Original Article

Qualitative And Quantitave Evaluation, Antibacterial And Antioxidant Activities Of Methanolic And Aqueous Extract Of Leaves And Roots Of (Glycyrrhiza Glabra L.) Growth In Algeria

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Abstract

The present study was carried out to assess the quality and quantity with the study of the antibacterial activity of phytochemicals present in the methanolic and aqueous extract of leaves and roots of the plane Glycyrrhiza glabra L. growth in Algeria. The phytochemicals detected were flavonoids, glycoside, tannins, saponin, terpenoids and Steroids. The quantitative analyses showed that leaves methanolic extract had the highest total phenolics content (TPC: 650,652 mg GEA/g), total flavonoids content (TFC: 184,023 mg QE/g) and total tannins content (TTC: 5.8 mg GAE/g). But roots methanolic extract had the highest total saponins content (TSC:191,183mg DE/g).

Also These extracts were tested in vitro against some gram positive bacterial strains such as Staphylococcus aureus ATCC 25923 and gram negative such as Escherichia coli (ATCC25922) Pseudomonas aeruginosa (ATCC 27853), Salmonella typhimurium (ATTC 13311), using the diffusion method in agar (antibiogram).

Methanolic and aqueous extract of the leaves and roots have shown considerable activity against most organisms tested, leaves methanolic and aqueous extract showed a high activity at a concentration of 100mg/ml compared to roots methanolic and aqueous extract at the same concentration, especially against $Gram^+$ strains. The diameters of inhibition zones ranged from 7 to 22 mm for all treatments. The values of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. And the CMB/CMI report made it possible to specify the mode of action of the substance. It was observed that the CMB / CMI of crude methanol extracted from the leaves and roots Glycyrrhiza glabra L. reached 2 , with the bacterial strain Escherichia coli (ATCC25922) it means has a bactéricidal effect. The methanolic extract of Glycyrrhiza glabra L. roots provided the highest antioxidant activity against free radical inhibition of DPPH. The results obtained in this study and the comparison with other works in the same field, allowed us to reveal the importance attributed by traditional medicine to Glycyrrhiza glabra L. as a source of natural antiseptics.

Keywords: Glycyrrhiza glabra L., Phytochemicals, antibacterial activity, antioxidant activity.

INTRODUCTION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are triterpenoid saponin, flavonoids, tannins, alkaloids, phenolic compounds [1]. Many of these indigenous medicinal plants are used as a spices and food plant. They are also sometimes added to foods meant for pregnant and nursing mothers for medicinal purposes [2,3]. Glycyrrhiza glabra L.is one of the useful medicinal plants.

Indeed, polyphenols are natural compounds widely distributed in the plant kingdom which have a growing importance, in particular thanks to their beneficial effects on health [4]. Their role as natural antioxidants and antibacterials is attracting more and more interest in the prevention and treatment of cancer, inflammatory and cardiovascular diseases [5]; they are also used as additives in the food, pharmaceutical and cosmetic industries [6]. Scientific research has been developed for the extraction, identification and quantification of these compounds from different sources such as agricultural and horticultural crops or medicinal plants [7,8].

Licorice (Glycyrrhiza glabra L.) is a perennial plant of the family (Fabaceae/ Papilionaceae), aromatic roots. It is native to southern Europe and Asia [9].

It is an herbaceous plant measuring 1 to 1,5 m high. It has large pinnate leaves composed of 9-17 leaflets small purple flowers arranged in the inflorescence. Its fruit is flat clove 2 to 3 cm long, containing numerous seeds. Licorice is called "sugar root" because the dose of sugar is 50 times greater than that of sucrose [10]. The roots are used as a folk medicine both in Europe and eastern countries. The main components are the triterpene saponins, glycyrrhizin and glycyrrhetic acid, which are believes to be partly responsible for anti-ulcer, anti-inflammatory, anti-diuretic, anti-epileptic, antiallergic and anti-oxidant properties of the plant as well as their ability to 'fight' low blood pressure. Furthermore, Glycyrrhiza glabra extracts have been shown to possess antidepressant like: memory-enhancing activities and producing anti-thrombotic effects [11].

The aim of this study is to evaluate the antibacterial and antioxidant activities of methanolic and aqueous extracts of the leaves and roots of Glycyrrhiza glabra L., which may be the basis for the synthesis of new antibiotics. Indeed, the increase in the emergence of bacterial strains resistant to multiple clinical diseases.

