Assessment of pesticidal potential of extracts and phytochemicals isolated from three Egyptian plants

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Received on: 1/11/2010 ***Accepted: 1/12/2010***

ABSTRACT

Integration use of chromatographic methods led to isolate fifteen compounds from three Egyptian plants, namely *Ambrosia maritima* L., *Achillea santolina* L. and *Adhatoda vasica* L. Five pseudoguaianolide sesquiterpenes, neoambrosin, damsinic acid, damsin, ambrosin, and hymenin, were isolated from the aerial parts of chloroform extract of *A. maritima*. A guaianolide sesquiterpene lactone, leucodin, and 6 flavonoids, artemetin, salvigenin, cirsimaritin, 5,7 dihydroxy-3`,4'dimethoxyflavone, santoflavone and eupatorin, were obtained from chloroform extract of the aerial parts of *A. santolina*. Further, three quinazoline alkaloids, vasicolinone, anisotine and vasicine, were isolated from the alkaloidal fraction of *A. vasica* ethanol extract. The chemical structures of the isolated compounds were determined by using spectral data of ^{1}H NMR, ^{13}C NMR, MS and UV. The bioactivities of the isolated extracts and compounds were evaluated against *Culex pipiens* L., *Theba pisana* Muller, *Eobania vermiculata* Muller and *Echinochloa crusgalli* L. The tested extracts and compounds showed variable toxicity towards the fourth instar larvae of *C. pipiens* with a flavonoid eupatorin (LC_{50}= 5.61 mg/l.) being the toxic compound. In addition, vasicine and damsin showed pronounced insecticidal activity with LC_{50} values of 88.88 and 104.87 mg/l, respectively. Out of sixteen extracts and compounds tested, neoambrosin, damsin and ambrosin exhibited the highest toxicity against the terrestrial snails *T. pisana* and *E. vermiculata*. The three compounds were more toxic than methomyl towards *T. pisana* after 24 h of treatment at 0.5 mg/snail. The phytotoxic experiments on barnyard grass, *E. crusgalli*, revealed that the sesquitepenes, neoambrosin, damsin and ambrosin, had strong seed germination inhibition, with complete suppression of germination at concentration of 500 mg/l. The majority of tested extracts and compounds significantly reduced the root and shoot growth of barnyard grass comparing with control; this effect was concentration-dependent. The inhibitory effect of extracts and compounds on root growth was greater than that of shoot growth. Sesquiterpenes inhibited seed germination and growth of both root and shoot more strongly than flavonoids and alkaloids. Correlation between bioactivity of the isolated compounds and chemical structure was discussed. The results obtained suggest that eupatorin, neoambrosin, damsin and ambrosin may serve as natural pesticidal compounds.

Key words: Pesticidal activity; Egyptian plants; Culex pipiens; Echinochloa crusgalli; terrestrial snails; sesquiterpenes; flavonoids; alkaloids

INTRODUCTION

Management of agricultural and public health pests over the past half century has been largely depending on the use of synthetic pesticides. However, the extensive use of these pesticides leads to adverse effects such as development of pesticide resistance, frequent pest out breaks, emergence of new pests and negative impact on natural enemies. In addition, increasing documentation of negative environmental and health impact of synthetic pesticides and increasingly stringent environmental regulation of pesticides have resulted in renewed interest in the development and use of bio-pesticides by agrochemical companies (Isman, 2000). Replacement of synthetic pesticides by bio-rational pesticides is an universally acceptable and practicable approach worldwide. Higher plant extracts and secondary metabolites are major class of bio-pesticides. Not only might certain secondary metabolites of the plant origin be a source of new pesticides, but also botanical derivatives may be more environmentally benign than synthetic pesticides (Arnason *et al.*, 1989). Higher plant extracts and their secondary metabolites are known to possess insecticidal, herbicidal, fungicidal, nematicidal, molluscicidal and rodenticidal properties (Jacobson, 1989; Duke, 1990).

Ambrosia maritima L. (Asteraceae) is a widely distributed weed in southern parts of Egypt, Sudan, Senegal, and neighboring countries. In Egypt, it is a popular medicinal plant for the treatment of renal colic and calculi. It acts also as antispasmodic, diuretic and useful in bronchial asthma, spasms and frequent urination (Ghazanfar, 1994). This plant showed promising potential as a molluscicidal agent (El-Sawy *et al.*, 1984). *Achillea santolina* L. (Asteraceae) is a herb growing wildly in western Alexandria. It is widely used in folk medicine as a tonic, anti-diabetic, anti-inflammatory, vermifugal and carminative. The plant also relieves stomach pain. Pharmacological screening of the plant showed antibacterial and antifungal activities (Ahmad *et al.*, 1995; Yazdanparast *et al.*, 2007). *Adhatoda vasica* L. (Acanthaceae) is a well-known plant drug in Ayurvedic and Unani medicine. It has been used for more than 3000 years for the treatment of various kinds of bronchial disorders including coughs, bronchial catarrh, asthma and bronchitis (Kapoor, 1990; Claeson *et al.*, 2000).

Culex pipiens L. (Diptera: Culicidae) is an important vector of several human pathogens, such as West Nile virus, Rift Valley Fever virus, and *Bancroftian flariasis* (Meegan *et al.*, 1980; Claire and Callaghan, 1999). This insect has a wide distribution throughout the tropical and subtropical areas. In Egypt, *C. pipiens* is the most common mosquito species in urban and rural areas, causing a health risk and nuisance to humans. The white garden snails, *Theba pisana* Muller, and the brown garden snails, *Eobania vermiculata* Muller, (Mollusca: Gastropoda: Helicidae) cause great damage to ornamental plants, vegetables and citrus trees (Miller *et al.*, 1988). They attack the plants and cause direct damage by feeding on the plants and indirect damage by creating wounds that allow plant pathogenic fungi to infect plants. Their mucus and shells can contaminate grains, vegetables and fruits (Godan, 1983; Barker, 2002). Nowadays, white garden snail is a serious agricultural pest in many areas of the world including Europe, USA, Mediterranean region and Australia particularly in wet seasons. In Egypt, these two snails are destructive agricultural animal pests to several economic crops including tree fruits, vegetables and ornamental plants (El-Okda, 1983). Barnyard grass (*Echinochloa crusgalli* L.), an annual grass, is widely spread throughout the world. It has been reported to cause problems in at least 61 countries and in at least 36 different crops (Holm *et al.*, 1991). It is a major weed in paddy fields as it competes with rice (*Oryza sativa* L.) and causes reduction in rice yield. It reduces crop yields by removing up to 80% of the soil nitrogen. Competition from 25 barnyard grass plants/m^2 can cause 50% reduction in rice yield (Chin, 2001).

No studies have been reported previously concerning the activity of *A. maritima*, *A. santolina* and *A. vasica* extracts, and the isolated sesquiterpenes, flavonoids and alkaloids from these plants against *C. pipiens*, *T. pisana*, *E. vermiculata* and *E. crusgalli*. Nevertheless, the molluscicidal activity of *A. maritima* extracts and its major constituents, damsin and ambrosin, against fresh water snails has been demonstrated (Shoeb and El-Emam, 1976; El-Sawy *et al.*, 1987 Abou Basha *et al.*, 1994). In the present study the extracts of the three Egyptian plants, *A. maritima*, *A. santolina* and *A. vasica*, were tested for their mosquitocidal, molluscicidal and herbicidal activities. Fifteen compounds of sesquiterpenes, flavonoids and alkaloids were isolated from the tested plants. Structure elucidation of the isolated compounds was described. In addition, the pesticidal properties of the isolated compounds were evaluated against *C. pipiens*, *T. pisana*, *E. vermiculata* and *E. crusgalli*.

1. General analytical and experimental procedures

Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ on a JEOL FX-600 spectrometer operating at 600 MHz for ^1H and 150 MHz for ^{13}C. Ultraviolet (UV) spectra were recorded in MeOH on a Hitachi U-3310 spectrophotometer. Mass spectra (EIS-MS) were measured on QSTAR-XL LC/MS. HPLC were performed on Waters μBondapak C_{18} column by using 30–40% H_2O/MeOH as solvent. Silica gel 70-230 mesh (Merck) and Sephadex LH-20 were used for open column chromatography.

2. Plant materials

Aerial parts of *Ambrosia maritima* L. (Asteraceae) and *Achillea santolina* L. (Asteraceae) were collected from Alfaium Governorate, south Cairo and Borg Elarab region, Alexandria, respectively. Leaves of *Adhatoda vasica* L. (Acanthaceae) were collected from Shalallat garden, Alexandria. The plant samples were collected in April 2009. The plants were identified with guidance of Flora of Egypt Book (Tackholm, 1974) and with assistance of Prof. Fath-Allah Zitoon of Alexandria University. Voucher specimens are deposited in Faculty of Agriculture, Alexandria University.

3. Test organisms

A *Culex pipiens* L. (Diptera: Culicidae) colony maintained in the laboratory of Mosquito Bioassay, Department of Economic Entomology, for more than 10 years was used. Mosquitoes were held at 26±2 °C, 70±5% RH, and a photo regime of 14:10 (light:dark) h. Adults of *Theba pisana* Muller (Mollusca: Gastropoda: Helicidae) (16 ± 0.5 mm shell diam) and *Eobania vermiculata* Muller (Mollusca: Gastropoda: Helicidae) (26±1mm shell diam.) terrestrial snails were collected from Faculty of Agriculture Garden, Alexandria, Egypt. The snails were acclimatized to laboratory conditions for one week and were fed on fresh lettuce leaves. Seeds of a field biotype barnyard grass, *Echinochloa crusgalli* L. (Poaceae) were obtained from Faculty of Agriculture Farm, Alexandria, Egypt. Uniform seeds were selected for the test while undersized and damaged seeds were discarded. Germination of the seeds was tested before use and was 70% after 5 days of sowing.

