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Acaricidal potential and phytochemical analysis of the indigenous *Centaurea aegyptiaca* L. against *Tetranychus urticae* Koch (Acari: Tetranychidae)

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Abstract
The acaricidal potential of *Centaurea aegyptiaca* L. (Family Asteraceae) extracts was assessed against adult females and one day old eggs of *Tetranychus urticae* Koch (Acari: Tetranychidae). Of five main *C. aegyptiaca* extracts, miticidal activity-directed separation, pet. ether, methylene chloride and ethyl acetate fractions. Application of various chromatographic methods for the most potent fractions and spectral measurements (EI-MS, ESIMS, 1D-NMR, 2D-NMR) have been resulted in isolation and structural identification of six secondary metabolites reported from this plant species for the first time named, *β*-amyrin (1), methyl 8α-(3,4-dihydroxy-2-methylene-butanoyloxy)-6α, 15-dihydroxy-elema-1,3,11(13)-trien-12-oate (2), *β*-Sitosterol-3-O-β-D-glucopyranoside (3), Syringaresinol (4), *p*-Hydroxy benzoic acid (5) and methyl 8α, 6α, 15-trihydroxy-elema-1 ,3 , 11 (13)-trien-12-oate (6). The sensitivity of adult females and one day old eggs of *T. urticae* to various fractions and isolated nature products revealed great variation in effectiveness in comparison with Azadirachtin (Okios 3.2% EC). Amongst the tested fractions and isolated compounds, ethyl acetate fraction, Syringaresinol (4) and methyl 8α, 6α, 15-trihydroxy-elema-1, 3, 11 (13)-trien-12-oate (6) were the most potent products using leaf-dipping technique against adult females after 3 and 7-days of exposure. While, pet. ether fraction and methyl 8α-(3,4-dihydroxy-2-methylene-butanoyloxy)-6α, 15-dihydroxy-elema-1,3,11(13)-trien-12-oate **(2)** exhibited the most toxic natural products to one day old eggs of *T. urticae.*

Keywords: acaricidal activity, phytochemical investigation, *tetranychus urticae*, *centaurea aegyptiaca*

Introduction
The two-spotted spider mite, *Tetranychus urticae* Koch, is a major economic pest in a wide range of crops including, but not limited to, cotton, vegetables**,** ornamentals, and many orchard crops around the world and cause considerable annual losses (Migeon *et al.* 2010) [19]. The polyphagus nature and short generation time make two-spotted spider mites an ideal pest and have potential to inflict yield losses when left unchecked under favorable environmental conditions (Wilson *et al*. 1987; Scott *et al*. 2013) [29, 25].
Two spotted spider mite damage can be classified as either direct or indirect (Brandenburg and Kennedy 1987) [5]. Direct effects include stippling, webbing, defoliation, leaf burning, and in extreme outbreaks plant death. Indirect effects of mite feeding may include decreased photosynthesis and transpiration. Coincidentally, outbreaks of *T. urticae* are closely associated with the use of various pesticides (Brandenburg and Kennedy 1987) [5].
The overuse application of various synthetic chemical acaricides lead to serious risks to non-target organisms including humans, beneficial terrestrial insects, domestic animals, birds, wildlife, fishes, other aquatic life, soil fauna and environmentally unacceptable (Mostafa *et al*. 2017; Mostafa *et al*. 2019; Dawidar *et al*. 2014) [20, 21, 8].
Plants manufacture a vast array of chemicals that could serve as sources or models for future insecticides with novel modes of action, highly effective and safe than synthetic ones such as the organophosphorus, carbamate and organochlorine insecticides (Helson 1992) [18].
Centaurea aegyptiaca L. (Family Asteraceae), known locally as Murrar Masry, is a biennial or short-lived perennial herb grows widely in the Egyptian desert, Sinai, Red Sea coastal strip, Gebel Elba (Bakr *et al*. 2016) [3]. Reviewing the previously phytochemical and biological investigation revealed the presence of sesquiterpene lactones (Dahmy *et al.* 1985; Sary *et al.* 2018, Sary *et al.* 2016) [12, 23, 24], phenolic acid esters and flavonoids (Bakr *et al.* 2016; Senosy *et al*. 2018) [3, 26] beside, *C. aegyptiaca* ethanol extract showed cytotoxic activities against hepatic and laryngeal carcinoma cell lines (Bakr *et al*. 2016) [3].
The main objective of the present study was to find out new leads could be used as natural pesticides against *T. urticae* and carry out an in-depth phytochemical investigation to characterize its main active ingredients using chromatographic and spectral analyses.

