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PHYTOCHEMICAL STUDY OF CROTALARIA THEBAICA (DEL.) DC. GROWING IN EGYPT

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ABSTRACT

From the chloroformic extract of Crotalaria thebaica (Del.) Dc. herb, three pyrrolizidine alkaloids, Spectabiline, Monocrotaline and Crosemperine as well as β-sitosterol glycoside and daidzein were isolated. From the methanol extract, two saponins were isolated and identified in the form of their methyl esters.

The identity of the isolated compounds was based on studies of their physical, chemical and spectral analysis including UV, IR, ^{1}H-NMR, ^{13}C-NMR and MS.

INTRODUCTION

Crotalaria thebaica (Del) Dc. belongs to family Leguminosae [1]. The genus *Crotalaria* is well known due to the interesting biological activities of its pyrrolizidine alkaloids [2-5]. Some of these alkaloids have carcinostatic effects [6] and hypotensive action [7]. Some *Crotalaria* species have wide use in folk medicine as diuretic, in treatment of sore throat and inflammation of mouth and to produce cooling sensation. The pyrrolizidine alkaloids have cytotoxic, liver toxic ef-

fects and fatal effects specially in Australia for grazing animals [8,9].

Reviewing the current literature, very little was mentioned about *Crotalaria thebaica* (Del) Dc., concerning the flok use and biological effects. Hence phytochemical study of this plant was thought to be interesting.

EXPERIMENTAL

General Experimental Procedure:

Melting points were uncorrected, ^{1}H and ^{13}C-NMR spectra were carried out in $CDCl_3$, CD_3OD, C_5D_5N at 400 MHz and 100 MHz, respectively. For column chromatography Amberlite IR A-45, (weak anion exchange resin) and silica gel (E. Merck) or wakogel C-200 (Japan) were used. Silica gel 60 F_{254} (E. Merck) and cellulose Art. 2331 E. Merck (Avicel) were used for TLC. UV analysis was carried out using Hitachi 550, double beam spectrophotometer (Japan). IR spectra were carried out using IR spectrometer, JASCO A-302 (Japan). ^{1}H and ^{13}C-NMR spectra were recorded by ^{1}H and ^{13}C-NMR Bruker AM-400 (West Germany) Mass spectra were carried out using MS spectrometer Hitachi-M-80 (Japan).

Plant Material:

The plant material used in this work consists of the aerial parts of *C. thebaica* (Del) Dc. The plant was collected from El-Hafafit in the Eastern desert in upper Egypt near Aswan in April 1987. The plant was kindly identified by Prof. Dr. Nabil El-Hadidy Professor of Taxonomy, Faculty of Science, Cairo University. The plant was dried and reduced to No. 40 powder, A voucher sample is kept in the Dept. of Pharmacognosy, Faculty of Pharmacy, Assiut University.

Solvent Systems:

1-Chloroform-methanol	(9:1).
2-Chloroform-methanol-water	(75:23:2).
3-Ethyl acetate-methanol-water	(80:19:1).
4-n-butanol-acetic acid-water	(6:3:1).
5-Acetone-pyridine-water	(3:1:1).

Extraction and Isolation:

The extraction of *Crotalaria thebaica* herb and the ioslation of its constituent are illustrated in the following flow sheet.

Flow Sheet for the Isolation of the Constituents of *Crotalaria thebaica* (DEL) DC.

Crotalaria thebaica herb (1.8 kg)

Extracted with hot MeOH (4 x3L)

Evaporated MeOH ext (113 g)

mixed with 500 ml H_2O

shake with hexane (5 x1.5L)

Hydro-methanol Fraction

Evap. Hexane Fraction

(23 g)

Part I

reduced with Zn/0.5 N H_2SO_4*

ext. with $CHCl_3$ (5x1.5)

Evaporated $CHCl_3$ Fr. (32 g)

Silica gel col. chr. (1:25) eluted with $CHCl_3$ and $CHCl_3$-MeOH in an increasing polarity

$CHCl_3$-MeOH (95:5)(3l)	$CHCl_3$-MeOH (93:7)(3l)	$CHCl_3$-MeOH (85:15)(3l)	$CHCl_3$-MeOH (80:20)(3l)	$CHCl_3$-MeOH (75:25)(3l)
Comp. I (46 mg)	Comp. II (145 mg)	Comp. IV (125 mg)	Comp. V (16 mg)	Comp. III (43 mg)

