

Isolation of Alkaloids from *Papaver rhoeas* (Papaveraceae) Wildly Grown in Iraq Amenah Ayad Lafta^{*1} and Maha N. Hamad^{*}

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Abstract

The plant *Papaver rhoeas*, which belongs to family Papaveraceae and known as common poppy is widely grown in Iraq. It was used in traditional medicine in wide range of diseases including inflammation, diarrhea, sleep disorders, treatment of cough, analgesia, and also to reduce the withdrawal signs of opioid addiction.

The project provide the first comprehensive research done in Iraq to study the phytochemical and the methods of extraction and separation of alkaloids from *Papaver rhoeas* widely grown in Iraq. The plant was harvested in April 2019 from Zurbatiya is an Iraqi town located at the northeast of Waist province in Iraq. The collected plant was washed thoroughly, dries under shade, and grounding in a mechanical grinder to fine powder. The plant was extracted by hot extraction method using Methanol then fractionation was done to separate alkaloids from chloroform Fraction by TLC and PTLC. The alkaloids were isolated and purified by PTLC then subjected to various analytical techniques for alkaloids identification such as UV, LC mass and IR. The result was indicated of three alkaloids (dihydrocodien, chelidonine and papaverrubine C) in *Papaver rhoeas* plant.

Keywords: *Papaver rhoeas*, Dihydrocodien, chelidonine, papaverrubine C.

عزل القلويدات من نبات الخشخاش المنثور البري في العراق

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الخلاصة

نبات الخشخاش والمعروف بالخشخاش المنثور الذي ينمو برياً في العراق ويستخدم في الطب الشعبي على مدى واسع لمعالجة الالتهابات، الاسهال، مشاكل النوم، معالجة السعال، مسكن الأم وتقليل اعراض الادمان. يعتبر هذا البحث اول بحث شامل في العراق لدراسة القلويدات الموجوده في نبات الخشخاش البري في العراق وطرق استخلاصها وفصلها. تم جمع النبات من قضاء زرباطيه في محافظة واسط في شهر شباط ٢٠١٩م وتم غسل وتجفيف النبات في الظل ثم طحنه بالمطحنه الميكانيكيه. تمت عملية الاستخلاص ب الطريقة الحاره ثم تمت عملية التجزئه لفصل القلويدات من طبقه الكلوروفورم بواسطة كروماتوغرافيا الطبقة الرقيقة وكروماتوغرافيا الطبقة التحضيرية وكروماتوغرافيا السائل والطيف الكتلي والاشعه تحت الحمراء والاشعه فوق البنفسجية وكانت نتيجة الكشف الكيمائي وجود القلويدات (الدايهيدروكواديين، الجليدونين وبابافروبين C) في نبات الخشخاش.

الكلمات المفتاحية: الخشخاش المنثور، الدايهيدروكواديين، الجليدونين، بابافروبين c.

Introduction

Poppy (*Papaver rhoeas* L.) figure 1 is a temperate native with a very wide distribution area, from Africa to temperate and tropical Asia and Europe⁽¹⁾. It grows in fields, beside roads, and on Grassland. *Papaver rhoeas* is a variable, erect annual, forming a long-lived soil seed bank that can germinate when the soil is disturbed. In the northern hemisphere it generally flowers in late spring (between May and October but if the weather is warm enough other flowers frequently appear at the beginning of autumn. It grows up to about 70 cm (28 in) in height⁽²⁾. The stems hold single which are large and showy, 5–10 cm (2–4 in) across with four petals that are vivid red, most commonly with a black spot at their base⁽³⁾. The plant has been used

for medicinal proposes a long time ago for treatment of a wide range of diseases including inflammation, diarrhea, sleep disorders, treatment of cough, analgesia, and also to reduce the withdrawal signs of opioid addiction⁽⁴⁾. Furthermore, it is known to claim intestinal and urinary irritation and to be useful in various conditions such as bronchitis, pneumonia, and rash⁽⁵⁾. Pharmacological studies have shown that the plant extract may have some radical scavenging properties⁽⁶⁾. Investigations also indicated that the *Papaver rhoeas* extract also possess properties of anti-ulcer genic⁽⁷⁾, Anti-nociception⁽⁸⁾, anti inflammatory effect⁽⁹⁾ and Stress amelioration effect⁽¹⁰⁾. This study was conducted for identification of alkaloids that extracted from *Papaver rhoeas*.