MATERIALS AND METHODS

1. Plant material collection and identification

Different parts (leaves and roots) of Glycyrrhiza glabra L.were collected from the Relizane region (North West of Algeria) in 2017. Sample collection was done in october for leaves and roots. The samples collected were identified.

The seeds and roots wereair dried at room temperature and kept in a dark-ambercolored bottle until processed. It was then finely grinded using a coffee grinder mill-type (for leaves and roots), to obtain a fine powder.

2. GPS coordinates

The coordinates of the region of Glycyrrhiza glabra L. (as google earth) are:

- Latitude: 35°44N
- Longitude: 0° 33 W
- Elevation: 139 m

3. Sources and maintenance of microorganisms

Bacterial strains of Gram$^+$:Staphylococcus aureusATCC25923. Bacterial strains of Gram$^-$: Escherichia coli ATCC25922, Salmonella typhimurium ATCC13311 and Pseudomonas aeruginosa ATCC27853. Bacterial strains are lots of ATCC (American Type Culture Collection), were obtained from the microbiology laboratory of the hospital Hakim Saâdan Biskra, Algeria, maintained bysubculture on nutrient agar medium favorable to their growth and incubated at 37°C for 24 h . So we obtain a bacterial suspension density of 10^6 colony forming units per milliliter (cfu/ml).

4. Extraction with methanol:

The methanolic extracts were extracted with methanol. 1 g of plant powder placed in a glass container (vial) with 10 ml of MeOH 70%, and then heated at 70°C for 5 minutes (This destroyed the plant tissue, preventing oxidation or enzymatic hydrolysis) [12].

The samples l macerate overnight (about 24 hours); after a first filtration on filter paper, and then evaporated (in the open air) and recovered with DMSO.

5. Extraction with water:

The aqueous extracts were extracted with water. 1g of plant powder placed in a glass container (vial) with 10 ml of H_2o 70%, and then heated at 70°C for 5 minutes (This destroyed the plant tissue, preventing oxidation or enzymatic hydrolysis) [13].

The samples 2 macerate overnight (about 24 hours); after a first filtration on filter paper, and then evaporated (in the open air) and recovered with distilled water.

6. Phytochemical screening

Phytochemical analysis of different constituents which have a pharmacological interest was performed on the powdered samples using standard methods as described by [14,15].

Detecting the different phytochemical families that exist in the plant by precipitation reactions or staining using specific to each family of compounds.

7. Quantitative phytochemical analysis

Phenolic compound have been extensively studied in the past 30 years. The root and leave of Glycyrrhiza species is one of the richest sources of biological active compounds such as phenolic, flavanoid, tannins and saponins compounds [16].

7.1. Total phenolic content

In order to determine the presence of phenolic content the Folin-Ciocalteu method was used.

The powder (30 g) was put in ethanol (80%, v / v; 360 mL) to macerate for 48 h. and then filtrate with vacuum filtration, refrigerated for 24 h then decanted and concentrated under reduced pressure the crude extracts of aerial part of the plant, were used for our analyzes.Total phenolic concentration was analyzed using Folin-Ciocalteu's method [17]. 0.125mL of the crude extract of the plant or gallic acide was added to 0.625 mL of Folin Ciocalteu. Resting for 5 min, 0.5 mL of sodium carbonate (7,5 g/L) was added , the mixture was shaken and leave to rest for 2 h. The absorbance of the samples was measured at 760 nm using UV–vis spectrophotometer. Concentrations were expressed as mg of gallic acid equivalent/g of lyophilized extract. The same procedure was used for making standard curve. All experiments were carried out in triplicates.

7. 2.Total flavonoids

The aluminum chloride colorimetric method was used for mesure the total flavonoids concentrations [18]. 500 µl of the extract samples were mixed with 500 µl methanol, 50µl of 10% AlCl3, 50µl of 1mol L^{-1} potassium acetate and 1.4mL water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of each reaction mixture was subsequently measured at 415 nm. The total flavonoid was calculated using quercetin as standard .Total flavonoids content was expressed as mg quercetin equivalents (mg QE)/g extract.

7.3. Determination of total tannins

500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipette out into a test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.1 N (HCl) and 0.008 M potassium ferro-cyanide. The absorbance was measured at 120 nm within 10 min. The total tannins,content of samples was determined in triplicates and the result was expressed as mg catechine equivalent per gram extract [19].