Part II

Evaporated (28 g)

treated with CH_2N_2

col. chr. on Amberlite (IR. A 45)(1:25)

eluted with

H_2O (4L)	H_2O-MeOH 3:2(4L)	H_2O-MeOH 1:1(4L)	H_2O-MeOH 2:3(4L)	MeOH (4L)
11.3 g	3.2 g	5.6 g	2.4 g	4.6 g

have the same spots on TLC

Silica gel col. chr.

eluted with $CHCl_3$-MeOH (70:30)(1.2 L)

Comp. VI (82 mg)	Comp. VII (23 mg)

* Tested for pyrrolizidine alkaloids using Mattock's reagent [10].

Complete Acid Hydrolysis of Saponins [11]:

Each isolated saponin (5 mg) was autoclaved in a sealed tube with 1-2 ml 2N trifluroacetic acid at 120°C/1 bar for 1.5 hours.

The aglycone was separated by addition of distilled water and subsequent shaking with chloroform. The remaining aqueous layer was evaporated and dissolved in the least possible volume of isopropyl alcohol. Chromatographic study of the aglycone and sugars was carried out using systems 2 and 4. Thymol-H_2SO_4 was used as spray reagent for sugars.

Characters of the Isolated Compounds:

Compound I: (46 mg)

Prisms (from acetone), m.p. 183-5°C. Its IR gave characteristic peaks at ν in cm^{-1} 3350 (OH), 1750 and 1720 (C=O), MS showed M^+ at m/z 367 and other peaks at 349, 324 and 280 Its ^{1}H-NMR and ^{13}C-NMR are listed in Tables 1 and 2.

Compound II: (145 mg)

Fine needles (methanol and chloroform) m.p. 200-202°C, IR spectrum showed peaks at ν in cm^{-1} 3350 (OH) and 1735 (C=O).

CIMS m/z (rel. int.%), 326 M^++1 (63), 325(25), 307 (M^+-H_2O) (1), 236(19) and 41(100).

Its ^{1}H-NMR and ^{13}C-NMR are listed in Tables 1 and 2.

Compound III: (43 mg)

Needle crystals (methanol) m.p. 118-121°C, IR in cm^{-1} 3450 (OH), 1750 and 1610 (C=O).

CIMS m/z (rel. int.%), 368 M^++1 (100), 324(6.1), 252(22), 236(28), 168(28), 152(24), 138(16), 113(28) and 110(26).

^{1}H-NMR and ^{13}C-NMR are listed in Tables 1 and 2.

Compound IV: (125 mg)

Fine needles (chloroform-methanol 4:1), m.p. 278-81°C. Acid hydrolysis of compound IV yielded one sugar and aglycone. The aglycone was identified as β-sitosterol (mp, mmp, IR and TLC using authentic sample), while the sugar was identified as glucose (TLC and PC using authentic sample).

Compound IV was identified as β-sitosterol-3-0-glucoside.

Compound V: (16 mg)

Fine neddles (methanol), m.p. 301-302°C.

UV λ MeOH 273(sh), 248(sh) and 304 nm.

+ NaOMe 328, 289(sh) and 260.

+ NaOAc 331, 310 and 254.

+ $AlCl_3$ + $AlCl_3$/HCl, no bathochromic shift.

MS, m/z (rel. int.%) 254(M^+, 100), 137(88), 118(47), 105(12), 89(16).

^{1}H-NMR (400 MHz, CD_3OD) δ 8.1(1H,S,H-2), 8.05(1,d, J=8.8 Hz,H-5), 7.36(2H,d, J=8.2 Hz, H-2`,H-6`), 6.93(1,d, J=8.8 Hz, H-6), 6.85(1H,S,H-8), 6.84(2H,d, =9.2 Hz, H-3`,H-5`).

^{13}C-NMR (100 MHz, CD_3OD) δ 178.2(S, C-4), 164.6(S, C-7), 159.8(S, C-4`), 158.7(S, C-8a), 154.6(d, C-2), 131.4(d, C-2`, C-6`), 128.6(d, C-5), 126.0(S, C-3), 124.4(S, C-1`), 118.3(S, C-4a), 116.5(d, C-6), 116.3(d, C-3`, C-5`), 103.3(d, C-8).

