Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

In vitro antibacterial and antitumor activities of some medicinal plant extracts, growing in Turkey

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ARTICLE INFO

Article history:
Received 28 March 2013
Received in revised form 17 April 2013
Accepted 7 May 2013
Available online 20 August 2013

Keywords:
Bioassays
Antibacterial
Antitumor
Medicinal plants

ABSTRACT

Objective: To investigate antibacterial and antitumor activities of 51 different extracts prepared with 3 types of solvents (water, ethanol and methanol) of 16 different plant species (*Ajuga reptans* (*A. reptans*) L., *Phlomis pungens* (*P. pungens*) Willd., *Marrubium astracanicum* (*M. astracanicum*) Jacq., *Nepeta nuda* (*N. nuda*) L., *Stachys annua* (*S. annua*) L., *Genista lydia* (*G. lydia*) Boiss., *Nuphar lutea* (*N. lutea*) L., *Nymphaea alba* (*N. alba*) L., *Vinca minor* (*V. minor*) L., *Stellaria media* (*S. media*) L., *Capsella bursa-pastoris* (*C. bursa-pastoris*) L., *Galium spurium* (*G. spurium*) L., *Onosma heterophyllum* (*O. heterophyllum*) Griseb., *Reseda luteola* (*R. luteola*) L., *Viburnum lantana* (*V. lantana*) L. and *Mercurialis annua* (*M. annua*) L.) grown in Turkey was conducted. **Methods:** Antibacterial activity was evaluated with 10 bacteria including *Streptococcus pyogenes* (*S. pyogenes*), *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*), *Escheria coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella typhimurium* (*S. typhimurium*), *Serratia marcescens* (*S. marcescens*), *Proteus vulgaris* (*P. vulgaris*), *Enterobacter cloacae* (*E. cloacea*), and *Klebsiella pneumoniae* (*K. pneumoniae*) by using disc diffusion method. Antitumor activity was evaluated with *Agrobacterium tumefaciens* (*A. tumefaciens*)-induced potato disc tumor assay. **Results:** Best antibacterial activity was obtained with ethanolic extract of *P. pungens* against *S. pyogenes*. Ethanolic and methanolic extract of *N. alba* and ethanolic extract of *G. lydia* also showed strong antibacterial activities. Results indicated that alcoholic extracts especially ethanolic extracts exhibited strong antibacterial activity against both gram-positive and gram-negative bacteria. Best antitumor activity was obtained with methanolic extracts of *N. alba* and *V. lantana* (100% tumor inhibition). Ethanolic extract of *N. alba*, alcoholic extracts of *N. lutea*, *A. reptans* and *V. minor* flowers, methanolic extracts of *G. lydia* and *O. heterophyllum* and ethanolic extract of *V. lantana* and aqueous extract of *V. minor* leaves exhibited strong tumor inhibitions. **Conclusions:** In near future works, identification of active components can be studied for plant extracts having strong bioactivity.

1. Introduction

Plants and their products for treatment of diseases have been used extensively by humans for many years[1]. Many higher plants have economically important compounds such as oils, resins, dyes, flavors and fragrances, pharmaceuticals, and pesticides. The antiseptic qualities of aromatic and medicinal plants and their extracts have been recognized since antiquity, while attempts to characterize these properties in the laboratory date back to the early 1900s[2]. However, most species of higher plants have never been described, much less surveyed for chemical or biologically active constituents, and new sources of commercially valuable materials remain to be discovered. More recently, medicinal plant products were gained great importance for use in medicine as natural products[3].

Ajuga reptans (*A. reptans*) has a mild narcotic, antirheumatic and astringent activity. It also has been used medicinally to treat bleeding from cuts and other wounds[4]. Flowering plant is effective as anti-diarrheal, anti-inflammatory, strengthening and diuretic[5]. The aerial

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Foundation: This study was supported by Abant Izzet Baysal University Research Foundation (Project no: BAP 2005.03.01.219).

part of *Phlomis pungens* has antibacterial and antifungal activity[6].

