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Evaluation of biological activity and antioxidant capacity of Turkish licorice root extracts

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Abstract

Glycyrrhiza glabra L. *(Licorice) is a well-known medicinal herb that grows in various parts of the world. In the current study, the antimicrobial activities of n-hexane and dichloromethane extracts from* G. glabra *root were investigated by using the disc-diffusion and microdilution-broth methods against human and fish pathogen microorganisms. The antioxidant activities of the extracts were determined with total antioxidant activity, DPPH activity, ferric ion reducing power and CUPRAC assay. The total phenolic compounds and flavonoids were also studied for the extracts. In antimicrobial screening,* G. glabra *root extracts showed potent antimicrobial activity against all the tested clinical and food-borne pathogenic microorganisms.* Streptococcus agalactiae *exhibited a higher sensitivity against the dichloromethane extract with the highest zone diameter (18.21 mm) and the lowest MBC value (1.41 mg/ml) of all the fish pathogens. On the other hand, the free radical scavenging activities of n-hexane and dichloromethane extracts were found as* IC_{50} *579.98 µg/ml and 111.49 µg/ml, respectively. The results showed that the root dichloromethane extract has a stronger antioxidant capacity compared with n-hexane extract. The findings indicated the potential use of the n-hexane and dichloromethane extracts from* G. glabra *root as biopreservatives as they demonstrated high antimicrobial and antioxidant activities.*

Keywords: Antibacterial, antifungal, human pathogen, fish pathogen, n-hexane, dichloromethane

Introduction

Glycyrrhiza glabra L. (licorice) is a general herb of Mediterranean and Asian region including Turkey. Root of this plant has several useful pharmacological properties such as antiinflammatory, anticancer and antimicrobial activities in addition to cardioprotective hepatoprotective and immunomodulatory effects [1]. It is widely used in food and pharmaceutical industries throughout the world.

Nowadays, multiple drug resistance has developed in human pathogenic microorganisms due to uses commercial antimicrobial drug randomly. This situation necessitated scientists to develop new herbal antimicrobial agents from various sources, such as medicinal plants [2].

Antibiotics were used to fish treatment against bacterial diseases. However, this cause development of bacterial resistance against used antibiotics in fish, environment and

sediments [3]. Increased public awareness of the negative effects caused by synthetic chemicals has led to the search for ‘‘green solutions’’, such as organic and synthetic chemical-free food products [4]. Organic fish production is based on materials from obtained natural sources. Nowadays, there has been increased interest in the possible use of traditional herbs as alternative treatments of bacterial diseases in aquatic animals [5].

Antioxidants prevent oxidative stress that may cause several degenerative diseases [6]. The most widely used synthetic antioxidants, such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT), have restricted use in food and have been suspected of being responsible for liver damage and carcinogenesis [7-9]. Less toxic and cost effective antioxidants from natural sources is necessary instead of synthetic antioxidants which have harmful side effects.

Antimicrobial and antioxidant activities of *G. glabra* root extracts from Turkey have been studied in previous researchers, but there are no reports about the effect of n-hexane and dichloromethane extracts from Turkish licorice root against food borne, clinical and fish pathogen microorganisms. In this study, we have also investigated the antioxidant capacity of these extracts of licorice root.

Materials and Methods

Plant materials and extraction procedure

Roots of *G. glabra* were purchased from a local market from Adana, Turkey. The root of *G. glabra* was washed with distilled water, and shade dried. The dried plant materials were then blended into powder using an electric blender for extraction. The powdered root was separately extracted with n-hexane (H) and dichloromethane (DCM) by using Soxhlet apparatus for 24 h. The extracts were concentrated by using a rotary evaporator and used for further test.

Microbial strains

Clinical and food borne pathogens were used to determine of antimicrobial activity: Thirteen bacteria (*Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* RSKK 863, *Micrococcus luteus* NRRL B-4375, *Bacillus subtilis* RSKK 244, *Escherichia coli* ATCC 11229, *Escherichia coli* ATCC 35218, *Escherichia coli* O157:H7, *Salmonella enteritidis* ATCC 13076, *Salmonella enteritidis* RSKK 171, *Shigella sonnei* Mu:57, *Pseudomonas aeruginosa* ATCC 27853, *Yersinia enterocolitica* NCTC 11175), and one yeast (*Candida albicans* ATCC 10231).

In vitro antibacterial activity against fish pathogens were also carried out on *Lactococcus garvieae, Streptococcus agalactiae* Pasteur Institute 55118, *Vibrio alginolyticus, Yersinia ruckeri*, *Vibrio anguillarum* (M1 and A4 strains, from two different companies), and *Aeromonas hydrophila* ATCC 19570).

Disc Diffusion Assay

The antimicrobial activity was determined by using disc diffusion method according to Murray et al. [10]. One hundred microlitres of suspension of the test microorganisms (adjusted to 0.5 McFarland) were spread on solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 15 µl (3.75 mg/disc) of the extracts and then placed on the inoculated plates. Afterwards, they were kept for 2 h in a refrigerator to enable prediffusion of the extracts into the agar. Then, the inoculated plates were incubated for 24 h and 48 h for bacterial and yeast strains, respectively. Antibiotic discs of Gentamicin (CN, 10 µg/disc), Amikacin (AK, 30 µg/disc) and

Ampicillin (Amp, 10 µg/disc) were also used as positive controls. The diameters of inhibition zones (mm) were used as a measure of antimicrobial activity.

