Compounds from *Ageratum conyzoides*: isolation, structural elucidation and insecticidal activity

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Abstract: This work aimed at identifying plant compounds with insecticidal activity against *Diaphania hyalinata* (L.) (Lepidoptera: Pyralidae), *Musca domestica* (L.) (Diptera: Muscidae), *Periplaneta americana* (L.) (Blattodea: Blattidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae). The plant species used were: basil (*Ocimum selloi* Benth.), rue (*Ruta graveolens* L.), lion's ear (*Leonotis nepetaefolia* L.), Jimson weed (*Datura stramonium* L.), 'baleeira' herb (*Cordia verbenaceae* L.), mint (*Mentha piperita* L.), wild balsam apple (*Mormodica charantia* L.) and billy goat weed (*Ageratum conyzoides* L.). Firstly, the insecticidal activities of hexane and ethanol plant extracts were evaluated against adults of *R. dominica*. Among them, only the hexane extract of *A. conyzoides* showed insecticidal activity. The hexane extract of this plant species was therefore fractionated by silica gel column chromatography to isolate and purify its bioactive chemical constituents. Three compounds were identified using IR spectra, ^{1}H NMR, ^{13}C NMR, HMBC and NOE after gel chromatography: 5,6,7,8,3′,4′,5′-heptamethoxyflavone, 5,6,7,8,3′-pentamethoxy-4′,5′-methylenedioxyflavone and coumarin. The complete assignment of ^{13}C NMR to 5,6,7,8,3′-pentamethoxy-4′,5′-methylenedioxyflavone was successfully made for the first time. 5,6,7,8,3′-Pentamethoxy-4′,5′-methylenedioxyflavone did not show any insecticidal activity against the four insect species tested. 5,6,7,8,3′,4′,5′-Heptamethoxyflavone showed low activity against *D. hyalinata* and *R. dominica* and was not toxic to *M. domestica* or *P. americana*. In contrast, coumarin showed insecticidal activity against all four insect pest species tested, with the following order of susceptibility: *R. dominica* < *P. americana* < *D. hyalinata* < *M. domestica* after 24 h exposure.

Keywords: botanical pesticide; insect control; secondary metabolites; bioactive compounds; *Ageratum conyzoides*

1 INTRODUCTION

Although recent innovations such as combinatorial chemistry enable 100 000 new chemicals per year to be screened compared with 15 000 previously, new compounds brought to the market are still decreasing at a rate of one per year.[1] In addition, the high cost of development, $100–200 million per product,[2] emphasizes the need for new tools of pest control.

Tropical plants are recognized sources of bioactive compounds, and less than 1% have been chemically investigated.[3] They can be used for pest control as plant extracts, horticultural oils (as either commercial or semi-commercial products) or as a source of molecules for pesticide synthesis (e.g. pyrethroids and neonicotinoids).

Ageratum conyzoides L. is an erect, herbaceous annual plant from the family Asteraceae (Compositae), native to tropical America, but with a distribution range in tropical and subtropical areas around the world.[4] In spite of several studies on the bioactivity of *A. conyzoides*, only a few of these have isolated and assessed the insecticidal activity of compounds from this plant, and these efforts have focused on human health pests and not crop and urban pests. Since such studies were carried out with either the crude extract or the essential oil of *A. conyzoides*, the potential use of this plant species for pest management as an insecticide requires further investigation.

The melonworm [*Diaphania hyalinata* (L.) (Lepidoptera: Pyralidae)], the American cockroach [*Periplaneta americana* (L.) (Blattodea: Blattidae)], the house fly [*Musca domestica* (L.) (Diptera: Muscidae)] and the lesser grain borer [*Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae)] represent four major orders of insect species of economic importance. *Diaphania hyalinata* is a key pest of Cucurbitaceae that is found in most countries of the Americas.[5,6] *Rhyzopertha dominica* is a cosmopolitan key pest of stored grains, and *M. domestica* and *P. americana* are important household pests throughout the world.[7,8] The economic importance of these four insect pest species,

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aided by their easy maintenance and handling, make them useful models for insecticide bioactivity assays.

