

Phytochemical Investigation of Aerial Parts of Iraqi *Cardaria draba*Alaa M. Abd^{*1} and Enas J. Kadhim^{**}^{*} Ministry of Health and Environment, Al- Diwaniyah, Iraq.^{**} Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Baghdad, Baghdad, Iraq.**Abstract**

Cardaria draba (L.) Desv. (Brassicaceae; syn. *Lepidium draba* (L). commonly known as Whitetop or hoary cress, is a perennial plant reproduces by seed and by horizontal creeping roots. Brassicaceae or Cruciferae family commonly known as the mustards family, contained flavonoids, alkaloids, saponins and a lot of dozens of glucosinolates. The aim of this research was to study chemical constituents of aerial parts of *Cardaria draba* since no phytochemical investigation had been studied before in Iraq for this plant.

Aerial parts of *Cardaria draba* were defatted by maceration in hexane for 48 h. The defatted plant materials were extracted using Soxhlet apparatus, with the aqueous methanol 90% as a solvent of extraction for 12 h, and fractionated with petroleum ether- chloroform – ethyl acetate- and n-butanol respectively. The n-butanol fraction was hydrolyzed with 10% HCl by reflux to break down the glycoside linkage. Flavonoids and phenolic acid compounds were isolated from hydrolyzed n-butanol fraction by preparative TLC to be then identified by HPLC, TLC, FTIR and melting point. The chromatographic and spectroscopic results showed the presence of luteolin, chlorogenic acid, caffeic acid, and resorcinol in aerial parts of *C. draba*.

Keywords: *Cardaria draba*, Flavonoids, Phenolic acid, High-performance liquid chromatography (HPLC) and Fourier Transform Infrared Spectroscopy (FTIR).

دراسة المحتوى الكيميائي للأجزاء الهوائية لنبات القانبري العراقيالاء ماجد عبد الرحيم^{*1} و ايناس جواد كاظم^{**}^{*} وزارة الصحة والبيئة، الديوانية، العراق^{**} فرع العقاقير والنباتات الطبية، كلية الصيدلة، جامعة بغداد، بغداد، العراق.**الخلاصة**

نبات القانبري العراقي من عائلة الخردليات وهو ينحوي على مركبات عديدة مثل الفلافونويدات والصابونيات. ينحوي النبات ايضا على كلاكوسيدات التي تحنوي على مركبات الكبريت.

يهدف البحث الحالي دراسة المكونات الفعالة للأجزاء الهوائية لنبات القانبري. حيث لم تتم دراسته من قبل في العراق. تم ازالة الدهون من الاجزاء الهوائية للنبات بواسطة تنقيعها لمدة ٤٨ ساعة في الهكسان. تمت عملية الاستخلاص للأجزاء المنزوعة الدهون في المحلول المائي للميثانول ٩٠% لمدة ١٢ ساعة و باستخدام جهاز السكسوليت ثم تمت عملية تجزئة بعدة مذيبات عضوية شملت بالتتابع: الايثر البترولي- كلوروفورم- خلات الاثيل و البيوتانول الاولى. تم كسر الاصرة الكلايكوسيدية باستخدام حامض الهيدروكلوريك ١٠% . تم التحري عن محتوى المركبات الفينولية والفلافونيدية وعزلها بتقنية طبقة السليكا التحضيرية من الجزء المتحلل (preparative TLC) ثم اجراء الكشف بمطياف الكثف تحت الحمراء والفصل الملون عالي الكفاءة (HPLC) والتأكد من نقاوتها بتقنية الفصل الملون بطبقة السليكا الرقيقة (TLC) واختبار درجة الانصهار للمركبات المعزولة. من نتائج الفصل الملون وكذلك نتائج تحليلات المطياف بينت وجود فلافونيد اللوتيونين والاحماض البسيطة الريزورسينول , حامضي الكلوروجينيك والكافاييك في الاجزاء الهوائية لنبات القانبري. الكلمات المفتاحية: نبات القانبري، الفلافونويدات والفينولات، كروماتوغرافيا السائل عالي الاداء، مطياف الاشعة تحت الحمراء.

Introduction

Cardaria draba (L.) Desv. (Brassicaceae; syn. *Lepidium draba* (L). commonly known as Whitetop or hoary cress, is a perennial plant reproduces by seed and by horizontal creeping roots⁽¹⁾. Brassicaceae or Cruciferae family commonly known as the mustards family, contained flavonoids, alkaloids, saponins and a lot of dozens of glucosinolates. They also have an enzymes called myrosinases which convert the glucosinolates into thiocyanates, isothiocyanate

and nitriles which are toxic to many organisms, and so that help protector against herbivores. The plant oil content is mostly produced from the seeds of various species⁽²⁾. The genus name arises from the Greek word kardia (heart), which refers to the heart shaped fruit of *C. draba*. although not all the fruit in this genus are heart shaped. Common names for *C. draba* are heart-podded, hoary cress, White-top, perennial peppergrass and in England it is known as hoary pepperwort, chalk weed and may be referred as whitlow pepperwort (devil's cabbage)⁽³⁾.

