

## Phytochemical investigation of *Suaeda baccata* (chenopodiaceae)

Al-Mohammadi S. Suhad \*

Al-Khateeb Ekbal

Al-shamma Ali

Received 24/8/2004 ; accepted 27/10/2004

### الخلاصة

تضمن البحث الحالي دراسة للمكونات الكيميائية لنبات الطرطيع *Suaeda baccata* للعائلة (Chenopodiaceae). هو نبات بري يغطي غالبية مدن العراق. بمراجعة المصادر وجد ان عدد كثير من الدراسات و البحوث العلمية تحدثت وبشكل مسهب عن كل اصناف هذا الجنس ما عدا صنف (baccata). ومن هنا اكتسبت هذه الدراسة أهميتها في كونها الاولى على هذه النبتة. أشارت الدراسات السابقة على احتواء بعض أجناس *Suaeda* على القلويدات, لذلك اقتصرنا هذه الدراسة على استخلاص القلويدات الموجودة في النوع *Suaeda baccata* والكشف عنها كيميائيا باستعمال محلول Mayer's reagent و Dragendorff's spraying reagent واستعمال تقنية كروماتوغرافيا الطبقة الرقيقة TLC و التي اثبتت وجود قلويد التراكونيلين (Trigonelline) و قلويد الكولين (Choline). تم إجراء الفحص التأكيدي لقلويد التراكونيلين (Trigonelline) بواسطة HPLC ودرجته الانصهار Melting point والتي طابقه المعايير القياسية لهذا المركب, اما بالنسبة لقلويد الكولين choline فلم نتمكن من فصل هذا المركب كبلورات بسبب قابليته الشديدة للتأكسد و التميع. وقد تم الكشف عنه باستخدام تقنية كروماتوغرافيا الطبقة الرقيقة TLC بواسطة ثلاثة أنواع من المحاليل الناقلة.

### ABSTRACT

This work covers some phytochemical studies of a wild plant, *Suaeda baccata* Forsk of the family Chenopodiaceae, which covers almost all the cities of Iraq. Literature survey revealed a number of publications concerning different species of *Suaeda*, but none at all concerned *Suaeda baccata*, therefore, a research on this plant will be of important value. Several reports indicated the presence of alkaloids in some *Suaeda* species, thus our investigation procedures emphasized the identification and isolation of the alkaloids that might be present in this plant. Preliminary investigation on the plant extract showed the presence of the alkaloids that were tested by both Mayer's reagent and Dragendorff's spraying reagent, and authenticated with standard compounds by TLC using several solvent systems that proved the presence of two alkaloids, trigonelline and choline. Trigonelline crystals were subjected to tests of purity as HPLC, melting point and mixed melting point, which matches with the standard trigonelline. Extract containing choline alkaloid was identified only by TLC using different solvent systems. Studies concerning its crystallization and identification by HPLC and melting point were left for further studies since choline is highly hygroscopic and oxidizable compound.

### INTRODUCTION :

*Suaeda baccata* is widely distributed in most cities of Iraq, to a degree that brings the attention of most people, why this shiny green plant is not edible by animals? This fact encouraged us to find out the content of this plant, since its content is not discovered yet. The Chenopodiaceae (goosefoot family) is a large family including about 102 genera, and 1400 species of low growing plants. Most grow naturally in soils containing much salt (halophytes)<sup>(1)</sup>.

\*Department of Pharmacognosy, College of Pharmacy, Baghdad University, Baghdad – Iraq.

The genus *Suaeda* includes a number of species, only seven species of this genus is present, and widely distributed in Iraq<sup>(2)</sup>. *Suaeda baccata* specie is distributed in south of Jazira District, Southern Desert District, Western Desert Central Alluvial Plain District and Eastern Alluvial District<sup>(2)</sup>. The literature survey indicated that different species of the genus *Suaeda* contain several different compounds, these compounds are: Alkaloids<sup>(3,4,5)</sup>, Acids<sup>(6)</sup>, Glycosides<sup>(3)</sup>, Proteins and amino acids<sup>(3,7,8,9)</sup>, Pigments<sup>(10)</sup>, and Sterols<sup>(4,11)</sup>. Since no previous work has been done on this Iraqi plant, it was found that study of the content of this plant would be of value.