Materials and Methods
Instruments
NMR spectra were recorded on 500 MHz (JEOL). Chemical shifts are given in δ (ppm) relative to TMS as internal standard material at Faculty of Science, Mansoura University. HSQC, HMBC, (H-H) COSY and NOESY were recorded at 500 MHz. EIMS spectra were recorded on a Ssimadzu GC/MS UB 1000 EX instrument (at the University of Cairo, Micro Analytical Center). High-resolution mass spectra were carried out on an API Q-STAR PULSAR i of applied biosystem by direct injection of the purified compounds.

Chemicals
Columns chromatography (CC) were established using silica gel F254 (230-400 mesh) or polyamide 6. Thin layer chromatography were done on silica gel (Kieselgel 60, F 254) of 0.25 mm thickness. Solvents of Petrolium ether, methylene chloride, ethyl acetate, butanol and methanol were purchased from Adwic Company, Azadirachtin (Okios

3.2% EC) as standard botanical pesticide.

Plant material

Centaurea aegyptiaca L. was collected in Dakahlia Governorate on the River Nile banks near Mansoura on April, 2018 and identified by Prof. Ibrahim A. Mashaly, Professor of Plant Ecology, Plant department, Faculty of Science, Mansoura University.

Extraction and isolation

The air dried whole plant of *Centaurea aegyptiaca* L. (6kg) was soaked in methanol (5 x 10 L) at room temp. The syrupy was filtered, concentrated and exhaustively partitioned successively with pet. Ether, methylene chloride, ethyl acetate and butanol to furnish pet. Ether (77.74 g), methylene chloride (36.52 g), ethyl acetate (24.86 g) and butanol (66.24 g) fractions. After defatting the petroleum ether fraction with cold methanol, petroleum ether fraction (31 g) was subjected to column chromatography over silica gel using petroleum ether/ethyl acetate solvent system as an eluent with gradient increasing in polarity. Nine sub-fractions were obtained based on their TLC pattern. Sub-fraction VIII, the 75:25 pet. Ether: ethyl acetate silica gel column fraction, was separated on PTLC using pet. Ether/ ethyl acetate 9:1 as an eluent to give compound (**1**) (R_f = 0.34). The methylene chloride fraction (25 g) was subjected to silica gel column chromatography using mixture of pet. Ether and ethyl acetate as eluent, with increasing polarities, the effluents were combined into eleven sub-fractions according to their TLC patterns. Sub-fraction V, the 40:60 pet. Ether: ethyl acetate silica gel column fraction, was further purified on silica gel preparative TLC developed by a mixture of methylene chloride /methanol (98:2 v/v) to yield a pure compound (2) (R_f = 0.38). Sub-fraction X, the 80:20 ethyl acetate: methanol silica gel column fraction, was purified by repetitive PTLC using (silica gel, methylene chloride/methanol 13:3) to obtain compound (3) (R_f = 0.71). The ethyl acetate fraction was chromatographed over polyamide S6 column using mixture of dist. H_2O, (dist.H_2O: methanol) and (methanol: ammonia) as eluent, with increasing polarities. The obtained effluents were carefully combined according to their TLC pattern to afford seventeen sub-fractions. Sub-fraction VII was chromate graphed on PTLC silica gel plates using ethyl acetate / methanol (98: 2) as a developing system to afford compound (4) (R_f = 0.87) and (5) (R_f= 0.83). Sub-fraction X was further developed over silica gel PTLC for extra purification by ethyl acetate/ methanol (95:5) to yield compound (6) (R_f = 0.76).

