Research Article

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A comparative study of biochemical, antimicrobial effects and phytochemical composition analysis of *Glycyrrhiza glabra* L. varieties root extracts

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Received: 30 April 2025/ Accepted: 20 May 2025

ABSTRACT: Plants are the significant global interest as alternative treatment sources with their biologically active compounds. This study compares the chemical composition and the antioxidant, antidiabetic, and antimicrobial properties of ethanol extracts of *G. glabra* L. two different varieties from different regions. The phytochemical compositions was determined using GC-MS. Additionaly, total phenolic (TPC), flavonoid (TFC) and triterpene (TTC) contents were determined. Glycyrrhizic acid contents were analysed by HPLC. *G. glabra* var. *glandulifera* (GF1) showed the highest antioxidant activity. All extracts had strong antidiabetic effects, besides GF1 showing the highest effect. The MIC values was determined against 8 bacterial and 1 yeast strain and values ranged from 2.500 to 0.500; 2.500 to 0.714; 2.500 to 0.714 for *G. glabra* var. *glabra* (GB), GF1, *G. glabra* var. *glandulifera* (GF2) respectively. Phytochemical studies have shown that TPC was 100.60±5.06, 127.90±0.30, 69.01±0.30 mg GAE /g extract; TFC was 80.07±0.15, 25.35±0.0, 16.58±0.31 mg KE/g and TTC was 217.30±6.05,172.40±2.17, 126.30±4.50 mg OE/g extract for GB, GF1, GF2, respectively. GF1 in particular has the highest glycyrrhizic acid content. This study will contribute to the creation of new treatment strategies and potential therapeutic agents in addition to the use of *G. glabra* L. in traditional treatments. Our study is also a preliminary study for future studies.

KEYWORDS: *Glycyrrhiza glabra* L; licorice root; antioxidant activity; antidiabetic activity; antibacterial activity; phytochemical content

1. INTRODUCTION

Licorice is a perennial plant belonging to the genus *Glycyrrhiza*. It is primarily classified into three main species: *Glycyrrhiza uralensis, Glycyrrhiza glabra* and *Glycyrrhiza inflata* [1]. Among these, *Glycyrrhiza glabra* L. (Fabaceae) is widely distributed across various regions, particularly in wet and humid environments. It grows widely in the Mediterranean, East Asia, Eastern Europe, the Middle East, Iran, Russia, Siberia, and Mongolia [2]. Additionally, it is abundantly present in Türkiye [3]. The *Glycyrrhiza* genus comprises more than thirty species worldwide, with six species naturally distributed in Türkiye [4,5]. Historically, it is known that it has been used for medical purposes since 400 BC in ancient Rome and as early as 2800 BC in traditional Chinese medicine. *G. glabra* is a plant of extensive scientific research due to its biologically active components and its long-standing traditional use [6]. Licorice extract, obtained from *G. glabra*, has been widely used in traditional Eastern medicine for the treatment of various complaints. In folk medicine, licorice has been utilized for wound healing, gastric ulcers, skin disorders, epilepsy, asthma, cough suppression, laxative effects, and detoxification [7-9]. Additionally, it is also commonly used as a natural sweetener [10]and is widely utilized as a flavoring agent in the beverage, confectionery, and alcohol industries [4].

How to cite this article: Gülmez G, Şen A, Servi H, Barak TH, Tekin F, Marzi M, Şener A. A comparative study of biochemical, antimicrobial effects and phytochemical composition analysis of *Glycyrrhiza glabra* L. varieties root extracts. J Res Pharm. 2025; 29(6): 2361-2372.

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ISSN: 2630-6344

https://doi.org/10.12991/jrespharm.1797864

To date, over 300 bioactive compounds have been identified in *G. glabra,* alongside its starch, saccharide, and mucilage content [10]. The primary bioactive constituents of *G. glabra* include phytochemicals such as flavonoids, which contribute to its yellow coloration, as well as triterpenic saponins, coumarins, and sterols [4,11,12]. Among these, glycyrrhizic acid, one of the most important active compounds and a triterpenoid saponin found in *G. glabra* roots, is known to be approximately 50 times sweeter than sucrose [13]. However, the concentration and composition of active constituents of *G. glabra* can vary significantly depending on the species, geographical region, plant maturity, soil pH, temperature, and harvesting and processing methods [14,15].

The pharmacological properties of *G. glabra* are attributed to its diverse bioactive compounds, which exert various biological and physiological mechanisms. These compounds have been demonstrated to exhibit expectorant, antioxidant, anti-ulcer, anticancer, anti-inflammatory, and antidiabetic activities [16]. Its antioxidant properties have been particularly associated with potential neuroprotective effects, suggesting its application as a natural therapeutic resource for neurodegenerative diseases [17]. Additionally, *G. glabra* has demonstrated antimicrobial activity, with its major constituent, glycyrrhizin, showing efficacy against viral infections such as Hepatitis C and HIV-1. Notably, it has been reported that glycyrrhizin has been shown to yield more effective results than a commonly used antiviral agent in the fight against the SARS virus [18,19]. Furthermore, in vitro and in vivo studies suggest that licorice root and its purified compounds have the potential to inhibit the initiation and progression of various malignancies [20,21]. Moreover, the phytoestrogenic properties of licorice root have been reported to exert suppressive effects on hormone-related cancers, including breast, endometrial, and prostate cancers [20,22].

