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Bioactive compounds from *Withania somnifera* dun and their toxicity against some piercing sucking pests

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Abstract

Piercing sucking pests are destructive to many strategic crops all over the world. Botanical pesticides can be used to control these pests. A new withanolide derivative **3** named sominone A ((20*R*,22*R*)-1*α*,3*β*,20,27-tetrahydroxywitha-5,24-dienolide) was isolated from the alkaloid fraction of the whole plant of *Withania somnifera*. In addition, there are three known compounds named withasomine **1**, methyl isoferulate **2**, and coagulin Q **4** were also isolated. The structures of isolated compounds were identified using different spectroscopic methods such as 1D, 2D NMR, and HRESIMS spectroscopy. The alkaloid fraction and the four isolated compounds were tested for their pesticidal activity against four piercing sucking pests (*Aphis craccivora* Koch, *Bemisia tabaci* Gennadius, *Nezara viridula* Linnaeus, and *Tetranychus urticae* Koch) that attack many strategic crops under laboratory conditions, along with azadirachtin (Okios 3.2% EC) as a positive control. The results showed that the alkaloid compound (withasomine **1**) was the most toxic to *A. craccivora*, *B. tabaci*, *N. viridula,* and *T. urticae,* with LC_{50} values of 15.44, 36.61, 85.11, and 128.28 ppm, respectively, compared with the control. Withanolide compounds had moderate effects on all tested pests. Biochemical parameters of six enzymes; *α*-esterase, *β*-esterase, chitinase, acetylcholinesterase, glutathione-*S*-transferase, and peroxidase of *A. craccivora* were estimated at the LC_{50} value of the most potent compound, withasomine **1** and the values were 38.83, 72.86, 31.45, 506.4, 2.62, and 251.0, respectively. The results demonstrated that all enzymes activity levels were increased compared with the control except a remarkable inhibition in AChE enzyme level was observed compared with control. Therefore, the alkaloid fraction of *W. somnifera* is a promising extract that contains many active compounds that can be used as a natural pesticide against many harmful pests in agriculture crops.

Keywords Withanolides, Pesticides, *Aphis craccivora*, *Bemisia tabaci*, *Nezara viridula*, *Tetranychus urticae*

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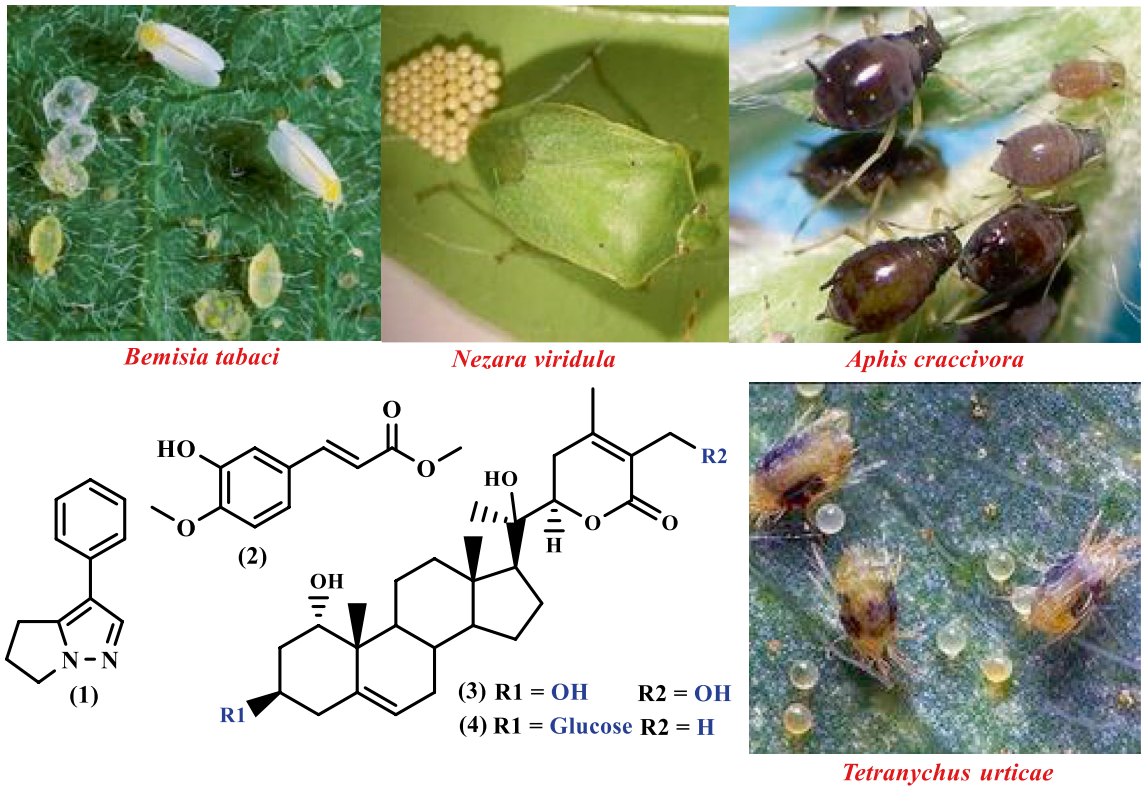
Graphical Abstract



Introduction

Natural pesticides of plant origin occupied an interesting position for scientists all over the world. This is due to the high safety rate of these compounds for humans and the environment in general, in addition to their rapid degradation in the environment. The excessive use of synthetic pesticides cause many harms to humans, animals and plants and led to insect resistance to these pesticides [1]. *Withania somnifera* Dun, belonging to the Solanaceae family, generally known as India ginseng, has been used as a food for promoting health and longevity by activating the organ systems and immune system [2, 3]. Therefore, using this type of extracts as pesticides to control pests, especially in vegetable crops, is safer for the environment and humans. Many natural product compounds can be used as pesticides, especially alkaloids [4]. Quinolizidine alkaloids like Anagyrine and Spartiene compounds have insecticidal activity against *Aphis gosspyii* and *Amrasca biguttula* [5]. Apart from the pesticidal activity, withanolides have other biological activities such as immune booster and anti-viral (COVID-19) [6]. The aphicidal activity of *W. somnifera* extracts was studied by Noureldeen et al. against rose aphid, *Macrosiphum rosae* [7]. *W. somnifera* is rich mainly with withanolides and quinolizidine alkaloids, in addition to phenols, steroids, saponins, flavonoids, and glycosides [8]. The metabolite profiling of *W. somnifera* root extract was studied by HPLC and different spectroscopic methods that yielded numerous withanolides [9]. Zhao et al. isolated many derivatives of withanolides, such as withanoside VIII, IX, X, and XI, from the roots of *W. somnifera* and studied their neurite outgrowth activity on a human neuroblastoma SH-SY5Y cell line [10]. Sominone and sominolide were isolated from *W. somnifera* by Atta-ur-Rahman et al. [11]. The alkaloid content of *W. somnifera* roots and callus was identified by GC/MS analysis, like withasomnine and somniferine [12]. Nine withanosides were isolated from *W. somnifera* roots by Misra et al. [13]. Yan et al. used Viscosalactone B, isolated from *W. somnifera,* as a natural LSD1 inhibitor for the treatment of prostate cancer [14]. Several insects are serious pests that cause damage to many agricultural crops such as *Aphis cracivora*, *Bemisia tabaci*, *Nezare viridalis* and *Tetrarhynchus urticae*. In addition to this, these insects can also serve as indirect vector of viral diseases [15, 16]. Because of these reasons, there is an urgent need to control these