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Plants belonging to this genus are reported to have wide applications in folk medicines, as an anthelmintic, antiscorbutic, purgative and expectorant effects⁽⁴⁾. The seeds have been used in treating flatulence and fish poison also used as a condiment. A decoction of the whole *C. draba* plant and seed is used as a diuretic in ethnomedicine in Iran. It has been reported that the edible species in Spain, rich in protein content higher than leaves of cabbage and spinach⁽⁵⁾. Some flavonoids and phenols isolated from *C. draba* exhibited antihypertensive, anti-inflammatory, antimicrobial, antioxidant and antiradical activity. In Iraq at Aldiwaniya city, *Cardaria draba* extract used to treat leishmaniasis (Baghdad sore) topically. Since there is no phytochemical investigation and separation of this naturally grown plant in Iraq; the current research for flavonoids and phenolic compounds were investigated. *C.draba* contains alkaloids, saponins and flavonoids. In total of 16 compounds; Isorhamnetin, quercetin and kaempferol were the most abundant flavonoids compounds while the most abundant phenolic compounds were sinapic acid, p-coumaric acid, caffeic acid and ellagic acid. Phenolics are mostly produced in plants as secondary metabolites via the shikimic acid pathway⁽⁶⁻⁸⁾. Phenolic compounds are present in aerial parts of plant. Plants have natural defense system against bacteria, insects, viruses and fungi, Phenolic compounds consider an important part of this system and they can switch plant hormones. Brassica species contain a wide and diverse range of polyphenols, namely the flavonoids and hydroxycinnamic acids, which serve as biochemical markers to differentiate members within different genera or even within the same species⁽⁹⁻¹¹⁾.

Chlorogenic and caffeic acids have antioxidant, anti-inflammatory, prevent diabetes, prevent premature aging, depigmentation, prevent neurodegenerative diseases, like Parkinson's disease, anti-hepatitis B virus activity also have been used to prevent sodium-selenite-induced cataract and reduce exercise-related fatigue⁽¹²⁻²³⁾.

Luteolin has anti-oxidant activity, anti-inflammatory, antimicrobial anticancer⁽²⁴⁻²⁹⁾. Resorcinol is an anesthetic found in throat lozenges also used as chemical intermediate for the synthesis of pharmaceuticals and other organic compounds⁽³⁰⁻³²⁾.

The dominant study objective is to investigate and isolate some flavonoids and phenols from n-butanol after hydrolysis fraction of *C.draba* grown in Iraq since there were no previous studies concerning the Iraqi species.

Experimental Section

Plant material

At the flowering stage, aerial parts of *Cardaria draba* were collected from Al Hamza city of Al-Diwaniyah, Iraq, In April 2018, identified at the Iraq natural history research center and museum ,university of Baghdad .

Extraction

Cardaria draba were air-dried for 3days. The aerial plant parts (75gm) were cut into small pieces, powdered and defatted by maceration in pure n-hexane for 48 hours, filtered through a whattman paper, shade dried, plant material was powdered then filled in the thimble and extracted with 700 ml of 90% methanol by a Soxhlet extractor for 12 h. This extract was concentrated using rotary evaporator. After complete evaporation of the solvent, dry extract was weighted and dissolved in 100 ml water, partitioned with 100 ml (3times) petroleum ether, chloroform, ethylacetate and butanol. Each fraction evaporated by rotary evaporator, each dry fraction weighted and revealed for preliminary test. The n-butanol fraction was hydrolyzed by reflux with 10% HCl, and then the hydrolyzed fraction was taken with n-butanol then dried for further investigation.

Screening of phytochemical components

To identify the phytochemical derivatives in the methanolic extract, general phytochemical screening was performed according to literature⁽³³⁾.

Flavonoids Test (Shinoda test)

Few amount of the extract were dissolved in 1mL of 50% methanol then dried on steam bath. Then a few drops of concentrated hydrochloric acid (HCl) were added followed by metallic magnesium. An orange or red color shows the presence of aglycone flavonoids.

Phenols Test: this test was done by adding few drops of 1% FeCl₃ to few milligrams of aqueous methanol plant extracts. Formation of dark greenish-blue color indicates the presence of phenols.

Alkaloids test by Dragendorff's reagent

The reagent is composed from two solutions, Solution A and B

Solution A contains 60mg of bismuthsubnitrate in 0.2 ml HCl

Solution B contained 600 mg KI in 1ml distilled water.

Few drops from plant extract was added to the mixture of solutions A and B, should give brown precipitate that indicate presence of alkaloids.

Saponins Test

In a test tube few milligrams of extract were added to 5 ml of H₂O. The solution was shaken strongly and observed for a stable persisting froth. Few drops of olive oil were mixed with the frothing and shaken vigorously then it was observed for the formation of an emulsion.

Test for tannins

In a test tube, few milligrams of the extract were boiled in 10 ml of water and filtered. A few drops of 0.1% ferric chloride (FeCl₃) were added and observed for a blue-black or brownish--green coloration.

Flavonoids and phenolic acid Compounds Isolation by Preparative TLC from the Hydrolyzed n-butanol fraction:

Flavonoids and phenolic compounds were isolated by preparative TLC from the hydrolyzed n-butanol fraction of *C. draba*.

