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**ORIGINAL PAPER**

# Biological activities of some endemic plants in Turkey

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## Abstract

*Two different bioassays (antibacterial and antitumor) were performed to evaluate the biological activities of 8 different Turkish endemic plants (Crocus abantensis T.Baytop & Mathew, Crocus ancyrensis (Herbert) Maw, Galanthus plicatus Bieb. subsp. byzantinus (Baker) D.A. Webb., Paronychia chionaea Boiss, Astragalus gymnolobus Fischer, Trifolium pannonicum Jacq. subsp.elongatum (Willd.) Zoh., Eryngium bithynicum Boiss and Convolvulus galaticus Rostan ex Choisy). For each plant, 3 different extracts (aqueous, methanol and ethanol) were prepared and totally 24 extracts were tested. The disc diffusion assay was used to screen for antibacterial activity. Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimurium, Serratia marcescens, Proteus vulgaris, Enterobacter cloacae and Klebsiella pneumoniae which are Gram-negative bacteria, and Staphylococcus aureus, Staphylococcus epidermidis and Streptococcus pyogenes which are Gram-positive bacteria were used. Best antibacterial activity was observed with T. pannonicum extracts. Ethanolic extract of T. pannonicum was better than aqueous and methanolic extracts against S. aureus, S. epidermidis, S. pyogenes, P. vulgaris and K. pneumonia. Antitumor activity was evaluated with potato disc diffusion bioassay. Best antitumor activity was observed with all extracts of G. plicatus. Generally, alcoholic extracts showed better antitumor activity than aqueous extracts. Alcoholic extracts of C. abantensis, C. ancyrensis, P. chionae and C. galaticus also exhibited strong antitumor activity.*

**Key words:** Antibacterial, Antitumor, *Astragalus gymnolobus*, *Convolvulus galaticus*, *Crocus abantensis*, *Crocus ancyrensis*, *Eryngium bithynicum*, *Galanthus plicatus*, *Paronychia chionaea*, *Trifolium pannonicum.*

## Introduction

Throughout the history, plants have been the most important source of medicines for human health [1]. World Health Organization estimates that up to 80% of the world's people rely on plants for their primary health care [2]. Plants have been a rich source of medicines because they produce a host of bioactive molecules, most of which probably evolved as chemical defenses against predation or infection [3]. Turkey has a rich plant diversity and higher endemism ratio comparing with Europe. According to last records [4], there are 8988 native plant species and endemism ratio is about 33.3% with 2991 endemic plant species. Having various climates, geomorphology and topographic structures of Anatolia, and taking place in the intersection of the three phytogeographical regions (Euro-Siberian, Mediterranean and Irano-Turanian) caused the extraordinary variety of habitats and ecosystem in Turkey [5]. The scientific verification of biological activity of endemic plants may be important to screen the potential value of endemics [6].

Generally, *Trifolium* spp. has been used in folk medicine to treat skin conditions [7]. Gođevac *et al.* [8] reported the antioxidant activity of *T. pannonicum*. Triterpene saponins in

the seeds and flavonoid glycosides in the aerial part of *T. pannonicum* were detected [8]. Roots and leaves of *Convolvulus* spp. are cholagogue, laxative and strongly purgative in traditional medicine [9, 10]. The aerial parts, roots and flowers of *C. arvensis* extracts have moderate diuretic, tranquillizing, hypoglycemic, antihemorrhagic, antibacterial, antifungal [11] and antitumor activities [12]. Traditionally, an infusion of the *Paronychia* leaves is known as aphrodisiac and diuretic. It is also used in the treatment of tuberculosis in folk medicine [9]. Inhibitory effect of *P. argentea* was found against *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* [13]. *Astragalus* spp. have been used in folk medicine to raise immune resistance, improve physical endurance and lower blood pressure [7]. *Astragalus* spp. have anti-inflammatory, analgesic, hypotensive, sedative, cardiotonic, hepatoprotective, antioxidative, antiviral and immunostimulant properties [14 - 18]. *Eryngium* spp. have been used in folk medicine as antispasmodic, aromatic, diaphoretic, diuretic, expectorant, stimulant, nervine and aphrodisiac [10]. The crushed leaves of *E. foetidum* are placed in the ear to treat pain, and are used for the local treatment of arthritic processes [19]. Küpeli *et al*. [20] reported the anti-inflammatory and antinociceptive activities of some members of *Eryngium* spp. in Turkey. Antibacterial activity of *E. foetidium* against *Helicobacter pylori* was recorded [21]. Some *Galanthus* spp. have been used traditionally in Bulgaria and Turkey for neurological conditions [22] besides as emmenagogue, digestive, resolutive and hearth strengthener [9, 10]. Galantamine isolated from *G. woronowii* is approved for the treatment of Alzheimer's disease, slowing the process of neurological degeneration [23]. Flowers of some *Crocus* spp. are consumed as a snack food and recorded as an antiseptic [24]. *Crocus* spp. have been used in the treatment of dysentery, measles, enlargement of the liver and gall bladder, urological infections, cough, stomach disorders, asthma and cardiovascular disorders [25]. Recent studies indicate its potential as an anticancer, antitumoral, cytotoxic, hypolipidaemic, anti-inflammatory and oxygenation enhancement agent [26 - 28].

