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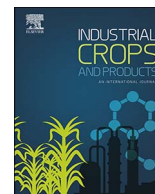# Insecticidal activity of Tithonia diversifolia and Vernonia amygdalina

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Insecticidal activity of *Tithonia diversifolia* and *Vernonia amygdalina*

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ARTICLE INFO

ABSTRACT

The diversity of synthetic pesticides has been reduced through regulation especially in the European Union, leading to a resurgence of interest in natural plant products for pest control. Here we investigated two Asteraceae species, *Tithonia diversifolia* and *Vernonia amygdalina* that are used by farmers in Africa in bio-rational pest control to determine the chemical basis of activity against pests of stored legumes and identify plant compounds with commercial potential. The cowpea beetle, *Callosobruchus maculatus*, an ubiquitous pest of African stored grain legumes, was exposed to extracts of both plant species at 10, 1 and 0.1% w/v and fractions of these extracts at representative concentrations. Extracts and fractions were toxic to recently emerged adults, but did not reduce oviposition by those females that survived. The sesquiterpene, tagitinin A, was isolated from one of the active fractions and identified using H^1 and C^{13}-NMR and shown also be toxic to *C. maculatus* and so partially explains the activity of the whole plant. Other compounds in the active fractions were identified, at least to structural class, using high resolution mass spectroscopy (HRESI-MS). Sequiterpenes and flavones were common to fractions from both plants. Stigmostane steroidal saponins were the most abundant secondary metabolites in *V. amygdalina*.

1. Introduction

Legume seeds provide food, are sold for profit and used for sowing subsequent crops, so it is essential for small holder farmers in developing countries to minimise insect damage during periods of seed storage (Sola et al., 2014). *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Bruchidae) is a significant pest of stored legumes throughout Africa (Abate and Ampofo 1996), the Middle East, India and South America, feeding on and contaminating the stored seeds (Tuda et al., 2006), especially cowpea, *Vigna unguiculata* L. Walp (Leguminosae) (Ehlers and Hall 1997). It is possible to control *C. maculatus* using synthetic pesticides e.g., Hill (1983) but these are expensive and require specialist equipment and training to be safely and effectively applied (Matthews et al., 2014). Synthetic pesticides can be toxic or have sub-lethal effects on the wider invertebrate community of beneficial insects, such as parasitoid wasps that can contribute to the control of bruchid pests (Van Alebeek 1996). A cost-effective and environmentally benign way of protecting crops is to use extracts or powdered plant materials of locally available insecticidal plants, and there are a number of examples of these being successfully employed to

kill insects and decrease crop losses (Hagemann et al., 1972; Stevenson et al., 2009; Mwine et al., 2011; Belmain et al., 2012; Stevenson et al., 2012; Amoabeng et al., 2013). More recently, field trials testing *Tithonia diversifolia* (Hemsl.) A.Gray (Asteraceae) and *Vernonia amygdalina* Delile (Asteraceae) against field pests of common beans (*Phaseolus vulgaris* L.) demonstrated that extracts were as effective at controlling pest insects as a synthetic pyrethroid (Mkenda et al., 2015a). Extracts of *T. diversifolia* and *V. amygdalina* consist of a range of insecticidal compounds, especially volatile and non-volatile terpenoids (Ganjian et al., 1983; Ambrósio et al., 2008; Adeniyi et al., 2010; Madkour et al., 2013; Mkenda et al., 2015a). Some data report that polyphenolic compounds in *T. diversifolia* extracts inhibit the glutathione-s-transferases of *C. maculatus* (Kolawole et al., 2011) and could explain the lethal effects of the plant extract. Determining the chemical basis of activity in pesticidal plants can inform methods for optimising their use and identify potential candidate compounds for commercialisation (Stevenson et al., 2016). Furthermore, experimentation with the extraction process can alter the yield of compounds and alter efficacy. As part of continuing work on optimising the use pesticidal plants, here, we determine the plant chemistry underlying the biological activity of *T. diversifolia* and

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V. amygdalina and discuss scope for improving application of these species for pest control in Africa.

2. Methods

2.1. Preparation of extracts and fractions

2.1.1. Extraction

Dried leaves from *Vernonia amydalina* and *Tithonia diversifolia* were obtained from the Kilimanjaro Region, northern Tanzania (Latitude 3°13′59.59″S Longitude 37°14′54″E) and voucher specimens were deposited at Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania. Powdered material of each plant was extracted in methanol at a rate of 100 mg of plant material per mL of solvent (10% w/v). Extracts were filtered and the solvent evaporated to yield 2.94 g of dried extract from 25.6 g of *V. amygdalina* and 1.93 g from 24.9 g of *T. diversifolia*. So, each gram (100 mg) of plant material yielded 114.8 mg (11.48 mg) and 77.4 mg (7.74 mg) of dried extract for *V. amygdalina* and *T. diversifolia*, respectively. Samples of dried extract were re-dissolved in methanol to 10% w/v equivalence for bioassay (11.48 and 7.74 mg mL^{-1}) and further diluted to 1% (1.15 and 0.774 mg mL^{-1}) and 0.1% (0.12 and 0.08 mg mL^{-1}) to determine dose effects. Stock-solutions of 100 mg mL^{-1} of dried extract in methanol were used for fractionation.