Compound VI: (82 mg)

Fine needles (methanol), m.p. 264-267°C.
IR at ν in cm^{-1} 3450 (OH) and 1750-1610 (C=O).
+ve FAB-MS, M^++1 at m/z 957 and M^++Na at m/z 979.
Acid hydrolysis of compound VI yielded aglycone and three sugars.
^{13}C-NMR of compound VI is listed in Table 3.

Compound VII: (23.4 mg)

Fine needles (methanol), m.p. 230-3°C.
IR showed peaks at ν in cm^{-1} 3460 (OH), 1760-1620 (C=O).
+ve FAB-MS, at m/z 927 (M^++1), 949 (M^++Na) and 965(M^++K).
Acid hydrolysis of compound VII yielded aglycone and three sugars.
^{13}C-NMR of compound VII is listed in Table 3.

RESULTS AND DISCUSSIONS

The chloroformic fraction of the methanolic extract of the aerial parts of *C. thebaica* (Del.) DC. gave positive results for the presence of pyrrolizidine alkaloids. When chromatographed over silica gel column, three alkaloids were separated, I, II and III in addition to one steroidal compound. IV and the other gave positive test for flavonoids (compound V).

From the aqueous methanolic extract, two compounds were isolated VI and VII.

Compound I:

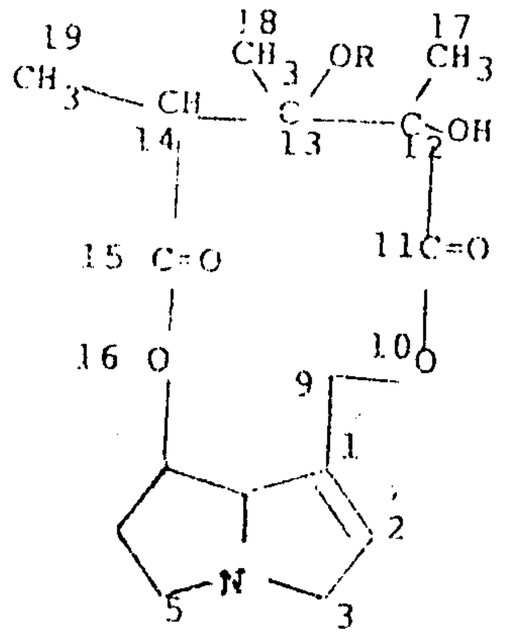
Its IR spectra showed characteristic bands at 3350 cm^{-1} (OH) and 1750-1720 (C=O).

MS showed M^+ at m/z 367 corresponding to the formula $C_{18}H_{25}NO_7$. other peaks 349 (M^+-H_2O) and 324 (M^+-CH_3-C=O).

The ^{1}H-NMR ($CDCl_3$) Table 1 shows the following characteristic signals at δ 1.33(3H,d, J=7.3 Hz) $\underline{CH_3}$-CH-), δ 1.40(3H,S $\underline{H_3}$C-C-OH), δ 1.71(3H,S $\underline{CH_3}$-C-O-C=O), δ 2.11(3H,S O-C-$\underline{CH_3}$) and δ 4.45 (equivalent H9 protons).

Also its ^{13}C-NMR Table 2 shows characteristic signals at δ 168.43(s) characteristic for -C=O of (O=C-CH_3) and δ 21.49(q) characteristic for -CH_3 group.

By comparing the spectral data of compound I (IR, MS, ^{1}H-NMR, ^{13}C-NMR) with that reported for the two membered ring pyrrolizidine alkaloid spectabiline, it was found that they are identical [12,13]. Accordingly compound I was identified as spectabiline.



Compound I R= C-CH_3
Compound II R= H

Compound II:

IR spectra showed characteristic band at 3350 cm^{-1} (OH) and 1735 cm^{-1} (C=O).

MS showed M^+ at m/z 325 corresponding to the chemical formula $C_{16}H_{23}NO_6$.

^{1}H-NMR (Table 1) shows the following characteristic signals at δ 1.23(3H,d, J=7.1 Hz CH_3-CH), δ 1.35 and 1.44(3H,S, CH_3-C-OH), δ 2.80(H6,q, J=7.1 Hz), δ 5.06(H7,m) and δ 6.04(H2,d. J=1.6 Hz).

From ^{1}H-NMR and ^{13}C-NMR (Table 1 and 2) it was found that compound II differs from compound I in the absence of the signals characteristic for the -C-CH_3.

