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COMPARISON BETWEEN RUTIN AND KAEMPFEROL CONTAIN IN ROOT AND LEA OF GLYCYRRHIZA GLABRA. L IN IRAQ

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ABSTRACT

Glycyrrhiza glabra (family leguminosae), commonly known as licorice, is an herbaceous perennial and has been used as a flavoring agent in foods and medicinal remedies for thousands of years. Licorice root has been widely used around the world to treat a cough since ancient times, it contains active compounds, including glycyrrhizin, Glycyrrhetinic acid, flavonoids, isoflavonoids, chalcones, Glycyrrhizin and Glycyrrhetinic acid are considered to be the most active components and are potent inhibitors of cortisol metabolism, due to their steroid-like structures.[1] The main types of flavonoid and compare amount between leaf and root significant part of Glycyrrhiza glabra. L. After drying, grinding, weighing, reflexes, rotary evaporator, wash with (hexane, n-butanol, and ethyl acetate), identification test, and HPTLC analysis, the result gives that root and leaf are rich in flavonoid (Rutin and Kaempferol) after comparing the result obtained suggested that root contain RUTIN and KAMPHEROL more than leaf extract.

KEYWORD: Glycyrrhiza glabra.L, leaf, root, rutin, kaempferol, HPTLC.

1-INTRODUCTION

Licorice root is the most widely use medicinal herbs worldwide and is the single most used herb in Chinese medicine today.[2]

The word" licorice" refers to the root of a plant called Glycyrrhiza glabra. It's native to Europe and Asia. Plant classified as a weed in those areas. The early Egyptians loved licorice root. They used it in tea as a cure-all concoction. Later, the licorice then imported to China where it becomes a significant important herb in Chinese medicinal tradition.[3]

Licorice, which belongs to the Leguminosae family, Magnoliopsida class, Glycrrhiza genus Glycyrrhiza glabra species.[4]

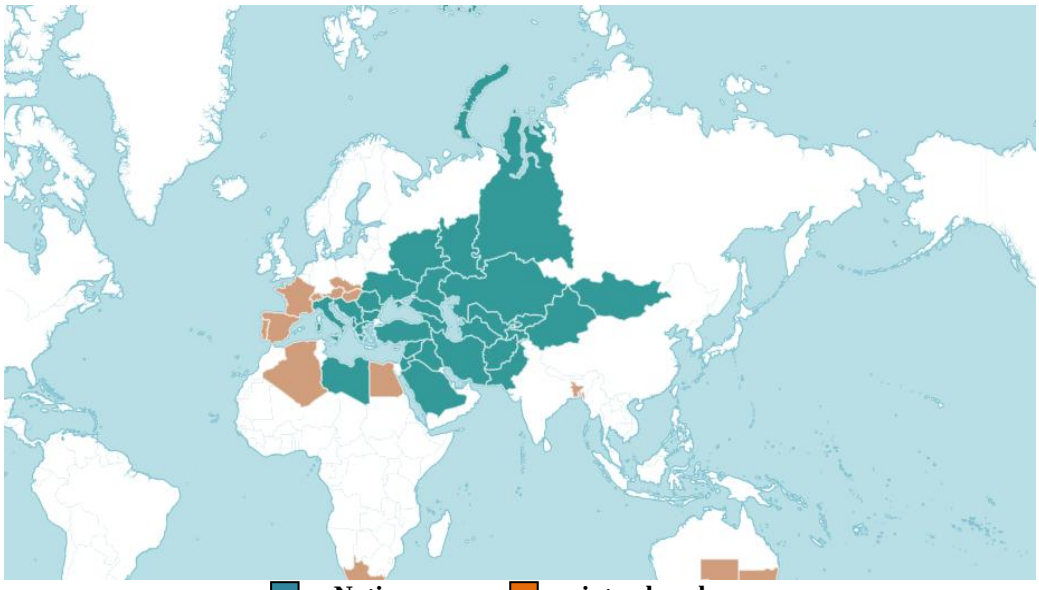


Figure 1: Distribution of Glycyrrhiza glabra. L.

As it point in figure1, Licorice distributed in Afghanistan, Albania, Bulgaria, Central European Rus, Cyprus, East Aegean Is., East European Russia, Greece, Iran, Iraq, Italy, Kazakhstan, Kirgizstan, Krym, Lebanon-Syria, Libya, Mongolia, North Caucasus, Pakistan, Palestine, Romania, Sardegna, Saudi Arabia, Sicilia, South European Russi, Tadzhikistan, Transcaucasus, Turkey, Turkmenistan, Ukraine, Uzbekistan, West Siberia, Xinjiang, Yugoslavia, and introduced to: Algeria, Austria, Bangladesh, Cape Provinces, Czechoslovakia, Egypt, France, Hungary, Maldives, New South Wales, Portugal, South Australia, Spain, Switzerland, Victoria.[5]

