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Phytochemical, Free Radical Scavenging and Antimicrobial Activities of *Glycyrrhiza glabra* L. rhizomes Collected from Algeria

Amel Benbott*, Saida Karouche, Camelia Mosbah, Sabah Boukeria, Abdlouahab Yahia

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Abstract

In the world of the pharmaceutical industry, plants are considered a major source of medicines due to the presence of bioactive compounds, which provide many physiological effects on the human body. Given the toxicity and unwanted side effects of synthetic molecules, there is an increasing demand for natural drugs. In this context, the present study focused on the phytochemical and biological study of extracts of the medicinal plant *Glycyrrhiza glabra* L.

The results of the phytochemical screening revealed the analyst plant contains active Chemical Compounds, except for the Imodals. Compounds also the extraction of phenolic using organic polarized solvents showed that the highest yield was with methanol extract (9, 2%) compared to the rest of the extracts. The quantitative estimate of these extracts showed that the methanol extract contains the largest quantity of phenols and flavonoids (50,37±2,14 mg AGE / g Ext) and (8,1±1,2 mg QE / g Ex) respectively. The biological study through the DPPH test showed that the extracts of the rhizomes of the plant *G. glabra* possess antioxidant capacity, as the methanol extract showed a high inhibition capacity estimated at (IC_{50}= 35±0.59 µg/ml). As for the anti-bacterial activity, it showed significant activity of *G.glabra* plant extracts against the tested bacterial strains, where the largest inhibition zone diameter was 19 mm with the methanol Extract against *Bacillus cereus* (ATCC 11778).

Keywords: *Glycyrrhiza glabra*, Polyphenols, Flavonoids, Antioxidant and Antibacterial activity

Amel Benbott*
Laboratory of Natural Substances, Bioactive Molecules and Biotechnological Applications, Larbi Ben M 'hidi University, Oum el Bouaghi, Algeria.
Biology Department, Nature, and Life Sciences Faculty, Larbi Ben M'hidi University Oum el Bouaghi, Algeria.
Laboratory of Natural Sciences and Materials, Mila University Center, Algeria.

Saida Karouche
Laboratory of Natural Substances, Bioactive Molecules and biotechnological applications, Larbi Ben M 'hidi University, Oum el Bouaghi, Algeria.
Biology Department, Nature, and Life Sciences Faculty, Larbi Ben M'hidi University Oum el Bouaghi, Algeria.

Camelia Mosbah
Laboratory of Natural Substances, Bioactive Molecules and Biotechnological Applications, Larbi Ben M 'Hidi University, Oum el Bouaghi, Algeria.

Sabah Boukeriab, Abdlouahab Yahia
Laboratory of natural sciences and materials, Mila university center, Algeria.

***E-mail:** malika1959@yahoo.fr

Introduction

The Algerian Sahara has exceptional floral biodiversity, consisting of more than 1,200 species (Ozenda, 1991), of which approximately 162 species are endemic to the north of the Sahara alone to which is added a secular tradition of the traditional use of plants. Among the desert plants, there is the legume family (Leguminosae) which represents the third largest subfamily compared to flowering plants, and consists of 650 genera and about 18,000 species (Kass & Wink, 1997), and contributes effectively to food, agriculture, industrial and pharmaceutical due to the abundance of these genera and species (Al-Rejaboo & Jalaluldeen, 2019; Alshehri, 2020).

Glycyrrhiza glabra belongs to the Fabaceae family, is a fairly common species in the Mediterranean region throughout Algeria especially in arid and semi-arid regions (Baba Aissa, 2011). It is a plant used for both culinary and medicinal purposes (Hayashi & Sudo, 2009). The roots and rhizomes of *G. glabra* are commonly used in Algerian society, as a remedy for the digestive system, stomach, and duodenal ulcers (Bardhan *et al*., 1978), As well as for the treatment of spasmodic pain of chronic stomach (Zadeh *et al.*, 2013). According to Bahmani *et al.* (2014), Licorice may reduce the symptoms of diabetes, such as frequent urination and polydipsia but cannot reduce blood glucose. The infusion prepared by the roots is considered to treat inflammatory diseases (Yang *et al*., 2017). According to the literature, Low bone mass, fractures, osteoporosis, bone defects, osteomalacia, osteogenesis imperfecta, bone disease, and periodontal illnesses have all been treated using *G. glabra* extract (Kumar *et al*., 2015).

