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PHYTOCHEMICAL AND BIOLOGICAL STUDIES OF *EMEX SPINOSA* (L.) CAMPD. GROWING IN EGYPT

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تم فصل ثلاثة عشر مركبا هي : الفا اميرين وبيتا سيتوستيرول وكريزوفانول وفسيون والوامودين و٦ ١٠ بالميتويل ٣ ١ بيتا سيتوستيرول جلوكوزيد وبيتا سيتوستيرول ٣ ١ بيتا جلوكوزيد والوامودين ٨ ١٠ بيتا جلوكوزيد وامودين ٨ ١ بيتا جلوكوزيد وتوراكريزون ٨ ١ بيتا جلوكوزيد وكافويل ٩ ١ بيتا جلوكوزيد وكامبيفرول ٣ ١ روتينوزايد وكورسيتين ٣ ١ روتينوزايد (روتين) من الاجزاء الهوائية لنبات ايمكس سبينوزا (لينيه) كمبد النامي في مصر وذلك باستخدام طرق الكروماتوجرافيا المختلفة. وقد تم التعرف علي المركبات بواسطة الطرق الطيفية المختلفة. تم تعيين الجرعات القاتلة لنصف عدد الفئران لخلاصات النبات المختلفة كما اجريت دراسة بيولوجية لخلاصات النبات المختلفة شملت تاثير الخلاصات علي البكتيريا والفطريات ومدي فاعليتها كمضاد للالتهابات ومسكن للالم ومخفض للحرارة وقد ثبت ان خلاصة خلات الايثيل هي الاكثر فاعلية كمضاد للبكتيريا. أما خلاصات خلات الايثيل والكلوروفورم فكان لها تاثير قوى كمضادات للالتهابات وخلاصة كل من خلات الايثيل والكحول الكلية فقد اثبتا فاعلية كمسكنين علاوة على انهما افضل الخلاصات تاثيرا كمخفض للحرارة.

Thirteen compounds; α-amyrin, β-*sitosterol, chrysophanol, physcion, aloe-emodin, 6´-O-palmitoyl-3-O-β-sitosterol glucoside,* β-*sitosterol-3-O-*β*-glucoside, aloe-emodin-8-O-*β*-glucoside, emodin-8-O-*β*-glucoside, torachryson-8-O-*β*-glucoside, caffoeyl-9-O-β-glucoside, kaempferol-3-O-rutinoside and quercetin-3-O-rutinoside (rutin) were isolated from the aerial parts of Emex spinosa (L.) Campd. growing in Egypt. Their structures were elucidated using different spectral techniques. The LD_{50} of various plant extracts were determined. Preliminary biological studies of different extracts revealed that the ethyl acetate extract was the most effective as antibacterial, ethyl acetate and chloroform extracts gave potent anti-inflammatory effects; whereas both ethyl*

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acetate and total alcohol extracts exhibited analgesic activities. The best extracts that gave antipyretic activity were ethyl acetate and total alcohol extracts.

INTRODUCTION

The genus Emex (F. Polygonaceae) comprises two species growing in Egypt viz.; *E. australis* Steinth. and *Emex spinosa* (L.) Campd. [syn. *Rumex spinosus* (L.) Campd].[1-3] *Emex spinosa* (L.) Campd is a common annual glabrous herb growing in sandy places around Alexandria, Mandara, Abukir, Siwa, Great Oasis, Pyramids of Giza, Basatin, Gebelahmar and Qubba. The plant is known locally as Figl-El-Gabl, Hanzab, Rukbet-El-Agus, Dirs-El-Agus or Hommeyd.[3] It is used in folk medicine as purgative, diuretic, a remedy for stomach disorders, dyspepsia, colic and the young leaf has been used as a spinach.[4&5] A variety of chemical constituents as flavonoids, anthraquinones, naphthoquinones, triterpenoids and sterols have been reported in the genus Emex.[1,2,4,6&7] Five anthraquinones and mixture of sterols were previously isolated and identified from the roots of *Emex spinosa* (L.) Campd. growing in Egypt.[6] It was, thus deemed of interest to continue the investigation of the aerial parts, never studied before for its phytoconstituents and biological activities.

EXPERIMENTAL

General Procedures

Electrothermal 9100 digital melting point instrument was used for determination of m.ps. JASCO Uvidec Model 320 spectrophotometer and Ultrospec 1000, UV/visible Spectrometer, Pharmacia Biotech, Cambridge for UV. Shimadzu infra red-470 spectrophotometer for recording infra red spectra. JEOL JMS 600 Hz. and JEOL GC mat Ionization Mode for EI-MS. JEOL TNM-LA 400 MHz FT NMR, Brüker FT 400 MHz and JEOL JNM ECA 500 MHz NMR Spectrometers for determination of ^{1}H-, ^{13}C-NMR using TMS as internal standard. Moderate pressure liquid chromatography (MPLC); CIG column system (22 mm i.d. x 30 cm, Kusano Scientific Co., Tokyo) was used for final purification. Silica gel (70-230 mesh) (Merck) and RP-18 silica, for c.c. Precoated silica gel $G_{60}F_{254}$ for TLC (Merck). Sheets of Whatman No. 1 filter paper (Whatman, Ltd., England) for paper chromatography. The following solvent systems were used:

I- n-Hexane - Ethyl acetate (9:1v/v)
II- n-Hexane - Ethyl acetate (8:2 v/v)
III- Chloroform- Methanol (9.5:0.5 v/v)
IV- Chloroform - Methanol (9:1 v/v)
V- Chloroform - Methanol (8.5:1.5 v/v)
VI-Ethyl acetate-Methanol (8.5:1.5 v/v)

VII- Chloroform – Methanol - Water (7.5:2.5:0.3 v/v)
VIII- n-Butanol - Acetic acid - Water (4:1:2 v/v)

Spots were visualized by spraying with 10% v/v H_2SO_4 in MeOH and/or 5% $AlCl_3$ in MeOH. Anthraquinones are visualized with 5% alcoholic potash.

Plant Materials

The aerial parts of *Emex spinosa* (L.) Campd. were collected during flowering stage in the period from September to June 2003 from the fields of Assiut governorate and kindly identified and authenticated by prof. Dr. M. M. M. Zarea, Professor of Taxonomy, Faculty of Science, Assiut University.