Considering the potential of plant species as a source of potential insecticides and the importance of these insect pests, the present work aimed at recognizing the insecticidal activity in extracts of eight plant species and at isolating and identifying the main bioactive compounds present in these extracts.

2 MATERIALS AND METHODS

2.1 Insect species and plant material

Toxicity bioassays were carried out with first-instar larvae of *D. hyalinata*, first-instar nymphs of *P. americana*, last-instar larvae of *M. domestica* and adults of *R. dominica*. *Rhyzopertha dominica* was reared on wheat. *Diaphania hyalinata, P. americana* and *M. domestica* were reared as described elsewhere.[6,8,9]

The following plants were subject to extraction and toxicity bioassays: basil (*Ocimum selloi* Benth.), rue (*Ruta graveolens* L.), lion's ear (*Leonotis nepetaefolia* L.), Jimson weed (*Datura stramonium* L.), 'baleeira' herb (*Cordia verbenaceae* L.), mint (*Mentha piperita* L.), wild balsam apple (*Mormodica charantia* L.) and billy goat weed (*Ageratum conyzoides* L.).

Samples (500 g) of the canopy of each plant species were collected within the campus of the Federal University of Viçosa, state of Minas Gerais, Brazil, where these plants are permanently cultivated. Each sample was placed in a 1 L Erlenmeyer flask with enough hexane to submerge the plant material. The solvent was removed under filtration after 48 h. Ethanol extraction was carried out by grinding the samples with the solvent and waiting for 48 h. The hexane and ethanol extracts were concentrated under low pressure and reduced temperature (<50 °C) and stored at low temperature for subsequent bioassays.

Ageratum conyzoides was selected for further extraction with hexane and fractionation and structural elucidation of its bioactive compounds. A total of 5.31 kg of leaves of *A. conyzoides* was used for this purpose. The solvent (hexane) was changed at intervals of 2 days for 45 days. The extraction continued until the solvent was colourless.

2.2 Gel chromatography and structural elucidation

The hexane extract of *A. conyzoides* was concentrated under low pressure and reduced temperature (<50 °C) and subjected to open column chromatography with silica gel 60 (70–230 mesh). Two columns were used in open column chromatography. The first column was eluted with pure hexane, hexane + diethyl ether (100 + 0.5 by volume), hexane + diethyl ether (50 + 50 by volume), pure diethyl ether and pure methanol. The second column was eluted with hexane + diethyl ether (100 + 10 by volume) and pure methanol. Seven fractions were obtained in the first column and four fractions in the second column. Thin-layer chromatography (TLC, silica gel 60 F254, 0.25 mm) spots were detected under UV (254 and 365 nm) and heating the plates to 100 °C after spraying with phosphomolybdic acid/ethyl alcohol. The IR spectra were recorded on potassium bromide in an infrared spectrometer (Paragon 1000 FTIR; Perkin Elmer, Wellesley, MA, USA) from 600 to 4000 cm^{-1}. The melting points (mp) were determined in MQAPF-301 apparatus (MicroQuímica Equip. Ltda, Palhoça, SC, Brazil). ^{1}H or ^{13}C NMR spectra, recorded in either a Bruker WM 400 (Bruker Optics Inc., Billerica, MA, USA) or a Varian Mercury 300 (Varian Inc., Palo Alto, CA, USA) spectrometer using deuterochloroform as solvent and tetramethyl silane as internal standard, were used to identify the isolated compounds. The NMR assignments were made by ^{1}H NMR, ^{13}C NMR, HMBC and NOE.[10]

2.3 Toxicity bioassays

Three sets of bioassays were carried out. The first was a set of screening bioassays to recognize the bioactive plant extracts using adults of *R. dominica*. The second set of bioassays aimed to select the most bioactive compounds of the most active plant extract for subsequent evaluation, using four insect pest species for assessing insecticide activity – the melonworm *Diaphania hyalinata*, the house fly *Musca domestica*, the American cockroach *Periplaneta americana* and again the lesser grain borer *R. dominica*. In the third set of bioassays, the relative toxicity of the selected compounds was assessed by establishing dose–mortality curves for the same four insect pest species used in the second set of bioassays.

