Zagazig J. Pharm. Sci., June 2003
Vol.12, No. 1, pp. 1-8

ISSN 1110-5089

CHEMICAL COMPOSITION AND ANTI-INFLAMMATORY ACTIVITY OF *VERBENA SUPINA* L; GROWING IN EGYPT

Mahmoud M. Abdel-Aal and Salah A. Ghareeb*
Department of Pharmacognosy and Department of Pharmacology*, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt

ABSTRACT:

Phytochemical investigation of the chloroform extract of *Verbena supina* L. afforded ursolic acid, oleanolic acid, 3β,24-dihydroxy-urs-12-en-28-oic acid and 3β,24-dihydroxy-olean-12-en-28-oicacid.The ethyl acetate extract yielded the phenylpropanoid acteoside. The isolated materials were identified on the bases of chromatographic spectroscopic (UV, IR, ^{1}H and ^{13}C-NMR and MS) evidences. Chloroform extract, ursolic acid and 3β,24-dihydroxy-urs-12-en-28-oic acid exhibited potent anti-inflammatory activities in comparison with hydrocortisone using carrageenan- induced hind paw edema model.

INTRODUCTION

The genus *Verbena* is represented in the Egyptian flora by two species, *Verbena supina* L and *Verbena officinalis* L.. *V. supina* L is a very common procumbent annual herb growing as a weed on canal banks in the Nile region and Western Mediterranean Costal region[1].

Many *Verbena* species are used in folk medicine as remedies for treatment of various diseases. In Australia, *V. bonariensis* has been suspected to cause abortion in bovine[2]. In South Africa, *V. officinalis* has been used as a popular medicine for fever, anemia, dropsy, and proved to be aphrodisiac, antineuralgic, anthelmintic, anti-inflammatory and useful for chronic bronchities, menstrual disorders and diuresis[2,3]. Several *verbena* species have been reported to contain iridoids, phenylpropanoids, flavonoids, triterpenoids and anthocyanins[3-13].

The severe side effects of synthetic anti-inflammatory drugs evoked us to search for new natural anti-inflammatory agents. The anti-inflammatory activity was previously reported for the chloroform extract of *V. officinalis*[3]. This activity was attributed to the presence of ursolic acid and oleanolic acid, that are usually similar in their pharmacological activity and occur naturally together[14].

To date, there are neither phytochemical nor pharmacological studies on the title plant. Therefore, a phytochemical study of *V. supina* L., is carried out in addition to a pharmacological evaluation of the chloroform extract and compounds **I** and **III** to validate the anti-inflammatory activity.

EXPERIMENTAL

Melting points were determined on SMP3 apparatus (UK) and were uncorrect. IR (KBr) spectra were run on a JASCO DT/IR-460 plus (Japan). UV spectra were measured on a UV-Visible Recording Spectrophotometer (Shimadzu UV-260, Japan). ^{1}H-NMR were obtained on a Varian Gemini 300 (at 300 MHz) Spectrometer; ^{13}C-NMR and 2D experiments (COSY and HETCOR) were run on AM-300 (at 75 MHz) spectrometer. Chemical shifts are given in δ ppm with TMS as an internal standard. MS were measured on a Finingan Mat 55 Q-700 Spectrometer (70 eV). Silica gel 60 (Merck) were used for CC and precoated TLC plates (Merck) were used.

Material and methods:

Plant material:

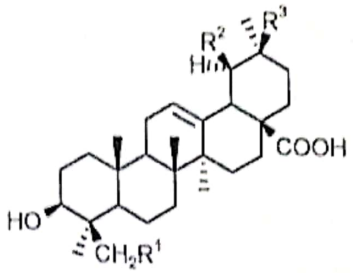
Verbena supina L aerial parts were collected from the neighborhood of the Faculty of Pharmacy, Zagazig University, in June and July 1998. The identity of the plant was confirmed by Dr. Husain Abdel-Baset, Associate Professor of Plant Taxonomy, Department of Botany, Faculty of Science, Zagazig University. A voucher specimen has been deposited at Pharmacognosy Department, Faculty of Pharmacy, Zagazig University, Zagazig, Egypt.

Extraction and Isolation:

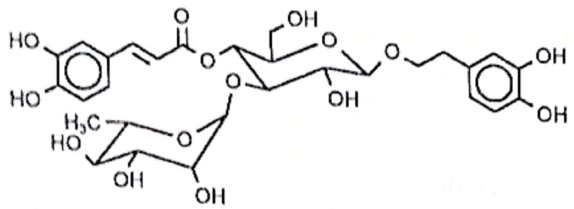
The air dried powdered aerial parts of *Verbena supina* L. (3 Kg) were exhaustively extracted with EtOH. The combined alcoholic extract was concentrated to 500 ml diluted with 500 ml of H_2O and extracted with chloroform then with ethyl acetate. Each of the chloroform and ethyl acetate extracts was distilled off under reduced pressure to obtain 153 and 24 g residue, respectively.

