Research Journal of Agriculture and Biological Sciences, 5(5): 649-659, 2009

Some Pharmacochemical Investigations on *Verbena Tenuisecta*

[1,2]Khaled A.A., [1]Adnan A.E. and [3]Salwa, M.N.

[1]Al Tahady University, Faculty of Science, Chemistry Department, Sirt, Libya, P.O. 674.
[2]National Research Center, phytochemistry Department, Dokki, Cairo, Egypt.
[3]National Research Center, pharmacology Department, Dokki, Cairo, Egypt

Abstract: The volatile constituents of the flowering buds of *Verbena tenuisecta Briq*. were prepared by hydrodistillation method and analyzed by GC/MS. It was found that, they consists of a mixture of thirteen compounds in which the main compounds are 1-octen-3-ol (52.87%), bicyclo[3.2.0]-heptan-2-one-6-hydroxy-5-methyl-6-vinyl (13.89%) and limonene (9.33%). Investigation of the flavonoidal constituents led to isolation of luteolin, apigenin from the chloroform fraction, while chrysoeriol-7-*O*-glucoside, chrysoeriol-7-*O*-rhamno-arabinoside and isorhamnetin-3-*O*-rhamno-glucoside were isolated from the butanol fraction of the methanolic extract. All compounds were identified by chromatographic and spectroscopic methods (UV, MS, ^{1}H and ^{13}C NMR spectroscopy). The analgesic, anti-inflammatory and atiulcerogenic effects of methanolic extract were determined. The obtained results reveled that, it exhibited significant effects.

Key words: *Verbenaceae*, volatile oil, flavonoids, anti-inflammatory and analgesic activity.

INTRODUCTION

Verbena tenuisecta Briq. is native to South America and it is now grown in Libya.[1] Some species of genus *Verbena* currently used in traditional Central and South American folk medicine against diarrhea, fever, gastrointestinal disorders and some sexually transmitted disease,[2] it is used as an anti-inflammatory topical applications[3]. The phytochemical investigations of some *Verbena* species led to isolation of various constituents include volatile oil,[4] sterols[5], triterpenoids[6], iridoids[7,8] and phenolic compounds (flavonoids[9,10], dihydrochalcone[11], anthocyanidin[12], phenylethanoids[7]).

MATERIALS AND METHODS

Plant Material: *Verbena tenuisecta* Briq. was collected from central garden at Sirte city, Libya in August 2005 during the flowering stage, the plant was kindly identified and authenticated by Dr. Mohamed N. Abohadra prof. of Taxonomy at Botany department, faculty of science, Al Fateh university, Tripoli, Libya. A voucher specimen was deposited at the herbarium of Botany department, faculty of science, Al Tahady University, Sirt, Libya.

Instruments:

i- GC/MS QP 1000E Schimadzu.
ii- Ultraviolet viewing lamp: At the long wavelength (366 nm).
iii- UV-Vis. Spectrophotometer 2401 Schimadzu in region of wavelength from 200 nm to 500 nm.
iv- Bruker NMR spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C.
v- Jeol MNR system 300.

Electron Impact Mass Spectroscopy (EI/MS) using GC/MS QP 1000 E Schimadzu.

Gas Chromatography Conditions:

Instrument	:TRASC GC, Splitless Mode.
Column	:DB-5 Capillary column (30 m, 0.25 mm internal diameter, 0.25 (µm film).
Temperature program	:Injector 50 C°, initial temp. 38 C°, Rate, 2 C°/min. to 200 C°, final temp. 200 C° for 5 min.
Flow gas	:Helium at 10 ml/min.

Mass Spectroscopy:

Instrument :TRACE DSQ.
Full scan 50-450, positive ion, Ion source 200 C°, mass transpher line 200 C°. Library :NIST.
The mass spectra were measured in EI scan Mode at (70 e.v.) from 50 to 450 mass units.

Corresponding Author: Khaled A.A., Al Tahady University, Faculty of Science, Chemistry Department, Sirt, Libya, P.O. 674.
E- mail: khabdelhady@yahoo.com
Tel.: 00218 91 40 30 557

Drugs and Chemicals:

- Indomethacin capful from Kahira pharm.&chem. Co., Cairo, Egypt.
- Carrageeenan from sigma –Alderich company, USA.
- Piroxicam from medical union pharm. Co., Abusultan, Ismalia, Egypt.

Hydrodist illation Extraction of the Volatile Constituents: About 350 g of the fresh plant material (flowering buds) of *V. tenuisecta* were subjected to water distillation in all-glass apparatus for about three hours according to Gunther method[13]. The trapped oil was removed after complete distillation and dried over anhydrous sodium sulphate to give pale yellow oil having a characteristic odor (0.02% v/w) and subjected to GC/MS.

Extraction and Fractionation of the Flavonoidal Constituents[14]: About 1.5 kg of the defatted powdered plant material (aerial parts of *V. tenuisecta*) was macerated with methanol (70%) till exhaustion. The alcoholic extract were evaporated in *vacuo* at about 45C° (85.7 g), dissolved in hot distilled water (600 ml), left over night in refrigerator and then filtered. The aqueous filtrate was extracted with successive portions of chloroform (4 x 400 ml) followed by butanol (5 x 500 ml). The solvents were dried; separately; over anhydrous sodium sulphate and evaporated in *vacuo* at 40 C° and 50 C° respectively. The chloroform and butanol free residues amounted to be 5 g and 18.3 g respectively.

Isolation of Flovonoidal Compounds: About 4 g of the chloroform extract were chromatographed on silica gel (60-120 mesh, BDH) column (5 x 80 cm) packed as aslurry in chloroform. Elution was affected with chloroform with increasing the polarity with methanol. Fractions of 50 ml each were collected; the chromatographic fractionation was monitored using PC and 20% acetic acid as a developing solvent. Further purification over Sephadex LH-20 column for the collected fractions (18-23) afforded compounds**1**and **2** while compound-**3** was obtained from fraction 37-45.

