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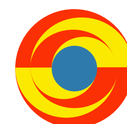

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First Report on Chemical Composition and Acaricidal Activity on the Cattle Tick *Rhipicephalus microplus* of Essential Oil from *Monanthotaxis parvifolia* (Oliv.) Verdc

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Abstract: The chemical composition of the essential oil obtained by hydrodistillation from leaves of *Monanthotaxis parvifolia* were analyzed by GC and GC/MS. Thirty compounds representing 99.9 % of oil were identified. This oil is rich in sesquiterpenes with oxygenated sesquiterpenes (87.1%) following by hydrocarbons compounds (10.8%). Caryophyllene oxide (50.5%), 14-hydroxy-9-epi-(E)-caryophyllene (7.6%) and β-eudesmol (7.3%) were the main components. The acaricidal activity of this oil against *Rhipicephalus microplus* was assessed by modified Larval Packet Test (LPT) and Adult Immersion Test (AIT) with oil concentrations of 5%, 10% and 20% (v/v). Mortality percentages of larvae were 89.51%, 82.18% and 75.01% respectively at 20%, 10% and 5% dilutions. *Monanthotaxis parvifolia* leave's essential oil has considerably reduced egg laying and induced a low reproductive index (0.067%) at 20% dilution.

Keywords: *Monanthotaxis parvifolia*, Acaricidal Activity, Essential Oil

1. Introduction

Rhipicephalus (Boophilus) microplus whose development cycle is monoxenous, is a one-host cattle tick which has a tropical and subtropical distribution. This major ectoparasite has a very important economic impact on cattle husbandry throughout its area of distribution. The control of cattle tick populations relies mainly on acaricides and the intensive use of such products has led to the development of resistance to most of the acaricide classes available on the market [1, 2, 3, 4].

Therefore, new alternatives are needed to improve or innovate the current strategies used for control of resistant tick populations, as well as to design acaricide resistance mitigation programs based on integrated pest management control.

Plant extracts with acaricidal activity provide a potential alternative as a substitute for synthetic acaricides currently used for tick control, since promising results have been obtained through testing some plant extracts against *R. microplus* [5, 6, 7, 8, 9].

The genus *Monanthotaxis* Baill. belongs to the family Annonaceae and represents several species that are indigenous to the rain forest of Africa [10]. This genus is of medicinal interest since such tumor inhibitor compounds as crotepoxide have previously been isolated from stem bark of *M. buchananii* [11] and from roots of *M. caffra* [12].

To date, no studies on chemical composition and acaricidal activity of essential oil from *Monanthotaxis parvifolia* (Oliv.)

Verdc., on *R. microplus* have been reported in the literature.

The present study attempts to determine for the first time, chemical composition and evaluate the efficacy of essential oil from *Monanthotaxis parvifolia* leaves (Oliv.) Verdc., from Benin against *R. (Boophilus) microplus* tick engorged females and larvae using AIT and LPT bioassays.

2. Materials and Methods

2.1. Plant Material

Leaves of *Monanthotaxis parvifolia* were collected in Bonou in Department Oueme (Benin). They were dried naturally on laboratory benches at room temperature until constant weight. Identification of the plant was made by Professor Akouegninou of National Herbarium of Benin in University of Abomey-Calavi where a voucher specimen was deposited under N°AA6706/HNB.

2.2. Methods

2.2.1. Essential Oil Extraction and Test Solutions Preparation

Essential oil were obtained from the leaves of the plant by hydrodistillation (4 h) using a Clevenger- type apparatus. The essential oil was dried over sodium sulfate and stored at 4°C until used. From this sample, dilutions were performed with Tween 20 (2%) and a mixture of trichloroethylene (solvent) and olive oil (2:1 ratio) to obtain solutions at 5%, 10% and 20% (v/v) respectively for AIT and LPT essays.

2.2.2. Gas Chromatography

GC analyses were performed on a Varian gas chromatograph, model CP-3380, with a flame ionization detector equipped with a silica capillary column: HP5 J&W Agilent (5%-phenyl-methylpolysiloxane) (30 m x 0.25 mm i.d. x 0.25 µm film); N_2 was the carrier gas at 0.8 mL/min; injection of 1 µL 1:10 CH_2Cl_2 solution, split ratio 1:100; injector temperature 220°C, detector temperature 250°C; temperature program 60-220°C at 3°C/min, then kept at 220°C during 20 min. The linear retention indices of the components were determined relative to the retention times of a series of *n*-alkanes with linear interpolation. The percentage composition of the essential oil was computed by the normalization method from the GC/FID peak areas on the HP5 capillary column, response factors being taken as one for all compounds.