The sesults obtained from compound II are identical with those reported for the alkaloid monocrotaline [12,13]. So that, compound II was identified as monocrotaline.

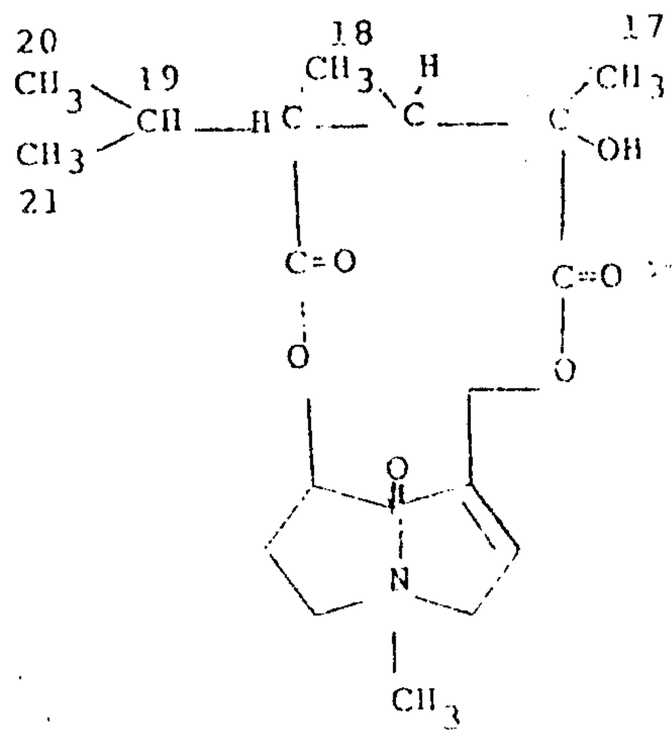
Compound III:

IR spectra showed characteristic band at 3450 cm^{-1} (OH) and 1750, 1610 (C=O).

MS showed M^+ at m/z 367 corresponding to the chemical formula $C_{19}H_{29}NO_6$. Other significant peaks at m/z 168, 152, 138, 113 and 110 which are characteristic for otonecine esters [21].

The ^{1}H-NMR spectrum of compound III Table 1 shows signals at δ 0.93, 0.97, 1.08, 1.42 and 2.20 due to four methyl groups and an N-CH_3 respectively. Multiplets δ 6.04, 5.09, 4.95 and 4.63 are expected for the C_2, C_7 and non equivalent C_9 protons respectively. The assignment of each proton in compound III was confirmed by performing decoupling experiments.

The ^{13}C-NMR of compound III Table 2) was found to be similar to that reported for the pyrrolizidine alkaloid crosemperine [21], which was previously isolated from three species of crotalaria [21-23].



Compound III

Compound IV:

From mp, mmp, IR and TLC after acid hydrolysis using authentic samples, compound IV was identified as β-sitosterol-3-0-glucoside.

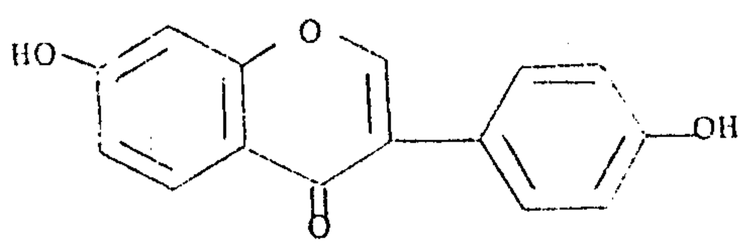
Compound V:

The UV absorption at 273 and 304 nm is characteristic for isoflavonoid [24]. Shifts of the two singlet signals for the H-2 and H-8 protons fell in the normal shift region for the isoflavonoid nucleus. The 7 oxygenation pattern of ring A could be derived from the two ortho coupled doublets at δ 8.05 and 6.93, which

was confirmed by studying the UV spectra with different complexing reagents [24].

The ^{1}H-NMR spectrum also exhibited an A_2B_2 pattern (δ 7.36 and 6.84, ^{13}C-NMR at δ 131.4 and 116.3) characteristic for P-substituted benzene ring.

From the above mentioned data, compound V was identified as 4',7-dihydroxy isoflovone. This compound was previously isolated from *Pueraria thamsonii* Benth [25].



