



FIELD STUDIES OF SELECTED PLANT ESSENTIAL OILS FOR ONCHOCERCIASIS CONTROL AND ELIMINATION

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Abstract

Black flies of the *Simulium damnosum* complex transmit both animal and human onchocerciasis (River blindness), a neglected tropical disease (NTD) of public health importance in Nigeria. Efforts have been made for decades to control and eliminate onchocerciasis, mostly focusing on the use of ivermectin chemotherapy targeted on the *Onchocerca* parasite in infected humans. However, studies have shown that vector control can help fast-track onchocerciasis elimination. The present study investigated the repellent potentials of essential oil extracts of *Eryngium foetidum* and *Eugenia uniflora* against black flies in the field using topical application on study volunteers in a repeated-measures experimental design. The chemical composition of these oils extracted by hydro-distillation were determined using GC-MS analysis. Essential oils of *E. foetidum* and *E. uniflora* showed 73.0% and 84.3% average repellency at 20% concentration respectively, after 5 hours of exposure. Repellence activities decreased with increase in time of exposure. The two plant oil extracts were more effective repellents when compared with a commercial product, N,N-Diethylbenzamide, which served as a positive control. The average repellence of the positive control was 61.5 % (P<0.05). The characterization of *E. foetidum* essential oil indicated an abundance of monoterpenes (29.2%), sesquiterpenes (25.0%) and triterpenes (4.2%), while *E. uniflora* was principally composed of sesquiterpenes (91.0%). The two plant oil extracts proved to be more effective repellents when compared with the positive control. These plants could be exploited in the development of plant-based, natural repellents against black flies. A synergy between Mass Drug Administration (MDA) with ivermectin and vector control using plant-based natural repellents from *E. foetidum* and *E. uniflora* would help realize onchocerciasis elimination targets faster than with MDA alone.

Keywords: repellence, black flies, essential oils, onchocerciasis, *Simulium damnosum*

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INTRODUCTION

Adult female black flies of the *Simulium damnosum* complex solely transmit onchocerciasis (river blindness) from one susceptible human to another while seeking blood meals (Adler, 2005; Chikezie *et al.*, 2018). The disease is caused by *Onchocerca volvulus* and over 120 million people are at risk (Tamarozi *et al.*, 2012), with about 99 % of all onchocerciasis cases occurring in sub-Saharan Africa (Noma *et al.*, 2014; WHO, 2019). The prevalence of onchocerciasis is directly related to the abundance, distribution pattern and contact between infective adult female flies and man (Atting *et al.*, 2005; Opara *et al.*, 2005; Busari *et al.*, 2021). Onchocerciasis is now a target for global elimination by the year 2045 (WHO, 2016).

Control of river blindness started in 1974 when the Onchocerciasis Control Programme in West Africa (OCP) was established. Synthetic larvicides applied to *S. damnosum* breeding sites was then the main strategy. Vector control interrupted transmission in virtually all the core savannah areas of the seven initial OCP countries (Hougard *et al.*, 2001; Boatin and Richards, 2006; Boatin *et al.*, 2012). However, it was discontinued for safety reasons, development of resistance to insecticides of choice, logistics, cost, among other reasons, leaving endemic African communities with no vector control (Chikezie *et al.*, 2022). The gains of the OCP provided a proof that vector control can help fast-track onchocerciasis control to the end of eliminating the disease. Weekly aerial larviciding of black fly breeding sites along approximately 700,000 km² of rivers in the savanna areas of West Africa with hyperendemic onchocerciasis from 1976 to 1989 led to a significant decrease in endemicity in these areas (Plaisier *et al.*,

1991; Hougard *et al.*, 1997). Studies conducted over two decades after the end of vector control suggests that 14 years of vector control may achieve long-term elimination of onchocerciasis, even in the absence of chemotherapy, provided the treated areas are not subjected to any contamination by exogenous parasites carried in infected humans or flies (Hougard *et al.*, 2001). Vector control is a valid method of interrupting transmission, especially if it complements Mass Drug Administration with Ivermectin (MDA-IVM) until parasite reservoirs within any given population is cleared (Awoke and Kasa, 2006).

