



Phytochemical Screening and Larvicidal Activities of Some Ethnobotanicals from North Eastern Nigeria against Culicine (Dipera: Culicidae) Mosquito

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Authors' contributions

This work was carried out in collaboration between the two authors. Author JSN designed the study, and he and author RSN performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Two of them managed both the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

This study was aimed to investigate the insecticidal activities of some selected ethnobotanicals against culicine mosquitoes. Various part of plant material collected were extracted using Soxhlet apparatus Methanol and Petroleum ether were used as solvents. Quantitative phytochemical Analysis for the crude extracts was carried out for the presence of azadrachtin, terpenoids, alkaloids, tannins, saponins, glycoside, steroids phenol and flavonoids. The larvicidal potentials of the various crude extracts were then tested against culicine mosquitoes.

More yields of phytochemical constituents (80.78 mg) were found in total of 900 g used for methanol extracts as compared to 57.64 mg found in 900 g used in petroleum ether extracts. All the extracts of methanol and petroleum ether of the different plant used showed larvicidal potentials against culicine 3rd instar larvae. The LC₅₀ of the methanol extracts (37.32, 38.52, 42.05, 45.91, 68.78, 126.56, and 141.73 ppm) of *E. globulus*, *O. kilimanscharicum*, *H. suaveolens*, Neem seeds, neem stem leave and orange peels respectively, are far better than their counterparts of petroleum

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ether extracts with LC₅₀ (100.25, 115.53, 145.88, 68.44, 114.55, 46.79, and 175.07 ppm). The larvicidal effect of individual isolates of alkaloids, tannin, saponins, azadirachtin, phenol and steroids demonstrated larvicidal potentials against 3rd instar larvae of culicine, where, terpenoids, flavonoids and glycoside showed no larvicidal potentials against culicine larvae. The larvicidal potential of each plant depend on the number of active ingredients and quantity available in each extract.

In conclusion, the present plant extracts have potentials for development of new and safe control products for culicine mosquitoes.

Keywords: Larvicidal; ethnobotanicals; culicine and phytochemical analysis.

1. INTRODUCTION

Mosquito (*Diptera: Culicidae*) is a family of small, midge-like flies which are considered by World Health Organisation (WHO), as the most dangerous insect pest to man [1]. There are many types of mosquitoes living in the tropical and sub-tropical regions of the world, such as *Aedes*, *Anopheles* and *Culex*. Female mosquitoes are generally considered as blood-eating pests. The female feeds on blood and on the process; they transmit extremely harmful human and livestock mosquito-borne illnesses [2]. Culicine Mosquitoes are the major vector of mosquito for the transmission of several communicable diseases, such as yellow fever, dengue fever, Filariasis and encephalitis [3], causing millions of deaths every year [4]. The diseases also cause allergic responses in humans that include local skin and systemic reactions such as angioedema as reported by [5]. *Aedes* and *Culex* are from family Culicidae and subfamily culicinae which are generally called culicine. *Aedes aegypti* (L) is generally known as a vector for an arbovirus, responsible for the cause of dengue fever, yellow fever, in the tropical and sub-tropical regions [5]. *Culex quinquefasciatus* is a vector of filariasis. Dengue fever, yellow fever and filariasis are all important public health problem as the number of reported cases continues to increase every year with report of some resistant and severe form of these diseases, such as dengue haemorrhagic fever and dengue shock syndrome or with unusual manifestations such as central nervous system involvement [5]. These diseases remain endemic in more than 100 developing tropical countries and their controls are major goal to improve worldwide health system. Among the available vector control methods, chemical method was decisively superior over other control strategies that have limited applicability in mitigating sporadic unpredictable outbreak of vector borne diseases. However, some culicine mosquitoes have shown resistance to deferent insecticides

used in the mosquito control, such as organochlorines, organophosphorous, pyrethroid and microbial insecticides throughout the world [4].