Marrubium astracanicum (*M. astracanicum*) has an antipyretic properties and used in the treatment of cold[7]. Some *Marrubium* species have hypoglycemic, anti-schistosomatic, antioxidant, vasorelaxant, abortifacient, hypertensive, antimicrobial and cytotoxic activities[8]. *Stachys annua* (*S. annua*) is used as antipyretic and in the treatment of cold[7]. *Genista lydia* (*G. lydia*) have been used to treat menopausal symptoms, and estrogen-related diseases such as prostate, breast, osteoporosis, and cardiovascular diseases due to its content daidzein known as a phytoestrogen because of its estrogenic activity in humans[9]. All the parts of *Nymphaea alba* (*N. alba*) have medicinal uses in traditional system of medicine. *N. alba* has antiseptic, sedative, cardiotonic and astringent properties. The leaves and roots of *N. alba* have been used in form of scrofulous ulcers and inflamed skin[10]. Anti-cancer[11] and antiprotozoal[12] activities of *N. lutea* have been reported. *Stellaria media* (*S. media*) has been used for skin diseases, bronchitis, rheumatic pains, arthritis and period pain[13]. *Capsella bursa-pastoris* (*C. bursa-pastoris*) has analgesic and anti-diarrheal properties. *C. bursa* pastoris is used for the treatment of stomach cramps, both internal and external bleedings, wounds, burns, premenstrual syndrome, malignant ulcers, stomach and uterine cancers, tumors and fibromas[14,15]. Galium species have diuretic, astringent, choleretic properties and are used in the treatment of some stomach, gout and epilepsy diseases[16]. The leaves of some Onosma species have been used for the treatment of wounds and distension[7]. *Reseda luteola* has antioxidant capacity due to its luteolin (flavonoid) content and also might be of interest for developing anticancer strategies[17]. *Viburnum lantana* (*V. lantana*) has powerful antinociceptive and slight anti-inflammatory activities[18]. *Vinca minor* (*V. minor*) has diuretic, laxative and febrifuge properties[5]. *V. minor* is used to enhance blood circulation, and to treat cardiovascular disorders due to its content vincamine[19]. Essential oil of *Nepeta nuda* (*N. nuda*) showed antibacterial activity against *Klebsiella pneumoniae* (*K. pneumoniae*) and *Salmonella typhi* (*S. typhi*)[20]. *Mercurialis annua* (*M. annua*) has been used in folk medicine as purgative, diuretic and antisyphilitic[14].

The aim of this study was to evaluate the antibacterial and antitumor activities of 16 different plant species found in Bolu, Turkey.

2. Materials and methods

2.1. Plant material and extraction

Aerial parts of sixteen plants were collected from Bolu, Turkey. Identification of species was made by using "Flora of Turkey and the East Aegean Islands"[21] and voucher specimens were deposited at the Abant Izzet Baysal University (AIBU) Herbarium, Bolu, Turkey. Collected plants were dried in oven at 40 ℃ and then ground into a powder. Water, ethanol and methanol were used as extraction solvents. Twenty grams from each plant sample were extracted with 200 mL water, ethanol and methanol separately at 40 ℃ in a water bath for 18 h. The extracts were then filtered. For aqueous extraction, frozen filtrate was lyophilized by using freeze-dryer at −65 ℃. For alcoholic extractions (ethanol and methanol), filtrates were evaporated under vacuum using rotary evaporator at 60 ℃ and then dissolved in 10 mL distilled water and lyophilized. For antibacterial and antitumor assays, lyophilized extracts were dissolved in sterile distilled water in order to obtain a final concentration of 100 mg/mL. All extracts were sterilized by filtering through a 0.22 μm filter (Millex®). Plant materials, voucher numbers, designation of treatments and yield (%) for each extraction were summarized in Table 1.

2.2. Antibacterial bioassay

The disc diffusion assay (Kirby-Bauer Method) was used to screen for antibiotic activity[22]. Ten bacterial strains were employed in the bioassay: *Escherichia coli* (*E. coli*) (ATCC 25922), *Pseudomonas aeruginosa* (*P. aeruginosa*) (ATCC 27853), *Salmonella typhimurium* (*S. typhimurium*) (ATCC 14028), *Serratia marcescens* (*S. marcescens*) (ATCC 8100), *Proteus vulgaris* (*P. vulgaris*) (ATCC 13315), *Enterobacter cloacae* (E. cloacae) (ATCC 23355) and *Klebsiella pneumoniae* (*K. pneumoniae*) (ATTC 13883) which are Gram-Negative bacteria and *Streptococcus pyogenes* (*S. pyogenes*) (ATTC 19615), *Staphylococcus aureus* (*S. aureus*) (ATTC 25923), and *Staphylococcus epidermidis* (*S. epidermidis*) (ATCC 12228) which are Gram-Positive 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 ℃. One bacteriological loop from each broth was streaked on Tryptic Soy Agar (TSA) plates and incubated for 2 d at 37 ℃. After 2 d, a single colony was removed and streaked on TSA plate and incubated at 37 ℃ for 2 additional days. The turbidity of each broth culture was adjusted with saline to obtain turbidity visually comparable to that of a 0.5 McFarland standard and then Mueller Hinton agar plates were inoculated by using cotton swabs.

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. Five different antibiotic 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) were used as positive controls. 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 ℃ incubator. After 16 to 18 h of

Table 1
Designation of studied plant extracts, their family and botanical names, used parts, and collection numbers.