2.1.2. Fractionation

An HPLC system consisting of a Waters 2695 separations module linked to a 2996 photodiodearray detector (PDAD) was used for fractionation of extracts. Aliquots of *T. diversifolia* extract (200 µL) were injected onto a Phenomenex Luna RP18 column (300 × 10 mm, length × i.d.; 10 µm particle size) and eluted at 4 mL min^{-1} using a linear gradient of 40%A: 10%B: 50%C (t = 0) to 90A: 10B: 0C (t = 20–25 min) returning to the starting conditions (t = 27 min), where A = methanol; B = 1% formic acid in acetonitrile and C = HPLC-water. The fractionation of *V. amygdalina* used a shorter column (150 mm) different initial conditions (A = 30%; B = 10%; C = 60%) and a non-linear gradient (Waters, curve = 7) to enhance the separation of compounds with similar retention times. The quantity of material injected and the proportion of each fraction in a 10% w/v extract was calculated. The fractions contributed from 0.17 (F2) to 0.50 mg mL^{-1} (F4) (*V. amygdalina*) and from 0.08 (F3) to 0.49 mg mL^{-1} (F1) (*T. diversifolia*)

2.2. Experimental

2.2.1. Insects

Callosobruchus maculatus were a wild Ghanaian strain, originally collected in 1995. They were housed in a temperature controlled room (28 ± 1 °C, 55% RH) that was kept in permanent darkness. Adults laid eggs on cowpea seeds *Vigna unguiculata* and 24–28 days later the next generation of adults emerged from the beans. The insects used for bioassays were 3–5 days post-emergence.

2.2.2. Bioassay procedure

Fractions and residue were dissolved to concentrations representing their proportions in 10, 1 and 0.1% w/v extracts of each plant species. Aliquots (75 µL) of compounds, extracts, methanol (negative control) or rotenone (1000 and 100 ppm, positive control) were evaporated onto vials (25 mL, nominal capacity) under a stream of air and with constant rotation of the vial. Insects (N = 5-12) were added to the vials, ensuring a ratio of at least 1:1 (male to female). 5 black eyed beans were added to each vial after 72 h. After a further 72 h mortality was recorded. The numbers of eggs laid on both the vials and the beans were counted and from these data the eggs laid per female were calculated. *ANOVA* followed by *Tukey's HSD* post hoc test (95% C.I.) were used to compare the mortality and eggs laid among and between treatments at equivalent concentrations (XLSTAT version 2015.1.03.16409).

2.3. Analyses

2.3.1. LC–MS

Accurate mass measurements of compounds detected in the extracts were obtained using an LTQ Orbitrap XL, linear ion trap/orbitrap hybrid mass spectrometer (Thermo Scientific, San Jose, California USA) with an electrospray ionisation source (Ion Max, Thermo Scientific) coupled to an "Acella 1250" UPLC system (Thermo Scientific). Samples were injected onto a Phenomenex Luna C18(2) column (150 × 3 mm i.d., 3 µm particle size) at 400 µL min^{-1} and eluted using a linear gradient of 90:0: 10 (t = 0 min) to 0:90:10 (t = 20–25 min), returning to 90:0:10 (t = 27–30 min). Solvents were water, methanol and 1% formic acid in acetonitrile, respectively. The column was maintained at 30 °C. Samples were scanned, using FTMS, from *m/z* 250–2000 in both
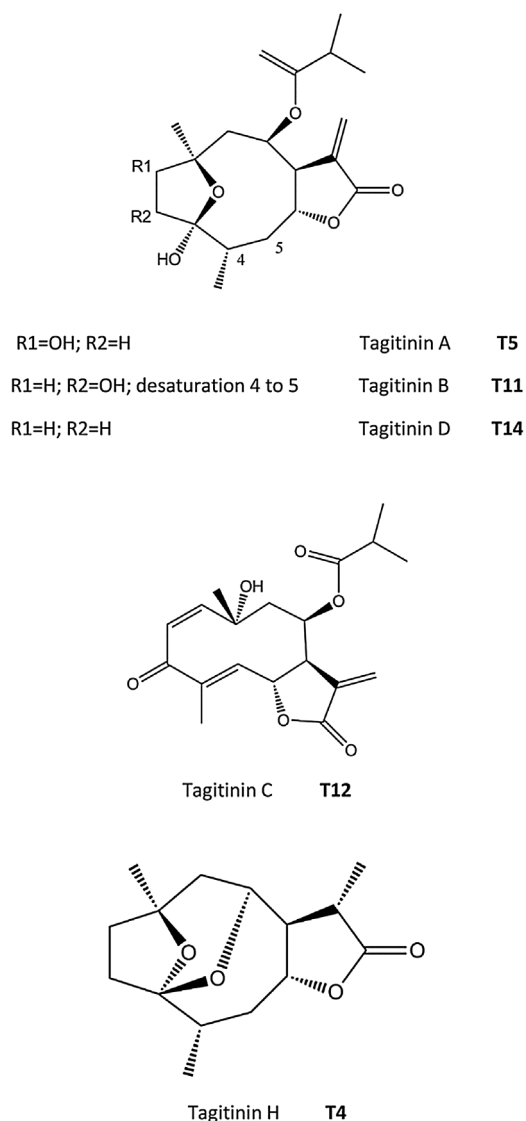
Table 1

