AJPS, 2016, Vol. 16, No.2

Date of acceptance: 8-5-2016

Detection of Nicotine in *Equisetum arvense* Grown Naturally in Iraq

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Abstract:

Equisetum arvense L, Family: Equisetaceae, common name hoarse tails. The plant is used traditionally to stop bleeding, heal ulcers and wounds, and treat tuberculosis and kidney problems.

The new approach is the investigation of cytotoxic and anti HIV activities of this plant. The aerial part of the plant was extracted to investigate the presence of alkaloids in it. Powdered plant was macerated with 2N HCl overnight.

The alkaloids were detected with Dragendorrf reagent and were found to be one spot. Investigation of the extracted alkaloids using GC/MS reveal the presence of nicotine as the single alkaloids found in the plant.

Key words: *Equisetum arvense, alkaloids, Nicotine.*

الكشف عن النيكوتين في نبات ذنب الخيل النامي طبيعياً الحال في العراق

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الخلاصة:

نبات كنباث الخيول والمعروف بإسم ذنب الخيل، يستعمل في الطب التقليدي لإيقاف النزيف، علاج التقرحات والجروح وعلاج أمراض التدرن والكليتين، أما في الطب الحديث فهناك أبحاث لتحديد فعالية النبات في إيقاف النموي الخلوي للأورام السرطانية والنشاط المضاد للفايروس المسبب لمرض نقص المناعة المكتسبة (الأيدز).

تم إستعمال الأجزاء الهوائية من النبات للكشف عن وجود القلويدات في النبات، حيث تم الإستخلاص عن طريق الإستخلاص الحامضي بإستعمال حامض الهيدروكلوريك، وتم إستعمال محلول دراجندورف والكروماتوكرافي الاعتيادي وكذلك الكروماتوكرافي الغازي.

أثبتت النتائج وجود القلويد نيكوتين في النبات وهو القلويد الوحيد الذي تم التحقق من وجوده في النبات.

الكلمات المفتاحية: *ذنب الخيل، القلويد، نيكوتين.*

Introduction:

Equisetum arvense L, Family: Equisetaceae, common name hoarse tails is a perennial herb with 0.1 m high. The plant passes a characteristic dark green color leaves and arrange in whorls shape (Figures 1and 2)[1].

Figure-1.

Figure-2.

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Phytochemical studies of this plant showed the presence of various compounds like flavonoids (isoquercetine and apeginin), caffeic acid and traces of the pyridine alkaloids (nicotine and palustrine)[2]. In addition to that the plant was reported to contain silicic acid and minerals[3].

The plant is used traditionally to stop bleeding, heal ulcers and wounds, and treat tuberculosis and kidney problems[4].

The new approach in using of this plant is the cytotoxic effects. It has been reported that crude proteins extracted from *Equisetum arvense* L. inhibit the proliferation of cultured cancer cells[5].

The collapse of mitochondrial trans-membrane potential, was all observed in cells cultured for 48 h with the herb extract of the plant[6].

Screening of some plant extracts for inhibitory effects on HIV-1 and its essential enzymes shows that water extract of aerial parts of *Equisetum arvense* possesses inhibitory effect on HIV-1 induced cytopathy[7].

Materials and Methods:

Plant material

Plant material was collected from Mkeshefa town in Salahaddin province, and was authenticated by the Iraqi National Herbs Center in Abughreb. The plant was collected during June, and dried in shade at room temperature.

Extraction

Powdered plant (50 g.) was macerated in 2N Hydrochloric acid (100 mL) for overnight and stirred gently by a magnetic stirrer. The extract was filtered by a Buchner funnel. Water (20 mL) was added and the pH of the extract was adjusted to 8-9 by ammonia solution. The extract partitioned with chloroform (30×3).

The combined organic layer was dried with anhydrous Sodium sulphate evaporated by a rotary evaporator to dryness. Alkaloids spot was detected by TLC after spray with Dragendorrf reagent and the type of alklaoids present was investigated by GC Ms analysis and

GC/MS analysis

GC/MS was Shimadzu apparatus GCMS-QP2010 Ultra. Injection Volume was 0.50.Column Oven Temp was 70.0 °C. Injection Temp was 240.00 °C. Injection Mode was Splitless. Sampling Time was1.00 min. Flow Control Mode was Pressure. Pressure was 100.0 kPa. Total Flow was 19.9 mL/minColumn Flow was 1.53 mL/min. Linear Velocity was 45.4 cm/sec. Purge Flow was 3.0 mL/min. Split Ratio was10.