Preparative silica gel GF254 plate of 20 cm×20 dimension with a layer thickness of 0.5cm. was reactivated by heating at 100°C for 15–20 min, then left to cool used for application.

Two mobile phase for n- butanol fraction after hydrolysis were used: first; (chloroform: methanol [90:10V/V]) and second mobile phase (chloroform: methanol: formic acid [87.5:10: 2.5]) placed in separated jars. The jars were lined with a filter papers closed tightly, and left for saturation.

Sample application was done by dissolving 0.5 g of the sample in absolute methanol and applied to the baseline of preparative TLC plate using capillary tubes.

The isolated flavonoid and phenolic compounds from n-butanol after hydrolysis fraction of the aerial parts were recognized by HPLC, TLC, FTIR and melting point.

By examination under UV light, the detection was done with wavelengths; 365 & 254 nm. By analytical TLC, the purity of each band was checked until a single spot had been obtained in corresponding to reference standard. The pure compounds were scraped and extracted from adsorbed silica by methanol to be analyzed by HPLC method.

Detection of Isolated Compounds By HPLC Analysis

HPLC analysis was achieved on pump system (Knauer, Bad Homburg, Germany), Model 8300 HPLC and an S-3210 photodiode-array detector (PDA), with a Water (150 × 4.6 mm i.d.) Eclipse XDR-C18 column, using a binary solvent system: solvent A: dd H₂O/ trifluoroacetic acid (97:3, v/v); and solvent B: acetonitrile. The following gradient program was used: 100–90% A from 0 to 10 min, 90–30% A from 10 to 32 min, 30–0% A from 32 to 45 min at a flow rate of 1 mL/ min⁽³⁴⁾.

Standard solutions for HPLC were: luteolin, resorcinol, caffeic acid and chlorogenic acid by dissolving 5 mg in 1 ml of HPLC methanol. Samples were firstly dried prepared for HPLC analysis then dissolving them in HPLC methanol and subjecting them to ultrasonication at (60% duty cycles for 25 min at 25°C then by centrifugation at 7500 rpm for 15 min). The pure upper layer of each sample was evaporated under vacuum. The residues were resuspended separately, in 1 ml of methanol HPLC grade, standardizing using vortex mixer, and passing them through 2.5 µm disposable filter, then vortex mixer, and passing them through (2.5 µm disposable filter, finally stored at 3-5°C). For HPLC analysis; 0.02 ml of the sample was injected.

Detection of Isolated Compounds by FTIR Analysis:

The isolated compounds were subjected to FTIR analysis to detect the functional groups of these compounds. The following condition and apparatus were used SHIMADZU 3800 FT-IR/ Japan in the pharmaceutical chemistry department at AL Mustansiriyah University / college of science.

Detection of Isolated Compounds by Melting point

Using melting point apparatus stuart melting point /SMP30

Results

Phytochemical investigation for methanolic extract of *C.draba*

Table 1 showed the major active constituents present in the *C.draba* extract.

Table1. Phytochemical Analysis of Aerial Parts of *Cardaria draba* Extract

Phytochemical components	Result
Alkaloid	+
Flavonoid	+
Phenols	+
Saponin	+
Tannin	-

The present study done for the *Cardaria draba* showed the presence of medicinally active constituents. *C. draba* phytochemical active compounds were qualitatively analyzed and the results are presented in Table 1. the positive and negative results, based on the presence or absence of color change. In this screening process, flavonoids, phenols, alkaloid and

saponins gave positive (+) results and tannin offered negative (-) result.

Flavonoids and phenolic acid Compounds Isolation by Preparative TLC from the Hydrolyzed n-butanol fraction:

TLC chromatogram for the hydrolyzed n-butanol fraction and non-hydrolyzed fraction showed in figure 1, which indicate the presence of chlorogenic acid, caffeic acid, luteolin and resorcinol in both fractions.