2.3.1 Bioassays with crude plant extracts

The stored extracts were diluted with either ethanol or hexane as solvent to a concentration of 20 mg mL^{-1}. Three replicates were used in this bioassay. Each replicate encompassed a petri dish (9 cm diameter) with a filter paper impregnated with 1 mL of the test extract. The controls were treated with solvent only (either ethanol or hexane). Ten adults of *R. dominica* were placed in each petri dish, which was maintained at 25 ± 0.5 °C, 75 ± 5% RH and 12 h photophase, and insect mortality was assessed after 4 and 24 h exposure to the impregnated filter paper. Mortality data were subjected to analysis of variance, and the averages were compared by the Scott–Knott groupment analysis test ($P < 0.05$).

2.3.2 Bioassays for selection of bioactive compounds

In this second bioassay, the compounds 5,6,7,8, 3′, 4′, 5′-heptamethoxyflavone, coumarin and 5,6,7, 8,3′-pentamethoxy-4′, 5′-methylenedioxyflavone, which were identified in fractionation of the most active plant extract, were topically applied at a dose of 10 mg g^{-1} fresh body mass to the four insect species. Three replicates were used in this bioassay. Each replicate encompassed a petri dish (9 cm diameter) with ten insects maintained at 25 ± 0.5 °C, 75 ± 5% RH and 12 h photophase. The control was treated with the

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same amount of solvent. Insect mortality was assessed at 6, 12, 24 and 48 h after treatment. Mortality data were subjected to analysis of variance, and the averages were compared by the Scott–Knott groupment analysis test ($P < 0.05$).

2.3.3 Dose–mortality curves

The third set of bioassays was carried out with the four insect pest species used in the previous set of bioassays to determine the potential insecticidal activity of the compounds obtained from the most bioactive plant extract. Four replicates were used in this bioassay. Each replicate encompassed a petri dish (9 cm diameter) with ten insects maintained at 25 ± 0.5 °C, 75 ± 5% RH and 12 h photophase. The insects were submitted to 5–8 increasing doses of the compound (plus a control with the application of the solvent only). Insect mortality was evaluated 6, 12 and 24 h after topical exposure. The data were corrected for control mortality[11] and probit analysis was carried out.[12]

2.3.4 Bioassay of positive control

This bioassay was carried out in order to compare results from commercial (synthetic) coumarin and the (natural) coumarin obtained from *A. conyzoides*. The objectives were to confirm the activity of coumarin and to exclude interference by contaminants from chromatography. The methodology was as stated in Section 2.3.2, but using commercial coumarin (synthetic; technical grade; 98% pure; Sigma-Aldrich Química Brasil, São Paulo, Brazil). The dose causing 90% mortality (LD_{90}) against *R. dominica* and *P. americana*, estimated from the dose–mortality curve, was used in this bioassay.

3 RESULTS

3.1 Bioactivity of plant extracts

Only the hexane extract from leaves of *A. conyzoides* showed insecticidal activity, causing 76.0 ± 2.35% ($n = 30$) and 88.7 ± 1.67% ($n = 30$) mean mortality ± SEM in adult *R. dominica* after 4 and 24 h exposure respectively. The mortality caused by the solvents was always negligible (<5%) in all sets of bioassays. The ethanol extract of *A. conyzoides* and the hexane and ethanol extracts from *R. graveolens, L. nepetaefolia, C. verbenacea, D. estramonium, M. charantia, O. selloi* and *M. piperita* did not show any insecticidal activity against *R. dominica*.

3.2 Isolation and structural elucidation

Extraction of 5.31 kg of *A. conyzoides* leaves with hexane yielded 86.13 g of crude extract. The crude extract, when stored at low temperature, generated an oily portion (77.37 g) and a crystallized portion (8.76 g). Compound **1** was isolated from the oily portion using the first column in the open column chromatography. This compound was collected in the second fraction. Compounds **2** and **3** were isolated from the crystallized portion in the second and fourth fraction respectively using the second column in the open column chromatography.