Results and Discussion:

TLC and Dragendroff's tests show the presence of an alkaloid with an Rf value slightly below 0.2, which is similar to that of alkaloids of this Genus as stated in the Plant Drug Analysis figure-3[8].

GC/MS analysis reveals the presence of the alkaloids nicotine only with 76.4% from the total extract. The other compounds may be present as impurities in the solvents and acids used in the extraction methods as none of them was representing a natural compounds founds in the plants (Table-1).

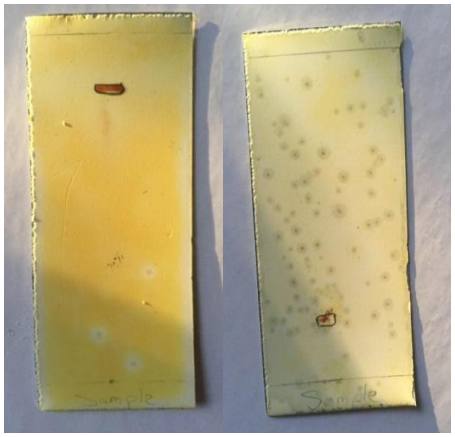

Figure-3: TLC analysis of the extracted alkaloid.

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Solvent system-1: Ethyl acetate: methanol: NH4OH (85:10:3).
Solvent system-2: Isopropanol: acetic acid: H2O (60:20:20).

The graphical results of the GC/ MS analysis for the alkaloid extract are represented in figure-4 which showed that the peak 3, the most intense peak, belongs to a compound that has the following fragmentation pattern comparing with literature (Figure-5). The obtained data, when compared to the data base of the (GC/MS) device, it showed that those data belong to the alkaloid Nicotine.

Table-1: GC/MS analysis of the alkaloids extracted from the plant

Name	Height %	Height	Area %	Area	R. Time	Number
Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S) (**Nicotine**)	**80.69**	**3486006**	**76.14**	**9448790**	**14.29**	**3-**

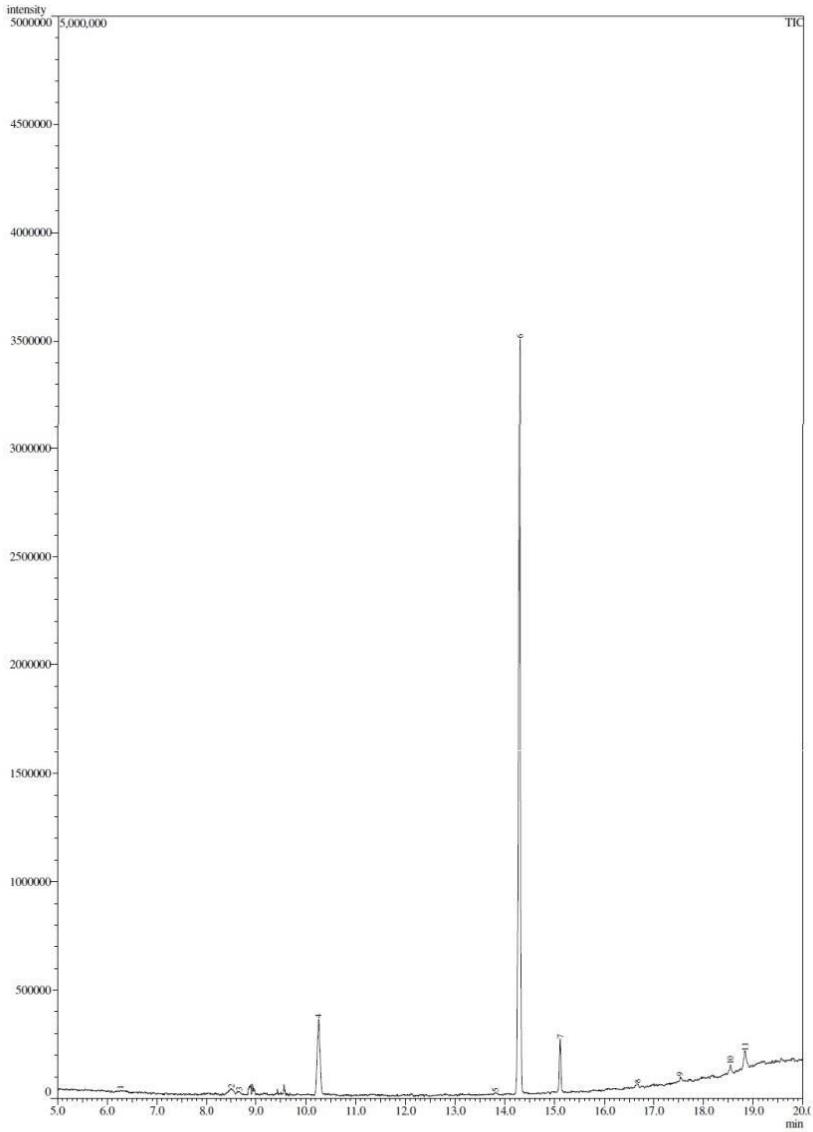

Figure-4: GC/MS analysis of the extracted alkaloids.