Mosquitoes control has been becoming increasingly difficult because of the indiscriminate uses of synthetic chemical insecticides and disturbs ecological balance. Majority of the chemical pesticides are harmful to man and domestic animals, some of which are not easily biodegradable and spreading toxic effects. The increased use of these chemical pesticides may enter into food chain, and thereby liver, kidney, heart and some vital organs may have irreversible damage that could eventually lead to deaths. Some of the chemical insecticides cause gene mutations that may lead to cancer development [6]. Moreover, mosquito control using chemical pesticides is very costly. In larval control, application of insecticides in ponds, wells and other water bodies may cause health hazard to human and larvivorus fishes. Nowadays, mosquito coils containing systemic pyrethroids and other organophosphorus compounds caused so many side effects, such as breathing problem, eye irritation, and headache, asthma, itching and sneezing to users. With the use of mosquito repellents, users experience ill health effects which sometimes require medical treatment. These problems have highlighted the need for the development of the new strategies for selective mosquito control. Botanicals can be used as alternative to synthetic insecticides or along with other insecticides vector control programs. Phytochemical are advantageous due to their eco-safety, target specific, no record of resistance development by insects, higher acceptability and suitability for rural areas. Phytochemicals can be obtained from the whole plant or specific part of plant by extraction with different types of solvents such as methanol, ethanol, petroleum ether, water chloroform, depending on the polarity of the phytochemicals. Some phytochemicals act as

toxicants (insecticides) both against adult and as well as against the larval stage of the mosquitoes, while others interfere with growth inhibition or with reproduction or produce an olfactory stimulus, thus acting as repellent or attractant [7].

Plants may be the source of alternative agents for control of mosquitoes because they are rich in bioactive chemicals that are active against a limited number of species including specific target insects and are biodegradable. They are potentially suitable for use in integrated pest management programs. In view of the recently increased interest in developing plant origin insecticides, this study was undertaken to assess the insecticidal potentials of *Azadirachta indica*, *Citrus senensis*, *Eucalyptus globulus*, *Hyptis suaveolens* and *Ocimum Kilimanscharicum* against culicine mosquitoes.

2. METHODOLOGY

2.1 Collection of Plant Materials

Fresh leaves, fruit peels and whole plants were collected in study area and identified at the Federal University Lokoja herbarium. The plants materials were shade dried, pounded into powdered form using mortar and pestle and stored in air tied polytene bags for soxhlet extraction (Table 1). The extraction of oil with methanol and petroleum ether were done using soxhlet extractor.

2.2 Extraction of Organic Molecules

Soxhlet apparatus were used for the plant extraction. The various plants extract for both methanol and Petroleum ether extracts were concentrated using water bath which removed the methanol and hexane component leaving behind only the components of the various extracts, which were used for toxicity bioassay [8].

2.3 Toxicity Bioassay

Lethal concentration determination of methanolic and petroleum ether extracts *Azadirachta indica*, *Citrus senensis*, *Eucalyptus globulus*, *Hyptissuaveolens* and *Ocimum Kilimanscharicum* were examined using modified method of [9]. One millilitre of various plant extracts was measured and emulsified with 3 drops of Tween±80 from a needle tip. The emulsified was made up to 1 litre with distilled water to form 1000 ppm stock solutions. For all the stock solutions, serial concentration was prepared. The ranges start from 50 ppm to 200 ppm. From each concentration, 250 ml of each extract was measured and introduced into separate labelled 500ml beakers. Twenty 4th instars larvae of Culicine mosquitoes were introduced to each beaker. Each treatment had five replicates. Mortality served as the end point of the test and results were used to determine the lethal concentration (LC₅₀) of the various plant extracts. The LC₅₀ is defined as the lethal concentration of the bioactive extracts that kills 50% of the test species. Larva was considered death if there was no moving or no response to gentle probing with a fine glass rod three times, 10 second each. Mortalities were recorded at after 36 hours for the various plant extracts and the control (only distilled water).

2.4 Quantitative Phytochemical Studies

The quantitative analysis was carried out through the use of spectrophotometer. The absorbance of the sample was measured with a spectrophotometer at 395 nm wavelength within 10 min [10].

2.5 Isolation of Individual Isolates

Isolation of Azadirachtin, saponins, steroids, alkaloids, tannins, terpenoids flavonoids and phenols by adopting the method of these researchers [11,12,13,14,15,16,17,18] respectively.

Table 1. Profile of test plants used traditionally as mosquito's repellent in north eastern Nigeria

Scientific Name	Family	Common Name	Plant part used
<i>Hyptissuaveolens</i>	Labiatae	Bush tea	Leaves
<i>Azadirachta indica</i>	Meliaceae	Neem	Leaves, seeds, bark
<i>Citrus sinensis</i>	Rutaceae	Orange	Leaves peals
<i>Eucalyptus globulus</i>	Myrtaceae	Eucalyptus	Leaves
<i>Ocimum kilimandscharicum</i>	Lamiaceae	Camphor basil	Whole plant

Azadirachtin was extracted from the powder of neem seed kernel by adopting method of [11].

