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Screening of Antimicrobial and Anticancer Potentials of Some Plant Extracts from Mugla Provience

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Abstract

Many phytochemicals from different plant species have potency in treatment and prevention of cancer. Currently, substantial research have being carried out in many laboratories the world over to discover new plant extracts with high anticancer and/or antimicrobial activities. In this study, seven different plant species *Glaucium flavum, Euphorbia falcata, Conyza canadensis, Chenopodium botrys, Catalpa sp., Quercus coccifera, Crataegus monogyna* from Mugla region were examined for their antimicrobial and anticancer capacities. Disc diffusion method was used to study antimicrobial potency of ethanol extracts against Gram-positive and Gram-negative bacteria. Anticancer activities of the plant extracts were tested against the six different cancer cell lines using the MTT assay. The fruit extract of *Q. coccifera* was found to be the most effective extract (13-15 mm 80µg/disc inhibition zone) against *S. albus, M. luteus and S.aureus*. In addition, *C. canadensis* exhibited antimicrobial activity only against *E.coli* and *B. subtilis* (10-14 mm 80µg/ml inhibition zone). Based on MTT assays, all of the plant extracts resulted in different degrees of anticancer activity on six different cancer cell lines. The most effective extracts were from *C. botrys, G. flavum, Catalpa* sp and *Q. coccifera* which caused more than threefold decrease in the cell proliferation of K562, PC-3 and MCF-7 cell lines compared to control. In conclusion, among the seven tested plant extracts, only *Q. coccifera* and *C. canadensis* showed anti-microbial capacity. On the other hand, 4 plant extracts have displayed high anticancer potency. Future studies related with cell death will elicit the mechanisms of anti-tumor activities of the plant extracts.

Key words: Plant extracts, Anti-microbial activity, Anti-cancer activity, Cancer cell lines

INTRODUCTION

It has been known that tremendous progress have been made in the understanding of microorganisms and their control so far. Many infectious diseases are treated with antibiotics and herbel remedies. However, pathogenic microorganisms such as *Streptococcus pyogenes, Sthaphlococcus aureus, Pseudomonas* and members of *Enterobacteriaceae* causing respiratory infection, diarrhea, urinary tract infections and sepsis became resistant to almost all of the known antibiotics [1] due to the indiscriminate use of antimicrobial drugs [2]. In addition, serious side effect of some of the antibiotics limits their medical use application. Therefore, such problems urge the need for development of new antimicrobial agents. Plants are the important candidates for the potential source of antibiotics.

Cancer is a multistep disease that represents one of the major causes of mortality worldwide. According to World Health Organization (WHO), global number of deaths will reached to 80% by 2030. Even though there is an increase in the number of potent chemotherapic anticancer agents, cancer stil remains a major cause of deaths in" the world. Most anticancer agents lacks the selectivity aganist cancer cells [3]. Therefore the development of new anticancer agents that are both safe and effective is required. From this point of view, terrestrial plants have been a practical approach to this problem. In fact, a variety of plant-derived anticancerous compounds are currently available for clinical use [4]. Plants have long history of use in the treatment of cancer. So far, more than 3000 plant species are used aganist cancer [5].

Due to its geographic location and climate Turkey is an important floristic center and nearly 10.000 natural plant species have been reported [6]. Environmental factors such as geographic location, climate, soil stucture, altitude and ecological conditions cause a change in the structure and formation of seconder metobolite of the same plant species [7]. Therefore, therapeutic potentials of seconder metabolites encourage the examination of antimicrobial and anticancer activities of the same plant species from different environmental and ecological conditions. The present study was conducted to screen and evaluate antimicrobial and anticancer potential of the ethanol extracts of 7 different plant species from Mugla Sıtkı Kocman (MSK) University campus.

MATERIALS AND METHODS

Plant Material

Plants were collected from MSK University campus in October. The plants were identified in Botanic Laboratory (Department of Biology, MSK University, Mugla) and then all the plants were air-dried. Aerial parts of plants were used for antimicrobial and anticancer activities.

Plant Extraction

The plant samples (leaves of *Glaucium flavum, Euphorbia falcata, Conyza canadensis, Chenopodium botrys, Tanacetum* sp, *Catalpa* sp; fruit or fruit skin of *Quercus coccifera*) were dried under shade and miled to a powder using a porcelain muller. Powdered plant materials (10 g) was soaked in absolute ethanol (96°, Fluka chemical) and placed in soxhlet apparatus for 10 hours to prepare the ethanolic extracts. The extracts were then filtered using Whatman filter paper no.1, concentrated in a rotary evaporator. Solvent was evaporated by keeping the extracts at 37 °C for 7 days. The powdered crude extracts were stored at 4 °C prior to use.