Authentic Materials

α-amyrin, β-sitosterol, β-sitosterol-3-O- β-glucoside, palmetic acid, kaempferol, quercetin and rutin were obtained from Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt. Authentic sugars; D-glucose, L-rhamnose, L-arabinose and L-galactose were provided by (El-Nasr Pharmaceutical and Chemical Co., Egypt) (ADWIC).

Extraction and Isolation

The air-dried powdered aerial parts (4.5 kg) of *Emex spinosa* (L.) Campd. were exhaustively extracted with methanol. Part of the concentrated combined methanolic extract (140 g) was successively fractionated with n-hexane, chloroform and ethyl acetate.

The n-hexane soluble fraction (15 g) was chromatographed over a silica gel column using n-hexane-EtOAc gradients (fractions, 100 ml, each, were collected). Fractions eluted with n-hexane-EtOAc (9.5: 0.5) and (9.0: 1.0) afforded compounds **E1** and **E2** (50 mg and 100 mg) identified as α-amyrin and β-sitosterol respectively. Fractions eluted with n-hexane-EtOAc (8.5:1.5) were purified by re-chromatography using $CHCl_3$-MeOH gradients, sub-fractions eluted with $CHCl_3$-MeOH (9.7:0.3), (9.5:0.5) and (9.3:0.7) gave respectively compounds **E3** (60 mg), **E4** (40 mg) and **E5** (50 mg).

The chloroform soluble fraction (10 g) was chromatographed over a silica gel column using gradient $CHCl_3$-MeOH. Fractions eluted with $CHCl_3$-MeOH (9.2: 0.8) and (9.0:1.0) afforded compounds **E6** (150 mg**)** and **E7** (500 mg), compound **E7** was identified as β-sitosterol-3-O-β-glucoside. Chromatography of the ethyl acetate soluble fraction (20 g) over a silica gel column using $CHCl_3$-MeOH (9.0: 1.0) and (8.7:1.3) as elutes yielded 50 and 100 mg of compounds **E8** and **E9**. Fractions eluted with $CHCl_3$-MeOH (8.5:1.5) were subjected to MPLC for final purification using RP-18 pre-packed column and MeOH-H_2O gradient. Sub-fractions eluted with MeOH-H_2O (7.0: 3.0) and (6.5: 3.5) afforded compounds **E10** (60 mg) and **E11** (70 mg); while fractions eluted with $CHCl_3$-MeOH (7.5:2.5) furnished compound **E12** (65 mg). Purification of fractions eluted with $CHCl_3$-

MeOH (7.0:3.0) was achieved by MPLC using RP-18 pre-packed column and MeOH-H_2O (4.0: 6.0) yielding compound **E13** (100 mg), identified as rutin.

Acid and alkaline hydrolysis

Acid (partial and complete) and alkaline hydrolysis of the glycosides were achieved according to the method described in literatures.[8&9]

Biological studies

These studies were performed on the different plant fractions: n-hexane, chloroform and ethyl acetate beside the total alcohl extract.

Experimental animals: Male albino mice (25 – 35g body weight) and male albino rats (wt. = 100 - 120g) were used. The animals were housed under standardized environmental conditions in the Pre-Clinical Animal House, Pharmacology Department, Faculty of Medicine, Assiut University. They were fed with standard diet and free access to tap water and kept under a 12/12 hours light/dark cycle.

Drugs: Indomethacin (El Nile Co., Egypt), Acetyl salicylic acid (Arab drug Co., Egypt) and ampicillin and nystatin discs (Oxoid Co.).

Micro-organisms: *Staphylococcus aureus, Escherichia coli, Klebsiella Pneumonia, Proteus vulgaris, Bacillus subtilis, Pseudomonas aeurginosa, Sarcina lutea, Enterobacter aeur-ginosa* and *Candida albicans* were obtained from Botany Department, Faculty of Science, Al-Azhar University. Assiut.

Characterization of the isolated compounds

Compound E3 (chrysophanol): yellow plates (MeOH), m.p 196-198°, R_f =0.63 (system II). UV (MeOH, nm), λ_{max}; 226, 243, 275, and 433. IR ν_{max} (KBr) cm^{-1}; 3520, 2928, 1667, 1650, 1628, 1613, 1560, 1077, 1053 and 906. EI-MS; m/z 254 $[M]^+$, 237, 226, 197, 152, 115, 92, 77 and 51.

Compound E4 (physcion): orange plates from (MeOH), m.p 201-203°, R_f =0.54 (system II). UV (MeOH, nm) λ_{max}; 223, 254, 286 and 430. IR νmax (KBr) cm-1; 3500, 2900, 1667, 1628, 1613, 1568, 1464, 1150, 1095 and 890. EI-MS; m/z 284 $[M]^+$, 254, 241, 226, 213, 198, 185, 167, 91, 77 and 51.

Compound E5 (aloe-emodin): orange needle crystals (MeOH), m.p 223-224°, R_f =0.48 (system III). UV (MeOH, nm) λ_{max}; 225, 256, 276, 284 and 431. IR ν_{max} (KBr) cm-1; 3430, 2918, 1666, 1620, 1612, 1575, 1077, 1053 and 863. EI-MS; m/z 270 $[M]^+$, 252, 241, 225, 213, 197, 185, 168, 139, 121 and 92.

Compound E6 (6´-O-palmitoyl-3-O-β-sitosterol-glucoside): yellow greasy substance (MeOH), R_f =0.46 (system IV). Part of this compound (40 mg) was acetylated by dissolving in equal volumes of pyridine and acetic anhydride, heated for 1.5 hr at 70°, then treated as usual; the acetate

obtained was crystallized from anhydrous chloroform and named compound **E6a**. The ^{1}H- and ^{13}C-NMR spectral data including DEPT experiment of E6a are listed in Table (2). Positive FAB-MS of **E6a** showed quessi molecular peak at m/z 963 $[M+Na]^{+}$ and other peaks characteristic for sterol moiety.