2.6 Data Analysis

Analysis of variance (ANOVA) was used to determine the significant differences between the mortality mean using Duncan multiple range test. The LC₅₀ and LC₉₀ values obtained true the use of probit analysis.

3. RESULTS

3.1 Quantitative Phytochemical Analyses

In this investigation, primary metabolites like glycosides, terpenoids, saponins, tannins, flavonoids, phenol, Alkaloids and steroids were quantitatively analysed. Table 2 shows the absence of phenol (0), terpenoid (0), tannin (0) and steroid (0) in the neem seed and absence of glycoside and steroid in neem stem. Maximum yield of azadirachtin (4.06 mg), Alkaloids (4.05 mg) was shown in methanol extract of neem seed. Also, the result showed the absence of azadirachtin in orange peel, *H. Suaveolens*, *O. killimanscharicum* and *E. gloublus*. Higher number of metabolites extracted by the methanol method (80.78 mg) was recorded when compared to petroleum ether method (57.64 mg). Higher number of metabolites was observed in the methanol extract of neem stem powder (16.68 mg), followed by methanol extract of neem leaf powder (13.68 mg), methanol extract of orange peels (12.63 mg), and the methanol extract of *Hyptis suaveolens*(12.58 mg), while petroleum ether extract showed lowest level of metabolites in *H. suaveolens* (4.14 mg).

The result of the probit analysis shows various degree of effectiveness of some plant extracts used against 3rd instar larvae of Culicine mosquitoes. LC₅₀ of petroleum ether extracts (100.25, 115.53, 145.88, 68.44, 114.55, 46.79 and 175.07 ppm) of neem seed, neem stem, neem leaf, *Ocimum kilimanscharicum*, orange peels, *Hyptis suaveolens* and *E. gloublus* respectively, were recorded and when these were compared with LC₅₀ of methanol extracts (45.91, 68.75, 126.56, 38.52, 141.73, 42.05 and 37.32ppm), the result proved that methanol extracts were more effective than petroleum ether extracts (Fig. 1). The figure also showed that *E. Globules* (37.32 ppm) of methanol extracts had the lowest concentration (LC₅₀) that killed 50% of the 3rd instar larvae of culicine mosquitoes, followed by *Ocimum killimanscharikum* (38.52 ppm), *Hyptis*

suaveolens (42.05 ppm), and neem seed (45.95 ppm) of the same methanol extracts, while orange peels (141.73 ppm) proved to be the most ineffective treatment agents of these extracts. The *E. globulus* of methanol extract showed that 37.32 ppm killed 50% and 93.12 ppm killed 90% of 3rd instar larvae of culicine larvae that were exposed to it, and this proved that methanol extracts are far better than petroleum ether extracts (175.07 and 676.27 ppm) of LC₅₀ and LC₉₀ respectively (Fig. 1). All the extracts of both methanol and petroleum ether showed high degree of effectiveness, when compared with control that showed 0.0% mortality of 3rd instar larvae of culicine.

Fig. 1, shows that all the treatment agents of methanol and petroleum ether extracts showed high significant differences ($p < 0.05$) to control against culicine. The control showed 0.0% mortality at all stages during the experiment. In general, LC₉₀ of methanol extracts (181.94, 239.29, 200.58, 145.40 and 160.70ppm), of neem seed, *O. killimanscharicum*, orange peels, *H. suaveolens* and *E. gloublus* respectively, are more effective than LC₉₀ of petroleum ether extracts (737.41, 435.747, 384.42, 251.56 and 426.03 ppm) of neem seed, *O. killimanscharicum*, orange peels, *H. suaveolens* and *E. gloublus* respectively. Neem stem (755.09 ppm,) and neem leaf (2484.34 ppm) of methanol extracts showed low toxicity effects against 3rd instar larvae of culicine when compared to petroleum extracts LC₉₀ of neem stem (478.95 ppm) and neem leaf (516.01) respectively.

3.2 Effects of Some Isolated Biochemical Compounds on Culicine 3rd Instar Larvae

The result of the larvicidal activity of the crude extracts of methanol and petroleum ether extracts of five ethnobotanical plants used against culicine have shown their potentiality against this subfamily of mosquito, which led to the investigation of the specific phytochemical ingredients that were responsible for the toxicity effects of this plant extracts.