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Figure 1. Iraqi *Papaver rhoeas*.

Materials and Methods

Collection of plant material

The arial parts of *Papaver rhoeas L.* (papaveracea) are collected from Zurbatiya which is an Iraqi township in the north east of Wasit province in Iraq , a border crossing with Iran in April (2019) The plant authenticated by Dr.sukiana Abbas Alewi in College of Sciences, University of Baghdad. The Plant parts cleaned and dried in shade for two weeks then the dried plant material was coarsely powdered using electrical grinder and weighed.

Extraction of alkaloids

About 200gm of the dried plant material was defatted by maceration in hexane for 24 hours then filtered and the dried defatted plant was placed in a soxhlet, and a sufficient amount of 85% methanol (1 L) was added to the apparatus for 14 hours until complete exhaustion was achieved. The alcoholic extract was filtered by filter paper and the filtrate was evaporated to dryness using rotary evaporator to obtain 50gm dark-greenish residue. The residue was suspended in about 70ml of 6% HCl (pH 4), and partitioned with chloroform (70ml x 3). The upper aqueous acidic layer was separated and basified by ammonium hydroxide (23-25%) added gradually by a dropper in room temperature with stirring until getting pH 10 then the basified aqueous layer partioned three times with equal volume of chloroform in separatory funnel. The lower chloroform layer was collected, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness.

The steps of alkaloids extraction were shown in the following scheme.