There are three species used as Licorice: Glycyrrhiza global. Glycyrrhiza uralensis Fisch, and Glycyrrhiza inflates Bat.[6]

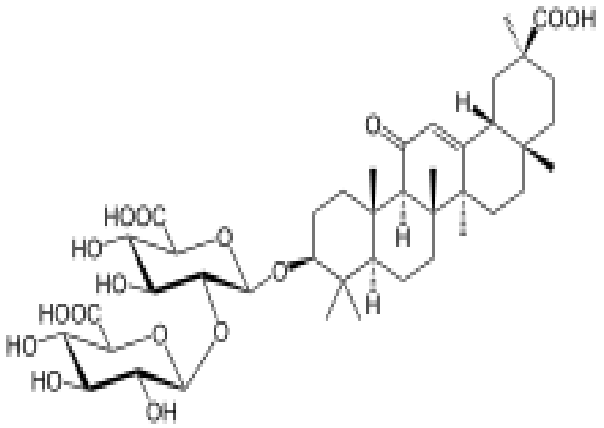


Figure 2: Glycyrrhizic acid structure.

Licorice root contains a variety of compound, including triterpenoids, polyphenols, and polysaccharides (starch, manners, and sucrose). Polyphenol includes phenolic acid, such as liquiritin, flavones, and flavans; chalcones; and isoflavonoids such as glabridin.[7,8,9] Also, the bright yellow color of the root is attributed to the flavonoid content especially liquiritin and isoliquiritin.[10] Plant gums, resins, and essential oil has been extracted; however, the root has been cultivated for the principle active glycoside glycyrrhizin.[11,12] The amount of glycyrrhizin varies from 7% to 10% or more depending on growing condition.[13] Glycyrrhizin, glycyrrhizin acid, and glycyrrhizinate amount of 10% to 25% of the root extract.[14] The ammoniated salt of glycyrrhizin has been manufactured to specification from licorice extract and use as a flavoring agent.[15] Carbenoxolone, a synthetic analog of glycyrrhizic acid, has using been used as a Pharmacological agent in the management of peptic ulcer,[16] Licorice is used for a variety of condition such as peptic ulcer, eczema, dyspepsia (indigestion, GERD), upper respiratory infections (cold, cough), weight loss menopause.[17]

Three extracts of different polarities of Glycyrrhiza glabra. L. leaves were characterized and evaluated for their antioxidant, anti-genotoxic and anti-inflammatory activity. The main components belong to the polyphenols family, being flavonoid and dihydro stilbene derivatives. The extracts had been investigating for their antioxidant, anti-genotoxic and anti-inflammatory activities, which are fundamental requirements of efficacious chemo-preventive agents.[18]

2- Method of work

2.1. Manually

1-Extraction of leaf

Take the leaf of Glycyrrhiza glabra.L and dry it, then grind it after grinding take 150 gm of glycyrrhiza glabra. L leaf powder and extract with 80% ethanol the volume use 350 ml (280 ethanol +70ml distill water) reflex for 3hrs after that make filtration to obtain the extract. Then put the extract in lottery evaporator to evaporate ethanol. Crud extract with water add hot water in separation final and take the lower layer after that we shake the lower layer with the following

1. Shake with hexane with drowning hexane.
2. Shake with ethyl acetate with drowning the ethyl acetate.
3. Lower layer shake & with n- butanol.

2-Extraction of root

Take the root of Glycyrrhiza glabra.L and dry it, then grind it Take 150 mg of Glycyrrhiza glabra.L root powder and extract with 80% ethanol the volume use 300(240 ml ethanol + 60 ml distill water) reflex for 3hrs after that make filtration to obtain the extract, then put the extraction in rotary evaporator in order to evaporate ethanol. Crud extract with water add hot water in separation final and take the lower layer after that we shake the lower layer with the following.

1. Shake with hexane with drowning hexane.
2. Shake with ethyl acetate with drowning the ethyl acetate.
3. Shake n-butanol with drowning the butanol.

After making separation of a thin layer with hexane, ethyl acetate and butanol we make the following tests to identify the contents of each extract.