Compound E8 (aloe-emodin-8-O-β-glucoside): yellow crystals (MeOH), m.p 224-226°. R_f =0.48 (system V). UV (MeOH, nm) λ_{max}; 222, 254 and 409. IR ν_{max} (KBr) cm^{-1}; 3230-3520, 2960, 1655, 1632, 1170, 1081, 1062 and 951. Positive FAB-MS of compound **E8** showed quessi molecular peak at m/z 455 $[M+Na]^{+}$ calculated for $C_{21}H_{20}O_{10}$.

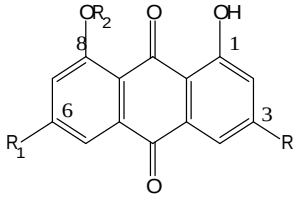
Compound E9: (emodin-8-O-β-glucoside): orange needle crystals (MeOH), m.p 190-192°. R_f =0.35 (system V). UV (MeOH, nm) λ_{max}; 212, 255, 283, 383, 424 and 429 nm. Positive FAB-MS of compound **E9** showed peak at m/z 455 $[M+Na]^{+}$, peak at m/z 433 $[M+1]^{+}$ calculated for $C_{21}H_{20}O_{10}$, other peak at m/z 271 $[(M+1) – sugar]^{+}$.

Compound E10 (torachryson-8-O-β-glucoside): yellowish amorphous powder (MeOH), m. p. 151-153°, R_f =0.43 (system V). UV (MeOH, nm) λ_{max}; 240, 314, 326 and 340. IR ν_{max} (KBr) cm^{-1}; 3420, 2960, 1635, 1190, 1150, 1085 and 980. The EI-MS of compound **E10** showed peak at m/z 246 $[M- glucose]^{+}$ and other peaks at m/z= 231 $[M-(glucose+CH_3)]^{+}$, m/z 203 $[M- (glucose+CO+CH_3)]$, m/z 185 $[M- (glucose+CO+CH_3+H_2O)]$.

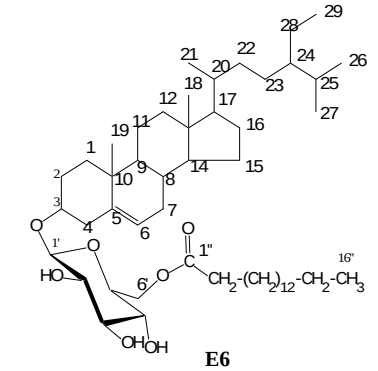
The ^{1}H-NMR spectra of compounds **E5**, **E8**, **E9** and **E10** are listed in Table (1), while the 13C-NMR spectra of compounds **E8**-**E10** are listed in Table (3).

Compound E11 (caffoeyl-9-O-β-glucoside): yellowish brown amorphous powder, R_f =0.52 (system VI). UV (MeOH, nm) λ_{max} 211, 247 and 333. IR ν_{max} (KBr) cm^{-1}; 3450, 2950, 1630, 1210, 1153, 1052 and 963. The ^{1}H- and ^{13}C-NMR spectral data including DEPT experiment (DMSO-d_6, 100 MHz) are listed in Table (4). Positive FAB-MS of compound **E11** showed peak $[M+Na]^{+}$ at m/z 365, and other peak at m/z 163 $[caffeoyl\ moiety]^{+}$.

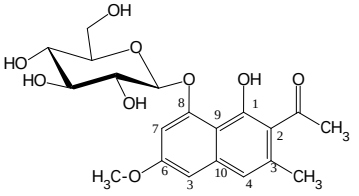
Compound E12 (kaempferol-3-O-rutinoside): yellow amorphous powder (MeOH), m.p 180–182°, R_f = 0.40 (system VII). IR ν_{max} (KBr) cm^{-1}; 3450, 2950, 1630, 1170, 1149, 1052 and 954. The UV spectral data of **E12** and **E13** in methanol as well as with different ionizing and complexing reagents are presented in Table (5) and their ^{1}H- and ^{13}C-NMR are listed in Tables (6 and 7). The positive FAB-MS of **E12** showed quessi molecular peak at m/z 617 $[M + Na]^{+}$ and other peak at m/z 287 $[(M+1)-(glucose+rhamnose)]^{+}$.



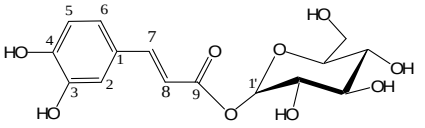
	E3	E4	E5	E8	E9
R	CH_3	CH_3	CH_2OH	CH_2OH	CH_3
R1	H	OCH_3	H	H	OH
R2	H	H	H	glc.	glc.



E6

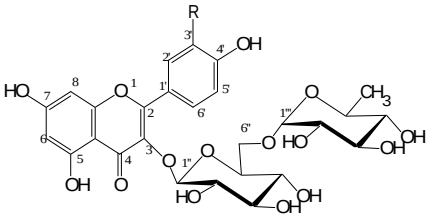


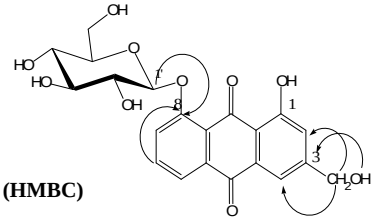
E10



E11

	E12	E13
R	H	OH





E8 (HMBC)

Fig. 1: Compounds isolated from *Emex spinosa* (L.) Campd. aerial parts.

Table 1: ^{1}H-NMR Spectral Data of Compounds **E5** & **E8** (DMSO-d_6, 400 MHz and **E9** & **E10** (DMSO-d_6, 500MHz).