Microorganisms Tested

Strains of pathogen microorganisms used in this study as follows. Two gram-negative bacteria, *Escherichia coli* (ATCC 11230), *Pseudomonas aeruginosa* (ATCC 29212); four gram positive bacteria, *Staphylococcus aureus* (ATCC 6538/P), *Bacillus subtilis* (ATCC 6633), *Micrococcus luteus* (NRRLB-4375) and *Streptomyces albus* (CIP104432). These strains were obtained from Dr. Nurettin Şahin (MSK University).

Antimicrobial Activity

Before the analysis, bacterial strains were cultured on Muller Hinton Agar (MHA, Merck) for 24 hr, at 37 °C. These microbial cultures were used as inocula for antimicrobial activity test of plant extracts. Antimicrobial activity was carried out using the disc diffusion method [8]. Briefly, inocula of bacterial cells was suspended in sterile physological saline solutions and homogenized on vortex until the density of the test suspension match the turbidity standart of $1x10^8$ cfu/ml (0.5 Mc Farland standart). Petri dishes with MHA medium were inoculated with 0.1 ml of the broth cultures of test bacteria. The petri dishes were rotated slowly to ensure a uniform distribution of microorganisms and left to solidify. After that, 20µl of each extract (4mg/ml) were absorbed onto the dishes. The plates were allowed to stand for 30 min at room temperature for proper diffusion of the extract. The bacteria were than incubated at 37 °C for 24 hr. At the end of incubation time, inhibition zones formed around the discs were measured in mm. In addition, discs of vancomycin (30µg/ml, Bioanalyse) and gentamycin (10µg/disc, Bioanalyse) were used as positive control, whereas the ethanol was used as negative control. Studies were performed in triplicate and developing inhibition zones and the results were expressed as average values.

Cell Lines and Culture Conditions

The colon carcinoma (CCC-221), prostate carcinoma (PC-3 and DU-145), chronic myeloid leukemia (K562), lung carcinoma (A549), breast adenocarcinoma (MCF-7) cell lines were obtained from Dr. Yusuf Baran (Izmir Institue of Technology, Turkey). All cell lines were maintained in RPMI 1640 medium (Biochrom, Germany) supplemented with 10% fetal bovine serum (FBS; Biochrom, Germany), penicilin (100U/ml) and strepromycin sulphate (100 mg/ml) (Biochrom, Germany). Cells were incubated at 37 °C in 5% CO_2, 95% air in a humified incubator.

Determination of Cytotoxicity by MTT

The anticancer activity of plant extracts was determined using MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-dipenyltetrazolium bromide] assay (Applichem, USA). This assay detects the reduction of MTT by mitochondrial dehydrogenase to blue formazan product which reflects the function of mitochondria and cell viability [9]. Exponentially growing cells at $2x10^4$ cells/ml were plated in duplicate into 96-well plates (Greiner, Germany) in 200µl of growth medium and incubated for 24 hr before the addition of extracts. Plant extract (4mg/ml) was dissolved in 10% DMSO and added to the cell culture at final concentration of 200µg/ml to be tested aganist six cell lines. Cells were incubated for 72 hr at 37 °C in 5% CO_2 incubator. After that, 10 µl of PBS containing 5 mg/ml MTT was added into each well. After 4 hr incubation, the medium was discarded and formazan blue crystals formed in the cells were dissolved in 100 µl DMSO. Reduced MTT was quanrified by reading the absorbance at 540 nm on a microplate reader. Antiproliferative effects of the tested extracts were determined by comparing the optical density of the treated cells aganist the optical density of the untreated cells.

Statistical Analysis

Each result is shown as the mean ± SD. Data were analysed by one-way ANOVA and then Dunnett post hoc test was performed to compare the findings among the groups. A difference was considered to have significance at $P<0.05$, $P<0.01$ and $P< 0.001$.

