

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

ABSTRACT

This study was aimed to investigate the insecticidal activities of some selected ethnobotanicals against culicine mosquitoes. Five mosquito repellent plants used by the indigenous people of north eastern Nigeria, were analysed for their effectiveness against culicine mosquitoes (Diptera: Culicidae). Methanol and petroleum ether extracts of the plants were investigated for their phytochemical compounds with insecticidal activities against culicine, at different concentration that ranged from 50-200ppm. The *insecticidal* effect was observed after every six hours for a period of 36hours. *H. suaveolens* of both methanol and petroleum ether extracts showed highest toxicity effect against culicine mosquitoes. The test plants contained phenol, alkaloids, flavonoids, tannins, azadrachtin, glycosides saponins, terpenoids and steroids.

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

Key words: Larvicidal, Ethnobotanicals, Culicine, and Phytochemical Analysis

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 (WHO, 2015). 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 (MMCO, 2013). Culicine Mosquitoes are the major vector of mosquito for the transmission of several communicable diseases, such as yellow fever, dengue fever, Filariasis and encephalitis (Caraballo, 2014), causing millions of deaths every year (Elumalai, *et al.* 2015). The diseases also cause allergic responses in humans that include local skin and systemic reactions such as angioedema as reported by Selvakumar *et al.*, (2012b). *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 (Selvakumar, *et al.*, 2012b). *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 (Selvakumar, *et al.*, 2012b). 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 (Elumalai, *et al.*, 2015).

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 (Patel, *et al.*, 2012). 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 larvivorous fishes. Nowadays, mosquito coils containing systemic pyrethroids and other organophosphorus compounds caused so many side effects, such as breathing problem, eye irritation, 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 (Gokulakrishnappa *et al.*, 2012a).

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 *Azadiracta indica*, *Citrus senensis*, *Eucalyptus globulus*, *Hyptis suaveolens* and *Ocimum Kilimanscharicum* against culicine mosquitoes

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 (Amusan *et al.*, 2005).

TOXICITY BIOASSAY

Lethal concentration determination of methanolic and petroleum ether extracts *Azadiracta indica*, *Citrus senensis*, *Eucalyptus globulus*, *Hyptis suaveolens* and *ocimum Kilimanscharicum* were examined using modified method of Lorke (1993). 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 1000ppm stock solutions. For all the stock solutions, serial concentration was prepared. The ranges start from 50ppm to 200ppm. From each concentration, 250ml of each extract was measured and introduced into separate labelled 500ml beakers. Twenty 4th instars larvae of *Anohpeline* 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).

RESULT

Quantitative Phytochemical Analyses

In this investigation, primary metabolites like glycosides, *terpenoids*, *saponins*, *tannins*, *flavonoids*, *phenol*, *Alkaloids* and steroids were quantitatively analysed. Table 1 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 (4.05mg) was shown in methanol extract of neem seed. Also, the result showed the absence of azadirachtin in orange peel, *H. Suaveolens*, *O. killimanscharikum* and *E. gloublus*. Higher number of metabolites extracted by the methanol method (80.78mg) was recorded when compared to petroleum ether method (57.64mg). Higher number of metabolites was observed in the methanol extract of neem stem powder (16.68mg), followed by methanol extract of neem leaf powder (13.68mg), methanol extract of orange peels (12.63mg), and the methanol extract of *Hyptis suaveolens* (12.58mg), while petroleum ether extract showed lowest level of metabolites in *H. suavelens* (4.14mg).

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 175.07ppm) 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 (Figure 1). The figure also showed that *E. Globules* (37.32ppm) of methanol extracts had the lowest concentration (LC50) that killed 50% of the 3rd instar larvae of *culicine* mosquitoes, followed by *Ocimum killimanscharikum* (38.52ppm), *Hyptis suaveolens* (42.05ppm), and neem seed (45.95ppm) of the same methanol extracts, while orange peels (141.73ppm) proved to be the most ineffective

treatment agents of these extracts. The *E. globulus* of methanol extract showed that 37.32ppm killed 50% and 93.12ppm killed 90% of 3rd instar larvae.

UNDER PEER REVIEW

Table 1 : Quantitative Phytochemical analyses of some mosquito repellent plants used by the indigenous people of north eastern Nigeria