The aim of this study was to evaluate the antibacterial and antitumour activities of 8 endemic plants found in Bolu, Turkey.

## Methodology

### Plant material and extraction

Studied five endemic plant species [*Crocus abantensis* T.Baytop & Mathew, *Crocus ancyrensis* (Herbert) Maw, *Galanthus plicatus* Bieb. subsp. *byzantinus* (Baker) D.A. Webb., *Paronychia chionaea* Boiss and *Astragalus gymnolobus* Fischer] were collected from Abant Lake, Bolu, Turkey and three endemic species [*Trifolium pannonicum* Jacq. subsp.*elongatum* (Willd.) Zoh., *Eryngium bithynicum* Boiss and *Convolvulus galaticus* Rostan ex Choisy] were collected from Abant Izzet Baysal University, Golkoy Campus, Bolu, Turkey in 2010. Identification of species was made by using "Flora of Turkey and the East Aegean Islands" [29] and voucher specimens were deposited at the Abant Izzet Baysal University (AIBU) Herbarium, Bolu, Turkey. All plant samples, collection times and numbers were presented in Table 1.

**Table 1.** Botanical and common names of studied endemic plants, their family, used parts, extract designations, extraction solvents, collection times, voucher numbers and yield (%) for each extraction.

[*]Yield (%) = Weight of extract (g) / 20 g of plant sample X 100.
[**]Plant specimens were collected by Arzu Ucar Turker.

| Family and plants species | Part used | Extract Designation | Extraction Solvents | Yield (%)* | Collection time | Voucher number |
|---|---|---|---|---|---|---|
| **IRIDACEAE** | | | | | | |
| *Crocus abantensis* T.Baytop & Mathew | | Ex 1a | Water | 20 | | |
| | Aerial | Ex 1b | MeOH | 61 | April, 2010 | AUT**-2010 |
| | | Ex 1c | EtOH | 22 | | |
| *Crocus ancyrensis* (Herbert) Maw | | Ex 2a | Water | 20 | | |
| | Aerial | Ex 2b | MeOH | 36 | April, 2010 | AUT-2011 |
| | | Ex 2c | EtOH | 34 | | |
| **AMARYLLIDACEAE** | | | | | | |
| *Galanthus plicatus* Bieb. | | Ex 3a | Water | 27 | | |
| subsp. *byzantinus* (Baker) D.A. Webb. | Aerial | Ex 3b | MeOH | 17 | March, 2010 | AUT-2012 |
| | | Ex 3c | EtOH | 17 | | |
| **CONVOLVULACEAE** | | | | | | |
| *Convolvulus galaticus* Rostan ex Choisy | | Ex 4a | Water | 26 | | |
| | Aerial | Ex 4b | MeOH | 10 | July, 2010 | AUT-2013 |
| | | Ex 4c | EtOH | 7 | | |
| **FABACEAE** | | | | | | |
| *Trifolium pannonicum* Jacq. | | Ex 5a | Water | 20 | | |
| subsp. *elongatum* (Willd.) Zoh. | Aerial | Ex 5b | MeOH | 9 | June, 2010 | AUT-2014 |
| | | Ex 5c | EtOH | 5 | | |
| **APIACEAE** | | | | | | |
| *Eryngium bithynicum* Boiss | | Ex 6a | Water | 17 | | |
| | Aerial | Ex 6b | MeOH | 12 | July, 2010 | AUT-2015 |
| | | Ex 6c | EtOH | 11 | | |
| **ILLECEBRACEAE** | | | | | | |
| *Paronychia chionaea* Boiss | | Ex 7a | Water | 24 | | |
| | Aerial | Ex 7b | MeOH | 10 | May, 2010 | AUT-2016 |
| | | Ex 7c | EtOH | 39 | | |
| **FABACEAE** | | | | | | |
| *Astragalus gymnolobus* Fischer | | Ex 8a | Water | 26 | | |
| | Aerial | Ex 8b | MeOH | 16 | June, 2010 | AUT-2017 |
| | | Ex 8c | EtOH | 14 | | |