RESULTS

Antimicrobial Activitiy

Ethanolic extracts of seven different plant species were tested against different microorganisms by means of disc diffusion method. Among the extracts, the extract from *Q. coccifera* fruit was the most effective on antimicrobial activity against six bacteria (Table 1). With this extract, the less antimicrobial activity with an inhibition zone of 7 mm was observed on *E. coli*, whereas the highest antimicrobial activity with an inhibition zone of 15 mm was detected on *S. albus.*

Antimicrobial activity of other extracts changed between 7 mm and 14 mm zone of inhibition.The extract from *C. canadensis* showed the highest antimicrobial activity with 14 mm zone of inhibition against the *B. subtilis*. Compared to Gram positive bacteria tested, Gram negative bacteria were less sensitive to the plant extracts because the highest inhibition zone was observed as 10 mm for both *E. coli* and *P. aeruginosa*. In addition, ethanol used as solvent exerted no effect on antimicrobial activitiy against the bacteria test.

Table 1. Antibacterial profile of various plant extracts

Bacterial strains	Zone of Inhibition (mm)								
	Antibiotic	Q.c*	Q.c**.	G.f	C.m.*	E.f	C.c	C.b	C
E.coli	13	7	8	7	8	10	10	9	9
B. subtilis	29	12	7	-	-	-	14	8	8
P.aeruginosa	24	10	8	8	-	-	8	7	7
S.albus	20	15	7	7	7	7	7	7	8
M.luteus	35	13	7	7	-	11	9	-	10
S.aureus	19	13	8	7	7	9	7	7	8

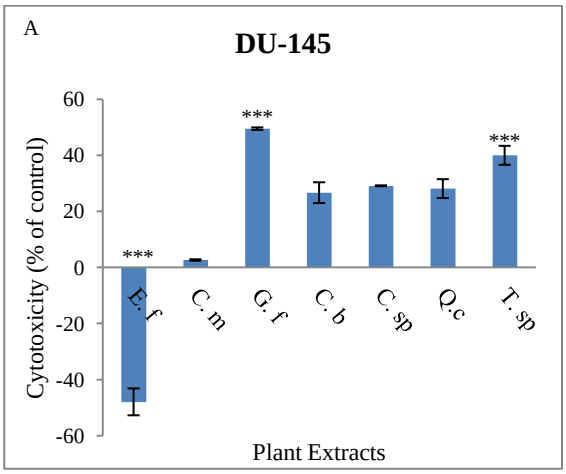
Antibacterial profile of various plant extracts. (-): No inhibition; **Q.c*. :** *Quercus coccifera* fruit; **Q.c**.** : *Quercus coccifera* fruit skin; **G.f:** *Glaucium flavum*; **C.m*.**:*Crataegus monogyna* fruit; **E.f** : *Euphorbia falcata*; **C.c**: *Conyza canadensis*; **C.b**: *Chenopodium botrys;* **C**: *Catalpa* sp. Results are the mean of three replicates and indicate the inhibition zone in mm.

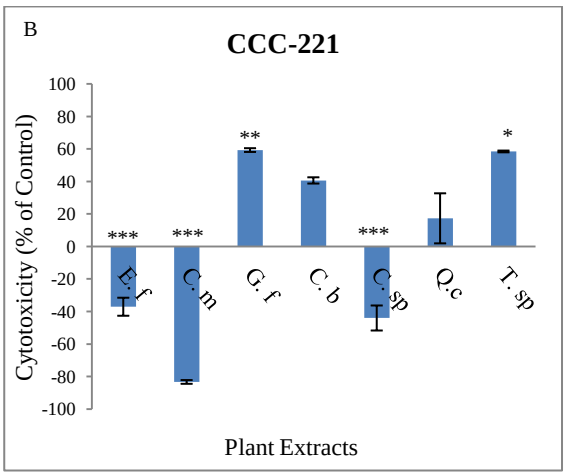
Cytotoxic Activity of Plant Extracts

The effect of the crude ethanolic extracts of seven plants on the growth of K562, PC-3, DU145, A549 and CCC cell lines was investigated by the MTT assay. For screening of the cytotoxic effect, the extracts were used at high concentration (200 µg/ml) because according to the US NCI plant screening program, a crude extract has an in vitro cytotoxic activity if the IC_{50} value is less than 20 µg/ml [10]. Control containing the appropriate volumes of blank solutions was included in the assay and the cytotoxic activity obtained from test extracts were normalized with that of the control.