Compound E5 *		Compound E8 *		Compound E9 **		Compound E10 **		Assignment
Chemical shift (δ) ppm, No of protons (Multiplicity)	J (Hz)	Chemical shift (δ ppm) No of protons (Multiplicity)	J (Hz)	Chemical shift (δ) ppm, No of protons (Multiplicity)	J (Hz)	Chemical shift (δ) ppm, No of protons (Multiplicity)	J (Hz)	
12.10, 1H (s)	-	12.87, 1H (s)	-	13.3, 1H (s)	-	9.45, 1H (s)	-	1-OH
12.09, 1H (s)	-	-	-	-	-	-	-	8-OH
7.81, 1H (d)	1.6	7.28, 1H (d)	7.6, 1.7	7.19, 1H (br.s)	-	-	-	H-2
7.36, 1H (d)	1.6	7.66, 1H (d)	7.6, 8.0	7.16, 1H (br.s)	-	7.05, 1H (br.s)	-	H-4
7.86, 1H (dd)	8.0, 1.2	7.89, 1H (dd)	8.0, 1.7	7.09, 1H (br.s)	-	6.95, 1H (br.s)	-	H-5
7.71, 1H (t)	8.0	7.86, 1H (dd)	1.6	6.86, 1H (br.s)	-	-	-	H-6
7.32, 1H (dd)	8.0, 1.2	7.71, 1H (dd)	1.6	-	-	6.87, 1H (br.s)	-	H-7
4.84, 2H (d)	5.2	4.62, 2H (d)	5.6	-	-	-	-	$C\underline{H}_2$-OH
-	-	-	-	2.37, 3H (s)	--	-	-	CH_3
-	-	-	-	-	-	3.79, 3H (s)	-	$OC\underline{H}_3$
-	-	-	-	-	-	2.47, 3H (s)	-	CO-$C\underline{H}_3$
-	-	-	-	-	-	2.18, 3H (s)	-	Ar-$C\underline{H}_3$
-	-	5.18, 1H (d)	7.6	4.98, 1H (d)	7.6	5.02, 1H (d)	7.1	H-1' (glc.)
-	-	3.23-3.72 (m)	-	-		3.16- 3.73 (m)	-	other sugar protons

*: at 400 MHz **: at 500 MHz

Table 2: ^{1}H- and ^{13}C-NMR Spectral Data of Compound **E6a** ($CDCl_3$, 400 and 125 MHz).

^{1}H-NMR Spectral Data of Compound **E6a** ($CDCl_3$, 400 MHz)				^{13}C-NMR Spectral Data of Compound **E6a** ($CDCl_3$, 125 MHz).			
Chemical shift (δ) ppm	No of protons Multiplicity)	Coupling constant (Hz)	Assignment	Carbon No.	Chemical shift (δ) ppm & Multiplicity	Carbon No.	Chemical shift (δ) ppm & Multiplicity
5.34	1H (br. s)	-	H-6	1	37.3 (t)	24	46.2 (d)
4.57	1H (d)	8.0	H-1' (glc.)	2	29.5 (t)	25	29.3 (d)
3.46	1H (m)	-	H-3α	3	80.2 (d)	26	19.1 (q)
2.03, 2.0, 1.98	each 3H (s)	-	3 X $C\underline{H}_3$-CO	4	39.1 (t)	27	19.9 (q)
1.58-1.23	(m)	-	C$\underline{H}$ and $C\underline{H}_2$ protons	5	140.4 (s)	28	23.2 (t)
0.96	3H (s)	-	Me-19	6	122.3 (d)	29	12.1 (q)
0.90	3H (d)	6.4	Me-21	7	32.0 (t)	2X $\underline{C}O$-CH_3	169.5 (s)
0.84	3H (d)	7.0	Me-26	8	32.2 (d)	$\underline{C}O$-CH_3	170.7 (s)
0.80	6H (m)	-	Me-29, 16"	9	50.3 (d)	2X CO-$C\underline{H}_3$	20.8 (q)
0.78	3H (d)	9.0	Me-27	10	36.8 (s)	CO-$C\underline{H}_3$	20.9 (q)
0.65	3H (s)	-	Me-18	11	21.2 (t)	**Glucose**	
				12	39.8 (t)	1'	99.8 (d)
				13	42.5 (s)	2'	71.7 (d)
				14	56.9 (d)	3'	73.1 (d)
				15	24.4 (t)	4'	68.8 (d)
				16	28.4t (t)	5'	71.9 (d)
				17	56.2 (d)	6'	62.2 (t)
				18	12.0 (q)	Fatty acid	
				19	19.5 (q)	C-1"	173.8 (s)
				20	36.3 (d)	C-2"	34.2 (t)
				21	18.9 (q)	C-3"- C-15"	22.8-29.8 (t)
				22	34.0 (t)	C-16"	14.2 (q)
				23	26.2 (t)		

Table 3: ^{13}C-NMR Spectral Data of Compounds **E8** (DMSO-d_6, 100 MHz) and **E9** & **E10** (DMSO-d_6, 125 MHz).

Carbon No.	Compound E8		Compound E9		Compound E10	
	Chemical shift (δ) ppm	Multiplicity	Chemical shift (δ) ppm	Multiplicity	Chemical shift (δ) ppm	Multiplicity
1	161.62	s	161.2	s	151.47	s
2	120.73	d	119.8	d	123.75	s
3	152.26	s	147.3	s	137.37	s
4	116.01	d	124.7	d	119.37	d
5	120.60	d	110.3	d	103.17	d
6	135.96	d	166.3	s	158.81	s
7	122.45	d	109.2	d	103.60	d
8	158.23	s	161.8	s	155.86	s
9	187.58	s	186.6	s	109.09	s
10	182.10	s	182.9	s	134.23	s
11	134.81	s	136.9	s	-	-
12	120.60	s	114.4	s	-	-
13	115.47	s	112.8	s	-	-
14	132.26	s	132.5	s	-	-
$\underline{C}H_2$-OH	62.05	t	-	-	-	-
CH_3	-	-	21.9	q	-	-
$\underline{C}O$-CH_3	-	-	-	-	205.36	s
Ar-$\underline{C}H_3$	-	-	-	-	19.97	t
CO-$\underline{C}H_3$	-	-	-	-	32.90	t
O$\underline{C}H_3$	-	-	-	-	55.86	t
Glucose						
1'	100.49	d	101.4	d	101.70	d
2'	73.29	d	73.7	d	73.88	d
3'	77.25	d	76.0	d	76.71	d
4'	69.52	d	70.1	d	70.40	d
5'	76.52	d	77.7	d	78.23	d
6'	60.61	t	61.1	t	61.23	t

Table 4: ^{1}H-NMR (DMSO-d_6, 400 MHz) and ^{13}C-NMR Spectral Data of Compound **E11** (DMSO-d_6, 100 MHz).

