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NEW QUATERNARY ALKALOID AND OTHER CONSTITUENTS BESIDE THE BIOLOGICAL ACTIVITIES OF *CADABA GLANDULOZA* F.

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تم فصل مركب قلوانى جديد سمى كدابين (أ) وكذا سبعة مركبات تم فصلها لأول مرة من النبات وهى بيتاسيتوستيرول ، بيتاسيتوسيترول جلوكوزيد ، كامبيفيرول-٣،٧-ثنائى ميثيل اثير ، كوارستين ، كامبيفيرول-٣-أ-جلوكوزيد ، كوارستين-٣-روتينوزايد وقلوانى ستاكدرين. وقد تم التعرف على التركيب البنائى لهذه المركبات بإستخدام الطرق الفيزيائية والطيفية المختلفة بالإضافة إلى ذلك تمت الدراسة البيولوجية على الخلاصة الكحولية ووجد أن لها تأثيراً كمضاد للإلتهابات ، ومسكناً وخافضاً للحرارة.

A new quaternary alkaloid named Cadabine A besides, β-sitosterol, β-sitosterol glucoside, kaempferol-3,7-dimethyl ether, quercetin, kaempferol-3-O-glucoside, quercetin-3-O-rutinoside and stachydrine which were isolated for the first time from the titled plant. Identification of these compounds was established by physical and spectral data (IR, UV, MS, ^{1}H-, ^{13}C and DEPT-NMR), as well as, by comparison with authentic samples. Moreover, some of the biological activities of the ethanolic extract were investigated.

INTRODUCTION

The plant *Cadaba glanduloza* F., family Capparaceae is highly viscid, low shrub with small inconspicuous flowers and closely packed, glandular-hispid, round leaves.[1] *Cadaba* species are used in folk medicine in the treatment of anthrax, relieve general body pain, in Egypt as antidote against poisoning, remedy for cough, fever, dysentery,[2] purgative, emmenagogue, antiphlogistic, edema of the lungs, hyperaemia and anthelmintic,[3] tonsilitis, abscesses and tumor cure.[4] Previous study of different *C. species* revealed the isolation and identification of the quaternary alkaloids stachydrine, 3-hydroxy stachydrine,[5,6] the spermidine alkaloids cadabicine,[7] cadabicine derivatives[8] and the dilactone cadabalone.[9] The flavonol rhamnocitrin and rhamnetin glycosides were isolated from *Cadaba glanduloza*.[10]

This paper deals with the isolation and structure elucidation of a new quaternary alkaloid, one known quaternary alkaloid, four flavonoids, β-sitosterol and β-sitosterol glucoside as well as, the study of the biological activities of the ethanolic extract of the plant.

EXPERIMENTAL

General experimental procedures

Melting points were uncorrected and determined by electrothermal model 550 spectrophotometer Thamson THN 60 ev. IR spectra were recorded in KBr using Unicam SP 1025 spectrometer. UV spectra were measured in MeOH and different ionizing and complexing reagents using a Uvidec 320 (Jasco, Tokyo, Japan) spectrophotometer with matched 1 cm quartz cells. ^{1}H- and ^{13}C-NMR spectra were run in DMSO-d_6 and CD_3OD at 400 and 100 MHz respectively using JEOL TNM-LA400 spectrometer and TMS as internal standard. EIMS and FABMS were recorded by JEOL, JMS 600 H. Column chromatography using silica gel G (E. Merck) and aluminum oxide (E. Merck). TLC was performed on silica gel 60 F_{254} (E. Merck). PC was performed using Whatman paper No. 3 and No. 1. Authentic sugars were obtained from Merck, Darmstadt, Germany, β-sitosterol, β-sitosterol glucoside, quercetin, kampferol-3-O-glucoside and rutin were obtained from Pharmacognosy Department, Faculty of Pharmacy, Assiut University.

Dragendorff's reagent was used for alkaloids detection, methanolic $AlCl_3$ for flavonoids and

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10% H_2SO_4 for sterols. The following solvent system were used.

I, II, III- Hexane-ethyl acetate (85:15), (70:30), (40:60)
IV, V- Chloroform-methanol (85:15), (80:20)
VI- Ethyl acetate-methanol (80:20)
VII- Chloroform-methanol-water (65:35:6.5)
VIII- n-Butanol-acetone-formic acid-water (60:17:8:15).

