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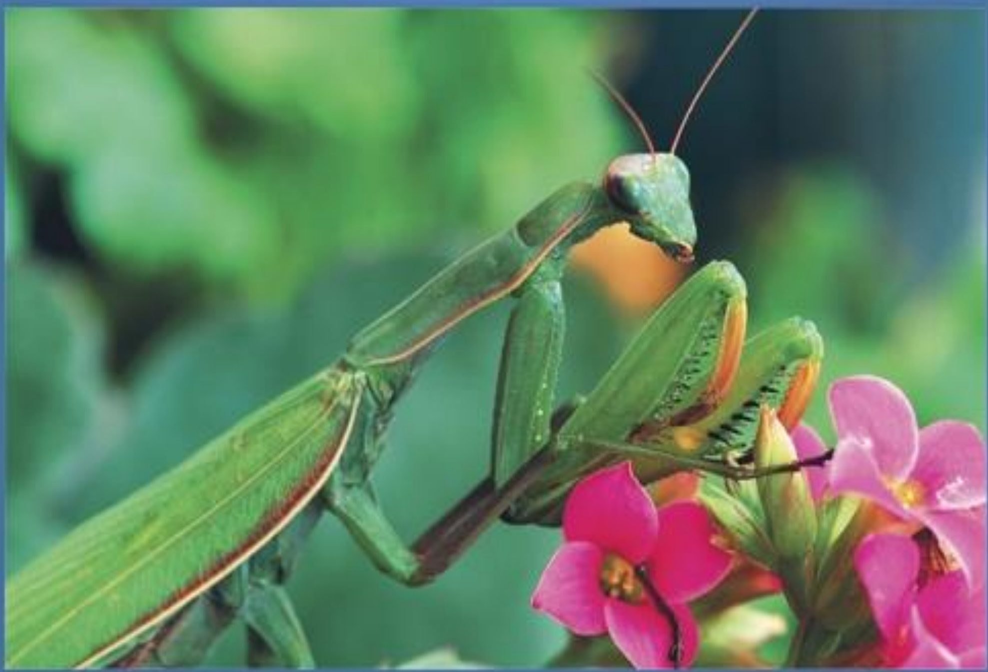

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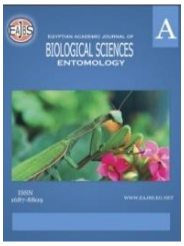

Chemical Composition and Repellent Activities of Formulated Creams from Essential Oils of Three Tropical Plants Against Adults of *Simulium damnosum* Sensu Lato

Monsuru Adebayo Adeleke [1*], Shola H. Awojide [2], Kehinde A. Oyewole[3], Chinonyelum Linda Oforka [4], Olabanji A. Surakat [1], Kamilu Ayo Fasasi [1*] and Chiedu Felix Mafiana [5].

1. Public Health Entomology and Parasitology Unit, Department of Zoology, Osun State University, P.M.B 4494, Osogbo, Nigeria.
2. Department of Pure and Applied Chemistry, Osun State University, P.M.B 4494, Osogbo, Nigeria.
3. Department of Mechanical Engineering, Osun State University, Osogbo.
4. Department of Zoology, University of Lagos, Nigeria.
5. National Open University of Nigeria, Abuja, Nigeria.

***E-mail:** monsuruadeleke@gmail.com

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ABSTRACT

Simulium damnosum sensu lato (Diptera: Simuliidae) commonly known as black fly transmits *Onchocerca volvulus*, the parasite which causes onchocerciasis (river blindness) in Africa. The medical and socio-economic importance associated with the biting of this fly is enormous, hence, the need to devise effective means of breaking man-fly contact. The present study examined repellent activities of formulated topical creams prepared from white soft paraffin and two concentrations (5.0% and 7.5%) of essential oils of three plants, namely *Monodora myristica*, *Eugenia caryophyllus* and *Xylopia aethiopica* against *Simulium damnosum* s.l. vectors of human onchocerciasis in Nigeria. The chemical characterization of the oils was determined using gas chromatography and mass spectrometry. Fly collectors that applied the formulated creams at both concentrations caught lower numbers of black flies compared with the control. The difference in the number of flies caught between each of the three essential oils and the control was statistically significant at 7.5% concentration, whereas only *E. caryophyllus* was significantly different from the control at 5.0% concentration. *Eugenia caryophyllus* had the highest repellent activities, with the protection of 47.1% and 50.6%, followed by *X. aethiopica* (37.1% and 36.1%), whereas *M. myristica* had the least (28.7% and 34.1%) for concentrations of 5.0% and 7.5%, respectively. Trans-13-octadecenoic acid, Eugenol and Alpha-pinene were found to be the principal chemical constituents in *M. myristica*, *E. caryophyllus* and *X. aethiopica*, respectively. The active agents in the essential oils could be useful in providing low-cost repellents to prevent human-vector contact in black fly-infested communities.

