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Original Research Article

Evaluation of Antibacterial and Antitumor Activities of Some Turkish Endemic Plants

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Abstract

Purpose: *To investigate the antibacterial and antitumor activities of the aerial parts of 8 different Turkish endemic plants (Phlomis russeliana, Phlomis armeniaca, Astragalus brachypterus, Astrantia maxima, Ptilostemon afer, Senecio castagneanus, Echium orientale and Arum euxinum).*
Methods: *Two different bioassays were performed to evaluate the antibacterial and antitumor activities of the endemic plants. For each plant, 3 types of extracts (aqueous, methanol and ethanol) were prepared, giving a total of 24 extracts tested. The disc diffusion assay was used to screen for antibacterial activity against 10 bacteria including Streptococcus pyogenes, Staphylococcus aureus, Staphylococcus epidermidis, Escheria coli, Pseudomonas aeruginosa, Salmonella typhimurium, Serratia marcescens, Proteus vulgaris, Enterobacter cloacea, and Klebsiella pneumoniae. Five different antibiotics (ampicillin, carbenicillin, chloramphenicol, erythromycin and tetracycline) were used as positive controls. Antitumor activity was evaluated using potato disc diffusion bioassay with camptothecin as positive control.*
Results: *The highest antibacterial activity was observed for all extracts of A. brachypterus against S. pyogenes (15.0 - 16.3 mm inhibition zone). The aqueous extract of S. castagneanus showed the strongest antibacterial activity against S. pyogenes (14.3 mm). Furthermore, alcohol extracts (ethanol and methanol) of P. russeliana exhibited moderate activity against S. epidermidis (9 and 8.5 mm, respectively) and S. pyogenes (9.5 mm). High antitumor activity was observed for all extracts of A. brachypterus (91.7 - 100 % tumor inhibition). In addition, ethanol extract of P. russeliana (75 % inhibition) exhibited strong antitumor activity.*
Conclusion: *The present study reveals the strong antibacterial and antitumor activities of A. brachypterus. However, the active components of the plant extracts needs to be identified in future studies.*

Keywords *Arum euxinum, Astragalus brachypterus, Astrantia maxima, Echium orientale, Ptilostemon afer, Phlomis armeniaca, Phlomis russeliana, Senecio castagneanus, Antibacterial, Antitumor.*

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INTRODUCTION

Medicinal plants are the richest bioresource of drugs in traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. 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. The scientific verification of the biological activity of endemic plants that are found exclusively in a particular area may be

important in screening for the potential value of these peculiar plants [2].

There are few studies on the biological activities of tested plants. Generally, *Echium* species have been used in folk medicine as diuretic, diaphoretic, febrifuge, expectorant, analgesic, vulnerary, sedative, anxiolytic and in the treatment of upper respiratory tract infections [3-6]. *Astragalus* species have been used traditionally to raise immune resistance, improve physical endurance and lower blood pressure [4]. *Astragalus* species have anti-inflammatory, analgesic, hypotensive, sedative, cardiotonic, hepatoprotective, antioxidative, antiviral and immunostimulant properties [7-11].

The roots of *Astragalus* species are used to treat leukemia and for wound healing in Turkish folk medicine [12]. *Phlomis* species have been used to treat various conditions such as diabetes, gastric ulcer, hemorrhoids, inflammation and wounds [13]. Demirci *et al* [14] revealed that *Phlomis* essential oils might be an alternative to conventional antimicrobials in various foods. Ozcelik *et al* [15] determined the antiviral, antibacterial and antifungal effects as well as cytotoxicity of selected Turkish *Phlomis* species. Caffeic acid-containing phenylpropanoid glycol-sides found in *P. armeniaca* showed activity against several kinds of cancer cells [16].

The aim of this study was to evaluate the antibacterial and antitumor activities of eight endemic plants found in Bolu, Turkey.

EXPERIMENTAL

Plant material and extraction

The aerial parts of the eight endemic plants were collected from Bolu, Turkey [17]. All plant samples and their treatments are presented in Table 1.