Mortality and total eggs laid by *C. maculatus* when exposed to different concentrations of fractions prepared from extracts of *Tithonia diversifolia* and *Vernonia amygdalina* for 6 days.[#]

Treatment [ppm for extracts]	LSD mean Mortality	LSD mean eggs laid, per female
EXTRACTS		
Tith 10% [11,480]	92.611 abcd	23.678 abc
Tith 1% [114.8]	92.424 abcd	20.270 abcde
Tith 0.1% [11.5]	77.027 def	17.507 abcdef
Vern 10% [7740]	90.238 abcd	19.378 abcde
Vern 1% [774]	89.841 abcd	15.735 abcdefg
Vern 0.1% [7.7]	82.567 abcde	20.105 abcde
FRACTIONS		
Tith F1 (Tagitinin A) 488 ppm	60.462 f	23.467 abc
Tith F1 (Tagitinin A) 48.8 ppm	100.000 a	17.027 abcdef
Tith F1 (Tagitinin A) 4.88 ppm	98.750 ab	13.088 bcdefg
Tith F2 95.2 ppm	98.571 ab	2.383 g
Tith F2 9.52 ppm	95.123 abcd	12.520 bcdefg
Tith F2 0.952 ppm	98.000 abc	17.113 abcdef
Tith F3 80.5 ppm	90.059 abcd	11.677 cdefg
Tith F3 8.05 ppm	85.844 abcd	9.233 defg
Tith F3 0.81 ppm	100.000 a	14.960 abcdefg
Tith F4 295.7 ppm	100.000 a	13.428 bcdefg
Tith F4 29.57 ppm	87.071 abcd	20.977 abcd
Tith F4 2.96 ppm	96.333 abcd	16.597 abcdef
Tith F5 260.1 ppm	95.500 abcd	13.292 bcdefg
Tith F5 26 ppm	93.389 abcd	18.543 abcdef
Tith F5 2.6 ppm	95.794 abcd	6.667 efg
Vern F1 265.1 ppm	91.813 abcd	20.183 abcde
Vern F1 26.5 ppm	89.143 abcd	16.793 abcdef
Vern F1 2.65 ppm	93.294 abcd	13.977 abcdefg
Vern F2 173.3 ppm	87.908 abcd	14.985 abcdefg
Vern F2 17.3 ppm	90.806 abcd	20.205 abcde
Vern F2 1.73 ppm	78.063 cdef	19.188 abcdef
Vern F3 179 ppm	84.149 abcde	23.045 abc
Vern F3 17.9 ppm	81.452 abcde	22.340 abcd
Vern F3 1.79 ppm	79.852 abcdef	18.155 abcdef
Vern F4 501.5 ppm	79.159 bcdef	20.692 abcd
Vern F4 50.1 ppm	83.357 abcde	24.185 abc
Vern F4 5.0 ppm	90.060 abcd	20.187 abcde
Vern F5 355.79 ppm	78.927 bcdef	27.630 a
Vern F5 35.58 ppm	86.440 abcd	20.823 abcd
Vern F5 3.56 ppm	65.204 ef	26.203 ab
Rotenone 1000 ppm	97.571 abc	15.020 abcdefg
Rotenone 100 ppm	97.778 abc	5.588 fg
Control	20.453 g	20.562 abcd
SEM	2.485	3.647
p > F	0	0
Significant	yes	yes

[#] Values with the same letter are not different Tukey's *post hoc* HSD-test (95% C.I.). Tith = *Tithonia*; Vern = *Vernonia*; F1, F2 etc = fraction number, followed by the concentration of the sample applied to the vials, as a w/v percentage equivalent for extracts, or in parts per million for the compound and fractions.

R1=OH; R2=H   Tagitinin A   **T5**

R1=H; R2=OH; desaturation 4 to 5   Tagitinin B   **T11**

R1=H; R2=H   Tagitinin D   **T14**

Tagitinin C   **T12**

Tagitinin H   **T4**

Fig. 1. Tagitinins identified in fractions from *T. diversifolia*[#].
[#] letters in bold after each compound name correspond to the compound number in Table 2.