7.4. Determination of total saponins

Estimated of total saponins content was determined by the method described by Makkar et al.[20] based on vanillinsulphuric acid colorimetric reaction with some modifications. About 50 µl of the extract was added with 250 µl of vanillin reagent (800 mg of vanillin in 10 ml of 99.5 % ethanol) was added. Then 2,5 mL of 72% sulphuric acid was added and it was mixed well. This solution was kept in a water bath at 60 ºC for 10 min. After 10 min, it was cooled in ice cold water and the absorbance was read at 544 nm. The values were expressed as diosgenin equivalents (mg DE/g extract) derived from a standard curve.

8. Antibacterial assay procedure

8.1. Inoculum preparation

The inoculum is prepared from conserved colonies. Revived strains was taken and homogenized in 5 ml of Mueller- Hinton broth and incubated for 3 to 5 hours at 37C°,inoculum was set to 10^6 colony forming units per milliliter (cfu/ml) [21].

8.2. Preparing Disks

The methanolic and aqueous extracts of leaves and roots were recovered by distilled water to obtain an initial solution concentration of C_0= 100mg/ml, from C_0 is conducting a series of dilutions. The sterile filter paper discs (6 mm diameter) were impregnated with 100 µl of different concentrations of each extract, finally preparing disks impregnated with sterile DMSO as control.

8.3. Diffusion method on agar medium

The antibacterial activity of different plant extracts was evaluated using the method of agar diffusion [22]. From colonies of 18 to 24 h, a bacterial suspension was made in sterile distilled water for each strain. The turbidity of this suspension is adjusted to 0.5 McFarland and then diluted 1/100. This gives an estimated inoculum to 10^6cfu/ml.

This inoculum is inoculated by flooding on Petri dishes containing Mueller-Hinton agar. Disks impregnated with various alkaloidal extracts of roots and seeds at concentrations were then delicately deposited on the surface of the agar. The Petri dishes left for 1 h at room temperature for pre-release substances, before being incubated at 37 ° C in an oven for 24 h. The antibacterial activity is determined by measuring the diameter of the inhibition zone around each disc [23].

8.4. MIC and MBC determination

The minimum inhibitory concentration (MIC) is the lowest concentration of a substance that prevents visible growth of a bacterium [24].

To determine the MIC and MBC respectively, must be prepared a series of dilutions with sterilized (100, 50, 25, 12.5, 6.25, 3.12,..... etc.) mg/ml. Also prepared for each bacterial strain, an inoculum whose turbidity was adjusted to 0.5 McFarland (either 10^8ufc/ml) and reduced to 10^6ufc/ml in the Mueller-Hinton broth double concentrated, then adding in hemolysis tubes, 1ml of each concentration and 1ml of bacterial inoculum. A growth control tube was prepared, containing 1ml of sterile DMSO and 1ml of inoculum, and a control tube containing 1ml of sterile distilled water and 1ml of sterile broth. And all treaties tube and two witnesses were incubated at 37°C for 24 hours. After incubation, examine bacterial growth in each tube, resulting in turbidity. The MIC of an extract vis-a- vis a given strain will be the smallest concentration showing no visible growth of germs. To determinethe MBC, we realize 24 hours earlier, a witness of bactericide by seeding in a streak on agar in Petri dishes, dilution 10°, 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} of the initial inoculum, corresponding respectively to 100%, 10%, 1%, 0.1% and 0.01% of survivors. After reading the MICs, subcultures are performed by streaking on fresh agar plates, tubes without visible growth. These subcultures were then incubated at 37°C and 24 h are compared with the control of bacterial streaks. The MBC is the lowest concentration which shows the growth of transplanted germ lower or equal to 0.01% of survivors.

9. Antioxidant activity in vitro

The antioxidant test by scavenging the DPPH radical was carried out according to the protocol of Bougandoura and Bendimerad [25]. 50 microliters of each extract were added to 2 ml of methanolic solution of DPPH (0.025 g/l). At the same time by combining 50 microliters of the solvent (methanol) with 2 ml of the methanolic solution of DPPH, a negative control was prepared. For each concentration, a blank was carried out and the absorbance was read at 515 nm after 30 min of incubation in the dark and at room temperature. Ascorbic acid was used as a positive control. The scavenging activity of $DPPH^{\bullet}$ was calculated using the equation:

$DPPH^{\bullet}$ scavenging effect (%) = [(Ac – As) / Ac] × 100

Ac is the absorbance of the control and As is the absorbance of the sample [26]. IC_{50} values indicate the concentration of the sample that removes 50% of DPPH free radicals.The results were calculated as IC_{50}.

