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Research Article

Phenolic Analysis and Antimicrobial Potential of *Glycyrrhiza glabra* L. Plant Root Extract

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Abstract

In many countries, traditional herbal medicines are gaining traction as potential alternative treatments for a variety of ailments. Medicinal plants have gained wider acceptance in recent years due to the belief that natural products are more effective and have fewer side effects than their synthetic counterparts. *Glycyrrhiza glabra* (*G. glabra*), commonly known as licorice root, a member of the Leguminoceae family, has been used as a medicinal plant in folk medicine since ancient times. A sugar-bearing plant, *G. glabra* contains five times more sugar than a typical plant. It contains many different bioactive sugars, including polysaccharides, flavonoids, terpenes, simple sugars, amino acids, and mineral salts. In research, we aimed to determine phenolic compounds by LC-MS/MS screening technique and to investigate its antimicrobial effect by MIC method using G. glabra methanol root extract. The inhibitory effect of *G. glabra* methanol extract on the growth of yeast (*C. albicans*) and pathogenic bacteria (*E. coli*, *P. aeruginosa, S. aureus*, and *B. subtilis*) was determined using the minimum inhibitory concentrations (MIC) method. A total of twenty-two compounds were identified as a result of the study. The main compounds detected in the sample were cynarin (11.62 µg g^{-1}), chlorogenic acid (8.75 µg g^{-1}), and p-coumaric acid (3.88 µg g^{-1}). The extract was determined to exhibit highly effective inhibition against *C. albicans* yeast, effective inhibition against *P. aeruginosa*, and mild inhibition against other bacteria. The findings showed that licorice root may be a natural alternative to drugs because it contains a large number of biochemicals, is selectively toxic, and inhibits the growth of some pathogenic bacteria.

Keywords: *Glycyrrhiza glabra*, chemical composition, antimicrobial

1. Introduction

The recent development of resistance and undesirable side effects to commercially available antibiotics has prompted researchers to seek alternative antibiotics. Studies of traditionally used medicinal plants have revealed a number of beneficial chemical compounds used in modern medicine to various ilness (Muteeb et al., 2023). Antimicrobial compounds in plants are generally found in phenolics and essential oils, which we call secondary metabolites. These compounds are also responsible for the plant's characteristic aroma and flavor. Antimicrobial activity depends on the plant species, composition, and concentration; the species and burden of the target microorganism; the composition of the food; and processing and storage conditions. (Mráz et al., 2025). Plants synthesize numerous defensive molecules to protect themselves from their enemies. These are called plant secondary metabolites, which have the potential to serve vital functions in plant structure, growth, and environmental response. Unlike primary metabolism, secondary metabolism refers to metabolic pathways and their associated small molecular products that are not essential for the organism's growth and reproduction (Al-Khayri et al., 2023). Research on this topic has shown that plant materials contain numerous bioactive phytochemical compounds with potent antioxidant and antimicrobial activity. Phenolic compounds are the most abundant bioactive compounds in plants. A class of metabolites known as phenolic compounds is produced by secondary pathways in plants (de Oliveira et al., 2025). Research progress on classification, sources and functions of dietary polyphenols for prevention and treatment of chronic diseases (Li et al., 2023). Polyphenolic compounds, classified as secondary metabolites, are extensively present in fruits and fruit-derived products throughout many plant parts, such as roots, stems, leaves, and flowers. The aforementioned compounds are mostly generated from l-phenylalanine via the phenylpropanoid pathway. This distribution confirms the nutritional value and functional characteristics of these compounds. Bioactive chemicals have a crucial role in safeguarding plants against many external factors, including physical, chemical, and microbiological influences (Amidžić Klarić et al., 2020; Hano and Tungmunnithum, 2020). The significance of polyphenols is growing, mostly due to their beneficial impact on human health. There is a growing recognition of the increasing importance of natural antioxidants in preventing and treating cancer (Rudrapal et al., 2022; Kowalski et al. 2024; Muchtaridi et al., 2024). Secondary metabolites are often characterized as the defensive mechanisms used by plants to combat pathogens that pose a threat to their well-being, including viruses, bacteria, fungi, protozoa, and insects, all of which are harmful agents. Metabolic processes may be categorized into three primary groups: phenols, alkaloids, and terpenes. In the field of medicine, phenolic compounds play a significant role by serving as antioxidants and possessing preventive effects against a wide range of diseases (Demain 2020; Irtegün et al., 2021). Due to their antioxidant properties, phenolic compounds, including stress-related phytochemicals, have been linked to the positive effects of fruit and vegetable consumption. Some studies suggest that fruits, vegetables, and beverages are the primary dietary sources of phenolic compounds. As dietary antioxidants, plant polyphenols may help prevent oxidative damage in both human health and disease. Phenolic chemicals, commonly found in plant-based foods and beverages and acting as natural antioxidants, are essential for nutrition and medical care (Swallah et al., 2020; Karadag et al., 2024; Roaa, 2020). The majority of medications used in contemporary medicine are derived from plants. Nevertheless, there has been a recent surge in interest in herbal remedies and traditional folk medicine. This may be attributed to several factors, including the presence of severe side effects, toxicity, and the emergence of drug resistance in microbes caused by the inclusion of other chemicals in the manufacture of chemical pharmaceuticals. Plant-derived extracts or active compounds are often recommended in some nations because of their efficacy, few adverse effects, and