Family and plants species	Colletion number	Part used	Extract	Designation	Yield (%)*
LAMIACFAE	AUT-1910	Aeiral	Water	Ex 1a	32.0
Ajuga reptans L.			EtOH	Ex 1b	6.0
			MeOH	Ex 1c	20.0
Phlomis pungens Willd. var. *pungens*	AUT-1913	Aeiral	Water	Ex 2a	4.4
			EtOH	Ex 2b	4.0
			MeOH	Ex 2c	10.0
Marubium astracanicum Jacq. subsp. *astracanicum*	AUT-1914	Aeiral	Water	Ex 3a	1.6
			EtOH	Ex 3b	3.5
			MeOH	Ex 3c	10.5
Nepeta mda L. subsp. *annua* var. *annua*	AUT-1929	Aeiral	Water	Ex 4a	30.9
			EtOH	Ex 4b	18.9
			MeOH	Ex 4c	25.7
Stachys annua L. subsp. *annua* var. *annua*	AUT-1930	Aeiral	Water	Ex 5a	13.5
			EtOH	Ex 5b	36.8
			MeOH	Ex 5c	32.0
Genista lydia Boiss. var. *lydia*	AUT-1926	Aeiral	Water	Ex 6a	29.6
			EtOH	Ex 6b	8.2
			MeOH	Ex 6c	22.5
NYMPHAEACEAE	AUT-1931	Leaves	Water	Ex 7a	14.6
nupharlutea (L.) Sm			EtOH	Ex 7b	16.8
			MeOH	Ex 7c	10.7
Nymphaea alba L.	AUT-1932	Leaves	Water	Ex 8a	8.4
			EtOH	Ex 8b	18.3
			MeOH	Ex 8c	9.0
APOCYNACEAE	AUT-1922	Leaves	Water	Ex 9a	20.9
Vinca minor L.			EtOH	Ex 9b	21.0
			MeOH	Ex 9c	28.0
		Flowers	Water	Ex 10a	33.5
			EtOH	Ex 10b	1.0
			MeOH	Ex 10c	4.5
CARYOPHYLLACEAE	AUT-1923	Aeiral	Water	Ex 11a	22.0
Stellaria media (L.) Vill.			EtOH	Ex 11b	3.0
subsp. media			MeOH	Ex 11c	6.0
BRASSICACEAE	AUT-1924	Aeiral	Water	Ex 12a	17.6
Capsella bursa-pastoris (L.) Medik.			EtOH	Ex 12b	15.5
			MeOH	Ex 12c	18.0
BUBIACEAE	AUT-1927	Aeiral	Water	Ex 13a	15.1
Galium spurium L.			EtOH	Ex 13b	20.3
			MeOH	Ex 13c	15.5
BORAGINACEAE	AUT-1925	Aeiral	Water	Ex 14a	25.2
Onosma heterophyllum Griseb.			EtOH	Ex 14b	31.0
			MeOH	Ex 14c	15.0
BESEDACEAE	AUT-1928	Aeiral	Water	Ex 15a	10.1
Reseda luteola L.			EtOH	Ex 15b	3.8
			MeOH	Ex 15c	12.0
CAPRIFOLIACEAE	AUT-1934	leaves and fruits	Water	Ex 16a	42.0
Viburnum lantana L.			EtOH	Ex 16b	32.6
			MeOH	Ex 16c	60.3
EUPHORBIACEAE	AUT-1935	Aeiral	Water	Ex 17a	72.4
Mercurialis annua L.			EtOH	Ex 17b	18.8
			MeOH	Ex 17c	19.8

*Yield (%) = Weight of extract (g) / 20 g of powdered plant sample × 100.

incubation, inhibition zone diameter (mm) was measured. All experiments were repeated three times.

2.3. Antitumor assay

Antitumor activity of all extracts was assessed with the potato disc method as modified by McLaughlin's group[23]. *Agrobacterium tumefaciens* (*A. tumefaciens*) (ATCC® 23341) was cultured on Yeast Extract Media (YEM) for 2-3 d at 28 ℃. 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[24]. 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* (*S. 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[25]. Plates were incubated at 28 ℃ in the dark for 2 weeks. After 2 weeks, discs were stained with Lugol's reagent (I_2-KI; 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 formula "% inhibition= [(solvent control mean - tested extract mean) / solvent control mean] · 100"[25].

2.4. 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 plant 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. At 30 min after inoculation, 0.1 mL of inoculum (bacteria + extract) was removed and inoculated on YEM media with spread plate technique. After 24 h incubation of inoculated plates at 28 ℃, colony counts were made. Also, bacterial growth was evidenced by growth across the plates[24].

2.5. 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).

3. Results

Fifty-one different extracts prepared with 3 types of solvents (water, methanol and ethanol) of 16 different plant species were tested in order to screen and show their potential as antibacterial and antitumor agents (Table 2 and 3).

Bacterial growth was generally sensitive to the reference antibiotics tested (Table 2). Inhibition zones varied from 54.3 mm for ampicillin and *S. pyogenes* to 8.3 mm for tetracycline and *S. epidermidis* (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.