Compound **1**, 5,6,7,8,3′, 4′, 5′-heptamethoxyflavone (Fig. 1), was isolated as a yellow solid (0.8 g) with mp 115.3–116.9 °C. Its IR spectrum was typical of non-phenolic flavones:[13] 2992, 2941 and 2838 cm^{-1} (ν C–H3), 2890 cm^{-1} (ν_s C–H3), 1643 cm^{-1} (ν C=O), 1589, 1570 and 1551 cm^{-1} (ν_s C=C arom.) and 1247 and 1040 cm^{-1} (ν_{as} C–O–C). The ^{13}C NMR spectrum gave rise to 22 carbon signals. Nine carbon signals were typical of the A and B ring of flavones: δ 161.00 (C-2), 107.86 (C-3), 177.56 (C-4), 148.49 (C-5), 144.25 (C-6), 151.61 (C-7), 138.12 (C-8), 147.75 (C-9) and 114.93 (C-10). Six carbon signals were typical of the C ring: δ 125.98 (C-1′), 100.51 (C-2′), 149.66 (C-3′), 138.42 (C-4′), 143.99 (C-5′) and 106.61 (C-6′). The signals of seven methoxy groups were present from δ 56 to 63. The ^1H NMR spectrum showed singlets at δ 6.63 (H-3), 7.15 (H-2′ and H-6′) and 3.92, 3.94, 4.01 and 4.10 respectively to 3H, 12H, 3H and 3H of the methoxy groups. The assignments of ^{13}C NMR and ^1H NMR signals are in agreement with the literature.[14,15]

Compound **2**, coumarin (Fig. 1), has a white needle-like appearance (5.28 g) with mp 66.7–68.9 °C. Its IR spectrum showed characteristic absorption bands at 3045 cm^{-1} (ν_{as} C–H), 1706 cm^{-1} (ν C=O), 1619, 1605 and 1562 cm^{-1} (ν C=C arom.) and 1259 and 1229 cm^{-1} (ν_s C–O). The ^{13}C NMR spectrum showed characteristic carbon signals from δ 160.52 (C=O), 116.5 (C=3), 143.3 (C=4), 153.71 (C-9) and 118.56 (C-10). The ^1H NMR spectrum showed two doublets from δ 5.59 and 6.90 (J 9.6 Hz) of two proton *cis*-olefinics of the coumarinic ring. The ^{13}C NMR spectrum was consistent with the published data.[16]

Compound **3**, 5,6,7,8,3′-pentamethoxy-4′, 5′-methylenedioxyflavone (Fig. 1), was isolated as a rose-blue solid (0.46 g), with mp 185–188.9 °C. Its IR spectrum was 3078 cm^{-1} (ν C–H arom.), 2970 and 2942 cm^{-1} (ν_{as} C–H_3), 2890 cm^{-1} (ν_s C–H_3), 1631 cm^{-1} (ν C=O), 1584, 1560 and 1551 cm^1 (ν C=C arom.), 1448 cm^{-1} (δ_{as} C–H_3), 1370 cm^{-1} (δ_s C–H_3), 1247 cm^{-1} (ν_{as} C–O–C) and 1040 cm^{-1} (ν_s C–O–C). The ^{13}C NMR spectrum showed 21 carbon signals, 15 of which were typical of flavones. The methylenedioxy group was observed at δ 102.45, and the signals from five methoxy groups were present from δ 56.82 to 62.38. The other non-hydrogenated C gave rise to δ 160.76 to 138.12. The complete assignment of ^{13}C NMR was made for this compound for the first time (Table 1). HMBC and NOE were used in the assignments (Table 1). The ^1H NMR spectrum showed a singlet at δ 6.09 from methylenedioxy (H-16), and the singlet at δ 6.57 was from H-3. The doublets at δ 7.11 and 7.06 (J 1.7 Hz) were from 6′-H and 2′-H. The methoxy groups (15 H) gave rise to δ 3.95 to 4.11. The assignments of ^{13}C NMR and ^1H NMR were confirmed by HMBC and NOE (Table 1).