affordability, despite the incomplete identification of their physiologically active components (Asadi-Samani et al., 2015; Al Sheikh et al., 2020; Aktepe and Baran, 2021; Jubair et al., 2021). Despite the considerable advancements in contemporary medicine, cancer remains one of the most lethal diseases globally in the present day. Therefore, it is essential to devise alternative pharmaceuticals that exhibit reduced adverse effects and enhanced efficacy in treating cancerous conditions. The bioactive constituents present in medicinal plants represent a potentially valuable resource in this regard (Beeby et al., 2020; Esmeeta et al., 2022). The medicinal plant known as licorice (*G. glabra* L.) is a member of the Leguminoceae family and has been used in public health practices since ancient times. It is cultivated in many temperate locations around the globe. Empirical investigations have shown that it has a substantial composition of proteins, polysaccharides, mineral salts, gums, pectin, sterols, and resins. Traditional medicine has long made use of the tiny perennial plant licorice, scientifically known as *G. glabra* L., for a wide variety of medical conditions, including but not limited to respiratory disorders, hyperdipsia, stomach ulcers, fever, epilepsy, paralysis, rheumatism, skin diseases, hemorrhagic illnesses, sexual debility, and jaundice. In addition, the *G. glabra* extracts contained various organic acids, and numerous volatile components (Bansal et al., 2020; Bansal et al., 2024). In contrast to its use in Europe, licorice is often included in traditional Chinese medicine as a supplementary herb inside a singular prescription. This practice serves as a distinctive "guide medication" that augments the efficacy of other constituents, mitigates toxicity, and enhances taste in over 50% of Chinese herbal formulations. Public health benefits from using this plant, which is pleasant, somewhat moist, calming, and immunostimulating. It can cleanse and safeguard the liver. The plant's roots have been used in several research because of their diverse qualities, including antibacterial, anti-inflammatory, analgesic, antipyretic, expectorant, antioxidant, and anti-anxiolytic effects (Al-Snafi et al., 2018; Babich et al., 2022). This study presents the first LC-MS/MS profile of *G. glabra* roots obtained from Adana province. The aim of our study is to discover the phenolics of *G. glabra* L. and to promote the use of the plant for medicinal purposes by examining these chemical components and antimicrobial properties.

2. Material and methods

2.1. Plant materials

The *Glycyrrhiza glabra* (licorice) has a wide distribution across many geographical areas, spanning from southeastern Europe to northwest Asia (Batiha et al., 2020; Wahab et al., 2021). Deep and sandy soils are beneficial to the licorice plant's extensive root system. Due to its preference for warm climates, this plant thrives in the Mediterranean environment, provided that its roots remain adequately moist. The *G. glabra* plant, which was obtained from the Adana region, was subjected to a drying process in a laboratory setting that was devoid of moisture. The item was kept in suitable conditions for future use. *G. glabra* plant, were described botanically and morphologically by Mardin Artuklu University Plants Department plant taxonomist Dr. Fatma Mungan Kılıç.

2.2. Preparation of plant methanol extract

A 30 g portion of dehydrated plant material was pulverized and transferred into a Soxhlet cartridge for extraction. A solvent consisting of 350 milliliters of methanol was added. Following the extraction process, the extracts were filtered using a porcelain filter. The crude extract for the experiment was obtained by using a rotary evaporator to remove the solvent from the extract. To prepare it for future usage, the crude plant extract was kept in a deep freezer at a temperature of -22 °C (Aktepe et al., 2021; Yılmaz et al., 2024).

