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Fumigant toxicity and phytochemical analysis of *Petiveria alliacea* (Linneaus) leaf and root bark oil on adult *Culex quinquefasciatus*

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Abstract

Background: Culex mosquitoes are a major vector of public health importance and have been implicated for the transmission of some parasitic diseases such as lymphatic filariasis and West Nile virus. It has also been reported to cause several problems which include developing resistance to synthetic insecticides, thus, necessitating the search for an insecticide of botanical origin which is bio-degradable, non-toxic, and readily available for man's use. This study aimed to evaluate the phytochemicals present in *Petiveria alliacea* and the fumigant efficacy of its oil extract against the adult stage of *Culex quinquefasciatus*.

Results: The result obtained shows that the oil extract of *Petiveria alliacea* at all concentrations had a significant effect on the adult mosquito for fumigant toxicity with percentage mortality range of 75.00–100% within a 2-h exposure period ($P < 0.05$) for the leaf extract and 81.67–100% mortality for the root bark extract. The synergistic effect of the leaf and root bark oil was also investigated. The lethal concentration (LC_{50}) of the leaf, root, and synergistic effect of leaf and root oil extract required to kill 50% of the adult *Culex quinquefasciatus* was 0.45 ml, 0.53 ml, and 0.47 ml, respectively. However, 2.20 ml, 1.194 ml, and 1.15 ml of the leaf, root, and leaf and root oil extract were required to kill 90% (LC_{90}) after a 2-h exposure period. A total of 29 organic compounds were isolated from leaf and root bark oil of the plant. The study has revealed that the leaves and root bark of *Petiveria alliacea* are rich in phytochemicals

Conclusion: These findings suggested *Petiveria alliacea* oil extract could be a good source of insecticide which may be used for the production of biopesticides. The present findings have important implications in the practical control of adult mosquito by using botanical insecticides. These plant extracts are easy to prepare, inexpensive, and safe for mosquito control which possesses enough insecticidal potential and can be used directly around human dwellings. The result suggests possible utilization of the cheap and readily available botanicals for possible control of mosquitoes as part of an integrated vector management programme.

Keywords: *Petiveria alliacea*, Bio-pesticide, *Culex quinquefasciatus*, Fumigant toxicity

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Background

Petiveria alliacea L. belongs to the family Phytolaccaceae which is considered to be the most primitive family of the Caryophyllales (Cronquist 2002). There are about 17 genera and 120 pan-tropical species in this group which are mostly found in North and South America (Barroso 2004).

Vector-borne diseases are among the major causes of illness and death in many developing countries. Mosquitoes (Diptera: Culicidae, Anophelinae) are the most important order in the animal kingdom which cause millions of deaths every year. This order is responsible for transmitting the most important vector-borne diseases including malaria, lymphatic filariasis, Japanese encephalitis, West Nile virus, and dengue fever as well as yellow fever and other forms of encephalitis (Barik et al. 2012). *Culex pipiens* and *Culex quinquefasciatus* are the two Culex species with the most important public health importance and are capable of transmitting bancroftian encephalitis caused by the West Nile virus and Japanese encephalitis. However, there are several other species of Culex that are of public health importance. The approach to combat this disease largely relied on interruption of the disease transmission cycle by either targeting the mosquito larvae through spraying of stagnant waters that serve as breeding sites or by killing the adult mosquitoes using insecticides. The vector-borne control strategies in Nigeria include the effective combination of insecticide-treated nets and indoor residual spraying which has been an adequate control measure. Mosquito resistance to the currently used insecticides and the emergence of multi-drug-resistant strains of parasites have escalated the malaria problem in the affected countries (Oduola et al. 2010).

Recently, there are limitations in the control measures used in the mitigation of malaria vectors, which includes insecticide resistance as well as impediments in achieving high scope (Killeen et al. 2002). *Culex quinquefasciatus* has also been reported by WHO to resist Organochlorines, Carbamates, Organophosphates, and Pyrethroids. In Nigeria, the susceptibility status of many mosquitoes into dichlorodiphenyl trichloroethane (DDT) and other classes of insecticides including organochlorine, organophosphate, carbamates, and recently pyrethroid in different zones has been well documented (Awolola et al. 2007). In South West Nigeria, the first case of pyrethroid resistance in *Anopheles gambiae*, a major malaria vector, in Nigeria was documented by Awolola et al. (2002) and since then the occurrence has been well established in this region (Kristan et al. 2003; Oduola et al. 2010). In North-central Nigeria, permethrin and DDT resistance in *Anopheles gambiae* has also been reported (Ndams et al. 2006; Olayemi et al. 2011). Thus, synthetic insecticides have caused several problems including the development of resistant insect strains, ecological imbalance, harm to mammals, and non-target arthropods. These setbacks of the hitherto developed insecticides have made researchers in these areas to effortlessly find and produce environmentally safe alternatives. As part of traditional healthcare, medicinal plants have been used in most parts of the world for thousands of years (Ajayi et al., 2010). The use of these plants by man in the treatment of ailments and diseases is a common practice in developing countries. However, botanical insecticides may serve as suitable alternatives to synthetic insecticides in the future, as they are relatively safe, bio-degradable, and readily available in many parts of the world. Researchers have resorted to plant extracts as potent sources of natural bio-insecticides (Bekele et al., 2014). Although insecticides of plant origin have been broadly used on agricultural pests, a very limited extent has been used against insect vectors of public health importance. Because of these, many of the reported tropical plants came under scrutiny, resulting in the extraction and characterization of their active components, which accounted for various uses by people. The most important constituents are alkaloids, terpenoids, steroids, phenols, saponins, and tannins, among others. Mosquito controls strategies, especially those that are effective, cheap, and environmentally non-hazardous, are needed. Hence, crude plant extracts have played an important role in this aspect. Diverse herbal products have been used as natural insecticides before the discovery of synthetic organic insecticides and a wide range of plants with larvicidal and adulticidal properties have been used against insects. Although various compounds of plant sources have been documented as insecticides and larvicides, there is still a vast scope for the discovery of more effective plant products particularly in the indigenous flora of lesser studied countries like Nigeria. However, this present research focuses on the fumigant toxicity of *Petiveria alliacea* leaf and root bark oil on the mortality of adult *Culex quinquefasciatus*; these plants have been said to possess various medicinal properties including antibacterial, antifungal, and anticancer constituents. The effectiveness of this plant powder against mosquito can determine how important it might be in our environments.

