# Qualitative and Quantitative analysis of *Lycium barbarum* L. Flavonoids from leaves

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## Abstract

There is no phytochemical study about Iraqi wild type *Lycium barbarum* L. and its active components. However ;*L.barbarum* as a traditional Chinese herb possessing vital biological activities , such as prevention of cancer and age-related macular degeneration, is widely used in Asian countries. The main active components of this plant have been identified as flavonoids, carotenoids especially zeaxanthin and polysaccharides, all of these components have been reported to be closely associated with the health-enhancing effect. In this study a method for investigating flavonoids from the leaves of wild type *L. barbarum* that are grown naturally in Iraqi deserts and then estimate the total quantity of these flavonoids as Quercetin. For qualitative assay classical chromatographic methods was applied; preparative thin layer chromatography (PTLC). The application employed different solvent systems for flavonoids separation. The best mobile phase for extracting and separation different flavonoids from *L. barbarum* leaves was chosen for (PTLC) in corresponding with standards to investigate each separated flavonoid for this methods. Total Flavonoids from leaves extract were calculated as Quercetin flavonoid represented the quantitative assay in this study. Results showed that the plant is rich with different flavonoids among them (rutin, quercetin, kaempferol, luteolin) and others. The technique with best solvent system for flavonoids separation is by preparative TLC chromatography with (Chloroform: Glacial acetic acid: Formic acid) in the ratio of (44:3.5:2.5). Iraqi wild type *Lycium barbarum* contain about (11.28 mg /g dried leaves ) total flavonoids as quercetin.

**Keywords: Lycium barbarum Flavonoids, Flavonoids TLC, PTLC, Quercetin, Kaempferol, Luteolin, Rutin.**

# التحليل النوعي والكميا لفلافونويدات نبات العوسج من الاوراق

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## الخلاصة

تضمنت هذه الدراسة طريقه للتحري عن الفلافونويدات لمستخلص أوراق العوسج البري والذي ينمو طبيعيا في الأراضي الصحراوية في العراق,ولتحديد كمية الفلافونويدات في النبات على أساس مادة الكوارستين القياسية وباستخدام طريقة تقليدية للتنقية في التحليل النوعي للمستخلص وهي طريقة كروماتوغرافي الطبقة الرقيقة التحضيرية وفي هذه الطريقة عدة أطوار متحركة جربت لفصل الفلافونويدات وتم اختيارأفضلها في الفصل وتجرى بعد كل اختبار تطبيق كروماتوغرافي الطبقة الرقيقة للنواتج للتحري عنها وبالمقارنة مع فلافونويدات قياسية. أجريت في هذه الدراسة تقيما كميا للفلافونويدات الكلية في مستخلص العوسج على أساس مادة الكوارستين القياسية, وأظهرت النتائج ان النبات غني بعدة فلافونويدات أهمها (الروتين, الكوارستين, الكامفيرول واللوتيولين)وغيرها.كانت أفضل تقنية وأفضل طور متحرك لفصل الفلافونويدات هي كروماتوغرافي الطبقة الرقيقة التحضيرية وباستعمال الطور المتحرك :كلوروفورم ( ٤٤ ):حامض الخليك الثلجي (٣.٥ ):حامض الفورميك (٢.٥) .أن العوسج العراقي البري يحوي ١١.٢٨ ملغم لكل غرام واحد من الأوراق الجافة مقدرة على أساس فلافونويد الكوارستين.

**الكلمات المفتاحية : فلافونويدات نبات العوسج ،كروماتوغرافي الطبقة الرقيقة كوارستين ، كامفيرول ، لوتيولين ، روتين .**

## Introduction

*Lycium barbarum* belongs to Solanaceae family is one of the important traditional Chinese medicinal plant species. It has been cultivated in Northwest China and Mediterranean region [(1)]. Fruits and leaves of *L. barbarum* are widely used as medicine vegetables and functional tea in China, Southeast Asia, Europe, and North America. The Chinese medicinal monographs recorded the plant as "nourishing liver and kidney, enhancing eyesight, enriching blood, invigorating sex, reducing rheumatism" [(2)]. More of its functions were reported as immunity improvement [(3)], anti-oxidant [(4)], anti-radiation effect [(5)], anticancer [(6)], enhancing hemopoiesis [(7)], anti-aging, and enhancing sex [(8)]. Phenolic compounds including flavonoids from plant foods proved

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to have effects on radical scavenging[9], antioxidation, and anticancer [10]. Flavonoids are the important active compounds present in the leaves of *L. barbarum*[11]. However, the main flavonoids of the leaves were still unknown. The aim of this study is to identify the main flavonoids in the leaves of Iraqi wild *L. barbarum* by using the proper technique for their separation and isolation.