10. Statistical analysis

All analyses were carried out in triplicate. Results were expressed in terms of means ± standard deviations. Statistical analyses (ANOVA) were performed with Minitab 13 software (Minitab Inc., France). Two-way analysis of variance was conducted to determine the significance of differences between analytical results at $p < 0.05$ significance level.

RESULTS

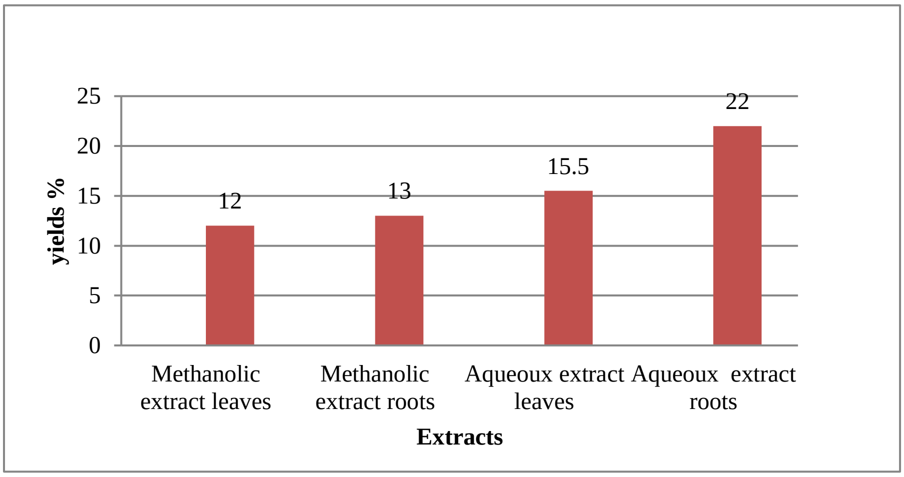

Figure1. Yields of the methanolic and aqueous extracts of leaves and roots

Extraction yields significantly differed ($p < 0.05$) depending on the powder organ classes and extraction solvent. (Figure 1) shows that the leaves yield of the aqueous extract (15.5%) is greater than the yield of the leaves methanolic extract (12%). And the yield of the roots of the aqueous extract (22%) greater than the yield of roots methanolic extract (13%).

Table 1. Phytochemicals screening of leaves of Glycyrrhiza glabra L.

Extract constituents	Extracts	Roots	Leaves
polyphenols	**M**	+++	+++
	A	++	+++
Saponins	**M**	+++	-
	A	+++	-
Steroids	**M**	+	+++
	A	-	+
Triterpenes	**M**	+++	+++
	A	+	++
Flavonoids	**M**	+++	+++
	A	+++	+++
Reducing compound	**M**	+++	+++
	A	++	++
Alkaloids	**M**	+++	+
	A	++	+
Catechic tannins	**M**	+++	+++
	A	+++	+

Gallic tannins	**M**	+++	+++
	A	+++	++
Glycosides	**M**	+++	+++
	A	+++	++

- Absent, + present, +++ = highly present, ++ = moderately present, M : methanolic, A : aqueous

Phytochemical study in (table.1), showed that the leaves methanolic and aqueous extract of plant Glycyrrhiza glaba L. contained flavonoids, alkaloids, tannins, glycosides, Steroids and triterpenes, and does not contain saponins. On the other hand, extracts from the roots contained flavonoids, alkaloids, tannins, glycosides, heterosides and triterpenes in addition to saponins.

Table 2. Levels of total phenolic (mg of Gallic acid equivalent per 1g extract), flavonoid contents (expressed as mg Quercetin equivalents/g extract), total tannins (expressed as mg catechine equivalent per gram extract) and total saponins expressed as mg diosgenin equivalents per gram extract) contents in methanolic and aqueous extracts of leaves and roots of Glycyrrhiza glabra L.

species	**Total phenolic (mgGAE/1g)**	**Total flavonoids (mg QE/g)**	**Total tannins (mg CTE/g)**	**total saponins (mg DE/g)**
Leaves methanolic extract	650,652 ± 5,45	184,023 ± 6,52	5,8± 0,4	/
Leaves aqueous extract	136,200 ± 8,99	101,687 ±5,70	6,2±0,5	/
Roots methanolic extract	389,266 ±5,77	142,015 ±5,46	5,33±0,3	191,183± 1,25
Roots aqueous extract	189,748 ± 9,71	143,135± 6,67	6,33±0,11	170,683± 1,60

The values are mean ± SD, are averages of three independent experiments.

