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PHYTOCHEMICAL AND BIOLOGICAL STUDY OF *VANGUERIA EDULIS* CULTIVATED IN EGYPT

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أدت تجزئة وتنقية الخلاصة الكحولية لأوراق وقلف الساق لنبات فانجيريا اديولس المنزرع فى مصر الى فصل ثلاثة عشر مركبا تم التعرف عليها كالتالى: خلات البيتا سيتوستيرول **(1)**، استجما ستيرول **(2)**، حمض البالمتيك **(3)**، سكوبوليتين **(4)** ، حمض البارا كوماريك **(5)**، حمض البروتوكاتيكويك **(6)**، سكيولتين **(7)**، إيثيل ١ ٠آ جلوكوزيل، ٤ ٠آ كافويل كينات **(8)**، كامبفيرول ٣ ٠آ رامنوزيد، ٧ ٠آ روتينوزيد **(9)**، بيتاسيتوستيرول **(10)**، كحول سيرلى **(11)**، حمض الفانيليك **(12)** وبيتاسيتوستيرول ٣ ٠آ جلوكوزيد **(13)**. وقد تم التعرف على هذه المركبات باستخدام خواصها الطبيعية والوسائل الكيميائية والطيفية المختلفة وأيضا بمقارنتها بالنتائج المنشورة سابقـا. المركبـان **8 و9 يفصلان** لأول مرة من مصدر طبيعى بينما بقية المركبات تفصل لأول مرة من جنس الفانجيريا.تم تعيين الجرعات القاتلة لنصف عدد الفئران للخلاصات المختلفة للنبات كما اجريت الدراسة البيولوجية التى شملت تأثير الخلاصات كمضادة للبكتيريا والفطريات والالتهابات وكخافضة للحرارة وأيضا تأثيرها على الجهاز العصبى المركزى.

Fractionation and purification of the alcoholic extract of the leaves and stem bark of Vangueria edulis cultivated in Egypt afforded thirteen compounds identified as: β-*sitosterol acetate* ***(1)****, stigmasterol* ***(2)****, palmitic acid* ***(3)****, scopoletin* ***(4)****, p-coumaric acid* ***(5)****, protocatechuic acid* ***(6)****, esculetin* ***(7)****, ethyl 1-O-glucosyl-4-O-(E) caffeoyl quinate* ***(8)****, kaempferol 3-O-rhamnoside, 7-O-rutinoside* ***(9)***, β-*sitosterol* ***(10)****, ceryl alcohol* ***(11)****, vanillic acid* ***(12)*** and β-*sitosterol-3-O-*β-*D-glucopyranoside* ***(13)****. Identification of these compounds has been established by physical, chemical and spectral data as well as comparison with authentic samples. Compounds* ***8*** *and* ***9*** *are firstly reported from a natural source while the rest of the compounds have been isolated for the first time from the genus Vangueria. The* LD_{50}*, antibacterial, antifungal, anti-inflammatory, antipyretic and the effect on CNS were studied.*

INTRODUCTION

Vangueria edulis (Vahl) Vahl Variety: *bainesii* Heirn (Rubiaceae) is a perennial shrub or a small tree not exceeds 6 meters in height, cultivated as an ornamental plant and known as Spanish tamarind[1]. According to the available literature, no report about the phytochemical constituents of this plant could be traced that encouragethis study which includes the isolation and identification of the chemical constituents in addition to the biological activities of the leaves and stem-bark of this plant.

MATERIAL AND METHODS

Experimental

General experimental procedure: Melting point (uncorrected)was recorded on Electrothermal 9100 Digital Instrument (England Ltd.). ESI-MS was recorded on Micromass® Quattro micro™ API mass spectrometer (Germany). ^{1}H-NMR (700, 600 and 400 MHz) and ^{13}C-NMR (175, 150 and 100 MHz) spectra were measured on Bruker AVANCE AVIII (Oxford), JOEL/ECP (Japan) and Varian unity INOVA spectrometers (England) respectively using TMS as an internal standard. UV spectra were determined

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on Ultrospec 1000, UV/visible Spectrometer, Pharmacia Biotech, Cambridge, England. Agilent spectrometer (USA) for GC/MS analysis. Column chromatography was performed on silica gel (70-230 mesh) and RP-18 (E-Merck, Germany), precoated silica gel $G_{60}F_{254}$ and RP-18 plates for TLC (E-Merck, Germany).

Plant material

The leaves and stem-bark of *Vangueria edulis* were collected at the flowering stage (May, 2008) from Al-Zohria garden, Cairo, Egypt and was kindly identified by Prof. Dr. Mo'men Mostafa Mahmoud, Professor of Taxonomy, Faculty of Science, Assiut University, Assiut, Egypt.

Extraction and isolation

The air-dried powdered leaves (3 kg) and stem-bark (1 kg) were separately extracted with 70% ethanoltill exhaustion. The combined extract was evaporated till dryness to give 300 g of leaf extract and 70 g of stem-bark extract. The obtained extracts were separately digested in small amounts of distilled water and successively extracted with *n*-hexane, $CHCl_3$, EtOAc(for both leaf and stem-bark) and finally *n*-butanol (for leaf)and dried over anhydrous sodium sulphate to yield (100 g, leaf; 0.6 g stem-bark), (2.5 g leaf; 3 g stem-bark), (160 g leaf; 3.5 g stem-bark) and (7 g leaf) for *n*-hexane, $CHCl_3$, EtOAc and *n*-butanol fractions respectively.

1- The leaf extract

Thirty grams of the dried hexane fraction was chromatographed over silica gel column (900 g) and gradiently eluted with hexane-EtOAc. Fractions (250 ml each) were collected and monitored using TLC [hexane:EtOAc (90:10) and (80:2) solvent systems]and 10% H_2SO_4 as spraying reagent; similar fractions were pooledto give six fractions (F-I to F-VI). F-III (5.5g) from hexane-EtOAc (9:1) and F-IV (8 g) from hexane-EtOAc (85:15) were separately re-chromatographed on silica gel CC using hexane-EtOAc gradient which afforded compounds **1** (60 mg) and **2** (150 mg) respectively. F-VI (3 g) eluted with hexane-EtOAc (7:3) afforded compound **3** (25 mg) after repeated silica gel CC using hexane-acetone gradient.

The dried chloroform fraction (2.5 g) was chromatographed over silica gel column (75 g) and eluted with $CHCl_3$-MeOH gradiently. Similar fractions were combined to give three fractions (F-I to F-III). F-I (1 g) eluted from pure $CHCl_3$ was re-chromatographed on silica gel CC using hexane-EtOAc gradient which afforded compound **4** (60 mg).