Methodology

Materials used

Common name: guinea henweed (Tarzon plant); fresh plant (*Petiveria alliacea*), dried leaves and root bark of *P. alliacea*; plant extract from leave and roots; distilled water; clean tray; filter paper/seiver; mortar and pestle; polyethylene bags; 100% ethanol; rotary evaporator which is used for crude extract; UV/V spectrophotometer; GC-MS; test tube; beaker; and micro-pipette

Plant material collection

Plant material was collected from Oke-Geu village located in Ondo East Local Government Area of Ondo

town, Ondo State, Nigeria. The plants were obtained precisely, at coordinates: 7°2′4″ N/4°53′24″ E and kept separately in polythene bags and brought to the laboratory for extraction after being air-dried for 30 days. The collections were made on March 15, 2019, and were authenticated by a botanist.

Extraction of *Petiveria alliacea* oil

The plant material was washed and air dried and kept in a polythene bag after which they were pulverized using a grinding machine (Model 2140J). For preparation of extract, approximately 550 g of plant powder (root, leaves) was taken and soaked in 2.5 l of absolute ethanol, respectively, after which the powder (leaf, root) was stirred separately in the solvent (ethanol) for 72 h for proper dissolution of the active ingredient present in the plant material. The extract was then filtered using a muslin cloth and extraction was done using a rotary evaporator separating machine (Rosena, model: SW200, Germany). The resulting extract contained both the solvent and oil. The oil was exposed to air, so that traces of the volatile solvent evaporated, leaving the oil extract. The resulting oil was kept in glass bottles and used for subsequent experiments.

Phytochemical screening

Phytochemical screening tests have been performed to detect the presence of bioactive components in the aforementioned plant extracts. The results for these tests were as follows:

Qualitative determination of phytochemicals

Test for alkaloids About 10 mg of each sample was taken and was dissolved in 2 mL of the Wagner's reagent. After dissolving both, the appearance of reddish-brown-coloured precipitates confirms the presence of alkaloids in the extracts.

Test for flavonoids About 10 mg of each sample was taken and few drops of diluted NaOH were added to each. The appearance of a yellow colour which disappears or becomes colourless after adding a few drops of diluted H_2SO_4 confirms the presence of flavonoids in the extracts.

Test for saponins About 10 mg of each sample was taken and diluted with 20 mL of distilled water. The test tubes were then shaken for 15 min by hand. Formation of foam on top of the test tube shows the presence of saponins in the extracts.

Test for steroids About 10 mg of each extract was taken and 1 mL of concentrated H_2SO_4 was added to each by the side walls of the test tube. The appearance of dark a reddish-green colour confirms the presence of steroids in the plant extracts.

Test for tannins About 10 mg of each sample was taken and was dissolved in 45% of the ethanol. The test tubes were then boiled for 5 min and 1 mL of 15% ferric chloride solution was added to each. The appearance of a greenish to black colour confirms the presence of tannins in the extracts.

Test for glycosides To 1 ml of each extract, a few drops of glacial acetic acid and ferric chloride and 3–4 drops of concentrated sulphuric acid were added. The appearance of a blue-green colour indicates the presence of glycosides.

Test for triterpenoids The test for triterpenoids is the same as that for steroids; the appearance of red, pink, or violet colour at the junction indicates the presence of triterpenoids.

Test for reducing sugar To 0.5 ml of extract solution, 1 ml of water and 5–8 drops of Fehling's solution was added to the test tube hot and observed for brick-red precipitate.

Test for anthraquinones Ten millilitres of benzene was added in 6 g of the Ephedra powder sample in a conical flask and soaked for 10 min and then filtered. Further, 10 ml of 10% ammonia solution was added to the filtrate and shaken vigorously for 30 s and a pink, violet, or red colour indicated the presence of anthraquinones in the ammonia phase.