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

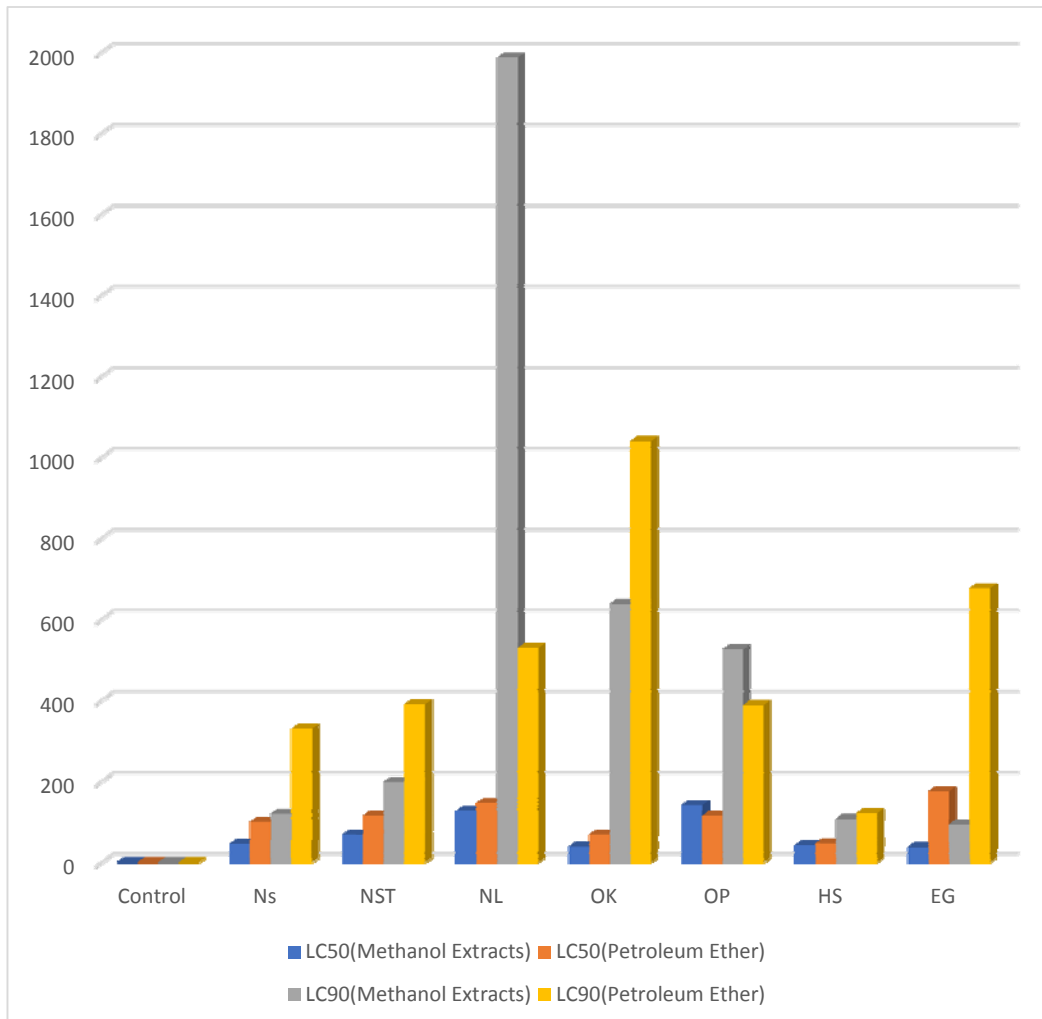


Figure 1: Effects of solvent used in extraction of extracts on *Anopheline* 3rd instar larvae

of *culicine* larvae that were exposed to it, and this proved that methanol extracts are far better than petroleum ether extracts (175.07 and 676.27ppm) of LC50 and LC90 respectively (**Figure 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*.

Figure 1, shows that all the treatment agents of methanol and petroleum ether extracts showed high significant differences ($p < 0.05$) to control against *anopheline*. 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. kilimanscharicum*, orange peels, *H. suaveolens* and *E. gloubilus* respectively, are more effective than LC90 of petroleum ether extracts (737.41, 435.747, 384.42, 251.56 and 426.03ppm) of neem seed, *O. kilimanscharicum*, orange peels, *H. suaveolens* and *E. gloubilus* respectively. Neem stem (755.09ppm,) and neem leaf (2484.34ppm) of methanol extracts showed low toxicity effects against 3rd instar larvae of *anopheline* when compared to petroleum extracts LC90 of neem stem (478.95ppm) and neem leaf (516.01) respectively.

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 *anopheline* and *culicine* have shown their potentiality against these subfamilies 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, 210.41ppm) of *azadirachtin*, steroids, Tannins, saponins, Alkaloids and phenols respectively, proved to have larvicidal potentials against the *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 2). 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.

Table 3: Effects of biochemical compounds on Culicine 3rd instar larvae

	Biochemical compounds									
	Control	Alkaloids	Azadirachtin	Flavonoids	Phenols	Steroids	Saponins	Taninns	Glycosides	Terpenoids
LC50	-	180.32	95.7	-	210.41	99.93	149	101	-	-
LC90	-	312.26	190	-	1069.11	110.11	300.28	193	-	-

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 (80.78mg) had demonstrated high occurrence of phytochemical constituents than the petroleum ether extracts (57.64mg). These may be responsible for high toxicity effects of the LC50 methanol extracts (45.91, 68.75, 126.56, 38.52, 42.05 and 37.32ppm) of neem seed, neem stem, neem leaf, *Ocimum kilimanscharicum*, *Hyptis suaveolens* and *E. gloubulus* 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 Dixon (2017) 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 Komalamisra *et al.* (2006) that reported

that petroleum ether extracts showed LC50 values between 11.2 and 18.84mg/L which are far better than the methanol extracts that showed LC50 values between 13.2 and 45.2mg/L.

High larvicidal activities against *Culex quinquefasciatus* is advantageous since the *Culex quinquefasciatus* 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 percent of *Culex quinquefasciatus* 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 *Culex quinquefasciatus*. This finding agrees with the recent reports of some researchers, that includes the report by Patel *et al.* (2012) 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 Moore, *et al.* (2007) that Eucalyptus based products has good *larvicidal* and repellent effect against *An. darlingi*.

The extracts of Eucalyptus globules (37.32ppm) with similar active ingredient exhibited higher potency on *Culex quinquefasciatus* than *Azadirachta indica* (45.91ppm) that contains the well-known insecticides compound *azadirachtin* and this agrees with the report of Obomanu *et al.* (2006), that extracts of *Lepidagathis alopecuroides* 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 (Ghosh *et al.*, 2012).

CONCLUSION

The secondary metabolites detected in the ethnobotanical extracts include alkaloids, phenol, *tannins*, *flavanoids*, *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 *azadirachta 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.

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