Thirty grams of the EtOAc fraction was transferred to a Buchner [14 (id)× 10 (l) cm] packed with silica gel; The elution started initially with pure $CHCl_3$ followed by $CHCl_3$-EtOAc gradiently, EtOAc and finally with EtOAc-MeOH. Similar fractions were pooled to give five fractions (F-I to F-V). F-II (8 g) eluted with $CHCl_3$:EtOAc (70:30) was chromatographed on silica gel CC and eluted gradiently with hexane-EtOAc giving 3 subfractions that by repeated silica gel CC, purification and crystallization afforded compounds **5** (45 mg), **6** (20) and **7** (25 mg). F-III (12 g) eluted with $CHCl_3$:EtOAc (30:70) was chromatographed on silica gel CC and eluted with $CHCl_3$-MeOH gradient giving 2 subfractions (F-IIIa and F-IIIb). F-IIIa was chromatographed over silica gel CC using *n*-hexane-EtOAc gradient, afforded compound **8** (70 mg) while F-IIIb was chromatographed over RP-18 CC using 30% MeOH afforded compound **9** (25 mg).

2- The stem-bark extract

Parts of the chloroform (2.5 g) and EtOAc extracts (30 g) wereseparately subjected to silica gel CC and gradiently eluted with hexane-EtOAc. Similar fractions were combined together(four fractions each). Fraction II eluted with *n*-hexane-EtOAc (90:10) of $CHCl_3$ extract after repeated silica gel CC yieldedcompound **10** (80 mg). EtOAc fraction II eluted with *n*-hexane-EtOAc (70:30)and fraction III eluted with *n*-hexane-EtOAc (50:50) after repeated silica gel CC yielded compounds **11** (30 mg), **12** (70 mg) and **13** (120 mg).

Compound (4): yellow crystals (MeOH). m.p. 206-208°C. ^{1}H-NMR ($CDCl_3$, 600 MHz): δ 6.20 (1H, *d*, 9.0 Hz, H-3), 7.55 (1H, *d*, 9.0 Hz, H-4), 6.84 (1H, *s*, H-5), 6.79 (1H, *s*, H-8), 3.88 (3H, *s*, OCH_3). ^{13}C-NMR (Table 1).

Table 1: ^{13}C-NMR spectral data of compounds **4-7** and **12** in CD_3OD (100 MHz).

C	Cpd. **4**$^{+}$	Cpd. **5**	Cpd. **6**	Cpd. **7***	Cpd. **12**
1	-	127.67 (*s*)	123.39 (*s*)	-	121.74 (*s*)
2	161.49 (*s*)	131.07 (*d*)	115.74 (*d*)	161.28 (*s*)	114.39 (*d*)
3	113.24 (*d*)	116.79 (*d*)	145.89 (*s*)	111.89 (*d*)	147.21 (*s*)
4	143.35 (*d*)	160.97 (*s*)	151.31 (*s*)	144.91(*d*)	151.18 (*s*)
5	107.55 (*d*)	116.79 (*d*)	117.71 (*d*)	112.73 (*d*)	112.37 (*d*)
6	150.18 (*s*)	131.07 (*d*)	123.88 (*d*)	143.36 (*s*)	123.83 (*d*)
7	144.08 (*s*)	146.64 (*d*)	170.61 (*s*)	150.93 (*s*)	168.69 (*s*)
8	103.13 (*d*)	115.71 (*d*)	-	103.07 (*d*)	-
9	149.76 (*s*)	167.00 (*s*)	-	148.96 (*s*)	-
10	111.44 (*s*)	-	-	111.16 (*s*)	-
OMe	56.38 (*q*)	-	-	-	54.97(q)

*Data in DMSO-d_6 + Data in $CDCl_3$ (150 MHz)

Compound (5): yellow crystals (MeOH). m.p. 131-133°C. ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.37 (2H, *d*, 8.4 Hz, H-2, 6), 6.73 (2H, *d*, 8.4 Hz, H-3, 5), 7.52 (1H, *d*, 16.0 Hz, H-7), 6.21 (1H, *d*, 16.0 Hz, H-8). ^{13}C-NMR (Table 1).

Compound (6): yellow crystals (MeOH). m.p. 178-180°C. ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.43 (1H, *d*, 2.0 Hz, H-2), 6.80 (1H, *d*, 8.0 Hz, H-5), 7.40 (1H, *dd*, 2.0, 8.0 Hz, H-6). ^{13}C-NMR (Table 1).

Compound (7): yellow crystals (acetone). m.p. 269-271°C. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 6.16 (1H, *d*, 9.4 Hz, H-3), 7.86 (1H, *d*, 9.4 Hz, H-4), 6.97 (1H, s, H-5), 6.73 (1H, s, H-8). ^{13}C-NMR (Table 1).

Compound (8): yellow amorphous powder. ESI-MS *m/z* (% rel. int.) 544 $[M]^+$ (45), 530 (97), 516 (100), 501 (28). ^{1}H-NMR (DMSO-d_6, 700 MHz): **Quinic acid:** δ 1.92 (1H, *dd*, 3.5, 13.5 Hz, H-2_{ax}), 2.10 (2H, *m*, H-2_{eq} and H-6_{eq}), 3.57 (1H, *m*, H-3), 5.02 (1H, *m*, H-4), 3.88 (1H, *m*, H-5), 1.76 (1H, *dd*, 9.5, 12.5 Hz, H-6_{ax}); **Caffeoyl moiety:** δ 7.02 (1H, *d*, 2.0 Hz, H-2'), 6.76 (1H, *d*, 8.8 Hz, H-5'), 6.97 (1H, *dd*, 2.0, 8.8 Hz, H-6'), 7.37 (1H, *d*, 15.9 Hz, H-7'), 6.01 (1H, *d*, 15.9 Hz, H-8'); **Glucose moiety:** δ 4.08 (1H, *d*, 7.6 Hz, H-1"), 3.42-4.39 (6H, *m*, H-2"-H-6"); **Ethyl ester**: 1.15 (3H, *t*, 7.0 Hz, C$\underline{H_3}$-1'"), 4.00 (2H, *q*, C$\underline{H_2}$-2'"). ^{13}C-NMR (Table 2).

Table 2: ^{13}C-NMR spectral data of compound **8** (DMSO-d_6, 175 MHz).