Three types of solvents - water, methanol (MeOH) and ethanol (EtOH)] were used for extraction. For aqueous extraction, each plant sample was extracted with water at 80 °C for 12 h. Water was removed from the extract by freeze-drying (Christ Alpha 1-2 LD Freeze dryer). For alcohol extractions, the plant samples were Soxhlet extracted with MeOH or EtOH at 60 °C for 12 h. The extracts were then vacuum evaporated. For bioassays, each residue was dissolved in sterile distilled water to obtain a final concentration of 100 mg/ml.

Antibacterial bioassay

The disc diffusion assay was used to screen for antibacterial activity [18]. *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 were used.

The turbidity of each broth culture of bacteria was adjusted to 0.5 McFarland standard and then Mueller Hinton agar plates were inoculated using cotton swabs. All extracts were sterilized by filtering through a 0.22 μm filter (Millex®) and sterile filter paper discs (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®) (Table 2). Water was used as a negative control. Inoculated plates 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.

Potato disc tumor induction assay

The antitumor activity of the extracts was assessed using potato disc method as modified by McLaughlin *et al* [19]. A suspension of *Agrobacterium tumefaciens* (ATCC® 23341) was standardized as determined by an absorbance value of 0.96 ± 0.02 at 600 nm [20]. All extracts and control solutions were filter sterilized.

Potatoes (*Solanum tuberosum* L.) were surface-sterilized by immersion in 10 % commercial bleach (Domestos®) for 20 min. Cylinders (10 mm diameter) were removed from the center of potato tissue using a cork borer and cut into 0.5 cm discs after excluding 1 cm end pieces. These discs were transferred to 24-well culture plates containing water-agar. Each disc was overlaid with 50 µl of appropriate inoculum. Plates were incubated at 28 °C in the dark. After 2 weeks, discs were stained with Lugol's reagent and tumors on each disc were counted. The experiments were repeated three times.

Bacterial viability testing

Bacterial suspension was serially diluted to 1 x 10^3 CFU. Bacterial viability was determined by incubating 1 ml of each plant extract with 1 ml of bacterial suspension. 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 the inoculated plates at 28 °C, colony counts were made [20].

Data analysis

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

RESULTS

Twenty-four different extracts prepared with three types of solvent (water, methanol and ethanol) of eight different endemic plant species were tested and the results are shown in Tables 1, 2 and 3. *S. pyogenes* was the most susceptible bacterium against tested extracts (Table 2). Good antibacterial activity was observed for all the tested extracts (water, EtOH and MeOH) of *A. brachypterus* against *S. pyogenes* (15, 17 and 16.3 mm, respectively). The aqueous extract of *S. castagneanus* also exhibited strong antibacterial activity against *S. pyogenes* (14.3 mm) while alcohol extracts of *S. castagneanus* and *P. russeliana* showed moderate antibacterial activity against *S. pyogenes* (8.8 and 9 mm). Moderate inhibition was also observed for alcohol extracts of *P. russeliana* against *S. epidermidis* (8.5 and 9 mm). All the tested extracts of *E. orientale* showed little inhibition against *K. pneumonia* (7.5 - 7.8 mm). Although *E. cloacae* was susceptible to only the ethanol extract of *E. orientale* (8.5 mm), this bacterium was not susceptible to other extracts (Table 2). All extracts of *P. afer, P. armeniaca* and *A. maxima* did not show any inhibitory activity against the pathogens (Table 2). *S. aureus, S. marcescens, S. typhimurium, P. aeruginosa, P. vulgaris* and *E. coli* were not sensitive to any of the extracts (data not shown).

Positive controls (reference antibiotics) generally showed antibacterial activity against the test microorganisms (Table 2). Since the final concentrations of all extracts were adjusted with distilled water, it was used as a negative control and the results show that there was no inhibition activity by the control solvent.

Good antitumor activity was observed with all extracts of *A. brachypterus* (91.7 - 100 %) (Table 3). The alcohol extracts of *P. russeliana* and aqueous extract of *P. armeniaca* also exhibited strong antitumor activity (61.1 - 75 %). Among the tested endemic plants, *A. maxima* showed least tumor inhibition (13.9 - 30.6 %). No tumor formation was observed with positive control, camptothecin (100% inhibition) (Table 3). The final concentrations of all the extracts were adjusted with distilled water which also served as negative control.

