

## Investigation of the Alkaloids of Two Ephedra Spp. Wildly Grown in Iraq

Ekbal Al-khateeb<sup>1\*</sup>, Hakeem Al-Ani<sup>2</sup>, Kalid Al-Kadi<sup>2</sup>, Essam D.F. Al-Obaidi<sup>2</sup>,  
Naeem Shalan<sup>1</sup>, Nasser Al-Rawi<sup>1</sup>

<sup>1</sup> Al-Ahliyya Amman University, Faculty of Pharmacy, Jordan.

<sup>2</sup> Al-Anbar University, Faculty of Pharmacy, Iraq.

### ABSTRACT

Studies covering different species of Ephedra had reported the presence of alkaloids in some species of the plant, but non on the Iraqi Ephedra, therefore a research on this plant might be of value in drug industry.

In a screening program for the Iraqi plants containing alkaloids it was found that Ephedra.alate and Ephedra.transitotia contain suitable amounts of alkaloids. According to these results, together with literature survey, it was deemed to carry out this phytochemical work, with emphasis on isolation and identification of the major alkaloids that might be present. This study covers some phytochemical investigation of two spp. Of wild plants of Ephedra which has wide medicinal reputation due to different pharmacological effects. The two plant spp. Are Ephedra alata Decnc and Ephedra transitoria Riedl. of the family Ephedraceae.

Detailed preliminary investigation on the dried, aerial plant showed the presence of alkaloidal amines that were tested by both dragendprff's and ninhydrine spraying reagents. The alkaloidal content of the plant extract was authenticated with standard compounds by TLC using several developing systems which proved the occurrence of one alkaloid amine, Ephedrine. However, only one of these developing systems indicated the presence of two major compounds, Ephedrine and Pseudoephedrine in the two plant spp. The extraction method of the plant material, fractionation, and isolation procedures of the alkaloids are fully described. Ephedrine crystals were subjected to tests purity using HPLC, UV, IR, M.P. and M.M.P. which matches with standard Ephedrine. Pseudoephedrine crystals were identified by M.P. and HPLC only

**Keywords:** HPLC, IR, UV, Mixed M.P., Extract of Ephedra species.

### 1. INTRODUCTION

The world of nature contains a large number of medicinal plants about 1500 economic plants have been recorded in Iraq<sup>(1)</sup>. One of these medicinal plant is *Ephedra* (ma huang) belong to the family ephedraceae. Ephedraceae contains the single genus *Ephedra*, about 40 species of shrubs. They occur in arid regions of the subtropics and tropics<sup>(2)</sup>.

*Ephedra L.* is a genus of about 30 species represented

in the drier parts in arid regions of the subtropics and tropics; there are four species in Iraq<sup>(3)</sup>, *Ephedra alata Decnc.*, *Ephedra transitoria Riedl.*, *Ephedra foliate Boissand*, and *Ephedra alteBoiss.* There are several local names have been recorded in Iraq and Middle east such as: *Iraq AL-Husan*, *Ailajun*, *Asabi AL-Arus*, *Alanda*, *Alda* and *Adam*<sup>(1-4)</sup>. The main constituents of *Ephedra* species are, alkaloids, flavonoids and essential oils<sup>(5-7)</sup>.

According to literature survey of *Ephedra* species, it was found that not all these species contain alkaloids, however if they contain, the chief compound is almost (-)-*Ephedrine* which represents from 40%-90% of the total alkaloids. A structurally related alkaloid the diastereoisomeric(+)-*Pseudoephedrine* is also present in

\* ekbal\_9783@yahoo.com

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some species of *Ephedra*, with or without *Ephedrine*<sup>(8,9)</sup>.