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INTRODUCTION

River blindness (human onchocerciasis) is a filarial infection caused by the nematode parasite, *Onchocerca volvulus,* which is transmitted by black flies of the genus *Simulium.* The disease constitutes a major public health problem in many riverine communities in sub-Saharan Africa and Latin America (Tekle *et al.,* 2012, Rodriguez-Pérez *et al.,* 2015). Between 1975 and 1995, the control of onchocerciasis along the West African coast was widely based on larviciding, with the introduction of ivermectin in a few communities in the early 1990s (Hodgkin *et al.,* 2007). Following the launch of the African Programme for Onchocerciasis Control (APOC) in 1995, the control focus shifted from vector to chemotherapy through the operational implementation of community-directed treatment with ivermectin (Opara *et al.,* 2007). The endemic communities were treated freely once or twice a year with ivermectin. Several authors have reported success in the control and elimination of onchocerciasis in communities earlier classified as hyper-endemic after repeated treatments with ivermectin (Adeleke *et al.,* 2010a, Diawara *et al.,* 2009, Tekle *et al.,* 2012).

APOC completed activities in 2015 and the onchocerciasis control programme in Africa is currently coordinated by the World Health Organization (WHO). In many areas in the region, the intense biting by black flies without protection threatens the possibility of recrudescence, even in communities where ivermectin distribution had been successful because the drug does not kill adult worms. Apart from the fear of recrudescence, the biting nuisance of the *Simulium* flies also imposes a socioeconomic burden on the affected communities (Usip *et al.,* 2006, Adeleke *et al.,* 2010b). Studies have shown that the biting nuisance of *S. damnosum* sensu lato, the major black fly species complex in Africa, is a serious problem in many rural communities along the Ogun–Osun River system in Nigeria, as it causes a reduction in productivity of the farmers and impedes socio-economic growth (Adeleke *et al.,* 2010b, Adeleke *et al.,* 2011). The high biting rates of the flies have also led to the emigration of people, especially youths, from the black fly-infested communities to urban areas.

The application of plant extracts for protection against hematophagous insects has a long history in many communities (Aisen *et al.,* 2004, Usip *et al.,* 2006, Hazarika *et al.,* 2012). Studies by Adeleke *et al.,* 2011 and Sam-Wobo *et al.,* 2011 documented varieties of plant extracts used for protection against black flies in rural communities in Southwestern Nigeria. Sam-Wobo *et al.,* 2011 showed that some crude extracts could offer about 70% protection against black flies. Therefore, exploration of the different blends of these extracts might offer a means to reduce human-black fly contact and improve the productivity and well-being of rural dwellers in southwestern Nigeria. In this study, we report the chemical composition and repellent activities of formulated creams from essential oils of three tropical plants against adult *S. damnosum* s.l.

MATERIALS AND METHODS

Selection of Plant Extracts:

A literature search was conducted to identify and document plant extracts that have proven evidence of toxicity against insect vectors in southwestern Nigeria and which had not been screened against *S. damnosum* s.l. Three plants, namely *Monodora myristica*, *Eugenia caryophyllus* and *Xylopia aethiopica*, were selected for the present study. The seeds of the plants were bought from Orisumbare market in Osogbo, Osun State, Nigeria, and confirmed in the Department of Plant Biology, Osun State University by a taxonomist using relevant keys. The seeds were air-dried and ground into powders.

Extraction of Essential Oils:

Essential oils from the ground powders of the seeds were extracted using the steam distillation method. The seed of *M. myristica* was ground to finer particles to increase its surface area, and then transferred into a 500 ml round bottom flask with the aid of a funnel; 200 ml of distilled water was then added. The solution was then mixed using a glass stirring rod. The heating mantle was connected to a power source and turned on; the flow of water through the condenser of the Clevenger commenced and was fitted into the round bottom flask and then placed on the heating mantle. The mixture was heated gradually, and extraction was allowed for 8 hours after which its extract was collected. The same procedure was used for the extraction of *X. aethiopica* and *E. caryophyllus* essential oils as described by (Fayemiwo *et al.*, 2014).