insect pests using extracts from botanical agents. In this study, *Withania somnifera* Dun plant, which contains different active compounds especially alkaloids, was studied for the insecticidal properties against piercing-sucking pests that are harmful to the agricultural crops.

Materials and methods

Chemicals and instruments

TLC silica gel Merck GF_{254} precoated plates (20 × 20 cm) on aluminum sheets, alumina (aluminum oxide active neutral LOBA CHEMIE PVT. LTD., India), methanol (MeOH), ethyl acetate (EA), methylene chloride (MC), petroleum ether (60-80°C) (PE), acetic acid and ammonia solution 25% were obtained from Edwic Company, Egypt. Spray reagent; Dragendorff's reagent. ^{1}H NMR, ^{13}C NMR, and 2D NMR analyses were measured using Bruker 400 MHz in CD_3OD and $CDCl_3$ with tetramethylsilane (TMS) as an internal standard. HRESI/MS spectrum in positive mode were carried out on an UPLC MS/MS "Agilent" 3100 "USA" with a TQ detector. A polarimeter, WXG-4, was used for optical rotation measurement. UV spectra were measured using the PG Instruments T80 + UV/vis spectrometer, UK. IR spectrum was recorded on ThermoFisher Nicolete IS10, USA.

Plant materials

Whole parts of the *Withania somnifera* plant, family Solanacease, were collected in August 2019 from Mansoura University Gardens, Mansoura City, Dakahlia, Egypt (31.04595°N–31.35402°E). The plant was identified by Dr. Maha El-Shamy, Associate Professor of Plant Ecology, Botany Department, Faculty of Science, Mansoura University, Egypt, according to Boulos [17].

Phytochemistry of *W. somnifera*

Extraction and liquid–liquid partitioning

The air-dried powder of the whole plant, *W. somnifera* (6 kg), was extracted with MeOH (5 L × 3), then evaporated at 40 °C under reduced pressure till dryness. The dried extract was kept in the refrigerator until use. The alkaloid fraction of the plant was isolated according to the acid–base method. MeOH extract was dissolved in a small amount of methanol, and then acetic acid was added till pH 3 with stirring for 24 h. The non-alkaloid fraction was removed by MC solvent using a separating funnel. The aqueous layer was basified with 25% ammonium hydroxide to a pH 11, and then the alkaloid fraction was extracted by MC and yielded 8 g.

Processing of alkaloid fraction of W. somnifera

The alkaloid fraction (7 g) was separated by neutral aluminum oxide column chromatography using PT/EA as a mobile phase. Then, 100% MC was used, and MeOH was added to increase the polarity gradually. Six subfractions were obtained; subfraction **I** (170 mg, at the eluent system MC/MeOH, 95:5) was purified using PTLC (silica gel, EA/PT, 1:2, R_f 0.41, 9 mg) to give compound **1**. Compound **2** was isolated from subfraction II (210 mg, MC/MeOH, 90:10) by PTLC (silica gel, MC, 100%, R_f 0.78, 14 mg). Subfraction **IV** (240 mg) that was obtained at the eluent system (MC/MeOH, 90:20) gave compound **3** after further purification using PTLC (silica gel, MC/MeOH, 9.5:0.5, R_f 0.44, 20 mg). Subfraction **VI** (430 mg, at the eluent system MC/MeOH, 70:30) gave compound **4** after purification by PTLC (silica gel, MC/MeOH, 4:1, R_f 0.41, 22 mg). Compounds **1**, **3**, and **4** gave an orange color on TLC with Dragendorff's reagent.

Characterization of separated compounds 1–4

The structural elucidation of the bioactive compounds (**1–4**) was carried out by different spectroscopic methods.

Compound 1: Pale yellow residue (9 mg), ^{1}H NMR (400 MHz, $CDCl_3$, δ_H, ppm, *J*, Hz): δ_H 7.82 (s, H-4), 7.45 (dd, *J* = 7.8, 1.2 Hz, 2H-2',6'), 7.36 (t, *J* = 7.8 Hz, 2H-3',5'), 7.19 (t, *J* = 7.8 Hz, H-4'), 4.19 (t, *J* = 7.5 Hz, 2H-5), 3.10 (t, *J* = 7.5 Hz, 2H-7), 2.69 (m, 2H-6).

Compound 2: Crystal sheets, m.p. 65–67 °; ^{1}H NMR (400 MHz, CD_3OD, δ_H, ppm, *J*, Hz): δ_H 7.60 (d, *J* = 15.9 Hz, H-7), 6.35 (d, *J* = 15.9 Hz, H-8), 7.17 (d, *J* = 1.8 Hz, H-2), 7.06 (dd, *J* = 8.2, 1.8 Hz, H-6), 6.80 (d, *J* = 8.2 Hz, H-5), 3.88 (s, 3H-11), 3.76 (s, 3H-10).

Compound 3: Amorphous powder (20 mg); $[\alpha]_D^{21}$ + 15.87 (*c* 0.036, MeOH); IR υ_{max} (film): 3309, 2940, 1696, 1601, 1456, 1385, 1133 and 1031 cm^{-1}; UV λ_{max} (MeOH): 428, 398, 284, 251 nm; ^{1}H and ^{13}C NMR ($CDCl_3$) data (Table 1); HRESIMS (positive-ion mode) *m/z* 475.3199 $[M+H]^+$ (calcd for $C_{28}H_{43}O_6$, 475.3060).

