Journal of Scientific and Innovative Research 2014; 3 (1): 28-34
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2014; 3(1): 28-34

Received: 18-01-2014
Accepted: 10-02-2014

Kriker Soulef
Department of Natural Sciences of Life, University Mohamed khaider, Biskra, Algeria

Yahia Abdelouahab
Centre universitaire Mila– Algeria

Boukhari Dalal
Department of Natural Sciences of Life, University Mohamed khaider, Biskra, Algeria

Correspondence:
Kriker Soulef
Department of Natural Sciences of Life, University Mohamed khaider, B.P 145 R.P, 07000 Biskra, Algeria
E-mail: soulefa2011@yahoo.fr

Effect of glycosides extract of the medicinal plant *Glycyrrhiza glabra* L. from the region of Mlilli (southeast of Algeria) on the growth of some human pathogenic bacteria

Kriker Soulef, Yahia Abdelouahab, Boukhari Dalal*

Abstract

Licorice is a perennial plant of the family Fabaceae, species Glycyrrhiza, and genre "*Glycyrrhiza glabra* L. "Takes from the region of Mlilli (southeast of Algeria,). The objective of this work is to study the effect of the glycoside extracted from the medicinal plant *Glycyrrhiza glabra* L. on three bacterial strains: *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Preliminary phytochemical study of the crude glycoside extract is based on the antibacterial test. This extract was tested in vitro by the method of agar diffusion (GN) and the diffusion in liquid medium (MH) on three bacterial species. The results show that our extract has an inhibitory effect on the Gram positive strain (*Staphylococcus aureus* ATCC23) and the Gram negative strain (*Pseudomonas aeruginosa* ATCC53), but no effect on *Escherichia coli* ATCC22 strain. From the results obtained, we suggest the use of licorice in daily live to treat from certain infectious diseases.

Keywords: *Glycyrrhiza glabra* L., Glycoside, Bacterial strains, MIC, MBC, Mlilli.

Introduction

Plants of the Fabaceae family, like licorice (*Glycyrrhiza glabra* L.), were the first to be cultivated. The oldest traces of these cultures date back over 10,000 years before Jesus Christ.[1]

The first traces of the use of licorice were in the burial chamber of Egyptian pharaoh Tutankhamun (1350 BC). It has found a description of the curative importance of licorice root. The softening effect of the preparations of liquorice in infections of the throat and bronchi was demonstrated for over 2,000 years.

Arab doctors prescribed it for cough and to reduce the inconvenience of laxatives.[2]

Licorice also refers to the root of this plant used in pharmacy and confectionery. The main components of this plant are: flavonoids, saponins, essential oils, tannins. In this work we concentrated on the active principle of "glycoside".[3]

The aim of the present study is to gather and synthesize different stages on our subject to know the effect of the Licorice extracted glycosides on some bacterial strains, and to determine their therapeutic interest.

Materials and Methods

Delineation of the study area

The plants used for the study were collected from the Mlilli area (wilaya of Biskra), its surface equal to 371.80 km^2, and its limits are:

- In the north: the common Hajeb.
- At the East: common Oumache.
- At South: El-Oued wilaya.
- At the West: province of Ouargla
- To the northwest: the common Bouchagroune