4. Extraction and isolation of sesquiterpenes (1-5) from *Ambrosia maritima*

The aerial parts (3 kg) of *A. maritima* were extracted with chloroform (8 l) at room temperature for one week to give 221 g of a crude extract. The resulting extract (160 g) was suspended in 600 ml of H_2O/MeOH (2:1) and extracted with hexane and chloroform (500ml×3), respectively. The chloroform fraction (50 g) was chromatographed on silica gel (600 g) with hexane/ethylacetate solvent system. Twenty-five fractions of 200 ml were collected. These fractions were pooled into five main fractions based on their TLC profiles. The second fraction (4.8g) eluted by 40% ethylacetate/hexane was further purified on silica gel column with

chloroform and 2.5% MeOH/chloroform as solvents to give 1.4 g of compound **1** and 1.6 g of compound **2**. Similar purification for the third fraction (11.5 g) yielded 7 g of compound **3**. The fourth fraction (8.1 g) was subjected to silica gel column and eluted with 2.5 and 5% MeOH/chloroform to afford 4.9 g of compound **4** and 1.7 g of compound **5**

Neoambrosin (1):

Colourless needels; ^{1}H NMR ($CDCl_3$): δ 5.97 (1H, t, *J* = 2.3 Hz, H-2), 3.16 (1H, dd, *J* = 2.3 and 22.9 Hz, H-3a), 2.80 (1H, dd, *J* = 2.3 and 22.5 Hz, H-3b), 4.43 (1H, d, *J* = 8.7 Hz, H-6), 3.40 (1H, m, H-7), 2.00~2.12 (2H, m, H-8 and H-9), 1.71~1.81 (2H, m, H-8 and H-9), 2.89~2.93 (1H, m, H-10), 6.25 (1H, d, *J* = 3.7 H-13a), 5.54 (1H, d, *J* = 3.2 Hz, H-13b), 1.17 (3H, d, *J* = 7.0 Hz, H-14), 1.18 (3H, s, H-15); ^{13}C NMR ($CDCl_3$): δ 148.9 (s, C-1), 124.1 (d, C-2), 39.7 (t, C-3), 213.8 (s, C-4), 58.5 (s, C-5), 79.6 (d, C-6), 43.2 (d, C-7), 23.6 (t, C-8), 29.9 (t, C-9), 38.5 (d, C-10), 138.5 (s, C-11), 169.7 (s, C-12), 119.7 (t, C-13), 20.9 (q, C-14), 14.6 (q, C-15); $C_{15}H_{18}O_3$; ESIMS (positive ion mode) *m/z* (rel. int.): 247.1720 (22) $[M+H]^+$ (calculated for $C_{15}H_{19}O_3$ 247.1329), 229.1585 (29), 211.1473 (24), 201.1584 (100), 183.1481 (33), 173.1148 (61), 159.1098 (69).

Damsinic acid (2):

Colourless needels; ^{1}H NMR ($CDCl_3$): δ 2.19 (1H, m, H-1), 1.57~1.63 (1H, m, H-2a), 1.65 (1H, m, H-2b), 2.26 (1H, m, H-3a), 2.07 (1H, m, H-3b), 2.16 (1H, dd, *J* = 7.7 and 17.4 Hz, H-6a), 2.46 (1H, dd, *J* = 8.3 and 19.7 Hz, H-6b), 2.73 (1H, m, H-7), 1.78 (1H, m, H-8a), 1.94 (1H, m, H-8b), 1.61~1.67 (2H, m, H-9a and H-9b), 1.87 (1H, m, H-10), 6.24 (1H, s, H-13a), 5.63 (1H, s, H-13b), 1.05 (3H, d, *J* = 7.3 Hz, H-14), 1.05 (3H, s, H-15); ^{13}C NMR ($CDCl_3$): δ 45.8 (d, C-1), 22.5 (t, C-2), 35.8 (t, C-3), 221.9 (s, C-4), 51.0 (s, C-5), 37.6 (t, C-6), 35.9 (d, C-7), 30.9 (t, C-8), 33.7 (t, C-9), 34.7 (d, C-10), 146.3 (s, C-11), 172.1 (s, C-12), 124.5 (t, C-13), 17.3 (q, C-14), 21.2 (q, C-15); $C_{15}H_{22}O_3$; ESIMS (positive ion mode) *m/z* (rel. int.): 251.1997 (7) $[M+H]^+$ (calculated for $C_{15}H_{23}O_3$ 251.1854), 233.1877 (90), 215.1761 (44), 187.1769 (100), 161.1578 (43), 145.1251 (49), 105.0873 (55).

Damsin (3):

Colourless needels; ^{1}H NMR ($CDCl_3$): δ 2.28 (1H, dd, *J* = 9.6 and 11.0, H-1), 1.60~2.13 (7H, m, 2H-2, 2H-8, 2H-9 and 1H-10), 2.45 (1H, ddd, *J* = 1.9, 8.7 and 17.8, H-3a), 2.23 (1H, m, H-3b), 4.55 (1H, d, *J* = 8.7 Hz, H-7), 3.35 (1H, m, H-7), 6.25 (1H, d, *J* = 3.2 Hz, H-13a), 5.57 (1H, d, *J* = 2.7 Hz, H-13b), 1.08 (3H, d, *J* = 7.3 Hz, H-14), 1.06 (3H, s, H-15); ^{13}C NMR ($CDCl_3$): δ 45.7 (d, C-1), 23.6 (t, C-2), 35.9 (t, C-3), 218.9 (s, C-4), 54.6 (s, C-5), 81.5 (d, C-6), 44.0 (d, C-7), 25.3 (t, C-8), 33.0 (t, C-9), 33.9 (d, C-10), 139.3 (s, C-11), 170.0 (s, C-12), 120.6 (t, C-13), 15.6 (q, C-14), 13.5 (q, C-15); $C_{15}H_{20}O_3$; ESIMS (positive ion mode) *m/z* (rel. int.): 249.1947 (12) $[M+H]^+$ (calculated for $C_{15}H_{21}O_3$ 249.1591), 231.1794 (100), 213.1687 (23), 203.1810 (40), 185.1679 (75).

Ambrosin (4):

Colourless needels; ^{1}H NMR ($CDCl_3$): δ 3.06 (1H, m, H-1), 7.58 (1H, dd, *J* = 1.8 and 5.9 Hz, H-2), 6.13 (1H, dd, *J* = 2.8 and 6.0 Hz, H-3), 4.68 (1H, d, *J* = 8.2 Hz, H-6), 3.50 (1H, m, H-7), 1.90 (1H, m, H-8a), 2.25 (1H, m, H-8b), 1.71 (1H, m, H-9a), 1.99 (1H, m, H-9b), 2.47 (1H, m, H-10), 6.27 (1H, d, *J* = 3.6 H-13a), 5.55 (1H, d, *J* = 3.2 Hz, H-13b), 1.05 (3H, d, *J* = 7.3 Hz, H-14), 1.18 (3H, s, H-15); ^{13}C NMR ($CDCl_3$): δ 47.2 (d, C-1), 163.7 (d, C-2), 130.4 (d, C-3), 210.6 (s, C-4), 55.6 (s, C-5), 79.8 (d, C-6), 44.0 (d, C-7), 24.1 (t, C-8), 29.0 (t, C-9), 33.1 (d, C-10), 137.6 (s, C-11), 170.2 (s, C-12), 119.5 (t, C-13), 17.0 (q, C-14), 16.7 (q, C-15); $C_{15}H_{18}O_3$; ESIMS (positive ion mode) *m/z* (rel. int.): 247.1503 (26) $[M+H]^+$ (calculated for $C_{15}H_{19}O_3$ 247.1334), 229.1576 (100), 211.1464 (35), 201.1576 (96), 183.1468 (42), 173.0913 (43), 159.1062 (32).

Hymenin (5):

Colourless needels; ^{1}H NMR ($CDCl_3$): 7.65 (1H, d, *J* = 6.0 Hz, H-2), 6.21 (1H, d, *J* = 5.5 Hz, H-3), 4.91 (1H, d, *J* = 9.6 Hz, H-6), 3.31 (1H, m, H-7), 1.72~1.84 (2H, m, H-8 and H-9), 2.02~2.18 (2H, m, H-8 and H-9), 2.40 (1H, m, H-10), 6.28 (1H, d, *J* = 3.7 H-13a), 5.60 (1H, d, *J* = 3.2 Hz, H-13b), 1.18 (3H, d, *J* = 5.5 Hz, H-14), 1.10 (3H, s, H-15); ^{13}C NMR ($CDCl_3$): δ 82.9 (s, C-1), 164.9 (d, C-2), 129.9 (d, C-3), 209.0 (s, C-4), 57.4 (s, C-5), 79.9 (d, C-6), 41.5 (d, C-7), 31.4 (t, C-8), 25.4 (t, C-9), 37.6 (d, C-10), 138.8 (s, C-11), 170.6 (s, C-12), 120.7 (t, C-13), 17.4 (q, C-14), 14.6 (q, C-15); $C_{15}H_{18}O_4$; ESIMS (positive ion mode) *m/z* (rel. int.): 263.1678 (5) $[M+H]^+$ (calculated for $C_{15}H_{19}O_4$ 263.1263), 245.1544 (60), 227.1412 (38), 217.1559 (100), 199.1425 (86), 189.0864 (88), 171.1424 (42), 121.0870 (34).

5. Extraction and isolation of a sesquiterpene (6) and flavonoids (7-12) from *Achillea santolina*

The air-dried aerial parts (2kg) of *A. santolina* were extracted with chloroform (10 l) at room temperature for 10 days to yield 106 g of a crude extract. The resulting extract was suspended in 500 ml of 10% H_2O/MeOH and extracted with hexane (500 ml × 3). The aqueous methanolic faction (35.3 g) was chromatographed on silica gel column (550 g) using hexane/ethylacetate solvent system. Fifty fractions of 200 ml were collected. The resulting fractions were pooled into five six fractions based on their TLC similarities. The second faction (frs 16~18, 4.9 g) eluted with 60% ethylacetate/hexane was further purified on Sephadex LH-20 column with MeOH/CH_3Cl (1:1) followed by recrystalization to give 2.6 g of compound **6**. The third fraction (frs 12~22, 2.3 g) eluted with 60% ethylacetate/hexane was subjected to HPLC with H_2O/MeOH (30~40%) to afford 196 mg of compound **7**, 43 mg of compound **8**, 23 mg of compound **9** and 18 mg of compound **10**. Similar

purification of the fourth faction (frs 23~31, 4 g), eluted with 80% ethylacetate/hexane, by HPLC gave 206 mg of compound **11** and 235 mg of compound **12**.

