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Detection of the bacterial activity of saponins and some mineral elements in the local aqueous extract of licorice

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ABSTRACT

The aim of the study was to detect and quantify saponins in the aqueous extract of licorice using HPLC technique. It was found that it contained the highest recorded concentration of Rhaoglucoglycyrrhizin in 335.37 µg/ml, while the lowest recorded concentration of Glycyrrhizin-20 methanoate µg /ml was 89.6. Also, the percentage of saponins in the aqueous and alcoholic extracts was 6.58% and 4.15%, respectively. The concentration of flavonoids and tannins in the aqueous extract of licorice were 18.99 and 6.28 ppm, respectively. The study also aimed to find the percentage of some major and minor metallic elements, including Fe, Na, Ca, K, Mg, Cu, Zn, Mn, and they were (1.382%, 2.04%, 5.5%, 5.38%, 4.186%, 1. 877%, 1.2%, 3.2%) respectively. The effect of the extract containing the saponins mentioned above was studied in two bacterial species (Serratia, Klesiella Pneumoniae) using brain diffusion agar and then the added concentrations (100, 50,25,12.5)mg / Ml of the extract. The transparent zone of the bacterial growth inhibitor showed a concentration of 100 mg / ml inhibitory zone for Klesiella Pneumoniae and 27 mm for Serratia .

Keywords: color reagents, aqueous extract, saponins, elements , Serratia, Klesiella Pneumoniae.

INTRODUCTION

Licorice herb (Glycyrrhiza glabra), which is used to treat many diseases, as licorice roots are the main commercial important source of drugs and medicines because they contain active substances such as (Glycyrrhiza) derived from the Greek word glykos meaning sweet and rhiza meaning root) ie sweet root [(1)]. A study in Japan showed that this substance is effective in treating chronic hepatitis and cirrhosis of the liver, as it was found that 75% of patients with liver failure had an increase in their liver activity after using a dose of 240 mg per day for only one month[(2)]. It has been observed that the herb has an effective role in activating the aldosterone-angiotensin-renin system, as it is believed that the soapy substances present in the licorice herb activate the effectiveness of aldosteron through its association with the receptors of the mineral cortex in the kidneys [(3)]. The study showed the use of licorice in the treatment of injuries Bacterial, viral and fungal, and that some of the compounds derived from it are of great importance in the treatment of some cancerous diseases. Glabridin acts as an anti-division of breast cells ex vivo, in addition to its use in the treatment of gastrointestinal ulcers. It increases the secretion of the mucous glands of the system that helps treat ulcers [(4)], and research has proven that the plant possesses an oxidant-anti-oxidant activity.[(5,6)]
The active substance in licorice is glycerite, and it has been proven that licorice contains sugary substances and mineral salts, the most important of which are potassium, calcium, magnesium, phosphorous, and soapy substances that cause foam when pouring its juice, and it also contains volatile oil. In the year (1955-1960) a steroid compound was separated and called glycerrhysic acid from the roots of the licorice plant. [(7)]

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Saponins are bioorganic compounds that are found in many plants and foods. These compounds are part of the group of phytosterols, saponins and are nutrients that have many health benefits such as lowering cholesterol, helping to control blood sugar levels and preventing the onset of cancer. It has anti-inflammatory, antioxidant, anticancer, immunostimulant, cytotoxic and antimicrobial properties [8].

Saponins are powerful antioxidants that protect cells from free radicals, and help prevent changes in DNA that can lead to diseases such as cancer. In addition, its antioxidant power also reduces the formation of atherosclerotic plaques in the blood vessels, which prevents problems such as heart attack and stroke. Saponins reduce cholesterol levels in the blood and liver, as they reduce the absorption of cholesterol from food in the intestine. In addition, they increase the excretion of cholesterol in the stool by increasing the elimination of bile acids [9,10].