## MATERIALS AND METHODS :

The plant material (aerial part) was collected during months of June, July, august, September and October 2001-2002. From the high ways of Baghdad city. The plant was identified by the Department of Pharmacognosy, college of Pharmacy, Baghdad; and authenticated by the National Herbarium of Iraq. Botany Directorate at Abu-Ghraib, Iraq. Five hundred grams of the dried aerial plant part were first defatted by 1.5L of n-hexane over night, and then extracted by maceration with 2L 96% ethanol for 24 hours. The ethanolic filtrate then was evaporated to dryness under reduced pressure at a temperature not exceeding 400C. The residue was then dissolved in 5ml of 5%hydrochloric acid solution. This solution was designated as fraction 2. The residual plant material left was re-extracted again by reflux with 1.5 liters of 96% ethanol for four hours. The ethanolic filtrate, after evaporation, refluxed with 10ml of 5% hydrochloric acid solution for two hours and then partitioned with 10ml of chloroform to give the acidic layer designated as Fraction 3.

The following scheme represents the extraction procedure

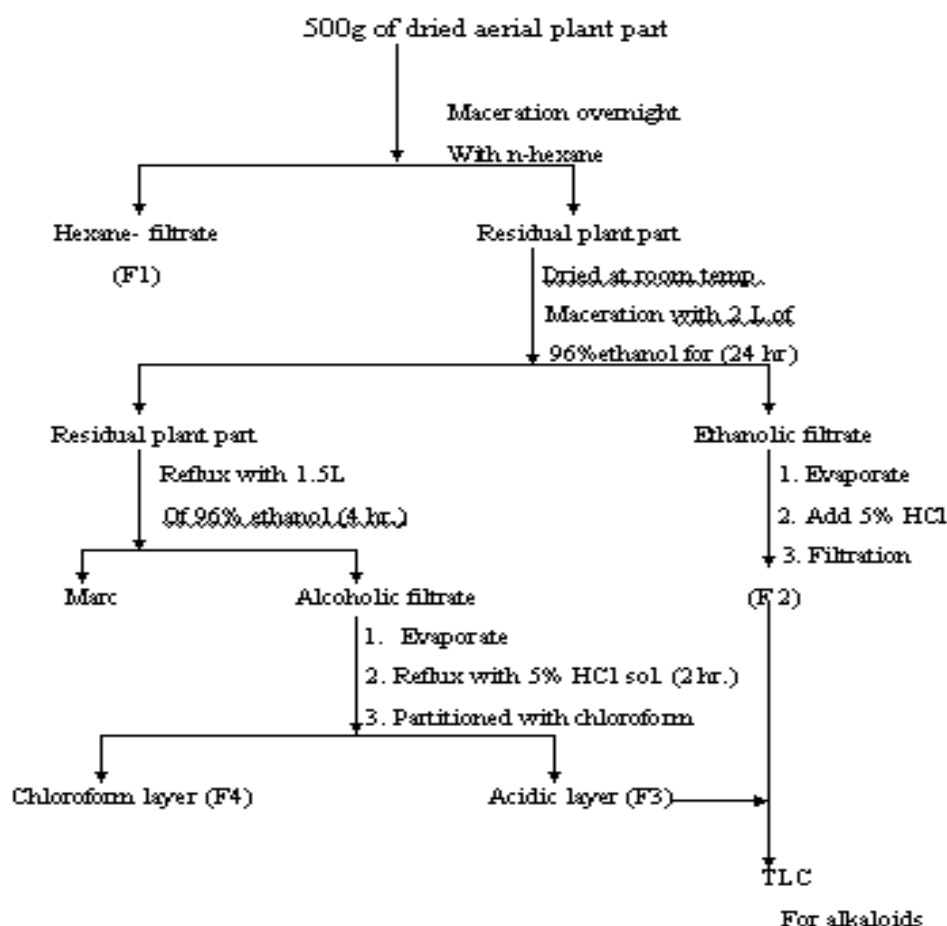


FIG 1 . SCHEMATIC PROCEDURE FOR THE ALKALOIDS EXTRACTION OF *SUAEDA BACCATA*

### Identification of the Alkaloids :

Identification was performed first by Mayer's reagent followed by TLC to fraction 1, and fraction 2, using silica gel G, Dragendorff's spray reagent<sup>(12)</sup>, standard trigonelline and choline, and different solvent systems that were:

Solvent (1): Methanol: Water: conc. HCl (50:50:2)

Solvent (2): Methanol: Water (50:50)

Solvent (3) n-Butanol: Glacial acetic acid: Water (100:20:50)

### Isolation of the Alkaloids :

Preparative TLC was performed by using plates of 20x20 cm, which was coated, with silica gel G (Merck) layers of 1mm thickness. The applied sample was fraction 2, which supposed to contain the two alkaloids, and the mobile phase was solvent 1.

Two separated bands were observed by making a groove in one side of the plates and identified with Dragendorff's reagent, according to references compounds, the lower band refers to trigonelline while the upper band refers to choline.

The silica zone containing each alkaloid was scrapped out, collected in a beaker and eluted with several portions of ethanol, then evaporated to dryness in vacuo to give yellow precipitate.

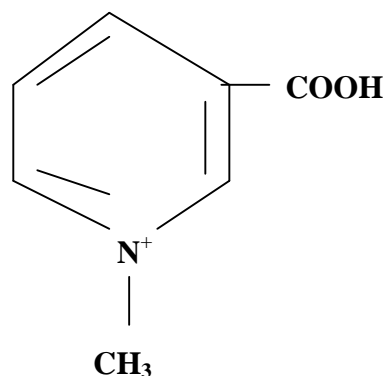
### RESULTS AND DISCUSSION :

Trigonelline alkaloid (3-Carboxy-1-methyl pyridinium hydroxide) with the following structural formula  $C_7H_7NO_2$ <sup>(13)</sup>. Trigonelline alkaloid Occurred in fraction (2) together with the choline alkaloid.

Choline alkaloid ((B-hydroxyethyl) trimethyl ammonium hydroxide) with the structural formula  $C_5H_{15}NO_2$ <sup>(14)</sup>.



