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Larvicidal Efficacy and GC-MS Analysis of *Hyptis suaveolens* Leaf Extracts against *Anopheles* Species

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Authors' contributions

This work was carried out in collaboration among all authors. Author YDD designed the study, managed the literature research and wrote the first draft of the manuscript. Authors CNA and DDP supervised and edited the work. Author AO performed the statistical analysis. Author FA played a role in GC-MS aspect of the work. Authors STJ and IRJ assisted in the lava scouting. Authors IRJ and SWK they played a role in the aspect of the phytochemical screening and author PML participated in the lava collection. All authors read and approve the final manuscript.

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ABSTRACT

Mosquitoes are the most important group of insects in terms of public health importance, which transmit serious human diseases. The continuous application of synthetic insecticides to control these mosquitoes causes development of resistance in vector species, and an adverse effect on environmental quality and non-target organisms including human health. Therefore, the use of active toxic agents from plant extracts as alternative mosquito control strategy cannot be over emphasized, as these are non-toxic, easily available at affordable prices, biodegradable and show

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target - specific activities against different species of vector mosquitoes. The aim of the present study was to evaluate the larvicidal efficacy of the aqueous and methanol leaf extracts of *H. suaveolens* against the fourth instars larvae of *Anopheles* species. Standard WHO protocols was adopted for the larvicidal bioassay. Larvicidal activity was evaluated at concentrations of 200, 400, 600, 800 and 1000 mg/L. The larval mortality was observed after 24 and 48hours bioassay. The results are mean of three replicates and the mortalities recorded were subjected to chi-square test and probit analysis to determine median lethal concentrations (LC_{50}). TheGC-MS analysis ofthe methanolic leaf extract of the plantrevealed the presence of twelve compounds on the chromatogram. Among the major compounds areOleic Acid (33.33%), Octadecanoic acid (13.52%), 1,3-Cyclohexadiene-1-methanol,alpha.,2,6,6-(10.42%), 1,3-Cyclopentanediol, trans (9.60%), n-Hexadecanoic acid (9.01%) and 4-Hepten-3-one, 4-methyl (7.0%).The phytochemical screening of the aqueous and methanol leaf extracts of the plant revealed the presence of Tannins, Terpenoids, Flavonoids, Steroids, Carbohydrates, Cardiac glycoside and resins. The LC_{50} values estimated were 316.22mg/Lfor methanol extract and 323.59 mg/L for aqueous extract. The differences between the two extracts were however not significantly different at $P>0.05$. The findings also revealed that mortality was concentration dependent and both extracts showed promising larvicidal activity against *Anopheles* species larvae. Therefore, the crude leaf extract of the plant is recommended to be used as alternative to synthetic larvicides and further research is needed to test the activity of the plant on non-target organisms.

Keywords: Mosquito; anopheles larvae; Hyptis suaveolens; extracts.

1. INTRODUCTION

Mosquitoes are insects of public health importance which transmit diseases such as dengue, chikungunya, Japanese encephalitis, filariasis and malaria, causing millions of deaths yearly [1]. The World Health Organization [2] reported that even when mosquitoes do not transmit diseases, they may cause great annoyance, making areas originally suitable for human and animal occupation not quite habitable. Among the insect-borne diseases, malaria is the mostprevalent. The World Health Organization [3], estimated the occurrence of 228 million malaria cases worldwide, with an estimated 405,000 deaths globally. Most of Sub-Saharan countries have stable endemic malaria because of the climatic conditions, which are ideal for the transmission, coincide with the species of *Anopheles gambiae*, *An. arabiense* and *An. funestus,* the most efficient vector mosquitoes in the world [4]. *Anopheles gambiae* are considered to be one of the world's most important human malaria vectors because of their susceptibility to the *Plasmodium* parasite, their preference for humans as a host and their indoor-feeding behavior [5]. Malaria is implicated in decreased learning capacity in children, students and trainees in the 5 – 25 age range and in the loss of economic productivity in the workforce age range 15 – 55 years [6]. One of the approaches for the control of these mosquito-borne diseases is the disruption of disease transmission through preventing the vector (mosquito) from biting the human beings [7]. This approach can be either through using mosquito nets, repellents, killing the mosquito or causing larval mortality in a large scale at the vector breeding sites. The latter approach is attractive target for pesticides because mosquitoes breed in water, and, thus, it is easy to deal with them in this habitat [8].

