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PHYTOCHEMICAL AND BIOLOGICAL STUDY OF *FICUS ELASTICA* ROXB. VAR. DECORA GROWING IN EGYPT.

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ABSTRACT:

Phytochemical investigation of light petroleum and ethyl acetate extracts of leaves and stem of *Ficus elastica* Roxb. var. decora (Family Moraceae) resulted in the isolation of a new natural compound viz.1-*O*-caffaeoyl-D-mannitol. Other separated compounds were moretenone, glutinol, moretenol, lupeol, β-sitosterol, sakuranin and kaempferol-3-*O*-rutinoside. The structures of these compounds were elucidated using spectroscopic and chemical evidences and by comparison with those reported in the literature. Biological screening was also carried out to reveal that the total alcoholic extract and the terpenoid fraction of the unsapnifiable matter (USM) showed significant anti-inflammatory and analgesic activities, while the total alcoholic extract showed a mild anti -diabetic activity. The anti-inflammatory activity of the terpenoidal fraction of USM was found to outweigh that of a marketed product.

INTRODUCTION

The genus *Ficus*-a member of the Moraceae family comprises nearly 2000 species of woody plants, trees, erect shrubs and climbers growing in tropical and subtropical regions[1,2]. Several plants belonging to the genus *Ficus* have been reported for their antimicrobial, antifungal, anthelmentic, anti-inflammatory and hypoglycemic activities[3-7]. The literature review showed that this genus rich in several compounds including sterols, triterpenes coumarins, flavonoids and alkaloids in addition to other miscell-aneous compounds[7,8]. Reviewing the literature, nothing was found concerning the phytochemical investigation of *Ficus elastica* Roxb. var. decora, therefore it is deemed necessary to investigate the phytochemical constituents of this plant to isolate its components and to identify them as possible as it could.

In this work we report the isolation of a new natural caffeic acid glycosidal derivative along with other known biologically interesting compounds. The total alcoholic extract and the terpenoidal fraction of unsaponifiable matter (USM) of the light petroleum fraction was subjected to certain pharmacological screening tests

EXPERIMENTAL

Plant Material:

Ficus elastica Roxb. var. decora was collected in April 2000 from the private garden of Faculty of Pharmacy, Zagazig University and was identified by Dr. Abdel Aziz Kamel Dawh, Horticulture Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt. Fresh cut leaves and steEIMS were extracted by maceration in ethyl alcohol.

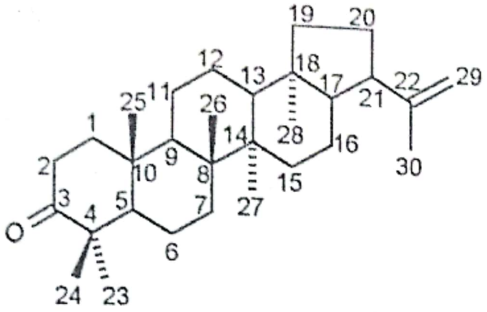
General Experimental Procedures :

Melting points were determined using: Digital, electro thermal LTD. UV spectra were obtained using: Schimadzu UV-260 spectrophotometer. IR spectra were recorded using: Jasko FTYIR- 460 plus and Bruker PT/IR. Mass Spectra were carried on: JEOL, 70 eV direct inlet using: NBA matrix, Varian MAT 311 A at 70eV and Carlo-Erba HRGC 4160 Finnigan MAT 4500 at 70 eV. GC was carried out on a Varian 3400 gas Chromatograph equipped with a fused silica column (DB5, 30m × 0.25mm I.D. 0.25 µm film thickness), J&W P/N: 122-5032 under the following conditions: Carrier gas: He with flow rate 2 ml /min.$^{-1}$; detector: FID; temp.: 300°C; inj.temp.: 250°C; split ratio: 1:10; oven temp.program: initial temp.: 50°C for 4 min., 50-90°C at 4°C min.$^{-1}$, 90-300°C at 10°C min^{-1} then hold for 10 min. ^{1}H and ^{13}C-NMR spectra were recorded on: AC Bruker operating at 300 MHz for ^{1}H-NMR and at 75 MHz for ^{13}C- NMR and varian MAT at 300 MHz for ^{1}H-NMR. Precoated TLC, Kieselgel 60 F254 (60-250), Merck and precoated TLC, cellulose (Merck).

Extraction and purification:

The total alcoholic extract was concentrated under reduced pressure to give a syrupy semi-solid residue which was then suspended in 1 litre of distilled water containing 1% ethanol and the suspension was partitioned into light petroleum, chloroform and ethyl acetate fractions to give 113, 10 and 25 g, respectively. The light petroleum fraction was subjected to saponification and preparation of the unsaponifiable matter (USM) and fatty acids.

About 19 g of the unsaponifiable matter were applied onto the top of a silica gel column (5 × 110 cm, 300 g, Merck). The elution was carried out with light petroleum and the polarity was increased with chloroform then methanol in a gradient elution technique. The effluent was collected in 200 ml fractions and monitored by TLC on silica gel plates (Merck) using light petroleum: chloroform (7 : 3) as a solvent system and identical fractions were pooled together. It afforded the isolation of compounds 1- 5. About 2 g of the unsaponifiable fraction was freed from hydrocarbons, acetylated and subjected to GC-MS and anti-inflammatory testing.



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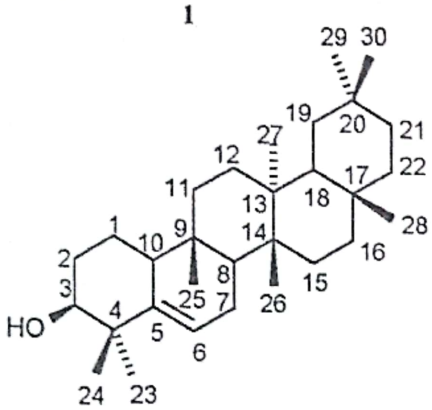

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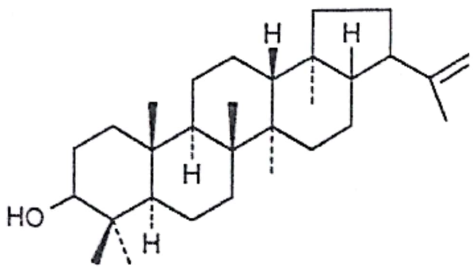

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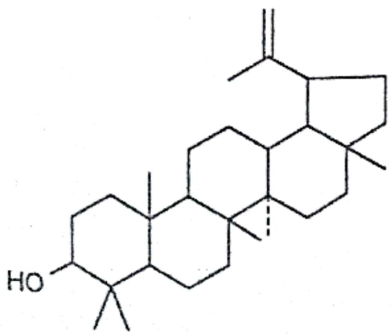

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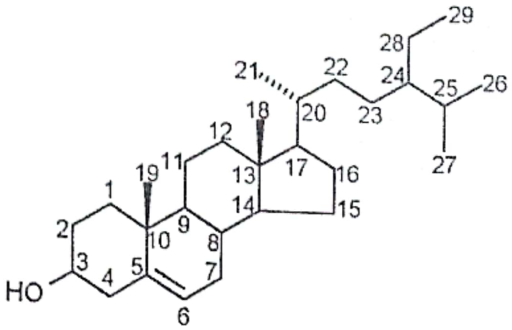

