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Effect of flavonoid extract of the medicinal plant (*Glycyrrhiza glabra* L.) in the region of Djamaa (south of Algeria) on the growth of some human pathogenic bacteria

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ABSTRACT

In this study we tested the effect of flavonoid extract of the medicinal plant *Glycyrrhiza glabra* L, on four pathogenic bacterial strains. *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*. After the extraction the yield is 1.2 g of a flavonoid from 100 g of roots of *Glycyrrhiza glabra* L with 1.2% percentage. The most used technique is the dissemination of the disc. After 24 hours incubation at 37 °C in the incubator. The result obtained is performed the inhibitory activity of the bacteria studied. Note the inhibitory activity decreased with decreasing of the concentration, the concentrate flavonoid has large inhibitory effect on both *Staphylococcus aureus* and *Enterococcus faecalis* but on the other bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) has less inhibitory effect and can see too that has no effect on *Escherichia coli* concentration in the 1/4 and 1/8 but on the other bacteria the inhibitory effect of the flavonoid extract is decreased with decreasing concentration.

Keywords: *Glycyrrhiza glabra* L, Flavonoid, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Staphylococcus aureus*

1. Introduction

Plants in general and in particular aromatic distinguished by two types of metabolism, the first primary metabolism which provides the building blocks with high quality (sugars, fats and proteins) and second secondary metabolism that produces metabolites in small quantities, but the application involved in many areas, including these secondary metabolites are flavonoids that explain in this research. *Glycyrrhiza glabra* L. is an herb measuring 1 to 1.5 m high. It has large pinnate leaves consist of 9-17 leaflets small purple flowers arranged in inflorescence. Its fruit is flat clove 2 to 3 cm long, containing numerous seeds [1]. The roots and rhizomes of licorice have complex physiological properties from different active ingredients that have been isolated and the chemical structure and Therapeutic actions are well known [2]. The aims of this work studied the effect of flavonoid extract of the medicinal plant *Glycyrrhiza glabra* L. on pathogenic bacteria, which are *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* and *Staphylococcus aureus*.

2. Materials and methods**2.1 Introducing the Djamâa area**

The plant is to be used is obtained from the region of Djamâa

- Place name: Djamâa
- Longitude: 6° 00' 09" E
- Area/state: El oued
- Country: Algeria
- Latitude: 33° 33' 10" N
- Feature description: town
- Population range of place: is between 5000 an10,000
- Country ISO code: DZ



Fig 1: Map of Djamâa

2. Plant Material:

To prepare the extract requires plant material, which is considered in this study on the underground part of medicinal plant *Glycyrrhiza glabra* L. roots, which sampled region of Djamâa, the variety is harvested, and then assembling, cleaning and drying up without light-air. The well dried roots are cut into small pieces and ground to powder is finally given last one used for the extraction of flavonoid extract.

3. Extraction of Flavonoid

We obtained the flavonoid extract from the powder of the roots of *Glycyrrhiza glabra* L. according to the protocol of extracting flavonoid with method of [4].

In this work we used the following protocol:

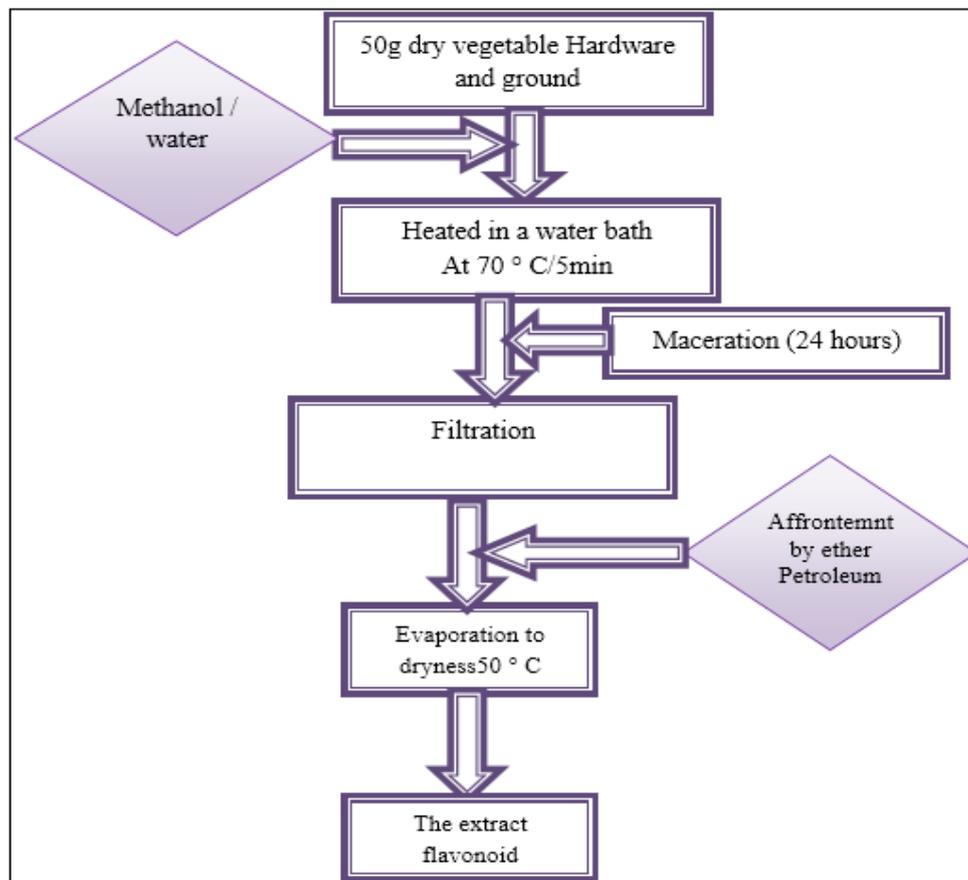


Fig 2: Protocol for the extraction of flavonoids [4].

4. Test the Flavonoid:

According to [3] on macerate 10g powder plant *Glycyrrhiza glabra* L. and added 150 ml of HCL acid is diluted (1%) overnight and then filtered, the filter takes 10ml and have no added sodium hydroxide until we see if mixture is gélosique. The appearance of light yellow color indicated the presence of flavonoid extract in the plant.

5. Microbiological tests

The strains used in tests that are pathogenic, received from the hospital Hakim Saadan Biskra (Algeria).

• *Escherichia coli*, • *Pseudomonas aeruginosa* ; • *Staphylococcus aureus*, • *Enterococcus faecalis*.

Depending on the methods used in the trial and according to the strains, we used the culture medium: Mueller-Hinton agar for bacteria used, which is most often used to test their sensitivity to antibacterial agents.

6. Antimicrobial activity

For bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus faecalis*), we tested their sensitivity by the method of discs or standard sensitivity.

Existing techniques is a technique by direct contact consist in the presence of flavonoid extract and bacteria, and then observe the growth of the latter. The contact may take place in agar; the most widely used technique is aromatogramme

6.1. Aromatogramme:

The aromatogramme is based on a technique used in medical bacteriology, called susceptibility or method by agar diffusion or disk method. This method has the advantage has to be flexibility in the choice of antibiotic tested, to apply to a large number of bacterial species, and have been extensively evaluated by 50 years of worldwide use . It is a method gelose medium agar made in a Petri dish [5].

6.1.1. Preparation of inoculums

A microbial colony are well insulated or two scraping using a sterile Pasteur pipette, and discharged into sterile nutrient broth containing microbes and kept in an incubator at 30 °C for 24 hours and then removed with the handle platinum quantity and placed in 5 ml of normal saline for 20 min.

6.1.2 The seeding

A sterile swab soaked in the bacterial suspension; wring it firmly by turning on the inner wall of the tube to discharge the maximum. Rub the swab knew the entire agar surface, dry, from top to bottom, in tight streaks. Repeat three times, rotating the Petri dish of 60° each time, do not forget to rotate the swab itself.

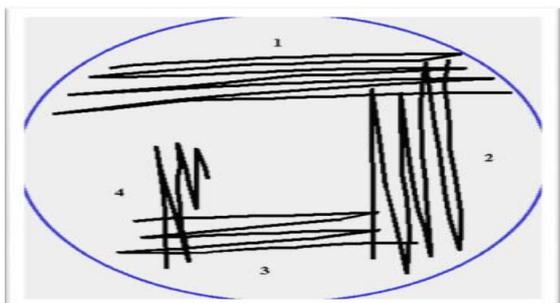


Fig 3: The seeding

Finish seeding from the swab on the periphery of the agar. In the case of seeding several Petri dishes must be recharged every time the swab.

6.1.3 Preparing Disks

The discs are used such as "blotting paper" cut with a diameter of 6mm, en suite they are sterile (sterilization at 120 ° C for 15 min by autoclaving). These drives are applied to the surface of agar medium with the determined distances sterile forceps, they are impregnated with different doses of flavonoid extract dilutions 1/2, 1/4, 1/8.par petroleum ether in this if used in an amount of 10µl discs.