According to [9], the most preferred target for mosquito control is larva stage, because larvae have low mobility in nature, as compared to adult stage, and hence making control to be easily localized in time and space. Controlling larvae that are relatively immobile and more concentrated in their aquatic breeding sites is easier and more efficient [10]. Larval control has the potential to reduce malaria transmission both in rural and urban settings [11]. It is now being reconsidered as a complementing intervention to current priorities such as insecticide-treated nets (ITNS) and access to early diagnosis and prompts treatment [12]. The control of immature aquatic stages of *Anopheles* mosquitoes may have particular promise in urban settings where large numbers of people can be protected in a relatively small area and rural settings with focal, seasonal breeding sites [13].

The primary public health intervention for reducing malaria transmission at the community level is through vector control and it is the only

intervention that can reduce malaria transmission from very high levels to close to zero [14]. Many approaches have been developed to prevent mosquito menace. One of such strategies has been mainly based on the use of synthetic insecticides to interrupt the disease transmission cycle by either targeting the mosquito larvae at breeding sites through spraying of stagnant water, by killing or repelling the adult mosquitoes [15]. Even though these are effective, they created many problems such as toxicity to human beings, harm to non-target population, long persistence in environment and entry in the food chain [16]. As a result of these problems, it is necessary to develop environmentally safe, biodegradable, economical and indigenous method for control of vector that can be used with minimum care by individuals and communities [17]. Plant products with potentials to act as insecticides or repellent can play an important role in the interruption of transmission of mosquito borne disease at the individual as well as community level [18]. The secondary metabolites in different plants make up a vast repository of compounds with a wide range of biological activities [19]. It is in view of an increasing interest in developing plant based insecticides that the present study was undertaken.

Hyptis suaveolens is a medium aromatic weedy shrub found in the tropics and sub-tropical region [20]. The plant has been reported to possess repellent activity against insect pests of stored grains [21]. Methanolic extract of *Hyptis suaveolens* possess insecticidal activity against *Sitophilus maculates* [22]. Fumes of the dried leaves are also used locally to repel mosquitoes and to control insect pests of stored grains. It was also reported by [23] that placing *H. suaveolens* branches or whole plants in houses was one of the most effective methods to repel malaria vector *An. gambiae* s.s Giles. Therefore, the crude leaf extracts of *Hyptis suaveolens* has been investigated in this study for its larvicidal potency against malaria vectors, *Anopheles* species.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out at the Research Laboratory of Zoology Department, University of Jos, Plateau State, Nigeria.

2.2 Procedures of Plant Collection and Extraction

Mature fresh leaves of *Hyptis suaveolens* were collected in May, 2016 at Rafin Saniye village in the Southern part of Pankshin (Latitude 9^0 20' 0'' North, and longitude 9^0 27' 0'' East) Local Government Area of Plateau state, Nigeria. The plant leaves were air-dried under shade in Zoology Department Research Laboratory; University of Jos, Nigeria at room temperature for 14days. The plant specimen was identified by a taxonomist Mr. Joseph Azila, at the Herbarium of Federal College of Forestry, Jos, Nigeria.

The plant extract was prepared by maceration method in which the dried leaves of the plant was ground with mortar and pestle and then sieved to get fine powder. 250 g of the plant powder was weighed into 500 ml conical flasks and soaked in 200 ml of methanol. This was left to stand overnight (24hours) and shaked for 3hours on a mechanical shaker. The content was filtered using a non-absorbent cotton wool on a Buchner funnel-flask using a vacuum pump. The residue was subjected to several parts of rinsing and filtration with fresh solvent to attain some level of maceration. The filtrate was evaporated to dryness using a rotary evaporator and a drying cabinet. The extract was then transferred into a sample container and preserved in the refrigerator. Same procedure was applied for the aqueous extract using distilled water as the extracting solvent and was concentrated to dryness using a water bath at control temperature of 60^0C.