There is need for an acceptable, environmentally, and non-target-organisms' friendly alternatives to synthetic insecticides. This is to enable the continuation of vector control which have proven to be effective. Any such alternative needs to be cheap and easily accessible. It should also require minimal or no technical skills for their effective deployment. Presently, plants represent the most acceptable source of safe pesticides and plant essential oils are known to affect the behavior of arthropods in many ways, including as repellents (Usip *et al.*, 2006; Camara *et al.*, 2015; Nalini *et al.*, 2017). Plant essential oils are natural products mostly present in aromatic and medicinal plants (Ayse and Ahu, 2025).

The biological activity of any given essential oil or plant extract depends mostly on the chemical composition of such extract. Essential oils with insecticidal and repellent potentials could bolster onchocerciasis elimination as potent vector control alternatives while preserving human and other non-targeted organisms and reducing negative impacts on the environment and biodiversity. A reduction in human vector contact through repellent action of plant

products, would significantly reduce the transmission of onchocerciasis.

Eryngium foetidum L. (Apiaceae) is a herb, indigenous to Central America and West Indies but can also grow in other parts of the world including Africa and Nigeria in particular (Wong et al., 1994). The leaves are similar to those of coriander sharing similar strong and pungent smell (Ochse and van Da Brink, 1977). *Eugenia uniflora* (Myrtaceae) on the other hand is a flowering plant native to tropical South America's east coast. It is often used in gardens as a hedge or screen (Janick and Paull, 2008).

The insecticidal and repellent activities of *E. foetidum* and *E. uniflora* against black flies has not previously been studied. However, their strong aromatic, antioxidant, antioxidative deterioration activities in food properties, phytochemical compositions and pharmacological potentials have all been studied (Martins et al., 2003; Wang et al., 2012; Thomas et al., 2017). This study investigated the repellence potentials of essential oils of *E. foetidum* and *E. uniflora* against adult black flies and determined their chemical composition.

MATERIALS AND METHODS

Study site

The study was carried out from July to September 2020 at Kwa Fall in Aningeje, Akamkpa Local Government Area of Cross River State, Nigeria and the immediate river emanating from it. This period was the peak period for black fly vectors in the area. Aningeje is an onchocerciasis endemic community (Chikezie et al., 2022) with high density of black fly vectors (Yuan et al., 2016).

Selection and collection of plant materials

Eryngium foetidum and *Eugenia uniflora* were selected for this study based on their phytochemical compositions, pharmacological potentials (Martins et al., 2003; Wang et al., 2012) and their widespread use in ethno-medicine. Fresh and

healthy leaves of *E. foetidum* and *E. uniflora* were collected from the medicinal farm of the Department of Pharmacognosy and Natural Medicines, University of Uyo, Uyo, Akwa Ibom State, Nigeria. Identification of the plants was carried out in the herbarium of the Department of Botany and Ecological Studies, University of Uyo with voucher numbers Chikezie, UUH, 4088 for *E. foetidum* and Chikezie, UUH, 4097 for *E. uniflora*.

Distillation of Essential oil

The hydro-distillation method was used to extract essential oils from plant materials in a Clevenger-type apparatus. Considering the volatility of some plant essential oils and other components, the extraction was conducted on fresh plants leaves in order to recover as much oils as possible. The plant materials were washed and chopped into tiny parts. One thousand grams (1,000 g) of plant materials were measured out using the triple beam balance, into a 5 L round-bottomed flask connected to a Clevenger apparatus. About 1,500 mL of water was introduced into the flask and heated to boil for 4 hours. After boiling, the volatile oils were collected into a sample bottle with tight screw caps. The oils were maintained at 4 °C until use. The volatile oil yield from the plants were obtained using the formula: mL/100g of fresh plant material (Xiao-Meng et al., 2018).

Determination of repellency potential

Eighteen trained fly collectors were recruited for this study. On the first day, 6 collectors were used for the study of the repellent potentials of *E. foetidum* essential oil at different concentrations [3 collectors each applying different concentrations of the essential oil (5 %, 10 %, 20 %), one collector applied N,N-Diethylbenzamide as positive control, one collector did not apply anything and served as a negative control and the last of the collectors applied liquid paraffin]. The procedure was repeated on the second day

using the same concentrations of a second plant, *E. uniflora* essential oil, while on the third day, the procedure was repeated using the same concentrations of a combination of the essential oils of the two plant (*E. foetidum* and *E. uniflora*).