2.2.3. Gas Chromatography-Mass Spectrometry

GC/MS analyses were performed using a Hewlett–Packard GC 5890 series II equipped with a HP5 (5%-phenyl-methylpolysiloxane) fused silica column (30 m x 0.25 mm; film thickness 0.25 µm) and a DB-Wax fused silica column (30 m x 0.25 mm; film thickness 0.25 µm) interfaced with a quadrupole detector (Model 5972) applying the same temperature program as for the GC/FID analyses with the apolar column; the temperature program was 70°C for 2 min, 70-220°C at 5°C/min, then kept at 220°C during 38 min using the polar column (calculation of the linear retention indices on this column by coinjection with a series of *n*-alkanes); injector temperature, 220°C; MS transfer line temperature, 250°C; carrier gas, helium at a flow rate of 0.6 mL/min; injection type, split, 1:10 (1 µL 10:100 CH_2Cl_2 solution); ionization voltage, 70 eV; electronmultiplier 1460 eV; scan range 35-300 amu; scan rate, 2.96 scan/s. The identification of the constituents was based on comparison of their relative retention indices with either those of authentic samples or with published data in the literature [13] and by matching their mass spectra with those obtained with authentic samples and/or the NBS75K, Wiley 7th NIST 98 EPA/NIH, and FFNSC 2 libraries spectra.

2.2.4. Acaricidal Bioassays

(i). Adult Immersion Test (AIT)

The modified AIT test proposed by Drummond and *al.* (1973) was used [14]. The engorged females were divided into three groups of ten females randomly. Three dilutions (20%, 10%, 5%, (v/v)) of oil were prepared using Tween 20 (2%). Then, each group of females was immersed for 2 min in oil dilutions. The control group was immersed for 2 min in a solution of Tween 20 (2 %). After, each female was and maintained individually in petri dishes (6 × 6 cm) to monitor oviposition (each tick = experimental unit). The experimental groups were maintained in a climate-controlled chamber (27 ± 1 °C and RH >80 ± 10 %). Daily observations were done on every day and death of ticks was confirmed by observing loss of motility and pedal reflex after exposing to light. The egg masses were weighed for the determination of percentage inhibition of oviposition on 15 days after treatment. The egg masses collected from each female were placed in a syringe with the distal end cut, then sealed with hydrophilic cotton and kept under the same temperature and humidity conditions described previously.

(ii). Larval Packet Test (LPT)

The modified LPT [15] was used to assess the acaricidal effect of essential oil on 14-to 21-day-old larvae. Briefly, three two-fold dilutions (20%, 10%, 5%, (v/v)) of oil were prepared using a mixture of trichloroethylene (solvent) and olive oil (2:1 ratio) to treat 7.5 × 8.5-cm filter papers that were placed for 2 h in a fume hood to allow the trichloroethylene to evaporate before being folded into packets using bulldog clips. Approximately 100 *R. microplus* larvae were placed into each treated filter paper packet, which was then sealed with additional bulldog clips and placed in an incubator (27°C, 85–86% RH, and a photoperiod of 12:12 [L/D]) for 24 h. Two replicates and a control (filter paper with trichloroethylene and olive oil) for each essential oil were used in two independent bioassays. After exposure to the respective dillutions, the numbers of live and dead larvae were counted to calculate the percentage of larval mortality [16].

2.2.5. Statistical Analysis

All assays were conducted at least three times with three different sample preparations. All data were

expressed as mean ± standard deviation (SD). Analysis of variance was performed using SAS. The 50 % lethal concentration (LC 50) for each formulation was calculated by the probit method [17], generated by the Probit POLOPC program (LeOra Software, 1987, Berkeley, CA, USA). A one-way ANOVA and unpaired Student's t-test were used for these analyses, and $p < 0.05$ was considered to be statistically significant.

3. Results and Discussion

3.1. Chemical Composition of the Studied Essential Oil

Composition of the oil extracted from *M. parvifolia* leaves as well as linear retention indices and percentage of the identified compounds are summarized in Table 1. Results showed that the major fraction of the oil was Oxygenated sesquiterpenes (88.5%) followed by (10.8%) of Sesquiterpene hydrocarbons. Main compounds are respectively caryophyllene oxide (50.5%), 14-hydroxy-9-epi-(E)-caryophyllene (7.6%) and β-eudesmol (7.3%).

Table 1. *Chemical composition of the essential oil hydrodistilled from leaves of Monanthotaxis parvifolia.*