In recent years, the focus of scientific research has shifted towards naturally derived bioactive compounds due to the adverse effects and potential resistance associated with pharmacological agents. Plants have garnered significant global interest as alternative therapeutic resources owing to their bioactive constituents, This study aims to investigate the chemical composition and the antioxidant, antidiabetic, and antimicrobial properties of two different *G. glabra* L. varieties as potential therapeutic agents. Furthermore, it seeks to elucidate the impact of variations in geographical growing regions and plant varieties on their chemical composition and biological activities.

2. RESULTS

2.1. The Antioxidant Activities of *G. glabra* L. Varieties Extracts

The IC_{50} values determined for the antioxidant activity of GB, GF1 and GF2 are shown in Table 1. The low IC_{50} values, identified as the concentration required to eliminate 50% ratio of the radical or inhibit enzyme activity by 50%, indicate high efficacy. According to the IC_{50} values, GF1 with IC_{50} values of 87.47 and 19.89 µg/mL showed the highest antioxidant activity against each $DPPH^{•}$ and $ABTS^{•+}$ by a big difference in values compared to the others. The GF1 also had the highest antioxidant capacity with 1677.92 µmol trolox equivalent value in cupric reducing capacity. The GF2 showed higher antioxidant activity than GB against each $DPPH^{•}$ and $ABTS^{•+}$ radical. In Cupric reducing capacity, GB showed higher antioxidant activity than GF2.

2.2. The Antidiabetic Activities of *G. glabra* L. Varieties Extracts

The GF1 and GB showed the highest α-glucosidase inhibitory activity with IC_{50} values of 67.28 and 68.36 µg/mL, respectively. Compared to the standard (Acarbose IC_{50}: 192.00 µg/mL), all extracts exhibited strong antidiabetic activity (Table 1).

Table 1. Comparison of the antioxidant, antidiabetic activity of the extracts of *G. glabra* L. varieties

Extracts*/ Standards	Antioxidant activity			Antidiabetic effects
	$DPPH^{•}$ inhibition activity IC_{50} (µg/mL)	$ABTS^{•+}$ inhibition activity IC_{50} (µg/mL)	Cupric reducing capacity µmolTE/ g extract	Alpha-glucosidase inhibition IC_{50} (mg/ mL)
GB	371.00 ± 0.14^{d}	74.22 ± 0.42^{d}	876.36 ±12.9^{b}	68.36 ± 0.60^{a}
GF1	87.47 ± 1.27^{b}	19.89 ± 0.45^{b}	1677.92±24.5^{a}	67.28 ± 0.04^{a}
GF2	119.10 ± 0.35^{c}	62.98 ± 1.33^{c}	589.09±31.2^{c}	157.90 ± 0.57^{b}
Ascorbic acid	17.60 ± 0.37^{a}			
Trolox		13.00 ± 0.21^{a}		
Acarbose				192.00 ± 1.00^{c}

* Each value was presented as mean ± standard deviation (n = 3). Different letter superscripts in the same

column indicate significant differences (P < 0.05).
Abbreviations: GB: *Glycyrrhiza glabra* var. *glabra* (Batman, TÜRKİYE), GF: *Glycyrrhiza glabra* var. *glandulifera* (GF1) (Malatya, TÜRKİYE), GF2: *Glycyrrhiza glabra* var. *glandulifera* (GF2) (Bingöl, TÜRKİYE)

2.3. The Antimicrobial Activities of *G. glabra* L. Varieties Extracts

The MIC values for *G. glabra* extracts was determined against 8 bacterial and 1 yeast strain as presented in Table 2. The MIC values ranged from 2.500 to 0.500; 2.500 to 0.714; 2.500 to 0.714 for GB, GF1, GF2, respectively. The inhibitory effect wasn't observed for *Acinetobacter baumannii* BAA 747 in all three extracts. In addition, GB and GF1 didn't show the inhibitory effect for *Pseudomonas auroginosa* ATCC 27853.

Table 2. Minimum inhibition concetrations (MIC) values of extracts of G. glabra L. varieties

Microorganisms	GB (mg/mL)	GF1 (mg/mL)	GF2 (mg/mL)
Escherichia coli ATCC 25922	1.666	1.666	1.255
Escherichia coli (NCTC12493	1.666	1.666	2.500
Enterococcus faecalis ATCC 29213	0.625	0.833	1.000
Staphylococcus areus ATCC 25923	0.625	0.833	0.714
Staphylococcus areus (NCTC 12493)	0.500	0.714	1.000
Klebsiella Pneumoniae ATCC 700603	2.500	2.500	1.666
Acinetobacter baumannii BAA 747	--	--	--
Pseudomonas auroginosa ATCC 27853	--	--	1.250
Candida albicans ATCC 10231	1.250	1.250	1.000

"--" No growth inhibition. Abbreviations: GB: *Glycyrrhiza glabra* var. *glabra* (Batman, TÜRKİYE), GF:*Glycyrrhiza glabra* var. *glandulifera* (GF1) (Malatya, TÜRKİYE), GF2: *Glycyrrhiza glabra* var. *glandulifera* (GF2) (Bingöl, TÜRKİYE)

2.4. Total Phenolic, Total Flavonoid and Total Triterpene Contents of *G. glabra* L. Varieties Extracts

TPC, TFC and TTC values of *G. glabra* extracts were expressed as gallic acid (GAE), quercetin (QE) and oleanolic acid (OE) equivalents, respectively in Table 3. The GB (80.07 mg/g, 217.30 mg/g) had the highest values for TFC and TTC, respectively. The GF1 (127.90 mg/mg) had the highest values for TPC.