Generally, ethanolic extracts of tested plants extracts gave better inhibitory activity against used bacteria (Table 2). Best antibacterial activity was obtained with ethanolic extract of *P. pungens* against *S. pyogenes* (35 mm). Ethanolic extract of *P. pungens* exhibited of a broad-spectrum activity against both gram-positive and gram-negative bacteria (Table 2 and 3). This activity against both types of bacteria may be indicative of the presence of broad-spectrum antibiotic compounds or simply general metabolic toxins. Furthermore, methanolic extract of *P. pungens* did not show any antibacterial activity against used bacteria in our study (Table 2). Similarly, Ismailoglu *et al*[6] reported that aerial part of this plant showed slight antibacterial activity against used bacteria. Antibacterial and antifungal activity of *P. pungens* may be due to its chemical composition including phenylpropanoid glycosides [6].

E. cloacae was most vulnerable to ethanolic extract of *G. lydia* (21.8 mm), *N. lutea* (15 mm) and *N. alba* (20.2 mm). Methanolic extract of *N. alba* (20 mm) was also showed strong antibacterial activity against this bacterium (Table 2). Tested extracts of *N. lutea* and *N. alba* exhibited similar antibacterial activity (moderate level) against used gram-positive bacteria (Table 2). Similarly, Turker *et al*[26] reported the strong antibacterial activity of *N. lutea* and *N. alba* against fish pathogens (*Aeromonas hydrophila*, *Yersinia ruckeri*, *Lactococcus garvieae*, *Streptococcus agalactiae* and *Enterococcus faecalis*).

Ethanolic extracts of *S. annua* against *S. aureus* (14 mm), *G. lydia* against *S. epidermidis* (13.5 mm) and *V. minor* leaves against *S. epidermidis* and *S. pyogenes* (15.3 mm and 14.8 mm, respectively) have strong antibacterial activities (Table

Table 2

Antibacterial activity of used plant extracts. Means with the same letter within columns are not significantly different at $P>0.05$

