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Comparative insecticidal activity of five Nigerian plant species against mosquito vectors in Yola, Adamawa state, Nigeria

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Abstract
Objective: To determine the mosquito insecticidal activities of methanol and petroleum ether of some ethnobotanicals against *Culicine* and *Anopheline* mosquitoes.
Methods: The insecticidal activities were assayed against two major group of mosquito species at various concentrations ranging from (0.5-2mg/ml) under the laboratory conditions.
Results: The result showed significant difference ($P< 0.01$) among the plant extracts. The Probit analysis showed that *H. suaveolens* (0.85mg) has the lowest confidence limit that gave LC50 against culicine. Higher insecticidal activities were observed in the methanol extracts than the petroleum ether extracts.
Conclusion: the present result suggest that the effective plant crude extracts have potentials to be used as an eco-friendly approach for the control of mosquito vectors. This present study provides the first report on the insecticidal activities of these plant's crude extracts against culicine and anopheline mosquitoes in general.

Keywords: comparative, insecticidal activity, mosquito vectors, Yola, Nigeria

1. Introduction
The approach to combat mosquito borne diseases is largely relied on interruption of disease transmission cycle through chemical, physical, Biological methods. Chemical methods used to be the best, but due to over dependence on them, had led to deleterious effects that includes development of resistance strains, destruction of non-target organisms and bio-accumulation of these synthetic insecticides in some organic tissues [1, 2]. The synthetic pesticides pose from mild public health problems that causes: skin irritation, eye irritation, nasal irritation to serious public health problems such as brain swelling in children, anaphylactic shock, low blood pressure [3, 4].
The control could be achieved mainly in two ways, either by targeting the mosquito larvae through in the stagnant waters that serve as the breading sites [5] or by killing the adult mosquito through use of synthetic chemical insecticides mainly organophosphates, Organochlorine and pyrethroids insecticides [6]. These insecticides are applied in two main ways: either as aerosol or liquid residual spray against adult mosquitoes' insides dwelling places or by spraying the insecticides on larvae breeding sites like drainage, ponds and grasslands [7].
The reason why World Health Organisation (WHO) failed malaria eradication campaign was mainly due to the fact that programme imposed a uniform vector control strategy (use of only chemical methods) in all countries, and ignoring the diversity of vectors and available natural control measures of nations [5]. For us to achieve sustainable mosquito control, it is important to have combinations of new approaches and specific tools for each region and country.
The use of plant products as alternative to synthetic insecticides in controlling mosquitoes is attracting attention worldwide because they are biodegradable, inexpensive and readily available as reported by National [8]. Some Plants products was in used before the advent of synthetic insecticides for control of insect pests in many tropical and subtropical countries like Nigeria, India and china [9].
So many researchers have investigated so many plants for their repellent properties against mosquitoes and many have shown high repellence potentials, such quelling, an insect repellent

produced in Asia, derived from extracts of the *Eucalyptus* and Lemmon grass has been evaluated against mosquitoes [10]. The essential oil obtained from *Vitex negudo* and flowers from *Lantana camara* have shown repellent activities against *Aedes aegyti* [11].

Apart from the repellence effects researchers have shown toxicity of so many plant families against the two subfamilies (culicine and anopheline) of mosquitoes that comprise all the vectors of mosquito-borne diseases. The researchers include: 12 has reported that the acetone extracts of *Nerium indicum* and *thunja orienthalis* had LC50 values of 200.89, 127.53ppm and 209 and 155.97ppm against 3rd instar of *Anopheles stephensi* and *Culex quiquefasciatus* respectively. 13 have reported earlier that methanol extract of *Datura metel* leaves have maximum anti-oxidants and has shown high toxicity against various mosquitoes' species of the genus anopheles and Culex. 14 reported that methanol extracts of *A. panicula, E. alba* and *C. halicacabum* are very effective against the 4th instar larvae of *anopheles stephensi* (LC50 79.68, 112.66, and 133.01ppm; LC90 158.66, 220.68, and 270.72ppm. 15 made a comparative estimation of the larvicidal potentials of three plants extracts on Aedes aegypti: he investigated the activities of the ethanol extracts of *A. indica, O. gratissimum* and *Citrus citratus* on *Aedes aegypti* larvae at 96hours of exposure. Mortalities were observed to increase with concentration. The larvae of Aedes aegypti exhibited differentials susceptibility to the extracts of three plants. 16 and 17 reported that mortality of plant extracts against mosquito larvae are concentration and time dependent which is the same with the report of 18 of the mortality of anopheles gambiae and *C. quinquefasciatus* for exposure to extracts of *Lepidagathis alopecuroides and A. indica.* 19 showed that over 90% mortality effect of *H. suaveolens* against *Aedes albopictus* and the same thing was also reported by 20 for the same plant but against anopheline species.

Screening locally available plant species for insecticidal activity will provide justification incorporation into the National Malaria Elimination Programme (NMEP) and contribute to the growing pool of knowledge on Botanicals that can serve as alternative to synthetic insecticides. For these reasons it is important to investigate the locally available plants species for their insecticidal activities in tropical countries like Nigeria with favourable environments for mosquito breeding and abundant natural vegetations that remain largely untapped for this reason.