Carbon No.	δ (ppm) and multiplicity	Carbon No.	δ (ppm) and multiplicity
Quinic acid			
C-1	72.5 (*s*)	C-7'	145.5 (*d*)
C-2	35.5 (*t*)	C-8'	114.3 (*d*)
C-3	69.5 (*d*)	C-9'	165.9 (*s*)
C-4	71.5 (*d*)	Glucosyl moiety	
C-5	67.2 (*d*)	C-1"	103.8 (*d*)
C-6	37.5 (*t*)	C-2"	73.5 (*d*)
C-7	173.5 (*s*)	C-3"	77.0 (*d*)
Caffeoyl moiety		C-4"	70.0 (*d*)
C-1'	125.8 (*s*)	C-5"	76.4 (*d*)
C-2'	114.9 (*d*)	C-6"	63.6 (*t*)
C-3'	148.9 (*s*)	Ethyl ester	
C-4'	146.0 (*s*)	C-1'"	14.3 (*q*)
C-5'	116.2 (*d*)	C-2'"	60.7 (*t*)
C-6'	121.7 (*d*)		

Compound (9): yellow amorphous powder. ^{1}H-NMR (CD_3OD, 400 MHz): δ 8.12 (2H, *d*, 8.8 Hz, H-2', 6'), 6.90 (2H, *d*, 8.8 Hz, H-3', 5'), 6.78 (1H, *d*, 2.0 Hz, H-8), 6.48 (1H, *d*, 2.0 Hz, H-6), 5.10 (1H, *d*, 7.6 Hz, glu-1), 4.52 (1H, *d*, 1.6 Hz, rham`.-1), 4.50 (1H, *d*, 1.6 Hz, rham-1), 3.2-3.9 (*m*, sugar protons), 1.26 (3H, *d*, 6.0 Hz, rham' 6-CH_3), 1.18 (3H, *d*, 6.4 Hz, rham 6-CH_3). UV/Vis. spectral data (Table 3). ^{13}C-NMR (Table 4).

Table 3: UV/Vis. spectral data of compound **9** in methanol and with different ionizing and complexing reagents.

Band	λ_{max} and $\Delta\lambda_{max}$ nm										
	MeOH	NaOMe		NaOAc		$NaOAc/H_3BO_3$		$AlCl_3$		$AlCl_3/HCl$	
	λ_{max}	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$
II	268	270	+2	268	-	266	-	274	+6	273	+5
I	343	384	+41	345	+2	343	-	393	+50	392	+49

Table 4: ^{13}C-NMR spectral data of compound **9** (CD_3OD, 100 MHz).

C- atom	δ (ppm) and ultiplicity	Carbon No.	δ (ppm) and multiplicity
2	158.01 (*s*)	rham -4	73.52[b] (*d*)
3	135.80 (*s*)	rham -5	71.38 (*d*)
4	179.76 (*s*)	rham -6	17.95[c] (*q*)
5	162.72 (*s*)	glu-1	100.65 (*d*)
6	99.81 (*d*)	glu -2	74.85 (*d*)
7	163.64 (*s*)	glu -3	78.01 (*d*)
8	95.71 (*d*)	glu -4	71.26 (*d*)
9	159.84 (*s*)	glu -5	77.91 (*d*)
10	104.83 (*s*)	glu -6	67.36 (*t*)
1'	122.39 (*s*)	rham '-1	101.14 (*d*)
2' and 6'	132.54 (*d*)	rham '-2	72.05 (*d*)
3'and 5'	116.23 (*d*)	rham '-3	72.92[a] (*d*)
4'	161.68 (*s*)	rham '-4	73.78[b] (*d*)
rham-1	101.75 (*d*)	rham '-5	69.68 (*d*)
rham -2	71.61 (*d*)	rham '-6	18.10[c] (*q*)
rham -3	72.25[a] (*d*)		

Compound (12): light yellow crystals (MeOH). m.p. 210-213°C. ^{1}H-NMR (CD_3OD, 400 MHz): δ 7.56 (1H, *dd*, 2.0, 8.0 Hz, H-6), 7.55 (1H, *br s*, H-2), 6.83 (1H, *d*, 8.0 Hz, H-5), 3.80 (3H, *s*, OMe). ^{13}C-NMR (Table 1).

Preparation for the fatty acids

Ten g of the *n*-hexane fractionof the leaves were saponified by refluxing with 50 ml of 0.5 N ethanolic KOH for 3 hrs on a boiling water bath. The alcohol was distilled off and the aqueous liquid was extracted with ether till exhaustion. The alkaline aqueous solution that remained after removal of the unsaponifiable matter was acidified with sulphuric acid and the liberated fatty acids were extracted with ether (3x50 ml). The combined ether extracts were washed with distilled water to remove any acidity and dried over anhydrous sodium sulphate. The solvent was distilled off under reduced pressure to give a viscous residue of the free fatty acids, which have yellowish brown colour. A part of the residue was subjected to esterification with methanol as follow:

One gram of the fatty acids was dissolved in 150 ml of 10% H_2SO_4 in MeOH[2] and then refluxed for 5 hrs. The solvent was distilled off and the residue was taken in 10 ml distilled water, the aqueous solution was rendered alkaline with dilute ammonium hydroxide, where an oily layer was separated and extracted with ether till exhaustion. The ethereal extracts were combined and distilled to give a yellowish-brown residue. A part of this residue was analyzed by GC.

Gas-liquid chromatography (GLC) of the fatty acid methyl esters

GLC analysis of the fatty acid methyl esters was performed using Agilent spectrometer. Gas chromatograph equipped

with flame ionization detector and fitted with 3% OV-17 on Carbowax HP 80/100 (6` x 1/8`` x 0.085``) SS column, programmed at 160°C for 2 min. then increase by 15°C/min. till 300°C and isothermal for 15 min. Injector and detectortemperature were 250°C and 320°C respectively.

Authentic materials

Authentic stigmasterol, β-sitosterol acetate, palmitic acid, β-sitosterol, ceryl alcohol and β-sitosterol glucoside from Pharmacognosy Dept., Faculty of Pharmacy, Assiut University.

Biological study

The biological studies were performed on the different fractions: *n*-hexane, chloroform, ethyl acetate, total ethanolic and aqueous extract of leaves and stem-bark in addition to the *n*-butanol fraction of the leaves.

1- Experimental animals

Male albino rats (100-120 g) and mice (20-25 g) were housed under standardized environmental conditions in the pre-clinical animal house, Pharmacology Department, Faculty of Medicine, Assiut University and fed with standard diet and allowed for free access to water.

2- Materials for biological study

Normal saline 0.9%, PEG-600 and Emmon's Sabouraud Dextrose Agar (El-Naser Pharmaceutical and Chemical Co., Egypt) (ADWIC). Indomethacin (El-Nile Co., Cairo, Egypt). Yeast. Gentamicin and clotrimazole (Memphis Co., Egypt). Carbamazepine (Novartis Co., Switzerland). Carrageenin, Pentylene tetrazole, 2% Tween 80 and Dimethylformamide (Sigma Chemical Co., St. Louis, USA). Bacterial strains:*Bacillus cereus*; *Escherichia coli*;*Klebsiella pneumonia*; *Micrococcus luteus*;*Pseudomonas aeruginosa*; *Staphylococcus aureus* and the fungus *Candida albicans* (Microbiology Department, Faculty of Medicine, Assiut University)

3- Preparation for toxicological study

Certain weights of the total ethanolic extracts of the leaves and stem-bark were suspended in saline solution by the aid of polyethylene glycol-600 (PEG-600, 3% w/v) and the volume was completed to 100 ml with saline solution.