About 15g of the butanol extract were chromatographed over a polyamide column (6x75). Elution started with distilled water followed with decreasing the polarity using methanol till methanol 100%. The fraction 19-23 containing compund-**4** were collected and rechromatographed over small column of Sephadex LH-20 eluted with methanol: water (85: 15) and collecting small fractions (10 ml each). The fractions containing compound-**4** were collected and the solvent was evaporated in *vacuo* till dryness at 45C°. The methanolic solution of this fraction was subjected to PPC in BAW 4:1:5 upper layer. The main zone (compound-**4**) was localized and eluted with methanol: water (80:20) to give compound-**4** in pure form after passing over Sephadex LH-20 column.

The fractions containing compound-**5** in semi pure form (PC, 20% acetic acid R_f = 0.59) were collected, the solvent was evaporated in *vacuo* till dryness at 45 C° and subjected to PPC using 15% acetic acid as an irrigating solvent. The main zone (compound-**5**) was localized eluted with methanol (80%) and passed through small Sephadex LH-20 column to afford compound-**5** in pure form.

Acid Hydrolysis: Abut 5 mg of the compound-**3** were dissolved in 25 ml of 2N HCl: MeOH (1: 1) and refluxed on boiling water bath for 2 hours. After complete hydrolysis, the solvent was evaporated and diluted with distilled water. The aglycone was extracted with ethyl acetate (3 x 50 ml). The ethyl acetate extract was washed with distilled water till free from acidity. After solvent evaporation the obtained aglycone was further purified by passing over a small Sephadex LH-20 column eluted with methanol to afford the aglycone in pure form, which was analyzed by MS.

The aqueous acidic solution after separation of the aglycone was neutralized with barium carbonate, filtered and evaporated till dryness. The residue was dissolved in isopropanol and the mixture of sugars was investigated by PC using phenol saturated with water as developing solvent against some authentic sugars. The chromatograms was dried and sprayed with aniline phathalate reagent and heated in an oven at 110 Cº for 5 min.[15]

Biological Study:

Animals: Sprague - Dawley strain rats or Swiss albino mice were obtained from the animal house colony from the National Research Center (NRC), Dokki, Cairo, Egypt. The average weights ranged between 100-120 gm for rats and 25 gm for mice. Food and water were provided ad libitum. The animals were grouped into groups of six animals each. The doses employed were based upon the human dose after conversion to that of rats[16] .

Acute Toxicity Determination: Animals were picked randomly and divided into groups, each of 6 mice of both sexes (25 gm). Doses were estimated in mg/100 g body wt. injected animals were observed for any possible toxicological symptoms for 24 hours.

Tests of Inflammation:

Carageenan-induced Paw Edema[17]: *V. tenuisecta* alcoholic extract was evaluated for anti-inflammatory activity using the carrageenan-induced paw edema

assay in rats according to reference 18. The animals were divided into four groups and the effect of oral administration of *Verbena* extract at doses of 50 or 100 mg/kg (0.5 ml, s.c., n = 6/ group) or indomethacin (10 mg/kg, s.c., 0.5 ml) given as a 60 min pretreatment was studied. The control group received saline (0.5 ml, n = 6 per group; s.c.). Paw edema was induced by sub-plantar injection of 100 µl of a 1% sterile carrageenan lambda in saline into the right hind paw. Contralateral paw received an equal volume of saline. Paw volume was determined immediately before carrageenan injection and at selected times thereafter using a plethysmometer (Ugo Basile, Milan, Italy). The edema component of inflammation was quantified by measuring the increase in paw volume (ml) at before carrageenan injection and at 1, 2, 3 and 4 h after carrageenan injection with respect to the pre-injection value for each animal. Edema was expressed as a percentage of change from control (pre-drug) values.

AntiNociceptive Activity: This activity was determined by measuring the responses of animals to thermal and chemical stimuli as follow:-

Hot-plate Assay (Thermal Stimulus)[18]: The hot plate test was performed on rats by using an electronically controlled hot plate (Ugo Basile, Italy) heated to 52 C° (± 0.1 C°). The cut-off time was 30s. Groups of rats (*n = 6* / group) were given *V. tenuisecta* alcoholic extract at doses of 50 or 100 mg/kg, saline (control), or indomethacin at 20 mg/kg 30 min prior to testing. The experimenter was blind to dose. Latency to lick a hind paw or jump out of the apparatus was recorded for the control and drug-treated groups. Pain thresholds were measured sequentially before and at 1, and 2h post-treatment.

Acetic Acid-induced Writhing (Chemical Stimulus)[19]: Rats were injected intraperitoneally with 0.6% aqueous acetic acid (10 ml/ kg) 60 min after oral administration of piroxicam (2.6mg/kg) or *Verbena* at 50 or 100 mg/kg or saline (control). Each mouse was then placed in an individual clear plastic observational chamber, and the total number of writhes made by each mouse was counted for 30 min after acetic acid administration.

Gastric Ulcerogenic Studies: Eighteen rats were fasted for 18 h before the experiment with water ad libitum. The animals were divided randomly into three groups. Gastric mucosal damage was evoked in rats by the administration of indomethacin (20 mg/kg, i.p.). The effect of *V. tenuisecta* (50 ort 100 mg/kg) administered at time of indomethacin injection was studied. Food and water were provided ad libitum. Rats were killed 24 h after drug administration, stomachs excised, opened along the greater curvature, rinsed with saline, extended on a plastic board and examined for mucosal lesions. The number and severity of mucosal lesions were noted and lesions were scaled[20].

