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Phytochemical Investigations of Iraqi *Abrus precatorius* Linn. Plant

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Abstract

The plant *Abrus precatorius*, which belong to Leguminosae (Fabaceae) family and known as Crab's eyes, Rosary pea with characteristic red and black seeds. It was used in folk medicine in India, China and East Asian countries for treatment of various diseases.

The plant was extracted by " general method of extraction" (Harborne, 1973) using 80% aqueous ethanol as a solvent of extraction by soxhlet apparatus. Preliminary qualitative phytochemical screening were performed on the crude ethanolic extract and revealed the presence of alkaloids, flavonoids ,terpenoids and phytosterols in Iraqi *Abrus precatorius* plant. Three different fractions were obtained from crude extract which are fraction one (chloroform fraction), fraction two (ethyl acetate fraction), and fraction three (petroleum ether fraction) which are represent alkaloids, flavonoids and steroids respectively. The alkaloid abrine was isolated from the chloroform fraction in pure form by using preparative thin layer chromatography (PTLC) and then subjected to different physico-chemical and specteral analytical techniques to identify its chemical structure: melting point (M.P.), thin layer chromatography (TLC), high performance liquid chromatography (HPLC) , fourier transforms infrared spectra (FT-IR) and elemental microanalysis (CHNO).

Keywords: ***Abrus precatorius*** **,Alkaloid abrine, HPLC, FT-IR, CHNO.**

دراسة كيميائية لنبات عين العفريت العراقي

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الخلاصة

نبات عين العفريت هو نوع من النباتات ينتمي الى العائلة البقولية وهو نبات مستزرع في شمال العراق وتحديدا في مدينة الموصل ويعرف ايضاً بالبازلاء الوردية مع بذور ذات اللون الاسود والاحمر التي يتميز بها. كان يستخدم في الطب الشعبي في الهند والصين وبلدان شرق آسيا لعلاج أمراض مختلفة. لم يخضع النبات لاي نوع من الدراسة التحليلة او التشخيصية لذلك يعتبر هذا البحث أول بحث أجري في العراق لدراسة المكونات الكيميائية النباتية التي لها اهمية طبية ونشاط الدراسة الدوائية الأولية (في المختبر) كمضاد للاكسدة. وفي هذه الدراسة تم استخلاص وفصل وعزل وتنقية بعض المركبات الكيميائية (القلويدات والستيرويد النباتي بيتا سايتوستيرول).

تم استخلاص النبات من خلال "الطريقة العامة للاستخراج"للعالم (هاربورن، ١٩٧٣) باستخدام ٨٠٪ من الإيثانول المائي كمذيب للاستخلاص بواسطة جهاز سوكسليت (Soxlet). وقد تم إجراء الفحوصات الكيميائية الأولية لنواتج الأيض الثانوية المختلفة من خلال اختبارات كيميائية محددة على مستخلص الايثانول الخام وقد كشف عن وجود قلويدات، فلافونويدات، تربينويدات و الستيرويدات في نبات القلقل الرجائي العراقي . وكذلك تم الحصول على ثلاثة اجزاء مختلفة من المستخلص الخام والتي هي الجزء الاول (جزء الكلوروفورم)، والجزء الثاني (جزء الايثيل اسيتيت)، والجزء الثالث (جزء الأثير البترولي) والتي تمثل قلويدات، الفلافونويدات والستيرويدات على التوالي. تم عزل قلويد ابرين (abrine) من جزء الكلوروفورم (الجزء الاول) في شكل نقي باستخدام كروماتوغرافيا الطبقة الرقيقة التحضيري (preparative TLC) وقد استخدمت التقنيات الفيزيائية والكيميائية والتحليلية الطيفية لتحديد تركيبها الكيميائي ووزنه الجزيئي ، وتشمل: نقطة الانصهار، كروماتوغرافيا الطبقة الرقيقة (TLC) ، الكروماتوغرافيا السائل عالي الاداء (HPLC) ، مطياف الاشعة تحت الحمراء (FT-IR) والتحليل الجزئي عنصري (CHNO).