Choline structure

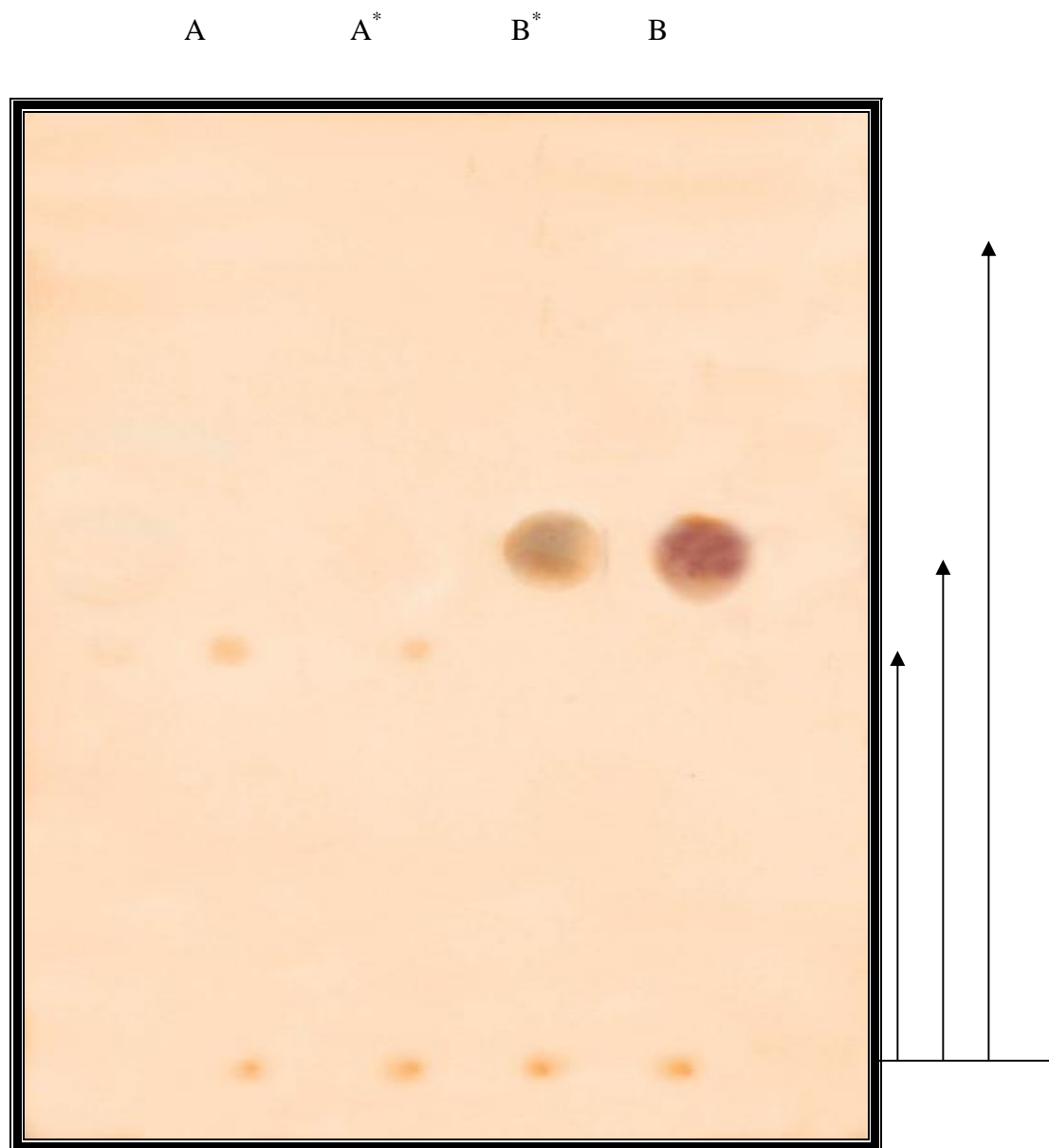


Trigonelline structure

The purity of the separated alkaloids was proved by use of TLC technique using three solvent systems that gave the following R<sub>f</sub> values with the standard trigonelline and standard choline.

Solvent system	Standard trigonelline	Isolated trigonelline	Standard choline	Choline extract
S1	0.608	0.6	0.76	0.752
S2	0.461	0.469	0.092	0.084
S3	0.177	0.166	0.133	0.122

The following diagram represents identification of both alkaloids using solvent system (1), and solvent system (2). ( Fig-2 & 3 respectively ).



**FIG 2 . REPRESENTS TLC OF THE ISOLATED TRIGONELLINE CRYSTALS AND CHOLINE EXTRACT TOGETHER WITH THE STANDARD COMPOUNDS, USING S1 AS A DEVELOPING SOLVENT SYSTEM.**