2. Methodology

2.1 Insecticidal effects

Laboratory study were carried out to determine the insecticidal properties of the candidate's plant species against of *Anopheline* and *culicine* adult mosquitoes. The required concentrations of different extracts (0.5, 1.0, 1.5, 2.0) were prepared through mixing up of stock extracts with variable amount of sterilized distilled water. The solution was impregnated on the filter papers (6.5cm) in diameter, with the help of pipette in plastic container and the untreated net covering the beakers were also impregnated. Equal amount of solvent (sterilized distilled water) were added on the filter papers without extracts and keep as control. The papers were allowed to dry for one hour at room temperature before testing. For each test, 20 laboratory-reared female *anopheline*' mosquitoes were placed into five separate laboratory cages (40 x 40cm) square. The filter papers impregnated with different extracts were placed into different cages. This procedure was replicated three times. The same procedure was repeated for the five ethnobotanical extracts for the *Anopheline* and *culicine*. Mortality served as the end point of the tests and results were used to determine the lethal concentration (LC50 and LC90), of the various extracts that were used. Mortalities were recorded at interval of 12, 24, 36 and hours for the various plant extracts and the control (only distilled water). The same procedure was repeated for *Culicine.*

2.2 Data analysis: Analysis of variance (ANOVA) were used to determine whether there is significant difference between the mean values. Then LC50 and LC90 values were obtained through use of probit analysis.

3. Result

3.1 Quantitative Phytochemical Analyses

In the present investigation, primary metabolites like glycosides, *terpenoids, saponins, tannins, flavonoids, phenol, Alkaloids* and steroids were quantitatively analysed. Table 1 shows number of metabolites extracted by the methanol method and petroleum ether method. The break down gave higher number of metabolites extracted by the methanol method (82.06mg) was recorded when compared to petroleum ether method (57.07mg). Higher number of metabolites was observed in the methanol extract of neem stem powder (16.68mg), followed by methanol extract of nee02m leaf powder (15.38mg), 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) and neem fruit (4.4mg).

Table 1, also showed absence of *phenol (0), terpenoid (0), tannin (0) and steroid (0)* in the neem fruit and absence of *glycoside* and steroid in neem stem. Maximum yield of alkaloids (4.05 mg) was shown in methanol extract of neem fruit followed by phenol (3.11mg) of methanol extract of *H. Suavelens.*

3.2 Effects of Petroleum Ether Extracts on Adult *Culicine*

The result of *adulticidal* activities of these plants obtained in the study confirms their potentials for the control of adult mosquito populations (Table 2). The results showed significant differences among all the treatment agents used ($P<0.01$). Neem seed, neem stem, neem leaves, *O. kilimanscharicum*, orange peels, *Hyptis suaveolens* and *Eucarlyptus globulus* have shown moderate mortality; however, the highest mortality was observed in *Hyptis suaveolens extracts.* The *culicine* mosquitoes due to exposure to petroleum ether extracts had values of LC50 = 1.37, 2.23, 2.70, 1.83, 2.31, 0.99 and 1.18mg and LC90 = 3.03, 5.81, 8.54, 15.45, 6.44, 3.51 and 3.54mg respectively. The results proved that *Hyptis suaveolens* (0.99) proved to be the most effective treatment agent used and neem stem (2.23mg) proved to be most ineffective treatment agents at LC50. The LC90 values revealed that neem seed (3.03mg) had the least concentration while *Ocimum kilimanscharicum* (15.45) has the maximum concentration needed.

3.3 Effects of Methanol Extracts on Adult *Culicine*

The *culicine* mosquitoes due to exposure to methanol extracts had values of LC50 = 1.09, 3.25, 5.11, 1.24, 2.50, 0.95 and

3.08mg and LC90 = 2.65, 42.87, 17.03, 5,51, 9.49, 2.87 and 1.14mg respectively (Table 3). The values of LC50 showed that *Hyptis suaveolens* (0.95mg) proved to the best, followed by neem seed (1.09mg) while Neem leave (5.11mg) proved to be less effective followed by neem stem (3.08) extracts. LC90 values, showed that neem seed (2.65mg) proved to be the best and neem stem proved to be the less effective.

3.4: Effects of Methanol Extracts on Adult *Anopheline*

The *anopheline* mosquitoes exposed to methanol extracts had LC50 = 0.97, 1.69, 2.45, 1.26, 2.50, 0.93 and 1.11 and LC 90 = 2.23, 6.85, 8.14, 4.89, 9.49, 2.55, and 2.90 respectively (Table 4). The LC50 values showed that *H suaveolens* (0.93), neem seed (0.97) had the lowest concentration while orange peels (2.50mg) and neem leave (2.45mg) had the highest concentration. LC90 values showed that neem seed proved to be the most effective treatment agents while neem leave proved to be the most ineffective treatment agent used.

3.5: Effects of petroleum ether extracts on adult *Anopheline*

The *anopheline* mosquitoes exposed to petroleum ether had LC50 = 1.30, 2.21, 2.70,1.73, 2.23,0.95 and 1.10 and LC90 = 2.68, 5.77, 8.54, 12.84, 5.90, 3.22, and 3.19 (Table 5). The P. values had significant different at $P< 0.001$. The tables also showed percentage confidence limits that were also calculated and the result proved that *Hyptis suaveolens* (0.85mg) as the most effective treatment agents used and neem leave (3.27) as the most ineffective treatment agents used for LC50. In all the four tables of the results of *Adulticidal* activities above, *Hyptis suaveolens* (0.99, 0.95, 0.93 0.95) of LC50 proved to be most effective treatment agents against both *anopheline* and *culicine* mosquitoes, followed by neem seed extracts which had LC50 of 1.37, 1.09, 0.97, and 1.30mg respectively.

