INTERNATIONAL JOURNAL OF PHARMACEUTICAL, CHEMICAL AND BIOLOGICAL SCIENCES

Available online at www.ijpcbs.com

Research Article

FIRST REPORT ON CHEMICAL COMPOSITION AND ACARICIDAL ACTIVITY ON THE CATTLE TICK *RHIPICEPHALUS* (*BOOPHILUS*) *MICROPLUS* OF *XYLOPIA PARVIFLORA* (A. RICH) BENTH LEAVE'S ESSENTIAL OIL IN BENIN, WEST AFRICA

Sedjro-Ludolphe Oronce Dedome[1], Guy Alain Alitonou[1*], Philippe Sessou[1,2], Fidele Paul Tchobo[1], Mahudro Yovo[1], Eric Yessinou[2], Souaibou Farougou[2], Felicien Avlessi[1], Chantal Menut[3] and Dominique Codjo Koko Sohounhloue[1]

[1]Laboratory of Study and Research in Applied Chemistry (LERCA), Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, Republic of Benin.
[2]Laboratory for Research in Applied Biology (LARBA), University of Abomey-Calavi, Republic of Benin.
[3]Institute of Biomolecules Max Mousseron, Team "Glyco and Nanocarriers for Therapeutic Targeting", Faculty of Pharmacy, Montpellier, France.

ABSTRACT

The essential oil extracted by hydrodistillation from the leaves of *Xylopia parviflora* (A. Rich.) Benth. was analyzed by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC–MS). Caryophyllene oxide (39.2%) was the major compound following by benzyl benzoate (9.7%), phytol (7.8%), humulene epoxide II (6.8%), (E)-14-hydroxy-9-epi-caryophyllene (4.9%), myrcene (4.3%), linalool (3.7%), (E)-β-caryophyllene (3.5%) and (Z)-nerolidol (3.5%). 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 88.27%, 80.29% and 78.94% respectively at 20%, 10% and 5% dilutions. LC_{50} value was 3.279%. *Xylopia parviflora* leave's essential oil has considerably reduced egg laying and induced a low reproductive index (0.7%) at 20% dilution. This study suggests the use of *Xylopia parviflora* oil to fight against *Rhipicephalus (Boophilus) microplus.*

Keywords: *Rhipicephalus microplus*, caryophyllene oxide, *Xylopia parviflora*, essential oil.

INTRODUCTION

In many parts of Africa in general and particularly in West Africa, ruminants are important speculation in animal production. These animals offer enormous zoo technical, economic and socio-cultural benefits[1]. Unfortunately, their productivity is very low because of many health problems affecting their growth and reproduction. Among the pathologies encountered, infestations by parasites are very accentuated especially during the rainy season. External parasites in general and ticks in particular weaken the body by taking blood and causes diseases such as cowdriose, theileriose, piroplasmose, babesiosis which are often difficult to diagnose [2].

Among the different species of ticks identified in Benin, the cattle tick *Rhipicephalus (Boophilus) microplus*, introduced in Benin following the importation of dairy cows of the Girolando breed from Brazil to Kpinnou rearing farm

currently represents one of the most resistant tick to acaricides in infested farms [3-6]

Manual de-labeling or the use of local medicinal plants are the most widely used methods in small-scale farmers, while the current application of synthetic acaricides is the most widely used method of intensive production systems to control these ectoparasites[1,2]. However, the consequences for man and his environment, the presence of acaricidal strains resistant to acaricides, and the scarcity and high cost of good quality synthetic products in local markets pose the problem of finding alternative solutions[7]. Some plants contain substances with therapeutic and antiparasitic properties in their organs (leaves, fruits, flowers, seeds)[1]. Essential oils in many of them are endowed with a wide range of biological properties (acaricides, insecticides, bactericides, fungicides) and have already been the subject of phytochemical and biological studies[8, 9].