The result of the probit analysis showed various degree of effectiveness of biochemical compounds used against 3rd instar larvae of culicine mosquitoes. The LC₅₀ of the biochemical compounds (95.7, 99.93, 101,149, 180.32, 210.41 ppm) of azadirachtin, steroids, Tannins, saponins, Alkaloids and phenols respectively, proved to have larvicidal potentials against the

Table 2. Quantitative phytochemical analyses of mosquito repellent plants used by the indigenous people of north eastern Nigeria [24]

Sample M/P	Phenol	Terpenois	Azadirachtin	Alkaloids	Saponins	Flavonoids	Glycosides	Steroids	Tannins	Total (M)	Total (P)
Neem seed (M)	0	0	4.06	4.05	2.05	0.98	0.16	0	0	11.3	
Neem seed (P)	0	0	3.71	2.94	1.39	0	0.07	0	0		8.11
Neem stem(M)	2.27	1.12	3.01	2.27	1.99	2.26	0	0	3.73	16.65	
Neem stem(P)	2.18	0	2.27	1.88	2.11	3.11	0	0	2.88		14.43
Neem leaf (M)	3.03	0.85	0.85	2.49	2.3	1.67	0.17	0.18	2.14	13.68	
Neem leaf (P)	1.77	0	0	1.64	1.89	2.38	0.11	0	2.81		10.60
Orange Peels(M)	2.97	0.33	0	1.26	0.64	4.06	0.24	0.11	3.02	12.63	
Orange Peels (P)	0	0.95	0	0.79	0.29	2.89	0.14	0.19	0		5.25
<i>H. suaveolens</i> (M)	3.11	0.08	0	1.8	1.16	3.19	0.31	0.33	2.6	12.58	
<i>H. suaveolens</i> (P)	0	0.16	0	1.12	0	2.47	0.18	0.21	0		4.14
<i>O. killimanscharikum</i> (M)	1.68	1.02	0	1.09	0.34	1.82	0.11	0.08	1.09	7.23	
<i>O. killimanscharikum</i> (P)	1.16	0.64	0	1.41	0	1.13	0.2	0.12	1.83		6.49
<i>E. gloubius</i> (M)	2.04	0.18	0	0.39	0.41	1.66	0.09	0.28	1.66	6.71	
<i>E. globules</i> (P)	2.89	0	0	1.06	0	0.76	0.21	0	3.7		8.62
Grand total (mg /100g)										80.78	57.64

Key: H= Hyptis, O= Occimum, E= Eucarlyptus, P= petroleum ether, M = Methanol extracts, Mg= Milligram

Table 3. Effects of biochemical compounds on Culicine 3rd instar larvae [24]

	Biochemical compounds									
	Control	Alkaloids	Azadirachtin	Flavonoids	Phenols	Steroids	Saponins	Taninns	Glycosides	Terpenoids
LC ₅₀	-	180.32	95.7	-	210.41	99.93	149	101	-	-
LC ₉₀	-	312.26	190	-	1069.11	110.11	300.28	193	-	-