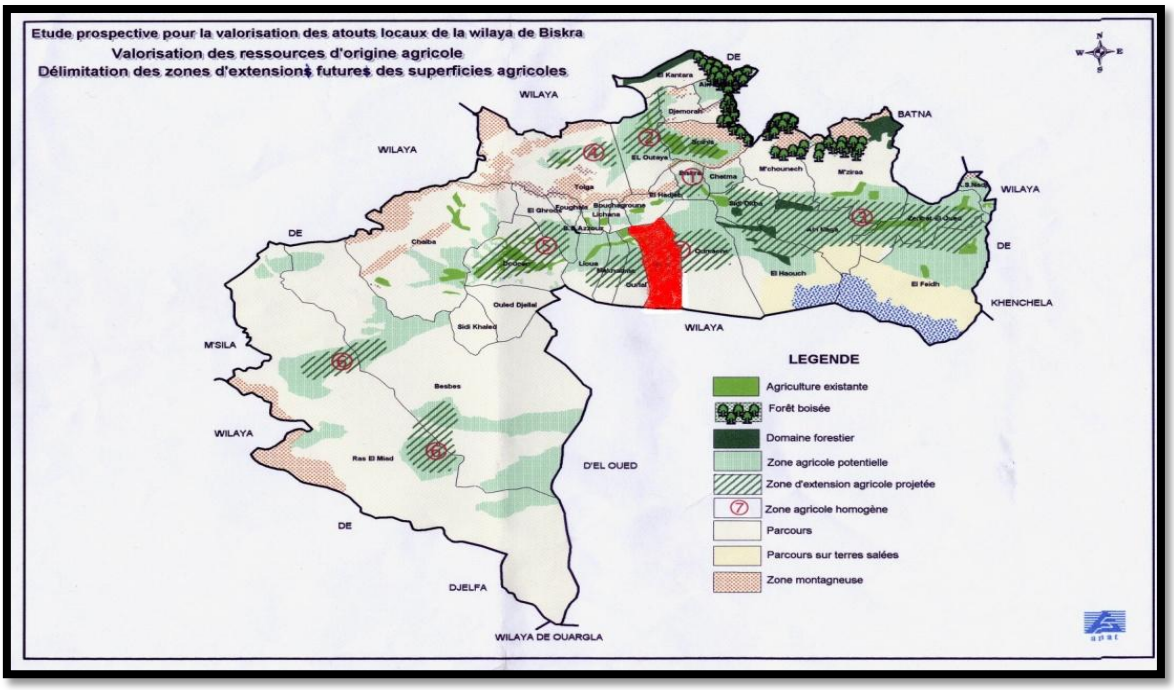


Figure 1: Location of the Common Mlilli in the wilaya of Biskra southeast of Algeria

Plant material

The plant material required for the glycosides extraction, in this study was the underground part of the medicinal plant; *Glycyrrhiza glabra* L. roots, which is sampled from the region of Mlilli, the variety is harvested, then assembled, cleaned and dried up without light-air.

The well dried roots are cut into small pieces and ground to powder which is finally used for the glycoside extraction.

Glycoside test

This test is used to detect the presence or the absence of the glycoside in *Glycyrrhiza glabra* L. (Test of Gonzloez, E.E and Delgado,J .N).[4]

Glycoside Extraction

When the plant *Glycyrrhiza glabra* L. contains glycoside, we have extracted this chemical, using the method of Balbaa S.

The yield of glycoside

Determining the efficiency of extraction by calculating the following equation:

$$\mathbf{R} = \frac{(\mathrm{M2} - \mathrm{M1}) \times 100}{\mathrm{E}}$$

Where R: performance.
M2: the weight of the balloon + extract.
M1: the weight of empty balloon.
E: the weight of the dry matter.

Microbial material

We used three bacterial strains from two different groups, Gram positive and Gram negative received from the hospital Hakim Saadan Biskra.

Gram positive bacteria: *Staphylococcus aureus* ATCC23 and Gram-negative bacteria are *Escherichia coli* ATCC22, *Pseudomonas aeruginosa* ATCC53.