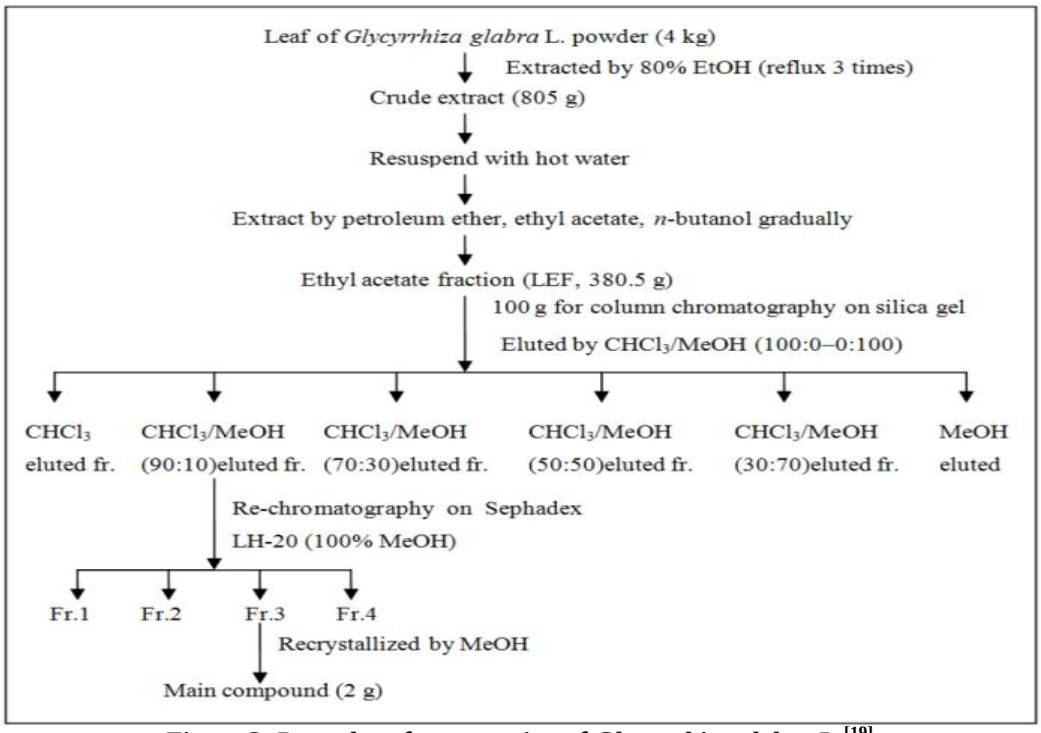


Figure 3: Procedure for extraction of Glycyrrhiza glabra L.[19]

2.2. Working test

1. Diterpenes
2. Phenol
3. Alkaloids
4. Flavonoids
5. Saponins (Foam test)
6. Terpenoid (Salkowski)
7. Glycoside (Benedict test)

1-Test for diterpenes

Powdered rosemary leaves (5gm), was extracted with ethanol by using soxhlet apparatus. Then the extract was concentrated to 2ml. Through evaporation by using a rotary evaporator. So the insoluble materials were being removed by filtration. Distilled water 4ml was adding to the solution, and the precipitate filter then the precipitated was treated with ethyl acetate and spotted on TLC plate and developed by elution with ethyl acetate: hex(1:4v/v) and. Chloroform: methanol (97:3) then sprayed with fecl3 solution10%w/v. The A yellow-green color of a spot was revealed to indicate the presence of diterpene.

2-Test for phenol

Alcoholic extract 0.5 ml was treat with treated with 3to 4drops of-ferric chloride appearance of intense green color indicated the presence of phenols.

3- Test for alkaloids

Alcoholic extract 3.0ml has acidifed with few drops of hydrochloric acid. Then a few drops of Wagner's reagent (that prepared by dissolving of iodine in potassium iodide) were added. The appearance of reddish brown precipitate indicates the presence of alkaloids.

4- Test for flavonoids

1-Alcoholic Koh (2-3ml) added to 1ml of methanolic extract of the plant. The yellow color is detected if flavonoids compounds are present.

2- Preliminary Tlc test for flavonoids Defatte methanolic extract 10ml concentrated under reduced pressure to about 3ml, and then 2ml of distilled water were added and partitioned with 20ml of ethyl acetate. Small amount of ethyl acetate layer was applied to investigate the flavonoids and separated on TLC plate coated with silica gel after development with chloroform 100-chloroform-ethyl acetate (60:40) and chloroform-methanol (96:4), the TLC plate was dried then the yellow spot revealed after spraying with aluminum chloride reagents (1gAlCl3 +100ml95% ethanol) and yellow/orange fluorescent spots were revealed under UV light at 366nm.

5-Test for saponins

Methanolic extract of the leaves 1ml was mixed with distilled water 5ml in the test tube and then shaken vigorously until persistent foam observed at least 1cm in height.

6- Test for terpenoids

(Salkowski test) Alcoholic extract of plant 5ml was mixed with chloroform 2ml and carefully added concentrated sulphuric acid 3ml a reddish brown color of an interface is formed to indicate the terpenoids.

7-Test for glycosides (Benedict's test)

Benedict's reagent 5 ml was added to the test tube to the methanolic extract (1ml), and then blend was heated in boiling water bath for 10 minutes. The green precipitate

appearance is a indication of the presence the reducing sugar.

Test for Flavonoid

The working layer after shaking with first hexane second with ethyl acetate third butanol The solution left who testing by ammonia and we find an intense color of yellow pigment which means the soluble flavonoid. The left part of Glycoside extracted boiling with five % HCL for 2hrs the set the free flavonoid.