R=H   homohesperitin   **T3**
R=glc-rha   homohesperitin-7-rutinoside   **V32**

R1= O-xyl; R2=H, R3=H; R4=H   luteolin-3 -xyloside   **V3**
R1=OCH₃; R2=H; R3=OH; R4=H   luteolin-3 -methyl ether   **V17**
R1=OH; R2=glc; R3=H; R4=H   cymaroside   **V2**
R1=H; R2=H; R3=OH; R4=H   luteolin   **V15**
R1=H; R2=OH; R3=H; R4=OCH₃   hispidulin   **T8**

Fig. 2. Flavones and related compounds tentatively identified from *T. diversifolia* and *V. amygdalina*[#].
[#] letters in bold after each compound name correspond to the compound number in Tables 2 and 3.

3. Results

3.1. Bioassays

Crude extracts of *T. diversifolia* and *V. amygdalina* were toxic to *C. maculatus* in a dose dependent manner, with *T. diversifolia* generally more toxic than *V. amygdalina* (Table 1). However, the crude extracts showed no significant effects on oviposition, even at the highest dose tested (Table 1; *ANOVA*, P > 0.05). All chemical fractions from both plant species were toxic in comparison to the untreated control. Many fractions showed comparably high mortality as that achieved with the standard, rotenone, particularly fractions 1, 2 and 5 from *T. diversifolia* and fractions 1, 2 and 4 from *V. amygdalina* (Table 1). Fractions 2 and 5 from *T. diversifolia* inhibited oviposition; however, no fractions from *V. amygdalina* reduced oviposition significantly (Table 1).

3.2. Characterisation of compounds

Fraction 1 (F1) from *T. diversifolia* recorded a single peak in the LC–MS chromatogram occurring after 10.1 min and which consisted of three major ions with *m/z* 413.18054 [M + HCOO]⁻; 781.36365 [2 M + HCOO]⁻ and 367.17517 [M − H]⁻, calculating for the molecular formula $C_{19}H_{28}O_7$ and corresponding to tagitinin A (Fig. 1). The NMR data were broadly in agreement with published data for tagitinin A in acetone-d_6 (Glaser et al., 2005), and in CDCl₃ (García et al., 2006). In acetone-d_6 no evidence of isomerisation was observed based on an additional 1D proton spectra after the acquisition of all the other spectra (1D ¹³C and 2D). The complete signal assignment is presented in Table S2.

Compounds were identified from other fractions of *T. diversifolia* and *V amygdalina* using accurate mass measurements (*m/z*) of major ions to calculate molecular formulae for compounds known from these or related species. Three classes of compounds were identified in fractions from *T. diversifolia*. Flavones: homohesperitin (F2) and hispidulin

2.3.2. NMR

NMR spectra were acquired in acetone-d_6 at 30 °C on a Bruker Avance 400 MHz instrument, with a 5 mm BBO probe. Standard pulse sequences and parameters were used to obtain one-dimensional ¹H and ¹³C spectra. A two-dimensional NOE spectrum was obtained in phase-sensitive mode with HDO presaturation and a mixing time of 800 ms. The Bruker microprograms were used for COSY (90 ° flip pulse), HSQC (multiplicity-edited) and HMBC (optimised to $^nJ_{CH}$ = 8 Hz) experiments, with appropriate adjustments in gradient-selection mode. Chemical shifts were referenced to tetramethylsilane as the internal standard.
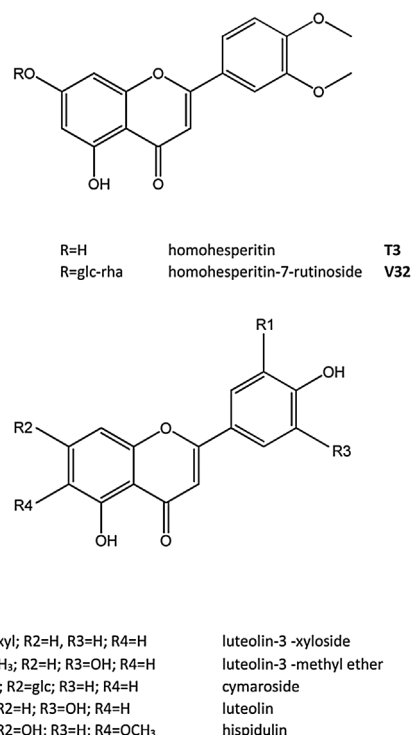
positive and negative modes. Samples were first matched with those reported from *T. diversifolia* and *V. amygdalina* in the Combined Chemical Dictionary (CCD, 2017), by using the *m/z* to calculate putative molecular formulae. Where compounds could not be matched to compounds known from these two species the search was extended first to the generic level, then to the Asteraceae and finally to other sources.

Table 2
Summary of analyses of fractions from *Tithonia diversifolia*.