Gas Chromatography and Mass Spectrometry (GC-MS):

Gas chromatography and mass spectrometry were performed on an Agilent 6890N instrument equipped with a flame ionization detector and HP-5MS (30m × 0.25mm × 0.25µm) capillary column. The essential oil components were identified on an Agilent Technologies 5973N mass spectrometer. A microsyringe was washed twice with acetone, and 2 µL of the essential oil was pulled into the syringe and then ejected to wash away the acetone. The chart recorder was set to an appropriate flow of 40 µL/min, the temperature of the detector (flame ionization detector) at 250°C, the injector at 200°C and the column at the range of 100-200°C. A duration of 60 minutes was set for the evolution to come to completion. The detector identified the various components of the essential oils at different retention times and sent a signal to the chart recorder, which resulted in the peaks of the chart paper.

Formulation of Repellent Creams:

The essential oils (0.250g and 0.375g) were added to a wide-mouth glass bottle and diluted with 1.5 ml of acetone overnight. The following day, 5g of white soft paraffin was added to a 100 ml beaker and melted in a water bath at 60°C. The aliquot was transferred into the molten white soft paraffin, mixed and used for repellency assays. The negative control was set up by pouring 1.5 ml of acetone in 5 g of melted white soft paraffin without any trace of essential oils.

Repellency Bioassay:

A-Preliminary Bioassay:

A preliminary bioassay was first conducted using two consenting human landing collectors each for experimental and control. Each of the human fly collectors was instructed to expose the lower part of their legs. The experimental human landing collectors applied the cream with extract formulations while the control human landing collectors applied the creams without extracts. The preliminary trials showed that concentrations below 5.0% of the three essential oils did not have appreciable repellent activities against black flies.

B.Experimental Bioassay:

Concentrations of 5.0% and 7.5% were further evaluated against the flies. Four consenting male fly collectors (25–40 years old) were used for the study. Three fly collectors served as experimental subjects (for the three formulated creams) and one served as the control. The experimental subjects applied the cream topically on both legs while the control applied the cream prepared without essential oil. The four fly collectors sat 5 m apart and exposed their lower legs on the bank of River Odo-Oba (Iwo Local Government, Osun State, Nigeria) from 8 AM to 5 PM daily for 9 days. The flies that landed on the exposed legs were collected, as described by Sam-Wobo *et al.,* 2011. The creams and sitting position of the fly collectors were rotated daily to eliminate bias due to individual attraction to *S. damnosum* s.l.

Ethical Approval and Consent to Participate:

Ethical approval for the study was obtained from Osun State Ministry of Health Ethical Review Board. Informed consent was obtained from the fly collectors after the details of the study were explained to them. The fly collectors are on annual ivermectin treatment coverage.

Data Analysis:

The percentage of protection of the formulated creams was calculated as described by Ansari *et al.*, (2005). The data were subjected to paired t-test to determine if there were significant differences in the degree of repellency of each cream in comparison with the control. Analysis of Variance (ANOVA) was used to test for differences in repellency among creams. Analysis was performed using Statistical Package for Social Science (SPSS) version 17.0.

RESULTS

The chemical components of the three essential oils used for the formulation of the tested creams are presented in Tables 1-3. *Monodora myristica* was found to have 18 chemical constituents with trans-13-octadecenoic acid having the highest percentage composition (25.2%) followed by Sabinol-cis (17.9%) and Linalool (9.1%). Of the 17 constituents in *E. caryophyllus*, Eugenol had the highest percentage composition (85.7%) followed by Eugenyl acetate (6.7%). The dominant chemical components of *X.* aethiopica were found to be β –Phellandrene (10.30%), α – Bisabolene (10.07%), α – Pinene (7.39%), Beta –ylangene (6.37%) and α-Farnesene, (E, E) -(5.10%) while the remaining 16 constituents occurred in low proportions.

Table 1: Chemical composition of constituents in the essential oil of *Monodora myristica* Kovats Retention Indices were calculated from our analysis with respect to a series of n-alkanes.