Leucoden (6):

Colourless needels; UV (MeOH) λ_{max} (ε): 255 (16000); ^{1}H NMR ($CDCl_3$): δ 6.16 (1H, qui, J = 1.3 Hz, H-3), 3.41 (1H, brd, J = 10.5 Hz, H-5), 3.62 (1H, t, J = 9.6 Hz, H-6), 1.94 (1H, dtd, J = 3.1, 10.1 and 13.0 Hz, H-7), 2.02 (1H, m, H-8α), 1.37 (1H, qt, J = 3.2 and 13.3 Hz, H-8β), 2.43 (1H, dt, J = 1.8 and15.2 Hz, H-9α), 2.34 (1H, m, H-9β), 2.24 (1H, q, J = 6.9 Hz, H-11), 1.26 (3H, d, J = 7.3 Hz, H-13), 2.43 (3H, s, H-14), 2.29 (3H, d, J = 1.2 Hz, H-15); ^{13}C NMR ($CDCl_3$): δ 131.8 (s, C-1), 195.8 (s, C-2), 135.4 (d, C-3), 169.9 (s, C-4), 52.5 (d, C-5), 84.1 (d, C-6), 56.3 (d, C-7), 25.9 (t, C-8), 37.5 (t, C-9), 152.1 (s, C-10), 41.0 (d, C-11), 177.5 (s, C-12), 12.9 (q, C-13), 21.5 (q, C-14), 19.7 (q, C-15); $C_{15}H_{18}O_3$; ESIMS (positive ion mode) *m/z* (rel. int.): 247.1455 (8) $[M+H]^+$ (calculated for $C_{15}H_{19}O_3$ 247.1334), 201.1390 (9), 173.1072 (100), 158.0826 (41), 145.1055 (76).

Artemetin (7):

Pale yellow powder; UV (MeOH) λ_{max} (ε): 346 (22000), 272 (19000), 255 (19000); ^{1}H NMR ($CDCl_3$): δ 6.51 (1H, s, H-8), 7.69 (1H, d, J = 2.3 Hz, H-2`), 7.00 (1H, d, $J$ = 8.2 Hz, H-5`), 7.73 (1H, dd, J = 1.8 and 8.7 Hz, H-6`), 3.87 (3H, s, 3-OMe), 3.93 (3H, s, 6-OMe), 3.97 (3H, s, 7-OMe), 3.98 (3H, s, 3`-OMe), 3,98 (3H, s, 4`-OMe); $^{13}$C NMR ($CDCl_3$): δ 155.9 (s, C-2), 138.8 (s, C-3), 178.9 (s, C-4), 152.3 (s, C-5), 132.2 (s, C-6), 158.7 (s, C-7), 90.3 (d, C-8), 152.7 (s, C-9), 106.6 (s, C-10), 122.9 (s, C-1`), 110.8 (d, C-2`), 148.8 (s, C-3`), 151.4 (s, C-4`), 111.3 (d, C-5`), 122.1 (d, C-6`) 60.9 (q, 3-OMe), 60.2 (q, 6-OMe), 56.3 (q, 7-OMe), 56.0 (q, 3`-OMe), 56.1 (q, 4`-OMe); $C_{20}H_{20}O_8$; ESIMS (positive ion mode) *m/z* (rel. int.): 389.1120 (4) $[M+H]^+$ (calculated for $C_{20}H_{21}O_8$ 389.1236), 373.0990 (32), 358.0835 (23), 331.0866 (100), 313.0743 (53).

Salvigenin (8):

Pale yellow powder; UV (MeOH) λ_{max} (ε): 329 (26000), 277 (20500); ^{1}H NMR ($CDCl_3$): δ 6.53 (1H, s, H-3), 6.57 (1H, s, H-8), 7.82 (2H, d, J = 8.9 Hz, H-2` and H-6`), 6.99 (2H, d, J = 8.9 Hz, H-3`and H-5`), 3.91 (3H, s, 6-OMe), 3.95 (3H, s, 7-OMe), 3.87 (3H, s, 4`-OMe), 12.52 (1H, s, 5-OH); $^{13}$C NMR ($CDCl_3$): δ 164.0 (s, C-2), 104.1 (d, C-3), 182.7 (s, C-4), 153.2 (s, C-5), 132.6 (s, C-6), 158.7 (s, C-7), 90.5 (d, C-8), 153.1 (s, C-9), 106.1 (s, C-10), 123.6 (s, C-1`), 128.0 (d, C-2`), 114.5 (d, C-3`), 162.6 (s, C-4`), 114.5 (d, C-5`), 128.0 (d, C-6`) 56.3 (q, 6-OMe), 60.8 (q, 7-OMe), 55.5 (q, 4`-OMe); $C_{18}H_{16}O_6$; ESIMS (positive ion mode) *m/z* (rel. int.): 329.0937 (0.7) $[M+H]^+$ (calculated for $C_{18}H_{17}O_6$ 329.0025), 313.0830 (7), 296.0818 (14), 268.0812 (100), 240.0878 (12).

Cirsimaritin (9):

Pale yellow powder; UV (MeOH) λ_{max} (ε): 333 (25000), 276 (18000); ^{1}H NMR ($CDCl_3$): δ 6.58 (1H, s, H-3), 6.60 (1H, s, H-8), 7.80 (2H, brd, J = 9.1 Hz, H-2` and H-6`), 6.95 (2H, brd, J = 8.7 Hz, H-3`and H-5`), 3.92 (3H, s, 6-OMe), 3.98 (3H, s, 7-OMe); ^{13}C NMR ($CDCl_3$): δ 164.8 (s, C-2), 103.1 (d, C-3), 182.7 (s, C-4), 153.2 (s, C-5), 132.0 (s, C-6), 158.6 (s, C-7), 90.7 (d, C-8), 152.3 (s, C-9), 105.7 (s, C-10), 121.9 (s, C-1`), 128.1 (d, C-2`), 115.9 (d, C-3`), 160.8 (s, C-4`), 115.9 (d, C-5`), 128.1 (d, C-6`) 60.6 (q, 6-OMe), 56.1 (q, 7-OMe); $C_{17}H_{14}O_6$; ESIMS (positive ion mode) *m/z* (rel. int.): 315.0775 (0.7) $[M+H]^+$ (calculated for $C_{17}H_{15}O_6$ 315.0868), 299.0745 (6), 282.0657 (16), 254.0654 (100), 226.0738 (16), 136.0176 (28).

5,7 Dihydorxy-3`,4`dimethoxyflavone (10):

Pale yellow powder; UV (MeOH) λ_{max} (ε): 339 (19000), 269 (15000), 242 (17000); ^{1}H NMR ($CDCl_3$): δ 6.58 (1H, s, H-3), 6.29 (1H, d, J = 1.8 Hz, H-6), 6.47 (1H, d, J = 1.8 Hz, H-8), 7.38 (1H, d, J = 1.8 Hz, H-2`), 7.01 (1H, d, $J$ = 8.7 Hz, H-5`), 7.56 (1H, dd, J = 1.8 and 7.8 Hz, H-6`), 3.97 (3H, s, 3`-OMe), 3.99 (3H, s, 4`-OMe); $^{13}$C NMR ($CDCl_3$): δ 164.1 (s, C-2), 103.9 (d, C-3), 182.3 (s, C-4), 161.4 (s, C-5), 99.5 (d, C-6), 164.1 (s, C-7), 94.1 (d, C-8), 157.0 (s, C-9), 103.8 (s, C-10), 123.5 (s, C-1`), 111.0 (d, C-2`), 144.5 (d, C-3`), 148.9 (s, C-4`), 113.5 (d, C-5`), 121.7 (d, C-6`) 55.7 (q, 3`-OMe), 55.8 (q, 4`-OMe); $C_{17}H_{14}O_6$; ESIMS (positive ion mode) *m/z* (rel. int.): 315.0967 (4) $[M+H]^+$ (calculated for $C_{17}H_{15}O_6$ 315.0868), 299.0731 (100), 271.0757 (26), 254.0682 (18), 242.0744 (12).

Santoflavone (11):

Pale yellow powder; UV (MeOH) λ_{max} (ε): 339 (25000), 276 (18000), 242 (20000); ^{1}H NMR ($CDCl_3$): δ 6.53 (1H, s, H-3), 6.58 (1H, s, H-8), 7.32 (1H, d, J = 3.0 Hz, H-2`), 6.95 (1H, d, $J$ = 10.5 Hz, H-5`), 7.50 (1H, dd, J = 3 and10.5 Hz, H-6`), 3.95 (3H, s, 6-OMe), 3.91 (3H, s, 7-OMe), 3.97 (3H, s, 3`-OMe), 3.97 (3H, s, 4`-OMe); $^{13}$C NMR ($CDCl_3$): δ 160.0 (s, C-2), 104.5 (d, C-3), 182.6 (s, C-4), 153.1 (s, C-5), 132.7 (s, C-6), 158.8 (s, C-7), 90.6 (d, C-8), 153.2 (s, C-9), 106.2 (s, C-10), 123.8 (s, C-1`), 108.8 (d, C-2`), 149.3 (s, C-3`), 152.3 (s, C-4`), 111.2 (d, C-5`), 120.1 (d, C-6`) 60.9 (q, 6-OMe), 56.3 (q, 7-OMe), 56.1 (q, 3`-OMe), 56.1 (q, 4`-OMe); $C_{19}H_{18}O_7$; ESIMS (positive ion mode) *m/z* (rel. int.): 359.1031 (2) $[M+H]^+$ (calculated for $C_{19}H_{19}O_7$ 359.1130), 343.0916 (24), 326.0906 (21), 298.0912 (100), 162.0741 (20).