Plant material

Leaves of *C. glanduloza* F. were collected in April (2000) from the rocky ground east of Jeddah, Saudi Arabia and identified by Prof. Dr. Abd El-Aziz Fayed, Prof. of Taxonomy, Faculty of Science, Assiut University. A voucher specimen is deposited in the Herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Egypt.

Extraction and isolation

2 kg of the powdered leaves were extracted (on cold) with alcohol 70%. The alcoholic extract was concentrated under reduced pressure to a syrupy consistency (58 g). 45 grams of the total extract were diluted with water and extracted successively and exhaustively with chloroform followed by ethyl acetate. The mother liquor was concentrated under reduced pressure to give (22 g) total extract. The chloroform soluble fraction (16 g) was chromatographed over silica gel column (750 g, 7x120 cm) and eluted with hexane-ethyl acetate gradient. The eluted fractions (100 ml each) were collected. Similar fractions (using systems I, II and III) were combined together. Three compounds were obtained; compound **1** (eluted with hexane-ethyl acetate 8:2), compound **2** (eluted with hexane-ethyl acetate 7:3) and compound **3** (eluted with hexane-ethyl acetate 6:4).

The ethyl acetate fraction was concentrated (7 g) and chromatographed over silica gel column (400 g, 5x120 cm) and eluted with chloroform-methanol gradient. The fractions eluted with chloroform-methanol (9:1) afforded compound **4**, while the fractions eluted with chloroform-methanol (85:15) were combined and subjected to PPC using HOAc-water (15:85) as a solvent system to give compound **5**. 10 g of total extract obtained for the mother liquor (22 g) were chromatographed over silica gel column (800 g, 5x180 cm). Elution was started with chloroform followed by chloroform-methanol gradient. The eluted fractions (250 ml each) were collected and similar fractions combined together (using systems VI and VII) to give three major groups A, B and C respectively.

Group A (1.5 g) (fractions eluted with chloroform-methanol 8:1) was subjected to PPC using 15% HOAc to afford compound **6**. Group B (6 g) (fractions eluted with chloroform-methanol 7.5:2.5) was chromatographed over an aluminum oxide column (E. Merck) (300 g, 5x120 cm) using chloroform-methanol (8:2) to give compound **7** as crystalline prisms from methanol. Group C (2.5 g) (fractions eluted with chloroform-methanol 7:3) was chromatographed over an aluminum oxide (E. Merck) column (200 g, 5x120 cm) using chloroform-methanol gradient to afford compound **8** as a gummy residue.

Acid hydrolysis

5 mg of each of the isolated glycosides were separately dissolved in 5 ml methanol to which 5 ml of 5% sulphuric acid were added and the mixture refluxed on a boiling water bath for 3 hours. The mixture was then cooled and the aglycone extracted with $CHCl_3$, purified and subjected to TLC and spectral studies. The aqueous phase was neutralized with barium carbonate, filtered and the filtrate examined by PC for the liberated sugar using solvent system VIII. The sugar moiety in the hydrolysate was identified as glucose in compounds **4** and **5**, glucose and rhamnose in compound **6**, while the aglycone was identified as β-sitosterol for compound **4**, kaempferol for compound **5** and quercetin for compound **6**.

Compound 1: White needles from methanol (180 mg), m.p 135-137°, R_f= 0.42, system I. The compound was identiifed as β-sitosterol by direct authentication (mmp and co-chromatography using system I).

Compound 2: Yellow amorphous powder (50 mg) from methanol-chloroform (1:1), m.p 246-247°, R_f= 0.46 in system II. The UV data with different ionizing and complexing reagents are shown in Table (1). ^{1}H-NMR (DMSO-d_6) δ: 3.78 (3H, s, OCH_3 at C-7), 3.81 (3H, s, OCH_3 at C-3), 6.22 (1H, d, J= 1.7 Hz, H-6), 6.41 (1H, d, J= 1.7 Hz, H-8), 6.8 (2H, d, J= 8.8 Hz, H-3`,5`) and 7.9 (2H, d, J= 8.8 Hz, H-2`,6`), EIMS, m/z (rel. int.%) 314 (M^+, base peak), 296 (15), 284 (14), 270 (30), 186

(4), 167 (8), 143 (13) and 121 (14). ^{13}C-NMR shown in Table (2).