2.5. Analyses of Glycyrrhizic Acid in *G. glabra* Extracts by HPLC

In the *G. glabra* extracts, the amount of glycyrrhizic acid was found to be the highest in GF1 with 5.29% (w/w), followed by GF2 (3.53%) and GB (0.07%) (Table 3).

Table 3. The content of the extracts of *G. glabra* L varieties

Extracts	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	TTC (mg OE/extract)	The total glycyrrhizic acid content (%, w/w)
GB	100.60 ± 5.06^{b}	80.07 ± 0.15^{a}	217.30 ± 6.05^{a}	0.07
GF1	127.90 ± 0.30^{a}	25.35 ± 0.00^{b}	172.40 ± 2.17^{b}	5.29
GF2	69.01 ± 0.30^{c}	16.58 ± 0.31^{c}	126.30 ± 4.50^{c}	3.53

* Results were expressed as gallic acid equivalent (GAE) for TPC, as quercetin equivalent (QE) for TFC, as oleanolic acid equivalent (OE) for TTC. Each value was presented as mean ± standard deviation (n = 3). Different letter superscripts in the same column indicate significant differences (P < 0.05).
Abbreviations: GB: *Glycyrrhiza glabra* var. *glabra* (Batman, TÜRKİYE), GF: *Glycyrrhiza glabra* var. *glandulifera* (GF1) (Malatya, TÜRKİYE), GF2: *Glycyrrhiza glabra* var. *glandulifera* (GF2) (Bingöl, TÜRKİYE), TPC: Total phenolic content, TFC: Total flavonoid content, TTC: The total triterpene content.

2.6. Chemical Composition of *G. glabra* Extracts by GC-MS

In the analysis of the chemical contents of *G. glabra* extracts, the major compounds were D-Pinitol, pentakis (9.0%), β-D-Glucopyranose (6.1%), β-D-Lactose, (isomer 1) (7.2%), D-(+)-Trehalose, octakis (7.2%), Sucrose (17.3%) in GB; D-(+)-Talofuranose, pentakis (isomer 2) (5.8%), D-Pinitol, pentakis (8.4%), β-D-Glucopyranose (7.8%), β-D-Lactose, (isomer 1) (6.0%), Sucrose (27.1%) in GF1 and D-Pinitol, pentakis (10.7%), β-D-Glucopyranose (5.0%), β-D-Lactose, (isomer 1) (12.0%), D-(+)-Trehalose, octakis (6.8%), Sucrose (28.0%), D-Lactitol, nonakis (6.2%) in GF2 (Table 4, Figure 1, Figure 1, Figure 3).

Table 4. Chemical composition of the extracts of *G. glabra* L varieties with GC-MS analysis

RT	RRI	RRI Lit.	Compounds	GB (%)	GF1 (%)	GF2 (%)
6.642	960	964	Silanamine, N,N'-methanetetraylbis[1,1,1-trimethyl	-	0.5	1.2
9.030	1019	1066	Lactic Acid	0.2	-	-
11.977	1097		L-Proline, TMS derivative	1.2	2.1	3.4
14.346	1265	1267	Pipecolic acid	0.1	-	-
14.841	1283	1296	Glycerol	1.2	1.1	1.3
14.984	1288	1290	Silanol, trimethyl-, phosphate (3:1)	1.4	-	-
15.299	1300	1302	L-Proline, 2TMS derivative	2.1	2.0	3.3
16.562	1348	1353	2-Butenedioic acid, (E)-	0.3	-	-
16.989	1364	1360	Pipecolic acid	0.4	-	-
20.377	1498		Malic acid	1.9	0.6	0.4
20.589	1506	1510	Hexanedioic acid	1.5	0.4	2.8
26.295			Phloretic acid	-	1.8	1.6
27.114	1795	1778	L-(-)-Sorbofuranose, pentakis	2.5	4.0	0.8
27.736	1826	1813	D-(-)-Tagatofuranose, pentakis (isomer 2)	4.0	3.9	3.2
27.915	1834	1841	D-(+)-Talofuranose, pentakis (isomer 2)	4.0	5.8	3.3
28.286	1852	1815	D-Pinitol, pentakis	9.0	8.4	10.7
28.574	1867	1852	Beta.-D-Galactofuranose, 1,2,3,5,6-pentakis-O-	2.0	2.2	1.0
29.516	1913	1924	α-D-Glucopyranose	4.6	4.7	3.0
30.244	1951	1969	D-Mannitol	1.1	1.0	-
30.367	1957	1937	D-Glucose	1.1	1.9	1.5
31.296	2005	1971	β-D-Glucopyranose	6.1	7.8	5.0
31.835	2034	2033	Palmitic acid	0.6	-	-
34.785	2196	2210	Linoleic acid	0.3	-	-
36.231	2280	2281	9-Tricosene, (Z)-	1.0	1.5	0.7
36.941	2322	2281	Sophocarpine	1.1	-	-
37.284	2343	2286	Matrine	3.0	-	-
37.606	2363		Sophoridine	2.1	-	-
41.284	2560	2529	β-D-Lactose, (isomer 1)	7.2	6.0	12.0
41.840	2616		((9-Methoxy-6a,11a-dihydro-6H-benzofuro[3,2-c]chromen-3-yl)oxy)	-	1.0	-
42.895	2627	2616	D-(+)-Trehalose, octakis	7.2	4.9	6.8
44.339	2679		Sucrose	17.3	27.1	28.0
45.010	2703	2736	D-Lactitol, nonakis	3.6	4.4	6.2
47.699	2775	2768	Aucubin, hexakis	0.6	1.1	-
			Total identified compounds	**88.7**	**93.7**	**96.2**

