

**Effect of methanolic extracts of different parts of
Licorice *Glycyrrhiza glabra* L. on growth of some
pathogenic bacteria and fungus *Candida albicans***

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Abstract

Methanolic extracts of different parts of *Glycyrrhiza glabra* L. (roots with rhizomes, stems, leaves and seeds), were tested for antibacterial and antifungal activities. Antibacterial study performed against six bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella dysenteriae*), and antifungal activity performed against one fungus (*Candida albicans*). The effect of the extracts was tested separately using Agar- well diffusion method. Indicated that investigated plant parts have potent activity against all microorganisms especially at 2.5 and 2.0 µg/ml. the inhibition zone in seeds extract reached 37 & 35 mm for bacteria *S. aureus* and *B. cereus* respectively, by compared with Streptomycin and also reached in seeds extract 20 mm for fungus by compared with Nystatin. Also the results showed that seeds extract have a good effect as antibacterial while the roots extract showed a best effects from other extracts as antifungus.

Key Words: *Glycyrrhiza Glabra*, Licorice, Antibacterial, Antifungal activity, Methanolic Extract.

تأثير المستخلص الميثانولي للأجزاء المختلفة لنبات السوس *Glycyrrhiza glabra* L. في نمو بعض أنواع البكتريا المرضية والفطر كانديدا *Candida albicans*

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المستخلص

اجريت الدراسة لمعرفة الفعالية التثبيطية للمستخلص الميثانولي للأجزاء النباتية المختلفة (الجزور مع الرايزومات والسيقان والاوراق والبذور) لنبات السوس (*Glycyrrhiza glabra* L.) تجاه 6 انواع بكتيرية متمثلة بـ *Staphylococcus aureus* و *Bacillus cereus* و *Escherichia coli* و *Pseudomonas aeruginosa* و *Salmonella typhi* و *Shigella dysenteriae* فضلاً عن خميرة الفطر كانديدا *Candida albicans* وذلك باستخدام طريقة الانتشار بالحفر اذ تم تقدير الفعالية التثبيطية للمستخلص بقياس معدل النمو القطري للبكتريا والفطر وظهرت النتائج وجود تأثير تثبيطي معنوي بالنسبة للاحياء المجهرية كافة قيد الدراسة، اذ بلغت اوجها في اعلى تركيزين (2.0 و 2.5 مايكروغرام/مل)، وبلغت منطقة التثبيط في مستخلص البذور (37 و 35 ملم) للبكتريا *S. aureus* و *B. cereus* على التوالي مقارنة بالمضاد الحيوي Streptomycin للمقارنة مع البكتريا وبلغت ايضاً في مستخلص البذور (20 ملم) للفطر كانديدا مقارنة بالمضاد الحيوي Nystatin وكذلك بينت نتائج المقارنة بين مستخلصات الاجزاء النباتية ان مستخلص البذور اعطى تأثيراً جيداً كمضاد للبكتريا بينما مستخلص الجزور اعطى افضل تأثير من بقية المستخلصات كمضاد للفطر.

الكلمات المفتاحية: *Glycyrrhiza Glabra* و Licorice و مضاد بكتيري و مضاد فطري و مستخلص ميثانولي.

Introduction

Liquorice or Licorice, (*Glycyrrhiza glabra* L.) Family: Leguminosae is a well known medicinal herb that grows in various parts of the world. It is a perennial plant, about 1.5 meters high with wrinkled woody, brown and very sweet taste roots. Leaves are unequally branched in 4-7 pairs. Flowers are violet colored, Pods are small and compressed with 3-5 brown seeds (17).

Because of the side effect and resistance that pathogenic microorganism formed against the antibiotic, much attention has been paid to extracts and biological active compounds isolated from plant species used in herbal medicine. Medicinal plants may offer a nature and new source of antimicrobial agents for use (16).

