Biotechnology & Biotechnological Equipment

ISSN: 1310-2818 (Print) 1314-3530 (Online) Journal homepage: https://www.tandfonline.com/loi/tbeq20

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To cite this article: Faruk Karahan, Cumhur Avsar, Ibrahim Ilker Ozyigit & Ismet Berber (2016) Antimicrobial and antioxidant activities of medicinal plant *Glycyrrhiza glabra* var. *glandulifera* from different habitats, Biotechnology & Biotechnological Equipment, 30:4, 797-804, DOI: 10.1080/13102818.2016.1179590

To link to this article: https://doi.org/10.1080/13102818.2016.1179590

Published online: 09 May 2016.

BIOTECHNOLOGY & BIOTECHNOLOGICAL EQUIPMENT, 2016
VOL. 30, NO. 4, 797–804
http://dx.doi.org/10.1080/13102818.2016.1179590

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ARTICLE; PHARMACEUTICAL BIOTECHNOLOGY

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Antimicrobial and antioxidant activities of medicinal plant *Glycyrrhiza glabra* var. *glandulifera* from different habitats

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ABSTRACT

In this study, the antimicrobial and antioxidant activities of root methanolic extracts of *Glycyrrhiza glabra* var. *glandulifera* (Waldst. & Kit.) Boiss. (Fabaceae) were investigated. Plant samples were collected from different habitats in the East Mediterranean part of Turkey. The plant extracts were evaluated for antimicrobial activities against nine bacterial and two yeast strains using disc-diffusion and minimum inhibitory concentration methods. The antioxidant activity was determined by using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The antimicrobial assays indicated that the plant root extracts were more effective against Gram-positive bacteria than against Gram-negative ones. In addition, the extracts had higher antimicrobial effect against *Candida* species than against bacteria. The extracts showed good antioxidant activity, with a median inhibitory concentration (IC_{50}) in the range of 588 ± 0.86 μg/mL to 2190 ± 1.73 μg/mL. Results indicated that different environmental conditions in each habitat might affect the contents of chemical compounds and biological activity in the natural licorice populations of. This study also supported the traditional use of licorice and as well as suggested that it may also be its beneficial role in the treatment of other infections. The obtained results indicated that different environmental conditions in each habitat might affect the contents of chemical compounds and the biological activity in the natural licorice populations.

ARTICLE HISTORY
Received 7 September 2015
Accepted 14 April 2016

KEYWORDS
Glycyrrhiza glabra; antimicrobial; antioxidant; Turkey

Introduction

The genus *Glycyrrhiza* includes well-known traditional medicinal plants growing in various parts of the world. The roots of the plants have been medically used by human beings over 4000 years. The *Glycyrrhiza* genus consists of about 30 species, of which 15 species have been studied so far.[1–3] The roots of *Glycyrrhiza glabra* (licorice), which is one of the most common species of this genus, contain glycyrrhizin, or glycyrrhizinic acid,[4] glabridin, glabrene, glabrol, licoflavonol, glycerol, licoricone, formononetin, phaseollinisoflavan, hispaglabridin A and B, 3-hydroxy glabrol, 3-methoxy glabridin,[5–11] glabranin isomer, narigenin and lupiwightenone.[12,13] Licorice root extracts have been shown to be beneficial in the treatment of eye diseases, throat infections, peptic ulcers, liver diseases, joint diseases, arthritic conditions, immunodeficiency,[14] cough, tuberculosis, respiratory diseases, cancer, diabetes, endocrine disorders,[15] kidney diseases,[16] bronchitis, asthma, psoriasis, eczema, haemorrhoids,[17] epilepsy, chronic hepatitis, heart diseases [3] and orodental diseases.[18] It has also been shown that extracts help regulate the estrogen–progesterone ratio [19–21] and the gastrointestinal system.[15] However, the effects of environmental variables on the antimicrobial and antioxidant activities have not been sufficiently studied in licorice. The objective of this work was to study the relationship between the ecological characteristics and the biological activities of methanolic extracts of licorice plants growing in different habitats.