Compound 4: Amorphous powder (22 mg),^{1}H-NMR (400 MHz, CD_3OD, δ_H, ppm, *J*, Hz): δ_H 3.80 (t, $W_{1/2}$ = 5.0 Hz, H-1), 1.82 (m, H-2a), 2.13(m, H-2b), 4.05 (m, H-3), 2.32 (m, H-4a), 2.46 (m, H-4b), 5.51 (d, *J* = 5.0 Hz, H-6), 1.60 (m, H-7a), 1.96 (m, H-7b), 1.49 (m, H-8), 1.70 (m, H-9), 1.52 (m, 2H-11), 1.41 (m, H-12a), 2.00 (m, H-12b), 1.14 (m, H-14), 1.16 (m, H-15a), 1.69 (m, H-15b), 1.67 (m, H-16a), 1.89 (m, H-16b), 1.79 (m, H-17), 0.87 (s, 3H-18), 1.01 (s, 3H-19), 1.26 (s, 3H-21), 1.99 (s, 3H-26), 1.85 (s, 3H-27), 4.24 (dd, *J* = 3.4, 13.2 Hz, H-22), 2.29 (dd, *J* = 18.1, 2.6 Hz, H-23a), 2.57 (t, *J* = 15.8 Hz, H-23b), 4.37 (d, *J* = 7.8 Hz, H-1'), 3.14 (t, *J* = 7.8 Hz, H-2'), 3.36 (m, H-3'), 3.25 (m, H-4'), 3.27 (m, H-5'), 3.64 (dd, *J* = 11.6, 4.9 Hz, H-6a), 3.85(d, *J* = 11.6 Hz, H-6b). ^{13}C-NMR (100 MHz, CD_3OD): δ_C 168.9 (C-26), 152.9 (C-24), 139.0 (C-5), 125.5 (C-6), 122.0 (C-25), 102.6 (C-1'), 82.7 (C-22), 78.0 (C-3'),77.9 (C-5'), 76.3 (C-20), 75.1 (C-2'), 74.9 (C-3), 73.5 (C-1), 71.7 (C-4'), 62.8 (C-6'), 58.1 (C-14),

Table 1 ^{1}H and ^{13}C NMR data for Sominone A (**3**) in $CDCl_3$ (δ) [ppm] (Multiplicity, *J* [Hz])

No	δ_H	δc
1	3.83 (t, $W_{1/2}$ = 5.0 Hz, 1H)	73.0
2	2.08 (m, 1H) 1.73 (td, *J* = 13.4, 1.6 Hz, 1H)	38.4
3	3.97 (m, 1H)	66.5
4	2.37 (m, 1H) 2.30 (m, 1H)	41.5
5	–	137.6
6	5.57 (d, *J* = 5.0 Hz, 1H)	125.5
7	2.19 (m, 1H) 1.61 (m,1H)	31.8
8	1.49 (m, 1H)	31.4
9	1.58 (m, 1H)	41.6
10	–	41.8
11	1.49 (m, 2H)	20.3
12	2.02 (m, 1H) 1.30 (m, 1H)	39.9
13	–	43.1
14	1.06 (m, 1H)	56.9
15	1.65 (m, 1H) 1.18 (dd, *J* = 11.1, 4.3 Hz, 1H)	24.0
16	1.95 (m, 1H) 1.53 (d, *J* = 4.7 Hz, 1H)	22.1
17	1.53 (d, *J* = 4.7 Hz, 1H)	54.8
18	0.86 (s, 3H)	13.8
19	1.02 (s, 3H)	19.6
20	–	75.3
21	1.28 (s, 3H)	20.9
22	4.28 (dd, *J* = 13.3, 3.3 Hz, 1H)	81.5
23	2.53 (dd, *J* = 17.1, 13.4 Hz, 1H) 1.98 (m, 1H)	31.8
24	–	153.3
25	–	125.8
26	–	166.3
27	4.39 (d, *J* = 12.5 Hz, 1H) 4.33 (d, *J* = 12.5 Hz, 1H)	57.4
28	2.06 (s, 3H)	20.2

55.8 (C-17), 42.7 (C-10), 43.9 (C-13), 42.5 (C-9), 39.1 (C-4), 37.6 (C-2), 40.9 (C-12), 32.8 (C-7), 32.2 (C-23), 32.6 (C-8), 23.0 (C-16), 25.0 (C-15), 20.5 (C-28), 21.1 (C-11), 20.8 (C-21), 20.0 (C-19), 14.3 (C-18), 12.4 (C-27) (Additional file 1).

Evaluation of the insecticidal activity

Rearing of tested pests

Aphis craccivora *Aphis craccivora* strains were collected from the farm of Faculty of Agriculture, Mansoura University, and they were known to be free from any contamination of insecticides. The aphids were maintained on cowpea plants under normal conditions in plastic greenhouse (3 × 2 × 2 m) [18].

Bemicia tabaci Adult whiteflies *(Bemicia tabaci)* were collected from the farm of Faculty of Agriculture, Mansoura University. The whiteflies were maintained on tomato plants in entomological cages (1.5 × 1.5 × 1.0 m) under normal conditions [18].

Nezara viridula The green stink bugs (*N. viridula*) were obtained from the farm of Faculty of Agriculture, Mansoura University, and reared on okra leaves (*Abelmoschus esculentus*) in the laboratory at a fixed temperature of 25 ± 2 °C, 65 ± 5% RH, and 12:12 (L:D) hr [19].

Tetranychus urticae The spider mites, *Tetranychus urticae* Koch, were collected from an infested castor tree. The collected pest was transferred to freshly cleaned castor leaves on moist cotton in petri dishes. They were kept under laboratory conditions (25 ± 2 °C and 60 ± 5% RH) and continued for two generations to be sensitive and homogenous before use in toxicity tests [20].

