous and ethanolic extracts of *F. officinalis* showed weak antibacterial activity against *S. marcescens* and *S. typhimurium* (between 7.8 and 9.5 mm). Similarly, Erdogrul (42) reported that *F. officinalis* did not show any antibacterial activity against the tested microorganisms (*Bacillus brevis*, *Bacillus megaterium*, *Bacillus subtilis*, *Micrococcus luteus*, *Mycobacterium smegmatis*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Streptococcus thermophilus*, *Pseudomonas fluorescens*, and *Yersinia enterocolitica*).

Methanolic extract of *E. vulgare* did not show antibacterial effect against any bacteria. However, aqueous extract of *E. vulgare* showed some antibacterial activity against *S. epidermidis*, *S. marcescens*, and *E. cloacae* (between 7 and 8.2 mm) (Table 2). Similarly, Kuruüzüm et al. (43) indicated that the methanol-soluble constituents of *E. vulgare* showed no detectable antimicrobial activity. Moreover, Allen et al. (44) reported that *E. vulgare* honey has no detectable antibacterial activity.

M. alba, *M. lupulina*, *X. annuum*, *G. palustre*, and *S. nigra* showed no inhibitory activity against any of the pathogens used in our study (data not shown). Similarly, Borchardt et al. (45) reported that *M. lupulina* was not active against the bacteria species used. In contrast, Hearst et al. (26) indicated strong antimicrobial effects of flowers and berries of *S. nigra* on various pathogens.

There is evidence that some plant extracts show a cell-type anticancer activity. Such plant extract activity may be attributed to the different classes of compounds that are found in the extract (5). The inhibition of *A. tumefaciens*-induced tumors (or crown gall) in potato disk tissue is an assay based on antimitotic activity that can detect a broad range of known and novel antitumor effects (4,32). The validity of this bioassay is predicted on the observation that certain tumorigenic mechanisms are similar in plants and animals. It was demonstrated that inhibition of crown gall tumor initiation on potato disk showed an apparent correlation with compounds and plant extracts known to be active in the 3PS (in vivo, murine leukemia) antitumor assay (4). Ferrigini et al. (31) showed that crown gall tumors on potato disks could routinely be employed as a comparatively rapid, inexpensive, safe, and statistically reliable prescreen for 3PS antitumor activity.

In the present study, methanolic extract of *M. alba* and aqueous extract of *F. officinalis* showed the best antitumor activity and were similar to the positive control, camptothecin (100% tumor inhibition) (Table 3). *M. officinalis* and *M. alba* contain flavones, volatile oils, resins, and tannins (46). There have been no records regarding the anticancer activity of *M. alba* and *F. officinalis* up to now; the current study provides the first report for this activity.

Among all tested extracts of *C. vulgare*, the best antitumor activity was observed with methanolic extract (85% tumor inhibition). The percentage inhibitions of all extracts of *C. vulgare* were more than 61% (Table 3). Dzhambazov et al. (1) reported that extracts from *C. vulgare* have a selective cytotoxic effect when incubated in vitro with normal and cancer cell lines. *C. vulgare* was found to be active only against MCF-7 cells in the present study with an LC_{50} of 60.4 µg/mL. Aqueous extract of *C. vulgare* showed strong antitumor activity when tested in vitro on A2058 (human metastatic melanoma), HEp-2 (epidermoid carcinoma, larynx, human), and L5178Y (mouse lymphoma) cell lines. Triterpenes and triterpenoid saponin ingredients (1) of *Clinopodium* species may contribute to the strong anticancer activities.