Compound 3: Yellow amorphous powder from methanol (30 mg), m.p 315-317°, R_f= 0.35 in system III. UV data are shown in Table (1). ^{1}H-NMR (DMSO-d_6) δ: 6.11 (1H, br.s, H-6), 6.29 (1H, br.s, H-8), 6.81 (1H, d, J= 8.5 Hz, H-5`), 7.52 (1H, d, J= 8.5 Hz, H-6`), 7.62 (1H, br.s, H-2`) and 12.2 (1H, s, OH at C-5).

Compound 4: White amorphous powder from $CHCl_3$-MeOH (150 mg), m.p 278-279°, R_f= 0.44 in system IV, IR (KBr, ν_{max}, cm^{-1}): 3450, 2940, 1365, 1600, 1070 and 1025. Acid hydrolysis afforded glucose as sugar part and aglycone identified as β-sitosterol (m.p, m.m.p and co-chromatography).

Compound 5: Yellow amorphous powder from methanol (15 mg), m.p 240-244°, R_f= 0.40 in system V. Its physical and spectral data are identical to kampferol-3-O-glucoside previously isolated from *Juglans nigra* L.[11]

Compound 6: Yellow amorphous powder from methanol (25 mg), m.p 190-193°, R_f= 0.60 in system VI. UV data are shown in Table (1). ^{1}H-NMR spectrum (DMSO-d_6) δ: 0.98 (3H, d, J= 5.84 Hz, CH_3 rhamnose), 3.21-3.70 (sugar protons), 4.38 (1H, br.s, H-1``` rhamnose), 5.10 (1H, d, J= 7.4 Hz, H-1`` glucose), 6.18 (1H, br.s, H-6), 6.37 (1H, br.s, H-8), 6.81 (1H, d, J= 8.5 Hz, H-5`), 7.51 (1H, br.d, J= 8.5 Hz, H-6`) and 7.53 (1H, br.s, H-2`). ^{13}C-NMR are shown in Table (2).

Compound 7: Crystalline prisms from methanol (250 mg), m.p 234-237° (anhydrous crystal), R_f= 0.35 in system VII. IR (KBr, ν_{max}, cm^{-1}): showed bands at 1630 for the carbonyl group of the carboxylate anion (COO^-). EIMS showed $[M]^+$ at m/z 143 and other fragment peaks at 129, 84 and 42. ^{1}H-NMR (DMSO-d_6) δ: 2.0 (2H, m, 2H-4), 2.20 (2H, m, 2H-3), 2.98 (3H, s, CH_3-7 or -8), 3.21 (3H, s, CH_3-8 or -7), 3.37 (1H, m, H_a-5), 3.57 (1H, m, H_b-5) and 3.81 (1H, t, J= 11.2 Hz, H-2). ^{13}C-NMR and DEPT NMR are shown in Table (3).

Compound 8: White gum (60 mg), R_f= 0.28 in system VII. IR (KBr, ν_{max}, cm^{-1}): 1730 (ester carbonyl group) and 1628 (carbonyl group of carboxylate antion). FABMS $[M+1]^+$ at 292, EIMS, M^+ at m/z 291, other peaks at m/z 247, 168, 123, 77 and 43. ^{1}H-NMR (CD_3OD) δ: 2.1 (2H, m, H_2-4), 2.50 (1H, m, H-5), 3.0 (1H, br.d, J= 10.0 Hz, H_a-6), 3.08 (3H, s, CH_3-8 or -9), 3.30 (3H, s, CH_3-9 or -8), 3.38 (2H, m, H_2-3), 4.1 (1H, dd, J= 10.0, 6.2 Hz, H_b-6), 4.50 (1H, t, J= 10.3 Hz, H-2), 7.52-7.62 (5 aromatic protons) and the signal of CH_2O benzyl was obscured under water signal. ^{13}C-NMR and DEPT NMR (Table 4).