MATERIALS AND METHODS

- Preparation of the aqueous extract:
  10 gm of dry ground licorice root was dissolved in 200 ml of distilled water for 24 hours with continuous stirring, then filtered. The extract concentrated and kept in refrigeration until use [11].
- Preparing the inductive reagents:

1. Detection of alkaloid: Alkaloid was detected by adding drops of Mayer's reagent to 1 ml of the extract to form a white to pink color [12]
2. Detection of sterols: adding some drops of sulfuric acid to 1 ml of the extract to give a red color [13]
3. Detection of saponites: Mix 1 ml with (1 cm^3) of (1% mercury chloride $HgCl_2$) to form a white precipitate [14]
4. Detection of turbines: Detection was carried out using turm-hill detection [15].
5. Detection of tannins and phenols using glacial acetic acid [16]
6. Detection of glycosides: using sodium hydroxide solution [17]
7. Detection of flavonoids using ferric chloride [18]

- Quantitative determination of saponins:
  according to the method used by [19]) with aqueous and alcoholic solutions only [19]
- Quantitative determination of flavonoids: An estimated weight of 25 g using a mixture of methanol and chlorform only [20]
- Quantitative determination of tannin: 10 gm of licorice root [21]
- Quantitative and qualitative determination of saponins in aqueous extract using HPLC only [22]
- Determination of mineral elements: some mineral elements were estimated by atomic abs Chemicals and instruments.
- Antibacterial activity
  The antibacterial potential of the prepared Samples [23] was investigated against Gram's negative and Gram's positive bacterial strains using agar well diffusion assay [24, 25]. About 20mL of on Muller–Hinton (MH) agar was aseptically poured into sterile Petri dishes. The bacterial species were collected from their stock cultures using a sterile wire loop [25]. After culturing the organisms, 6 mm-diameter wells were bored on the agar plates using of a sterile tip. Into the bored wells, different concentrations of aqueous extract of licorice were used. The cultured plates containing aqueous extract of licorice and the test organisms were incubated overnight at 37°C before measuring and recording the average the zones of inhibition diameter [26,27].
- Statistical analysis.
  Data were statically analysis using Graphpad prism program [28]. Data are represented as mean ± SD of three experiments. Indicate statistically significant difference at $p<0.05$ [29,30].

RESULTS AND DISCUSSION

It is evident from table (1) that the aqueous extract of licorice contains alkaloids, saponins, tannins and glycosides, which are secondary metabolites.

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TABLE 1: Phytochemical screening of the aqueous extract of licorice

Phytochemical	**Test**	**Result**
Alkaloids	Alkaloids	+
Phytosterol	Salkowski	-
	Libermann-Burchard	
Saponins	$HgCl_2$	+
Terpenoids	Trim-Hill	-
phenolic compounds and tannins	Ferric chloride	+
Glycosides	Alkaline reag.	+

(+) positive detection
(-) negative detection
It was found through the current study that the percentage of saponins in the aqueous extract was 6.58%, while that of the alcoholic extract was 4.15%. It was found through the current study that the concentration of flavonoids is 18.99 ppm, while the concentration of tannin is 6.23 ppm

TABLE 2: shows the percentage of saponins isolated from licorice using different solvents

Saponins	**Solvents**	**% saponins**
	Aqueous	6.58%
	Alcoholic	4.15%

TABLE 3: Concentration of flavonoids and tannins isolated from licorice

Licorice	**Extracts**	**Con. Ppm**
	Flavonoids	18.99
	Tannin	6.28

Figure (1) showed the standard form of saponins separated by HPLC using a separation column C18,4.6mm x150mm,5µ.