2.3 Phenolic component determination by LC-MS/MS

The commercially obtained substances for examination included ammonium formate, formic acid, methanol, and liquid chromatography-grade acetonitrile from

Sigma-Aldrich (Germany). Before LC-MS/MS analysis, all solutions underwent filtration using a nylon membrane filter (*Captiva premium*) that was covered by polypropylene. The filter had a diameter of 25 mm and a pore size of 0.45 µm. A 100 mg dried plant extract of *G. glabra* was prepared in methanol at a concentration of 10 ml for the experiment. Subsequently, the resultant solution was diluted to a concentration of 2 mg mL^{-1} with the addition of 50% solvent (MeOH) to water of superior quality. A membrane filter with a diameter of 0.45 mm was used to filter the resulting solution for LC-ESI-MS/MS analysis. The chromatographic separation was conducted using a Poroshell 120 EC-C18 column, which has dimensions of 100 mm x 4.6 mm x 2.7 mm. The mobile phase of carrier A was composed of mobile phases containing 0.1% formic acid, 5 mM ammonium formate, and methanol. The B mobile phase consisted of mobile phases with 0.1% formic acid and 5 mM ammonium formate (İpek et al., 2024, Hamid et al., 2004).

2.4 Antimicrobial assay

The effectiveness of methanol extract derived from the *G. glabra* plant has been seen against significant gram-negative pathogens (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853) as well as gram-positive microorganisms (*S. aureus* ATCC 29213 and *B. subtilis* ATCC 11774) that pose a threat to human health. The minimum inhibitory concentration (MIC) approach was used to assess the presence of *Candida albicans* (ATCC 10231). The study was conducted using 96-well microplates. A start volume of 0.5 ml of Mueller Hinton broth (Roswell Park Memorial Institute medium/RPMI) was added to find the minimum inhibitory concentration (MIC) values without putting microorganisms into the microplate wells. The extracted solution was introduced into the microorganisms cultured on microplates (Baran et al., 2022). After that, a volume of culture media equal to one hundred microliters was extracted from each well of the microplate, and it was then transferred to the subsequent well to produce dilutions. To obtain the MIC (minimum inhibitory concentration) values of the plates, they were placed in an oven in which the temperature was set to 37 ºC for twenty-four hours.

3. Results and discussion

3.1. Analysis of phenolic components in *G. glabra* root methanol extracts via LC–ESI–MS/MS

Phenolic compounds are among the most common byproducts of secondary metabolism in plants. All plant cells produce them via the acetate-malonate and shikimate pathways. They produce polyphenols, including flavonoids, oligomeric and polymeric phenolic compounds, and phenylpropanoids. Biological activity and function are influenced by phenolic diversity (Zagoskina et al., 2023). Through plant and human food chains, many of these chemicals interact with reactive oxygen species to provide antioxidant effects. The potent biological activity of phenolic compounds allows them to be used as beneficial drugs and dietary supplements. Plant polyphenols, in addition to their medicinal value for public health, are also vital for functional nutrition. Promising directions in plant polyphenol research and development are suggested (Zhang et al., 2022). The study used the conventional extraction method to obtain the plant extract. The study yielded a methanol extract of 4.30 g from *G. glabra* root. The analysis of the phenolic components in this extract was conducted using an LC-MS/MS instrument at a concentration of aqueous solution. The examination revealed the presence of 22 phenolic components in the methanol extract. The amounts of cynarine (11.62 µg g^{-1}), chlorogenic acid (8.75 µg g^{-1}), p-coumaric acid (3.81 µg g^{-1}), and trans-ferulic acid (3.48 µg g) were significantly higher, as shown in Table 1 and Figure 1.

Table 1. Phenolic component analysis of methanol extracts from *G. glabra* L. using LC-MS/MS

Quantitation results				
	Phenolic components	Retention Time	Final Conc	µg g^{-1}
1	Protocatechuic acid	2.852	0.04	µg g^{-1}
2	Gentisic acid	3.298	0.03	µg g^{-1}
3	4-Hydroxybenzoic acid	4.622	1.88	µg g^{-1}
4	Chlorogenic acid	5.315	8.75	µg g^{-1}
5	4-Hydroxybenzaldehyde	5.773	1.39	µg g^{-1}
6	Vanillic acid	6.013	1.96	µg g^{-1}
7	Caffeic Acid	6.090	0.34	µg g^{-1}
8	Syringic acid	7.008	1.26	µg g^{-1}
9	P-coumaric acid	8.522	3.81	µg g^{-1}
10	Salicylic Acid	8.728	1.64	µg g^{-1}
11	Polydatine	9.839	0.20	µg g^{-1}
12	Trans-ferulic acid	9.586	3.48	µg g^{-1}
13	Quercimeritrin	10.809	0.04	µg g^{-1}
14	Coumarin	10.399	0.14	µg g^{-1}
15	Cynarin	11.187	11.62	µg g^{-1}
16	Hyperocide	11.694	0.12	µg g^{-1}
17	Quercetin-3-glucoside	11.926	0.42	µg g^{-1}
18	Quercetin-3-D-xyloside	12.505	0.87	µg g^{-1}
19	Kaemerol-3-glucoside	13.361	0.01	µg g^{-1}
20	Trans-cinnamic acid	14.330	0.27	µg g^{-1}
21	Biochanin A	20.545	0.29	µg g^{-1}
22	Diosgenin	30.535	0.05	µg g^{-1}

