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Gynandropsis gynandra essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) repellents

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Abstract

The repellency of the essential oil of the previously reported anti-tick pasture shrub *Gynandropsis gynandra* and identified constituents of the oil were evaluated against the livestock tick, *Rhipicephalus appendiculatus*. In a tick-climbing repellency bioassay, the oil of *G. gynandra* exhibited a repellency effect which at the highest treatment levels was higher than that of the commercial arthropod repellent *N*,*N*-diethyltoluamide. Twenty eight compounds were identified in the *G. gynandra* oil by GC, GC-MS and coinjection with authentic samples. Carvacrol was found to occur in largest quantity (29.2%), followed by *trans*-phytol (24.0%), linalool (13.3%), *trans*-2-methylcyclopentanol (7.2%) and *β*-caryophyllene (4.4%). *m*-Cymene, nonanal, 1-*α*-terpineol, *β*-cyclocitral, nerol, *trans*-geraniol, carvacrol, *β*-ionone, *trans*-geranylacetone, and nerolidol were the most repellent components against *R. appendiculatus*. Methyl isothiocyanate which occurred in the *G. gynandra* oil at a relative percentage of 2.1 and which was not tested in the bioassay due to its toxicity may also contribute significantly to the repellency of the oil. The repellency of the oil of *G. gynandra* supported earlier findings by other workers that *G. gynandra* repelled *R. appendiculatus* ticks.

Keywords: *Gynandropsis gynandra*; Capparidaceae; *Rhipicephalus appendiculatus*; Repellents; Tick; Essential oil; Anti-tick pasture; Brown-ear tick

1. Introduction

The livestock (brown ear) tick, *Rhipicephalus appendiculatus* Neumann (Acari: Ixodidae) is a pest of major economic importance in Africa where it is the vector of the pathogen, *Theileria parva* (Apicomplexa: Theileriidae) which causes East Coast fever in animals. Control of *R. appendiculatus* is currently accomplished almost entirely by short-interval use of synthetic acaricides which are applied in plunge dips, dusts, or sprays, or released from plastic tags and collars. These methods are not very satisfactory due to the high cost of acaricides, the logistics of repeated treatment, the development of acaricide-resistant tick strains and contamination of milk, meat and the environment by the acaricides.

The use of anti-tick plants which repel, immobilize or kill the free-living stages of ticks, has been proposed as a possible tick control method (Beesley, 1982; Sutherst, Jones, & Schnitzerling, 1982; Sutherst & Wilson, 1986; Wilson & Sutherst, 1986; Sutherst, Wilson, Reid, & Kerr, 1988; Wilson, Sutherst, & Kerr, 1989; Dipeolu, Mongi, Punyua, Latif, Amoo, & Odhiambo, 1992; Malonza, Dipeolu, Amoo, & Hassan, 1992). Molasses grass, *Melinis minutiflora*, has been shown to reduce tick survival (Menendenz, 1924; Thompson, Roa, & Romero, 1978), and some tropical pasture legumes of the genus *Stylosanthes*, produce sticky secretions that immobilize and kill larvae of ticks (Sutherst et al., 1982; Sutherst & Wilson, 1986; Wilson & Sutherst, 1986; Sutherst et al., 1988; Wilson et al., 1989; Zimmerman, Garris, & Beaver, 1984). The tick-repellent and acaricidal properties of the East African shrub *Gynandropsis gynandra* (Capparidaceae) have also been demonstrated and the plant proposed for use as an anti-tick pasture plant (Dipeolu et al., 1992; Malonza et al., 1992).

G. gynandra was shown to exhibit repellent and acaricidal properties to larvae, nymphs and adult *R.*

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appendiculatus and *Amblyomma variegatum* ticks (Malonza et al., 1992). Field investigations indicated that ticks were not found at distances up to 2–5 m from the plant in areas where the plant was predominant (Malonza et al., 1992). *G. gynandra* is a branched and rather stout annual herb that grows up to 1 m tall and is common in the tropical and subtropical climatic regions (Elfers, Graham, & Dewolf, 1964; Grainage & Ahmed, 1988). The leaves are used as food for humans and as a vegetable, and are reported to have a high leaf protein content (Imbamba, 1973). The oil has been used as a repellent against headlice, *Pediculus humanus capitis* (Siphunculata: Pediculidae) and as a general vermicide in hairdressing (Mitchell & Breyer-Brandwijk, 1962; Jacobson, 1975) while the seeds and oil have been reported to be anti-nematode (Usher, 1973). The petroleum ether extract at 2% concentration was reported to cause 100% mortality to insect pests of the cruciferous painted bug, *Bagrada cruciferanum* (Verma & Pandey, 1982). The plant has also been used in traditional medicine for the treatment of rheumatism, headache, epileptic fits, stomachache, conjunctivitis, stiffneck, scurvy, earaches and severe infection of threadworms (Mitchell & Breyer-Brandwijk, 1962; Kokwaro, 1976). The whole plant has been reported to be a fish poison, alkaloids being stipulated to be the active principles (Smolenski, Silinis, & Farnsworth, 1975). There is, however, no previous report on the identification of constituents of the essential oil of *G. gynandra* and on tests of the repellency of the oil and its constituents to arthropods.