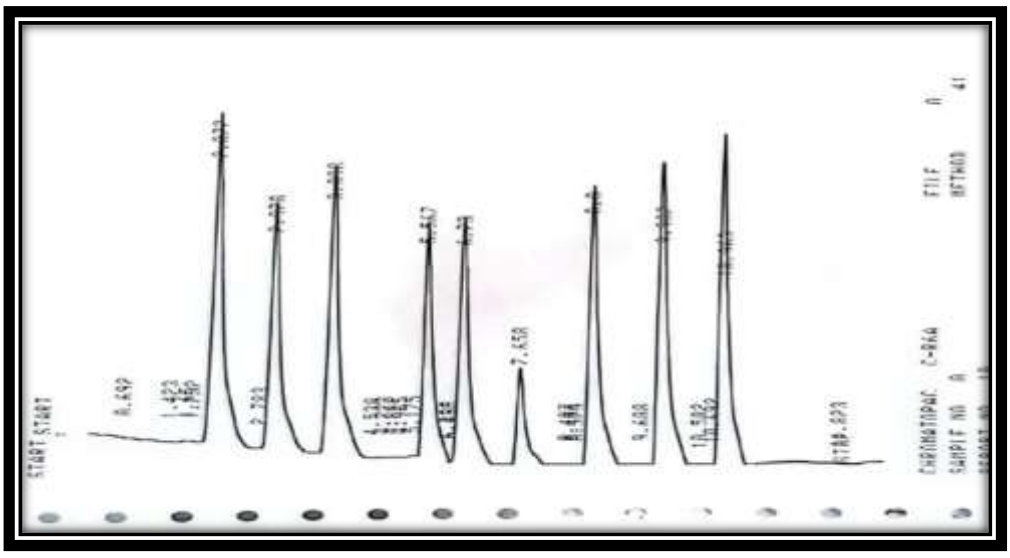

Figure 1: The standard form of saponins.

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Table 4: shows the standard saponins, the retention time and the separated beam area by HPLC device.

Seq	Sapoinine	R.t	Aare	Con.µg/ml
	30-yrrhydroxyglycyrrhizin	2.07	69104	50 µg/ml
	24-hydroxyglucoglycrrhizin	2.97	94627	50 µg/ml
	Glycyrrhizin-20 methanoate	3.89	59352	50 µg/ml
	Rhaoglycyrrhizin	5.65	49002	50 µg/ml
	11-deoxorhaoglycyrrhizin	6.73	51061	50 µg/ml
	Rhaoglucoglycyrrhizin	7.65	20004	25 µg/ml
	Rhaogalactoglycyrrhizin	8.8	60053	50 µg/ml
	11-deoxo-20- & glycyrrhizin	9.90	65399	50 µg/ml
	Glucoliquiritin apioside	10.96	72510	50 µg/ml

Figure (2) showed the two saponins separated from licorice, the time of detention and the area of the bundle separated by the HPLC using a separation column C18,4.6mm x150mm,5µ.

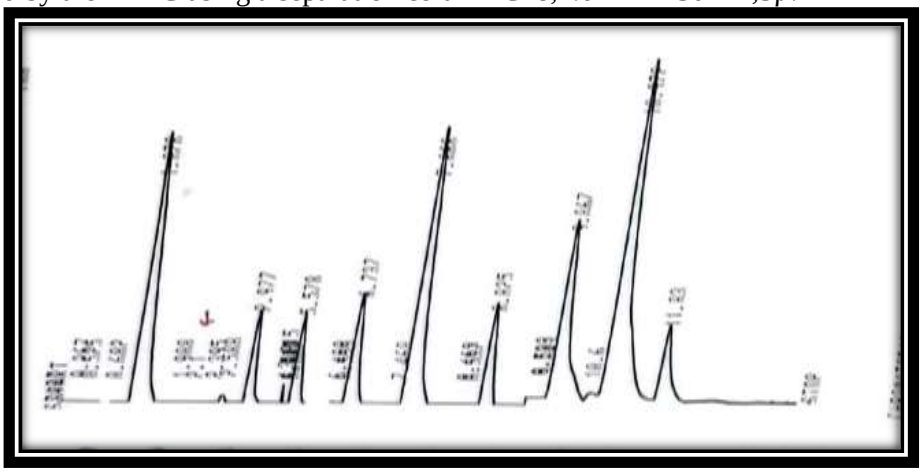

Figure 2: saponins separated from aqueous extract of licorice root.