Abbreviations: GB: *Glycyrrhiza glabra* var. *glabra* (Batman, TÜRKİYE), GF: *Glycyrrhiza glabra* var. *glandulifera* (GF1) (Malatya, TÜRKİYE), GF2: *Glycyrrhiza glabra* var. *glandulifera* (GF2) (Bingöl, TÜRKİYE)

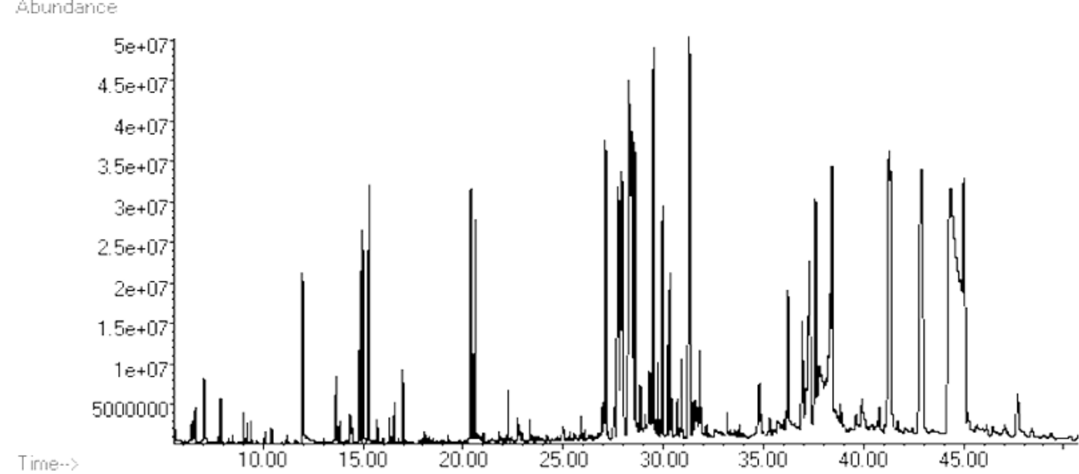

Figure 1. GC-MS chromatogram of *G. glabra* var. *glabra* extract (GB)

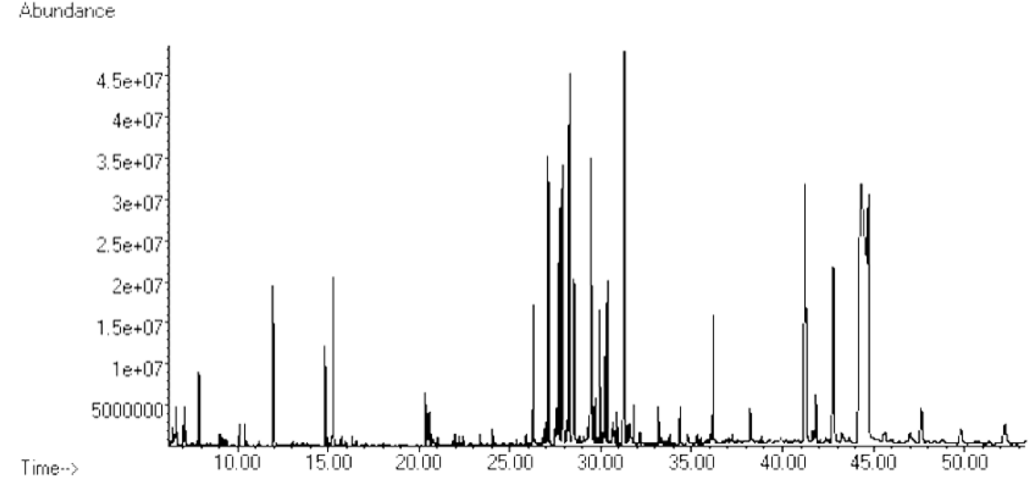

Figure 2. GC-MS chromatogram of *G. glabra* var. *glandulifera* extract (GF1)