Determination of the LD_{50}

The LD_{50} of the total ethanolic extracts were determined according the reported method[3]. The experiment was carried out in two phases; the first phase involved an initial dose finding procedure, in which the animals were divided into three groups (3 rats, each). Doses of 10, 100 and 1000 mg/kg were administered i.p., one dose for each group. The treated animals were monitored for 24 hrs for mortality and general behavior. The second phase, based on the result of the above step; four different doses were chosen and administered i.p. to four groups (1 rat, each). The treated animals were again monitored for 24 hrs. The LD_{50} was calculated as the geometric mean of the lowest dose showing death and the highest dose showing no death[3&4]. The symptoms of the toxicity are characterized by irritability, writhing, hypothermia, loss of motor co-ordination, sedation and deep sleep followed by death.

4- Preparation for antimicrobial study

200 mg of the extracts were separately taken and reconstituted in a least amount of dimethylformamide (DMF) and the volume was completed to 2 ml with DMF (100 mg/ml). DMF served as a negative control.

Antibacterial activity

The tested bacterial strains were inoculated into Muller Hinton broth medium and incubated for 3-6 hrs at 35°C in a shaker water bath until the culture attained a turbidity of 0.5 McFarland units. The final inoculums were adjusted to $5x10^5$ cfu/ml. Antibacterial screening was done by a modified agar-well diffusion method[5]. One ml of the standard suspension (5×10^5 cfu/ml) of each test bacterial strain was spreads evenly on Muller Hinton Agar (MHA) plates and allowed to dry at room temperature. 10 mm-diameter wells were bored and a 100 µl of each plant extract was pippetted into the wells. After 1 hr, they were incubated at 37°C for 24 hrs and the bacterial growth inhibition zone diameter was measured. Gentamicin (5 µg/ml) was included as a positive control while dimethylformamide (DMF) as a negative control.

Antifungal activity

The activity was evaluated by the agar dilution method using Emmon's Sabouraud Dextrose Agar (ESDA) medium. 100 mg/ml of the test extracts were prepared in dimethylformamide (DMF). Plates were inoculated with 0.05 ml of the fungal suspensions and incubated at 30°C until macroscopically visible growth appeared in the control. Clotrimazole (5 µg/ml) was included as a positive control while ESDA incorporating only DMF was used as a negative control.

Determination of the minimal inhibitory concentration (MIC)

The MIC was determined by the modified agar-well diffusion method[5].

5- Preparation for pharmacological study

One gram of the extracts were separately taken and solubilized in 0.9% normal saline with the aid of 2% tween 80 and the volume was completed to 10 ml with normal saline (100 mg/ml).

Anti-inflammatory activity

Winter *et al*.'s method was used[6]. Thirteen groups (5 rats, each) were used. The pedal inflammation was induced in rat paws by injection of 0.1 ml, 1% carrageenan suspension in 0.9% NaCl solution into the sub-plantar tissue of the right hind paw. At the beginning of the experiment, the paws thickness was measured in mm using Varinier caliber. The first group was injected i.p. by 2% tween 80 in normal saline (negative control) while the second group was injected by indomethacin (8 mg/kg) as a positive control. Other groups were separately injected i.p. with the different fractions of the leaves and stem-bark(400 mg/kg of the body weight). After 30 minutes, the inflammation was induced by injection of the carrageenan suspension in the right paw while the left one was injected by an equal volume of saline solution. The difference between the thicknesses of the two paws was taken as a measure of edema. The anti-inflammatory efficacy of the tested fractions was estimated by comparing the magnitude of paw swelling in the pretreated animals with those induced by in control animals receiving saline. The measurement was carried out at 1, 2, 3, 4, 5 hrs after injection of the inflammatory agent. The percentage of edema was calculated as follows: (R-L)x100/L while the% of inhibition was calculated as follows: $(V_0-V_t)x100/V_0$, where: R: the right paw thickness; L: the left paw thickness ; V_0: the average paw thickness of control group and V_t: the average paw thickness of the treated group.

Antipyretic activity

Thirteen groups (5 rats, each) were used and the rectal temperature was recorded with a thermometer. Hyperthermia was induced by subcutaneous injection of 20% (w/v) aqueous suspension of yeast in a volume of 10 ml/kg. The first group was kept as a negative control injected i.p. by 2% Tween 80 in 0.9% normal saline. The second group was injected by indomethacin (8 mg/kg) as a positive control. The other groups were separately injected i.p. with the different fractions at doses of 400 mg/kg body weight. Rectal temperatures were taken after 1, 2, 3 and 4 hrs from administration of tested fractions[7].

Activity on the central nervous system

The activity of the different fractions and the total ethanolic extracts of both leaves and stem-bark on the central nervous system were evaluated by performing assays of their effect on motor co-ordination (Rota-rod test) and pentylene tetrazole induced convulsion[(8-10)].

Assay of the effects on motor co-ordination (Rota-rod test)

Boissier's method[8&9] was used where mice were placed on a Rota-rod and those staying on the rod for longer than three minutes were selected for the experiment. Twelve groups each of five mice were placed on the rod 30 and 60 minutes after i.p. injection of the control or the test fractions at dose (400 mg/kg). The time that they stayed on the rod was recorded.

Effect on pentylene tetrazole (PTZ) induced convulsion

Thirteen groups (5 mice, each) were used. The first group (control group) was i.p. injected with convulsive agent alone. The second group was i.p. injected with (PTZ) 30 minutes after i.p. injection of 5 ml/kg Carbamazepine as a standard anticonvulsant drug. Other groups were i.p. injected with the test fractions (400

mg/kg) 30 minutes before the i.p. injection of PTZ. Latencies to the onset of clonic, tonic and the mortality were evaluated during 30 min after PTZ injection[10].

6- Statistical analysis

All the results were expressed as mean ± standard error of the mean. The significance in results from control mice was calculated using the student's t-test. *P< 0.05 or less considered as positive result.

RESULTS AND DISCUSSION

Identification of the isolated compounds

All compounds were isolated from the alcoholic extract of the air-dried leaves and stem-bark of *Vangueria edulis.* The identification of compounds **1**, **2**, **3**, **10**, **11** and **13**as β-sitosterol acetate, stigmasterol, palmitic acid, β-sitosterol, ceryl alcohol and β-sitosterol glucoside respectively were established through different physical, chemical and spectral data together with co-chromatography with authentic samples. To the best of our knowledge all compounds were isolated here for the first time from the genus *Vangueria* except compounds **8** and **9** which are new compounds.

Compound (4)

The ^{1}H-NMR spectral data (experimental section) revealed the presence of aromatic compound from the signals at δ 6.79 and 6.84 (each 1H, s) and at δ 6.20 and 7.55 (each 1H, *d*, *J* = 9.0 Hz), these signals were indicative of two aromatic and two *Cis*-olefinic protons, respectively. A singlet peak at δ 3.88 (3H) with δ_c 56.38 indicated the presence of aromatic methoxy group. The^{13}C-NMR spectral data (Table 1) confirmed the aromatic structure from the signals at δ 113.24 (d), 143.35 (d), 107.55 (d), 150.18 (s), 144.08 (s), 103.13 (d), 149.76 (s) and 111.44 (s) including a signal for δ-lactone function at δ 161.49 (s). The ^{1}H-NMR and ^{13}C-NMR spectral data indicated the presence of coumarin nucleus[11] where the signals at δ_H 6.84, 6.79, 6.20 and 7.55 with δ_C107.55, 103.13, 113.24 and 143.35 were assigned for H-5, H-8, *Cis*-olefinic protons H-3 and H-4 of the coumarin nucleus, respectively. The position of the methoxy group was deduced from the chemical shifts in the ^{1}H-NMR and ^{13}C-NMR data suggested that compound **4** is scopoletin[11].