Percentage repellency was calculated using the formula:

$$PR(\%) = \left[\frac{(N_c - N_t)}{(N_c + N_t)} \right] \times 100$$

Where N_c is the number of insects in the negative control half and N_t is the number of insects in the tested half (WHO, 1991).

Bioassay for the repellency of *Eryngium foetidum* and *Eugenia uniflora* on *Simulium* vectors

The repellence activity of essential oil extracts was investigated against adult female black flies using the human landing collection (HLC) technique as described by Usip *et al.* (2006). To determine the repellence activity of each of the oil extracts, a stock oil solution of 20% v/v of pure oil was made with liquid paraffin. Further dilutions were made from this stock using liquid paraffin (v/v). Three concentrations, 20 % v/v, 10 % and 5 % v/v were used for the repellence bioassays. Aliquots of 2.5 ml were applied topically on both legs and forearms of each test individually. The test experiments were separated by at least 5 meters from each other to minimize the chances of cross effects of the various extracts. Fly collection followed the protocol of WHO, (1991). The study was carried out during the established peak period of agricultural activities in the village since this is known to be a major predisposing factor to bites from the black fly vectors in the area. The fly landing rate was recorded at one-hourly intervals during the test period.

Determination of chemical composition of essential oil

The identification of active compounds present in the essential oils were carried out using Gas Chromatography—Mass Spectrometry (GC-MS) Agilent system (GC 7890B, MSD 5977A, Agilent Tech), coupled to a model 7890 N gas chromatograph, Triple Quad 7000 A model mass detector and an Agilent ChemStation data system. The GC column was an HP-5 ms fused silica capillary with a (5% phenyl)-methyl polysiloxane stationary phase (30 m x 250 μ m x 0.25 μ m). Helium carrier gas was used with a column head pressure of 9.7853 psi and flow rate of 1.2 mL/min. Inlet temperature and MSD detector temperature was 250 $^{\circ}$ C. The following G-C oven temperature program was adopted as follows: 50 $^{\circ}$ C initial temperature, held for 5 minutes; increased at 6 $^{\circ}$ C/minute to 190 $^{\circ}$ C for 20 minutes; increased 7 $^{\circ}$ C/minute to 290 $^{\circ}$ C for 15 minutes; increased 7 $^{\circ}$ C/minute to 300 $^{\circ}$ C for 10 minutes. The sample was dissolved in dichloromethane (split ratio 10:1; split flow 12 mL/minute). Total ion chromatogram peaks were used to determine the percentage composition of each compound present in the essential oils. The components were identified by comparison of their mass spectra with those available in the NIST-1998 MS library. The retention indices (RI) of separated compounds were determined relative to the retention time of homologous n-alkane series (C₉-C₂₄) analyzed at the same GC-MS parametric values as with the essential oils, with linear interpolation on the HP-5 ms column. The calculated RI of separated compounds was compared with available published data for the determination of elution order and

identification of compounds (Adams, 2007; Hazarika et al., 2012).

Data analysis

Percentage repellence was calculated from the average number of bites received by individuals in a treatment group relative to that of the negative control experiments. Significant means were separated using LSD. Repellency data were analyzed using SPSS version 18.

RESULTS

The repellent activities of *E. foetidum* and *E. uniflora* essential oils against black flies at varied concentrations of 5 %, 10 % and 20 % were compared with N,N-Diethylbenzamide as a positive control and the untreated as negative control over a 5-hour exposure time (7 am – 11 am). Table 1 shows that the negative control had zero repellent activity, while the positive control had a 61.48 % average repellency. At 5 %, 10 % and 20 % concentrations, *E. foetidum* had an average repellent activity of 67.4 %, 68.3 % and 74.2 % respectively. On the other

hand, *E. uniflora* essential oils at 5 %, 10 % and 20 % concentrations exhibited an average repellent activity of 50.10 %, 63.6 % and 84.3 % respectively. Generally, there were no significant differences ($P > 0.05$) between the repellence activities of the plant oils and the positive control at the various concentrations tested except the essential oil of *E. uniflora* which had a significantly lower repellent property at 5 % concentration. The mean percentage repellency of the oil were all higher than that of the positive control (61.48 %) except the essential oil of *E. uniflora* at 5 % concentration.