Biological bioassays

A. craccivora The tested plant samples were examined as insecticides by the spray method on cowpea leaves. Five concentrations of the alkaloid fraction (10, 20, 40, 80 and 160 ppm) and their isolated compounds (compound **1**: 5, 10, 20, 40 and 80; **2**: 20, 40, 60, 80 and 100; **3** and **4**: 50, 100,150, 200 and 250 ppm) were prepared using Tween 80 with distilled water for each treatment (four replicates per concentration). Ten aphids in each replicate were transferred on a disc of cowpea leaf (4.0 cm in diameter) placed in petri dishes over agar (1.5%) before 30 min of treatment. Finally, each treatment was sprayed with 2 ml of each tested solution, then covered and kept at room temperature. Whereas, the control treatment was sprayed with distilled water containing Tween 80. Mortality was count after 24 h. Mortality% were corrected by using Abbott equation [21]. The LC_{50}'s, LC_{90}'s values and their confidence limits were obtained according to Finney [22], in addition to the slope of regression lines (LC-P line). Also, the toxicity index was measured by the sun equation [23].

Bemisia tabaci nymphs Adults of *B. tabaci* (20 insects per cage) were collected and placed in cages containing tomato plant leaves. After 15 days, sections of tomato leaves (2 cm in diameter) containing 30 of the 3rd nymphs of *B. tabaci* were transferred over 2% agar medium into petri dishes. Five concentrations (four replicates for each one) of the *W. somnifera* alkaloid fraction (15, 30, 60, 120 and 240 ppm) and their isolated compounds (compound

1: 10, 20, 40, 80 and 160; **2**: 25, 50, 75, 100 and 125; **3** and **4**: 75, 150, 225, 300 and 375 ppm) were prepared. Only 2 ml of diluted solution were sprayed on each treatment, and water was used only in the control treatment. Mortality was accounted for after 24 h of treatment [24].

Nezara viridula Five concentrations (4 replicates for each one) of the alkaloid fraction (100, 200, 300, 400 and 500 ppm) and its isolated compounds (compound **1**: 25, 50, 100, 200 and 400; **2**: 50, 100, 200, 400 and 800; **3** and **4**: 150, 300,450, 600 and 750 ppm) were prepared. In each replicate, 10 adults of *N. viridula* were transferred to okra leaves in a glass jar. Each treatment was sprayed with 2 ml of test solution, and the control treatment was sprayed with distilled water containing Tween 80 and then covered. The mortality was recorded after 24 h [19].

Acaricidal activity

The effects of *W. somnifera* alkaloid fraction and its isolated compounds were evaluated against adult female *T. urticae* by the leaf-dip technique using five concentrations (200, 400, 600, 800 and 1000 ppm) and (compound **1**: 50, 100, 150, 200 and 250; **2**: 100, 200, 300, 400 and 500; **3** and **4**: 250, 500, 750, 1000 and 1250 ppm), respectively according to Dawidar [25].

Biochemical investigation of *A. craccivora*

The most toxic compound (**1**) at LC_{50} value was used for measuring enzyme activities. Fifty individuals of *A. craccivora* were sprayed with one milliliter of compound (**1**) solution, in addition to a control treatment that sprayed with distilled water only. After 24 h of treatment, the live aphids were weighed and kept frozen in a suitable tube, according to Elhefni et al. [18]. The insect enzymes α-esterase (α-EST) and β-esterases (β-EST) were determined using α- and β-naphthol acetate as substrates, respectively. The mixture was incubated for exactly 15 min at 27 °C then 1 ml of diazoblue colour reagent was added. The change in colour was read at 600 and 555 nm for α- and β-naphthol, respectively. The activity was expressed as µg α- or β-naphthol released /min/g body weight [26]. Acetylcholinesterase (AChE) activity was measured by Simpson et al. method [27] using acetylcholine bromide (AChBr) at a level of 6×10^{-3} M and the reading was measured at 515 nm. Peroxidase (POD) activity was determined by Hammerschmidt et al. method. The reading was taken at 420 nm to a mixture of Pyrogallol (0.05 M) and 100 µl aphid enzyme extract. The enzyme activity was expressed as change in absorbance/min/g sample [28]. Glutathione-*S*-transferase (GST) was determined by added 25 *µl* of the substrate 1-chloro 2,4-dinitrobenzene solution (CDNB) to reaction mixture (1 ml of phosphate buffer, pH 6.5, 100 µl of glutathione (GSH) and 200 µl of aphid extract). The absorbance was taken at 340 nm and the enzyme activity was expressed as mmol/min/mg protein [29, 30]. Chitinase (CTase) was assayed according to the method described by Ishaaya and Casida [31]. The reaction mixture consisted of 0.12 ml (0.2 *M*) phosphate buffer (pH 6.6); 0.3 ml 0.5% colloidal chitin; 0.18 ml aphid extract. After 60 min. incubation at 37 °C, enzyme activity was terminated by adding 1.2 ml 3,5-dinitrosalycilic acid reagent (DNSA). The reaction mixture was heated at 100 °C for 5 min then cooled and diluted with 1.2 ml distilled water. Undigested chitin was sedimented by centrifugation for 15 min. at 6000 rpm and the absorbance was measured at 550 nm. Chitinase activity is expressed as µg N-acetylglucosamine released/g body weight/min. All analyses were assessed in the Analysis Unit of the Plant Protection Research Institute, Agriculture Research Center. Total protein contents were determined according to Bradford [32].

Statistical analysis

Data were analyzed using MINITAB®software program (version Minitab®21.4.1). Statistical differences between the two means was calculated using two-sample T-test. Differences are significant when $P<0.05$. The LC_{50} and LC_{90} values of all treatments were determined using probit analysis.

Results and discussion

Identification of compounds (1–4)

Compound **1** (Fig. 1) was isolated as a pale yellow residue that gave a positive result with Dragendorff's reagent. ^{1}H NMR data showed the presence of a monosubstituted benzene ring at δ_H 7.45 (dd, $J=7.8$, 1.2 Hz, 2H-2', 6'), 7.36 (t, $J=7.8$ Hz, 2H-3',5'), 7.19 (t, $J=7.8$ Hz, H-4'). As well as the down-field proton of the pyrazole ring at δ_H 7.82 (s, H-4). Also, three different aliphatic methylene groups appeared at δ_H 4.19 (t, $J=7.5$ Hz, 2H-5), 3.10 (t, $J=7.5$ Hz, 2H-7), and 2.69 (m, 2H-6). These data were in agreement with those identified before as withasomine [33]. Compound **2** was isolated as a crystal sheets with m.p. 65–67 °. ^{1}H NMR analysis indicated the presence of the ABX system at δ_H 7.17 (d, $J=1.8$ Hz, H-2), 7.06 (dd, $J=8.2$, 1.8 Hz, H-6), 6.80 (d, $J=8.2$ Hz, H-5), in addition to two olefinic trans protons at δ_H 7.60 (d, $J=15.9$ Hz, H-7), and 6.35 (d, $J=15.9$ Hz, H-8). The above data indicated its identity as methyl isoferulate **2**, which was confirmed by comparing their data with the isolated data by Bowden et al. [34].