Results indicated that the cytotoxic activity of the plant extracts changed according to cell lines used (Figure 1). The most effective plant extracts on cytotoxicity were found to be *G. flavum* and *C. botrys* (Figure 1). The level of cytotoxicities with these plant extracts were around 78%, 74% and 73% on PC-3, K562 and MCF-7 cell lines, respectively (Figure 1 C,D,F). According to the cytotoxicity on DU-145 and CCC-221 cell lines, *G. flavum* was better than that of *C. botrys* because *G. flavum* resulted in 50% and 60% cytotoxicity, whereas *C. botrys* caused 25% and 30% cytotoxicity on these cell lines, respectively (Figure 1 A,B). In addition, *Tanacetum* sp gave rise to cytotoxicity in all cell lines and the level of cytotoxicity changed between 63% and 39%. Likewise, the extract from *C. coccifera* caused cytotoxic activity in 5 out of 6 cell lines. On the other hand, the less effective plant extract on the cytotoxicity was *C. monogyna* which caused cytotoxicity less than 45% in all cell lines (Figure 1).

Unlike other plant extracts, the extracts from *E. falcata*, *C. monogyna* and C*atalpa sp.* induced cell proliferation instead of cytotoxicity for some of the cell lines. For example, *E. falcata* resulted in 50%, 40% and 10% increase in the proliferation of DU145, CCC-221 and A549 cell lines, respectively, whereas the highest increase (66%) in cell proliferation was observed with the extract from *C. monogyna* in the CCC-221 cell line (Figure 1,B).