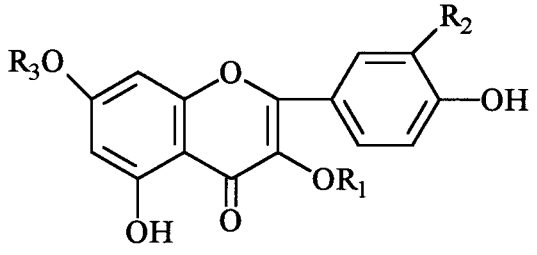
Biological screening

1- Analgesic effect

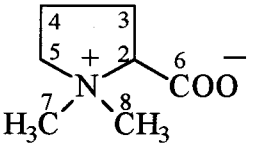
The analgesic effect of the ethanolic extract was studied using the hot plate method as described by Jacobs and Bosovsk.[12] Fifteen mice of either sex each weighing 20-28 g were divided into 3 groups each of 5 animals. The first group was left as control. The second group was injected subcutaneously with indomethacin (10 mg/kg) and considered as standard. Animals of the last group were injected subcutaneously with the ethanolic extract (50 mg/kg). After treatment each mouse was placed on a hot plate thermostatically controlled at 55.5°. The time elapsed until the mouse jumped or leaked its paw was considered as the reaction time for the analgesic activity. This parameter was recorded at 30, 60, 120, 180 and 240 min after administration of the extract. The results are listed in Table (5).

2- Anti-inflammatory effect

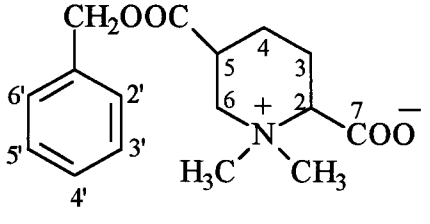
The anti-inflammatory effect was done according to the method described by Vinegar,[13] where pedal inflammation in rat paws was induced by subcutaneous injection of Kaolin (1 ml, 10%). Fifteen adult rats of both sexes weighing from 200-250 g were equally divided into 3 groups and inflammation was induced in the right paw of all animals. At the beginning of the test, the paw's thickness were measured in mm. Thereafter, the first group was kept as control, non-treated, while indomethacin was injected subcutaneously to the second group at a dose of 8 mg/kg of body weight one hour after induction of inflammation. The ethanolic extract was injected subcutaneously to the third group at a dose of 100 mg/kg of body weight one hour after induction of inflammation. Finally, the paw thickness of all groups were measured at 30, 60, 120, 180 and 360 min following administration. The percent of inhibtion of paw oedema was estimated according to the



	R_1	R_2	R_3
Compound **2**	CH_3	H	CH_3
Compound **3**	H	OH	H
Compound **6**	glucose-rhamnose	OH	H



Compound **7**



Compound **8**

Table 1: UV data of the isolated flavonoids **2, 3** and **6**.

Comp.	λ_{max} MeOH	+NaOMe	+NaOAc	+NaOAc H_3BO_3	+$AlCl_3$	+$AlCl_3$/ HCl
2	267 350	268 288 (sh) 390	266 398	265 349	275 302 (sh) 397	275 300 (sh) 398
3	254 267 371	272 410	275 384	258 389	270 438	265 410
6	255 168 356	263 402	274 365	260 370	270 425	267 396

Table 2: ^{13}C-NMR of compound **2** and **6**.

Carbon No.	Compound **6** (DMSO-d_6) δ (ppm)	Compound **2** ($CDCl_3$) δ (ppm)
2	156.4	156.0
3	133.3	137.5
4	177.4	177.9
5	161.2	159.6
6	98.7	97.1
7	164.1	164.6
8	93.6	91.34
9	156.6	156.6
10	104.0	105.2
1`	121.2	120.4
2`	115.2	129.5
3`	144.7	115.1
4`	148.4	161.0
5`	116.3	115.1
6`	121.6	129.5
1``	101.2	OCH_3 at C-7 55.1
2``	74.1	OCH_3 at C-3 59.3
3``	76.4	--
4``	70.5	--
5``	75.9	--
6``	67.0	--
1```	100.7	--
2```	70.2	--
3```	70.4	--
4```	71.8	--
5```	68.2	--
6```	17.8	--

Table 3: ^{13}C-NMR and DEPT of compound 7 in DMSO-d_6.

Carbon No.	δ (ppm)	DEPT
2	75.8	CH
3	25.3	CH_2
4	18.3	CH_2
5	65.7	CH_2
N^+-CH_3-7*	51.1	CH_3
N^+-CH_3-8*	44.6	CH_3
C=O	166.6	C

* Interchangeable signals.

Table 4: ^{13}C- and DEPT NMR of compound **8** (CD_3OD).

Carbon No.	δ (ppm)	DEPT
2	74.5	CH
3	25.6	CH_2
4	20.0	CH_2
5	40.02	CH
6	67.1	CH_2
1`	133.3	C
2`	129.8	CH
3`	132.3	CH
4`	132.3	CH
5`	132.3	CH
6`	129.8	CH
7`	69.0	CH_2
7-$\underline{C}OO^-$	167.5	C
-$\underline{C}OOCH_2$	169.2	C
N^+-CH_3-8*	53.8	CH_3
N^+-CH_3-9*	47.1	CH_3

* Interchangeable signals.

