Original Research Article

Comparative Toxicity effect of Some plant extracts Against larvae of *Anopheline* (*Diptera: Culicidae*) in the North Eastern Nigeria

ABSTRACT

Owing to the adverse effect of synthetic pesticides, there has been increasing need to search for natural and environment friendly pesticides of plant origin as agents for control of vector of mosquito-borne diseases. This study was aimed to investigate the larvicidal activities of North eastern botanicals against vector of mosquitoes-borne diseases. Five mosquito repellent plants used by the indigenous people of north eastern, Nigeria were analysed for their effectiveness against Anopheline mosquitoes. The methanol and petroleum ether were analysed for their phytochemical properties. The methanol and petroleum ether extracts were investigated for phytochemical compounds with *larvicidal* activities against *anopheline* 3rd instar larvae through using deferent extracts of methanol and petroleum ether of different concentration that ranged from 50-200ppm. The *larvicidal* were observed after every six hours for total period of 36hours. The extracts of *Hyptis suaveolens* and *Azadirachta indica* of both methanol and petroleum ether extracts showed high toxicity effects against *anopheline*. 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 mosquitoes. As natural occurring insecticides, these plants derived materials that could be useful as an alternative for synthetic insecticides.

Key words: Larvicidal, Insecticidal, Ethnobotanicals, Anopheline 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 (1). There are many types of mosquitoes living in the tropical and sub-tropical regions of the world, such as *Aedes*, Anopheles and *Culex* (2). Female mosquitoes are generally considered as blood-sucking pests. According Michigan Mosquito Control Organization (MMCO), only few mosquito species are harmless or even useful to humanity, but most are considered as a nuisance, or even deleterious human. The female feeds on blood and in the process, they transmit extremely harmful human and livestock mosquito-borne illnesses (3). Mosquito-borne illness are diseases caused by bacterial, viruses or parasites transmitted by mosquitoes. World Health Organization (WHO) reported that the diseases transmitted by mosquitoes include: malaria, dengue, *filariasis*, *zika* virus *chikungunya*, yellow fever, encephalitis (4).

Mosquito-borne diseases contribute significantly to morbidity and mortality in most tropical countries.

Despite the advances in techniques and products used for their control, mosquito continue to pose serious public health problems and some of the methods are compounding more problems due to environmental pollution that has a connection to human health hazard. Various products used for mosquito control have varying degree of effectiveness especially chemicals which are hazardous to environmental ecosystem and human being. The chemical products are costly and sometimes not available in some interior villages. Result of mosquito repellent based on chemicals have remarkable profile, but some are toxic against the skin, nervous system, eye irritation, nasal irritation and some result to worse problem such as brain swelling in children, anaphylactic shock, low blood pressure (5). Insects repellent of plant origin has minimal environmental effects and as well as provide a means of personal protection that could go a long way in reducing burden due to mosquito and environmental pollution. The use of plant derivatives in mosquito protection instead of chemicals could reduce the costs and environmental health effects (6). The obvious benefits would provide public health protection and an environmentally safer alternative. The significant effects could be worldwide because of the existence of these types of plants in countries where production could be possible done simply and economically to provide a viable form of protection against disease vectors (7). The inference derived from this study will go a long way in reducing burden due the pesticide and burden due to the mosquito borne disease.

Methodology

Plant selection

For this study, a survey was carried out and five (5) plant species were selected from five states of North-eastern region (Adamawa, Borno, Gombe and Tataba) of Nigeria (Plate 1). Twenty people were interview from each Local Government Area, for the types of plants and

Plate 1: Map of Study area showing the Local Government Area Visited

Keys	
	Map of Nigeria showing north eastern region
	Adamawa
	Bauchi
	Borno
	Gombe
	Taraba

parts of plants that were used against mosquitoes in the localities. Three local government areas from each state, one from each geopolitical zone were covered.

Plant selection in the study area, were based on interviewing members of the community to specify the indigenous plant species known for their use in the regular control of mosquitoes and other insects in their localities. This species of plants was selected because of their popularity among the local and were ranked following the application of weighted criteria as described by (5). The most mentioned plants were considered first before the other ones. Those that were mentioned by less than six (6) people were not included in the test plants. The plants were further selected by combining the ethno botanical leads and chemotaxonomic evidence (popularity of plants already used as insecticides by local people and documented evidence of insecticidal constituents in the family to which the candidate species belongs), highly promising plant were identified confirmed as *Hyptis suaveolens, Azadirachta indica, Eucarlyptus globulus, Citrus sinensis,* and *Ocimum kilimandscharicum,* in Federal University Lokoja Herbarium. Following this procedure, essential information for further evaluation of larvicidal and insecticidal activities of the few priority plants were tested against mosquito species. Table 1, showed the profile of test plants used traditionally as mosquito's repellent in north eastern Nigeria.