Compound (5)

The doublet signals at δ_H 7.37 and 6.73 (each 2H, *J* = 8.4 Hz) with δ_C 131.07 (*d*, 2C) and 116.79 (*d*, 2C) respectively in the ^{1}H- and ^{13}C-NMR spectral data (experimental section and Table1) in addition to signals at δ_C 127, 67 and 160.97 indicated the presence of *p*-disubstituted benzene ring while thedoublet signals atδ_H 7.52 and 6.21 (each 1H, *J* = 16.0 Hz) with δ_C 146.64 and 115.71 indicated the presence of *trans*-olefinic protons. These data in addition to a carbonyl carbon at δ_C 167.00 and comparing with the literature[12&13], indicated that the compound is *p*-coumaric acid.

Compound (6)

Compound **6** gave a greenish-brown colour with $FeCl_3$ suggesting its phenolic nature. The ^{1}H-NMR spectral data (experimental section) revealed the presence of trisubstituted benzene ring with an ABX system from the three signals at δ 7.43 (1H, *d, J* = 2.0 Hz), 7.40 (1H, *dd, J* = 2.0, 8.0 Hz) and 6.80 (1H, *d, J* = 8.0 Hz). The ^{13}C-NMR spectral data (Table 1) confirmed the aromatic structure from the 6 signals at δ 123.39 (s), 117.71 (d), 145.91 (s), 151.31 (s), 115.74 (d) and 123.88 (d) in addition to a signal for a carbonyl group at δ 170.61 (s). The spectral data and the literature[14], indicated that compound **6** is protocatechuic acid.

Compound (7)

The ^{1}H- and ^{13}C-NMR spectral data (experimental section and Table1) revealed the presence of aromatic compound from the signals at δ_H 6.73 and 6.97 (each 1H, *s*) and δ_H 6.16 and 7.86 (each 1H, *d, J* = 9.4 Hz) with δ_C 103.07 (d), 111.89 (d), 112.73 (d) and 144.91(d) respectively indicating two aromatic andtwo*Cis*-olefinic protons and carbons. These data in addition to the remaining five signals in the ^{13}C-NMR data (Table1) are similar to that of compound **4** except for the absence of the methoxy signal in both ^{1}H- and ^{13}C-NMR data indicating that the compound is esculetin and confirmed by comparing with the published data[15].

Compound (8)

The ^{1}H-NMR spectral data (experimental section) showed the presence of*trans*-caffeoyl moiety from the three aromatic protons with an ABX system at δ 7.02 (1H, *d, J* = 2.0 Hz), 6.97 (1H, *dd, J* = 2.0, 8.8 Hz) and 6.76 (1H, *d, J* = 8.8 Hz); two *trans* olefinic protons with an AB system and large coupling constant at δ 7.37 and 6.01(each 1H, *d, J* = 15.9 Hz) and a singlet carbon at δ_C 165.9[16&17]. It also showed an ethyl group as a triplet at δ_H 1.15 and quartet at δ_H 4.00; seven protons in a typical accordance with previously reported literature data of C-4 substituted quinic acid[17] and a doublet proton at δ 4.08 with large coupling constant 7.6 Hz which assigned for glucopyranosyl moiety in β-configuration[18&19], The ^{13}C-NMR data (Table 2) confirmed the presence of the caffeoyl moiety from the nine signals in the region from 114.3 ppm to 165.9 ppm[17&20], and the glucose moiety from the six signals at δ_C 103.8 (d), 77.0 (d), 76.4 (d), 73.5 (d), 70.0 (d) and 63.6 (t). The quinic acid moiety was deduced from the two methylenes at δ_C 35.5 and 37.5, three oxymethines at δ_C 67.2, 69.5, and 71.5, one quaternary carbon at δ_C 72.5 and one carboxyl group at δ 173.5 in addition to the ethyl group from the two signals at δ_C 60.7 (t) and 14.3 (q). The assignment of the compound was established through 2D NMR spectroscopy (^{1}H-^{1}H COSY, HSQC and HMBC).^{1}H-^{1}H COSY showed cross peaks as follow: H-3 with H-4; H-5 with H-6; H-7′ with H-8′; H-5′with H-6′ and CH_3-1‴ with CH_2-2‴. The attachment between glucose, and ethyl groups to the quinic acid was deduced from HMBC where a correlation peak was observed between H-1″ at δ_H 4.08 and C-1 at δ_C 72.5; H-2‴ at δ_H 4.00 and C-7 at δ_C 173.5. The ESI-MS confirmed the structure which showed a quasi-molecular ion peak at *m/z* 544 $[M]^+$; other peaks at *m/z*530, 516 (base peak) and 502.Based on the above mentioned data, compound **8** was assigned as ethyl 1-*O*-glucosyl-4-*O*-(E) caffeoyl quinate.

Compound (9)

The UV spectral data of compound **9** (Table 3) showed two absorption bands at λ_{max} 343 nm and 268 nm characteristic for flavonoids[21]. A bathochromic shift in band I (+41 nm) in presence of NaOMe indicating the presence of a free hydroxyl group at C-4' while the bathochromic shift in band I (+50 nm) of the $AlCl_3$complex that not affected by conc. HCl (+49) indicating the presence of a free hydroxyl group at C-5 andabsence of *O*-dihydroxy groups. This is confirmed by the absence of bathochromic shift in band I upon the addition of $NaOAc/H_3BO_3$ mixture. The absence of bathochromic shift in band II after addition of NaOAc indicated that hydroxyl group at C-7 is blocked or absent. The ^{1}H-NMR spectraldata (experimental section) confirmed the flavonoid skeleton from the signals at δ 6.48 and 6.78 (each l H, *d, J* = 2.0 Hz) assignable to*meta*-coupled protons H-6 and H-8 of ring A, respectively; which were at rather lower field than those of kaempferol indicating blocked OH group at C-7[22]; also, signals at δ 6.90 and 8.12 (each 2H, *d, J* = 8.8 Hz) indicatedthe presence of *p*-disubstituted benzene ring. These data indicated the aglycone moiety as kaempferol[22&23]. The ^{1}H-NMR data also showed three anomeric proton at δ 5.10 (1H, *d, J* = 7.6 Hz), δ 4.52 (1H, *d, J* = 1.6 Hz) and δ 4.50 (1H, *d, J* = 1.6 Hz) which in addition to the signals at δ 1.26 (3H, *d, J* = 6.0 Hz) and δ 1.18 (3H, *d, J* = 6.4 Hz) suggesting the presence of one glucose and two rhamnose moieties, respectively in β-configuration of glucose from the large coupling constant and α-configuration of the rhamnose moieties[24]. A low field broad singlet at δ 12.39 representedthe chelated 5-OH group of flavonoid nucleus[25]. The ^{13}C-NMR spectral data (Table 4) displayed 31 carbon signals equivalent to 33 carbons. Thirteen signals equivalent to fifteen carbon atoms were assigned for the kaempferol aglycone while the remaining eighteen signals were assigned for the three sugars most probably the glucose at δ_C 100.65 for the anomeric carbon and two rhamnose at δ_C 101.75, 101.14 with their C-6 methyls at δ_C 17.95 and 18.10. The carbon signal at δ 158.01which assigned to the C-2 of the aglycone was shifted to lower field comparing with kaempferol by ca. 8 ppm indicating glycosylated C-3 hydroxyl group. The attachment of sugars was deduced as follow: one rhamnose moiety was attached to C-6 of the glucose indicated from the downfield shift of C-6 of glucose comparing with free glucose moiety while the other rhamnose unit in different position from the normal chemical shift values of rutinose signals. The presence of the rutinose moiety at