This study also showed that repellent activities increased with increasing concentrations and reduced with increase in exposure time. In addition, the repellence activity of the two plant oils at 10 % and 20 % concentrations showed great potentials, with up to 2 hours of 100 % repellency. A synergistic study of the repellence effect of *E. foetidum* and *E. uniflora* gave a higher repellency time of 3 hours of 100 % repellency than individual plant oil which gave 2 hours of 100 % repellency each.

Table 1. Repellent activities of essential oil extracts of *Eryngium foetidum*, *Eugenia uniflora*, combined oils and controls against black flies of the *S. damnosum* complex. Means along row with the different letter(s) are significant at P<0.05.

Sample	Repellent %					Mean±S.E.	p Value
	0 h	1 h	2 h	3 h	4 h		
E. foetidum							
5	100.00±0.00a	83.00±0.10b	69.20±0.80c	52.60±0.20d	34.15±0.85e	67.79±7.65	<0.001*
10	100.00±0.00a	100.00±0.00a	64.80±4.80b	52.20±2.10c	34.85±3.35d	70.37±8.71	<0.001*
20	100.00±0.00a	100.00±0.00a	73.70±0.70b	58.05±1.95c	43.35±1.35d	75.02±7.52	<0.001*
E. uniflora							
5	100.00±0.00a	59.40±1.10b	43.25±0.15c	28.35±1.75d	21.30±0.70e	50.46±9.35	<0.001*
10	100.00±0.00a	100.00±0.00a	53.65±3.35b	44.70±3.60c	28.45±1.85d	65.36±9.84	<0.001*
20	100.00±0.00a	100.00±0.00a	78.15±0.55b	74.70±0.20c	67.70±0.70d	84.11±4.47	<0.001*
E. foetidum and E. uniflora							
5	100.00±0.00a	100.00±0.00a	48.60±3.10b	45.75±0.25b	45.90±1.10b	68.05±8.72	<0.001*
10	100.00±0.00a	100.00±0.00a	79.15±9.05b	49.40±6.50c	40.80±0.40c	73.87±8.45	0.001*
20	100.00±0.00a	100.00±0.00a	100.00±0.00a	58.50±3.70b	49.85±1.75c	81.67±7.56	<0.001*
Negative Control	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	NA
Positive Control	100.00±0.00a	100.00±0.00a	43.35±1.35b	40.05±0.65c	23.10±1.10d	61.30±10.78	<0.001*
Liquid Parafin	23.70±3.10a	22.35±2.25ab	20.90±0.90ab	16.80±0.80bc	11.75±0.25c	19.10±1.57	0.026*

Means with different alphabet(s) along the same row are statistically significant at p≤0.05.

Chemical Composition of Essential Oils

Figures 1 and 2 show the gas chromatogram of the essential oils of *E. foetidum* and *E. uniflora* respectively, with the various components of the oil passing through the column and reaching the mass spectrometer detector at different times as shown on the x - axis. The peaks that are shown correspond to the time at which each of the components reached the detector. The y-axis, or the area of the peak reflects the amount of each specific analyte that is present, based on the number of counts taken by the mass spectrometer detector at the point of retention.

Eryngium foetidum essential oil characterization showed that it contained a

total of twenty-five (25) compounds (Table 2). It was rich in monoterpenes (29.2 %), sesquiterpenes (25.0 %) and Triterpenes (4.2 %). Two single most abundant chemical compounds found in the essential oil of *E. foetidum* in this study were 2-Dodecenal, (E)- (39.32 %) and Cyclododecane (13.32 %). A total of thirty-four (34) chemical compounds were recorded from the essential oil of the second plant, *E. uniflora* (Table 3). The essential oil *E. uniflora* was found to be principally composed of sesquiterpenes (91.0 %). In the case of *E. uniflora*, the two single most abundant chemical compounds were Benzofuran, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dim (28.41 %) and gamma.-Elemene (8.63 %).