***β*-amyrin (1)**

White amorphous powder; EI-MS, m/z (rel. int): 426 (10%) [M^+], 411 (7) [M^+-CH_3], 408 (3) [M^+-H_2O], 393 (3) [M^+-CH_3-H_2O], 218 (100%) [$C_{16}H_{26}$], 208 (3) [$C_{14}H_{24}O$], 207 (10) [$C_{14}H_{23}O$], 203 (48) [$C_{15}H_{23}$], 189 (14) [$C_{14}H_{21}$]. ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H 0.79 (3H, s, H-24); 0.83 (3H, s, H-28); 0.87 (6H, s, H-29, H-30); 0.94 (3H, s, H-25); 1.00 (3H, s, H-26); 1.14 (3H, s, H-23); 1.26 (3H, s, H-27); 3.19 (1H, dd, J =5.5, 10.7 Hz, H-3); 5.19 (1H, t, J =4.4 Hz, H-12).

Methyl 8*α*-(3, 4-dihydroxy-2-Methylene-Butanoyloxy)-6*α*, 15-dihydroxy-elema-1, 3, 11(13)-trien-12-oate (2)

Colourless oil; ESI-MS (positive mode) m/z 455 [M+ 2Na-H]$^+$ (Calcd for $C_{21}H_{30}O_8$, 410).^{1}H-NMR (DMSO-d_6): δ_H 5.72 (1H, dd, J = 17.5, 10.8 Hz, H-1); 4.87 (1H, br.d, J = 17.5 Hz, H-2a); 4.89 (1H, br.d, J = 10.8 Hz, H-2b); 5.21 (1H, br.s, H-3a); 4.79 (1H, br.s H-3b); 1.78 (1H, d, J = 10.7 Hz, H-5); 4.00 (1H, t, J = 10.7 Hz, H-6); 2.62 (1H, t, J = 10.7 Hz, H-7); 5.21 (1H, m, H-8); 1.52 (1H, d, t = 12.3 Hz, H-9α); 1.67 (1H, dd, J = 12.3, 4.4 Hz, H-9β); 6.18 (1H, br.s, H-13a); 5.81 (1H, br.s, H-13b); 1.10 (3H, s, H-14); 3.82 (1H, d, J = 15.3 Hz, H-15a); 3.72 (1H, d, J = 15.3 Hz, H-15b); 6.00 (1H, br.s, H-3′a); 5.81 (1H, bd.s, H-3′b); 4.30 (1H, dd, J = 6.3, 3.1 Hz, H-4′); 3.35 (1H, dd, J = 15.5, 6.3 Hz, H-5′a); 3.13 (1H, dd, J = 15.5, 6.3, Hz, H-5′b); 3.67 (3H, s, -OCH_3); ^{13}C-NMR (DMSO-d_6): δ_C 147.55 (C-1); 110.88 (C-2); 109.57 (C-3); 147.39 (C-4); 54.82 (C-5); 69.85 (C-6); 54.69 (C-7); 71.04 (C-8); 43.24 (C-9); 39.77 (C-10); 138.55 (C-11); 166.44 (C-12); 128.05 (C-13); 18.53 (C-14); 65.31 (C-15); 164.96 (C-1'); 142.01 (C-2'); 124.47 (C-3'); 70.08 (C-4'); 65.38 (C-5'); 51.60 (-OCH_3).

***β*-Sitosterol-3-O-β-D-glucopyranoside (3)**

Colourless crystals substance; ^{1}H NMR (DMSO-d_6): δ_H 3.51 (1H, m, H-3), 5.33 (1H, d, J =1.4 Hz, H-6), 0.65 (3H, s, H-18), 0.95 (3H, s, H-19), 0.90 (3H, d, J =6.3 Hz, H-21), 0.79 (3H, d, J = 7.3 Hz, Me-26), 0.76 (3H, d, J = 7.3 Hz, H-27), 0.81 (3H, t, J = 6.8 Hz, H-29), 4.21 (1H, d, J = 7.8 Hz, H-1'). ^{13}C-NMR (DMSO-d_6), δ_C 36.83 (C-1), 29.27 (C-2), 76.74 (C-3), 40.02 (C-4), 140.45 (C-5), 121.19 (C-6), 31,42 (C-7), 31.37 (C-8), 49.60 (C-9), 36.21 (C-10), 20.60 (C-11), 38.31 (C-12), 41.86 (C-13), 56.17 (C-14), 23.86 (C-15), 27.79 (C-16), 55.43 (C-17), 11.78 (C-18), 19.1 (C-19), 35.49 (C-20), 18.61 (C-21), 33.35 (C-22), 25.44 (C-23), 45.14 (C-24), 28.71 (C-25), 18.94 (C-26), 19.71 (C-27), 22.60 (C-28), 11.67 (C-29), 100.80 (C-1$^{'}$), 73.44 (C-2$^{'}$), 76.74 (C-3$^{'}$), 70.07 (C-4$^{'}$), 76.9 (C-5$^{'}$), 61.06 (C-6$^{'}$).