position-7 was deduced from the upfield shift of anomeric proton of the glucose moiety comparing with C-3 substitution[26] and the presence of rhamnose moiety at position-3 was deduced from the downfield shift of the anomeric carbon comparing with C-7 substitution[23&27]. From the above mentioned data, compound **9** is identified as kaempferol 3-*O*-rhamnoside 7-*O*-rutinoside

Compound (12)

The ^{1}H- and ^{13}C-NMR spectral data (experimental section and Table 1) showed the presence of aromatic compound with an ABX system similar to compound **6** except for the presence of additional singlet signal at δ_H 3.80 (3H) with δ_C 54.97 (q) indicating the presence of aromatic methoxy group. The ^{13}C-NMR spectral data (Table 1) also showed a signal for a carbonyl function at δ 168.69. From the previous data and the comparison with the reported ones[28], compound **12** was identified as vanillic acid.

Biological studies

- The LD_{50} results (Table 5) revealed that the total extracts of the leaves and stem-bark are safe enough to be used.

Table 5: The LD_{50} of the total ethanolic extracts of the leaves andstem-bark.

Extract	LD_{50} (g/kg)
Total extract of the leaves	3.8
Total extract of the stem-bark	3.8

- The different fractions of the leaves and stem-bark had inhibiting effects on several test organisms, especially Gram-negative bacteria and *Candida albicans* (Table 6). Concerning Gram-positive bacteria; *Bacillus cereus* was sensitive to all the fractions with the exception ofthe *n*-butanol fraction of the leaf. The most active leaf fraction was the $CHCl_3$, while the highly active stem-bark fractions were *n*-hexane, $CHCl_3$ and EtOAc.

Table 6: Inhibition zone diameter (IZD) and MICs (given in brackets) of the total ethanolic extracts and different fractions of the leaves and stem-bark[a,b].

Group	Dose	Inhibition zone diameter (mm/sample)						
		(G^+) bacteria			(G^-) bacteria			fungus
		BC	ML	SA	KP	EC	PA	CA
Positive control								
Gentamicin	5	14 (0.15)	12 (2.50)	10 (1.00)	12 (0.25)	12 (0.30)	10 (0.15)	-
Clotrimazole	5	-	-	-	-	-	-	14 (4.00)
Leaves								
Total eth. Ext.	100	8 (50)	7 (55)	4 (40)	17 (15)	10 (50)	11 (55)	10 (20)
n-Hexane Fr.	100	8 (6.25)	7 (75)	6 (37)	12 (25)	9 (60)	-	9 (35)
$CHCl_3$ Fr.	100	11 (35)	-	6 (25)	15 (20)	9 (25)	10 (50)	20 (14)
EtOAc Fr.	100	9 (65)	-	4 (40)	18 (10)	15 (40)	12 (60)	20 (13)
n-Butanol Fr.	100	-	-	5 (36)	14 (25)	10 (75)	-	9 (30)
Aqueous Fr.	100	8 (75)	-	6 (40)	13 (30)	10 (15)	-	14 (18)
Stem-bark								
Total eth. Ext.	100	8 (75)	-	6 (40)	12 (22)	-	-	22 (15)
n-Hexane Fr.	100	10 (65)	-	9 (35)	12 (24)	-	-	14 (30)
$CHCl_3$ Fr.	100	12 (55)	8 (50)	10 (33)	13 (24)	10 (20)	9 (25)	21 (18)
EtOAc Fr.	100	11 (50)	8 (25)	14 (20)	15 (20)	10 (25)	11 (25)	18 (18)
Aqueous Fr.	100	9 (75)	8 (75)	-	9 (35)	11 (35)	10 (25)	14 (55)

- = no effect.

[a] Abbreviations of the test organisms: BC, *Bacillus cereus*; CA, *Candida albicans*; EC, *Escherichia coli*; KP, *Klebsiella pneumonia*; ML, *Micrococcus luteus*; PA, *Pseudomonas aeruginosa*; SA, *Staphylococcus aureus*.

[b] Dose of positive control and their MICs given in µg/ml, while dose of different extracts and fractions and their MICs given in mg/ml.

Micrococcus lu teus was insensitive to most fractions except total ethanolic extract and *n*-hexaneof the leaves and the $CHCl_3$, EtOAc and the aqueous of the stem-barkwhich showed low activity. *Staphylococcus aureus* was sensitive to all the fractions with the exception of aqueous fraction of the stem-bark. The highly active fractions were the $CHCl_3$ and EtOAc fractions of the stem-bark. Concerning Gram-negative bacteria; *Klebsiella pneumonia* was sensitive to all fractions. The highly active leaf fractions were the EtOAc, the total ethanolic extract and $CHCl_3$, while the highly active stem-bark fractions were the $CHCl_3$ and EtOAc. Ethyl acetate of the leaf also was the highly active fraction against *Escherichia coli* while the total ethanolic extract and *n*-hexane fraction of the stem-bark were ineffective. Other fractions have varying activity. *Pseudomonas aeruginosa*was insensitive to *n*-hexane, *n*-butanol and aqueous fractions of the leaf in addition to the total ethanolic extract and *n*-hexane fraction of the stem-bark. Other fractions give a moderate activity. All extracts of both leaves and stem-barkinhibited the growth of *Candida albicans.* The highly active leaf fractions werethe $CHCl_3$ and EtOAc, while the highly active stem-bark fractions were the total ethanolic extract, $CHCl_3$ and EtOAc. The antibacterial and antifungal activities of the chloroform and ethyl acetate fractions of the leaves may be attributed to coumarins and other phenolic compounds[29].