Gas chromatography-mass spectrometry (GC-MS) analysis

GC-MS analysis Gas chromatography-mass spectrometric (GC-MS) analysis was performed using Varian 4000 GC-MS system (Agilent Technologies, Santa Clara, CA, USA) equipped with an HP-5MS capillary column (30 m × 0.25 mm × 0.25 μm) (Agilent J&W Scientific, Folsom, CA) and split (50:1) injection system. The GC oven was programmed from 100 °C held for 4 min to a final temperature of 300 °C at the rate of 4 °C/min and held isothermally at the final temperature of 240 °C for 10 min. Helium at a constant flow rate of 1.5 mL/min was used as a carrier gas and running time of 49 min. A total of 1 μl aliquot of the sample was injected automatically. The samples were analysed in the full scan mode. The electron ionization energy of 70 eV, source temperature of 250 °C, and a solvent delay of 5 min was employed. These compounds were identified based on their mass spectrum, molecular weight, and fragment ions obtained from the mass spectrum. These parameters were matched

with those of reference compounds obtained from the National Institute of Standards and Technology 2011 database which were incorporated into the computer system of the equipment.

All the samples and replicates were continuously injected as one batch in random order to discriminate technical from biological variations. Additionally, the prepared pooled samples were used as quality controls (QCs), which were injected at regular intervals throughout the analytical run to provide a set of data from which the repeatability can be assessed.

Sample preparation

Samples were determined by following the procedure earlier described by Sharma et al. (2016). Organic acids were extracted from 50 mg of the oven-dried (80 °C, 24 h) by adding 0.5 ml of 0.5 NHCl and 0.5 ml of methanol. After that, the samples were shaken for 3 h followed by centrifugation at 12,000 rpm for 10 min. To the supernatant, 300 μl of methanol and 100 μl of 50% sulphuric acid were added followed by overnight incubation in a water bath at 60 °C. The mixture was cooled down to 25 °C, and 800 μl of chloroform and 400 μl of distilled water were added to it followed by vortexing for 1 min. The lower chloroform layer was used to estimate the volatile organic compounds and organic acids using GC-MS

Collection and culture of mosquito species

The wild larvae of *Culex quinquefasciatus* were collected from three regions, namely Oke-Aro, Igoba, and Oba-ile, Akure, Ondo State, Nigeria. The mosquitoes were identified by an entomologist in the Department of Biology, Federal University of Technology, Akure, Nigeria. The culture maintained was under laboratory conditions of 27 ± 1 °C and 70 ± 5% R.H. The larvae were fed on a diet of yeast/biscuit powder. Newly formed pupae were collected and transferred from the trays to a cup containing water and placed in screened cages (60 × 30 × 45 cm) where the adult emerged. The male and female adults were continuously provided with 10% sucrose solution in a jar with cotton wool. On day four post emergence, the adult females (identified by the presence of the long tiny proboscis) were deprived of sugar for 12 h then provided with a shaved albino rat (*Rattus norvigicus*) placed in resting cages overnight for blood feeding. Wet filter paper was placed on the corners for egg laying and the lifecycle was repeated.

Bioassay test

To the treatment set, 1%, 2%, 3%, 4%, and 5% of the oil extract will be taken from the stock solution and added to 10 ml of solvent after which they were tested on the mosquito's species. Twenty adult Culex mosquitoes were introduced into the containers with different treatments and covered with a lid. Each experiment was replicated thrice after which adult mortality was recorded after 30 min, 60 min, 90 min, and 120 min. The control experiment was also replicated thrice.

Percentage mortality

The percentage mortality was calculated by using the formula (1), and corrections for mortality when necessary were done using Abbot's (1925) formula (2)

$$\%_{\mathrm{MORTALITY}} = \frac{N_{\text{dead adult}}}{N_{\text{adult introduced}}} \times 100$$

where $\%_m$ is the percentage mortality, N_{da} is the number of dead adults, and N_{ai} is the number of adults introduced.

Corrected percentage of mortality

$$P_{\mathrm{T}} = \frac{P_{\mathrm{O}} - P_{\mathrm{C}}}{100 - P_{\mathrm{C}}} \times 100$$

where

- P_{T} = corrected mortality (%)
- P_{O} = observed mortality (%)
- P_{C} = control mortality (%)

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) to check for significance using Duncan's new multiple range test at $P < 0.05$ and then the mortality of each bioassay will be subjected to Probit analysis to determine 50% lethal concentration (LC_{50}) and the 90% lethal concentration (LC_{90}) using SPSS (Statistical Package for Social Sciences) version 20.0.

Fumigant effect of leaf and root bark oil of *P. alliacea* on the mortality of adult *Culex quinquefasciatus*

The oil extract of leaf and root bark was prepared in 10 ml ethanol to give 1%, 2%, 3%, 4%, and 5% concentrations, respectively, which are then poured into a petri dish of 9-cm diameter and 3-cm depth. Whatman's no. 1 filter paper of dimension 2 cm by 2 cm was formed into strips and then soaked into each concentration, after which was allowed to air dry for 2 h. The treated filter papers were then placed inside a plastic container of dimension 15 cm diameter and 12 cm depth. Twenty adult mosquitoes (about 48 h old) were introduced into the container with the treated filter papers and then covered with a lid. Each experiment was replicated three times after which the adult mortality was recorded after 30 min, 60 min, 90 min, and 120 min. The control and experiment were also replicated thrice.