Syringaresinol (4)

White residue; ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 6.58 (4H, s, H-2, 6, 2', 6'), 4.73 (2H, d, J= 4.3 Hz, H-8, 8'), 4.29 (2H, dd, J= 9.1, 6.8 Hz, H-9b, 9'b), 3.90 (12H, s, 4 (-OCH_3)), 3.90 (2H, m, H-9a 9'a), 3.10 (2H, m, H-7,7').

***p*-Hydroxy benzoic acid (5)**

White residue; ^{1}H-NMR (CD_3OD): δ_H 7.84 (2H, d, J = 8.6 Hz, H-2/H-6); 6.75 (1H, d, J = 8.6 Hz, H-3/H-5).

Methyl 8*α*, 6*α*, 15-trihydroxy-elema-1,3,11(13)-trien-12-oate (6): White residue; ^{1}H-NMR (CD_3OD): δ_H 5.71 (1H, dd, J = 17.2, 11.1 Hz, H-1); 4.93 (1H, br.d, J = 17.2 Hz, H-2a); 4.95 (1H, br.d, J = 11.1 Hz, H-2b); 5.28 (1H, br.s, H-3a); 4.90 (1H, br.s H-3b); 1.78 (1H, d, J = 10.8 Hz, H-5); 4.08 (1H, t, J = 10.8 Hz, H-6); 2.26 (1H, t, J = 10.8 Hz, H-7); 4.07 (1H, m, H-8); 1.44 (1H, d, t = 12.0 Hz, H-9α); 1.68 (1H, dd, J = 12.0, 4.4 Hz, H-9β); 6.29 (1H, d, J = 1.3 Hz, H-13a); 5.70 (1H, d, J = 1.3 Hz, H-13b); 1.08 (3H, s, H-14); 3.94 (1H, d, J = 14.8 Hz, H-15a); 3.84 (1H, d, J = 14.8 Hz, H-15b); 3.70 (3H, s, -OCH_3).

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 colony was isolated from heavily infested castor oil plant leaves and reared on fresh one. These leaves were cleaned and placed on moisten cotton wool pad in Petri dishes. This colony was left for one year under the precious conditions in order to get a homogenous and sensitive colony. Spider mites 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* individuals.

Assessment of acaricidal activity

In this respect, laboratory experiments are conducted to evaluate the activity of tested plant extracts and its isolated compounds against *T. urticae* two different stages (eggs and adult females). The leaf-dip technique was used (Dittrich, 1969) [11].

Mortality percentages were determined and corrected by using Abotts (1925) [1] formula and they are statistically analyzed to estimate LC_{50}, LC_{90} and slope values according to Finney (1971) [14]. 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) [27] equation.

$$\text{Toxicity index} = \frac{LC_{50} \text{ of compound A}}{LC_{50} \text{ of compound B}} \times 100$$

Where: A is the most effective compound; B is the tested compound

Results and Discussion

The increasing serious problems of resistance and residue to pesticides as well as contamination of the biosphere associated with large-scale use of broad spectrum synthetic pesticides have led to the need for effective biodegradable pesticides with greater selectivity. This awareness has created a world-wide interest in the development of alternative strategies**,** including the discovery of newer botanical pesticides (Dayan *et al.* 2009) [9].

The action of botanical origin natural products are actually being studied as alternative eco-friendly pesticides to manage the crop pest, *Tetranychus urticae* Koch (Mostafa *et al*. 2017; Dawidar *et al*. 2014) [20, 8].

Processing of the air dried whole plant of *Centaurea aegyptiaca* furnished four main fractions pet. ether, methylene chloride, ethyl acetate and butanol fractions (*cf.* Materials and Methods). Of these four main *C. aegyptiaca* fractions, miticidal activity-directed separation, pet. ether, methylene chloride and ethyl acetate which were the most potent fractions against adult females and one day old eggs of *T. urticae* using leaf-dipping technique (Tables 1 and 3).

