*Egypt. J. Chem.* **Vol. 64,** No. 1 pp. 341 - 357 (2021)

**Egyptian Journal of Chemistry**

**http://ejchem.journals.ekb.eg/**

# Eco-Friendly Secondary Metabolites from *Conyza dioscoridis* Against *Spodoptera littoralis*

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**Abstract**

2[ry] metabolites of bioactive chloroform/ methanol extract of *Conyza dioscoridis* using chromatographic techniques and spectroscopic analysis led to identify five major compounds; *β*-amyrin-3-acetate, *β*-lupeol-3-acetate, *β*-amyrenone, dotriacontane and 5,4′-dihydroxy-6,7-dimethoxyflavone. The percentage of cumulative mortalities during pupal and adult stages reached to 76.6% and 83.3%, respectively after feeding 4[th]larval instar of *Spodoptera littoralis* on *C. dioscoridis* crude extract with insecticidal activity in a dose-dependent manner. Whereas, *β*-amyrenone, lupeol acetate and 5,4′-dihydroxy-6,7-dimethoxyflavone suppressed 50, 60 and 73.3% of 4[th] instar larvae of *S. littoralis* at concentration 0.3, 0.5 and 0.5%, respectively. Also, the extract showed marked decreasing in acetyl cholinesterase activity, total lipids and protein contents. The GC/MS analysis of volatile oil of *C. dioscoridis* aerial part led to identify 63 sensory metabolites that represent 93.68% of total volatile constituents. The oil was characterized by a high percentage of oxygenated sesquiterpenes (36.00%) and sesquiterpene hydrocarbons (21.09%), in addition phenylated and aliphatic hydrocarbons (15.43% and 14.58%, respectively). *C. dioscoridis* exhibited acute toxicity on both sexes, reduced adult longevity, oviposition deterrents and reduced fertility on *S. littoralis*. So, it used as new natural target insecticidal agent for *S. littoralis*, biodegradable alternatives to chemical insecticides and can used as a natural tool in pest management program.

*Keywords*: *Conyza dioscoridis*; triterpenes; methoxyflavone; sesquiterpenes; insecticidal; *Spodoptera littoralis*

## 1. Introduction

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisd) (family: Noctuidae), is *one* of the most destructive agricultural lepidopterous polyphagous *pests* where it could *damage at least* 29 hosts from ornamental and *vegetable crops* in Egypt **[1].** As one of the most important global economic problems, the protection of crops from pests is urgently needed. The chemical pesticides are continuously used leading to negative effects on human health as well as animals and food consumers. For protection of crops, many different countries search for natural alternatives to chemical dangerous pesticides using botanical pesticides with minimal costs and ecological side effects **[2].** Several plants extracts or isolated active compounds showed toxic effects against number of economically important insects among of them *S. littoralis* **[3, 4].** The plants are considered as one of the richest sources of diversity secondary metabolites that can be used as pest control agents. These metabolites can effect on various biochemical components in insects such as proteins, and lipids, thereby altering the internal metabolism of the insect, causing suppression of their activity or mortality **[5].**Among of these plants *Conyza dioscoridis* that affect the life cycle of the insects of *S. littoralis* (Boisd) which showed potency against egg stage and different larval instars with all

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Receive Date: 11 August 2020, Revise Date: 07 September 2020, Accept Date: 13 September 2020
DOI: 10.21608/EJCHEM.2020.38753.2798

concentrations adversely affected the egg viability, and unhatchability **[6].**

*Conyza dioscoridis* (L.) Desf. (Synonym:*Pluchea dioscoridis*(L.) DC), along with other names; *Baccharis dioscoridis* L. and *Conyza odora* Forssk., belongs to family Asteraceae **[7]**. It distributed in Egypt along Nile River, Mediterranean Sea, Eastern & Western Deserts and Sinai and it is known as mosquito tree due to repellent effect on mosquito **[8]**.

*C. dioscoridis* is used in folk medicine for rheumatic pains, colic, cold as well as in treatment epilepsy in children **[9].** Several bioactivity studies of *C. dioscoridis* extract exhibited antinociceptive, antidiarrheal, antioxidant, antiulcerogenic, anti-inflammatory, antipyretic, antihyperglycemic, cytotoxicity versus brine shrimp and colon carcinoma cell line, larvicidal activity against *Culex pipiens* and antimicrobial activity **[9-12]**. Also, *C. dioscoridis* crude extract proved herbicidal activity on some perennial weeds as well as insecticidal effect against many insects **[13, 14]**. Referring to safety of *Conyza dioscoridis*, **Elshamy el al., (2015)** proved that different extracts did not produce any obvious toxic symptoms or mortality on rats till dose 5 gm/kg animal body weight **[11].** Many phytoconstituents have been reported in the different parts of *C. dioscoridis* "*P. dioscoridis*" such as thiophenacetylenes, sesquiterpene derivatives including; sesquiterpene lactones, eudesmane derivatives, eudesmanolides and costic acid derivatives in addition phenolic compounds such as sulfated flavonoids, non-sulfated flavonoids and phenolic acids **[11, 12, 15].** Various studies on the volatile constituents of *C. dioscoridis* leaves have been reported which composed mainly of sesquiterpene hydrocarbons and oxygenated sesquiterpenes **[10, 16]**.

So our study is targeting to suggest eco-friendly natural agents have ability to prohibit population of *S. littoralis* with more safety to human health, environment and ecosystem. Referring to the insecticidal activity of *C. dioscoridis*, the aim of our current study is evaluation of insecticidal properties of chloroform/ methanol extract and the volatile oil prepared from *C. dioscoridis* aerial parts to suppress the population of $4^{th}$ larval instars of *S. littoralis* under laboratory conditions. As well as the biochemical changes of acetylcholine esterase activity and total lipids and protein contents were determined. In addition, characterization of bioactive $2^{ry}$ metabolites and volatile constituents that may contribute to the insecticidal effect using different chromatography techniques and spectroscopic analysis.

## 2. Material and Methods

### Plant material

*Conyza dioscoridis* aerial parts (Fam. Asteraceae) were collected from plants growing wild along the Cairo–Suez road (east of Cairo) in flowering stage in April. The identity of the plant was confirmed by Prof. Dr Mona Marzouk, NRC, Cairo. Voucher specimen was kept in NRC Herbarium (CAIRC) under number 1126. Aerial parts containing flowers were dried, and then coarsely powdered and stored in polyethylene plastic bags in a dry place.

### General procedure

All solvents are analytical grade. Nuclear magnetic resonance (NMR) experiments were recorded on a Bruker spectroscopy: 400 MHz ($^{1}$H NMR) and 100 MHz ($^{13}$C NMR). UV spectra were obtained using Shimadzu model-2401 CP spectrophotometer (Shimadzu Inc., Tokyo, Japan). Gas chromatography/mass spectrometry analysis (GC/MS) analysis was carried out using a Finnigan SSQ 7000 (ThermoFinnigan, San Jose, California, USA) GC/MS spectrophotometer equipped with library software Wiley 138 and NBS 75 under the following conditions: DB-5-fused silica capillary column, 30 m in length, 0.32 mm ID, and with a film thickness of 0.25 μm; carrier gas, helium at a flow rate of 10 ml/min; temperature programmed to 60–260°C at a rate of 4°C/min, chart speed: 0.5 cm/min, ionization voltage 70 eV, and detector: flame ionization detector.