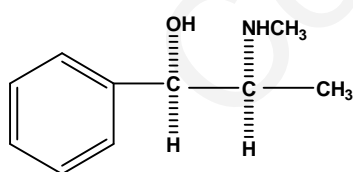
**Table 1. Different types of solvent systems used in the experiments**

S <sub>1</sub>		S <sub>2</sub>		S <sub>3</sub>		S <sub>4</sub>		S <sub>5</sub>		S <sub>6</sub>		S <sub>7</sub>	
Methanol	100	Methanol	50	Methanol	50	Chloroform	50	Ethanol	40	Methanol	40	<i>n</i> -Butanol	40
HCl	2.0	Water	50	Ethanol	50	Methanol	50	Water	10	Acetone	10	Acetic acid	10
-----	---	HCl	2.0	HCl	2.0	-----	----	HCl	-----	-----	---	Water	50

**Table 2. Represents R<sub>f</sub> values of sample as compared with the standard reference in different solvent systems**

Solvent System	S <sub>1</sub>	S <sub>2</sub>	S <sub>3</sub>	S <sub>4</sub>	S <sub>5</sub>	S <sub>6</sub>
<b>Standard Ephedrine</b>	0.75	0.74	0.64	0.60	0.60	0.84
<b>Sample</b>	0.75	0.74	0.64	0.60	0.60	0.84

*Ephedra* or ma hung is the entire plant or over ground of *Ephedra spp.* Which are distributed throughout the world<sup>(10)</sup>. The characters "Ma" means in Chinese as astringent and "hung" means yellow, probably referring to the taste and color of drug. It has been used as a medicine in China for more than 5000 years for the treatment of asthmatic conditions<sup>(8,11)</sup>. The *Ephedrine* compound which is an alkaloid can be obtained from the stems of various species of *Ephedra*. The pure alkaloid was obtained by extraction of the plant material or by chemical synthesis<sup>(9)</sup>.



**(-)-Ephedrine**

The above structure contains two dissimilar asymmetric centers, so that four optically active isomers and two racemic forms are possible. The (erythro) racemate is called *Ephedrine* and (threo) racemate is known as pseudoephedrine (*ψ*-*Ephedrine*)<sup>(5)</sup>.

The Merck chemical company was able to manufacture synthetic Ephedrine which differs from the natural levorotatory form of the alkaloid in that it is racemic, i.e. optically inactive, because it is made up of two enantiomorphous isomers<sup>(8)</sup>. The natural drug causes fewer heart complaints (e.g. palpitations). Hence the preparation of synthetic *Ephedrine* has not diminished the importance of the natural drug form<sup>(11)</sup>.

The biosynthesis of *Ephedrine* has been studied using labeled precursors showed that they are derived from the common aromatic amino acid L-phenylalanine or tyrosine such as *Ephedrine*, the main alkaloid in species of *Ephedra*<sup>(12, 13)</sup>.

The various sympathomimetic drugs including ephedrine may act on ( $\alpha$ ) and ( $\beta$ ) receptors, or they may act indirectly by releasing endogenous catechol amines. Some also have a mixed action<sup>(8,17)</sup>. The concept was based on the order of activity of series of sympathomimetic drugs at various effectors, and was greatly strengthened when specific blocking agents were developed for each receptor. *Ephedrine* is essentially a sympathomimetic drugs. When given by mouth, (in therapeutic doses as the Hydrochloride Ephedrine) it

constricts the peripheral vessels, thus raising the blood pressure. It relaxes the bronchioles and decreases the tone and peristaltic movements of the intestine, but contracts the uterus. It contracts the sphincter but relaxes the depressor muscle of the bladder, dilates the pupil, and stimulates the central nervous system. The prolonged administration of ephedrine has no cumulative effect but tolerance may develop<sup>(16,17,18)</sup>.

The aim of this research was directed towards the isolation, identification, and determination of the quantity of the most important alkaloids present in *Ephedra alata* and *Ephedra transitoria* due to their economical values in drug industry.

## EXPERIMENTAL

### Plant Materials:

The plant material of *Ephedra alata* was collected from South East sector of the desert region of Iraq and West of Karbala. The plant material of *Ephedra transitoria* was collected from Falluja. These plants were identified by the department of Pharmacognosy/College of Pharmacy/University of Baghdad and authenticated by National Herbarium of Iraq Botany Directorate at Abu-Ghraib.

### Procedures:

1. The plant were collected during June up to October and were cleaned and dried at room temperature in the shade for about 10 days and was pulverized by mechanical mills and weighed.

