



REGULAR ARTICLE

ANTI-BACTERIAL EFFECTS OF GLYCOSIDES EXTRACT OF *GLYCYRRHIZA GLABRA* L. FROM THE REGION OF DJAMÂA (SOUTH-EAST OF ALGERIA)

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ABSTRACT

The natural extracts of plants contain a variety of biologically active molecules. In this context, we attempted to evaluate the antimicrobial activity of some extracts prepared from the root of *Glycyrrhiza glabra* L., which grows in the region of Djamaa (south of Algeria). The qualitative analysis of these extracts revealed the presence of plant glycosides, which is confirmed by a quantitative analysis based on the detection test of glycosides, who demonstrated that the extracts are rich in these molecules, where the content of glycoside reached (10 %) for the ethanol extraction. The evaluation of the glycoside extract antimicrobial activity revealed a strong effect of inhibition on different strain of bacteria, when it was responsible for a large zone of inhibition of the bacterial strain *Pseudomonas aeruginosa*, compared to *Escherichia coli* and *staphylococcus aureus*. From these results, we can say that the glycoside extracts have different antibacterial effects depending on the species.

Keywords: *Glycyrrhiza glabra*, Antimicrobial activity, Glycoside, *Escherichia coli*, *Pseudomonas aeruginosa*, *S. aureus*

INTRODUCTION

For a long time, natural remedies and medicinal plants were mostly the main or the only treatment used, despite the development of the pharmaceutical industry that has allowed modern medicine to treat. In Africa, the behavior varies, may be because of the persistence of witchcraft. Millions of people use primarily and sometimes exclusively traditional medicine, because it remains the most affordable and it seems effective. Other prefers the accidental medicine, because they associate medicine traditional superstition [1]. And number of diseases that lead often to death [2].

Medicinal plants are medicinal say, when their bodies have pharmacological activities, which may lead to therapeutic uses. It uses usually a portion of the plant: root, leaf, flower and seed depending on the richest in active principle [3].

The purpose of this study is the investigation of the effect of the glycoside extracted from *Glycyrrhiza glabra* L. of Djamaa area (south of Algeria). on three bacterial strains which are: *Escherichia coli*, *Pseudomonas aeruginosa* and *staphylococcus aureus*.

MATERIALS AND METHODS

Study area

Plant material

The underground part of the medicinal plant, *Glycyrrhiza glabra* L. was collected from the region of Djamaa, and dried up without light-air.

The well dried roots were cut into small pieces and ground to powder and used for the glycoside extraction.

Glycoside test

The presence or absence of the glycoside in *Glycyrrhiza glabra* was tested by following standard methods [4].

Yield of glycoside

The quantity of raw glycosides in licorice

This study aims to measure a quantity of glycosides in licorice roots in the region of Djamaa, the ball was weighed for a first time empty before the extraction and for a second time after the extraction, the difference between the weights is the weight of the crude glycosides.

Extraction of glycoside

When the plant *Glycyrrhiza glabra* L. contains glycoside, we have extracted this chemical, using the standard methods [5, 6].

Microbiological tests

To study the antibacterial effect of glycoside three bacterial strains belonging to different families were chosen: *Pseudomonaceae*, *Enterobacteriaceae*, *Staphylococaceae*, received from the hospital Hakim Saadan Biskra (Algeria). Some characteristics of the bacterial strains used are presented in the following table:

Received 13 November 2017; Accepted 28 December 2017

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Place name: Djamâa Latitude: 33 ° 33' 10" N
 Longitude: 6 ° 00' 09" E Feature description: town
 Area/state: El oued Population range of place: is between 5000 and 10,000
 Country: Algeria Country ISO code: DZ

Fig. 1: Location map of the study area and sampling sites

Table 1: The strains tested

The bacterial strain	Gram coloration
<i>Staphylococcus aureus</i> ATCC25923	gram positive
<i>Pseudomonas aeruginosa</i> ATCC27853	gram negative
<i>Escherichia coli</i> ATCC25922	gram negative