Minimal Bactericidal (MBC) or Fungicidal Concentration (MFC)

The micro-dilution method with serially dilution (2 folds) was used to determine MBC or MFC values of the extracts according to Chandrasekaran and Venkatesalu [11]. Some modifications were made to the method. The extracts were studied for microorganisms which are sensitive to the extracts in the disc diffusion assay. The extracts were added to growth broth medium to get a final concentration of 90.00 mg/ml, and serially diluted (2 folds) to reach 0.36 mg/ml. The final volume in each tube was 100 µl. 2.5 µl of each tested microorganism was transferred to each microtube. A positive control (2.5 µl inoculum and 100 µl growth medium) and a negative control (2.5 µl of extract and 100 µl growth medium) were included in each micro tube. The contents of the tubes were mixed by pipetting and were incubated for 24 h. The MIC (minimum inhibitory concentration) was defined as the lowest concentration of an antimicrobial agent that inhibits the visible growth of a microorganism [12]. However, the tested plant extracts in the study were colored, and the visible growth could not observed and so, 5 µl samples from all tubes were plated on solid growth medium to confirm microbial growth. The lowest concentration of the extract that did not permit any visible bacteria and fungal colony growth on the agar plate recorded as MBC or MFC values in the study.

Assays for total phenolic and flavonoid content

The total phenolic content in the extracts was determined with the Folin–Ciocalteu method according to Slinkard and Singleton [13]. The extracts were dissolved in methanol, and 0.2 ml of extract solution (1 mg/ml) was added into the test tube containing 1 ml of Folin-Ciocalteu's reagent and 2 ml of Na_2CO_3 (7.5%). The final volume was adjusted to 7 ml with deionized water. After incubation for 2 h at room temperature, the absorbance against blank was measured at 765 nm with an UV-Vis Spectrophotometer (HITACHI U-2000). The total phenolic content was expressed in terms of mg gallic acid equivalents (GAE) /g dry extracts.

The total flavonoid content in root and flower extracts was estimated spectrophotometrically according to Arvouet-Grand et al. [14]. 1 ml of 2% aluminium trichloride ($AlCl_3$) methanolic solution was mixed with the same volume of extract solution (1 mg/ml). After 10 min, absorption readings at 415 nm were taken against a blank sample consisting of a 1 ml extract solution with 1 ml methanol without $AlCl_3$. Quercetin was used as the standard and the total flavonoid content was expressed in mg quercetin equivalent (QE) /g per dry extract.

Determination of total antioxidant capacity

The phosphomolybdenum method according to Prieto et al. [15] was used to determine the total antioxidant capacity of extract. The extracts were dissolved in methanol (2 mg/ml), and 0.3 ml of each extract was added to 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 min. After the mixture had cooled to room temperature, the absorbance of the solution was measured at 695 nm against a blank. The antioxidant activity was expressed as the number of equivalents of ascorbic acid (mg AAE/g).

1,1-Diphenyl-2-picrylhydrazy (DPPH) radical-scavenging activity

The free radical scavenging activity of the extracts was determined with slight modifications of the method described by Sarikurkcu et al. [16]. 0.5 ml of test samples at different concentrations was mixed with 3 ml 6.10^{-5} M of a methanol solution of DPPH. The reaction mixture was incubated at room temperature in the dark. The scavenging activity on the DPPH radical was

determined by measuring the absorbance at 517 nm after 30 min. The inhibition activity was determined by using following equation:

$$I(\%)= 100 \times (A_0-A_1)/A_0$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract or standard. BHT (butylated hydroxytoluene) was used as positive controls.

Ferric ion reducing power

The reducing power was obtained according to the method of Oyaizu [17] with slight modifications. Various concentrations of extracts were mixed with 2.5 ml of 0.2 M phosphate buffer and potassium ferricyanide (1%). The mixture was incubated at 50ºC for 20 min. After the incubation, 2.5 ml of 10% trichloroacetic acid was added. 2.5 ml of the reaction mixture was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 0.1%). The solution absorbance was measured at 700 nm. The increasing absorbance of the reaction mixture indicates an increasing in reducing power. The same procedure was applied with BHT.

Cupric Ion Reducing Antioxidant Capacity (CUPRAC) Assay

The cupric ion reducing capacity of extracts was determined according to Apak et al. [18]. One milliliter each of 10 mM $CuCl_2$, 7.5 mM neocuproine, and NH_4Ac buffer (1 M, pH 7.0) solutions were added into a test tube. Then, 0.5 ml of different concentrations of the extract was mixed, and the total volume was brought up to 4.1 ml with deionized water. After 30 min incubation at room temperature, the mixture absorbance at 450 nm was recorded against a blank. The same procedure was applied with BHT.

