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A New Method for the Isolation and Purification of Trigonelline as Hydrochloride from *Trigonella foenum-graecum L.*

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Abstract:

Separation of Trigonelline, the major alkaloid in fenugreek seeds, is difficult because the extract of these seeds usually contains Trigonelline, choline, mucilage, and steroidal saponins, in addition to some other substances. This study amis to isolate the quaternary ammonium alkaloid (Trigonelline) and choline from fenugreek seeds (*Trigonella-foenum graecum L.*) which have similar physiochemical properties by modifying of the classical method. Seeds were defatted and then extracted with methanol. The presence of alkaloids was detected by using Mayer's and Dragendorff's reagents. In this work, trigonilline was isolated with traces of choline by subsequent processes of purification using analytical and preparative TLC techniques. Further identification was done by using HPLC, IR and MP. Pure Trigonelline was isolated from the seeds of *Trigonella-foenum graecum* excluding other alkaloid like choline. In this study, a new, fast and convenient method for isolation and purification of Trigonelline from fenugreek seeds has been established. Unlike other methods, this one excludes all the non-alkaloidal components from the fenugreek seeds extract.

Key words: Choline, Hplc, IR, M.p, Trigonelline.

Introduction:

Fenugreek has several uses ranging from primary ones that include treatment of high cholesterol, diabetic retinopathy, gastric disorders, lung infections, excessive mucus, and sore throat, and secondary uses including abscesses, anemia, asthma, boils, bronchitis, cancer, swollen eyes, fevers, gallbladder problems, heartburn, inflammation, sinus problems, ulcers, uterine problems, and water retention (1–4). Composition of fenugreek is similar to cod liver oil which is rich in phosphates, lecithin, nucleoalbumin, and organic iron. Also, it contains trimethylamine, neurin, and betain which tend to stimulate appetite by their action on the nervous system, or can produce a diuretic effect.

Trigonelline (1-Methylpyridinium-3-carboxylate) is an alkaloid with chemical formula C_7H_7NO= (Fig. 1), it is a product of the metabolism of niacin (vitamin B_3) and it has been isolated from fenugreek seeds (*Trigonella foenum-graecum*, hence the name) (5). Trigonelline is a polar hydrophilic alkaloid that is also extracted from many other plant species, such as, *Allium sepapea*, *Coffea* sp, *Pissum sativum*, *Glycine max*, and *Lycopersicon esculentum* (6).

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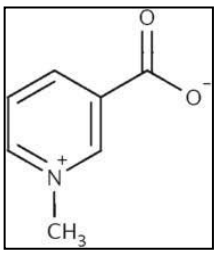


Figure 1. Chemical structure of Trigonelline alkaloid

Trigonelline is considered as the most important metabolite of fenugreek, found to be very effective in treating diabetes, antihypertensive, and decreasing blood cholesterol (7–11) Other study suggested that Trigonelline has dose dependent neuroprotective and antiapoptotic effects (12). Among other studies, Trigonelline incorporated chitosan nanoparticles and effectively inhibited the invasion of tumor cells (13).

Using ethanol, Trigonelline, choline, mucilage, and steroidal saponins, in addition to some other substances will be extracted. Thus, handling of such extract and isolation of Trigonelline will be difficult. The aim of this study is to establish a new method for the isolation of Trigonelline from the total seeds extract.

Materials and Methods:

Isolation of Trigonelline from Fenugreek Seeds .

A five hundred (500) gm of authenticated fenugreek seeds was crushed by mortar and defatted for 48 hrs with petroleum ether in a soxhlet apparatus. The mixture was allowed to cool and then filtered. Defatted seeds (Marc I) were allowed to dry at room temperature for 12 hrs to obtain 462 gm which was macerated in 500 ml methanol for 4 hours with gentle shaking from time to time.

The mixture was then filtered and a fresh 500 ml of methanol was added again to the seeds and left overnight. Most of the solvent was evaporated under vacuum, and subjected to the test of alkaloids, using Mayer's and Dragendorff's reagents. It was noticed that the extract contains some dyes and traces of gummy substances. These substances were precipitated by the addition of excess acetone (acetone, methanol 3:1) which can be easily removed by filtration. Acetone was evaporated then activated charcoal was added to the extract and heated gently for 5 minutes to get a clear solution. The mixture was acidified with 5% HCl (pH 4) and refluxed for 2 hrs and then partitioned with chloroform.