2.3. HPTLC Analysis

After all these identification tests have been done, make HPTLC analysis of a separated mixture of the component of root and leaf and identify the individual, ingredient of the mix. HPTLC had a similar approach and employed the same physical principle of TLC (adsorption chromatography, the mobile phase flows through because of capillary action. Ingredient move according to their affinity towered adsorbent. Ingredient with more affinity towered the stationary phase will travels slower. The component with lesser affinity to stationary phase will go faster. Thus the elements separated on a chromatographic plate.[20]

The chromatographic condition

A- The mobile phase: will use is (acetic acid: formic acid: ethyl acetate: water) (2:2:30:4).

B-Stationary phase: HPT LC plates silica gel 60 F254 (Merck), 10 x 10cm or 20 x 10cm.

C-Sample application: 2uL test solution and 5uL standard is applied as 8mm bands, min, 2mm apart, 8mm from a lower edge of a plate.

D- Development: 20x20 cm or 20 x10 cm Twin Trough Chamber, saturated for 20 min (filter paper), 5mL (respective 10mL) development solvent per trough, development distance 70mm from lower edge of plate.

The plate dried with a hairdryer (cold air) for 5min.

These apparatus uses to complete the HPLC analysis.
1-Semi-auto / Sample Applicator: (Camag Linomat 5): For spot / line application, spray on technique, four modes of applications---Quantitative analysis, micro-preparative, in-situ and superimpose 10-method storage, stand-alone or PC control.

2-TLC Scanner & Data evaluation
Computer controlled Scanner / Densitometer for automatic spectrum scanning for identification and purity check.

3- Automatic Developing Chamber with Humidity Control (Camag ADC -2).

Automatic Developing Chamber for fully automatic development of TLC/HPTLC plates 20 x 10 cm and 10 x 10 cm (glass, plastic, aluminum).

4-Photodocumentation under GLP-(Camag Visualiser)

12 bit, high-resolution industrial camera (4096 grey level resolution. Image of the highest quality A fixed focus for total reproducibility.[21]

3-RESULT AND DISCUSSION

3.1. Result

Table 1: Glycyrrhiza glabra root In this table shows the effect of identification tests (phenol, diterpene, flavonoids, saponin, terpenoid, glycoside, alkaloids), that made for rotary root, root, root extract, n-butanol, ethyl acetate, hexane as shown below.

Part	Phenol	Flavonoid	Diterpin	Saponin	Terpenoid	Glycoside	Alkaloides
Rotary root	Positive	Positive	Positive	Positive	Positive	Positive	Positive
root	Negative	Positive	Positive	Negative	Positive	Negative	Positive
n-Butanol	Positive	Positive	Positive	Positive	Negative	Positive	Negative
Ethyl acetat	Negative	Positive	Positive	Negative	Positive	Negative	Positive
hexan	Negative	Positive	Positive	Negative	Positive	Positive	Negative

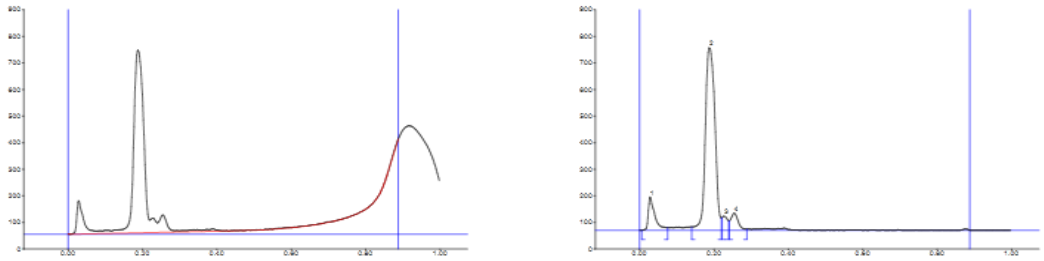
Table 2: Glycyrrhiza glabra leaf In this table shows the result of identification tests (phenol, diterpene, flavonoids, saponin, terpenoid, glycoside, alkaloids), that made for rotary leaf, leaf, leaf extract, n-butanol, ethyl acetate, hexane as shown below.

Part	Phenol	Flavonoid	diterpin	Saponin	terpenoid	glycoside	Alkaloides
Rotary leaf	Positive	Negative	Positive	Positive	Positive	Positive	Positive
Leaf	Positive	Positive	Positive	Positive	Negative	Positive	Positive
n-Butanol	Negative	Positive	Negative	Negative	Negative	Positive	Positive
Ethyl acetat	Positive	Negative	Positive	Negative	Positive	Negative	Positive
hexan	Negative	Negative	Positive	Negative	Positive	Negative	Positive

As a result, obtained from HPTLC analysis of Glycyrrhiza glabra.L leaf and root extract that shows the most type of flavonoid in leaf and root is Rutin and kaempferol in two wavelengths 254 and 366.