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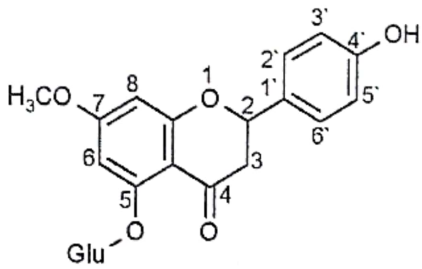

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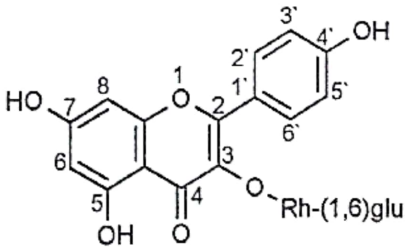

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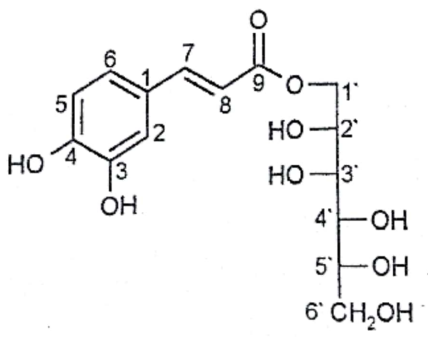

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About 25 g of the ethyl acetate fraction were chromatographed over a silica gel column (5 × 110 cm, 300 g, Merck), then gradiently eluted with chloroform containing increasing proportions of methanol. The effluent was collected in 250 ml fractions and monitored on silica gel plates (Merck) using ethyl acetate : formic acid : water (12 : 1 : 1). It afforded the isolation of compounds **6- 8**.

Compound (1): Colorless needles (chloroform-methanol; 30 mg), m.p. 249-251°C; R_f 0.73 [solvent system: petroleum ether: chloroform (7:3)]; IR(KBr) ν_{max} cm^{-1}: 2928, 2866, 1712, 1648, 1458, 1386 and 1110; EIMS, m/z (relative abundance %) : 424 (M+;42), 409(20), 381(4), 368(8), 341(1), 313(28), 302(8), 273(16), 245(12), 218(48),205(100) 189(44), 161(28), 139(5), 121(34), 109(42), 95(35), 81(28), 68(21) and 55(17). ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$) data are summarized in Tables (1 and 2)

Table (1): Selective ^{1}H-NMR ($CDCl_3$, 300 MHz) spectral data of compounds **1-3**.

H-No.	1	2	3
3	--	3.46, brs	3.2, dd (11.1, 5.4)
6	--	5.63, d (6.0)	--
23	1.18, s	1.14, s	0.98, s
24	0.88	1.04, s	0.97, s
25	0.80, s	0.85, s	0.76, s
26	0.93, s	1.10, s	0.94, s
27	0.97, s	0.99, s	0.82, s
28	0.73, s	1.16, s	0.68, s
29	4.70, brs	0.95, s	4.70, d (1.5)
29`	4.50, brs	--	4.69, d (1.5)
30	1.69, s	1.01, s	1.68, s

Coupling constants (J, Hz) are given in parenthesis.

Compound (2): White flacks (chloroform-methanol; 45 mg), m.p. 195-197°C; R_f 0.53 [solvent system : petroleum ether: chloroform (7:3)]; IR (KBr) ν_{max} cm^{-1}: 3326, 2927, 2866.1654, 1465, 1382 and 1132; EIMS m/z(relative abundance %): 426 (M^+ ; 6), 411(3), 393(5), 299(3), 286(6), 276(9), 274(100), 259(97), 254(21), 231(18), 218(13), 205(62), 189(20), 152(14), 173(24), 137(28), 134(42), 119(30), 95(36), 81(20), 69(36) and 57(24); ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$) readings are summarized Tables(1and 2)

Compound (3): White needles (chloroform-methanol; 900 mg), m.p. 236-237°C, R_f 0.36 [solvent system: chloroform: methanol (7:3)]; IR (KBr) ν_{max} cm^{-1}.: 3415, 2943, 2865, 1656, 1385 and 1103; EIMS: m/z(relative abundance %): 426(M^+; 36), 411(12), 393(6), 370(2), 259(1), 218(5), 207(68),198(100), 135(13), 107(12) and 81(9); ^{1}H-NMR and ^{13}C-NMR values are summarized in Tables (1and 2)

Table (2): ^{13}C-NMR ($CDCl_3$, 75 MHz spectral date of compounds **1- 3**.

C-No.	1	2	3
1	38.15, t	18.64, t	38.70, t
2	35.01, t	22.60, t	33.34, t
3	218.22, s	76.33, d	78.99, d
4	42.98, s	38.95, s	47.88, s
5	54.90, d	145.25, s	55.08, d
6	20.26, t	122.06, d	18.39, t
7	32.75, t	27.66, t	32.63, t
8	41.51, s	47.42, s	38.84, s
9	49.77, d	35.10, d	50.37, d
10	35.99, s	49.68, d	37.10, s
11	20.24, t	34.00, t	21.04, t
12	21.04, t	28.00, t	23.88, t
13	48.22, d	40.82, s	48.67, d
14	41.51, s	37.83, s	38.84, s
15	32.75, t	29.70, t	32.63, t
16	20.24, t	36.00, t	20.85, t
17	53.09, d	30.08, s	53.83, d
18	42.98, s	43.05, d	47.88, s
19	42.54, t	35.00, t	40.15, t
20	28.18, t	28.24, s	27.37, t
21	49.95, d	30.00 t	48.67, d
22	150.88, d	40.00, t	148.19, s
23	31.77, q	25.40, q	28.01, q
24	15.77, q	28.90, q	16.69, q
25	17.94, q	16.30, q	15.36, q
26	14.65, q	20.10, q	16.60, q
27	18.01, q	18.30, q	15.88, q
28	14.47, q	13.78, q	15.10, q
29	109.37, t	35.50, q	109.44, t
30	18.67, q	32.03, q	19.65, q

Compound (4): White needles (chloroform-methanol; 12 mg); m.p. 223-224°C; R_f 0.31 [solvent system: light petroleum: chloroform (7:3)] IR (KBr) ν_{max} cm^{-1}: 3428, 2936, 2867, 1654, 1465, 1382 and 1107; EIMS: m/z(relative abundance %): 426 (M^+ ;45), 411(12), 383(68), 247(20), 231(8), 218(4), 207(24), 203(10), 189(54), 177(30), 161(64), 121(100), 93(80) and 55(68).