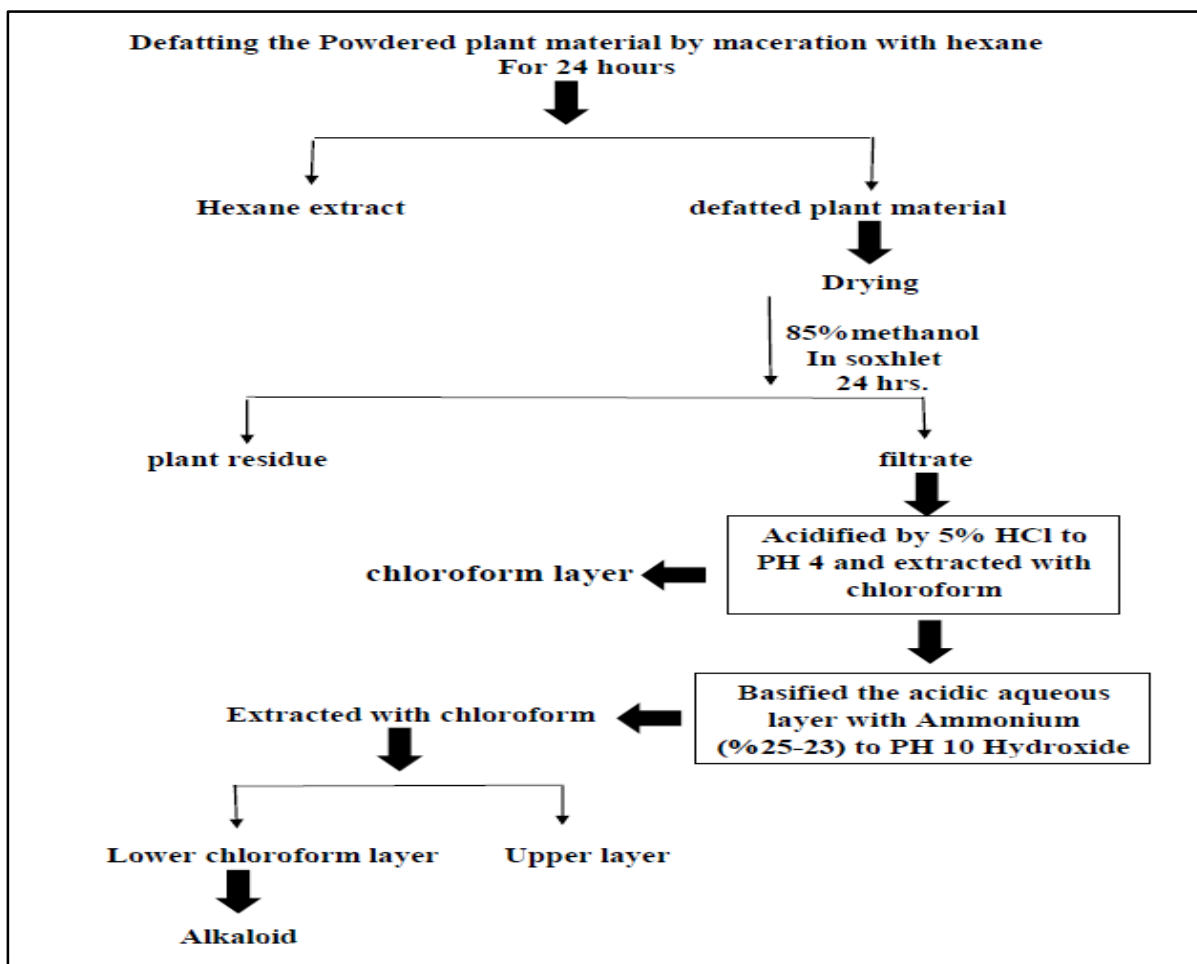


Figure 2. Steps of alkaloids extraction.

Identification, isolation and purification of alkaloids from *Papaver rhoeas* plant

1. Preliminary phytochemical screening of alkaloid compound for the methanolic plant extract using dragendorff and Mayer reagent

2. Thin layer chromatography (TLC):- few milligrams from the extracted alkaloids was re suspended with 1 ml absolute methanol then applied on an analytical TLC plate pre coated with silica gel 0.25mm using the mobile phase: Cyclohexane: Chloroform: Diethyl amine (70:20:10)⁽¹²⁾.

3-Isolation and purification of alkaloid compounds by preparative thin layer chromatography :- A readymade pre coated silica gel glass plate with 0.5mm thickness was placed in oven for 5 minute for activation. At 100C then the plate was placed into glass jar contained 100 ml mobile phase then closed tightly, and left for saturation for about one hour far from sunlight and air current, After development, the plates were taken out of the jar then left at room temperature to dry then the bands were determined and scrubbed by needle under UV light using a wavelength of 254.

5- LC mass (Liquid chromatography–mass spectrometry):- The analytical LC-MS was performed using Agilent System Joined to an Applied Bio systems API 2000 mass spectrometer.

6- FT-IR (Fourier-transform infrared spectroscopy):-Fourier transform infrared spectroscopy is a technique for material analysis it offers an qualitative analysis of the sample. FTIR identified chemical bands in molecules, the range of scanning 4000-400 cm-1.

7- UV (Ultraviolet–visible spectroscopy):-

Identification of isolated compound was done by measuring the absorbance by measuring their UV absorption at 240nm

Results

Preliminary Identification of Alkaloid n *Papaver Rhoëas* Plant by preparative thin layer chromatography

Isolation and purification of alkaloids was carried out by using preparative TLC, in jar contains: Cyclohexane: Chloroform: Diethyl amine (70:20:10) as mobile phase,

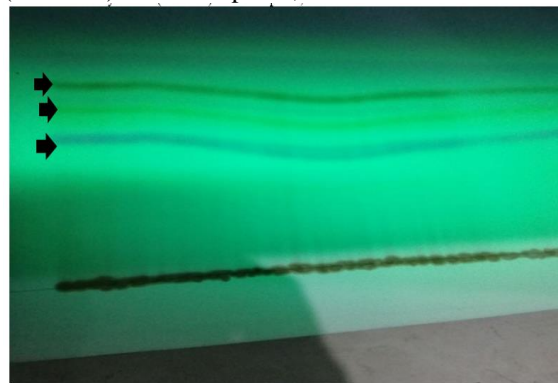


Figure 3. Preparative TLC chromatogram for *Papaver rhoeas* alkaloids on silica G F254 plate using mobile phase Cyclohexane: Chloroform: Diethylamine (70:20:10) under U.V light

Identification of the isolated compounds by LC-Mass Technique

Identification of compound A

LC mass (Liquid chromatography–mass spectrometry) for compound A are shown in figure 4 below