الكلمات المفتاحية :نبات عين العفريت ، القلويد أبرين ، كرومرتوغرافيا السائل عالي الاداء، مطياف الاشعة تحت الحمراء ، التحليل الجزئي العنصري

Introduction

The *Abrus precatorius* Linn. of the Leguminosae family is woody twinning plant widely distributed in India [(1)]. And it is distributed in the north of Iraq certainly in Al-Mousul city and it's authenticated at first time in Iraq. It's known as Jequirity bean, rosary pea, and Crab's Eye. *Abrus precatorius* (figure 1) is a slender, twining vine with a woody base. It is supported generally on other plants or a fence. The leaves are pinnate and glabrous**.** The fruit of Abrus plant **,** a pea-shaped pod about 1.5 inches long, splits open as it dries to reveal (3-5) hard-coated, brilliant scarlet, pea-sized seeds with a small enamel-black spot at the point of attachment (hilum). [(2, 3)]. It is used traditionally to treat eye diseases, gastrointestinal aliments and as abortifacient**.** [(4-8)].

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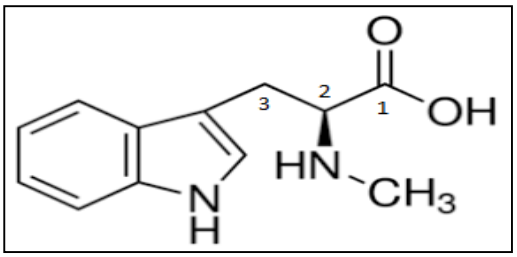
Several studies have shown that the extracts of *Abrus precatorius* at different concentrations have antimicribial activity against G + ve and G –ve bacteria and that attributed to widespread use of the plant as local remedy for a variety of ailments ranging from ulcers to bronchitis. [9]

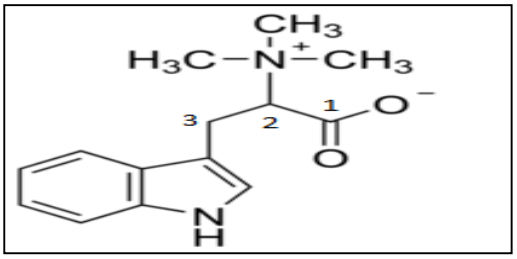
While other studies have been revealed that the petroleum ether extract of *Abrus precatorius* Linn (PEEAP) possessed significant antitumor activity. [10] Furthermore, it have antidiabetic [11], antioxidant [12], antifertility [13] and anti-inflammatory [14] effects.

Abrus precatorius are known as ones of the most toxic plant worldwide especially the seeds. The toxic principle contained in *Abrus precatorius* has been found to be the toxalbumin abrin [15]**.** The estimated human fatal dose is 0.1 to 1 microgram/kg and ingestion of one to two crushed seeds is sufficient to cause death. [16] Various alkaloids of indole type have been reported in *Abrus precatorius* [17] **.**which are abrine, hypaphorine and trigonelline, figure (2). Abrus plant contains other phytochemicals like flavonoids and phytosterols (beta-sitosterol) which have various pharmacological activities. [18, 19]

Figure 1. Iraqi *Abrus precatorius* plant [20]



A



B

Figure 2. A-Chemical structure of abrine, (2S)-3-(1H-indole-3-yl)-2-(methylamino) propionic acid, ($C_{12}H_{14}O_2N_2$) [21], B-Figure (1.4): Chemical structure of hypaphorine, (3S)-3-(1H-indole-3-yl)-2-(trim ethyl azaniumyl) propionate, ($C_{14}H_{18}O_2N_2$) [22].

Material and methods

Plant collection

The plant was authenticated by Dr. Ibrahim Saleh/ Head of Pharmacognosy department/ College of Pharmacy/ Al-Mustanserya University.

The seeds and aerial parts were collected during October (2016) from Al- Mosul city which is located at the north of Iraq then it dried at room temperature in the shade then pulverized by mechanical mills and weighed.

Extraction and fractionation of different active constituents: [23]

Shade-dried coarsely powdered plant (seeds and aerial parts) (500gm) were defatted by maceration with hexane for 24 hr then allowed to dry at room temperature. The defatted plant materials was extracted with 80% ethanol (2 L) in soxhlet apparatus until complete exhaustion.