Results and Discussion

Antimicrobial activity

In vitro inhibitory effects of *G. glabra* root extracts were studied against the 14 clinical and food-born pathogenic microorganisms (Table 1). The root DCM and n-hexane extracts showed different degrees of inhibition against food-borne and clinical pathogens. In the current study, the range of inhibition zone of the microorganisms varied from 19.87-8.80 mm. For the n-hexane root extract, the two maximum inhibition zones were observed against *M. luteus* (NRRL B-4375, 19.58 mm) and *S. aureus* (ATCC 25923, 19.29 mm), and the minimum inhibition zone was noticed against *S. enteritidis* (ATCC 13076, 8.80 mm). *S. aureus* is a major clinical pathogen. During the past decade, this bacterium has developed resistance to many commonly used antibiotics [19]. In this study, the root extracts of *G. glabra* showed very high activity against *S. aureus* and can be used for phytotherapy.

The DCM extract of the root has shown significant antimicrobial effect on the studied microrganisms. The highest inhibition zone was determined as 19.87 mm against *B. subtilis* (RSKK 244), while the minimum inhibition zone was found against *S. enteritidis (*ATCC 13076) as 9.14 mm. The extracts of the roots of *G. glabra* have shown magnificent antimicrobial effect. Some of these plant extracts were more effective than traditional antibiotics (Amikacin (30 µg/disc), Gentamicin (10 µg/disc) and Ampicillin (10 µg/disc)) at combating the pathogenic microorganism strains studied (Table 1).

The disc diffusion assay is not enough to decide whether the activity type is lethal or static. The disc diffusion test should be followed by determination of MIC and MBC or MFC values [20]. However, the tested plant extracts in the study were colored, and the visible growth could not be observed for MIC and so MBC or MFC values were determined. The MBC and MFC values are shown in Table 1. The plant root extracts of MBC/MFC values varied from 0.71 to 45.00 mg/ml.

The antimicrobial properties of *G. glabra* are reported from different parts of the world due to its medicinal importance. In their studies, Ateş and Erdoğrul [21] researched the

Table 1. Antimicrobial activity of *G. glabra* root extracts against test microorganisms

Test microorganisms	MBC[a] or MFC[b] (mg/ml)		Inhibition zone diameter[c] (mm)		Antibiotics Inhibition zone diameter (mm)		
	n-Hexane	DCM	n-Hexane	DCM	Amp	CN	AK
B.cereus RSKK 863	0.71	0.71	17.33±0.40	17.74±0.12	38.11±0.02	13.39±0.16	22.28± 0.05
E. coli O157:H7	45.00	45.00	9.51±0.44	9.90±0.02	27.51±0.13	12.22±0.03	22.31±0.10
S. sonnei Mu:57	22.50	11.25	9.78±0.32	10.95±0.58	33.54±0.08	11.51±0.01	13.57±0.02
M. luteus NRRL B-4375	0.71	0.71	19.58±0.63	17.00±0.21	34.68±0.07	11.06±0.05	13.24±0.12
Y. enterocolitica NCTC 11175	22.50	0.71	13.50±0.95	10.45±0.43	13.90±0.05	16.96±0.01	24.38±0.15
E. coli ATCC 11229	0.71	22.50	9.96±0.08	11.40±0.88	25.27±0.03	10.78±0.07	20.82±0.09
P. aeruginosa ATCC 27853	45.00	45.00	9.25±0.25	12.35±0.36	-[d]	16.60±0.09	19.55±0.05
S. aureus ATCC 25923	0.71	0.71	19.29±0.71	16.26±0.10	36.76±0.30	15.40±0.07	17.35±0.09
E.coli ATCC 35218	45.00	22.50	10.70±0.44	11.69±0.09	21.56±0.13	11.33±0.16	15.27±0.10
S. enteritidis ATCC 13076	45.00	45.00	8.80±0.20	9.14±0.31	25.96±0.38	10.53±0.05	14.62±0.04
L. monocytogenes ATCC 7644	0.71	0.71	16.11±0.21	16.74±0.25	32.21±0.41	20.06±0.15	19.14±0.06
B. subtilis RSKK 244	0.71	0.71	13.28±0.12	19.87±0.21	37.57±0.08	15.35±0.04	16.86±0.07
S.enteritidis RSKK 171	11.25	22.50	10.39±0.15	10.77±0.11	31.27±0.11	12.05±0.14	15.67±0.09
C. albicans ATCC 10231	0.71	22.50	9.33±0.05	10.44±0.56	-	-	-

[a]: Minimal Bactericidal Concentration (MBC)
[b]: Minimal Fungicidal Concentration (MFC)
[c]: Diameter of the inhibition zone including disc diameter. Values are reported as means ± SD.
[d]: Indicates no antimicrobial activity

antimicrobial activity of alcohol, ethyl acetate, acetone and chloroform root extracts of *G. glabra* from Turkey against 13 microorganisms by using the disc diffusion method (at the concentration of 20 µl extract/disc). The results showed that the extracts showed various degrees of antimicrobial activity but had no antimicrobial activity against *Y. enterocolitica.* In our study; n-hexane and DCM extracts showed activity against *Y. enterocolitica*. In one of their studies, Sultana et al. [22] used the disc diffusion method and found that the methanolic extract of *G. glabra* plant from Bangladesh showed potent antimicrobial activity against all test microorganisms (11 strain) except *P. aeruginosa*. In their study, *S. aureus* was found the highest susceptible bacteria among the tested microorganisms. In another study, Sedighinia et al. [23] indicated that good antibacterial activity was determined from *G. glabra* root ethanolic extract against six oral pathogens by using disc and well diffusion method at the concentration of 3.125-100 mg/ml. In their study, MBC value was found as 50 mg/ml for *S. aureus*. In our study, MBC values of n-hexane and DCM extracts for *S. aureus* ATCC 25923 were determined as 0.71 mg/ml.