As the pet. ether, methylene chloride and ethyl acetate Fractions were proved to be highly effective against *T. urticae*, it is recommended to study in details the chemical constituents responsible for that activity.

So, isolation and structure elucidation of its constituents were performed.

The use of diverse chromatographic (CC and PTLC) and spectrophotometric (EI-MS, ESI-MS, ^{1}H-NMR, ^{13}C-NMR, H-H COSY, NOESY, HSQC and HMBC) techniques for the bioactive fractions have led to the identification of six secondary metabolites belonging to different classes; three terpenes (one triterpene (1), two elemanolides sesquiterpenes (2, 6)), two shikimates (4, 5) and one sterol glycoside (3).

Compound (1) was isolated as a white amorphous powder from the pet. Ether fraction, its molecular formula was determined on the basis of EI-MS with M^{+} ion peak at m/z 426 (10%) corresponding to $C_{30}H_{50}O$. The identity of the compound was established using ^{1}H-NMR spectrum which gave the same characteristic signals to *β*-amyrin (1) as reported previously by Virgilio *et al.* (2015) [28].

Compound (2) was isolated as a colourless oil from the methylene chloride fraction, the positive ion ESI-mass spectrum showed quasi-molecular ion $[M+ 2Na- H]^{+}$ at m/z 455, indicating a molecular weight of 410 (calcd for $C_{21}H_{30}O_8$). The ^{1}H-NMR, ^{13}C-NMR, H-H COSY, NOESY, HSQC and HMBC spectra of compound (2) were clearly indicated the presence of elemanolide sesquiterpene class from its typical low-field signals, and all the observed spectral signals are typically matched methyl 8α-(3,4-dihydroxy-2-methylene-butanoyloxy)-6α, 15-dihydroxy-elema-1,3,11(13)-trien-12-oate (2) which was assigned before from *Centaurea paui* by Cardona *et al.* (1997) [7].

Methylene chloride fraction afforded another isolated white powder, compound (3), which showed ^{1}H and ^{13}C-NMR characteristic signals for sterol glycoside and confirmed by comparing its spectra data to be *β*-sitosterol-3-O-β-D-glucopyranoside (3) which was reported previously by Gohar *et al*. (2000) [16].

Careful examination of ^{1}H-NMR spectra of compounds (4 and 5) which was isolated from ethyl acetate fraction revealed that it belongs to shikimate class. A furofuran-type lignin was identified as syringaresinol (4) by comprising its ^{1}H-NMR data with those reported in the literature by Gohari *et al.* (2011) [17].

Also, ^{1}H-NMR spectrum of (5) possessing a substitution pattern of AA'BB' system assigned to *p*-hydroxy benzoic acid which was confirmed by matching its spectra with those reported by Riaz *et al.* (2014) [22].

Analysis of ^{1}H-NMR data of compound (6) displayed signals ascribable to another elemanolide which was closely related to (2) except for the absence of signals related for 3,4-dihydroxy-2-methylene-butanoyloxy moiety so, compound (6) is elucidated as methyl 8α, 6α, 15-trihydroxy-elema-1,3,11(13)-trien-12-oate (6), the known previously published natural product by Cardgna *et al.* (1992) [6]. All the above elucidated six compounds reported for the first time from *C. aegyptiaca.*

