Mansoura Journal of Chemistry Vol. 36 (1), June, 2009.

CHEMICAL CONSTITUENTS AND MITICIDAL ACTIVITY OF ECBALLIUM ELATERIUM AND SCHINUS EREBINTHIFOLIUS AGAINST TETRANYCHUS URTICAE KOCH

A. M. Dawidar [a,*], M. Abdel-Mogib [a], M. E. El-Naggar[b], M. E. Mostafa[b].

a: Chemistry Dept., Fac. of Sci., Mansoura Univ., Mansoura, Egypt.
b: Plant Protection Research Institute, Agriculture Research Center, Egypt.

(Received: 13 / 12 / 2008)

ABSTRACT

In a survey of eleven plant extracts to miticidal activity against the two spotted spider mites *Tetranychus urticae* Koch; the extract of *Ecballium elaterium* (*Cucurbitaceae*) was found to be the most effective as natural acaricide, followed by the essential oil of *Schnius terebenthifolius* (*Anacardiaceae*). The mortality percentages were obtained at a concentration of 7000 ppm after 7 days of treatment.

Repeated chromatographic separation and structure elucidation have resulted in identification of a phytosterol, 24-ethyl-5α-cholesta-7,22,25-trien-3β-ol and a flavanoid glycoside, rutin. The composition of the essential oil of *S. terebenthifolius* was determined by the GC/MS technique.

Keywords: Miticidal activity; *Tetranychus urticae* Koch; *Ecballium elaterium*; *Schnius terebenthifolius*; 24-ethyl-5α-cholesta-7,22,25-trien-3β-ol; rutin.

INTRODUCTION

Ecballium elaterium (*cucurbitaceae*) is a medicinal plant, known as squirting cucumber, in folk's medicine since the Mediterranean civilization *era*. Therefore, the plant was subjected to extensive biological **[Agil *et al.*, (1995)]**, medicinal **[Toker *et al.*, (2003)]** and chemical studies **[Salim *et al.*, (1996)]**. For example the juice of the fruit is antirheumatic, cardiac and purgative **[Chittendon *et al.*, (1951)]**, in

addition to paralysis and shingles treatment **[Organ (1963)]**. Family *Cucurbitaceae* is characterized chemically by the presence of tetracyclic triterpenoids, referred to as cucurbitanes **[Nurit & Alfred (1990)]**. The roots, leaves and fruits of *E. elaterium* contain the free cucurbitacines; cucurbitacine H, C, G, D, E, I, R, L, B, anhydro-22-deoxo-3epiisocucurbitacin D, hexanorcucurbitacin I, 16-deoxy-Δ^{16}-hexanor cucurbitacin O **[Seifert & El-gamal (1977)]**. The leaves, stems and roots of *Ecballium elaterium* (*Cucurbitaceae*) contain in addition to cucurbitacins: 4-demethyl sterols, 24-ethyl-5α-cholesta-7,22,25-trien-3β-ol, 24-ethyl-5α-cholesta-7,22-dien-3β-ol, 24-ethyl-5α-cholesta-7,25-dien-3β-ol, 24-ethyl-5α-cholesta-5,25-dien-3β-ol, 24-ethyl-5α-cholesta-7,24-dien-3β-ol, 24-ethyl-5α-cholesta-5,22-dien-3β-ol (stigmasterol) **[Hyalands & Oskoui (1979)]**, cycloeucalenol **[Oskoui (1986)]**, 3β-hydroxystigmasta-7,16,25(26)-triene (elasterol) **[Cozalez, & Panizo (1967)]**. This is in addition to flavoniods glucosides as rutin and kaempferol 3-O-rutinoside **[Imperato (1980)]**, triterpenoid glycosides as 2-O-β-D-glucopyranosyl cucurbitacin B and 2-O-β-D-glucopyranosyl cucurbitacin D **[Seifert & El-gamal (1977)]** and shikimates as hydroquinone, 4-hydroxyacetophenone, 4-hydroxy-3-methoxy-acetophenone (acetovanillone), 2-nitroquinol,4-hydroxyl-phenyl-bis-epoxylignan **[Rao & Lavie (1979)]**.

Schinus terebenthifolius (*Anacardiaceae*) essential oil was reported as antibacterial agent **[Siddique *et al.*, (1995)]** and antifungal agent **[Siddique *et al.*, (1996)]**. The essential oil of *S. terebenthifolius* was studied for its composition and characterization by several workers. For example **[Saleh (1988)]** reported that the major components of the oil were, the sesquiterepenens β-bulnesene, β-elemene, β-patchoulene , β-caryophyllene and β-elemol . On the other hand, **[Pieribattesti *et al.*, (1981)]** detected the monoterepenenes camphene, β-phellandrene and γ-terpinene, α-pinene (26.5%) α-phellandrene (22.3%) and careen. **[Shafiq *et al.*, (1994)]** identified terpens as α-pinene (43.20%), camphene (0.42%), β-pinene (2.29%), sabinene (1.91%), α-phellandrene(18.85), 3-carene (0.27%), *p*-cymene (0.84%), γ-terpinene (0.76%), terpinolene (1.07%) and β-caryophyllene (0.41%) from the whole oil.

Phytophagous mites, *Tetranychus urticae* (Koch), are among the major pests attacking cotton, fruit trees and vegetables in Egypt.