Compound Present	Retention Time (Mins)	% Composition	Retention Index
Camphene	3.12	0.75	950
Linalool	3.50	9.11	1086
Sabinol-cis	5.47	17.87	1139
Sabinol	5.75	3.08	1142
Cis-beta-Terpineol	6.53	1.16	1189
Carvacrol acetate	8.18	1.31	1373
γ -Cadinene	10.34	0.30	1513
δ -Cadinene	10.56	0.51	1523
α-CadineneT1n3	10.78	0.81	1533
Caryophyllene oxide	11.91	1.09	1580
Cadinol	12.88	1.21	1637
alpha.-Cadinol	13.14	1.14	1651
β-Bisabolol	13.92	0.52	1672
α-Bisabolol, epi-T1n3	14.02	2.73	1685
n-Hexadecanoic acid	17.51	7.66	1968
trans-13-Octadecenoic acid	19.71	25.18	2133
9-Octadecenoic acid (Z)-, 2-hydroxyethyl ester	30.83	2.58	2096

Table 2: Chemical composition of constituents in the essential oil of *Eugenia caryophyllus* Kovats Retention Indices were calculated from our analysis with respect to a series of n-alkanes

Compounds present	Retention time	% Composition.	Retention Index
δ Carene	3.20	0.21	998
Ethanone,1-(2,5-dihydroxyphenyl)	3.36	0.09	1041
Fenchol, endo-T1n3	3.72	0.52	1100
β-Terpineol, cis-	4.70	0.83	1129
Cyclohexanol, Terpine-1-ol	4.59	1.07	1134
Isoborneol	4.78	0.55	1158
Borneol	5.02	0.72	1166
Benzenemethanol, alpha	6.87	0.47	1270
Terpinen-4-ol acetate	7.34	0.82	1302
α-Terpinyl acetate	7.75	0.17	1347
Tricyclo [5.4.0.0(2,8)] undec-9-ene,2,6,6,9-tetramethyl	9.52	0.51	1350
eugenol	13.62	85.71	1357
Caryophyllene	14.70	1.30	1407
α-Caryophyllene	15.30	0.16	1419
β-Farnesene, (E)-	16.40	0.16	1449
eugenyl acetate	17.10	6.15	1484
n-Hexadecanoic acid	17.48	1.32	1955

Table 3: Chemical composition of constituents in the essential oil *Xylopia aethiopica* Kovats Retention Indices were calculated from our analysis with respect to a series of n-alkanes

Compound Present	Retention Time (Mins	% Compound	Rentention Index
α - Pinene	4.51	7.39	936
3-Carene	4.70	2.22	1007
β -Phellandrene	5.30	10.30	1021
Gamma -Terpinene	5.71	2.29	1050
p-Menth-8-en-1-ol	7.26	1.80	1114
alpha. -Cubebene	10.38	1.95	1352
α -copaene	10.91	2.87	1376
Beta -ylangene	12.38	6.37	1416
gamma. -Elemene	13.97	1.85	1436
β-Farnesene, (E)-	14.40	1.39	1455
Gamma -Muurolene	15.10	1.47	1473
Aromadendrene	16.43	2.47	1439
Viridiflorene	17.41	2.82	1488
α-Farnesene, (E, E)-	18.36	5.10	1496
Calamenene, trans-	19.79	2.24	1512
β-Sesquiphellandrene	20.10	2.10	1513
Calamenene, cis-T1n3	20.59	2.77	1522
γ- Bisabolene	20.98	1.44	1525
α - Bisabolene	24.92	10.07	1540
Caryophyllene oxide	21.79	1.51	1570
Cis-Z-. alpha. -Bisabolene epoxide	24.80	1.54	1674

The results of the repellent activities of the three formulated creams are presented in Tables 4 and 5. The fly collectors that applied the formulated creams (5.0% and 7.5% concentration of essential oils) caught a lower number of black flies compared with the control. The difference in the number of flies caught between each of the three essential oils and the control was statistically significant at a concentration of 7.5% ($p<0.05$) whereas only *E. caryophyllus* differed significantly from the control at a concentration of 5.0%.

The concentration of 7.5% of essential oils in the formulated creams showed a higher percentage of protection than did the concentration of 5.0% except in *X. aethiopica*. *Eugenia caryophyllus* had the highest repellent activities against *S. damnosum* s.l., with the protection of 47.1% and 50.6% followed by *X. aethiopica* (37.1% and 36.1%), whereas *M. myristica* had the least (28.7% and 34.1%) for concentrations of 5.0% and 7.5%, respectively. Even though the number of flies caught varied among the three formulated creams for both concentrations, the difference was not statistically significant ($p>0.05$).

Table 4: Comparison of repellency and percentage of protection at 7.5% concentration of the essential oils in the formulated creams.

Cream formulation applied	Number of *S. damnosum* s.l. caught by experimental collectors	Number of *S. damnosum* s.l. caught by control	Percentage protection	p-value
Cream with *Monodora myristica*	173	263	34.2	0.049
Cream with *Eugenia caryophyllus*	130	263	50.6	0.001
Cream with *Xylopia aethiopica*	168	263	36.1	0.001

P less than 0.05 is significant.