TABLE 5: The separated saponin concentration, retention time, and top area of the aqueous extract of licorice.

Seq	Sapoinine	R.t	Eare	Con. µg /ml
	30-yrrhydroxyglycyrrhizin	2.1	5000	180.08 µg
	24-hydroxyglucoglycrrhizin	2.97	42638	214.79 µg
	Glycyrrhizin-20 methanoate	3.89	21273	89.6 µg
	Rhaoglycyrrhizin	5.65	36740	187.44 µg
	11-deoxorhaoglycyrrhizin	6.73	37010	181.2 µg
	Rhaoglucoglycyrrhizin	7.65	56860	335.37 µg
	Rhaogalactoglycyrrhizin	8.8	28733	119.6 µg
	11-deoxo-20- & glycyrrhizin	9.90	61096	233.5 µg
	Glucoliquiritin apioside	10.96	92637	319.39 µg

TABLE 6: shows the percentage of some mineral elements in licorice.

Minerals	%
Fe	1. 3824
Na	2.04
Ca	5.5
K	5.38
Mg	4. 186
Cu	1. 877
Zn	1.2
Mn	3.2

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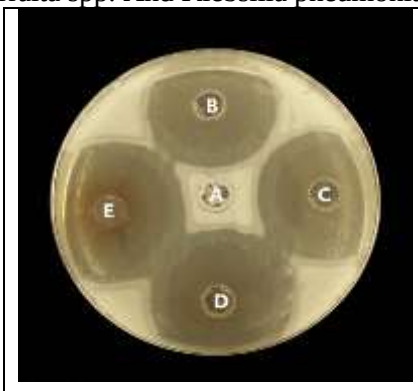
Result of antibacterial activity with different concentration shown by the figures below all details explained by table (6)

TABLE 6: Explain the antibacterial activity of aqueous extract of licorice

Antibacterial analysis (Inhibition Zone)						
sample		A	B	C	D	E
Serratia	aqueous extract of licorice	6	24	25	26	27
Klesiella Pneumoniae		6	19	21	23	26

Figure (3,4) shows the antibacterial activity Antibacterial activity of aqueous extract of licorice against Serraita spp. And Klebsilla pneumoniae.



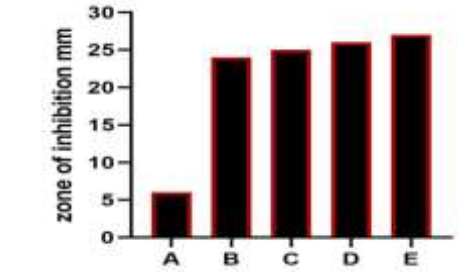
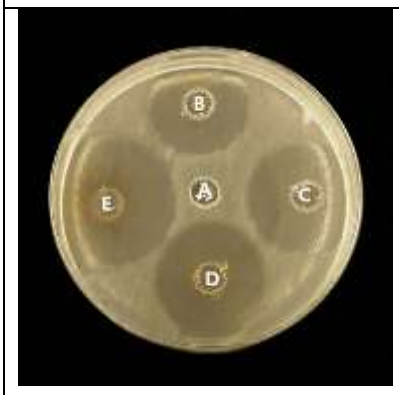


Figure. 3: Antibacterial activity of aqueous extract of licorice against Serraita. A, control negative . B, 12.5%. C,25 %. D,50 %. E, 100 %



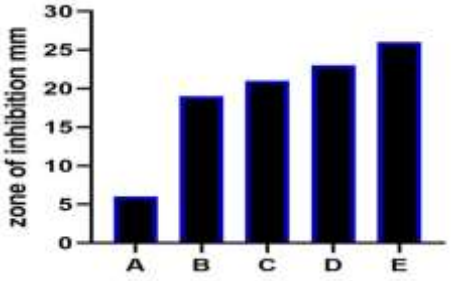


Figure 4: Antibacterial activity of aqueous extract of licorice against Klebsilla pneumoniae. A, control negative . B, 12.5%. C,25 %. D,50 %. E, 100 %