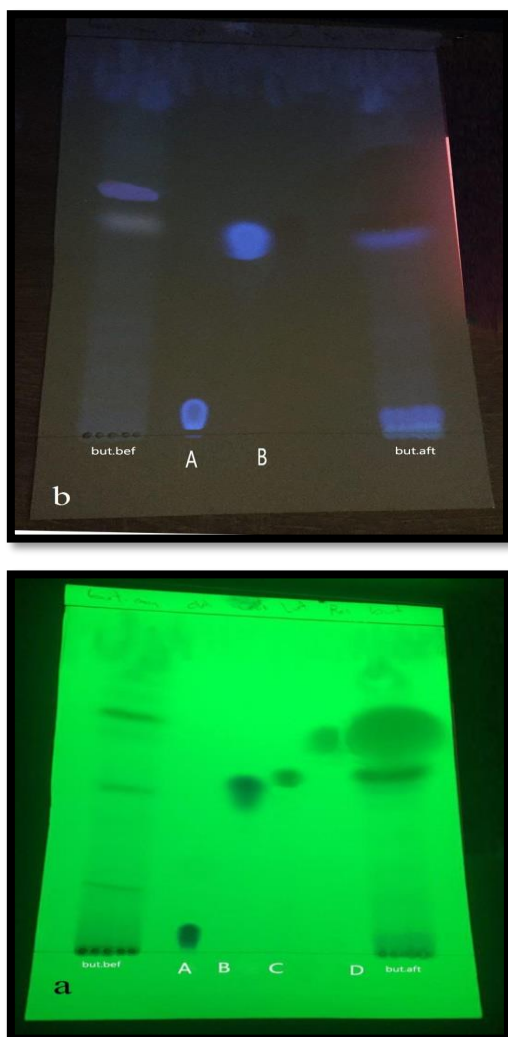


Figure 1-TLC Chromatogram at 254nm(a), at 365nm(b) for n-butanol fraction before hydrolysis(left),and after hydrolysis(right); A:chlorogenic acid B: caffeic acid C: luteolin D :resorcinol, mobile phase: CHCl₃:M₂OH : Formic acid (87.5 : 10: 2.5)

For the preparative TLC, bright lines were investigated which had been scrapped for isolated each compound,figure (2,3).

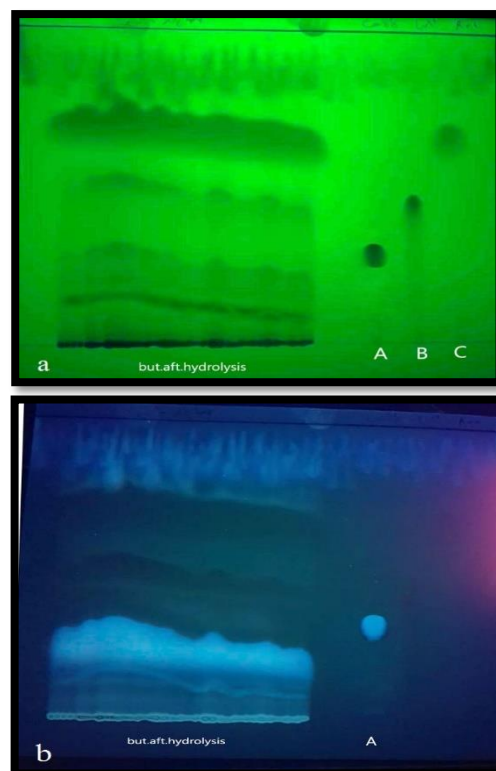


Figure .2. Preparative TLC chromatogram of n-butanol hydrolyzed fraction at a: 254 and b:365, A : caffeic acid B: luteolin C : resorcinol , mobile phase CHCl₃:MeOH (90 :10)

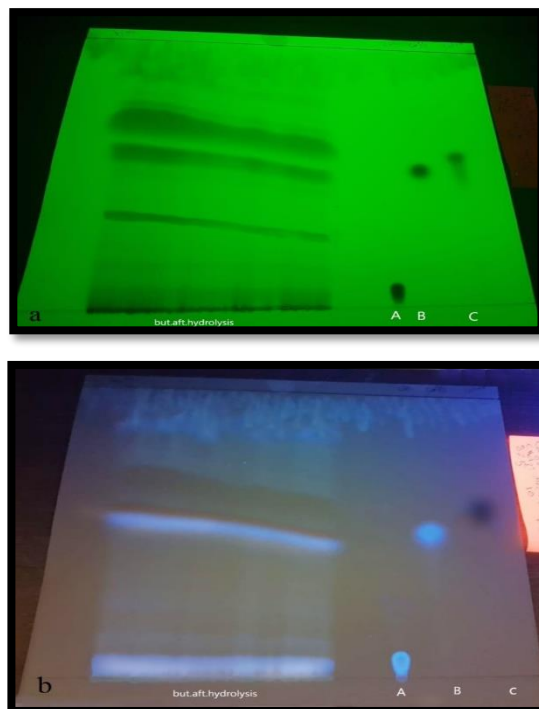


Figure 3- Preparative TLC chromatogram of n-butanol hydrolyzed fraction at a: 254 & b:365, A :Chlorogenic acid B:caffeic acid. C :luteolin, mobile phase CHCl₃:MeOH: formic acid (87.5 :10 :2.5)

HPLC Chromatogram for the hydrolyzed n-butanol fraction

HPLC analysis were performed for the n-

butanol fraction (Figure 4) and each isolated compound as shown in Figures 5, 6, 7 and 8.