Materials and methods

Plant materials

Specimens (roots and rhizomes) of *Glycyrrhiza glabra* var. *glandulifera* (Waldst. & Kit.) Boiss. were collected from 15 different localities of the East Mediterranean part of Turkey (Figure 1 and Table 1). The botanical identification was carried out by Dr V. Altay from the Department of Biology, Faculty of Sciences and Arts, Mustafa Kemal University, based on Davis.[22]

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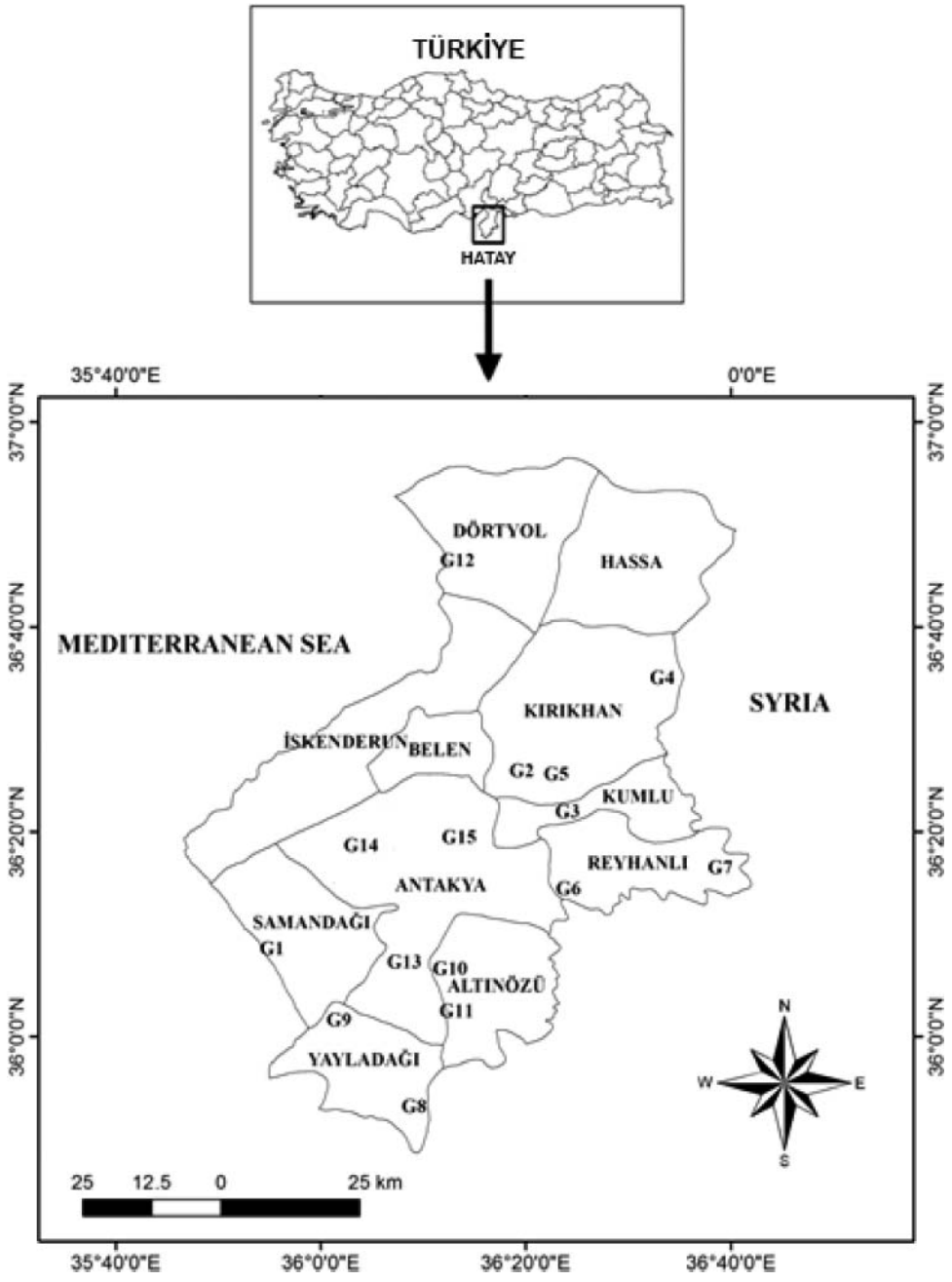

Figure 1. The study area.