### Preparation of plant crude extract

Dried powdered of *C. dioscoridis* aerial part was extracted at a solid to solvent ratio of 1:10 (w/v) with chloroform/ methanol (1:1) till exhaustion. The solvents were evaporated under reduced pressure at 40℃.

### Preparation of volatile constituents

The fresh *C. dioscoridis* aerial part was cut to small pieces and then hydro-distilled in modified Likens–Nickerson apparatus using *n*-pentane. The volatile constituents were characterized using GC/MS analysis. The identification of volatile oil constituents was performed depending on spectral fragmentation pattern compared with those of published data **[17]** and a library database [Wiley (Wiley Institute, USA) and NIST (National Institute of Technology, USA)].

Quantitative determination of volatiles was recorded from gas chromatogram peaks area measurements.

**Colum chromatography**

The chloroform/ methanol extract (10 g) was chromatographed on silica gel column and eluted with a mixture of solvents including *n*-hexane and chloroform with increasing polarity. Fractions of 100 ml were collected and that exhibited similar TLC profiles, were combined together to afford five main sub fractions A (100% hexane), B (80% hexane/ chloroform), C (60% hexane/ chloroform), D (40% hexane/ chloroform) and E (100% chloroform). Further, purification was performed on Silica gel 60 $F_{254}$ using developing system benzene: ethyl acetate (95:5, 8:2 and 7:3 v/v) and sulfuric acid reagent (20% in ethanol) used for detecting terpenoids. While chloroform: methanol (9:1v/v) used for detecting flavonoids using ammonia and $AlCl_3$ reagents. The purification give compounds 1, 2, 3, 4 and 5 (10, 13, 19, 26, and 15 mg, respectively). The chromatographic spot of compounds 1, 2, 3 and 4 turned purple when sprayed with 20% sulfuric acid**.** Meanwhile, compounds 2, 3 and 4 gave responded positively to the Lieberman-Buchard test for triterpenes

**Compound 1**: Colorless crystals isolated from sub-fraction (A), $R_f$: 0.70 (benzene: ethyl acetate- 95: 5 v/v). EI-MS (70 eV) showed $[M]^+$ at *m/z* 450 corresponding to a molecular formula of $C_{32}H_{66}$ with base peak fragment at *m/z* 57 and other principle fragments 449, 435, 421, 393, 379, 365, 351, 337, 309, 295, 281, 253, 225, 113, 99, 85, 71, 57. $^1$H-NMR ($CDCl_3$, 400 MHz) showed chemical shifts at δ 0.82 (6H, *t, J* = 8 Hz, $2CH_3$), 1.18 (60H, br *s*, $30CH_2$).

**Compound 2**: Isolated from sub-fraction (B) as Amorphous powder of m.p. 202-204 ºC, $R_f$: 0.71 (benzene: ethyl acetate - 8: 2 v/v).EI-MS (70 eV) showed $[M]^+$ at *m/z* 468 corresponding to a molecular formula of $C_{32}H_{52}O_2$ with fragments at *m/z* 218 (b.p.) base peak, 393, 365, 273, 249, 203 and 189. $^1$H-NMR (CDCl3, 400 MHz), *δ* 5.39 (1H, d , *J*= 4.4 Hz, H-12), 0.72 (3H, s, H-23), 0.97(3H, s, H-24), 0.93(3H, s, H-25), 0.95 (3H, s, H-26), 1.04 (3H, s, H- 27), 0.83(3H, s, H-28), 0.88(3H, s, H-29), 0.89(3H, s, H-30), 2.05 (1H, s, C<u>H</u>$_3$CO), 4.52 (1H, dd, *J*= 6.32, 10, H-3).$^{13}$C NMR(100 MHz, $CDCl_3$), δ 39.73(C-1), 27.78(C-2), 80.9(C-3), 39.63(C-4), 56.04(C-5), 18.19(C-6), 33.94(C-7), 38.30(C-8), 48.7(C-9), 36.16(C-10), 23.70(C-11), 122.64(C-12), 145.3(C-13), 42.21(C-14), 28.91(C-15), 27.98(C-16), 32.92(C-17), 56.79(C-18), 40.50(C-19), 41.09(C-20), 31.87(C-21), 42.18(C-22), 29.71(C-23), 15.90(C-24), 16.05(C-25), 16.51(C-26), 23.70(C-27), 28.91(C-28), 17.71(C-29), 21.43(C-30), 170.53, 21.47(OAc)

**Compound 3:**Isolated from sub-fraction (B) as white crystals, $R_f$: 0.65 (benzene: ethyl acetate- 8: 2 v/v).EI-MS(70 eV) showed $[M]^+$ at *m/z* 468 corresponding to a molecular formula of $C_{32}H_{52}O_2$, with fragments at *m/z*189 (b.p.), 408, 393, 365, 273, 249, 229, 218, 207 and 203. The $H^1$NMR ($CDCl_3$, 400 MHz) spectrum gave seven methyl signals at δ1.04 (3H, s, H-23), 0.83 (3H, s, H-24), 0.88 (3H, s, H-25), 0.80 (3H, s, H-26), 0.97 (3H, s, H- 27), 4.51(1H, br s, H-28), 4.66 (1H, br s, H-28), 0.91 (3H, s, H-29), 1.69 (3H, s, H-30),2.07(1H, s, C<u>H</u>$_3$CO), and 4.62 (1H, dd, *J*= 6.32, 10, H-3).$^{13}$C NMR(100 MHz, CDCl3): δ 38.45(C-1), 27.95(C-2), 80.98(C-3), 38.13 (C-4), 55.94 (C-5), 18.78 (C-6), 34.39(C-7), 41.9 (C-8), 50.34(C-9), 37.05(C-10), 21.02(C-11), 25.41(C-12), 38.13(C-13), 42.04(C-14), 27.78(C-15), 36.61(C-16), 47.63(C-17), 48.66(C-18), 47.99(C-19), 154.63 (C-20), 40.01 (C-21), 29.67(C-22), 28.25(C-23), 16.36 (C-24), 16.34(C-25), 16.51 (C-26), 14.7(C-27), 18.19 (C-28), 19.31(C-29), 109.14 (C-30), 171.01, 21.32(OAc).

**Compound 4:** Isolated from sub-fraction (C) as white crystals of m. p. 167-169 °C, $R_f$: 0.70 (benzene: ethyl acetate - 8:2 v/v). EI-MS(70 eV) showed $[M]^+$ at *m/z* 424 which corresponded to a molecular formula of $C_{30}H_{48}O$ with fragments at *m/z* (rel. int) 424 $[M]^+$ (16), 409 (8), 355 (20), 327 (16), 281 (40), 218 (100), 205 (24), 203 (59), 189 (20), 163 (14), 133 (24), 119 (22) and 55 (60).