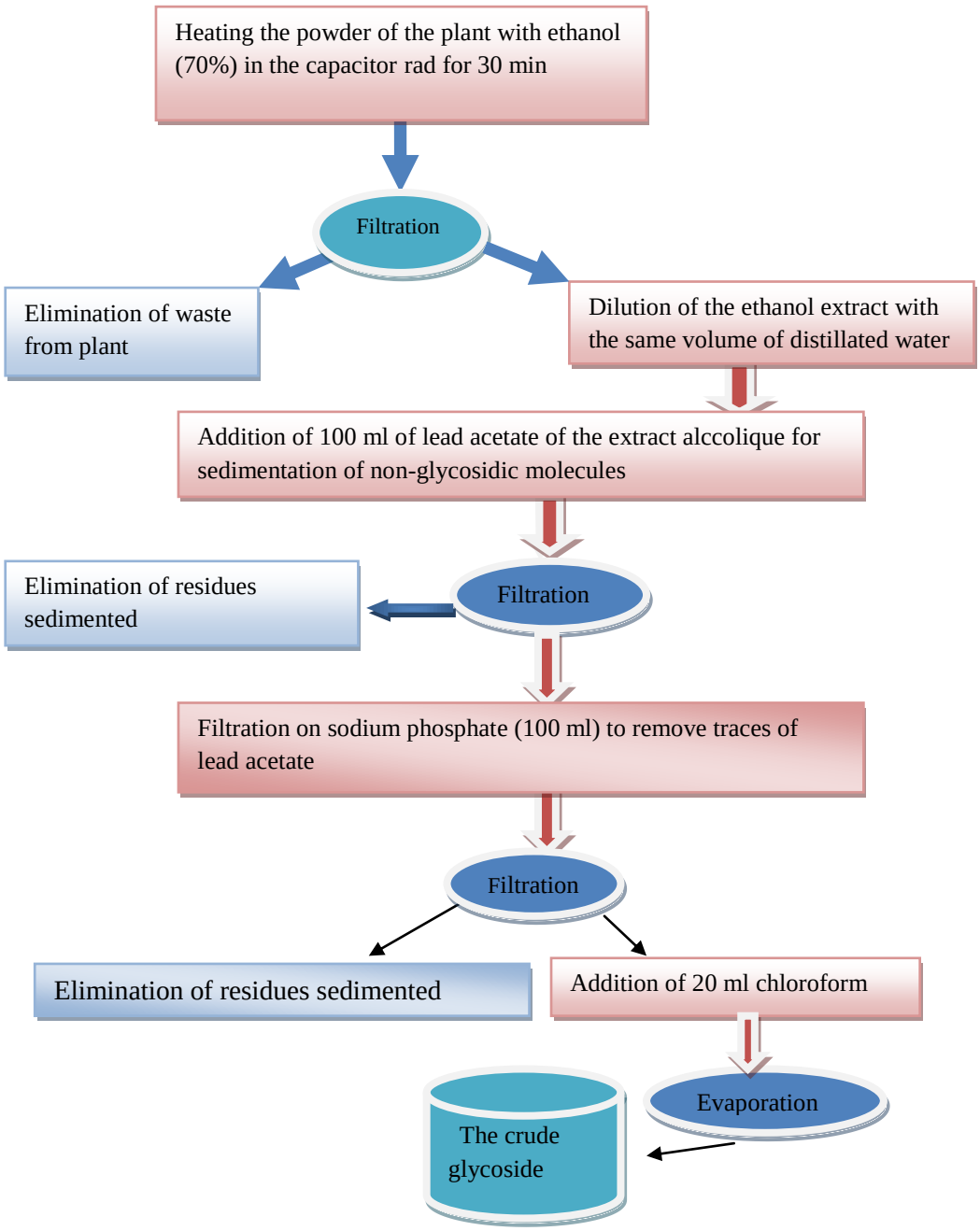


Figure 2: Steps for extraction of glycoside

The choice has been focused on these organisms for their potential pathogenicity in one hand and to define action presumed glycoside tested other.

Aantibacterial activity

The antibacterial activity of different plant extracts was evaluated by the method of diffusion in agar medium (disc method) and micro dilution method for the determination of MIC (minimum inhibitory concentration) and MBC(minimum bactericidal concentration).

Sensitivity test: The culture medium used MH (Muller Hinton), is the medium most used for testing the antibacterial agents susceptibility.

The knead boxes were sterilized at first then left for 15 minutes to solidify.

The strains were tested by the extracts solubilised in DMSO (Diméthylsulfoxyde) is a witness to the end compared with the effect of our extract.

Preparation of inoculums

Samples from the well-separated colonies of bacterial species involved were collected using a sterile platinum loop and homogenized in 10 ml of nutrient broth and then brought to the incubation for (18-24) hours at 37°C.

Seeding of bacteria

Bacterial seeding was performed using a swab, by immersion in the bacterial suspension, then by seeding the colonies on the entire surface of the kneaded boxes from top to bottom, in striated tight.

Preparation of Discs

The discs were prepared by a Whatman No 3 paper cut into 6 mm diameter, and then sterilized in a Pasteur oven. Using a 10 µl micropipette, the discs were flooded by the extracts with different concentrations: 1/2, 1/4 and1/8, then, left to dry for 15 min before use. Once the MH boxes were seeded, the disks soaked with each extract were delicately placed on the surface of the agar using sterile forceps.

Reading the results

The results were observed; after 24 h, by measuring the diameters of clear halos around the discs or the inhibition zones using a ruler. The larger the area is, the greater the germ is sensitive

The determination of MIC and MBC

The determination CMI is important in diagnostic laboratories to confirm the microorganism's resistance against antimicrobial agent and also to monitor the activity of new antimicrobial agents.

***Preparation of tubes**

In a series of 08 test tubes of 1500µl we put; 50 µl of bacterial suspension, 400 µl of the plant extract dissolved in DMSO in different concentration and the rest BGT (culture medium). For example the C5 concentration is 1,25 % and C1 is 20%, the last tube is noted C8 as control tube. Then the tubes were incubated at 37 °C for 24 hours in an oven.