Compound **3** (Fig. 1) was isolated as an amorphous powder that gave a positive test with Dragendorff's [35]. ^{13}C NMR (Table 1) and HSQC (Hetereonuclear Single Quantum Coherence) spectra indicated signals for 28 carbon atoms, including 4 methyl, 9 methylene, 8

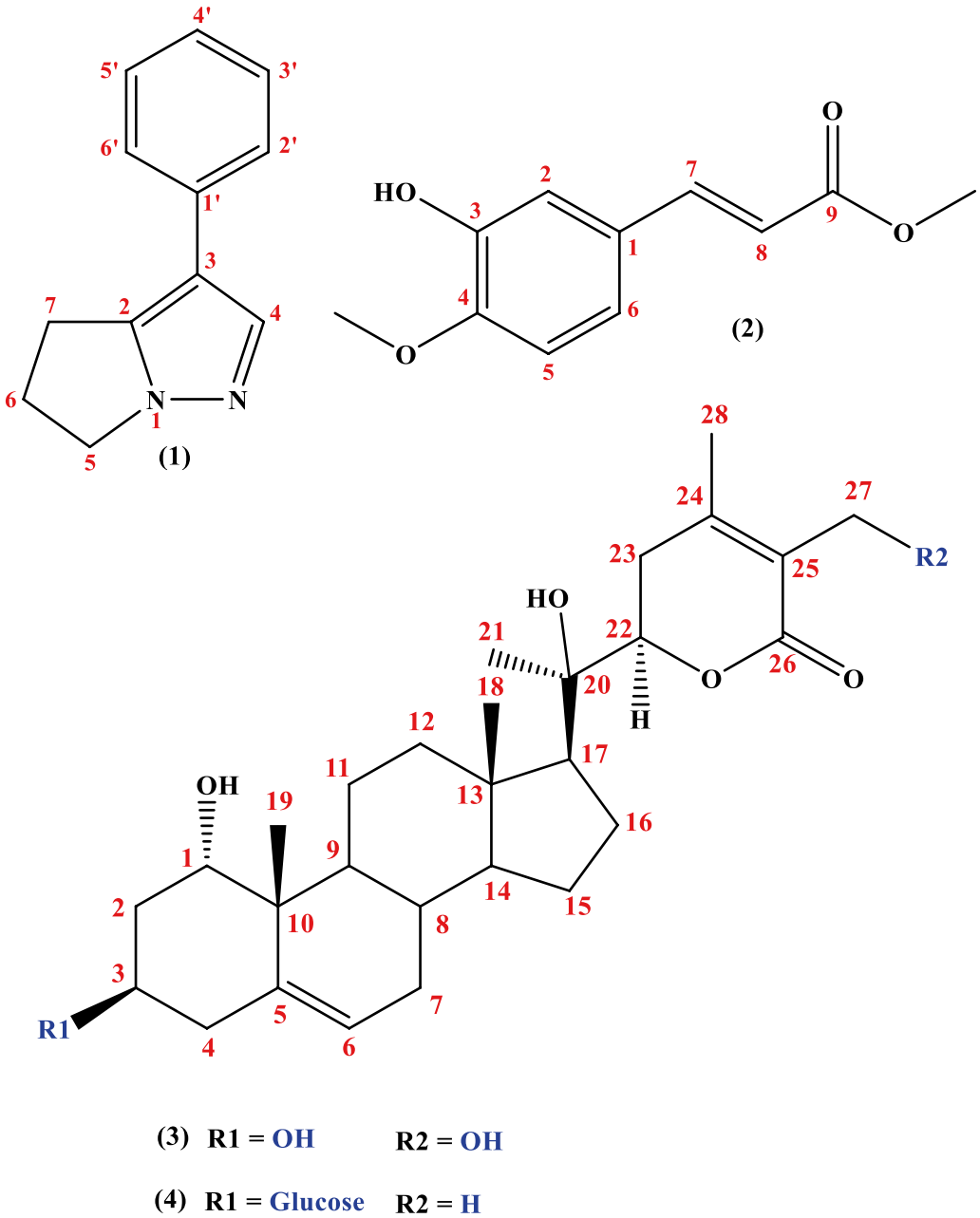


Fig. 1 Chemical structure of compounds (**1**–**4**)

methine groups, and 7 quaternary carbon atoms containing one carbonyl group at δ_C 166.3 ppm. The HRESIMS spectrum of compound **3** showed a $[M+H]^+$ peak at *m/z* 475.3199, in agreement with the molecular formula $C_{28}H_{43}O_6$. IR analysis indicated the presence of an unsaturated six-membered lactone ring at 1696 and 1601 cm^{-1}. The ^1H NMR spectrum (Table 1) of compound **3** showed four proton singlets for quaternary methyl groups at δ_H 0.86, 1.02, 1.28, and 2.06 ppm for the C-18, C-19, C-21, and C-28 protons, respectively. Two doublet signals at δ_H 4.39 and 4.33 ppm (J = 12.5 Hz) were ascribed to methylenic protons of C-27. A methine proton signal was assigned at δ_H 4.28 (dd, J = 13.3, 3.3 Hz) that related to the proton of C-22 for lactone moiety. The above observations supported the presence of a tetracyclic steroidal skeleton with a lactone substituent [11].

Two oxygenated methine protons appeared at δ_H 3.83 (t, $W_{1/2}$ = 5.0 Hz, H-1) and 3.97 (m, H-3) related to C-1

and C-3, respectively. The stereochemistry of the C-l (α) and C-3 (β) hydroxyl groups was assigned by comparing the coupling constant values and chemical shifts with the reported before withanolide lα, 3β, 20-trihydroxy (20*R*, 22*R*)-witha-5,24-dienolide [11, 36]. Also, the down-field proton signal at δ_H 5.57 (d, J=5.0 Hz) was ascribed to the proton of C-6. The cross peaks between (H-3, 3.97) and (H-2, 1.73 and 2H-4, 2.37, 2.30), as well as the cross peaks between (H-22, 4.28) and (H-23, 2.53), were assigned by the H–H COSY spectrum (Fig. 2). The HMBC (Heteronuclear Multiple Bond Correlation) spectrum (Fig. 2) confirmed the correlation of 2H-27 at δ_H 4.33 and 4.39 ppm with C-24, C-25, and C-26 at δ_C 153.3, 125.8, and 166.3 ppm, respectively. As well, the proton at δ_H 3.83 (H-1) was connected to C-3 and C-5 at δ_C 66.5 and 137.6 ppm, respectively. Also, the positions of the four methyl groups were confirmed by HMBC analysis (Fig. 2). Therefore, the above-mentioned data led to the assignment of structure (**3**) ((20*R*,22*R*)-1α,3β,20,27-tetrahydroxywitha-5,24-dienolide) to sominone A.