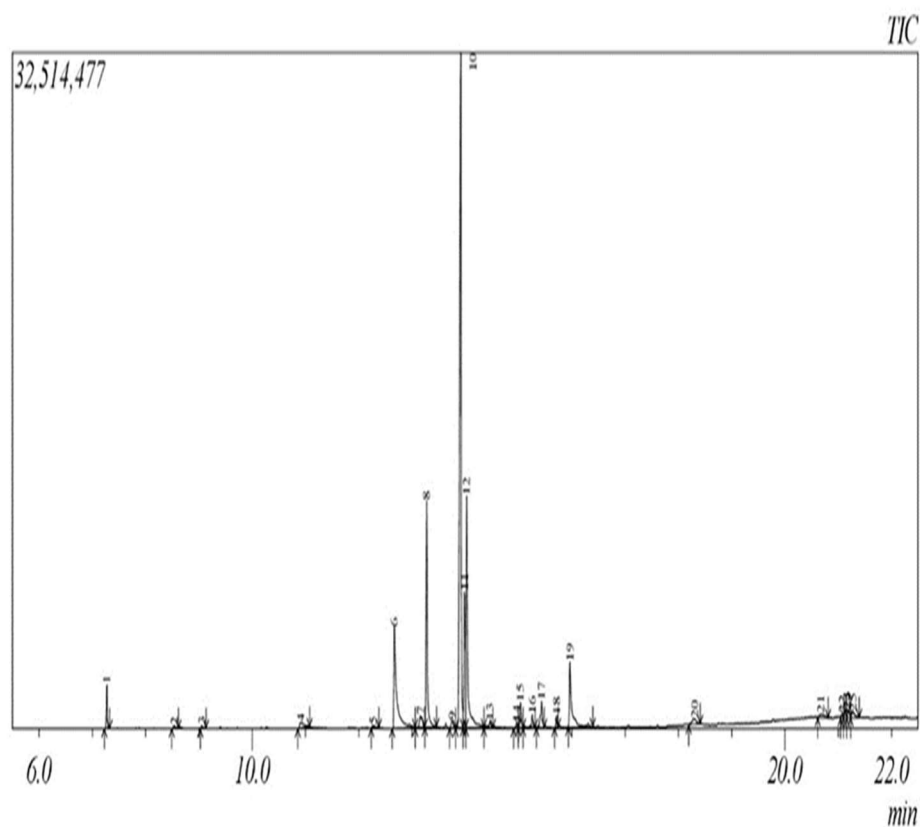


Figure 1. Chromatogram of GC-MS analysis of *E. foetidum* essential oil.

The peaks correspond to the time at which each of the components reached the detector. The y-axis reflects the amount of each specific analyte that is present.

Table 2. Chemical composition of Oil extract of *E. foetidum* after GC-MS analysis

Peak	Retention Time	Area %	Molecular Formula	Molecular Weight	SI	Name
1	7.272	1.67	C10H16	136	98	(1R)-2-Trimethylbicyclo[3.1.1]hept-2-ene.
2	8.534	0.22	C10H14	134	91	1,3,8-p-Menthatriene
3	9.053	0.18	C10H16	136	91	.gamma.-Terpinene
4	10.910	0.98	C10H20O	156	93	Decanal
5	12.264	0.28	C10H12O	148	84	Benzaldehyde, 2,4,6-trimethyl-
6	12.671	10.32	C10H12O	148	93	Benzaldehyde, 2,4,5-trimethyl-
7	13.159	1.19	C10H18O	154	88	cis-4-Decenal
8	13.276	10.34	C12H24O	184	98	Dodecanal
9	13.749	0.56	C12H22O	182	84	2-Dodecenal, (E)-
10	13.913	38.76	C12H22O	182	94	2-Dodecenal, (E)-
11	13.987	7.20	C12H24O	184	93	trans-2-Dodecen-1-ol
12	14.026	13.32	C12H24	168	96	Cyclododecane
13	14.462	0.54	C15H24	204	79	Naphthalene,1,2,3,5,6,7,8,8a-octahydro-1,8a-dimethyl-7
14	14.961	0.26	C15H26O	222	85	1,6,10-Dodecatrien-3-ol,3,7,11-trimethyl-, (E)-
15	15.038	1.32	C15H24O	220	79	Isoshyobunone
16	15.257	0.91	C15H24O	220	91	Caryophyllene oxide
17	15.433	1.85	C15H26O	222	91	Carotol.
18	15.713	0.56	C15H24O	220	77	Alloaromadendrene oxide-(1)
19	15.967	5.51	C17H32O	252	94	8-Hexadecenal, 14-methyl-, (Z)-
20	18.300	0.99	C32H52O2	468	63	9,19-Cycloergost-24(28)-en-3-ol,4, 14-dimethyl-, acetate
21	20.677	0.65	C33H58OSi	498	60	Silane, [[[3.beta.)-lanosta-9(11),24-dien-3-yl]oxy]trimet
22	21.083	0.28	C26H44	356	39	1-(cyclopropacyclododecan-1-ylidene)cyclopropacyclod
23	21.152	0.53	C27H45N3O3	459	28	N-Methyltomatidine
24	21.167	0.78	C28H47NO2	429	31	N-Methyltomatidine
25	21.275	0.78	C30H48O2	440	49	3-Oxoallobetulane