Statistical Analyses: Paw edema experiment data are expressed as mean ± S.E. The results of carrageenan-induceds are expressed as a percentage of change from control (pre-drug) values. Differences between vehicle control and treatment groups were tested using one and two-way ANOVA followed by multiple comparisons by the Duncan's multiple comparison tests. When there were only two groups a two-tailed Student's t test was used. A probability value less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSIONS

Results: The GC/MS of the volatile oil of the plant showed that, it is a mixture of thirteen compounds. The identified compounds represent several chemical classes, *vis.* : alcohols (60%), bicyclic monoterpenes (16.55%), monocyclic monoterpenes (11.95%), aromatics (6.69%), aldehydes (3.25%), acyclic monoterpenes (0.59%). Also, the results showed that 1-octen-3-ol (52.87%), bicyclo [3.2.0] heptan-2-one-6-hydroxy-5-methyl-6-vinyl (13.89%) and limonene (9.33%) are the main compounds. These data were in agreement with that reported by Mohammad *et al*[4] where they investigated the volatile oil of *V. officinalis* in 2003 and identified 1-octen-3-ol as one of the main compounds in addition to Chalchat and Garry[21] were identified limonene in the volatile oil of the same plant in 1996.

Discussion: The GC/MS data of the volatile oil were in agreement with that reported by Mohammad *et al*[4] where they investigated the volatile oil of *V. officinalis* in 2003 and identified 1-octen-3-ol as one of the main compounds in addition to Chalchat and Garry[21] were identified limonene in the volatile oil of the same plant in 1996.

The Study of Flovonoids Led to Identification of Fine Compounds as Follow:

Compound-1, *Luteolin*: The chromatographic behavior of this compound proved that it is an aglycone. The UV absorption data showed that it is a flavone type with *ortho* dihydroxy system in ring B in addition to a free OH at C-7.

The EI-mass spectrum of compound-1 showed a molecular ion peak [M^+] at m/z =286 (43.5%) which corresponding to the molecular formula $C_{15}H_{10}O_6$. Another important peaks at m/z = 285[M^+ - H],

(100%), m/z = 258 [M^+ - CO], (41.9 %) and m/z = 264 (M^+ - H_2O) (32.3%).

The fragmentation pathway of compound-1 undergoes Retero Diel's Alder reaction (RDAR) giving rise to fragments at m/z = 152 [$A_1^{\cdot+}$] (46.7%) and 134 [$B_1^{\cdot+}$] (16.1 %) and [$B_2^{\cdot+}$ - H] at 137 (43.5 %).
The ^{1}H-NMR data showed signals at δ in ppm 7.5 (1H, d, J = 8 Hz, H-2'), 7.4 (1H, d, J = 9 Hz, H-6'), 6.92 (1H, d, J = 8.5 Hz, H-5'), 6.55 (1H, s, H-3), 6.44 (1H, d, J = 3 Hz, H-8) and 6.2 (1H, d J = 3 Hz, H-6), which are in agreement with those reported for luteolin [14] while ^{13}C-NMR data were confirm the flavone nature of compound-1 where C-4 appears at 180.65 ppm and the other data were coincided with that reported for Luteolin as shown by Kumari *et. al.* [22]. All these data were coincided with that reported for Luteolin, so compound-**1** could be identified as Luteolin. This compound was previously isolated from other *Verbena* species as reported by Zaghloul *et al.*[9] and Al-Azizi *et al.*[23]

Compound-2, *Apigenin*: The UV absorption data of compound-2 proved that it is a flavone type with a free OH group at C-7. The EI-mass spectrum showed a molecular ion peak [$M^{\cdot+}$] at m/z = 270; (13.7 %) which correspond to the molecular formula $C_{15}H_{10}O_5$. The most important fragments at m/z = 242 [M^+- CO], (8.6%) and that result from Retero Diel's Alder reaction (RDAR) at m/z = 152 [$A_1^{\cdot+}$] (27.5%), m/z = 118 [$B_1^{\cdot+}$] (17.2%) and m/z = 120 [B_2 -H] (8.6 %) are indicated the presence of only one OH group in ring-B and the other OH groups are at ring-A.

These data were in agreement with that reported for apigenin. So, we can identify compound-**2** as Apigenin. Chen *et al.*[24] isolated recently this compound from *V. officinalis* in 2006.

Compound-3, *Chrysoeriol-7-O- glucoside*: The chromatographic behavior of this compound in different solvent systems proved that it is a monoglycosidic in nature. The UV absorption spectrum of the compound-3 in spectroscopic methanol displayed band-I at 337 nm, which indicates the flavone nature of this compound. A bathochromic shift 52 nm was noticed in band-I with increasing intensity in on addition of NaOMe indicates the presence of a free OH group at C-4'.

The EI-mass spectrum of compound-3 figure (28) showed a molecular ion peak [M^+] at m/z = 462 (16.3%) which constituted with the molecular formula $C_{22}H_{22}O_{11}$. The presence of a peak at m/z = 300 indicates the sugar is hexose [M^+ - 162]. There are another important fragments at m/z = 446 [M^+ - CH_3], m/z = 416 [M^+ - (OH + OCH_3)], m/z = 405 [(M^+ + 1) - (CO + OCH_3)] and m/z = 286 [M^+ - (CH_3 + hexose moiety)]. The fragmentation pathway of compound-3 undergoes RDAR giving rise at m/z = 152, m/z = 149, m/z = 151. From this fragmentation pathway we can say that the methoxy group was present at C-3' in ring-B.

The mass spectrum of the obtained aglycone of compound-3 after acid hydrolysis displayed a molecular ion peak at m/z = 300 [$M^{\cdot+}$] (33.3%) corresponding to the molecular formula of $C_{16}H_{12}O_6$ also the peaks at m/z = 272 [$M^{\cdot+}$ - CO], m/z = 286 [$M^{\cdot+}$ - CH_3] and m/z = 152 [A_1] confirm the identity the chrysoeriol.
Finally, the chromatographic,acid hydrolysis and the available spectroscopic data substantiated that compound-**3** is Chrysoeriol-7-*O*-glucoside[13]. The compound was isolated before from other *Verbena* species by Michal *et al.*[10].

Compound-4, *Chrysoeriol-7-O- rhamno-arabinoside*: The UV absorption spectrum of compound-**4** showed that it is a flavone type with afree OH at C-4'.