Table 1: Quantitative Phytochemical analyses

Sample M/P	*Phenols*	*Terpenoids*	*Azadirachtin*	*Alkaloids*	*Saponins*	*Flavonoids*	*Glycosides*	*Steroids*	*Tannins*	Total (M)	Total (P)
Neem fruit (M)	0	0	4.06	4.05	2.05	0.98	0.16	0	0	10.85	
Neemfruit(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	16.68	
Neem stem(P)	2.18	0	2.27	1.88	2.11	3.11	0	0	2.88		14.40
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.suaveolens(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		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.globulus(P)	2.89	0	0	1.06	0	0.76	0.21	0	3.7		8.62
Neem leaf(M)	3.03	0.85	0.85	2.49	2.3	1.67	0.17	0.18	2.14	15.38	
Neem leaf(P)	1.77	0	0	1.64	1.89	2.38	0.11	0	2.81		10.49
Grand total (mg /100g)										82.06	57.07

Keys H= *Hyptis* C= *Citrus* O= *Occimum* E= *Eucarlyptus* A= *Azadirachta* P= petroleum ether M = Methanol extracts Mg= Milligram

Table 2: Effects of Petroleum Ether Extracts on Adult *Culicine*

Extracts	Concentration Mg/ml	LC50(Mg)	LC99(Mg)	F. values	Pr(>F)
NS	Control				
	0.5	1.37	3.03	964.66	2e-16***
	1.0	1.24-1.52	2.51-4.07		
	1.5				
	2.0				
NST	Control				
	0.5	2.23	5.81	173.57	2e-16***
	1.0	2.03-2.55	4.52-8.56		
	1.5				
	2.0				
NL	Control				
	0.5				
	1.0	2.70	8.54	65.16	2e-16***
	1.5	2.34-3.38	5.95-15.53		
	2.0				
OK	Control				
	0.5				
	1.0	1.83	15.45	103.56	2e-16***
	1.5	1.58-2.24	9.09-37.29		
	2.0				
OP	Control				
	0.5				
	1.0	2.31	6.44	142.12	2e-16***
	1.5	2.08-2.69	4.88-9.93		
	2.0				
HS	Control				

	0.5				
	1.0	0.99	3.51	366.55	2e-16***
	1.5	0.89-3.51	2.81-4.87		
	2.0				
EG	Control				
	0.5				
	1.0	1.18	3.54	638.79	2e-16***
	1.5	1.07-1.31	2.86-4.84		
	2.0				

HS= *Hyptis* Suaveolens OP= Orange peels O = *Occimum Kilimanscharicum*, EG= *Eucalyptus Globulus*, NS= neem seed, NST =neem stem, NL = neem leaf, Pr (>F)= Fcalculated less than F tabulated.

Table 3: Effects of Methanol Extracts on Adult *Culicine*

Extracts	Concentration Mg/ml	LC50(Mg/ml)	LC99(Mg/ml)	F. Values	Pr(>F)
NS	Control				
	0.5	1.09	2.65	584.47	2e-16***
	1.0	(0.977-1.213)	(2.210-3.493)		
	1.5				
	2.0				
NST	Control				
	0.5	3.245	42.87	429.71	2e-16***
	1.0	2.46-5.41	17.68-241.69		
	1.5				
	2.0				
NL	Control				
	0.5				
	1.0	5.11	17.03	117.54	2e-16***
	1.5	3.27-13.91	31.21-2550		
	2.0				
OK	Control				
	0.5				
	1.0	1.24	5.51	115.52	2e-16***
	1.5	1.10-1.41	3.97-9.30		
	2.0				
OP	Control				
	0.5				
	1.0	2.50	9.49	130.28	2e-16***
	1.5	2.14-3.20	6.28-19.13		
	2.0				
HS	Control				
	0.5				
	1.0	0.95	2.87	144.7	2e-16***
	1.5	0.85-1.05	2.37-3.79		
	2.0				
EG	Control				
	0.5				
	1.0	3.08	1.14	601.43	2e-16***
	1.5	2.54-4.10	1.03-1.26		
	2.0				

HS= *Hyptis* Suaveolens OP= Orange peels O= *Occimum Kilimanscharicum*, EG= *Eucalyptus Globulus*, NS= neem seed, NST =neem stem, NL = neem leaf, Pr(>F)=Fcalculated less than F tabulated.

Table 4: Effects of Methanol Extracts on Adult *Anopheline*

Extracts	Concentration Mg/ml	LC50(Mg)	LC99(Mg)	F.Values	Pr(>F)
NS	Control				
	0.5	0.97	2.23	563.38	2e-16***
	1.0	0.89-1.06	1.94-2.68		
	1.5				
	2.0				
NST	Control				
	0.5	1.69	6.85	186.98	2e-16***
	1.0	1.54-1.90	5.18-10.25		
	1.5				
	2.0				
NL	Control				
	0.5				

	1.0	2.45	8.14	158.80	2e-16***
	1.5	2.11-3.08	8.01-15.44		
	2.0				
OK	Control				
	0.5				
	1.0	1.26	4.89	114.04	2e-16***
	1.5	1.11-1.45	3.57-8.25		
	2.0				
OP	Control				
	0.5				
	1.0	2.50	9.49	142.58	2e-16***
	1.5	2.14-3.10	6.29-19.13		
	2.0				
HS	Control				
	0.5				
	1.0	0.93	2.55	559.65	2e-16***
	1.5	0.83-1.03	2.12-3.34		
	2.0				
EG	Control				
	0.5				
	1.0	1.11	2.90	664.50	2e-16***
	1.5	0.99-1.24	2.38-3.94		
	2.0				

HS= *Hyptis* Suaveolens OP= Orange peels O= *Occimum Kilimanscharicum*, EG= *Eucalyptus Globulus*, NS= neem seed, NST =neem stem, NL = neem leaf, Pr(>F)=Fcalculated less than F tabulated**.**