In a survey for some local plants possessing acaricidal activity against *T. urticae* (Koch), several trails for application of botanicals as environmentally safe natural pesticides were made. We report here our

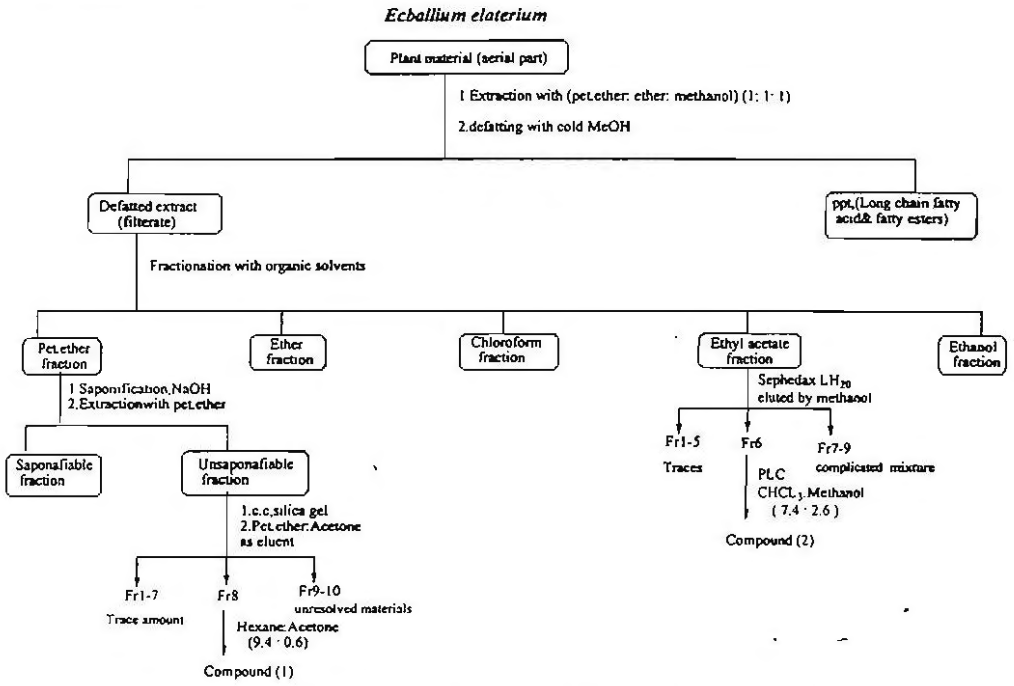
results of testing eleven plant extracts as miticides against *T. urticae* (Koch), as well as the chemical constituents of *E. elaterium* and the essential oil of *S. terebenthifolius*.

RESULTS AND DISCUSSION

The aerial parts of *E. elaterium* was tested among eleven different wild plants against larvae and adult females of *T. urticae* which were extracted with a mixture of organic solvents; pet. ether/ether/methanol (1:1:1) at room temperature and processed according to scheme 1. Moreover, the essential oil of the leaves of *Schinus terebenthifolius* (*Anacardiaceae*) was obtained by steam distillation. All the extracted plant materials were investigated for their miticidal activity phytophagous mites, *T. urticae* (Koch), the mortality percentages were obtained at a concentration of 7000 ppm after 7 days, and the results are shown in (Table 1), which showed that *E. elaterium* is the most active as miticide followed by *H. muticus* **[Dawidar *et al.*, (2008)]** then *S. terebenthifolius*.

The crude extract of *E. elaterium* which proved to be the most active miticidal agent was subjected for further fractionation according to scheme 1. The susceptibility of the larvae of *T. urticae* to various plant fractions revealed a great variation in effectiveness after 7 days of treatments (table 2). The LC_{50} values were 0.0018, 0.532, 18.44, 68.81, 86.89 and 1418.62 ppm for chloroform fraction, crude extract, pet. ether fraction, ethyl acetate, ether fraction and ethanol fraction, respectively. The descending order of the larvicidal activity at the LC_{90} level was the same as LC_{50} level. The slopes of the toxicity lines were calculated to be fluctuated and increased from 0.515 for the chloroform fraction to 1.678 for the ethanol fraction. The other fractions lines came between these two fraction lines.

The toxicity index of the LC_{50} values, as shown in (table 2), could be showed that chloroform fraction was the most effective plant fraction against larvae of *T. urticae* after 7 days of treatment, followed by crude extract, pet. ether fraction, ethyl acetate fraction and ether fraction. Ethanol fraction was the least effective. This reflects the distribution of the active ingredients between the different fractions.



Scheme (1): Processing of *Ecballium elaterium* (aerial part)

In case of *S. terebenthifulius* essential oil extract, the LC_{50} and LC_{90} values were 1340 and 9950 ppm, respectively after 3 days of treatment, (table 2).

Table (3) showed the efficiency of the tested *E. elatrium* fractions against the adults of *T. urticae* after 7 days of treatment. The chloroform fraction was the most effective at LC_{50} level followed by crude extract, pet. ether fraction, ethyl acetate fraction, ether fraction and ethanol fraction. The LC_{50} values of the tested fractions were 0.0094, 1.162, 26.997, 51.86, 299.57 and 1468.63 ppm, respectively.

At LC_{90} level the potency arrangement was the same as obtained at LC_{50} level except of a switch in position between pet. ether fraction and ether fraction.

The classification according to toxicity index showed that chloroform fraction was the most effective fraction against the adults of *T. urticae* after 7 days of treatment followed by crude extract, pet. ether fraction, ethyl acetate fraction and ether fraction while ethanol fraction was the least toxic one.

Table (3) indicated that the LC_{50} and LC_{90} of *S. terebenthifolius* essential oil fraction against adults of *T. urticae* after 7 days of treatment were 1350 and 6840 ppm, respectively.

Table (1): Acaricidal screening of some Egyptian wild desert plants.