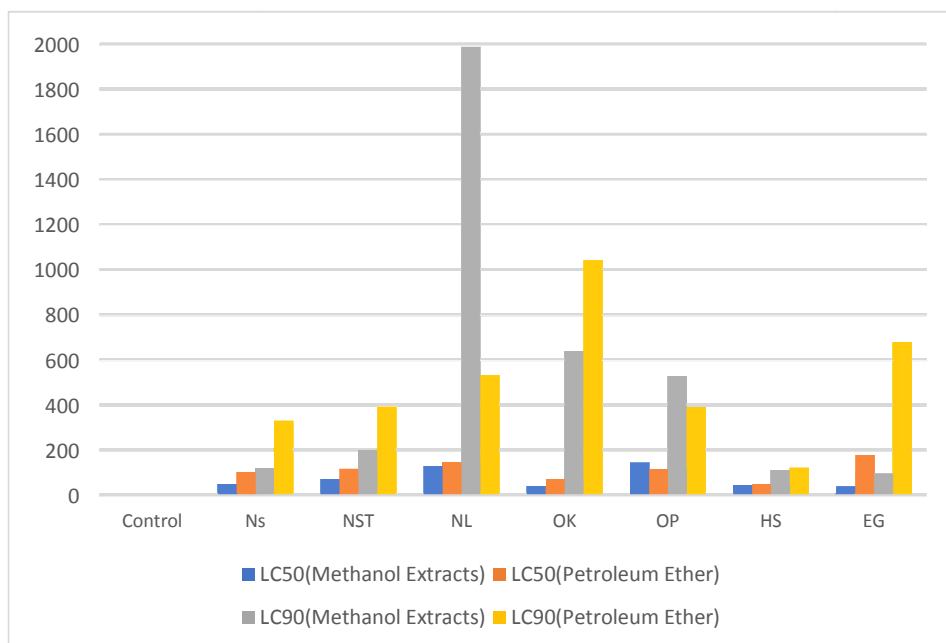


Fig. 1. Effects of solvent used in extraction of extracts on Culicine 3rd instar larvae

culicine mosquito larvae, while some biochemical compounds (Flavonoids, Glycosides and terpenoids) showed no any toxicity effects against this group of mosquitoes. The result showed that azadirachtin (99.93ppm) proved to be the most effective treatment agents, followed by steroid and tannins (Table 3). The LC₉₀ (110.11, 190, 193, 300.28, 312.26, 1069.11) of steroids, azadirachtin, Tannins, saponins, and phenols respectively, also showed the toxicity effect against culicine mosquitoes and steroid proved to be the most effective treatment agent used.

4. DISCUSSION

This present study has demonstrated the biological activity of methanol and petroleum ether extract. In this study primary metabolites of methanol extracts and petroleum ether of five ethnobotanicals commonly used in North eastern Nigeria, as mosquito repellent (*Azadirachta indica*, *Occimum Kilimanscharicum*, *Eucalyptus globulus*, *Hyptis suaveolens* and *Citrus senensis*) have demonstrated promising result against 3rd instar larvae of culicine. The result revealed that all these plants have high phyto-chemical constituents that are capable of controlling diverse species of mosquitoes. The result has shown diverse phyto-profile with reference to solvents, where methanolic extract proved to be the most effective treatment agent used against

3rd instar larvae. The phytochemical analysis results showed that methanol extracts (80.78 mg) had demonstrated high occurrence of phytochemical constituents than the petroleum ether extracts (57.64 mg). These may be responsible for high toxicity effects of the LC₅₀ methanol extracts (45.91, 68.75, 126.56, 38.52, 42.05 and 37.32 ppm) of neem seed, neem stem, neemleaf, *Ocimum kilimanscharicum*, *Hyptis suaveolens* and *E. globulus* respectively, than their counterparts of Petroleum ether extracts (100.25, 115.53, 145.88, 68.44, 46.79, and 175.07ppm). This finding is in agreement with the findings of [19] that reported that methanol as a solvent has higher extracting power than hexane, chloroform and petroleum ether. This finding is contrary to the report of [20] that reported that petroleum ether extracts showed LC₅₀ values between 11.2 and 18.84 mg/L which are far better than the methanol extracts that showed LC₅₀ values between 13.2 and 45.2 mg/L.

High larvicidal activity observed against culicine is advantageous since the culicine are vector of yellow fever and filariasis among other diseases in sub-saharan Africa. The *Eucalyptus globulus* (37.32) proved to have the list concentration of methanol extracts that killed 50 per cent of culicine of 3rd instar larvae exposed to it. This may be attributed to the combine effects of these

phytochemical constituents (Tannins, Phenol, saponins, terpenoids, alkaloids, and steroids) found in the methanol extracts of *E. globulus*, because their individual isolates have demonstrated high larvicidal potentials against culicine. This finding agrees with the recent reports of some researchers, that includes the report by [6] who have tested the methanol root extracts of *Balanites aegyptiaca* against the *Aedes aegypti* larvae and was found to be median lethal concentration (LC₅₀=289.59ppm) and the report by of [21] that Eucalyptus based products has good larvicidal and repellent effect against *An. darlingi*.

The extracts of Eucalyptus globules (37.32 ppm) with similar active ingredient exhibited higher potency on culicine than *Azadirachta indica* (45.91 ppm) that contains the well-known insecticides compound azadirachtin and this agrees with the report of [22], that extracts of *Lepidagathisal opecuroides* with similar active ingredients, also showed higher potency than *Azadirachta indica*. Insecticides of plant origin are usually composed of synergy botanical chemical compounds act concertedly, unlike conventional insecticides which are based on a single active ingredient. This normally makes it difficult for mosquitoes to developed resistance against the plant-based insecticides [23].