The alcoholic extract was evaporated under reduced pressure at a temperature not exceeding 40 ˚C to give a dark brown residue designated as a crude fraction.

Crude fraction was acidified with hydrochloric acid (5%) to pH 2 and partitioned (three times) with ethyl acetate to get two layers (aqueous acidic and ethyl acetate layer). The aqueous acidic (A-1) layer was then separated and basified with equal volume of ammonium hydroxide 15% to pH 10 and extracted with chloroform in the separatory funnel (three times) to get two layers, the chloroform layer which was separated and dried by addition of anhydrous sodium sulfate powder then evaporated under reduced pressure at a temperature not exceeding 40°C to give brownish residue designated as fraction 1 (F-1) and aqueous basic layer designated as (A2).

The ethyl acetate layer of the original alcoholic extract (crude fraction) was evaporated to dryness under reduced pressure and basified with 300ml of sodium hydroxide 5% to pH 10 and extracted with chloroform in the separatory funnel to get two layers, the aqueous basic layer and chloroform layer.

The aqueous basic layer was separated, evaporated to dryness and acidified with 5% hydrochloric acid to pH 2 then extracted with ethyl acetate to get fraction designated as fraction 2 (F-2) .Chloroform layer was also separated and evaporated to dryness under reduced pressure then partitioned with methanol 80% and petroleum ether to get two layers methanol 80% and petroleum ether fraction which is designated as fraction 3 **(F-3),** (Figure 3). Each fraction was tested using specific chemical test.

Identification of alkaloids in Abrus pretacorius plant extract

1. Preliminary phytochemical screening of alkaloids in the *Abrus precatorius* plant using the ethanolic extracts from plants using Mayer's, Wagner's and the Dragendorff's reagents to identify the alkaloids. [23-25].

2. Thin layer chromatography (TLC**):**

In this qualitative identification using a ready-made aluminum plates of silica gel GF_{254} and using 3 different developing solvent systems for detection the plant alkaloids in fraction one (F-1) comparing with alkaloid standard(s) and detection by spraying with Dragendorff's spray reagent, and they are listed in the table (1):

Table 1. Developing solvent systems were used in identification of expected alkaloids in (F-1) of *Abrus precatorius* plant extract

No.	Composition	References
$S2_k$	Chloroform: acetone: diethyl amine (50 : 40 :10)	**26**
$S4_k$	Dichloromethane: methanol: water: formic acid: diethyl amine (72.3 : 25 : 2.5 : 0.1 : 0.1)	**27**
$S5_k$	Toluene: ethyl acetate: diethyl amine(70 : 20 : 10)	**28**

3. Qualitative estimation of fraction one (F-1) by high performance liquid chromatography (HPLC) :

The expected alkaloids in (F-1) were separated by HPLC method and identified by comparison with standard compounds using ODS C 18 column (250 x 4.6 mm , 5µm particle size) and acetonitrile -0.1 % H_3PO_4 +0.1g sodium sulphonate (35:65) as a mobile phase with flow rate 1.2 ml / min, and UV detector at 254 nm at room temperature[29].

Isolation and purification of alkaloid from (F-1)

Isolation of alkaloid was carried out by using preparative TLC. fraction one (F-1) was applied on a number of preparative TLC plates as a concentrated solution in streak using capillary tube on each plate , then the plates placed inside glass tank which contained the S5K (Toluene: ethyl acetate: diethyl amine (70 : 20 : 10)) solvent system.

The detection was done using Dragendroff's reagent. The band had been scrapped off, eluted with chloroform, and then filtered. The filtrate evaporated to dryness, in vacuo to give off-white powder, for more purification the iolated compound was dissolved in a hot ethylacetate. The hot solution was filtered and the filtrate was concentrated and cooled to give solid precipitate of (expected abrine alkaloid).