Eupatorin (12):

Pale yellow powder; UV (MeOH) λ_{max} (ε): 340 (15000), 275 (11000), 242 (12000); ^{1}H NMR ($CDCl_3$): δ 6.61 (1H, s, H-3), 6.59 (1H, s, H-8), 7.42 (1H, d, J = 1.8 Hz, H-2`), 6.98 (1H, d, $J$ = 8.2 Hz, H-5`), 7.47 (1H, dd, J = 1.8 and 8.7 Hz, H-6`), 3.91 (3H, s, 6-OMe), 3.98 (3H, s, 7-OMe), 3.99 (3H, s, 4`-OMe); ^{13}C NMR ($CDCl_3$): δ 164.8 (s, C-2), 104.0 (d, C-3), 183.1 (s, C-4), 153.6 (s, C-5),

130.5 (s, C-6), 159.1 (s, C-7), 91.1 (d, C-8), 152.6 (s, C-9), 111.3 (s, C-10), 124.0 (s, C-1`), 106.1 (d, C-2`), 146.7 (s, C-3`), 151.1 (s, C-4`), 112.9 (d, C-5`), 119.1 (d, C-6`) 56.1 (q, 6-OMe), 56.5 (q, 7-OMe), 61.0 (q, 4`-OMe); $C_{18}H_{16}O_7$; ESIMS (positive ion mode) *m/z* (rel. int.): 345.0846 (2) $[M+H]^+$ (calculated for $C_{18}H_{17}O_7$ 345.0974), 330.0841 (14), 312.0769 (21), 284.0774 (100), 269.0582 (30),148.0554 (15).

6. Extraction and isolation of alkaloids (13-15) from *Adhatoda vasica*

Air-dried and powdered leaves of *A. vasica* (2.8 kg) were extracted with ethanol (11 l, 99%) under reflux for one hour. The solvent was evaporated under reduced pressure to yield 172 g of a crude dark extract. The extract was dissolved in 1 l HCl (3.6%) and extracted with chloroform (1 l × 4). The aquatic layer was made alkaline with concentrated NH_4OH (pH = 9~10) and extracted with chloroform (1 l × 6). The combined chloroform layers were dried under vacuum to give 31.1 g of crude alkaloidal extract. The alkaloidal fraction (15.3 g) was fractioned by silica gel column (300 g) with mixtures of chloroform and methanol. Thirty- four fractions of 200 ml were collected and combined into six main fractions based on their TLC profiles. The second fraction (frs 6 and 7, 0.9 g) was subjected to several purification steps starting with column chromatography on silica gel eluted with 1% MeOH/CH_3Cl, then with column chromatography on Sephadex LH-20 eluted with MeOH, then with preparative thin layer chromatography (PTLC) developed with 5% acetone/chloroform and finally with HPLC using 35% H_2O/MeOH to give 13.6 mg of compound **13**. Similar purification for the second fraction (frs 13-15, 0.9 g) on Sephadex LH-20, PTLC and HPLC afforded 5 mg of compound **14**. The third fraction (frs 16-32, 9 g) was purified on Sephadex LH-20 column eluted with MeOH followed by recrystallization in MeOH to give 5g of compound **15**.

Vasicolinone (13)

White amorphous powder; UV (MeOH) λ_{max} (ε): 297 (4000), 259 (9000), 220 (30000); ^1H NMR ($CDCl_3$): δ 4.15 (1H, dt, *J* = 8.3 and 14.9 Hz, H-1α), 4.42 (1H, ddd, *J* = 4.1, 9.1 and 31.3 Hz, H-1β), 2.73 (1H, m, H-2α), 2.24 (1H, ddd, *J* = 8.7, 13.8 and 16.9 Hz, H-2β), 5.04 (1H, t, *J* = 9.1 Hz, H-3), 7.61 (1H, dd, *J* = 1.4 and 8.2 Hz, H-5), 7.69 (1H, td, *J* = 1.4 and 6.9 Hz, H-6), 7.41 (1H, td, *J* = 1.4 and 8.2 Hz, H-7), 8.31 (1H, td, *J* = 1.4 and 8.3 Hz, H-8), 7.09~ 7.16 (2H, m, H-12 and H-14), 7.26~7.33 (2H, m, H-13 and H-15), 2.62 (6H, s, $N(CH_3)_2$); ^{13}C NMR ($CDCl_3$): δ 45.1 (t, C-1), 29.8 (t, C-2), 45.6 (d, C-3), 161.8 (s, C-3a), 149.5 (s, C-4a), 127.2 (d, C-5), 133.9 (d, C-6), 126.0 (d, C-7), 126.2 (d, C-8), 120.5 (s, C-8a), 161.3 (s, C-9), 136.7 (s, C-10), 153.2 (s, C-11), 121.6 (d, C-12), 129.1 (d, C-13), 125.0 (d, C-14), 128.4 (d, C-15), 46.1 (2C, q, $N(CH_3)_2$); $C_{19}H_{20}N_3O$; ESIMS (positive ion mode) *m/z* (rel. int.): 307.1761 (19) $[M+H]^+$ (calculated for $C_{19}H_{21}N_3O$ 307.1686), 274.1534 (9), 262.1519 (10), 186.1088 (11), 121.1095 (100).

Anisotine (14):

White amorphous powder; UV (MeOH) λ_{max} (ε): 327 (6000), 311 (6000), 297 (7000), 248 (18000), 213 (46000); ^1H NMR ($CDCl_3$): δ 4.14 (1H, m, H-1α), 4.41 (1H, m, H-1β), 2.72 (1H, m, H-2α), 2.29 (1H, m, H-2β), 3.38 (1H, m, H-3), 7.72 (1H, m, H-5), 7.72 (1H, m, H-6), 7.45 (1H, td, *J* = 1.4 and 7.8 Hz, H-7), 8.31 (1H, d, *J* = 8.2 Hz, H-8), 7.83 (1H, d, *J* = 2.2 Hz, H-11), 6.68 (1H, d, *J* = 8.7 Hz, H-14), 7.30 (1H, dd, *J* = 2.3 and 8.7 Hz, H-15), 3.82 (3H, s, OMe-17), 2.91 (3H, d, *J* = 5.1 Hz, NCH_3-18); ^{13}C NMR ($CDCl_3$): δ 44.7 (t, C-1), 29.9 (t, C-2), 49.1 (d, C-3), 124.7 (s, C-3a), 149.3 (s, C-4a), 127.4 (d, C-5), 134.0 (d, C-6), 126.3 (d, C-7), 126.3 (d, C-8), 120.5 (s, C-8a), 161.1 (s, C-9), 109.9 (s, C-10), 130.8 (d, C-11), 149.3 (s, C-12), 151.3 (s, C-13), 111.5 (d, C-14), 134.2 (d, C-15), 168.8 (s, C-16), 51.4 (q, C-17) 29.6 (q, C-18); $C_{20}H_{19}N_3O_3$; ESIMS (positive ion mode) *m/z* (rel. int.): 350.1640 (11) $[M+H]^+$ (calculated for $C_{19}H_{20}N_3O_3$ 350.1506), 318.1392 (100), 290.1603 (40), 234.1078 (12), 185.0903 (53).

Vasicine (15):

White amorphous powder; UV (MeOH) λ_{max} (ε): 285 (7000), 248 (7000), 216 (20000); ^1H NMR ($CDCl_3$): δ 3.20 (1H, dt, *J* = 6.9 and 9.1 Hz, H-1α), 3.39 (1H, m, H-1β), 2.35 (1H, m, H-2α), 2.09 (1H, td, *J* = 6.0 and 12.9 Hz, H-2β), 4.75 (1H, dd, *J* = 6.4 and 7.8 Hz, H-3), 7.14 (2H, m, H-5 and H-6), 6.96 (1H, td, *J* = 2.7 and 7.3 Hz, H-7), 6.85 (1H, d, *J* = 7.8 Hz, H-8), 4.56 (2H, s, H-9a and b); ^{13}C NMR ($CDCl_3$): δ 48.3 (t, C-1), 28.9 (t, C-2), 70.0 (d, C-3), 164.0 (s, C-3a), 142.4 (s, C-4a), 123.7 (d, C-5), 128.4 (d, C-6), 124.1 (d, C-7), 125.8 (d, C-8), 119.0 (s, C-8a), 47.1 (t, C-9); $C_{11}H_{12}N_2O$; ESIMS (positive ion mode) *m/z* (rel. int.): 189.1112 (18) $[M+H]^+$ (calculated for $C_{11}H_{13}N_2O$ 189.1028), 171.1154 (65), 169.1028 (31), 154.0914 (22), 144.1028 (23), 118.0828 (100).

7. Bioassay against the fourth instar larvae of *Culex pipiens*

The toxicity of the extracts and isolated compounds was tested against the fourth instar larvae of *C. pipiens* by the method recommended by the World Health Organization (WHO, 1981) with some modification. Stock solutions of the tested extracts and/or compounds were prepared in acetone. In preliminary trail, the tested extracts and compounds were evaluated at 500 mg/l. The extracts and compounds caused mortality higher than 50% were further tested in a series of at least eight concentrations (from 1 to 500 mg/l). Groups of 20 mosquito larvae were separately put into 200-ml glass cups containing 100 ml of distilled water. The

tested extract and/or compound solutions in acetone were added to each cup to give the desired concentration. The control was prepared with distilled water containing the maximum amount (1 ml) of acetone in the test samples. Three replicates for each concentration and control treatment were carried out. Malathion (95%, Kafr Elzayat Pesticides and Chemicals Co, Egypt) was used as a reference insecticide. Larval mortalities were recorded after 24 h of treatment.

