# AQUEOUS AND ALCOHOLIC EXTRACTS FROM *GLYCYRRHIZA GLABRA* AND THEIRS ACTIVITY AGAINST BACTERIA AND RHABDOMYO SARCOMAS

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Active substances such as Saponins (41.1 %), volatile oils (2.5 %) and Tannins (13.2 %) are isolated from *Glycyrrhiza glabra* plant, and some mineral elements such as sodium (203 ppm), calcium (176 ppm) and potassium (181 ppm) respectively from the *Glycyrrhiza glabra* seeds. The concentration of the mineral elements was measured by using Flame Spectrometer. Also a study of anti-bacterial activity of the extracts was made using two types of pathogenic bacteria viz., Escherichia Coli and aurous Staphylococcus showed the ability of inhibition for all different extracts by vary inhibition diameters for different active substances, concentrations and bacteria. One type of cancer cellular line used to study the effect of *Glycyrrhiza glabra* extracts were studied on the growth of *Rhabdomyo sarcomas* (RD) cell line in human by using *in vitro* system and compared with anticancer drug cisplatin (cis-pt) as a positive control. The cancer cell were treated with different concentration for each of the three treatments and cis-pt after 72 h exposure time. The cytotoxic activity was tested by inhibition rate as parameter. The results showed significant differences ($p < 0.05$) for each three treatments when the inhibition rates were increased. There was strong correlation between the three treatments and the different concentrations in comparison with cisplatin.

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## Introduction

Liquorice or licorice is the root of *Glycyrrhiza glabra* from which a somewhat sweet flavor can be extracted. The liquorice plant is a legume (related to beans and peas) that is native to southern Europe and parts of Asia. It is not botanically related to anise, star anise, or fennel, which are sources of similar flavouring compounds.

**Figure 1.** Glycyrrhiza glabra

The source of scent of liquorice root is from a complex and variable combination of compounds present in the root, of which anethole is a minor component (0-3 % of total volatiles). Much of the sweetness in liquorice, which is obtained by boiling liquorice root and evaporating most of the water, is due to glycyrrhizin, which has 30–50 times the sweetness of sugar. The sweetness is very different from that of sugar, being less instant and longer lasting. Liquorice extract is marketed both in solid and syrup form.

The isoflavene glabrene and the isoflavane glabridin, found in the roots of liquorice, are xenoestrogens. [1]

**_Glycyrrhiza glabra_ from Koehler's Medicinal-Plants**

The compound glycyrrhizic acid, found in liquorice, is now routinely used throughout Japan for the treatment and control of chronic viral hepatitis, and there is a possible transaminase-lowering effect.[2] Hepatoprotective mechanisms have been demonstrated in mice.[3] Recent studies indicated that glycyrrhizic acid disrupts latent Kaposi's sarcoma (also demonstrated with other herpesvirus infections in the active stage), exhibiting a strong anti-viral effect.[4] The Chinese use liquorice to treat tuberculosis.[5]

Liquorice affects the body's endocrine system as it contains isoflavones (phytoestrogens). It might lower the amount of serum testosterone slightly [6], but whether it affects the amount of free testosterone is unclear. Consuming liquorice may prevent the development of hyperkalemia in persons on hemodialysis.[7] Large doses of glycyrrhizinic and glycyrrhetinic acid in liquorice extract can lead to hypokalemia and serious increases in blood pressure, a syndrome known as apparent mineralocorticoid excess. These side effects stem from the inhibition of the enzyme 11-hydroxysteroid dehydrogenase (type 2) and subsequent increase in activity of cortisol on the kidney. Cortisol acts at the same receptor as the hormone aldosterone in the kidney and the effects mimic aldosterone excess, although aldosterone remains low or normal during liquorice overdose. To decrease the chances of these serious side effects, deglycyrrhizinated liquorice preparations are available. The disabling of similar enzymes in the gut by glycyrrhizinic acid and glycyrrhetinic acid also causes increased mucus and decreased acid secretion.