Preparation of plant extracts

The plant roots were shade-dried at room temperature and then ground into powder. About 20 g of each powdered sample was extracted with 250 mL of methanol for 24 h. The extracts were filtered and concentrated under vacuum to obtain crude extracts.

Micro-organisms

The test organisms included six Gram-positive bacteria [*Staphylococcus aureus* ATCC 6538, *Enterococcus faecalis* ATCC 51299, *Micrococcus luteus*, *Bacillus cereus* 7064, vancomycin-resistant *Enterococcus* (VRE) and methycilline resistant *Staphylococcus aureus* (MRSA)]; three Gram-negative bacteria (*Escherichia coli* ATCC 11293, *Pseudomonas aeruginos* and *Klebsiella pneumoniae*) and two yeast species (*Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019). They were all obtained as pure cultures from the Molecular Biology and Microbiology Laboratory, Department of Biology, Faculty of Arts and Science, Sinop University, Turkey.

Evaluation of antimicrobial activity

The antimicrobial activity of each methanol extract sample was evaluated by using the disc-diffusion method.

Table 1. List of the localities, habitat types and altitudes of *G. glabra* var. *glandulifera* samples.

Code	Localities	Altitude (m)	Lat (N)	Long (E)	Direction	Climate type
G1	Samandağ—Çevlik coast, roadside	5	36° 03' 02"	35° 57' 56"	South	Rainy Mediterranean
G2	Kırıkhan—Topboğazı village, water channels	96	36° 27' 17.8"	36°18' 49.6"	East	Semi-arid Mediterranean
G3	Kumlu—Uzunkavak village, wheat field	93	36° 28' 34.5"	36° 27' 38"	West	Semi-arid Mediterranean
G4	Kırıkhan—Acarköy, water channels	94	36° 31' 49"	36° 25' 03"	North-east	Semi-arid Mediterranean
G5	Kırıkhan—Öz Soğuksu village, hill slope	127	36° 27' 55.5"	36° 19' 06.8"	East	Semi-arid Mediterranean
G6	Reyhanlı—Konuklu village, water channels	80	36° 15' 26.5"	36 ° 27' 19.3"	South-east	Semi-arid Mediterranean
G7	Reyhanlı—Alakuzu village, roadside	181	36° 17' 14"	36° 38' 23"	South-east	Semi-arid Mediterranean
G8	Yayladağı—Üçırmak village, olive field	124	35° 58' 40.2"	36° 11' 37.4"	South-east	Rainy Mediterranean
G9	Yayladağı—Karaköse town, olive field	370	36° 01' 54.5"	36° 01' 17.6"	North-east	Rainy Mediterranean
G10	Altınözü—Tepehan village, roadside	430	36° 08' 38"	36° 13' 2.1"	South-east	Low-rain Mediterranean
G11	Altınözü—Akamber village, wheat field	282	36° 05' 34.5"	36° 14' 11.5"	North-east	Low-rain Mediterranean
G12	Erzin—Burnaz, sand dune	7	36° 55' 32"	36° 02' 21.5"	South-west	Rainy Mediterranean
G13	Harbiye—Sofular village, wheat field	461	36° 06' 26"	36° 10' 24.7"	South-east	Rainy Mediterranean
G14	Antakya—İskenderun highway, roadside	95	36° 16' 35.2"	36° 11' 25"	West	Rainy Mediterranean
G15	Antakya—Paşaköy, hill slope	110	36° 22' 09.6"	36 ° 14' 09.7"	South-west	Rainy Mediterranean