The EI-mass spectrum of compound-4 showed a molecular ion peak [M^+] at m/z = 578 (20.6%) which constituted with the molecular formula $C_{27}H_{30}O_{14}$. The presence of a peak at m/z = 300 (578 - 278) means the aglycone may be Chrysoeriol and the sugar moiety is a disaccharide [pentose + deoxyhexose (132 + 146 = 278)].

About 4 mg of compound-4 were subjected to acid hydrolysis. The aglycone was identified as chrysoeriol, which, means the sugars are attached at C-7 (confirmed by UV measurements).The partial acid hydrolysis proved that, the presence of chrysoeriol 7-*O*-rhamnoside that means rhamnose attach directly to the flavonoid ring. The identified sugars are arabinose and rhamnose. This means that the two sugars were present at the same position on the aglycone. MS confirmed the identity of the chrysoeriol.

Finally, the chromatographic and the available spectroscopic data substantiated that compound-**4** is Chrysoeriol-7-*O*-rhamno-arabinoside. But the position of attachment between the two sugars and the aglycone still unknown because it require further investigation by NMR measurements but we have small amount from this compound. A similar compound known as Chrysoeriol-7-*O*-rhamno-glucoside was isolated from *V. bipinnatifida* by Michale *et al.* [10] in 2001.

Compound-5, *Isorhamnetin-3-O-rhamno-glucoside*: The UV absorption spectrum of the compound-5 showed peak-I in methanol at 348 nm (flavone type structure or highly substituted flavonol at C-3)[14]. A bathochromic shift (53 nm) was noticed in band-I with increasing in intensity on addition of NaOMe indicating the presence of free OH group at C-4'.

The $AlCl_3$ spectrum showed a bathochromic shift (38 nm) in peak-I relative to methanol indicate the presence of a free OH group at C-5. Moreover the absence of an *ortho*-dihydroxy system was confirmed through the $AlCl_3$/HCl spectrum where, there is no hypsochromic shift was observed in peak-I. The presence of free OH group at C-7 was confirmed, where there is a bathochromic shift (9 nm) in peak-II was noticed in NaOAc spectrum. Also, the absence of an *ortho*-dihydroxy system was confirmed through NaOAc/H_3BO_4 spectrum where there is no bathochromic shift in band-I relative to methanol.

The ^{1}H-NMR spectrum (DMSO) of compound-5 showed signals at δ in ppm 7.95 (1H, d, J = 7.5 Hz, H-2'), 7.55 (1H, d, J = 8.5 Hz, H-6'), 6.85 (1H, d, J = 8 Hz, H-5'), 6.35 (1H, d, J = 3.5 Hz, H-8), 6.25 (1H, d, J = 3.5 Hz, H-6) and 3.95 (3H, s, OCH_3-C3'). Two anomeric protons appears as doublet at δ = 5.2 and 4.55 ppm attributed to glucose and rhamnose respectively. The CH_3 group protons of rhamnose appear as doublet at 1.2 ppm.

The ^{13}C-NMR spectrum of compound-5 in DMSO showed the most important signal of flavonol type structure where C-4 appears at 179.34 ppm, and other data were summarized in table (2).[25]

The EI-mass spectrum of compound-**5** showed a molecular ion peak [M^+] at m/z = 624 (20.5%) which corresponding to the molecular formula $C_{28}O_{16}H_{32}$. which confirm the presence of isorhamnetin as an aglycone m/z = 316 (23%) and two sugar moieties as hexose and deoxyhexose.

The acid hydrolysis revealed the presence of rhamnose and glucose as sugars and isorhamnetin as an aglycone.

Also the identity of (the aglycone) isorhamnetin was confirmed by its mass spectrum which displayed a molecular ion peak at m/z = 316 [$M\cdot^+$] corresponding to molecular formula of $C_{16}H_{12}O_7$ and other peaks at m/z = 315 [$M\cdot^+$ - H], m/z = 288 [$M\cdot^+$ - CO], m/z = 286 [$M\cdot^+$ - OCH_3], m/z = 152 [ring A fragment], characteristic for isorhamnetin.

Finally, the chromatographic and the available spectroscopic data substantiated that compound-5 is isorhamnetin-3-*O*-rhamno-glucoside.

Finally, the isolated flavonoids were in accordance with that reported for *verbena* genus except compound-**5**, which isolated for the first time from this genus[9,26,27] but there is some of investigators isolated flavonol type structures like quercetin, kaempferol, penduleten, Artemetin and their glycosides from various *Verbena* species[28].

Acute Toxicity: Oral administrations of different doses of the alcoholic extract of *V. tenuisecta* up to 500 mg/100 g b.wt. to mice induced no toxic effect and all treated animals were alive 24 hours later.

Effect of *V. Tenuisecta* Alcoholic Extract on Carrageenan-induced Paw Edema: The results revealed that oral administration of 50 mg/kg, *V. tenuisecta* extract reduced paw edema by -21.4%, 28% at 3 and 4 h post-carrageenan at 100 mg/kg 25.3%, -27.8, -29.4, -35.5% decrease in paw edema was seen at 1, 2, 3 and 4h post-carrageeena respectively the non steroidal anti-inflammatory agent indo-methacin given at 10 mg/kg reduced paw edema by -12.5%, -19.6%, -31.8%, 33.3% at1, 2, 3 and 4h post-carrageeena respectively as shown in table (3) and fig.(1), these data were in agreement with that reported by Deepak and Handa[6,29] where they reported that, the chloroform extract of *V. officinalis* exhibited antiinflammatory activity (56%) at a dose = 500 mg kg $^{-1}$ body weight when compared with 79% for ibuprofen at 50 mg kg $^{-1}$ b. wt. This activity may be due to the presence of phenolic compounds which have antioxidant potential as inflammation involves oxidative damage.

Hot-plate Test: The data in table (4) and fig. (2) Proved that, *V. tenuisecta* caused an increase in hot-plate latency indicating the analgesic effect of the extract.