As it inhibits *Helicobacter pylori*, it is used as an aid for healing stomach and duodenal ulcers, and in moderate amounts may soothe an upset stomach. Liquorice can be used to treat ileitis, leaky gut syndrome, irritable bowel syndrome and Crohn's disease as it is antispasmodic in the bowels.[8]

The compound carbenoxolone, derived from liquorice, indicated that it inhibited 11-hydroxysteroid dehydrogenase (type 1), an enzyme that is highly expressed in liver and fat tissues, which plays a role in metabolism, and in the brain, where the same enzyme is involved in stress response that has been associated with age-related mental decline.[9]

Excessive consumption of liquorice or liquorice candy is known to be toxic to the liver[10] and cardiovascular system, and may produce hypertension[11] (acquired pseudohyperaldosteronism) and edema.[12] In occasional cases, blood pressure has increased with excessive consumption of liquorice tea, but such occasions are rare and reversible when the herb is withdrawn.[13] Most cases of hypertension from liquorice were caused by eating too much concentrated liquorice candy.[14] Doses as low as 50 grams (2 oz) of liquorice daily for two weeks can cause a significant rise in blood pressure.[15]

## Experimentals

(Cis-platin) (10 mg/20ml) was provided by Ebew (Austria). *Glycyrrhiza glabra* was obtained from the local market. *Glycyrrhiza glabra* was grounded and kept at a laboratory temperature until use. 40 g of powdered *Glycyrrhiza glabra* was placed in a conical flask containing 200 mL of distilled water and stirred with a magnetic blender for 30 minutes and then centrifuged for 15 minutes. The supernatant liquid was kept at 35 ℃ in an electric furnace until the extract was obtained hich was the suitably diluted to give 5, 10, 15, 20 and 25 % solutions,

Alcoholic extract was obtained from putting 50 g of *Glycyrrhiza glabra* powder in an extraction unit (Soxhlet) and 350 ml of 80 % ethanol was added and boiled at 40 ℃ for 12 hours . The alcoholic extract was then obtained by using Vacuum Rotary Evaporator at 35 °C.[16] The extract was the diluted to desired dilutions with 80 % ethanol.[17]

#### Isolation of active components

#### A: Tannins

Tannins were isolated from *Glycyrrhiza glabra* by adding 75 ml of distilled water to 0.5 g of *Glycyrrhiza glabra* powder. The mixture was placed in the boiling water bath for 30 minutes. It was then centrifuged at 200 cycle $min^{-1}$ for a period of 20 minutes .The solution was next transferred to a 100 mL flask and made up to the mark with distilled water. A 20 mL of of 4 % lead acetate was added and the mixture was continuously shaken for some time before filtering it. The sludge was dried at 70 °C in an electric furnace.[18]

#### B: Saponins

To 10 g of *Glycyrrhiza glabra* powder was added (50 mL of 20 % ethanol and then heated at 55 °C using a water bath for half an hour with constant stirring. It was then filtered and the filtrate was added to 100 ml of ethanol. This solution was then heated at 90 $^{o}$C in a water bath till the final volume was reduced to about 40 mL. It was then filtered and the filtrate was transferred to 20 mL of ether in a separating funnel. The ether mixture was shaken for some time and allowed to stand till the water and ether layers separated. The water layer was separated and added to 10 mL of n-butanol and shaken vigourously. The n-butanol was then evaporated in the water bath. The resulting solution was dried to get saponins.[17,18]

#### C: Volatile oils

Volatile oils were extracted from 5 g of *Glycyrrhiza glabra* powder mixed with 150 ml of ether in a Soxhlet flask. The extraction process was carried out for a period of 24 hours when the volatile oils were separated from the ether solvent.[19]

#### D: Determination of ash content

Ash content was estimated by burning 2 g of *Glycyrrhiza glabra* powder in an oven at 550 °C till a the powder turned into a gray italics white and then the weight of the form a second time to calculate the percentage of ash.[20]

#### E: Determination of moisture

The percentage of moisture was estimated from 2 g of *Glycyrrhiza glabra* powder which was heated at 60 ℃ in an electric furnace for a period of 24 hours. It was then taken out and weighed again to estimate the proportion of moisture.[20]

#### F: pH measurement

10 g of *Glycyrrhiza glabra* powder was blended with 100 ml of distilled water using magnetic stirrer for 10 minutes. The pH of the supernatant liquid wasmeasured using pH-Meter.