- Potent anti-inflammatory activity compared with indomethacin (Tables 7, 8) was observed with the $CHCl_3$ and EtOAc fractions of the leaves with maximum effect being obtained after 2 hrs. and continues for 4 hrs. The aqueous fraction of the leaves and stem-bark, $CHCl_3$ and EtOAc of the stem-barkshowed maximum effect after 2 hrs. Other fractions showed varying effects while total ethanolic extract and *n*-butanol of the leaves showed non-significant effect. The activity of the $CHCl_3$ and EtOAc fractions may be attributed to scopoletin which has anti-inflammatory activity[30]. Esculetin and scopoletin showed marked activity as inhibitors of eicosanoid-release from ionophore-stimulated mouse peritoneal macrophages[31] in addition, scopoletin is a specific inhibitor of the production of inflammatory cytokines in mast cells which explain its beneficial effect in the treatment of chronic inflammatory diseases[30]. The anti-inflammatory activity of leaf *n*-hexane and stem-bark chloroform fractions may be also attributed to the presence of the sterols[32-34].
- The total ethanolic extracts and all fractions of both leaves and stem-bark have no antipyretic activity (Table 9).

Table 7: The anti-inflammatory activity of the total ethanolic extracts and different fractions of the leaves and stem-bark on carrageenan induced edema in rats.

Group	Dose mg/kg	Thickness of the right paw (mm) after injection(Mean ±S.E.), n= 5				
		1 hr	2 hr	3 hr	4 hr	5 hr
Control	-	8.30±0.054	8.20±0.054	8.30±0.068	8.43±0.063	8.45±0.041
Indomethacin	8	5.30±0.070***	4.80±0.080***	4.30±0.060***	4.30±0.030***	4.20±0.060***
Leaves						
Total eth. ext.	400	7.38±0.043***	7.94±0.044**	8.10±0.128	7.96±0.064***	7.50±0.028***
n-Hexane Fr.	400	7.26±0.050***	7.69±0.022***	6.08±0.059***	6.25±0.095***	7.71±0.100***
$CHCl_3$ Fr.	400	6.36±0.043***	5.49±0.031***	5.31±0.085***	5.63±0.024***	8.30±0.091
EtOAc Fr.	400	5.66±0.043***	5.33±0.063***	5.15±0.067***	5.80±0.054***	7.86±0.070***
n-Butanol Fr.	400	7.32±0.040***	7.56±0.017***	7.48±0.033***	7.49±0.028***	8.16±0.064**
Aqueous Fr.	400	6.46±0.057***	5.78±0.050***	7.15±0.028***	7.25±0.035***	7.56±0.017***
Stem-bark						
Total eth. ext.	400	6.60±0.026***	6.30±0.064***	7.30±0.054***	8.25±0.050*	8.40±0.050
n-Hexane Fr.	400	6.38±0.024***	6.00±0.061***	6.02±0.058***	8.40±0.076	8.70±0.030
$CHCl_3$ Fr.	400	5.96±0.078***	5.70±0.090***	7.34±0.041	8.62±0.026	8.90±0.020
EtOAc Fr.	400	6.38±0.062***	5.59±0.103***	7.47±0.035	8.14±0.050*	8.27±0.055*
Aqueous Fr.	400	5.54±0.036***	5.42±0.016***	7.20±0.054**	8.50±0.010	8.56±0.070

S.E. = standard error n = Number of animals

Differences with respect to the control group were evaluated using the student's t-test (*p< 0.05, **p< 0.01, *** p< 0.001)

Table 8: The inhibitory effect of the total ethanolic extracts and different fractions of the leaves and stem-bark on carrageenan induced edema in rats.

Group	Dose mg/kg	% of inhibition				
		1 hr	2 hr	3 hr	4 hr	5 hr
Control	---	---	---	---	---	---
Indomethacin	8	27.40	35.10	42.60	46.20	49.30
Leaves						
Total eth. ext.	400	12.04	3.17	2.40	5.57	11.24
n-Hexane Fr.	400	12.53	6.21	26.74	25.86	8.87
$CHCl_3$ Fr.	400	23.37	33.04	36.02	33.21	12.04
EtOAc Fr.	400	31.80	35.00	37.95	31.19	6.98
n-Butanol Fr.	400	11.80	07.80	09.87	11.15	3.43
Aqueous Fr.	400	22.16	29.51	13.85	13.99	10.53
Stem-bark						
Total eth. ext.	400	20.48	23.17	12.04	2.13	5.00
n-Hexane Fr.	400	23.13	26.82	27.46	0.35	---
$CHCl_3$ Fr.	400	28.19	30.48	11.56	---	---
EtOAc Fr.	400	23.13	31.82	10.00	3.44	2.10
Aqueous Fr.	400	33.25	33.90	13.25	---	---

Table 9: The antipyretic activity of the total ethanolic extracts and the different fractions of the leaves and stem-bark on yeast induced pyrexia in rats.

Group	Dose mg/kg	Average rectal temperature (°C) ± S.E., n = 5			
		1 hr	2 hr	3 hr	4 hr
Control	---	39.90±0.014	39.90±0.020	39.64±0.080	39.98±0.141
Indomethacin	8	36.62±0.132***	35.71±0.150***	35.84±0.091***	35.62±0.070***
Leaves					
Total eth. ext.	400	39.80±0.038*	39.85±0.040	39.92±0.051	40.24±0.046
n-Hexane Fr.	400	39.94±0.078	39.82±0.036	39.80±0.042	39.78±0.058
$CHCl_3$ Fr.	400	39.96±0.016	39.85±0.084	39.68±0.043	40.56±0.075*
EtOAc Fr.	400	39.98±0.040	39.92±0.075	39.70±0.091	39.80±0.020**
n-Butanol Fr.	400	39.84±0.046	39.86±0.046*	39.74±0.046	40.28±0.051
Aqueous Fr.	400	39.86±0.033	39.90±0.057	39.66±0.046	40.84±0.041
Stem-bark					
Total eth. ext.	400	39.86±0.083	40.18±0.057	39.90±0.020	41.15±0.026
n-Hexane Fr.	400	40.30±0.089	40.00±0.088	40.42±0.043	41.22±0.029
$CHCl_3$ Fr.	400	39.90±0.046	39.96±0.026	39.76±0.036	40.38±0.110
EtOAc Fr.	400	39.90±0.024	40.66±0.041	39.86±0.022	40.54±0.076
Aqueous Fr.	400	39.84±0.050	39.96±0.017	40.14±0.083	40.12±0.055

S.E. = standard error
Differences with respect to the control group were evaluated using the students t-test (*$p<0.05$,**$p<0.01$).

- The total ethanolic extracts as well as the other fractions of both leaves and stem-bark attained a central nervous system depressant activity (Table 10). The highly active leaf fractions were *n*-butanol, *n*-hexane and $CHCl_3$ while the highly active stem-bark fractions were aqueous, total ethanolic extract and *n*-hexane.