Treatments	Mean diameter of inhibitory zones (mm ± SE)									
	S. aureus	*S. epidermidis*	*S. pyogenes*	*S. marcescens*	*S. typhimurium*	*P. aeruginosa*	*P. vulgaris*	*K. pneumonia*	*E. cloacae*	*E. coli*
Ex 1a	–	7.0±0.0^{i}	–	9.3±0.3^{e}	9.2±1.2^{d}	7.8±0.2^{fg}	9.5±0.2^{f}	–	8.3±0.2^{j}	–
Ex 1b	–	7.0±0.0^{i}	–	8.2±0.2^{f}	8.3±0.9^{e}	11.7±0.6^{cd}	8.0±0.0^{hi}	–	8.0±0.3^{j}	9.2±0.2^{f}
Ex 1c	–	7.0±0.0^{i}	–	–	–	–	–	–	–	–
Ex 2a	7.0±0.0^{m}	8.0±0.4^{h}	–	8.0±0.0^{f}	–	9.2±0.2^{e}	7.5±0.2^{i}	–	7.8±0.2^{j}	9.7±1.0^{ef}
Ex 2b	8.0±0.0^{l}	8.2±0.2^{h}	35.0±1.8^{e}	8.2±0.2^{f}	8.0±0.9^{e}	8.5±0.2^{ef}	8.2±0.2^{h}i	–	8.8±0.2^{j}	–
Ex 2c	–	–	–	–	–	–	–	–	–	–
Ex 5a	–	–	–	–	–	–	–	–	–	–
Ex 5b	14.0±0.3^{f}	–	–	–	–	–	–	–	–	–
Ex 5c	–	11.0±0.0^{fg}	–	–	–	–	–	–	–	–
Ex 6a	–	–	–	–	–	–	–	–	–	–
Ex 6b	10.5±0.8^{gh}	13.5±0.9^{e}	–	–	–	–	12.0±0.0^{e}	11.7±0.2^{c}	21.8±0.2^{e}	–
Ex 6c	–	–	10.8±0.3^{hi}	–	–	–	–	–	–	–
Ex 7a	9.0±0.3^{jk}	11.3±0.3^{f}	11.5±0.2^{gh}	–	–	–	9.0±0.0^{fg}	–	12.0±0.5^{h}	–
Ex 7b	9.2±0.4^{ij}	10.2±0.3^{g}	9.8±0.2^{jk}	–	–	12.3±0.7^{c}	–	–	15.0±1.7^{g}	–
Ex 7c	10.0±0.0^{hi}	10.5±0.2^{fg}	10.7±0.2^{hi}	–	–	–	–	–	–	–
Ex 8a	7.0±0.0^{m}	8.5±0.3^{h}	10.3±0.2^{ij}	–	–	–	–	–	10.2±0.2^{ij}	–
Ex 8b	9.8±0.2^{ij}	10.3±0.2^{g}	10.0±0.0^{ij}	–	–	–	–	–	20.2±0.3^{f}	–
Ex 8c	9.2±0.8^{ij}	10.3±0.2^{g}	9.0±0.4^{k}	–	–	–	–	–	20.0±0.0^{f}	–
Ex 9a	–	–	–	–	–	–	–	–	–	–
Ex 9b	11.3±0.2^{g}	15.3±0.3^{d}	14.8±0.2^{f}	–	–	–	9.0±0.0^{fg}	–	–	9.3±0.2^{f}
Ex 9c	8.2±0.5^{kl}	8.5±0.5^{h}	–	–	–	–	–	–	–	–
Ex 11a	–	–	–	–	–	–	–	–	–	–
Ex 11b	–	–	–	–	–	–	9.0±0.0^{fg}	7.3±0.2^{h}	–	–
Ex 11c	–	–	–	–	–	–	–	–	–	–
Ex 12a	–	–	–	–	–	–	–	–	–	–
Ex 12b	–	–	–	–	–	7.0±0.0^{g}	–	9.0±0.0^{g}	–	–
Ex 12c	–	–	–	–	–	–	–	–	–	–
Ex 13a	–	–	–	–	–	–	–	–	–	–
Ex 13b	–	–	12.2±0.6^{g}	–	–	–	–	–	–	–
Ex 13c	–	–	–	–	–	–	–	–	–	–
Ex 14a	–	–	–	–	–	–	–	–	–	–
Ex 14b	–	–	–	–	–	–	8.3±0.2^{gh}	10.5±0.2^{e}	–	–
Ex 14c	–	–	–	–	–	–	–	–	–	–
Ex 15a	–	–	–	–	–	–	–	7.5±0.2^{h}	–	–
Ex 15b	–	–	–	–	–	–	–	7.5±0.2^{h}	–	–
Ex 15c	–	–	–	–	–	–	–	–	–	–
Ex 16a	–	–	–	–	–	–	–	–	–	–
Ex 16b	–	–	–	–	–	–	–	–	–	–
Ex 16c	7.7±0.2^{lm}	7.0±0.0^{i}	7.5±0.2^{l}		–	–	–	–	–	–
Ampicillin (10 mg)	42.7±0.4^{a}	22.3±1.1^{c}	54.3±0.7^{a}	18.5±0.9^{d}	29.3±0.6^{a}	8.3±0.2^{ef}	23.0±0.6^{c}	10.0±0.6^{f}	28.0±0.0^{d}	20.7±0.2^{d}
Carbenicillin (100 mg)	41.7±0.4^{b}	24.0±1.0^{b}	53.5±0.9^{a}	20.5±0.2^{c}	27.0±0.4^{b}	18.3±0.6^{b}	34.0±0.6^{b}	8.7±0.2^{g}	34.7±0.4^{a}	25.7±1.2^{c}
Chloramphenicol (30 mg)	25.3±0.4^{e}	30.7±0.4^{a}	36.7±0.6^{d}	30.0±0.0^{a}	27.3±0.8^{b}	12.0±0.5^{cd}	21.6±0.7^{d}	29.0±0.6^{b}	29.3±0.2^{c}	28.3±1.1^{b}
Erythromycin (15 mg)	26.7±0.6^{d}	31.3±0.8^{a}	41.3±0.8^{c}	9.3±0.2^{e}	10.3±0.2^{c}	11.0±0.0^{d}	9.3±0.2^{f}	11.0±0.4^{d}	9.0±0.0^{ij}	10.3±0.7^{e}
Tetracycline (30 mg)	33.3±0.8^{c}	8.3±0.2^{h}	50.5±0.2^{b}	28.3±0.6^{b}	28.7±0.8^{a}	21.7±2.6^{a}	35.6±1.5^{a}	29.7±0.2^{a}	30.3±0.2^{b}	29.7±0.6^{a}
Water	–	–	–	–	–	–	–	–	–	

2).

Generally, the gram-positive bacteria commonly seem to be more susceptible to the inhibitory effects of the plant extracts than the gram-negative bacteria do. Susceptibility of gram-positive bacteria may come from their cell wall structure consisting of a single layer, but the gram-negative cell wall is a multi-layered structure and quite complex [26]. On the contrary, *E. cloacae* which is gram-negative bacterium seemed to be more susceptible to used extracts of *G. lydia*, *N. lutea* and *N. alba* than gram-positive bacteria (Table 2).

Tested extracts of *M. astacanicum*, *N. nuda*, *V. minor* flowers, *R. luteola* and *M. annua* did not show any inhibitory activity against used bacteria (data not shown). On the other hand, Alim *et al*[20] demonstrated the antibacterial activity of essential oil of *N. nuda* against *K. pneumoniae* and Salmonella typhi.

Although tested extracts of *V. minor* flowers did not show antibacterial activity against used bacteria, especially ethanolic extract of *V. minor* leaves exhibited moderate inhibitory activity against *S. epidermidis* (15.3 mm), *S. pyogenes* (14.8 mm) and *S. aureus* (11.3 mm) (Table 2). Tested extracts of *A. reptans* have no significant or weak antibacterial activity against used bacteria (around 8-9 mm). Ethanolic extract of *A. reptans* showed better inhibitory activity against *P. aeruginosa* whose inhibition zone was 11.7 mm (Table 2).