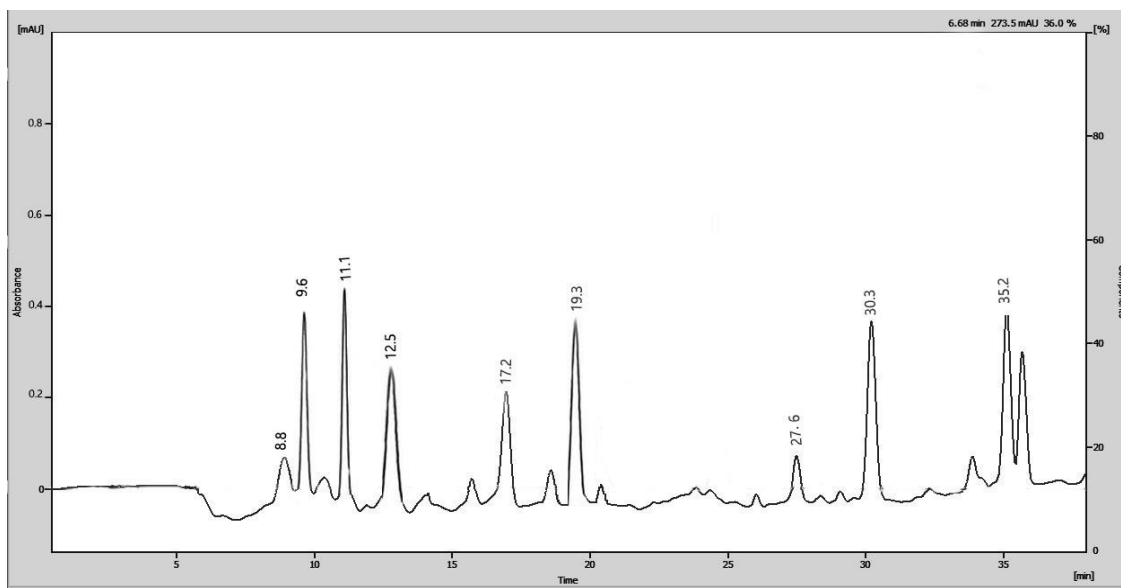


Figure 4. High performance liquid chromatogram (HPLC) analytical of but. aft. hydrolysis fraction.

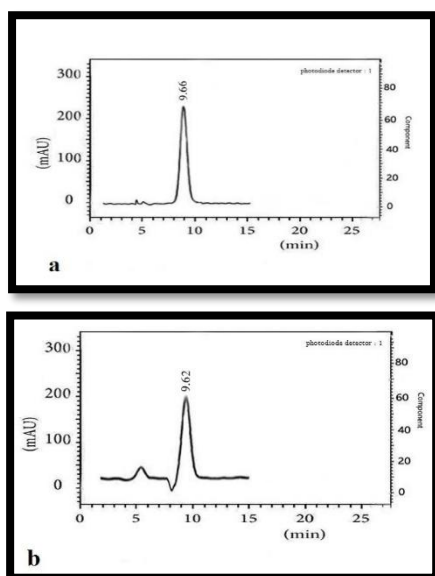


Figure.5. a: HPLC of standard chlorogenic acid, b: HPLC of isolated chlorogenic acid

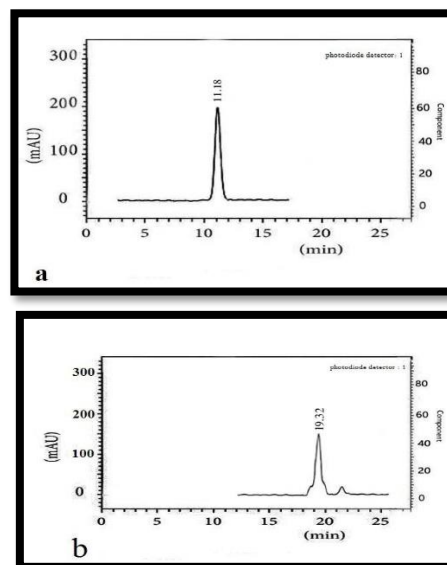


Figure 6 .a : Hplc of standard caffeic acid, b: Hplc of isolated caffeic acid.

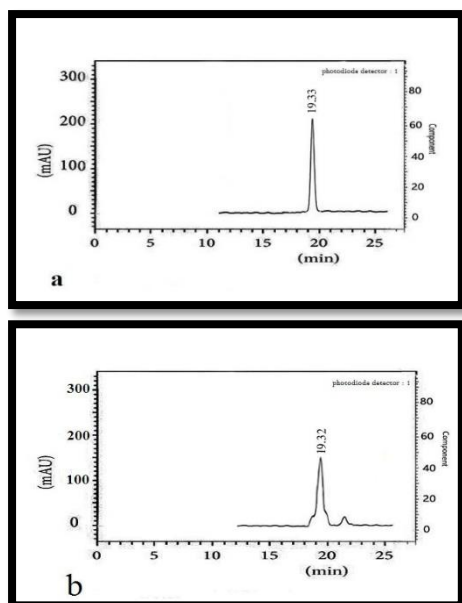


Figure 7 .a:Hplc of standard luteolin
b: Hplc of isolated luteolin.

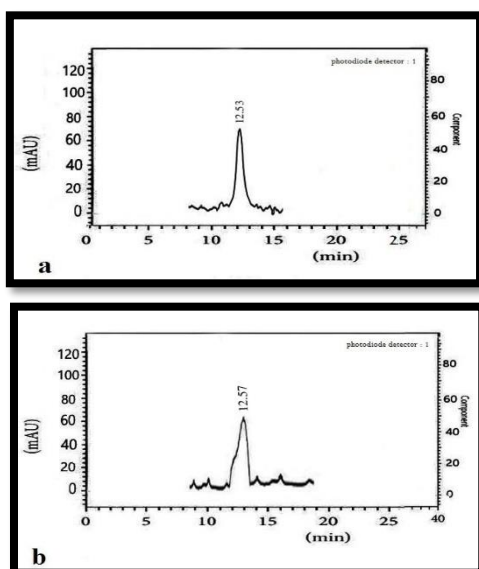


Figure 8. a: HPLC of standard resorcinol b
: HPLC of isolated resorcinol

TLC for the isolated compounds

This was performed to insure the purity of the isolated compound which were isolated by scraping the isolate bands of the preparative TLC.