The n-hexane and DCM root extracts of *G. glabra* were also screened against 7 fish pathogenic microorganisms are shown in Table 2. All microorganisms were found to be sensitive to the extracts. The n-hexane extract showed significant antimicrobial activity against *S. agalactiae* (Pas.Inst. 55118) with the zone of inhibition 16.58 mm of all the tested fish pathogens. *G. glabra* DCM root extract exhibited various antimicrobial activities against six fish pathogens but not showed against *V. alginolyticus.* The highest antimicrobial activity was found in the DCM extracts as 18.21 mm against *S. agalactiae* (Pas.Inst. 55118). The n-hexane extract, which had a higher antimicrobial activity zone against fish pathogens in the root extracts, also showed better antibacterial activities against *S. agalactiae* (Pas. Inst. 55118) when compared with standard AK (Table 2). The MBC values of the root extracts varied from 1.41 mg /ml to 22.50 mg/ml for n-hexane and DCM root extracts. *S. agalactiae* (Pas. Inst. 55118) had the highest inhibition zones and the lowest MBC values (1.41 mg/ml) against both of n-hexane and DCM extracts.

Table 2. Antibacterial activity of *G.glabra* root extracts against different bacterial fish pathogens

Microorganisms	MBC[a] (mg/ml)		Inhibition zone diameter[b] (mm)		Inhibition zone diameter[b] (mm) Antibiotics		
	n-hexane	DCM	n-hexane	DCM	Amp	CN	AK
L. garviae	5.63	1.41	13.23±0.05	16.47±0.13	33.10±0.12	15.19±0.10	10.30±0.08
Y. ruckeri	22.50	22.50	10.99±0.16	8.72±0.09	32.30±0.15	18.85±0.05	18.69±0.12
V.anguillarum M1	5.63	22.50	10.57±0.07	9.64±0.11	9.02±0.04	12.38±0.09	9.46±0.12
V.anguillarum A4	11.25	22.50	11.66±0.16	10.23±0.15	9.40±0.11	15.13±0.15	12.07±0.13
V.alginolyticus	5.63	-	14.18±0.08	-[c]	13.57±0.09	15.06±0.07	15.03±0.03
S. agalactiae Pas.Inst. 55118	1.41	1.41	16.58±0.11	18.21±0.25	37.46±0.12	19.72±0.08	16.15±0.08
A.hydrophila ATCC 19570	11.25	1.41	9.91±0.25	9.63±1.79	11.20±0.06	19.03±0.09	30.57±0.11

[a]: Minimal Bactericidal Concentration (MBC)
[b]: Diameter of the inhibition zone including disc diameter. Values are reported as means ± SD.
[c]: Indicates no antimicrobial activity.

Goudarzi et al. [24] studied the antimicrobial activity of ethanolic root extract of *G. glabra* from Iran against *L. garvieae* by using disc diffusion method in the range of 62.5 and 1000 µg/ml. While there was no activity of the ethanol extract at these concentrations against *L. garvieae*, in our study antimicrobial activity at 3.75 mg/disc was recorded from n-hexane and DCM root extracts as 13.23 mm and 16.47 mm, respectively. In their study, they also indicated MIC (minimal inhibitory concentration) and MLC (minimum lateral concentration) as >1000 µg/ml. Similarly, in our study, MBC values for n-hexane and DCM extracts for *L. garviae* were determined as 5.63 mg/ml and 1.41 mg/ml, respectively. In the study by Pirbalouti et al. [25], ethanolic root extract of *G. glabra* from Iran was tested for antimicrobial activity against a major fish pathogen *Streptococcus iniae*. In their study, the extract (at 100 µg/disc) concentration showed antimicrobial activity 13.67 mm by using disc diffusion method. They indicated MIC value for *S. iniae* as >1000 µg/ml which is a result similar to Goudarzi et al. [24] and our results.

Antioxidant capacity

Several methods have been used to determine antioxidant activity of plant extracts. Our study present four methods to evaluate antioxidative activity of *G. glabra*, namely, total antioxidant capacity, DPPH radical-scavenging activity, ferric ion reducing power and CUPRAC assay. The total phenolic and flavonoid contents of the extracts were also determined.

Total phenolic (TPC) and flavonoid (TFC) content

Phenolic compounds have been extensively studied in the past 30 years. They have one or more aromatic rings bearing hydroxyl groups that are potentially able to act as reducing agents, hydrogen donating antioxidants and singlet oxygen quenchers [26, 27]. The root of *Glycyrrhiza* species is one of the richest sources of biological active compounds such as phenolic and flavanoid compounds [28]. In order to determine the presence of phenolic content the Folin-Ciocalteu method was used and the total phenolic content of the extracts is presented in Table 3. In the root extracts, the phenolic compound of DCM extract was found higher (214.36 mg GAE/g extract) than n-hexane extract (86.86 mg GAE/g extract). According to our findings, total phenolic content of DCM root extract was higher than root aqueous and ethanol extracts from Turkish liquorice (*G. glabra* L.) [29]. Cakmak et al. [30] was also observed lower phenolic content (114.33 mg GAE/g) of methanolic root extract from *G. echinata* L. than DCM extract in our study. However, in their investigation, the level of phenolic content in the ethanolic extract was found higher than n-hexane extract in this study. Pratibha et al. [31] observed a lower phenolic content (73.51 mg GAE/g) from DCM extract of *G. glabra* root and stolon when compared to DCM extract in our study.