The objective of our study was to estimate the number of polyphenols and flavonoids in *Glycyrrhiza glabra* L as well as the antioxidant and antimicrobial properties of these active compounds in the rhizomes parts.

Materials and Methods

Plant Material Collection and Identification

The rhizomes of the *G.glabra* plant were obtained from the Ghardaïa region in southern Algeria (Latitude: 32° 29' 24.81" N, Longitude: 3° 40' 25.8276"E) in 2021. The plant was identified by Dr. Y. Halis Researcher in Scientific and Technical Research Center for Arid Areas (Touggourt) Algeria. The plant materials were cleaned and dried at room temperature for 15 days then ground into a powder, weighed, and stored in clean glass jars.

Phytochemical Screening

The phytochemical screening is carried out in the extracts prepared from the plant tested to demonstrate the presence of certain secondary metabolites. The analytical tests carried out are based on precipitation or coloring responses employing reagents that are particular to each chemical family **(Table 1)**. The different chemical groups have been characterized according to the methods described by Trease and Evans (2002).

Table 1. Primary qualitative tests in Phytochemical screening

Chemical group	Identification reagents	Indicator
Flavonoids	Hydrochloric alcohol	light yellow color
Saponosides	Moss index> 2 cm	The appearance of a foam Persistent
Polyphenols	Ferric chloride FeCl3 (1٪)	Blackish blue or dark green color
Sterols and triterpenes	Acetic anhydride Chloroform Sulphuric acid	Brownish red ring
Coumarins	NH_4OH (10%) HCl (10%)	Examined under ultra-violet light, fluorescence intensity
Tannins	Ethyl alcohol 50٪ $FeCl_3$ (1٪)	the green or blue-green color
Volatile oils	Ethereal solution Ethanol	Aroma
Reducing compounds	Fehling's solution (A/B)	Red precipitate
Imodols	Ammonia (10%)	Red color

Preparation of Phenolic Extracts from Rhizomes of the G. glabra

According to the Markham (1982) protocol, the different types of extracts were prepared from the pulverized rhizomes (100 g) using 1L of increasing polarity solvents (dichloromethane, ethyl acetate, butanol, methanol). At the end of the extraction, the four organic extracts were concentrated under vacuum at Rotavapor at temperatures of 35°C, 40°C and 50°C respectively. Each extract's dried sample was weighed, and the yield of soluble components was estimated using the equation: Yield (%) = [Final weight of dried extract / initial weight of licorice powder] x100 to determine the total phenol and flavonoid content as well as antioxidant activity, the extracts were dissolved in methanol (1 mg/ml). The experiment was carried out three times.

Determination of Total Polyphenols

The Folin-Ciocalteau method was used to determine the concentration of total phenols content in dry extracts of *G.glabra* rhizomes (Singleton & Rossi, 1965). 1 ml of Folin-Ciocalteu reagent (10%) was added to 200 µl of each extract and incubated for 4 minutes at room temperature. Then, 800 µl of sodium bicarbonate (7.5 %) was added to each mixture, after the second incubation of 2 hours, the total polyphenols content was measured at λ max = 765 nm with a spectrophotometer of CECIL2041 UV-VS. the gallic acid was used as a stander and the results were expressed in milligrams of equivalents of gallic acid per 100 g of dry matter (mg GAE / g Ext)

Determination of Total Flavonoids

The total flavonoids content was measured by the colorimetric method of aluminum chloride ($AlCl_3$) (Bahorun *et al.*, 1996). 1 ml of each extract tested as well as the reference compound (standard) were added to 1 ml $AlCl_3$ solution (2% dissolved in methanol). The mixture was left to react for 10 min and the absorption was read at λ max = 430 nm. The flavonoid concentrations were deduced from the calibration curve established with quercetin. The results were measured in milligrams of quercetin equivalents per 100 grams of dried material (mg EQ / 100 g Ext) (Saptarini & Herawati, 2019).

DPPH Radical Scavenging Assay

The varied extracts were tested for the antioxidant potential by using the technique outlined by Mansouri *et al*. (2005) with some modification. 300 µl of the extract were added to 1300 µl of DPPH solution while the negative control is formed by blending 300 µl of methanol with 1300 µl of the DPPH solution.