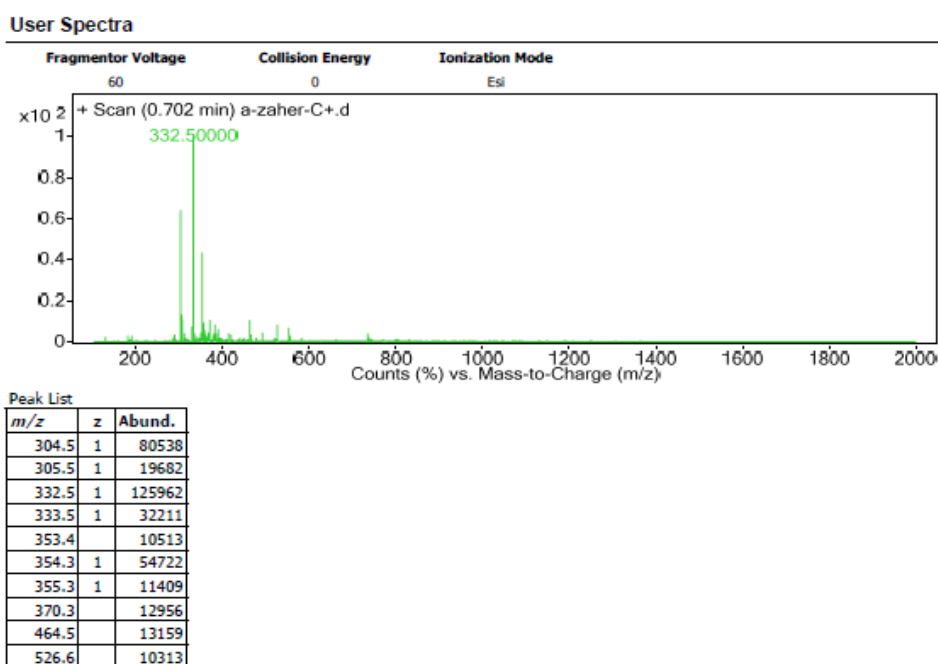


Figure 4. LC mass for compound A.

The molecular ion peak at m/z 304 $[M]^+$ was nearly correspond to a molecular formula of dihydrocodeine ($C_{18}H_{23}NO$) which is 301, also the abundance of peak 304 is (80538) which is the second highest one between other ions as shown in figure 4 above.

Depending on the analysis above, the expected chemical structure for

The isolated compound A is demonstrated in figure 5 below

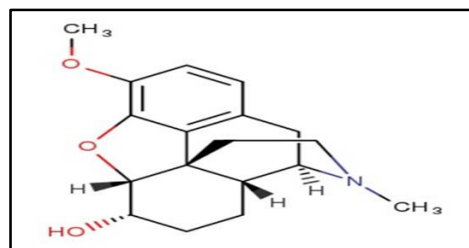


Figure 5. Chemical structure of compound A (dihydrocodeine).

The λ max spectrum and FTIR chart for compound A were shown in figure 6, 7 and table 1

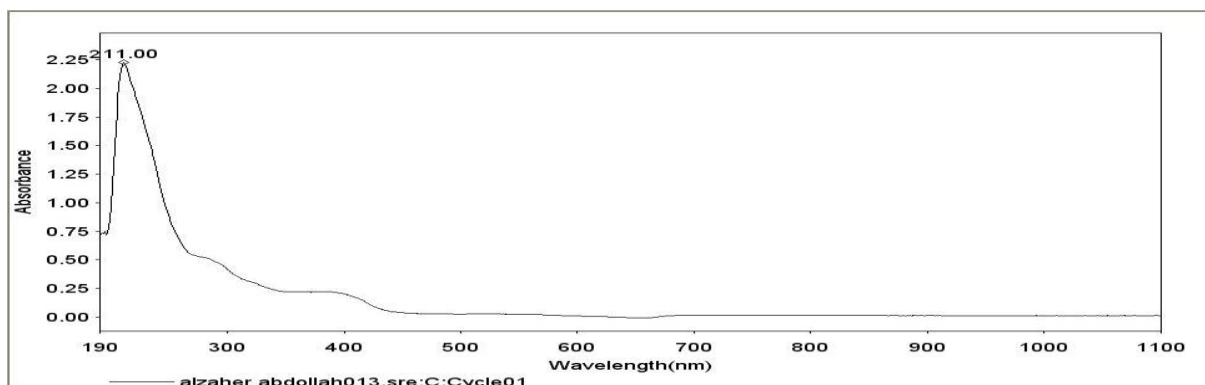


Figure 6. The λ max spectrum for dihydrocodeine.

The λ max spectrum for the extracted alkaloid was 211 nm which is a typical

spectrum for dihydrocodeine alkaloid 211 nm⁽¹³⁾.