DISCUSSION

The results of the current study are consistent with what was found by Sahi and Khudair [31,32], where the latter found that the aqueous extract of licorice contains saponins and flavonoids, while the latter noted that the aqueous extract of licorice did not contain alkaloids. The results of the current study do not agree with Hamza [33], where the latter found that the alcoholic extract contains flavonoids, saponins and resins, while it does not contain alcoedes. Also, the results of the current study agree with Ayoub [34], where he indicated that the aqueous extract contains flavonoids, saponins, glycosides and tannin.

Also, the results of the current study are consistent with the result of Kumar et al. [35] who found that the aqueous extract contains saponins, tannins and flavonoids. While it does not contain alkaloids.

It was found through the current study that the percentage of saponins in the aqueous extract was 6.58%, while that of the alcoholic extract was 4.15%. The results of the current study agree with Al-Ajimi [36], where the latter found that the percentage of saponins in licorice is (4-24)% in the aqueous extract.

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The current study agrees with what was reached by Newall et al [37]. The results of the current study do not agree with what was found by Mohammed [38], where the latter found that the concentration of flavonoids in the aqueous extract is 2.3ppm and the reason for this may be due to the type of environment in which the plant was grown. The results of the current study are in agreement with Roth [39], where the latter found that the aqueous extract contained flavonoids and phenolic compounds. The roots of licorice contain multiple compounds, the most important of which is saponin, known as glycerin It has a sweet taste that is more than fifty times more sugary than the sugar produced from sugar cane and bee honey. It is found in the form of calcium and potassium salts at a rate of 15%-2, and when hydrolyzed or enzymatically decomposed, a compound known as and Liquiritin Chalcones ,Flavans, Flavones, Glabridin [40]. The roots contain flavonoids that give the roots a bright yellow color, gums and 15% Resinous Oils and other substances, including 2-6% asparagine, mannitol and atropine [41]. Atropine, coumarins, choline, betaine and hormone-related substances Progestogen and steroids similar to adrenocorticotropic hormone [42]. From the quantitative estimation of the aqueous extract of licorice, it was found that it contained many saponins. It contains the highest 30-yrrhydroxyglycyrrhizin,24-hydroxyglucoglycerrhizin, Glycyrrhizin-20 methanoate, Rhaoglycyrrhizin, 11-deoxorhaoglycyrrhizin, Rhaoglucoglycyrrhizin, quirkyrhaoglycyrrhizin 180, , 214.79, 89.6, 187.44, 181.2, 335.37, 119.6, 233.5, 319.39) in µg /ml units, respectively .The highest recorded concentration was Rhaoglucoglycyrrhizin 335.37 µg /ml, while the record concentration of Glycyrrhizin-20 methanoate was 89.6 µg /ml. The results of the current study are in agreement with the findings of Andrisano et al. [43] that licorice contains the soapy compounds glycyrrhetic acid and 24-hydroxy-18 beta-glycyrrhetic acid. Finally, the alcoholic extract of licorice contains glycyrrhizin using HPLC.

The results of the current study agree with Mahmoud et al. [38], where the latter found that licorice contains Glycyrrhizin and Glycyrrhetinic acid, Glabridin. The results of the study agree with what was found by Ayoub [34], where the latter found that licorice contains major and minor essential mineral elements. Through the current study, it was found that the percentage of calcium is 5.5%, potassium is 5.38%, and magnesium is 4.186%, while Ayoub found that the percentage of potassium is 2.9%, while calcium is 2.1% and magnesium 1.3%[34].

CONCLUSION

The method of separation of saponins aqueous extract from licorice by HPLC technique, the concentration of saponins in the licorice extract was found to be close after comparing with different concentrations of the standard material . It was found that it contains some metallic elements, it has the ability to inhibition some types of bacteria that infect the respiratory system.

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