Collected plants were dried in the oven at 40 °C and then ground into a powder. Three different solvents [water, methanol (MeOH) and ethanol (EtOH)] were used for extraction. For aqueous extraction, 20 g from each plant sample were extracted with 200 ml water at 80 °C in a waterbath for 12 hours. The extract was then filtered and lyophilized. For alcoholic extractions, 20 g of plant sample were Soxhlet extracted with 350 ml MeOH or EtOH at 60 °C for 12 hours. The extract was then vacuum evaporated. For antibacterial assay, 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 were summarized in Table 1.

**Antibacterial bioassay**

The disc diffusion assay (Kirby-Bauer Method) was used to screen for antibacterial activity [30]. The microorganisms used *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 and *Streptococcus pyogenes* (ATCC® 19615), *Staphylococcus aureus* (ATTC® 25923) and *Staphylococcus* 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 °C. One bacteriological loop from each broth was streaked on Tryptic Soy Agar (TSA) plates and incubated for 2 days at 37 °C. After 2 days, a single colony was removed and streaked on TSA plate and incubated at 37 °C for 2 additional days. The turbidity of each broth culture

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

**Potato disc tumor induction bioassay**

Antitumour activity of extracts was assessed with potato disc method as modified by McLaughlin's group [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 x $10^9$ 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 then immersed in 20% commercial bleach 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 [33]. Plates were incubated at 28 °C 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] X 100" [33 - 35].

**Bacterial viability testing**

Standardized bacterial suspension (1 x $10^9$ CFU of *A. tumefaciens* in PBS) was serially diluted with PBS to 1 x $10^3$ CFU. Bacterial viability was determined by incubating 1 ml of each plant extract with 1 ml of bacterial suspension (1 x $10^3$ 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 °C, colony counts were made. Also, bacterial growth was evidenced by growth across the plates [32].

**Data analysis**

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

## Results and Discussion

Twenty-four different extracts prepared with three kinds of solvent (water, methanol and ethanol) of eight different endemic plant species were tested in order to screen and show their potential as antibacterial and antitumor agents (Table 2 and 3). 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 [30]. Best inhibitory activity was obtained with *T. pannonicum*. All 3 kinds of extracts (water, MeOH and EtOH) obtained from *T. pannonicum* exhibited strong antibacterial activity against *S. epidermidis* and *S. pyogenes* (Table 2). Antibacterial activity of *T. pannonicum* may explain why *Trifolium* spp. is used in folk medicine to treat skin conditions (caused by *S. epidermidis* and *S. pyogenes*). Generally, ethanolic extract of *T. pannonicum* was better than aqueous and methanolic extracts of this plant against *S. aureus*, *S. epidermidis*, *S. pyogenes*, *P. vulgaris* and *K. pneumonia*. Although *E. cloacae* was susceptible to methanolic extract of *T. pannonicum*, this bacterium was not susceptible to aqueous and ethanolic extracts (Table 2). Only ethanolic extract of *T. pannonicum* showed inhibition against *K. pneumonia* (Table 2). Antibacterial activity of aqueous, methanolic and ethanolic extracts of *T. pannonicum* was greater than reference antibiotic tetracycline against *S. epidermidis* (Table 2). *T. pannonicum* extracts were active against both Gram-positive and Gram-negative bacteria. Especially, methanolic and ethanolic extracts of *T. pannonicum* exhibited of a 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 [36].