Compound	Retention time	m/z, FTMS (relative intensity)	Molecular formula of compound and identification	Fraction(s)	Additional information
T1	8.31	187.09773⁻	$[C_9H_{15}O_4]^-$, not identified	5	
T2	9.64	241.10835 [M-H]⁻	$[C_{12}H_{17}O_5]^-$, not identified	5	
T3	9.82	315.05048 [M-H]⁻	$C_{17}H_{16}O_6$, homohesperitin	2	UV = 235, 250sh, 270sh, 346 nm
T4	9.99	267.12198 [M+H]⁺	$C_{15}H_{22}O_4$, tagitinin H	5	
T5	10.08	413.18170 [M+FA-H]⁻	$C_{19}H_{28}O_7$, tagitinin A	1, 2	
T6	10.52	413.18112 [M+FA-H]⁻	$C_{19}H_{28}O_7$, 2α-hydroxyrotundin or 1,4-epoxy-1,3,8-trihydroxy-11(13)-germacren-12,6-olide; (1α,3β,6β,8α,10α)-form, 8-O-(2-Methylpropanoyl).	2	1,4-epoxy- isolated from *Picrasma javanica* (Simaroubaceae)
T7	10.59	411.16559 [M+FA-H]⁻	$C_{19}H_{26}O_7$, 4,8,10-Trihydroxy-3-oxo-11(13)-guaien-12,6-olide; (1α,4β,5α,6α,8β,10β)-form, 8-O-(2-Methylpropanoyl)	4	
T8	11.16	299.05508 [M-H]⁻	Hispidulin	3	UV = 236, 273sh, 335 nm
T9	11.41	413.18167 [M+FA-H]⁻	$C_{19}H_{28}O_7$, 2α-hydroxyrotundin or 1,4-epoxy-1,3,8-trihydroxy-11(13)-germacren-12,6-olide; (1α,3β,6β,8α,10α)-form, 8-O-(2-Methylpropanoyl).	2	1,4-epoxy- isolated from *Picrasma javanica* (Simaroubaceae)
T10	11.53	409.15063 [M+FA-H]⁻	$C_{19}H_{24}O_7$, 8-Hydroxy-4-oxo-3,4-seco-11(13)-guaiene-3,10:12,6-diolide; (1α,5α,6α,8β,10α)-form, 8-O-(2-Methylpropanoyl)	5	
T11	11.84	411.16574 [M+FA-H]⁻	$C_{19}H_{26}O_7$, tagitinin B	2	
T12	11.87 and 11.91	393.15503 [M+FA-H]⁻	$C_{19}H_{24}O_6$, tagitinin C	3, 4, 5	
T13	12.17	425.18112 [M+FA-H]⁻	$C_{20}H_{28}O_7$, 2-O-methyl tagitinin B, tirotundifolin E, tithonin or 3,10-epoxy-1,3,8-trihydroxy-4,11(13)-germacradien-12,6-olide; (1β,3α,4Z,6α,8β)-form, 3-Me ether, 8-O-(2-methylpropanoyl)	2	
T14	12.59	351.14456 [M-H]⁻	$C_{19}H_{28}O_6$, tagitinin D	3	
T15	12.84 and 12.86	379.17642 [M-H]⁻	$C_{20}H_{28}O_7$, 2-O-methyl tagitinin B, tirotundifolin E, tithonin or 3,10-epoxy-1,3,8-trihydroxy-4,11(13)-germacradien-12,6-olide; (1β,3α,4Z,6α,8β)-form, 3-Me ether, 8-O-(2-methylpropanoyl)	2, 4	
T16	14.17 to 14.20	381.175328 [M-H]⁻	$C_{20}H_{28}O_7$, 2-O-methyl tagitinin B, tirotundifolin E, tithonin or 3,10-epoxy-1,3,8-trihydroxy-4,11(13)-germacradien-12,6-olide; (1β,3α,4Z,6α,8β)-form, 3-Me ether, 8-O-(2-methylpropanoyl)	2, 3, 4	

Table 3
Summary of analyses of fractions from *Vernonia amygdalina*.