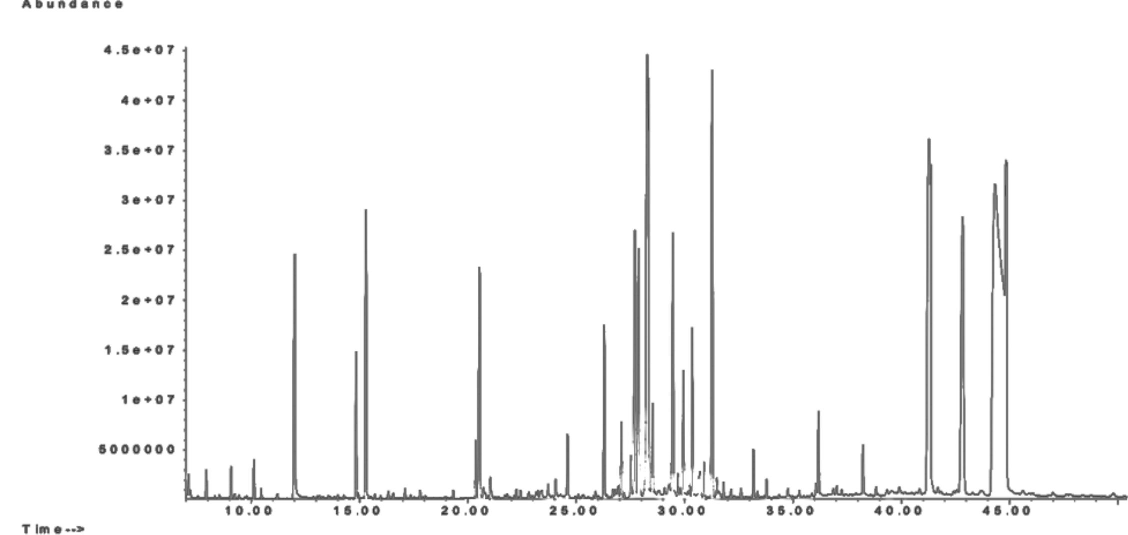

Figure 3. GC-MS chromatogram of *G. glabra* var. *glandulifera* extract (GF2)

3. DISCUSSION

The plants with their various biological compounds are the focus of global research areas for the treatment of diseases. This study aimed to reveal the phytochemical contents and antioxidant, antidiabetic and antimicrobial effects of the *G. glabra* L. varieties and to determine the effect of the differences in regions and varieties on these factors.

The plants have high antioxidant properties with their functional components such as phenols (such as phenolic acids, hydroxylated flavonoids), flavonoids and saponins [23]. In our study, that was demonstrated that the different varieties of *G. glabra*'s extracts had different antioxidant capacities. GF1 and GF2 had higher antioxidant activity than GB against the DPPH and ABTS radicals. It was also observed GF1 had higher antioxidant activity than GB in cupric reducing capacity. In addition, the differences in antioxidant activity were observed in samples collected from different regions within the same varieties. GF1 from Malatya had higher activity than GF2 from Bingöl in all antioxidant activity tests. In the study conducted Visavadiya et.al., it was shown that aqueous and ethanolic extracts of *G. glabra* had 43.6 mg/mL and 28.3 mg/mL IC_{50}; 77.3 and 57.2 mg/mL IC_{50} values against DPPH and ABTS radicals, respectively [24]. The previous study reported that the *G. glabra* aqueous extract from Egypt had 29.92 mg/g GAE against DPPH radical [25]. In another study, it was shown that the IC_{50} values of *G. glabra* methanol extracts obtained from various regions (Rayen, Eghlid, Kalat, Zanjan) varied between 35.25 and 59.39 µg/mL [26]. The current study found better results than previous studies. Different regions may have an effect on differences in the antioxidant activity, as well as different solvents used in extraction. In our study, ethanol was preferred for contributing to pharmacological agents that can be developed in the future. due to it was suitable for human consumption and had high activity in Jo et. al. study [36]. GF1 exhibited higher antioxidant activity compared to other *G. glabra* extracts. When the chemical content of GF1 was examined, it was seen that it had a high glycyrrhizic acid content. It has also been found to have high TPC. Glycyrrhizic acid and phenolic compounds were previously reported to have antioxidant activity [27,28]. Therefore, Glycyrrhizic acid and phenolic compounds, together with other components found in the extract, may be responsible for the antioxidant effect of GF1.

The α-glucosidase inhibitors found in natural sources such as fruits and vegetables may be helpful in controlling hyperglycemia and diabetes. In this study, we demonstrated that all extracts exhibited strong antidiabetic activity compared with acarbose (IC_{50}:192 µg/mL) which was used as a standard. GF1 has shown the highest activity with 67.28 µg/mL IC_{50} value. In a study, α-glucosidase inhibition effect of *G. glabra* aqueous extracts was reported. According to the result of this report, *G. glabra* aqueous extracts from Siddha Medicine store has shown <50 inhibitory activity in 10, 25, and 50 µg/mL concentrations and shown >50% inhibitory activity in 100 and 500 µg/mL concentrations [29]. In another study, antidiabetic activities of *G. glabra* ethanol extracts of different geographical origins were showed with alpha-amylase inhibition. The IC_{50} values of *G. glabra* ethanol extracts were reported between 67.11 µg/mL to 120.6 µg/mL from Syria, Egypt, America, Pakistan, India, Palestine, Georgia, and Morocco [30]. Furthermore, Yang et.al., has presented that the *G. glabra* ethanol extracts also had an antidiabetic effect in vivo studies [31]. In the current study, similar to the results in the literature, *G. glabra* extracts, especially GF1, exhibited significant antidiabetic activity. Previous studies have reported that glycyrrhizic acid, which is found in the highest concentration in GF1, has significant antidiabetic activity [32]. Therefore, it can be considered that glycyrrhizic acid, as well as other compounds, is significantly responsible for the antidiabetic effect of GF1.