At 50 mg/kg 34.7 & 57.6% increase in hot plate latency at 1/2 and 1h post-treatment was seen compared with the pre-drug basal values (0 time). Higher dose (100 mg/kg) of the extract caused 59.6 & 68% increase in hot plate latency at 1/2 and 1h post-treatment Indomethacin caused 28.9 & 53.6% increase in hot-plate latency at 1/2 and 1h post-treatment, which means the extract is more potent analgesic than indomethacin.

The analgesic effect of the methanolic extract may be attributed to the presence of flavonoids and iridoids as reported by Calvo *et al.*[3,30] in 2006& 1997, also suggests that the analgesic effect may be mediated via supraspinal mechanism[31].

Acetic Acid-induced Writhing: Marked inhibition of the number of abdominal constrictions by 52.6% & 89.2% compared with the control group was seen when *V. tenuisecta* extract was administered at 50 and 100 mg/kg, respectively. These values were significantly higher than that obtained with piroxicam which inhibited the writhing response by 35.1% as shown in table (5) and fig. (3) These data were in accordance with that reported by Calvo in 2006[3] where he proved that the prepared topical ointment containing *V. officinals* exhibited an analgesic effect at doses 1-3%.

Number and Severity of Gastric Lesions: The oral administration of *V. tenuisecta* at 50 and 100 mg/kg together with indomethacin caused a dose-dependent reduction in the number and severity of gastric mucosal lesions caused by injection of indomethacin in rats. An

Table 1: GC/MS data of volatile oil of *V. tenuisecta*

Peak No.	R_t (Min)	Relative %	Mass spectral data			Compounds
			M^+	B.P.	Fragments	
1	4.18	1.25	92	91	90(6), 65(8), 63(6), 39(8).	Toluene.
2	7.38	0.59	106	91	77(15), 51(8), 39(8).	1, 6-Heptadien-3-yne, 5-methyl.
3	12.51	0.77	136	93	121(115), 94(8), 79(23), 77(29).	α-pinene.
4	13.29	52.87	128	57	111(2), 110(4), 72(17), 67(8).	1-Octen-3-ol.
5	15.75	9.33	136	93	107(19), 92(33), 68(73), 67(31).	Limonene.
6	17.74	1.32	142	57	99(6), 83(19), 70(18), 56(29).	2-Nonen-1-ol
7	21.23	1.89	166	93	135(23), 105(21), 91(32), 77(17).	3(7)-Carene, 4-hydroxymethyl.
8	24.84	5.03	174	55	126(3), 83(23), 69(48), 14(85).	1, 10-Decanediol.
9	43.98	2.62	180	41	123(15), 96(77), 81(79), 55(96).	Cyclododecene, 1-methyl.
10	44.66	3.25	180	109	135(15), 124(59), 81(48), 68(75).	8, 10- dodecadienal.
11	44.97	1.75	202	82	184(15), 98(96), 69(82), 55(54).	1, 12-dodecandiol.
12	46.81	5.44	220	205	177(18), 163(4), 145(2), 77(52).	2, 4,6-triisopropylphenol.
13	56.91	13.89	166	97	111(4), 98(6), 55(23).	Bicyclo [3.2.0] heptan-2-one, 6-hydroxy-5-methyl-6-vinyl.

Table 2: ^{13}C-NMR data of compound-1&5

Carbon No.	Compound -1	Comound-5		
2	164.19	158.31	rhamnose	
3	102.84	136.55	1"	94.87
4	181.65	179.34	2"	71.99
5	161.46	162.82	3"	73.80
6	98.84	101.83	4"	78.08
7	163.89	165.89	5"	75.88
8	93.84	99.92	6"	17.94
9	157.27	158.72	glucose	
10	103.67	105.59	1"	105.87
1'	121.48	123.72	2"	74.95
2'	113.37	114.54	3"	73.80
3'	145.75	150.76	4"	73.08
4'	149.71	148.28	5"	79.01
5'	116.06	115.90	6"	67.33
6'	118.95	122.85		
OCH_3	-	56.89		

Table 3: Anti-inflammatory effect of *V. tenuisecta* extract at on carrageenan-induced rat paw edema

Groups	After 1 hr		2 hrs		3 hrs		4 hrs	
	% edema X+S.E	% edema inhibition	% edema X+S.E	% edema inhibition	% edema X+S.E	%edema inhibition	% edema X+S.E	% oedema inhibition
Control	79.1 ± 5	-	97.0±6.4		110.0 ± 5.8	-	113.0 ± 8.0	-
V. tenuisecta extract 50mg/kg	82.45 ± 1.7	±4.2	95.85 ±5.6	-1.2	86.42 ± 5.6*	-21.4	81.32 ± 5.6*	-28
V. tenuisecta extract 100 mg/kg	59.1 ± 1.9*	-25.3	79.99 ±2.3*	-27.8	77.65 ± 6.5*	-29.4	72.9 ± 6.0*	-35.5
Indomethacin 10mg/kg	69.15 + 7.1	-12.5	78.0 +5.0*	-19.6	75.0 + 6.0*	-31.8	75.3 + 6.2*	-33.3

Data are presented as mean ± SEM, Significant change from control values at respective time
Points are denoted by * P < 0.05 (one way ANOVA & Duncan test)

Table 4: Analgesic effect of *V. tenuisecta* extract (hot plate test)

Group	Pre-drug value	1 hr		2 hrs	
	X^- +SEM	X^- +SEM	% of change	X^- +SEM	% of change
Control Saline	20.97±2.03	21.85±1.7	-	20.85±2.1	-
V. tenuisecta extract 50mg/kg	20.68±0.47	27.85±.0.86*	34.7	32.6±2.1	57.6
V. tenuisecta extract 100mg/kg	19.33±2.4	30.85±3.49*	59.6	32.48±2.2*	68
Indomethacin	18.33+0.69	23.5+1.66*	28.9	28.01+1.2*	53.6

· Data are presented as mean ± SEM, % of change from basal (pre-drug) value for each group . Value is denoted by * P < 0.05 (one way ANOVA & Duncan test)