The methanol extract of the plant contains a significant amount of the phytochemical cynarine, which exhibits many pharmacological characteristics, notably antioxidant activity and the ability to scavenge free radicals. Although cynarin is chemically a derivative of caffeic acid, it is usually studied under the larger heading of dicaffeoylquinic acid derivatives or general polyphenols rather than as "pure" phenolic acids (Petropolus et al., 2018). Cynarine is notable for its anticholesterolemic, anticarcinogenic, antibacterial, anti-inflammatory, and diuretic properties (Naveed et al., 2018; Erikel et al., 2019; Lu v et al., 2020). Cynarin: Its capacity to promote liver health and display choleretic and cholagogue properties is among its best-known effects. This facilitates the liver's removal of toxins and aids in digestion. *G. glabra* extract's high cynarin content indicates that this plant may be used to treat liver conditions in traditional medicine (Sharma et al., 2021). Chlorogenic acid, which is the second most prevalent compound in our research, is an ester derived from caffeic acid and quinic acid. These compounds are often found in many dietary sources, including coffee and tea. Chlorogenic acid, known for its notable antioxidative properties, has been demonstrated in scientific studies to possess anti-inflammatory effects, regulate lipid and glucose metabolism in certain genetic metabolic disorders, protect the liver, lower cholesterol levels, lower blood pressure, protect the gastrointestinal system, protect the cardiovascular system, and protect the nervous system (Khalaf et al., 2010; Jan et al., 2022). Both cynarin and chlorogenic acid are strong antioxidants that fight free radicals to shield cells from oxidative harm. Strong antioxidant capabilities are anticipated in areas including cardiovascular health, anti-inflammation, and general cellular protection in extracts with a high total phenolic content, which is mostly given by these two substances (Gil-Martínez et al., 2025). Our results are supported by polyphenolic investigations conducted on *G. glabra* (Stompor-Gorący et al., 2021; Kaur et al., 2022). P-coumaric acid, which is sometimes referred to as 4-hydroxycinnamic acid, was determined to be the third most intensive polyphenolic ingredient found in the plant extract. Produced in vegetables, fruits, and grains, it is a phenolic molecule that has very potent pharmacological effects. It is noteworthy that p-coumaric acid and its derivatives have qualities that are antibacterial, antioxidant, anti-carcinogenic, antiarrhythmic,

antiangiogenic, cardio-protective, hepato-protective, and antidiabetic (Yo et al., 2009). As our fourth-ranked molecule with a high concentration, trans-ferulic acid plays a significant role in the treatment of a variety of conditions, including inflammation, cardiovascular illnesses, certain bacterial and viral infections, and diabetes, with a primary focus on neurodegenerative disorders (Lee et al., 2009).