Compound (5): White needles (chloroform-methanol; 30 mg); m.p. 136-137°C; R_f 0.26 [solvent system: light petroleum: chloroform (7:3)]; IR (KBr) ν_{max} cm^{-1}: 3428, 2936, 2867, 1654,1465, 1382 and 1107; EIMS: m/z(relative abundance%): 415 (M^++1, 59.4), 414(35.3,M^+), 397(33.1), 382(31.0), 330(38.1), 275(6.2), 255(26.1), 229(10.8), 213(25.2), 201(7.2), 187(11.1), 175(6.5), 161(20.7), 147(17.1), 145(27.6), 132(6.6), 107(36.5), 95(40.4), 69(41.0), 57(78.9), 55(100).

Compound (6): Reddish-brown crystals (ethyl acetate-methanol; 15 mg); m.p. 204-205°C; R_f 0.54 [solvent system : ethyl acetate: formic acid: water

(12:1:1)] ; IR (KBr) ν_{max} cm^{-1} 3444, 2951, 2843, 1657, 1641, 1382, 1207 and 1031; UV, λ_{max} nm: Me OH: 280 , 335 (sh), 391 (sh); + $NaOCH_3$: 290 , 335 (sh) , 406 (sh); +$AlCl_3$: 283, 345(sh), 406(sh); +$AlCl_3$+ HCl : 280, 325 (sh) , 391 (sh); +Na OAc : 280, 335(sh), 407(sh); +Na OAc + H_3BO_3 : 285, 335 (sh), 398, (sh); EIMS: m/z(relative abundance %): 286 (M^+;10) 195(3), 167(16), 164(30), 159(9), 151(21), 150(28), 139 (31), 133(16), 119(14), 110(100) and 93(22). Acid hydrolysis revealed the presence of glucose (Solvent system : *n*-butanol : acetic acid : water (4 : 1 : 5).

Compound (7): Brownish sandy crystals (ethyl acetate-methanol; 50 mg); m.p. 206-207°C; R_f 0.16 (solvent system : ethyl acetate: formic acid : water (12 : 1 : 1); IR(KBr) ν_{max} cm^{-1}: 3500- 3250, 2927, 2866, 1715, 1634,1558, 1456, 1385, 1362, 1181, 1093 and 1036; UV, λ_{max} nm : Me OH: 265, 290 (sh) , 352; +$NaOCH_3$: 273, 324 (sh), 404; +$AlCl_3$: 270, 300 (sh) , 357, 395; +$AlCl_3$ + HCl : 268,300 (sh), 351,400 (sh); +Na OAc : 269,359; +Na OAc+ H_3BO_3: 264, 295 (sh), 362; EIMS: m/z(relative abundance %):286 (M^+-sugar; 49), 258(31), 136(17), 134(13), 121(37) , 118 (23), 108(16), 93(27) and 69(95); ^{1}H-MMR (300 Mz, DEIMSO): δ 7.90 (2H, d, *J*=8.8 Hz, H-2`,6), 6.8 (2H, d, *J*=8.8 Hz, H-3 , 5), , 6.3 (1H, d, *J* = 1. 96 Hz, H-8) , 6.11(1H, d, *J*=1.96 Hz, H-6), anomeric protons at δ 5.01 (1H, d, *J*=7.5 Hz, H-1`` Rh) and 4.87 (1H, brs, H-1``` gl), 1.02 (3H, d, *J*=6.08 Hz, CH_3-Rh) and 3.18-3.72 (other sugar protons) ^{13}C-NMR spectral data are summarized in Table(2). Acid hydrolysis revealed the presence of rhamnose and glucose. UV (aglycone) λ_{max} nm : MeOH: 289, 359; + $NaOCH_3$: 286,340 (sh), 412; + $AlCl_3$: 289, 350 (sh), 418; +$AlCl_3$ + HCl : 288, 350(sh),421; + NaOAc : 289, 358, 365; + NaOAc+ H_3BO_3 : 288, 365.

Table (3) : ^{13}C-NMR data of compound 7 (75 MHz, DEIMSO).

C-No.	δ-value	C-sugar	C-No.	δ-value
2	146.8	Rh	1``	102.39
5	132.4		2``	72.05
4	179.35		3``	72.19
5	161.47		4``	73.85
6	100.0		5``	69.69
7	162.9		6``	17.99
8	94.9	glu	1```	104.57
9	159.31		2```	75.67
10	104.60		3```	78.01
1`	122.7		4```	71.35
2`, 6`	130.37		5```	77.15
4`	149.80		6```	68.53
3`,5`	116.13			

Table (4): ^{1}H-NMR and ^{13}C-NMR spectral date of compound **8** (300, 75 MHz, DEIMSO)

H & C No.	δ-value				
	^{1}H-NMR	^{13}C-NMR	H& C-No.	^{1}H-NMR	^{13}C-NMR
1	–	125.41, s	1`	4.1 (2H, brs)	72.80, t
2	7.04 (1H, brs)	114.66, d	2`		72.27, d
3	--	145.74, s	3`		71.51, d
4	–	148.58, s	4`	3.3-3.7 (6H, m)**	70.96, d
5	6.7 (1H, d, *J*=8.1Hz)	115.83, d	5`		69.95, d
6	6.95 (1H, brd, *J*=8.1 Hz)	121.26, d	6`		60.25, t
7	7.4 (1H, d, *J*=15.8Hz)	144.77, d			
8	6.2(1H,d, *J*=15.8Hz)	115.10, d			
9	–	166.34, s			

* Multiplicities were determined on basis of DEPT and HETCOR experiments.

** Overlapped.

Compound (8): Reddish-brown crystals (ethyl acetate- methanol; 500 mg): m.p. 246-247°C; R_f 0.37 [solvent system, ethyl acetate : formic acid : water (12 : 1:1)]; IR. □$_{max}$ cm^{-1} 3402, 2926, 2868, 1714, 1645, 1558, 1506, 1456, 1219, 1108 and 1072; UV λ_{max} nm : Me OH: 230, 295 (sh), 327; +$NaOCH_3$: 228, 306 (sh), 376; +$AlCl_3$: 230, 300 (sh), 337; +$AlCl_3$ + HCl : 230, 300 (sh), 329; +Na OAc: 227, 299(sh), 328; +Na OAc + H_3BO_3 : 227, 260, 297(sh), 349; EIMS , m/z (relative abundance %): 180 (M^+–Sugar; 14), 163(M^+ – OH; 46) 136 ($M^+ - CO_2$; 68), 110 [($M^+ - CO_2$) – C_2H_2; 52], 44 (100); FAB-EIMS: m/z (relative abundance %): 391 (M+ 2Na + H; 57) $^+$, 345 (M^++H; 2), 329 [(M^++2H) –OH; 30]$^+$, 307(70), 136[(M^+ +H)-mannitol; 100] ; ^{1}H- and ^{13}C-NMR spectral data are summarized in Table (4). Acid hydrolysis revealed the presence of mannitol (PC and solvent system : *n*-butanol: acetic acid: water (4: 1: 5).

RESULTS AND DISCUSSION

Column chromatography of the unsapenifiable fraction of the light petroleum extract afforded compounds **1-5** and they all gave a positive Liebermann-Burchard's test[9] for sterols and/or triterpenes. Compounds **6-8** were isolated from the ethyl acetate fraction by repeated column chromatography.