**Compound 5:** Isolated from sub-fraction (E) as white needle crystals, $R_f$: 0.48 ($CHCl_3$: MeOH - 9.5: 0.5 v/v), UV: violet, UV/ $NH_3$: yellow, $AlCl_3$: yellow. UV ($\lambda_{max}$) spectral analysis (nm): methanol: 214, 273, 335; NaOMe: 284, 355; $AlCl_3$: 264, 278, 305 (s), 350; $AlCl_3$/ HCl: 261, 279, 305 (s), 354; NaOAc: 271, 336, 391; NaOAc/ $H_3BO_3$: 273, 336.$^1$HNMR (DMSO, 400 MHz): δ 7.9 (2H, d, *J*=8.8 Hz, H-2′, 6′), 6.91 (2H, d, *J* = 8.7 Hz, 3′, 5′), 6.94 (1H, s, H-8), 6.83 (1H, s, H-3), 3.93 (3H, s, 7-OMe) and 3.73 (3H, s, 6-OMe).

**Preparation of the tested formulated extract:**

Chloroform/ methanol extract was formulated by mixing three drops of the emulsifier (tween-80) with water to obtain a concentration 5% of extract as stock solution. Serial concentrations 0.3, 0.5, 0.8, 1, 3 and

5% from the formulation were prepared for biological treatments. Compounds 3, 4, and 5 were mixed with a drop of the emulsifier (tween-80) and water to obtain a concentration 0.3, 0.5 and 0.5%, respectively.

**Test insect:**

A laboratory strain of *Spodoptera littoralis* was reared on castor leaves under controlled conditions (25±2˚C and 65±5% R.H.), away from any insecticidal contaminations. The new moult of the fourth instars larvae (25–30 mg) were chosen from the stock culture for bioassay study.

**Bioassay technique**

**Larval/ pupal toxicity test**

Newly molted 4th instars larvae were allowed to feed for 48 hrs on treated leaves. Three replicates (each containing ten larvae) were used for tested extract and the control group was fed on untreated leaves. Mortality counts were recorded daily till adult emergence and corrected according to Abbott's formula, (1925) **[18]**.

$$\text{Corrected Mortality \%} = \frac{\text{Observed mortality \%} - \text{Control mortality \%}}{100 - \text{Control mortality}} \; x\; 100$$

The cumulative percent mortality was calculated for different criteria where: IPF "Cumulative percent inhibition till pupal formation" and IAE "Cumulative percent inhibition till adult emergence".

The Lethal concentration of the different criteria cause mortality 50% ($LC_{50}$) and 90 % ($LC_{90}$) of insects, and the slope values were calculated from the regression lines according to **Finney, (1971) [19]**.

**The adult reproductive effect**

The effect of tested formulated extract on some biological aspects of *S. littoralis* after treating 4th instars larvae with the $LC_{50}$ value of the cumulative mortality till adult emergence (0.3%) (Table 3).The treated larvae were observed daily until reaching the adult stage.

The adults obtained from treated larvae were crossed with those obtained from untreated larvae as follow: (treated ♀ x treated ♂), (untreated ♀ x treated ♂), (treated ♀ x untreated ♂) and (untreated ♀ x untreated ♂). Each pair of adult was kept in glass jar and fed on 20 % sugar solution. Oviposition took place on strips of paper hanged in the jars. The egg patches were calculated daily, placed in clean glass jars and kept to hatch.

The number of eggs deposited by each female, percent egg hatch and longevity of each sex in addition number of matting were determined. The percent of female fecundity was calculated according to **Crystal and Lachance (1963) [20].**

$$\text{\% Fecundity} = \frac{\text{Number of eggs/ Treated larva}}{\text{Number of eggs/ Control larva}} \; x\; 100$$

$$\text{\% Hatchability} = \frac{\text{Number of hatched larvae}}{\text{Total number of eggs}} \; x\; 100$$

$$\text{Sterility index} = 100 - \left[\frac{\text{Treatment egg hatch}}{\text{Untreatment egg hatch}}\right] x\; 100$$

Also, dead females were dissected and the number of spermatophores in bursa copulatrix was counted. The data were analyzed using analysis of variance (ANOVA) with Duncan's new multiple range test to separate treatment means at the $p < 0.05$ level **[21]**.

**Biochemical studies**

**Treatment**

4th instars larvae of *S. littoralis* were treated with the $LC_{50}$ (0.3%) of the tested extract. After 72 h of treatment the larvae were kept under freezing conditions at -20ºC.

**Tissue preparation**

The untreated larvae "control" and treated larvae were homogenized separately in distilled water (1 g insect body/5 ml water) using a mortar for 3 min. The homogenates were centrifuged at 3000 rpm for 15 min under a cooling centrifuge. The deposits were discarded and the obtained supernatants were used for biochemical determinations acetylcholine esterase activity (AchE), total lipids and total protein.

**Acetylcholine esterase activity (AchE) in insect assay:**

The activity of acetyl cholinesterase was determined using acetylthiocholine iodide as Substrate. A 10 ul aliquot of the obtained supernatant was added to 1.5 ml of 5,5- dithiobis-2-nitrobenzoic acid (DTNB) in 52 mM phosphate buffer, pH 7.2. After mixing and incubation, 50 ul of a 156 mM solution of a thioester, acetylthiocholine iodide was added. Enzyme activity was recorded as the increase in optical density due to conversion of dithiobisnitrobenzoate "DTNB" to 5-thio-2-

nitrobenzoic acid. The reaction was monitored spectrophotometrically at 412 nm **[22]**.

**Total lipids assay:**

Total lipids were determined according to **Knight et al., (1972) [23].**

**Total protein determination:**

Total protein was determined by Colorimetric method using Biuret reagent according to **Gornall et al. (1949**) **[24]**. Briefly, 0.025 ml of supernatant and 1 ml of biuret reagent in a test tube agitated and left to stand for 30 min. The color intensity is directly proportional to the protein concentration. It is determined by measuring the increase in the absorbance at 550 nm.

## 3. Results and discussion

Hydro-distillation of the fresh aerial parts of *C. dioscoridis* yielded 0.24% w/w (calculated to fresh weight) as dark yellow with a characteristic odor. GC-MS revealed at least 70 components, 63 compounds could be identified which represented 93.68% of total volatile constituents (**Table 1**). The oil was characterized by a high percentage of sesquiterpene alcohols (36.00% of total volatile constituents), followed by sesquiterpene hydrocarbons (21.09%), phenylated hydrocarbons (15.43%), saturated hydrocarbon (14.58%) and unsaturated hydrocarbon (1.11%). The major components of sesquiterpene alcohols were *α*-cadinol (8.47%), elemol (5.95%), caryophylla-4(12),8(13) diene-5-*β*-ol (4.55%) and *β*-eudesmol (3.98%) which was mostly compatible with constituents of hexane extract obtained from *C. dioscoridis* shoots **[11]**. On the other hand, modheph-2-ene (4.85%) was identified previously in oil of C. *bonariensis* aerial part **[25**]. 5-phenylundecane (5.59%) represented the principle hydrocarbons. As to our knowledge, this is the first report for phenylated and aliphatic hydrocarbons in *conyza species*. On the other hand, the variation of chemical composition of oils obtained from the same organs of *C. dioscoridis* was noted in different reports **[26]**. Therefore, further studies needed to reveal whether this variation in the chemical composition is attributed to ecological factors, ontogenesis stages and time of collection, etc., or to probable existence of chemo-types among *C. dioscoridis*.