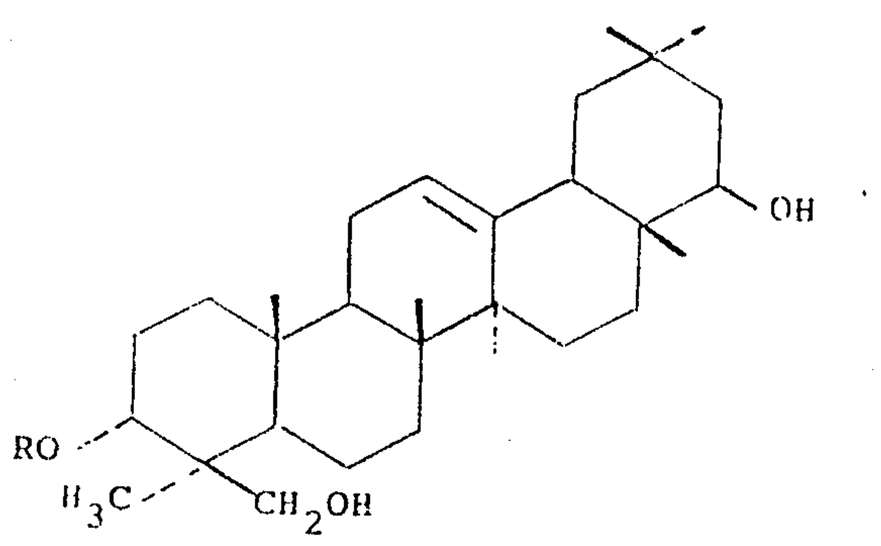
compound V

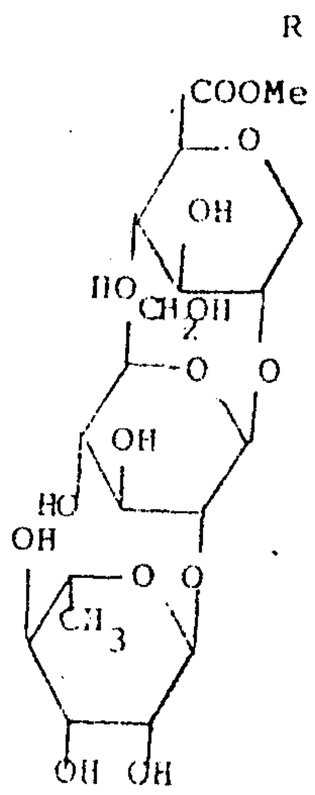
Compound VI:

The ^{1}H-NMR of compound VI showed 7 singlets for seven methyls. It also indicated three anomeric proton signals at δ 4.95(d, J=7.3 Hz), δ 5.78(1H,d, J=7.6 Hz) and δ 6.29(s) for D-glucuronic acid, D-galactose and L-rhamnose. Acid hydrolysis of compound VI yielded glucuronic acid, galactose and rhamnose (PC and TLC using authentic sugars) as well as aglycone which was identified as soyasapogenol-B (From IR, ^{1}H-NMR, ^{13}C-NMR and MS) [26-28].

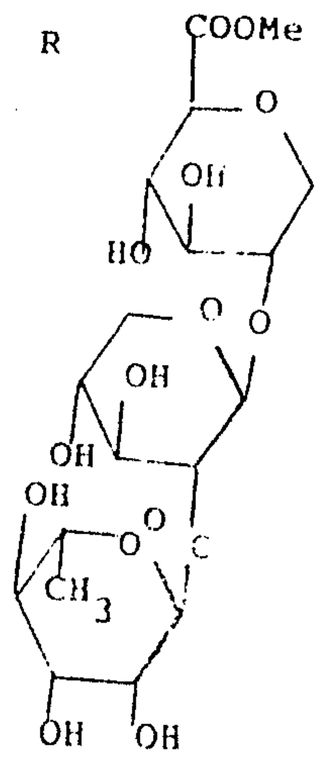
The ^{13}C-NMR of compound VI Table 3 shows signals for three pyranoside anomeric carbons at δ 105.5 (glucuronide C-1), 101.08 (galactoside C-1) and 102.4 (rhamnoside C-1), thus the glycosidic configuration in compound VI was suggested to be α-pyranoside for L-rhamnoside linkage and β-pyranoside for D-galactoside and D-glucuronide linkages [29,30].