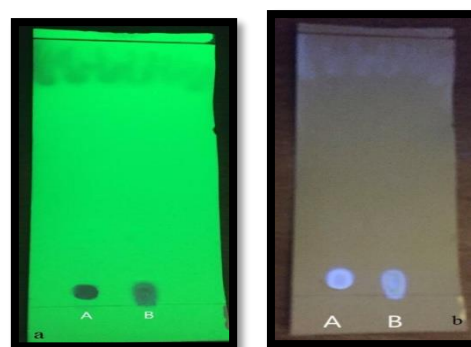


Figure 9. Thin- layer chromatography for chlorogenic acid standard A and isolated chlorogenic acid B, on Gf₂₅₄ silica gel detection under uv light a: at 254 nm and b: 366 nm.

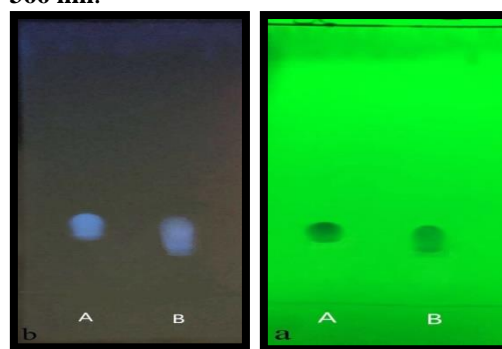


Figure10: Thin- layer chromatography for caffeic acid standard A and isolated caffeic acid B, detected under uv light (a): at 254 nm and (b): at 366 nm.

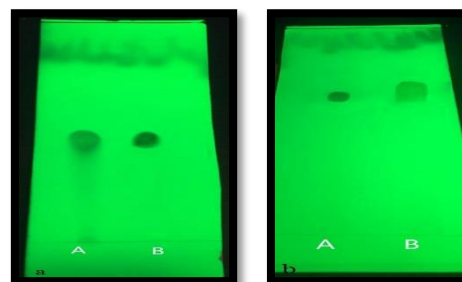


Figure.11. Thin- layer chromatography for (a): luteolin isolated A and standard luteolin B, (b) resorcinol standard A and isolated resorcinol (B) on GF₂₅₄ silica gel revealing under 254 nm uv light

FTIR Analysis for the isolated compounds

The FTIR spectral analysis of separated chlorogenic acid compound show peaks at 3600, 3400-3200, 1680, 1640, 1600, 1500, 1400, 1280. (Figure12)

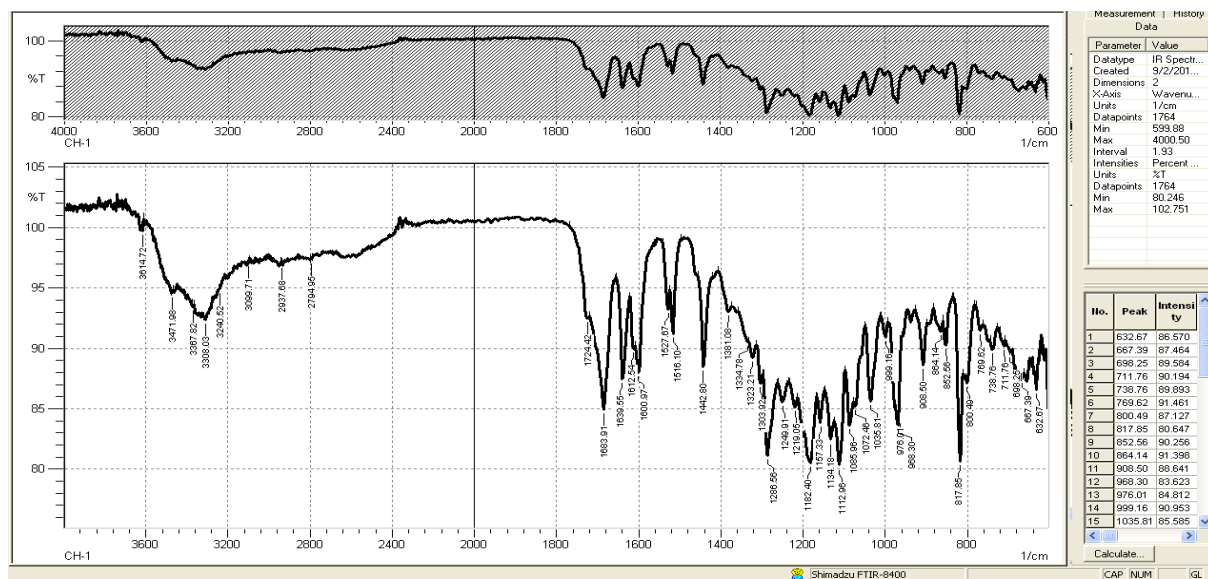


Figure12. FTIR spectrum of isolated chlorogenic acid.