Regarding to composition of chloroform/ methanol extract of *C. dioscoridis*, the tested extract was chromatographed on silica gel led to the isolation of five major compounds including three triterpenes; *β*-amyrin-3-acetate, *β*-lupeol-3-acetate, *β*-amyrone along with dotriacontane hydrocarbon as well as one methoxyflavone; 5,4′-dihydroxy-6,7-dimethoxyflavone.

The $^{1}$H-NMR spectrum of compound 1 showed a six-proton triplet signal at δ 0.82 with *J*= 8 assigned to two chain end methyl groups. A signal at δ 1.18 integrating for 60 protons indicated 30 methylene groups in a nearly identical environment. In addition, mass spectral data of compound 1 showed typical hydrocarbon pattern for straight-chain alkane, giving molecular weight at *m/z* 450 $[M]^{+}$ corresponding to dotriacontane. According to our knowledge dotriacontane is isolated for the first time from this *conyza* species. Previously, triacontane was isolated previously from *Conyza filaginoides* **[27]**.

While, the $^{1}$H-NMR spectrum of **compound 2** showed the presence of eight methyl singlet signals resonating at δ 0.72, 0.83, 0.88, 0.89, 0.93, 0.95, 0.97 and 1.04 and one olefinic proton resonating at δ 5.39 (d, *J*= 4.4 Hz), in addition the mass spectra showed fragments at m/z 218 as base peak and m/z 203 generated due to a retro-Diels Alder fragmentation indicative of the presence of double bond at position 12 in ring C confirmed that compound 2 possesses an oleanane type triterpenoid. Moreover, the presence of two methyl singlet signals for C29 and C30 in addition peak intensity at *m/z* 203 was more abundant than twice of that at *m/z* 189 which characterized for *β*-amyrin **[28, 29]**. Also, $^{1}$H and $^{13}$C NMR spectrum showed the sharp singlet signal at δ 2.05 and signals at 21.47 and 170.53 ppm together with deshielded signal of an oxygenated proton to δ 4.62 and 80.98 which is indicative to substitution of the hydroxyl group with an acetate group at position 3. On the basis of these findings and by comparison with the literature **[28 - 30]**, therefore, the **compound 2** was assigned as β-amyrin acetate. According to our knowledge this compound is isolated for the first time from this *conyza* species.

The $^{1}$H-NMR spectrum of compound 3 displayed seven methyl singlet signals resonating at δ 0.80, 0.83, 0.88, 0.91, 0.97, 1.04 and 1.69. In addition, two broad singlet signals at δ 4.51 and 4.66 and at δ 154.63 and 109.14 for exocyclic olefinic protons of C-30 together with characteristic fragments base peak

at m/z189 (b.p.) suggested that the compound 3 possesses a lupeol-type triterpenoid. Moreover, a sharp singlet signal resonated at δ 2.07 and doublet of doublet signal at δ 4.62 as well as δ 170.01 and 21.32 for a carbonyl of an ester group and δ 80.98 for the oxygenated carbon atom normally assignable to C-3 were appeared in $^{13}C$ –NMR spectrum. By comparing aforementioned data and mass data of **compound 3** is closely agreement to that reported for lupeol acetate **[31]**. It was previously reported from *C. dioscoridis* leaves and roots **[9]**.

The mass spectra of **compound 4** showed peak ion at *m/z* 424 in addition to fragments at *m/z* 409 $[M\text{-}CH_3]^+$, 218[RDA], 203 $[218\text{-}CH_3]^+$, 205 $[C_{14}H_{21}O]^+$ and 189 $[218\text{-}CH_2CH_3]^+$ significantly implied the compound 4 is typical for pentacyclic triterpene with Δ 12-oleane skeleton. By comparison of EI-MS spectrum fragmentation pattern of **compound 4** and melting point with literature data of **[32]**, it could be tentatively identified as to be *β*-amyrenone (12-oleanene-3-one). According to our knowledge this compound is isolated for the first time from this *conyza* species.

Insight on **compound 5**, UV spectrum displayed absorption maxima at 273 and 335 nm for a typical flavone type. Shift reagents proved the presence of free OH group at carbon 5 with 6 oxygenation, no free OH group in position 7 and absence of *ortho*-dihydroxy groups in ring B. $^1H$-NMR spectrum displayed two doublets in the aromatic region resonating at 7.9 (2H, d, *J*= 8.8 Hz), 6.91 (2H, d, *J* = 8.7 Hz) for H-2′& 6′ and 3′&5′, respectively, whereas, two singlet methine resonating at δ 6.94 and 6.83 for H-8 and H-3, respectively. Moreover, two methoxylated protons appear as singlet signals at δ 3.93 for 7-OMe and 3.73 for 6-OMe. By comparison of the literature data with Shafiq et al., (2014) **[33]**, compound **5** was assigned as 5,4′-dihydroxy-6,7-dimethoxyflavone. It is worthy to be mention that it is the first time for isolation of 5,4′-dihydroxy-6,7-dimethoxyflavone from *Conyza* species. While other methoxy-flavones; conyzatin (5,7-dihydroxy-3,8,3′,4′,5′-pentamethoxyflavone) and 5,7-dihydroxy-3,8,4′-trimethoxyflavone were identified in *Conyza stricta* **[34]**.

**Insecticidal activity of the chloroform/ methanol formulated extract of *Conyza dioscoridis* aerial parts**

The effect of *C. dioscoridis* tested formulated extract against Egyptian 4th instars *S. littoralis* larvae showed mortality in all tested concentrations 0.3 - 5% with dose dependent manner **(Table 2**). The insecticidal efficacy of the tested extract exerted extended effects through pupal and adult stages where it showed cumulative inhibition for pupae (IPF) and adult (IAE) up to 76.6% and 83.3%, respectively.

Moreover, the insecticidal activity of the isolated compounds; *β*-amyrenone, lupeol acetate and 5, 4′-dihydroxy-6,7-dimethoxyflavone were evaluated separately against 4th instar larvae of *S. littoralis* at concentration 0.3, 0.5, and 0.5%, respectively where they suppressed 50, 60 and 73.3% of cumulative till adult emergence, respectively (**Table 2**). They exhibited more insecticidal effect than that of test extract in comparing with the same concentration. Moreover, 5, 4′-dihydroxy-6,7-dimethoxyflavone was the most effective compound against *S. littoralis*.