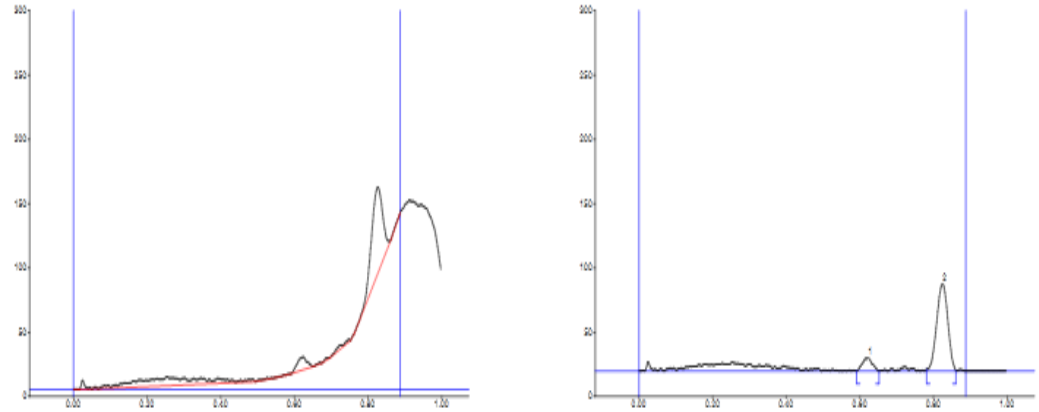
Wavelengths 254

Track 1, ID: Standard1



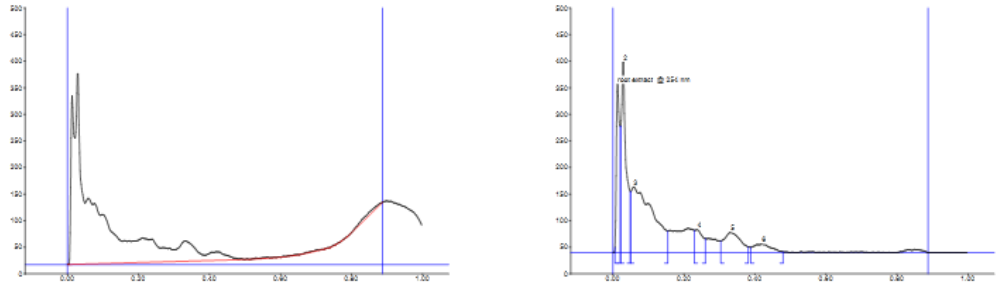
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	0.2	0.03	126.0	13.49	0.08	10.3	1753.7	10.14	unknown *
2	0.14	13.3	0.19	688.1	73.68	0.22	47.1	13869.2	80.19	unknown *
3	0.22	48.5	0.23	54.3	5.82	0.24	36.1	616.5	3.56	unknown *
4	0.24	36.4	0.26	65.6	7.02	0.29	5.1	1055.5	6.10	unknown *

Track 4, ID: Standard1

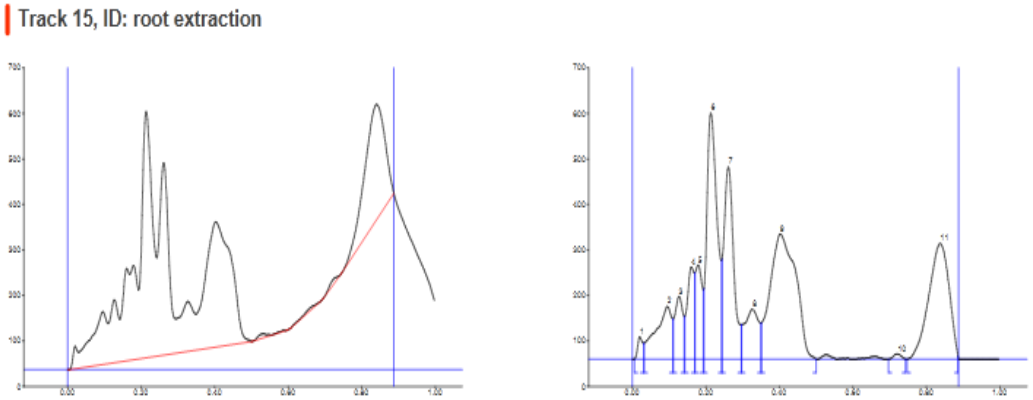


Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.59	0.0	0.62	10.1	12.95	0.65	0.0	213.0	11.75	unknown *
2	0.78	1.3	0.83	67.7	87.05	0.86	0.2	1599.4	88.25	unknown *