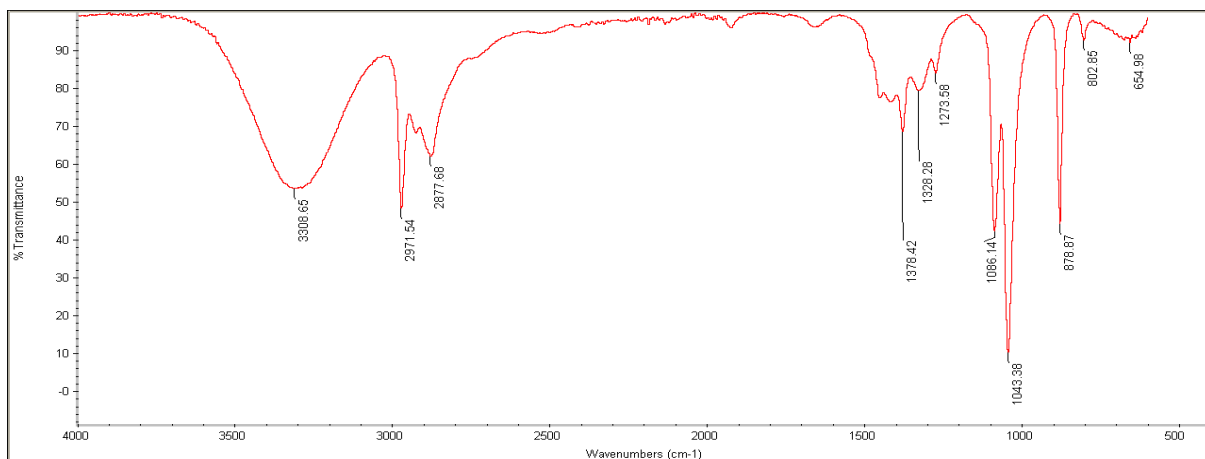


Figure 7. IR spectrum for compound A

Table 1 .The FTIR spectrum regions indicated the major functional groups in compound A

IR band of compound A	Interpretation
3308 ,2971	O-H stretching of phenol and carboxylic.
2877	Asymmetric and Symmetric stretching of CH_2
1378	O-H bending of phenol
1273	C-O-C stretching of ether
1066,1043	C-H bending of aromatic (in plane)
887,802,654	C-H and C=C bending of aromatic in and out and in-plane

Finally, the data obtained from IR, UV, LC/MS of the isolated compound A were identical with the Data of dihydrocodeine, which indicate that compound A could be Dihydrocodeine alkaloids⁽¹⁴⁾.

Identification of compound B

The LC mass (Liquid chromatography–mass spectrometry) for compound A are shown in figure 8

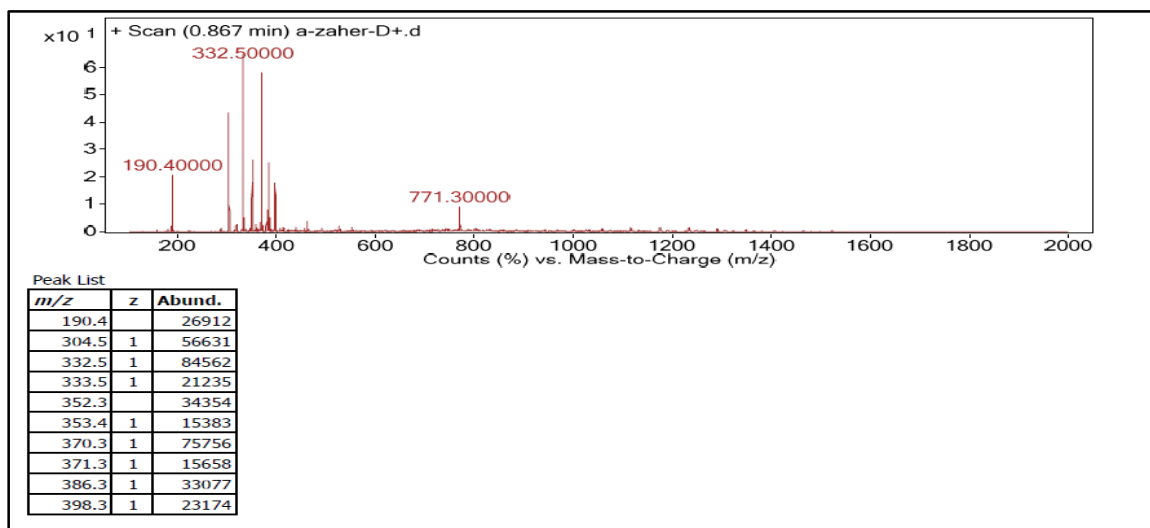


Figure 8. LC mass for compound B.