^{1}H-NMR Spectral Data of Compound **E11** (DMSO-d_6, 400 MHz)				^{13}C-NMR Spectral Data of Compound **E11** (DMSO-d_6, 100 MHz).					
Chemical shift (δ) ppm,	No of protons (Multiplicity)	Coupling constant (Hz)	Assignment	Carbon No.	Chemical shift (δ) ppm	Multiplicity	Carbon No.	Chemical shift (δ) ppm	Multiplicity
7.58	1H (d)	15.8	H-7	1	125.40	s	Glucose.		
7.08	1H (d)	2.0	H-2	2	114.86	d	1'	94.29	d
7.04	1H (dd)	8.2, 2.0	H-6	3	145.69	s	2'	72.55	d
6.79	1H (d)	8.2	H-5	4	148.83	s	3'	77.86	d
6.29	1H (d)	15.8	H-8	5	115.85	d	4'	69.56	d
5.46	1H (d)	7.9	H-1' (glc.)	6	121.80	d	5'	76.50	d
3.1-3.68	(m)	-	Other sugar protons	7	146.49	d	6'	60.64	t
				8	113.38	d			
				9	165.42	s			

Table 5: UV Spectral Data of Compounds **E12** and **E13** with Different Ionizing and Complexing Reagents.

Comp.	Band	**λ_{max} and $\Delta\lambda_{max}$ nm**										
		MeOH	NaOMe		$AlCl_3$		$AlCl_3$/HCl		NaOAc		NaOAc/H_3BO_3	
		λ_{max}	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$	λ_{max}	$\Delta\lambda$
E12	II	257	282	+25	267	+10	265	+8	272	+15	262	+5
	I	347	400	+53	397	+50	395	+48	372	+25	353	+6
E13	II	255	287	+32	275	+20	265	+10	270	+15	266	+8
	I	357	420	+62	423	+66	400	+43	381	+24	372	+15

Table 6: ^{1}H-NMR Spectral Data of Compounds **E12** (DMSO-d_6, 400 MHz) and **E13** (DMSO-d_6, 300 MHz).

Assignment	**E-12**		**E-13**	
	(δ) ppm (No. of proton, multiplicity)	J (Hz)	(δ) ppm (No. of proton, multiplicity)	J (Hz)
5-OH	12.56, (1H s)	-	12.57, (1H s)	-
H-6	6.21, (1H d)	2.0	6.18, (1H d)	1.8
H-8	6.42, (1H d)	2.0	6.37, (1H d)	1.8
H-2'	7.99, (1H d)	8.9	7.54, (1H d)	2.1
H-3'	6.89, (1H d)	8.9	-	-
H-5'	6.89, (1H d)	8.9	6.84, (1H d)	9.3
H-6'	7.99, (1H d)	8.9	7.51, (1H dd)	2.1, 9.3
H-1" (glc.)	5.31, (1H d)	7.5	5.33, (1H d)	7.0
H1'"(rham.)	4.45, (1H br. s)	-	4.37, (1H br. s)	-
Other sugar protons	3.06-3.70 (m)	-	3.2-3.75 (m)	-
CH_3, rham.	0.99, (3H d)	6.2	0.98, (3H d)	6.0

Table 7: ^{13}C-NMR Spectral Data of Compound **E12** (DMSO-d_6, 100 MHz).

Carbon No.	Chemical shift (δ) ppm	Multiplicity	Carbon No.	Chemical shift (δ) ppm	Multiplicity
2	156.58	s	glucose		
3	133.27	s	1"	101.41	d
4	177.50	s	2"	74.24	d
5	161.24	s	3"	76.42	d
6	98.86	d	4"	69.99	d
7	164.43	s	5"	75.79	d
8	93.87	d	6"	66.96	t
9	156.91	s	rham.		
10	103.97	s	1"'	100.83	d
1'	120.95	s	2"'	70.40	d
2'	130.49	d	3"'	70.66	d
3'	115.18	d	4"'	71.88	d
4'	159.96	s	5"'	68.31	d
5'	115.18	d	6"'	17.77	q
6'	130.94	d			

Biological Studies

Determination of LD_{50}

Weighed amounts of the different extracts (total alcohol, n-hexane, chloroform and ethyl acetate) were dissolved in distilled water with the aid of 2% Tween 80. Subsequent dilutions were made in saline. Control solution was made using 2% of tween (Negative control). The LD_{50} of different extracts of *Emex spinosa* was determined according to the standard method.[10]

Antibacterial and Antifungal activities

Certain weights each of the different plant extracts were dissolved in dimethyl sulphoxide (DMSO) to obtain different concentrations; as 5, 10, 25, 50, 75 and 100 mg/ml. The inocula of the organisms were prepared by growing the cells on nutrient agar at 37° for 24 hr, for bacteria and dextrose broth at 28° for 72hr, for fungi. The test plates were prepared as mentioned in literature[11] and the well technique[12-16] for determination of the minimal inhibitory concentration (MIC).

Anti-inflammatory, Antipyretic and Analgesic activities

Preparation of extracts for administration

Weighed amounts (2, 4 and 8 g each) of n-hexane, chloroform and ethyl acetate fractions and total alcohol extract were solubelized in normal saline with the aid of 2% Tween 80 to obtain concentrations of 20, 40 and 80 mg/ml. Each extract was subjected to preliminary pharmacological screening as anti-inflammatory, analgesic and antipyretic activities.

Both the anti-inflammatory and antipyretic activities were done according to the methods described

in literatures.[14,17-20] The results were listed in Tables 8 & 9. For analgesic activity, the hot-plate test was used.[20-24] The results are listed in Table (10).

Statistical Analysis

Data were analysed using the student's t-test and the values were expressed as mean ± S.E. (n= 5 animals)., *P< 0.05 or less considered as positive result.

Table 8: Results of anti-inflammatory activity and inhibitory effects of the different extract of *E. spinos* on yeast induced edema in rats.