## Material and methods

***Extraction of flavonoids from L.barbarum leaves***

A quantity of 25 g. from *L.barbarum* dried leaves were defatted by soxhlet for 10 hours using 300 ml n-hexane, then reflected for another 10 hours after filtration using 200 ml of 2M Hydrochloric acid(HCl) solution. The filtrate was cooled and transferred to a saperotary funnel. The aglycone moiety was extracted by organic solvent like ethyl acetate. The collected ethyl acetate layers were washed with distilled water to get rid of the excesses acid then evaporated to dryness by rotary evaporator at 40ºC. The dried residue was weighted then re-dissolved in 30 ml 50% ethanol solution for qualitative and quantitative evaluation[12].

***A-Qualitative Assay*:**

*Thin layer chromatography mobile phase:* To choose the most proper mobile phase that separate the extracted *L.barbarum* flavonoids efficiently ,different solvent mixtures were used as follow:

Toluene: Ethylacetate: Formic acid
36:12:5----------(A)

*n*-Hexane: Ethylacetate: Glacial acetic acid
31:14:5----------(B)

*n*-Hexane: Ethylacetate: Glacial acetic acid
30:20:1.5----------(C)

Chloroform: Glacial acetic acid: Formic acid
44:3.5:2.5---------(D)

*n*-Butanol: Glacial acetic acid: Distilled water
20:5:25 ----------(E)

onc.HCl: Glacial acetic acid: Distilled water
1.2:12:4 ----------(F)

The chromatography was performed on silica gel Gf254 aluminum plates and a spot of 0.1mg/ml ethanol solution from each rutin, kaempferol, quercetin and luteolin standard solutions. After running of the mobile phase, the best system was chosen for preparative TLC chromatography to separate different flavonoids in comparison with standards[13].

***B-Quantitative Assay***

For calculating the total flavonoids obtained as Quercetin: Quercetin standard solutions were prepared at concentration (1,0.5,0.25 and 0.1)mg/ml in 50% ethanol. Transfer 1ml from each standard solution and from the re-dissolved residue into a glass tubes then 0.75 ml of 5% sodium nitrite solution was added, mixed well and stand at room temperature for 5 min. To all tubes 1.5 ml was added of 10% $AlCl_3$ dissolved in 50% ethanol, shacked well and kept stand at room temperature for another 5 minutes, at last 5ml of 1N NaOH solution was added to all tubes and the absorbance of all tubes were read at 510nm. A plotted curve was applied represented the absorbent of each tube (the Y axis )verses the concentration of standard solutions (the X axis). Total flavonoid concentration can be calculated as Quercetin from the equation of straight line that concluded from the plotted curve [14-17].

***C-Preparative TLChromatography:***

About 2ml from the leaves of *L.barbarum* extracted flavonoids was applied as straight line on silica glass plate of 0.75 cm thickness with aid of syringe of 25G needle was done with the same standard mentioned above. The preparative silica plates was scraped for each separated band to detect type of flavonoid [18].