Table 2. Antibacterial activity of used plant extracts. Means with the same letter within columns are not significantly different at P>0.05.

| | Mean diameter of inhibitory zones (mm ± SE) | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Treatments** | ***S. aureus*** | | ***S. epidermidis*** | | ***S. marcescens*** | | ***S. pyogenes*** | | ***S. typhimurium*** | | ***P. aeruginosa*** | | ***P. vulgaris*** | | ***K. pneumonia*** | | ***E. cloacae*** | | ***E. coli*** | |
| Ex 1a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 1b | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 1c | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 2a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 2b | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 2c | 7.00 ± 0.00 e | | - | - | - | - | - | - | - | - | - | - | - | - | 7.50 ± 0.50 c | | - | - | - | - |
| Ex 3a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 3b | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 3c | - | - | 7.25 ± 0.25 g | | - | - | 12.50 ± 0.50 f | | - | - | - | - | 8.25 ± 0.62 d | | 7.25 ± 0.25 c | | - | - | - | - |
| Ex 4a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 4b | - | - | - | - | - | - | 7.00 ± 0.00 h | | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 4c | - | - | - | - | - | - | 11.00 ± 1.15 fg | | - | - | 7.00 ± 0.00 d | | 7.75 ± 0.47 d | | - | - | 7.50 ± 0.28 e | | - | - |
| Ex 5a | - | - | 10.75 ± 1.03 ef | | - | - | 8.50 ± 0.28 gh | | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 5b | 7.00 ± 0.00 e | | 10.75 ± 0.25 ef | | - | - | 18.00 ± 0.40 e | | - | - | - | - | 7.00 ± 0.00 d | | - | - | 7.50 ± 0.28 e | | - | - |
| Ex 5c | 7.50 ± 0.28 e | | 11.50 ± 0.86 e | | - | - | 19.75 ± 0.75 e | | - | - | - | - | 7.25 ± 0.25 d | | 7.75 ± 0.75 c | | - | - | - | - |
| Ex 6a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 6b | - | - | - | - | - | - | 9.50 ± 0.28 gh | | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 6c | - | - | - | - | - | - | 8.25 ± 0.25 gh | | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 7a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 7b | - | - | - | - | - | - | 7.75 ± 0.75 h | | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 7c | - | - | - | - | - | - | 7.00 ± 0.00 h | | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 8a | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 8b | - | - | - | - | - | - | 7.00 ± 0.00 h | | - | - | - | - | - | - | - | - | - | - | - | - |
| Ex 8c | - | - | - | | - | - | 7.00 ± 0.00 h | | - | - | - | - | - | - | - | - | - | - | - | - |
| Chloramphenicol (30 µg) | 26.00 ± 0.57 d | | 29.75 ± 1.18 b | | 27.75 ± 0.62 a | | 33.75 ± 1.31 d | | 27.75 ± 1.31 a | | 10.75 ± 1.49 c | | 20.50 ± 1.25 b | | 28.50 ± 0.95 a | | 30.50 ± 0.64 b | | 27.25 ± 0.85 b | |
| Tetracycline (30 µg) | 31.50 ± 0.28 c | | 9.25 ± 0.25 f | | 23.00 ± 0.91 c | | 43.75 ± 2.39 ab | | 26.25 ± 1.31 b | | 18.50 ± 1.32 b | | 30.75 ± 1.88 a | | 27.75 ± 1.31 a | | 28.75 ± 1.03 c | | 29.00 ± 0.70 a | |
| Ampicillin (10 µg) | 39.00 ± 2.48 b | | 19.00 ± 1.00 d | | 14.25 ± 1.25 d | | 46.25 ± 2.39 a | | 26.25 ± 0.75 b | | - | - | 21.00 ± 2.38 b | | - | - | 27.00 ± 1.08 d | | 20.75 ± 0.47 d | |
| Carbenicillin (100 µg) | 40.50 ± 0.28 a | | 23.75 ± 0.62 c | | 25.25 ± 0.47 b | | 43.00 ± 2.64 b | | 24.00 ± 1.29 c | | 23.25 ± 2.49 a | | 31.75 ± 1.65 a | | - | - | 32.50 ± 1.70 a | | 22.75 ± 1.03 c | |
| Erythromycin (15 µg) | 25.25 ± 0.25 d | | 32.50 ± 2.25 a | | 10.75 ± 1.18 e | | 38.25 ± 1.65 c | | 11.50 ± 0.28 d | | 18.50 ± 5.54 b | | 11.00 ± 0.57 c | | 12.75 ± 0.85 b | | - | - | 15.25 ± 2.13 e | |
| Water | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

Turker *et al*. [6] reported the antibacterial activities of our studied endemic plants on common fish pathogens (*Aeromonas hydrophila, Yersinia ruckeri, Streptococcus agalactia, Lactococcus garvieae* and *Enterococcus faecalis*) using the same plant extracts. Similarly, the alcoholic extracts of *T. pannonicum* among all of the plant extracts showed a broad antibacterial spectrum against *A. hydrophila, Y. ruckeri, S. agalactia, L. garvieae* except *E. faecalis* [6].