Table 5: Comparison of repellency and percentage of protection at 5.0% concentration of the essential oils in the formulated creams.

Cream formulations applied	Number of *S. damnosum s.l.* caught by experimental collectors	Number of S. *damnosum* s.l. caught by control	Percentage protection	p-value
Cream with *Monodora myristica*	171	240	28.7	0.090
Cream with *Eugenia caryophyllus*	127	240	47.1	0.006
Cream with *Xylopia aethiopica*	151	240	37.1	0.127

P less than 0.05 is significant.

DISCUSSION

Black flies are a serious public health problem in sub-Saharan Africa and Latin America due to their biting nuisance, with annoying bites and disease transmission (Ubachukwu 2004; Adeleke 2010a). There is, therefore, a need for effective measures to control human-black fly contact. The formulated creams from essential oils of *M. myristica*, *E. caryophyllus* and *X. aethiopica* used for the creams exhibited repellent activities against *S. damnosum* s.l. These plants have been used as insecticides against mosquitoes and stored product pests (Hunag *et al.*, 2012; Adewole *et al.*, 2013; Fayemiwo *et al.*, 2014). To the best of our knowledge, this is the first report of the repellent activities of these essential oils against *S. damnosum* s.l.

The variation in the degree of repellency exhibited by the essential oils at the two tested concentrations might be attributed to the composition and abundance of its chemical constituents that are known to possess insecticidal properties. Earlier studies have shown that the bioactivity of the plant extracts against mosquitoes is determined by the chemical composition and relative abundance of its active elements in the extracts (Adewole *et al.,* 2013; Fayemiwo *et al.,* 2014). The chemical composition of the three essential oils analyzed in the present study varied slightly in the number of chemical constituents and percentage of abundance, as compared with previous reports (Ansari *et al.,* 2005; Fayemiwo *et al.,* 2014). The cream formulated with *E. caryophyllus* exhibited the highest repellency and protection against *S. damnosum* s.l. Eugenol, which is the principal constituent in the essential oil of the plant (85%), has insecticidal properties and high toxicities against mosquitoes (Ansari *et al.,* 2005). The insecticidal properties of major constituents in other essential oils, such as trans-13-Octadecenoic acid, Sabinol-cis, Linalool, Alpha-pinene, 3-Carene, and Eucalyptol, have also been reported by other researchers (Adewole *et al.,* 2013; Murilloa *et al.,* 2014).

The three tested cream formulations did not have dermal reactions on the fly collectors during or after the experiments, using the model proposed by Buck (1974). This result is expected, as plant-based repellents are known for their safety and low toxicity to humans (Usip *et al.* 2006; Spero *et al.,* 2008). Plant-based repellents also potentially offer cheaper, readily available, and more environmentally friendly alternatives to synthetic chemical-based repellents. Studies on the use of essential oils of plant derivatives have been known to exhibit highly repellent actions against haematophagous insects but are safe for human use. Ansari *et al.,* 2005, Usip *et al.,* 2006 and Sam-Wobo *et al.,* 2011 all reported repellent activities of extract of O*cimum* leaves against either mosquitoes or black flies. Meanwhile, Adeleke *et al.,* 2011 reported the wide use of *Ocimum* leaves and lime and orange bark as repellent in a study in Ogun State, southwestern Nigeria. Therefore, the development of low-cost plant-based repellent creams would probably be widely accepted in many black fly-infested communities in Sub-Saharan Africa.

Conclusion

The present study strongly highlights the chemical composition and development of repellent creams from essential oils of *M. myristica*, *E. caryophyllus* and *X. aethiopica* against *S. damnosum* s.l. black flies. The active ingredients with repellent activities in the essential oils should be isolated and assessed further against *S. damnosum* s.l. These active ingredients can then be incorporated into creams for use as repellents in breaking human-blackfly contact in onchocerciasis endemic communities. All of these will contribute towards attaining the United Nations Sustainable Development Goal 3 (Good health and well-being) and the WHO Global Vector Control Response targets by the year 2030.

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Authors' Contributions:

MAA, SHA and CLO designed and conducted the field experiments. MAA, OAS, CFM, KAF, and CLO wrote the manuscript. SHA and KAO isolated the compounds in the botanicals and prepared the creams. MAA, SHA, KAF, CLO, and OAS revised the manuscript. All authors read and approved the final manuscript.

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