Fiore et al. (47) reported in vitro antitumor activity of the methanol crude extracts of 6 *Salvia* species using the MTT test on human tumor cell lines. All MeOH crude extracts of the 6 *Salvia* species exhibited marked antiproliferative activity against all tumor cell lines. Similarly, in the present study, the highest antitumor activity of *S. tomentosa* (88%) and *S. verticillata* (86%) was shown with methanolic extracts. Among all extracts of *M. pulegium*, the best antitumor activity was observed with aqueous extracts (94%). Better antitumor activity was obtained with aqueous and methanolic extracts (82% and 88%, respectively) of *M. lupulina* than with ethanol extract (55%). Among all extracts of *G. officinalis*, aqueous extract showed the best antitumor activity (98%). Literature data indicated that 2 phytoestrogens

Table 3. Antitumor activity of plant extracts, positive control (camptothecin), and negative control (water). Means with the same letter within columns are not significantly different at P > 0.05.

Treatments	Extract	Mean number of tumors (± SE)				% Tumor inhibition
Water (negative control)		48.63	±	3.53	o	-
Camptothecin (positive control)		0.00	±	0.00	a	100
C. vulgare	Water	19.25	±	2.12	k,l,m	61
	EtOH	9.25	±	1.23	c,d,e,f,g	82
	MeOH	7.42	±	1.05	a,b,c,d,e,f	85
S. verticillata	Water	10.13	±	1.75	c,d,e,f,g	80
	EtOH	9.39	±	1.13	c,d,e,f,g	82
	MeOH	6.84	±	0.85	a,b,c,d,e,f	86
S. tomentosa	Water	25.33	±	3.85	m	49
	EtOH	11.75	±	2.81	e,f,g,h,i	76
	MeOH	6.42	±	0.86	a,b,c,d,e,f	88
M. pulegium	Water	3.21	±	0.80	a,b,c,d	94
	EtOH	11.00	±	1.45	e,f,g,h,i	78
	MeOH	7.08	±	1.42	a,b,c,d,e,f	86
M. officinalis	Water	15.92	±	2.86	g,h,i,j,k,l	67
	EtOH	11.88	±	1.57	e,f,g,h,i	76
	MeOH	8.25	±	1.68	b,c,d,e,f	84
M. alba	Water	8.29	±	1.60	b,c,d,e,f	84
	EtOH	9.88	±	1.78	c,d,e,f,g	80
	MeOH	0.17	±	0.12	a	100
M. lupulina	Water	8.63	±	1.74	i,j,k,l,m,n	82
	EtOH	21.63	±	2.20	l,m	55
	MeOH	6.08	±	0.86	j,k,l,m,n,o	88
G. officinalis	Water	1.00	±	0.84	a,b	98
	EtOH	8.54	±	1.32	i,j,k,l,m,n	82
	MeOH	6.33	±	1.89	a,b,c,d,e,f	88
X. annuum	Water	17.50	±	1.84	i,j,k,l	63
	EtOH	7.00	±	1.23	a,b,c,d,e,f	86
	MeOH	7.42	±	1.13	a,b,c,d,e,f	86
C. intybus	Water	13.67	±	2.45	e,f,g,h,i,j	71
	EtOH	6.21	±	1.17	a,b,c,d,e,f	88
	MeOH	6.00	±	1.25	a,b,c,d,e,f	88
P. lanceolata	Water	22.63	±	4.60	l,m	53
	EtOH	10.71	±	1.90	d,e,f,g,h	78
	MeOH	5.08	±	1.18	a,b,c,d,e	90
P. major	Water	19.33	±	2.84	k,l,m	61
	EtOH	35.29	±	5.86	n	29
	MeOH	35.21	±	4.65	n	29
F. officinalis	Water	0.46	±	0.27	a	100
	EtOH	17.33	±	2.99	h,i,j,k,l	65
	MeOH	12.58	±	2.58	e,f,g,h,i,j,k	73
G. palustre	Water	13.04	±	2.56	e,f,g,h,i,j	73
	EtOH	6.92	±	0.98	a,b,c,d,e,f	86
	MeOH	1.33	±	0.33	a,b	98
E. vulgare	Water	9.00	±	1.72	c,d,e,f,g	82
	EtOH	18.21	±	2.64	i,j,k,l	63
	MeOH	2.42	±	0.68	a,b,c	96
S. nigra	Water	9.50	±	2.02	c,d,e,f,g	80
	EtOH	18.38	±	2.32	i,j,k,l	63
	MeOH	10.42	±	1.85	c,d,e,f,g	80