In the present study, the in vitro antimicrobial effect of *G. glabra* extracts was assessed against the 3 gram-positive bacteria, 5 gram-negative bacteria and 1 yeast strain. *G. glabra* extracts, especially GB and GF1, were found to have good antibacterial activity against *Enterococcus faecalis* ATCC 29213, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus aureus* (NCTC 12493) strains. *G. glabra* extracts were found to have good antimicrobial activity against three strains, namely *Enterococcus faecalis* ATCC 29213, *Staphylococcus aureus* (NCTC 12493) and *Staphylococcus aureus* ATCC 25923. GF1 was effective against three strains while GB and GF2 were effective against all strains except the last one. A previous study revealed that glycyrrhizin, which is highly abundant in GF2 in the current study, exhibits significant antimicrobial activity against these strains. Therefore, this compound, together with other secondary metabolites present in the extracts, may be responsible for the effect of the extracts, especially GF1. Our results showed that each three *G. glabra* varieties extracts had the lowest MIC value against the *S. areus.* While GB and GF1 had the highest MIC value against the *K. pneumoniae* ATCC 700603, GF2 had the highest MIC value against *E. coli* (NCTC12493. *S. aureus* is related to a wide range of significant health problems such as soft tissue and skin infections, sepsis, endocarditis and food poisoning. In addition to this bacterium is an important reason for pneumonia,

surgical infections, bloodstream infections etc. [33]. Owing to the increasing prevalence of antibiotic-resistant strains such as *S. aureus*, the results of studies are important to show the inhibitory effect of natural sources such as *G. glabra* against these bacteria. In a previous study reported the antimicrobial effect of *G. glabra* ethanol extracts. According to the result of this study, *G. glabra* ethanol extract had good activity against *E. coli* ATCC25922, *Salmonella* spp, *S. epidermidis* EMCC1353. On the contrary, the sensitivity was observed against *K. pneumonia* ATCC12296, *Candida albicans* EMCC105 and *A. flavus* EMCC 274 [25]. The reports demonstrate the majority of antimicrobial effect of *G. glabra* owing to isoflavonoid components [34].

The scientific researches have demonstrated that the flavonoids and phenolic compounds have significants roles against chronic and acute diseases and for antioxidant properties. In our study, we observed the differences of TPC and TFC between GB, GF1 and GF2. While GF1 had the highest level of TPC with 127.90 mg GAE/g extract, GF2 had the lowest level of TPC with 69.01 mg GAE/g extract. For TFC, GB had the highest level (80.07 mg QE/g extract) and GF2 had the lowest level (16.58 mg QE/g extract). In Hamad et.al., study, TPC was reported 7.88 mg GAE/g extract of aqueous extract of *G. glabra* from Egypt. In the same study DPPH radical scavenging activity was reported lower value than us [25]. Another report showed that *G. glabra* ethanol extract had 47.41 mg GAE/g extract of TPC and 17.47 mg QE/g extract of TFC while *G. glabra* ethanol extract had 36.50 mg GAE/g extract of TPC and 12.75 mg QE/g extract of TFC [24]. In the other hand, different properties could be observed to phenolic, flavonoid content, and antioxidant activity depending on the solvent of extraction, solvent concentration, the origin of the plant, and the harvesting time of the plant.

When the phytochemical content revealed by GC-MS analysis of *G. glabra* extracts was evaluated in terms of major components, D-Pinitol, β-D-Glucopyranose, β-D-Lactose and Sucrose were found in all three extracts, while D-(+)-Trehalose was found in GB and GF2, D-(+)-Talofuranose was found only in GF1, and D-Lactitol was found only in GF2. Moreover, a previous study by Akhtar et al. investigated the phytochemical profile of *Glycyrrhiza glabra* root methanolic extract using GC-MS and reported mome inositol, a sugar alcohol, as the major constituent [35]. These findings are consistent with our results, in which sucrose, another sugar-based compound, was identified as the predominant ingredient. This similarity supports the reproducibility of sugar-rich profiles in *G. glabra* root extracts and reinforces the validity of our GC-MS analysis. In addition, when these extracts were compared in terms of glycyrrhizic acid content by HPLC, the highest rate was seen in GF1 (5.29%), followed by GF2 (3.53%) and GB (0.07%). Hayashi et al. reported that the glycyrrhizic acid content of *G. glabra* roots varied between 4.76% and 6.13%. The glycyrrhizic acid content of GF1 was observed to be in agreement with these values [36]. When *G. glabra* extracts were analyzed in terms of total triterpene content, the highest content was observed in GB (217.30 mg/g), followed by GF1 (172.40 mg/g) and GF2 (126.30 mg/g). Haleem et al. found the total triterpene saponin amount of *G. glabra* to be 239.64 mg in g extract, equivalent to diosgenin. This value was found to be close to the value of GB in our current study [37]. The reason for these differences in chemical contents may be due to the differences in the varieties of the plant species and the soil and climatic conditions of the places where they grow.

4. CONCLUSION

In this study, two different *G. glabra* L. varieties plant collected from different regions were used and compared their chemical composition and antioxidant, antidiabetic, and antimicrobial properties. Our results showed that not only did different varieties have different results, but also the same variety samples collected from different regions had differences in terms of antioxidant, antidiabetic, antibacterial effects and phytochemical contents. Although GF1 and GF2, which are the same varieties compared to GB, had a closer effect on each other in most of the studies, GF1 was the more effective sample. GF1 showed the highest antioxidant activity. All three samples had strong antidiabetic effects, with GF1 showing the highest effect. Physiochemical studies have shown that GF1 in particular has a high glycyrrhizic acid content.