### 2. Extraction method:

Extraction was carried out by using three different methods with different solvents, then checking the extracts by TLC using different developing systems, these preliminary investigation were carried out to select the best solvent, method of extraction and the best developing system.

The following extraction method was the best method for general extraction and isolation of *Ephedrine*<sup>(19)</sup>.

A quantity of 250gm of powdered plant material

(aerial part) was moistened with diluted sodium carbonate solution. The mixture were dried and soaked in cold benzene (2.5L) three times for 4-6 hours and filtered. Benzene extract was partitioned with three times equal volume dilute 5% HCl and the acid solution clarified. After adding enough solid potassium carbonate to the acid solution, the alkaloids were partitioned three times with chloroform. The chloroform layer concentrated, dried over anhydrous sodium sulphate and evaporated to dryness. The residue was yellow color (21.2gm).

3. TLC plates were used for routine work, ready made from glass plates 20x20 cm and 0.25mm thickness of silica gel GF<sub>254</sub>. Seven solvent systems (v/v) were used as in the table 1<sup>(21)</sup>:

### 4. Separation and Purification of the Alkaloids:

i) Quantity of 20gm of the residue of each plant extracts of *Ephedra alata* & *Ephedra transitoria* were dissolved separately in a minimum amount of chloroform and applied together with standard reference ephedrine on a number of preparative TLC plates using silica gel/Gf<sub>254</sub>(0.75mm thickness)<sup>(21)</sup>, and developed in (S<sub>1</sub>) solvent system. The solvent was allowed to rise to height of 15cm from the base line. One major band was observed under UV-light (254nm). This band was scrapped off then eluted with methanol and filtered. The filtrate evaporated to dryness under vacuum to give a yellowish product (500mg) for *alata* species and (608mg) for *transitoria* plants. The two products represent mixture of alkaloids.

ii) Another developing system S<sub>7</sub> was used and showed the presence of three compounds in the crude extract. Two of the separated compounds have the same color and R<sub>f</sub> values of ephedrine and pseudoephedrine. Therefore another quantity (250gm) of the *E.alata* was extracted using the general extraction method to give a residue of (22.5gm) and (21.3gm) from *E.transitoria* using same quantity (250gm).

A quantity (20gm) of this residue was separated by preparative TLC plates of silica gel Gf<sub>254</sub> as in the previous step using in this case standard reference

ephedrine ( $R_1$ ) and pseudoephedrine ( $R_2$ ) and developed in ( $S_7$ ) solvent system. Two major bands (I & II) were observed under UV light(254nm) each band was removed and eluted separately with methanol and filtered. The filtrate were evaporated to dryness under vacuum to get a yellowish residue of each alkaloid base (22%) and (32%) from *E.transitoria*.

### iii) Alkaloid salt:

The isolated alkaloid bases were converted to their hydrochloride salts by passing HCl gas into a flask containing the dried residue of the alkaloid base<sup>(15)</sup>. The salt of alkaloid obtained were crystallized then re-crystallized out of ethanol-ether to give crystals of *Ephedrine. HCl* and by repeat the same procedure to give *Pseudoephedrine. HCl*. All samples were subjected to spectral analysis such as HPIC, IR, & UV.

## RESULTS AND DISUSSION

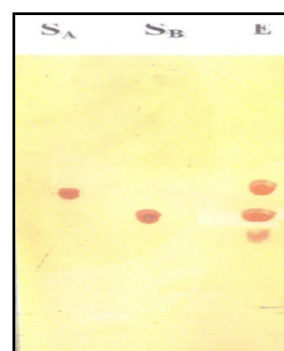
Three deferent extraction procedures were performed for qualitative as well as quantitative examination of the crude extracts, and the general method was chosen as the best one for the extraction of the plant materials. Seven developing system ( $S_1$ - $S_7$ ) were used for the analysis of the extracts , All the solvents systems mentioned here are efficient developing systems and the  $R_f$  values of sample were compared with standard reference in these solvents as shown in f table 2

Only ( $S_7$ ) showed the presence of three compounds in the crude extracts obtained. One method was tried for separation and purification of the extracted crude residue obtained from *Ephedra alata* and *Ephedra transitoria*. Preparative TLC was used for separation and further purification of the crude residue while the charcoal used for partial purification. From this experiment, the total yields of alkaloids isolated out of 250gm for each extracts of alata and transitoria species in addition to their percentage are shown in table 3.