The molecular ion peak at m/z 370 $[M]^+$ and 371 which represent M and $M+H$ respectively that correspond to a molecular formula of Papaverrubine C (Epiporphyroxine) 370, also the abundance of peak 370 is (75756) which is the second highest peak between other ions. Depending on the analysis above, the expected chemical structure for the isolated compound is demonstrated in figure 11.

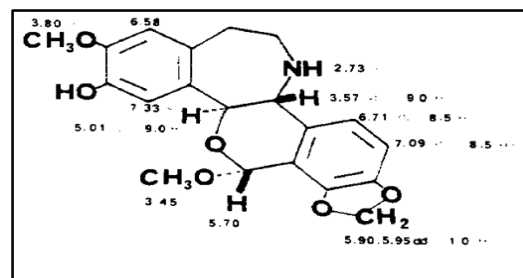


Figure 9. chemical structure of compound B indicated Papaverrubine C (Epiporphyroxine).

The λ_{max} spectrum and FTIR chart for compound B were shown in figure 10, 11 and table 2.

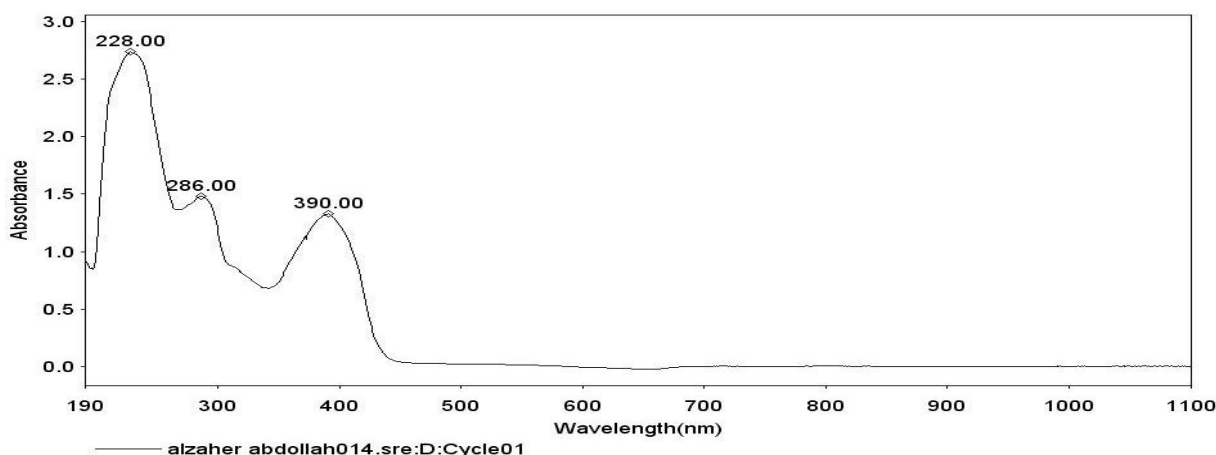


Figure 10. The λ_{max} spectrum for Papaverrubine C compound.

The λ_{max} spectrum for the extracted alkaloid was 232 and 285 nm which is a typical

spectrum for Papaverrubine alkaloid 285 nm⁽¹⁵⁾.

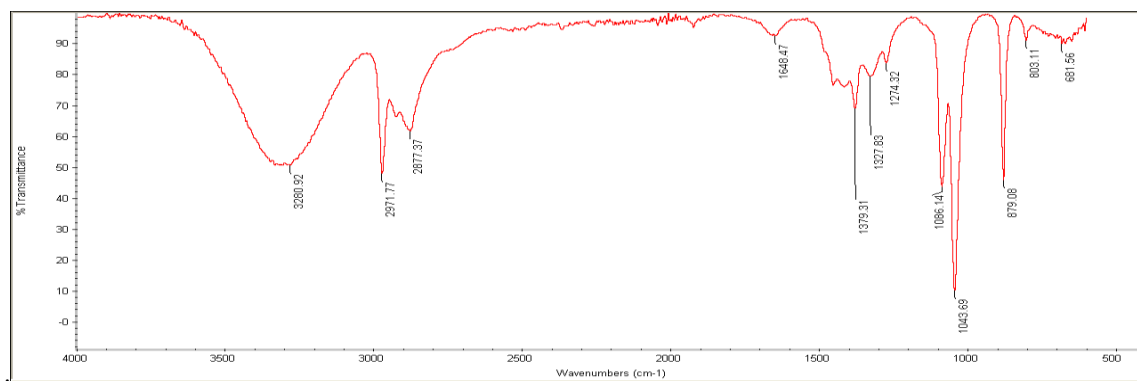


Figure 11. IR spectrum for compound B

Table 2. The FTIR spectrum regions indicated the major functional groups in compound B