Quantitative Analysis in (table 2), showed that extracts of this plant contained high quantity of flavonoids and total phenolic content. Total polyphenols content are significantly vary according to the extracts ($p > 0.05$).In the root extracts, the phenolic compound of methanolic extract was found higher (389,266 mg GAE/g extract) than aqueous extract (189.748 mg GAE/g extract). And in the leave extracts, the phenolic compound of methanolic extract was found higher (650,652 mg GAE/g extract) than aqueous extract (136,200 mg GAE/g extract).

Statistically, total flavonoid contents in studied extracts (table 2) were similar ($p > 0.05$).The level of flavonoid content in the methanolic and aqueous extract of the root was determined as 142,015 mg QE/g extract and 143,135 mg QE/g extract, respectively and the concentration of flavonoids in the methanolic and aqueous extract of the leaves was 184,023 mg QE/g extract and 101,687 mg QE/g extract, respectively .

The results of condensed tannins contents displayed in (table 2) revealed a significant difference ($p < 0.05$) between all extracts.The tannins content of the extracts was determined and found to be 5,8 mg CTE/g in leaves methanolic extract, 6,2 mg CTE/g in leaves aqueous extract, 5,33 mg CTE/g in roots methanolic extract and 6,33 mg CTE/g in roots aqueous extract (Table 3).

Table 2 presents the total saponins contents of the extracts with significant difference ($p < 0.05$).The total saponins content of the samples were found to be highest in roots methanolic extract (191,183 mg DE/g) followed by roots aqueous extract (170,683 mg DE/g) (Table 3). But the methanolic and aqueous extract of the leave does not contain saponins, because [27] showed that Saponosides are found only in the root.

Table 3. Results of the microbial activity of methanolic and aqueous extracts (leaves and roots) of the plant Glycyrrhiza glabra L.

Microorganisms	Extract		Diameters of inhibition (mm)

		plant parts	0 mg/ml	100 mg/ml	50 mg/ml	25 mg/ml	12.5 mg/ml	6.25 mg/m
Escherichia coli 25922	Methanolic	Leaves	6 (-)	20 (+++)	17(++)	17(++)	15(++)	13 (+)
		Roots	6 (-)	15 (++)	12 (+)	10 (+)	10 (+)	8 (-)
	Aqueous	Leaves	6 (-)	16 (++)	11 (+)	10 (+)	10 (+)	8 (-)
		Roots	6 (-)	10 (+)	9 (+)	9 (+)	9 (+)	8 (-)
Staphylococcus aureus 25923	Methanolic	Leaves	6 (-)	22 (+++)	19 (++)	15(++)	14 (+)	13 (+)
		Roots	6 (-)	15 (++)	14 (+)	13 (+)	13 (+)	12 (+)
	Aqueous	Leaves	6 (-)	9 (+)	9 (+)	6 (-)	6 (-)	6 (-)
		Roots	6 (-)	10 (+)	8 (-)	7 (-)	7 (-)	6 (-)
Pseudomonas aeruginosa 27853	Methanolic	Leaves	6 (-)	10 (+)	10 (+)	9 (+)	9 (+)	7 (-)
		Roots	6 (-)	6 (-)	6 (-)	6 (-)	6 (-)	6 (-)
	Aqueous	Leaves	6 (-)	10 (+)	9 (+)	9 (+)	7 (-)	7 (-)
		Roots	6 (-)	6 (-)	6 (-)	6 (-)	6 (-)	6 (-)
Salmonella typhimurium 13311	Methanolic	Leaves	6 (-)	12 (+)	10 (+)	10 (+)	9 (+)	8 (-)
		Roots	6 (-)	6 (-)	6 (-)	6 (-)	6 (-)	6 (-)
	Aqueous	Leaves	6 (-)	12 (+)	10 (+)	10 (+)	9 (+)	8 (-)
		Roots	6 (-)	11(+)	7 (-)	7 (-)	7 (-)	6 (-)

The values + and - represent the degree of sensitivity of the strains according to [28]: Not sensitive or resistant (-): diameter <8mm; Sensitive (+): 9 mm <diameter <14 mm; Very sensitive (++): 15mm <diameter <19mm. Extremely sensitive (+++): diameter> 20 mm

The results of the antimicrobial activity of extracts of leaves and roots of Glycyrrhiza glabra L. are presented in (Table 3). We observe that the sensitivity tests show the effect of methanolic and aqueous extracts from leaves and roots on different bacterial strains, giving varying diameters depending the tested strains.