FTIR spectral analysis of isolated caffeic acid
(compound show peaks at 3400, 3250- 3200,

3000, 2900, 2800, 2500, 1640, 1620, 1600,
1530, 1440, 1290 (Figure 13).

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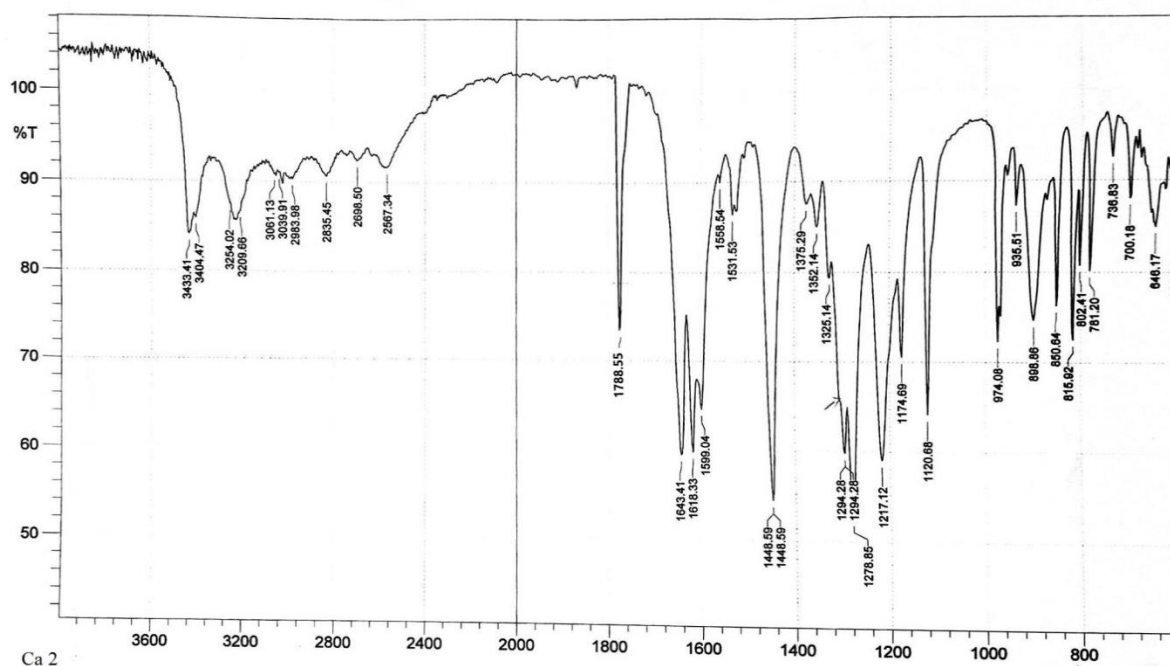


Figure13. FTIR spectrum of isolated caffeic acid.

FTIR spectral analysis of isolated luteolin
compound show peaks at 3400-3000, 2700,

2600,1650, 1600, 1550, 1500, 1500,1400 ,1300
,1200 (Figure 14).

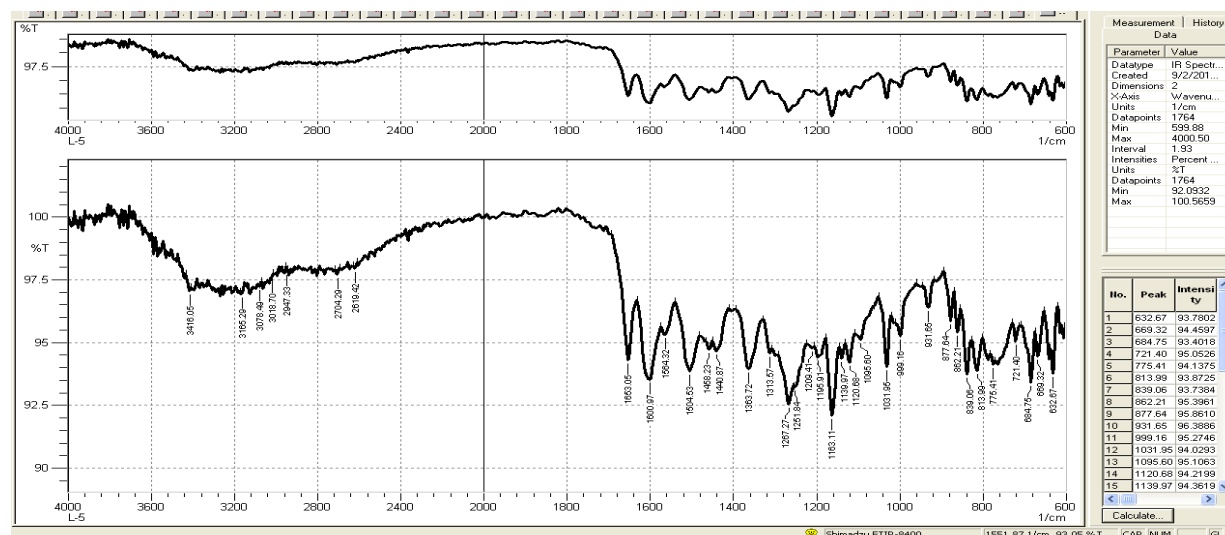


Figure14. FTIR spectrum of isolated luteolin.