**Table 3. The total weight of alkaloids isolated from the two plants**

Plant Name	Month	Isolated Alkaloids	
		Wt.(mg)	%
<i>E. alata</i>	July	500	0.2
	October	550	0.22
<i>E. transitoria</i>	July	608	0.24
	October	801	0.32

Qualitative investigation of the crude extract which has been obtained from aerial part of the plants *Ephedra alata* and *Ephedra transitoria* indicated the presence of one compound in each plant extract. This compound has similar color and  $R_f$  values of the standard reference ephedrine HCl on TLC plates using the six different developing systems ( $S_1$ - $S_6$ ) when detected by Dragendorff's spraying reagent<sup>(21)</sup>. However the solvent system ( $S_7$ ) was able to separate the crude extracts in to three components in which two of these components gave orange color on the TLC plate when detected by dragendorff's reagents, in addition to the superimposed of the  $R_f$  value of the ephedrine and pseudoephedrine (HCl) with two components as shown in figure 1.



**Figure 1. TLC of each (*E.alata*, *E. transtoria*species) obtained by the general extraction method using Silica gel GF<sub>254</sub> as adsorbent and ( $S_7$ ) as developing system( $S_A$ =Standard *ephedrine*,  $S_B$ =Standard *pseudoephedrine*,  $E$ = *Ephedra alata*)**