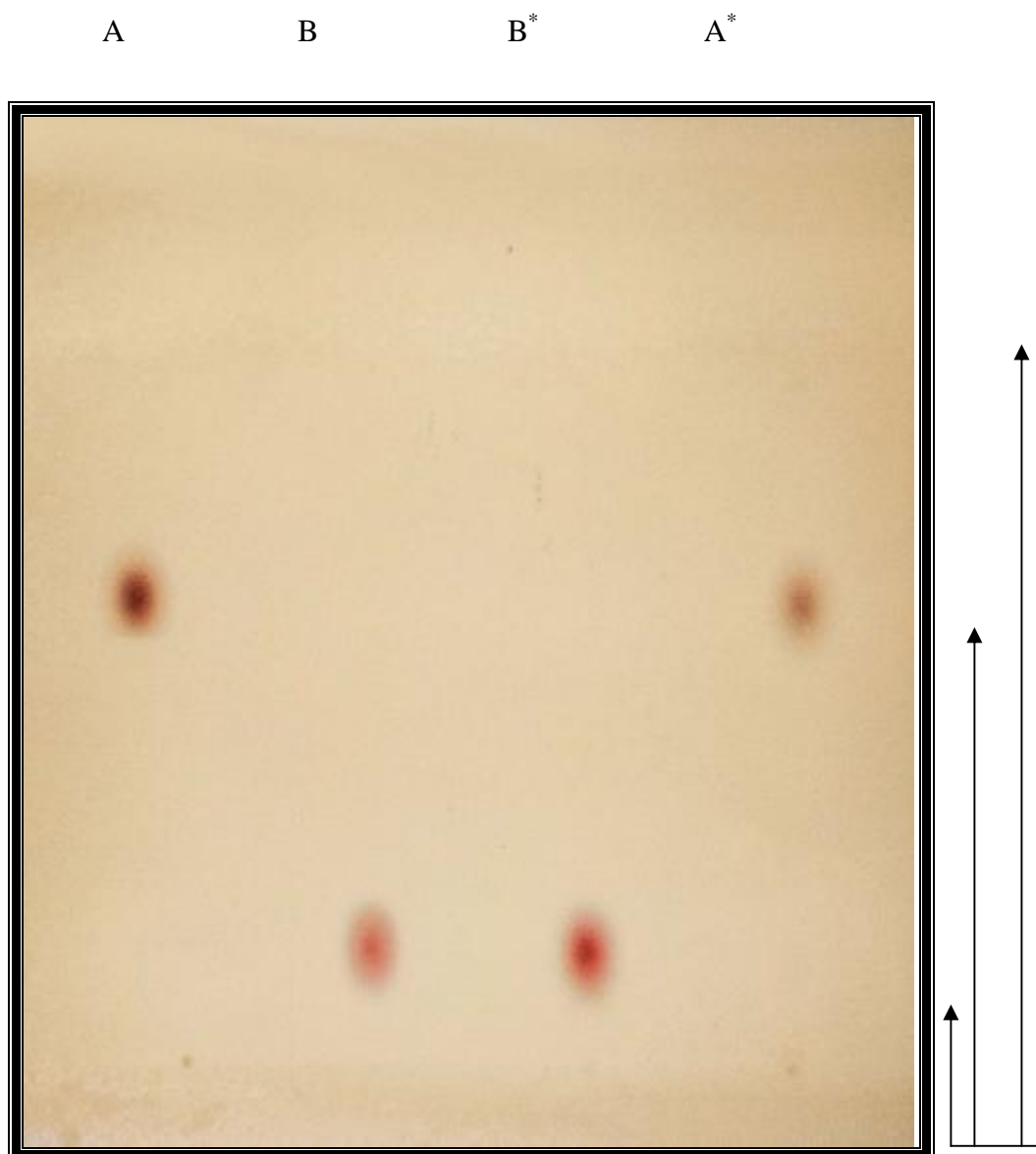
S1 (Methanol: Water conc. HCl 50:50:2)

A : Trigonelline Standard.

A\* : Isolated Trigonelline.

B : Choline Standard.

B\* : Choline extract.