Thirty grams of the chloroform residue were applied onto the top of a silica gel column (500 g, Merck). The elution was carried out with petroleum ether and the polarity was increased with chloroform then methanol in a gradient elution technique. The effluent was collected in 500 ml fractions and monitored by TLC on silica gel plates (Merck) using $CHCl_3$: MeOH (9:1) as a solvent system and identical fractions were pooled together. It afforded the isolation of compounds **I** (80 mg), **II** (10 mg) **III** (70 mg) and **IV** (30 mg).

About twenty four grams of the EtOAc residue were chromatographed over a silica gel column (300 g, Merck), then gradiently eluted with $CHCl_3$ containing increasing proportions of 90 % MeOH. The effluent was collected in 150 ml fractions and monitored on silica plates (Merck) using (EtOAc : 90% MeOH 9:1). Identical fractions were pooled together. It afforded the isolation of compound **V** (70 mg).



	R^1	R^2	R^3
I	H	CH_3	H
II	H	H	CH_3
III	OH	CH_3	H
IV	OH	H	CH_3



V

Compound I: Amorphous white powder, m.p. 279 - 280°C (MeOH). IR ν_{max} cm^{-1} : 3411 (OH), 1695 (C=O of COOH), 1455, 1387. EIMS m/z (rel. int.%): 456 $[M]^+$ (26), 438 $[M-H_2O]^+$ (10), 410 $[M-HCOOH]^+$ (4), RDA ions : 248 (100), 207 (24), 203 (32), 289 (10), 147 (4), 133 (24). ^1H and ^{13}C-NMR, Tables 1 and 2, respectively.

Compound II: Amorphous white powder, m.p. 298-300°C (MeOH). IR ν_{max} cm^{-1} : 3390 (OH), 1700 (CO of COOH), 1460, 1390. EIMS m/z (rel. int.%) 456 $[M]^+$ (4), 439 $[M-OH]^+$ (6), 410 $[M-HCOOH]^+$ (2), RDA ions : 248 (100), 207 (20), 203 (58), 175 (36), 133 (8). ^1H-NMR, Table 1.

Compound III: Amorphous white powder, m.p. 286-288°C (MeOH). IR ν_{max} cm^{-1} : 3396 (OH), 1692 (CO of COOH), 1461, 1379. EIMS m/z (rel. int.%) 454 $[M-H_2O]^+$ (1%), 248 (100), 203 (68), 189 (14), 175 (24), 145 (8), 133 (10). ^1H and ^{13}C-NMR, Tables 1 and 2, respectively.

Compound IV: Amorphous white powder, m.p. 276-278°C (MeOH). IR ν_{max} cm^{-1} : 3380 (OH), 1689 (CO of COOH), 1461, 1381. EIMS m/z (rel. int.%) 454 $[M-H_2O]^+$ (2), 248 (100), 203 (58), 189 (12), 175 (19), 145 (5), 133 (7). ^1H and ^{13}C-NMR, Tables 1 and 2, respectively.

Compound V: Brownish yellow flacks, m.p. 260-262°C (MeOH). IR ν_{max} cm^{-1} : 3407 (OH), 1692 (C=O),1610, 1520. EIMS m/z (rel. int.%) 154 [(M-sugar)-cinnamate]$^+$ (92), 137 [Aglycone-OH] (100), 163 [cinnamate-OH] (5), 136 [cinnamate-CO_2] (98). ^1H and ^{13}C-NMR, Table 3.

Table 1: Selected ^1H-NMR spectral data for compounds **I-IV** (DMSO- δ)*.

H	I	II	III	IV
3	3.0 brs	4.07, dd (4.6, 12)	4.4, dd (5.7, 11.1)	3.5[b]
12	5.1 brs	5.2, brs	5.3, brs	5.2, brs
18	2.1, d(11.4)	2.72, m	2.1, d (12)	2.78[b]
23	0.86, s	1.09, s	0.73, s	0.6, s
24	0.88, s	0.81, s	3.5, d (11.2)	3.2[b]
			3.6, d (11.2)	3.4[b]
25	0.67, s	0.87, s	0.91, s	0.90, s
26	0.74, s	0.67, s	0.81, s	0.70, s
27	1.02, s	1.20, s	1.20, s	1.10, s
29	0.80, d(6.3)	0.87, s	0.65, d(6.2)	0.80, s
30	0.89, d(5.6)	0.81, s	0.99, d (6.7)	0.88, s

*Assignments on basis of chemical shifts and ^1H, ^{13}C, 2D experiments (COSY and HETCOR); Coupling constants (J, Hz) are given in parenthesis.
[b] Signal pattern are unclear due to overlapping.

Table 2: ^{13}C-NMR spectral data for compounds **I**, **III** and **IV** (DMSO, δ).