#### G: Qualitative detection of components

The components of *Glycyrrhiza glabra*, such as semi-alkaloids, carbohydrates, saponin and flavonoids, lipids, proteins, and tannins, were qualitatively detected from the extract of Glycyrrhiza glabra as follows.[20,21]

#### Study of the activity against bacteria

The sensitivity of bacteria, *Escherichia Coli* and *Aurous Staphylococcus* bacteria (isolated from *Glycyrrhiza glabra* and diagnosed in the culture laboratory of Children's Hospital in Ramadi) was measured using the Agar-well diffusion method as described by Kirby Baauer.[22] We also used the Mueller Hinton agar to test the sensitivity of bacteria from *Glycyrrhiza glabra* extracts, prepared using the process as per instructions of the company. It was then placed in Petri dishes which were put in an incubator at 37 °C for 24 hours. The inhibition diameter was then measured (inhibition zone)[22] in each hole by a ruler.

#### Preparation of standard solutions of isolated substances from *Glycyrrhiza glabra*:

A series of solutions containing 5%, 10%, 15% 20% 25% mg $mL^{-1}$ of different extracts were prepared.

**Study of cytotoxic effect on cancer cell line**

One type of cancer cell lines was used to study the impact of the extracts from *Glycyrrhiza glabra* powder on the growth of cells in laboratory to know the specifications of extracts to act as an antitumor ( work was carried out at the Department of Cancer Research in Biotechnology Research Center, University of Nahrain).

All solutions were prepared at the same center and culturing tissues were studied in vitro under optimum conditions by the same center. The growth media used in tissue culture technique was MEM (Minimum Essential Media), provided by Fetal Calf Serum (10 %) to form a confluent monolyer, then Subculture to discard the previous growth medium and the cell washed with sterilized phosphate buffer solution (PBS) by autoclave at 121 $^{o}$C for 15 min and addition 2-3 min and moving the culture flask kindness. The trypsin-versene solution to discard and cells incubated at 37 $^{o}$C until the cell separation from ground flask, added new growth media and redistribution of cells at the microtiter and incubated at 37 $^{o}$C.[23]

**Statistical Analysis**

Data were analyzed by ANOVA. Investigation of differences between cis-platin and the relation with other groups by toward using the statistical program (SPSS) within significant level ($p<0.05$) [24].

## Result and discussion

The results of the phytochemical (screening of plant materials ) studies of the *Glycyrrhiza glabra* are presented in Table 1.

**Table 1.** Chemical substances effective in *Glycyrrhiza glabra*

| Active Compounds | Reagents | Indicators | Results |
|---|---|---|---|
| Alkaloids | Draggendorff | orange | + |
| Tannins | $FeCl_3$, Lead acetate | Greenish blue solution, ge-latinous ppt. | + |
| Intense Tannins | Lead acetate | Light Brown ppt. | + |
| Flavonoids | Ammonia solution | Yellow solution | + |
| Amino acids | Alnhidran | purple | + |
| Phenols | Potassium ferrocyanide | Greenish blue ppt. | + |
| Resins | Aq. HCl | Turbid | - |
| Terpenoids | Salkowski | dark red | + |
| Saponins | $HgCl_2$ | white ppt. | + |
| Carbohydrates | α-naphthol | Purple | + |
| Loco antho-cyanidins | Aq. HCl | Red ppt. | + |
| Steriods | Terpenoids reagent after 1 d | Blueish solution | + |
| Glycosides | Benedict | red precipitate | + |

(+) indicate the positive test; (-)indicate the positive test

According to the results shown in Table 1. the aqueous extract of *Glycyrrhiza glabra* plant contains flavonoids, phenols, terpenoids and tannins, etc.