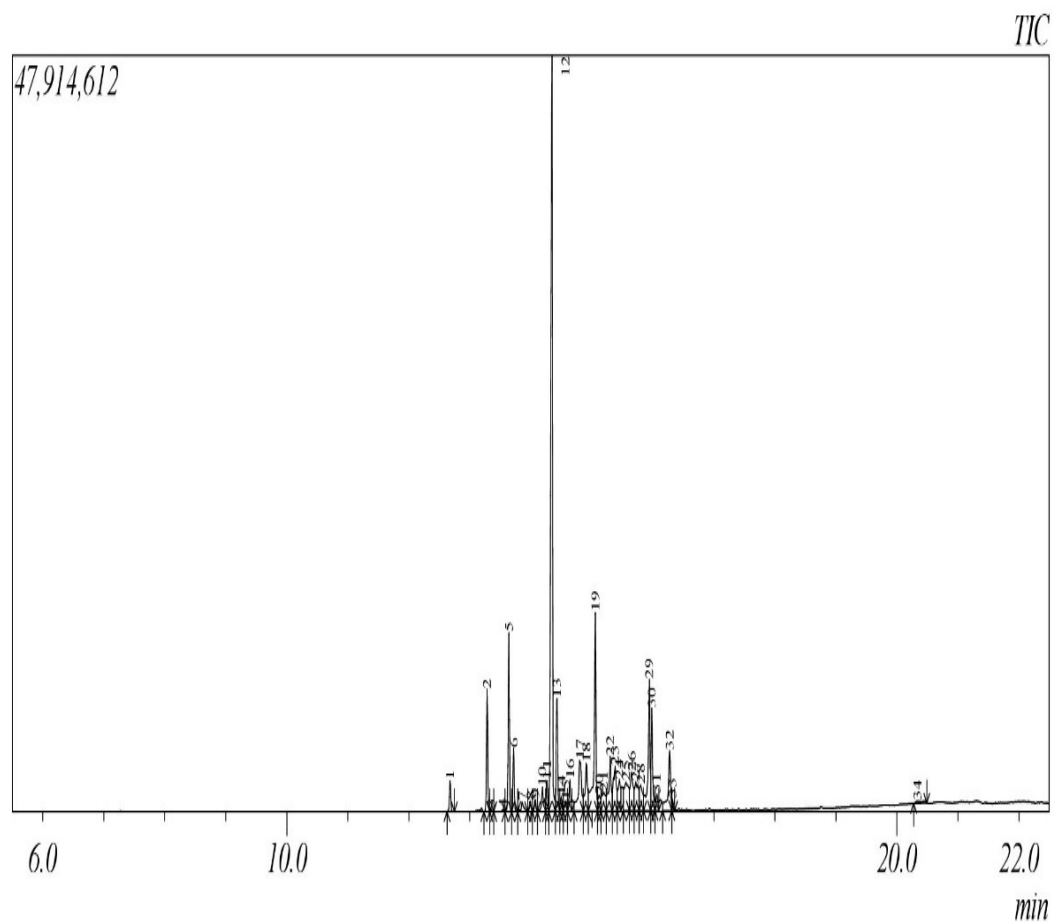


Figure 2. Chromatogram of GC-MS analysis of *E. uniflora* essential oil. The peaks correspond to the time at which each of the components reached the detector. The y-axis reflects the amount of each specific analyte that is present.