Table 5: Effect of *V. tenuisecta* extract on number of abdominal constrictions induced in mice by ip injection of 0.6% acetic acid

Group	Number of contraction X^- + SEM	% of change
Control	57.0±6	-
V. tenuisecta extract 50mg/kg	27.0±1.3*	- 52.6
V. tenuisecta extract 100mg/kg	6.17±1.0*	-89.2
Piroxicam 2.6mg/kg.	37.0+2.1*	-35.1

Table 6: Effect of *V. tenuisecta* extract on number and severity of gastric mucosal lesions

Group	Dose mg/kg	Number of lesions/ rat/ X^- ± SEM	% of change	Severity of lesion/ rat X^- ± SEM	% of change
Indomethacin (control)	10 mg/kg	9.4±0.6	-	12.2±0.9	-
V. tenuisecta extract	50mg/kg	6.5±0.7*	-31	8.2±0.7*	-32.8
V. tenuisecta extract	100mg/kg	5.0+0.4**	-46.8	5.3+0.5**	-56.6·

Data are presented as mean ± SEM, % of change are calculated vs control group. * P < 0.05 ** P < 0.01 (student's t test)

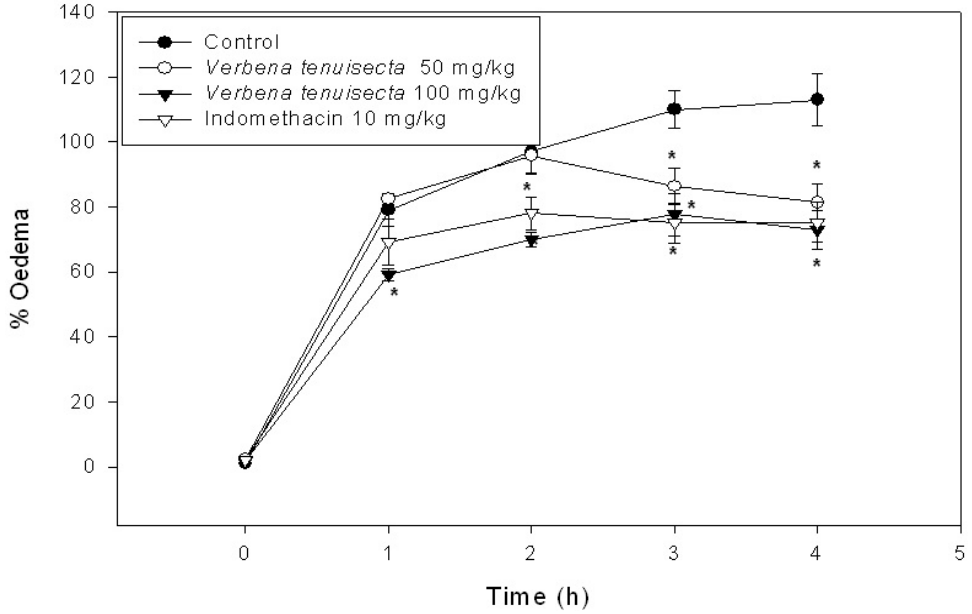


Fig. 1: Effect of *V. tenuisecta* extract oncarrageenan-induced rat paw edema

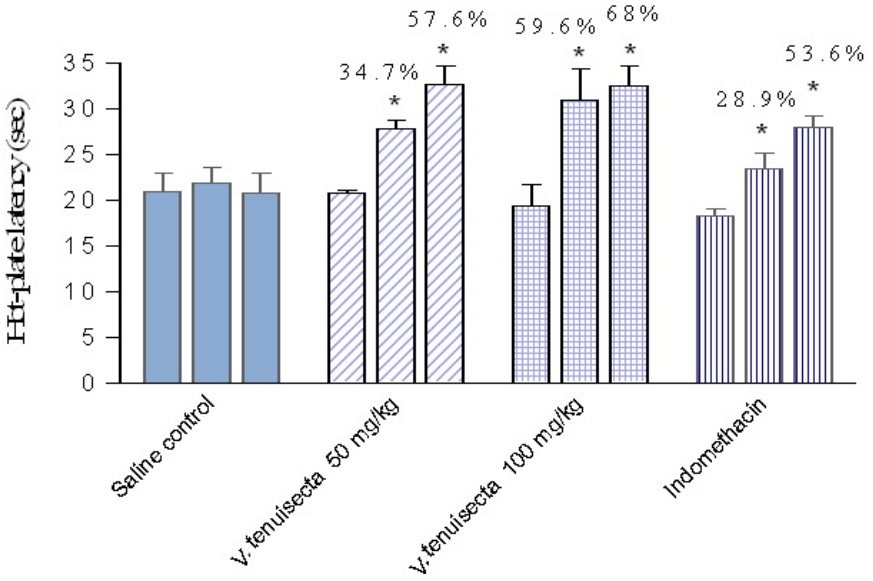


Fig. 2: Effect of *V. tenuisecta* extract on the thermal pain response in the hot-plate test in mice

Table 7: Effect of *V. tenuisecta* extract on floating time (Forced immobility test)

Group	Seconds "immobile" time out of 6 minutes $\bar{X} \pm$ SEM
Control	225 ± 6.7
V. tenuisecta extract 50mg/kg	220 ± 4.8
V. tenuisecta extract 100mg/kg	234.2 ± 2.9