This study will contribute to the creation of new treatment strategies and potential therapeutic agents in addition to the use of *G. glabra* L. in traditional treatments. Our study is also a preliminary study for future studies.

5. MATERIALS AND METHODS

5.1. Plant Material and Preparation of Extracts

A total of three roots from two different varieties of *G. glabra* L. were freshly supplied from the Gene Bank of the GAP International Agricultural Research and Training Center Directorate of Republic of Türkiye Ministry of Agriculture and Forestry. These samples were *G. glabra* var. *glabra* (GB) (Batman, TÜRKİYE), the

others were of *G. glabra* var. *glandulifera* (GF1) (Malatya, TÜRKİYE), *G. glabra* var. *glandulifera* (GF2) (Bingöl, TÜRKİYE) taxa collected from different regions (Figure 4). The taxonomy was identified by the botanist Assist. Prof. Dr. Ahmet Doğan, member of Faculty of Pharmacy, University of Marmara. A few dried plant samples were deposited in the Herbarium of the Faculty of Pharmacy, Marmara University (Mare No: GB: 23769, GF1: 23768, GF2:23770)

The cleaned roots were dried in the research laboratory of the Department of Pharmacognosy, Faculty of Pharmacy, Marmara University. Later on, the dried roots were weighed and powdered. The powdered plant roots were transferred to the erlenmeyer and macerated with 80% ethanol for 48 hours, and the solutions were filtered with filter paper. The reason for using 80% ethanol in the extraction is that it was determined to have high activity in a previous study on these plant species [38]. The 80% ethanol was added to the remaining residue and this process was repeated until the solvent color lightened. The solvents of the obtained filtrates were evaporated under vacuum at a temperature not exceeding 40°C using a rotary evaporator. The extracts were kept at +4°C until analysis.

Figure 4. A: *Glycyrrhiza glabra* var. *glabra* (GB) (Batman, TÜRKİYE), B: *Glycyrrhiza glabra* var. *glandulifera* (GF1) (Malatya, TÜRKİYE), C: *Glycyrrhiza glabra* var. *glandulifera* (GF2) (Bingöl, TÜRKİYE)

5.2. In Vitro Antioxidant Activity

The ABTS and DPPH radical scavenging activities of the extracts were determined according to Zou et al. [39]. Ascorbic acid and trolox were used as standards. Results were shown as IC_{50} (µg/mL).

5.3. Cupric Reducing Capacity (CUPRAC)

The cupric reducing antioxidant capacity of the extract was measured according to Apak et al. [40]. Trolox was used as a standard, and total antioxidant capacity (TAC) values were expressed as the µmol trolox equivalent per g extract.

5.4. In Vitro Antidiabetic Activity

The in vitro antidiabetic effect of 80% ethanol extracts of *G. glabra* was evaluated through inhibition of alpha-glucosidase enzyme according to the method described by Ramakrishna et al. with slight modifications [41,42]. Ten µL of the 80% ethanol extracts of *G. glabra* L. varieties in DMSO at different concentrations (Stock concentration range: 5000-9.77 µg/mL), 40 µL of 0.1 M sodium phosphate buffer (pH 6.9), 100 µL of *α*-glucosidase (obtained from *Saccharomyces cerevisiae*) prepared in buffer were mixed. The mixtures were incubated at 25°C for 10 minutes. In the next step, 50 µL of 5 mM *p*-nitrophenyl-*α*-D-glucopyranoside prepared in buffer was added. The mixture was incubated again at 25°C for 5 minutes. The absorption at 405 nm was measured before and after incubation. Acarbose was used as a standard.

The inhibition percentage in terms of alpha-glucosidase was calculated according to the following equation:

$$\% \text{ inhibition} = [(A_{control} - A_{sample}) / A_{control}] \times 100$$

A dose-response curve was plotted to determine the inhibition concentrations (IC_{50}) values with using the Graphpad Prism5 program. All tests and analyses were performed in triplicate.

5.5. Antimicrobial Assays

The microorganisms used in this study included gram-positive bacteria *E. faecalis* (ATCC 29213), *S. areus* ATCC (25923), *S. areus* (NCTC 12493); gram-negative bacteria *E. coli* (ATCC 25922), *E. coli*

(NCTC12493), *K. Pneumoniae* (ATCC 700603), *A. bannanni* (BAA 747), *P. auroginosa* (ATCC 27853) and one clinical isolated yeast strain *Candida albicans* (ATCC 10231).

Bacterial strains used to determine the antimicrobial activity of 80% ethanol extracts of *G. glabra* varieties were incubated at 37°C for 18-24 hours, while the yeast strain was incubated at 27°C for 48 hours. The inocula of bacterial strains were prepared for 0.5 McFarland standard solution, containing approximately 10^6 CFU/mL for bacterial strains and 10^7 CFU/mL for yeast strains [43]. The minimum inhibitory concentration (MIC) values for extracts of *G. glabra* varieties were determined utilizing the broth microdilution method, following the approach presented by Kowalska–Krochmal and Dudek–Wicher [44]. The MIC corresponds to the lowest concentration of the plant sample required to inhibit bacterial growth after incubation. Mueller–Hinton broth (MHB) was used as a medium for microbial strains. Fifty µL of the microbial inoculum was added to each well of a 96-well microplate. Stock solution of extracts were prepared in DMSO at a concentration of 5 mg/mL. Serial dilutions were made by adding 100 µL of extract stock solutions to the first wells. The plate was incubated as explained above. After incubation, microbial growth was evaluated spectrophotometrically. The positive control comprised MHB containing the test microorganisms. DMSO was used for a negative control. The findings were presented in mg/mL, and the experiments were performed in triplicate.