DISCUSSION

Kirby-Bauer test (disc diffusion method) is the most widely used standard method for antibacterial bioassay. It is currently performed by National Committee for clinical laboratory standards on disc diffusion susceptibility testing [18]. Gram-positive bacteria (*S. epidermidis* and *S. pyogenes*) were more susceptible to plant extracts than Gram-negative bacteria (Table 2). 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 [2].

E. orientale extracts showed barely inhibitory activity against *K. pneumonia* and *E. cloacae*. Eight bacteria were not sensitive to *E. orientale* extracts in our study. Similarly, Mansouri [21] reported that ethanolic extract of *Echium amoenum* had no antibacterial activity against *S. aureus* isolates. Morteza-Semnani *et al* [22] demonstrated that *Echium italicum* essential oil exhibited concentration-dependent antimicrobial activity on all microorganisms tested. Kuruuzum-Uz *et al* [23] isolated kaempferol, uridine, lactic acid and rosmarinic acid from *E. vulgare* and compounds showed no detectable antimicrobial activity against *E. coli*, *Bacillus subtilis*, *S. aureus* and *Candida albicans*. *E. vulgare* contains pyrrolizidine alkaloids, allantoin, alkannins and mucilage. The alkannins are antimicrobial and allantoin help wounds to heal [4].

Strong antibacterial activity of *A. brachypterus* may explain why *Astragalus* species is used in folk medicine to treat skin conditions (caused by *S. pyogenes*) [12]. All tested extracts of *A. brachypterus* showed strong inhibition against only *S. pyogenes* in our study (Table 2). Similarly, alcoholic extracts of *Astragalus gymnolobus* exhibited little inhibition against only *S. pyogenes* [2]. *A. gymnolobus* extracts were also effective only on *Aeromonas hydrophila* among tested five different fish pathogens [24]. The moderate antibacterial activities of some members of *Astragalus* species (*A. siculus*, *A. gummifer*, *A. membranaceus*, *A. malanophrurius* and *A. verrucosus*) were recorded against Gram-positive and Gram-negative bacteria [10,25]. *A. siculus* showed antibacterial activity against *S. aureus, S. epidermidis, Streptococcus faecalis,*

Table 1: Family, botanical names, collection numbers, yields (%) and extraction solvents of tested endemic plants. Means with the same letter within columns are not significantly different at p>0.05.

Family	Botanical names	Collection #	Treatment	Yield (%)
Asteraceae	*Ptilostemon afer* (Jacq.) Greuter subsp. *eburneus*	AUT-1948	W	14.7
			E	7.0
			M	15.9
	Senecio castagneanus DC.	AUT-1952	W	14.0
			E	15.0
			M	15.3
Lamiaceae	*Phlomis russeliana* (Sims) Bentham.	AUT-1946	W	6.2
			E	8.6
			M	20.0
	Phlomis armeniaca Willd.	AUT-1954	W	4.4
			E	8.9
			M	16.9
Fabaceae	*Astragalus brachypterus* Fischer	AUT-1947	W	8.6
			E	7.6
			M	13.2
Apiaceae	*Astrantia maxima* Pallas subsp. *haradjianii* (Grintz.) Rech.	AUT-1949	W	16.0
			E	17.5
			M	20.0
Boraginaceae	*Echium orientale* L.	AUT-1950	W	8.2
			E	4.6
			M	10.6
Araceae	*Arum euxinum* R. Mill	AUT-1951	W	9.7
			E	30.8
			M	39.3

Note: *W = aqueous extract; E = ethanol extract M = methanol extract*

Proteus mirabilis, Citrobacter freundii, P. aeruginosa and *Klebsiella oxytoca* [25].

All extracts of *S. castagneanus* showed strong antibacterial activity against only *S. pyogenes* in our study (Table 2). On the other hand, Albayrak *et al* [26] evaluated the methanol extracts of six *Senecio* species growing in the Black Sea region of Turkey (*S. pandurifolius*, *S. trapezuntinus*. *S. integrifolius*, *S. hypochionaeus*, *S. hypochionaeus* and *S. lorentii*). *K. pneumoniae* was the most sensitive microorganism to the all extracts examined while *E. coli* and *Candida albicans* were the most resistant one.