8. Bioassay against the land snails, *Theba pisana* and *Eobania vermiculata*

The activity of isolated extracts and compounds was evaluated on adult snails of *T. pisana* and *E. vermiculata* as described by Hussein *et al.*, 1994 and El-Zemity and Radwan, 1999 with some modifications. The isolated extracts and compounds were prepared in acetone and tested at one dose of 0.5 mg/snail against *T. pisana* and 1.0 mg/snail against *E. vermiculata*. These doses contained in 20µl of acetone solution in the case of *T. pisana* and 40µl in the case of *E. vermiculata* were gently applied on the surface of the snail body inside the shell using a micropipette. Three replicates (ten snails in each one) of each concentration were used. Control snails were treated with the same volumes of acetone. The treated snails were placed in 0.3 L glass jars. The jars were covered with cheese cloth fastened by rubber bands to prevent escape of snails and to ensure proper ventilation. The snails were fed on fresh lettuce leaves. Methomyl (98%, Wako, Japan) was used at same concentrations as reference molluscicide for land snails. The mortality percentages were recorded after 24 and 48 hours of treatment.

9. Barnyard grass seed germination and seedling growth bioassay

The isolated extracts and compounds were tested for their inhibitory effects on barnyard grass germination and subsequent seedling growth. Stock solutions of the test extracts and compounds were prepared in acetone. Then, three concentrations (100, 250 and 500 mg/l) of each extract and compound were prepared by dilution with distilled water. The concentration of acetone in the final test solution was 1% v/v. Control treatments were an aqueous solution of acetone (1% v/v). Three replicates, each of 10 seeds, were prepared for each treatment using glass Petri dishes (9 cm diam.) lined with Whatman No. 2 filter paper. Six milliliters of each test solution were added to each petri dish. The petri dishes were placed in the bottom of 0.1-mm-thick polyethylene bags (15 × 30 cm) that were expanded to contain air and then closed at the top with rubber bands to prevent the loss of moisture and placed in a germination cabinet at 26±2 °C with a 12-h photoperiod. Germination percentages and lengths of root and shoot were determined 5 d after sowing. The percentages of growth inhibition of root and shoot lengths were calculated from the following equation: $I(\%) = [1 - T/C] \times 100$; T is the length of treatment (cm) and C is the length of control (cm).

10. Statistical analysis

Mortality of the larvae of *C. pipiens* was calculated after 24 h of treatment as a mean of three replicates. The mortality data were subjected to Probit analysis (Finney, 1971) to obtain the LC_{50} values, using SPSS 12.0 (SPSS, Chicago, IL, USA). The LC_{50} values were considered significantly different, if the 95% confidence limits did not overlap. Mortality percentages of *C. pipiens* larvae and *T. pisana* and *E. vermiculata* adults, and germination percentages, root lengths and shoot lengths of *E. crusgalli* were subjected to one-way analysis of variance followed by Student–Newman–Keuls test (Cohort software Inc. 1985) to determine significant differences among mean values at the probability level of 0.05.

RESULTS AND DISCUSSION

1. Isolation and structure elucidation of sesquiterpenes, flavonoids and alkaloids

Repetitive column chromatography of chloroform fraction of *A. maritima* chloroform extract gave five pseudoguaianolide sesquiterpenes, namely neoambrosin (**1**), damsinic acid (**2**), damsin (**3**) ambrosin (**4**) and hymenin (**5**). The combination use of silica gel and shephadex columns with HPLC of water/methanol fraction of *A. santolina* chloroform extract led to isolate a guaianolide sesquiterpene lactone, leucodin (**6**) and 6 flavonoids (artemetin (**7**), salvigenin (**8**), cirsimaritin (**9**), 5,7 dihydorxy-3`,4`dimethoxyflavone(**10**), santoflavone (**11**) and eupatorin (**12**)). The alkaloidal fraction of *A. vasica* ethanol extract was subjected to silica gel and Sephadex columns, followed by HPLC to yield three quinazoline alkaloids; vasicolinone (**13**), anisotine (**14**), vasicine (**15**). The chemical structures (Figure 1) of these compounds were determined based on their spectral data of ^{1}H NMR, ^{13}C NMR, MS and UV. The obtained spectral data of the isolated compounds were identical with those reported in the literature (Mesquita *et al.*, 1986; Iskander *et al.*, 1988; Ali *et al.*, 1989; Joshi *et al.*, 1994; Nagaya *et al.*, 1994; Grayer *et al.*, 1996; Balboul *et al.*, 1997; Gu and weng, 2001; Yoshioka *et al.*, 2004).

2. Insecticidal activity of extracts and isolated compounds against *Culex pipiens*

The results of the preliminary test on the toxicity of the extracts and compounds against the fourth instar larvae of *C. pipiens* were shown in Table 1. In this assay, the extracts and compounds were evaluated at one concentration of 500 mg/l. Among the tested extracts *A. vasica* alkaloidal fraction was the most effective with complete mortality of *C. pipiens* larvae followed by *A. santolina* extract which caused 43.3% mortality.

Two sesquiterpenes (neoambrosin (**1**) and damsin (**3**), and two flavonoids (santoflavone (**11**) and eupatorin (**12**)) and the alkaloid vasicine (**15**) caused complete mortality of the larvae at this concentration. The lethal concentration values (LC_{50}) as well as other statistical parameters generated by linear regression analysis for the extracts and compounds that cuased mortality higher than 50% are presented in Table 2. Eupatorin (**12**) revealed the strongest toxicity with LC_{50} of 5.61 mg/l. The alkaloid vasicine (**15**) showed good insecticidal activity since LC_{50} value was 88.88 mg/l. Among the tested sesquiterpenes, damsin (**3**) had the highest activity, followed by neoambrosin (**1**) while hymenin (**5**) was the less effective one. The tested extracts and compounds exhibited variable degrees of toxicities. In general the pure compounds were more toxic than the crude extracts. This variable toxicity was also appeared among each group of compounds. For example, sesquiterpenes **1** and **3** had a good toxicity while **6** had very week toxicity. Similarly, flavonoid **12** was highly toxic comparing with **11**.

Comparing the toxicity of the tested sesquiterpenes (**1**, **3** and **5**) against the larvae of *C. pipiens* with those of sesquiterpene lactones costunolide and parthenolide, isolated from *Magnolia grandiflora* L., revealed that costunolide was more toxic than the tested sesquiterpenes but parthenolide had a comparable toxicity (Abdelgaleil, 2005). No studies have been reported on insecticidal activity of the tested extracts and compounds against the larvae of *C. pipiens*. However, the larvicidal activity of some plant extracts, essential oils and phytochemicals against *C. pipiens* have been demonstrated (Ju *et al.*, 1998; Al-Doghairi *et at.*, 2004; Cao *et al.*, 2004; Traboulsi *et al.*, 2005; Abdelgaleil 2006; Michaelakis *et al.*, 2007; Radwan *et al.*, 2008).

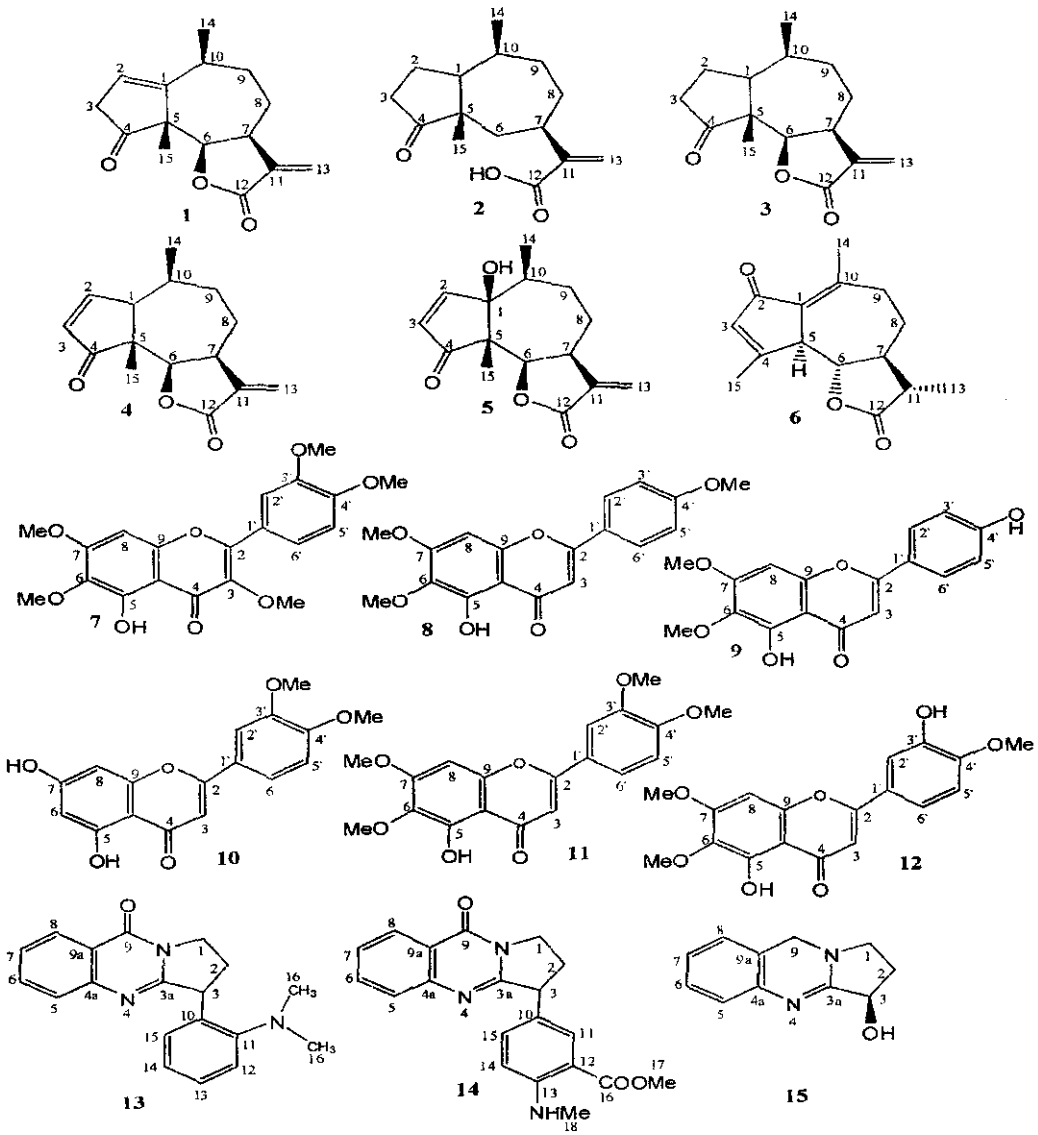

Fig. 1: Chemical structure of compounds isolated from *Ambrosia maritima* (1-5), *Achillea santolina* (6-12) and *Adhatoda vasica* (13-15).