[23] All bacteria were maintained at −20 °C in nutrient agar (NA) and all yeast, in Sabouraud dextrose agar (SDA, Difco) containing 20% (v/v) glycerol. Before testing, the bacteria were transferred to nutrient broth (NB) and the fungi, to Sabouraud dextrose broth (SDB, Difco), and were cultured overnight at 37 °C. Then, the turbidity was adjusted to equivalent to 0.5 McFarland standards (approximately 10^8 CFU/mL for bacteria and 10^5 or 10^6 CFU/mL for fungi). Aliquots of 100 μL of micro-organisms were spread over the surface of NA plates. Sterile Whatmann No. 1 filter paper discs (6 mm) were soaked with 25 μL of extract residue diluted in the corresponding extract solvent (1000 μg/1 mL of 12.5% dimethyl sulfoxide (DMSO)) and placed on the surface of the freshly inoculated medium. The media were incubated for 24 h at 37 °C. Antibiotic susceptibility discs including bacitracin (0.04 U), ceftazidime (30 μg), imipenem (10 μg), novobiocin (5 μg), polymyxin B (300 U), tetracycline (30 μg), ampicillin (10 μg) and cycloheximide were used as control, and negative controls were 12.5% DMSO, methanol and deionized water. The antimicrobial activity was evaluated by measuring the diameter of the inhibition zones. The experiment was performed in triplicate. The values were expressed as means with standard deviations (±SD).

The minimum inhibitory concentration (MIC) was determined by the serial tube dilution method. Methanol extracts of plants (1000 mg) were dissolved in 12.5% DMSO to obtain initial stock solutions. The stock solutions were stirred into 0.9 mL of NB (for bacteria) and SDB (for fungi) in glass tubes in order to adjust to concentrations of 1000−0.001 mg/mL. All tubes were inoculated with 100 μL of standardized inoculums of each organism and incubated for 24 h at 37 °C (for bacteria) and 48−72 h at 25 ± 1 °C (for fungi). The MIC of the extracts was taken as the lowest concentration that showed no growth. The experiment was performed in duplicate.

Determination of free-radical-scavenging activity

The free-radical-scavenging activity assay of the plant extracts was determined by the DPPH (1,1-diphenyl-2-picrylhydrazyl) method.[24,25] The powdered root samples were dissolved to a concentration of 1000 μg/mL in ethanol. Solutions of different concentrations (1000, 500, 250, 125 and 62.5 μg/mL) were prepared by the serial dilution technique. One millilitre of ethanol extract solution of each concentration was mixed with 4 mL of a DPPH-ethanol solution (0.1 mmol/L). Thirty minutes later, the absorbance was measured at 517 nm (Helios Alpha, Thermo Scientific). The DPPH-radical-scavenging activity was calculated by using the following equation:

$$\%\ \text{inhibition} = [(A_B - A_S)/A_B] \times 100$$

where A_B is the absorbance of the positive control and A_S is the absorbance of the sample containing the tested extract. Ascorbic acid was used as a standard or positive control. Reaction mixture without a sample was used as the negative control.

All experiments were run in triplicate. The concentration providing 50% inhibition (IC_{50}) was calculated from the resulting graph plot by interpolation.

Data analysis

All experiments were carried out in triplicates and values are expressed as means with standard deviations (±SD). Graphics were drawn using MS Office Excel 2007.

Results and discussion

Ethnopharmacological studies suggest that natural chemical compounds isolated from medicinal plants have been used to treat the adverse effects of

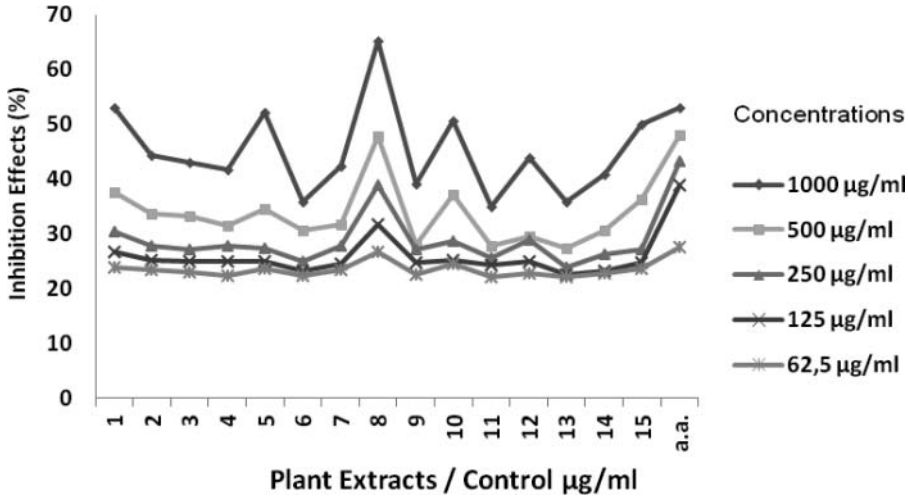

Figure 2. DPPH free-radical-scavenging activity of methanolic extracts (samples 1−15) as compared to ascorbic acid (AA) as a standard.

several bacterial, fungal and viral infectious by all civilizations from ancient times to the present.[26−31] Previous investigations have reported that *G. glabra* is a valuable medicinal plant due to its antimicrobial and antioxidant properties.[32−34] In this study, we compared the antioxidant and antimicrobial potential of root extracts from *G. glabra* collected from different localities in Turkey.