Table 3. Total phenolic, flavonoid and antioxidant capacity of *G. glabra* extracts

Extracts	TPC[a] (mg GAE/g)	TFC[b] (mg QE/g)	TAC[c] (mg AAE/g)
n-Hexane	86.86±2.57[d]	3.92±0.02	74.92±9.79
DCM	214.36±12.54	12.43±0.41	159.15±8.16

[a] TPC: Total phenolic content
[b] TFC: Total flavonoid content
[c] TAC: Total antioxidant capacity
[d] Values are reported as means ± SD

The level of flavonoid content in the n-hexane and DCM extract of the root was determined as 3.92 mg QE/g extract and 12.43 mg QE/g extract, respectively is presented in Table 3. Tohma and Gulçin [29] found that the aqueous and ethanol extracts of *G. glabra* root had higher flavonoid content (5.1 µg QE/mg and 4.2 µg QE/mg, respectively) than n-hexane extract but lower than DCM extract in this study. Cakmak et al. [30] observed the flavonoid content of *G. echinata* methanolic root extract as 116.54 mg RE/g. Li et al. [32] studied the flavonoid content of ethyl acetate, hexane, chloroform, water and n-butanol extracts of *G. uralensis* root and found that it varied from 3.601 to 66.546 mg RE/g.

Determination of total antioxidant capacity (TAC)

The phosphomolybdenum method is based on the reduction of Mo (VI) to Mo (V) with the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. The phosphomolybdenum method is quantitative since the antioxidant activity is expressed as the number of equivalents of ascorbic acid [15]. TAC was reported as ascorbic acid equivalents and is shown in Table 3. The total antioxidant capacities in the n-hexane and DCM root extracts were found as 74.92 and 159.15 mg AAE/g extract. As a result, DCM extract showed higher total antioxidant content than n-hexane extract. In our study, the n-hexane extract was found lower TAC than the methanolic root extract of *G. echinata* (161.18 mg AAE/g), but the DCM extract was determined to close *G. echinata* ethanol extract [30]. Tohma and Gulçin [29], using ferric thiocyanate method, reported the total antioxidant activity of the aqueous and ethanol extracts from *G. glabra* root as 88.6% and 80.1%, respectively.

DPPH radical scavenging activity

1,1-diphenyl-2-picrylhydrazyl radical (DPPH) is used by most of the researchers to determine antioxidant activity. DPPH is a free radical that keeps its stability in aqueous or methanolic solutions. It accepts an electron or hydrogen ion to become a stable diamagnetic molecule [33]. The change in absorbance of DPPH radicals was monitored and at the concentration of 400 µg/ml, the DCM extract of the root of *G. glabra* showed higher DPPH scavenging activity (92.58%) than n-hexane extract (37.53%). The radical scavenging of BHT was determined as 91.19% at 200 µg/ml concentration (Figure 1). IC_{50} values of n-hexane and DCM extracts are 579.98 ± 26.53 µg/ml and 111.49 ± 3.26 µg/ml, respectively.

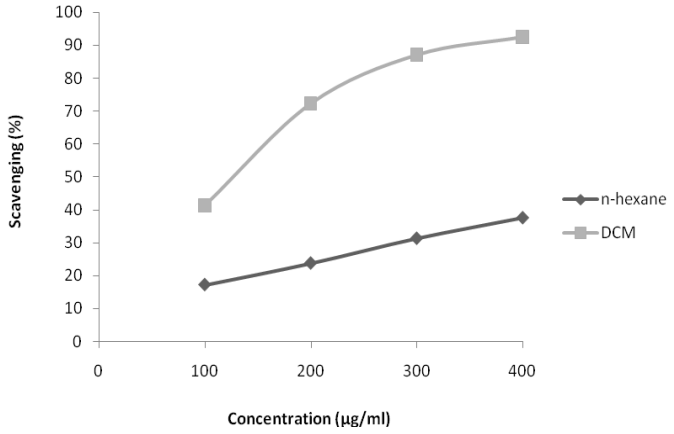

Fig. 1. DPPH scavenging activity of *G. glabra* root extracts

Tohma and Gulçin [29] reported that DPPH radical scavenging activities of *G. glabra* aqueous and ethanol root extracts were 52.2% and 54.4% at 30 µg/ml concentration. Cakmak et al. [30] found that DPPH radical scavenging activity for methanolic root extract (IC_{50} 184.99 µg/ml) of *G. echinata* was lower when compared to n-hexane extract but higher than

DCM extract in this study. A lower IC_{50} value indicates a higher antioxidant activity [34]. Pratibha et al. [31] indicated DPPH radical scavenging activity of *G. glabra* root and stolon methanolic extract as IC_{50} 51.07 µg/ml. In our study, the n-hexane and DCM extracts showed a dose-dependent radical scavenging activity. However, none of the extracts exhibited scavenging activity higher than the standard BHT. The radical scavenging values depend on locality, polarity of extraction solvents and used plant parts.