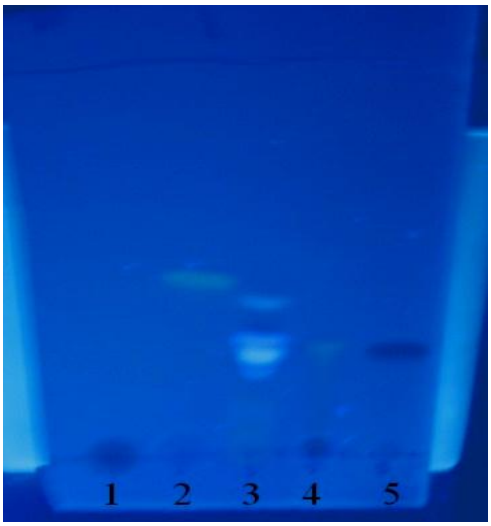
## Results

***Extraction of Flavonoids***

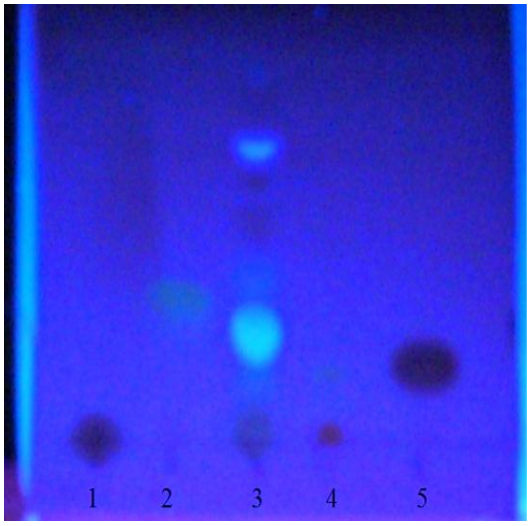
The dried residue from 25g leaves extract has a weight of 0.41g including the total flavonoids in the leaves which then was dissolved in 30 ml of 50%v/v ethanol for qualitative and quantitative investigations.

***A-Qualitative Determination****:*

Chromatographic analysis for flavonoids was done, during comparison of different mobile phases, it was found that mobile phase (D)is the proper mobile phase as long as it gave good separation of the components, as shown in Figure(1).

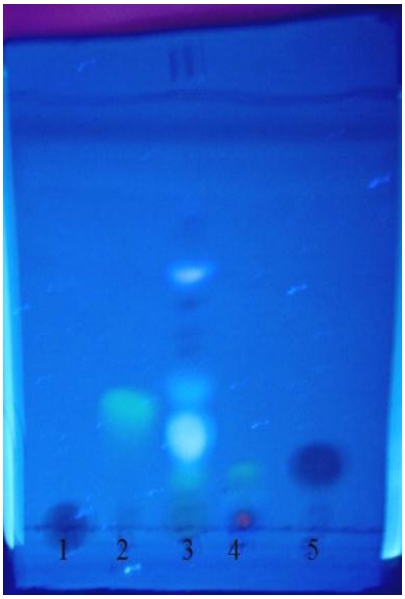



**Mobile Phase(A)**

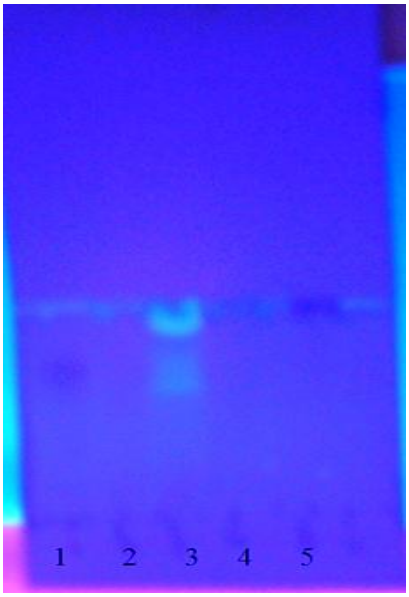



**Mobile Phase (B)**


**Right: For Mobile Phase(C).Left: For Mobile Phase(F).**




**Mobile Phase (D)**




**Mobile Phase (E)**

**Figure(1):- TLC Chromatogram read under 254nm uv chamber with mobile phase A,B,C,D and E of standard solutions include :Rutin(1), Kaempferol(2) , Quercetin(4), Luteolin(5) and L.barbarum leaves flavonoids extract (3).**

**Table(1):- $R_f$ Values for different flavonoid standards and the extracted flavonoid**

| No. | $R_f$ value for Flavonoid | Mobile Phase | | | | | |
|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F |
| 1 | Rutin | baseline | Baseline | Baseline | Baseline | 0.66 | Baseline |
| 2 | Kaempferol | 0.48 | 0.42 | 0.44 | 0.3 | 0.6 | 0.28 |
| 4 | Querecetin | 0.30 | Baseline | 0.32 | 0.15 | 0.6 | 0.08 |
| 5 | Luteolin | 0.27 | 0.22 | 0.26 | 0.16 | 1 | 0.14 |
| 3 | flavonoids Leave extract | All spots are present + other flurescent spots of 0.85 $R_f$ value. | | | | | |

***B-Quantitative Assay***

The absorption of Quercetin standard solutions and the re-dissolved flavonoid extract solution were summarized in table-2- .