Table 3. Chemical composition of Oil extract of *E. uniflora* after GC-MS analysis

.	Retention Time	Area %	Molecular Formula	Molecular Weight	SI	Name
1	12.678	0.92	C ₁₅ H ₂₄	204	93	Cyclohexene, 4-ethenyl-4-methyl-3-(1-methyleth
2	13.284	3.34	C ₁₅ H ₂₄	204	94	Cyclohexane, 1-ethenyl-1-methyl-2,4-bis(1-meth
3	13.347	0.10	C ₁₅ H ₂₄	204	83	1,4-Methano-1H-indene, octahydro-4-methyl-8-m
4	13.543	0.11	C ₁₅ H ₂₄	204	86	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octa
5	13.641	4.84	C ₁₅ H ₂₄	204	96	Caryophyllene
6	13.719	1.85	C ₁₅ H ₂₄	204	94	gamma.-Elemene
7	13.859	0.63	C ₁₅ H ₂₄	204	93	Alloaromadendrene
8	13.992	0.35	C ₁₅ H ₂₄	204	95	Humulene
9	14.073	0.41	C ₁₅ H ₂₄	204	94	Alloaromadendrene
10	14.191	1.28	C ₁₅ H ₂₄	204	88	2-Isopropenyl-4a,8-dimethyl-1,2,3,4,4a,5,6,7-oct
11	14.263	1.02	C ₁₅ H ₂₄	204	94	1,6-Cyclodecadiene, 1-methyl-5-methylene-8-(1-
12	14.347	28.41	C ₁₅ H ₂₀ O	216	94	Benzo-furan, 6-ethenyl-4,5,6,7-tetrahydro-3,6-dim
13	14.429	4.02	C ₁₅ H ₂₄	204	93	Guaia-1(10),11-diene
14	14.499	0.61	C ₁₅ H ₂₄	204	90	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimeth
15	14.575	0.57	C ₁₅ H ₂₄	204	89	.gamma.-Muurolene
16	14.640	1.39	C ₁₅ H ₂₄	204	91	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimeth
17	14.803	3.95	C ₁₅ H ₂₄	204	92	1H-Cycloprop[e]azulene, 1a,2,3,4,4a,5,6,7b-octa
18	14.912	2.62	C ₁₅ H ₂₄	204	95	Selina-3,7(11)-diene
19	15.057	8.63	C ₁₅ H ₂₄	204	93	gamma.-Elemene
20	15.120	0.96	C ₁₅ H ₂₆ O	222	77	1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-m
21	15.196	1.63	C ₁₅ H ₂₄ O	220	79	1H-Cycloprop[e]azulen-7-ol, decahydro-1,1,7-tri
22	15.301	3.12	C ₁₅ H ₂₆ O	222	92	Globulol
23	15.383	3.16	C ₁₅ H ₂₆ O	222	89	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-
24	15.451	1.77	C ₁₅ H ₂₆ O	222	89	2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-octahyd
25	15.557	3.00	C ₁₅ H ₂₆ O	222	77	1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-m
26	15.650	2.57	C ₁₅ H ₂₆ O	222	86	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-
27	15.720	2.50	C ₁₈ H ₃₀	246	73	d-Norandrostane (5.alpha.,14.alpha.)
28	15.799	1.87	C ₁₅ H ₂₆ O	222	87	.tau.-Muurolol
29	15.942	6.08	C ₁₆ H ₂₄	216	74	Cyclobutane, tetrakis(1-methylethylidene)-
30	15.984	3.29	C ₁₅ H ₂₀ O	216	69	Cyclopentanone, 2,5-dicyclopentylidene-
31	16.065	0.92	C ₁₅ H ₂₆ O	222	83	1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methyl
32	16.274	3.40	C ₁₅ H ₂₂ O	218	94	3,7-Cyclodecadien-1-one, 3,7-dimethyl-10-(1-methylet
33	16.325	0.30	C ₁₅ H ₂₆ O	222	89	1-Naphthalenol, decahydro-1,4a-dimethyl-7-(1-methyl
34	20.337	0.37	C ₃₂ H ₅₂ O ₂	468	57	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, acet

DISCUSSION

The public health and socio-economic consequences associated with the bites of

black flies are enormous. The control or prevention of man-fly contact is a veritable way of preventing onchocerciasis

transmission and its attendant morbidity and herbal repellents can provide safe, eco-friendly and inexpensive means of protecting inhabitants of endemic areas from black fly bites. Topical application of essential oils extracted from plants significantly reduced the biting rates of black flies. The findings of this study agree with the reports of Usip *et al.* (2006), Hazarika *et al.* (2012) and Adeleke *et al.* (2023), each of which reported protection from different plants extracts at various concentration and in different areas.