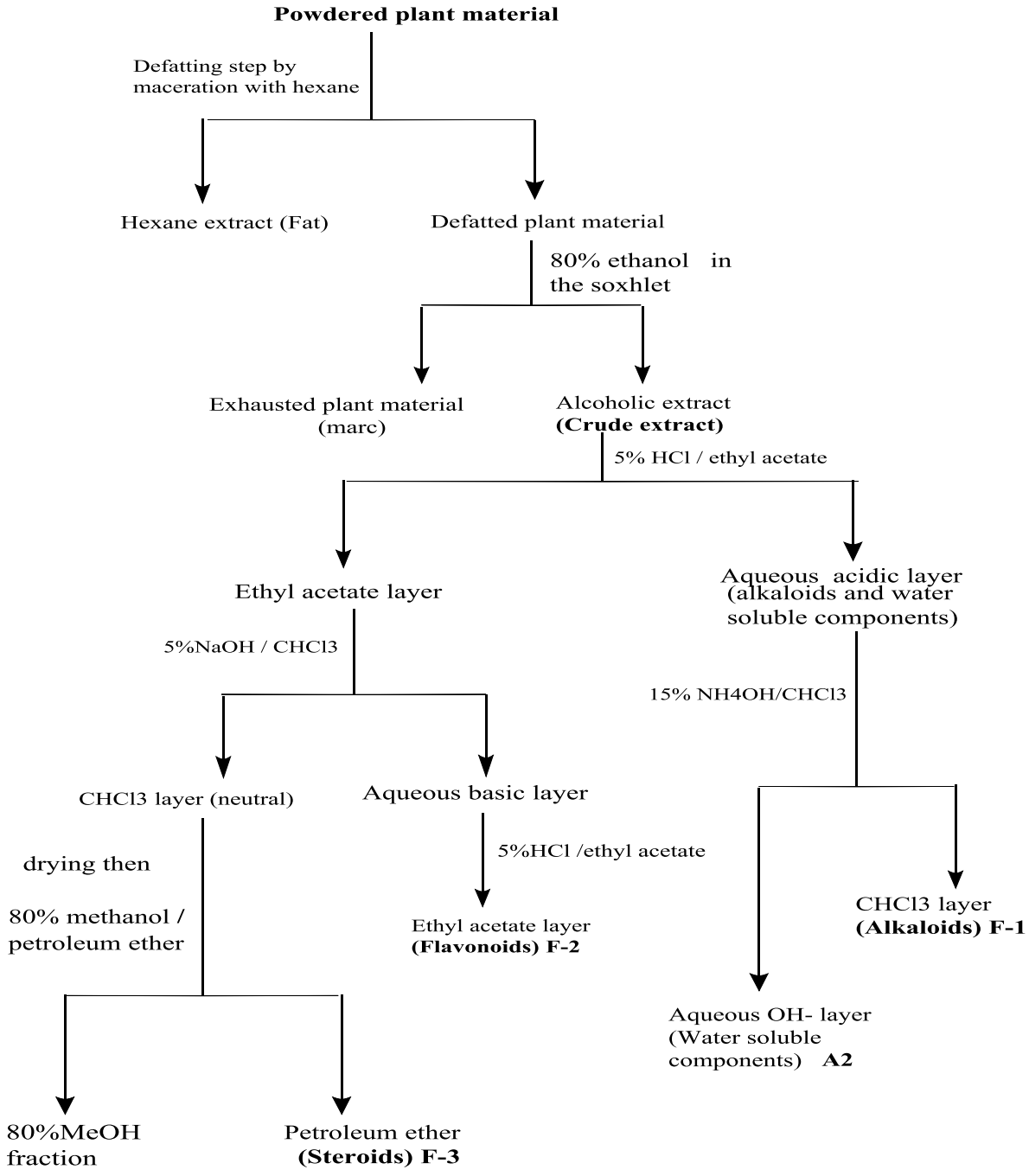


Figure 3. General scheme for separation of different plant constituents [23]

Results and discussion

Extraction method

The method of extraction mostly depend on the critical input parameters; understanding the nature of plant matrix; chemistry of bioactive compounds and scientific expertise. Since different plant parts contain different chemical classes of active constituents, alkaloids (basic compounds), flavonoids (acidic compounds) and steroids (neutral compounds) so the fractionation based on the conversion of basic compound to its salt by aqueous mineral acids, and when the salt of an alkaloid is treated with hydroxide ion, nitrogen gives up a hydrogen ion and the free amine is liberated which is taken or extracted by specific organic solvent like (chloroform) to get free alkaloids **(F-1)** leaving quaternary alkaloids and water soluble compounds in the aqueous layer **(A-2).**

Table 2. Percentage of different fractions obtained from plant extraction of *Abrus precatorius* L.