5.6. The Phytochemical Composition Analysis

5.6.1.The total phenolic content (TPC)

The total phenolic content of the extracts was determined by a colorimetric assay using solution Folin-Ciocalteau (Merck, Germany) based on the procedure described by Gao et al. with adapted to a 96-well microplate format [45,46]. Ten µL of the extracts, 20 µL of Folin-Ciocalteau, 200 µL of ultrapure water and 100 µL of 15% Na_2SO_4 were mixed. Following, the absorption was measured at 540 nm. Gallic acid was used as a standard, and TPC was expressed as the mg gallic acid equivalent per g powder ethanol extract.

5.6.2.The total flavonoid content (TFC)

The total flavonoid content of the extracts was determined based on the procedure described by Zhang et. al. with adapted to a 96-well microplate [46,47]. One hundred and twenty µL of ultrapure water, 25 µL of the extracts, 7,5 µL of 5% sodium nitrite were mixed and incubated at 25°C for 6 minutes. After that, 15 µL of 10% aluminum chloride hexahydrate was added and incubated again at 25°C for 5 minutes. Fifty µL of 1M NaOH was added to the solution and made up to 250 µL with ultrapure water. Then the absorption of the solutions was measured at 510 nm. Quercetin was used as a standard, and TFC of the extracts was expressed as mg quercetin per g extract.

5.6.3.The total triterpene content (TTC)

The total triterpene content of the extracts was determined based on the procedure described by Chang et al. with adapted to a 96-well microplate [48]. Ten µL of each *G. glabra* extract in methanol at 6 different concentrations (Stock concentration range: 5000-9.77 µg/mL) was mixed with 15 µL of vanillin-glacial acetic acid solution (5% w/v) and 50 µL of perchloric acid solution. The plate was incubated at 60 °C for 45 min and then cooled to room temperature in an ice-water bath. Then, 225 µL of glacial acetic acid was added and the absorbance of the solutions was measured at 548 nm. For the standard curve graph, absorbances corresponding to each concentration were measured using oleanolic acid (1200-37.5 µg/mL). Oleanolic acid was used as a standard, and the results were expressed as mg oleanolic acid equivalent per g extract (mg OE/g plant extract).

5.7. Quantitative Determination of Glycyrrhizic Acid by HPLC

Schimadzu LC-20A / Prominence device was used for the HPLC analysis. The system consists of an LC-20 AT Pump Unit (configured with low-pressure gradient unit), a DGU-20A3 Degasser Unit, an SPD-20A UV Detector, a manual injection valve (7725I), a CTO-10ASVP Column Furnace, a CBM-20 Alite System Control Unit, an LC-Solution Data Operating Program, a Reservoir Tray. HPLC conditions: DAD detector; purospher Star RP-18 (250 mm x 4.0 mm column, 5 µm); pump low pressure, mobile phase: 40% acetonitrile (A), 60% water (0.1% TFA) (B); detection wavelength: 250 nm; column temperature: 30°C; flow rate: 0.5 mL/min; injection volume: 20 µL; peak time: 14.59 min.; system control and data analysis: LC solution software (Shimadzu). Sample preparation: Sample: 0.5 gram, dilution rate: 0.5 gram/1000 mL (dilution rate in GF2: 0.5 g/100 mL.

5.8. GC-MS Analysis

The derivatization was performed with using trimethylsilyl (-TMS) based on the procedure described by Medina et al., and Pandya et al. [49,50]. After derivatization, the *G. glabra* varieties extracts were analyzed on the Agilent 5975 GC-MSD (Santa Clara, CA) system, HP Innowax FSC column (60 m x 0.25 mm, 0.25 µm film thickness) with helium mobile phase (1.0 mL/min). The GC oven temperature was held at 60 °C for 10 minutes and increased to 220 °C at a rate of 4 °C/min, kept constant at this temperature for 10 minutes and increased again to 240 °C at a rate of 1 °C/min. The samples were injected in splitless mode. The injection temperature was set to 250 °C. The mass spectrometer will be set to 70 eV ionization energy. The mass spectrometer scan range was set to m/z 35-450. The commercial Wiley GC/MS Library and NIST17 mass spectrum libraries were used for the identification of components in the extracts.

5.9. Statistical Analysis

Statistical significance between groups was analyzed using one-way analysis of variance (ANOVA) with Tukey's post hoc test, performed using GraphPad 9.0 software. A *p*-value less than 0.05 was considered statistically significant.

Acknowledgements: The authors would like to thank Assist. Prof. Dr. Ahmet DOĞAN for helping in identification of the plant material.

Author Contributions: Gizem Gülmez: Investigation, Conceptualization, Designing the Experiments, Analyzing the Data, Writing - Original Draft. Ali Şen: Project Administration Investigation, Writing. Hüseyin Servi: Investigation. Analyzing the Data. Timur Hakan Barak: Designing the Experiments. Fetullah Tekin: Designing the Experiments Analyzing the Data. Mahdi Marzi: Designing the Experiments, Analyzing the Data Azize Şener: Supervisior, Project administration, Editing. All authors read and approved this version for submission.

Conflict of Interest: The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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