Bacterial growth was generally sensitive to the reference antibiotics tested (Table 2). Inhibition zones varied from 46.25 mm for ampicillin and *S. pyogenes* to 9.25 mm for tetracycline and *S. epidermidis*. 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 (Table 2).

All endemic plant extracts were not effective against *S. marcescens, S. typhimurium* and *E. coli* which are Gram-negative bacteria (Table 2). In our study, Gram-positive bacteria (*S. aureus, 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 [37].

*C. abantensis* did not show any inhibitory activity against all used pathogens. Similarly, Ilcim *et al*. [38] reported that *Crocus chrysanthus* was not active against their used bacteria species (*Bacillus megaterium, B. brevis, K. pneumoniae, E. coli, Enterobacter aerogenes, P. aeruginosa, S. aureus*, and *Listeria monocytogenes*). However, ethanolic extract of *C. ancyrensis* showed just a little activity (7 and 7.5 mm) against only *S. aureus* and *K. pneumonia* (Table 2).

Generally all alcoholic extracts (MeOH and EtOH) except *C. abantensis* and C. *ancyrensis* showed inhibition against *S. pyogenes* (Table 2). *S. pyogenes* was the most susceptible bacterium.

Alcoholic extracts (MeOH and EtOH) of *E. bithynicum, P. chionaea* and *A. gymnolobus* showed inhibition against only *S. pyogenes* in our study (Table 2). Ndip *et al*. [21] recorded the antibacterial activity of *E. foetidium* against *Helicobacter pylori*. Inhibitory effect of *P. argentea* was also found against *Bacillus subtilis, S. aureus, E. coli* and *P. aeruginosa* [13]. Moderate antibacterial activities of some members of *Astragalus* spp. (*A. siculus, A. gummifer, A. membranaceus, A. malanophrurius* and *A. verrucosus*) were recorded against Gram-positive and Gram-negative bacteria [17, 39]. *A. siculus* showed antibacterial activity against *S. aureus, S. epidermidis, Streptococcus faecalis, Proteus mirabilis, Citrobacter freundii, P. aeruginosa* and *Klebsiella oxytoca* [39].

Alcoholic extracts of *C. galaticus* exhibited antibacterial activity against *S. pyogenes, P. aeruginosa, P. vulgaris* and *E. cloacae* in our study (Table 2). Similarly, antibacterial activity of *Convolvulus arvensis* was recorded by Awaad *et al*. [11].

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 [32, 34]. 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 [32, 40].

Ferrigini *et al*. [31] 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.

First, all extracts' antitumor activity were tested at 100.000 mg/l (Table 3). Then, extracts having best tumor inhibition (1b, 2b, 2c, 3a, 3b, 3c, 4b, 4c, 5b, 5c and 7b) were tested at 10.000 mg/l, 1000 mg/l and 100 mg/l, respectively (Table 4). Best antitumor activity was observed with all extracts (water, MeOH and EtOH) of *G. plicatus* at 100.000 mg/l (Table 3). As the concentration of all *G. plicatus* extracts decreased from 10.000 mg/l to 100 mg/l, % tumor inhibition was decreased from 100% (3a), 96.6% (3b) and 97.2% (3c) to 0% (Table 4). There are no records about anticancer activity of *Galanthus* spp. up to now. Generally, *Galanthus* spp. has been used traditionally in Bulgaria and Turkey for neurological conditions [22]. Moreover, methanolic extracts of *C. abantensis* (1b) and *P. chionae* (7b), methanolic and ethanolic extracts of *C. ancyrensis* (2b and 2c), *C. galaticus* (4b and 4c) and *T. pannonicum* (5b and 5c) showed strong antitumor activity at 100.000 mg/l (Table 3). Methanolic extract of *C. ancyrensis* (2b) also exhibited strong antitumor activity at 10.000 mg/l. But, tumor inhibition was decreased to 27% at 1000 mg/l and to 18.5% at 100 mg/l (Table 4). Furthermore, methanolic extract of *P. chionae* (7b) exhibited moderate antitumor activity (57.7% tumor inhibition) at 10.000 mg/l (Table 4).