Biological activities of essential oils including acaricidal properties are well documented. Several interesting results have been obtained using the Larval Packet Test (LPT) and Adult Immersion Test (AIT) against the cattle tick *R. (B.) microplus*. Acaricidal essential oils can derive from different plant families as Conifers, Apiaceae, Lamiaceae, Liliaceae and Verbenaceae[9-12]. As far as we know, no investigations have been made on the chemical study and acaricidal activity of the volatile oil obtained from leaves of *Xylopia parviflora*.

The present study has objective to study chemical composition and evaluate the efficacy of essential oil from leaves of *Xylopia parviflora* from Benin against *R. (B.) microplus* tick engorged females and larvae using AIT and LPT bioassays.

MATERIALS AND METHODS

Plant material

Leaves of *Xylopia parviflora* were collected in Bonou, Departments of Oueme (Benin). They were dried naturally on laboratory benches at room temperature until constant weight.

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 to obtain solutions at 5%, 10% and 20% (v/v) for biological essays.

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 oils 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.

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; electron multiplier 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.

Evaluation of acaricidal activities of the essential oil

Adult Immersion Test (AIT)

The modified AIT test proposed 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.

Eggs laid by these ticks were collected and weighed. The inhibition of oviposition was evaluated after weighing the eggs as follows: reproductive index (RI) = weight of eggs laid (g)/weight of females (g); percentage inhibition of oviposition (%IO) = [RI (control group) – RI (treated group)] × 100/RI (control group) [15].

Larval Packet Test (LPT)

The modified LPT [16] was used to assess the acaricidal effect of essential oil on 14-to 21-day-old larvae. Briefly, 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 essential oil were used in three independent bioassays. After exposure to the respective dilutions, the numbers of live and dead larvae were counted to calculate the percentage of larval mortality [17].

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 [18]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.

RESULTS AND DISCUSSION

Chemical composition of the studied essential oil

The chemical composition of the essential oil analyzed by GC-FID and GC-MS is given in Table 1. Results showed that sesquiterpens (74.8%) with oxygenated (65.5%) occuring in higher levels than hydrocarbon compounds (9.3%) were the major fraction of the essential oil obtained from the leaves of *Xylopia parviflora*. As shown by their proportions given in Table 1, the most abundant components in *Xylopia parviflora* leaves essential oil were caryophyllene oxide 39.2%, benzyl benzoate 9.7%, phytol 7.8%, humulene epoxide II 6.8% and (E)-14-hydroxy-9-epi-caryophyllene 4.9% while the minor components were myrcene, linalool, E-(β)-caryophyllene, (z)-nerolidol and *trans*-sabinene hydrate.

Acaricidal activities of the essential oil

Larval Packet Test

The larvicidal activity of the essential oil studied is presented in Table 2. The oil didn't produce 100% mortality of larvae at concentrations tested. The *X. parviflora* essential oil produced respectively 88.27%, 80.29% and 78.94% mortality respectively at 20%, 10% and 5% dilutions against the 14-to-21 day-old *R. microplus* tick larvae. The LC_{50} value of this essential oil on larvae was 3.279%.

Adult immersion test

The results of adult immersion test are shown in Table 3. Essential oil from *X. parviflora* at the 20% dilution caused a significant reduction in the egg mass weight (0.012g) and reproductive index (0.7 %) of the cattle tick *R. (B.) microplus* in comparison with the control group (0.303 g and 24.5 %, respectively).

The study revealed that the chemotype of *Xylopia parviflora* leave's essential oil was Caryophyllene oxide/benzyl benzoate which is different of that from fruits essential oils. In fact, several studies conducted in Cameroon and Chad on the chemical composition of *Xylopia parviflora* fruits established that this oil was dominated by monoterpenes hydrocarbons mainly β-pinene and α-pinene[19-21]. We can conclude that essential oil of leaves from *Xylopia parviflora* dominated by sesquiterpenes is qualitatively different from that of fruits in majority dominated by monoterpenes hydrocarbons. 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*. Biocidal activities on females and larvae of *R. microplus* were observed with oxygenated monoterpenes as pulegone[22], thymol and Carvacrol[12, 25]. Sesquiterpenes (hydrocarbons and oxygenated) have also demonstrated acaricidal activity and synergistic effect against *R. (B.) microplus*[9, 23, 24]. Moreover, monoterpenes hydrocarbons as sabinene, α-pinene, limonene or β-pinene have shown lethal effect in larval immersion test[26]. The non-strong acaricidal activity observed may due to the low proportion of compounds with acaricidal potential.