Ferric ion reducing power

The reducing capacity of compound Fe^{3+}/ferrecyanide complex to the ferrous form may serve as a significant indicator of its antioxidant capacity [35]. Figure 2 shows the dose response curves for the reducing power of the extracts from *G. glabra* root extracts. In this study, the reductive ability of n-hexane and DCM root extracts and standard antioxidant (BHT) was measured by investigating the $Fe^{+3} \rightarrow Fe^{+2}$ transformations according to the method of OYAIZU [17]. The result for ferric ion reducing power (A_{700} nm 0.51) was found in the n-hexane extract while the values for ferric ion reducing power was found in the DCM extract (A_{700} nm 1.37) at the concentration 1000 µg/ml. BHT, at 62.50 µg/ml the concentration, exhibited remarkably higher reducing power (A_{700nm} 0.91) than the extracts. CAKMAK et al. [30] reported ferric ion reducing power activity as EC_{50} 582.14 and 432.63 µg/ml for *G. echinata* methanolic root and aerial part extracts, respectively. In the another study, reducing power of methanol, ethanol and water extracts from *G. glabra* root from India was found lower than our extracts in this study, at concentration of 1 mg/ml [36].

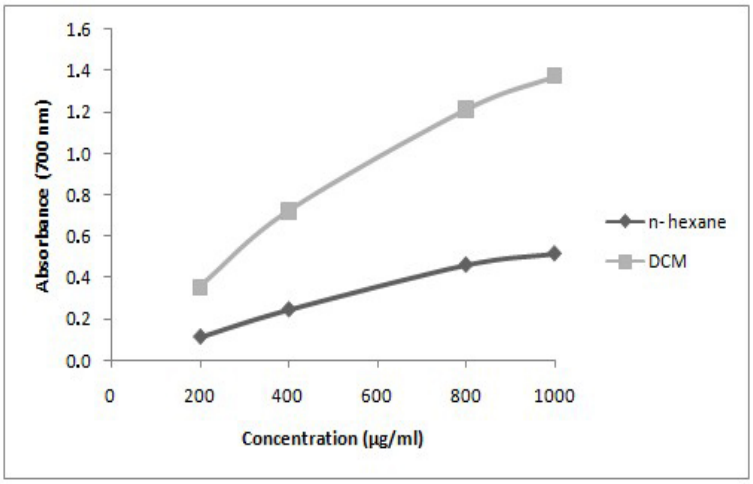

Fig. 2. Ferric ion reducing power of *G. glabra* root extracts

CUPRAC assay

The CUPRAC assay used the copper (II)-neocuproine reagent as the chromogenic oxidizing agent. The method is based on the measurement of absorbance at 450 nm by formation of stable complex between neocuproine and copper (I) [37]. The cupric ion reducing power of extracts was dependent on the concentration of extract (Figure 3). According to CUPRAC data of *G. glabra* root, the DCM extract (A_{450nm} 1.51) showed higher reducing power activity than n-hexane extract (A_{450nm} 0.67) (at concentration 800 µg/ml). BHT, at 62.50 µg/ml the concentration, exhibited remarkably higher cupric ion reducing power (A_{450nm} 0.42±0.01) than the extracts. Cakmak et al. [30] found that cupric reducing antioxidant capacity was lower for *G. echinata* methanolic root and aerial part extracts when compared to our results for DCM extract at 400 µg/ml concentration. However, in their study, CUPRAC of these extracts was higher than n-hexane extract in our study. In their study, Tohma and Gulçin [29] reported that

cupric reducing antioxidant capacities at 30 µg/ml concentration were OD_{450} 0.496 and 0.572 for the root aqueous and ethanol extracts of *G. glabra*, respectively.