Compound **4** was isolated as an amorphous powder that gave an orange color with Dragendorff's [35]. ^{13}C NMR (Table 1) and HSQC analyses proved the presence of 34 carbon atom signals, including 5 methyl, 9 methylene, 13 methine groups, and 7 quaternary carbon atoms containing one carbonyl group at δ_c 168.9 ppm. ^{1}H NMR analysis (*c.f.* experimental) indicated that compound 4 is similar to compound 3, but a methyl group singlet signal was found at δ_H 1.85 (s, 3H-27) instead of the oxygenated methylene group, as well as a glucose moiety connected to β-OH of C-3 of the withanolide at δ_H 4.05 (m, H-3). The HMBC spectrum indicated a correlation between the anomeric proton of glucose at δ_H 4.19 (d, J=7.8 Hz, H-1') and the C-3 of withanolide at 74.9 ppm. The above-mentioned data were in agreement with the structure of Coagulin Q [37, 38]. Compounds **2**, **3**, and **4** were not alkaloid compounds but contained α, β-unsaturated ester especially withanolide derivatives that behave like alkaloids and gave positive results with Dragendorff's reagent [35].

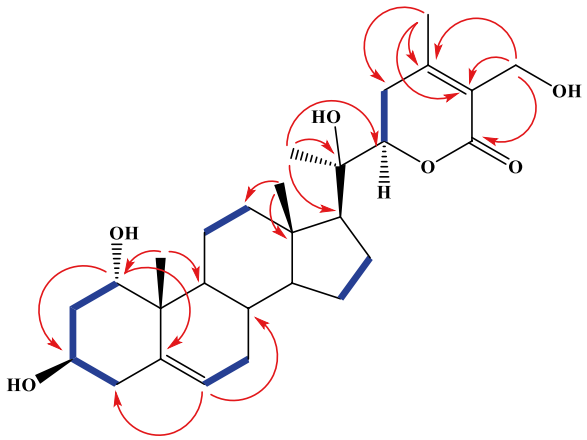


Fig. 2 HMBC (⌒) and COSY (▬) correlations for compound **3**

Insecticidal activity

The effects of the alkaloid fraction of *W. somnifera* and their isolated compounds (**1–4**) (Fig. 1), as well as Azadirachtin (Okios 3.2% EC), against *A. craccivora*, *B. tabaci*, *N. viridula,* and *T. urticae,* were tested and compared. Generally, it is clear that the alkaloid fraction and their compounds have a toxicity effect against the four pests with different ratios (Tables 2, 3, 4, and 5). Results in Table 2 showed that withasomine **1** was the most potent against *A. craccivora,* with a LC_{50} value of 15.44 ppm, compared to alkaloid fraction and the other compounds. The LC_{50} values of treatments in Table 3 indicated that withasomine **1** and Azadirachtin were the most effective against *B. tabaci* with LC_{50} values of 36.61 and 36.78 ppm and against *N. viridula* with LC_{50} values of 85.11 and 98.26 ppm, respectively.

As shown in Table 5, the most toxic compound was found to be withasomine **1** toward *T. urticae,* with a LC_{50} value of 128.29 ppm. It is clear that withasomine **1**, azadirachtin, isoferulate **2**, and the alkaloid fraction have strong toxicity against *A. craccivora*, *B. tabaci,* and *N.*

Table 2 Toxicity of *W. somnifera* alkaloid fraction and isolated compounds on *A. craccivora* after 24 h under laboratory conditions

Treatment	LC_{50} (ppm)	Confidence limit 95% (ppm)		LC_{90} (ppm)	Confidence limit 95% (ppm)		Slope ± *S.E*	Toxicity index (%)at LC_{50} value
		Lower	Upper		Lower	Upper		
Alkaloid fraction	28.97	20.35	39.15	164.32	103.61	378.01	1.70 ± 0.29	53.30
Withasomine **1**	15.44	11.12	20.67	81.48	52.26	178.69	1.77 ± 0.29	100
Isoferulate **2**	55.80	47.42	65.92	140.34	108.30	219.81	3.20 ± 0.49	28.67
Sominone A **3**	137.15	111.43	168.51	431.17	303.87	878.62	2.58 ± 0.49	11.26
Coagulin Q **4**	130.55	107.61	156.25	362.87	271.52	625.54	2.89 ± 0.50	11.83
Azadirachtin (Okios 3.2% EC)	27.40	17.56	45.64	178.28	83.30	2003.89	1.58 ± 0.44	56.35

Table 3 Toxicity of *W. somnifera* alkaloid fraction and isolated compounds on *B. tabaci* after 24 h under laboratory conditions

Treatment	LC_{50} (ppm)	Confidence limit 95% (ppm)		LC_{90} (ppm)	Confidence limit 95% (ppm)		*Slope* ± *S.E*	Toxicity index (%)at LC_{50} value
		Lower	Upper		Lower	Upper		
Alkaloid fraction	47.19	35.82	60.83	189.62	131.93	343.36	2.12 ± 0.31	77.58
Withasomine **1**	36.61	26.06	50.61	228.80	135.37	612.10	1.61 ± 0.28	100
Isoferulate **2**	69.93	57.93	84.36	197.02	144.78	356.76	2.85 ± 0.51	52.35
Sominone A **3**	193..83	161.06	229.71	508.75	389.73	825.57	3.06 ± 0.51	18.89
Coagulin Q **4**	199.67	164.14	240.54	572.14	421.52	1028.16	2.80 ± 0.50	18.34
Azadirachtin (Okios 3.2% EC)	36.78	27.22	49.93	184.33	118.04	401.99	1.83 ± 0.29	99.54

Table 4 Toxicity of *W. somnifera* alkaloid fraction and isolated compounds on *N. viridula* after 3 days under laboratory conditions