Compound (1) was proved to be a terpenoid from its positive response to Liebermann-Burchard's test. The IR spectrum of compound **1** displayed an intense band at 1712 cm^{-1} indicating the presence of carbonyl group in addition to an olefinic band as indicated by the presence of a band at 1648 cm^{-1}. The occurrence of two bands at 1458 and 1386 cm^{-1} suggested the presence of *gem*-dimethyl groups. The EIMS spectrum of compound **1** showed a molecular ion peak at m/z 424 which was in agreement with the molecular formula $C_{30}H_{48}O$ and indicating the presence of a pentacyclic triterpenoid[11]. The mass fragmentation pattern showed fragments at m/z 218, 189 and 205, characteristic for hopane derivatives[11]. Other fragments at m/z 409 and 381 indicated the loss of methyl group and isopropyl group, respectively. The ^{1}H-NMR data indicated the presence of seven tertiary methyl group singlets at δ 1.18 (H-23), 0.88 (H-24), 0.80 (H-25), 0.93 (H-26), 0.97 (H-27), 0.73 (H-28) and 1.69 (H-30). It also showed two olefinic broad singlets at δ 4.7 and 4.5 assigned for H-29 and H-29'. Other multiplet peaks were displayed and corresponding to the rest of protons and was confirmed by comparison with the reported data[12-14]. The ^{13}C-NMR spectrum of this compound showed signals corresponding to thirty carbon atoms indicating the presence of a pentacyclic triterpenes compound. The ^{13}C-NMR spectrum confirmed the presence of seven methyl groups at δ 31.77, 15.78, 17.94, 14.65, 18.01, 14.47 and 18.67 assigned to C-23 to 28 and 30, respectively. The signal at 218.22 was attributed to the carbonyl group at C-3 and that at δ 150.88 assigned to C-22 . Assignment of other carbons is shown in Table (2) and was confirmed by comparison with the reported data for moretenone that indicated the presence of hopane triterpenoid derivative[12,15]. Evidently, compound **1** was confirmed to be moretenone thorough the previous informations and comparison with the available published data for moretenone[12,13,20]

Compound 2 gave positive Liebermann-Burchard's test indicating its steroidal or triterpenoidal nature[9]. IR spectrum showed absorption bands at 3428 cm^{-1} for an alcoholic function, 2936 and 2867 cm^{-1} (CH stretching), a band at 1654 cm^{-1} (C=C) in addition to two bands at 1465 and 1382 cm^{-1} (*gem*-dimethyl) signifying an unsaturated steroid or triterpenoidal alcohol. The EIMS showed a molecular ion peak at m/z 426 corresponding to the molecular formula $C_{30}H_{50}O$ for a pentacyclic triterpenoid[11] . The base peak at m/z 274 (retro Diels- Alder cleavage in ring B), the strong peak at m/z 259 (274 - Me) in addition to remarkable ions at m/z 245, 205, 152, 134, 81 and 69 indicating Δ^5- unsaturated glutinan skeleton with no further substitution in any of the rings C or D or E[16]. This was confirmed by the arise of an olefinic proton signal at δ 5.62 (d, J = 6.0 Hz), eight tertiary methyl signals at δ 1.14, 1.04, 0.85, 1.10, 0.99, 1.16, 0.95 and 1.01 assigned to H- 23 to 30, respectively and a methine proton at δ 3.5 brs for H-3 in the ^{1}H-NMR spectrum. The ^{13}C-NMR resonance signals of compound **2** Table 2 were similar to that of glutinone except for the absence the signal to the carbonyl group at C-3 [19] and the arise of a signal at δ 76.33 characteristic for the secondary alcoholic function at C-3. On the bases of the above spectral data, chemical evidence and comparison with the previously reported data, compound **2** was confirmed to be glutinol (gult-5-en-3β-ol)[10,18].

Compound (3): The IR spectrum of compound **3** showed several bands corresponding to alcoholic OH, olefinic carbon as well as *gem*-dimethyl groups. The EIMS spectrum displays a molecular ion peak at m/z 426 in addition to other fragments at m/z 411, 383, 218, 189, and 207 that are characteristic for triterpenoid of the hopane type[11,20]. The ^{1}H-NMR spectrum of compound **3** showed singlets corresponding to seven tertiary methyl groups at δ 0.98, 0.97, o.67, 0.94, 0.82, 0.68 and 1.68 assigned for H-23 to 28 and 30, respectively. It also showed two doublets for olefinic protons at δ 4.70 (J=1.5 Hz) and 4.69 (J=1.5 Hz), in addition to a doublet of doublet at δ 3.2 (1H, J=11.1, 5.4 Hz) for H-3 indicating the presence of a secondary -OH group at C_3. The ^{13}C-NMR spectrum exhibited signals for seven tertiary methyl groups at δ 28.01, 16.69, 15.36, 16.60, 15.88, 15.10 and 19.65 assigned for C- 23 to 28 and 30, respectively, a signal at δ 78.99 representing C_3 with -OH group and another one at δ 148.19 corresponding to an olefinic carbon at C_{22}. The arrangement of methyl groups in ^{13}C-NMR spectrum was confirmed by HETCOR experiment. Other primary, secondary and quaternary carbon signals confirmed the suggested hopane-derivative [15]. The above mentioned spectral data of compound **3** were comparable with those of compound **1** except for the characteristic differences between moretenone and moretenol. The ^{1}H-NMR signal of compound **3** at δ 3.2 (dd, J=11.1, 5.4 Hz, H-3), carbon resonance at δ 78.99 (C-3) and the presence of a broad band at 3415 cm^{-1} in IR spectrum in addition to direct comparison (IR, EIMS and mp.) with the previously published data[12,13,20] gave clear evidence that compound **3** is moretenol.

Compound (4): The IR spectrum of this compound exhibited an absorption band at 3428 cm^{-1} (-OH) and 1107 cm^{-1} for (C-O) indicating the presence of a secondary hydroxyl group, another band at 1654 cm^{-1} for the unsaturation and two bands at 1465 and 1382 cm^{-1} for (-CH_2 and CH_3 bending, respectively). The EIMS spectrum of compound 4 showed a molecular ion peak at m/z 426 indicating a triterpenoidal compound $C_{30}H_{50}O$. The spectrum also showed fragments at m/z 411 and 383 corresponding to [M-Me]$^+$ and [M-isopropyl]$^+$, respectively. Other significant peaks at m/z 218, 207,203,189 characteristic for lupane type petacyclic triterpenes[11]. The structure of this compound was confirmed to be lupeol from the above mentioned data, direct comparison with authentic sample (mp., mmp., Co-TLC) and comparison with the reported data for lupeol[13].

Compound (5): The IR spectrum showed the presence of a hydroxyl band at 3422 cm^{-1} and C-O band at 1107 cm^{-1} in addition to a band at 1654 cm^{-1} (C=C) indicating the presence of an unsaturated alcoholic compound. The EIMS spectrum showed a molecular ion peak at m/z 414 with other significant fragments at m/z 396, 382, 330 303, 273 and 255 indicating a compound with a Δ^5 unsaturated steroidal skeleton[11]. The above mentioned data of compound 5 suggested the presence of β-sitosterol. This was confirmed by direct comparison (IR, EIMS, Co-TIC, mp. and mmp.) with authentic sample of β-sitosterol.