Table 5: Analgesic activity of the ethanolic extract of *C. glanduloza*.

Animal group	Tested compound or extract	Reaction time after administration in min				
		30	60	120	180	240
Group I (control)	untreated	1.383 ±0.296	1.456 ±0.233	1.273 ±0.225	1.152 ±0.257	1.041 ±0.244
Group II	Indomethacin	1.265* ±0.231	4.133* ±0.236	5.680* ±0.223	6.676* ±0.340	4.043* ±0.285
Group III	ethanolic ext. (50 mg/kg)	1.142 ±0.354	2.905* ±0.339	3.711* ±0.268	4.337* ±0.259	3.353* ±0.291

Values represent the mean ± S.E (n = 5)
* Significant difference from control values at $P < 0.05$

formula: % inhibition= $(V_o - V_t) / V_o \times 100$, where V_o: is the average paw thickness of control group and V_t: is the average paw thickness of the treated group. The results are listed in Table (6).

3- Antipyretic activity

The anti-pyretic activity was studied by inducing pyrexia in groups of mice using 20% yeast aqueous suspension. Fifteen mice of either sex each weighing 20-25 g were injected subcutaneously with 20% yeast aqueous suspension to induce pyrexia, then divided into 3 groups each of 5 animals. The first group was left as control. The second groups was injected subcutaneously in a dose of 5 mg/kg indomethacin and considered as a standard for comparison. Animals of the last group were injected subcutaneously 50 mg/kg of the ethanolic extract of the plant to be tested. The rectal temperature was recorded before the beginning of the experiment, immediately before and 30, 60, 120, 180 and 240 min after treatment with the drug.[14] The results are listed in Table (7).

Table 6: Anti-inflammatory effect of the ethanolic extract of *C. glanduloza*.

Tratment	% increase in paw volume (mean ± S.E) after time				
	½ hr	1 hr	2 hrs	3 hrs	4 hrs
Control group	59.30±3.53	65.5±3.94	84.55±5.49	88.90±6.73	89.56±6.65
Indomethacin (mg/kg)	55.40±2.57 (6.5)	60.23*±2.75 (8.74)	62.54*±5.39 (26.03)	68.17*±4.29 (23.31)	73.58*±4.71 (17.84)
Ethanolic ext. (50 mg/kg)	60.57±4.33 (2.14)	63.5±2.07 (3.05)	57.5*±2.07 (31.99)	49.26*+±2.91 (44.58)	40.53*+±2.17 (54.77)

Values represent the mean ± S.E (n = 5)
* Significant difference from control values at P< 0.05
+ Significant difference from indomethacin treated group
Values in parenthesis represent the percentage of oedema inhibition.

Table 7: Anti-pyretic effect of the ethanolic extract of *C. glanduloza*.

Treatment	Rectal temperature in °					
	0 min	30 min	60 min	120 min	180 min	240 min
Control	37.6 ±0.349	38.0 ±0.357	38.5 ±0.293	39.3 ±0.337	38.5 ±0.282	37.9 ±0.391
Indomethacin	37.2 ±0.319	37.0 ±0.352	36.8* ±0.306	36.2* ±0.273	35.9* ±0.304	35.4* ±0.362
Ethanolic ext.	38.5 ±0.329	38.5 ±0.278	38.4 ±0.309	37.7* ±0.355	37.5* ±0.352	37.1* ±0.378

Values represent the mean ± S.E (n = 5)
* Significant difference from control values at P< 0.05

RESULTS AND DISCUSSION

Compound 1: was identified as β-sitosterol by comparison of its physico-chemical data such as m.p and co-chromatography with reference sample.

