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

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 were 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 yield of phytochemical constituents (80.78mg) were found in total of 900g usedfor methanol extracts as compared to 57.64mg found in 900g 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 141.73ppm) of *E. globulus*, *O. kilimanscharicum, H. suaveolens*, Neem seeds, neem stem leave and orange peels respectively, are far better than their counterparts of petroleum ether extracts with LC50 (100.25, 115.53, 145.88, 68.44, 114.55, 46.79, and 175.07ppm).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.

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, pyrothroid 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 larvivorus fishes. Nowadays, mosquito coils containing systemic pyrothroids 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 *Azadiractaindica*, *Citrussenensis*, *Eucarlyptus globulus, Hyptissuaveolens and OcimumKilimanscharicum*against culicine mosquitoes

Methodology

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.*

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

Scientific Name	***Family***	***Common Name***	***Plant part used***
Hyptissuaveolens	*Labiatae*	*Bush tea*	*Leaves*
Azadiractaindica	*Meliaceae*	*Neem*	*Leaves, seeds, bark*
Citrus sinensis	*Rutaceae*	*Orange*	*Leaves peals*
Eucalyptus globulus	*Myrtaceae*	*Eucalyptus*	*Leaves*
Ocimumkilimandscharicum	Lamiaceae	*Camphor basil*	*Whole plant*

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 living 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*Azadiractaindica*, *Citrussenensis, Eucarlyptus globulus, Hyptissuaveolens and ocimumKilimanscharicum*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 introduce 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. Larvawas 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).

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 (Gupta *et al*., 2013).

isolation of individual isolates

Isolation of Azadirachtin, saponins, steroids, alkaloids, tannins, terpenoids flavonoids and phenols by adopting the method of these researchers**(**Sinha, et al. (1999, Majinda, (2012, Ahmed, *et al*. 2013, Liu et al., 2015, Sruthi, 2016Chappel, 2016, Li-Jing et al., 2016Mumper, 2010) respectively.

Azadirachtin was extracted from the powder of neem seed kernel by adopting the of Sinha, et al. (1999).

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

RESULT

Quantitative Phytochemical Analyses

In this investigation, primary metabolites like glycosides, *terpenoids, saponins, tannins, flavonoids, phenol*, *Alkaloids* and steroids were quantitatively analysed. Table 2shows 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 azadiractin 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 *Hyptissuaveolens*(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,*Ocimumkilimanscharicum*, orange peels, *Hyptissuaveolens* 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 *Ocimumkillimanscharikum*(38.52ppm), *Hyptissuaveolens (*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.

Table 2 : Quantitative Phytochemical analyses of somemosquito 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)
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
H. suaveolens (M)	3.11	0.08	0	1.8	1.16	3.19	0.31	0.33	2.6	12.58	
H. suaveolen s(P)	0	0.16	0	1.12	0	2.47	0.18	0.21	0		4.14
O. killimanscharikum (M)	1.68	1.02	0	1.09	0.34	1.82	0.11	0.08	1.09	7.23	
O. killimanscharikum (P)	1.16	0.64	0	1.41	0	1.13	0.2	0.12	1.83		6.49
E. gloublus (M)	2.04	0.18	0	0.39	0.41	1.66	0.09	0.28	1.66	6.71	
E. globules (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: sH= *Hyptis,* O= *Occimum,* E= *Eucarlyptus,* P= petroleum ether, M = Methanol extracts, Mg= Milligram