CONCLUSION

Results of this study showed that *Xylopia parviflora* leave's essential oil contained mainly sesquiterpenes. Acaricidal activity assessed by Larval Packet Test (LPT) and Adult Immersion Test (AIT) revealed an average potential 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*.

ACKNOWLEDGMENTS

The authors are thankful to the Government of Benin for its financial support through the Scholarship fellowship initiated by the Ministry of Higher Education and Scientific Research.

Table 1: Chemical composition of the essential oil from leaves of *Xylopia parviflora* (A. Rich.) Benth

N° Pic	KI	Compounds	%
1	979	sabinene	0.2
2	991	myrcene	4.3
3	1023	p-cymene	0.2
4	1029	β-phellandrene	0.4
5	1036	*cis*-β-ocimene	0.3
6	1065	*cis*-sabinene hydrate	0.2
7	1089	6,7-epoxymyrcene	0.2
8	1098	linalool	3.7
9	1101	*trans*-sabinene hydrate	2.2
10	1179	rosefuran epoxide	1.0
11	1184	dihydrolinalool	0.8
12	1190	α-terpineol	0.4
13	1279	p-menth-1-en-7-al	1.4
14	1367	α-copaene	1.1
15	1380	geranyl acetate	0.4
16	1428	(E)-β-caryophyllene	3.5
17	1463	α-humulene	1.0
18	1468	germacrene D	0.5
19	1474	allo-aromadendrene	0.8
20	1497	δ-selinene	0.5
21	1498	epi-cubebol	0.2
22	1501	valencene	0.1
23	1507	γ-patchoulene	0.2
24	1510	β-bisabolene	0.6
25	1516	γ-cadinene	0.4
26	1519	cubebol	0.4
27	1526	δ-cadinene	0.6
28	1561	(Z)-nerolidol	3.5
29	1593	caryophyllene oxide	39.2
30	1618	humulene epoxide II	6.8
31	1645	caryophylla-4(12),8(13)-dien-5-ol	1.1
32	1652	allo-epoxide-aromadendrene	0.5
33	1665	(Z)-14-hydroxy-caryophyllene	0.7
34	1677	(E)-14-hydroxy-9-epi-caryophyllene	4.9
35	1770	benzyl benzoate	9.7
36	2098	phytol	7.8
Monoterpene hydrocarbons			5.4
Oxygenated monoterpenes			9.9
Sesquiterpene hydrocarbons			9.3
Oxygenated sesquiterpenes			65.5
Aromatic compounds			9.7
Total			99.8
KI Kovats retention indices relative to n-alkanes (C_9-C_{20}) on a DB_1 capillary column (5%-phenyl-methylpolysiloxane)			

Table 2: Mortality percentage of *Rhipicephalus microplus* tick larvae exposed to different concentrations of *Xylopia parviflora* essential oil

Essential oil concentration (%)	*X. parviflora*
20	88.27±1.93
10	80.29±3.06
5	78.94±0.95
Yield (%)	0.38
LC_{50} (IC 95%)	3.279 (2.040 - 4.372)

Table 3: Mean female weight before oviposition (g), egg mass weight (g), reproductive index (RI) of engorged females of *Rhipicephalus microplus* treated with different concentrations of essential oil from the leaves of *Xylopia parviflora* under laboratory conditions (27 ± 1 °C and RH >80 ± 10 %) and percentage inhibition of oviposition (%IO)

Oil Concentration (%)	Female weight before oviposition (g)	Egg mass weight (g)	RI	%IO
20	1.665±0.049	0.012±0.003	0.007±0.001	97.069±0.653
10	1.548±0.101	0.143±0.025	0.092±0.014	62.331±5.887
5	1.711±0.191	0.346±0.099	0.199±0.038	18.589±15.643