There have been very important values with the strain of Gram$^+$. The highest diameter of inhibitory zone was recorded by S. aureus of 22mm with the methanolic extract in leaves at a concentration of 100mg/ml. The smaller diameter of inhibition zone was recorded with aqueous extract 7mm, at a concentration of 6.25mg/ml of the aqueous extract in roots (Figure 2). The bacterial strains of Gram$^-$ shows that the highest diameter of the inhibitory zone was registered with E. coli 20 mm, for the methanolic extract in leaves at a concentration of 100mg/ml, and the smallest diameter of the inhibitory zone was recorded with Salmonella typh 7mm at a concentration of 6.25mg/ml for the aqueous extract in roots. The (Table 4) show that methanolic and aqueous extracted from the roots and leaves were active in all bacterial strains. It is found that the increase of the diameters of inhibitory zones is directly related to the increase of the concentration of crude extracts.

Table 4. Determining the values of MIC and MBC of methanolic and aqueous extracts (leaves and roots) and the Ratio of MBC / MIC.

Concentrations (mg/ml) Microorganisms	**Extracts**	MIC (mg/ml)	MBC (mg/ml)	MBC / MIC
E. coli ATCC 25922	M. Roots	3.125 ±0.006	6.25±0.011	2
	A. Roots	6.25± 0.02	50 ±0.21	8
	M. Leaves	1.563± 0.003	3.125±0.321	2
	A. Leaves	3.125± 0.001	12.5±0.002	4
S. aureus ATCC 25923	M. Roots	3.125 ±0.004	12.5± 0.125	4
	A. Roots	12.5± 0.025	50±0.014	4
	M. Leaves	1.563± 0.009	6.25 ±0.058	4
	A. Leaves	50±0.014	100 ±0.025	2
Salmonella typhimurium ATCC 13311	M. Leaves	12.5± 0.008	50±0.005	4
	A. Leaves	12.5 ±0.005	/	/
	A. Roots	12.5 ±0.0147	50±0.023	4
P.aeruginosa ATCC 27853	M. Leaves	12.5± 0.002	/	/
	A. Leaves	12.5 ±0.001	/	/

A : aqueous extract. **M :** methanolic extract.

The Ratio MBC / MIC [29]

- MBC / MIC < 4 effect is bactericidal.
- MBC / MIC > 4 effect is bacteriostatic
- MBC /MIC ≥32: resistant

The MBC / MIC report made it possible to specify the modality of action of the substance [30].

(Table 4) show the magnitude of the differential impact of methanolic and aqueous extracts of leaves and roots of this plant on different bacterial strains.

The methanolic extract of the leaves and roots has a bactericidal activity on the E.coli bacteria, and equal to 2 . However the aqueous extract of the root has a bacteriostatic activity on the E.coli bacteria, greater than 8.

The methanolic extract of the leaves has a bacteriostatic activity on Staphylococcus aureus bacteria, greater than 4. However the aqueous extract of the leaves has a bactericidal activity on the Staphylococcus aureus bacteria, equal to 2. While that the methanolic extract of the leaves and the aqueous extract of the root has a bactericidal activity on the Salmonella typh. bacteria, equal to 4. But P. aeruginosa was more resistant to different extracts of Glycyrrhiza glabra L.

Results of the antioxydant activity

In the present study, the leaves extract of Glycyrrhiza glabra exhibited rich scavenging effects on DPPH, while the roots extract had the lowest (Table 5). Both roots and leaves extracts of Glycyrrhiza glabra exhibit a percent inhibition of DPPH• radical less than ascorbic acid. Moreover, the methanolic extract showed higher antioxidant activity than the aqueous extract.

Extracts	methanolic extract of roots	aqueous extract of roots	methanolic extract of leaves	aqueous extract of leaves	ascorbic acid
IC_{50} (mg/ml)	0.58±0.01	2.28±0.02	1.74 ± 0.03	2.02 ± 0.01	0.033±0.03

DISCUSSION

Plants are a source of natural bioactive compounds with high medicinal activities for the treatment of various diseases [31].The use of plants for local remedies is a traditional custom . And Algeria is rich in high quality medicinal plants. This study provides support to traditional and alternative use of Glycyrrhiza glabra L. from Relizane (North West of Algeria) against various infections.