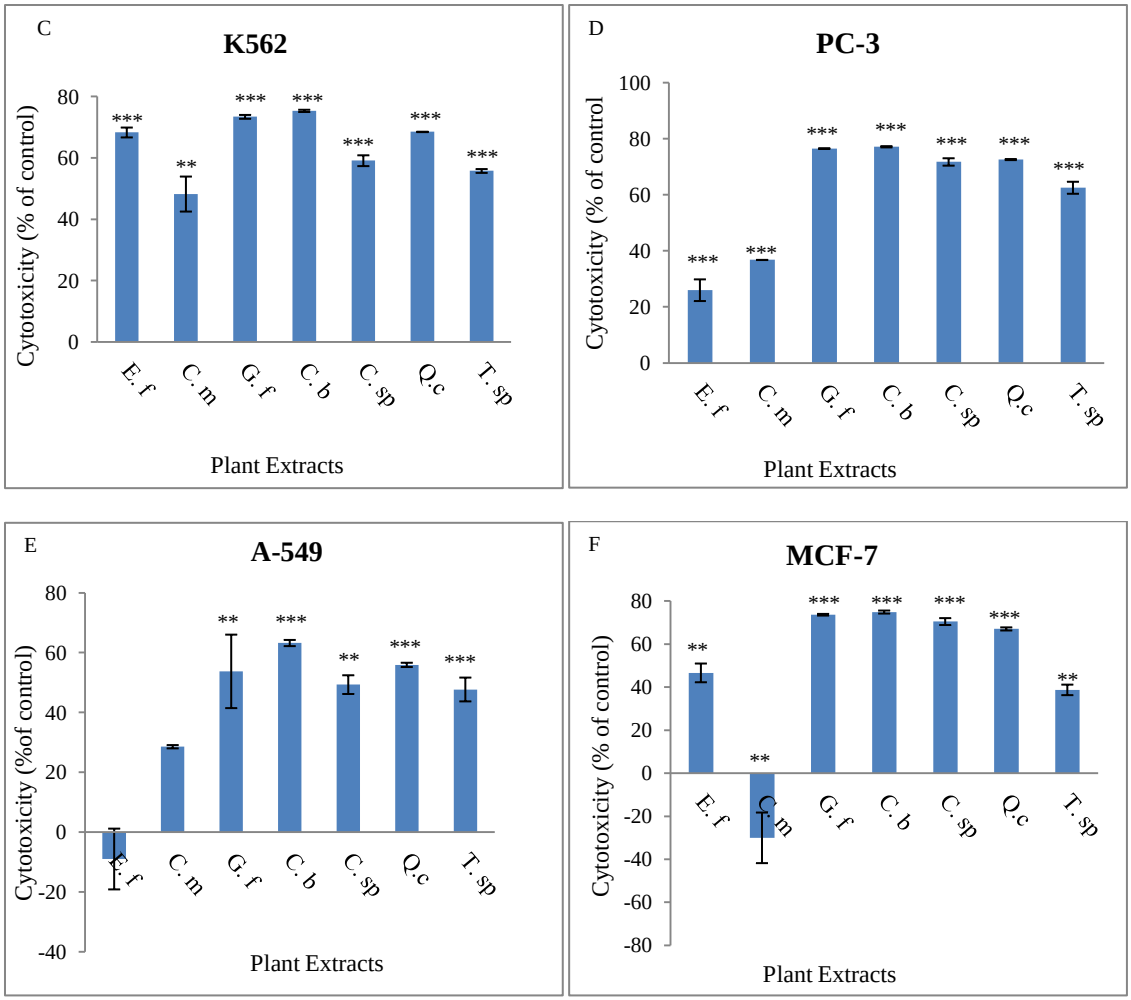


Figure 1. Cytotoxicity of plant extracts on cell proliferation. Cytotoxicity of plant extracts on cell proliferation. The cells were plated onto 96-well plates and tretaed with or without (control) each plant extract (200µg/ml) for 72 hr. Cytotoxic effect of the plant extracts was determined based on MTT assay. Data are means (±SD) of three replicates. *: P<0.05; **: P<0.01; ***:P<0.001. **Q.c**. : *Quercus coccifera* fruit skin; **G.f**: *Glaucium flavum*; **C.m.**:*Crataegus monogyna* fruit; **E.f** : *Euphorbia falcata*; **C.c**: *Conyza canadensis*; **C.b**: *Chenopodium botrys;* **C**: *Catalpa sp.*

DISCUSSION

Plants have long history of use in traditional medicine. The majority of recent research focused on antimicrobial and anticancer activities of plant extracts. First, ethanolic extracts of plant species were examined for their antimicrobial activity in this present study. Compared to other plant extracts, the extract from fruit of *Q. coccifera* exhibited the most effective antimicrobial activity against the test organisms. In addition, antimicrobial activity of fruit extract was also shown on *Candida albicans* [11]. Similarly, methanolic extract from different species of *Quercus* exerted both antimicrobial and antifungal activity [12]. However, antifungal activity was found to be higher than the antibacterial activity. They also reported that the efficacy of each species on antimicrobial activities changed according to chemical profile of the plants.

Conyza belongs to *Asteraceae* family and it is used for a variety of pharmocological applications. We found that ethanolic extract of *C. canadensis* at the concentration of 80 µg/disc was the most effective extract on the antimicrobial activity against *B. subtilis, E. coli, M. luteus* and *P. aeruginosa* with the zone of inhibition 14, 10, 9 and 8 mm, respectively. Shah et al. [13] studied antimicrobial activity of *C. canadensis* and found that methanolic extract of the plant at 18 mg/disc demonstrated maximum activity against *E. coli, P. aeruginosa* and *S. aureus* with the zone of inhibition 14, 11 and 11 mm, respectively. Results of these studies indicate that ethanolic extract of this plant is more effective than methanolic extract in regard to antimicrobial activity because the ethanolic extract at 80 µg/disc gave rise to similar results with methanolic extracts at 18 mg/disc.

In this present study, no antibacterial activity against *B. subtilis* and *P. aeruginosa* was detected with the extract of *E. falcata*, whereas the growth inhibition of *E. coli*, *S. albus* and *S. aureus* was observed with the inhibition zone ranging from 9 to 11 mm (Table 1). In the study of Kirbag et al., [14] methanolic extract of different *Euphorbia* species exerted antimicrobial activity with the MIC values between 31.2 and 1000 µg. In addition, Sundaram et al. [15] indicated a significant antimicrobial activity especially in *Proteus vulgaris* and *S. aureus* with the ethanolic extract of *E. heterophylla*. These findings indicate that different extracts or species of *Euphorbia* have antimicrobial potential against certain bacteria.

Catalpa is a tropical plant. Biological properties of ethyl ether, butanol, and aqueous fractions of this plant

were investigated and found that no antimicrobial and antitumoral effects were detected [16]. However, certain level of antimicrobial activity ranging from 7 to 10 mm inhibition zone against test bacteria was observed in this present study. This result may imply that antimicrobial content of *Catalpa* leaves is available in the ethanolic extract of the plant.