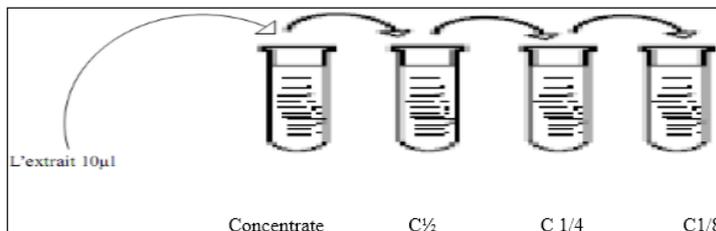


Fig 4: dilution of flavonoid extract

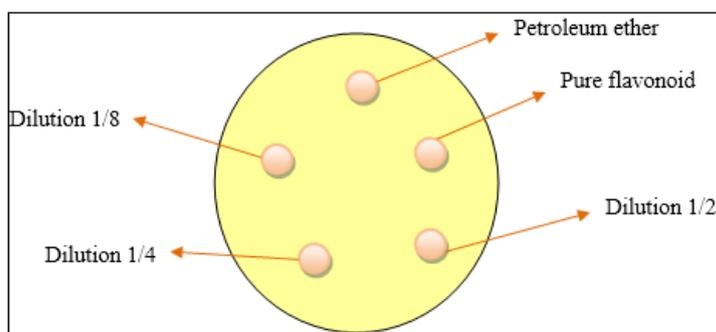


Fig 5: The Petri dish with the discs

6.2 Incubation and reading:

The Petri dishes used were incubated in an incubator at 37 ° C for 24 hours. Reading results is done by measuring the zone of inhibition of bacteria vis-à-vis the flavonoids extracts, the area represented by an aureole formed goshawks each disc or no growth is observed.

7. Results and Discussion

In the horizon of this part of results and discussion, we will present and synthesize the results of various tests of antibacterial activity of the flavonoid extract of medicinal plant *Glycyrrhiza glabra* L. The effect of flavonoids extracts been evaluated by the sensitivity of the bacteria are *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*. This effect can be measured from the zone of inhibition observed that after the test. The choice has been focused on these bacteria for its pathogenicity and to determine the spectrum action of the flavonoid extract. The antimicrobial and therefore anti-infective activity of flavonoids has been demonstrated in numerous studies. Disinfectants can be considered as antibiotics according to their role and their influence on bacterial growth. Antibacterial antibiotics are molecules that selectively inhibit certain metabolic pathways of bacteria, usually without toxic effects of exercise to higher organisms. This property distinguishes antiseptic [6].

7.1 Yield of the Flavonoid Extract:

Extract recovered after evaporation to dryness under reduced pressure and were weighed to determine the dry weight resulting, this extract contains flavonoids, the yield was determined relative to 100 g of plant material (*Glycyrrhiza glabra* L) rendering powder undergoing gentle extraction at room temperature, and the yield is

1, 2g (1, 2%).

This value is consistent with those reported by some studies. While [7] obtained in a yield of 0.26%. According to [8] Flavonoids represent about 0.65 to 2% of the chemical composition of the drug.

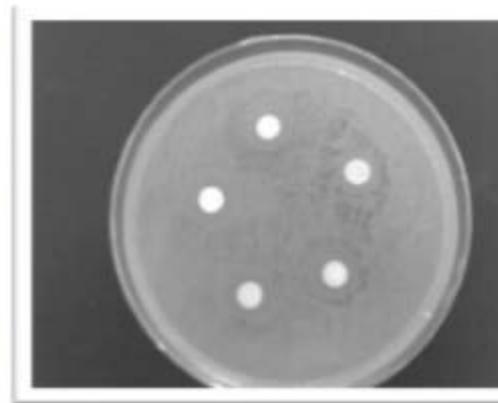
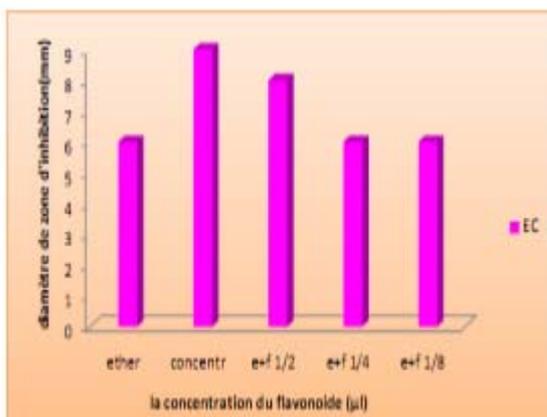


Fig 6: Histogram of the effect on *Escherichia coli*. **Photo 1:** zones of inhibition of the flavonoid on *Escherichia coli*

The flavonoid extract antibacterial activity, according [9] say that this activity is mainly due to the ability of these molecules to inhibit the expression of DNA and the synthesis of some membrane proteins and enzymes of microorganisms. Showed that the

flavonoid extract are able to inhibit the growth of different types of bacteria: *Escherichia coli* [10].