Dried plant weight	Crude extract weight	Weight of fractions			Percentage %		
		F1 alkaloids	F2 flavonoids	F3 sterols	F1 alkaloids	F2 flavonoids	F3 sterols
500 gm	50 gm	3 gm	2.4 gm	4.8 gm	0.6%	0.48%	0.96%

Preliminary qualitative phytochemical analysis

The results of phytochemical analysis of crude ethanolic extract are given in (table 3).

***Preliminary identification of alkaloid in Abrus plant by chromatography*A- TLC:**

Thin layer chromatography of different fractions F- 1 confirms the presence of indolic alkaloid abrine in fraction-1, and that alkaloid appeared as single spot by using the mobile phase systems **($S2_k$, $S4_k$ and $S5_k$)**. The spot of alkaloid have the same retention factor (R_f) value comparing with its corresponding standard after detection by the dragendorff's sparying reagent as shown in the figures from (4 to 6).

Table 3: Phytochemical screening of alkaloids in the crude ethanolic extract of *Abrus precatorius* plant.

Alkaloids	Dragendorff's reagent	Orange ppt	+
	Mayer's reagent	White ppt	+
	Wagner's reagent	Reddish brown ppt	+

(+) represent the presence of alkaloids

Table 4. The R_f values of abrine alkaloid in (F-1) in *Abrus precatorius* extract, and standard in different developing solvent systems using TLC.

Mobile phases	R_f value of abrine standard	R_f value of abrine alkaloid in (F-1)
$S2_k$	0.4	0.38
$S4_k$	0.58	0.6
$S5_k$	0.24	0.23

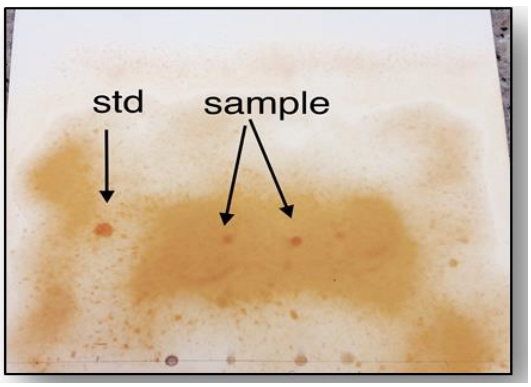


Figure 4. TLC chromatogram of F-1 for *Abrus pretacorius* plant using silica gel GF_{254nm} as adsorbent and $S2_K$ [Chloroform: acetone: diethylamine (50: 40:10)] as a mobile phase. Detecting by spraying with dragendorrf's reagent.
Std: Abrine standard.
Sample: fraction 1 (F-1).

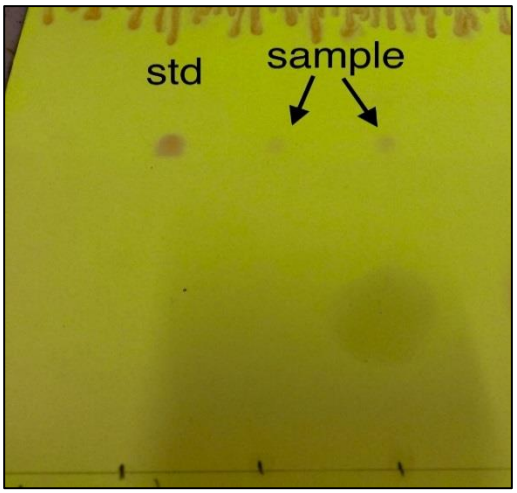


Figure 5. TLC chromatogram of F-1 for *Abrus pretacorius* plant using silica gel GF_{254nm} as adsorbent and $S4_K$ [Dichloromethane: methanol: water: formic acid: diethylamine (72.3: 25: 2.5: 0.1: 0.1)] as a mobile phase. Detecting by spraying with dragendorrf's reagent.
Std: Abrine standard.
Sample: fraction 1 (F-1).