Table 5: Effects of petroleum ether extracts on adult *Anopheline*

Extracts	Concentration Mg/ml	LC50(Mg)	LC99(Mg)	F.Values	Pr(>F)
NS	Control				
	0.5				
	1.0	1.30	2.68	823.43	2e-16***
	1.5	1.16-1.46	2.23-3.67		
	2.0				
NST	Control				
	0.5				
	1.0	2.21	5.77	881.25	2e-16***
	1.5	2.02-2.52	4.50-8.47		
	2.0				
NL	Control				
	0.5				
	1.0	2.67	8.54	145.07	2e-16***
	1.5	2.34-3.38	5.95-15.53		
	2.0				
OK	Control				
	0.5				
	1.0	1.73	12.84	155.54	2e-16***
	1.5	1.52-2.07	8.02-27.38		
	2.0				
OP	Control				
	0.5				
	1.0	2.23	5.90	288.33	2e-16***
	1.5	2.02-2.55	4.58-8.74		
	2.0				
HS	Control				
	0.5				
	1.0	0.95	3.22	190.76	2e-16***
	1.5	0.84-1.05	2.60-4.44		
	2.0				
EG	Control				
	0.5				
	1.0	1.10	3.20	391.81	2e-16***
	1.5	0.98-1.22	2.58-4.41		
	2.0				

HS= *Hyptis* Suaveolens OP= Orange peels O= *Occimum Kilimanscharicum*, EG= *Eucalyptus Globulus*, NS= neem seed, NST =neem stem, NL = neem leaf, Pr(>F) =F. calculated less than F tabulated.

3.6 Percentage mortality of *Culicine* exposed to petroleum ether and methanol extracts by time

The various plant extracts showed *adulticidal* effects on *culicine.* The lowest concentrations (0.5mg) of neem seed, neem stem, neem leaves, *O. kilimanscharicum, citrus senensis, Hyptis suaveolens and E. globulus* of methanol extracts showed lower *adulticidal* effect (0, 10, 15, 16.5, 0, 23.5 and 16.5%) respectively, in *culicine* exposed to it within 12hrs of the test, and also had mortality means (26.5, 15, 26.5, 26.5, 10, 31.5 and 26.5%) respectively, that was recorded after 36hours of observation **(**Table 6**)**. At this dose *Hyptis suaveolens* (31.5%) proved to be the most effective treatment agents. There was significant different (P<0.001) in percentage mortality between treated and untreated mosquitoes. At highest concentration 2.0mg, highest percentage mortalities (98.5, 40, 36.5, 90, 41.5 98.5 and 91.5%) was observed for neem seed, neem stem, neem leaves, *O. kilimanscharicum, citrus senensis Hyptis suaveolens and E. globulus* respectively. At the highest dose (2.0mg), *Hyptis suaveolens* (98.5%) and neem stems (98.5%) proved to be the most effective treatment agents against *culicine*, followed by *Eucarlyptus globulus* (91.5%) and *Ocimum kilimanscharicum* (90%). The least among all the treatment agents at this dose were observed in in neem leaves.

The table 6, also demonstrated the effects of petroleum extract of the various plants against the adult *culicine*. The percentage mortality of *culicine* insects increased significantly with increase in the concentration of the extract. The *culicine* mortalities range between 0-98.5%, according to the concentration doses.

The percentage mortality also increased significantly with increase in the duration of exposure. The lowest concentrations (0.5mg) of neem seed, neem stem, neem leaves, *O. kilimanscharicum, citrus senensis, Hyptis suaveolens and E. globulus* of petroleum extracts showed lower *adulticidal* effect (0, 0, 0, 11.5, 0 18.5, 0%) respectively, in *culicine* population exposed to it, within 12hrs of the test but mortality percentage (15, 0, 0, 25, 0, 31.5 and 26.5) increased a little higher as it was recorded after 36hours of observation (Table 5).

At this dose *Hyptis suaveolens* (18.5%) proved to be the most effective treatment agents followed by *O. kilimanscharicum* (11.5), while neem seed (0), neem stem (0), neem leaves (0), Orange peels (0) and *Eucarlyptus globulus* (0) showed no significant difference to control. There was significant different (P<0.001) in percentage mortality between treated and untreated mosquitoes. At highest concentration 2.0mg, highest percentage mortalities (83.5, 41.5, 41.5, 65.5, 36.5 88.5 and 81.5%) was observed for neem seed, neem stem, neem leaves, *O. kilimanscharicum, citrus senensis Hyptis suaveolens and E. globulus* respectively. At this dose (2.0mg), *Hyptis suaveolens* (88.5%) proved to be the most effective treatment agents against *culicine*, followed by *Eucarlyptus globulus* (81.5%) and neem seeds (83.5%). *O. kilimanscharicum* (65.5%), neem leaves (41.5%) and neem stem that showed moderate mortality. The least among all the treatment agents at this dose were observed in orange peels.