The isolation of various chemical constituent of roots and rhizomes of Liquorice has been previously reported which include glycyrrhizin, glycyrrhizic acid, glycyrrhetol, glycyrol, glabrin A and B, glabrolide, isoglabrolide, glabridin, glabrene, glabrol, glabranin isomer, licoricone, isoliquirtigenin, isoflavones, licoflavonol, phaseollin isoflavan, formononetin, paratocarpin, hemileiocarpin, coumarines and triterpene sterols (7; 14; 23).

Liquorice has been used for centuries as an herbal therapeutic substance for its wide ranging therapeutic properties including relief of rheumatic, cold coughs, hoarseness, sore throat, bronchitis and other painful illnesses. Its healing effects on the gastric ulcers, prophylactic as well as therapeutic drug for major body ailments at any age group irrespective of sex (26; 17). Treatment of early Addison disease (9) and liver disease (20), and pulmonary, skin diseases (3) have been detected. It also has been shown promise as a memory enhancing agent in mice (10).

The plant has been clinically employed for its antibacterial (4; 11; 13; 21; 28), antifungal (12; 22), antioxidant (19), antihyperglycemic (18), antimalarial effect (6), antiviral and Immunostimulatory (2), antiallergic (16), antiulcer activity (5), hepatoprotective and antihepatocarcinogenic effects (15), anticonvulsant activity (31), anti-inflammatory (25) and anticarcinogenic effect (3).

The main objective of the present study was to investigate and compare the antimicrobial activity of methanolic extracts of roots, stems, leaves and seeds of *Glycyrrhiza glabra* on some bacterial and fungal diseases.

Materials and Methods

Plant materials:

The whole plants (roots with rhizomes, stems, leaves and seeds) were collected from the campus of the University of Baghdad, Baghdad, Iraq in November 2011. The plant was authenticated by a botanist in the Department of Biology, College of Science for women, University of Baghdad. The plants were cleaned and air dried under shade at room temperature, and powdered with a mechanical grinder to obtain a coarse powder.

Preparation of plant Extract:

Hundred gram of the prepared powder sample from different part of plant were refluxed with 1000 ml of Methanol 99 % at 70 °C for 48 hrs using Soxhlet apparatus. The liquid extract was separated from solid residue by vacuum filtration through whatman filter paper (No. 1). Filtered methanolic was then concentrated to small volume using rotary evaporator, followed by drying in freeze dryer to get constant mass which kept in air tight container at 4 °C. The resulting methanol extract was then used for antibacterial and antifungal activities (1).

Screening for antibacterial and antifungal activities:

The antibacterial and antifungal activities of the extract was investigated by employing 24 hrs cultures of two Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*) and four Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Shigella dysenteriae*) and the fungi (*Candida albicans*). All organisms mentioned above were obtained from the laboratories of Al- Yarmook hospital, Baghdad, Iraq.

The effect of the extracts was tested separately using Agar - well diffusion method. The medium was sterilized by autoclaving at 121 °C for 15 min. (15 lb/in²). About 30 ml of the medium (Muller-Hinton agar medium) with the respective strains of bacteria and fungi was aseptically transferred into each sterilized Petri plate. The plates were left at room temperature for solidification. In each plate a single well of 5 mm diameter was made using a sterile cork borer. The extracts were tested at various concentrations 0.5, 1, 1.5, 2 and 2.5 µg/ml. The samples and the control were placed in 5 mm diameter well. Antibacterial assay plates were incubated at 37±2 °C for 24 hrs

to allow maximum growth of bacteria, antifungal assay plate were incubated at 28 ± 2 °C for 24 hrs to allow maximum growth of the fungi. Standard disc (5 mm diameter) with Streptomycin (5 µg/disc) was used as a positive control for antibacterial activity, whereas Nystatin (5 µg/disc) was used as a positive control for antifungal activity. Distilled water was used as a negative control for both antibacterial and anti microbial activities. Antimicrobial activity was evaluated by measuring the diameter of the zone of inhibition expressed in (mm) around the well. The assay was repeated three times and the mean diameter was considered (8).