$LC_{50}$ and $LC_{90}$ values of the *C. dioscoridis* tested extract against IPF and IAE of *S. littoralis* were recorded in **Table (3)**. $LC_{50}$ of the tested extract were 0.3 and 0.8% for IPF and IAE, respectively

**Effect of Chloroform/ methanol formulated extract on the reproductive potential of *S. littoralis***

The extract of *C. dioscoridis* aerial parts was evaluated for its effect on reproductive capability of *S. littoralis* which previously treated in 4th instars larvae with $LC_{50}$ (0.3%). A significant reduction of imaginal longevity in both sexes was observed as shown in **Table (4**). The treated adults with $LC_{50}$ of tested extract reduced adult longevity with average of 7.2 and 6.6 days for males and 7.6 and 7.4 days for females, respectively, whereas untreated adults survived 9.6 and 10.6 days for ♂ and ♀, respectively.

The number of mating terminated by spermatophore deposited in bursa copulatrix varied in all experimental series (**Table 4**). The reduction in the number of formed spermatophores transferred by treated males was affected and had lower number after treatment (1 and 1.2) compared to control (2.6).

Regarding to oviposition efficiency of *S. littoralis*, a clear significant difference between treated and untreated insect was found in the number of deposited eggs, whether only one or both mated parents were derived from treated larvae. As shown in **Table (5**), untreated females deposited an average

of 1230 eggs/♀ whereas treated females deposited 480 and 690 eggs/♀.

Concerning to egg hatchability (fertility), it was significantly reduced when at least one of the parents was derived from treated larvae, and was more pronounced when males were treated. The hatchability in replicates of untreated was 92.6% whereas some egg patches from the treated pairs did not hatch at all. The percent hatchability in all reciprocal crosses ranged between 53.59 to 62.70%. The reduction of hatchability to 53.59 % may be caused by either defects in the differentiation of Oocytes and sperms or the inhibition of spermatogenesis or inactive spermatozoa which led to no fertilization and no hatchability of eggs as well as can be attributed to permeability of the tested extract through egg membranes that prevent hatching by interfering with embryonic cuticle synthesis **[35, 36]**. **Jeong et al.,(2001)** observed the reduction of fertility with the profound abnormalities in spermatogenesis of *Heliocoverpa assulta* male treated with sap of *Nerium indicum* because of reduction of levels of the two major polyamines; spermidine and spermine in the testes due to direct inhibition on the enzymes used in the conversions of putrescence to spermidine and then to spermine **[37]**.

$LC_{50}$ of tested extract led to reduction of both fecundity and egg hatchability of treated adults causing sterility in comparison with untreated adults. The sterility index ranged between 62.22 to 78.38% indicated that both sexes are responsible for the sterilant action.

**Biochemical study:**

$LC_{50}$ of chloroform/ methanol formulated extract of *C. dioscoridis* aerial parts inhibited acetylcholine esterase activity to 49.17% where the activity decreased AChE in insect tissue to 35.19 µ mole/ ml/ g tissue in comparing with untreated insect 69.97 µ mole/ ml/ g tissue (**Table 6**). Elevated detoxification enzymes activity in insect tissues is often associated with enhanced detoxification of allelochemicals **[38]**. Among the detoxification enzymes, AChE is a key enzyme that terminates nerve impulses by catalyzing the hydrolysis of the neurotransmitter acetylcholine in the nervous system **[39]**.

Regarding to total lipids and total protein contents**,** $LC_{50}$ of tested extract caused inhibition of total lipids and protein contents of 4$^{th}$ instars larvae of *S. littoralis* post 72 hrs treatment to 58.61% and 80.39 %, respectively, in comparing with untreated 4$^{th}$.

*Spodoptera littoralis* (Boisd.), is one of the most destructive agricultural lepidopterous pests in many countries, targeting many economically crucial crops and vegetables as well as ornamental and orchard trees because of direct result of extensive larvae feeding **[40].** So, EPPO "European and Mediterranean Plant Protection Organization" has assigned *S. littoralis* as A2 quarantine pest **[41].** For controlling this economic insect, several chemical synthetic pesticides including organochlorines "polychlorinated biphenyls, dibenzo-p-dioxiins and organochlorine pesticides", neurotoxic insecticides "carbamates and organophosphates" known as acetylcholinesterase (AChE) inhibitors and insect development inhibitions "Chlorantraniliprole" were applied **[41-44].** The massive application of these chemical pesticides led to increasing agriculture production and decreasing the incidence of endemic and epidemic diseases. Nevertheless, over reliance on synthetic pesticides is disappointed due to their adverse effects on human health, environment, and development of resistant pests and pathogen strains. The organochlorine compounds exert many toxic effects on human health, such as, endometriosis, infertility, cancer of male and female reproductive system, developmental toxicity, neurotoxicity and immunotoxicity. Moreover, the continuous use of synthetic pesticides has increased the risk of ozone depletion, carcinogenic, teratogenic and mutagenic effects in non-targets and cross- and multi-resistance in insects **[45].** The accumulation of neurotoxic insecticides increases stimulations that lead to behavioral changes, asphyxia, hyperactivity, and death **[43].** Recently, various researches focus on botanical insecticides as developmental insecticides alternatives to chemical insecticides. According to United States Food and Drug Administration "FDA", botanical pesticides are environmentally less harmful as it is easily biodegradable **[46].** Botanical extracts have different mechanisms of action as repellent, insecticidal, antifeedants, growth inhibitors, oviposition inhibitors, ovicides, and growth-reducing effects on a variety of insects **[47]**. Lengai et al., (2020) reviewed phytochemical activity and role of botanical pesticides and the challenges facing their adoption and utilization for sustainable agricultural crop production [**48].**

Our current study findings indicated that *C. dioscoridis* "*Pluchea dioscoridis*" chloroform/

methanol extract inhibited pupal formation "IPF" and adult emergence "IAE" of *S. littoralis* (Boisd) with $LC_{50}$ were 0.3 and 0.8%, respectively and its toxicity showed a dose dependent manner. Interestingly, the isolated compounds; *β*-amyrenone, lupeol acetate and 5,4′-dihydroxy-6,7-dimethoxyflavone exhibited mortality % more than that of the tested extract at the same concentration.

Moreover, our study was extended to insecticidal mechanism of action of *C. dioscoridis* extract on *S. littoralis* (Boisd) obtained from treated larvae. It showed a significant reduction of imaginal longevity in sexes, the number of formed spermatophores, the number of deposited eggs, hatchability and fecundity. The obtained results coincide with **Mogahed and Mohanna, (1992**) and **Amer and Rasmy (1994**) who reported that crude extract of *Pluchea dioscoridis* had potent effect on the egg viability, and unhatchability of the Egyptian cotton leaf warm *S. littoralis* (Boisd) which was positively correlated with the extract concentration **and a**lso exhibited pronounceable toxic effects on adults and eggs of the two-spotted spider mite *Tetranychus urticae* **[6, 49**]. Moreover, **El-Lakwah *et al.* (1998)** found that petroleum ether extract of *Pluchea dioscoridis* leaves was more effective on *Sitophilus oryzae* adults than acetone extract **[50]**. Destruction of epithelial cells of *S. littoralis* were observed with different plant extracts among of them *C. dioscoridis* which caused slight and severe disintegration of the epithelium, fading of the boundaries of epithelial cells and detachment of epithelial cells **[51].**

Data obtained from the biochemical effect revealed that the tested extract at dose 0.3% showed marked inhibition acetylcholine esterase activity (AchE) as well as decreased total protein content and total lipids content of 4$^{th}$ larval instars of *S. littoralis*. Similar observations were also obtained by many authors for neem extract and other plant extracts against *S. littoralis* and *S. gregaria* **[52]**. Treatment the late instars larvae and adults of *S. litura* with *Azadirachta indica* and *Thymus vulgaris* oils showed highly significant inhibition in AchE **[53]**.