Compound	Retention time	*m/z*, FTMS (relative intensity)	Molecular formula of compound	Fraction	Additional information, UV absorption in nm
V1	5.34	363.14377 $[M + H]^+$	$C_{19}H_{22}O_7$, 11,13-dihydrovernodalin	1	
V2	6.78	449.10696 $[M + H]^+$	$C_{21}H_{20}O_{11}$, cynaroside (luteolin-7-glucoside)	1, 3	UV = 235, 253, 266sh, 348 nm
V3	6.91	461.07196 $[M-H]^-$	$C_{21}H_{18}O_{12}$, luteolin (3′, 5 or 7-hexuronide)	1	UV = 243, 347 nm
V4	7.59	377.12354 $[M-H]^-$	$C_{19}H_{22}O_8$, vernodalinol	1	UV = 290sh, 326 nm
V5	7.68	515.11890 $[M-H]^-$	$C_{23}H_{32}O_{13}$, dimethyl, dihexosyl ester of caffeic acid	1	UV = 290sh, 328 nm
V6	8.17	859.43347 $[M + FA-H]^-$	$C_{41}H_{66}O_{16}$, a vernocuminoside	5	
V7	8.25	425.14484 $[M + FA-H]^-$	$C_{19}H_{24}O_8$, seven possible configurations known from *Vernonia* spp.	1	
V8	8.55	859.43384 $[M + FA-H]^-$	$C_{41}H_{66}O_{16}$, a vernocuminoside	5	
V9	8.66	429.17676	$[C_{20}H_{29}O_{10}]^-$, not identified	2	
V10	8.7	437.14417 $[M + FA-H]^-$	$C_{20}H_{24}O_8$, vernodalol	1	
V11	9.10, 9.11	709.34448 $[M + FA-H]^-$	$C_{35}H_{52}O_{12}$, vernonioside D	4, 5	
V12	9.40	843.60394 $[M + FA-H]^-$	$C_{41}H_{66}O_{15}$, a vernocuminoside	4, 5	
V13	9.48	665.27881 $[M + H]^+$	$C_{35}H_{52}O_{12}$, a vernonioside	4	
V14	9.66, 9.69	843.47894 $[M + FA-H]^-$	$C_{41}H_{66}O_{15}$, a vernocuminoside	4, 5	
V15	9.75	285.03992 $[M-H]^-$	$C_{15}H_{10}O_6$, luteolin	2	UV = 236, 253sh, 266sh, 348 nm
V16	9.97	693.35400 $[M + FA-H]^-$	$C_{35}H_{52}O_{11}$, six possible configurations known from *Vernonia*, mainly vernoniosides but including a vernoamyoside.	4	
V17	9.99	299.04455 $[M-H]^-$	$C_{16}H_{12}O_6$, luteolin methyl ether	2	UV = 235, 283, 335 nm
V18	10.75	735.36096	$[C_{38}H_{55}O_{14}]^-$, not identified	3	UV = 236, 243 nm
V19	10.93	633.25232 $[M + H]^+$	$C_{35}H_{52}O_{10}$, a vernonioside	4	
V20	10.98	651.37311 $[M + H]^+$	$C_{35}H_{54}O_{11}$, a vernonioside	3	UV = 236, 250, 330 nm. Possible saturation of a single bond to increase MW by two hydrogens, from known compounds, such as vernonioside A_4
V21	11.1, 11.12	693.34894 $[M + FA-H]^-$	$C_{35}H_{52}O_{11}$, a vernonioside	2, 3	UV = 240, 250 nm
V22	11.36, 11.38	677.35510 $[M + FA-H]^-$	$C_{35}H_{52}O_{10}$, a vernonioside	3, 4, 5	UV = 240, 243 nm
V23	11.61	677.35425 $[M + FA-H]^-$	$C_{35}H_{52}O_{10}$, a vernonioside	3	UV = 243, 246 nm
V24	11.73	825.42908 $[M + FA-H]^-$	$C_{41}H_{64}O_{14}$, a vernonioside	5	
V25	11.95	675.33826 $[M + FA-H]^-$	$C_{35}H_{50}O_{10}$, a vernonioside	3	UV = 240, 246 nm
V26	12.07	911.42392 $[C_{45}H_{67}O_{19}]^-$ (100) 681.38623 $[M-H]^-$ (10)	$C_{36}H_{58}O_{12}$, a vernonioside	5	Possible saturation of a single bond to increase MW by two hydrogens, from known compounds, such as vernonioside B_2
V27	12.28	677.47559 $[M + FA-H]^-$	$C_{35}H_{52}O_{10}$, a vernonioside	4	UV = 238, 243 nm
V28	12.37	411.16620 $[M-H]^-$	$C_{19}H_{24}O_{10}$, four possible 12, 6-germacronolides	2	
V29	12.50	725.37634 $[M + FA-H]^-$	$C_{36}H_{56}O_{12}$, a vernonioside	5	
V30	12.84	675.49408 $[M + H]^+$	$C_{37}H_{54}O_{11}$, a vernonioside	4	
V31	13.32	677.47314 $[M + FA-H]^-$	$C_{35}H_{52}O_{10}$, a vernonioside	4, 5	UV = 238, 243 nm
V32	14.41	661.35931 $[M + FA-H]^-$	$C_{29}H_{36}O_{15}$, homohesperitin 7-rutinoside	5	

(F3) (Fig. 2); sequiterpene lactones: tagitinins A (F1 and F2), B (F2), C (F3, 4 and 5), D (F3) and H (F5) (Fig. 1) together with germacren-12,6-olides (F2 to F5) and a guiaiene-12,6-olide (F4) (Table 2). Ten compounds were tentatively identified in fractions of *V. amygdalina*. These compounds included sesquiterpenes (11,13-dihydrovernodalin, F1) (Fig. 3); sesquiterpene lactones (vernodalinol and vernodalol, F1) (Fig. 3); flavones (cynaroside, F1 and F3; luteolin hexuronide, F1; luteolin and luteolin methyl ether, F2; homohesperitin 7-rutinoside, F5) (Fig. 2) and a dimethyl, dihexosyl ester of caffeic acid (F1) (Fig. 4; Table 2). Due to the structural similarity between many of the remaining compounds in F3 to F5 they were categorized into structural classes, such as vernoniosides (F3 to F5) and less polar vernocuminosides/vernoamyosides (F4 and F5) (Fig. 5; Table 2).