The Figure (1) has shown that the yield of the aqueous extracts of Glycyrrhiza glabra L. is superior and better than the methanol extracts.

According to [32]: explain that the yield is not relative; It depends on the method and conditions under which the extraction was carried out. In addition, [33] describe in general the origin of the fluctuations in yields due to several factors, such as the harvest period of the plants, the extraction method used, environmental factors (altitude, soil type), Geographical origin.

According to [34], the yield figures vary according to the origin of the sample. Also [35] says that the weight of the crop depends on the nature of the soil and the variety.This differentiation could also be explained by [36] choice of Harvest period as it is essential in terms of yield and quality of plant extract, climate, geographical area of the plant organ used, drying period, extraction method. These factors can have a direct impact on plant extract yields.

Phytochemical studies of Glycyrrhiza glabra L. showed that the major components were flavonoids,alkaloids, saponins, tannins, glycosides, terpenoids and steroids, however the absence of saponins in leaves compared with the roots which was rich in saponins. Our results are in agreement with many researchs [37]. According to [27] the Saponosides are found only in the root. A large number of components have been isolated from licorice roots 40 to 50% of the total dry weight of Glycyrrhiza glabra L is represented by a water-soluble, biologically active solution [38].

According to our findings, total phenolic content of root methanolic and aqueous extract was higher than root n-hexane and DCM extracts from Turkish liquorice (G.glabra [39]. [40] was also observed lower phenolic content (114,33 mg GAE/g) of methanolic root extract from Glycyrrhiza echinata L. than methanolic and aqueous 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 his study. [41] observed a lower phenolic content (73,51 mg GAE/g) from DCM extract of Glycyrrhiza glabra root and stolon when compared to methanolic and aqueous extract in our study. [42] found that the aqueous and ethanol extracts of Glycyrrhiza 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 methanolic and aqueous extract in this study. [40] observed the flavonoid content of Glycyrrhiza echinata methanolic root extract as 116,54 mg RE/g. [43] studied the flavonoid content of ethyl acetate, hexane, chloroform, water and n-butanol extracts of Glycyrrhiza uralensis root and found that it varied from 3,601 to 66,546 mg RE/g.

Whatever the mode of extraction, the water records the highest contents of condensed tannins followed by the methanol which extracts the tannins weakly.Water and acetone have the ability to solubilize proanthocyanidins that are not soluble in methanol [44, 45]. But the problem according to Rosales [46] and Jokić et al. [47] is that water and acetone, especially at high temperatures, also extract unwanted substances such as proteins, lipids and non-phenolic dyes that cause interference when dosing tannins. The extraction of condensed tannins depends on their chemical nature, the solvent used and the operating conditions [48].

Saponins are bioactive compounds with a wide range of medicinal properties, including hypo-cholesterolemic, anticarcinogenic, anti-inflammatory, antimicrobial and antioxidant activities [49]. Saponins are found in a number of plant-derived foods such as legumes, and are present in many medicinal plants, including licorice [50].

Previous researchers have also reported that licorice root contains more than 3% Saponins [51]. And the Saponosides are found only in the root of Glycyrrhiza glabra [27].

These classes saponin, tannins, flavonoids are known to have activity against pathogens and therefore aid the antimicrobial activities of medicinal plants [52].

Differences such as this may be attributed to, the chronological age of the plant, percentage humidity of the harvested material, situation and time of harvest, and whether the method of extraction was a possible source of variation for the chemical composition, toxicity and bioactivity of the extracts [53].

Antimicrobial activities of the two parts (leaves and roots) of Glycyrrhiza glabra L.against tested bacteria have shown that Gram-negative strains were more resistant compared to the Gram positive ones. Similar findings have been reported by other authors, [54, 55, 56,57] who found that The methanolic extracts of Glycyrrhiza glabra L. showed antibacterial activity against Gram-positive (S. aureus, B. megaterium and B. subtilis with zone of inhibition of 22 mm, 14 mm, 16 mm respectively) and Gram-negative (E. coli, P. aeruginosa and S. paratyphi with an area of inhibition of 16 mm, 8 mm and 10 mm respectively). Due to the presence of glabrene, licoisoflavone B, isolicoflavonol, gancaonin I in this plant. And according to [58] the majority of the antimicrobial effects of licorice are due to isoflavonoid components, particularly paglabridine and B4'-O-methylglabridine, glabridine, glabriol and 3-hydroxyglabrol.