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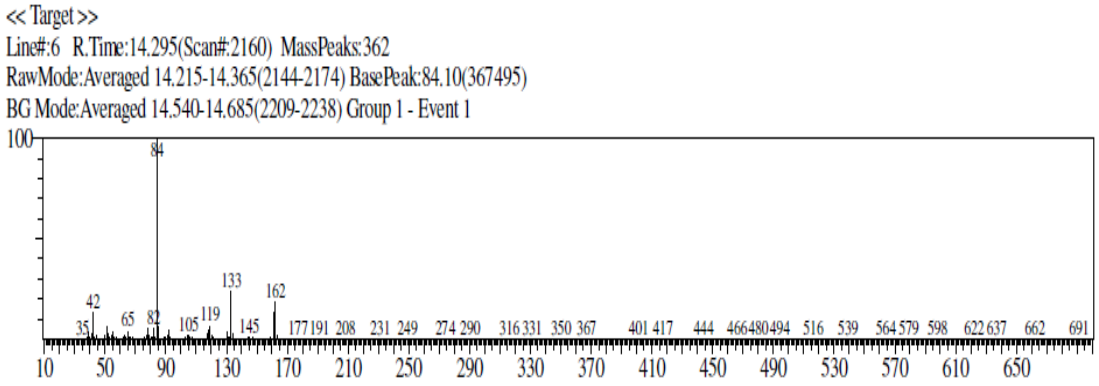

Figure-5: Fragments of the extracted alkaloid.

Interpretation of the fragmentation pattern:

The base peak has an m/z value = 84, and explained by the cleavage of the two nitrogenous heterocycles, which is confirmed by the adjacent peak 78 as in figure-6.

Regarding the second peak in intensity, the 133, its explanation is as in figure-7 [9].

The alkaloid nicotine is reported to be found in traces in the plant *Equisetum arvense*[2].

These results showed that Nicotine in the Iraqi plant is found in higher concentrations comparing to another countries. This may be attributed to the type of Iraqi soil which results in a fact that Iraqi plants are a rich source of alkaloids[10]. Although nicotine causes tolerance and addiction; it can be useful as a tool in the aiding of smoking sessation[11].

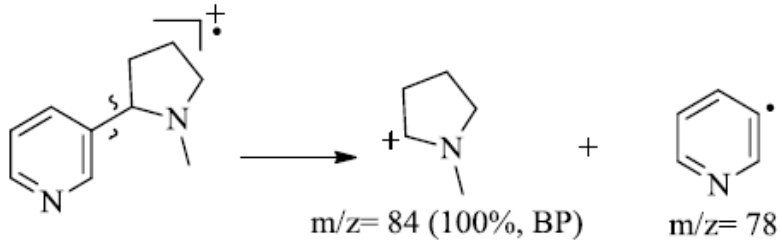

Figure-6: Interpretation of the fragments.

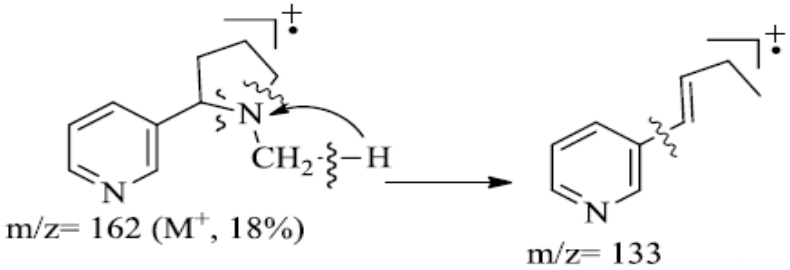

Figure-7: interpretation of fragment (m/z, 133).

Conclusion:

Equisetum arvense of Iraq contains nicotine which is a pyridine-pyrolidine alkaloid. The presence of this alkaloid was confirmed by GC/MS analysis and its fragmentation pattern was identical to that reported in the literature.

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