4. Discussion

The data presented here showed that extracts of *T. diversifolia* and *V. amygdalina* were toxic to *C. maculatus,* and that this activity was at least in part explained by sesquiterpene lactones in the whole extracts and chromatographically separated fractions. Biological activity in these plant species has been reported before where extracts of *T. diversifolia* suppressed populations of a range of insects and fungal pathogens under field conditions (Owolade et al., 2004), common bean field pests (Mkenda et al., 2015b), leaf-cutting ants (Castaño-Quintana et al., 2013) and deterred feeding of *Chlosyne lacinia* (Geyer) (Lepidoptera: Nymphalidae) larvae (Ambrósio et al., 2008). *V. amygdalina* oils and extracts have been shown to be toxic to stored product pests (Asawalam

et al., 2008; Adeniyi et al., 2010) and both repellent and toxic to *Spodoptera exempta* (Walker) (Lepidoptera: Noctuidae) larvae (Ganjian et al., 1983).

Our research showed that methanol extracts of both plant species were equally effective against bruchids whereas earlier work (Mkenda et al., 2015a) reported extracts of *T. diversifolia* to be less insecticidal than extracts of *V. amygdalina* on a range of field pests of common beans. Besides the different target pest species, the observed differences may be partly explained by the method of extraction. Mkenda et al. (2015b) extracted plants in water with 0.1% soap for field crop application whereas in the present work methanol was used and may have extracted biologically active non-polar compounds from *T. diversifolia* more effectively. Fractionation produces mixtures of compounds of varying polarity that are usually as toxic as the complete extract at equivalent concentrations. Extracts and fractions are broadly as effective as the known insecticidal compound, rotenone which was used as a positive control. It has been reported that 150 different compounds have been isolated from *T. diversifolia* (Zhao et al., 2012). In the Combined Chemical Dictionary, 22 compounds are listed from *T. diversifolia* and 14 from *V. amygdalina* (CCD, 2017) although this generally excludes the more ubiquitous compounds, such as the luteolin derivatives that we have identified. The extracts present pest insects with multiple chemical challenges. Furthermore, there is evidence that extracts are useful as antimicrobials (Erasto et al., 2006; Orsomando et al., 2016) and are toxic to parasitic protozoa (De Toledo et al., 2014; Abay et al., 2015) which would make them more useful to rural communities, particularly as they are easily cultivated. *T. diversifolia* is a
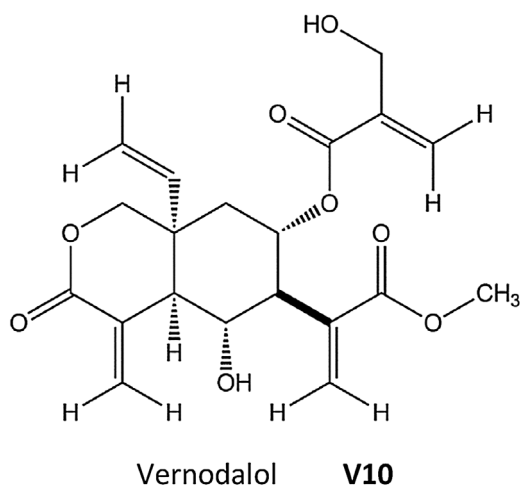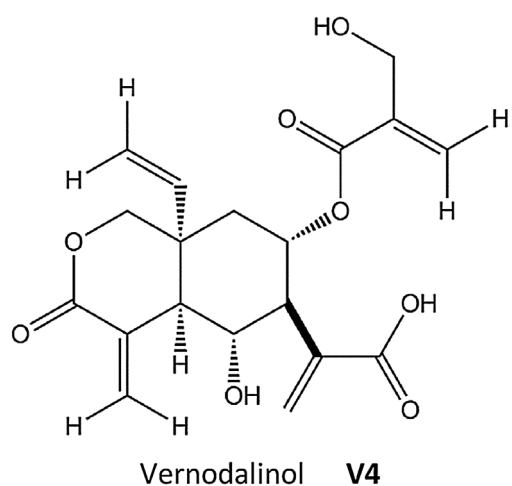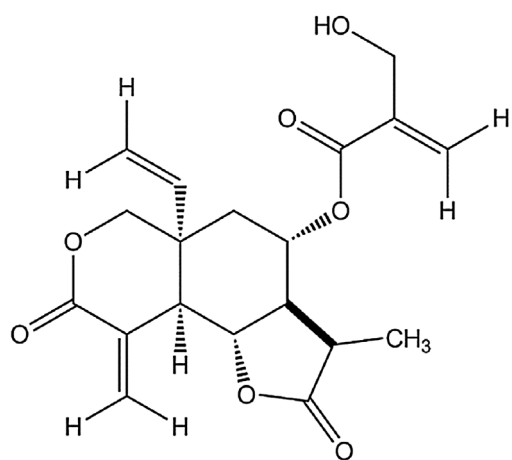
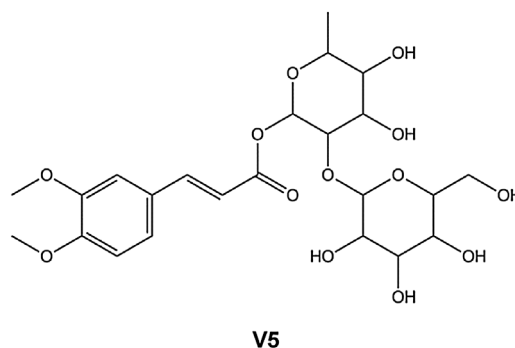11, 13-dihydrovernodalin   **V1**