Table 1: Mortality percentages of the fourth instar larvae of *Culex pipiens* after 24 hours of treatment with extracts and compounds isolated from *Ambrosia maritima*, *Achillea santolina* and *Adhatoda vasica* at concentration of 500 mg/l[a]

Extract/Compound	Mortality (%) ± SE[b]	Extract/Compound	Mortality (%) ± SE
A. maritima extract	20.0 ± 2.89 f	Ambrosin (**4**)	86.7 ±1.67 b
A. maritima chloroform fraction	26.7 ± 3.34 e	Hymenin (**5**)	60.0 ± 0.0 c
A. santolina extract	43.3 ± 3.34 d	Leucoden (**6**)	40.0 ± 0.0 d
A. santolina H_2O/MeOH fraction	30.0 ± 2.89 e	Santoflavone (**11**)	100.0 ± 0.0 a
A. vasica extract	13.3 ± 1.67 g	Eupatorin (**12**)	100.0 ± 0.0 a
A. vasica alkaloidal fraction	100.0 ± 0.0 a	Vasicine (**15**)	100.0 ± 0.0 a
Neoambrosin (**1**)	100.0 ± 0.0 a	Malathion	100.0 ± 0.0 a
Damsinic acid (**2**)	20.0 ± 0.0 f	Control	0.0 ± 0.0 h
Damsin (**3**)	100.0 ± 0.0 a		

[a] Data are expressed as means ± SE from experiments with three replicates of 20 larvae each.
[b] Means sharing the same letter are not significantly different at the 0.05 probability level.

Table 2: Comparative toxicity of extracts and compounds isolated from *Ambrosia maritima*, *Achillea santolina* and *Adhatoda vasica* against the fourth instar larvae of *Culex pipiens* after 24 hours of treatment

Extract/Compound	LC_{50}[a] (mg/l)	95% Confidence limits		Slope ± S.E.[b]	Intercept ± S.E.[c]	$(\chi^2)^d$
		Lower	Upper			
A. vasica alkaloidal fraction	190.79	70.0	314.52	4.04 ± 0.35	- 9.21 ± 0.83	9.44
Neoambrosin (**1**)	126.27	118.35	134.80	8.34 ± 0.82	-17.52 ± 1.71	0.10
Damsin (**3**)	104.87	93.23	116.98	3.01 ± 0.26	- 6.09 ± 0.55	3.74
Ambrosin (**4**)	162.82	146.65	180.11	2.93 ± 0.23	- 6.47 ± 0.51	4.90
Hymenin (**5**)	427.25	388.25	491.05	3.49 ± 0.82	- 9.19 ± 2.13	0.64
Santoflavone (**11**)	126.59	90.63	204.13	0.92 ± 0.28	- 1.93 ± 0.58	0.73
Eupatorin (**12**)	5.61	5.04	6.12	5.24 ± 0.65	-3.93 ± 0.54	0.24
Vasicine (**15**)	88.88	53.44	151.82	3.27 ± 0.24	- 6.39 ± 0.47	19.27
Malathion	0.38	0.16	0.51	3.55 ± 0.35	1.48 ± 0.11	13.12

[a] The concentration causing 50% mortality.
[b] Slope of the concentration-mortality regression line.
[c] Intercept of the regression line.
[d] Chi square.

3. . Molluscicidal activity of extracts and isolated compounds against terrestrial snails *T. pisana* and *E. vermiculata*

Table 3 presents mortality percentages of *T. pisana* after 24 and 48 h of treatment with the tested extracts and compounds at dose of 0.5 mg/snail. The results showed that neoambrosin (**1**), damsin (**3**) and ambrosin (**4**) were the most toxic compounds without significant differences between them. They caused mortality percentages of 86.7, 80.0 and 80.0 after 24 h, respectively. The three compounds were more toxic to the snail than methomyl, a reference moluscicide, after 24 h of exposure. *Achillea santolina* H_2O/MeOH fraction had the highest toxic effect among the tested extracts. With increasing the time of exposure, the toxicity of all of the tested extracts and compounds to the snail increased with exception of neoambrosin (**1**) and santoflavone (**11**). The later compound was not toxic to the snail at the dose of 0.5 mg/snail after two exposure times. Similarly, the sesquiterpenes neoambrosin (**1**), damsin (**3**) and ambrosin (**4**) showed the highest mortality percentages after 48 h of exposure; however they were less toxic than methomyl.

On the other hand, damsin (**3**) and ambrosin (**4**) caused the highest mortality percentages (86.7) for *E. vermiculata* after 24 h at dose of 1.0 mg/snail (Table 4). The toxicity of the two compounds was similar to methomyl at this time of exposure. After 48 h, damsin (**3**) was most toxic compound (mortality = 93.3%). Moreover, ambrosin (**4**) had a strong toxicity compound (mortality = 86.7%). All of the tested compounds and extracts were more toxic to the snail after 48 h than 24 h. Molluscicidal activity of sesquiterpenes neoambrosin (**1**), damsin (**3**) and ambrosin (**4**) against both snails was more potent than sesquiterpene lactones of costunolide and parthenolide (Abdelgaleil, 2005). In addition, these compounds (**1**, **3** and **4**) were more toxic than monoterpenoids (α-terpineol, pulegone, anisole, thymol and eugenol) against *T. pisana* (El-Zemity, 2001). However, they were less active than monoterpenoids ((-)-camphor, (-)-carvone, 1-8-cineole, cuminaldehyde, (L)-fenchone, geraniol, (-)-limonene, (-)-linalool, (-)-menthol) evaluated against this snail (Abdelgaleil, 2010).

Table 3: Mortality percentages of *Theba pisana* adult snails after 24 and 48 hours of treatment with extracts and compounds isolated from *Ambrosia maritima*, *Achillea santolina* and *Adhatoda vasica* at dose of 0.5 mg/snail[a]

Extract/Compound	Mortality (%)[b]	
	24 h	48 h
A. maritima extract	33.3 ± 6.7 bcde	40.0 ± 0.0 d
A. maritima chloroform fraction	46.7 ± 6.7 bc	80.0 ± 5.8 b
A. santolina extract	33.3 ± 6.7 bcde	60.0 ± 0.0 c
A. santolina H_2O/MeOH fraction	53.3 ± 6.7 b	73.3 ± 6.7 bc
A. vasica extract	6.7 ± 3.3 fg	40.0 ± 0.0 d
A. vasica alkaloidal fraction	40.0 ± 0.0 bcd	73.3 ± 6.7 bc
Neoambrosin (**1**)	86.7 ± 6.7 a	86.7 ± 6.7 ab
Damsinic acid (**2**)	13.3 ± 3.3 efg	33.3 ± 6.7 d
Damsin (**3**)	80.0 ± 0.0 a	86.7 ± 6.7 ab
Ambrosin (**4**)	80.0 ± 5.8 a	86.7 ± 6.7 ab
Hymenin (**5**)	40.0 ± 5.8 bcd	60 ± 0.0 c
Leucoden (**6**)	20.0 ± 5.8 defg	26.7 ± 3.3 d
Artemetin (**7**)	26.7 ± 6.7 cdef	40.0 ± 0.0 d
Santoflavone (**11**)	0.0 ± 0.0 g	0.0 ± 0.0 e
Eupatorin (**12**)	13.3 ± 6.7 efg	26.7 ± 6.7 d
Vasicine (**15**)	20.0 ± 5.8 defg	33.3 ± 6.7 d
Methomyl	53.3 ± 6.7 b	100.0 ± 0.0 a
Control	0.0 ± 0.0 g	0.0 ± 0.0 e

[a] Data are expressed as means ± SE from experiments with three replicates of 10 snails each.
[b] Means within a column sharing the same letter are not significantly different at the 0.05 probability level.

Table 4: Mortality percentages of *Eobania vermiculata* adult snails after 24 and 48 hours of treatment with extracts and compounds isolated from *Ambrosia maritima*, *Achillea santolina* and *Adhatoda vasica* at dose of 1.0 mg/snail[a]

Extract/Compound	Mortality (%)[b]	
	24 h	48 h
A. maritima extract	20.0 ± 0.0 de	33.3 ± 6.7 cde
A. maritima chloroform fraction	20.0 ± 5.8 de	26.7 ± 3.3 cdef
A. santolina extract	23.3 ± 3.3 d	43.3 ± 6.7 bc
A. santolina H_2O/MeOH fraction	26.7 ± 6.7 d	26.7 ± 6.7 cdef
A. vasica extract	0.0 ± 0.0 e	6.7 ± 3.3 f
A. vasica alkaloidal fraction	6.7 ± 3.3 de	40.0 ± 5.8 bcd
Neoambrosin (**1**)	70.0 ± 5.8 b	83.3 ± 3.3 a
Damsinic acid (**2**)	6.6 ± 3.3 de	20.0 ± 0.0 def
Damsin (**3**)	86.7 ± 3.3 a	93.3 ± 6.7 a
Ambrosin (**4**)	86.7 ± 3.3 a	86.7 ± 3.3 a
Hymenin (**5**)	56.7 ± 3.3 c	56.7 ± 3.3 b
Leucoden (**6**)	13.3 ± 6.7 de	13.3 ± 6.7 ef
Artemetin (**7**)	6.7 ± 3.3 de	13.3 ± 6.7 ef
Santoflavone (**11**)	6.7 ± 3.3 de	13.3 ± 6.7 ef
Eupatorin (**12**)	6.7 ± 3.3 de	13.3 ± 6.7 ef
Vasicine (**15**)	6.7 ± 3.3 de	23.3 ± 6.7 cdef
Methomyl	86.7 ± 3.3 a	100.0 ± 0.0 a
Control	0.0 ± 0.0 e	6.7 ± 3.3 f

[a] Data are expressed as means ± SE from experiments with three replicates of 10 snails each.
[b] Means within a column sharing the same letter are not significantly different at the 0.05 probability level.