Antioxidant activity

The results for the DPPH-radical-scavenging activity of the methanolic extracts of licorice roots are shown in Figure 2. The IC_{50} values of the extracts are given in Figure 3. The IC_{50} values of the extracts were found to be between 588 ± 0.86 µg/mL and 2190 ± 1.73 µg/mL and that of the control (ascorbic acid) was determined to be 745 ± 0.05 µg/mL.

According to observed experimental data in terms of IC_{50}, the plant designated as number 8 showed higher antioxidant activity (IC_{50} of 588 ± 0.86 µg/mL) compared to the control and the other studied plant extracts. In addition, the antioxidant activity of the plant extracts numbers 1, 5, 10 and 15 was close to that of the control (Figure 3). These results support the data reported by Chopra et al.,[3] who observed that methanolic extracts of licorice roots displayed good antioxidant activity (IC_{50} = 359.45 µg/mL), which is in a similar range to the IC_{50} determined in our study. Al-Bachir and Al-Adawi [34] also demonstrated that licorice roots powder showed antioxidant activity with an IC_{50} value of 87.152 µg/mL versus 22.78 µg/mL for ascorbic acid.

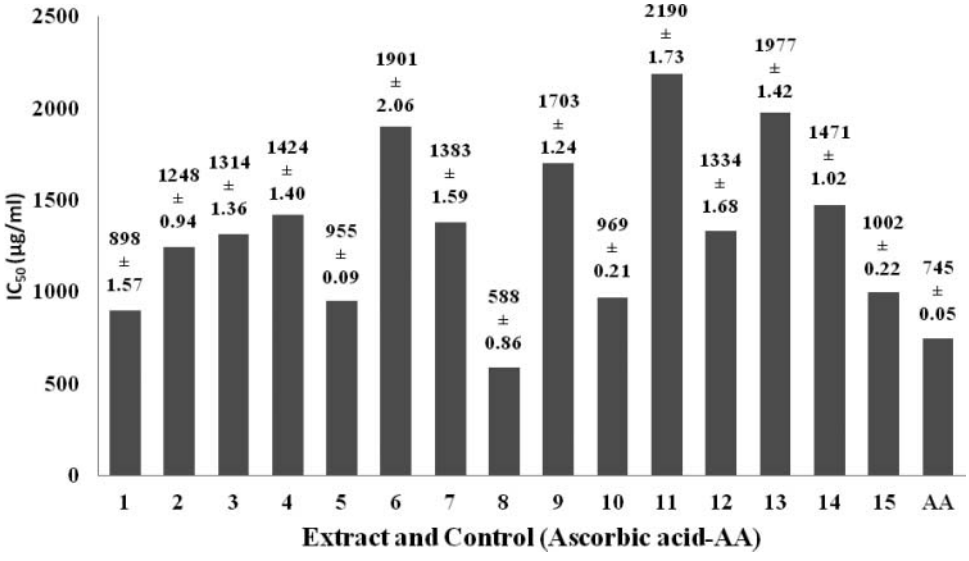

Figure 3. IC_{50} values of the plants methanol extracts collected from 15 sampling points (samples 1−15) as compared to ascorbic acid (AA).

Table 2. Inhibition zones (mm) of the methanolic plant extracts collected from 15 different localities against tested micro−organisms using disc−diffusion method.