Extraction

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 removes the methanol and hexane component living behind only the components of the various extracts, which were used for toxicity bioassay (8). These plants were screened for their various active ingredients (qualitative and quantitative analysis.

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

Scientific Name	Family	Common Name	Plant part used
Hyptissu aveolens	Labiatae	Bush tea	Leaves
Azadiracta indica	Meliaceae	Neem	Leaves, seeds, bark
Citrus sinensis	Rutaceae	Orange	Leaves peals
Eucalyptus globulus	Myrtaceae	Eucalyptus	Leaves
Ocimum kilimandscharicum	Lamiaceae	Camphor basil	Whole plant

Phytochemical Screening

This was carried out using the procedure described by (9) and (10). Test were carried out on *Azadirachta indica* (seed, Leaf and stem), Eucalyptus globulus (leaf), *Occimum Kilimanscharicum* (whole plant), *Citrus senensis* (orange peels), samples to detect the presence or absence groups of phytochemical compounds such as alkaloids, glycosides, flavonoids terpenoids, saponins, phenols, *Azadrachtin*, steroids, and tannins

TOXICITY BIOASSAY

Third instar larvae of anopheline were collected from the rice fields and some natural water bodies in Yola, Adamawa State, Nigeria. Larvicidal bioassay were also carried out in insectary prepared for the course of this study in Yola, Adamawa state.

The larvicidal activities of these extracts were evaluated in static Bioassays. The method adopted from that described by World health organisation (WHO), (11). 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-200ppm. From each concentration, 250ml of all extracts was measured and introduce into separate labelled 500ml of specimen bottles. Twenty 3rd instars larvae of Anopheline mosquitoes were introduced to each beaker. Each treatment has five replicates. Mortality served as the end point of the test and result were used to determine the lethal concentration (LC_{50} and LC_{90}), of the various plant extracts. Larvae was considered dead if there is no moving or no respond to gentle probing with a fine glass rod three times, 10 second each. Mortalities were recorded after 36 hours for the various plant extracts and the control (only distilled water).

Data Analysis

Data obtained from this investigation were subjected to analysis using Duncan Multiple Range Test for analysis of variance. The lethal concentrations (LC_{50} and LC_{90}) of the extracts were determined by Probit analysis (12) using SPSS Version 17

RESULT

The result of the **Table 2**, showed that out of the three hundred (300) people interviewed about ethnobotanicals that were being used as agent of mosquito repellent within their localities, 145(48.3%) of people interviewed showed that *Hyptis suaveolens* was being used within their localities, as mosquito repellent. Followed by *Eucarlyptus globulus* 60(20%) and *Azadirachta indica* 45(15%). *Citrus senensis* 20(6.67%) showed to be the most unpopular plant for mosquito repelling in the region. The result also shows that *Hyptis suaveolens* has the highest percentage (58.3%, 66.67, 50and 50%) of plant used against mosquitoes in four states of North eastern Nigeria that includes Adamawa, Bauchi, Gombe and Taraba respectively, followed by *Eucarlyptus globulus* (42.70, 25 16.7, and 16.7%) of Taraba, Gombe, Adamawa, and Borno respectively. The locals in Borno Sate used *Azadirachta indica* (66.67%) more than any other plant products in the area as mosquito repellent.

Table 2: Percentage of plants in use per states of North-eastern Nigeria

State Location	*H. suaveolens* N0. (%)	*C.senensis* No. (0)	*O. kilimanscharicum* No. (%)	*E. globulus* No. (%)	*A. inndica* No. (%)
Adamawa	35(58.3)	0(0)	10(16.6)	10(16.6)	5(8.3)
Borno	10(16.6)	0(0)	0(0)	10(16.6)	40(66.6)
Bauchi	40(66.7)	0(0)	20(33.4)	0(0)	0(0)
Gombe	30(50)	15(25)	0(0)	15(25)	0(0)
Taraba	30(50)	5(8.3)	0(0)	25(42.7)	0(0)
Total	145(48.3)	20(6.67)	30(10)	60(20)	45(15)

Qualitative Phytochemical Analysis

In this study, primary metabolites of different solvent extracts of some ethnobotanical used against mosquito in North eastern Nigeria, were analysed qualitatively **(Table 3)**. The extracts of different plants showed diverse phyto-profile with reference to solvents. Methanol and petroleum ether were used as solvents for the extraction. Methanol extracts demonstrated maximum occurrence of phyto- constituents (54/63) such as *flavonoid, Saponins, glycosides, tannins, terpenoids, steroids, alkaloids, Azadirachtin and phenol.* The Extracts derived from neem fruits showed presence of glycosides, *alkaloids, saponins and flavonoids* while *steroid, tannin, phenol and terpenoid* were absent. In the case of petroleum ether extracts, the result showed low occurrence of phyto-constituents (39/63) and absence of *terpenoids* were observed in all the neem plant extracts. Methanol extracts, of orange peels (8), *Hyptis suaveolens,* (8), *O. killimanscharicum* (8), *E. gloublus* (8) and neem leaves (8) showed all the metabolites under observation are present.