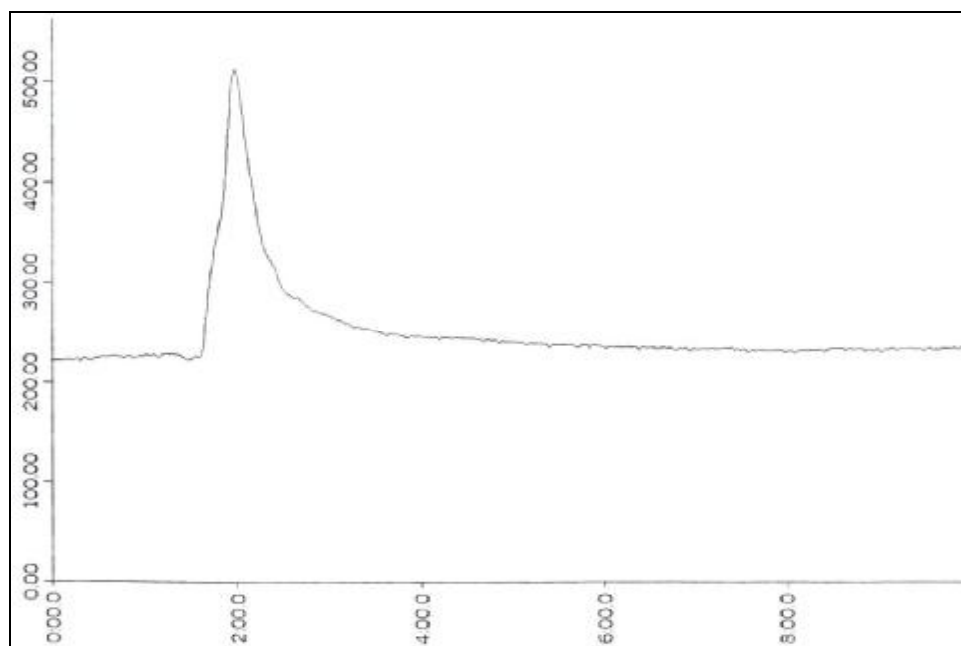
**FIG 3 . REPRESENTS TLC OF THE ISOLATED TRIGONELLINE CRYSTALS AND CHOLINE EXTRACT TOGETHER WITH THE STANDARD COMPOUNDS, USING S2 AS A DEVELOPING SOLVENT SYSTEM.**

S2 (Methanol: Water 50:50).  
 A : Trigonelline Standard.  
 A\* : Isolated trigonelline.  
 B : Choline standard.  
 B\* : Choline extract.

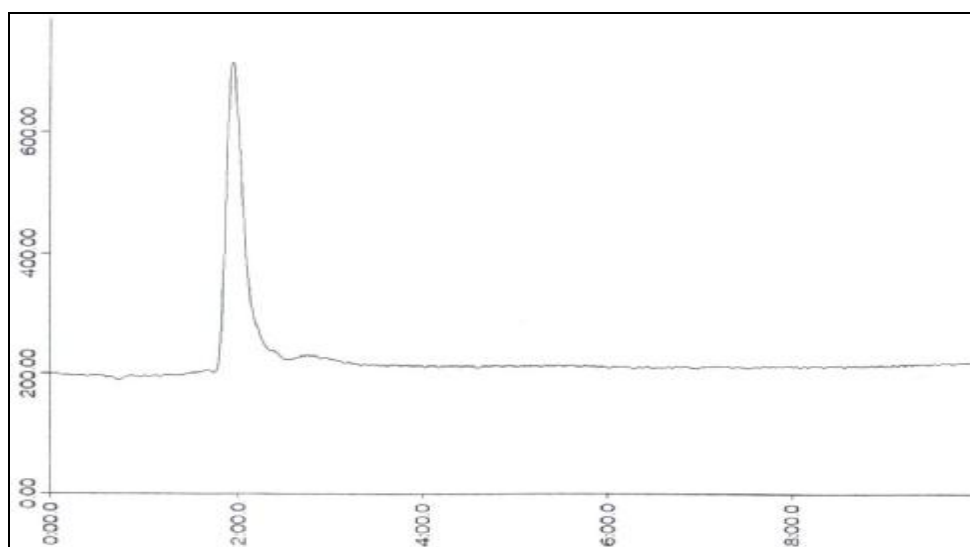
The isolated trigonelline crystals had a sharp melting point that match with the standard, and a mixed melting point of 260°C. Further more the trigonelline crystals were subjected to HPLC analysis (table-1) in which the retention time of both the isolated trigonelline and the standard trigonelline were identical. ( Fig-4 & 5 ) .

**Table 1 . Conditions used for HPLC of trigonelline alkaloid**

Item	Condition
Mobile Phase	Methanol-Water 50:50
Attenuation	4
Flow rate	1.5 ml/min
Absorbance	254nm



*FIG 4 . HPLC OF THE ISOLATED TRIGONELLINE HCL CRYSTALS*



*FIG 5 . HPLC OF THE STANDARD TRIGONELLINE HCL*

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