Track 10, ID: laef extraction



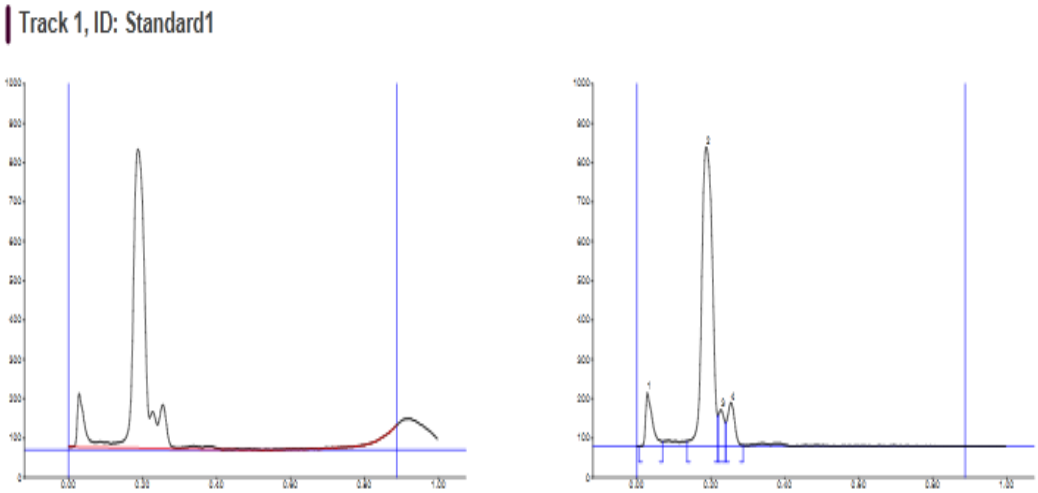
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	10.3	0.01	317.3	35.35	0.02	238.0	2357.2	15.56	root extract
2	0.02	238.1	0.03	358.5	39.94	0.05	115.0	3968.8	26.20	unknown *
3	0.05	115.2	0.06	123.8	13.79	0.16	40.8	5953.4	39.31	unknown *
4	0.23	42.3	0.24	43.5	4.84	0.26	26.6	801.3	5.29	unknown *
5	0.30	21.9	0.33	38.3	4.26	0.38	10.9	1412.3	9.32	unknown *
6	0.39	10.2	0.42	16.2	1.81	0.48	1.9	652.7	4.31	unknown *



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	0.2	0.02	49.5	2.12	0.03	35.4	534.1	0.85	unknown *
2	0.03	35.8	0.10	116.3	4.99	0.11	88.7	3914.4	6.21	unknown *
3	0.11	88.9	0.13	138.2	5.93	0.14	93.6	2395.6	3.80	unknown *
4	0.14	94.0	0.16	203.3	8.73	0.17	189.1	3107.7	4.93	unknown *
5	0.17	190.6	0.18	207.0	8.89	0.19	151.8	2984.9	4.74	unknown *
6	0.20	156.2	0.21	540.8	23.22	0.24	216.5	11862.9	18.82	unknown *
7	0.24	220.2	0.26	422.1	18.12	0.30	76.0	8811.2	13.98	unknown *
8	0.30	76.1	0.33	110.0	4.72	0.35	79.3	3282.3	5.21	unknown *
9	0.35	79.9	0.40	275.4	11.83	0.50	0.3	14989.7	23.78	unknown *
10	0.70	0.1	0.72	12.0	0.51	0.74	0.2	190.5	0.30	unknown *
11	0.75	0.2	0.84	254.6	10.93	0.89	7.0	10954.6	17.38	unknown *

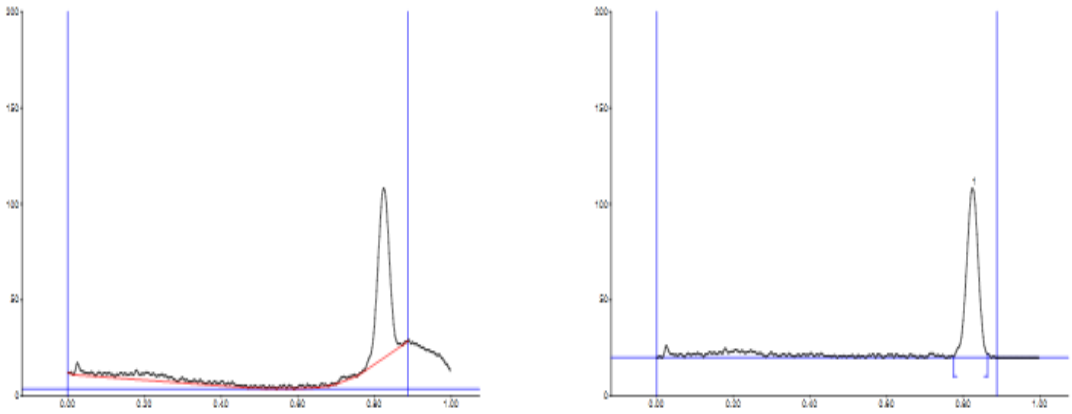
Figure 4: in this figure, we use standers ROUTINE and KAMPHROL root and leaf extract of glycyrrhiza glabella (in Wavelengths 254).