No	Scientific name	Plant family	Plant part	Biological activity mortality %
1	*Hyoscyamus muticus*	*Solanaceae*	l	90
			s	86.66
			fr	73.33
			r	76.6
2	*Zygophyllium coccinum*	*Zygophyllaceae*	l	20
			s	50
3	*Salavia deserti*	*Labiatae*	wa	30
			r	36.67
4	*Ecabillum elaterium*	*Cucurbitaceae*	l	100
			s	66.66
			fr	90
5	*Artemisia monosperma*	*Compositae*	l	10
6	*Peganm harmala*	*Zygophyllaceae*	l	40
			s	31.03
			fr	53.33
			r	40
7	*Achillea fragrantissima*	*Compositea*	l	30
			s	23.33
			r	50
8	*Caltropis procera*	*Asclepidiaceae*	l	50
			s	46.6
			fr	13.33
9	*Haloxylon salicornnicum*	*Chenopodiaceae*	l	36.66
			s	43.33
10	*Deverra tortousa*	*Umbelliferae*	s	30
			r	45
11	*Schinus terebenthifolius*	*Anacardiaceae*	l	83

l. leaves; s, stems; fr, fruits; r, roots; wa, whole plant above ground.

Table (2): Toxicity of plant extracts against larvae of *T. urticae* after 7 days of treatment.

Plant species (Family)	Plant extract	LC_{50} (ppm) and confidence limits at 95%	LC_{90} (ppm) and confidence limits at 95%	Slope	Toxicity index at LC_{50} value
E. elaterium (*Curcurbitaceae*)	crude extract	0.532 0.171 0.847	3.502 2.299 9.063	1.565±0.392	0.338
	pet. ether fraction	18.436 9.19 36.97	397.45 198.19 797.06	0.961±0.459	0.0098
	ether fraction	86.894 42.87 176.13	3182.562 1570.11 6450.95	0.82±0.543	0.0021
	chloroform fraction	0.0018 0.00049 0.00657	0.54 0.148 1.973	0.515±0.291	100.00
	ethyl acetate fraction	68.806 50.41 149.49	757.617 439.93 1304.71	1.23±0.624	0.0026
	ethanol fraction	1418.618 633.562 2078.912	8233.617 5250.33 24055.18	1.678±0.41	0.0001
S. terebenthifolius (*Anacardiaceae*)	essential oil fraction	1340 470 2070	9950 5870 42300	1.473±0.396	——

Table (3): Toxicity of plant extracts against adults of *T. urticae* after 7 days of treatment.

Plant species (Family)	Plant extract	LC_{50} (ppm) and confidence limits at 95%	LC_{90} (ppm) and confidence limits at 95%	Slope	Toxicity index at LC_{50} value
E. elaterium	crude extract	1.162 0.245 2.276	21.70 11.52 89.63	1.008±0.242	0.809
	pet. ether fraction	26.997 10.98 66.37	4999.51 3253.9 19666.22	0.565±0.375	0.035
	ether fraction	299.573 166.287 410.032	1385.99 1006.89 2540.31	1.926±0.387	0.0031
	chloroform fraction	0.0094 0.000087 0.036	0.651 0.274 4.973	0.696±0.209	100.00
	ethyl acetate fraction	51.863 22.27 120.792	2163.97 929.19 5039.59	0.791±0.304	0.0180
	ethanol fraction	1470 460 2260	12030 6570 92470	1.403±0.418	0.0006
S. terebenthifolius	essential oil fraction	1350 110 2410	6840 4830 15710	1.821±0.604	——

According to scheme 1, a natural compound was isolated from the unsaponifiable fraction of pet. ether. This compound was designated as compound 1 and gave green colour with Libermann-Burchardt test, characteristic for phytosterols. The IR spectrum showed the presence of hydroxylic group due to the absorption band at 3426 cm^{-1}, and a carbon-carbon double bond band at 1647 cm^{-1}.

The mass spectrum showed M^+ at m/z 410 (11%) corresponding to [$C_{29}H_{46}O$]. The ^{1}H-NMR revealed the presence of two angular methyl protons signals at δ 0.54 and δ 0.80 ppm (each s for H-18,H-19), an olefinic proton signal at δ 5.15 ppm (brs , H-7) and a multiplet at δ 3.59 ppm (1H for H-3) of a Δ^7-3β-hydroxy sterol and not Δ^5-3β-hydroxysterol. This was in agreement with 24-ethyl-5α-cholesta-7, 22.25-trien-3β-ol, which was isolated and characterized from *E. elaterium* by **[Hyalands & Oskoui (1979)]**.

Compound 2 was isolated from the ethyl acetate fraction. The UV spectrum revealed the absorption band at λ_{max} in MeOH at 258 and 278 nm. in addition of sodium acetate bathochromic shift which leads to absorption bands at 276 nm, shoulder at 322 nm and 400 nm. ^{1}H-NMR spectral data were characteristic of a flavonoid glycoside. Ring A was apparently 5.7-disubstituted as shown by two meta-oriented protons at δ 6.13 ppm (1H, d. J=2.4 Hz. H-6) and δ 6.34 ppm (1H, d. J=2.4 Hz. H-8). On the other hand, the observation of ABX system at δ 7.50 ppm (1H, d, J=3.8 Hz. H-2'), δ 6.80 ppm (1H. d. J=8.4 Hz. H-5') and δ 7.52 ppm (1H, dd. J=8.4. 3.8 Hz. H-6') suggested a 3',4'-disubstituted ring B. A 3-O-substituted flavonol structure was deducible from anomeric proton at δ 5.27 ppm (1H, d, J=6.8 Hz), characteristic for glucosyl moiety. another monosaccharide residue could be determined as rhamnose from its chemical shift value δ 4.34 ppm (1H, brs) as the anomeric proton, and its methyl group at δ 0.96 ppm (3H. d. J=6.1Hz. H-6'''). This was found to be in agreement with rutin, characterized by **[Ying (2000)]**.