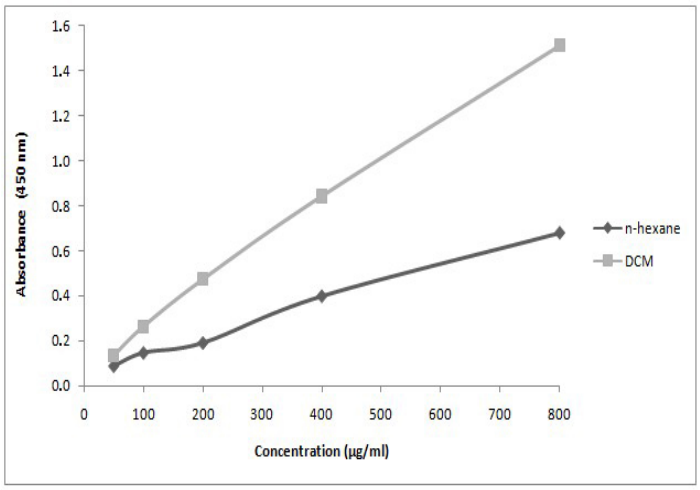

Fig. 3. Cupric ion reducing power of *G. glabra* root extract

Conclusions

In this study, the antimicrobial effect of the root n-hexane and DCM extracts of *G. glabra* from Turkey, which is a significant plant in our country and in the world, against clinical and food borne human and fish pathogens were determined. The antioxidant capacity of this plant root extracts was discovered as well. Most researchers have been used ethanol and methanol as solvents to extract almost all of the proposed antimicrobial and antioxidant agents in order to prepare the basis to monitor different antimicrobial and antioxidant agents. According to the results of the present study, n-hexane and DCM extracts of *G. glabra* root showed a better or close some antimicrobial and antioxidant activities to results, which were used ethanol and methanol as solvents to extract. In the our results, the DCM root extract of *G. glabra* showed better antioxidant activity than n-hexane extract and also had a higher total phenolic and flavonoid content. The findings indicated the potential use of the n-hexane and DCM extracts from *G. glabra* root as biopreservatives as they demonstrated high antimicrobial and antioxidant activities.

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References

[1] M.N.ASL, H. HOSSEINZADEH, Review of pharmacological effects of *Glycyrrhiza* sp. and its bioactive compounds. *Phytother. Res.*, 22: 709-724, 2008.
[2] İ. KARAMAN, F. ŞAHIN, M. GÜLLÜCE, H. ÖĞÜTÇÜ, M. ŞENGÜL, A. ADIGÜZEL, Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. *J. Ethnopharmacol.*, 85: 231-235, 2003.
[3] M. THAKARE, Pharmacological screening of some medicinal plants as antimicrobial and feed additives, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA, 2004, p. 73.
[4] A. ABUTBUL, A. GOLAN-GOLDHIRSH, O. BARAZANI, D. ZILBERG, Use of *Rosmarinus officinalis* as a treatment against *Streptococcus iniae* in Tilapia (*Oreochromis* sp.). *Aquaculture*, 238: 97-105, 2004.
[5] S. DIREKBUSARAKOM, Y. EZURA, M. YOSHIMIZU, HERUNSALEE N., Efficacy of Thai traditional herb extracts against fish and shrimp pathogenic bacteria, *Fish Pathol.*, 1998; 33: 437–441.