Compound 2: The UV data (Table 1) showed a free OH at C-4` (NaOMe), absence of free OH at C-7 (NaOAc) and absence of ortho-dihydroxy group in ring B ($AlCl_3$, $AlCl_3$/HCl). The $^1$H-NMR data revealed two doublets at δ 6.8 (J= 8.8 Hz, H-3`,5`), 7.9 (J= 8.8 Hz, H-2`,6`) in accord with a B-ring substituted only at the 4`-position.[15,16] The ^{1}H-NMR also showed two doublets meta coupling at δ 6.22 and 6.41 (J= 1.7 Hz) characteristic for H-6 and H-8, two sharp singlets at δ 3.76 and 3.81 assigned for methoxyls at C-7 and C-3 respectively. Its MS exhibited a molecular ion peak at m/z 314 consistent with the molecular formula $C_{17}H_{14}O_6$ for a flavonoid with two methoxy and two hydroxyl groups. The ^{13}C-NMR data (Table 2) showed that the signals at δ 59.3 and 55.1 assigned for methoxy group at C-3 and C-7, confirmed by appearance of C-6 and C-8 at 97.1 and 91.3 respectively.[17] From the above data, it could be concluded that compound **2** is 3,7-dimethoxy kaempferol.

Compound 3: UV data (Table 1) showed a flavonol structure with free OH at C-4` (NaOMe), ortho-dihydroxy in ring B ($AlCl_3$/HCl and NaOAc/H3$BO_3$) and free OH at C-7 (NaOAc). The $^1$H-NMR spectral data showed two doublets at δ 6.81 (J= 8.5 Hz) and 7.52 (J= 8.5 Hz), each integrated for one proton, and assigned for H-5` and H-6` respectively. Other signals at δ 6.11, 6.29, 7.62 and 12.5 (broad singlets) were assigned for H-6, H-8, H-2` and 5-OH respectively. From these data, as well as, comparison with the published data[18] and m.m.p, co-chromatography with authentic sample, compound **3** was identified as quercetin.

Compound 4: was identified as β-sitosterol glucoside by comparing its physical (m.p, m.m.p) and also through hydrolysis of this glycoside (see experimental). Further confirmation was carried out by co-chromatography with authentic sample.

Compound 5: The physical, chemical and spectral data (UV and ^{1}H-NMR) with those reported[19] and also through acid hydrolysis of this compound (see experimental). The chromatographic study of the sugar in the aqueous fraction revealed that the sugar appeared as a single spot corresponding to authentic glucose using system VIII (R_f= 0.41). Further confirmation was carried out using authentic sample isolated from *J. nigra* L.[11] Compound **5** was identified as kaempferol-3-O-glucoside.

Compound 6: The UV data (Table 1) showed a free OH at C-4` (NaOMe), ortho-dihydroxy group in ring B ($AlCl_3$/HCl and NaOAc/H_3BO_3) and free OH at C-7 (NaOAc shift). ^{1}H-NMR spectrum showed two anomeric protons at δ 5.10 and 4.38 for glucose and rhamnose respectively indicating a (1→6) rutinoside linkage.[19] Moreover, the sugar sequence was confirmed by ^{13}C-NMR data which were in agreement with those reported for qucertin-3-O-rutinoside. Acid hydrolysis of compound **6** gave quercetin, glucose and rhamnose identified by CO-PC and CO-TLC with authentic samples. Comparison of its UV, ^{1}H-NMR and ^{13}C-NMR spectra with published data[17,20-22] in addition to co-chromatography with an authentic sample concluded that compound **6** is quercetin-3-O-rutinoside.

Compound 7: Obtained as crystalline prisms from methanol and gave orange colour with Dragendorff's reagent. The anhydrous crystals melt at 234-237°.[23] The IR spectrum (KBr, ν_{max}, cm^{-1}) showed absorption at 1630 cm^{-1} for carbonyl group of carboxylate anion (COO^-). The EIMS showed $[M]^+$ at m/z 143 corresponding to the molecular formula $C_7H_{13}O_2N$. ^{1}H-NMR spectrum showed signals at δ 2.0 (2H, m), 2.20 (2H, m), 3.37 (1H, m), 3.57 (1H, m), 3.81 (1H, t, J= 11.2 Hz for H_2-4, H_2-3, H-5a, H-5b and H-2 respectively), showed also two signals at 2.98 (3H, s) and 3.21 (3H, s) assigned for two methyl groups (7,8). The ^{13}C-NMR and ^{13}C-DEPT NMR spectra (Table 3) indicated that compound **7** contained two CH_3, three CH_2, one CH and one quaternary carbonyl carbon (C=O). The ^{1}H-NMR and ^{13}C-NMR were closly related to those reported for stachydrine.[24] Furthermore, the ^{13}C-DEPT NMR data (Table 3) confirmed the above structure of compound **7** as the quaternary alkaloid stachydrine.