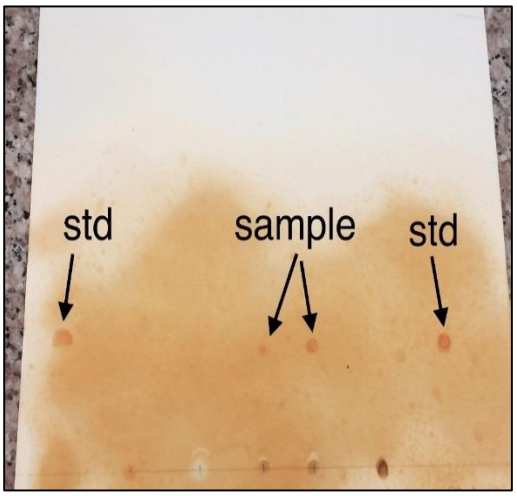


Figure 6. TLC chromatogram of (F-1) for *Abrus pretacorius* plant using silica gel GF_{254nm} as adsorbent and $S5_K$ [Toluene: ethyl acetate: diethyl amine (70: 20: 10)] as a mobile phase. Detecting by spraying with dragendorrf's reagent.
Std: Abrine standard.
Sample: fraction one (F-1).

B- HPLC analysis

The results gained from HPLC analysis method:

1-The retention time of alkaloids (A, B and C) in (F-1) (figure 7) match with the retention time of standard alkaloids (1 trigonelline, 2 Abrine and 3 hypaphorine) (figure 8) respectively.

2-An abrine alkaloid have the high percent among the other alkaloids.

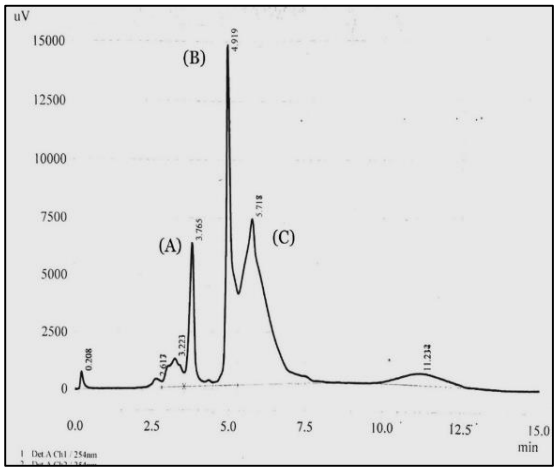


Figure 7. HPLC chromatogram of fraction one (F-1).

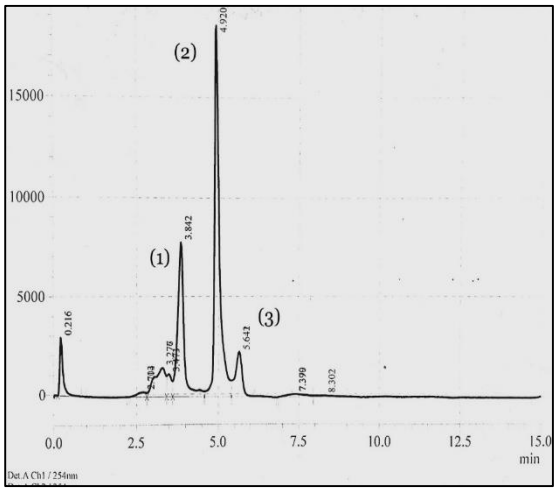


Figure 8. HPLC chromatogram of alkaloid standards.