GC-EIMS analysis of the terpenoid rich fraction of the unsaponifiable matters (USM) revealed the presence of β-sitosterol, β-sitosterol acetate, β-amyrine-β-amyrine acetate, α-amyrine acetate, lupeol acetate, germanicol and moretenol as major components (80.16%). These compounds were identified by comparing their mass spectral data with those reported in literature [13].

Analysis of the fatty acids methyl esters was carried out by GLC and identified by comparing their retention times to those of the authentic samples. The results revealed the presence of capric, lauric, myristic, palmetic, stearic, oleic, linoleic and linolenic acids.

Compound (6): The IR spectrum of this compound revealed the presence of a peak at 3444 cm^{-1} (OH), a peak at 1657 cm^{-1} for γ-pyrone carbonyl and another one at 1641 cm^{-1} for (C=C). The UV spectrum in methanol showed a band at 335 nm as a shoulder to band II at 280 nm, suggested a flavanone or dihydroflavonol[21]. $NaOCH_3$, showed a 10 nm bathochromic shift in band **II** suggesting the presence of a hydroxyl function in ring A. The addition of NaOAc showed no bathochromic shift in band II indicating the absence or substituted -OH group[21,22]. The EIMS spectrum exhibited a molecular ion peak at m/z 286 in accord with a flavanone containing two hydroxyl groups and a methoxy group[21]. The fragment ion peak at m/z 167 indicated the presence of a methoxy and hydroxy functions in ring A, while the fragment ion peak at m/z 119 suggesting a hydroxyl function in ring A. Moreover, the fragments at m/z 167, 151 , 133 and 119 indicated the isomerization of the compound into chalcone that was confirmed by the UV spectrum in $NaOCH_3$ that shows band **I** at 400 nm as a shoulder[21]. Acid hydrolysis indicated the presence of glucosyl moiety that was indicated by PC and Co-PC with authentic sample of glucose. Furthermore, comparison of the physical characters, m.p, color reaction of compound 6 and its aglycone together with comparing the above mentioned spectral data with corresponding previously reported data[21,23] suggested that compound 6 was identified as Sakuranin.

Compound (7): The IR Spectral data showed a hydroxyl stretching band at 3500-3250 cm^{-1} with several peaks at 1181, 1093 and 1036 cm^{-1} for C-O indicating several -OH groups. Also it showed an absorption band at 1715 cm^{-1} for C=O besides a band at 1634 cm^{-1} for C=C. The UV spectrum in methanol indicated a flavonol skeleton[21,22]. NaOMe produces 52 nm bathochromic shift in band **I** without decrease in the intensity indicating the presence of C'_4–OH subsitution. A band at 342 nm with $NaOCH_3$ indicated free C_7-OH group. The bathochromic shift (43 nm) with $AlCl_3$ in band **I** indicated the presence of free C_5-OH group and the absence of free C_3-OH group which was confirmed by addition of HCl[22] . The EIMS Spectrum of the aglycone (after splitting of sugar moiety) showed parent ion peak at m/z 286 ($C_{15}H_{10}O_6$), representing a flavonol skeleton with three hydroxyl groups[21]. Mass fragmentation showed an ion at m/z 121 indicating the presence of one OH group in ring B which was confirmed by an ion at m/z 136 in addition to the presence of C_3-OH group. The presence of two hydroxyl groups in ring A was confirmed by the ion at m/z 108 and this fragmentation pattern is characteristic for kaempferol. The ^{1}H–NMR spectrum of compound 7 showed two doublets : one at δ 7.9 (2H, *J*=8.8 Hz) for H-2`, 6` and another one at δ 6.8 (2H, *J*=8.8 Hz) H-3`, 5` which are characteristic for 4`–substituted B-ring. It also showed two doublets with *J*=1.69 Hz at δ 6.3 and 6.11 corresponding to H-8 and H-6 respectively[21,24,25]. The glycosidic nature was confirmed by the presence of two anomeric proton signals; one doublet at δ 5.01 (*J*=7.5 Hz) for 1``-H rhamnosyl proton and another broad singlet at δ 4.87 for glucosyl proton. Other sugar proton signals appeared at δ 3.18 – 3.72 that is concomitant with the literature[21]. This was confirmed by ^{13}C-NMR spectral data Table (3) which were identical with those previousely reported for kaempferol 3-*O*-rhammose (1-6) glucose[26]. Acid hydrolysis gave kaempferol which was identified by mp. 279- 281°C, Co-TIC and UV[22]. The sugar moieties were identified as rhamnose and glucose by PC using authentic sugars.

Compound (8) : Compound 8 exhibited a blue flowrescence under UV lamp indicating the presence of a coumarin or a simple phenolic compound[27]. IR spectrum showed the presence of hydroxyl group(s) (3402 cm^{-1}), a carbonyl group (1714 cm^{-1}), a double bond (1645 cm^{-1}) and an aromatic ring bands at 1558, 1540 and 1506 cm^{-1}. The UV spectral data in methanol showed an absorption band at 327 nm with a shoulder at 295 nm. The ortho dihydroxy system at C_3, C_4 was confirmed by a bathochromic shift with $AlCl_3$ (+10 nm) and Na OAc + H_3BO_3 (+22nm)[21]. FAB-MS (Pos.) of compound 8 showed molecular ion peak sat m/z 391 $[M+H+2Na]^+$,345 $(M^+ +1)$ and 163 $[(M^+ +1) - 182]^+$ while the EIMS showed a molecular ion peak at 180 and characteristic ion peaks due to caffeic acid at m/z 163 and 136[28,29] suggesting a caffeic acid in glycosidal combination with mannitol as a sugar moiety. ^{1}H-NMR showed a pair of doublets at δ 7.4 (d, *J*=15.8 Hz) and 6.2 (d, *J*=15.8 Hz) assignable to a

trans-olefine system and aromatic protons at δ 7.04 (brs), 6.7 (d, J=8.1) and 6.95 (d, J=8.1) characteristic for ortho dihydroxy system indicating the presence of caffeoyl moiety[28]. The sugar signals in the ^{1}H-NMR at δ 4.1 brs (2H, H-1`) and δ 3.3 - 3.7 (6H, H-2`- 6`) assignable to mannitol moiety[30]. According to the FAB-MS, EIMS and ^{1}H-NMR data in addition to the arise of four CHOH groups at δ 69.95, 70.96, 71.51 and 72.27 and two CH_2OH groups with large chemical shift differences at δ 60.25 and 72.80[30] (^{13}C-NMR and DEPT), compound 8 was confirmed to contain a caffeic acid moiety linked to D-mannitol. Acid hydrolysis indicated the presence of mannitol as a sugar part, which was identified by PC and Co-PC with authentic D-mannitol. On basis of spectral data, chemical evidence and comparison with the previously reported data for a number of similar compounds[28-30]. The structure of compound 8 was established as 1-*O*-caffeoyl-D-mannitol. According to the available literature, this is the first report for the isolation of this compound from genus *Ficus* and from nature.