REFERENCES

1. Pamo TE, Tapondjou l, Tendonkeng F, Nzogang JF, Djoukeng J, Ngandeu F and Kana J R. Effet des huiles essentielles des feuilles et des extrémités fleuries de Cupressus lusitanica sur la tique (Rhipicephalus lunulatus) à l'Ouest-Cameroun. Revue de l'Académie des Sciences du Cameroun. 2003; 3(3):169-175.
2. Lhoste P, Dolle V, Rousseau J and Soltner D. Manuel de Zootechnie des régions chaudes. Les systèmes d'élevage. Collection précis d'élevage. Ministère de la Coopération, Paris. 1993;288p.
3. De Clercq EM, Vanwambeke SO, Sungirai M, Adehan S, Lokossou R and Madder M. Geographic distribution of the invasive cattle tick Rhipicephalus microplus, a country-wide survey in Benin. Exp App Acarol. 2012;58:441-452.
4. Madder M, Adehan S, De Deken R, Adehan S and Lokossou R. New foci of Rhipicephalus microplus in West Africa. Exp Appl Acarol. 2012;56(4):385-390.
5. Yessinou RE, Akpo Y, Adoligbe C, Adinci J, Assogba MN, Koutinhouin B, Youssao IAK and Farougou S. Resistance of tick Rhipicephalus microplus to acaricides and control strategies. Journal of Entomology and Zoology Studies. 2016;4(6):408-414.
6. Adéhan SB, Biguezoton A, Adakal H, Dossa F, Dougnon TJ, Yessinou E, Sessou P, Aboh AB, Youssao AKI, Assogba N, Mensah GA, Madder M and Farougou S. Acaricidal Activity of Ethanolic and Volatile Extracts of The Leaves of Selected Plants Used in Veterinary Pharmacopeia on The Larvae of Rhipicephalus microplus in Benin. Alexandria Journal of Veterinary Sciences. 2016;49 (1):1-11.
7. Wharton RH. Les maladies du bétail transmises par les tiques et leurs vecteurs : résistance aux acaricides. Revue Mondiale de Zootechnie, FAO. 1976 ;20:8-15.
8. Ling B, Zhang M, Kong C, Pang X and Liang G. Chemical composition of volatile oil from Chromolaena odorata and its effect on plant, fungi and insect growth. Ying Yong Sheng Tai Xue Bao. 2003;14(5):744-6.
9. Lebouvier N, Hue T, Hnawia E, Lesaffre L, Menut C and Nour M. Acaricdal activity of essential oils from five endemic conifers of New Caledonia on the cattle tick Rhipicephaalus (Boophilus) microplus Parasitol Res. 2013;112(4):1379-1384.
10. Martinez VM, Rosario CR, Castillo HGA, Flores FJM, Lopez RJ, Hernandez GR and Lugo CEC. Acaricidal effect and chemical composition of essential oils extracted from Cuminum cyminum, Pimenta dioica and Ocimun basilicum against the cattle tick Rhipicephalus (Boophilus) microplus (Acari : Ixodidae). Parasitol Res. 2011;108:481-487.
11. Martinez VM, Rosario CR, Castillo HGA, Flores FJM, Alvarez AH and Lugo CEC. Acaricidal effect of essential oils from Lippia graveolens (Lamiales