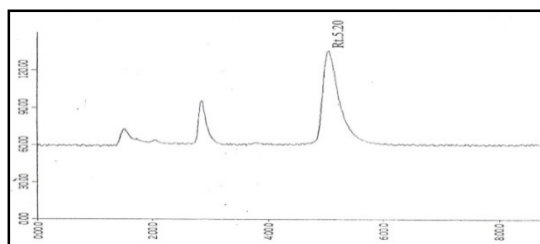


Figure 2. HPLC chromatogram for isolated Ephedrine HCl

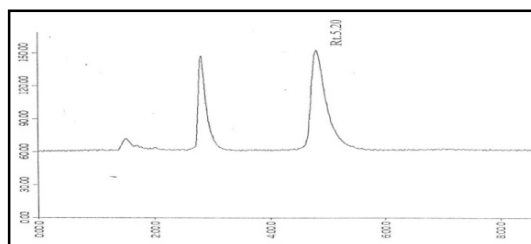


Figure 3. HPLC chromatogram for standard Ephedrine HCl

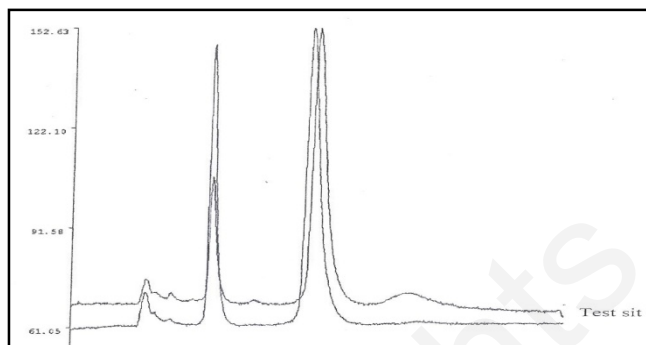


Figure 4. Potentiating HPLC chromatogram for isolated and standard Ephedrine HCl

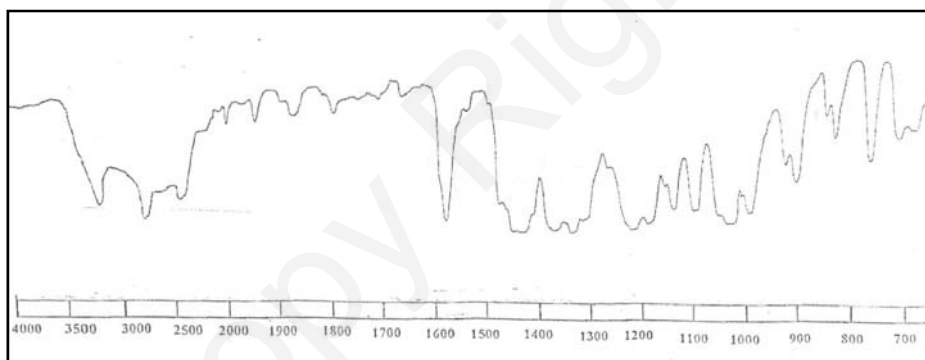


Figure 5. IR-Spectrum for isolated Ephedrine HCl

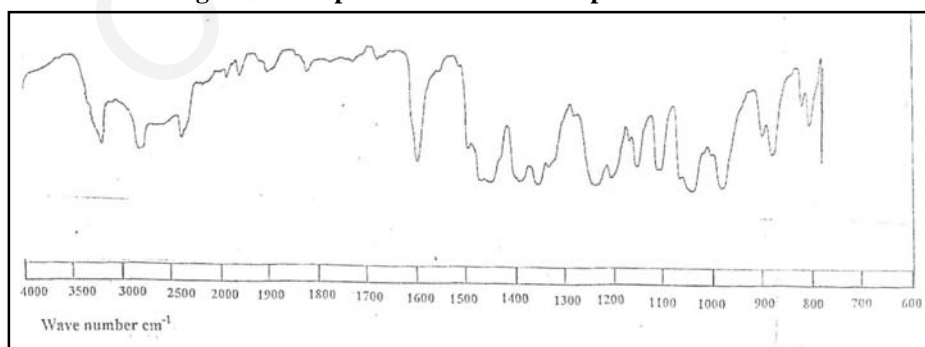


Figure 6. IR-Spectrum for standard Ephedrine HCl

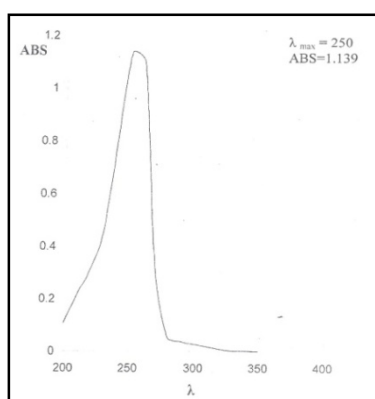


Figure 7. UV-Spectrum for isolated Ephedrine HCl

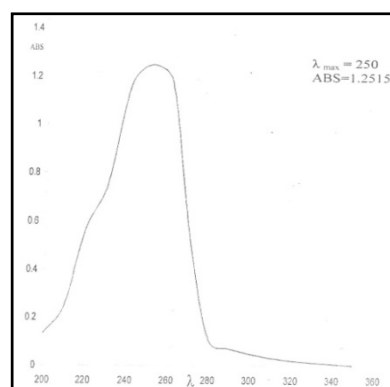


Figure 8. UV-Spectrum for standard Ephedrine HCl

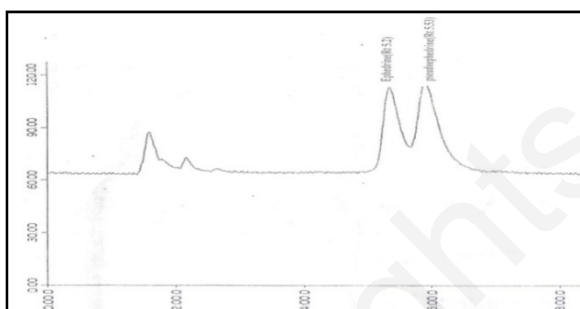
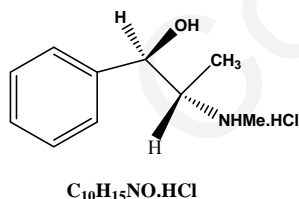


Figure 9. HPLC chromatogram for the mixed isolated Ephedrine HCl and pseudoephedrine HCl

These experimental results obtained are due to the fact that *Ephedrine* and *Pseudoephedrine* are diastereoisomers having almost the same physic-chemical properties<sup>(22)</sup>.