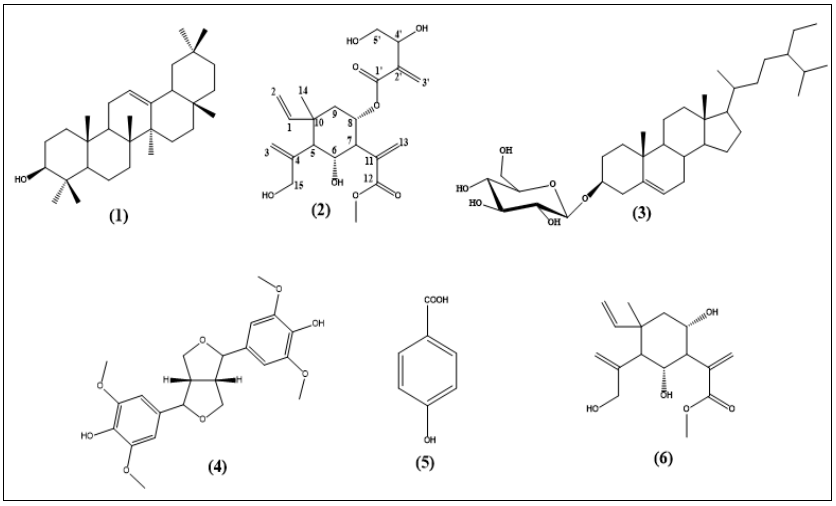

Fig 1

Acaricidal potintial of *C. aegyptiaca* fractions and isolated compounds to adult females of *Tetranychus urticae* Koch after 3 and 7-days of post treatment.

In the light of results presented in table 1, adult females of *T. urticae* showed different degrees of susceptibility to the five botanical extracts after 3 and 7-days of post application using leaf-dipping technique in comparison with azadirachtin (Okios 3.2% EC) under laboratory conditions. The potency arrangement at LC_{50} level, was as the following; ethyl acetate, methylene chloride, crude methanol extract, butanol, pet. Ether fractions and azadirachtin, respectively after 3-days of treatments. The LC_{50} values were: 364.08, 381.45, 422.81, 1168.50, 1700.83 and 1839.91 ppm, respectively.

Taking the toxicity index into consideration after 7-days of treatment ethyl acetate fraction was also the most toxic against adult females of *T. urticae* followed by methylene chloride, crude methanol extract, azadirachtin, butanol and pet. ether fractions with LC_{50} values 73.09, 99.95, 109.98, 392.62, 400.43 and 406.98 ppm, respectively.

The insecticidal activity of any extract from any natural source closely depend upon the chemical composition of its secondary metabolites and the concentrations of active ingredients. Following these active principles phyto chemically to isolate pure appreciable identified substances amounts and test all individually to confirm their toxicity nature is the main goal of this study.

The results obtained in table (2) showed the efficiency of the tested *C. aegyptiaca* isolated compounds against the adult females of *T. urticae* after 3 and 7- days of treatment. Syringaresinol (4) which belong to furofuran-type lignin isolated from ethyl acetate fraction (most effective fraction) was the most effective at LC_{50} level followed by elemane (2), elemane (6), *p*- hydroxy benzoic acid (5), *β*-sitosterol-3-O-*β*-D-glucopyranoside (3), *β*- amyrine (1) and finally azadirachtin after 3 days of treatment. The LC_{50} values were 806.16, 925.11, 1005.50, 1630.42, 1732.72, 1878.24 and 1839.91 ppm, respectively.

After 7-days of former application, elemane (6) exhibited a high degree of efficiency followed by syringaresinol (4), azadirachtin, *p*- hydroxy benzoic acid (5), elemane (2), *β*-sitosterol-3-O-β-D-glucopyranoside (3) and *β*- amyrine (1). The LC_{50} values of these tested isolated compounds were: 17.62, 78.32, 392.62, 204.84, 267.87, 369.45 and 712.64 ppm, respectively.

It is obviously clear from the above toxicity assay that syringaresinol (4) and the sesquiterpene elemane (6) were the most effective compounds and considered to be one of the active ingredients which isolated from the most potent ethyl acetate fraction after 7-days of treatment.

Previously studies by Born *et al*. (2012) [4] suggested that terpenes, can be employed for the development of new pesticides and become prototype agrochemical agents, also antifeedant activity against the African armyworm *Spodoptera exempta* was recordrd by application two elemanolide lactones isolated from the ether extract of *Vernonza amygdalina* by using a leaf-disk assay (Ganjian *et al*. 1983) [15].

Table 1: Toxicity of plant fractions against adult females of *T. urticae* after 3 and 7- days of treatment.