A prerequisite for potato disc tumor induction assay is that the extract or substance being tested should not have antibacterial activity toward *A. tumefaciens* [41]. 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 $1 \times 10^3$ 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 [42, 43], 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 $9.2 \times 10^3$ to $13 \times 10^3$ CFU) except *T. pannonicum* extracts. All tested extracts other than *T. pannonicum* did not affect the viability of the bacterium. Thus, observed inhibition of tumor formation for these extracts was on the formation of tumors (Table 3). On the other hand, *T. pannonicum* extracts affected on the viability of the bacterium and *A. tumefaciens* bacterial growth was not observed across the plates. So, it was understood that inhibition of crown gall formation on potato disc is caused by decreasing the viability of the *A. tumefaciens* for *T. pannonicum* extracts and it was not suitable to evaluate the antitumor activity of *T. pannonicum* extracts (5b and 5c) with potato disc bioassay (Table 3 and 4). Although the results herein did not prove the anti-tumor activity of *T. pannonicum* extracts, anticancer activity of this plant should be tested using different cancer cell lines in the future. Because, Godevac *et al*. [8] recorded the antioxidant activity of *T. pannonicum*.

**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 | 76.81 | ± | 2.91 | fg | - |
| Camptothecin | 0 | ± | 0 | | 100 |
| Ex 1a | 68.64 | ± | 2.72 | f | 10.6 |
| Ex 1b | 10.04 | ± | 1.86 | b | 86.9 |
| Ex 1c | 33.33 | ± | 2.49 | c | 56.6 |
| Ex 2a | 57.10 | ± | 2.31 | e | 25.7 |
| Ex 2b | 0 | ± | 0 | a | 100 |
| Ex 2c | 0.06 | ± | 0.04 | a | 99.9 |
| Ex 3a | 0.12 | ± | 0.12 | a | 99.8 |
| Ex 3b | 0 | ± | 0 | a | 100 |
| Ex 3c | 0 | ± | 0 | a | 100 |
| Ex 4a | 69.77 | ± | 2.97 | f | 9.2 |
| Ex 4b | 0 | ± | 0 | a | 100 |
| Ex 4c | 0.97 | ± | 0.43 | a | 98.7 |
| Ex 5a | 71.08 | ± | 3.96 | f | 7.5 |
| Ex 5b | 13.08 | ± | 1.31 | b | 83.0 |
| Ex 5c | 10.50 | ± | 1.96 | b | 86.3 |
| Ex 6a | 80.10 | ± | 3.42 | g | 0.0 |
| Ex 6b | 74.70 | ± | 3.34 | fg | 2.7 |
| Ex 6c | 57.12 | ± | 2.88 | e | 25.6 |
| Ex 7a | 72.35 | ± | 3.70 | fg | 5.8 |
| Ex 7b | 6.37 | ± | 1.31 | a | 91.7 |
| Ex 7c | 59.27 | ± | 3.40 | e | 22.8 |
| Ex 8a | 89.33 | ± | 3.99 | h | 0.0 |
| Ex 8b | 44.45 | ± | 3.88 | d | 42.1 |
| Ex 8c | 47.41 | ± | 4.28 | d | 38.3 |