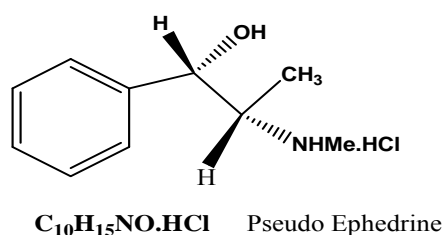
The converted salt Ephedrine hydrochloride was identified from its M.P, M.M.P. and  $R_f$  values by comparison with the standard reference Ephedrine hydrochloride.



It was crystallized out of ethanol-ether as needle shape, white crystals with a melting point of 217-220°C and M.M.P. of 216-219°C compared to the values of standard compound<sup>(14)</sup>. The compound was identified by HPLC technique using the mobile phase phosphoric acid(0.2M):diethyl amine(0.1M):diluted methanol(10%)

which show one peak as in figures(2-4). The I.R. spectra were measured using KBr disc and compared with the standard compound as in figures (5 & 6). The stretching vibration of (O-H) showed a broad peak at 3500 $cm^{-1}$  due to intermolecular hydrogen bonding and the spectrum clearly indicates the (C-H) stretching vibration peak at 2980 to 2890 $cm^{-1}$  for the alkyl group (CH<sub>3</sub>), also the strong and sharp peak at 1600 $cm^{-1}$  indicated the presence of amine salt(secondary amine). The presence of the absorption peaks at region 1600, 1580, 1500, and 1450 $cm^{-1}$  indicated the presence of (C=C) aromatic ring<sup>(23)</sup>.

The UV spectra of the compound showed  $\lambda_{max}$  at 250nm in aqueous acid solution as in figures (7&8). The second compound isolated from both plants was crystallized out of ethanol-ether as white crystals with M.P. of 182-185°C having similar TLC results of the standard *Pseudoephedrine* was analyzed by HPLC. The spectrum in figure (9) shows that they have different retention times.



## REFERENCES

- (1) Chakravarty, H. L. Plant Wealth of Iraq, Baghdad, Botany Directorate of Agriculture of a grain reforms. 1976; 1, 219.
- (2) Trease, G.E. Evans, W.C. *Pharmacognosy*, 15<sup>th</sup>. Edition 2002.
- (3) Townsend and Guest, E. Flora of Iraq. 1966; 2, Pp: 84-86.
- (4) AL-Rawi, A. Chakravarty H .L. Medicinal Plants of Iraq. 1964; Baghdad, Ministry of Agriculture.
- (5) Wilson and Gisvold, *Textbook of Organic Medicinal and Pharmaceutical Chemistry*, 12<sup>th</sup> edition, 2011, P. 531.
- (6) Zhgenti, N. Soobshch, Akad, NaukGruz, *Chemical Composition of Ephedra Procera Growing in SSR*. C.A. 1967 66: P.92413.
- (7) JiLi, Z. Xu, G. Pan, G. Yang, Coustituents of Essential Oils from Stems of Ephedra Sinica. *Journal Article*, 1997; Pp: 489-512.
- (8) Bruneton, J. *Pharmacognosy, Phytochemistry of Medicinal Plants*. 1995; P. 712.
- (9) Manske, R. H. F. Holmes, H. L. *The Alkaloids Chemistry and Physiology*. 1959; P.349.
- (10) Rudolf, V. *Herbal Medicine*. 2000: Pp: 208-209.
- (11) Naka, K. Goto, T. Shoito, Shinsaku, N. *Natural Product Chemistry*. 1975; 2: P.301.
- (12) Bernfeld, P. *Biogenesis of Natural Compounds*. 1963; Pp: 955-956.
- (13) O'Neil, J. *Merck Index*. 15<sup>th</sup> Edition, 2013.
- (14) Clark, *Isolation and Identification of Drugs*. 1986; P. 584.
- (15) Gaud, R. S. *The Pharmaceutical Codex*. 11<sup>th</sup> Edition, 1979; Pp: 325-326.
- (16) Rang, H. P. Dale, M. M. Ritter, J. M. *Pharmacology*. 2012; 7<sup>th</sup> edition, Churchill Livingstone, Bristol, UK.
- (17) James, E. F. Reynolds, Martindale, *The Extra Pharmacopia*, 1982; Pp: 2011-2056.
- (18) Chou, K. K. J. *Biol.Chem*. 1929; 70, P. 109.
- (19) Wagner, H. and Bladt S. *Plant Drug Analysis*. 200; 2<sup>nd</sup> edition, Germany, Springer.
- (20) Egon, S. *Thin Layer Chromatography*. 1965; 1<sup>st</sup> edition, P. 873.
- (21) Shibata H. Nabe, T. Yamamura, H. Osaka, J. L- Ephedrine is a Major constituent of Chinese Medicine. 2000; *Journal-of inflamm-Res.*, 49(8): 394-403
- (22) National Institute of Standards and Technology. 2011. By the U.S. secretary of commerce on behalf of the United States of America.