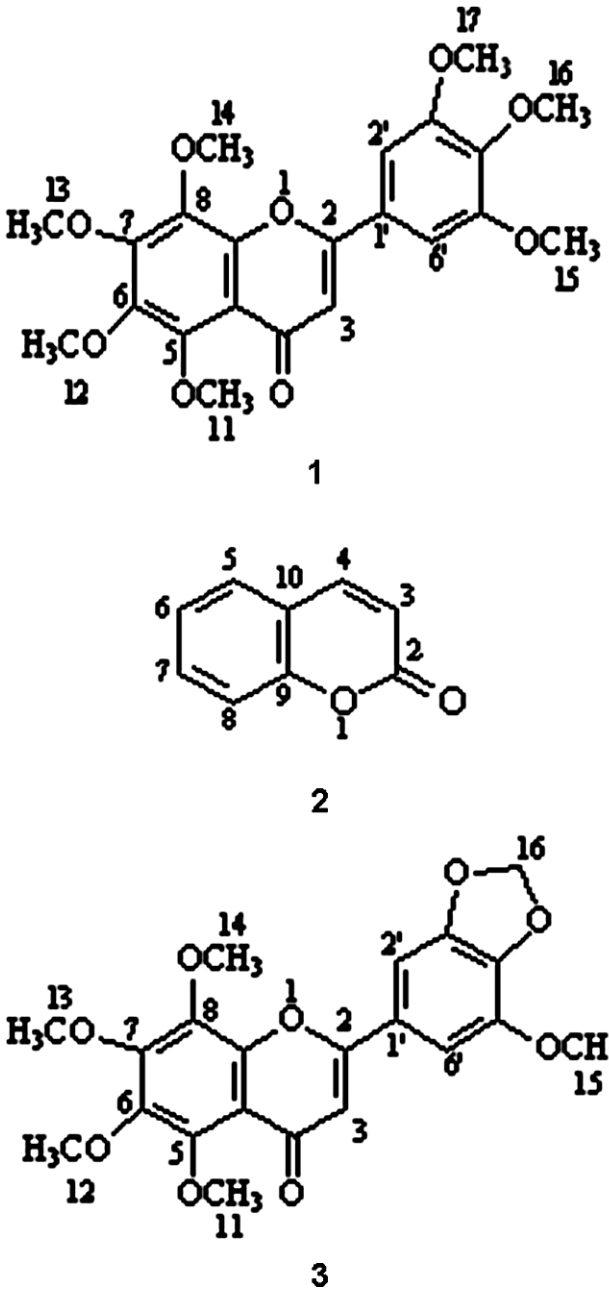

Figure 1. Structures of compounds isolated from *A. conyzoides*: 5,6,7,8,3′, 4′, 5′-heptamethoxyflavone (**1**), coumarin (**2**) and 5,6,7,8,3′-pentamethoxy-4′, 5′-methylenedioxyflavone (**3**).

The ^{1}H NMR spectrum agrees with data previously reported by Quijano *et al*.[17]

3.3 Bioactivity of the isolated compounds

5,6,7,8,3′, 4′, 5′-Heptamethoxyflavone showed low insecticidal activity against *D. hyalinata* and *R. dominica*, but no activity was detected against *M. domestica* and *P. americana* at 48 h after topical application (Table 2). Coumarin was toxic to *M. domestica, P. americana, R. dominica* and *D. hyalinata* at 6, 12, 24 and 48 h after topical application. In contrast, 5,6,7,8,3′-pentamethoxy-4′, 5′-methylenedioxyflavone was not toxic to these insect pests.

Table 1. ^{13}C NMR, ^{1}H NMR, HMBC and NOE from 5,6,7,8,3′-pentamethoxy-4′, 5′-methylenedioxyflavone

C atom	^{13}C NMR	^{1}H NMR	HMBC C to H	NOE[a]
2	160.76		3, 2′, 6′	
3	107.41	6.53		2′,* 6′*
4	177.37		3	
5[b]	148.49		11, 3	
6[b]	144.25		12	
7	151.61		13	
8	138.12		14	
9	147.75			
10	114.93		3	
11	62.38	3.91		
12	62.11	3.92		
13	61.78	4.07		14
14	61.95	3.98		13, 2′+ 6′+
15	56.82	3.95		
16	102.45	6.05		
1′	125.98		3, 2′, 6′	
2′	100.51	7.06 (*J* 1.7 Hz)	6′	3, 14
3′	149.66		16, 2′, 3′	
4′	138.42		16, 2′, 3′	
5′	143.99		2′, 3′	
6′	106.61	7.11 (*J* 1.7 Hz)	2′	3, 14, 15

[a]* and + denote equal intensity.
[b] This assignment can be inverted.