4. Effect of extracts and isolated compounds on seed germination of barnyard grass

The results of the inhibitory effect of six extracts and ten compounds on seed germination of barnyard grass at three concentrations of 100, 250 and 500 mg/l are shown in Table 5. Despite the decreasing in germination percentages caused by the majority of tested extracts and compounds, there was no significant inhibition in germination compared with control at 100 mg/l. Neoambrosin (**1**) was the only compound which exhibited significant reduction in seed germination at 250 mg/l. It caused a strong inhibition of germination with seed germination percentage of 13.3. Three sesquiterpenes, neoambrosin (**1**), damsin (**3**) and ambrosin (**4**), inhibited the seed germination completely (germination percentage = 0) at 500 mg/l. Moreover, damsinic acid (**2**) exhibited significant inhibition in seed germination at this concentration. Out of 16 extracts and compounds 4 sesquiterpenes, neoambrosin (**1**), damsinic acid (**2**), damsin (**3**) and ambrosin (**4**), were promising seed germination inhibitors against barnyard grass. Both tested flavonoids and alkaloids had no significant inhibition of seed germination. In our previous studies, sesquiterpene lactones costunolide and parthenolide reduced the seed germination of wheat, lettuce radish, onion and wild oat (Abdelgaleil and Hashinaga, 2007; Abdelgaleil *et al.,* 2009). It has been also reported that artemisinin and its sesquiterpene analogues decreased the seed germination of lettuce, rye, and arabidopsis (Dayan *et al.,* 1999). Sesquiterpene lactones tagitinin A and B isolated from Mexican sunflower [*Tithonia diversifolia* (Hemsley) A. Gray] also inhibited seed germination of radish, cucumber, and onion (Baruah *et al.,* 1994). Batish *et al.* (2002) found that a sesquiterpene lactone parthenin caused seed germination reduction of wild oat.

5. Effect of extracts and isolated compounds on root and shoot growth of barnyard grass

The inhibitory effect of the tested extracts and isolated compounds on root growth of barnyard grass treated with concentrations of 100, 250 and 500 mg/l is shown in Table 6. For the majority of tested compounds and extracts, the percentage of inhibition increased with the extract and/or compound concentration. At concentration of 100 mg/l, ambrosin (**4**) exhibited the highest inhibitory effect as it caused 93.5% inhibition of root growth. Among the tested extracts, *A. santolina* H_2O/MeOH fraction was the most effective one with inhibition percentage of 51.6. All of the tested extracts and compounds except for *A. vasica* extract, *A. vasica* alkaloidal fraction, artemetin (7) and vasicine (**15**) significantly inhibited the growth of roots at concentration of 250 mg/l. Neoambrosin (**1**) and ambrosin (**4**) caused complete inhibition of root growth at this concentration. Similarly, damsin (**3**) showed a strong inhibitory effect as it inhibited the root growth by 98.6%. *Ambrosia maritima* chloroform fraction was the most effective extract while *A. vasica* extract was the less effective one. At concentration of 500 mg/l, all of the tested extracts and compounds significantly reduced the root growth except *A. vasica* extract. Complete inhibition of root growth was observed when seed treated with neoambrosin (**1**), damsin (**3**) and ambrosin (**4**). It is noteworthy the extracts and compounds of *A. maritima* had more inhibitory effect than the extracts and compounds of *A. vasica* and *A. santolina.*

Table 5: Effect of extracts and compounds isolated from *Ambrosia maritima*, *Achillea santolina* and *Adhatoda vasica* on barnyard grass, *Echinochloa crusgalli*, germination 5 d after sowing[a]

Extract/Compound	Germination (%) ± SE[b]		
	100 mg/l	250 mg/l	500 mg/l
Control	73.3 ± 3.3 a	73.3 ± 3.3 a	73.3 ± 3.3 ab
A. maritima extract	63.3 ± 3.3 a	63.3 ± 3.3 a	73.3 ± 3.3 ab
A. maritima chloroform fraction	76.7 ± 6.7 a	66.7 ± 6.7 a	56.7 ± 6.7 abc
A. santolina extract	63.3 ± 6.7 a	66.7 ± 3.3 a	63.3 ± 3.3 abc
A. santolina H_2O/MeOH fraction	56.7 ± 6.7 a	70.0 ± 5.7 a	53.3 ± 3.3 bc
A. vasica extract	70.0 ± 5.8 a	60.0 ± 5.7 a	70.0 ± 5.6 ab
A. vasica alkaloidal fraction	60.0 ± 5.8 a	70.0 ± 5.7 a	66.7 ± 3.3 ab
Neoambrosin (**1**)	63.3 ± 3.3 a	13.3 ± 6.7 b	0.0 ± 0.0 d
Damsinic acid (**2**)	56.7 ± 6.7 a	66.7 ± 3.3 a	43.3 ± 6.7 c
Damsin (**3**)	63.3 ± 3.3 a	60.3 ± 5.7 a	0.0 ± 0.0 d
Ambrosin (**4**)	63.3 ± 3.3 a	53.3 ± 6.7 a	0.0 ± 0.0 d
Hymenin (**5**)	63.3 ± 3.3 a	60.0 ± 5.7 a	63.3 ± 3.3 abc
Leucoden (**6**)	56.7 ± 3.3 a	63.3 ± 6.7 a	66.7 ± 6.7 ab
Artemetin (**7**)	80.0 ± 5.8 a	76.7 ± 6.7 a	56.7 ± 6.7 abc
Santoflavone (**11**)	66.7 ± 6.7 a	63.3 ± 3.3 a	70.0 ± 5.7 ab
Eupatorin (**12**)	73.3 ± 3.3 a	63.3 ± 3.3 a	80.0 ± 5.7 a
Vasicine (**15**)	70.0 ± 5.8 a	70.0 ± 5.7 a	70.0 ± 5.7 ab

[a] Data are expressed as means ± SE from experiments with three replicates.
[b] Means within a column sharing the same letter are not significantly different at the 0.05 probability level.

Table 6: Effect of extracts and compounds isolated from *Ambrosia maritima*, *Achillea santolina* and *Adhatoda vasica* on barnyard grass, *Echinochloa crusgalli*, root growth[a]

Extract/Compound	100 mg/l		250 mg/l		500 mg/l	
	Root length (cm)[b]	I (%)[c]	Root length (cm)	I (%)	Root length (cm)	I (%)
Control	3.1 ± 0.16 b	0.0	3.1 ± 0.16 a	0.0	3.1 ± 0.16 a	0.0
A. maritima extract	2.9 ± 0.06 b	6.5	1.9 ± 0.31 bcd	38.7	0.9 ± 0.20 e	71.0
A. maritima chloroform fraction	3.0 ± 0.09 b	3.2	0.9 ± 0.13 e	71.0	0.4 ± 0.03 f	87.1
A. santolina extract	2.7 ± 0.29 b	12.9	1.9 ± 0.18 bcd	38.7	1.5 ± 0.15 cd	51.6
A. santolina H_2O/MeOH fraction	1.5 ± 0.12 d	51.6	1.7 ± 0.29 bcde	45.2	0.9 ± 0.15 e	71.0
A. vasica extract	2.4 ± 0.10 bc	22.6	3.0 ± 0.33 a	3.2	3.4 ± 0.09 a	- 9.7
A. vasica alkaloidal fraction	4.0 ± 0.29 a	- 29.0	2.5 ± 0.15 ab	19.4	2.2 ± 0.20 b	29.0
Neoambrosin (**1**)	0.7 ± 0.13 e	77.4	0.0 ± 0.0 f	100.0	0.0 ± 0.0 f	100.0
Damsinic acid (**2**)	1.8 ± 0.03 cd	41.9	1.0 ± 0.23 e	67.7	0.2 ± 0.13 f	93.5
Damsin (**3**)	0.5 ± 0.07 e	83.9	0.1 ± 0.06 f	96.8	0.0 ± 0.0 f	100.0
Ambrosin (**4**)	0.2 ± 0.03 e	93.5	0.0 ± 0.0 f	100.0	0.0 ± 0.0 f	100.0
Hymenin (**5**)	2.2 ± 0.20 bcd	29.0	1.1 ± 0.15 de	64.5	0.2 ± 0.07 f	93.5
Leucoden (**6**)	2.3 ± 0.28 bc	25.8	1.4 ± 0.18 cde	54.8	1.4 ± 0.14 cd	54.8
Artemetin (**7**)	2.7 ± 0.35 b	12.9	2.4 ± 0.15 ab	22.6	2.3 ± 0.23 b	25.8
Santoflavone (**11**)	2.2 ± 0.23 bcd	29.0	2.0 ± 0.23 bc	35.5	1.9 ± 0.12 bc	38.7
Eupatorin (**12**)	2.9 ± 0.10 b	6.5	1.7 ± 0.17 bcde	45.2	1.9 ± 0.12 bc	38.7
Vasicine (**15**)	2.7 ± 0.23 b	12.9	2.5 ± 0.15 ab	19.4	1.3 ± 0.20 de	58.1

[a] Data are expressed as means ± SE from experiments with three replicates.
[b] Means within a column sharing the same letter are not significantly different at the 0.05 probability level.
[c] I = Inhibition.

On the other hand, the inhibitory effect of the tested extracts and compounds on shoot growth of barnyard grass is presented in Table 7. As was the case with root growth, ambrosin (**4**) showed the highest inhibitory effect on shoot growth at concentration of 100 mg/l with inhibition percentage of 45.3. The extracts of *A. santolina* and *A. vasica* revealed the highest inhibitory effect while *A. vasica* alkaloidal fraction was the least effective among the tested extracts. At concentration of 250 mg/l, neoambrosin (**1**) was the most potent inhibitor for shoot growth followed by ambrosin (**4**) and damsin (**3**). All of the tested extracts and compounds significantly reduced the shoot growth at 500 mg/l. At this concentration, neoambrosin (**1**), damsin (**3**) and ambrosin (**4**) caused complete inhibition of shoot growth.