Characterization and identification of the isolated abrine alkaloid:

1-Thin Layer Chromatography (TLC):

The isolated alkaloid appeared as single spot having the same color and R_f value as the corresponding abrine standard as shown in the figure (9). R_f value **0.19 and 0.18** for abrine standard and isolated abrine respectively**.**

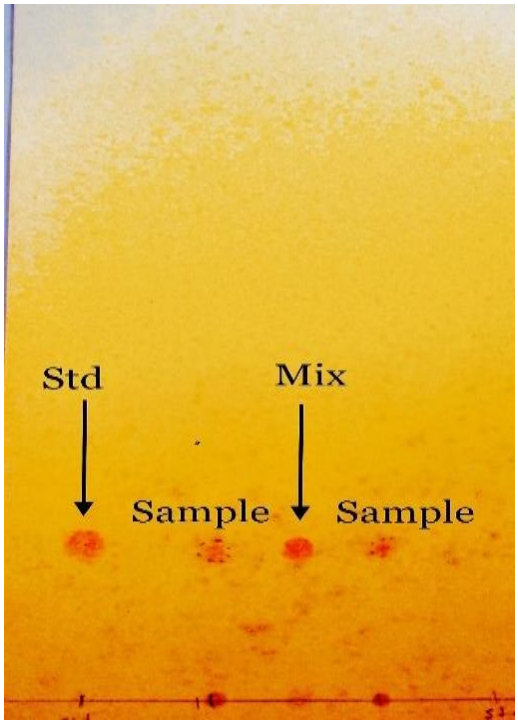


Figure 9. TLC of the band (sample) isolated by preparative TLC from fraction-1 (F-1) using silica gel GF_{254nm} as adsorbent and S_{5K} as a mobile phase. Detection by dragendorff's spraying reagent.
Std : Abrine standard.
Mix: abrine standard and sample mixture.

2-Melting point (M.P.)

The isolated alkaloid was characterized from its sharp melting point of 273 – 277℃ compared with standard alkaloid melting point 275 – 280 ℃.

3- HPLC analysis

The isolated alkaloid was identified by comparison its retention time (4.101 min) with the retention time of abrine standard (4.087 min) as shown in the figures (10 and 11).

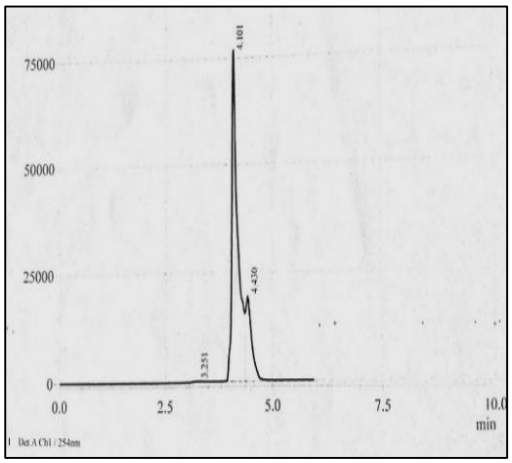


Figure 10 . HPLC chromatogram of isolated alkaloid .

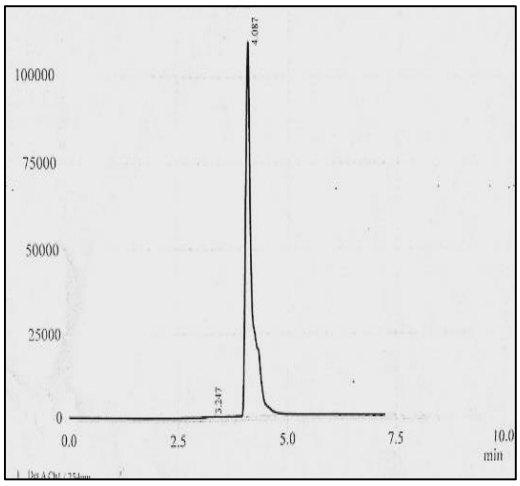


Figure 11. HPLC chromatogram of abrine standard alkaloid.

FTIR

The IR spectrum of isolated alkaloid showed the characteristic bands which are reported for abrine standard as shown in (table 5) and figure (12).