	Micro−organisms and inhibition zones (mm)										
Plant extracts	*B. cereus* ATTC 7064	*C. krusei* ATCC 6258	*C. parapsilosis* ATCC 22019	*E. coli* ATCC 11293	*E. faecalis* ATCC 51299	*K. pneumoniae* ESBL (+)	MRSA	*M. luteus*	*P. aeruginosa*	*S. aureus* ATCC 6538	VRE
1	12 ± 0.05	20 ± 1.1	25 ± 0.4	–	11 ± 0.3	–	15 ± 1.1	–	–	14 ± 0.5	12 ± 0.2
2	15 ± 0.6	19 ± 0.5	24 ± 0.5	–	10 ± 0.1	–	13 ± 0.1	–	–	13 ± 0.1	11 ± 0.2
3	16 ± 0.1	21 ± 0.4	26 ± 0.5	–	12 ± 0.7	–	14 ± 0.2	–	–	14 ± 1.1	13 ± 0.2
4	11 ± 0.2	20 ± 1.2	24 ± 0.5	–	10 ± 10	–	14 ± 0.2	–	–	14 ± 0.6	11 ± 0.1
5	16 ± 0.6	16 ± 0.5	25 ± 0.6	–	10 ± 0.1	–	14 ± 0.0	–	–	14 ± 0.2	12 ± 0.2
6	20 ± 2.2	17 ± 0.2	26 ± 1.1	–	10 ± 0.2	–	14 ± 0.1	–	–	19 ± 0.3	11 ± 0.0
7	11 ± 0.5	20 ± 0.0	23 ± 0.8	–	12 ± 0.3	–	13 ± 0.2	–	9 ± 0.1	13 ± 0.2	11 ± 0.1
8	17 ± 1.6	15 ± 0.1	21 ± 0.2	–	11 ± 10	–	14 ± 0.6	–	–	14 ± 0.2	13 ± 0.4
9	13 ± 0.7	23 ± 0.7	26 ± 0.1	–	13 ± 1.1	–	14 ± 0.5	–	–	14 ± 0.1	10 ± 0.2
10	16 ± 0.5	21 ± 0.2	26 ± 0.5	–	14 ± 0.7	–	15 ± 0.4	–	–	16 ± 0.2	15 ± 1.1
11	13 ± 0.0	21 ± 0.2	27 ± 0.4	–	11 ± 0.1	–	14 ± 0.2	–	–	16 ± 0.3	12 ± 0.5
12	10 ± 0.1	20 ± 0.3	26 ± 1.2	–	10 ± 0.0	–	11 ± 0.1	–	–	15 ± 0.2	9 ± 0.2
13	13 ± 0.2	17 ± 0.5	24 ± 2.1	–	10 ± 0.2	–	13 ± 0.2	–	–	13 ± 0.1	13 ± 0.1
14	11 ± 10	20 ± 1.6	25 ± 1.6	–	12 ± 0.5	–	13 ± 0.0	–	–	14 ± 0.6	12 ± 0.2
15	11 ± 0.2	19 ± 0.5	22 ± 0.1	–	10 ± 0.0	–	13 ± 10	–	–	12 ± 0.0	11 ± 0.1
DMSO	–	–	–	–	–	–	–	–	–	–	–
Bac		*	*	–	–	–	–		–	–	–
Nov	10 ± 0.1	*	*	–	15 ± 0.2	9 ± 0.1	24 ± 0.7		–	29 ± 0.4	10 ± 0.5
Tet		*	*	26 ± 1.3	23 ± 0.5	24 ± 0.6	10 ± 0.2		17 ± 0.1	40 ± 1.4	13 ± 0.9
Amp		*	*	–	35 ± 1.2	–	16 ± 0.5		–	42 ± 1.6	24 ± 0.5
Imp		*	*	28 ± 0.5	34 ± 1.2	26 ± 0.7	50 ± 2.3		14 ± 0.3	50 ± 1.6	30 ± 1.3
Poly B		*	*	11 ± 0.4	–	15 ± 0.5	11 ± 0.2		22 ± 1.0	11 ± 0.1	–
Cef		*	*	19 ± 0.2	20 ± 1.1	19 ± 0.4	23 ± 0.8		26 ± 1.4	25 ± 0.6	8 ± 0.10
Cyc	*	43 ± 1.2	40 ± 1.1	*	*	*	*	*	*	*	*

Note: (-) no effect; (*) not tested; Bac, bacitracin; Nov, novobiocin; Tet, tetracycline; Amp, ampicillin; Imp, imipenem; Poly B, polymyxin B; Cef, ceftazidime; Cyc, cycloheximide.