Statistical analysis:

The results of antibacterial and antifungal activity were analyzed according to (SAS, 2004), Significant differences between mean values were determined by Duncan's Multiple Range Tests, at a level of $P < 0.01$ (29).

Results and Discussion

The methanolic extracts of *Glycyrrhiza glabra* was screened against six test bacteria and one test fungi, all organisms were found to be sensitive to the extracts. (Table, 1) shows Significant results of the roots extract as antimicrobial activity especially in 2.5 and 2.0 µg/ml against *S. aureus* and *B. cereus* with the zone of inhibition 35, 33 mm, respectively, when compared with the control except *P. aeruginosa* in which the highest inhibition was by the antibiotic Streptomycin and 1.5 µg/ml concentration with no significant differences between them.

The inhibition of microorganism by stems, leaves and seeds extracts exhibited a strong activity against all species of bacteria and fungi under study and all the concentrations especially in 2.5 and 2.0 µg/ml in compared with the control negative control and positive control (Streptomycin and Nystatin) for both bacteria and fungi (Tables 2, 3 and 4).

(Table, 5) shows the antimicrobial activity of different plant parts, the seeds extracts gave excellent effect as antibacteria, while the roots extract was better than the others as antifungal.

The majority of Antimicrobial effects from licorice are due to isoflavonoid compounds particularly hispaglabridin and methyl

glabridin, glabridin, glabriol and hydroxyglabrol and other compounds (7; 13; 30).

In this study methanolic extracts of *Glycyrrhiza glabra* showed good inhibition against all the tested pathogens, and can be used as raw materials for phytotherapy. The present study provides further evidence about the leaves and seeds antimicrobial activity of *Glycyrrhiza glabra*. The findings of this present study agreed with earlier studies of antibacterial activity (16; 24; 27; 30) and antifungal activity (12; 16).

Table (1): Antimicrobial and antifungal activity of methanolic extract from the roots of *Glycyrrhiza glabra*.

Concentrations	Methanol. Extr (Inhibition zone -mm)								LSD Value
	0.0	0.5	1.0	1.5	2.0	2.5	St.	Ny.	
Microorganisms									
Bac. Gram +Ve									
<i>S. aureus</i>	5	18	22	28	31	35	27	-	6.382 *
<i>B. cereus</i>	5	15	20	25	29	33	23	-	6.303 *
Bac. Gram -Ve									
<i>E. coli</i>	5	13	18	23	28	31	21	-	5.493 *
<i>P. aeruginosa</i>	5	10	15	29	22	28	30	-	6.155 *
<i>S. typhi</i>	5	12	17	21	25	30	14	-	5.284 *
<i>Sh. dysenteriae</i>	5	14	19	23	28	31	19	-	4.827 *
Fungi									
<i>C. albicans</i>	5	8	10	13	15	18	-	11	4.251 *

St. / Ny. =Positive control, St. = Streptomycin, Ny. = Nystatin. 0.0 = Negative control (D.W.), * (P<0.01).

Table (2): Antimicrobial and antifungal activity of methanolic extract from the stems of *Glycyrrhiza glabra*.

Concentrations Microorganisms	Methanol. Extr (Inhibition zone -mm)								LSD Value
	0.0	0.5	1.0	1.5	2.0	2.5	St.	Ny.	
Bac. Gram +Ve									
<i>S. aureus</i>	5	16	20	25	30	34	26	-	6.377 *
<i>B. cereus</i>	5	14	18	22	27	31	23	-	5.938 *
Bac. Gram -Ve									
<i>E. coli</i>	5	11	16	20	25	29	22	-	4.728 *
<i>P. aeruginosa</i>	5	9	13	17	21	25	31	-	5.229 *
<i>S. typhi</i>	5	11	15	20	24	29	14	-	4.217 *
<i>Sh. dysenteriae</i>	5	12	16	21	25	30	19	-	4.885 *
Fungi									
<i>C. albicans</i>	5	6	9	11	13	15	-	12	3.638 *

St./ Ny. =Positive control, St.= Streptomycin, Ny.= Nystatin. 0.0 = Negative control (D.W.), * (P<0.01).