Treatment	LC_{50} (ppm)	Confidence limit 95% (ppm)		LC_{90} (ppm)	Confidence limit 95% (ppm)		*Slope* ± *S.E*	Toxicity index (%)at LC_{50} value
		Lower	Upper		Lower	Upper		
Alkaloid fraction	273.92	225.61	331.22	790.49	577.59	1453.13	2.78 ± 0.50	31.07
Withasomine **1**	85.11	63.99	111.29	375.03	252.38	729.61	1.99 ± 0.30	100
Isoferulate **2**	203.91	158.05	263.50	792.10	545.72	1456.76	2.17 ± 0.31	41.74
Sominone A **3**	399.86	334.73	472.45	1021.10	786.31	1637.26	3.15 ± 0.52	21.28
Coagulin Q **4**	440.08	366.68	532.57	1224.52	895.86	2246.73	2.88 ± 0.52	19.34
Azadirachtin (Okios 3.2% EC)	98.26	70.44	132.88	550.67	343.85	1287.89	1.71 ± 0.28	86.62

Table 5 Toxicity of *W. somnifera* alkaloid fraction and isolated compounds on *T. urticae* after 3 days under laboratory conditions

Treatment	LC_{50} (ppm)	Confidence limit 95% (ppm)		LC_{90} (ppm)	Confidence limit 95% (ppm)		*Slope* ± *S.E*	Toxicity index (%)at LC_{50} value
		Lower	Upper		Lower	Upper		
Alkaloid fraction	467.79	356.03	586.11	1784.56	1195.73	4327.56	2.20 ± 0.46	27.42
Withasomine **1**	128.29	104.86	154.36	370.47	273.93	659.20	2.78 ± 0.49	100
Isoferulate **2**	266.69	222.77	315.88	691.05	529.22	1123.24	3.10 ± 0.52	48.10
Sominone A **3**	606.02	487.11	732.91	1836.41	1345.69	3352.52	2.66 ± 0.48	21.17
Coagulin Q **4**	641.48	522.85	774.01	1883.55	1383.57	3412.49	2.74 ± 0.49	20.00
Azadirachtin (Okios 3.2% EC)	749.85	392.26	3765.36	21,305.20	4069.86	85,416.21	0.88 ± 0.26	17.11

viridula, but withanolides, sominone A, and coagulin Q have moderate effects. In the case of *T. urticae,* withasomine **1** and isoferulate **2** were more potent than other treatments.

Salamatullah (2022) found that the hydroethanol extract of *Withania adpressa* has insecticidal activity on *Callosobruchus maculatus* [39]. A methanol extract of *W. somnifera* was used as an aphicide against the rose aphid *Macrosiphum rosae* [6]. *W. somnifera* root extract was toxic to Fall armyworm, *Spodoptera frugiperda* [40], and *Spodoptera litura* pupae and larvae [41]. *Withania coagulans*, a plant from the same genus as *W. somnifera,* was tested as an insecticide against the green peach aphid, *Myzus persicae* [42], and *B. tabaci* [43]. Al-Ani et al. explained the important role of alkaloids as insecticides [44]. Phenolic compounds (ferulic acid) can be used to protect the plants from all kinds of pests [45].

Effect of withasomine (1) on *A. craccivora* enzyme activity

The effects of the most potent alkaloid compound, withasomine **1**, on *A. craccivora* enzymes (α, β-EST, CTase, AChE, GST, and POD) were measured (Table 6 and Fig. 3). The activity of α-EST and β-EST with values of 38.83 and 72.86 µg, respectively, slightly increased with

Table 6 Activity of defensive enzymes of *Aphis craccivora* adults treated with LC_{50} of withasomine **1**

Treatments	*α*-esterase activity (*μg α*-naphthol/ min/g b wt.) ± *SE*	*β*-esterase activity (*μg β*-naphthol/ min/g b wt.) ± *SE*	Chitinase Activity (*μg* NAGA /min/g b wt.) ± *SE*	Acetyl choline esterase activity (ug AchBr /min/gm b wt.) ± *SE*	GST activity (mmol sub.conjugated /min/mg protein) ± *SE*	Peroxidase activity (Δ mO.D./min/mg protein) ± *SE*
Withasomine **1**	38.83 ± 6.14 ^{ns}	72.86 ± 5.45^{ns}	31.45 ± 3.25*	506.4 ± 3.29**	2.62 ± 0.05**	251.0 ± 2.08*
Control	31.13 ± 3.54	64.03 ± 6.54	10.53 ± 0.67	643.8 ± 3.18	1.92 ± 0.04	242.3 ± 1.45
T-value	1.09	1.04	6.30	−30.02	0.74	−3.41
P-value	0.36	0.38	0.02	0.00	0.00	0.04

SE Standard error; Statistical differences between the two means was calculated using two-sample T-test. Differences are significant when P < 0.05, *ns* Non-significant