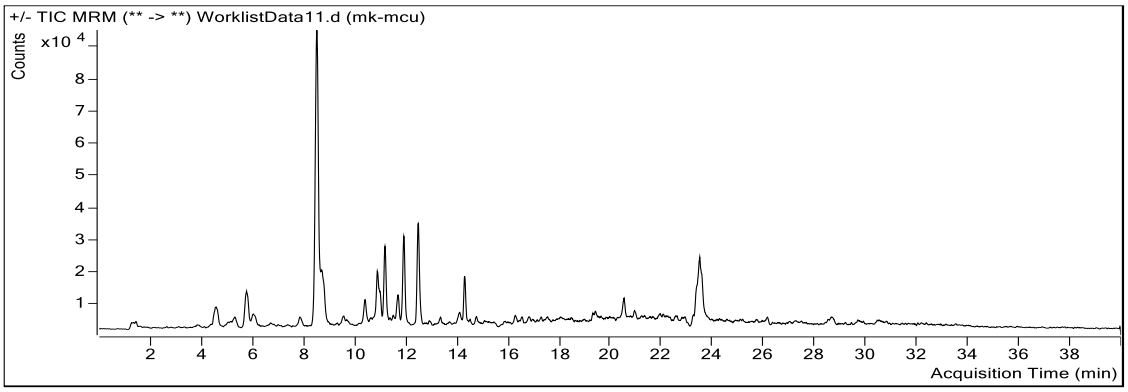


Figure 1. Standard chromatograms of methanol extracts of *G. glabra* root

3.2. Antimicrobial assay results

The ability of the methanol extract from *G. glabra* roots to inhibit bacterial strains and yeast was tested by treating it with pathogenic microorganisms. The plant's antibacterial capabilities were assessed using the minimum inhibitory concentration (MIC) technique on microbial strains including *E. coli*, *P. aeruginosa*, *S. aureus*, *B. subtilis*, and *C. albicans*. According to the results of the experiment, the extract was very effective against *C. albicans* but not so much against *P. aeruginosa*. It did not show any antimicrobial activity against other species (Table 2) (Aktepe et al., 2023).

Table 2: Inhibitory effect of methanol extract of *G. glabra* L. on pathogenic microorganisms

Organism	***G. glabra* L. (mg mL^{-1})**	***Antibiotic (ppm)**
E. coli (ATCC25922)	7.87	2
P. aeruginosa (ATCC27853)	1.96	2
S. aureus (ATCC 29213)	15.75	0,5
B. subtilis (ATCC 11774)	3.93	1
C. albicans	0.98	1

* Vancomycin was used for gram-positive bacteria, Colistin for gram-negative bacteria, and Fluconazole for yeasts

The antimicrobial properties of *G. glabra* plant extracts were examined in a review conducted by Anagha et al. (Anagha et al., 2014). The findings of four separate studies showed that the plant extract had the most effective antimycotic activity against *C. albicans* with 0.98 µg g^{-1}, and secondarily had an antimicrobial inhibitory effect against P. aeruginosa with 1.96 µg g^{-1}, Rahimmalek et al. (2025) demonstrated in their study that chlorogenic acid disrupts cell membrane integrity, causing damage, while also preventing biofilm formation and inhibiting fungal morphological changes (hyphae formation). In their antimicrobial experiment, Liu et al. (2025) demonstrated that it caused cell membrane damage in *V. parahaemolyticus*. In our study, the amount of chlorogenic acid was found to be quite high. In a study conducted by Raut et al. (2024), *C. albicans* showed a concentration-dependent decrease in cellular ergosterol in the presence of vanillic acid, suggesting that inhibition of ergosterol biosynthesis may be a potential target. At the same time, Liu et al. (2016) proved that salicylic acid, Yan et al. (2023) proved that ferulic acid, and Tsivileva et al. (2022) proved that coumarin had antifungal

effects. These studies support the strong antimycotic effect of the plant extract on *C. albicans*. Researchers have discovered new antimicrobial properties in plants by examining some of the key mechanisms by which pathogens develop antimicrobial resistance. However, this problem continues to grow. Therefore, further research on licorice and similar plants would be appropriate. (Teodoro et al., 2015). Furthermore, the study revealed the presence of potent antibiotic activity against *P. aeruginosa*. Previous research has shown notable antibacterial properties in the plant extract, which aligns with our investigation of the *C. albicans* yeast and other bacterial strains (de Oliveira et al., 2013). Previous studies using licorice root extract have shown its efficacy in inhibiting *B. subtilis*, as seen in our investigation, as well as effectively inhibiting *E. coli* and *S. aureus* bacteria, in contrast to the findings of our study (Gupta et al., 2014; Eshghi 2019).

4. Conclusion

G. glabra L. root is one of the plants that is considered significant since it has economic worth and is used in traditional medicine all over the globe. Our research was carried out with the purpose of determining the bioactive components that are present in the methanol extract of the root of *G. glabra*, as well as assessing antibacterial activities of the extract. As a result of our research, we have shown that the licorice plant has a substantial amount of bioactive components. As a result of the resistance of harmful bacteria to antibiotics, the treatment of cancer illnesses, which are a health issue and one of the main causes of mortality in the world, is now experiencing a variety of difficulties. To this day, researchers in this sector are still looking for novel medications. Based on the findings of our investigations, we have come to the conclusion that licorice contains bioactive chemicals that are beneficial in treating a variety of disorders and may be tested using pharmacological methods. In addition to having a high economic value as a meal, licorice root has the potential to be an excellent source for cancer therapies and new antimicrobial research intended to combat the antibiotic resistance of bacteria that have lately appeared. Researchers have discovered new antimicrobial properties in plants by examining some of the key mechanisms by which pathogens develop antimicrobial resistance. However, this problem continues to grow. Therefore, further research on licorice and similar plants would be appropriate.

Declaration of Author Contributions

All authors contributed to this present work: Conceptualization was done by A.B; methodology was presented by A.B, supervision was provided by

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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