Alcohol extracts of *P. russeliana* exhibited moderate antibacterial activity against *S. epidermidi*s, *S. pyogenes* and *K. pneumonia* in our study. Demirci *et al* [14] tested *P. russeliana* essential oil against common food borne bacteria such as *E. coli*, *P. aeruginosa*, *S. aureus* and *S. typhimurium*. They observed weak to moderate minimum inhibitory concentrations. *P. armeniaca* extracts did not show inhibitory activity against any of the tested pathogens in our study. However, Ozcelik *et al* [15] evaluated the antibacterial activity of petroleum ether and methanol extracts of seven *Phlomis* species (*P. armeniaca*, *P. bourgaei*, *P. leucophracta*, *P. lunariifolia*, *P. lycia*, *P. pungens* var. *pungens*, and var. *hirta*). Methanol extracts of some *Phlomis* species (*Phlomis bruguieri*, *Phlomis herba-venti* and *Phlomis olivieri*) exhibited antibacterial effects against *E. coli*, *K. pneumonia*, *S. aureus*, *Staphylococcus sanguis* and *P. aeruginosa* [13].

The inhibition of *A. tumefaciens*-induced tumors in potato disc tissue is an assay based on antimitotic activity and can detect a broad range

of known and novel antitumor effects [19,20]. The validity of this bioassay is predicated 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 [20].

A prerequisite for potato disc tumor induction assay is that the extract or substance being tested should not have antibacterial activity toward *A. tumefaciens* [20]. 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. 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 x 10^3 to 13 x 10^3 CFU). All the extracts did not affect the viability of the bacterium. Thus, the observed inhibition of tumor formation for these extracts was on the formation of tumors.

Good tumor inhibition was observed with all the test extracts of *A. brachypterus* in our study. Triterpene saponins ingredient of *Astragalus* species may contribute to the strong immunomodulatory and anticancer activities

Table 2: Antibacterial activities of used plant extracts. Means with the same letter within columns are not significantly different at $p > 0.05$.

		Mean diameter of inhibitory zone (mm ± SEM)			
Botanical names	**Treatment**	***S.epidermidis***	***S.pyogenes***	***K. pneumonia***	***E. cloacae***
	W	- -	- -	- -	- -
P. afer	E	- -	- -	- -	- -
	M	- -	- -	- -	- -
	W	- -	14.3 ± 0.9 fg	- -	- -
S. castagneanus	E	- -	8.8 ± 1.0 i	- -	- -
	M	- -	9.0 ± 0.7 i		
	W	- -	- -	- -	- -
P. russeliana	E	9.0 ± 0.9 de	9.5 ± 0.6 i	- -	- -
	M	8.5 ± 0.9 e	9.5 ± 0.3 i	8.8 ± 0.3 d	- -
	W	- -	- -	- -	- -
P. armeniaca	E	- -	- -	- -	- -
	M	- -	- -	- -	- -
	W	- -	15.0 ± 0.0 efg	- -	- -
A. brachypterus	E	- -	17.0 ± 0.9 e	- -	- -
	M	- -	16.3 ± 0.3 ef	- -	- -
	W	- -	- -	- -	- -
A. maxima	E	- -	- -	- -	- -
	M	- -	- -	- -	- -
	W	- -	- -	7.8 ± 0.3 ef	- -
E. orientale	E	- -	- -	7.5 ± 0.3 ef	8.5 ± 0.9 d
	M	- -	- -	7.5 ± 0.3 ef	- -
	W	- -	- -	- -	- -
A. euxinum	E	- -	- -	- -	- -
	M	- -	- -	- -	- -
	Ampicillin	40.0 ± 2.9 a	53.5 ± 1.2 a	12.5 ± 0.9 b	27.8 ± 0.5 c
	Carbenicillin	41.5 ± 4.9 a	48.3 ± 1.0 b	8.3 ± 0.5 de	33.8 ± 0.9 a
Controls	Chloramphenicol	36.0 ± 2.3 b	37.0 ± 1.7 d	30.0 ± 1.2 a	29.5 ± 0.9 b
	Erythromycin	35.8 ± 2.5 b	37.8 ± 1.3 d	11.0 ± 0.4 c	8.8 ± 0.8 d
	Tetracycline	11.0 ± 0.6 cde	39.8 ± 1.8 c	29.3 ± 0.5 a	29.3 ± 0.8 b

Note: *W = aqueous extract; E = ethanol extract M = methanol*

Table 3: Mean number of tumors observed with used plant extracts. Means with the same letter within columns are not significantly different at $p > 0.05$.