Table 2 and Fig. 1 show the percentage of active contents that were isolated from *Glycyrrhiza glabra* and the percentage of saponins, tannins and volatile oils are (41.1 %, 2.5 % and 13.2 %) respectively, where it is noted that the percentage of moisture (2.1 %) , ash (1.6 % ) and pH (6.35).

The percentage values in the plant depend on several factors, including climatic related to different temperatures and different seasons of the year as the high temperature leads to the loss of more water, and that the decline reduces the loss of water from the plant.[17]

Total carbohydrates = 100 - (% moisture +% crude protein

+% crude fat +% ash content)

The percentage of protein and carbohydrates about 96.3%. which is consistent with the calculated ratios of previous studies.[25]

**Table 2.** Percentage of active combatants in Glycyrrhiza glabra

| Active combatants | Percentage |
|---|---|
| Volatile oils | 2.5 % |
| Tannins | 13.2% |
| Saponins | 41.1% |
| Moisture | 2.1% |
| PH | 6.35 |
| Ash | 1.6% |

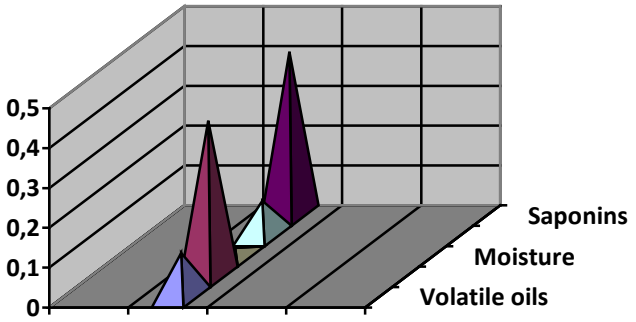


**Fig. 1.** The percentage of extracted material

Table 3 and Fig. 2. show the amount of mineral elements in *Glycyrrhiza glabra* where the result showed that contains sodium (203) ppm, calcium (176) ppm and potassium (181) ppm, all are important functional and metabolic metals for body.[24]

**Table 3.** The amount of mineral elements in *Glycyrrhiza glabra* measured by Flame Spectrometer.

| Concentration (ppm) | Symbol | Element |
|---|---|---|
| Sodium | Na | 203 |
| Calcium | Ca | 176 |
| Potassium | K | 181 |

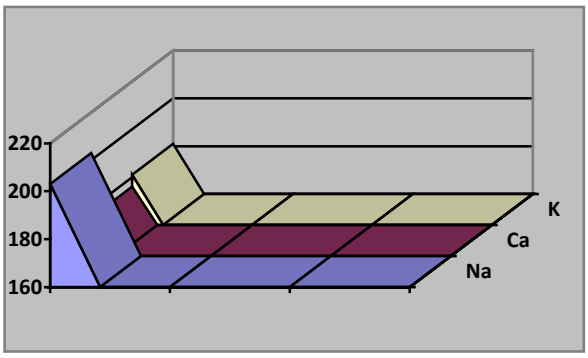


**Figure 2.** Shows the percentages of elements in the extract of *Glycyrrhiza glabra*

Tables 4 and 5 shows a the anti-bacterial activity results of *Glycyrrhiza glabra* extracts. The activity have been studying for these extracts separately, in different concentrations and using two types of pathogenic bacteria *Escherichia Coli* and *Aurous Staphylococcus*. The water extracts of *Glycyrrhiza glabra* shown higher activity at 25 mg $ml^{-1}$, where inhibition diameter was 15 mm for *Staphylococcus aurous* and 12 mm for *Escherichia Coli,* followed by the rest of the varying concentrations and rates Table 4.