The MBC was determined by sprawl of 0.l ml of each tube concentration greater than or equal to the MIC on solid medium. From the MIC, the lowest concentration that leaves only 0.01% bacterial survives from the initial suspension after 24 hours of incubation, corresponding to the CMB.[6]

The antibacterial effect was found to be bactericidal or bacteriostatic depending on the MBC / MIC ratio. If MBC / MIC <4 the effect is bactericidal. If MBC / MIC> 4 the effect is bacteriostatic.[7]

Results and discussion

The antibacterial activity of glycoside product is estimated in terms of the diameter of the zone of inhibition around disks containing different concentrations of the extract glycoside test vis-à-vis the origins of hospital pathogenic germs (*Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*) after 24 hours of incubation at 37 ° C.

Test glycoside

Result of this powder with Fehling's solution is the appearance of the red color. So there is a glycoside in this powder.

The yield of glycoside

We extracted the glycoside from the medicinal plant *Glycyrrhiza glabra* L. the region of Mlilli, 3.2% (0.9 g per 30 g of dry matter), the difficulty of extracting sufficient quantity glycoside can be explained by the following reasons: the origin of the plant, drying time, mode and the duration of the extraction of glycoside. This amount is hold between percentages 2-15%.[8]

Diffusion method in agar

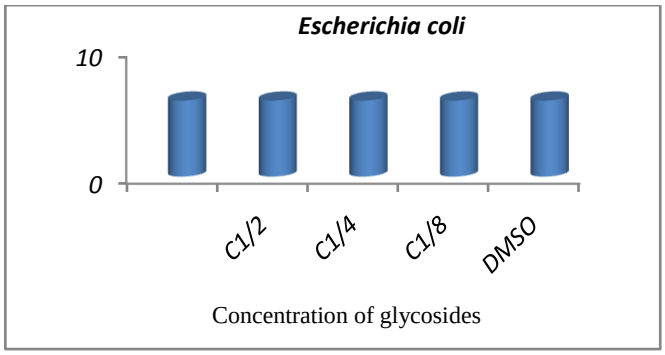
This test allowed highlighting the antibacterial effect of the extract, which was tested against the bacterial strains: *Escherichia coli* ATCC22, *Staphylococcus aureus* ATCC23 and *Pseudomonas aeruginosa* ATCC53. The results of the sensitivity of the strains with the four dilutions are shown in table 1.

Table 1: Diameter of the zones of inhibition (mm) showing the antimicrobial activity

The dilutions / the strains	Pure	DMSO	1 /2	1/4	1/8
Escherichia coli	06	06	06	06	06
Pseudomonas aeruginosa	15	06	11	08	07
Staphylococcu saureus	12	06	07	06	06

Effect of glycoside on the bacterium Escherichia coli

a)



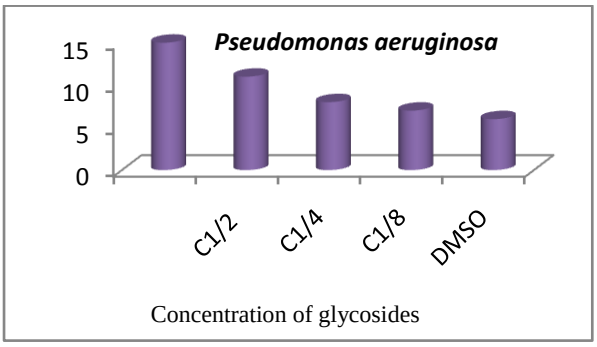
b)

Figure 3: a) Effect of the glycoside on *Escherichia coli* **b)** Histogram represents inhibition areas of glycoside extract against *Escherichia coli*

The results in Figure 3 show no inhibitory effect of glycoside extract of *Glycyrrhiza glabra* L. on the bacterium *Escherichia coli*. Develop low inhibition zones vis-a-vis *Escherichia coli*. We can say that the pathogenic *Escherichia coli* are resistant.