Plant extract	3 days					7 days				
	LC_{50} (ppm) and confidence limits at 95%	**LC_{90} (ppm) and confidence limits at 95%**	**Slope ± SE**	**X^2**	**Toxicity index**	**LC_{50} (ppm) And confidence limits at 95%**	**LC_{90} (ppm) and confidence limits at 95%**	**Slope ± SE**	**X^2**	**Toxicity index**
Crude extract (MeOH)	422.81 255.79 838.06	5380.59 1827.78 343199.81	1.160±0.356	0.01	86.12	109.98 63.62 159.94	757.85 431.38 2669.86	1.529±0.334	0.20	66.46
Pet. ether	1700.83 1030.77 3991.56	36955.71 10231.73 1917953.30	0.959±0.256	2.65	21.41	406.98 259.17 547.29	1840.36 1220.41 4319.35	1.956±0.406	0.15	17.96
Methylene chloride	381.45 176.34 791.85	9567.09 2680.61 935391.21	0.916±0.273	0.55	95.45	99.95 31.70 156.76	549.17 368.03 1388.53	1.732±0.455	0.21	73.13
Ethyl acetate	364.08 238.93 970.84	2563.27 964.33 48837.45	1.512±0.398	0.63	100.00	73.09 43.95 99.49	337.25 224.83 791.35	1.930±0.412	0.30	100.00
Butanol	1168.50 676.78 6873.72	12212.54 3223.53 2486276.77	1.258±0.390	1.60	31.16	400.43 249.78 709.41	4215.75 1630.81 104839.95	1.254±0.359	0.04	18.25
Azadirachtin (Okios 3.2% EC)	1839.91 963.49 20671.30	15141.58 3770.56 5252410.27	1.400±0.446	0.45	19.79	392.62 267.75 605.07	2730.53 1334.81 18088.50	1.522±0.368	0.73	18.62

Table 2: Toxicity of isolated compounds against adult females of *T. urticae* after 3 and 7- days of treatment.

	Compounds	3 days					7 days				
		LC_{50} (ppm) and confidence limits at 95%	LC_{90} (ppm) and confidence limits at 95%	Slope ± SE	X^2	Toxicity index	LC_{50} (ppm) and confidence limits at 95%	LC_{90} (ppm) and confidence limits at 95%	Slope ± SE	X^2	Toxicity index
Pet. ether	*β*- amyrine (1)	1878.24 809.23 43038.00	51465.64 6838.59 253180759.90	0.891±0.283	0.69	42.92	712.64 375.09 4874.66	29648.21 4529.31 99848688.98	0.792±0.255	0.07	2.47
Methylene chloride	Elemane (2)	925.11 382.17 14964.86	37449.05 4486.06 70804082.22	0.797±0.231	1.26	87.14	267.87 133.87 1411.66	12410.59 1985.87 4556995.97	0.769±0.210	2.74	6.58
	β-Sitosterol-3-O-β-D-glucopyranoside (3)	1732.72 771.56 31002.78	45766.79 6478.69 130405651.70	0.901±0.282	1.05	46.53	369.45 224.90 774.08	7580.87 2266.00 276097.17	0.977±0.255	1.57	4.77
Ethyl acetate	Syringaresinol (4)	806.16 362.23 11543.56	14860.26 2475.09 11488213.67	1.013±0.301	1.32	100.00	78.32 37.75 127.93	1106.86 475.05 10556.66	1.114±0.277	0.49	22.50
	p- hydroxyl Benzoic acid (5)	1630.42 726.78 31217.22	48423.71 6516.86 226742295.50	0.870±0.277	0.99	49.45	204.84 101.74 333.75	2829.47 1214.99 26516.82	1.124±0.277	0.30	8.60
	Elemane (6)	1005.50 413.41 26721.29	19585.56 2812.77 50784908.42	0.994±0.310	0.30	80.18	17.62 1.87 36.15	390.81 188.75 3839.19	0.952±0.280	0.24	100.00
Azadirachtin (Okios 3.2% EC)		1839.91 963.49 20671.30	15141.58 3770.56 5252410.27	1.400±0.446	0.45	19.79	392.62 267.75 605.07	2730.53 1334.81 18088.50	1.522±0.368	0.73	18.62

Acaricidal potintial of *C. aegyptiaca* fractions and isolated compounds to one day eggs of *T. urticae* Koch.