Wavelengths 366



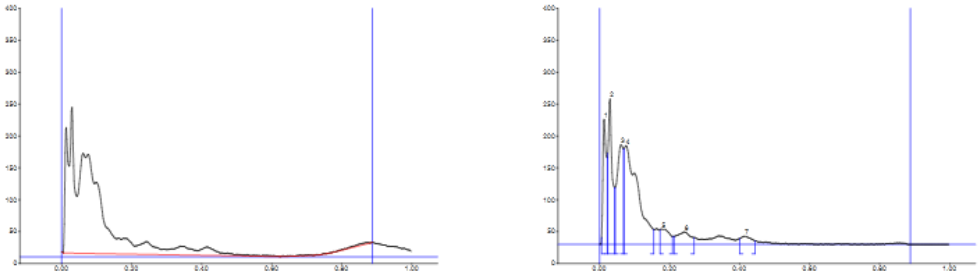
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	0.1	0.03	135.9	12.36	0.07	12.5	1939.1	8.96	unknown *
2	0.14	13.0	0.19	760.0	69.09	0.22	77.2	16858.4	77.90	unknown *
3	0.22	78.7	0.23	92.7	8.42	0.24	60.9	1128.8	5.22	unknown *
4	0.24	61.3	0.26	111.5	10.13	0.29	3.1	1714.7	7.92	unknown *

Track 4, ID: Standard1



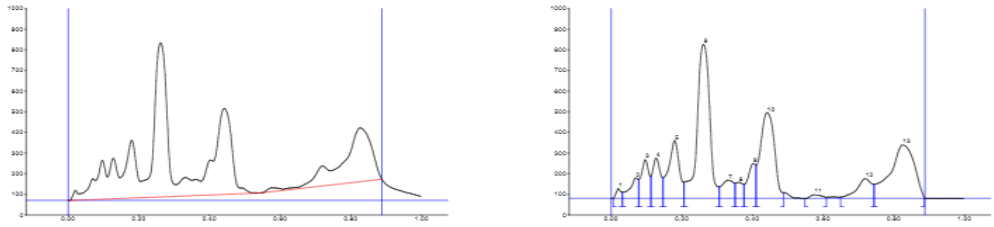
Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.78	0.4	0.83	88.4	100.00	0.87	1.9	2097.6	100.00	unknown *

Track 10, ID: laef extraction



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	11.5	0.01	196.9	24.73	0.02	136.3	1599.4	13.23	unknown *
2	0.02	143.4	0.03	228.8	28.74	0.04	89.5	2054.8	17.00	unknown *
3	0.04	89.6	0.06	156.8	19.70	0.07	150.2	2243.8	18.57	unknown *
4	0.07	150.5	0.08	155.3	19.51	0.16	24.0	4752.1	39.32	unknown *
5	0.17	23.2	0.18	24.7	3.10	0.21	12.8	516.1	4.27	unknown *
6	0.21	13.0	0.24	20.2	2.53	0.27	11.1	605.9	5.01	unknown *
7	0.40	9.7	0.42	13.3	1.67	0.44	6.2	313.8	2.60	unknown *

Track 15, ID: root extraction



Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %	Assigned substance
1	0.01	0.7	0.02	48.5	1.80	0.03	30.7	529.6	0.72	unknown *
2	0.03	30.9	0.07	100.9	3.75	0.08	92.4	1896.5	2.59	unknown *
3	0.08	93.2	0.10	188.7	7.01	0.11	106.9	3212.5	4.38	unknown *
4	0.11	110.2	0.13	196.3	7.29	0.15	100.5	3433.0	4.68	unknown *
5	0.15	100.6	0.18	280.5	10.42	0.21	78.7	6718.0	9.16	unknown *
6	0.21	79.4	0.26	746.8	27.74	0.31	57.0	20639.9	28.15	unknown *
7	0.31	57.1	0.33	89.3	3.32	0.35	74.8	2322.9	3.17	unknown *
8	0.35	74.9	0.36	78.7	2.92	0.38	68.5	1272.6	1.74	unknown *
9	0.38	68.6	0.40	169.3	6.29	0.41	163.9	2947.1	4.02	unknown *
10	0.41	164.0	0.44	417.3	15.50	0.49	28.0	12817.7	17.48	unknown *
11	0.55	0.1	0.58	19.2	0.71	0.61	5.3	464.5	0.63	unknown *
12	0.65	7.3	0.72	96.7	3.59	0.74	69.0	3205.5	4.37	unknown *
13	0.75	68.6	0.83	260.3	9.67	0.89	5.0	13874.0	18.92	unknown *

Figure 5: in this figure, we use standers ROUTINE and KAMPHROL root and leaf extract of glycyrrhiza glabella (in Wavelengths 366).