## CONCLUSION

From this study we conclude that the two plant species, *Ephedra alata* and *Ephedra transitoria* grown wildly in Iraq contain the alkaloidal amines *ephedrine* and *pseudoephedrine* (0.25% and 0.38%) respectively. These percentages could be increased by a high degree of manipulative skill in extraction.

## مسح القلويدات لنوعين من الافيدرا اللذين ينموان برياً في العراق

إقبال الخطيب<sup>1</sup>، حكيم العاني<sup>2</sup>، خالد القاضي<sup>2</sup>، عصام العبيدي<sup>2</sup>، نعيم شعلان<sup>1</sup>، نصير الراوي<sup>1</sup>

<sup>1</sup> كلية الصيدلة والعلوم الطبية، جامعة عمان الأهلية، الأردن.

<sup>2</sup> كلية الصيدلة، جامعة الأنبار، العراق.

### ملخص

شملت الدراسة جنس ال (Ephedra) (علندة) وبنوعية (alata, transitoria) الذي ينمو برياً في الأراضي العراقية. إن نبات ال (Ephedra) له استعمالات طبية عديدة لما يتميز به من تأثيرات مختلفة على جسم الإنسان. هناك العديد من الأدبيات العلمية حول هذين النوعين من النبات (علندة) في مختلف أنحاء العالم ولكن لا توجد أي دراسة حول هذين النوعين المنتشرين في العراق مما جعل التوجه نحو دراسة هذا الجنس من النبات له قيمة بحثية من الناحية الاقتصادية في الصناعة الدوائية. ضمن المسح العام لنبات (علندة) الذي يحتوي على مركبات القلويدات، تم التركيز على مادتي ال (pseudoephedrine, ephedrine) حيث وجد أن النباتين يحتويان على كمية مناسبة من القلويدات الأمينية (Alkaloidal amine) وعلى هذا الأساس وعلاوة على ما ذكر في المصادر العلمية ارتأينا التوجه والتركيز على فصل مادة ال القلويدات وتنقيتها وحساب كميتها. في هذا البحث تم ذكر ومناقشة المسح العام للقلويدات الأمينية لمسحوق النبات (جميع أجزاء النبات عدا الجذور) ومن ثم تم استخلاص وتحليل المستخلص لنبات (علندة) وباستعمال عدة مذيبات كوسيط ناقل. عند تطبيق تقنية كروماتوغرافيا الطبقة الرقيقة ومن ثم التحقق من وجود مادة القلويدات الأمينية للنبات بنوعية ومقارنتها مع المادة القياسية تم تثبيت طريقة الاستخلاص للنبات وتجزئته وفصل مادة القلويدات الأمينية بشكل تفصيلي. واعتماداً على نتائج التحليل للمادة المفصولة من النبات والمتضمنة قياسات ال (M.M.P., M.P., HPLC, IR) ومقارنتها مع المادة القياسية وجد أنها مطابقة لمادة ال (ephedrine) أما مادة (pseudoephedrine) فقد تم التعرف إلى بعض خواصها مقارنة مع المادة القياسية.

الكلمات الدالة: أنواع الافيدرا، HPLC، UV.

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