After 30 minutes of incubation in the dark, the absorbance measurement was taken against a blank at 517 nm. The same method was followed for all the dilutions. The ascorbic acid was used as a reference compound and the results expressed as anti-free radical activity or inhibition of free radicals in percentages (I%) using the formula below:

% inhibition = [(absorbance of control - absorbance of the sample)/ absorbance of control]×100 (1)

The IC 50 value was computed as the concentration of extract that inhibited the 50% of DPPH radical

Evaluation of the Antibacterial Activity

Sources and maintenance of microorganisms Bacterial strains of Gram+: *Bacillus cereus* ATCC 11778 and *Staphylococcus aureus* ATCC25923; Bacterial strains of Gram-: *Escherichia coli* ATCC25922 and *ATCC 27853 Pseudomonas aeruginosa (American Type Culture Collection),* obtained from the Pasteur Institute of Algiers, maintained by subculture on nutrient agar medium favorable to their growth and incubated at 37 ° C for 24^h. So we obtain a bacterial suspension colony-forming units per milliliter (cfu/ml) density of 10^6 cfu/ml

Preparing Disks

The phenolic extracts of rhizomes were recovered by dissolved in DMSO 2% (dimethyl-sulfoxide) A series of dilutions are carried out from C0 to reach an initial solution concentration of C 0 = 100mg/ml. 100 µl of each extract was impregnated into the sterile filter paper discs (6 mm diameter).

Concentrations of Each Extract

Diffusion Method on Agar Medium

The antibacterial activity of different plant extracts was evaluated using the method of agar diffusion (Benbott *et al.*, 2012). For each strain, a bacterial suspension was produced in sterile distilled water from colonies that had grown for 18 to 24 hours. This suspension's turbidity is corrected to 0.5 McFarland before being diluted to 1/100. This produces a 10 6 cfu/ml inoculum estimate. This inoculum is inoculated by flooding Mueller-Hinton agar-coated Petri plates. Various concentrations of impregnated disks (12.5 %, 25 %, 50 %, and 100 %) were then placed on the agar's surface. Before being incubated at 37 ° C in an oven for 24 hours, the Petri plates were placed at room temperature for 1 hour to allow for pre-release chemicals. The diameter of the inhibition zone surrounding each disc is used to measure antibacterial activity.

Results and Discussion

Phytochemical Screening

The results of the phytochemical tests are shown in **Table 2**.

Table 2. Results of detection of secondary metabolism products of *G. glabra* root extracts

Extract Metabolites	Result
Phenols	+
Volatile oils	+
Tannins	+
Flavonoids	+
Reducing compounds	+
Saponins	+
Comarins	+
Triterpenes and Sterols	+
Imodols	-

+: Presence**, -:** Absence

The results of phytochemical analysis of *G. glabra* rhizomes showed the presence of numerous chemical compounds, such as phenols, Volatile oils, tannins, flavonoids, Reducing compounds, saponins, coumarins, triterpenes, and sterols. On the other hand, we note the absence of the imodols. The presence of these active compounds in the roots of *G. glabra* indicates the importance of this plant in traditional and modern medicine, some of these results are close to those obtained by Rizzato *et al*. (2017) and Wang *et al*. (2015a).

The Yield of Phenolic Compounds in Various Extracts

The findings of this investigation revealed that the optimum yield of phenolic compounds in *G. glabra* rhizomes was with the methanol extract (9.2 ± 0.21 %) knowing that the extraction was done with 80% methanol The dichloromethane had the lowest yield. extract (5.8 ± 0.13%) **(Table 3)**. There is a correlative relationship between the polarity of the solvent and its solubility due to the solubility nature of these compounds in the solvents (Benbott *et al*., 2018). Note that the yield of the methanol extract is high compared to the rest of the extracts. Indeed, alcoholic solvents can increase the permeability of cell walls by facilitating the extraction of more polar, middle, and low polar molecules.