Group	Dose mg/kg	Thickness of the right paw (mm) after injection (mean±S.E.), n=5 and Percentage of inhibition									
		1hr		2hr		3hr		4hr		5hr	
Control	-	7.6±0.11	-	7.9±0.14	-	8.0±0.10	-	8.3±0.16	-	8.5±0.18	-
Indomethacin	8	5.0±0.19**	34.2	4.5±0.17**	43	4.4±0.22**	45.1	4.3±0.10**	46.9	4.1±0.26**	49.4
Total extract	100	6.4±0.11**	15.8	6.0±0.22**	24	6.9±0.26**	13.8	7.6±0.19*	06.1	7.8±0.17	03.7
	200	5.5±0.16**	27.6	5.2±0.18**	34.2	6.4±0.12**	20.0	7.4±0.25**	08.6	7.5±0.18*	07.4
	400	5.2±0.18**	31.6	4.6±0.25**	41.8	5.9±0.21**	26.3	6.8±0.30**	16.0	7.1±0.22**	12.3
n-Hexane fraction	100	6.7±0.20**	11.8	6.3±0.16**	20.2	7.0±0.25*	12.5	7.7±0.15*	04.9	7.8±0.32	03.7
	200	5.9±0.15**	22.4	5.5±0.17**	30.4	6.7±0.19**	16.3	7.5±0.16*	07.4	7.6±0.16*	06.2
	400	5.6±0.19**	26.3	5.1±0.11**	35.4	6.5±0.20**	18.8	7.1±0.15**	12.3	7.4±0.11*	08.6
Chloroform fraction	100	6.3±0.12**	17.1	5.8±0.16**	26.6	6.6±0.21**	17.5	7.4±0.10*	08.6	7.8±0.25	03.7
	200	5.2±0.14**	31.6	5.1±0.13**	35.4	5.9±0.26**	26.3	6.9±0.12**	04.8	7.2±0.27**	11.1
	400	4.5±0.18**	40.8	4.1±0.23**	48.1	6.6±0.11**	30.0	6.2±0.25**	23.5	6.8±0.14**	16.0
Ethyl acetate fraction	100	6.2±0.22**	18.4	5.7±0.16**	27.8	6.5±0.25**	18.8	7.3±0.28**	09.9	7.6±0.29*	06.2
	200	4.6±0.11**	39.5	4.4±0.20**	44.3	5.7±0.13**	28.8	7.0±0.15**	13.6	7.2±0.17**	11.1
	400	3.9±0.17**	48.7	3.8±0.15**	51.9	5.1±0.22**	36.3	6.0±0.19**	25.9	6.6±0.26**	18.5

Data are expressed as mean ± S.E., n=5 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).

Table 9: Results of antipyretic activity of the different extracts of *E. spinosa* on yeast-induced fever in rats.

Groups	Dose	Average rectal temperature (°C) ± S.E., n= 5				
	mg/kg	1 hr	2 hr	3 hr	4 hr	5 hr
Control	-	38.5±0.20	38.7±0.13	38.8±0.09	38.8±0.18	38.6±0.35
Indomethacin	8	36.6±0.24**	36.5±0.05**	36.5±0.15**	36.4±0.24**	36.4±0.09**
Total extract	100	37.5±0.13**	37.1±0.19**	37.4±0.13**	37.5±0.27*	37.6±0.20*
	200	36.4±0.24**	36.3±0.06**	36.9±0.09**	37.3±0.16**	37.3±0.07**
	400	36.3±0.33**	36.2±0.22**	36.5±0.11**	36.9±0.29**	37.2±0.14**
n-Hexane Fraction	100	37.1±015**	37.1±0.09**	37.3±0.16**	37.5±0.15*	37.6±0.12*
	200	36.6±0.20**	36.3±0.19**	36.8±0.18**	37.4±0.34**	37.6±0.26*
	400	36.2±0.21**	35.9±0.40**	36.6±0.24**	37.3±0.18**	37.4±0.11**
Chloroform Fraction	100	36.9±0.30**	37.1±0.25**	37.2±0.9**	37.6±0.08*	37.6±0.10*
	200	36.4±0.09**	36.3±0.33**	36.9±0.25**	37.4±0.15**	37.6±0.41*
	400	36.1±0.15**	35.9±0.17**	36.5±0.29**	37.3±0.23**	37.3±0.38**
Ethyl acetate Fraction	100	36.7±0.32**	37.1±0.23**	37.4±0.10**	37.2±0.38**	37.6±0.17*
	200	36.2±0.1**	36.1±0.18**	36.7±0.20**	37.1±0.24**	37.4±0.19**
	400	36.0±0.13**	35.9±0.16**	36.6±0.17**	36.9±0.18**	37.2±0.21**

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).

Table 10: Results of central analgesic activity of the different extarcts of *Emex spinosa* on heat- induced pain in mice.

Groups	Dose mg/kg	Average reaction time (sec.) ± S.E.	PV	S
Control	-	b11.53 ±0.25 a 11.25 ±0.14	2.4	-
ASA	100	b 11.28 ±0.32 a 13.62 ±0.24	17.18	NS
Total extract	100	b 11.14±0.17 a 13.46±0.45	17.20	NS
	200	b 11.25±0.38 a 14.70±0.60	23.40	0.05
	400	b 11.55±0.25 a 16.20±0.44	28.71	0.05
n-Hexane fraction	100	b 11.15±0.33 a 12.50±0.32	10.80	NS
	200	b 11.35±0.56 a 13.45±0.68	16.28	NS
	400	b 11.15±0.27 a 13.86±0.44	19.54	NS
Chloroform fraction	100	b 11.36±0.51 a 12.85±0.35	11.60	NS
	200	b 11.24±0.96 a 14.75±0.64	18.25	NS
	400	b 11.53±0.70 a 14.55±0.86	20.80	NS
Ethyl acetate fraction	100	b 11.48±0.18 a 14.95±0.54	23.21	0.05
	200	b 11.43±0.73 a 16.75±0.56	31.76	0.05
	400	b 11.52±0.83 a 18.23±0.78	36.81	0.05

PV: Percentage of variation. b: mean reaction time before treatment.
a: mean reaction time after treatment. S: degree of significance.
NS: non-significant. S.E.: Standard error. ASA= Acetyl salicylic acid

RESULTS AND DISCUSSION

The methanolic extract of *Emex spinosa* (L.) Campd. aerial parts afforded thirteen compounds labeled **E1-E13** (Fig. 1).

The identification of compounds **E1**, **E2**, **E7**, and **E13** as α-amyrin, β-sitosterol, β-sitosterol-3-O-β-glucoside and rutin was established through different physical, chemical and spectral data together with co-chromatography with authentic samples. β-sitosterol, β-sitosterol-3-O-β-glucoside and rutin were previously isolated from the roots of this plant,[6] but this is the first report of the isolation of α-amyrin from the genus Emex.