From the above mentioned data, compound VI was found to be identical with 3-O-(α-L-rhamnopyranosyl (1→ 2) β-D-galactopyranosyl (1→2) β-D-glucuronopyranosyl)-soyasapogenol B (Saponin methyl ester), which previously isolated from Soya bean [27].





Compound VI



Compound VII

Compound VII:

Its ^{1}H-NMR spectrum is exactly similar to that of compound VI in the up field region. In the down field region its ^{1}H-NMR showed signals for three anomeric protons at δ 6.24(1H,s), 5.58(1H,d, J=7.4 Hz) and 4.95(H,d, J=7.4 Hz) suggesting the presence of L-rhamnose, D-xylose and D-glucuronic acid.

Acid hydrolysis of compound VII yielded aglycone, identified as soyasapogenol B (From mp, IR, ^{1}H-NMR, ^{13}C-NMR and MS) [26-28] and three sugars were identified as D-glucuronic acid, D-xylose, and L-rhamnose (PC and TLC).

The +ve FAB-MS of the glycoside revealed M+1 at m/z 927 and peaks at m/z 949 (M^++Na) and m/z 965 (M^++K).

Based on the above mentioned data compound VII is 3-O[α-L-rhamnopyranosyl (1-- 2)-β-D-xylopyranosyl (1-- 2)-β-D-glucuronopyranosyl] soyasapogenol B which was previously isolated from *Wistaria brachybotrys* [31].

The above mentioned compounds are isolated for the first time from *Crotalaria thebaica* (Del.) DC. growing in Egypt.

Table 1: ^{1}H-NMR Data of the Isolated Compounds I, II & III in $CDCl_3$ (400 MHz).

Proton No.	Compound I δ ppm (Hz)	Compound II	Compound III
2	6.90,d(1.7)	6.04,d(1.6)	6.04,brs
3	4.52,d(18.0)	3.91,dt(16.2 & 1.7)	3.42,dt(18.8 & 1.8)
3`	3.73,d(18.0)	3.48,dd(16.2 & 4.6)	3.19,dt(18.8 & 2.7)
5	3.99,m	3.24,m	[2.18,2H,m]
5`	3.10,m	2.61,m	
6	2.18,(m)	[2.08,2H,m]	2.28,1H,m
6`	2.50,(m)		2.10,1H,m
7	5.67,(m)	5.06,m	5.09,dd(4.6 & 6)
8	5.16,(m)	4.42,m	------
9	4.45,d(11.9)	4.90,d(11.95)	4.95,d(11.7)
9`	4.40,d(11.9)	4.68,dd(11.95 & 0.9)	4.63,dt(11.7 & 1.1)
13	-----	-----	2.05(m)
14	3.02,q(7.2)	2.80,q(7.1)	2.45,dd(8.3 & 0.7)
17	1.71,3H,s	1.44,3H,s	1.42,3H,s
18	1.40,3H,s	1.35,3H,s	1.08,3H,d(7.6)
19	1.33,3H,d(7.3)	1.23,3H,d(7.1)	1.91,1H,m
20	-----	-----	0.97,3H,d(6.7)
21	-----	-----	0.93,3H,d(6.6)
N-CH_3	-----	-----	2.20,3H,s
CH_3-C=O	2.11,3H,s	-----	-----

Table 2: ^{13}C-NMR of the Isolated Compounds I, II and III in $CDCl_3$ (100 MHz).

Carbon No.	Compound I	Compound II	Compound III
1	132.00 (s)	132.73 (s)	135.24 (s)
2	130.63 (d)	134.14 (d)	134.98 (d)
3	58.25 (t)	60.54 (t)	65.98 (t)
5	54.08 (t)	53.63 (t)	53.16 (t)
6	32.90 (t)	33.54 (t)	34.23 (t)
7	70.42 (d)	75.01 (d)	76.51 (d)
8	78.71 (d)	76.80 (d)	188.67 (s)
9	59.16 (t)	61.21 (t)	57.76 (t)
11	169.32 (s)	173.95 (s)	177.20 (s)
12	77.40 (s)	78.80 (s)	77.60 (s)
13	85.56 (s)	77.21 (s)	42.73 (d)
14	43.63 (d)	44.38 (d)	50.30 (d)
15	173.51 (s)	173.51 (s)	172.68 (s)
17	16.96 (q)	21.91 (q)	29.85 (q)
18	17.14 (q)	17.70 (q)	12.63 (q)
19	14.21 (q)	13.62 (q)	27.07 (d)
20	-.-	-.-	20.70 (q)
21	-.-	-.-	19.89 (q)
$N-CH_3$	-.-	-.-	41.89 (q)
$O=C.CH_3$	168.43 (s)	-.-	-.-
$O=C.CH_3$	21.49 (q)	-.-	-.-