Dose–mortality curves for 5,6,7,8,3′, 4′, 5′-heptamethoxyflavone and 5,6,7,8,3′-pentamethoxy-4′, 5′-methylenedioxyflavone against *M. domestica, P. americana, R. dominica* and *D. hyalinata* were not obtained owing to their low or lack of activity against these species.

The toxicity of coumarin was higher in evaluations at 24 h than at 6 and 12 h of exposure. The coumarin dose–mortality curve for *P. americana* showed the steepest slopes, suggesting a higher homogeneity of response of this species to this compound compared with the other species (Table 3). The slopes obtained for *R. dominica, D. hyalinata* and *M. domestica* were generally smaller and similar to each other. The increasing order of susceptibility to coumarin was *R. dominica* < *D. hyalinata* < *P. americana* < *M. domestica* for 12 h exposure and *R. dominica* < *P. americana* < *D. hyalinata* < *M. domestica* for 24 h exposure.

The mortality results obtained with the bioassays of positive control using the same dose of the commercial (synthetic) coumarin and the (natural) coumarin obtained from the hexane extract of *A. conyzoides* were very similar. The mortality of *R. dominica* was $92.00 \pm 0.27\%$ and $96.00 \pm 0.18\%$ to synthetic and natural coumarin respectively after 24 h exposure. The mortality of nymphs of *P. americana* was $95.00 \pm 2.13\%$ and $94.50 \pm 1.97\%$ to synthetic and natural coumarin respectively after 24 h exposure.

4 DISCUSSION

The only plant species showing insecticidal activity in the present study was the billy goat weed *A. conyzoides*,

Table 2. Mortality of *Rhyzopertha dominica, Diaphania hyalinata, Musca domestica* and *Periplaneta americana* 6, 12, 24 and 48 h after topical application of 10 mg g^{-1} of 5,6,7,8,3′, 4′, 5′-heptamethoxyflavone (**1**); coumarin (**2**) or 5,6,7,8,3′-pentamethoxy-4′, 5′-methylenedioxyflavone (**3**) extracted from leaves of *Ageratum conyzoides* (only solvent was used in the control)

	Mortality (%) (± SEM)[a]			
Insect species	Control	**1**	**2**	**3**
		6 h after topical application		
M. domestica	0.00 bA	0.00 bA	23.33 (±3.33) aB	0.00 bA
P. americana	0.00 bA	0.00 bA	66.16 (±2.36) aA	0.00 bA
R. dominica	0.00 bA	3.33 (±3.33) bA	16.67 (±4.67) aC	0.00 bA
D. hyalinata	0.00 bA	6.67 (±6.67) bA	63.33 (±8.82) aA	0.00 bA
		12 h after topical application		
M. domestica	0.00 bA	0.00 bB	23.33 (±3.33) aC	0.00 bA
P. americana	0.00 bA	0.00 bB	80.44 (±3.53) aA	0.00 bA
R. dominica	0.00 bA	3.33 (±3.33) bB	23.33 (±8.82) aC	0.00 bA
D. hyalinata	0.00 cA	13.33 (±6.67) bA	70.00 (±5.77) aB	0.00 cA
		24 h after topical application		
M. domestica	0.00 bA	0.00 bB	26.67 (±6.67) aD	3.33 (±3.33) bA
P. americana	0.00 bA	0.00 bB	81.37 (±0.37) aA	0.00 bA
R. dominica	0.00 bA	6.67 (±3.33) bB	43.33 (±14.53) aC	0.00 bA
D. hyalinata	0.00 cA	16.67 (±3.33) bA	70.00 (±7.40) aB	0.00 cA
		(48 hours after topical application)		
M. domestica	0.00 bA	0.00 bB	33.33 (±3.33) aC	3.33 (±3.33) bA
P. americana	0.00 bA	0.00 bB	83.01 (±2.56) aA	0.00 bA
R. dominica	0.00 cA	13.33 (±6.67) bA	68.89 (±11.6) aB	0.00 cA
D. hyalinata	0.00 cA	20.00 (±5.77) bA	68.18 (±7.40) aB	0.00 cA

[a] Means followed by the same lower-case letter in a row or by the same upper-case letter in a column are not significantly different by the Scott–Knott groupment analysis test at $P < 0.05$.