We reinvestigated the composition of the essential oil of *S. terebenthifolius* by the GC/MS technique. The mass spectra were compared with the corresponding compounds from NIST library. Our results were reported in (table 4) and the structures of the identified compounds are shown in Chart 1.

The toxicity of the isolated compounds against larvae of *T. urticae* were presented in (table 5). Compound 1 (24-ethyl-5α-cholesta-7,22,25-trien-3β-ol) was the most effective isolated compound against larvae of *T. urticae* after 7 days of treatment followed by compound 2

(rutin). The LC_{50} values were 171.327, 189.492, respectively for larvae after 7 days of treatment. In case of adult females of *T. urticae*, rutin (2) was the most effective isolated compound followed by, 24-ethyl-5α-cholesta-7,22,25-trien-3β-ol (1). The LC_{50} values were 138.317, 313.183, respectively.

Table (4): Chemical constituents of the essential oil *S. terebenthifolius* leaves.

No.	Component	R_t, min	Area %	M.F	m/z (ret. int. %)
Monoterpene					
1	4-Carene (3)	7.97	0.55	$C_{10}H_{16}$	136 (15) $[M^+]$, 121 (5) $[C_9H_{13}]^+$, 93 (100) $[C_7H_9]^+$, 91 (60) $[C_7H_7]^+$, 77 (35) $[C_6H_5]^+$, 65 (10) $[C_5H_5]^+$, 51 (5) $[C_4H_3]^+$
2	Sabinene (4)	9.13	0.82	$C_{10}H_{16}$	136 (10) $[M^+]$, 121 (7) $[M-CH_3]^+$, 93 (100) $[C_7H_9]^+$, 77 (36) $[C_6H_5]^+$, 43 (14) $[C_3H_7]^+$
3	Sabinene hydrate (5)	7.64	0.55	$C_{10}H_{18}O$	154 (1) $[M^+]$, 136 (14) $[M-H_2O]^+$, 111 (2) $[M-C_3H_7]^+$, 93 (100) $[M-H_2O, C_3H_7]^+$, 77 (31) $[C_6H_5]^+$, 65 (7) $[C_5H_5]^+$, 43 (52) $[C_3H_7]^+$
4	L-Borneol (6)	8.42	2.19	$C_{10}H_{18}O$	154 $[M^+]$, 139 (7) $[M-CH_3]^+$, 121 (7) $[M-CH_3, H_2O]^+$, 110 (9) $[M-CH_3, CO, H]^+$, 95 (71) $[C_7H_{11}]^+$, 40 (100) $[C_3H_4]^+$
5	L-Cryptone (7)	8.77	8.22	$C_9H_{14}O$	138 (7) $[M^+]$, 96 (57) $[C_7H_{12}]^+$, 81 (17) $[C_6H_9]^+$, 67 (17) $[C_5H_7]^+$, 40 (100) $[H_2C=C=CH_2]^+$,
6	Ocimenol (8)	8.87	2.74	$C_{10}H_{18}O$	154 (1) $[M^+]$, 136 (24) $[M-H_2O]^+$, 121 (17) $[M-H_2O, CH_3]^+$, 93 (100) $[C_7H_9]^+$, 59 (71) $[C_3H_7]^+$, 41 (57) $[C_3H_5]^+$
7	β-Citronellol (9)	9.50	0.55	$C_{10}H_{20}O$	156 $[M^+]$, 138 (5) $[M-H_2O]^+$, 123 (12) $[C_9H_{15}]^+$, 109 (10) $[C_8H_{13}]^+$, 69 (71) $[C_5H_9]^+$, 55 (34) $[C_4H_7]^+$, 41 (100) $[C_3H_5]^+$
8	E-3(10)-Caren-2-	10.18	0.55	$C_{10}H_{16}O$	152 (5) $[M^+]$, 137 (5) $[M-$

	ol (10)				$CH_3]^+$, 119 (5) $[M-CH_3, H_2O]^+$, 109 (40) $[C_7H_9O]^+$, 69 (5) $[C_5H_9]^+$, 55 (17) $[C_4H_7]^+$, 41 (55) $[C_3H_5]^+$, 40 (100) $[C_3H_4]^+$
Sesquiterpenes					
9	β-Elemene (11)	11.92	1.1	$C_{15}H_{24}$	204 $[M^+]$, 189 (12) $[M-CH_3]^+$,147 (19) $[C_{11}H_{15}]^+$, 107 (36) $[C_8H_{11}]$, 93 (83) $[C_7H_9]^+$, 41 (100) $[C_3H_5]^+$
10	Caryophyllene (12)	13.31	3.01	$C_{15}H_{24}$	204 (2) $[M^+]$, 189 (5) $[M-CH_3]^+$, 147 (12) $[C_{11}H_{15}]^+$, 133 (31) $[C_{10}H_{13}]^+$, 55 (17) $[C_4H_7]^+$, 41 (100) $[C_3H_5]^+$
11	Germacrene B (13)	12.49	27.4	$C_{15}H_{24}$	204 $[M^+]$, 189 (7) $[M-CH_3]^+$, 161 (24) $[C_{12}H_{17}]^+$, 147 (12) $[C_{11}H_{15}]^+$, 121 (68) $[C_9H_{13}]^+$, 67 (40) $[C_5H_7]^+$, 41 (100) $[C_3H_5]^+$
12	Germacrene D (14)	13.13	3.01	$C_{15}H_{24}$	204 (33) $[M^+]$, 189 (13) $[M-CH_3]^+$, 161 (95) $[M-C_3H_7]^+$, 147 (15) $[C_{11}H_{15}]^+$, 133 (10) $[C_{10}H_{13}]^+$, 119 (38) $[C_9H_{11}]^+$, 105 (100) $[C_8H_9]^+$, 43 (35) $[C_3H_7]^+$
13	4,6-Eudesmadiene (15)	13.21	0.55	$C_{15}H_{24}$	204 (18) $[M^+]$, 189 (15) $[M-CH_3]^+$, 175 (13) $[C_{13}H_{19}]^+$, 161 (33) $[M-C_3H_7]^+$, 147 (28) $[C_{11}H_{15}]^+$, 133 (25) $[C_{10}H_{13}]^+$, 119 (23) $[C_9H_{11}]^+$, 41 (100) $[CH_3\text{-}C{=}CH_2]^+$
14	1(10)-Aromadendrene (16)	13.33	1096	$C_{15}H_{24}$	204 (23) $[M^+]$, 189 (38) $[M-CH_3]^+$, 175 (15) $[C_{13}H_{19}]^+$, 161 (35) $[C_{12}H_{17}]^+$,147 (28) $[C_{11}H_{15}]^+$,133 (35) $[C_{10}H_{13}]^+$,119 (28) $[C_9H_{11}]^+$, 41 (100) $[CH_3\text{-}C{=}CH_2]^+$