C	I	III	IV
1	38.9	38.7	33.3
2	27.0	27.5	22.7
3	76.8	76.8	76.7
4	38.9	41.6	42.4
5	52.4	46.8	50.6
6	18.0	17.0	18.8
7	33.0	32.5	33.0
8	40.1	40.0	40.1
9	46.8	46.8	47.0
10	38.4	38.4	37.0
11	21.1	23.3	23.4
12	124.6	124.6	121.6
13	138.2	138.2	143.8
14	41.6	41.6	42.4
15	28.0	28.0	28.0
16	24.0	24.0	25.6
17	47.5	47.9	48.9
18	54.8	52.4	45.4
19	39.2	39.2	39.2
20	39.1	39.0	39.0
21	30.0	30.5	30.4
22	36.5	36.5	36.5
23	28.0	32.5	13.1
24	15.2	65.0	64.1
25	16.1	15.2	15.3
26	16.9	16.1	17.0
27	23.1	23.3	25.3
28	178.2	178.3	178.6
29	17.2	16.9	25.1
30	23.3	21.1	26.0

Table 3: ^{1}H and ^{13}C-NMR spectral data for compound V (CD_3OD, δ)*.

No	H	C
Aglycne 1	-	131.58
2	6.54, d (2.1)	117.17
3	-	146.16
4	-	144.70
5	6.57, d (8.1)	116.58
6	6.97, dd (8.1, 2.1)	121.31
α	3.26 - 4.03, m	72.16
β	2.79, t (7.5)	36.62
Caffeic acid-1`	-	127.75
2`	7.05, d(2.1)	115.34
3`	-	148.01
4`	-	149.78
5`	6.75, d (8.1)	114.81
6`	6.68, dd (8.1, 2.1)	123.20
α`	6.27, d (15.9)	116.38
β`	7.59, d (15.9)	146.85
C=O	-	168.32
glucose 1``	4.37, d (7.8)	104.27
2``	3.26 - 4.03, m	76.27
3``		81.67
4``	4.9, t (9.6)	70.71
5``	3.26 - 4.03, m	76.12
6``		62.46
Rhannose 1```	5.19, d (1.5)	103.03
2```	3.26 - 4.03, m	72.41
3```		72.27
4```		73.88
5```		70.45
6```	1.09, d (6)	18.48

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

Anti-inflammatory activity:

Thirty six adult male albino rats weighing 150-180 g body weight were used in this study. Rats were distributed into six groups (n=6) and were kept in separate cages on equal light cycle, 12 hr day and 12 hr night . They were left free on excess food and water *ad Libitium*. The animal groups were distributed as follows:

Gp.1: received the solvent (10 % gum acacia, 0.3 ml / 100 g b.w.) and served as control.

Gp.2: received hydrocortisone (Solu-Cortef®-UPJON) in a dose 200 mg/kg, injected intraperitoneally.

Gp.3 to gp.6: received the chloroform extract in a dose of 200 and 400 mg/kg, compound **I** (200mg/kg) and compound **III** (200 mg/kg).

The chloroform extract and compounds **I** and **III** were prepared as suspension in 10 % gum acacia and injected intraperitoneally.

Inflammation was induced according to Winter *et al* method[15]. Carrageenan was injected in a dose of 0.05 ml (1% in saline solution) in the subplanter region of the left hind paw after 1.5 hr of the injection of the solvent (control) and the tested samples. The difference in the sizes of edema were measured in the control, hydrocortisone and the tested samples at 0.5, 1.5, 2.0, 3.0, 4.0 and 6.0 hr after injection of carrageenan.

Statistical calculations:

The percentage thickness of the hind paw edema of rats were expressed as mean value ± S.E.M. (n=6) at different time intervals. Results reported as anti-inflammatory effects, were calculated as percentage inhibition of inflammation (A %) and recorded as the total area under the curve in comparison with the control group receiving only the solvent, 1.5 hr before injection of carrageenan[16]. The significance of the drug-induced changes was estimated using Student's t-test[17]. Relative potencies of the chloroform extract and compounds **I** and **III** versus hydrocortisone as a standard anti-inflammatory was calculated.

RESULTS AND DISCUSSION

The alcoholic extract of *V. supina* L was subjected to fractionation with $CHCl_3$ and EtOAc, followed by classical chromatographic isolation techniques as described in the experimental section. This has led to the isolation of compounds **I-V**.