Table 3. The yield of phenols in each extract of *G.glabra*

Extracts	Aspect	Color	Yield (%)
Dichloromethane	Pasty	brown	5.9± 0. 18
Ethyl acetate	Crystals	Golden yellow	6.8± 0.23
Butanol	Powdery	Light brown	8.4± 0.49
Methanol	Pasty	Dark brown	9.2 ±0.65

Content of Total Phenolic and Flavonoid Compounds

Table 4 reports the contents of phenolic compounds in the extracts of the rhizomes of *G. glabra*. A considerable phenolic content was observed by the extracts tested, while the methanol extract has the highest concentration 50.37 ± 2.14 mg EAG / g E. On the other hand, we find that the content of flavonoids in the butanoic extract of the rhizomes of the plant *G. glabra* is higher compared to the other extracts.

Table 4. Total phenols and flavonoids content of *G. glabra* rhizomes

Extracts	Total Phenols (mg EAG / g Ext)	Total flavonoids (mg EQ / g Ext)
dichloromethane	4.90 ±1.25	0.19± 0.011

ethyl acetate	11.95±0.94	1.01±0.19
Butanol	28.14±1.24	4.02±0.8
Methanol	50.37±2.14	8.1±1.2

Our results differ from those of Asan-Ozusaglam and Karacoka (2014), which obtained higher levels of phenolic compounds in dichloromethane than those obtained from aqueous and ethanolic extracts of Turkish licorice root.

Several factors can have an impact on the distribution of phenolic compounds in the fractions, such as climatic and environmental parameters.

DPPH Free Radical-Scavenging Activity

The results of the change in the inhibition percent of DPPH as a function of the concentration of the extracts and the standard are shown in **Figures 1** and **2.**

We find that the extracts exert an inhibitory power of the free radical DPPH. This power gradually increases with increasing concentration of extracts as well as standard.

The greatest percentage of inhibition was recorded by the methanol extract at a concentration of 0.1 mg / mL (94.04% ± 0. 14). According to Tohma and Gulçin (2010), the DPPH radical inhibition percent of *G. glabra* aqueous and ethanol root extracts at a concentration of 0.03 g/ml were 52.2 % and 54.4%, respectively. These percentages of inhibitions allowed us to calculate the IC $_{50}$ and compare the effectiveness of the extracts. We recall that the lower the value of the IC $_{50}$, the more the extract is powerful against free radicals.

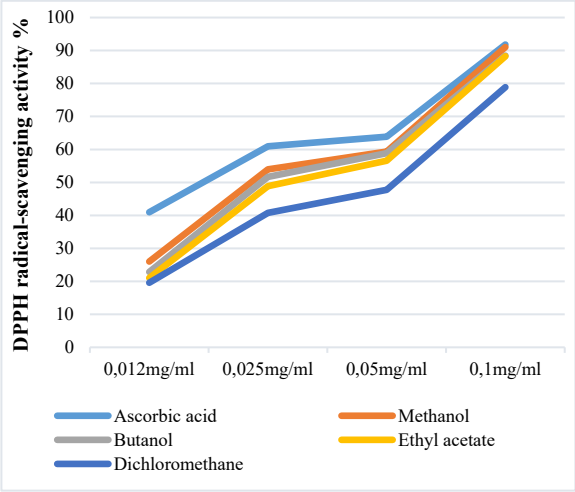

Figure 1. DPPH Free radical scavenging activities of *G. glabra* rhizome phenol extracts and Ascorbic acid

According to the values obtained, the methanol extract has the lowest IC $_{50}$ compared to that of the other extracts, and therefore the best activity with an IC $_{50}$ of approximately 0.035 ± 0.24 mg / mL followed by the butanol extract and the ethyl acetate extract. Whereas, the highest

IC $_{50}$ was recorded by dichloromethane extract which means the lowest antioxidant activity.

However, when compared with the standard antioxidant, the extracts tested were found to be moderately active.

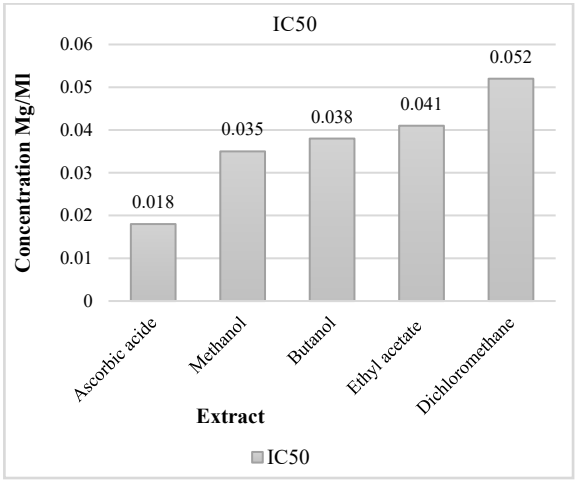

Figure 2. IC50 values of ascorbic acid and phenolic extracts in each phase

Our results are in agreement with the study conducted by Sharma *et al.* (2013) which indicated that the methanol extract of the roots of *G. glabra* was more effective as a free radical scavenger and had good reducing power.