15	α-Cadienene (17)	13.39	0.55	$C_{15}H_{24}$	204 (18) $[M^+]$, 189 (5) $[M-CH_3]^+$, 175 (3) $[C_{13}H_{19}]^+$, 161 (43) $[M-C_3H_7]^+$, 133 (8) $[C_{10}H_{13}]^+$, 119 (20) $[C_9H_{11}]^+$,105 (100) $[C_8H_9]^+$, 43 (20) $[C_3H_7]^+$, 41 (50) $[CH_3-C=CH_2]^+$
16	γ-Muurolene (18)	13.57	0.82	$C_{15}H_{24}$	204 (15) $[M^+]$, 189 (8) $[M-CH_3]^+$, 161 (100) $[M-C_3H_7]^+$, 133 (20) $[C_{10}H_{13}]^+$,119 (13) $[C_9H_{11}]^+$, 41 (38) $[C_3H_5]^+$, 91 (50) $[C_7H_7]^+$
17	δ-Cadinene (19)	13.68	0.82	$C_{15}H_{24}$	204 (30) $[M^+]$, 189 (10) $[M-CH_3]^+$, 161 (100) $[M-C_3H_7]$, 119 (33) $[C_9H_{11}]^+$, 147 (5) $[C_{11}H_{15}]^+$, 105 (43) $[C_8H_9]^+$, 91 (38) $[C_7H_7]^+$, 43 (5) $[C_3H_7]^+$, 41 (45) $[CH_3-C=CH_2]^+$
18	Eudesma-3,7(11)-diene (20)	13.93	1.37	$C_{15}H_{24}$	204 (30) $[M^+]$, 189 (40) $[M-CH_3]^+$, 175 (5) $[C_{13}H_{19}]^+$, 161 (100) $[C_{12}H_{17}]^+$, 133 (15) $[C_{10}H_{13}]^+$, 122 (40) $[C_9H_{14}]^+$, 107 (40) $[C_8H_9]^+$, 91 (40) $[C_7H_7]^+$, 41 (53) $[CH_3-C=CH_2]^+$
19	α-Aromadendrene (21)	14.13	8.22	$C_{15}H_{24}$	204 (20) $[M^+]$, 189 (15) $[M-CH_3]^+$, 175 (3) $[M-C_{13}H_{19}]^+$, 161 (40) $[C_{12}H_{17}]^+$, 147 (23) $[C_{11}H_{15}]^+$, 133 (23) $[C_{10}H_{13}]^+$, 105 (48) $[C_8H_9]^+$, 91 (58) $[C_7H_7]^+$, 41 (100) $[C_3H_5]^+$
20	γ- Gurjuene (22)	14.6	0.55	$C_{15}H_{24}$	204 (20) $[M^+]$, 189 (20) $[M-CH_3]^+$, 175 (5) $[C_{13}H_{19}]^+$,161 (70) $[C_{12}H_{17}]^+$, 147 (30) $[C_{11}H_{15}]^+$, 105 (68) $[C_8H_9]^+$, 41 (100) $[CH_3-C=CH_2]^+$
21	10-Aromadendranol (23)	14.48	7.12	$C_{15}H_{24}O$	220 $[M^+]$, 219 (3) $[M-H]^+$, 202 (18) $[M-H_2O, H]^+$, 187 (23) $[M-H_2O, CH_3, H]^+$,