Although *S. aureus* showed sensitivity to ethanolic extract of *S. annua* in our study, Digrak *et al* [27] reported that there was no antibacterial activity of *S. annua* against *S. aureus* and some human disease bacteria. On the other hand, Turker *et al*[26] reported that alcoholic extracts (methanol and ethanol) of *S. annua* showed strong antimicrobial activity against fish pathogens (Aeromonas hydrophila and Yersinia ruckeri). Some tested extracts of *S. media*, *C. bursa-pastoris*, *G. spurium*, *O. heterophyllum*, *R. luteola*, *V. lantana* and *M. annua* exhibited weak inhibition against some of used bacteria (Table 2). Park *et al*[28] reported that isolated shepherins (antimicrobial peptites) from root of *C. bursa* pastoris have antibacterial activity against gram-negative bacteria.

Strong antitumor activity was observed with *A. reptans*, *G. lydia*, *N. lutea*, *N. alba*, *V. lantana*, *O. heterophyllum* and *V. minor* with *Agrobacterium tumefaciens*-induced potato disc tumor assay (Table 3). A prerequisite for this assay is that the extract or substance being tested should not have antibacterial activity toward *A. tumefaciens*[24]. Inhibition of crown gall formation on potato discs is caused by two effects: by anti-tumorogenesis or 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 plant extracts with 1×10^3 colony-forming units (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[29], 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* + plant extracts) in terms of colony counts (ranged from 9.2×10^3 to 13×10^3 CFU). All tested extracts did not affect the viability of the bacterium. Thus, observed inhibition of tumor formation for these extracts was on the formation of tumors and not on the viability of the bacterium.

Since final concentrations of all extracts were adjusted with distilled water, it was used as a negative control and no inhibition was observed with water. Tumor formation was not observed with positive control camptothecin (100% inhibition).

Best antitumor activity was obtained with methanolic extract of *N. alba* and *V. lantana* (100% inhibition) similar to positive control camptothecin. Alcoholic extracts (ethanol and methanol) of *N. lutea* and *N. alba* showed similar antitumor activity (between 99.4%-100% tumor inhibition). All tested extracts of *V. minor* leaves and flowers exhibited strong antitumor activities (between 80.4%-96.6% tumor inhibition). Similarly, strong antitumor activity was observed with all tested extracts of *A. reptans* (between 83.2%-99.2% tumor inhibition). Alcoholic extracts (ethanol and methanol) of *G. lydia* (81.7% and 99.3%, respectively), ethanolic extract of *V. lantana* (90.9%) and methanolic extract of *O. heterophyllum* (91.8%) also showed strong tumor inhibition (Table 3).

Table 3

Mean number of tumors observed with used plant extracts at a concentration of 100 000 mg/L. Means with the same letter within columns are not significantly different at $P>0.05$.

Treatments	Mean number of tumors (±SE)	% Tumor inhibition
Water	80.83 ± 5.02^{v}	–
Camptothecin	0 ± 0^{a}	100.0
Ex 1a	13.58 ± 2.02^{defghijk}	83.2
Ex 1b	0.67 ± 0.20^{ab}	99.2
Ex 1c	3.63 ± 0.74^{abcd}	95.5
Ex 2a	25.00 ± 3.51^{lmno}	69.1
Ex 2b	11.46 ± 2.02^{cdefghi}	85.8
Ex 2c	11.58 ± 1.91^{cdefghi}	85.7
Ex 3a	21.92 ± 3.65^{ijklmno}	72.9
Ex 3b	9.08 ± 1.91^{abcdefg}	88.8
Ex 3c	10.08 ± 1.65^{abcdefgh}	87.5
Ex 4a	20.88 ± 5.36^{hijklmn}	74.2
Ex 4b	19.54 ± 4.35^{ghijklmn}	75.8
Ex 4c	12.71 ± 2.85^{cdefghij}	84.3
Ex 5a	31.25 ± 4.77^{opr}	61.3
Ex 5b	19.58 ± 2.04^{ghijklmn}	75.8
Ex 5c	18.0 ± 2.21^{fghijkl}	77.7
Ex 6a	37.83 ± 3.68^{prs}	58.2
Ex 6b	14.83 ± 2.73^{efghijkl}	81.7
Ex 6c	0.54 ± 0.26^{ab}	99.3
Ex 7a	15.25 ± 3.36^{efghijkl}	81.1
Ex 7b	0.50 ± 0.25^{ab}	99.4
Ex 7c	0.46 ± 0.26^{ab}	99.5
Ex 8a	8.96 ± 2.03^{abcdefg}	88.9
Ex 8b	0.13 ± 0.12^{a}	99.8
Ex 8c	0 ± 0^{a}	100.0
Ex 9a	6.38 ± 1.11^{abcde}	92.1
Ex 9b	11.54 ± 1.00^{cdefghi}	85.7
Ex 9c	14.83 ± 2.04^{efghijkl}	81.6
Ex 10a	15.88 ± 1.64^{efghijkl}	80.4
Ex 10b	6.04 ± 1.78^{abcde}	92.5
Ex 10c	2.75 ± 1.20^{abc}	96.6
Ex 11a	22.67 ± 1.99^{jklmno}	71.9
Ex 11b	24.08 ± 4.45^{klmno}	70.2
Ex 11c	18.17 ± 2.70^{fghijklm}	77.5
Ex 12a	46.08 ± 3.60^{st}	42.9
Ex 12b	57.00 ± 5.15^{u}	29.5
Ex 12c	46.08 ± 4.98^{st}	42.9
Ex 13a	28.88 ± 3.52^{nop}	64.3
Ex 13b	11.29 ± 2.50^{cdefghi}	86.0
Ex 13c	15.08 ± 2.23^{efghijkl}	81.3
Ex 14a	32.17 ± 6.11^{opr}	60.2
Ex 14b	24.38 ± 3.34^{lmno}	69.8
Ex 14c	6.58 ± 1.59^{abcde}	91.8
Ex 15a	47.54 ± 4.77^{st}	41.2
Ex 15b	48.25 ± 4.94^{t}	40.3
Ex 15c	78.92 ± 7.07^{v}	2.4
Ex 16a	10.96 ± 2.66^{bcdefgh}	86.4
Ex 16b	7.33 ± 2.0^{8abcdef}	90.9
Ex 16c	0 ± 0^{a}	100.0
Ex 17a	28.46 ± 2.94^{mnop}	64.8
Ex 17b	12.71 ± 2.82^{cdefghij}	84.3
Ex 17c	39.0 ± 3.22^{rst}	51.8