During the course of this study, the results showed that the biochemical effect of each plant extract differs from plant to plant, and also depend on the extraction solvent used for the extraction. This revealed that active ingredients differ from plant to plant, and the extracting power of each solvent also differs. This led to the search for the possible active ingredients that were responsible for variation in larvicidal and adulticidal effects.

The phytochemical constituents isolated, observed for their larvicidal effects during the course of this study, includes Alkaloids, Azadirachtin, flavonoids, saponins, steroids, tannins, terpenoids and phenols. During the experiment Alkaloids, Azadirachtin, saponins, steroids, tannins, terpenoids and phenols have demonstrated larvicidal potentials against subfamily (Culicine), while terpenoids, glycosidase and flavonoids have shown non-significant difference to control.

In general crude extracts of *Hyptissuaveolens* proved to be the most effective treatment agent used during the experiment against 3rd instar

larvae of culicine for both methanol and petroleum ether extracts. Methanol and petroleum ether extracts demonstrated their high potentials against culicine with LC₅₀ (42.05 and 46.78 ppm) respectively. This finding may be attributed to higher concentration of phenol (3.11), alkaloids (1.8), saponins (1.16) and tannins (2.6) as observed in quantitative phytochemical analysis table above, as their individual isolates has demonstrated high larvicidal effects on culicine. These isolates (tannins, saponins, alkaloids and phenols) have demonstrated high larvicidal effect against culicine 3rd instar larvae, with LC₅₀ (101, 149, 180.32 and 210ppm) respectively.

The LC₅₀ and LC₉₀ values of the crude extracts (42.05 and 46.78ppm) and (79.67 and 53.07ppm) compared to the individual effect of isolated compounds, showed that combining effect of the crude extract gave better result than any individual isolate.

The crude extracts of neem seeds, has also demonstrated high larvicidal potentials against culicine mosquitoes. The petroleum ether and methanol extracts showed high larvicidal potentials against these subfamilies of mosquitoes. The LC₅₀ (45.91 ppm) and LC₉₀ (119.83 ppm) prove to be second most effective treatment agent used against these group of mosquitoes after *Hyptis suaveolens* with LC₅₀ (42.05 ppm) and LC₉₀ (107.15 ppm). The reason for high larvicidal effects of neem seed extracts may be linked to high concentration of azadirachtin (4.06 mg), alkaloids (4.05 mg) and saponins (2.05 mg) of the extracts, and their high larvicidal effects of their individual isolates. These biochemical constituents showed high larvicidal potentials against culicine mosquitoes with LC₅₀ (95.7, 180.32 and 149 ppm) respectively.

The methanol crude extract of *E. globulus* proved to be the most effective treatment agent used against culicine mosquitoes, with LC₅₀ (37.32 ppm) and LC₉₀ (92.12 ppm). This high larvicidal potential effect may be associated with the presence of phenol (2.04mg), tannins (1.66 mg), saponins (0.49 mg) alkaloids (0.39 mg) and steroids (0.28 mg), because the individual effect of these biochemical compounds has shown high effectiveness against culicine 3rd instar larvae. These biochemical constituents (alkaloids, phenol, steroids, saponins tannins) has LC₅₀ of (142.3, 150.32, 139.62, 190.3 103.6ppm) respectively.

5. CONCLUSION

The secondary metabolites detected in the ethnobotanical extracts include alkaloids, phenol, tannins, flavonoids, glycosides, saponins, steroids, azadirachtin and terpenoids, in methanol extracts of orange peels, *Hyptis suaveolens*, *Ocimum kilimanscharicum*, neem leaves and *Eucalyptus globulus*. Terpenoids, phenol, steroids and tannins were absent in neem stem and neem seeds. Azadirachtin were only present in *azadirachtica indica* products. The petroleum ether extracts showed low metabolites extracted.

The extracts exhibited larvicidal effects on the mosquitoes exposed to them at different concentration. The larvicidal effects varied from plant to plant and from part to part. Generally, *E. globulus* extracts proved to be most effective treatment agent used, followed by *Hyptis suaveolens* extract and *neem seeds extracts*. The most ineffective treatment agent observed was neem leaves and orange peels extracts. This research may serve as scientific basis lend credence to the claim by the local populace that these plants materials have some metabolites that mosquitoes are not comfortable with which cause their repellence. It justifies the claim that the selected plants are efficacious in the management of mosquito populations. It also concludes that potency of these plants is dependent on the solvent of extraction and the dose administered which may vary with the target mosquito

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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