In the present study, at 20 % essential oil concentration, the two plants significantly repelled black flies for more than 4 hours during peak biting periods. This suggests that topical applications of these essential oils could provide effective protection from black flies bites during active biting periods. West African black flies mostly exhibit a bimodal biting activity (Opara *et al.*, 2005; Kiplang'at and Mwangi, 2014; Mafiana, 1988; Adewale *et al.*, 1999; Opara *et al.*, 2008) with early morning and late evening peaks. These periods correspond with times of human outdoor activities in the study area. It is possible that early morning and late evening applications of these essential oils might reduce human contact with black flies in the community, thereby reducing onchocerciasis transmission. The implication of this finding is that in endemic communities undergoing treatment with ivermectin, vector – man contact could be reduced below the thresholds under which transmission of the disease is interrupted. Furthermore, reduction in black fly biting nuisance would provide succor to people living in onchocerciasis endemic areas where black fly bites are a scourge on its own. Similar observation have been made by (Usip *et al.*, 2006; Hazarika *et al.*, 2012, Adeleke *et al.*, 2011; Sam-Wobo *et al.*, 2011). They all found different plant extracts to be effective control agents for various insect vectors at different concentrations and locations.

Of interest in the present study is the percentage repellence activity obtained from the two essential oils at 20 % concentration which were higher than the repellence from the positive control after 5 hours of exposure. This is most likely due to the presence of bioactive compounds present in the oils. Also, in the present study, a synergistically enhanced repellency, providing a total repellence of up to 3 hours was observed when the essential oils of *E. foetidum* and *E. uniflora* were mixed and applied at 20 % concentration. This observation was in line with those of Kiplang'at and Mwangi, (2014) who reported that the reinforcement of neem oil with sweet basil oil and *Lemon eucalyptus* oil resulted in a superior repellent activity. This synergistic effect could be as a result of the different modes of action of various components present in each of these oils. Also, Chokechaijaroenporn *et al.* (Chokechaijaroenporn *et al.*, 1994) found that a combination of volatile compound with a non-volatile substance blocked insect attack both on the air and the skin surface. In yet another study, improved repellent activity of neem oil was obtained by combining the oil with sweet basil and *Lemon eucalyptus* oils (Oyedele *et al.*, 2002).

Generally, terpenes have demonstrated strong repellent potentials against insect vectors (Choi *et al.*, 2002; Tripathi *et al.*, 2009; Tyagi, 2016), and were richly present in the oils studied. It is possible that any other compounds found in the essential oils may have also contributed and potentiated their overall repellent effects on the simuliids. Plant essential oils and their constituent compounds can play a key role in onchocerciasis elimination. This approach to onchocerciasis elimination can be self-sustaining, with minimal research inputs. Sustainability and affordability are important requirement for any tools that are to be deployed for elimination of diseases. Local

people can readily acquire and apply such plant-based repellents even if it means buying it from local markets. It can also be possible for households to plant *E. foetidum* and *E. uniflora* in and around their dwelling places for the purpose of repelling flies including disease vectors such as black flies and mosquitoes. This can also be adopted for use in diverse environments.

CONCLUSION

This study revealed the repellent potentials of essential oils from *E. foetidum* and *E. uniflora* against black flies. The essential oils holds a potential as effective repellents against black flies. Results of the present work show that these essential oils can effectively serve as an alternative to the few locally available commercial repellents. The plants tested contained well known insecticidal and repellent compounds that accounted for the reduced man-black fly contact. This is a grey and yet important area that could impact onchocerciasis control and elimination, not just in the study area but even in other areas beyond this particular study area. Improvement in chemotherapeutic coverage as well as a functional vector control approach will support and fast-track onchocerciasis elimination. Plant based repellents could reduce the discomfort posed by black fly bites as well as preserve the environment and other non-targets organisms. However, there is need to test these natural products in different geo-climates with different black fly species to really ascertain their suitability in other places.

Ethical approval

Ethical approval was obtained from the Ethical Review Board of the Cross River State

Ministry of Health with the reference number CRS/MH/HREC/020/VOL.V1/204. Care was taken to ensure that all fly collectors who had not been treated in the past six months before the study were treated by the community-based ivermectin distributor and properly documented

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