Least antitumor activities (less than 50% tumor inhibition) were obtained with all extracts of *C. bursa-pastoris* and *R. luteola* (Table 3). On the contrary, Samy *et al*[17] reported that luteolin isolated from *R. luteola* prevented chemical induced carcinogenesis in rats and has great potential for cancer prevention and therapy. Furthermore, Bekker *et al*[15] noted that *C. bursa* pastoris has been used for the treatment of some cancer types.

Alcoholic extracts of *N. lutea* and *N. alba* exhibited better tumor inhibition than aqueous extracts in our study. Similarly, Matsuda *et al*[11] found that *N. lutea* extracts have anticancer activity on human leukemia cell (U937), human fibroblast (HT1080), and mouse melanoma cell (B16F10) lines. Antitumor activities of leaves and flowers of *V. minor* were compared. Aqueous extract of *V. minor* leaves was better than alcoholic extracts. On the other hand, alcoholic extracts of *V. minor* flowers were better than aqueous extract (Table 3). Khanavi *et al*[30] studied cytotoxic effects of *V. minor* alkoloids on Colon carcinoma (HT-29), colorectaladenocarcinoma (Caco-2), breast ductalcarcinoma (T47D) and Swiss mouse embryo fibroblast (NIH 3T3) cell lines. They found that alkoloids affected the growth and proliferation of these cell lines was found[30].

Antitumor activity of alcoholic extracts of *P. pungens* was better than aqueous extract of it and showed strong tumor inhibition (85%). Ismailoglu *et al*[6] studied the phenylpropanoid glycosides isolated from *P. pungens* and reported that some phenylpropanoid glycosides could inhibit proliferation of some tumor cells.

The inhibition of *A. tumefaciens*-induced tumors (or crown gall) in potato disc tissue is an assay based on antimitotic activity and can detect a broad range of known and novel antitumor effects[24]. 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 disc showed an apparent correlation with compounds and plant extracts known to be active in the 3PS (*in vivo*, murine leukemia) antitumor assay[24]. Ferrigini *et al*[23] showed that crown gall tumors on potato discs could routinely be employed as comparatively rapid, inexpensive, safe, and statistically reliable prescreen for 3PS antitumor activity.

4. Discussion

Generally, the gram-positive bacteria commonly seem to be more susceptible to the inhibitory effects of the plant extracts than the gram-negative bacteria do. Susceptibility of gram-positive bacteria may come from their cell wall structure consisting of a single layer, but the gram-negative cell wall is a multi-layered structure and quite complex[26]. On the contrary, in the present study, *E. cloacae* which is gram-negative bacterium seemed to be more susceptible to used extracts of *G. lydia*, *N. lutea* and *N. alba* than gram-positive bacteria (Table 2). In our study, ethanolic extract of *P. pungens* showed a broad-spectrum activity against both gram-positive and gram-negative bacteria. This activity against both bacteria may be indicative of the presence of broad spectrum antibiotic compounds or simply general metabolic toxins[27-32]. Furthermore, methanolic extract of *P. pungens* did not show any antibacterial activity. Similarly, Ismailoglu *et al*[6] reported that aerial part of this plant showed slight antibacterial activity against used bacteria. Antibacterial and antifungal activity of *P. pungens* may be due to its chemical composition including phenylpropanoid glycosides[6].