3.7 Percentage mortality of *Anopheline* exposed to methanol extracts by time

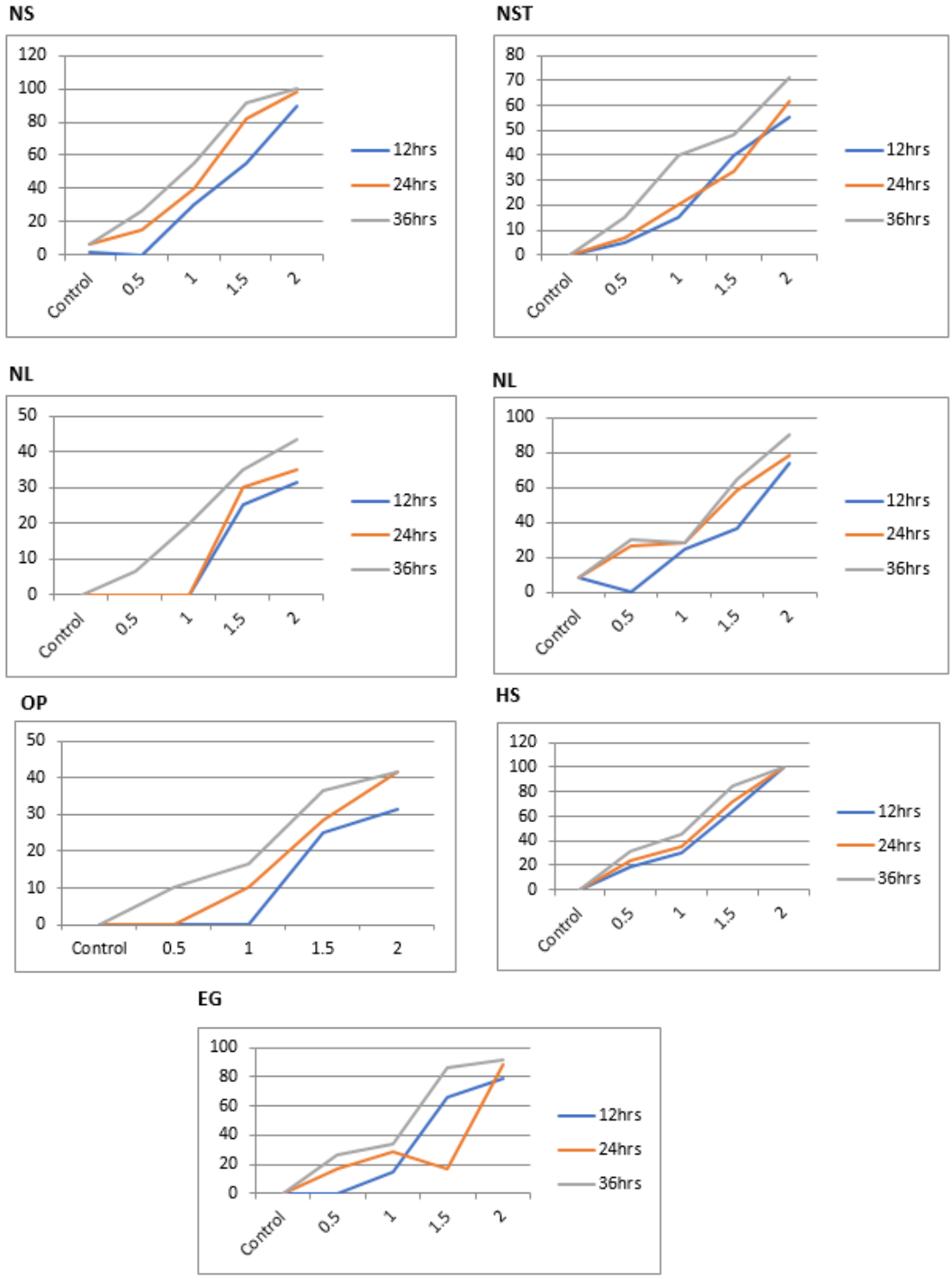
The data presented in Figure 1, exhibits the effects of methanol extract of ethno-botanicals against the adult *anopheline* with different concentrations (0.5-2mg). The percentage mortality of *anopheline* insects increased significantly with increase in the concentration of the extract. The *anopheline* mortalities range between 0-100%, according to the concentration doses that ranged between 0.5-2mg, respectively, and percentage mortality also increased significantly with increase in the duration of exposure. At lowest concentrations (0.5mg) of neem seed, neem stem, neem leaves, *O. kilimanscharicum, citrus senensis, Hyptis suaveolens and E. globulus* of methanol extracts showed lower *adulticidal* effect (0, 5, 0, 0, 0 18.5, 0%) respectively, against *anopheline* population exposed to it, within 12hrs of the test and as the time of exposure increased the mortality percentage (15, 6.5, 0, 8.5, 0, 23.5 and 16.5) also increased as observed after 24hrs. The highest mortality percentages (26.5, 15, 6.5, 8.5, 10, 26.5) were observed after 36hours in figure 1.

Table 6: Percentage mortality of *culicine* exposed to petroleum ether and methanol extracts by time

Samples	Dose(mg)	Methanol (%mortality/h)			P. ether (%mortality/h)		
		12h	24h	36h	12hrs	24hrs	36hrs
Ns	0.0	0	0	0	0	0	0
	0.5	0	15	26.5	0	0	15
	1.0	15	20	36	0	13.3	26.5
	1.5	65	75	90	55	63.5	73.5
	2.0	83.5	93.5	98.5	75	80	83.5
NST	0.0	0	0	0	0	0	0
	0.5	10	13.5	15	0	0	0
	1.0	15	21.5	26.5	0	8.5	18.5
	1.5	26.5	33.5	35	26.5	28.5	33.5
	2.0	38.5	40	40	35	40	41.5
NL	0.0	0	0	0	0	0	0
	0.5	15	26.5	26.5	0	0	0
	1.0	18.5	20	20	0	8.5	20
	1.5	17.5	25	28	16.5	23.5	25
	2.0	33.5	35	36.5	35	40	41.5
OK	0.0	0.0	0	0	0	0	0
	0.5	16.5	26.5	26.5	11.5	21.5	25
	1.0	28.5	28.5	28.5	25	26.5	26.5
	1.5	58.5	58.5	65	31.5	45	50
	2.0	75.5	78.5	90	51.5	58.5	66.5
Op	0.0	0.0	0	0	0	0	0
	0.5	0	0	10	0	0	0
	1.0	10	10	16.5	0	10	10.5
	1.5	28.5	28.5	36.5	25	28.5	36.5
	2.0	41.5	41.5	41.5	31.5	36.5	36.5
Hs	0.0	0.0	0	0			0
	0.5	23.5	23.5	31.5	18.5	23.5	31.5
	1.0	35	35	65	30	35	45
	1.5	70	70	85	65	68.5	85

	2.0	96.5	96.5	98.5	85	86.5	88.5
Eg	0.0	0.0	0	0	0	0	0
	0.5	16.5	16.3	26.5	0	10.5	26.5
	1.0	28.5	28.5	61.5	15	28.5	30
	1.5	76.5	76.5	80	61.5	76.5	80
	2.0	88.5	88.5	91.5	75	80	81.5

Keys: Hs= *Hyptis* Suaveolens OP= Orange peels Ok= *Occimum Kilimanscharicum*, Eg= *Eucalyptus Globulus*, NS= neem seed, NST =neem stem, NL = neem leaf, %=percentage, H= hour.