[59] is found that the strains of Pseudomonas aeruginosa prove to be the least resistant in his study, this is linked to its great ability to develop resistances to many antimicrobial agents, from where his involvement in frequent hospital infections.

The results of [60] showed that leaf and root extracts can inhibit the growth of certain gram-positive bacteria. The higher inhibitory effect was with methanolic extracts compared to aqueous extracts. Our results are in agreement with the results of [61] who found that the extracts of Glycyrrhiza glabra L. showed strong activity against the tested bacteria with the exception of the aqueous root extracts which exhibited a low zone of inhibition against these bacteria. And according to [62] Glycyrrhiza glabra L. the hydro alcoholic extract is more active and concentrates the antibacterial active ingredients contained in the plant better than the aqueous extracts. Where [32] states that it is important to note that the method used (choice of solvents) and the conditions under which extraction is carried out (hot or cold), all affect the total content of phenols and flavonoids and therefore affects the biological activities mediated by these metabolites.

Results of MIC and MBC confirme the antibacterial results and showed that Staphylococcus aureus was more sensitive to this plant compared to Pseudomonas aeruginosa which was more resistant to Glycyrrhiza glabra L. extracted with different mode of extraction.[63] shows that the MIC showing growth inhibition observed for bacteria in liquid culture also suggests that the Glycyrrhiza glabra extract at a concentration of 5-10 mg / ml is mainly of bacteriostatic and non-bactericidal nature.

According to [61] CMI and CMB values in most cases, the leaf extracts have a better activity than the root extracts. This activity may be due to materials extracted from various solvents. [37] says that the CMI and CMB value of the methanol extract was 3.125 mg / ml for S. aureus, 1.56 mg / ml for St. Agalactiae and 12.5 mg / ml for E. coli. While the aqueous extract having higher MIC and MBC values, 6.25 mg / ml for S. aureus, 3.125 mg / ml for St. Agalactiae and the result was negative for E. coli.

The antioxidant capacity of the different extracts was determined from the IC_{50}; this is the concentration necessary to reduce 50% of the DPPH radical. The lower of the IC_{50} value indicated the higher antioxidant activity of a compound [64]. Antioxidant molecules such as ascorbic acid, tocopherol, flavonoids and saponins have been shown to reduce and decolorize DPPH due to their ability to donate hydrogen [25]. For the radical scavenging mechanism, the reaction between the antioxidant and DPPH depends on the structural conformation of the antioxidant. Some compounds react rapidly with DPPH, reducing a number of DPPH molecules equal to the number of hydroxyl groups [65]. The polyphenols contained in the extracts of G. glabra are probably responsible for the antioxidant activity of these extracts. This is in agreement with the work carried out by [9]. High phenolic compounds present in Glycyrrhiza glabra L. are responsible for its strong antioxidant activity due to its free radical scavenging, metal ion chelation, hydrogenation, anti-lipid peroxidation and reduction activities. Studies have reported that licorice flavonoids have 100 times stronger antioxidant activity compared to the antioxidant activity of vitamin E [66].

CONCLUSION

This work aims to investigate the phytochemical, antibacterial and antioxidant activities of Glycyrrhiza glabra L. from Relizane region (North West of Algeria) which is a significant plant in our country and in the world, against clinical and food borne human and fish pathogens were determined. This study showed that Glycyrrhiza glabra L. is very rich on flavonoids, alkaloids, saponins, tannins, glycosides, terpenoids and steroids. Also Our results indicate that both the aqueous and methanolic root extract of Glycyrrhiza glabra are a good source of phenolics, flavonoids, tannins and saponins compound.

The antibacterial activities have shown Glycyrrhiza glabra L. as a potential source of antimicrobial drug against various pathogenic organisms. This is particularly important to combat the recent trend towards the emergence of multi-drug resistant organisms. Further studies are however necessary for the development of new antimicrobials from this plant.

The study of the antioxidant activity of extracts from the species Glycyrrhiza glabra L. according to the DPPH free radical trapping method showed that the two methanolic and aqueous extracts of the roots and leaves have moderate antioxidant activity. These extracts could therefore constitute an alternative to certain synthetic additives.

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CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest in the manuscript

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