We have recently reported on a study of the repellency of *C. monophylla* essential oil and its identified constituents against the livestock tick, *R. appendiculatus* and the maize weevil, *Sitophilus zeamais* (Ndungu, Lwande, Hassanali, Moreka, & Chhabra, 1995). *C. monophylla* belongs to the same family (Capparidaceae) as *G. gynandra*. In the present study, we report on the identification of constituents of the *G. gynandra* essential oil and on the repellency of the oil and authentic samples of the identified constituents against the livestock (brown ear) tick, *R. appendiculatus*.

2. Results and discussion

The oil of *G. gynandra* obtained by hydrodistillation showed repellency against the livestock tick, *R. appendiculatus*, in our tick-climbing repellency bioassay (Ndungu et al., 1995).

Table 1 gives the percentage repellency of *G. gynandra* oil at four different treatment levels. At the higher treatment levels (0.1 and 0.01 μl) the percentage repellency of the oil was comparable to that of the commercial arthropod repellent *N*,*N*-diethyl-toluamide (DEET). However, as the treatment level decreased, the percentage repellency of the oil was slightly lower than that of DEET.

The essential oil of *G. gynandra* was analyzed by GC and GC-MS. The constituents of the essential oil were identified by MS and their identity confirmed by comparison of their mass spectra and retention times with those of authentic samples. Of the compounds identified (Table 1), carvacrol occurred in largest quantity (29.2%) followed by *trans*-phytol (24.0%), linalool (13.3%), *trans*-2-methyl cyclopentanol (7.2%) and *β*-caryophyllene (4.4%).

Authentic samples of the compounds identified in the volatile oil of *G. gynandra* were tested for repellency against *R. appendiculatus*. Table 1 gives the percentage repellencies of the compounds against *R. appendiculatus* at four different treatment levels. However, methyl isothiocyanate, which occurred in the oil at a relative percentage of 2.1, was not tested in the tick-climbing repellency bioassay due to its toxicity. Of the compounds tested, the most active components were *m*-cymene, nonanal, 1-α-terpeneol, α-cyclocitral, β-cyclocitral, nerol, *trans*-geraniol, carvacrol, α-ionone, *trans*-geranyl acetone, nerolidol and cedrene, all of which had repellencies comparable to that of DEET at the higher treatment levels (Table 1). Next in hierarchy was benzaldehyde, phenyl acetaldehyde, β-ocimene, linalool, phenyl acetonitrile and methyl salicylate.

The results of this study support the previous report by Malonza et al. (1992) that *G. gynandra* is repellent to *R. appendiculatus*. The fact that the repellency of the oil of *G. gynandra* is comparable to that of the commercial arthropod repellent, DEET, at all the treatment levels except the lowest one (0.001 μl) indicates that this plant, in fact, has strong tick repellent properties. The essential oil of *G. gynandra* is also more repellent than that of the related species, *C. monophylla*, the repellency of which we have recently reported on (Ndungu et al., 1995).

Methyl isothiocyanate which occurred in the *G. gynandra* oil at a relative percentage of 2.1 was not tested in the tick repellency bioassay due to its toxicity. However, in view of the fact that it has been found to be toxic to other arthropods (Seck et al., 1993), it is likely to contribute significantly to the repellency of the *G. gynandra* oil to *R. appendiculatus*. Nevertheless, the repellent compounds identified from the *G. gynandra* oil in this study must be playing a dominant role in the repellency of the plant to *R. appendiculatus*. Methyl isothiocyanate has also been reported to be liberated by the shrub *Boscia senegalensis* (Pers) Lam. (Capparidaceae) and to be responsible for the toxicity of *B. senegalensis* to several stored grain pests (Seck et al., 1993).