:Verbenaceae), Rosmarinus officinalis (Lamiales : Lamiaceae), and Allium sativum (Liliales : Liliaceae) against Rhipicephalus (Boophilus) microplus (Acari : Ixodidae). J Med Entomol. 2011;48:822-827.
12. Gomes AG, de Oliveira MCM, de Oliveira Souza TS, Zeringota V, Calmon F, da Silva RM, Daemon E, da Silva RWG, Gilvandete MPS and de Carvalho MG. Chemical composition and acaricidal activity of essential oil from Lippia sidoides on larvae of Dermacentor nitens (Acari: Ixodidae) and larvae and engorged females of Rhipicephalus microplus (Acari: Ixodidae) Parasitol Res. 2012; 111:2423.
13. Adams RP. Identification of essential oil components by Gas Chromatography/Mass Spectrometry. 2007; 4th edn; Allured Publishing Corporation Carol Stream. IL.
14. Drummond RO, Ernest SE, Trevino JL, Gladney WJ and Graham OH. Boophilus anulatus and Boophilus microplus: laboratory test of insecticides. J Econ Entomol. 1973;66:130–133.
15. FAO. Resistance Management and Integrated Parasite Control in Ruminants. Guidelines. Animal Production and Health Division, FAO. 2004;25–77.
16. Stone BF and Haydock KP. A method for measuring the acaricide susceptibility of the cattle Boophilus microplus. (CAN). Bull. Entomol. Res. 1962;53:563-578.
17. Aguirre EJ and Aburto S. Determinación de las concentraciones discriminantes como medio de diagnóstico de susceptibilidad en garrapatas. B. microplus. In: IV Reunión Anual de Parasitología Veterinaria, México, D.F., 2–6 de septiembre de 1983;610.
18. Finney DS. Probit analysis. 3rd edn. Cambridge University Press, Cambridge. 1971.
19. Lamaty G, Menut C, Bessiere JM, Zollo PHA and Fekam. The essential oil of Xylopia parviflora (A. Rich) Benth. from Cameroon. Journal of Essential Oil Research.1989;1(5):247-8.
20. Woguem V, Fogang HPD, Maggi F, Tapondjou LA, Womeni HM, Quassinti L, Bramucci M, Vitali LA, Petrelli D and Lupidi G. Volatile oil from striped African pepper (Xylopia parviflora, Annonaceae) possesses notable chemopreventive, antiinflammatory and antimicrobial potential. Food Chemistry. 2014;149:183-189.
21. Bakarnga-Via I, Hzounda JB, Fokou PV,Tchokouaha LR, Gary-Bobo M, Gallud A, Garcia M, Walbadet L, Secka Y and Dongmo PM. Composition and cytotoxic activity of essential oils from Xylopia aethiopica (Dunal) A. Rich, Xylopia parviflora (A. Rich) Benth.) and Monodora myristica (Gaertn) growing in Chad and Cameroon. BMC complementary and alternative medicine. 2014; 14(1):125.
22. Ribeiro VL, Dos Santos JC, Bordignon SA, Apel MA, Henriques AT and von Poser GL. Acaricidal properties of the essential oil from Hesperozygis ringens (Lamiaceae) on the cattle tick Riphicephalus (Boophilus) microplus. Bioresour Technol. 2010;101:2506–2509.
23. Ribeiro VL, Rolim V, Bordignon S, Henriques AT, Dorneles GG, Limberger RP and von Poser G. Chemical composition and larvicidal properties of the essential oils from Drimys brasiliensis Miers (Winteraceae) on the cattle tick Rhipicephalus (Boophilus) microplus and the brown dog tick Rhipicephalus sanguineus. Parasitol Res. 2008;102:531–535.
24. Ribeiro VL, dos Santos JC, Martins JR, Schripsema J, Siqueira IR, von Poser GL and Apel MA. Acaricidal properties of the essential oil and precocene II obtained from Calea serrata (Asteraceae) on the cattle tick Rhipicephalus (Boophilus) microplus (Acari: Ixodidae). Vet Parasitol. 2011;179:195–198.
25. de Oliveira Souza TS, Zeringóta V, de oliveira CMM, Calmon F, Maturano R, Amorin GG, Faza A, de Carvalho MG and Daemon E. Assessment of the acaricidal activity of carvacrol, (E)-cinnamaldehyde, trans-anethole, and linalool on larvae of Rhipicephalus microplus and Dermacentor nitens (Acari: Ixodidae). Parasitol Res. 2013;112:1461.
26. Apel MA, Ribeiro VL, Bordignon SA, Henriques AT and von Poser G. Chemical composition and toxicity of the essential oils from Cunila species (Lamiaceae) on the cattle tick Rhipicephalus (Boophilus) microplus. Parasitol Res. 2009;105:863–868.