Synergistic effect of leaf and root bark oil of *P. alliacea* on the mortality of adult *Culex quinquefasciatus*

Equal concentration of the plant part oil (root and leaf) are measured up to 1%, 2%, 3%, 4%, and 5% respectively. The extract is agitated together to ensure a uniform mixture. Whatman's no. 1 filter paper of dimension 2 cm by 2 cm were formed into strips and then soaked into each concentration, after which was allowed to air dry for 2 h. The treated filter papers when then place inside a plastic container of dimension 15 cm diameter and 12 cm depth. Twenty adult *Culex quinquefasciatus* were introduced into the container with treated filter paper and then covered with the lid. Each experiment was replicated three times after which the adult mortality was recorded after 30 min, 60 min, 90 min, and 120 min, respectively. The control experiment was also replicated thrice.

Results

Fumigant toxicity of leaf oil of *P. alliacea* on the mortality of adult *Culex quinquefasciatus*

The fumigant effect of leaf oil extract on adult mortality of *Culex quinquefasciatus* is presented in Table 1. At 30 min post-treatment, all concentrations of the leaf oil extract (1%, 2%, 3%, 4% and 5%) caused 10.00–33.33% adult mortality. The leaf oil extract at 60 min for all concentrations caused 31.67–71.67% adult mortality, while specifically at 4–5% concentration, 63.33–71.67% mortality was recorded. At 90 min post-treatment period, 91.67–100% mortality was recorded at 4–5% concentration, while 61.67–81.67% mortality was recorded at 1–3% post-treatment period. At 120 min 75.00–88.00% mortality was recorded at 1–2% concentration, however, at 3–5% concentration 93.33–100% adult mortality was recorded and were not significantly ($P > 0.05$) different except for 1% (0.1 ml) which had 75.00% adult mortality but are different from the control.

Fumigant toxicity of root bark oil of *P. alliacea* on the mortality of adult *Culex quinquefasciatus*

The fumigant effect of root oil extract on adult mortality of *Culex quinquefasciatus* is presented in Table 2. At 30 min post-treatment, all concentrations caused 11.67–40.00% adult mortality, however, at 3% concentration, 25.00% mortality was recorded which is significantly different ($P < 0.05$) from all other concentrations. At 60 min post-treatment period, 61.67–81.68% mortality was recorded at 3–5% concentration, respectively, while 36.67–53.33% mortality was recorded at 1–2% concentration. 91.67–100% mortality was first recorded at 90 min post-treatment interval at 3–5% concentration and 66.67–81.67% at 1–2% concentration but were significantly ($P < 0.05$) different from each other. One hundred percent mortality was recorded after 120 min at 3–5% concentration; however, at 1–2% concentration, 81.67–90.00% mortality was recorded and was significantly different ($P < 0.05$) from each other. Meanwhile, at all concentrations, they are significantly different from the control.

Fumigant toxicity of synergistic application of leaf and root bark oil of *P. alliacea* on the mortality of adult *Culex quinquefasciatus*

The synergistic effect of leaf and root bark of *P. alliacea* on the mortality of adult *Culex quinquefasciatus* is presented in Table 3. At all grammes of powder concentration, no adult mortality was recorded at 30 min post-treatment periods except from 5 g that caused adult mortality of 5.00% and was significantly ($P < 0.05$) different from all others. At 60 min post-treatment periods, 1–2 g powder concentration caused 8.33–11.67% adult mortality and were not significantly ($P > 0.05$) different from each other while at 3–4 g powder concentration 30.00–31.67% adult mortality was recorded but were not significantly ($P > 0.05$) different from each other. At 5 g powder concentration, 46.67% adult mortality was recorded which was significantly ($P < 0.05$) different from all others. At 90 min post-treatment periods, 1–5 g powder concentration caused 18.33–63.33% adult mortality and were all significantly ($P < 0.05$) different from each other as well as the control. At 120 min post-treatment period, 1–5 g powder concentration caused 25.00–75.00% adult mortality which were all significantly ($P < 0.05$) different from one another as well as the control.

Table 1 Fumigant toxicity of leaf oil extract of *P. alliacea* on the mortality of adult *Culex quinquefasciatus*

Concentration (%)	%Mortality after			
	30 min	60 min	90 min	120 min
1	10.00 ± 0.00^{b}	31.67 ± 0.33^{b}	61.67 ± 0.33^{b}	75.00 ± 0.00^{b}
2	16.67 ± 0.33^{c}	40.00 ± 0.00^{c}	73.33 ± 0.33^{c}	88.33 ± 0.67^{c}
3	23.33 ± 0.33^{d}	53.33 ± 0.67^{d}	81.67 ± 0.33^{d}	93.33 ± 0.88^{cd}
4	23.33 ± 0.33^{d}	63.33 ± 0.67^{e}	91.67 ± 0.33^{d}	93.33 ± 0.88^{cd}
5	33.33 ± 0.33^{e}	71.67 ± 0.33^{f}	100 ± 0.00^{f}	100 ± 0.00^{d}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is the mean ± standard error of three replicates. Mean followed by the same alphabet are not significantly ($P > 0.05$) different from each other using Duncan's multiple range test

Table 2 Fumigant toxicity of root bark oil extract of *P. alliacea* on the mortality of adult *Culex quinquefasciatus*