Compound 8: White gum and responded positively to Dragendorff's test. The IR spectrum displayed an intense absorption bands at 1730 cm^{-1}

characteristic for an ester carbonyl group[25] and at 1628 cm^{-1} for carbonyl group of the carboxylate anion (COO^-). The molecular formula of compound **8** was deduced to be $C_{16}H_{21}O_4N$ from DEPT NMR, FABMS and EIMS. The positive FABMS spectrum showed a significant peak at m/z 292 corresponding to $[M+1]^+$ and EIMS at m/z 291 corresponding to $[M]^+$. The ^{1}H-NMR spectra showed signals at δ 2.10 (2H, m), 2.38 (2H, m), 2.50 (1H, m), 3.0 (1H, br.d, J= 10.0 Hz), 4.1 (1H, dd, J= 10.0, 6.2 Hz) and 4.50 (1H, br.t, J= 10.3 Hz) assigned for H_2-4, H_2-3, H-5, H_a-6, H_b-6 and H-2, respectively. The ^{1}H-NMR spectra also showed signals at δ 3.08 (3H, s) for CH_3-9 or 8, 3.30 (3H, s) for CH_3-8 or 9 and signals from 7.62-7.52 (5H, m) indicating the presence of monosubstituted aromatic ring. The ^{13}C- and ^{13}C-DEPT NMR spectra (Table 4) indicated that compound **8** contained two CH_3, four CH_2, seven CH carbons as well as one quaternary and two carbonyls carbons. The signals at δ_c 129.8, 132.3 and 133.3 integrated for six carbons, one for quaternary carbon and five for CH carbons confirmed the presence of of monosubstituted benzyl moiety.[26] The spectral data indicated that compound **8** is a carboxyhomostachydrine with additional benzyl group. The CH_2 groups at δ 69.0 and 67.0 were assigned for $\underline{C}H_2$ of benzyl moiety and C-6 of homostachydrine respectively. The position of the carboxylation of homostachydrine was suggested to be at C-5 since the proton of this carbon appeared as multiplet (1H, m) and protons at C-6 appeared as broad doublet at δ 3.0 (1H, J= 10.0 Hz, H_a-6) and doublet of doulbet at δ 4.1 (1H, dd, J= 10.0 and 6.2 Hz, H_b-6). This was confirmed from ^{13}C- and DEPT NMR spectral data (Table 4). From all the above mentioned data compound **8** was suggested to be a new homostachydrine derivative given the name cadabine A.

Results of biological screening

1- Analgesic activity

Results in Table (5) revealed that the subcutaneous injection of indomethacin (10 mg/kg) into mice produced significant analgesic effect at ($P< 0.05$) at 60, 120, 180 and 240 min. following administration. Similarly, the subcutaneous injection of the ethanolic extract of *C. glanduloza* (50 mg/kg) into mice led to significant analgesic effect ($P< 0.05$) at all the tested time intervals except at 30 min.

Statistical analysis of the results produced by both indomethacin and the extract of *C. glanduloza* showed that the extract was found to possess lower analgesic effect than indomethacin. The difference was found to be significantly lower at the time intervals of 60, 120, 180 and 240 minutes as it is shown in Table (5).

2- Anti-inflammatory activity

Using the rat paw oedema method, the subcutaneous injection of the extract of *C. glanduloza* into rats in a dose of 100 mg/kg was able to show significant anti-inflammatory effect at 1, 2, 3 and 4 hours after injection (Table 6).

The subcutaneous injection of the standard drug indomethacin showed significant anti-inflammatory properties at the four tested time intervals (1, 2, 3 and 4 hours) following its injection (Table 6).

Compared with indomethacin, the extract of *C. glanduloza* was able to bring a significant differnece ($P< 0.05$) in its anti-inflammatory action at 3 and 4 hours following administration (Table 6).

3- Antipyretic effect

Using the yeast suspension method for inducing hyperpyrexia, the subcutaneous injection of *C. glanduloza* extract was found to cause significant antipyretic effect ($P< 0.05$) 120, 180 and 240 minutes following its administration as it is obvious in Table (7). However, the subcutaneous injection of the standard drug, indomethacin in a dose of 8 mg/kg produced significant lowering of body temperature ($P< 0.05$) at all tested time intervals. Results shown in Table (7) demonstrate that the extract as antipyretic agents is less effective when compared with indomethacin.

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