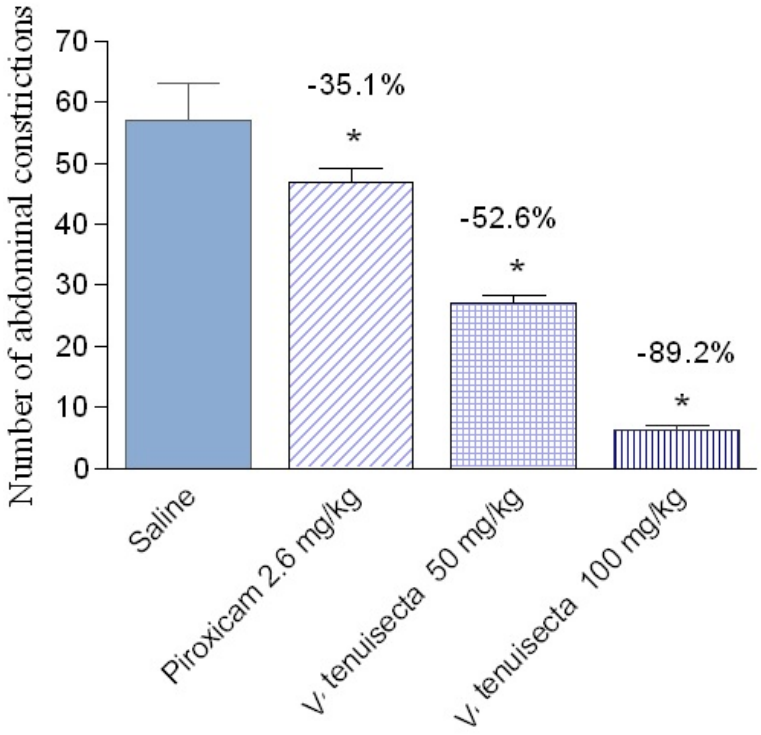


Fig. 3: Effect of *V. tenuisecta* extract on the number of abdominal constrictions induced in mice

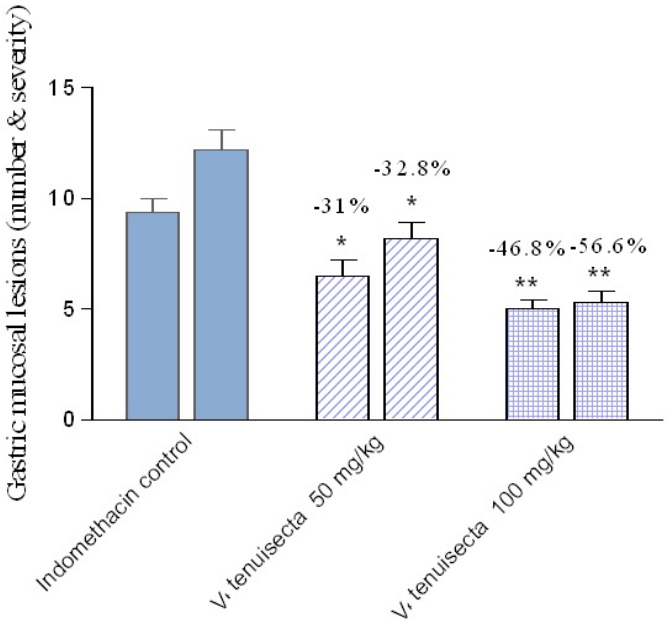
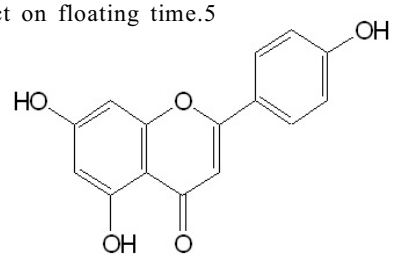


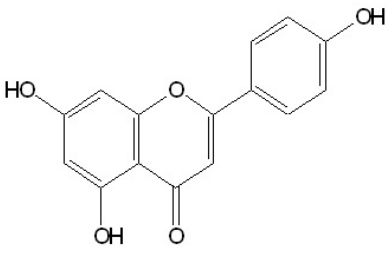
Fig. 4: Effect of *V. tenuisecta* extract on the number and severity of gastric mucosal lesions induced by the administration of indomethacin in rats.

increasing number of evidence indicates a gastro protective effect for antioxidants , including flavonoids against experimentally induced ulcers[32].

Effect of *V. tenuisecta* Extract on Floating Time (Forced Immobility Test): The data in table(7) showed no effect on floating time.5