Compound **I** proved to be a triterpenoid from its positive response to Liebermann Burchard test[18]. It showed IR absorption bands at 3411 cm^{-1} for an alcoholic function and at 1695 cm^{-1} due to a carbonyl function of an acid. The EIMS showed M^+ ion peak at m/z 456, corresponding to the molecular formula $C_{30}H_{48}O_3$. The presence of significant peak at m/z 248 and 203 resulting from retro-Diels-Alder cleavage of ring C suggested a structure of Δ^{12}-unsaturatd triterpen-28-oic acid[19,20]. The ^{1}H-NMR spectrum displayed seven methyl signals (Table 1), two of which appeared as doublets at δ 0.80 and 0.89 (for H-29 and H-30, respectively), an olefinic proton (δ 5.1 brs, H-12) and a methine proton (δ 3.0, H-3) in addition to a signal at δ 2.1, d, J = 11.4 Hz (H-18)[19,21-25]. This was confirmed by the appearance of characteristic C-12 and C-13 resonance at δ 124.6 (d) and 138.2 (s), respectively, in the ^{13}C-NMR spectrum. Comparing these data with the published data for ursolic acid[22,23] confirmed structure **I**. Ursolic acid has previously been isolated from *V. officinalis*[23].

Compound **II** gave positive Liebermann-Burchard's test indicating its steroidal or triterpenoidal nature. The analysis of its ^{1}H-NMR (Table 1) and EIMS spectra, comparison with the reported data[26,27] and TLC comparison with authentic sample of

oleanolic acid established the identity of compound **II** as oleanolic acid.

EIMS of compound **III** showed RDA fragmentation peaks at m/z 248, 203, 189, 175 and 133 characteristic for Δ^{12}-pentacyclic triterpenoids[7] and a molecular ion peak at m/z 454 (M^+-H_2O) corresponding to the molecular formula $C_{30}H_{48}O_4$. The ^{1}H and ^{13}C-NMR data (Tables 1& 2) showed very close similarity with those of **I** except for the presence of two doublets at δ 3.5 and 3.6 (J = 11.2) in addition of a ^{13}C-NMR signal at δ 65 attributed to an axial hydroxy methylene group assigned for 24-CH_2OH[7]. Comparing these data with published data[3,15-21] for similar compounds confirmed that compound **III** is 3β,24-dihydroxy-urs-12-en-28-oic acid.

The spectral data of compound **IV** was comparable with those of **III** except for the characteristic differences between oleanane and ursane skeletons[3]. Carbon resonance of **IV** at δ 45.4 (C-18), 121.6 (C-12) and 143.8 (C-13), the down field shift of H-18 at δ 2.78 and the presence of six tertiary methyl groups in ^{1}H-NMR (Table 1) in addition to molecular ion peak at m/z 454 $(M-H_2O)^+$ in EIMS gave clear evidence for an oleanan skeleton with a molecular formula $C_{30}H_{48}O_4$ .The spectral data were in accordance with reported data for 3β,24-dihydroxy-olean-12-en-28-oic acid[3,24,28].

IR spectrum of compound **V** showed an absorption band at 1692 cm^{-1} characteristic for a conjugated carbonyl ester that was confirmed by a down field signal at δ 168.32 in ^{13}C-NMR corresponding to C=O group. Acid hydrolysis and PC of the sugar moiety afforded glucose and rhannose. ^{1}H-NMR, ^{13}C-NMR (Tables 1& 2) and MS spectra indicated that α-L-rhamnose is the terminal sugar unit and β-D-glucose is the central one[29]. The ^{1}H-NMR spectrum showed two ABX systems assigned for two ortho-dihydroxy aromatic rings, one belongs to a caffeic acid moiety, and the other to 3,4-dihydroxyphenylethanol moiety. The chemical shifts for the acyl moiety signals were in accordance with those reported for caffeic acid and its derivatives[29,30]. The ^{1}H-NMR signals at δ 6.27 and 7.59 (d, J = 15.9, each) indicated trans configuration of two olefinic protons of the caffeic acid moiety. From the previous data and by comparison with the reported data[12,13,31,32], it can be concluded that compound **V** is actoside.

Anti-inflammatory activity:

The anti-inflammatory effects (Fig. 1-3) of the chloroform extract and compounds **I** and **III** started at 30 minutes after injection of carrageenan and terminated after 6 hours. There was no significant differences between the anti-inflammatory effect of the two tested doses of chloroform extract viz. 200 mg/kg and 400 mg/kg. The recorded inhibition of inflammation induced by the chloroform extract (200 mg/kg) was 84.3 %, 58.0 %, 75.4 %, 74.9 %, 91.3 % and 87 % in comparison with that of the control group at 0.5, 1.5, 2.0, 3.0, 4.0 and 6.0 hr after injection of carrageenan. The maximum anti-inflammatory effect was reached after 4 hr in the recorded peak (Fig. 1). Compounds **I** and **III** showed lower anti-inflammatory activity than that induced by the chloroform extract. The percentages inhibition of inflammation induced by compound **I** were 34.7, 42.0,53.9, 74.0, 49.0 and 47.5; while those induced by compound **III** were 34.7, 18.5, 58.8, 48.9, 35.9 and 23.6 at 0.5, 1.5, 2.0, 3.0, 4.0 and 6 hr after injection of carrageenan (Fig. 3).