2.3 Phytochemical Screening

The phytochemical screening was carried out as described by [24,25]. The procedures are briefly described below.

2.3.1 Test for tannins

About 2 ml of the extract was stirred with 2 ml of distilledwater and few drops of $FeCl_3$ solution were added. The formation ofa green precipitate was an indication for the presence of tannins.

2.3.2 Test for saponins

5 ml of extract was shaken vigorously with 5 ml of distilledwater in a test tube and warmed. The

formation of stable foam wastaken as an indication for the presence of saponins.

2.3.3 Test for flavonoids

To 1 ml of extract was added 1 ml of 10% lead acetatesolution. The formation of a yellow precipitate was taken as apositive test for flavonoids.

2.3.4 Test for terpenoids

2 ml of the extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid wasthen added and heated for about 2 min. A greyish colour indicatesthe presence of terpenoids.

2.3.5 Tests for steroids

A red colour produced in the lower chloroform layer when 2 ml ofextract was dissolved in 2 ml of chloroform and 2 ml concentrated sulphuric acid added indicates the presence ofsteroids.

2.3.6 Test for alkaloids

3 ml of the extract was stirred with 3 ml of 1% HCl on a steambath. Mayer's and Wagner's reagents were then added to themixture. Turbidity of the resulting precipitate was taken as evidencefor the presence of alkaloids.

2.3.7 Tests for carbohydrates

To 3 ml of the extract was added about 1 ml of iodinesolution. A purple colouration at the interphase indicates thepresence of carbohydrates.

2.3.8 Tests for glycosides

Salkowski's test: 2 ml of the extract was dissolved in 2 ml ofchloroform. 2 ml of sulphuric acid was added carefully and shakengently. A reddish brown colour indicates the presence of a steroidal ring (that is, a glycone portion of glycoside).

2.3.9 Test for resin

2 ml of the extract and 2 ml of acetic anhydride were mixed together. A drop of concentrated sulphuric acid was added to the mixture. A purple or violet colour indicates the presence of resin.

2.3.10 Test for balsams

2 ml of the extract and 2 ml ethanol were mixed together and two drops of alcoholic ferric chloride solution was added. A dark green colouration indicates the presence of balsams.

2.4 Larvae Collection

Anopheles larvae were collected from stagnant water at the edges of river Timshing in Rafin saniye village of Pankshin Local Government Area of Plateau State, Nigeria using a dipper. The larval stage of *Anopheles* species were distinguished from those of *Culex* species using morphological differences [26].

2.5 Gas Chromatography and Mass Spectrometry (GC – MS) Analysis

The GC-MS analysis of the methanol extracts of the *H. suaveolens* was carried out on GCMS- QP 2010 plus, at National Research Institute for Chemical Technology (NARICT) Zaria, Nigeria. REF TEK column was used for the analysis. The sample was injected with split less mode. Helium gas was used as a carrier gas. The database used for the identification of the compounds was National Institute Standard and Technology MS library.

2.6 Preparation of the Concentrations of the Extracts and Larvicidal Bioassay

A Range Finding Test (RFT) was conducted to obtain definitive toxic concentration for bioassay [27]. Five concentrations (200, 400, 600, 800 and 1000 mg/L) of the leaf extracts with control as 0.00 mg/L were prepared.

The larval bioassay was carried out according to the WHO standard procedure [28]. The tests were conducted in plastic containers. From the extracts, concentrations of 200, 400, 600, 800 and 1000mg/L were prepared. The fourth instars larvae of *Anopheles* species were placed in each test solution using a dropper to study the larvicidal property as per the following procedures. Twenty healthy fourth larval instars of *Anopheles* species were released in each concentration of both treatment and the replicates with extract solution and control experiments without extracts were run in parallel. The larvae in each solution were then left for 24hours and 48hours, the numbers of dead larvae were counted after 24hours and 48hours of exposure and the percentage mortality were recorded from the average of three replicates. Larvae were considered dead if appendages did not move when probed with needle. Larvae incapable of rising to the surface or not showing

the characteristic diving reaction when water was disturbed, were considered moribund and added to the dead larvae for calculation of percentage mortality.