Antimicrobial and antioxidant capacities of extracts from *Crataegus monogyna* berries were determined by Benmalek et al. [17]. Flavanol contents extracted by ethyl ether were shown to be active on the growth of *P. aeruginosa* but not on that of *S. aureus*. It was suggested that this strong antibacterial activity could be due to the effect of flavanols because they inhibit the enzyme responsible for synthesis of compounds for cell growth. Ethanolic extract of the plant in this current study did not show any inhibitory effect on *P. aeruginosa*, implying that only flavanols extracted by ethyl ether may have entry into the cell wall of Gram negative bacteria.

Different biological activities were listed for the different species of *Chenopodium*. Essential oil isolated from aerial parts of *C. botrys* exhibited important antibacterial and antifungal activity [18]. We also showed the antibacterial effect of *C. botrys* against all bacteria tested, except *M. Luteus*.

A large number of studies in the literature indicate that plants are important source of anticancer agents [4]. Even though plants represent a tremendous diversity on the earth, only a small portion of them has been explored. Therefore, anticancer activitiy of plants have been extensively investigated. In this present study, anticancer activity of seven plant species was examined. Ethanolic extract from fruit skin of *C. coccifera* exerted cytotoxic effect especially on PC-3, K562, and MCF-7 cell lines (Figure 1). Similar to our results, Şöhretoğlu et al. [19] reported a high antiproliferative activity of methanolic extract of leaves from *Q. cerris*, *Q. aucheri* and *Q. macranthera* subsp. *sysprenses* on Hep-2 carcinoma cell line. Even though the extracts used in these studies were from different parts of the plant, results indicate that both fruit skin and leaves contain anticancer substance.

Chenopodium species are well known for its medical applications [18]. On the basis of recent studies, cytotoxicity was one of the different biological activities attributed to this plant. Various species of the genus possess differential cytotoxic activities. For example, *C. anthelminticum* showed antitumor activity against different cell lines such as HL60, MDA-MB-231, CCRF-CEM, whereas *C. ambrosioides* caused tumor induction in the liver of *Bufo regularis*. Unlike *C. ambrosioides*, *C. botrys* caused a high level of cytotoxicity in PC-3, MCF-7 and K562 cell lines in the present study. Similarly, anticancer activities of *C. quinoa* leaves extracts were reported on rat prostate cancer cell lines AT-2 and MAT-LyLu [20].

Glaucium flavum belongs to *Papaveraceae* family and aerial parts of this plant is very rich in alkaloids. One of the recent studies indicate that methanolic extract of *G. flavum* roots decreased the tumor growth and angiogenesis *in vivo* and protopine was found to be a major alkaloid for these activities [21]. On the other hand, a main anticancer activity was attributed to boccoline in their following study [22]. Even though we used different cancer cell lines to determine the anticancer activity of *G. flavum* leaves extract, we also found that the crude extract of this plant leaves was higly active against cell growth of PC-3, MCF-7, and K562 cell lines. Further research may indicate the type of active compound responsible for anticancer activity in the leaves.

Some *Euphorbia* species have been used to treat several diseases in Turkey [23] and curative properties of *Euphorbias* are due to presence of various secondary metabolites in these plants. Different extracts of *Euphorbias* have been tested for their anticancer activities and found that chloroform and ethylacetate extract inhibited cell proliferation in a dose and time dependent manner at the cocentration of 50-200 µg/ml [24]. Unlike previous study, we used ethanolic extract of *E. falcata* and showed that proliferation of K562 cell line decreased by 68% compared to untreated cells.

Taken together, these findings indicate that plant extracts from the same genus or from the same species exhibit different biological effects depending on the different test methods, type of solvents for extraction, test microorganisms and cell lines, and variation in the chemical profiles of the plants due to the origin of the plant species. Therefore, it is diffucult to compare the studies with each other. Because different geographical locations and agroclimatic conditions cause the difference in the seconder metabolites of the plants belonging the same taxa [7], it is important to search and identify the most effective componenets of plants from different environments.

In conclusion, we secreened the antimicrobial and anticancer activities of seven different plant species in the present study. The highest antibacterial activity was observed with the extract from *Q. coccifera* fruit. On the basis of cytotoxicity analysis, three plant species *C. botrys*, *Q. coccifera* and *G. flavum* demonstrated promising anticancer activities against especially PC-3, MCF-7 and K562 cancer cell lines. In addition, the most sensitive cell line to the extract from *E. falcata* was K562. Further work will continue to identify effective antibacterial and anticancer components of these plant extracts. Finally, mechanisms potentially responsible for anticancer activity will be determined.

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