Table (3): Antimicrobial and antifungal activity of methanolic extract from the leaves of *Glycyrrhiza glabra*.

Concentrations Microorganisms	Methanol. Extr (Inhibition zone -mm)								LSD Value
	0.0	0.5	1.0	1.5	2.0	2.5	St.	Ny.	
Bac. Gram +Ve									
<i>S. aureus</i>	5	14	18	23	28	32	26	-	6.449 *
<i>B. cereus</i>	5	12	17	22	27	30	24	-	6.142 *
Bac. Gram -Ve									
<i>E. coli</i>	5	9	14	18	23	28	22	-	5.382 *
<i>P. aeruginosa</i>	5	8	13	17	19	23	30	-	5.483 *
<i>S. typhi</i>	5	8	12	16	20	25	13	-	4.746 *
<i>Sh. dysenteriae</i>	5	9	13	18	22	27	18	-	4.452 *
Fungi									
<i>C. albicans</i>	5	5	7	9	10	12	-	11	3.253 *

St./ Ny.= Positive control, St.= Streptomycin, Ny.= Nystatin. 0.0= Negative control (D.W.), * (P<0.01).

Table (4): Antimicrobial and antifungal activity of methanolic extract from the seeds of *Glycyrrhiza glabra*.

Concentrations	Methanol. Extr (Inhibition zone -mm)								LSD Value
	0.0	0.5	1.0	1.5	2.0	2.5	St.	Ny.	
Microorganisms									
Bac. Gram +Ve									
<i>S. aureus</i>	5	19	24	29	33	37	27	-	6.326 *
<i>B. cereus</i>	5	16	22	26	31	35	22	-	5.847 *
Bac. Gram -Ve									
<i>E. coli</i>	5	14	19	24	28	32	21	-	5.644 *
<i>P. aeruginosa</i>	5	12	17	21	25	30	31	-	5.352 *
<i>S. typhi</i>	5	13	18	22	27	31	15	-	4.869 *
<i>Sh. dysenteriae</i>	5	14	20	24	29	33	19	-	5.037 *
Fungi									
<i>C. albicans</i>	5	9	12	15	17	20	-	10	4.319 *

St. / Ny. =Positive control, St. = Streptomycin, Ny. = Nystatin. 0.0 = Negative control (D.W.), * (P<0.01).

Table (5): Antimicrobial and antifungal activity of methanolic extracts from the roots, stems, leaves and seeds of *Glycyrrhiza glabra*.

Concentrations	Methanol. Extr (Inhibition zone -mm)							LSD Value
	Roots	Stems	Leaves	Seeds	Control-	St.	Ny.	
Microorganisms								
Bac. Gram +Ve								
<i>S. aureus</i>	26.8	25.0	23.0	30.0	5	24.50	-	5.427 *
<i>B. cereus</i>	24.4	22.4	21.6	27.2	5	21.50	-	4.321 *
Bac. Gram -Ve								
<i>E. coli</i>	22.6	20.2	21.0	24.8	5	16.50	-	3.311 *
<i>P. aeruginosa</i>	20.8	17.0	20.4	24.8	5	20.25	-	4.573 *
<i>S. typhi</i>	21.0	19.8	17.2	22.6	5	12.25	-	4.734 *
<i>Sh. dysenteriae</i>	23.0	20.8	19.6	25.0	5	15.25	-	5.024 *
Fungi								
<i>C. albicans</i>	13.4	10.8	9.6	9.8	5	-	7.50	2.868 *

St. / Ny. =Positive control, St. = Streptomycin, Ny. = Nystatin. 0.0 = Negative control (D.W.), * (P<0.01).

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