Keys: Hs= *Hyptis* Suaveolens OP= Orange peels Ok= *Occimum Kilimanscharicum*, Eg= *Eucalyptus Globulus*, NS= neem seed, NST =neem stem, NL = neem leaf, %=percentage, H= hour.

Fig 1: Percentage mortality of *Anopheline* exposed to methanol

3.8 Percentage mortality of *Anopheline* exposed to P. ether extracts by time

The data arranged in table 7, shows the percentage mortality of *anopheline* mosquitoes that were exposed to petroleum ether extracts of neem seed, neem stem, neem leaves *O. kilimanscharicum* orange peels, *H. suaveolens* and *Eucalyptus globulus*. The results showed that lower mortality percentage (0, 0, 0, 11.5, 0, 18.5 and 0) were observed at the lower concentrations in the first 24hrs but as the time of exposure increased to 36hrs, the mortality percentages also increased (0, 0, 0, 25, 0, 31.5 and 26.5) respectively. The neem seed, neem stem, neem leaves and orange peels showed no significant difference to control because there was no mortality recorded against them.

The result also showed that at highest concentration (2.0mg) high percentage mortality (98.5, 41.5, 31.5 78.5, 31.5, 95 and 90%) respectively, was observed. Neem seed (98.5%) proved to be the most effective treatment agent followed by *H. suaveolens* (95%) and *E. gloublus* (90%). The most ineffective treatment agents were observed in neem leaves (31.5) and orange peels (31.5).

3.9 Comparative study of methanol and petroleum ether extracts against *anopheline*.

At highest concentration 2.0mg, highest percentage mortalities (90, 55, 31.5, 73.5, 31.5, 100 and 78.5%) was observed for neem seed, neem stem, neem leaves, *O. kilimanscharicum*, *Citrus senensis*, *Hyptis suaveolens and E. globulus* respectively after 12hrs of exposure. At the highest dose (2.0mg), *Hyptis suaveolens* (100%) proved to be the most effective treatment agents against *anopheline*, which showed 100% mortality after just 24hrs of exposure followed by *neem seed* extracts of 100% mortality after 36hrs of exposure.

The data presented in figure 2, exhibit the comparative effects of methanol extracts and P. ether extracts of ethno-botanicals used against the adult *culicine* with different concentrations (0.5-2mg). The percentage mortality of *culicine* insects increased significantly with increase in the concentration of the extracts. The results at lower dose (0.5mg) revealed that petroleum ether extracts have lower mortality (0, 0, 0, 25, 10.5, 31.5, and 26.5%) percentage as compared to lower doses of methanol extracts mortality (26.5, 15, 6.5, 30, 10 31.5, and 26.5%) percentage.

Even at the highest dose methanol extracts still proved to be better with mortality percentage (100, 71.5, 43.5, 90, 41.5, 100 and 91.5%) than Petroleum ether extracts with mortality percentage (98.5, 41.5, 31.5, 78.5, 31.5, 95 and 90%) respectively. The results showed significant difference ($P<0.05$) between solvents used for the extraction.

3.10 Effects of some isolated biochemical compounds on Anopheline and culicine 4th instar larvae

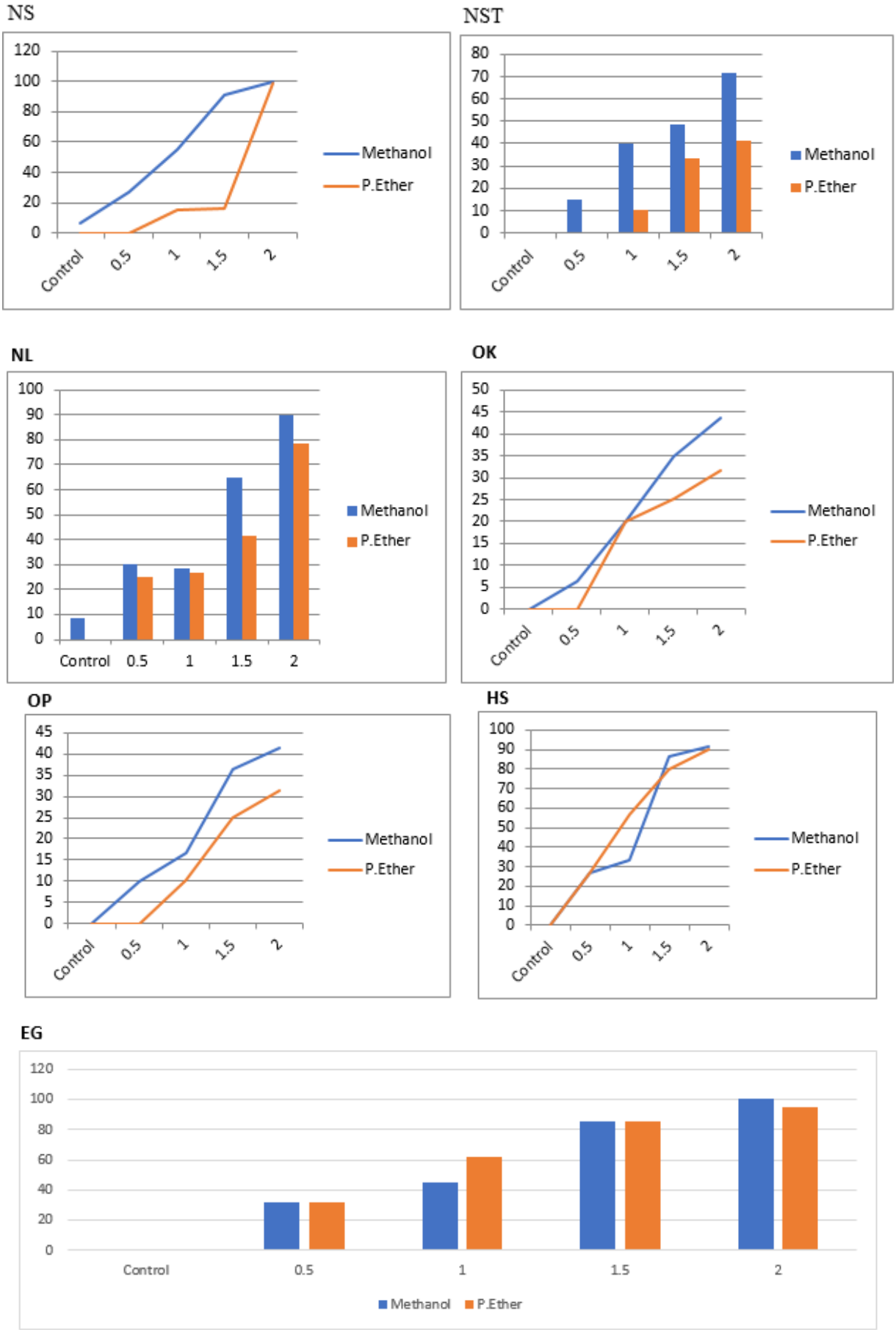
The result of the probit analysis showed various degree of effectiveness of biochemical compounds used against 4th instar larvae of culicine mosquitoes. The LC50 of the biochemical compounds (95.7, 99.93, 101,149, 180.32, 210.41ppm) of *azadirachtin*, steroids, Tannins, saponins, Alkaloids and phenols 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 8).