Although, the inhibitory effects of some sesquiterpenes on the root and shoot growth have been described (Baruah *et al.*, 1994; Batish *et al.*, 2002; Abdelgaleil and Hashinaga, 2007; Abdelgaleil *et al.*, 2009), there were no reports on inhibitory effects of flavonoids and alkaloids. In this study, the sesquiterpenes (**1-6**) were more potent growth inhibitors for barnyard grass than flavonois and alkaloids. It was also observed that the inhibitory effects of the tested extracts and compounds on root growth were greater than on shoot growth. These results are consistent with those reported elsewhere for other sesquiterpenes and plant extracts (Chung and Miller, 1995; Turk and Tawaha, 2002; Abdelgaleil *et al.*, 2009). This finding might be expected, because it is likely that roots are the first to absorb the allelochemical compounds from the environment (Turk and Tawaha, 2002). The results of phytotoxicity experiments indicated that the inhibitory effect of tested extracts and compounds on root and shoot growth was greater than that on germination. Similarly, Leather and Einhellig (1985) demonstrated that bioassays determining seedling growth are usually more sensitive than those measuring germination.

The structure–activity relationship investigation of the tested compounds revealed some interesting findings. For example, eupatorin (**12**) with hydroxyl group in the side ring at C3` was more toxic than santoflavone (**11**) with methoxy group at the same position against the larvae of *C. pipiens*. Sesquiterpenes neoambrosin (**1**), damsin (**3**), ambrosin (**4**) and hymenin (**5**) with α-methylene-γ-lactone are more active than damsinic acid (**2**) in which the lactone ring is opened and formed carboxyl group and leucoden (**6**) in which the methelene moiety is converted to methyl group against all of the tested pests. Similarly, Rodriguez *et al.* (1976) stated that the presence of α-methylene-γ-lactone is essential for potent bioactivity of sesquitrpenes. In addition, the introduction of hydroxyl group at C1 in hymenin (**5**) decreased the bioactivity comparing with ambrosin (**4**). This finding is in a good agreement with those reported on the antifungal activity of sesquiterpene melampolides and sesquiterpene lactones in which the least polar compounds showed the greatest activity (Inoue *et al.*, 1995; Barrero *et al.*, 2000). Moreover, comparison of neoambrosin (**1**) with ambrosin (**4**) indicated that the double bond position, either C1–C2 or C2–C3, has no effect on activity.

Table 7: Effect of extracts and compounds isolated from *Ambrorsa maritima*, *Achillea santolina* and *Adhatoda vasica* on barnyard grass, *Echinochloa crusgalli*, shoot growth[a]

Extract/Compound	100 mg/l		250 mg/l		500 mg/l	
	Shoot length (cm)[b]	I (%)[c]	Shoot length (cm)	I (%)	Shoot length (cm)	I (%)
Control	5.3 ± 0.22 a	0.0	5.3 ± 0.22 a	0.0	5.3 ± 0.22 a	0.0
A. maritima extract	4.6 ± 0.23 abc	13.2	3.9 ± 0.10 cd	26.4	3.7 ± 0.07 d	30.2
A. maritima chloroform fraction	4.6 ± 0.20 abc	13.2	4.4 ± 0.31 bc	17.0	2.7 ± 0.03 e	49.1
A. santolina extract	4.2 ± 0.20 bcd	21.0	4.9 ± 0.07 ab	7.5	4.3 ± 0.12 bc	18.9
A. santolina H_2O/MeOH fraction	4.3 ± 0.15 bcd	18.9	4.4 ± 0.18 bc	17.0	3.5 ± 0.29 d	34.0
A. vasica extract	4.2 ± 0.03 bcd	21.0	4.3 ± 0.09 bc	18.9	3.8 ± 0.17 cd	28.3
A. vasica alkaloidal fraction	4.9 ± 0.17 ab	7.5	4.1 ± 0.21 cd	22.6	3.9 ± 0.21 cd	26.4
Neoambrosin (**1**)	3.8 ± 0.15 cd	28.3	0.4 ± 0.22 f	92.5	0.0 ± 0.0 f	100.0
Damsinic acid (**2**)	3.7 ± 0.10 cd	30.2	3.5 ± 0.09 d	34.0	2.5 ± 0.13 e	52.8
Damsin (**3**)	3.6 ± 0.12 d	31.1	1.8 ± 0.17 e	66.0	0.0 ± 0.0 f	100.0
Ambrosin (**4**)	2.9 ± 0.23 e	45.3	1.6 ± 0.09 e	69.8	0.0 ± 0.0 f	100.0
Hymenin (**5**)	4.4 ± 0.30 bcd	17.0	4.2 ± 0.03 cd	21.0	2.7 ± 0.12 e	49.1
Leucoden (**6**)	4.4 ± 0.21 bcd	17.0	3.6 ± 0.10 d	32.1	3.6 ± 0.15 d	32.1
Artemetin (**7**)	3.9 ± 0.21 cd	26.4	3.9 ± 0.07 cd	26.4	3.5 ± 0.17 d	34.0
Santoflavone (**11**)	4.3 ± 0.03 bcd	18.9	4.5 ± 0.25 bc	15.1	4.5 ± 0.17 b	15.1
Eupatorin (**12**)	4.6 ± 0.15 abc	13.2	3.8 ± 0.03 cd	28.3	3.7 ± 0.09 d	30.2
Vasicine (**15**)	4.3 ± 0.29 bcd	18.9	4.0 ± 0.23 cd	24.5	3.9 ± 0.15 cd	26.4

[a] Data are expressed as means ± SE from experiments with three replicates.
[b] Means within a column sharing the same letter are not significantly different at the 0.05 probability level.
[c] I = Inhibition.

From these correlations, it is obvious that the bioactivity of these classes of compounds may vary because of the presence of specific chemical groups in the molecule.

In summary, fifteen compounds of sesquiterpenes, flavonoids and alkaloids have been isolated from the extracts of *A. maritima*, *A. santolina* and *A. vasica*. Although these compounds have been previously isolated, this is the first report of the investigation of their bioactivity against *C. pipiens*, *T. pisana*, *E. vermiculata* and *E. crusgalli*. The results show that eupatorin (**12**) is a promising toxicant to mosquito larvae. The sesquiterpenes neoambrosin (**1**), damsin (**3**) and ambrosin (**4**) had marked toxicity against the terrestrial snails *T. pisana* and *E. vermiculata*. These compounds had also a pronounced inhibitory effect on seed germination and seedling growth of *E. crusgalli*. Based on these findings, compounds **1**, **3**, **4** and **12** might be used as potential natural pesticides.

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الملخص العربى

تقييم الخصائص الإبادية للمستخلصات والمركبات المعزولة من ثلاث نباتات مصرية

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تم عزل ١٥ مركباً بإستخدام طرق الفصل الكروماتوجرافى المختلفة من ثلاث نباتات مصرية هى *Ambrosia maritima* و *Achillea santolina* و *Adhatoda vasica* . خمس مركبات من pseudoguaianolide sesquiterpenes هى neoambrosin و damsinic acid و damsin و ambrosin و hymenin تم عزلها من من مستخلص الكلورفورم للمجموع الخضرى لنبات الدمسيسة (*Ambrosia maritima*). مركب guaianolide sesquiterpene lactone وهو leucodin وست مركبات flavonoids وهى artemetin و salvigenin و cirsimaritin و 5,7 dihydorxy-3`,4`dimethoxyflavone و santoflavone و eupatorin تم عزلها من مستخلص الكلوروفورم للمجموع الخضرى لنبات *Achillea santolina* . بالإضافة إلى ثلاث مركبات من quinazoline alkaloids تم عزلها من مستخلص الإيثانول لأوراق نبات *Adhatoda vasica* . التركيب الكيماوى للمركبات المعزولة تم التعرف عليه بإستخدام أجهزة التحليل الطيفى مثل الرنين النووى المغناطيسى وجهاز مطياف الكتلة وجهاز الاسبكتروفوتوميتر. النشاط الحيوى للمستخلصات والمركبات المعزولة قيم على العمر اليرقى الرابع لبعوضة *Culex pipiens* وقوقع الحدائق الأبيض وقوقع الحدائق البنى وحشيشة الدنيبة. المستخلصات والمركبات المعزولة أظهرت درجات مختلفة من السمية ضد العمر اليرقى الرابع للبعوض وكان مركب eupatorin أعلى المركبات فاعلية حيث كانت قيمة LC_{50} تساوى ٥،٦١ مجم/لتر. من بين المستخلصات و المركبات المختبرة الستة عشر كانت مركبات neoambrosin و damsin و ambrosin أعلى سمية ضد قوقع الحدائق الأبيض وقوقع الحدائق البنى. المركبات الثلاثة كانت أعلى سمية من مبيد الميثوميل على القوقع الأبيض بعد ٢٤ ساعة من المعاملة. أظهرت إختبارات السمية النباتية على حشيشة الدنيبة أن مركبات sesquitepenes وهى neoambrosin و damsin و ambrosin سببت تثبيطاً عالياً لإنبات البذور وقد كان التثبيط كاملاً عند تركيز ٥٠٠ مجم/لتر. معظم المستخلصات والمركبات المختبرة أحدثت خفضاً معنوياً فى نمو الجذور والمجموع الخضرى مقارنة بالكنترول وكان هذا التأثير معتمداً على التركيز. وكان تثبيط نمو الجذور أعلى من تثبيط نمو المجموع الخضرى. مركبات الـ sesquitepenes كانت أكثر فاعلية فى خفض الإنبات وتثبيط نموالمجموع الخضرى والجذرى من flavonoids و alkaloids. العلاقة بين النشاط البيولوجى والتركيب الكيماوى للمركبات المعزولة تم منقاشتها. النتائج المتحصل عليها تنبىء بإمكانيه إستخدام مركبات مثل eupatorin و neoambrosin و damsin و ambrosin كمبيدات طبيعية.