2.7 Calculation of Mortality Rate

The mortality rate was calculated using the formula:

$$\text{Observed mortality} = \frac{\text{Total number of dead larvae}}{\text{Total number of larvae used}} \text{ x } 100 \quad \text{equation(1)}$$

2.8 Interpretation of Susceptibility Test Results

The interpretation was based on WHO bioassay protocol [29] which states that:

- Mortality in the range of 98 – 100% indicates susceptibility.
- Mortality in the range of 80 – 97% is suggestive of the existence of resistance and further investigation is needed.
- Mortality less than 80% indicates resistance.

2.9 Statistical Analysis

Data obtained were analyzed using R Console software (Version 3.2.2). Proportions of mortality rate of *Anopheles* species larvae in relation to concentrations of aqueous and as well as methanol extracts were compared using Pearson's Chi-square test. It was also used to compare proportions of mortality rate of *Anopheles* species larvae between extracts for each concentration. The average larval mortality data were subjected to probit analysis for calculating LC_{50}. Results with P-values < 0.05 were considered to be statistically significant.

Fig. 1. The plant sample, *Hyptis suaveolens*

3. RESULTS

3.1 Phytochemical Screening of *H. suaveolens* from Methanol and Aqueous Extracts

Phytochemical screening analysis showed that *H. suaveolens* has seven (7) active ingredients from both methanol and aqueous extracts with the exception of saponins and alkaloids (Table 1). Terpenoids was strongly positive in methanol extract while flavonoid was strongly positive in aqueous extract. Cardiac glycosides' was at trace level in methanol extract but not detected in aqueous extract. Resins were at positive level in aqueous extract but not detected in methanol extract. Steroids were at trace level in both methanol and aqueous extracts.

3.2 Mortality Rate of *Anopheles species* Larvae a Cross the Concentrations of Extracts of *H. suaveolens* in Relation to Time

3.2.1 Aqueous extract

The control had no dead *Anopheles* species larvae at both 24 hours and 48 hours of the bioassay whereas the aqueous extracts treatments showed varying levels of mortality across concentrations (Table 2). At the end of 24hrs and 48hours exposure time respectively, mortality rate increased in ascending order of concentrations. The 1000 mg/L concentration recorded the highest mortality rate of *Anopheles* species larvae of 95% followed by 800 mg/L with 80%, 600 mg/L had 63.5%, 400 mg/L showed 55% while 200 mg/L was 25% .The LC_{50} of aqueous extract obtained at the end of 48 hours exposure time of the assay was 323.5 mg/L. There was significant difference in the mortality rate of *Anopheles* species larvae across concentrations of aqueous extract (χ^2 = 116.8, df = 5, P < 0.05).

The *Anopheles* larvae were tolerant (mortality rate <80%) to concentrations of aqueous extract between 200 to 600 mg/L while they showed possible tolerance (mortality rate 80% to 97%) between 800 to 1000 mg/L concentrations.

3.2.2 Methanol extract

The control had no dead *Anopheles* species larvae at both 24 hours and 48 hours of the bioassay whereas the methanol extract treatments showed varying levels of mortality

across concentrations (Table 3). At the end of 24hrs and 48hrs exposure time respectively, mortality rate increased in ascending order of concentrations. The 1000 mg/L concentration recorded the highest mortality rate of *Anopheles* species larvae of 100% followed by 800 mg/L with 86.7%, 600 mg/L had 65%, 400 mg/L showed 43.4% while 200 mg/L was 36.7% .The LC_{50} of methanol extract obtained at the end of 48 hours exposure time of the assay was 316.22 mg/L (Table 5). There was significant difference in the mortality rate of *Anopheles* species larvae across concentrations of methanol extract (χ^2 = 119.78, df = 5, P < 0.05).

The *Anopheles* species larvae were tolerant (mortality rate <80%) to concentrations of methanol extract between 200 to 600 mg/L while they showed possible tolerance (mortality rate 80% to 97%) to 800 mg/L concentration, but were susceptible (mortality rate 100%) to concentration of 1000 mg/L.