					159 (100) $[C_{12}H_{15}]^+$, 145 (33) $[C_{11}H_{13}]^+$, 131 (53) $[C_{10}H_{11}]^+$, 117 (25) $[C_9H_9]^+$, 131 (53) $[C_{10}H_{11}]^+$, 117 (25) $[C_9H_9]^+$, 41 (100) $[C_3H_5]^+$
22	4, 4, 11, 11-Tetramethyl- 7-tetracyclo [6, 2, 1, 0(3, 5)0(3, 9)] undecanol (24)	14.40	2.19	$C_{15}H_{26}O$	222 $[M^+]$, 204 (40) $[M-H_2O]^+$, 189 (40) $[M-H_2O, CH_3]^+$, 175 (8) $[C_{13}H_{19}]^+$, 161 (100) $[C_{12}H_{17}]^+$, 147 (20) $[C_{11}H_{15}]^+$, 119 (30) $[C_9H_{11}]^+$,91 (75) $[C_7H_7]^+$, 41 (100) $[C_3H_5]^+$
23	Eudesmol (25)	14.27	4.11	$C_{15}H_{26}O$	222 (3) $[M^+]$, 204 (33) $[M-H_2O]^+$,189 (50) $[M-H_2O, CH_3]^+$, 175 (5) $[C_{13}H_{19}]^+$, 161 (100) $[C_{12}H_{17}]^+$, 107 (5) $[C_8H_{11}]^+$, 93 (13) $[C_7H_9]$, 91 (58) $[C_7H_7]^+$, 81 (100) $[C_6H_9]^+$
24	τ-Cadinol (26)	15.18	5.48	$C_{15}H_{26}O$	222 (1) $[M^+]$, 204 (23) $[M-H_2O]^+$,189 (15) $[M-H_2O, CH_3]^+$, 161 (100) $[M-C_3H_7]^+$, 133 (10) $[C_{10}H_{13}]^+$, 119 (25) $[C_9H_{11}]^+$,91 (15) $[C_7H_7]^+$, 43 (95) $[C_3H_7]^+$,41 (50) $[C_3H_5]^+$

Table (5): Toxicity relation between the isolated compounds to larvae and adult females of *T. urticae* after 7 days of treatment.

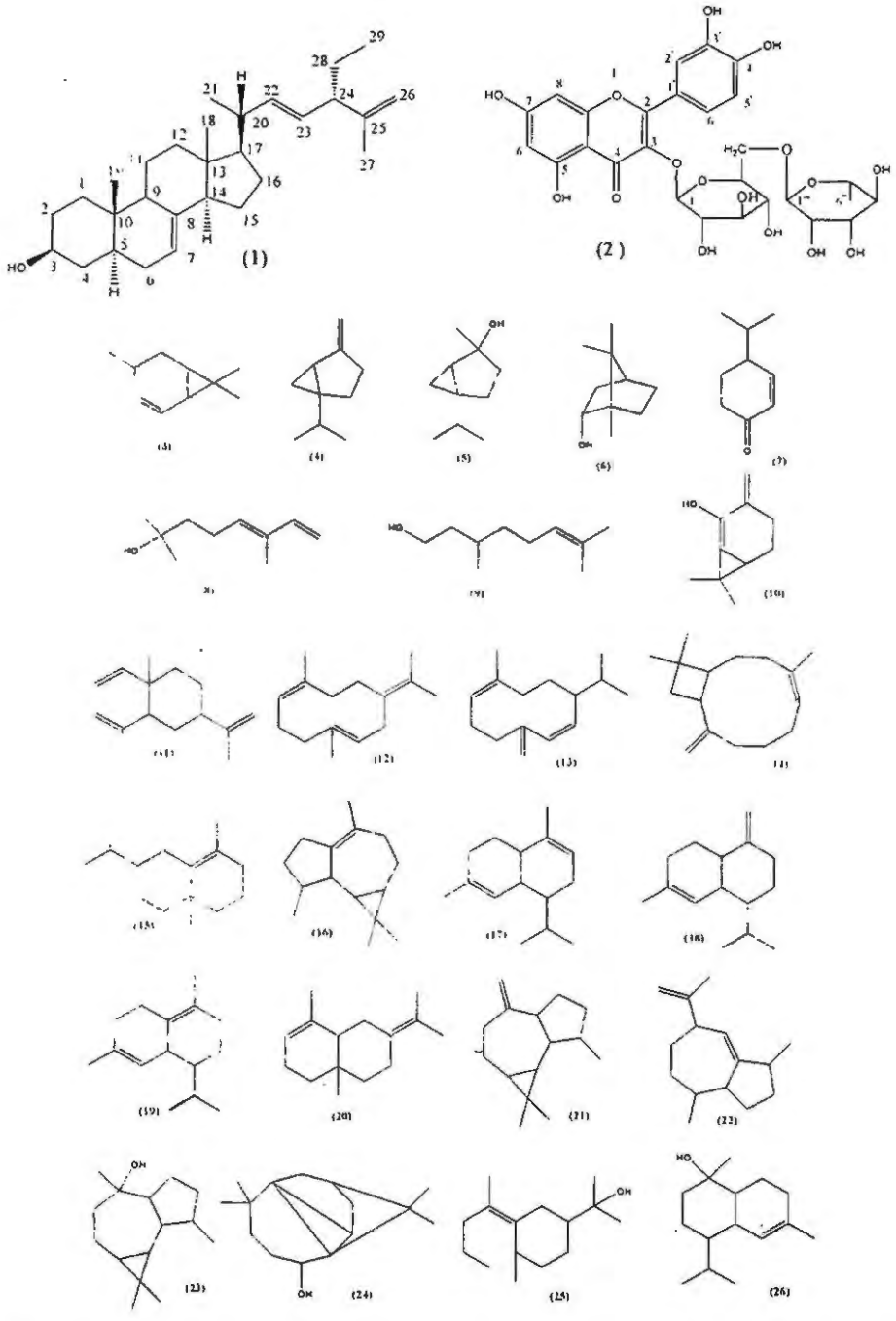


Chart (1): Structural formulas of isolated and characterized compounds.