Diffusion method

a-completion of the transplant

We realized transplanting for all three strains (*Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*) from pre-cultivated frozen 3 colonies scraped using a Pasteur pipette, and then the discharge tube sterile saline. A drop of bacterial suspension prepared on agar in a casting box was needed, and then seeded with a Pasteur pipette. The prepared boxes were incubated at 37°C for 18h to 24h

b-the seeding

This was done after the preparation of the inoculums, with a swab a bacterial colonie is taken and put in 2 ml of physiologic water; sterile swab is dipped in bacterial suspension. Fleet then swabs the entire surface of Miller-Hinton (MH), up and down, tight streak.

c-Preparation of dilutions

A set of dilution was prepared in 3 sterile glass Tubes (1/2, 1/4, 1/8), The first tube contains 100 µl of glycosidic extract and 100 µl of diluents (DMSO), Taking 100 µl from the first tube to the second and from the second to the third. Each time we complete with the same volume of DMSO.

d-Preparation of Discs

Discs of 6 mm diameter were produced from watman papers. These discs were sterilized in an autoclave for 20

min at 120 °C in order to improve that the inhibition will be due to the extract not to any contamination in the disc.

e-Application of Discs

We used a clamp to 5 discs in the Petri dishes, using a micropipette on each disc soaks 10 µl of extract in the first disc, 10 µl in the second disk that contains the 1/2 dilution, 10 µl of 1/4, 10 µl of 1/8, and 10 µl DMSO.

After applying the Petri dishes are left for 20 min at room temperature, and then incubated at 37 °C for 24 h.

f-Reading a susceptibility

After incubation, the discs were surrounded with areas that do not present any bacterial growth. These areas correspond to the inhibition zone.

Preparation dilutions of CMI

CMI is the lowest concentration of an antibiotic that inhibits any visible culture of a bacterial strain after 18 h of cultivation at 37 °C. This value characterizes the bacteriostatic effect of an antibiotic [7].

The method for determining the CMI is the smallest concentration of glycoside which can completely inhibit bacterial growth after incubation 18h to 24h.

Determination of CMB

The CMB was determined by following standard methods [8].

RESULTS AND DISCUSSION

After the glycoside test, the glycoside presence was detected by the appearance of a red color.

The yield of glycoside: R = 10% from 30g.

Method distribution (disk method):

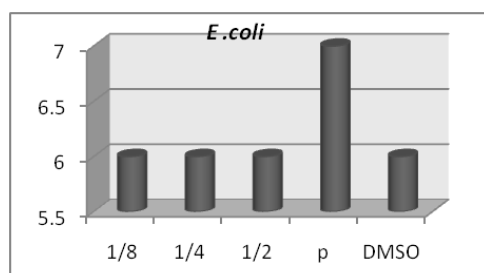
The results of the glycoside's antimicrobial activity; were obtained by measuring the inhibition zone diameters (in mm) of the growth of the tested bacteria, are reported in the table (02).

Table 2: The diameter of inhibition zone

The dilutions the strains	1/8(6.25 mg/ml)	1/4 (12.5 mg/ml)	1/2 (25 mg/ml)	Pure (50 mg/ml)	DMSO
<i>Escherichia coli</i>	06	06	06	07	06
<i>Pseudomonas aeruginosa</i>	07	08	11	18	06
<i>Staphylococcus aureus</i>	06	07	09	11	06



a)

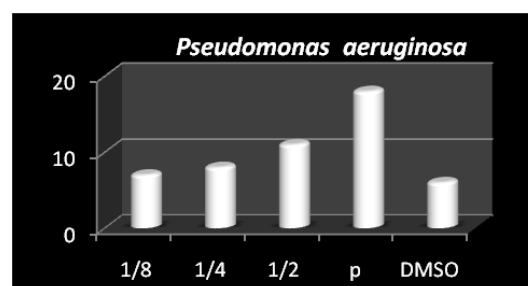


b)