As in the case of *C. monophylla* (Ndungu et al., 1995), the repellent action of the *G. gynandra* oil is due

Table 1
Composition of essential oil from *Gynandropsis gynandra* Oliv. and mean percentage repellencies (±S.E) of some of the identified compounds, the oil of *G. gynandra*, and *N*,*N*-diethyltoluamide (DEET) to *Rhipicephalus appendiculatus*

Compound	Relative (%)	Treatment (μl)			
		0.1	0.01	0.001	0.0001
G. gynandra		98.9 ± 0.0^{a}	89.8 ± 0.0^{a}	70.5 ± 3.6^{a}	50.5 ± 0.0^{b}
DEET		84.0 ± 3.9^{a}	82.8 ± 3.6^{a}	75.6 ± 4.5^{b}	70.5 ± 3.6^{a}
Methyl isothiocyanate	2.1	–	–	–	–
trans-2-Methyl cyclopentanol	7.2	33.4 ± 2.0^{fg}	28.6 ± 2.0^{gh}	24.1 ± 1.5^{efg}	20.8 ± 1.1^{fgh}
cis-3-Hexen-1-ol	1.0	26.5 ± 2.1^{h}	22.0 ± 1.6^{i}	14.8 ± 2.5^{i}	7.2 ± 2.2^{i}
trans-2-Hexen-1-ol	1.0	41.2 ± 4.0^{de}	25.4 ± 3.0^{hi}	19.2 ± 2.9^{hi}	17.9 ± 2.9^{h}
Heptan-2-one	0.3	45.1 ± 4.0^{d}	27.8 ± 3.0^{gh}	14.5 ± 1.4^{gi}	11.8 ± 1.7^{gh}
Anisole	0.4	-6.6 ± 2.2^{i}	0.0 ± 0.0^{k}	0.0 ± 0.0^{j}	0.0 ± 0.0^{l}
Benzaldehyde	0.4	71.5 ± 3.1^{b}	40.7 ± 2.5^{de}	0.0 ± 0.0^{j}	0.0 ± 0.0^{l}
2,4,5-Trimethyl-thiazole	0.4	40.0 ± 1.3^{d}	25.7 ± 2.0^{hi}	20.3 ± 1.8^{ghi}	26.2 ± 0.9^{cde}
Phenyl-acetaldehyde	0.7	87.9 ± 1.4^{a}	33.3 ± 1.4^{fg}	$25.6 \pm 1.4d^{efg}$	19.2 ± 1.2^{h}
m-cymene	0.6	90.0 ± 0.0^{a}	83.4 ± 2.0^{b}	31.7 ± 2.5^{def}	24.2 ± 1.0^{efg}
d-Limonene	0.3	27.2 ± 2.6^{gh}	16.2 ± 1.2^{j}	5.1 ± 1.4^{j}	1.7 ± 1.1^{kl}
β-Ocimene	0.4	77.8 ± 2.9^{b}	40.0 ± 1.7^{de}	$25.7 \pm .1.7d^{efgh}$	5.5 ± 3.1^{ijk}
Nonanal	0.6	90.0 ± 0.0^{a}	85.3 ± 2.0^{ab}	31.0 ± 2.5^{def}	6.6 ± 3.5^{ij}
Linalool	13.3	85.0 ± 0.4^{a}	37.2 ± 1.8^{ef}	26.7 ± 1.6^{efgh}	19.4 ± 1.4^{h}
Phenyl acetonitrile	0.6	84.9 ± 2.2^{a}	40.7 ± 1.6^{de}	32.8 ± 4.2^{de}	4.9 ± 2.7^{ijk}
Methyl salicylate	0.7	87.7 ± 1.6^{a}	29.7 ± 2.6^{gh}	14.9 ± 3.6^{i}	1.9 ± 1.4^{jkl}
1-α-Terpeneol	3.3	89.9 ± 0.0^{a}	89.9 ± 0.0^{a}	68.2 ± 3.7^{b}	37.4 ± 2.9^{b}
β-Cyclocitral	0.9	90.0 ± 0.0^{a}	86.8 ± 1.7^{ab}	51.5 ± 2.7^{c}	36.2 ± 1.1^{b}
Nerol	1.0	90.0 ± 0.0^{a}	90.0 ± 0.0^{a}	65.2 ± 4.8^{b}	29.4 ± 1.4^{bcd}
trans-Geraniol	3.1	90.0 ± 0.0^{a}	86.4 ± 2.9^{ab}	37.2 ± 2.8^{d}	30.4 ± 1.0^{bc}
Carvacrol	29.2	89.9 ± 0.0^{a}	77.0 ± 4.9^{b}	26.3 ± 2.8^{fgh}	25.3 ± 2.4^{dbec}
α-Ionone	1.1	90.0 ± 0.0^{a}	84.9 ± 2.2^{b}	35.9 ± 2.1^{de}	20.8 ± 0.8^{fgh}
β-Caryophyllene	4.4	27.8 ± 3.2^{gh}	15.3 ± 0.7^{j}	3.7 ± 2.0^{j}	1.6 ± 1.6^{kl}
trans-Geranyl-acetone	0.4	90.0 ± 0.0^{a}	84.7 ± 2.3^{b}	35.7 ± 1.8^{de}	34.0 ± 1.8^{bc}
β-Ionone	2.4	48.7 ± 7.1^{cd}	39.3 ± 2.6^{de}	36.9 ± 1.7^{de}	38.3 ± 1.9^{b}
Tridecanal	0.1	35.7 ± 2.8^{ef}	30.0 ± 1.0^{gh}	21.5 ± 1.1^{ghi}	16.5 ± 1.0^{h}
Nerolidol	0.1	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	98.3 ± 0.7^{a}	30.2 ± 2.1^{bc}
trans-Phytol	24.0	48.4 ± 1.8^{cd}	39.9 ± 2.9^{de}	34.8 ± 2.6^{de}	
Cedrene	0.1	86.7 ± 1.6^{a}	65.1 ± 2.2^{c}	29.4 ± 2.9^{defg}	25.5 ± 1.3^{def}