Table 3: Qualitative Phytochemical Analysis

Sample(M/P)	Phen	Terp	Aza	Alk	Sap	Flav	Glyc	Ster	Tan	Total(M)	Total(P)
Neem seed	-	-	+	+	+	+	+	-	-	5	
	-	-	+	+	+	-	+	-	_		4
Neem stem	+	+	+	+	+	+	-	-	+	8	
	+	-	+	+	+	+	-	-	+		6
Neem leaf	+	+	+	+	+	+	+	+	+	9	
	+	-	+	+	+	+	+	+	+		8
O.kilimanscharicun	+	+	-	+	+	+	+	+	+	8	
	+	+	-	+	_	+	+	+	+		7
Orange peels	+	+	-	+	+	+	+	+	+	8	
	-	+	-	+	+	+	+	-	-		5
Hyptis suaveolens	+	+	-	+	+	+	+	+	+	8	
	-	+	-	+	-	+	+	+	-		5
E. globulus	+	+	-	+	+	+	+	+	+	8	
	+	-	-	+	-	+	+	_	+		5
Grand total										54/63	39/63

Keys: P. petroleum ether extract, M. methanol extracts, Phen= phenol, Glyc= glycosidae, Tan= tanins, Ster= steroids Terp= terpenoids, Flav =flavonoids, Alk= Alkaloids and sap =saponins.

Note: Total observation made was 63 for the 7 sample plants used for methanol extract and the same observation were made for petroleum ether extracts

Effects of solvent used in extraction of extracts on *Anopheline* 3rd instar larvae.

Figure I, shows that all the treatment agents of methanol and petroleum ether extracts showed high significant differences to control against *anopheline*. The control showed 0 mortality at all stages during the experiment. In general, LC_{90} 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 LC_{90} 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. LC90 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 LC_{90} of neem stem (478.95ppm) and neem leaf (516.01ppm) respectively.

The LC50 methanol extracts (63.91, 77.76, 66.88, 53.07 and 56.67ppm) of neem seed, *O. kilimanscharicum*, orange peels, *H. suaveolens and E. gloublus* respectively. These results showed higher effectiveness against 3rd instar of *anopheline* than the LC50% petroleum ether extracts (193.20, 126.86, 111.18, 79.67 and 123.60ppm) of neem seed, *O. kilimanscharicum*, orange peels, *H. suaveolens an d E. gloublus* respectively.