Effect of glycoside on the bacterium Pseudomonas aeruginosa

a)



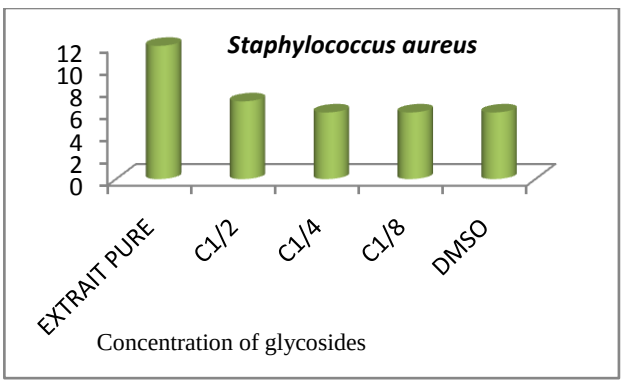
b)

Figure 4: a) Effect of the glycoside on *Pseudomonas aeruginosa* **b)** Histogram represents inhibition areas of glycoside extract vis-a-vis *Pseudomonas aeruginosa*

The results presented in Figure 4 indicate that the glycoside *Glycyrrhiza glabra* L. Develop strong inhibition zones vis-à-vis *Pseudomonas aeruginosa*, so we may say there is an inhibitory effect of this extract on the pathogenic bacterium *Pseudomonas aeruginosa*.

The effect of the glycoside on the Staphylococcus aureus strain

a)



b)

Figure 5: a) Effect of the glycoside on *Staphylococcus aureus* **b)** Histogram represents inhibition areas of glycoside extract vis-a-vis *Staphylococcus aureus*

The results presented in figure 5 indicate that the *Glycyrrhiza glabra* L. extracted glycoside develop an average inhibition zones vis-à-vis *Staphylococcus aureus*, so we may say there is an inhibitory effect of this extract on the pathogenic bacterium *Staphylococcus aureus*.

Determination of MIC

Analysis of the experimental data compared with the control growth showed that, there is a progressive decrease in the bacterial colonies number studied in experimental tubes (tubes turbidity), accompanied with the increase of the extract concentration, as represented in table 2.

Table 2: The minimum inhibitory concentration (MIC)

Dilution / Strains bacteries	T	1 /2	1/4	1/8	1/16	1/32	1/64	1/128
Escherichia coli ATCC	-	-	-	-	-	-	-	-
Pseudomonas aeruginosa ATC53	-	-	-	-	+	+	+	+
Staphylococcus aureus ATCC23	-	-	-	-	-	-	-	-

+: There is an inhibition.
- : There is no inhibition

MIC values are almost the same in both *E. coli* and *S. aureus* strains, but different in the pseudo strain. The values differ depending on the efficiency of the extract and the strain tested. The best observed MIC value is 2.5mg/µl.

Determination of MBC

The passages of tubes have concentrations ≥ CMI allows the determination of the CMB values by reading the results of the box where there is no growth is the CMB as it is shown in the figure 6.

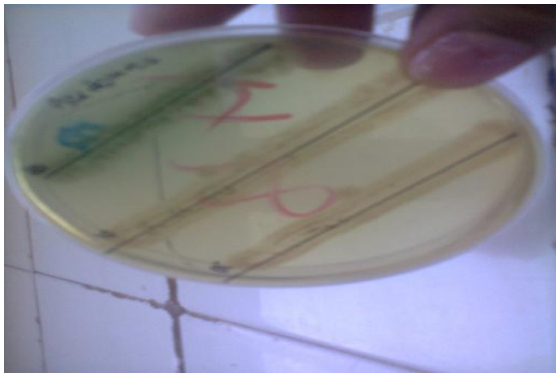


Figure 6: MBC of extract vis-à-vis the strain *Pseudomonas aeruginosa*

Table 3: Values of MIC and MBC

Concentration / Strains bacteries	CMI	CMB	CMB/CMI
Escherichia coli ATCC22	/	/	/
Pseudomonas aeruginosa ATCC53	2.5	/	/
Staphylococcus aureus ATCC23	/	/	/

According to the MBC / MIC ratio:

If MBC / MIC <4 the effect is bactericidal.

If MBC / MIC> 4 the effect is bacteriostatic.[7] So the effect is bacteriostatic.

Pseudomonas aeruginosa and *Staphylococcus aureus* were given with a glycoside inhibition zone of 15 mm and 12 mm respectively, so they are sensitive to the glycoside, while *E. coli* showed more resistance to the glycoside, it gave a 6 mm inhibition zone.