The susceptibility of 1- day old egg stage of *T. urticae* to *C. aegyptiaca* extracts represented in table (3), revealed that azadirachtin exhibited a high degree of efficiency followed by pet. ether, ethyl acetate, methylene chloride, butanol and the least one crude extract (MeOH). The LC_{50} values were 378.54, 716.12, 1017.50, 1116.79, 1121.03, 2308.00 ppm, respectively. Comparing the slopes values (table 3), methylene chloride fraction recorded the flattest toxicity line (0.604±0.152) which indicated that mortality is not concentration-dependent while, azadirachtin had the steepest one (1.299±0.213) which indicated that the toxicity is a concentration dependent in this case. The other fraction lines came between these two fractions. Table (4) showed the susceptibility of 1-days old eggs of *T. urticae* to *C. aegyptiaca* isolated compounds. Data revealed that azadirachtin was the most toxic followed by elemane (2), elemane (6), *p*- hydroxy benzoic acid (5), syringaresinol (4), *β*- amyrine (1) and *β*-sitosterol-3-O-β-D-glucopyranoside (3). The LC_{50} values were 378.54, 467.26, 850.00, 1034.06, 1103.38, 1149.59 and 1624.50, respectively.

From the foregoing ovicidal results, pet. ether fraction and elemane (2) exhibited the most toxic natural products to one day old eggs of *T. urticae* also, elemanolide sesquiterpenes (2) and (6) considered to be the ovicidal active ingredients when compared with their own fractions (methylene chloride and ethyl acetate fractions). Our results were in agreement with those obtained by Farag *et al*. (1993) [13], who recorded that the hexane extract (low polar fraction) was more effective against eggs of *T. urticae* than ethanol extract (high polar fraction). Also, it was reported that *β*-amyrin (triterpene) which isolated from the crude petroleum ether extract of *Abrus precatorius* seeds was the most toxic compound tested against the egg stage and spraying females with the LC_{25} for *β*-amyrin caused a significant reduction in fecundity and the viability of resulting eggs (Dimetry *et al*. 1990; Amer *et al*. 1989) [10, 2].

Table 3: Toxicity of plant fractions against one day old eggs of *T. urticae*.

Plant extract	LC_{50} (ppm) and confidence limits at 95%	LC_{90} (ppm) and confidence limits at 95%	Slope ± SE	X^2	Toxicity index
Crude extract (MeOH)	2308.00 1348.30 4718.30	107925.86 30509.13 1430306.69	0.768±0.141	5.34	16.40
Pet. ether	716.12 334.56 1219.36	11542.85 5885.49 37766.85	1.062±0.186	1.53	52.86
Methylene chloride	1116.79 470.91 2467.60	147869.97 27405.05 17290662.47	0.604±0.152	0.18	33.90
Ethyl acetate	1017.50 441.39 2246.09	42881.62 10987.84 2045138.33	0.789±0.203	0.85	37.20
Butanol	1121.03 387.89 2815.21	81659.01 15999.85 19631676.42	0.688±0.198	0.71	33.77
Azadirachtin (Okios 3.2% EC)	378.54 189.02 624.22	3672.39 2092.78 8968.53	1.299±0.213	1.374	100.00

Table 4: Toxicity of isolated compounds against one day old eggs of *T. urticae*.

Plant extract	Isolated compounds	LC_{50} (ppm) and confidence limits at 95%	LC_{90} (ppm) and confidence limits at 95%	Slope ± SE	X^2	Toxicity index
Pet. ether	*β*- amyrine (1)	1149.59 297.45 3171.68	13515.64 4388.25 739830.09	1.197±0.384	0.321	32.91
Methylene chloride	Elemane (2)	467.26 67.79 1046.53	8297.28 3144.20 186255.31	1.026±0.315	0.02	81.01
	β-Sitosterol-3-O-β-D glucopyranoside (3)	1624.50 378.04 4397.81	57407.65 13824.80 11754908.36	0.828±0.260	0.72	23.30
Ethyl acetate	Syringaresinol (4)	1103.38 442.39 2409.49	17067.81 6249.51 172669.26	1.078±0.251	0.56	34.31
	p- hydroxyl Benzoic acid (5)	1034.06 340.61 3370.51	22599.94 5594.16 3389372.96	0.957±0.295	1.40	36.61
	Elemane (6)	850.00 495.77 1363.19	4494.74 2525.18 13469.74	1.772±0.350	2.00	44.54
Azadirachtin (Okios 3.2% EC)		378.54 189.02 624.22	3672.39 2092.78 8968.53	1.299±0.213	1.37	100.00

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