Comparing the various physical, chemical and spectral data of anthraquinones **E3**, **E4**, and **E5** with those reported in literatures[7,25&26] proved their identity with chrysophanol, physcion and aloe-emodin respectively, previously isolate from the roots of the plant.[6]

Compound E6: gave a positive colour reaction for sterols and/ or triterpenes, and positive Molish's test, indicating its glycosidic nature.[27&28] Acetylation of compound **E6** afforded compound **E6a**.The ^{1}H- and ^{13}C-NMR spectral data of compound **E6a** suggested the presence of β-sitosterol-3-O-β-glucoside and fatty acid moiety[29-31] based on the following evidence; ^{1}H-NMR spectral data (Table 2) showed signal at δ 5.34 (1H, br.s) assigned to olefinic proton at C-6 and signal at δ 3.46 (1H, m) assigned to H-3α. The coupling constant of the anomeric sugar proton at δ 4.57 (1H, d, J = 8.0 Hz) indicated the β- configuration of the sugar moiety. The spectrum revealed also ten methyl groups, one assigned to terminal methyl group of fatty acid moiety at δ 0.80, three for acetyl groups at δ 2.03, 2.00 and 1.98 and six methyl groups related to the sterol moiety. These results were confirmed through ^{13}C-NMR spectral data (Table 2) that revealed signals at δ 20.8 (2X$\underline{CH_3}$-CO) and 20.9 ($\underline{CH_3}$-CO) for three acetyl groups, six methyl groups of the steroidal moiety at δ 12.0, 12.1, 18.9, 19.1, 19.5, and 19.9 and signal at δ 14.2 for a terminal methyl group of fatty acid as well as signals at δ 140.4 and 122.3 for the C5-6 double bond. Moreover the signals at δ 173.8, 34.2, 22.8-29.8 and 14.2 attributed to C1" (CO), C2", cluster of methylene groups (C3"-C15") and terminal methyl (C16") confirmed the presence of the fatty acid moiety.[9&29-34] The attachment of the palmitoyl moiety at C-6' of the glucose was deduced from the downfield shift of C-6' (at δ 62.2).[9&31] Alkaline hydrolysis of compound **E6** yielded fatty acid moiety and steroidal glycoside. These were identified as palmitic acid and β-sitosterol-3-O-β-glucoside through (co-chromatography using systems I and IV). Acid hydrolysis of the steroidal glycoside proved the presence of β-sitosterol and one glucose unit identified by (co-chromatography with authentic samples using PC for the sugar). The identity of **E6** established as 6´-O-Palmitoyl-3-O-β-sitosterol-glucoside was based on the above chemical and spectral evidences.

Compound E8: was identified as aloe-emodin-8-O-β-glucoside, $C_{21}H_{20}O_{10}$. It gave positive tests for an anthraquinone glycoside.[35] The UV and IR spectra pointed to the presence of free OH and both chelated and non-chelated carbonyl groups. The ^{1}H-NMR spectrum (Table 1) displayed signals for five aromatic protons, two being *meta*-coupled at δ 7.66 and 7.28 (each 1H, d, J=1.6 Hz) and three protons for ABX system at δ 7.89 (1H, dd, J= 7.6, 1.7 Hz), 7.86 (1H, dd, J= 7.6, 8.0 Hz) and 7.71 (1H, dd, J= 8.0, 1.7 Hz)

attributed for the H-5, H-6 and H-7 respectively in monosubstituted ring A, all these proton resonances were confirmed through 2D NMR spectrum suggesting an anthra-quinone with a mono- and disubstituted rings A and C respectively.[36] Both ^{1}H and ^{13}C-NMR spectra revealed signals at δ_H 12.87 and δ_C 161.6 for a peri-OH at C-1, δ_H 4.62 (2H, d, J= 5.6 Hz) and δ_C 62.05 for hydroxymethylene group at C-3, as well as a signal for an anomeric sugar proton in a β-configuration, at δ_H 5.18 (1H, d, J=7.6 Hz) and δ_C 100.49. Moreover the appearance in the ^{13}C-NMR spectrum of signals at δ_C 187.58 and 183.1 attributed to chelated and non-chelted carbonyls proved the 1, 8-dihydroxylated pattern of the compound. The location of hydroxymethylene at C-3 was confirmed from HMBC experiment (Fig. 1) through correlation of CH_2 signal at δ_H 4.62 with those of C-2, C-3 and C-4, on the other hand, H-6 and H-1' correlated with C-8, confirming the linkage of the sugar unit to the C-8 hydroxyl. Finally acid hydrolysis of **E8** furnished an aglycone identical with **E5** previously identified as aloe-emodin and a glucose unit, thus confirming the above structure.

Compound E9: was proved to be emodin-8-O-β-glucoside, $C_{21}H_{20}O_{10}$. It showed a close similarity to **E8**, mass of the aglycone being at m/z 270. However, the ^{1}H-NMR spectrum showed two differences: (i) two pairs of *meta*-coupled aromatic protons indicating disubstituted rings A and C. (ii) the appearance of an aromatic CH_3 group at δ 2.37 in place of the CH_2OH. On the other hand, in the ^{13}C-NMR spectrum of **E9**, the C-6 signal at δ_C 166.3 was shifted from that of **E8** (δ_C 135.95), pointing to its oxygenation. Confirmation of the structure was further supported by acid hydrolysis of **E9**, that gave an aglycone which spectral data were in complete accordance with those reported for emodin.[37&38]

Compound E10: the bluish-green colour given with $FeCl_3$ (T.S.) and positive Molish's test suggested a phenolic glycoside.[39] The UV and IR spectral data pointed to a hydroxynaphthalene skeleton having a chelated carbonyl group.[40] ^{13}C-NMR spectral data showed twenty signals assigned to twenty carbon atoms of which 4 were oxygenated at δ 151.4, 158.81, 155.83 and 205.35 (CO-CH_3), in addition to those of the sugar part. ^{1}H- and ^{13}C-NMR spectra exhibited respectively an aromatic methyl at δ_H 2.18 and δ_C 19.97, a methyl of the acetyl group at δ_H 2.47 and δ_C 32.9, methoxyl group at δ_H 3.79 and δ_C 55.85 and a signal at δ_H 5.02 (1 H, d, J= 7.1 Hz) and δ_C 101.7 assigned to an anomeric sugar proton in β-configuration. ^{1}H-NMR spectrum revealed also the presence of three aromatic protons as broad signals at δ_H 7.05, 6.95 and 6.87. The EI-MS spectrum showed peak at m/z 246 [M- glucose] $^{+}$, peaks at m/z 231 [M- (glucose+CH_3)]$^{+}$, m/z 203 [M-(glucose+CO+CH_3)] and at m/z 185