Table 3. Toxicity of coumarin extracted from leaves of *Ageratum conyzoides* against *Rhyzopertha dominica, Diaphania hyalinata, Musca domestica* and *Periplaneta americana*

Insect species	Slope (± SE)	LD_{50} (mg g^{-1}) (95% FL)	LD_{90} (mg g^{-1}) (95% FL)	χ^2	Probability
		6 h after topical application			
R. dominica	2.76 (±0.25)	39.72 (35.90–45.00)	115.19 (88.89–173.90)	6.27	0.09
		12 h after topical application			
R. dominica	2.87 (±0.18)	20.82 (18.70–23.08)	58.04 (49.40–71.65)	3.17	0.37
D. hyalinata	2.65 (±0.07)	3.80 (3.27–4.34)	11.54 (9.95–13.78)	0.75	0.86
M. domestica	1.62 (±0.20)	2.28 (1.73–3.44)	14.07 (7.81–36.00)	2.97	0.60
P. americana	3.19 (±0.27)	3.05 (3.16–3.96)	8.82 (7.07–12.33)	0.85	0.66
		24 h after topical application			
R. dominica	2.37 (±0.26)	11.82 (10.07–13.59)	42.94 (34.67–50.30)	6.93	0.07
D. hyalinata	2.14 (±0.09)	2.21 (1.75–2.64)	8.72 (7.31–10.92)	0.52	0.92
M. domestica	2.60 (±0.10)	1.18 (0.98–1.52)	3.67 (2.56–6.29)	1.18	0.76
P. americana	3.38 (±0.07)	2.49 (2.29–2.70)	5.15 (4.54–6.08)	1.89	0.61

and such activity was observed only in the hexane extract, not in the ethanol extract. The insecticidal activity of *A. conyzoides* extract has previously been reported against *Culex quinquefasciatus* Say (Diptera: Culicidae).[18] Fagoonee and Umrit[19] observed the presence of precocenes I and II in the acetone-diethyl ether extract of *A. conyzoides* and reported ovarian inhibition in *Dysdercus flavidus* Signoret (Heteroptera: Pyrrhocoridae). However, the insecticidal activity of the hexane extract against *R. dominica* is not due to the precocenes, since these compounds have antijuvenile hormone activity interfering with insect moulting and reproduction, while the insecticide mortality observed in the present study was acute and quickly apparent (within 24 h), unlike what would be expected with precocenes.

Three compounds were identified in the bioactive fractions of the hexane extract of *A. conyzoides*: two flavonoids (5,6,7,8,3′, 4′, 5′-heptamethoxyflavone and 5,6,7,8,3′-pentamethoxy-4′, 5′-methylenedioxyflavone) and coumarin. Flavonoids are a major class of phytochemicals found in plants, with beneficial action to humans, including antimicrobial, pharmacological and antioxidant activity, besides adversely affecting insect pests.[20] These compounds have been mainly reported as antifeedant and growth inhibitors in insects, probably because of their interference with endocrine regulation.[20] Maysin, a C-glycosil flavone,

has been implicated in maize resistance to the corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), and in this case is thought to interfere with amino acid metabolism in the insect gut.[21] The flavone meliternantin (3,5-dimethoxy-3′, 4′,6,7-bismethylenedioxyflavone) is a feeding deterrent that reduces the growth rate and food consumption rate, and inhibits 50% of feeding by *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae) at 125 ppm.[22]

Flavonoids possess a catecholic B-ring responsible for their toxic activity to insects.[23] The activity varies according to the chemical structure of these compounds.[24] Thus, 5,6,7,8,3′-pentamethoxy-4′, 5′-methylenedioxyflavone did not show toxicity, while 5,6,7,8,3′, 4′, 5′-heptamethoxyflavone was toxic to *R. dominica* and *D. hyalinata*. The structural diversity of this group emphasizes the need for structure–activity studies to guide the search for higher toxicity to insects. The diversity in mode of action and the range of effects of these compounds against insects are likely to slow down the evolution of resistance to them.