(sativan and medicarpin) isolated from leaves of *G. officinalis* were cytotoxic against human breast cancer (48). Moreover, antiviral, antiinflammatory, antimutagenic, and anticarcinogenic activities of the phytoestrogens have been previously shown (48). Recently, it was reported that phytosterols induced apoptosis in human colon cancer cells by targeting different signaling pathways (49). In the present study, the antitumor activity of the aqueous extract from *G. officinalis* can be attributed to the alkaloids (galegine) and flavonoids (phytoestrogens) found as major compounds in *G. officinalis*. Alcoholic extracts of *X. annuum* (86%) exhibited stronger antitumor activity than the aqueous extract (63%).

Strong antitumor activity was observed with all tested extracts of *C. intybus*. Alcoholic extracts (88% tumor inhibition) were better than aqueous extracts (71%). Similarly, Hazra et al. (50) reported tumor inhibitory activity of the ethanolic extract of *C. intybus* against Ehrlich ascites carcinoma in mice. Moreover, Conforti et al. (5) showed significant anticancer activity of *C. intybus* on melanoma. *C. intybus* is an important antiproliferative agent that includes both phenolic compounds and phytosterols and can be used in cancer therapy as a supplementary agent (5). Several studies evaluated the relationships between the antiproliferative activity of plant products and their phenolic content. A correlation exists between the structural oxidation state and the position, number, and nature of the substituents of the polyphenolic compounds and their antiproliferative effects (51). Since phenolics act as antiproliferative agents through the cell cycle, they may liquidate the tumor cells by this mechanism (5).

Among all extracts of *P. lanceolata* and *P. major*, the best antitumor activity was observed with methanolic and ethanolic extracts of *P. lanceolata* (90% and 78%, respectively). Alcoholic extracts of *P. major* were not as effective as alcoholic extracts of *P. lanceolata* (Table 3). In the Arabian region, medicinal usage of *P. major* and *P. lanceolata* as antitumor remedies is well established (52). Concerning all tested extracts of *G. palustre*, alcoholic extract showed better antitumor activity than aqueous extract, and the best tumor inhibition was observed with methanolic extract (98%). The aqueous, ethanol, and methanol extracts of *E. vulgare* exhibited significant inhibition of grown gall tumors on potato disks (82%, 63%, and 96%, respectively). Jaric et al. (24) reported that organic extracts of *E. vulgare* partially inhibited the growth of tumor cells. All tested extracts of *S. nigra* showed moderate tumor inhibition. Aqueous and methanolic extracts (80%) were better than ethanolic extract (63%) (Table 3).

The use of most medicinal plants discovered by traditional societies has not been supported by scientific data. Simple bench top bioassays can provide initial screening data. However, bioassays must be rapid, convenient, reliable, inexpensive, sensitive, and require little material. Furthermore, they should be easy to apply in-house by chemists, botanists, and other scientists who lack the resources or expertise to carry out more elaborate bioassays (53).

Antibacterial and antitumor activities of 48 different extracts obtained from 16 different plants grown in Bolu, Turkey, were evaluated. Results obtained herein revealed the strong antibacterial activities of *M. officinalis* and the strong antitumor activities of *M. alba* and *F. officinalis*. Future studies should focus on fractionation of the extracts of *M. officinalis*, *M. alba*, and *F. officinalis* in order to identify the active components. The anticancer activity of *M. alba* and *F. officinalis* should be studied using different cancer cell lines in the future. With these results, there is some scientific justification to view the tested plants as medicinal plants. In the future, identification of active components can be studied in plant extracts having strong bioactivity.

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