IV- Pharmacological Activities

The pharmacological study includes screening of the anti-inflammatory activity of the total alcoholic extract and the terpenoidal fraction of USM of the plant in addition to the analgesic activities of the total alcoholic extract.

A. Anti-inflammatory Activity :

The anti-inflammatory activity of the total extract and that of the terpenoidal fraction of the USM were studied on rats after injection of carrageenan and compared with both aspirin and cortisone using hind paw odema method[31].

As shown in Table 5 and Figs (1, 2) both aspirin and the total extract significantly decreased the thickness of oedema of the hind pow compared to the control group as indicated by reduction of the total area under the curve (AUC). The injection of the terpenoidal fraction of the USM induced a significant reduction of the size of the hind paw oedema in comparison with both control and hydrocortisone (Solu-Cortef®-UPJOHN) treated groups. This effect was also similar to hydrocortisone in its duration of action as shown in Table 6 and Figs 3, 4. These results showed that the terpenoidal fraction has 1.25 as the relative potency of hydrocortisone.

A comparative study of the anti-inflammatory activity of the terpenoidal fraction and a marketed pharmaceutical product (Mebo®; an ointment containing 0.25% β-sitosterol) was also done according to the method described by Alpermann[32]. It was found that the tested terpenoidal fraction has more potent anti-inflammatory characters than that of the tested product, where the former reduced the hind paw thickness significantly by 27.4% while the later caused only 19.5 % reduction as shown in Table 7

B. Analgesic Activity :

Writhing method[33] was used for the determination of the analegesic activity of the total extract on mice and *p*-benzoquinone is used to induce writhing.

The results given in Table 8 showed that the total extract in a dose of 200 mg/kg exerted an analgesic effect after both oral and intrapretoneal administrations. The extract exhibited 38% and 40% analgesia compared to control rats, respectively, however, it showed 48% and 50% analgesic effect in comparison to aspirin providing 0.48 and 0.50 relative potencies of aspirin, respectively. It could be concluded that the total extract has half the activity of aspirin as an analgesic agent.

C- Anti-diabetic activity :

Eighteen adult male diabetic rats were used in this study .Rats were divided into three groups (n=6).the first group received gum acacia mucilage (6%) and served as a control. The second and third groups received the extract (200 mg/kg) and glibinclamide (5 mg/kg), respectively. Diabetes was induced in rats by intraperitoneal injection of streptosotozin (STZ) in a single dose of 38 mg/kg. Rats became diabetic after 5 days of injecting STZ. Rats having blood glucose range from 180-350 mg/dl were considered diabetic and subjected to the current study. Diabetic rats were given the solvent, extract and glibinclamide suspended in gum acacia (6%) orally by gavages for two weeks .Blood glucose was determined at the end of this period using glcomen-glyco® (blood glucose meter,18 sensor strips) .

As shown in Table 9, the total extract of *Ficus elastica* Roxb.var decora induced 14% reduction of blood glucose level (BGL) of diabetic rats as compared to the values before treatment. Glibinclamide induced 40 % reduction in BGL in rats. On the other hand, the effect of the total extract was 0.35 of the effect of Glibinclamide. Thus ,the total extract of *Ficus elastica* Roxb.var decora induces a smooth reduction in BGL in diabetic rats and thus having a mild anti-diabetic activity.

Conclusion: *Ficus elastica* Roxb.var decora, that is an ever green widely distributed plant, represents an excellent anti-inflammatory candidate with analgesic characters and an added value of anti-diabetic activity.

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Table 5: Effect of the total extract of *Ficus elastica* Roxb. var. decora and aspirin on the thickness of the hind paw edema induced by injection of carrageenan in rats.

Item	Thickness of edema after carrogeenan (mm)						AUC	% effect	Relative potency to aspirin
	1hr.	2hrs.	4hrs	6hrs	10hrs	24hrs			
Control	0.924	1.32	1.902	1.904	2.184	1.09	42.962	100	--
	±0.137	±0.12	±0.071	±0.209	±0.212	±0.247	±3.522		
Aspirin	0.692	0.54	1.024	1.684	1.238	0.97	29.39	68.4	1
	±239	±0.126	±0.282	±0.095	±0.129	±0.212	±2.223		
Total extract	0.708	0.868	1.43	1.536	1.216	0.696	28.654	66.7	1.03
	±0.099	±0.152	±0.118	±0.207	±0.121	±0.145	±2.411		

- These results expressed as ±SE of mean
- AUC: Area Under the Curve

Table 6: Effect of the terpenoidal fraction of the light petroleum extract of *Ficus elastica* Roxb. var. decora and hydrocortisone on the thickness of the hind paw edema induced by injection of carrageenan in rats.

Item	Thickness of edema after carrogeenan (mm)					AUC	% effect	Relative potency to Hydrocortisone
	1hr.	2hrs.	4hrs	6hrs	10hrs			
Control	1.38	2.16	2.43	2.38	2.105	20.17	100	--
	±0.067	±0.2	±0.252	±0.11	±0.228	±1.522		
Hydrocortisone	0.9	1.65	2.25	2.39	1.64	16.33	80.96	1
	±0.104	±0.219	±0.186	±0.14	±0.142	±1.79		
Terpenoidal fraction	0.257	0.933	1.47	1.783	1.607	13.03	64.6	1.25
	±0.09	±0.162	±0.105	±0.118	±0.141	±0.889		

- These results expressed as ± SE of mean
- AUC: Area Under the Curve

Table 7: Anti-inflammatory screening of 0.25% ointment prepared from the terpenoidal fraction of the light petroleum extract of *Ficus elastica* Roxb. var. decora compared with Mebo® using the hind paw thickness method.

Item	Hind paw thickness (mm)				
	Before administration	After administration			
		After 2 hours	% Reduction	After 4 hours	% Reduction
Control	5.49 ± 0.28	5.92 ± 0.52	--	5.94 ± 0.52	--
Mebo®	5.38 ± 0.235	5.02 ± 0.152	6.69	4.33* ± 0.089	19.4
Terpenoidal fraction 0.25%	5.786 ± 0.232	5.24 ± 0.102	9.43	4.196* ± 0.038	27.4

* Significantly different at $P < 0.05$.
- Data represented as mean ± SE.

Table 8: The protective analgesic effect of total extract of *Ficus elastica* Roxb. var. decora given orally and intraperitonealy (200 mg/kg) and aspirin given orally (100 mg/kg) against P-benzoquinone induced writhing in mice.

Item / Group	Control	Aspirin	Extract	
			Oral	IP
Number of mice	10	10	8	10
Number of animals writhing	10	2	5	6
Number of animals exerting analgesia	0	8	3	4
% response to control	0.00	80	38	40
% response to aspirin	-	100	48	50
Relative potency to aspirin	-	1	0.48	0.5

Table 9: Effect of *Ficus elastica* Roxb. var. decora extract (200 mg/kg) and glibinclamid (5 mg/kg) on blood glucose levels of STZ-diabetic rats.