Concentration (%)	%Mortality after			
	30 min	60 min	90 min	120 min
1	11.67 ± 0.33^{b}	36.67 ± 0.33^{b}	66.67 ± 0.33^{b}	81.67 ± 0.33^{b}
2	16.67 ± 0.33^{c}	53.33 ± 0.67^{c}	81.67 ± 0.33^{c}	90.00 ± 0.00^{c}
3	25.00 ± 0.00^{d}	61.67 ± 0.33^{d}	91.67 ± 0.33^{d}	100.00 ± 0.00^{d}
4	31.60 ± 0.33^{e}	76.67 ± 0.33^{e}	100.00 ± 0.00^{e}	100.00 ± 0.00^{d}
5	40.00 ± 0.00^{f}	81.67 ± 0.33^{e}	100.00 ± 0.00^{e}	100.00 ± 0.00^{d}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is the mean ± standard error of three replicates. Mean followed by the same alphabet are not significantly ($P > 0.05$) different from each other using Duncan's multiple range test

Lethal dosage effect of *Petiveria alliacea* oil extract after 120-min post-treatment period

As shown in Table 4, the oil extract of the leaf, root, and synergistic application of root and leaf tested on adult *Culex quinquefasciatus* within 120 min post-treatment periods gave a lethal concentration [LC_{50} (LCL–UCL)] values of 0.45 (0.199–0.678), 0.53 (0.301–0.714), and 0.47 ml (0.205–0.664), respectively; however, the [LC_{90} (LCL–UCL)] gave values of 2.20 (1.797–2.760), 1.43 (1.194–1.695), and 1.15 ml (0.909–1.379), respectively.

Phytochemical analysis

Preliminary phytochemical analysis for leaves and root bark of *Petiveria alliacea* are tabulated in Table 5.

When performed qualitative tests for phytochemicals in *Petiveria alliacea*, the number of phytochemicals shows positive results in their specific tests. Though some were found in abundance while some in little amount. In the present study, the phytochemical screening of *P. alliacea* leaf showed positive results for phenol, saponins, tannins flavonoids, glycosides, alkaloids, and steroids. The root bark showed the presence of phytochemicals like flavonoids, tannins, saponins, steroids, phenol, glycosides, and reducing sugar. The analysis indicated that the phytochemicals found in both parts are the same except for reducing sugar found in the root bark.

Table 6 shows the chemical compounds present in the root bark of the plant, with the varying retention time and %peak area. Fourteen compounds were isolated after the analysis. Piperine (11.92%), 9,12,15-octadecatrienoic acid, methyl ester (12.83%), and 1,1-dimethyldecahydronaphthalate (15.02%) were the compounds seen to have the highest proportion, while other compounds like phytol, hexadecanoic acid, and N,N,dimethylalanine among others were also reported. However, Table 7 shows the chemical compounds present in the leaf oil of the plant. A total amount of 15 compounds were isolated with their varying chemical structure, retention time, and %peak area. 1,2,3-propinetriol,1-acetate (22.36%), phytol (21.10%), n-hexadecanoic acid (8.43%), and 1-hexadecanoic acid (8.39%) were the compound with the highest proportion. Other compounds isolated include stigmasterol, pentadecanoic acid, and squalene.

A chromatogram showing the level of volatile organic compounds of leaf extract and root bark extract alongside its retention time against intensity can be seen in Figs. 1 and 2. However, various retention times can be seen in Tables 6 and 7 above for compound identification.

Table 3 Fumigant toxicity of synergistic application of *P. alliacea* root bark and leaf oil extract on the mortality of adult *Culex quinquefasciatus*

Concentration (%)	%Mortality after			
	30 min	60 min	90 min	120 min
1	23.33 ± 0.33^{b}	41.67 ± 0.33^{b}	71.67 ± 0.33^{b}	86.67 ± 0.33^{b}
2	30.00 ± 0.00^{c}	56.33 ± 0.33^{c}	81.67 ± 0.33^{c}	96.67 ± 0.33^{c}
3	40.00 ± 0.00^{d}	71.67 ± 0.33^{d}	96.67 ± 0.33^{d}	100.00 ± 0.00^{d}
4	40.00 ± 0.00^{d}	80.00 ± 0.00^{e}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
5	56.67 ± 0.33^{e}	86.67 ± 0.33^{f}	100.00 ± 0.00^{d}	100.00 ± 0.00^{d}
Control	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}

Each value is the mean ± standard error of three replicates. Mean followed by the same alphabet are not significantly ($P > 0.05$) different from each other using Duncan's new multiple range test

Table 4 Lethal concentration effect of *Petiveria alliacea* oil extract after 120 min post-treatment period

Oil extract	LC_{50} (ml)	95% confidence limit		LC_{90} (ml)	95% confidence limit		Slope	χ^2
		LCL	UCL		LCL	UCL		
Leaf	0.45	0.199	0.678	2.20	1.797	2.760	1.853	4.811
Root	0.53	0.301	0.714	1.43	1.194	1.695	2.991	6.232
Root and leaf	0.47	0.205	0.664	1.15	0.909	1.379	3.318	1.248