Both pathogenic strains: *Pseudomonas aeruginosa* and *Staphylococcus aureus* are sensitive to the glycoside of *Glycyrrhiza glabra* L. that is to say there is an inhibitory effect of our extract "glycoside" on these bacteria, it is the same result of Ates *et al* (2003).[9]

For the Escherichia coli no inhibitory effect of the glycoside extract on this strain. This is the same result of Erdogul O.T *et al* (2002)[10] Nitaliker *et al* (2010)[11] and Sultana *et al* (2010)[12] indicated that the extract of *Glycyrrhiza glabra* L "glycoside" is able to inhibit bacterial growth of all bacteria especially Gram-positive bacteria such as *Staphylococcus aureus*. This result agrees with the result of Ates *et al* (2003).[9]

According to Al-dahriy *et al*. (2010)[13], Effect glycoside seed *Prosopis farcta* on bacteria abstracts in the series ordained by the concentration of inhibition as follows:

Pseudomonas aeruginosa > *Staphylococcus aureus* > *Escherichia coli*, which agrees with our result.

The ability of *Glycyrrhiza glabra* L. extract to inhibit is due to its constituent "Glycyrrhizin", which plays an inhibitory role on the bacterial growth by inhibiting the RNA nucleic acid synthesis.[14]

Conclusion

The results found in this study, are warning that the glycoside of *Glycyrrhiza glabra* L. has a very large and diverse antibacterial activity. Indeed, they attack a large number of bacteria with different intensity depending on the organism and the ecosystem in which it is located. The glycoside is capable of inhibiting the growth of various kinds of bacteria such as: *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

References

1. Brenckmann. F: Grains de vie, Paris, Arthaud, 1997, pp159.

2. Bruneton. J: Pharmacognosie, phytochimie. plantes médicinales, 3ème Ed. Paris, Lavoisier, 1999, pp 102.

3. Delphine CAEL. Contribution a l'étude de la réglisse (*Glyccyrrhiza Glabra* L.): Ses utilisations thérapeutiques et alimentaires. Diplôme d'Etat de Docteur en Pharmacie, université Henri Poincaré, Nancy. 2009, pp 20, 29-30, 39.

4. Gonzloez, E.E; Delgado, Phytochemical investigation of Amphipterygium adstringens. J. N. J. Pharm. sci. 1962; 51: 786-790.

5. Balbaa, S. I: Médicinal plant constitents. PHD. In collaboration Egyptation, 3th Edition. Dar El kotab, 1981, pp 246-248, 258-260.

6. Moroh JLA, Bahi C, Djè K, Loukou YG, Guédé-Guina F, Étude de l'activité antibactérienne de l'extrait acétatique (EAC) de Morinda morindoides (Baker) milne-redheat (rubiaceae) sur la croissance in-vitro des souches d'*Escherichia coli*, Bull de la SR des Scien de Liège, 2008; 77: 44–61.

7. Kone, W.M., Kamanzi Atindehou, K., Terreaux, C., Hostettmann, K., Traore, D., Dosso,M. Traditional medicine in North Cote-d'Ivoire screening of 50 medicinal plants for antibacterial activity. J. Ethnopharmacologie, 2004; 93: 43-49.

8. PETIT A.C.toxicité et utilisation de quelle que Fabacéaes alimentaire et médicinales. Diplôme d'état de docteure en pharmacie, univ. Henri Poincaré. Nancy, France. 2011, 164.

9. Ates, D. A. and Erdogrul, O. T, "Antimicrobial activities of various medicinal and commercial plant extracts", Turk. J. Biol, 2003; 27: 157-162.

10. Erdogul O.T: Antibacterial activities of some plant extracts used in folk medicine. Pharmaceutical Biol. 2002; 40: 269-273.

11. Nitaliker. M. M, Munde. k. C, Dhore.B. V, Shikalgar.S.N, Studies of Antibacterial activities of *Glycyrrhiza Glabra* L. Root extract. J.Pharmatech, 2010; 2(1): 899-901.

12. Sultana. S, Afroza. H, Kaiser. H,Kaniz. F. U. and Sumon, R., Antimicrobial, cytotoxic and antioxidant activity of methanolic extract of *Glycyrrhiza glabra* L., Agric. Biol, J. N. Am, 2010; 1(5): 957-960.

13. Al-dahriy.A,Al-ubaid.R,Al-Ani.W,Al-gylani.M., Studying some chemical of Seeds the Prosopis farcta and their anti bacterial effects, College of Education for pure sciences, 2010; 8(4): 182-193.

14. Kim H. K, Park, Y, Kim.H.N, Choi.B. H, Jeong, H. G, Jeong, G, Lee. H. Hahm, K.S., Antimicrobial mechanism of β-glycyrrhetinic acid isolated from liquorice, *Glycyrrhiza glabra* L, J. Biotec. Letters, 2004; 24(22) 1899-1902.