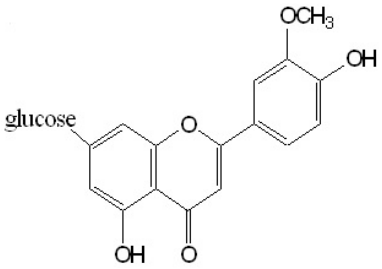


Luteolin



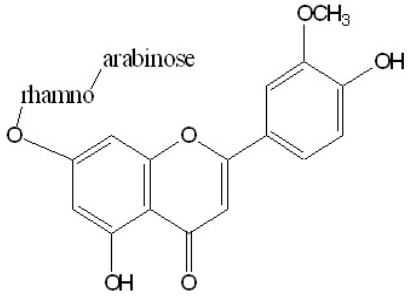
Apigenin

Compound-2



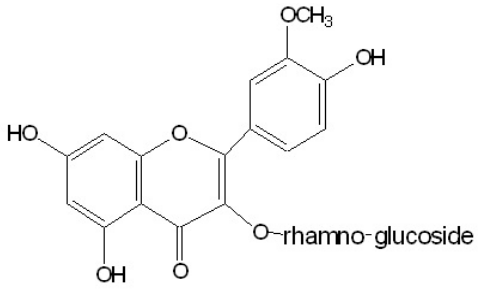
Chrysoeriol-7-*O*-glucoside

Compound-3



Chrysoeriol-7-*O*-rhamno-arabinoside

Compound-4



Isorhamnetin-3-*O*-rhamno-glucoside

Compound-5

REFERENCES

1. Jafri, M.H. and A. Elgadi, 1980. "Flora of Libya" Verbenaceae, Revolution Printing Press, Tripoli, Libya, 82.
2. Gamboa, I.C. and O. Castro, 2004. Iridoids from the aerial parts of *Verbena littoralis.* Phytochem., 65: 2369-72.
3. Calvo, M.I., 2006. Antiinflammatory and analgesic activity of the topical preparation of *Verbena officinalis.* J. Ethnopharm., 107: 380-82.
4. Mohammad, R.S.A., M. Mahmoud, S. Alireza, 2003. volatile constituents of *Verbena officinalis* (Vervain) Iranian J. Pharm. Res., 1(2): 39-42.
5. Li, Y., M. Ishibashi, M. Satake, X. Chen, Y. Oshima and Y. Ohizumi, 2003. Sterol and triterpenoid constituents of *Verbena littoralis* with NGF potentiating activity. J. Nat. Prod., 66(5): 696-698.
6. Deepak, M. and S.S. Handa, 1998. 3 24-dihydroxy –urs-12-en-28 oic acid from *Verbena officinalis.* Phytochem., 49(1): 269-71.
7. Li, Y., M. Ishibashi, M. Satake, Y. Oshima and Y. Ohizumi, 2003. A new iridoid glycoside with NGF potentiating activity, gelsmoil6-trans-caffeoyl-1-glucoside from *Verbena littoralis.* Chem. Pharm. Bull., 51(9): 1103-05.
8. Ono, M., K. Oishi, H. Abe, C. Masuoka, M. Okawa, T. Ikeda and *et al*, 2006. new iridoid glycosides from the aerial parts of *Verbena brasiliensis*. Chem. Pharm. Bull., 54(10): 1421-24.
9. Zaghloul, A.M., 1995. flavonoid from *Verbena peruviana*. Mansoura J. Pharm. Sci., 11(1): 43-54.
10. Michael, H.N., J.Y. Salib and M.S. Ishak, 2001. New methoxy flavone from *V. bipinnatifida*. Pharmazie, 56(4): 348-49.
11. Li, Y.S., K. Matsunaga, R. Kato and Y. Ohizumi, 2001. Verbenachalcone, a novel dimeric dihydrochalcon with PGF from *Verbena littoralis*. J. Nat. Prod., 64(6): 806-08.
12. Toki, K., N. Saito, N. Terahara and T. Honda 1995. Pelargonidin-3-glucoside 5-acetylglucoside in *Verbena* flowers.Phytochem., 40(3): 939-40.
13. Khaled, A.A., 1997. M.Sc. Thesis, Chemistry Dept., Faculty of science, Cairo University, Egypt.
14. Mabry, T.J., K.R. Markham and M.B. Thomas, 1970. "The Systematic Identification of Flavonoids" , Springer-Verlag, Berlin, First edition.
15. Markham, K.R., 1982. "Techniques of flavonoid identification", Academic Press, New York, Firest edition.
16. Paget, G.E. and J.M. Ranes, 1964. In "Evaluation of drug activates Pharmaceutics" Eds. Laurence, D.R. and Baharach, A.L. London, Academic press, pp: 1-135.
17. Winter, C.A., E.A. Risley and G.W. Nuss, 1962. Caragennan induced edema in hind of rats as an assay for antiiflamatory drugs. Proc. Soc. Exp. Bio. Med., 111: 544- 52.
18. Woolfe, G. and A.D. MacDonald, 1944. The evaluation of the analgesic action of pethidine hydrochloride, J. pharmacol. Exp. Ther., 80: 300-301.
19. Witkin, L.B. and A.J. Plumer, 1961. Pharmacology of 2- aminoindane hydrochloride A potent non narcotic analgesic. J. pharmacol. Exp. Ther., 133: 400-408.
20. Ibrahim, L.F., S.A. Kawatshy, A.R. Baiuomy, M.M. Shabana and S.I. ElNegoumy, 2007. A comparative study of the flavonoids and some biological activities of two *Chenopodium* species. Chem. Nat. Comp., 4(1): 24-2.
21. Chalchat, J.C. and R.P. Garry, 1996. Chemical composition of the leaf oil of V. officinalis. J. Essent. Oil Res., 8(4): 419-20.
22. Kamari, G.N., L.J.M. Rao and N.S.P. Rao, 1986. Proc. Indian, Acad., 79: 171.
23. El-Azizi, M.M., A.B. Singah, M.M. El-Hamouly, and E.A. Cenady, 2000. Investigation of flavonoidal constituents of *V. bipinnatifida* Al-Azhar J. Pharm. Sci., 25: 238-46.
24. Chen, G.M., J.Y. Zhang and H.M. Liu, 2006. Chemical constituents of *V.officinalis.* Zhong Yao Cai, 29(7): 677-79.
25. Markham, K.R., B. Ternai, R. Stanley, H. Geiger, and T.J. Mabry, 1978. ^{13}C nmr studies of flavonoids-III Naturlly occurring flavonoid glycosides and their acylated derivatives. Tetrahedron, 34: 1389.
26. Tian, J., Y.M. Zhao and X.H. Luan, 2005. Studies on the chemical constituents in herb of *V. officinalis.* Zhongguo Zhong Yao Za Zhi, 30(4): 268-69.

27. Carnat, A., O. Chavignon, A. Heitz, R. Wylde and J.L. Lamaison, 1995. Luteolin -7- diglucouronoid, the major flavonoid from *Aloysia triphylla* and *V. officinalis*. Planta Med., 61(5): 490.
28. Makboul, A.M., 1986. Chemical constituents of V. officinalis. Fitoterapia, 57(1): 50-51.
29. Deepak, M. and S.S. Handa, 2000. Antiinflammatory activity and chemical composition of *V. officinalis*. Phytother. Res., 14: 463-65.
30. Charles, D.A.I., K.S. Vidyalakshimi, R.V. Hannah, G.V. Rajamanickam, and G.P. Dubey, 2006. Antiinflammatory activity of *Plumbago capensis*. Pharcog. Mag., 2(8): 239-43.
31. Calvo, M., A. Julian and M. Fernandez, 1997. Identification of the major compounds in extracts of *V. officinalis* (verbenaceae) by HPLC with column derivatization. Chromatographia, 46(5-6): 421-44.
32. Kudahewa, D.D., D.T. Abeytunga and W.D. Ratnasooriya, 2008. Antinoceseptive activity of *Pleurotus cystidiosus*, an edible mashroom in rats. Pharmcog. Mag., 4(13): 37-41.