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.

The data arranged in table 9, showed various degree of effectiveness of biochemical compounds us against 4th instar larvae of anopheline mosquitoes. The LC50 of the biochemical compounds (100, 112.92, 139.62, 142.3, 150.32, 190.3ppm) of *azadirachtin*, Tannins, steroids, Alkaloids, phenols and saponins 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 tannins and steroid and (Table 8).

Table 7: Percentage mortality of *Anopheline* exposed to petroleum ether

Plants extracts	Concentration	Percentage mortality/hours		
		12hrs	24hrs	36hrs
Ns	Control	0	0	0
	0.5	0	0	0
	1.0	0	0	15
	1.5	0	13.5	16.5
	2.0	75	80	98.5
NST	Control	0	0	0
	0.5	0	0	0
	1.0	0	8.5	10.5
	1.5	26.5	31.5	33.5
	2.0	35	40	41.5
NL	Control	0	0	0
	0.5	0	0	0
	1.0	0	8.5	20
	1.5	16.5	20	25
	2.0	28.5	31.5	31.5
OK	Control	0	0	0
	0.5	11.5	28.5	25
	1.0	25	26.5	26.5
	1.5	31.5	45	41.5
	2.0	53.5	55	78.5
Op	Control	0	0	0
	0.5	0	0	0
	1.0	0	10	10.3
	1.5	16.5	20	25
	2.0	28.5	31.5	31.5
Hs	Control	0	0	0
	0.5	18.5	23.5	31.5
	1.0	30	35	61.5
	1.5	65	70	85
	2.0	85	86.5	95
Eg	Control	0	0	0
	0.5	0	16.5	26.5
	1.0	15	28.5	56.5
	1.5	56.5	76.5	80
	2.0	75	80	90



Keys: HS= *Hyptis* Suaveolens OP= Orange peels O= *Occimum Kilimanscharicum*, EG= *Eucalyptus Globulus*, NS= neem seed, NST =neem stem, NL = neem leaf,

Fig 2: Comparative Study of Methanol and Petroleum Ether Extracts Against *Anopheline*.

Table 8: Effects of biochemical compounds on Culicine 4th instar larvae

	Biochemical compounds									
	Control	**Alkaloids**	***Azadirachtin***	**Flavonoids**	**Phenol**	**steroids**	**Saponins**	**Tannins**	**glycosides**	**Terpenoids**
LC50	-	180.32	95.7	-	210.41	99.93	149	101	-	-
LC90	-	312.26	190	-	1069.11	110.11	300.28	193	-	-

Table 9: Effects of biochemical compounds on Anopheline 4th instar larvae

	Biochemical compounds									
	Control	**Alkaloids**	***Azadirachtin***	**Flavonoids**	**Phenol**	**steroids**	**Saponins**	**Tannins**	**glycosides**	**terpenoids**
LC50	-	142.3	100	-	150.32	139.62	190.3	103.6	-	-
LC90	-	305.60	210.36	-	350	290.33	401.31	129.65	-	-

The LC90 (210.36, 230.09, 290.33, 305.60, 350, 401.31ppm) of *azadirachtin*, Tannins, steroids, and phenols and saponins respectively, also showed the toxicity effect against anopheline mosquitoes and steroid proved to be the most effective treatment agent used.

4.1 Discussion

The results showed significant difference $P<0.05$) among the treatment plants used. The *culicine* mosquitoes exposed to methanol extracts had values of LC50 = 1.37, 2.23, 2.70, 1.83, 2.31, 0.99 and 1.18mg. The result proved that *H. suovelens* (0.99) is the most effective treatment plant used and this may be as a result of high amount of phenol (3.11mg) as observed in the qualitative analysis as it has shown high larvicidal effect of LC50(150.32ppm). While neem stem proved to be the most ineffective treatment agent used.