The chloroform extract in doses 200 mg/kg and 400 mg/kg (Figs. 4 & 5) showed 44.65 %.hr and 41.89 %.hr total area under the curve (AUC), respectively. Moreover, the chloroform extract (200 mg/kg) showed 44.65 %.hr (AUC), while compounds **I** and **III** showed 80.46%.hr and 107.65 %.hr AUC, respectively (Fig. 5). The chloroform extract also recorded 74.3% inhibition in the AUC in comparison with the control group (Table 4). In the meanwhile, both compounds **I** and **III** showed 53.8% and 38.1 % inhibition in the AUC as compared with the control group, respectively (Table 4). Hydrocortisone recorded 49.6% inhibition in the AUC in comparison with the control group (Table 4).

Accordingly, the anti-inflammatory activity of the injected chloroform extract, compounds **I** and **III**, and hydrocortisone can be arranged in the following rank order: chloroform extract > compound **I** > hydrocortisone >compound **III**. They can be arranged according to their relative potencies (RP) to hydrocortisone (Table 4) as follows: chloroform extract (1.5), compound **I** (1.08), hydrocortisone (1.0) and compound **III** (0.77).

The present study showed that the chloroform extract induced a significant anti-inflammatory activity which was greater than that of hydrocortisone. The anti-inflammatory effect of the chloroform extract may be due to the presence of ursolic acid and oleanolic acid which was reported to be potent anti-inflammatory agent[14]. The anti-inflammatory effect of the chloroform extract is biphasic. The second phase was more pronounced than the first one and started after three hours from the injection of carrageenan and reached its peak after four hours. These results explain that the chloroform extract inhibits prostaglandin synthesis in the second phase of inflammation rather than blocking of histamine release in the first phase. Carrageenan was reported to induce biphasic inflammatory events[33]. The initial phase was attributed to the release of histamine and serotonin while, the second phase to prostaglandin release[34].

The potent activity of the chloroform extract in the second phase of inflammation is due to the suppression of mononuclear leukocyte migration into the inflamed tissues[34]. Ursolic acid (in the chloroform extract) not only inhibit leukocyte elastase but also 5-lipoxygenase and cyclo-oxygenase activity[35,36]. Moreover, it was reported to inhibit the concanavalin A-induced histamine release[37]. This

may also explain the less potent anti-inflammatory effect of the chloroform extract in the initial phase of carrageenan induced inflammation. The obtained anti-inflammatory effect of the chloroform extract is most probably due to the structure similarity of ursolic acid and oleanolic acid to the adrenal cortical hormones.

Table 4: The anti-inflammatory activity of hydrocortisone, the chloroform extract and compounds I & III on carrageenan- induced hind paw edema.

Treatment	Total AUC (%.hr) ± S.E	% Reduction in AUC	Relative potency to hydrocortisone
Solvent (control)	173.83 ± 24.46	--	--
Hydrocortisone (200 mg/kg)	87.60 ± 16.65	49.60	1.00
Chloroform ext (200 mg/kg)	44.65 ± 4.67	74.3	1.49
Chloroform ext (400 mg/kg)	41.89 ± 4.13	75.9	1.53
Compound I (200 mg/kg)	80.46 ± 23.11	53.8	1.08
Compound III (200 mg/kg)	107.65 ± 16.62	38.1	0.77

AUC = area under the curve; S.E. = standard error.

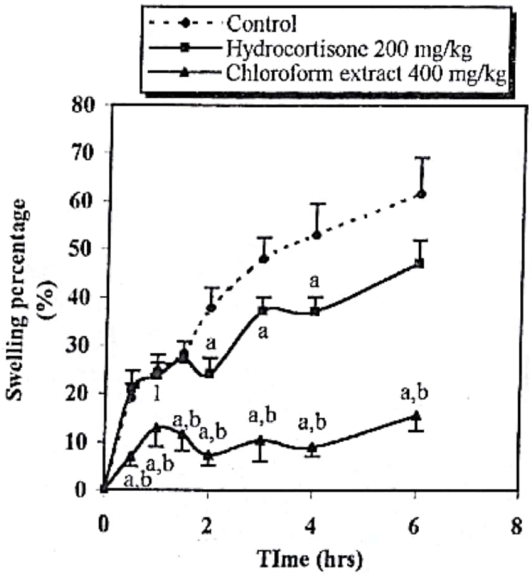


Fig. 1. Swelling percentage in the rat hind paw after injection of carrageen (1% solution) in normal rats and rats pretreated with 200 mg/kg Hydrocortisone and 400 mg/kg chloroform extract of *V. supina*.

a: Significantly different from control Group at P< 0.05.