Lc_{50} lethal concentration that kills 50% of the exposed adult, *Lc_{90}* lethal concentration that kills 90% of the exposed adult, *LCL* lower confidence Limit, *UCL* upper confidence limit, Slope, *χ^2* chi-square

Discussion

Mosquitoes (Diptera: Culicidae) presents an array of insect which more than any group poses the greatest challenge to human and veterinary health as a vector of diseases (Goddard et al., 2002). There are over 4500 species of mosquito distributed throughout the world in about 34 genera: most of which belong to the Anophelinae and Culicidae family (Ghosh et al. 2013). *Culex quinquefasciatus* have been implicated in the transmission of lymphatic filariasis, West Nile virus, and brancroftian encephalitis (Yerpude et al. 2013). These diseases not only cause mortality or morbidity among humans but also cause a social, cultural, environmental, and economic loss of society (Ghosh et al. 2013). *Culex pipiens* and *Culex quinquefasciatus* are the two Culex species with the most important public health importance. The approach to combat these diseases largely relied on the disease transmission cycle by either targeting the mosquito larvae through spraying their breeding sites or by killing the adult mosquito using insecticides (Ekloh et al., 2013). However, due to the problem of high cost and development of resistance in many vector mosquito species to several synthetic insecticides, interest has been received in exploring the pest control potential of plants (Grainge and Ahmed, 1988). Also, economic and environmental concerns have encouraged a tendency recently towards the use of soft pesticides (Awad 2003).

Table 5 Qualitative Phytochemical Screening of *Petiveria alliacea* leaf and root bark

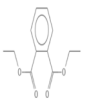
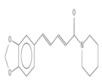
Phytochemical Compound	Leaf	Root bark
Phenol	++	++
Flavonoids	++	++
Tannins	+	++
Alkaloids	++	++
Saponins	++	+
Steroids	++	++
Glycosides	++	++
Triterpenoids	–	–
Reducing sugar	–	+

"+" indicates weak present, "++" indicates strong present, and "–" indicates absent

The assessment of the botanicals leaf oil extract of *Petiveria alliacea* shows that all concentration (1%, 2%, 3%, 4%, and 5%) achieved 75.00–100% adult mortality after 120 min exposure period while the root bark oil extract caused 81.67–100% mortality after 120 min exposure time. However, the synergistic effect of the root and leaf oil caused 86.67–100% mortality. The observation of the leaf oil extract was similar to the report of Akinneye et al. (2019) that ethanolic extract of *Zingiber officinale* killed 80.00–100% of adult Anopheles mosquito after 90 min post-treatment period. The mortality of the adult *Culex quinquefasciatus* in the study could be as a result of the strong pungent odour of the leaf oil. The phytochemical analysis of the leaf oil indicated the presence of phenols, flavonoids, tannins, alkaloids saponins, steroids, glycosides, triterpenoids, and other bioactive compounds. Phytol, 1,2,3-propanetriol,1-acetate, squalene, stigmasterol, benzoic acid, 2-benzoyl hydrazide, 1-hexadecanol, and n-hexadecanoic acid among others constitute the pungent smell of the leaf which might be responsible for the mortality of the adult Culex mosquitoes. However, 1,2,3-propanetriol,1-acetate (22.36%), phytol (21.10%), 1-hexadecanol (8.59%), and n-hexadecanol (8.43%) shows the highest portion of bioactive compounds observed. Moreover, it has been reported that plants with pungent odour have high bioactivity against insects (Dupriez and De Leneer 1998). Additionally, ethanol was found easier to penetrate the cellular membrane to extract the intracellular ingredients from the plant material.

The root bark oil caused 81.67–100% mortality and was highly toxic against adult *Culex quinquefasciatus* possibly due to the presence of some more toxic active compounds such as 9,12,15-octadecatrienoic acid-methyl ester, phenols, N, N-dimethylaniline, phytol, diethyl phthalate, 1,1-dimethyldecahydronaphthalate, piperine, and hexadecanoic acid among others with 1,1-dimethyldecahydronaphthalate (15.02%), 9,12,15-octadecatrienoic acid-methyl ester (12.83%), hexadecanoic acid, methyl ester (8.65%), and piperine (11.92%) constituting the highest portion of the bioactive compounds. However, piperine, along with its isomer chavicine, is the alkaloid responsible for the pungency of black pepper and long pepper. It has been used in some forms of traditional medicine. Helen (1977) reported that 95% ethanolic extract of ground black pepper was highly toxic to the rice

Table 6 GC-MS analysis of volatile organic compounds present in the root bark extract of *Petiveria alliacea*