The results with the commercial (synthetic) coumarin closely resembled the effect of the (natural) coumarin extracted from *A. conyzoides,* confirming its activity against the tested insect pest species, which was higher than the activity of the two flavonoids obtained in the bioactive fractions of the hexane extract of this plant species. The difference in coumarin toxicity to *D. hyalinata*, *M. domestica*, *P. americana* and *R. dominica* is due to the differential susceptibility of these species. Such variation in insecticidal activity against different insect species is frequent and it was expected.

The active compound ryanodine, extracted from plants of the genus *Ryania*, has an LD_{50} of $0.39\,\mu g\,g^{-1}$ on adults of *M. domestica* and several of its chemical analogues have LD_{50} from 0.11 to $100\,\mu g\,g^{-1}$.[25] Coumarin was less toxic to *R. dominica* ($11.82\,\mu g\,g^{-1}$) than deltamethrin ($0.3\,ng\ g^{-1}$),[26] but the authors were not able to find any information about the toxicity of coumarin and related compounds to *D. hyalinata*. Although coumarin and its related compounds are not used as insecticides, their lower toxicity to humans[27] and significant insecticidal activity, capable of enhancement through quantitative structure–activity relationship studies, make them potential insect control agents for pest management.

There is a lack of studies on the mode of action of coumarins, but surangin B has been subjected to such investigation. The coumarin surangin B is an inhibitor of mitochondrial electron transport, probably targeting cytochrome c oxidoreductase (complex III) and cytochrome b, leading to a reduction in ATP synthesis and bioenergetic muscle disruption.[28] Respiration in the cricket *Acheta domestica* L. (Orthoptera: Gryllidae) and blowfly *Phaenicia sericata* Meig (Diptera: Calliphoridae) flight muscle mitochondria are blocked by surangin B.[28]

Coumarins and mainly furanocoumarins inhibit the cytochrome P450 detoxication enzymes, disrupting the insect detoxication capability.[29,30] Coumarins have a reversible inhibition of cytochrome P450, and furanocoumarins show reversible or irreversible inhibition.[30] Letteron *et al*.[29] suggested that the coumarin xanthotoxin is metabolically activated on the outer double bond on the furan ring to form an extremely unstable radicaloid species. Cytochrome-P450-mediated radicaloid formation requires oxidation of xanthotoxin. The unstable radicaloid may bind covalently to the active site of the P450 or be released from the active site.

Other compounds with potential pesticidal activity are also likely to be present in *A. conyzoides* and they certainly deserve attention, as does coumarin as a potential pest management tool.

5 CONCLUSION

The present work identified three compounds in the bioactive fractions of the hexane extract from leaves of *Ageratum conyzoides*; hexane and ethanol extracts from seven other plant species did not show any insecticidal activity. Among these three compounds from *A. conyzoides,* identified as 5,6,7,8,3′-pentamethoxy-4′, 5′-methylenedioxyflavone, 5,6,7,8,3′, 4′, 5′-heptamethoxyflavone and coumarin, only the last two showed insecticidal activity. 5,6,7,8,3′, 4′, 5′-Heptamethoxyflavone was less active and showed insecticidal activity only against *D. hyalinata* and *R. dominica*. In contrast, coumarin was active against all four insect pest species tested. The increasing order of susceptibility to coumarin was *R. dominica* < *D. hyalinata* < *P. americana* < *M. domestica* at 12 h and *R. dominica* < *P. americana* < *D. hyalinata* < *M. domestica* at 24 h. Therefore, coumarin is a potential pest management tool likely to have its insecticidal activity improved through organic synthesis guided by quantitative structure–activity relationship studies.

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