The identified compounds are listed in order of elution from the non polar GC column. Mean values with the same letters within the same treatment level are not significantly different at the 5% level (adjusted mean test on transformed mean values).

to an additive effect of the different constituents of the oil with different individual levels of repellency. As such, the blend as a whole rather than any specific components, might constitute a more effective agent with a sufficiently broad spectrum of activity for use as a general purpose arthropod repellent.

3. Experimental

3.1. Plant material

Aerial parts of *G. gynandra* were collected from Buruburu, Nairobi. A voucher specimen (Voucher No. 92/6, Ndakala and Mathenge) is deposited at the Herbarium of the University of Nairobi.

3.2. Plant extraction

The fresh aerial parts were cut into small pieces and hydrodistilled using a Clevenger-type apparatus to yield the essential oil (0.2%).

3.3. Gas–liquid chromatography (GC) of volatile oil

GC analyses were performed with a Hewlett Packard HP 5890A gas chromatograph equipped with a flame ionization detector (240°C). Two fused silica capillary columns (Hewlett Packard, 50 m × 0.32 mm

ID), one coated with methyl silicone (0.17 μm film thickness) and the other with Carbowax 20 M (0.32 μm film thickness) were used with nitrogen as the carrier gas. All GC analyses were performed in the splitless mode with the injector temperature at 220°C. For the methyl silicone-coated column, oven temperature was programmed from 45°C isothermal for 5 min, to 180°C at 5°C/min, then to 280°C at 20°C/min where it was maintained for 15 min, while for the Carbowax 20 M-coated column, it was programmed from 60°C isothermal for 7 min, to 120°C at 5°C/min, then to 220°C at 20°C/min where it was maintained for 10 min. Peak areas were calculated using a Hewlett Packard HP3393A integrator.

3.4. Gas chromatography-mass spectrometry (GC-MS) of volatile oil

GC-MS analyses were performed with a VG Masslab 12-250 quadruple gas chromatograph-mass spectrometer. Chromatographic separations were achieved using a fused silica capillary column (Hewlett Packard, 50 m × 0.2 mm ID) coated with methyl silicone (0.32 μm thickness). All GC-MS analyses were made in the splitless mode with helium as the carrier gas.

The GC column was temperature-programmed as in the case of GC analysis. Compounds were identified by their EI (electron impact) mass spectral data, order of elution and relative GC retention times, and by comparison of their mass spectra and GC retention times to those of authentic samples. The chirality of the compounds was however not taken into account when making identifications and when investigating the repellency activity of authentic compounds.

3.5. Synthetic chemicals

β-Cyclocitral and *trans*-2-methylcyclopentanol were obtained from Sigma Chemical, Poole, Dorset, UK, and the rest of the synthetic samples from Aldrich Chemical, Gillingham, Dorset. All the samples were over 95% pure.

3.6. R. appendiculatus ticks

These were reared at the International Centre for Insect Physiology and Ecology (ICIPE), Nairobi. The ticks were maintained by feeding on the ears of rabbits according to the methods described by Bailey (Bailey, 1960).

3.7. Tick-climbing repellency bioassay

A climbing bioassay was used to test for *R. appendiculatus* repellency of plant extracts and synthetic compounds, details of which have been reported recently by us (Ndungu et al., 1995). Percentage repellency (R) was calculated using the equation $R = [(N_c - N_t)/(N_c + N_t)] \times 100$, where N_c and N_t are the numbers of ticks above the filter paper strips on the control and treated glass tubes, respectively.

3.8. Statistical analysis

Due to the nature of the sampling technique, a repeated-measure analysis of variance was applied to angular transformed percentages to test for differences in response due to dosages, repellents and their interactions. Means comparison was based on the least squares method.

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