Peak #	RT	Name of compound	Formula of compound	MW	% Peak Area	% wt.	m/z	Structure
1	0.48	Phenol	C_6H_6O	94	5.04	3.23	39, 66, 94	
2	2.03	N,N-dimethylaniline	$C_8H_{11}N$	121	9.39	2.38	77, 120, 121	
3	4.00	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O$	270	8.65	12.66	74, 87, 270	
4	7.62	Bicyclo3.1.1heptanes, 2, 6, 6-trimethyl-	$C_{10}H_{18}$	138	8.54	3.66	55, 95, 138	
5	9.53	Heptadecane, 2,6,10,14-tetramethyl-	$C_{21}H_{44}$	296	5.01	8.67	43, 57, 296	
6	11.40	Diethyl Phthalate	$C_{12}H_{14}O_4$	222	6.27	5.52	149, 177, 222	
7	15.06	9, 12, 15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292	12.83	13.08	79, 108, 292	
8	15.84	Phytol	$C_{20}H_{40}O$	296	6.26	6.10	71, 123, 296	
9	19.92	Hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.20	9.02	43, 55, 186	
10	20.98	1,1-dimethyldecahydronaphthalene	$C_{12}H_{22}$	166	15.02	14.24	55, 71, 196	
11	23.40	Piperine	$C_{17}H_{19}NO_3$	285	11.92	14.52	115, 201, 285	
12	24.28	(Z)6,(Z)9-Pentadecadien-1-ol	$C_{15}H_{28}O$	224	5.16	5.02	55, 67, 224	
13	27.55	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	4.50	5.72	60, 73, 242	
14	37.92	Squalene	$C_{30}H_{50}$	410	1.80	2.03	69, 81, 410	

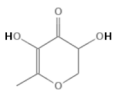
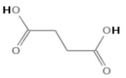
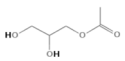
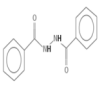
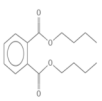
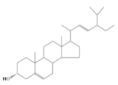
RT retention time, *MW* molecular weight, *%wt*. weight percent, *m/z* mass to charge ratio

weevil (*Sitophilus oryzae*) when they were used on surface treatment of wheat infested by the insect. Dibenzyl trisulfide has often been reported severally in the literature to be present in the root bark of *Petiveria alliacea*. It is one of the major active organic components with high insecticidal properties as reported by Schmelzer and Gurib-Fakim (2008). It was reported by Schmelzer and Gurib-Fakim (2008) that this organic compound was effective against sweet potato weevil (*Cylas formicarius*). Schmelzer and Gurib-Fakim also reported that the activity of this compound was strikingly higher than commercial acarides including dimethoate, lindane, and carbaryl. However, in this study, this compound was not isolated which might due to some factors like climate, the geographical location of where the plant was obtained for this study, and the equipment used in determining the phytochemical present in the plant oil extract. The synergistic effect of the leaf and root bark oil shows more insecticidal effectiveness due to the reaction of some active compounds found in the leaf and root oil possibly resulting in the formation of more complex chemical compounds which might have led to the high toxicity. Plants oil is commonly used for insect control because they are relatively bioactive against virtually all stages of the life cycle of insects (Aranilewa et al. 2006). The effectiveness of oil extract might also be attributed to the presence of active ingredients such as tannin, saponin, and alkaloids in the plants (Akinkulolere et al. 2011). Also, Fafioye et al. 2004 reported that the ethanolic oil extract of *Parkia biglobasa* and *Raphia vinifera* were more potent against the juvenile of *Clarias garapinus* than the aqueous form. This could be a result of the polarity, volatility, and power of the ethanol to dissolve more of the active ingredients of the plant than the aqueous solution. The study revealed that the root oil was very effective against adult *Culex quinquefasciatus*. However, the synergistic effect of the root and leaf oil after being evaluated shows more effectiveness. The lethal concentration (LC_{50}) of the leaf, root, and synergistic effect of leaf and root oil extract required to kill 50% of the adult *Culex quinquefasciatus* after 120 min was 0.45 ml, 0.53 ml, and 0.47 ml, respectively. However, 2.20 ml, 1.194 ml, and 1.15 ml of the leaf, root, and leaf and root oil extract were required to kill 90% (LC_{90}) after a 2-h exposure period. This result agrees with work carried out by Afolabi et al. 2018 where the extract of *Ocimum gratisimum* and *Datura stramonum* showed adulticidal activity against *Anopheles gambiae* at LC_{50} and LC_{90} value of 2.35–4.75 mg/l and 0.82–2.38 mg/l, respectively.

Conclusion

Due to the toxic action against adult *Culex quinquefasciatus*, the oil extract of root and leaf of *P. alliacea* show potentiality to suppress the population of *Culex quinquefasciatus*. The extract of *P. alliacea* is promising for

Table 7 GC-MS analysis of volatile organic compounds present in the leaf extract of *Petiveria alliacea*

Peak #	RT	Name of compound	Formula of compound	MW	% Peak Area	% wt.	m/z	Structure
1	2.02	Naphthalene	$C_{10}H_{28}$	128	2.70	5.76	51, 64, 128	
2	4.61	Phytol	$C_{20}H_{40}O$	296	21.10	16.14	71, 123, 296	
3	9.45	4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl	$C_6H_8O_4$	144	1.86	2.09	43, 44, 144	
4	21.62	Butanedioic acid	$C_4H_6O_4$	118	2.45	3.64	45, 74, 118	
5	24.74	1,2,3-propanetriol, 1-acetate	$C_5H_{10}O_4$	134	22.36	16.07	43, 103, 134	
6	26.16	Benzoic acid,2-benzoylhydrazide	$C_{14}H_{12}N_2O_2$	240	3.92	3.81	77, 105, 240	
7	26.41	1-Hexadecanol	$C_{16}H_{34}O$	242	8.39	9.58	55, 97, 242	
8	29.72	Dibutyl phthalate	$C_{16}H_{12}O_4$	278	3.05	4.11	149, 205, 278	
9	32.26	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	8.43	9.32	43, 73, 256	
10	32.89	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	6.09	6.45	57, 74, 298	
11	39.00	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.47	0.71	43, 73, 242	
12	40.08	Squalene	$C_{30}H_{50}$	410	5.83	6.15	69, 81, 410	
13	48.24	Tetracontane	$C_{40}H_{82}$	563	3.82	4.91	57, 71, 563	
14	54.61	Pentadecanoic acid	$C_{15}H_{30}O_2$	242	0.47	2.41	60, 73, 242	
15	61.46	Stigmasterol	$C_{29}H_{48}O$	412	3.91	4.38	55, 83, 412	