IR band of compound B	Interpretation
3280, 2971	O-H stretching of phenol and carboxylic
2877	Asymmetric and Symmetric stretching of CH ₂
1648	C=C stretching of alkene
1379	O-H bending of phenol
1068.1043	C-H bending of aromatic (in plane)
879,803,681	C-H and C=C bending of aromatic in and out and in-plane

Finally, the data obtained from IR, UV, LC/MS of the isolated compound B were identical with the Data of papaverrubine C (Epiporphyroxine), which indicate that compound B could be papaverrubine C (Epiporphyroxine) which is Rhoeadines/papaverrubines type of alkaloids^(16,17).

Identification of compound C

The LC mass (Liquid chromatography–mass spectrometry) for compound C are shown in figure 12.

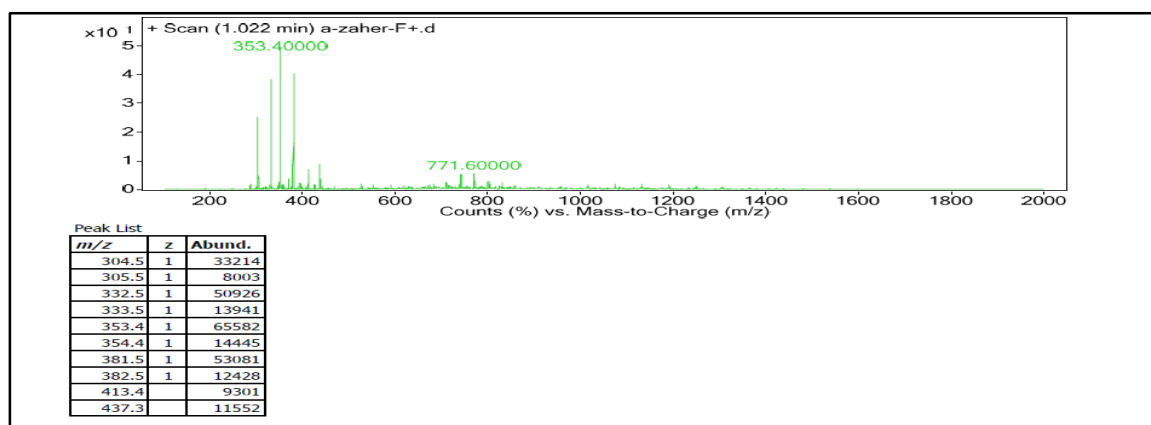


Figure 12. Lc mass diagram for compound C.

The molecular ion peak at m/z 353 [M]⁺ and 354 which represent M and M+H⁺ respectively that correspond to a molecular formula of chelidonium (C₂₀H₁₉NO₅, also the abundance of peak 353 is (65582) which is the highest peak between other ions. Depending on this analysis, the expected chemical structure for the isolated compound is demonstrated in figure 13

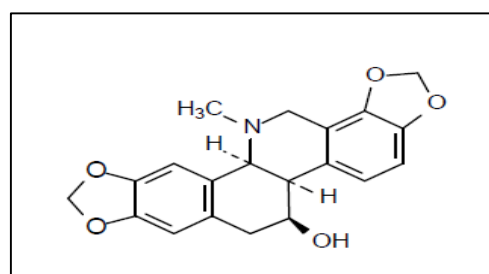


Figure 13. chemical structure of chelidonium.

The λ_{max} spectrum and FTIR chart for compound C were shown in figure 14, 15 and table 3.