[M-(glucose+CO+CH_3+H_2O)] supporting the structure. Acid hydrolysis of compound **E10** yielded aglycone and sugar moieties. The sugar was identified as glucose (PC suing system VIII) while the physical and spectral data of the aglycone were identical to those reported for torachryson.[40-43] Accordingly compound **E10** was identified as torachryson-8-O-β-glucoside.

Compound E11: gave positive Molish's and colour reaction with $FeCl_3$ (T.S.) suggesting its glycosidic and phenolic nature.[39] ^{1}H-NMR spectrum (Table 4) displayed signals for a trisubstituted benzene ring and a sugar moiety. The signals of ABX system at δ 7.08 (1 H, d, J= 2.0 Hz), 7.04 (1H, dd, J= 8.2, 2.0 Hz) and 6.79 (1H, d, J= 8.2 Hz) for three aromatic protons, alongside with two doublets at δ 7.58 and 6.29 with J value = 15.8 Hz attributed to *trans* olefenic protons, were characteristic for caffeoyl derivatives.[44] ^{13}C-NMR spectral data (Table 4) showed fifteen signal ascribable to fifteen carbons of which three were oxygenated in the aromatic region. Signals of glucose were observed in the both ^{1}H- and ^{13}C-NMR spectra. Although the upfield shift of carbonyl group at δ 165.42 indicated that, the glucose unit is attached to the carboxyl group of caffeoyl moiety in an ester linkage.[44] The FAB mass spectrum showed peak $[M+Na]^+$ at m/z 365, and at m/z 163 confirming the presence of caffeoyl moiety. The ^{1}H-^{1}H COSY spectral data clarified the connectivity of each proton in **E11**. The olefinic signal at δ 7.58 (H-7) displayed connectivity to the proton signal at δ 6.29 (H-8). The signal at δ 7.04 (H-6) was connected to both proton signals at δ 7.08 and 6.79 (H-2 and H-5). Furthermore, the methine signal at δ 5.46 displayed cross peak with the protons at δ 3.1-3.68 (other sugar protons). The aglycone obtained from acid hydrolysis of **E11** showed crromatographic and spectral data identical to those reported for caffeic acid.[44-48] Accordingly **E11** was identified as caffoeyl-9-O-β-glucoside. This is the first report of this compound in the genus Emex.

Compound E12: different colour reactions and spectral analysis pointed to a flavonol glycoside.[35&48&49] The UV and ^{1}H-NMR spectra (Tables 5 and 6) showed a typical flavonol structure with a 4'-OH group. Each of ^{1}H and ^{13}C-NMR spectra showed two anomeric protons at δ_H 5.31 (1H, d, J= 7.5 Hz) and δ_C 101.41 and δ_H 4.45 (1H, br.s) and δ_C 100.83, indicating its bioside nature. The appearance of a doublet at δ_H 0.99 (3H, d, J= 6.2 Hz) and δ_C 17.77, confirmed the presence of rhamnose moiety. The large coupling constant (J = 7.5 Hz) of anomeric proton of glucose indicated its β-configuration while the rhamnose was in α- configuration. This assignment was confirmed from 2D ^{1}H-^{1}H COSY. The downfield shifts of C-6" at δ_C 66.96 of glucose, suggesting that, the interglycosidic linkage between glucose and

rhamnose was (1'''→ 6''). Acid hydrolysis of compound **E-13** yielded glucose and rhamnose (confirmed by PC). The resulting aglycone was identified as kaempferol (co-chromatography with authentic sample using system II). The above data were found to be identical with those reported for kaempferol-3-O-rutinoside.[50&51] Compounds **E6, E8-E12** are reported for the first time from the genus Emex.

The LD_{50} of the different plant extracts were 5, 6, 6 and 4 g/kg for total alcohol, n-hexane, chloroform and ethyl acetate extracts respectively. This is an important parameter, as the doses used in biological investigation must be lower than these levels.

All plant extracts exhibited variable antibacterial activity against Gram. +ve and Gram. -ve bacteria but a weak antifungal activity. The most effective was the ethyl acetate extract, it showed strong activity against *Sarcina lutea*, *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Enterobacter aeurginosa* and *Proteus vulgaris* at doses 5, 10, 10, 10, 25, 25 mg/ml respectively but has no effect on *Escherichia coli* and *Pseudomonas aeroginosa.*

The total alcoholic, n-hexane and chloroform extracts gave maximum activity against *Enterobacter aeurginosa* at dose 10, 10 and 25 mg/ml for each. Moreover, the alcohol extract was significantly effective against *Sarcina lutea* at dose 5mg/ml.

Results of anti-inflammatory activity of the different extracts of Emex spinosa (L.) Campd. on yeast induced edema in rats (Table 8) revealed that; both ethyl acetate and chloroform extracts were the most potent, their activities was continues for 5 hr.

All four extracts showed a well-marked antipyretic activity at doses 100, 200 and 400 mg/kg (Table 9). They reduced yeast-induced fever compared with reference compound indomethacin (8 mg/kg) with maximum activity after 2 hours. The best extracts were ethyl acetate and total alcohol extracts respectively at dose 200 mg/kg for each, they control the hyperthermia for 5 hr without decrease in activity.

Results of analgesic activity (Table 10) showed that the polar fractions (ethyl acetate as well as total alcohol extract) exhibited central analgesic activities at doses 100, 200 mg/kg respectively, since they exerted a significant protective effect on thermal painful stimuli, which characteristic for central analgesics as morphine.[23&52] Although acetyl salicylic acid has no central analgesic activity but due to lack of opiate as reference material, ASA was used in this experiment as previously reported.[20&23]

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