b: Significantly different from hydrocortisone Group at P< 0.05

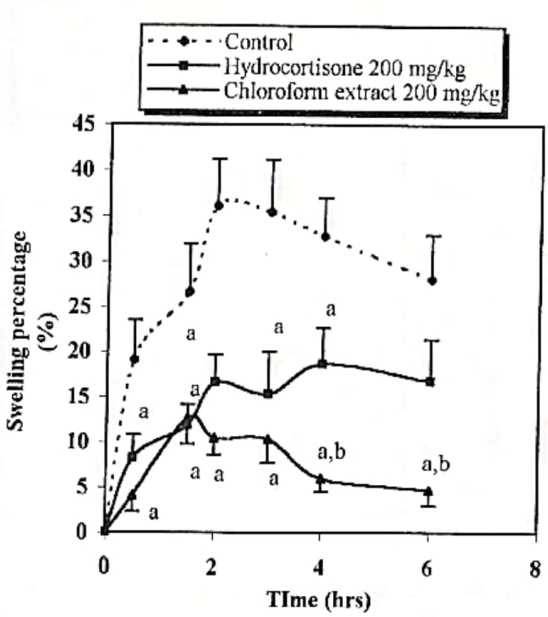


Fig. 2. Swelling percentage in the rat hind paw after injection of carrageen (1% solution) in normal rats and rats pretreated with 200 mg/kg Hydrocortisone and 200 mg/kg chloroform extract of *V. supina*.

a: Significantly different from control Group at P< 0.05.

b: Significantly different from hydrocortisone Group at P< 0.05

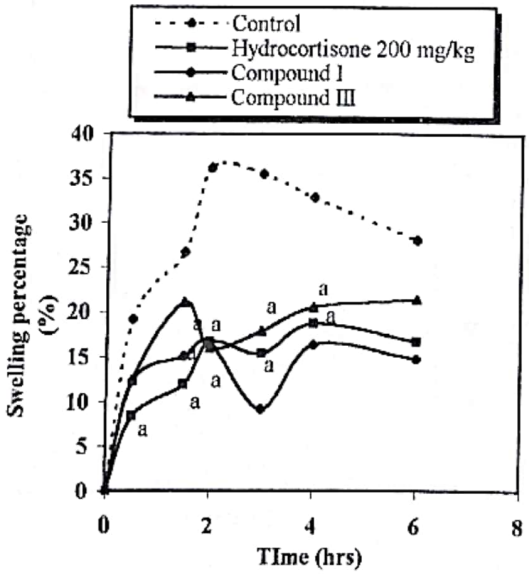


Fig. 3. Swelling percentage in the rat hind paw after injection of carrageen (1% solution) in normal rats and rats pretreated with 200 mg/kg Hydrocortisone, 200 mg/kg compound I and 200 mg/kg compound III.

a: Significantly different from control Group at P< 0.05.

b: Significantly different from hydrocortisone Group at P< 0.05

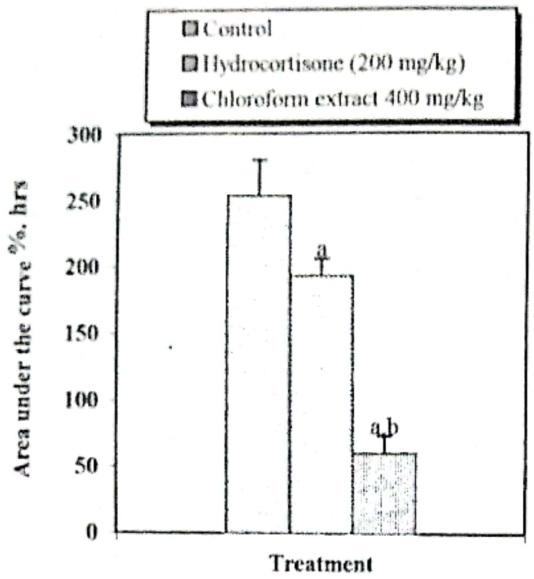


Fig. 4. The calculated area under the swelling percentage-time curves under the effect of carrageen (1% solution)injection in normal rats and rats pretreated with 200 mg/kg Hydrocortisone and 400 mg/kg chloroform extract of *V. supina*.

a: Significantly different from control Group at P< 0.05.

b: Significantly different from hydrocortisone Group at P< 0.05 .