Fig 7: Histogram of the effect on *Staphylococcus aureus*. **Photo 2:** zones of inhibition of the flavonoid on the *Staphylococcus aureus*

The flavonoid extract is inhibits the growth of *Staphylococcus aureus*. The recognition is performed by [11] that flavonoid extract have a very broad antibacterial activity and very diverse. Indeed, they attack a large number of bacteria with different intensity depending on the organism and the ecosystem in which it is located: flavonoids extracts are able to inhibit the growth of various types of bacteria: *Staphylococcus aureus*.

7.2. The effect of the extract on *Pseudomonas aeruginosa*:

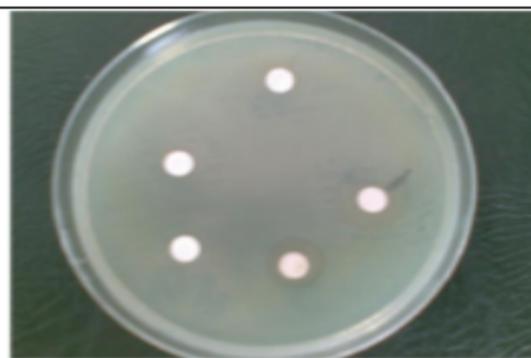
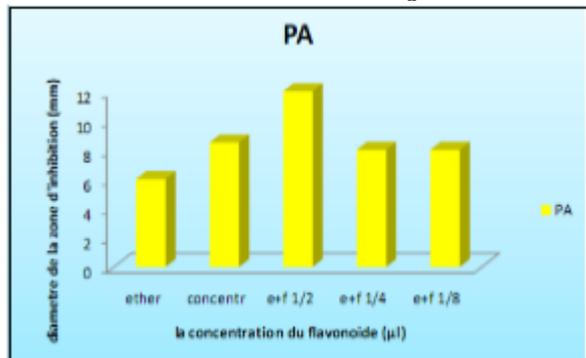


Fig 8: Histogram of the effect on *Pseudomonas aeruginosa*. **Photo3:** zones of inhibition of the flavonoid on *Pseudomonas aeruginosa*.

Pseudomonas aeruginosa is naturally resistant to most antibiotics more employees, chemotherapy is often difficult [12]. Acquired resistance of micro-organisms has always been a concern. It

appears in some bacteria naturally sensitive data to an antibiotic [13].

7.3 The effect of the extract on *Enterococcus faecalis*:

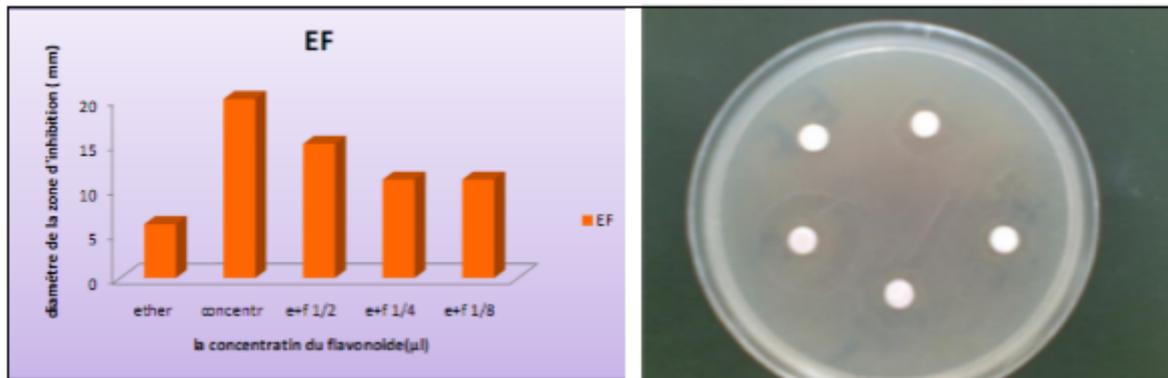


Fig 9: Histogram of the effect on *Enterococcus faecalis*. **Photo 4:** zones of inhibition of the flavonoid on *Enterococcus faecalis*.

The inhibitory effect of flavonoid extract on *Enterococcus faecalis* is achieved by [14, 15, 16] that flavonoids extracts have a very broad antibacterial activity and very diverse.

Indeed, they attack a large number of bacteria with different intensity depending on the organism and the ecosystem in which it is located: flavonoids extracts are able to inhibit the growth of *Enterococcus faecalis*, *Enterobacter cloacae*, *Heliotropium sinuatum*, *Proteus mirabilis*.

8. Conclusion

Medicinal plants represent an inexhaustible source of substance and bioactive natural compounds such as flavonoids. The study of their antibacterial properties on four bacterial germs "*Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*". The flavonoid extract from the plant *Glycyrrhiza glabra* L is proven very good antibacterial activity, another word said that the flavonoid extract inhibits bacterial growth of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Enterococcus faecalis*. This result shows that the use of the medicinal plant *Glycyrrhiza glabra* L and / or flavonoid extract generates particularly important antibacterial effects.

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