Regarding to reduction of total protein content and total lipids**, Rawi et al., (2011)** demonstrated that marked decrease in total lipids and total protein contents in the 4$^{th}$ instar larvae of *S. littoralis* post treatment with methylene chloride extract of *Azadirachta indica* and *Citrullus colocynthis* **[52].** Similar results were observed on hemolymph protein of *S. littoralis* and *Agrotis ipsilon* after treatment with methanolic extract of *Melia azedarach* **[54]**. The reduction of the total protein and lipid contents in the fifth instar larvae of lesser mulberry pyralid (*Glyphodes pyloalis*) and third instars larvae of *X. luteola* was observed after treatment with *Artemesia annua* L **[55, 56]**. Lipids and protein are the major cell components which played the most important role in all biological processes including reproduction **[57]**. Many of the botanical extracts showed anti-feedant effect on insects as well as reduced feeding efficiency causing reduction of some of the vital components like proteins in the body **[58]**. Ultimately, insects die due to reduce energy metabolism **[58]**. In the current study, total lipid and total protein of treated larvae with 0.3% of *C. dioscoridis* extract were considerably reduced compared to the untreated larvea. Several reports showed reduction of protein and lipid levels in insect tissues by botanical extracts which may lead to decrease in fecundity of adults **[59]**. Overall, the depletion of energy reserves could be due to a reduction in plant consumption, or to high mobilization of these primary metabolites to compensate the metabolic stress produced by the toxic effects of the plant extracts **[60]**. It may be also explained that the insects may be degrade proteins to amino acids in order to permit them enter into the TCA cycle as a keto acid for compensation for diminish energy caused by stress **[61].**

Many study reported that the insecticidal activity including to larvicides, adulticides and ovicides may be attributed to phenolics, triterpenes and sesquiterpenes contents **[46].** Due to their lipophilic properties, triterpenes and sesquiterepenes might facilitate their permeability through egg membranes or insect tissues where they destroy the reproductive tissues or inhibit some vital enzymes **[6].** Pentacyclic triterpenes play a role in plant defense which have insect antifeedant effects and also have wide pharmacological activities **[62]**. In spite of several study proved anti-insect properties of pentacyclic triterpenes, their mechanism of action remain unclear. Amyrins and their derivatives have been described as defensive substances against phytophagous insects

**[62]**. α-Amyrin acetate produced 52.63–57.89% mortality in *Spodoptera litura* **[63]**. β-Amyrin palmitate acts as an insect growth inhibitor and exhibits chemosterilant properties. On the hand, oleanolic and ursolic acids have fluidity-modulating effects on liposomal membranes **[62].** Lupeol acetate and β-amyrin acetate are exhibited antifeedant effects in choice tests on *L. decemlineata* and thier activity depended on the presence of the acetate group at C-3 **[62]**. **Mallavadhani et al. (2003)** found that 3-O-fatty acid ester derivatives of ursolic and oleanolic acids were stronger antifeedants to *S. litura* when compared with their parent acid **[63]**.

Flavonoids, are a major class of secondary metabolites found in plants, has beneficial effect on human health, and besides adversely affecting on insect pests. They have antifeedant effect and growth inhibitors in insects probably because of their interference with endocrine regulation **[64]**. Although few study on the insecticidal of flavonoids, **Morimoto et al., (2003) [64]** found that the insecticidal activity varies according to chemical structure of flavonoids which depending on:

- 6-Position-substituted derivative of 2-phenyl flavonoids that showed strong insect antifeedant activity against common cutworm.
- A hydroxyl group as a substituent on any of the positions tended to increase the activity but increasing hydrophilic substituent led to decrease the activity.
- A catecholic B-ring responsible for their toxic activity to insects.
- The bulky B-ring was a disadvantage for the antifeedant activity.
- The charge on C (3) and C (5) of the flavonoid was important for the insecticidal activity**.**

Concerning to volatile constituents, the obtained volatile oil from hydro-distillation of C. *dioscoridis* aerial parts principally composed from oxygenated sesquiterpenes which composed mainly α-cadinol, elemol, α- and β-eudesmol and caryophylla-4(12),8(13) diene -5β-ol. The different studies referred the promising insecticidal effect of volatile oils obtained from plants to the higher contents of oxygenated mono- and sesquiterpenes **[66-69].** Unfortunately, the insecticidal activity of obtained volatile oil from C. *dioscoridis* aerial parts couldn't evaluate here against the 4$^{th}$ larval instars of *S. littoralis* but various study recorded insecticidal potency of oils obtained from different parts of *C. dioscoridis*. Our results agreed with **Grace (2002)** identified 36 components in the volatile oil of *Pluchea dioscoridis* leaves where oxygenated sesquiterpenes (26.4%) and sesquiterpene hydrocarbons (39.4%) represented the main constituents in the oil which showed a marked mosquito larvicidal activity against *Culex pipiens* **[10]**. Also, **El-Hamouly and Ibraheim (2003)** reported that the leaves of *Pluchea dioscoridis* containing 3-5% volatile oil, where were consisted mainly of sesquiterpene hydrocarbons (mainly β-maaliene and α-elemene) and oxygenated sesquiterpenes (mainly α-cadinol, muurolol and caryophyllene oxide isomer) **[16]**. Also, C. *dioscoridis* leaves showed toxicity and antifeedant effect against 1$^{st}$ larval instar of *Pectinophora gossypiella* which referred to the presence α-cadinol, caryophyllene oxide, β-eudesmol and α-selinene **[70].** *C. Aegyptiaca* oil proved their efficacy as larvicides, adulticides, ovicides and repellents against different species of mosquitoes **[71]**. **Rosselli et al., (2012)** found that sesquiterpenes have an elemane skeleton showed moderate antifeedant activity against larvae of *Spodoptera littoralis* **[72].** Yeom et al., (2015) demonstrated that the insecticidal activity of β-phellandrene against German cockroach "*Blattella germanica*" correlates with its ability to inhibit AChE **[73].**

## 4. Conclusion

Our study revealed that *Conyza dioscoridis* was rich source of diverse of bioactive 2$^{ry}$ metabolites with promising insecticidal properties targeting *Spodoptera littoralis*. β-amyrin-3-acetate, *β*-amyrenone, and 5,4′-dihydroxy-6,7-dimethoxyflavone isolated here, for the first time from genus conyza, had toxic effect on *S. littoralis* more than that of the tested extract.