FTIR spectral analysis of isolated resorcinol compound show peaks at 3400-3000, 2880,

2600, 1600, 1400, 1370, 1290, 1100 (Figure15).

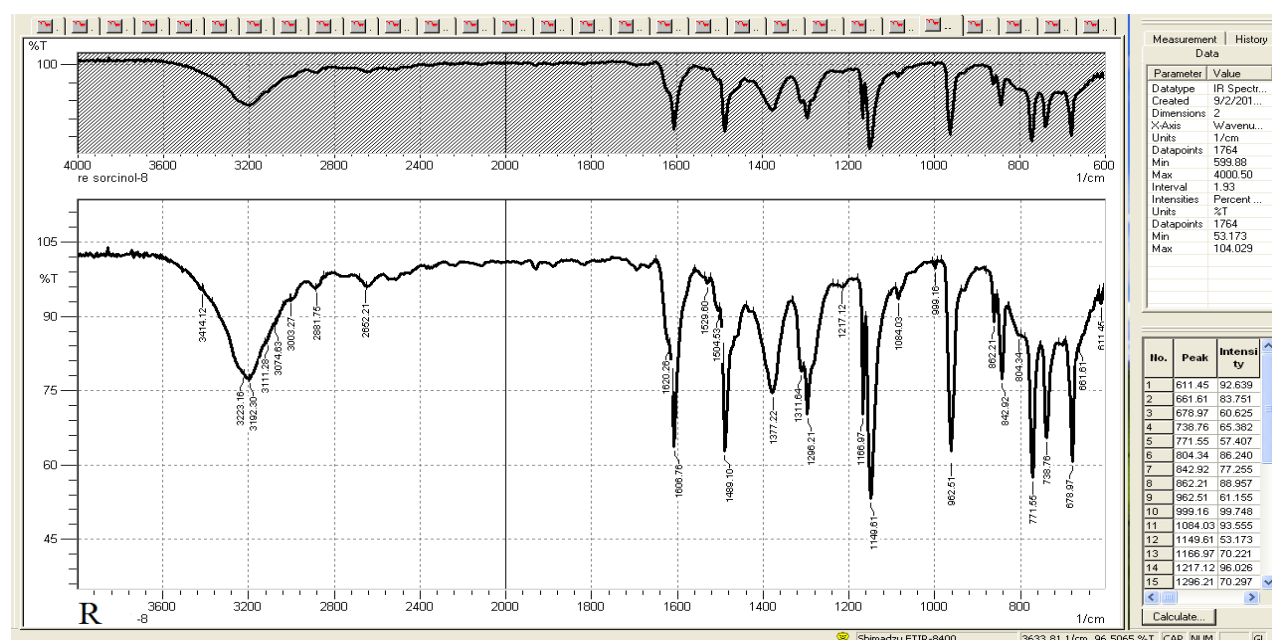


Figure15. FTIR spectrum of separated resorcinol.

Melting point

CH1 compound melt at 205- 208 °c which match standard chlorogenic acid.

Ca 2 compound melt at 221-224 °c which match standard caffeic acid.

L5 compound melt at 267-270 °c which match standard luteolin.

R8 compound melt at 108-111 °c which match standard resorcinol.

Discussion

Natural products have at all times been a preferred choice of all as they play a great role in discovering new medicines. During

extraction, solvents drawn-out into the solid plant material then solubilize compounds with similar polarity. Through standard procedure plant's chemical constituent extraction and separation depend on selective solvents. Flavonoids have an important role in the healthcare since they are a major class of natural compounds, broadly distributed in plants and numerous traditional medicine systems of the world.

The preliminary phytochemical analysis confirmed the presence of alkaloids, phenols, and flavonoids. In (IR) spectral analysis, firstly

the peak at 2947.33 cm^{-1} showed C-H stretching due to $-\text{CH}_2$, the peak at 3300.10 cm^{-1} , a broad band is most probably the result of O-H stretching vibrations of phenol -OH group. The peak at 1697.41 cm^{-1} indicates the presence of $(-\text{C}=\text{O})$ carbonyl group. The peak at 1606.76 cm^{-1} showed the presence of $-\text{CH}=\text{CH}$ group. The peak at 1643 & 1508.38 cm^{-1} revealed the presence of benzene ring. In addition to hplc, melting point and the above results approve that the isolated compound is chlorogenic acid.

Peak at 3100-3400, broadly band is utmost possibly the result of (O-H) stretching vibrations of phenol (OH) group, The peak at 1606.76 cm^{-1} showed the presence of $(-\text{CH}=\text{CH})$ group. The peak at 1643 & 1508.38 cm^{-1} revealed the presence of benzene ring. In addition to hplc, melting point and the directly above results approve that the isolated compound is resorcinol.

The importance of this study, is the first study which confirms the presence of chlorogenic acid, caffeic acid, luteolin and resorcinol in the Iraqi species of *C. draba*.

Conclusion

The results of this study exhibited the presence of phenols, i.e., chlorogenic, resorcinol and caffeic acid and flavonoids, i.e., luteolin and in butanol fraction after hydrolysis.

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