A Thin layer chromatography (TLC) was used to evaluate the presence of Trigonelline HCl and traces of choline, by using mobile phases (Methanol, water, 50:50 v/v). Trigonelline HCl was then isolated by preparative TLC and recrystallized from ethanol 90%. The detection of Trigonelline HCl was carried out by using, HPLC IR,and MP,

Results and Discussion:

About 0.5 gm of Trigonelline HCl has been isolated from 500 gm fenugreek seeds. The result according to different methods of identification were obtained as follow:

Table 1. HPLC represents the conditions used for trigonelline HCl

Item	Condition
Mobile phase	Methanol-Water 50:50
Attenuation	4
Flow rate	1.5
Absorbance	254

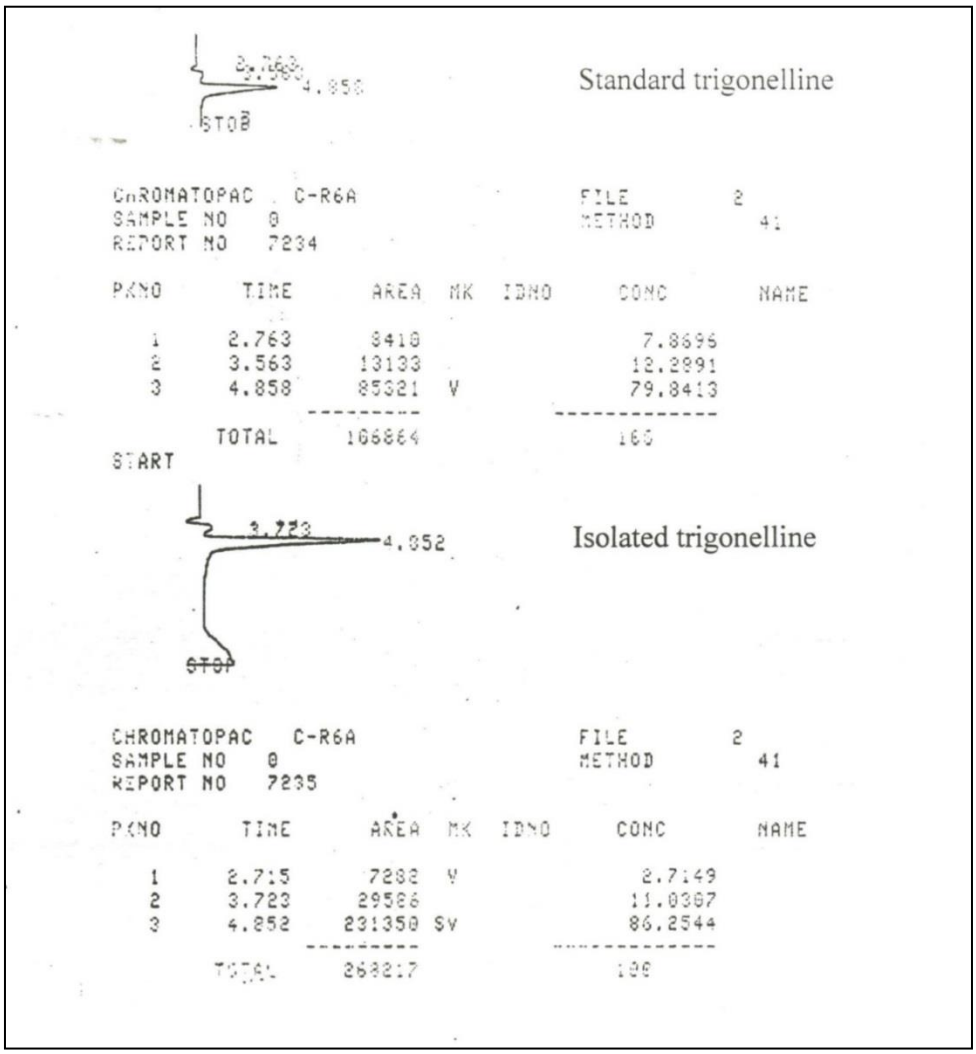


Figure 2. The fingerprint for standard and isolated trigonelline HCl which were identical according to their retention time.