This is the first study conducted on the insecticidal activity and the mechanism of action of *C. dioscoridis* extract and isolated compounds to limit *S. littoralis* population. Interestingly, *C. dioscoridis* extract had adverse effects on *S. littoralis* such as survival, fecundity, oviposition, pupae and adults development, and also acts as metabolic and acetylcholine esterase inhibitors against 4$^{th}$ instars of larvae due to effect to phenolics, terpenoids and other bioactive compounds.

Population growth as well as the pests "*S. littoralis*" which has negatively affecting on quantity and quality is major challenge to agricultural production. According to the insecticidal effect, easily biodegradable and safe for human health, the present findings may encourage more applied researches to evaluate *C. dioscoridis* extract and/or tested compounds in semi-field and field trial for controlling *S. littoralis* and other pests beside for reducing the use of synthetic insecticides.

## 5. Conflicts of interest

The authors declare that there are no conflicts of interest

## 6. Acknowledgment

Authors would thank National Research Centre for doing this work in NRC lab

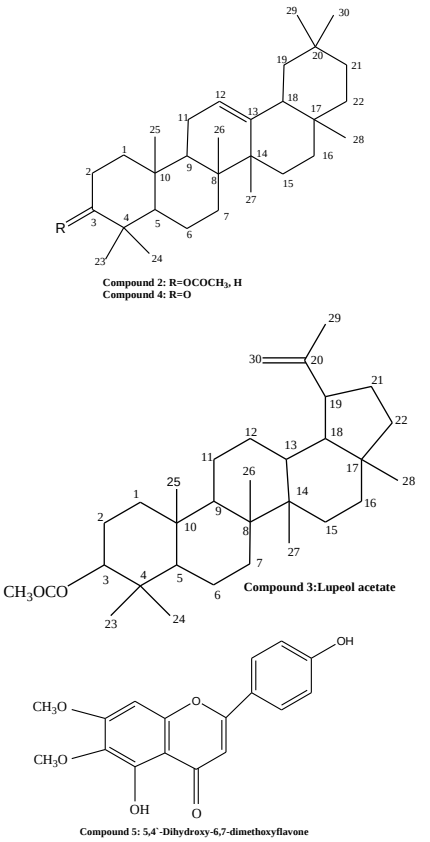


**Figure 1: Structures of isolated compounds**

**Table (1): Chemical composition of volatile constituents obtained from fresh *Conyza dioscoridis* aerial parts**

| Compounds | Formula | Retention time/min | Area % | Base peak | Molecular weight |
|---|---|---|---|---|---|
| **Oxygenated monoterpens** | | | **0.67** | | |
| *p*-Menth-3-en-9-ol | $C_{10}H_{18}O$ | 13.00 | 0.31 | 123 | 154 |
| 4-Terpineol | $C_{10}H_{18}O$ | 13.54 | 0.36 | 71 | 154 |
| **Sesquiterpen hydrocarbons** | | | **21.09** | | |
| δ-Elemene | $C_{15}H_{24}$ | 19.03 | 1.54 | 41 | 204 |
| α-Cubebene | $C_{15}H_{24}$ | 20.46 | 0.24 | 105 | 204 |
| Modheph-2-ene | $C_{15}H_{24}$ | 20.24 | 4.85 | 189 | 204 |
| α -Isocomene | $C_{15}H_{24}$ | 20.70 | 3.28 | 162 | 204 |
| β-Isocomene | $C_{15}H_{24}$ | 21.03 | 1.93 | 108 | 204 |
| β-Caryophyllene | $C_{15}H_{24}$ | 21.45 | 1.35 | 69 | 204 |
| β-Copaene | $C_{15}H_{24}$ | 21.98 | 0.31 | 161 | 204 |
| Muurola-3,5-diene (cis) | $C_{15}H_{24}$ | 22.26 | 0.26 | 161 | 204 |
| α-Humulene | $C_{15}H_{24}$ | 22.48 | 0.21 | 93 | 204 |
| Germacrene D | $C_{15}H_{24}$ | 22.79 | 1.08 | 161 | 204 |
| Cadina-1,4-diene (cis) | $C_{15}H_{24}$ | 23.74 | 0.41 | 119 | 204 |
| Epizonarene | $C_{15}H_{24}$ | 23.87 | 0.38 | 161 | 204 |
| γ-Cadinene | $C_{15}H_{24}$ | 24.35 | 2.31 | 161 | 204 |
| δ-Cadinene | $C_{15}H_{24}$ | 24.65 | 2.57 | 161 | 204 |