Treatment	Blood glucose level mg/dl		Effect %	Relative potancy
	Before treatment	After treatment		
Control	256 ± 21.32	241 ± 19.13	- 5.8	--
Glibinclamide	275 ± 25.11	165 ± 13.18	- 40	1
Total extract	236 ± 22.19	203 ± 18.85	- 14	0.35

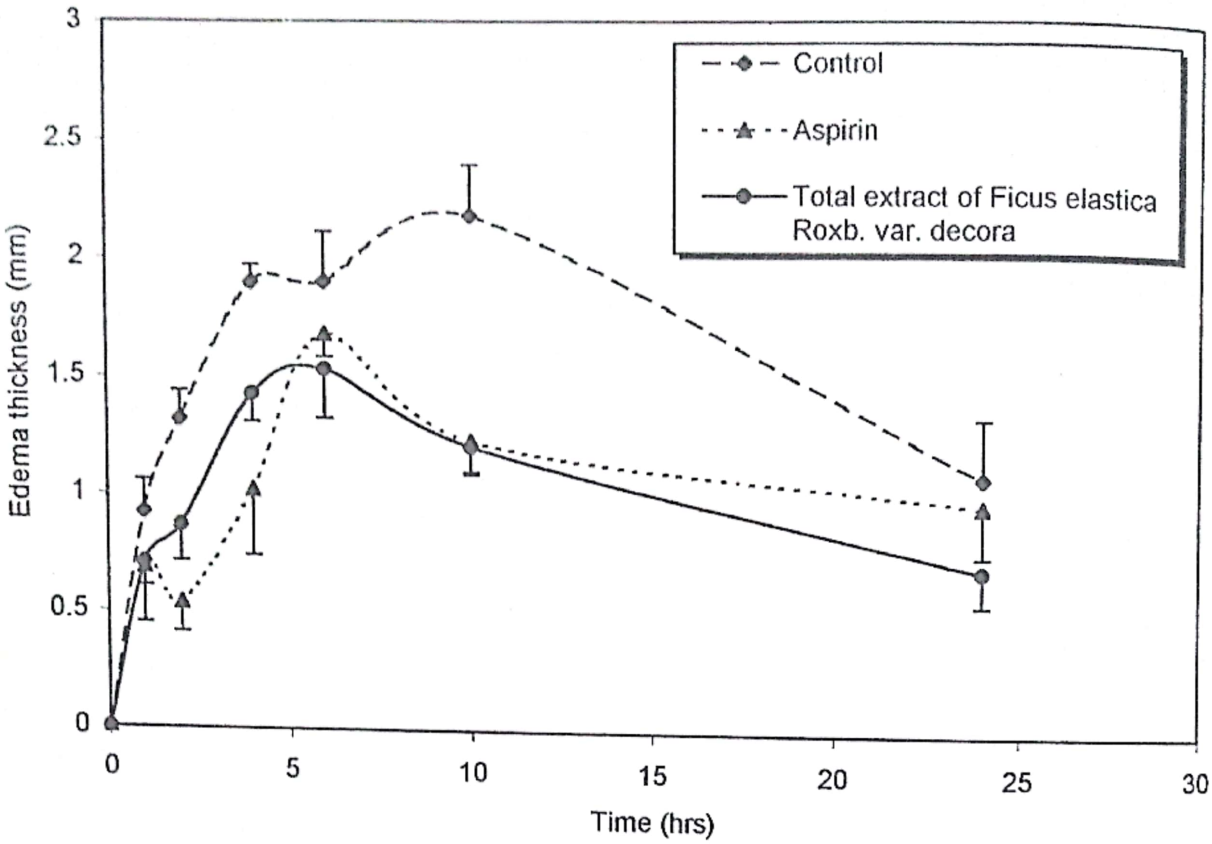

Fig. (1): Effect of total extract of *Ficus elastica* Roxb. var. decora and aspirin on the thickness of hind paw oedema induced by injection of carrageenan in rats.

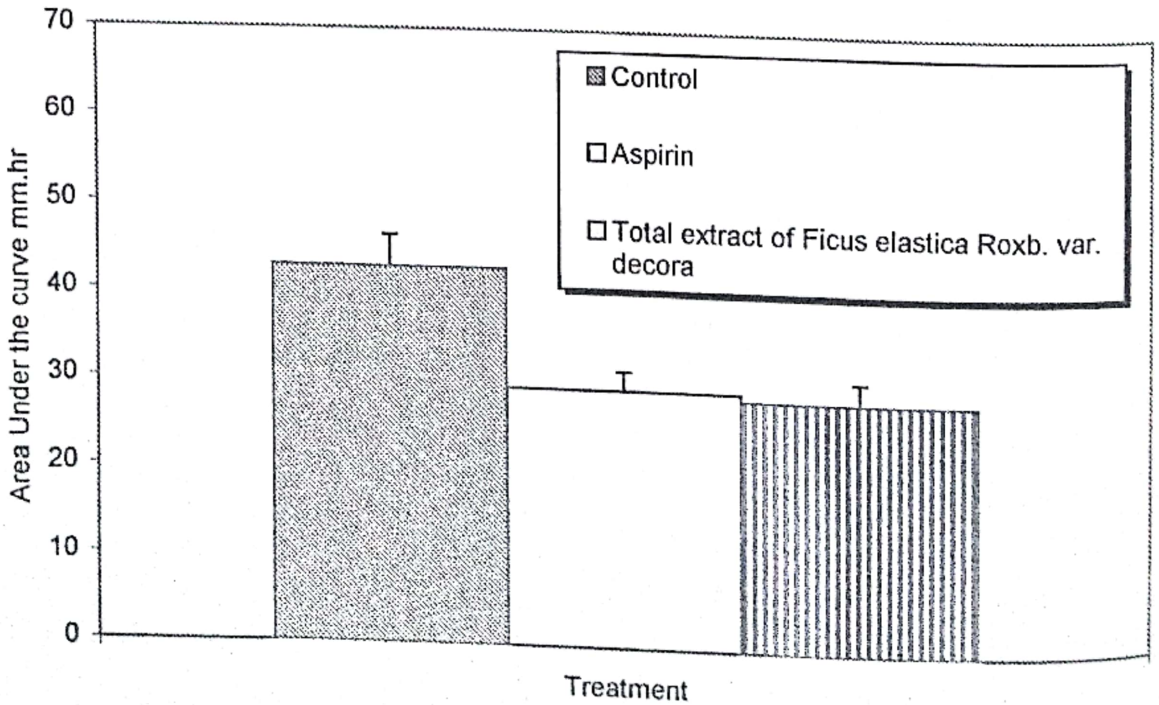

Fig. (2): Effect of total extract of *Ficus elastica* Roxb. var. decora and aspirin on the thickness of hind paw oedema induced by injection of carrageenan in rats.