** High significant

* Significant

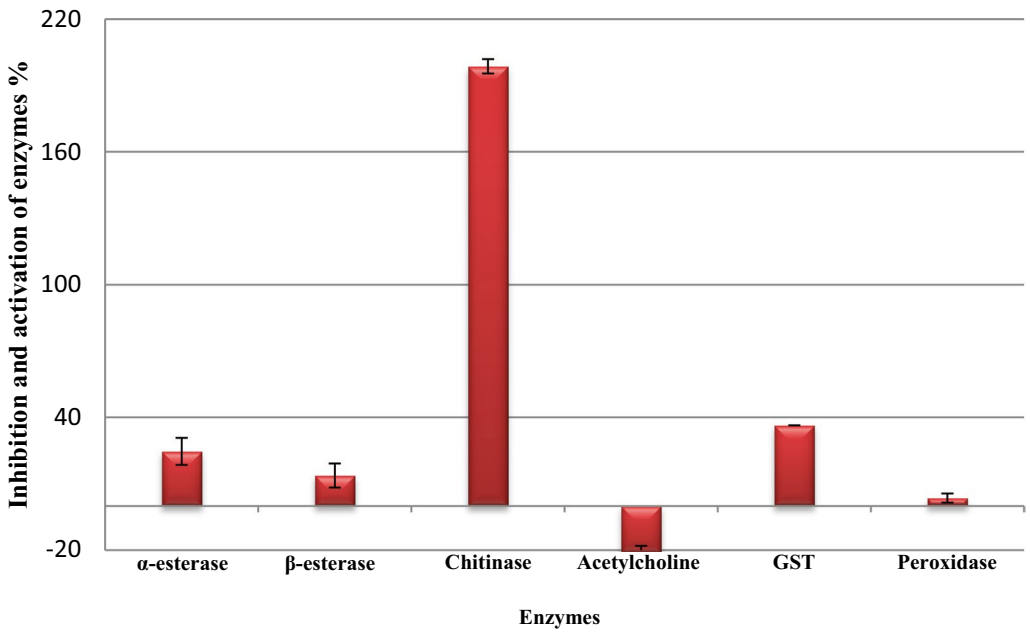


Fig. 3 Enzymes activity of *A. craccivora* at LC_{50} of withasomine **1**

no significant difference after 24 h of treatment when compared with the control. On the contrary, the activity of the GST enzyme increased with highly significant difference compared with the control (2.62 and 1.92 mmol, respectively). *α*, *β*-EST, and GST enzymes have important roles in the elimination of strange compounds like insecticides from insects through metabolic processes [46, 47]. These results were in agreement with the biochemical studies that proved an increase in the detoxifying enzymes activity (*α*, *β*-EST, and GST) as a result of the increased insect resistance to insecticides [47, 48]. Also, POD activity (251.0) increased, with significant difference compared to the control (242.3). The POD enzyme is known as an antioxidant enzyme that acts as a protective cellular system by producing H_2O from H_2O_2. This reaction decreases the damage to the biomembrane caused by reactive oxygen species [49, 50]. The activity of CTase (31.45 μg) increased with significant compared to the control (10.53 μg). Previous studies proved that the development of insect resistance to pesticides mainly depends on the thickness of the chitin layer [51]. Increasing the CTase activity led to chitin hydrolysis, in addition to an increase in the cell membrane permeability; therefore, the soluble proteins and sugars were reduced. These results are in agreement with the effect of tea saponins on *Ectropis oblique* [51, 52].

The activity of AChE showed a significant reduction compared to the control group (506.4 and 643.8 μg AchBr/min/g b wt.), respectively. Inhibition of AChE indicates an interaction between AChE and the

bioactive compounds. Phenolic compounds and alkaloids affect the nervous system of insects and inhibit AChE activity [7]. These results were in agreement with the previous studies [53]. Therefore, it is clear to us from the results that withasomine **1** is the active substance that has the greatest effect on the pests under study.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-024-00880-z.

Additional file 1. Supplemental data. **Figure S1.** ^{1}H NMR (400 MHz) spectrum of withasomine **1** in $CDCl_3$. **Figure S1a.** Expanded ^{1}H NMR (400 MHz) spectrum of withasomine **1** in $CDCl_3$. **Figure S1b.** Expanded ^{1}H NMR (400 MHz) spectrum of withasomine **1** in $CDCl_3$. **Figure S2.** ^{1}H NMR (400 MHz) spectrum of methyl isoferulate **2** in CD_3OD. **Figure S2a.** Expanded ^{1}H NMR (400 MHz) spectrum of isoferulate **2** in CD_3OD. **Figure S3.** ^{1}H NMR (400 MHz) spectrum of sominone A **3** in $CDCl_3$. **Figure S3a.** Expanded ^{1}H NMR (400 MHz) spectrum of sominone A **3** in $CDCl_3$. **Figure S3b.** Expanded ^{1}H NMR (400 MHz) spectrum of sominone A **3** in $CDCl_3$. **Figure S4.** ^{13}C NMR (100 MHz) spectrum of sominone A **3** in $CDCl_3$. **Figure S4a.** Expanded ^{13}C NMR (100 MHz) spectrum of sominone A **3** in $CDCl_3$. **Figure S4b.** Expanded ^{13}C NMR (100 MHz) spectrum of sominone A **3** in $CDCl_3$. **Figure S5.** H–H COSY spectrum of sominone A **3** in $CDCl_3$. **Figure S6.** Edited HSQC spectrum of sominone A **3** in $CDCl_3$. **Figure S6a.** Expanded Edited HSQC spectrum of sominone A **3** in $CDCl_3$. **Figure S6b.** Expanded Edited HSQC spectrum of sominone A **3** in $CDCl_3$. **Figure S7.** HMBC spectrum of sominone A **3** in $CDCl_3$. **Figure S7a.** Expanded HMBC spectrum of sominone A **3** in $CDCl_3$. **Figure S7b.** Expanded HMBC spectrum of sominone A **3** in $CDCl_3$. **Figure S8.** UV spectrum of sominone A **3**. **Figure S9.** IR spectrum of sominone A **3**. **Figure S10.** HRESIMS spectrum of sominone A **3**. **Figure S11.** ^{1}H NMR (400 MHz) spectrum of coagulin Q **4** in CD_3OD. **Figure S11a.** Expanded ^{1}H NMR (400 MHz) spectrum of coagulin Q **4** in CD_3OD. **Figure S11b.** Expanded ^{1}H NMR (400 MHz) spectrum of coagulin Q **4** in CD_3OD. **Figure S12.** ^{13}C NMR (100 MHz) spectrum of coagulin Q **4** in CD_3OD. **Figure S12a.** Expanded ^{13}C NMR (100 MHz) spectrum of coagulin Q **4** in CD_3OD. **Figure S13.** H–H COSY spectrum of coagulin Q **4** in CD_3OD. **Figure S14.** Edited HSQC spectrum of coagulin Q **4** in CD_3OD. **Figure S14a.** Expanded Edited HSQC spectrum of coagulin Q **4** in CD_3OD. **Figure S14b.** Expanded Edited HSQC spectrum of coagulin Q **4** in CD_3OD. **Figure S15.** HMBC spectrum of coagulin Q **4** in CD_3OD. **Figure S15a.** Expanded HMBC spectrum of coagulin Q **4** in CD_3OD. **Figure S15b.** Expanded HMBC spectrum of coagulin Q **4** in CD_3OD. **Figure S15C.** Expanded HMBC spectrum of coagulin Q **4** in CD_3OD.

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Author contributions
The chemistry experiments were performed by AR. Experimental planning and data analysis were performed by ARE, MAT and HHE. ARE collected the plant materials and performed the insecticidal activity. Conceptualization and supervision performed by ARE, MAT and HHE. The manuscript was written by ARE. All authors reviewed the manuscript.

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests
The authors declare that they have no competing interests.

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