Botanical name	Treatments	Mean Number of Tumors (±SE)				% Tumor inhibition
	W	19.6	±	3.1	defg	44.4
P. afer	E	13.8	±	4.3	cde	61.1
	M	14.7	±	2.6	cde	58.3
	W	14.3	±	2.5	cdef	61.1
S. castagneanus	E	21.5	±	3.4	efgh	38.9
	M	14.9	±	3.4	cdef	58.3
	W	22.4	±	2.4	efghi	38.8
P. russeliana	E	8.7	±	1.3	bc	75.0
	M	14.4	±	3.6	cde	61.1
	W	11.0	±	2.0	bcd	69.4
P. armeniaca	E	20.0	±	2.6	defg	44.4
	M	15.6	±	2.6	cdef	55.6
	W	3.1	±	1.1	ab	91.7
A. brachypterus	E	3.0	±	0.6	ab	91.7
	M	0.0	±	0.0	a	100.0
	W	31.3	±	3.2	ij	13.9
A. maxima	E	29.5	±	3.5	hij	16.7
	M	24.8	±	2.8	fghi	30.6
	W	28.1	±	2.5	ghij	22.2
E. orientale	E	26.0	±	3.4	ghij	27.8
	M	13.8	±	2.5	cde	61.1
	W	16.7	±	2.7	cdef	52.8
A. euxinum	E	22.6	±	2.2	efghi	36.1
	M	21.3	±	3.4	efgh	41.7
Controls	Water	35.8	±	4.5	j	-
	Camptothecin	0.0	±	0.0	a	100

Note: *W = aqueous extract; E = ethanol extract M = methanol*

[12]. Similarly, strong antitumor activity of *Astragalus membranaceous* was recorded with some studies [27-29]. Rittenhouse *et al* [27] reported that *A. membranaceous* may exert its antitumour activity by abolishing tumor-associated macrophage suppression. Tin *et al* [28] proposed that members of *Astragalus* may possess anti-tumorigenic potential in certain cancer cell types. The anti-carcinogenic effects of *Astragalus* saponin extracts were investigated in HT-29 human colon cancer cells and the results indicated that this extracts could be an effective chemotherapeutic agent in colon cancer treatment. Cho and Leung [29] isolated bioactive fractions from the roots of *A. membranaceous.* One of the fractions exhibited potent anti-tumor effects both *in vitro* and *in vivo*. Cho and Leung [30] also reported the immunomodulating and immunorestorative effects of same fraction. They concluded that A. *membranaceous* could exhibit both *in vitro* and *in vivo* anti-tumor effects, which might be achieved through activating the anti-tumor immune mechanism of the host [29,30]. On the other hand, strong antitumor activity was not observed with *Astragalus gymnolobus* [2].

P. armeniaca showed moderate antitumor activity in our study and Saracoglu *et al* [16] reported that phenyl propanoid caffeic acid, phenylethyl alcohol and phenylethylalcohol glycosides isolated from *P. armeniaca w*ere found to show cytotoxic activity against several kinds of cancer cells. Alcohol extracts of *P. russeliana* exhibited stronger antitumor activity than alcoholic extracts of *P. armeniaca*. Conversely, antitumor activity of aqueous extract of *P. armeniaca* was better than aqueous extract of *P. russeliana* in our study.

CONCLUSION

The antibacterial and antitumor activities of 24 different extracts obtained from eight different endemic plants grown in Turkey were evaluated. Results obtained herein revealed the strong antibacterial and antitumor activities of *A. brachypterus*. Future studies should

focus on fractionation of the extracts of *A. brachypterus* in the hope of identifying active components. Furthermore, antiproliferative activity studies of *A. brachypterus* should be carried on using different types of cancer lines in the future.

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