EXPERMINTAL

Instrumentation

NMR spectra were recorded on a 500 MHz JEOL FTNMR spectrometer. Chemical shifts are given in δ (ppm) relative to TMS as internal standard material and the coupling constant (*J*) are in Hz. IR spectra were recorded on Mattson 5000 FT-IR spectrometer. GC/MS analysis of the volatile fractions were performed on a Varian GC interfaced to Finnegan SSQ 7000 Mass selective Detector (SMD) with ICIS V2.0 data system for MS identification of the GC components. The column used was DB-5 (J&W Scientific, Folosm, CA) cross-linked fused silica capillary column (30 m. long, 0.25 mm. internal diameter) coated with poly dimethylsiloxane (0.5 μm film thickness). The oven temperature was programmed from 50°C for 3 min., at isothermal, then heating by 7°C /min. to 250°C and isothermally for 10 min., at 250°C. Injector temperature was 200°C and the volume injected was 0.5μl. Transition-line and ion source temperature were 250°C and 150°C, respectively. The mass spectrometer had a delay of 3 min. to avoid the solvent plead and then scanned from m/z 50 to m/z 300. Ionization energy was set at 70 eV. (Agriculture Research Center, Dokki, Cairo).

EI-MS were recorded on a Kratos MS-25 instrument. TLC was performed on silica gel (Kieselgel 60, F 254) of 0.25 mm thickness.

The Plant material

Ten Egyptian wild plants and one cultivated plant representing nine different families were collected by the researcher and identified by prof. Dr. Ibrahim Mashaly, Department of Botany, Faculty of Science, Mansoura University. All the selected plant species were collected at April, (2004) about 3-10 km from Al-Arish airport except *Hyoscyamus muticus* from Al-Aresh city, *Ecabillum elaterium* from El-Shekh Zowaied and *Schinus terebenthifolius* from the garden of Mansoura University campus (table 1).

Processing of E. elaterium aerial parts

The aerial parts of *E. elaterium* (550 g) were processed according to scheme 1, where the following five fractions were obtained; pet. ether (9.45 g), ether (1.27 g),chloroform (0.5 g), ethyl acetate (1.8 g) and ethanol (6.3 g).

The pet. ether fraction (9.45 g) was saponifiêd using alcoholic aqueous sodium hydroxide and re-extracted by pet. ether to give the

unsaponifiable fraction (3.73 g), which was subjected to column chromatography using silica gel. Elution of the column was performed by using a series of eluents from pet. ether/acetone combinations of increasing polarity. The effluents were combined into ten fractions according to their TLC patterns. Fraction 8, which was eluted by hexane/acetone (7:3) and further purified by PTLC using pet. ether/acetone (9.4 : 0.6, v/v) (R_f = 0.16), afforded compound 1.

The ethyl acetate fraction (1.8 g) was purified on sephadex LH-20, eluted with MeOH, the effluents were combined into nine sub fractions based on their TLC pattern. Sub fraction 6 was further purified using preparative TLC developed in a mixture of $CHCl_3$/ MeOH (7.4: 2.6, v/v), (R_f = 0.19) to afford compound 2.

Processing of Schinus terebenthifolius leaves

Shinus terebenthifolius fresh leaves (400 g) were processed by means of steam distillation in order to obtain the volatile oil fraction (0.8 g. 0.2% dry weight). The volatile oil fraction was separated by GC/MS. Table (4) reports the composition of the oil.

Maintance of spider mite colony

Colony of spider mite *Tetranychus urticae* (Koch) was reared under laboratory condition (25±2 °C and 60±5 % R.H) at plant protection research institute branch. dakahlia Governorate. This study colony was isolated from heavily infested castor oil plant leaves. Spider mite colony was reared on castor oil leaves. The leaves were cleaned and placed on moisten cotton wool pad in Petri dishes and the colony was left for one year under the precious conditions in order to get a homogenous and susceptible colony. Spider mite individual were transferred to the leaves by the aid of fine camels hair brush. Breeding leaves were changed twice weekly at the summer and once weekly at the winter. Adding water was done twice daily to prevent escaping of *T. urticae* indiveduals.

Assessment of miticidal activity

In this respect, laboratory experiments are conducted to evaluate the activity of various tested plant extracts against *T. urticae* mobile stages (larvae and adult females). The leaf-dip technique method described by **[Dittrich (1962)]** was used as the following.

All extracts were formulated as emulsion in water containing 0.3% triton X-100.The emulsions were used immediately after preparation. Serial concentrations of each extract were prepared for each

tested stage. Castor oil leaf discs (2 cm diameter), were dipped in each concentration for 10 seconds, and left to dry. Discs were placed onto cotton wool pads in Petri dishes (12 cm in diameter). Ten *T. urticae* individuals of the same age were transferred to treated castor oil leaf-discs by using camel hair brush with the aid of stero-micoscope. All of treatments were left under laboratory condition. Each treatment was replicated three times in addition to control. Controls were dipped in the solvent mixture only. Observations were taken after 7-days. Those mites that crawled off the leaf disc and drowned in the wet cotton were disregarded. The indication of mortality was chosen as the failure of mites to respond positively by leg movement followed light brooding with a fine brush **[Ismail (1997)]**. Mortality percentages were determined and corrected by using **Abot's formula (1925)** formula and they are statistically analyzed to estimate LC_{50}, LC_{90} and slope values according to **[Finney (1971)]**. Toxicity index was computed for different extracts and their isolated compounds by comparing these materials with the most effective extracts or isolated compounds using **[Sun's (1950)]** equation.

REFERENCES

Abbott, W.s., A method of computing the effectiveness of an insecticides. J. Econ. Entomol. 18: 625-627(1925).

Agil, M.A., Risco. S., Miro, M., Navarro, M.C., Ocete, M.A. and Jimeneze. Analgesic and antipyretic effects of *Ecballium elaterium* (L.) A. Richard. Extract in rodents, J. Phytotherapy Research, 9 (2), 135-138 (1995).

Chittendon, F., RHS Dictionary of Plants plus Supplement, Oxford University Press, London, (1951).

Cozalez, B.R. and Panizo, F.M., An. Real Soc. Espan. Fis. Quim., Ser. B, 63 (12), 1123-36 (1967).