**Table (2) :- Quercetin standard solutions adsorption at different concentration & the extracted solution**

| Concentration(mg/ml) Quercetin | Absorbance (nm) |
|---|---|
| 0.1 | 0.091 |
| 0.25 | 0.189 |
| 0.5 | 0.306 |
| 1 | 0.513 |
| The extracted solution | 0.800 |

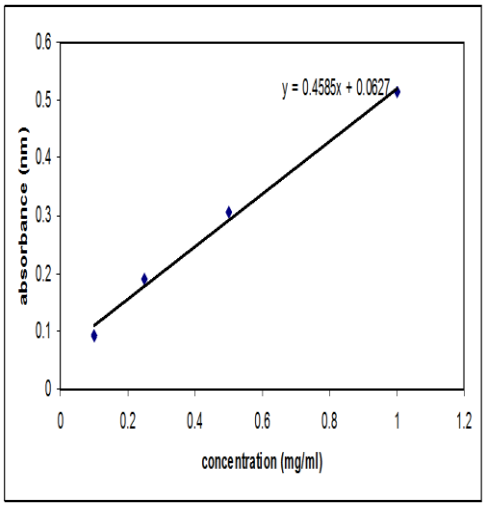



**Figure (2):- Quercetin standard curve for different concentration and the absorbance at 510 nm.**

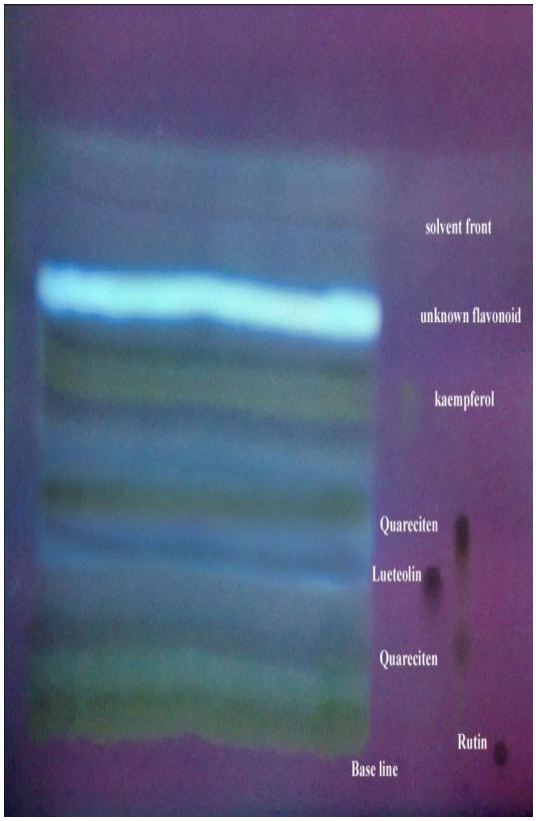
When plotting a standard curve figure(1), the total flavonoid concentration can be calculated as Quercetin by applying the equation from the above curve as follow:-Y = 0.4585 X + 0.0827

When Y is the absorbance at 510 nm, and X is the concentration of the flavonoids in mg/ml.

Total flavonoid concentration in 25g dried leaves of *L.barbarum* is about 281 mg as Quercetin (11.28 mg /g dried leaves).

***C-Preparative TL Chromatography:***

For PTLC results figure (3) showed that different flavonoids were separated as straight lines indicated by different $R_f$ values. Five layers were scraped and eluted with ethanol; some were detected as Luteolin, Quercetin(gives two spots), Kaempferol and Rutin ,others were still unknown .




**Figure(3):-Prerarative TLC chromatogram for the leaves extract showed five seperated layers represented different flavonoids and spots for different standards**.

## Discussion

Many studies(13-19)included total amount of flavonoid in *L. barbarum* leaves or fruits based on Quercetin equivalents, Catechin equivalents or Rutin equivalents and different results were reported between the wild type and cultivated type. Jing *et al*., (2009) study indicated the contents of total flavonoids (21.25mg/g) of cultivated *L.barbarum* leaves were much higher than those in wild *L.barbarum* leaves (17.86 mg/g) (11). Although the present study shows lower flavonoid content (11.28mg/g) due to the bad conditions the wild plant grows in, the plant leaves considered to be suitable source for medicine and functional tea. The qualitative assay of the leaves through simple chromatographic method showed different flavonoids content and with aid of standard solutions most of these flavonoids could be separated and isolated while in one study by Stephen *et al.,(*2010) on fruits about 52 phenolic acid and flavonoids were resolved some could be identified , others still unknown(15). The purpose of use different mobile systems in TLC application is to optimize and find the one that shows the greatest differences in identification characteristics between substances as the

retention factor ($R_f$) value. Solvent system (D) was chosen for this purpose. Good results for flavonoids separation had been shown with PTL chromatography in this study, although Silica gel G60 had the ability to absorb 50% of material separate made them lost during assay procedure, it was simple and yielded enough separated products with low cost.

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