[6] A. HELEN, K. KRISHNAKUMAR, P.L. VIJAYAMMAL, K.T. AUGUSTI, Antioxidant effect of onion oil (*Allium cepa* Linn.) on the damage induced by nicotine in rats as compared to alphatocopherol, *Toxicol. Lett.*, 2000; 116: 61–68.
[7] H.C. GRICE, Safety evaluation of butylated hydroxytoluene (BHT) in the live, lung and gastrointestinal tract, *Food Chem. Toxicol*., 1986; 24: 1127–1130.
[8] N.S. HETTIARACHCHY, K.C. GLENN, R. GNANASAMBANDAM, M.G. JONHNSON, Natural antioxidant extract from fenugreek (*Trionella foenumgraecum*) for ground beef patties, *J. Food Sci.*, 1996; 61: 516–519.
[9] M. SENEVIRATHNE, S. KIM, N. SIRIWARDHANA, J. HA, K. LEE, Y. JEON, Antioxidant potential of Ecklonia cava on reactive oxygen species scavenging metal chelating, reducing power and lipid peroxidation inhibition. *Food Sci. Technol. Int.*, 12(1): 27–38, 2006.
[10] P.R. MURRAY, E.J. BARON, M.A. PFALLER, F.C. TENOVER, R.H. YOLKE, Manual of Clinical Microbiology, 6th ed, ASM Press, Washington, 1995.
[11] M. CHANDRASEKARAN, V. VENKATESALU, Antibacterial and antifungal activity of *Syzygium jambolanum* seeds. *J. Ethnopharm.*, 91: 105–108, 2004.
[12] J.M. ANDREWS, Determination of minimum inhibitory concentrations. *J. Antimicrob. Chemother.,* 48: 5-16, 2001.
[13] K. SLINKARD, V.L. SINGLETON, Total phenol analysis: Automation and comparison with manual methods. *Am. J. Enol. Viticult.*, 28: 49-55, 1977.
[14] A. ARVOUET-GRAND, B. VENNAT, A. POURRAT, P. LEGRET Standardisation d'un extrait de propolis et identification des principaux constituants. *J. Pharm. Belg.*, 49: 462–468, 1994.
[15] P. PRIETO, M. PINEDA, M. AGUILAR, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphor molybdenum complex: Specific application to the determination of vitamin E. *Anal. Biochem.*, 269: 337-341, 1999.
[16] C. SARIKURKCU, B. TEPE, D. DAFERERA, M. POLISSIOU, M. HARMANDAR, Studies on the antioxidant activity of the essential oil and methanol extract of *Marrubium globosum* subsp. *globosum* (*Lamiaceae*) by three different chemical assays, *Bioresource Technol.*, 99: 4239-4246, 2008.
[17] M. OYAIZU, Studies on products of browning reactions: Antioxidative activities of browning reaction prepared from glucosamine. *Jpn. J. Nutr.*, 44: 307-315, 1986.
[18] R. APAK, K. GUCLU, M. OZYUREK, S.E. KARADEMIR, E. ERCAG, The cupric ion reducing antioxidant capacity and polyphenolic content of some herbal teas. *Int. J. Food. Sci. Nutr.*, 57: 292-304, 2006.
[19] M.M. MARANANAN, B. MOREIAR, S. BOYLE-VAVRA, R.S. DAUM, Antimicrobial resistance in Staphylococci. *Infect. Dis. Clinic North. Am.*, 11: 813-837, 1997.
[20] E.M. ALTUNER, T. ÇETER, E. BAYAR, S. AYDIN, F. ARICI, G. SÜLEYMANOĞLU, A. EDIS, Investigation on antimicrobial effects of some moss species collected from Kastamonu region. *Commun. Fac. Sci. Univ. Ank. Series C*, 23(1-2): 33-43, 2011.
[21] D.A. ATEŞ, Ö.T. ERDOĞRUL, Antimicrobial activities of various medicinal and commercial plant extracts. *Turk. J. Biol.*, 27: 157-162; 2003.
[22] S. SULTANA, A. HAQUE, K. HAMID, K.F. URMI, S. ROY, Antimicrobial, cytotoxic and antioxidant activity of methanolic extract of *Glycyrrhiza glabra. Agr. Biol. J. N. Am.*, 1(5): 957-960, 2010.
[23] F. SEDIGHINIA, A.S. AFSHAR, S. SOLEIMANPOUR, R. ZARIF, J. ASILI, K. GHAZVINI, Antibacterial activity of *Glycyrrhiza glabra* against oral pathogens: an *in vitro* study. *Avicenna Journal of Phytomedicine*, 2(3): 118-124, 2012.
[24] M.A. GOUDARZI, B. HAMEDI, F. MALEKPOOR, R. ABDIZADEH, A. GHASEMI PIRBALOUTI, M. RAISSY, Sensitivity of *Lactococcus garvieae* isolated from rainbow trout to some Iranian medicinal herbs. *J. Med. Plants Res.*, 5(14), 3067-3073, 2011.
[25] A.G. PIRBALOUTI, V.N. BROUJENI, M. MOMENI, F.M. POOR, B. HAMEDI, Antibacterial activity of Iranian medicinal plants against *Streptococcus iniae* isolated from rainbow trout (*Oncorhynchus mykiss*). *Arch Biol. Sci. Belgrade*, 63 (1): 59-66, 2011.
[26] C.A. RICE-EVANS, N.M. MILLER, G. PAGANDA, Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radic. Bio.l Med.*, 20: 933-956, 1996.
[27] R. MATTEI, R.F. DIAS, F.B. ESPINOLA, E.A. CARLINI, S.B.M. BARROS, Guarana (*Paullinia cupana*): Toxic behavioral effects in laboratory animals and antioxidant activity *in vitro. J. Ethnopharmacol.,* 60*:* 111-116, 1998.
[28] K. ROTH, Licorice spirals. *Chemie in unserer Zeit*, 38: 202-207, 2004.
[29] H.Ş. TOHMA, İ. GULÇIN, Antioxidant and radical scavenging activity of aerial parts and roots of Turkish liquorice (*Glycyrrhiza glabra* L.). *Int. J. Food Prop.*, 13: 657-671, 2010.
[30] Y.S. CAKMAK, A. AKTUMSEK, A. DURAN, Studies on antioxidant activity, volatile compound and fatty acid composition of different parts of *Glycyrrhiza echinata* L. *EXCLI J.*, 11: 178-187; 2012.

[31] N. PRATIBHA, D. SUSHMA, K. GUPTA RAJINDER, Screening for Antioxidant and Antibacterial potential of common medicinal plants in the treatment of Acne. *Int. J. Drug Dev. & Res.,* 4 (1): 65-71, 2012.
[32] M. LI, Y. XU, W. YANG, J. LI, X. XU, X. ZHANG, F. CHEN, D. LI, In vitro synergistic antioxidant activities of solvent-extracted fractions from *Astragalus membranaceus* and *Glycyrrhiza uralensis.* LWT - *Food Sci. Technol.*, 44: 1745-1751, 2011.
[33] J.R.T. SOARES, C.P. DINIS, A.P. CUNHA, L.M. AMEIDA Antioxidant activity of some extracts of *Thyms zygis. Free Rad. Res.,* 26: 469-478, 1997.
[34] YAMAGUCHI T., TAKAMURA H., MATOBA T. AND TERAO J., HPLC method for evaluation of the free radical scavenging activity of foods by using 1, 1-diphenyl-2-picrylhydrazyl, *Biosci. Biotechnol. Biochem.*, 62: 1201-1204, 1998.
[35] A. YILDIRIM, A. MAVI, A. KARA, Determination of antioxidant and antimicrobial activities of *Rumex crispus L*. extracts, J. Agric. Food Chem., 49: 4083-4089, 2001.
[36] B. DAYANANDA, A.K. RAGHAVAN, F. KHANUM, B.A. SNGH, In vitro antioxidant and free radical scavenging activity of *Glycyrrhiza glabra* root extracts. *Journal of Herbal Medicine and Toxicology*, 4 (1): 97-102, 2010.
[37] M. OZYUREK, K. GUCLU, E. TUTEM, K.S. BASKAN, E. ERCAG, S.E. CELİK, S. BAKİ, L. YILDIZ, Ş. KARAMAN, R. APAK, A comprehensive review of CUPRAC methodology. *Anal. Meth.*, 3: 2439-2453, 2011.