| | | | | | |
|---|---|---|---|---|---|
| α-Cadinene | $C_{15}H_{24}$ | 25.00 | 0.37 | 161 | 204 |
| **Oxygenated sesquiterpens** | | | **36.00** | | |
| Cubebol | $C_{15}H_{26}O$ | 24.85 | 2.27 | 161 | 222 |
| Elemol | $C_{15}H_{26}O$ | 25.44 | 5.95 | 59 | 222 |
| Caryophyllene oxide | $C_{15}H_{26}O$ | 26.35 | 0.85 | 41 | 220 |
| β-Copaen-4-α-ol | $C_{15}H_{26}O$ | 26.46 | 0.64 | 159 | 220 |
| Cubenol | $C_{15}H_{26}O$ | 27.23 | 2.25 | 161 | 222 |
| Caryophylla-4(12),8(13) diene -5β-ol | $C_{15}H_{24}O$ | 27.93 | 4.55 | 136 | 220 |
| Cedrelanol | $C_{15}H_{24}O$ | 28.06 | 0.79 | 161 | 222 |
| β-Eudesmol | $C_{15}H_{26}O$ | 28.33 | 3.98 | 59 | 222 |
| α-Eudesmol | $C_{15}H_{26}O$ | 28.44 | 3.28 | 59 | 222 |
| α-Cadinol | $C_{15}H_{26}O$ | 28.46 | 8.47 | 95 | 222 |
| **α-S**antalol | $C_{15}H_{24}O$ | 28.83 | 0.99 | 93 | 220 |
| Germacra-4(15),5,10(14)-trien-1-α-ol | $C_{15}H_{24}O$ | 29.23 | 1.64 | 41 | 220 |
| cis-Nuciferol | $C_{15}H_{24}O$ | 36.04 | 0.34 | 119 | 218 |
| **Unsaturated hydrocarbon** | | | **1.11** | | |
| 1-Hexadecene | $C_{16}H_{32}$ | 26.59 | 0.49 | 55 | 224 |
| 1-Octadecene | $C_{18}H_{36}$ | 32.00 | 0.62 | 41 | 252 |
| **Saturated hydrocarbon** | | | **14.58** | | |
| Heneicosane | $C_{21}H_{44}$ | 41.11 | 0.35 | 57 | 296 |
| Docosane | $C_{22}H_{46}$ | 41.58 | 0.38 | 57 | 310 |
| Tricosane | $C_{23}H_{48}$ | 43.69 | 1.15 | 57 | 324 |
| Tetracosane | $C_{24}H_{50}$ | 45.71 | 0.96 | 57 | 338 |
| Pentacosane | $C_{25}H_{52}$ | 47.69 | 1.93 | 57 | 338 |
| Hexacosane | $C_{26}H_{54}$ | 49.58 | 2.17 | 57 | 366 |
| Heptacosane | $C_{27}H_{56}$ | 51.38 | 2.14 | 57 | 380 |
| Octacosane | $C_{28}H_{58}$ | 53.14 | 1.96 | 57 | 394 |
| Nonacosane | $C_{29}H_{60}$ | 55.16 | 1.81 | 57 | 408 |
| Triacontane | $C_{30}H_{62}$ | 57.62 | 1.15 | 57 | 422 |
| Hentriacontane | $C_{31}H_{64}$ | 60.15 | 0.58 | 57 | 436 |
| **Phenylated hydrocarbon** | | | **15.43** | | |
| 5-Phenyldecane | $C_{16}H_{26}$ | 25.03 | 0.38 | 91 | 218 |
| 2-Phenyldecane | $C_{16}H_{26}$ | 26.83 | 0.65 | 105 | 218 |
| 5-Phenylundecane | $C_{17}H_{28}$ | 27.84 | 5.59 | 91 | 232 |
| 3-Phenylundecane | $C_{17}H_{28}$ | 28.64 | 0.80 | 91 | 232 |
| 2-Phenylundecane | $C_{17}H_{28}$ | 29.64 | 1.18 | 105 | 232 |
| 6-Phenyldodecane | $C_{18}H_{30}$ | 30.34 | 0.94 | 91 | 246 |
| 5-Phenyldodecane | $C_{18}H_{30}$ | 30.48 | 0.81 | 91 | 246 |
| 4-Phenyldodecane | $C_{18}H_{30}$ | 30.78 | 0.96 | 91 | 246 |
| 3-Phenyldodecane | $C_{18}H_{30}$ | 31.34 | 0.63 | 91 | 246 |
| 2-Phenyldodecane | $C_{18}H_{30}$ | 32.33 | 0.77 | 105 | 246 |
| 6-Phenyltridecane | $C_{19}H_{32}$ | 32.90 | 0.89 | 91 | 260 |
| 5- Phenyltridecane | $C_{19}H_{32}$ | 33.05 | 0.54 | 91 | 260 |
| 4- Phenyltridecane | $C_{19}H_{32}$ | 33.38 | 0.50 | 91 | 260 |
| 3- Phenyltridecane | $C_{19}H_{32}$ | 33.93 | 0.48 | 91 | 260 |
| 2-Phenyltridecane | $C_{19}H_{32}$ | 34.88 | 0.31 | 105 | 260 |
| **Miscellaneous compound** | | | **4.80** | | |
| 3-Hexen-1-ol | $C_6H_{12}O$ | 4.22 | 2.15 | 67 | 100 |
| Nonanal | $C_9H_{18}O$ | 11.18 | 0.34 | 57 | 142 |
| Phytol | $C_{20}H_{40}O$ | 39.67 | 0.78 | 71 | 296 |
| 1-Octadecanol | $C_{18}H_{38}O$ | 40.72 | 1.31 | 57 | 270 |
| Octadecanol acetate | $C_{20}H_{40}O_2$ | 43.42 | 0.22 | 43 | 312 |

**Table (2): Insecticidal activity of the formulated crude extract of *Conyza dioscoridis* aerial parts and isolated compounds against 4th instars larvae of *Spodoptra littoralis* fed on treated leaves.**

| Criteria | Mortality (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Crude extract | | | | | | *β*-amyrenone | Lupeol acetate | 5, 4′-dihydroxy -6,7-dimethoxy flavone |
| Concentrations (%) | 0.3 | 0.5 | 0.8 | 1 | 3 | 5 | 0.3 | 0.5 | 0.5 |
| IPF | 36.6 | 40 | 50 | 53.3 | 60 | 76.6 | 40 | 42.6 | 52 |
| IAE | 46.6 | 60 | 60 | 66.6 | 70 | 83.3 | 50 | 60 | 73.3 |

IPF: Cumulative percent inhibition till pupal formation
IAE: Cumulative percent inhibition till adult emergence

**Table (3): The toxicity of the formulated crude extract of *Conyza dioscoridis* aerial part on 4th instars larvae of *Spodoptera littoralis* at different criteria**

| Criteria | $LC_{50}$ | | | $LC_{90}$ | | | Slope |
|---|---|---|---|---|---|---|---|
| | Value | Lower | Upper | Value | Lower | Upper | |
| IPF | 0.8 % | 0.5 | 1.09 | 36.19 % | 14.86 | 193.75 | 0.77 |
| IAE | 0.3 % | 0.128 | 0.488 | 20.84 % | 8.79 | 124.37 | 0.69 |

IPF: Cumulative percent inhibition till pupal formation
IAE: Cumulative percent inhibition till adult emergence

**Table (4): Average longevity and mating frequency of adults of *Spodoptera littoralis* emerged from larvae treated with formulated crude extract of *Conyza dioscoridis* aerial part**

| Treatment | Pairing | Longevity (days) | | Number of mating |
|---|---|---|---|---|
| | | ♂ | ♀ | |
| Control | U ♀ x U ♂ | 9.6 a | 10.6 a | 2.6 a |
| Tested crude extract | T ♀ x T ♂ | 7.2 b | 7.6 c | 1c |
| | T ♀ x U ♂ | 9.2 a | 7.4 c | 1.8 b |
| | U ♀ x T ♂ | 6.6 b | 9.4 b | 1.2 bc |
| LSD 0.05 | | 1.16 | 1.20 | 0.73 |

Means followed by the same letter in the same column are not significantly different ($P< 0.05$; Duncan's new multiple range test).

**Table (5): The reproductive potential of *Spodoptera littoralis* treated in the 4th instar larvae with formulated crude extract of *Conyza dioscoridis* aerial parts**

| Treatment | Pairing | Eggs/♀ | Fecundity% | % Egg hatchability | Sterility index |
|---|---|---|---|---|---|
| Control | U♀ x U♂ | 1230 a | 100 | 92.61 a | --- |
| crude extract | T ♀ x T ♂ | 480 c | 39.02 | 53.59 b | 78.38 |
| | T ♀ x U ♂ | 690 b | 56.09 | 62.70 b | 62.22 |
| | U ♀ x T ♂ | 718 b | 58.37 | 57.26 b | 63.62 |
| LSD 0.05 | | 200.69 | - | 13.76 | - |

Means followed by the same letter in the same column are not significantly different (P< 0.05; Duncan' s new multiple range test)

**Table (6): Enzymatic activity of fourth instars larvae of *S. littoralis* treated with crude extract of *Conyza dioscoridis* aerial part**

| Enzyme Samples | *C. dioscoridis* extract | | Control | |
|---|---|---|---|---|
| | Activity | % Inhibition | Activity | % Inhibition |
| Acetylcholine esterase (µ mole/ ml/ g tissue) | 35.19±6.77 | 49.71 | 69.97±4.1 | --- |
| Total protein (mg/ g tissue) | 36.20±5.9 | 80.39 | 184.6±25.8 | --- |
| Total lipid (mg/ g tissue) | 23.06±8.2 | 58.61 | 55.72±12.64 | --- |

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