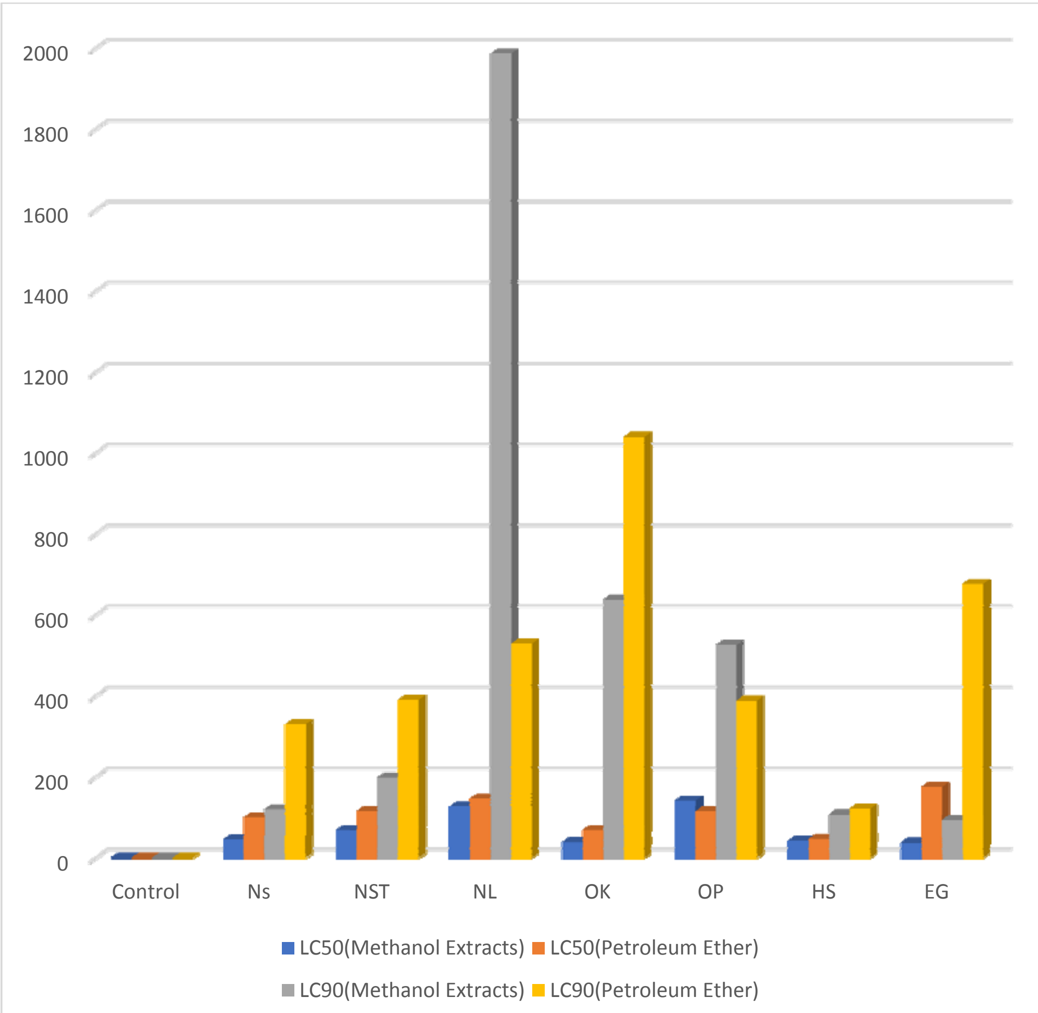

Figure 1: Effects of solvent used in extraction of extracts on *Culicine* 3rd instar larvae

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 *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. kilimanscharicum*, 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 426.03ppm) of neem seed, *O. kilimanscharicum*, orange peels, *H. suaveolens and E. gloublus*respectively.Neem stem (755.09ppm,) and neem leaf (2484.34ppm) of methanol extracts showed low toxicity effects against 3rd instar larvae of *culicine* 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 culicine have shown their potentiality against these 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, 210.41ppm) 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 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.

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	-	-

DISCUSION

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 (*Azadirachtaindica*, *OccimumKilimanscharicum*, *Eucalyptus globulus, Hyptissuavelens and Citrus senensis)* have demonstrated promising result against 3rdinstar 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 toxicityeffects 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,*Ocimumkilimanscharicum*,*Hyptissuaveolens* and *E. gloublus* respectively, thantheir 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 *culicine* is advantageous since the culicine are vector of yellow fever and filariasis among other diseases in sub-saharan Africa. The *Eucarlyptus globulus* (37.32) proved to have the list concentration of methanol extracts that killed 50 percent of *culicine* of 3rdinstar 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 Patel *et al.* (2012) who have tested the methanol root extracts of *Balanitesaegyptiaca*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 culicine than*Azadirachtaindica*(45.91ppm)that contains the well-known insecticides compound *azadirachtin* and this agrees with the report of Obomanu*et al.* (2006), that extracts of *Lepidagathisalopecuroides* with similar active ingredients, also showed higher potency than *Azadirachtaindica.*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).

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 ingredient that were responsible for variation in larvicidal and adulticidal effects.

The phytochemicals constituents isolated and 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 46.78ppm) 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 (45.91ppm) and LC90 (119.83ppm) prove to be second most effective treatment agent used against these group of mosquitoes after *Hyptissuaveolens*with LC50 (42.05ppm) and LC90 (107.15ppm). The reason for high larvicidal effects of neem seed extracts may be linked to high concentration of *azadirachtin* (4.06mg), alkaloids (4.05mg) and saponins (2.05mg) 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 149ppm) respectively.

The methanol crude extract of *E. globulus* proved to be the most effective treatment agent used against culicine mosquitoes, with LC50 (37.32ppm) and LC90 (92.12ppm). This high larvicidal potential effect may be associated with the presence of phenol (2.04mg), tannins (1.66mg), saponins (0.49mg) alkaloids (0.39mg) and steroids

(0.28mg), because the individual effect of these biochemical compound 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.

CONCLUSION

The secondary metabolites detected in the ethnobotanical extracts include alkaloids, phenol, *tannins, flovanoids, glycosides, saponins, steroids, azadirachtin and terpenoids,* in methanol extracts of orange peels, Hyptissuaveolens, Ocimumkilimanscharicum, neem leaves and *Eucalyptus globulus*. T*erpenoids, phenol, steroids and tannins* were absent in neem stem and neem seeds. *Azadirachtin* were only present in *azadirachtaindica*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 *Hyptissuaveolens* 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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