The inhibition action of alcohol extract is due to it contained *Glycyrrhiza glabra*, flavonoids, tannins, and include some of phenolic compounds which have a biological influence on many bacteria races due to the presence of hydroxyl groups ($-OH^-$), as it have the ability to form hydrogen bonds between hydroxyl group in these compounds and water molecules in bacterial cell, were water is (90 %) of weight and that will disables dynamic actions in bacterial cell.[26] These compounds as phenolic compounds have the ability to coagulation the bacterial cell proteins and destroy enzymes involved in the manufacture of necessary amino acids to increase cell division.[27]

**Table 4.** Effect of aqueous extract of *Glycyrrhiza glabra* in different concentrations on growth of pathogenetic bacterial races

| Conc. in mg $ml^{-1}$ | Inhibition diameter | |
|---|---|---|
| | *Aurous Staphylococcus* | *Escherichia Coli* |
| 25 | 12 | 13 |
| 20 | 11 | 10 |
| 10 | 9 | 9 |
| 10 | 7 | 7 |
| 5 | 4 | 5 |

**Table 5.** Effect of alcoholic extract of *Glycyrrhiza glabra* in different concentrations on growth of pathogenesis bacterial races

| Conc. in mg $ml^{-1}$ | Inhibition diameter | |
|---|---|---|
| | *Aruginosa Pseudomonas* | *Escherichia Coli* |
| 25 | 12 | 15 |
| 20 | 10 | 12 |
| 10 | 8 | 10 |
| 10 | 7 | 8 |
| 5 | 5 | 6 |

In general, from Tables 4 and 5 for all extract and in all prepared concentrations the influence almost equal against bacteria *Aurous Staphylococcus* and *Escherichia Coli*. It found that inhibition diameter for aqueous extract is bigger than for the rest of the extracts of other due to the varying rate of active materials in different extracts.

**Study of Cytotoxic Effect**

Cancer cell lines were used to study the effect of Glycyrrhiza glabra extracts on the growth of cells in laboratory to know the specifications of extracts as anti-tumors. Cancer cell line type mice transformed cell line (RD) used with different concentrations comparable with anticancer drug cisplatin as a positive control after 72 h. exposure time.[30]

In this method, we calculate the proportion of cells number within the optimal conditions for growth without the addition of extracts so the output is the control group (control). Then extracts are added for the purpose of knowing their effects on cell growth in elected lines.

Extracts were divided into three groups, first group included a hot water extract, second group included hot alcoholic extract, third group Cis-Pt.

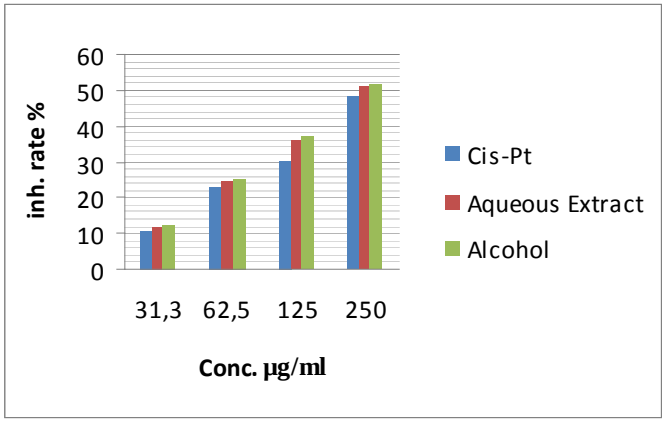


**Figure 3.** The comparison of inhibition rates between three treatments with cis-pt drug in cell line (RD)

**Table 6.** Inhibition effect on cancer line (RD) with different concentrations of extract and cis-pt after 72 h exposure time.

| Treatment conc. µg $ml^{-1}$ | Inhibition rates % | | |
|---|---|---|---|
| | Cis-Pt | Aqueous Extract | Alcohol Extract |
| 31.25 | 10.55 | 11.98 | 12.10 |
| 62.5 | 22.95 | 24.88 | 25.49 |
| 125 | 30.1 | 35.65 | 36.99 |
| 250 | 47.97 | 50.76 | 51.45 |

The result statistically analyzed by one way ANOVA. the following results as Fig. 3. which demonstrates the impact of compounds on cells number ratio when using cell line (RD), it is clear that hot alcoholic extract have the greatest influence on the proportion of growth cell number and the effect was significantly ($p$ <0.05). This result is identical to those published in literature.[28,29]

Also the effect of aqueous extract was significant effect (P <0.05) but the percentage of inhibition - as in the scheme 4- less effect than alcoholic extract Table (6).

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