According to Biondi *et al.* (2003). The dihydrostilbene derivates found in G. glabra leaves have been shown to have high antioxidant activity. On the other hand, *G. glabra* also contains licochalcones B and D, which have a significant DPPH radical scavenging action as well as the capacity to suppress microsomal lipid peroxidation (Biondi *et al*., 2003; Sharma *et al*., 2018).

Determination of the Antibacterial Activity

The sensitivity of the strains to extracts of the plant of *G. glabra* was achieved by the technique of diffusion in an agar medium. The results of the diameters of the zones of inhibition of extracts from the rhizomes of the *G. glabra* plant against Gram-positive and Gram-negative bacteria are shown in **Table 5**.

The results reveal variable responses depending on the strains tested and the concentration of the extract studied. The highest antibacterial activity against *B. subtilus* was recorded with the methanol extract at the highest concentration with an inhibition diameter of 19±0.8 mm, compared to butanol extract with an 11±0.3 mm inhibition diameter. Concerning the E. *coli* and *P. aeruginosa* bacterial strains, the diameter of the inhibition zones is between 6. ±0.1 and 9±0.37mm.

Table 5. Determination of the activity of methanolic extract and butanol against four reference bacterial strains

Tested bacteria	Inhibition zone diameter in (mm) at different concentrations of methanol and butanol extracts in (mg/ml)							
	Methanol extract of the roots				Butanol extract of the roots			
	1	1/2	1/4	1/8	1	1/2	1/4	1/8
B. subtilus ATCC 6633	19±0.8	10±0.9	9±0.78	8±0.0	11±0.3	10±0.4	8±0.3	6±0.5
S. aureus ATCC 25923	13±0.32	12±0.54	8± 0.13	7± 0.7	10 ± 0.23	9±0.17	7±0.74	6± 0.3
P. aeruginosa ATCC 27853	8±0.24	8±0..5	7±0.9	6±0.61	6. ±0.1	-	-	-
E. coli ATCC 25922	9±0.37	9±0.15	8±0.12	6±0.4	8±0.64	8±0.3	7±0.09	-

The results of the study were in agreement with Jafari-Sales *et al.* (2018), who recorded the highest antibacterial activity with *S. aureus* and *B. cereus* strains with inhibition diameters of 19.5 mm and 18.8 mm respectively, at a concentration of 400 mg/ml and the lowest bacterial activity against *P. aeruginosa* is 10.6 nm. According to the literature, *G. glabra* has antibacterial activity against Gram-positive and Gram-negative microorganisms such as *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans*, and Bacillus subtilis (Gupta *et al*., 2008; Wang *et al*., 2015b). Secondary metabolites, such as saponins, alkaloids, and flavonoids, are responsible for the antibacterial action seen (Wang *et al.*, 2015b). In 2014, Ahn *et al.* have documented that Liquorice inhibits bacterial caries produced by Streptococcus mutans and Streptococcus sobrinus. According to research. *G. glabra* has been shown to have antibacterial activity against Mycobacterium. Many other researchers have indicated that glabridin is the active component (Simmler *et al*., 2013). Licoisoflavone and licochalcone A were previously found as antitubercular phenolic compounds (Chakotiya *et al*., 2017).

Conclusion

We can confirm that the rhizomes of the *Glycyrrhiza glabra* L. plant grown in the region of Ghardaia in southern Algeria are rich in active substances such as tannins, saponins, terpenes, phenols, volatile oils, etc..., and have a relatively high percentage of total phenols and flavonoids in the methanolic extract compared to other extracts.

The licorice plant is a potentially effective natural remedy for diseases caused by free radicals and diseases caused by antibiotic-resistant strains of bacteria and can be used as a safe alternative to food additives.

All of these findings remain a first step in the search for materials from a natural, biologically active source.

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