The retention time for standard Trigonelline HCl 4.858 min and for Isolated Trigonelline HCl 4.852 min

The IR spectra for the isolated and standard Trigonelline HCl (Figs. 3a and 3b) were superimposable which confirm the purity of the isolated Trigonelline HCl.

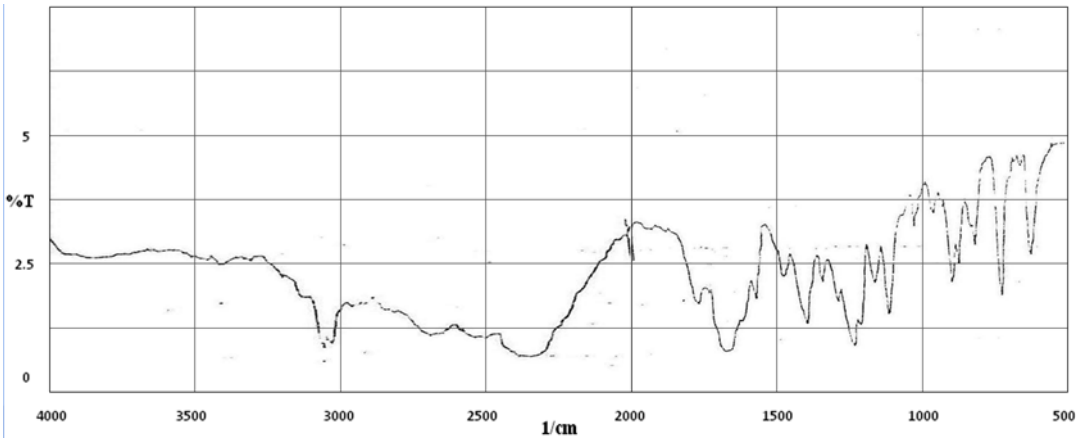


Figure 3a. IR spectrum of isolated Trigonelline HCl

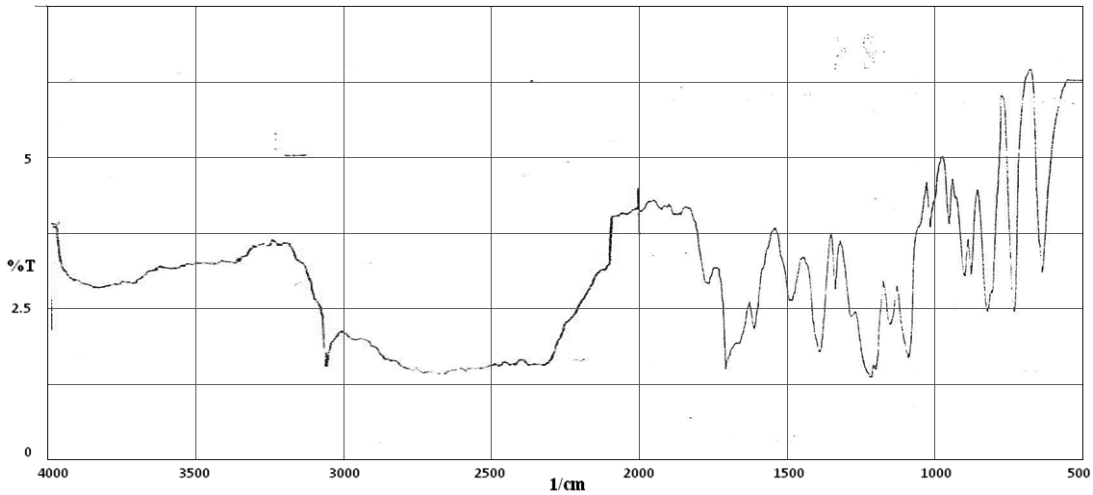
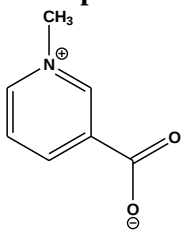


Figure 3b. IR spectrum of standard Trigonelline HCl

Table 2. Characteristic Infrared Functional groups absorptions (cm^{-1}) of isolated Trigonelline compound