- The *n*-hexane and *n*-butanol fractions of the leaves; $CHCl_3$, *n*-hexane fractions of the stem-bark (400 mg/kg) have a potent anticonvulsant activity against PTZ induced convulsion comparing with carbamazepine while EtOAc, $CHCl_3$ and aqueous fractions of the leaves showed a weak activity (Table 11).

Table 10: The effect of the total ethanolic extracts and different fractions of the leaves and stem-bark on motor co-ordination of the mice.

Group	Dose mg/kg	Time on the rod (sec.) (mean± S.E.), n=5	
		0.5 hr	1 hr
Control	---	94.20±1.090	105.40±1.188
Leaves			
Total eth. ext.	400	80.20±0.712***	87.20±0.384***
n-Hexane Fr.	400	48.40±0.220***	36.80±0.900***
$CHCl_3$ Fr.	400	48.60±0.228***	57.80±0.384***
EtOAc Fr.	400	56.40±0.230***	63.80±0.517***
n-Butanol Fr.	400	45.40±0.819***	21.00±0.316***
Aqueous Fr.	400	50.40±0.540***	73.80±0.328***
Stem-bark			
Total eth. ext.	400	41.00±1.250***	51.40±0.756***
n-Hexane Fr.	400	42.20±0.450***	45.00±1.440***
$CHCl_3$ Fr.	400	62.00±0.460***	58.20±0.620***
EtOAc Fr.	400	104.60±0.641	68.40±0.414***
Aqueous Fr.	400	37.80±0.497***	20.60±0.670***

S.E. = standard error n= Number of animals

Differences with respect to the control group were evaluated using the students T- test (***p<0.001).

Table 11: The effect of the total ethanolic extracts and different fractions of the leaves and stem-bark on pentylene tetrazole induced convulsion and death.

Group	Dose mg/kg	Time of clonic convulsion (min) (mean± S.E), n=5	Time of tonic convulsion (min) (mean± S.E), n=5	Time until death (min)(mean± S.E), n=5
Control	------	0.56±0.009	0.63±0.005	0.92±0.009
Standard	100 mg	3.50±0.175**	10.32±0.312**	14.21±0.212**
Leaves				
Total eth. Ext.	400 mg	1.04±0.007***	1.21±0.010***	1.41±0.009***
n-Hexane Fr.	400 mg	7.49±0.021***	14.06±0.014***	17.32±0.199***
$CHCl_3$ Fr.	400 mg	1.12±0.009***	1.42±0.008***	2.74±0.007***
EtOAc Fr.	400 mg	1.94±0.004***	2.11±0.005***	2.68±0.010***
n-Butanol Fr	400 mg	3.22±0.015***	5.50±0.022***	5.82±0.013***
Aqueous Fr.	400 mg	1.82±0.018***	2.44±0.004***	2.95±0.015***
Stem-bark				
Total eth. Ext.	400 mg	0.77±0.004***	1.21±0.006***	1.39±0.040***
n-Hexane Fr.	400 mg	2.95±0.007***	5.06±0.010***	5.51±0.010***
$CHCl_3$ Fr.	400 mg	5.07±0.010***	14.58±0.061***	16.72±0.185***
EtOAc Fr.	400 mg	0.45±0.009	0.71±0.006***	0.82±0.004
Aqueous Fr.	400 mg	0.88±0.006***	1.04±0.006***	1.42±0.011***

S.E. = standard error n= Number of animals

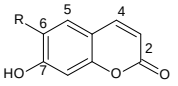
Differences with respect to the control group were evaluated using the students t- test(***p<0.001).

Fatty acids analysis

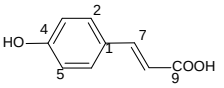
- GC/MS analysis of the fatty acids methyl esters showed the presence of thirty fatty acids (Table 12). The saturated and unsaturated fatty acids represent 69.10% and 28.36% respectively. The main components were methyl palmitate (44.70%), 9, 12, 15-octadecatrienoic acid methyl ester (12.11%), methyl stearate (10.54%), 8, 11-octadecadienoic acid, methyl ester (8.88%) and eicosanoic acid, methyl ester (6.00%).

Table 12: Results of GLC analysis of fatty acids methyl esters of the leaves.

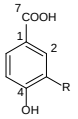
Peak No.	Fatty acids methyl esters	Base peak (*m/z*)	Rt/min	Relative area %
1	Dodecanoic acid, methyl ester	74.00	7.869	0.18%
2	Nonanedioic acid, monomethyl ester	44.00	8.149	0.10%
3	Methyl myristate	74.10	10.139	3.09%
4	Unknown	44.00	10.294	0.0001%
5	Unknown	88.10	10.895	0.06%
6	Pentadecanoic acid, methyl ester	74.10	11.227	0.09%
7	9-Hexadecenoic acid, methyl ester	55.00	12.046	0.37%
8	9-Dodecenoic acid, methyl ester	55.10	12.191	0.20%
9	Methyl palmitate	74.10	12.253	44.70%
10	Unknown	88.10	12.916	0.97%
11	Methyl margarate	74.10	13.237	1.07%
12	4,4- dimethylpimelate	44.00	13.548	0.08%
13	8,11-Octadecadienoic acid, methyl ester	67.10	13.890	8.88%
14	9,12,15-Octadecatrienoic acid, methyl ester	79.00	13.942	12.11%
15	11-Octadecenoic acid, methyl ester	55.10	14.004	0.09%
16	Methyl stearate	74.10	14.181	10.54%
17	Linoleic acid methyl ester	67.10	14.502	0.30%
18	Linolenic acid methyl ester	79.10	14.554	0.41%
19	Nonadecanoic acid, methyl ester	74.10	15.092	0.62%
20	Pentadecyl cyclohexanecarboxylate	129.10	15.144	2.22%
21	Unknown	55.10	15.756	0.32%
22	Eicosanoic acid, methyl ester	74.10	15.963	6.00%
23	Unknown	99.10	16.160	1.07%
24	Heneicosanoic acid, methyl ester	74.10	16.792	0.87%
25	Unknown	44.00	17.020	0.05%
26	Docosanoic acid, methyl ester	74.10	17.600	2.73%
27	Tricosanoic acid, methyl ester	74.10	18.388	0.97%
28	Tetracosanoic acid, methyl ester	74.00	19.269	1.60%
29	Pentacosanoic acid, methyl ester	74.10	20.295	0.24%
30	Unknown	44.00	21.528	0.07%



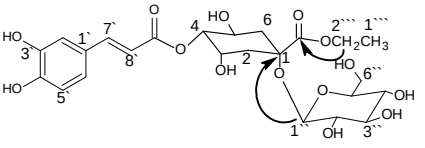
4 R= OCH_3
7 R= OH



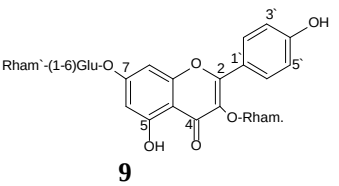
5



6 R= OH
12 R= OCH_3



8 HMBC



9

The structures of the isolated compounds

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