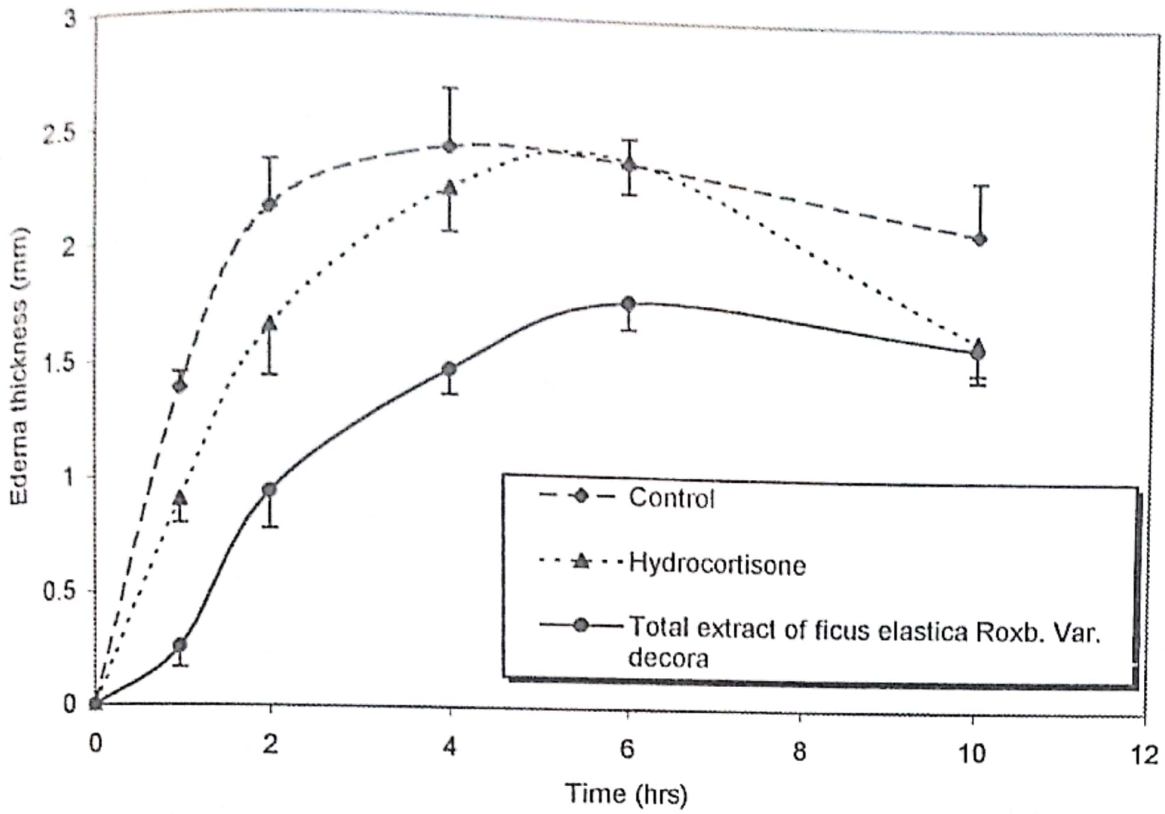

Fig. (3): Effect of otal extract of *Ficus elastica* Roxb. var. decora and hydrocortisone on the thickness of hind paw oedema induced by injection of carrageenan in rats.

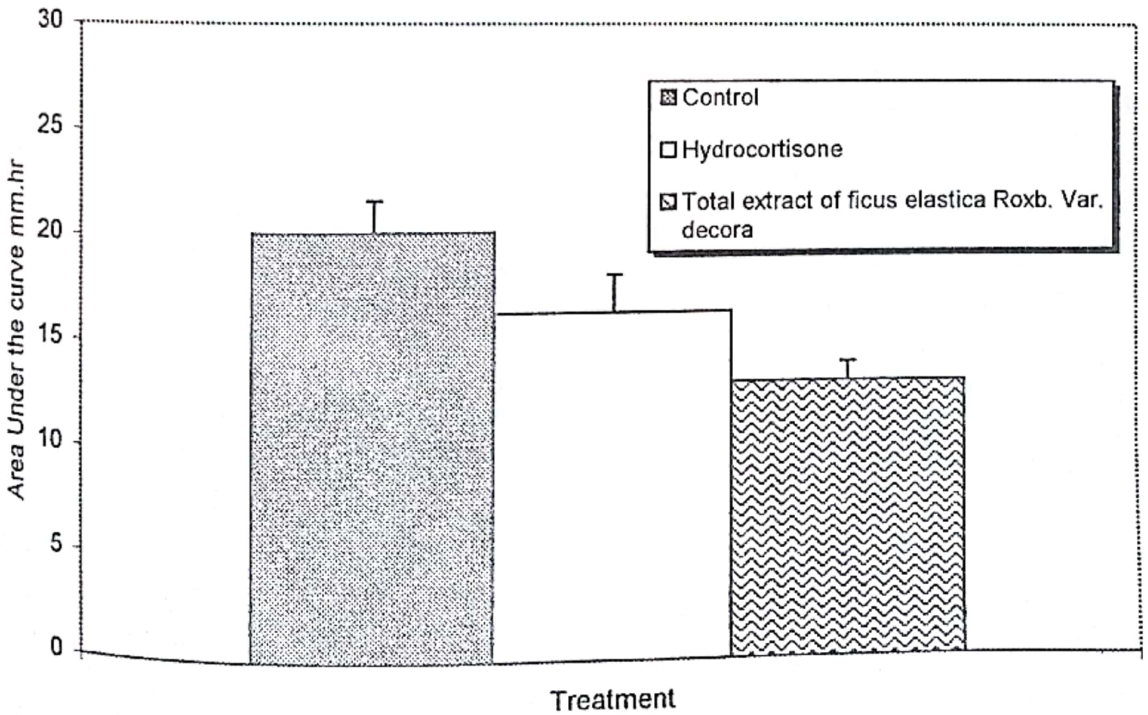

Fig. (4): Effect of otal extract of *Ficus elastica* Roxb. var. decora and hydrocortisone on the thickness of hind paw oedema induced by injection of carrageenan in rats.

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دراسة كيميائية وبيولوجية لنبات فيكس الاستيكا (ديكورا) المنزرع فى مصر

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فـــى هـــذه الدراسة تم فصل المركب الجديد ١-O – كافيويل- د- مانيتول بالاضافة إلى المركبات المعروفة: مورتيـــنون، جلوتينول ، مورتينول، لوبيول، بيتا-سيتوستيرول ، ساكيورانين، كامفيرول- ٣-O – روتينوسيد من الجـــزء الغـــير متصـــبن لخلاصـــة الايثر البترولى وخلاصة خلات الايثيل لأوراق وسيقان نبات فيكس الاستيكا (ديكـــورا) مـــن العائلـــة التوتـــية وتم التعرف على المركبات المفصولة بالطرق الطيفية والخواص الكيميائية لتلك المركبات.

وأثبتـــت الدراســـة البيولوجـــية أن للخلاصـــة الكحولية والجزء التربينى من الجزء غير المتصبن تأثير فعال كمضـــاد للالتهاب ومسكن للألم. بينما كان للخلاصة الكحولية تأثير متوسط كخافض للسكر فى الدم. وبمقارنة تأثير الجزء التربينى كمضاد للالتهاب بمستحضر تجارى فاق الجزء التربينى هذا المستحضر.