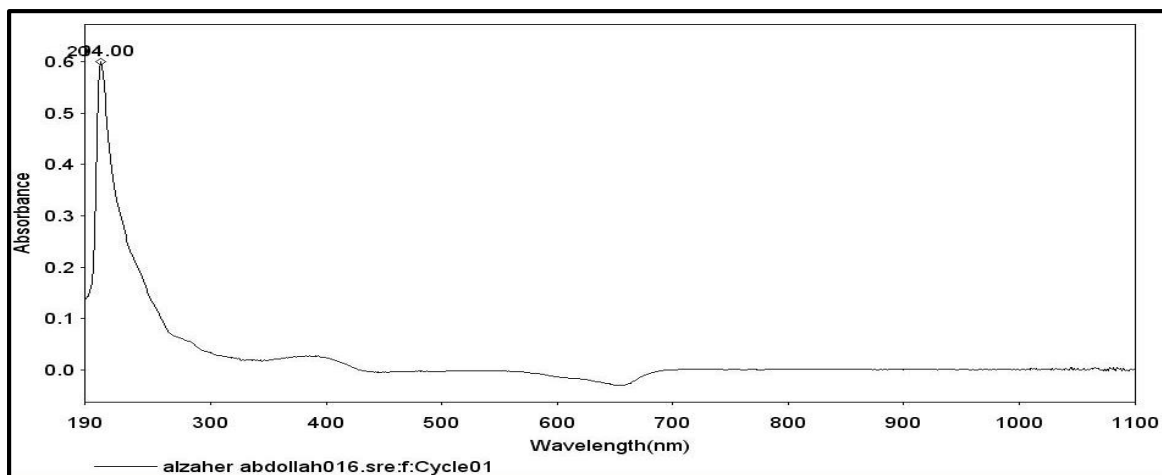


Figure 14. The λ max spectrum for compound C

The major absorption maxima are 201 which is the same that also observed for chelidonium at 204.

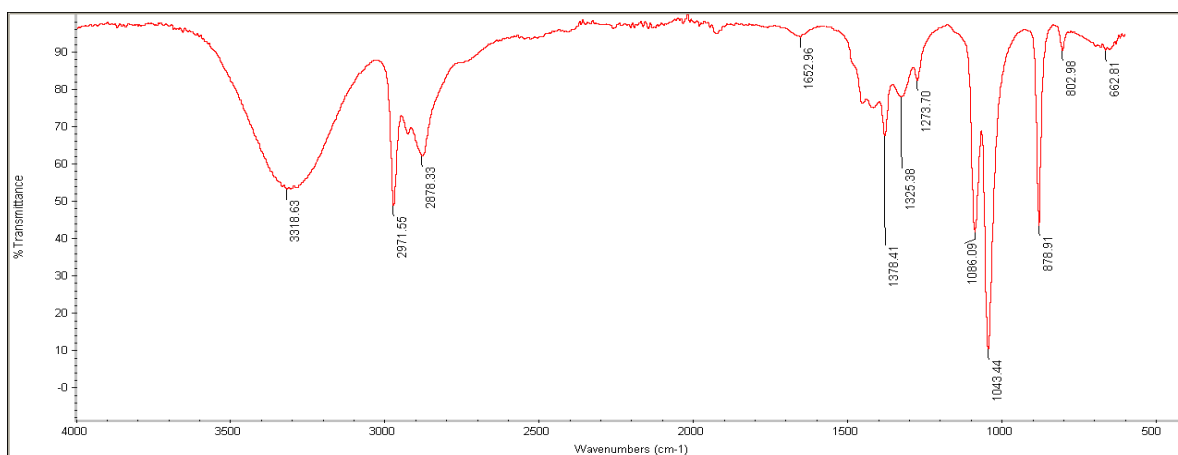


Figure 15. IR spectrum of chelidonium.

Table 3 .The FTIR spectrum regions indicated the major functional groups in compound C.

IR band of compound C	Interpretation
3318,2971	O-H stretching of phenol and carboxylic
2878	Asymmetric and Symmetric stretching of CH ₂
1652	C=C stretching of alkene
1378,1325	O-H bending of phenol
1273	C-O stretching of alkyl aryl ether
1086,1043	C-H bending of aromatic (in plane)
878,802,662	C-H and C=C bending of aromatic in and out and in-plane

Finally, the data obtained from IR, UV, LC/MS of the isolated compound C were identical with the Data of chelidonium, which indicate that compound

C could be chelidonium which is isoquinoline type of alkaloid.

Conclusion

Phytochemical investigation of wild Iraqi plant *Papaver rhoeas* was done to the whole plant and the results include the presence of different type of alkaloids[Dihydrocodeine (Morphinans),papaverrubine C (Rhoeadines/ papaverrubines), chelidonium (isoquinoline)] and these types detected by LC mass,UV,IR .

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