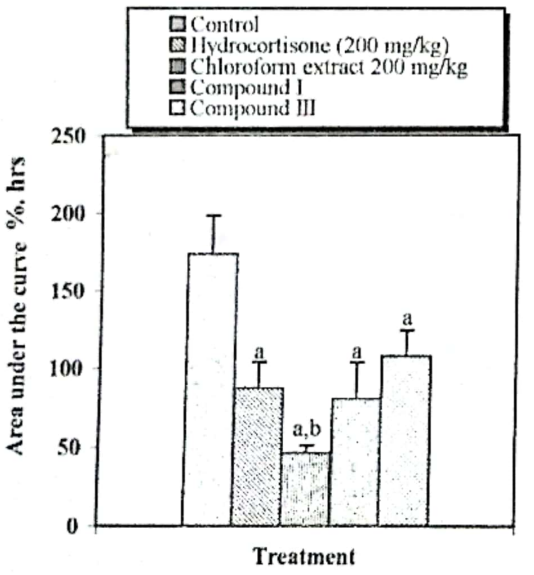


Fig. 5. The calculated area under the swelling percentage-time curves under the effect of carrageen (1% solution)injection in normal rats and rats pretreated with 200 mg/kg Hydrocortisone, 200 mg/kg chloroform extract of *V. supina*, 200 mg/kg Compound I and 200 mg/kg Compound III.

a: Significantly different from control Group at P< 0.05.

b: Significantly different from hydrocortisone Group at P< 0.05

The mechanism of action of steroidal anti-inflammatory agent is the blocking of all pathways of eicosanoid metabolism by stimulating the synthesis of lipocortins which inhibit phospholipase A_2 activity and prevent the release of arachidonic acid[38]. Moreover, they also inhibit both cellular and fluid egress from the vascular space to the site of inflammation in addition inhibition of cellular functions involved in the inflammation process[39].

Results of the present study also revealed that compounds **I** and **III** have less potent anti-inflammatory activity than that of the chloroform extract. However, compound **I** is similar to hydrocortisone and more effective than compound **III**.

Finally, the obtained results indicated that the potent anti-inflammatory activity of the chloroform extract may be due to a synergistic effect between ursolic acid and oleanolic acid, and their derivatives constituted in the extract. The chloroform extract of *Verbena supina*L. can be suggested as a potent anti-inflammatory in a pharmaceutical product rather than the isolated compounds.

REFERENCES

1. Täckholm, V.; "Students' Flora of Egypt", 2nd Ed., Cairo University, Co-operative Printing Company, Beirut, p. 454 (1974).
2. Watt, J.M. and Breyer-Brandwijk; M.G., "Medicinal and Poisonous Plants of Southern and Eastern Africa" 2nd Ed., E. and S. Livingstone LTD. Edinburgh and London, p. 1045 (1962).
3. Deepak, M. and Handa, S.S.; **Phytochemistry**, 49, 269 (1998).
4. Franke, A. and Rimpler, H.; **Photochemistry**, 26, 3015 (1987).
5. Terahara, N.; Shioji, T.; Toki, K.; Saito, N. and Honda, T.; **Phytochemistry**, 28, 1507 (1989).
6. Toki, K.; Terahara, N.; Saito, N.; Honda, T. and Shioji, T.; **Phytochemistry**, 30, 671 (1991).
7. Toki, K.; Yamamoto, T.; Terahara, N.; Saito, N.; Honda, T.; Inoue, H. and Mizutani, H.; **Phytochemistry**, 30, 3828 (1991).
8. Toki, K.; Saito, N.; Terahara, N. and Honda, T.; **Phytochemistry**, 40, 939 (1995).
9. Toki, K.; N. Saito, K.; H. Kuwano, K.; Terahara, N. and Honda T.; **Phytochemistry**, 38, 515 (1995).
10. Carnat, A.; Chavignon, O.; Heitz, H.; Wylde, R. and Lamaison, J.; **Planta Med.**, 61, 490 (1995).
11. Zaghloul, A.M.; **Mans. J. Pharm. Sci.**, 11, 43 (1995).

12. Zaghloul, A.M.; Amer, M.M.; El-Domiaty, M. and Salama, O.; **Alex. J. Pharm. Sci.**, 9, 129 (1995).

13. Lahloub, F.M.; Salama, O.M. and Mansour, E.S.; **Bull. Fac. Pharm. Cairo Univ.**, 28, 75 (1990).

14. Lui, J.; **J. Ethnopharmacol.**,49,57 (1995).

15. Winter, C.A.; Risley, E.A. and Nuss, G.W.; **J.Pharmacol .Exper. Therap.**, 141 ,369 (1963).

16. Backhouse, N.; Delporte, C.; Negrete, R.; Munoz, O. and Ruiz, R.; **Int.J.Pharmacog.**, 32,239 (1994).

17. Bowman, W.C. and Rand, M.J.; " Textbook of Pharmacology "; 2nd Ed.Black, Oxford, pp.13-18,22 (1980)

18. Leibermann, C. and Burchard, H.; **Chem. Zentr.**, 61, 1 (1890).

19. Budzikiewiez, H.; Jerassi, C.D. and Williams, D.H.; "Structure Elucidation of Natural Products By Mass Spectrometry"; Vol. II, Holden-Day, INC, San Francisco, London, Amsterdam, p. 122 (1964).