Appendix

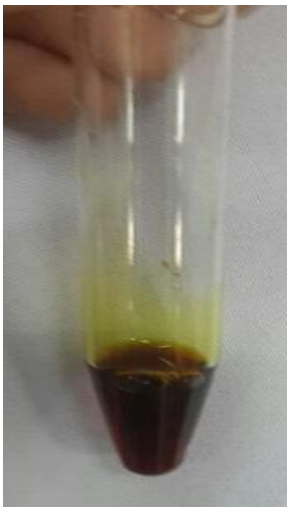
Test 1: Saponin

Rotary Root

Leaf Saponin

Test 2: Flavonoid

Rotary Root



Leaf Flavonoid

Test 3: Terpenoid

Leaf Terpenoid

Root Terpenoid

Test 4: Pheno.

Leaf Phenol

Rotary Root Phenol

Test 5: Benedict Test

Rotary Leaf Benedict

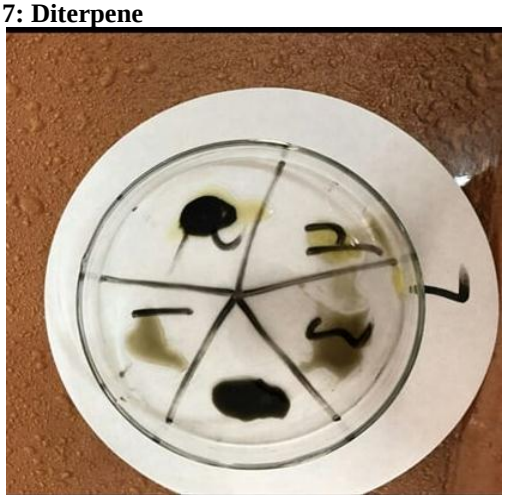
N-Buraol Root Benedict

Test 6: Alkaloid

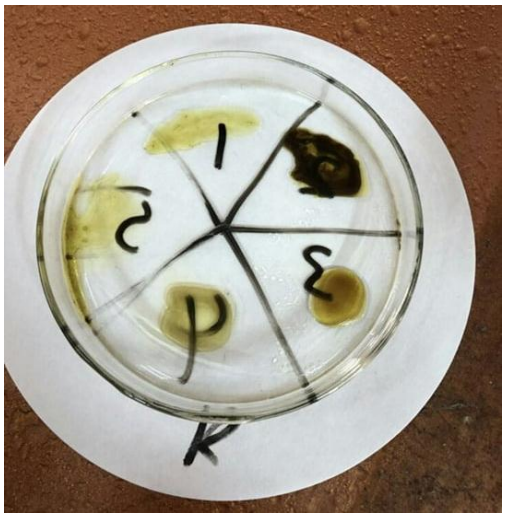
Leaf Alkalod

Root Alkaloid

Test 7: Diterpene



Leaf Diterpene



Diterpene Root

Apparatus Used to Complete HPTLC

Linomat 5. CAMAG

TLC Visualizer 2 CAMAG

Automatic developing chamber ASDC 2 CAMAG

3.2 DISCUSSION

Depend on the test for identification for Glycyrrhiza glabra root and leaf that Based the HPTLC analysis result for flavonoid for leaf and root that show rotary leaf & ethyl acetate contain rutin5.16%, 21.33% respectively and apigenin 49.38% and 55.91% respectively; n-butanol contain rutin11.89% kaempferol 11.32% hexane comprise kaempferol 27.79%.

While leaf extras not contain one of flavonoid in this wavelengths 254.

while rotary root contain kaempferol 17.87% ethyl acetate, root extract contain rutin 7.33%,4.74% & kaempferol 23.63%,17.38% respectively hexane contain rutin 6.26% & n-butanol root not contain any flavonoid in this wavelengths 254. in 366 wavelengths for leaf show rotary leaf contain rutin 5.69%, contain apigenin 43.38%, ethyl acetate contain rutin 3.13% and quercetin 38.84% n-butanol contain rutin 14.08% kaempferol 5.93%, hexane contain apigenin 51.66%, but leaf extract contains rutin only 4.27%. while rotary root

contain 10.46% kaempferol 16.67% ethyl acetate, root rutin 8.96% & kaempferol 22.55, n-butanol contain rutin 13.66% & kaempferol 1.93%, hexane contain rutin 8.12%, root extract rutin 9.16% & kaempferol 18.92%.

After comparison between area % of leaf and root and depending on the RF max of stander, rutin, kaempferol, apigenin, quercetin, and luteolin that shows the root rich in rutin kaempferol more than leaf and leaf contain quercetin and apigenin not found in the root this result in plan native in Iraq in two wavelengths use 254 and 366.

While in comparison with another plant native in chine show leaf more flavonoid than root and give another type of flavonoid this is pinocembrin, and liquiritin also have amount differ leaf rich with pinocembrin while rooting of Glycyrrhiza glabra rich with liqiritin.

Thus different in two results as different in environment condition as soil season in which plant native and collecting.

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