Dawidar. A. M.: Abdel-Mogib, M.; EL- Naggar. M. E. ; Mostafa. M. E. Acaricidal activity and chemical constituents of *Hyoscyamus muticus* against *Tetranychus urticae* Koch. submitted to Rev. Latinoamer.Quim.

Dittrich. V., J. Econ. Entomol. 55: 633-648 (1962).

Finney. D.J. probit Analysis. A Statistical treatment of the sigmoid Response curve. 7th Ed. Cambridge Univ. Press. Cambridge. England. (1971).

Hyalands. P. J. and Oskoui. M.T., Planta Med., 37 (1), 3744 (1979).

Imperato. F., Five plants of the family cucurbitaceae with flavonoid patterns of pollens different from those of corresponding stigmas, Experientia. 36 (10). 1136-7 (1980).

Ismail, S.M.M., Selectivity and joint action of *Malia azadarach* L fruit extracts with certain acaricides to *Tetranychus urticae* Koch and *Stethorus gilvifrons* Mulsant., Annals of Agric. Sc., Moshtohor, 35(1), 605-618 (1997).

Nurit. B.N. and Alfred. M.M., Cucurbitacins protect cucumber tissue against infection by *Botrytis cinerea*, Phytochemistry, 29 (3), 787-791 (1990).

Organ, J., Gourds, Faber, South Africa, (1963).

Oskoui, M.T., Toxicity and Partial Characterization of *Ecballium elaterium* Fruit Juice, Planta Med., 2, 159, (1986).

Pieribattesti, J.C., Conan, J.Y., Grondin, J., Vincent, E.J. and Guerere, M., Ann. Falsif. Expert. chim. Toxicol., 74 (793), 11-16 (1981).

Rao, M.M. and Lavie, D., The constituents of *Ecballium elaterium* L-XXII: Phenolics as minor components, Tetrahedron. **30**, 3309-3313 (1974).

Saleh, M.A., Arab Gulf Journal of Scientific Research, B Agricultural and Biological Sciences. 6 (2). 219-226 (1988).

Salim, M.L., Ammar, H.A., Abderruhman, S. and El-Remawy, H.A., Phytochemical screening and antimicrobial activity of wild Jordanian plants from Al-Balq'a. Bull. Fac. Pharm. (Cairo univ.), 34 (3). 235-238 (1996).

Seifert, K. and El-gamal, M.H., *Ecballium elaterium*: an in vitro source of cucurbitacins *Pharmazie*, **32** (10), 605-6 (1977).

Shafiq, M.M., Shahid, M. and Abdul Pesir, S., Sci. Int. (Lahore), 6 (4), 351-352 (1994).

Siddique, R.R., Zafar, U., Chaudhry, S.S and Ahmad, H., Antimicrobial activity of essential oils from *Schinus terebinthifolius*, *Cypress sempervirens*, Citrus limon, *Ferula assafoetida*. Part I *Pak.*, J. Sci. Ind. Res., 38 (9-10), 358-361 (1995).

Siddiqui, R.R., Ahmad, H., Shakoor, C.S., Ehteshamuddin, A.F.M. and Shireen, S., Antimicrobial activity of essential oils. Part II, Pak. J. Sci. Ind. Res., 39 (1-4), 43-47 (1996).

Sun, Y.P., Toxicity indexes an improved method of comparing the relative toxicity of insecticides. J.Econ. Entomol. 43: 45-53 (1950).

Toker, G., Memisoglu, M., Toker, M.C. and Yesilada, E., Callus formation and cucurbitacin B accumulation in *Ecballium elaterium* callus cultures, Fitoterapia, 74, 618-623 (2003).

Ying, M.C., Phytochemical Studies on Chienese Medicinal plants: *Hyoscyamus niger*, *Euptelea pleiospermum*, and *Rorippa Montana*, Ph.D, Hong Kong University of Science and Technology, (2000).

الملخص العربى

تم دراسة بعض المركبات العضوية التي تم فصلها من بعض النباتات الصحراوية المصرية واختبار سميه خلاصتها الطبيعية كمبيدات طبيعية على الأكاروس الأحمر ذو البقعتين.Tetranychus Urticae (Koch)، والتى تعد من أهم الآفات التي تصيب نباتات القطن والمحاصيل الزراعية المختلفة حيث تسبب لها أضرار بالغة، وبدراسة نباتات فاقوس الحمار والفلفل العريض والتى أعطت خلاصتها نتائج ايجابية ضد الأكاروس تم فصل وتعريف المركب الاستيرويدى (١) من خلاصه الايثر البترولي بعد إجراء عملية التصبن ومشتق فلافنيود جلوكوسيدى الريوتن (٢) من خلاصه الايثل اسيتات وباختبار سمية كلا من الخلاصات والمركبات العضوية المفصولة تبين أن مستخلص الكلورفورم من بين الخلاصات لنبات فاقوس الحمار كان الأقوى تأثيرا على الأطوارغير الكاملة (اليرقة) والإناث البالغة للأكاروس الأحمر ذو البقعتين بعد مرور سبعه أيام من إجراء المعاملة حيث كانت قيم التركيز القاتل لنصف عدد الأفراد ٠,٠٠١٨ و٠,٠٠٩٤ جزء من المليون على الترتيب، ثم يليه الزيت الطيار لنبات الفلفل العريض حيث كانت قيم التركيز القاتل لنصف عدد الأفراد لكلا من اليرقات والإناث البالغة للأكاروس الأحمر ذو البقعتين بعد مرور سبعه أيام من إجراء المعاملة ١٣٤٠ و١٣٥٠ جزء من المليون على الترتيب .