20. Haberlein, H. and Tschiersch, K.; **Phytochemistry**, 35, 765 (1994).

21. Siddiqui, B.S.; Farahat, F.; Begum, S. and Siddiqui, S.; **Planta Med.**, 63, 47 (1997).

22. Santos, G.G.; Alves, J.C.N.; Robilla, J.M.L.; Duart, A.P.; Lihgow, A.M. and Urones, J.G.; **Phytochemistry**, 44, 1309 (1997).

23. Siddiqui, H.; Siddigui, B.S.; Begum, S. and Naeed, A.; **Phytochemistry**, 29, 3615 (1990).

24. El-Domiaty, M.; El-Shafae, A.M.and Abdel-Aal, M.; **Alex. J. Pharm. Sci.**, 13, 1 (1999).

25. Afifi, M.S.;M.Sc. Pharm. Science Thesis, Cairo Univer., p. 45 (1969).

26. Afifi, M.S.; Amer, M.M.; Zaghloul, A.M.; Ahmed, M.M.; Kinghorn, A.D. and Zaghloul, M.G.; **Mans. J. Pharm. Sci.**, 17, 66 (2001).

27. Durham, D.J.; Liu, X.; Michael, R. and Richards, R.; **Phytochemistry**, 36, 1469 (1994).

28. Miranda, R.P.; Delgado, G. and Vivar, A.R.; **J. Nat. Product**, 49, 225 (1986).

29. Sawada, H.; Miyakoshi, M.; Isoda, S.; Ida, Y. and Shoji, J.; **Phytochemistry**, 34, 1117 (1993).

30. Birkofer, L.; Kaiser, C. and Thomas, U.; **Z. Naturforsch. Theil**, B23, 1051 (1968).

31. Kelley, C.J.; Mahajan, J.R.; Brooks, L.C.; Neurbert, L.A.; Brenneman, W.R. and Carmack, M.J.; **J. Org. Chem.**, 40, 1804 (1975).

32. Zimin, L. and Zhongjian, J.; **Phytochemistry**, 30, 1341 (1991).

33. Vinegar, R.; Schreiber, W. and Hogo, R.; **J.Pharmac.Exp.Ther.**, 166,96 (1969).

34. DiRosa, M. and Willoughby, D.A.; **J.Pharm.Pharmac.**, 23, 297 (1971).

35. Safayhi, H.; Rall, B.; Sailer, E. and Ammon, H.; **J. Pharmacol. and Exper. Therap.**, 281, 460 (1997).

36. Najid, A.; Simon, A.; cook, J.; Chable-Rabinovitch, H.; Delage, C.; Achulia, A. and Rigound, R.; **FEBS**, 299,213 (1992).

37. Tsuruga,T.; Chun,Y.; Ebizuka,Y.and Sankawa,U.; **Chem.Pharm.Bull.**, 39, 3276 (1991).

38. Katzung, G.B.; " Basic and Clinical Pharmacology"; 5th Ed.Appleton and Lange, A Publishing division of Prentice Hall pp.263-277 (1992).

39. Spiegel, T.M.; " Practical Rheumatology"; Printed by A.Wiley, Medical publication, John Wiley and Sons, USA, pp.161-183 (1983).

Received: May 7, 2003
Accepted: May 29, 2003

المحتوى الكيميائى والتأثير المضاد للالتهابات لنبات فربينا سوبينا الذى ينمو فى مصر

محمود محمد عبد العال، صلاح عبد المنعم غريب*

قسم العقاقير وقسم الفارماكولوجى* - كلية الصيدلة- جامعة الزقازيق- مصر

نتج عن الدراسة الكيميائية لنبات فربينا سوبينا فصل حمض الأورزوليك وحمض الأوليانوليك بالإضافة إلى مركب ٣ بيتا،٢٤- داى هيدروكسى- أورز- ١٢- اين- ٢٨- أويك أسيد ومركب ٣ بيتا، ٢٤- داى هيدروكسى- أوليان- ١٢- اين- ٢٨- أويك أسيد من خلاصة الكلوروفورم. كما تم فصل مركب أكتيوسيد (فينيل بروبانويد) من خلاصة خلات الايثيل. وتم التعرف على المركبات المفصولة باستخدام الأشعة فوق البنفسجية والأشعة تحت الحمراء والرنين النووى المغناطيسى ومطياف الكتلة.

وأثبت المسح الفارماكولوجى بأن خلاصة الكلوروفورم وحمض الأورزوليك ومركب ٣ بيتا،٢٤- داى هيدروكسى- أورز- ١٢- اين- ٢٨- أويك أسيد لهم تأثير قوى ضد الالتهابات المحدثة فى الجرذان باستخدام الكراجينان مقارنة بالهيدروكورتيزون. وكذلك أثبتت الدراسة أن خلاصة الكلوروفورم لها تأثير مضاد للالتهاب أقوى من الهيدروكورتيزون والمركبات المفصولة من تلك الخلاصة.