Table 3: ^{13}C-NMR of compounds VI & VII in C_5D_5N (100 MHz).

	aglycone part		Sugar moiety	
C. No.	Comp. VI	Comp. VII	Compound VI	Compound VII
1	38.6 (t)	38.7 (t)	β-D-glucuronopyranosyl	β-D-glucuronopyranosyl
2	26.5 (t)	26.5 (t)	1` 105.5	105.5
3	91.4 (d)	91.2 (d)	2` 78.2	78.1
4	43.9 (s)	44.0 (s)	3` 76.5	76.5
5	56.2 (d)	56.2 (d)	4` 74.7	73.3
6	18.9 (t)	18.9 (t)	5` 76.9	76.5
7	33.2 (t)	33.3 (t)	6` 170.4	170.3
8	40.0 (s)	39.9 (s)	$O=C-CH_3$ 52.1	52.1
9	47.9 (d)	47.9 (d)		
10	36.5 (s)	36.5 (s)	β-D-galactopyranosyl	β-D-xylopyranosyl
11	24.1 (t)	24.1 (t)	1`` 101.08	107.5
12	122.4 (d)	122.4 (d)	2`` 77.0	77.6
13	144.8 (s)	144.9 (s)	3`` 73.5	76.9
14	42.3 (s)	42.3 (s)	4`` 71.2	70.5
15	26.7 (t)	26.7 (t)	5`` 76.6	66.9
16	28.7 (t)	28.7 (t)	6`` 61.7	-.-
17	38.0 (s)	38.0 (s)	----------	-.-
18	45.3 (d)	45.3 (d)	α-L-rhammopyranosyl	α-L-rhamnopyranosyl
19	46.6 (t)	46.8 (t)	1``` 102.4	101.9
20	30.9 (s)	30.9 (s)	2``` 72.3	72.4
21	42.3 (t)	42.4 (t)	3``` 72.7	72.8
22	75.6 (d)	75.6 (d)	4``` 74.3	74.4
23	23.6 (q)	23.0 (q)	5``` 69.4	69.5
24	63.6 (t)	63.5 (t)	6``` 18.9	18.9
25	15.8 (q)	15.8 (q)	----------	-.-
26	17.0 (q)	17.0 (q)	----------	-.-
27	25.7 (q)	25.7 (q)	----------	-.-
28	28.7 (q)	28.7 (q)	----------	-.-
29	33.3 (q)	33.3 (q)	----------	-.-
30	21.2 (q)	21.2 (q)	----------	-.-

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دراسه كيميائيه لنبات الكروتالاريا ثابيكا "دل" الذى ينمو فى مصر

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نبات الكروتالاريا ثابيكا يتبع فصيلة البقوليات وجنس الكروتالاريا مشهور باحتوائه على قلوانيات من نوع البيرولليزيدين وهذا النوع من القلوانيات له تاثير مخفض للضغط ويوقف نمو الخلايا السرطانية.

وباستقصاء المراجع المتوافرة وجد ان نبات الكروتالاريا ثابيكا لم تجر عليه اى دراسات لذا رؤى من الضرورى اجراء دراسة كيميائية على هذا النبات.

وقد اسفرت هذه الدراسة عن فصل ثلاثة قلوانيات من نوع البيروليزيدين هى قلوانيات اسبكتابلين، احادى كروتالين وكروسمبرين. بالاضافة الى ٣- اسيتوستيرول جلوكوزيد و٤، ٧-داى هيدروكسى ايزوفلافون. وقد أمكن كذلك فصل اثنين من الصابونينات.

وجميع هذه المواد تفصل لأول مرة من نبات الكروتالاريا ثابيكا وقد أمكن التعرف على المواد المفصولة باستخدام الطرق الطبيعية والطيفية المختلفة مثل الاشعة فوق البنفسجية ودون الحمراء وكذلك الرنين النووى المغناطيسى بأنواعه البروتونى والكربونى ودى الاحداثيات المزدوجة وكذلك باستخدام مطياف الكتلة.

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