**Table 4.** Mean number of tumors of extracts having best antitumor activity at different concentrations (10.000 mg/l, 1.000mg/l and 100 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 | 76.81 | ± | 2.91 | fgh | - |
| Camptothecin | 0 | ± | 0 | | 100 |
| Ex 1b (10.000) | 98.42 | ± | 9.45 | hıj | 0.0 |
| Ex 1b (1000) | 108.42 | ± | 6.31 | ıj | 0.0 |
| Ex 1b (100) | 111.75 | ± | 3.25 | j | 0.0 |
| Ex 2b (10.000) | 3.17 | ± | 0.80 | a | 93.2 |
| Ex 2b (1000) | 34.25 | ± | 8.01 | bcd | 27.0 |
| Ex 2b (100) | 38.33 | ± | 7.62 | bcd | 18.5 |
| Ex 2c (10.000) | 38.17 | ± | 4.66 | bcd | 18.7 |
| Ex 2c (1000) | 39.85 | ± | 8.33 | bcd | 15.3 |
| Ex 2c (100) | 72.75 | ± | 12.73 | efgh | 0.0 |
| Ex 3a (10.000) | 0.0 | ± | 0.0 | a | 100 |
| Ex 3a (1000) | 22.83 | ± | 6.35 | ab | 51.5 |
| Ex 3a (100) | 53.08 | ± | 12.91 | cdef | 0.0 |
| Ex 3b (10.000) | 1.58 | ± | 1.15 | a | 96.6 |
| Ex 3b (1000) | 26.50 | ± | 6.59 | abc | 43.6 |
| Ex 3b (100) | 91.50 | ± | 7.36 | hıj | 0.0 |
| Ex 3c (10.000) | 1.25 | ± | 0.39 | a | 97.2 |
| Ex 3c (1000) | 20.08 | ± | 4.14 | ab | 57.2 |
| Ex 3c (100) | 48.58 | ± | 7.79 | bcde | 0.0 |
| Ex 4b (10.000) | 61.75 | ± | 8.74 | defg | 0.0 |
| Ex 4b (1000) | 71.75 | ± | 13.58 | efgh | 0.0 |
| Ex 4b (100) | 60.92 | ± | 12.66 | defg | 0.0 |
| Ex 4c (10.000) | 39.17 | ± | 8.55 | bcd | 16.6 |
| Ex 4c (1000) | 85.42 | ± | 7.99 | ghıj | 0.0 |
| Ex 4c (100) | 97.58 | ± | 15.13 | hıj | 0.0 |
| Ex 5b (10.000) | 84.75 | ± | 6.73 | ghıj | 0.0 |
| Ex 5b (1000) | 96.58 | ± | 13.21 | hıj | 0.0 |
| Ex 5b (100) | 83.25 | ± | 11.71 | ghı | 0.0 |
| Ex 5c (10.000) | 72.50 | ± | 9.64 | efgh | 0.0 |
| Ex 5c (1000) | 77.58 | ± | 10.59 | fgh | 0.0 |
| Ex 5c (100) | 56.58 | ± | 7.31 | defg | 0.0 |
| Ex 7b (10.000) | 19.92 | ± | 3.34 | ab | 57.7 |
| Ex 7b (1000) | 34.58 | ± | 6.09 | bcd | 26.4 |
| Ex 7b (100) | 62.75 | ± | 13.14 | defg | 0.0 |

Generally, alcoholic extracts (MeOH and EtOH) of all endemic plants exhibited better % tumor inhibition than aqueous extracts (Table 3). *E. bithynicum* and *A. gymnolobus* did not show any antitumor activity (Table 3). No tumor formation was observed with positive control camptothecin (100% inhibition) (Table 3). Final concentrations of all extracts were adjusted with distilled water. Therefore, water was used as a negative control.

Anticancer, antitumoral and antioxidant activities of *Crocus sativus* (saffron) have been reported by some studies [26, 27, 44]. Similarly, strong antitumor activity was observed with ethanolic extracts of *C. abantensis* (1b) and *C. ancyrenis* (2b) and 2c) in our study (Table 3 and 4). Main biologically active metabolites of *Crocus sativus* include crocins, crocetin, picrocrocin, β-carotene and safranal, that is the main component of the essential oil and is

responsible for the characteristic saffron aroma [26]. Crocin derivatives and mainly crocin suppress tumour growth, while safranal and crocetin were proved to possess antileukaemic activity [26]. Tumor incidence and histopathological studies proves crocetin is a potent antitumour agent [44].

Although antitumor activity of *Astragalus membranaceous* was recorded with some studies [45, 46, 47], we did not observe strong antitumor activity with *A. gymnolobus* (8a, 8b and 8c) (Table 3). Rittenhouse *et al*. [45] reported that *A. membranaceous* may exert its antitumour activity by abolishing tumor-associated macrophage suppression. Tin *et al*. [46] 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 [47] 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 [48] 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 [47, 48].

Alcoholic extracts of *C. galaticus* (4b and 4c) exhibited strong antitumor activity at 100.000 mg/l (Table 3). Similarly, Habibinya *et al*. [12] recorded the antitumor activity of *C. arvensis.*

## Conclusions

Antibacterial and antitumor activities of 24 different extracts obtained from 8 different endemic plants grown in Turkey were evaluated. Results obtained herein revealed the strong antibacterial activities of *T. pannonicum* and strong antitumor activities of *G. plicatus*. Future studies should focus on fractionation of the extracts of *T. pannonicum* and *G. plicatus* in hopes of identifying active components. Anticancer activity of *G. plicatus* should be studied using different cancer cell lines in the future. The scientific verification of antibacterial and antitumor activity of 8 endemic plants exposed the potential value of these endemics in Turkey.

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