V5

Fig. 4. Dimethyl-, dihexosyl caffeic acid tentatively identified in fractions from *V. amygdalina*[#].

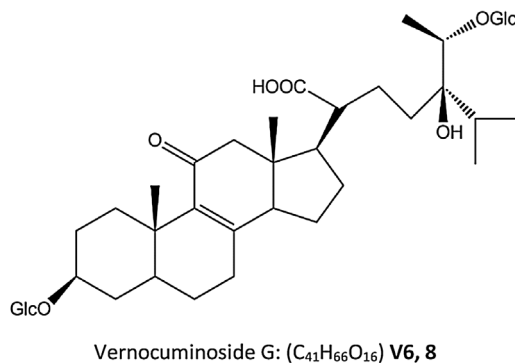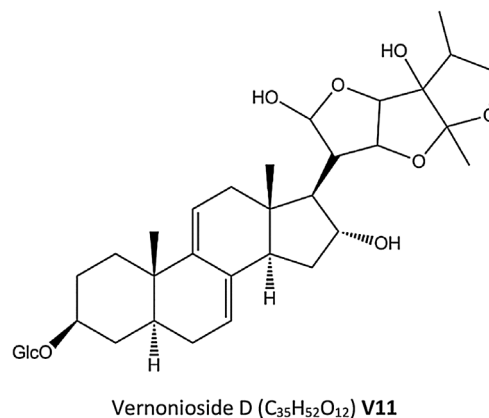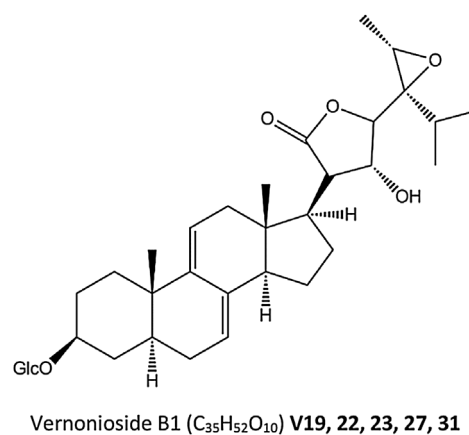[#] letters in bold correspond to the compound number in Table 3.

Vernodalinol   **V4**

Vernonioside B1 ($C_{35}H_{52}O_{10}$) **V19, 22, 23, 27, 31**

Vernonioside D ($C_{35}H_{52}O_{12}$) **V11**

Vernodalol   **V10**

Fig. 3. Sesquiterpene lactones tentatively identified in fractions from *V. amygdalina*[#].

[#] letters in bold after each compound name correspond to the compound number in Table 3.

globally invasive weed (Yang et al., 2012) and a particular problem in Africa (Henderson 2007) where its collection and use as a pesticide could help reduce its environmental impacts. Alternatively, flowering field margin plants can be important for providing food and refuge for beneficial insects (Mkenda et al., 2015b; Gurr et al., 2017), and pesticidal plants grown in field margins could support ecosystem services of natural enemies and pollinators.

Vernocuminoside G: ($C_{41}H_{66}O_{16}$) **V6, 8**

Fig. 5. Vernoniosides and a vernocuminoside tentatively identified in fractions from *V. amygdalina*[#].

[#] letters in bold correspond to the compound numbers in Table 3.

5. Conclusion

Along with evidence from earlier published work (Ambrósio et al., 2008; Mkenda et al., 2015b, b), we conclude that the biological activity of the sesquiterpene tagitinin A presents a potential target molecule for commercialisation (Dutta et al., 1986) However, the non-target toxicity of this compound must not be overlooked, where further research is required to ensure safety (Passoni et al., 2013). Pesticidal plants can vary in their efficacy due to genetic or environmental differences (Belmain et al., 2012; Stevenson et al., 2012). A combination of chemotyping plants together with laboratory and field trials could help determine the conditions that maximise the quality and quantity of insecticidal components. This approach, emphasised by Isman and Grieneisen (2014) would help explain variability, while increasing efficacy and uptake of effective pesticidal plants by farmers.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.indcrop.2017.08.021.

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