Turker *et al*[26] reported the strong antibacterial activity of *N. lutea* and *N. alba* against fish pathogens (Aeromonas hydrophila, Yersinia ruckeri, Lactococcus garvieae, Streptococcus agalactiae and Enterococcus faecalis). In our study, also we observed that *N. lutea* and *N. alba* exhibited moderate antibacterial activity against used gram-positive bacteria.

Although *S. aureus* showed sensitivity to ethanolic extract of *S. annua* in our study, Digrak *et al*[33] reported that there was no antibacterial activity of *S. annua* against *S. aureus* and some human disease bacteria. On the other hand, Turker *et al*[26] reported that alcoholic extracts (methanol and ethanol) of *S. annua* showed strong antimicrobial activity against fish pathogens (Aeromonas hydrophila and Yersinia ruckeri). In our study, Some tested extracts of *C. bursa-pastoris* exhibited weak inhibition against gram-negative bacteria (*K. pneumoniae* and *P. aeruginosa*) (Table 2). Similarly, Park *et al*[34] reported that isolated shepherins (antimicrobial peptites) from root of *C. bursa* pastoris have antibacterial activity against gram-negative bacteria. Alim *et al*[20] demonstrated the antibacterial activity of essential oil of *N. nuda* against *K. pneumoniae* and *S. typhi*. On the contrary, tested extracts of *N. nuda* did not show any inhibitory activity against used bacteria.

The inhibition of *A. tumefaciens*-induced tumors (or crown gall) in potato disc tissue is an assay based on antimitotic activity and can detect a broad range of known and novel antitumor effects[24]. 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 disc showed an apparent correlation with compounds and plant extracts known to be active in the 3PS (*in vivo*, murine leukemia) antitumor assay[24]. Ferrigini *et al*[23] showed that crown gall tumors on potato discs could routinely be employed as comparatively rapid, inexpensive, safe, and

statistically reliable prescreen for 3PS antitumor activity.

A prerequisite for this assay is that the extract or substance being tested should not have antibacterial activity toward *A. tumefaciens*[24]. Inhibition of crown gall formation on potato discs is caused by two effects: by anti-tumorogenesis or decreasing the viability of the *A. tumefaciens*. In the present study, viability tests were carried out with all extracts to distinguish between these possibilities. Bacterial viability was determined by incubating plant extracts with 1×10^3 colony-forming units (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[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* + plant extracts) in terms of colony counts (ranged from 9.2×10^3 to 13×10^3 CFU). All tested extracts did not affect the viability of the bacterium. Thus, observed inhibition of tumor formation for these extracts was on the formation of tumors and not on the viability of the bacterium.

In our study, least antitumor activities (less than 50% tumor inhibition) were obtained with all extracts of *C. bursa-pastoris* and *R. luteola*. On the contrary, Samy *et al*[17] reported that luteolin isolated from *R. luteola* prevented chemical induced carcinogenesis in rats and has great potential for cancer prevention and therapy. Furthermore, Bekker *et al*[15] noted that *C. bursa* pastoris has been used for the treatment of some cancer types.

Matsuda *et al*[11] found that *N. lutea* extracts have anticancer activity on human leukemia cell (U937), human fibroblast (HT1080), and mouse melanoma cell (B16F10) lines. Similarly, in our study, also we observed strong tumor inhibition of the extacts of *N. lutea*.

Khanavi *et al*[36] studied cytotoxic effects of *V. minor* alkoloids on Colon carcinoma (HT-29), colorectaladenocarcinoma (Caco-2), breast ductalcarcinoma (T47D) and Swiss mouse embryo fibroblast (NIH 3T3) cell lines. They found that alkoloids affected the growth and proliferation of these cell lines was found[36]. Similarly, in our study, tested extracts of *V. minor* leaves and flowers exhibited strong tumor inhibition.

Ismailoglu *et al*[6] studied the phenylpropanoid glycosides isolated from *P. pungens* and reported that some phenylpropanoid glycosides could inhibit proliferation of some tumor cells. Similarly, in our study, strong antitumor activity was observed with all tested extracts of *P. pungens*.

In conclusion, antibacterial and antitumor activities of 51 different extracts obtained from 16 different plants grown in Turkey were evaluated. Strong antibacterial activity was obtained with ethanolic extract of *P. pungens* against *S. pyogenes*. Ethanolic extracts of *N. lutea* and *N. alba* also showed strong antibacterial activities against *E. cloacae*. Strong antitumor activities of *A. reptans*, *G. lydia*, *N. lutea*, *N. alba*, *V. lantana*, *O. heterophyllum* and *V. minor* were observed. With these results, tested plants have some scientific justification as a medicinal plant. In the future, identification of active components can be studied for plant extracts having strong bioactivity. Future studies should focus on fractionation of the extracts in hopes of identifying active components. Anticancer activity of these plants should be studied using different cancer cell lines in the future.

Conflict of interest statement

We declare that we have no conflict of interest.

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