N° Pic	RI	Components	Relative percentage %
1	939	α-thujene	0.3
2	984	sabinene	0.3
4	1383	α-copaene	0.5
5	1430	(E)-β-caryophyllene	2.6
6	1469	γ–muurolene	1.9
7	1477	γ-himachalene	0.8
8	1506	germacrene-D	2.1
9	1531	(E)-calamenene	1.7
10	1552	α-calacorene	1.2
11	1556	M= 220	0.5
12	1563	elemol	1.7
13	1595	caryophyllene oxide	50.5
14	1604	guaiol	0.7
15	1621	humulene epoxide II	3.7
16	1637	M= 220	1.1
17	1642	γ-eudesmol	2.0
18	1648	caryophylla-4(12), 8(13)-dien-5α-ol	3.1
19	1654	α-muurolol	1.1
20	1662	β-eudesmol	7.3
21	1668	14-hydroxy-(Z)-caryophyllene	1.9
22	1668	trans-calamenen-10-ol	1.0
23	1681	14-hydroxy-9-epi-(E)-caryophyllene	7.6
24	1685	khusinol	1.4
25	1691	Eudesma-4(15), 7-dien-1β-ol	0.7
26	1699	10-nor-Calamenen-10-one	0.1
27	1716	cis-thujopsenal	0.8
28	1772	benzyl benzoate	1.4
29	1919	(5E, 9E)-farnesyl acetone	1.2
30	1954	phytol	0.7
Monoterpene hydrocarbons			0.6
Sesquiterpene hydrocarbons			10.8
Oxygenated sesquiterpenes			88.5
Total			99.9

RI; Retention Index relative to n-alkanes (C_9-C_{20}) on a DB_1 capillary column (5%-phenyl-methylpolysiloxane)

3.2. Acaricidal Activities of the Essential Oil

The acaricidal activity of leaves essential oil from *Monanthotaxis parvifolia* is presented in Table 2, Table 3 and Table 4. *Monanthotaxis parvifolia* leaves essential oil induces a mortality of 89.51, 82.18 and 75.01 % of *R. (B.) microplus* larvae respectively at the concentrations of 20%, 10% and 5%. The LC_{50} values of this essential oil were 0.948 % and 15.925% respectively on larvae and engorged female of *R. (B.) microplus*. As showed by Table 4, essential oil from *M. parvifolia* at the 20% dilution caused a significant reduction in the egg mass weight (0.103g) and reproductive index (0.067 %) in comparison with the control group (0.303 g and 24.5 %, respectively). This caused a strong percentage inhibition of oviposition (72.299±13.997).

Table 2. *Mortality percentage of R. (B.) microplus tick larvae exposed to different concentrations of M. parvifolia essential oil.*

Essential oil concentration %	Mortality percentage
20	89.51±1.42
10	82.18±6.33
5	75.01±3.11

Table 3. *Dose-response data of R. (B.) microplus against essential oil of Monanthotaxis parvifolia using adult immersion test (AIT) and larval packet test (LPT).*

Test	LC_{50} (%) (95% CI)	LC_{90} (%) (95% CI)
LPT	0.948 (0.121 - 2.021)	22.065 (15.326 - 53.072)
AIT	15.925 (10.982-33.827)	96.065 (41.435-956.529)

Table 4. *Mean female weight before oviposition (g), egg mass weight (g), RI and %OI values.*

Oil Concentration %	Female weight before oviposition (g)	Egg mass weight (g)	RI	%IO
20	1.515±0.033	0.103±0.054	0.067±0.034	72.299±13.997
10	1.665±0.280	0.218±0.143	0.123±0.072	49.824±29.262
5	1.437±0.114	0.253±0.055	0.175±0.026	28.595±10.538
Control	1.241±0.078	0.303±0.005	0.245±0.011	-

RI: reproductive index of engorged females treated with dilutions of essential oil from under laboratory conditions (27 ± 1 °C and RH >80 ± 10 %)
%IO: percentage inhibition of oviposition

Investigation of the chemical composition of *Monanthotaxis parvifolia* leave's essential oil revealed that this oil is rich in sesquiterpenes and has caryophyllene oxide/14-hydroxy-9-epi-(E)-caryophyllene (50.5/7.6) as chemotype. To date, there are no data on the chemical composition of *Monanthotaxis parvifolia* leave's essential oil and globally of the genus *Monanthotaxis*. However, study on the chemical composition of *Monanthotaxis capea* organs (leafs, bark and fruits) essential oils have revealed that these oils have a chemical composition dominated by phenylbutane

derivatives in range of 97–57% [18]. The average acaricidal activity obtained in the present study may be linked to the content of this oil in sesquiterpenes. In fact, according to literature, several terpene derivates are mentioned to be toxic against the cattle tick *R. (B.) microplus*. Sesquiterpenes (hydrocarbons and oxygenated) have demonstrated acaricidal activity and synergistic effect against *R. (B.) microplus* [9, 19, 20]. The non-strong acaricidal activity observed may due to the low proportion of compounds with acaricidal potential.

4. Conclusion

Chemical composition and acaricidal activity on the cattle tick *R. (B.) microplus* of essential oil from leaves of *M. parvifolia* were investigated. This study showed that oxygenated sesquiterpenes dominated chemical composition of this oil. This essential oil didn't exhibit strong acaricidal activity but has considerably reduced egg laying and reproductive index on cattle tick *Rhipicephallus (Boophilus) microplus*. This result suggests the use of this oil to control *R. (B.) microplus* population.

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