RT retention time, *MW* molecular weight, *%wt*. weight percent, *m/z* mass to charge ratio

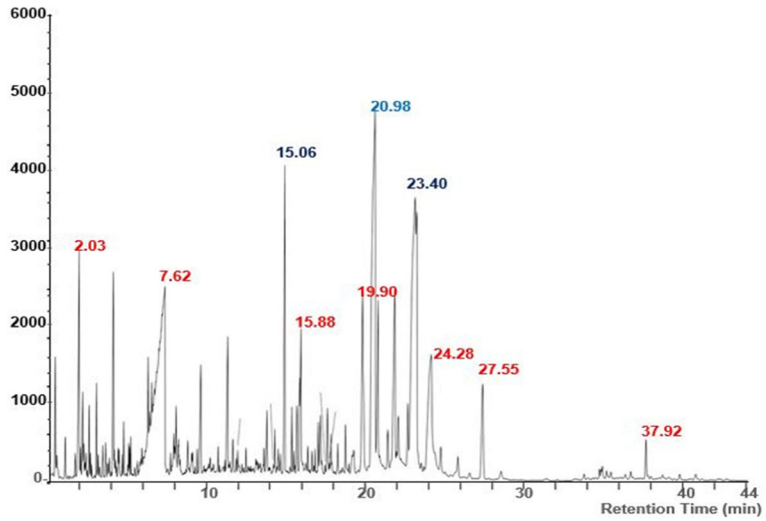


Fig. 1 A chromatogram showing the volatile organic compound present in the root bark of *P. alliacea*

use in the control of filariasis vector. The present findings have important implications in the practical control of adult mosquito by using botanical insecticides. These plant extracts are easy to prepare, inexpensive, and safe for mosquito control which possesses enough insecticidal potential and can be used directly around human dwellings. The result suggests possible utilization of the cheap and readily available botanicals for possible control of mosquitoes as part of an integrated vector management programme. However, large-scale harvesting from nature will not be sustainable in the long run and hence cultivation in marginal areas could be considered an option. The phytochemical screening of the leaf and root of *P. alliacea* showed the presence of alkaloids, flavonoids, saponins, steroids, phenols, tannins, terpenoids, glycosides, and reducing sugar. The presence of these phytochemicals in the leaf and root oil of *Petiveria alliacea* confers them for their insecticidal value.

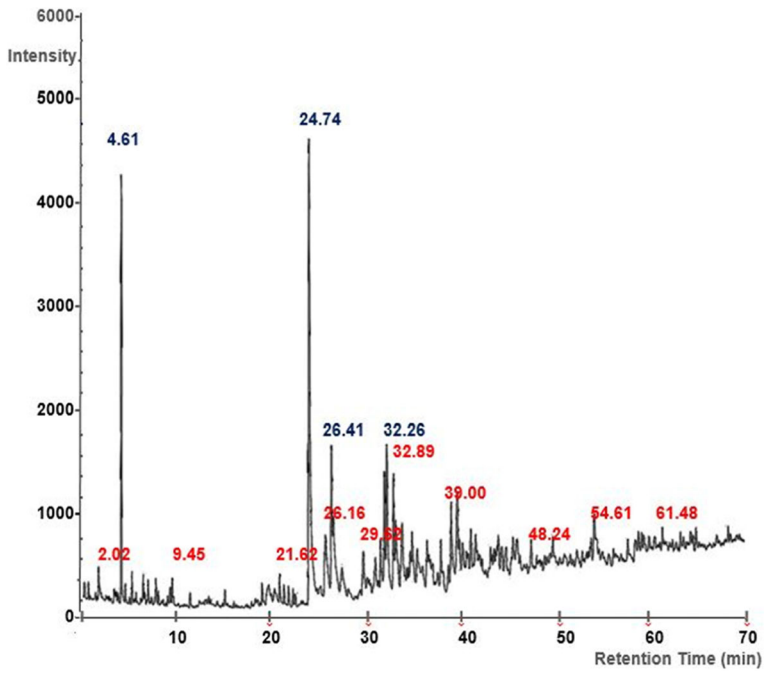


Fig. 2 A chromatogram showing the volatile organic compound present in the leaf of *P. alliacea*

Abbreviations
DDT: Dichlorodiphenyl trichloroethane; R.H.: Relative humidity; WHO: World Health Organization

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Authors' contributions
Dr. J.O. Akinneye designed the experiment and also helped in determining the concentration used for the work, while Akintan, M. O. carried out the laboratory works, statistical analysis of the research, and the interpretation of results. The author(s) read and approved the final manuscrpit.

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