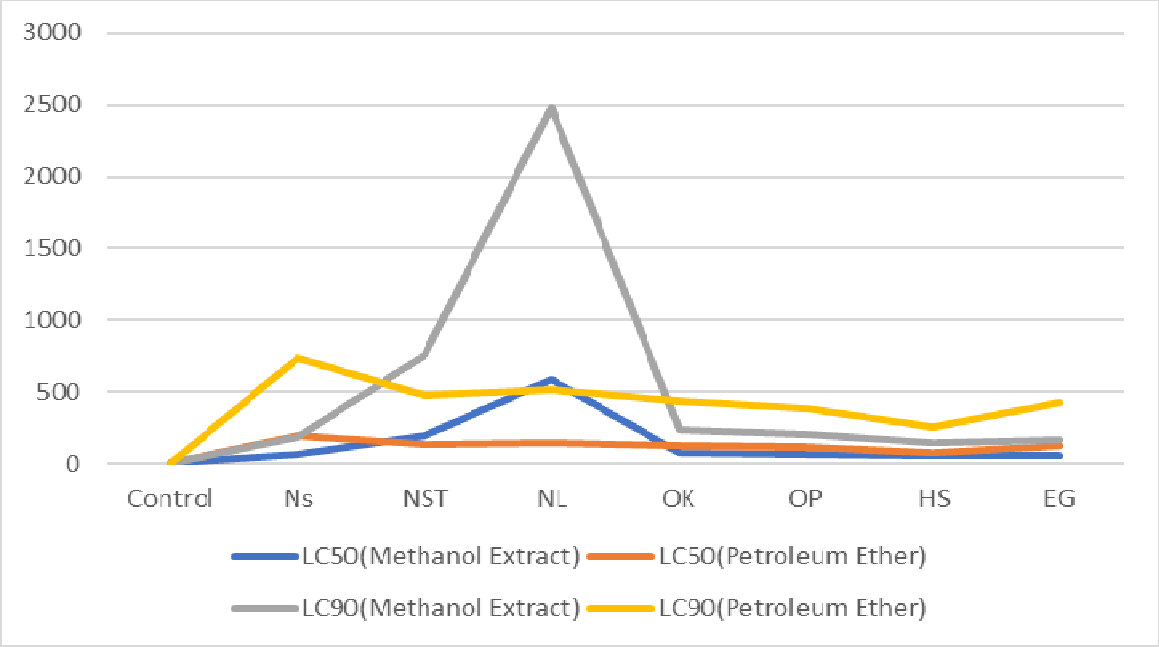

Figure 1: Effect of solvent extracts on LC50 and LC90 for *Anopheline* 3rd instar larvae post 36h of treatment.

DISCUSION

Ethnobotany are well known as the study of region's plants and their practical uses through the traditional knowledge of local culture and people. The health benefits of ethno-botanical are mainly accounted to the presence of many active phytochemical constituents in various parts of these plants. The present study was conducted with an objective to identify the best extraction solvent, which can be used to obtain the maximum amount of the phytochemicals from the shade dried ethnobotanicals used against mosquitoes in the North eastern Nigeria.

The present study, started with ethnobotanicals survey in north eastern Nigeria, and investigations revealed that *Hyptis suaveolens, Eucarlyptus globulus, Citrus senensis and Ocimum kilimanscharicum,* were popularly used, by some local populace. The current study showed that *Hyptis suaveolens* [145 (48.3%] proved to the most popular plant in the region that has been used as mosquito repellent. The findings of this Present study agree with the works of. (13) and. (14). The result of the survey showed that *Hyptis suaveolens* has the

highest percentage (58.3, 66.67, 50.0, 50.0%) of plants that are used in Adamawa, Bauchi, Gombe and Taraba respectively, followed by *Eucarlyptus globulus* (41.67, 25 16.7, and 16.7%) of Taraba, Gombe, Adamawa, and Borno respectively. The locals in Borno prefers using *Azadirachta indica* (66.67%) more than any other plants product in the area as mosquito repellent. Their preference is based on how it worked for them.

Results have shown that all the effect of the treatment agents of methanol and petroleum ether extracts used against anopheline larvae mosquitoes are significantly different from each other. The LC50 methanol extracts (63.91, 77.76, 66.88, 53.07 and 56.67ppm) of neem seed, *O. kilimanscharicum*, orange peels, *H. suaveolens and E. gloublus* respectively, showed higher larvicidal effect than petroleum ether with LC50 (193.20, 126.86, 111.18, 79.67 and 123.60). LC90 values of methanol extracts (181.94, 239.29, 200.58, 145.40 and 160.70), of neem seed, *O. kilimanscharicum*, orange peels, *H. suaveolens and E. gloublus* respectively, are also more effective than LC90 of petroleum ether extracts (737.41, 435.747, 384.42, 251.56 and 426.03) of neem seed, *O. kilimanscharicum*, orange peels, *H. suaveolens and E. gloublus* respectively. This agrees with the reports of (15) and (16) that showed methanolic extracts as the best treatment agent against many mosquito species than some of the extracts from other solvents. This may be attributed to high polarity effect of methanol than the petroleum ether as reported by (17)

In the case of Neem stem (755.09ppm,) and neem leaf (2484.34ppm) of methanol extracts, opposite is the case, they showed low toxicity effects against 3rd instar larvae of *anopheline* when compared to petroleum extracts with LC_{90} of neem stem (478.95ppm) and neem leaf (516.01) respectively and this is contrary to the reports of (16) and (15), that reported higher larvicidal effect of methanolic extracts than the petroleum ether extracts but is in agreement with the report of Komalamisra et al. (18) where petroleum ether extracts of some Thai plants that showed LC_{50} values between 11.2 and 18.84mg/L which are far better than the methanol

extracts that showed LC_{50} values between 13.2 and 45.2mg/L. The difference in the amount phytochemical constituents extracts of different plants by different solvent may depend on the physiology and the polarity effect of the individual solvents used.

CONCLUSION

The extracts exhibited *larvicidal* effects on the 3rd instar larvae exposed to them at different concentration. The *larvicidal* effects are concentration dependent. Generally, *Hyptis suaveolens* extracts proved to be most effective treatment agent used, followed by neem seed extract and *Ocimum kilimanscharikum*. 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 this plants material has some metabolites that mosquitoes are comfortable with it which causes 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 depended on the solvent used for the extraction and the dose administered.

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