The *culicine* mosquito exposed to petroleum ether extracts had values of LC50= 1.09, 3.25, 5.11, 1.25, 2.50, 0.95 and 3.08mg respectively, the LC50 values showed that *Hyptis suovelens* (0.95mg) proved to be the best, may be as a result of presence (*alkaloids, phenol, steroid, saponins, and tannins*). These biochemicals have LC50 of (142.3, 150.32, 139.62, 190.3 103.6ppm) respectively, while neem leaves (5.11mg) prove to be the most less effective treatment agent used, this could be attributed to absence of terpenoid and steroids as observed in qualitative phytochemical analysis.

The *anopheline* mosquitoes exposed to methanol extracts, had LC50=0.97, 1.69, 2.45, 1.26, 2.50, 0.93 and 1.11mg. *H. suaveolens* (0.93mg) and neem seed (0.97mg) extracts had the lowest concentration that killed 50% of the *anopheline* exposed to them, this implies that, they are the most effective treatment agent used. Neem seed high effective power may be due high concentration of *azadirachtin* (4.06mg) and alkaloids (4.05mg) as observed in the quantitative phytochemical analysis earlier. These two phytochemical constituents have demonstrated high larvicidal effect against anopheline mosquito with LC50 (100 and 142.3ppm) respectively. This study agrees with the findings of 21 and 15 that showed *azadirachtin* component of neem products as the major component that is responsible for its toxic effects against insects. They also reveal that azadiractin is also attributed to the cause of reproduction defects, antifeedancy, growth reduction, increase in mortality and abnormal and delay in moults.

Anopheline exposed to petroleum ether had the LC50 = 1.30, 2.21, 3.70, 1.73, 2.23, 0.97and 1.10mg. The result of confidence limits that were calculated proved that *Hyptis suaveolens* (0.85mg) as the most effective treatment agent used while neem leaves (3.27mg) as the most ineffective treatment agent. The insects' mortality was observed under 12, 24 and 36hours. The result of insecticidal activities clearly indicated that the percentage mortality is directly proportional to the concentration of the extracts. In all the observations for the *adulticidal* activities, the results showed that *Hyptis suaveolens* (0.99, 0.95, 0.93 and 0.95) prove to be the most effective treatment agent against both *anopheline* and *culicine*, followed by the neem seed extracts which had LC50 = 1.37, 1.09, 0.97 and 1.30mg respectively. The present findings showed high effective of neem seed extracts against adult of both *culicine* and *anopheline* mosquitoes and agrees with some of the previous findings, such as the findings of 19 and 20 that showed over 90% mortality effect against *Aedes albopictus* and *anophelese* respectively.

The percentage mortality of both culicine and anopheline mosquitoes due to their period of exposure to methanol and petroleum ether of plants extract proved that the longer the exposure period the higher the mortality. The increase of mortality with time suggests that the effect is not authoumatic, it needs time for reaction to occur. The control group showed no significant difference ($P> 0.5$) to each other. Percentage mortality of the treated doses showed significant difference ($p< 0.5$) to each other. At 0.5 doses *H. suaveolens* (18.5%) proved to be the most effective treatment agent against anopheline followed by *O. kilimanscharicum* (11.5%). All other plants extract showed 0% mortality after 12hrs of exposure. All neem seeds products showed 0% mortality after exposure to petroleum ether extracts of 0.5mg and 1.0mg. Neem seed extracts also showed 0% mortality at to the petroleum ether extracts of 1.5mg dose, which is contrary to what is observed at the highest dose 2.0mg where neem seed (98.5%) prove to be the most effective treatment agents followed by *H. suaveolens* (95%) after 36hrs of exposure. The lower mortality effect of the first 12hrs and high mortality effect observed after 36hrs of exposure prove the toxicity effect is not automatic but gradually. This agrees with the findings of 16 and 17 that reported that mortality due to use of the plants extracts against mosquito larva is time dependent. This is also in agreement with the report of 18 that larval mortality of *An. gambiae* and *Cx. quinquefasciatus* exposed to crude extracts of *Lepidagathis alopecuroides* and *Azadirachta indica* increased with time of exposure and concentration.

A comparative estimation of the adulticidal potentials of two solvents were tested on culicine and anopheline. The activities of the ethanol extracts of *A. indica, O. Kilimanscharicum,* and *Citrus senensis, Hyptis suaveolens* and E. globulus were tested on culicine and anopheline for 36hours of exposure. The result generally showed that methanolic extracts had higher percentages mortalities (98.5, 40, 36., 90, 41.5, 98.5 and91,5%) against adult culicine mosquitoes than the percentage mortalities (83.5, 41.5, 41.5, 66.5, 36.5, 88.5, 81.5%) of petroleum ether after treatment with neem seed, neem stem, neem leaf, *Ocimum kilimanscharicum,* orange peels, *H. suaveolens and E. globulus* respectively. This finding agrees with report of Govnidarajan (2011) that reported that methanol extracts of *A. panicula, E. alba* and *C. halicacabum* are very effective against the 4th instar larvae of *anopheles stephensi* (LC5079.68, 112.66, and 133.01ppm; LC90 158.66, 220.68, and 270.72ppm. The result also showed that the higher the exposure period the higher the mortality that is also in agreement with the report of 15.

4.1.1 Conclusion

All the plant products used for this study have shown their potential as adult mosquito insecticides with *Hyptis suaveolens* proved to be most effective insecticides. Community-wide use of products from these plants have potential to substitute synthetic chemicals used as mosquito insecticides that are hazardous to man and some non-Targeted organisms. These ethnobotanicals are readily available in the study area and the country at large. Therefore, there is need to conduct more research on these plants, to also evaluates their potentials against other vectors of public health concern.

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