O-H	C=N	C-N	=C-H	-C-H	C-H	=CH2	C-C
3086(s,b) 3055(s,b) 1334(w) 972,925(m)	1990(w) 1940(w) 1593(m) 671(s)	864	3028(m) 1639 1509(m)**	2445(m) 2407(m)	1477 1292* 1249* 844(s)	1392(m) 902(m)	1029(m)

C-C=O	O=C-O	C-O	C-O-C	C=O
1890(w)	1707(s)#	1176(s)	1122(s)	752(s)

Peak intensity: w=Weak, m=Medium, s=Strong, b=Broad
Carboxylic acid
*****Multiple, Depend on which types of bonding
** Aromatic ring

The other result for identification was the melting point of the isolated Trigonelline HCl which was 259°C while trigonelline HCl standard was 260°C, and for a mixture of equal quantities of standard trigonelline and isolated Trigonelline HCl .

was 260°C. This indicate the overlap of both compounds and which are identical.

The result according to thin layer chromatography depending on Rf values for the compounds (isolated trigonelline HCl., choline, standard trigonelline HCl and choline which were identical for standard and the isolated compounds after spraying with Dragendorffs reagent. Table (3) represents the Rf value for all compounds using the mobile phase (methanol: water 50:50)

Table 3. Rf value for trigonelline HCl standard, isolated trigonelline HCl, choline standard and choline sample.

Item	Rf value
Trigonelline HCl standard	0.61
Isolated trigonellineHCl	0.61
Choline standard	0.11
Isolated choline	0.11

Conclusion:

Many studies approved that Trigonelline alkaloid has a therapeutic potential for the treatment of diabetes and central nervous system diseases (14). Studies have also confirmed its anticancer, antipyretic and antiulcer effects (3). It was important to establish a protocol for the isolation and purification of Trigonelline from its plant sources. In this study, a new, fast and convenient method for isolation and purification of Trigonelline from fenugreek seeds has been established. Unlike other methods where nicotinic acid is present with Trigonelline (15–18), this method excludes all the non-alkaloidal components from the fenugreek seeds extract. In addition, the Trigonelline and choline alkaloids are separated and isolated by TLC and preparative TLC Techniques. One more thing to consider, there are significant variations exhibited among fenugreek biotype for growth habit, flowering time, seed colour, seed size, biomass and seed yield which affect the yield of Trigonelline (19).

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Conflicts of Interest: None.

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طريقة جديدة لعزل وتنقية ترايكونللين هايدرو كلوريد من بذور الحلبة

اقبال حسن الخطيب

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الخلاصة:

ان فصل مادة الترايكوننللين وهو القلويد الرئيسي في نبات الحلبة يعتبر معقدا وذلك لاحتواء نبات الحلبة على العديد من المواد الاخرى كمادة الكولين، والمادة الهلامية، والستيرويد الصابوني ومواد اخرى. هدفت هذه الدراسة الى عزل مادة الترايكوننللين عن بقية المواد، والكولين من بذور الحلبة واللذان يشتركان في صفات فيزياوية وكيميائية متقاربة وبطريقة محورة عن الطريقة التقليدية.

تم عزل المواد الدهنيه (الزيوت الثابته) من بذور الحلبه باستعمال مذيب لاقطبي وبعدها تم استخلاص المواد الفعاله الأخرى من البذور عن طريق استخدام الميثانول حيث تم التاكد من وجود القلويد باستعمال كاشف دراجندروف و ماير. ثم عزل وتنقية الترايكونلين مع القليل من الكولين عن طريق اجراء خطوات متعاقبة من التحليل والفصل باستعمال تنقية TLC . تم تنقية والتعرف على الترايكونلين باستعمال تنقية MP, IR, HPLC. عزل الترايكونلين النقي من بذور نبات الحلبة من القلويدات مثل الكولين. في هذه الدراسة تم استعمال طريقة عزل وتنقية الترايكونلين من بذور الحلبة وهذه الطريقة لاتشبه الطرق الاخرى التي يستخلص معها المركبات الغير قلوية من مستخلص نبات الحلبة.

الكلمات المفتاحية: الكولين، HPLC ، IR ، MP ، الترايكونلين.