Fig. 2: a) Effect of the glycoside on *Escherichia coli*
b) Histogram represents inhibition areas of glycoside extract



a)



b)

Fig. 3: a) Effect of the glycoside on *Pseudomonas aeruginosa*
b) Histogram represents inhibition areas of glycoside extract



a)

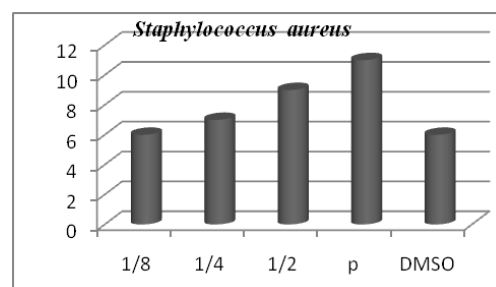


Fig. 4: a) Effect of the glycoside on *Staphylococcus aureus*
b) Histogram represents inhibition areas of glycoside extract

3.3. Méthode dilution: The MIC results are shown in the following table:

Table 3: The minimum inhibitory concentration

Dilutions the strains	Pure(50 mg/ml	1/2	1/4	1/8	1/16	1/32	1/64	1/128	DMSO
<i>Escherichia coli</i>	+	-	-	-	-	-	-	-	-
<i>Pseudomonas aeruginosa</i>	+	-	-	-	-	-	-	-	-
<i>Staphylococcus aureus</i>	+	-	-	-	-	-	-	-	-

+: there is inhibition.-: there is no inhibition

The MIC results for all three strains, there is bacterial growth in most tubes (presence of turbidity due to the bacterial multiplication) with exception of concentration in the tube (1/2).

-The minimum inhibitory concentration: MIC = 25 mg/ml for all of the three strains.

3. 4. The result of CMB:



Fig. 5: Photo showing the CMB of the three strains tested

-From the fig. (5), the results indicate that there is no bacterial bactericidal effect that is to say there is a bacteriostatic effect.

Also, we found that there is a glycoside antibacterial effect of the three strains tested, but with a different degrees of inhibition. The glycoside has an effect on *Pseudomonas aeruginosa* more than *Escherichia coli* and *Staphylococcus aureus*. According to previous study [9], interpretations are under agreement with us, they discover that the *Pseudomonas aeruginosa* is most inhibited by the glycoside extracted *Glycyrrhiza glabra* L but *E. coli* is not inhibited. According to earlier report [10], *Pseudomonas aeruginosa* variation in sensitivity between different antibiotics, they said: "The activity of these antibiotics is not regular and must always be specified by DST".

According to Fadia et al. [11], the ethanoic extracts from *Glycyrrhiza glabra* characterized by a high inhibitory potency of the previous three strains that have especially as Gram+*Staphylococcus aureus*. Vivek et al. [12] used extract of *Glycyrrhiza glabra* as a treatment against gastric ulcer and inflammation of the bladder because they learned that *Glycyrrhiza glabra* in general is a good inhibitor against germs. At the end, interpreting the results it's to say that the glycosidic extract from *Glycyrrhiza glabra* has antibacterial effect and also Kim et al. [13] reported that extracts of *Glycyrrhiza glabra* antimicrobial due to the presence of glycyrrhizin which inhibits bacterial growth by preventing to produce RNA.

CONCLUSION

All microbial strains tested (bacteria) are inhibited by glycosidic extract, except *Escherichia coli* which is resistant to the extract. All the results obtained *in vitro* are only the first step in the search for natural substances and biologically active source. Further tests will be needed and should be able to confirm the performance put in evidence.

ACKNOWLEDGMENT

The authors would like to thank Department of Natural and Life Sciences El hadjeb in Biskra (Algeria) for providing the facilities for the research.

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