Table 5. The characteristic bands frequencies from FT-IR spectrum of isolated [30]

Functional group	Group frequency wave number (in cm^{-1})	Assignment
O-H	3527	O-H stretching of carboxylic acid
N-H	3452	N-H stretching (2° amine)
C=C-H	3039	C-H stretching of aromatic alkene
C-H	2947, 2891,2810	Asymmetric and symmetric stretching of CH_2 and CH_3
C=O	1735	C=O stretching of carboxylic acid (dimer)
C=C	1585,1614	C=C stretching of aromatic alkene and N-H bending is included
C-H and C=C	821,810,738	C-H and C=C bending of aromatic (out and in-plane)

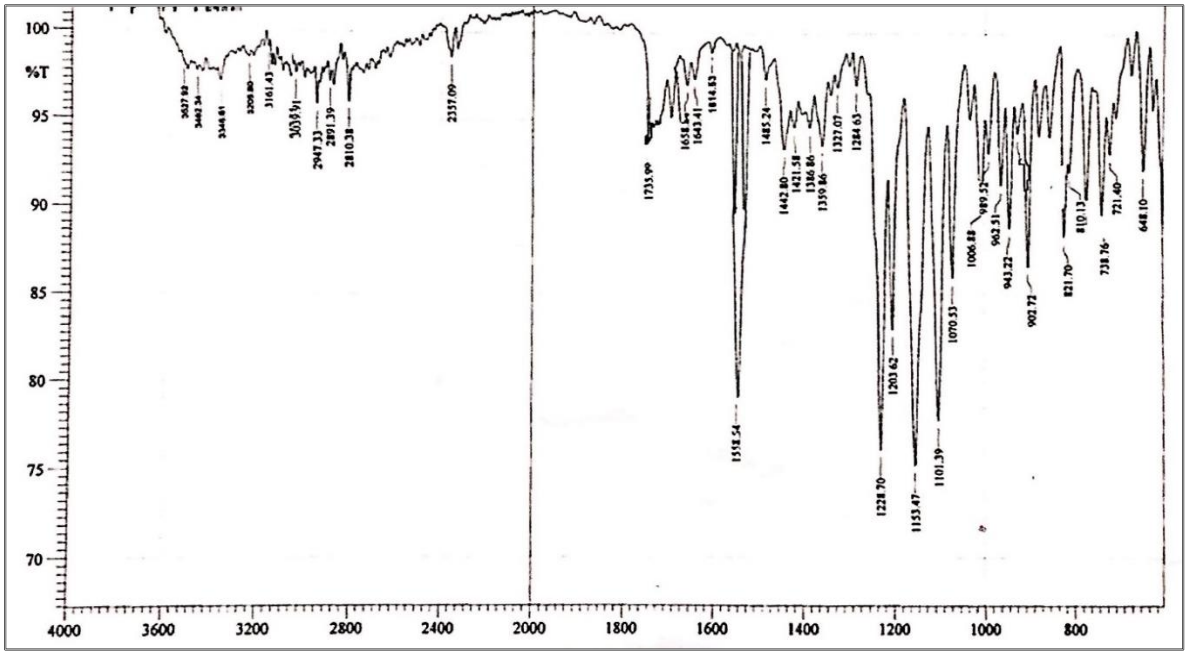


Figure 12.IR spectrum of isolated alkaloid.

1. Elemental microanalysis CHNO

The isolated alkaloid was subjected to elemental microanalysis to confirm its chemical structure. The result was listed in the table (6) which is demonstrated the different percentage of carbon, hydrogen, oxygen and nitrogen found in the compound.

Table 6. Elemental Microanalysis of the isolated alkaloid.

Element	Calculated %	Found %
C	66.09	65.49
H	6.04	5.99
N	13.22	12.91
O	14.99	14.59

All the above data coincide with that reported for abrine which might indicate that the isolated compound is the alkaloid abrine.

Conclusions

The following points were drawn based on previous findings:

1. Phytochemical screening of new Iraqi plant *Abrus precatorius* was done for seeds and aerial part of plant and the results include the presence of alkaloids.

2. The abrine is the only alkaloid was detected and isolated by preparative TLC. The other alkaloidal constituents may be present in low percent so they was demonstrated their presence by HPLC analytical method comparing with their corresponding standards.

3. Most of the results of this study are consistent with the results of international researches which carried out on this plant.

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