Antimicrobial activity

The antimicrobial activities of the plant extracts collected from different habitats against nine bacteria and two yeasts using the disc-diffusion and broth microdilution (MIC) procedures are presented in Tables 2 and 3. According to the results of the disc-diffusion assays, all the plant methanolic extracts inhibited the growth of *B. cereus*, *E. faecalis*, *K. pneumonia*, MRSA, *S. aureus*, VRE, *C. krusei* and *C. parapsilosis*. However, there was no activity against *E. coli*, *K. pneumoniae* and *M. luteus*. Our results that the three tested Gram-negative bacteria were more resistant to the extracts than the tested Gram-positive bacteria and yeasts could most likely be explained by the differences in the cell wall structure, since the lipopolysaccharide layer in the outer membrane of Gram-negative bacteria is known to serve as a strong permeability barrier to many environmental substances.

Interestingly, in our study, the plant extract designated as number 7 was the only extract that showed activity against *P. aeruginosa* (9 mm, Table 2). Our results indicated that the extracts had higher antimicrobial activity against *Candida* species than against the tested bacterial strains. Moreover, the antimicrobial activity of some plant extracts was found to be higher than that of

Table 3. MIC values (μg/mL) of the methanolic plant extracts collected from 15 different localities against different micro−organisms, using the broth microdilution method.

Plant extract	*B. cereus* ATCC 7064	*C. krusei* ATCC 6258	*C. parapsilosis* ATCC 22019	*E. coli* ATCC 11293	*E. faecalis* ATCC 51299	*K. pneumoniae* ESBL (+)	MRSA	*M. luteus*	*P. aeruginosa*	*S. aureus* ATCC 6538	VRE
1	100	100	10	–	1000	–	100	–	–	1000	1000
2	1000	100	100	–	≥1000	–	1000	–	–	1000	1000
3	100	100	100	–	1000	–	1000	–	–	100	100
4	1000	100	100	–	≥1000	–	100	–	–	1000	1000
5	100	100	100	–	1000	–	1000	–	–	1000	1000
6	100	100	100	–	≥1000	–	1000	–	–	100	1000
7	1000	100	100	–	1000	–	1000	–	≥1000	1000	1000
8	100	1000	100	–	≥1000	–	≥1000	–	–	1000	1000
9	100	100	100	–	100	–	1000	–	–	1000	1000
10	100	100	10	–	100	–	100	–	–	100	100
11	100	100	10	–	≥1000	–	1000	–	–	100	1000
12	≥1000	100	100	–	≥1000	–	≥1000	–	–	1000	≥1000
13	1000	1000	100	–	≥1000	–	1000	–	–	1000	1000
14	≥1000	100	100	–	1000	–	1000	–	–	100	1000
15	≥1000	1000	100	–	≥1000	–	1000	–	–	≥1000	≥1000

Note: (-) no effect.

some standard commercial antibiotics. Notably, extract number 10 showed more potent activity against *B. cereus*, MRSA, *S. aureus* and VRE than novobiocin, tetracycline, polymyxin B and ceftazidime (Table 2).

In addition, our data demonstrated a good correlation between the disc-diffusion results and the MIC test. The MIC values of different extracts against Gram-positive bacteria were found to be in the range of 100 to ≥1000 μg/mL. The most effective extract was No. 10 (MIC value of 100 μg/mL or less) against all the tested micro-organisms. In addition, the MIC values of the extracts against the yeasts used in the test were found to be in the range of 10−1000 μg/mL. All the extracts showed high anti-yeast activity against *C. parapsilosis*, ranging from 10 to 100 μg/mL (Table 2).

These results are in agreement with previous reports that *G. glabra* methanolic extracts have antibacterial and antioxidant activity. For example, Chopra et al. [3] reported that methanolic extracts of licorice roots showed moderate antibacterial activity. In another study, Sultana et al. [2] determined that *G. glabra* methanolic extracts exhibited antibacterial activity against Gram-positive (*S. aureus*, *Bacillus megaterium* and *B. subtilis*) and Gram-negative (*E. coli*, *P. aeruginosa* and *Salmonella paratyphi*) bacteria. Al-Bachir and Al-Adawi [34] reported relatively high total aerobic plate counts, including coliform, *E. coli* and *Klebsiella* spp., after treatment with licorice roots powder. Gupta et al. [14] also found that the root extracts of *G. glabra* showed activity against *Mycobacterium tuberculosis* $H_{37}Ra$ and $H_{37}Rv$ at a concentration of 500 μg/mL.

Imakiire et al. [35]. and Fukai et al. [9] demonstrated that compounds extracted from *G. glabra* and *G. uralensis* have good antibacterial activity against *Helicobacter pylori*. Fukai et al. [10] determined that 19 flavonoids isolated from *G. glabra*, *G. inflata* and *G. uralensis* were active against methycilline sensitive *Staphylococcus aureus* (MSSA), MRSA, *M. luteus*, *B. subtilis*, *E. coli*, *K. pneumoniae* and *P. aeruginosa*. In our study, however, we could not confirm any effect against *E. coli*, *K. pneumonia*, *M. luteus* and *P. aeruginosa* (except for one extract). We hypothesize that these different results might be due to differences in the ecological conditions under which the collected plants have grown.

The effects of environmental conditions on plant growth and development, reproduction and distribution are well known in plant ecology.[36−39] Many studies have been carried out about the relationships among plant chemical contents, biological activity and environmental variables in natural and cultivated plant species.[40−42] This information has been used to determine the medicinal value and economic importance of plant products.[43]

Based on the results from our study on the antimicrobial and the antioxidant activities of methanolic extracts obtained from *Glycyrrhiza* growing in different habitats, it could be summarized that the plant genetic and environmental factors have important effects on the production and the quality of medicinal plants.[44] Previous reports have demonstrated that geographic variations result in changes in the content of some metabolites, e.g. glycyrrhizin.[45] For example, Hosseini et al. [46] studied the effects of soil and climatic conditions on the content of glycyrrhizic acid in various Iranian populations and reported that the glycyrrhizin content was significantly correlated with temperature, suggesting that temperature is an important factor affecting the amount of the glycyrrhizin acid in licorice. The same authors [46] also reported that some physicochemical soil properties are important regarding the accumulation of active compounds in natural populations of *G. glabra*. Similarly, Zhang et al. [38] and Zhou,[39] reported that soil variables had a significant effect on the content of glycyrrhizic acid in *Glycyrrhiza* spp. Glycyrrhizin has also been reported to vary in the range of 1.96%−2.24% in the roots of *G. glabra* from Italian populations.[47] In addition, many previous studies reported that light intensity influences the biosynthesis of secondary metabolites. [48,49] In this context, our results showed that the three extracts whose antioxidant properties were the highest were obtained from populations (designated as population 1, 8 and 10) that are similar in terms of climate type (rainy Mediterranean climate and low-rain Mediterranean climate), cardinal direction, habitat properties and geographical coordinates (Table 1). Also, the extracts from populations 6, 8 and 10 had the highest antimicrobial effects and their habitats (water channel, field and roadside) were under the anthropogenic pressure. Among our extracts, those that showed the highest antimicrobial and antioxidant effects originated from populations in the south-east direction. Therefore, our results indicate that there is a correlation between light intensity and the biological effects of the studied plants. In addition, it could be speculated that the observed differences in the antioxidant and antimicrobial properties of *G. glabra* populations could possibly be attributed to different environmental conditions in each habitat, which might affect the contents of active chemical compounds in natural licorice populations differently.

Conclusions

Significant differences were observed in the antimicrobial and antioxidant activities of root extracts of *G. glabra* var. *glandulifera* from different habitats. The extracts with most potent antioxidant properties were obtained from populations originating from regions with similar types of climate (rainy Mediterranean climate and low-rain

Mediterranean climate), whereas the extracts that showed the strongest antimicrobial effect originated from habitats under anthropogenic pressure. Thus, it could be suggested that the differences in the biological activities might be attributed, at least in part, to the influence of environmental factors.

Acknowledgments

The authors are grateful to Dr V. Altay, Department of Biology, Faculty of Science and Art, Mustafa Kemal University for providing identifications of plant taxa.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This study was supported by the Scientific Research Commission of Mustafa Kemal University [grant number 1204Y0130].

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