3.3 Comparison of Mortality Rate in Relation to Extracts of *H. suaveolens* at the End of 48 Hours Exposure Time

Mortality rate of *Anopheles* species larvae was higher in all concentrations of methanol extract over the aqueous extract with the exception of 400 mg/L concentration in which aqueous extract had higher mortality rate (Table 4). However, there was no significant difference (P > 0.05) in the mortality rate of *Anopheles* species larvae between extracts of *H. suaveolens* for each concentration.

3.4 Preliminary Characterization of *H. suaveolens* Methanol Leaf Extracts Using Gas Chromatography and Mass Spectrometry (GC-MS)

The composition and identification of the main compounds (Table 5) present in the methanol leaf extracts of *H. suaveolens* revealed the presence of twelve compounds on the chromatogram. These compounds were identified through mass spectrometry attached with the gas chromatography. The gas chromatography revealed the concentrations of the various compounds as a function of retention time while the peak heights showed the relative concentrations of the components present in the leaves of the plant *H. suaveolens*. On the other hand, the mass spectrometer analyses the compounds at different times to identify their structures as well as their nature. The highest peak area is recorded in peak seven, having 33.33% with 21.378 minutesretention time (RT),and the compound name is Oleic Acid while the lowest is recorded in peak eleven having 0.56% with 24.810 minutesas retention time(RT) and the compound name is Decyl fluoride. The gas chromatography and mass spectrometry (GC-MS) spectrum of the extract revealed the presence of long chains of hydrocarbons.

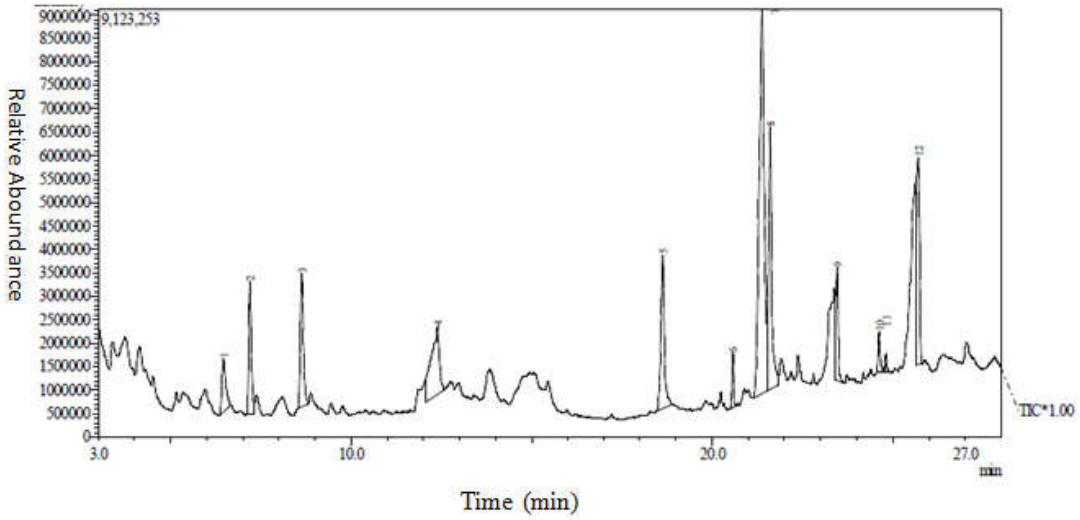

Fig. 2. Showed GC-MS chromatogram of the methanol extract of *H. suaveolens*. Twelve compounds were detected in the extract. The highest peak area is recorded in peak seven, having 33.33% with 21.378 minutes retention time (RT) and the compound name is oleic acid while the lowest is recorded in peak eleven having 0.56% with 24.810 minutes as retention time(RT) and the compound name is decyl fluoride

Table 1. Phytochemical screening of active ingredients of *H. suaveolens* from methanol and aqueous extracts

S/No	Secondary metabolite	*H. suaveolens* Methanol extract	*H. suaveolens* Aqueous extract
1	Tannins	+	++
2	Saponins	-	-
3	Alkaloids	-	-
4	Terpenoids	+++	+
5	Carbohydrates	+	++
6	Cardiac glycosides	+	-
7	Balsams	++	+
8	Resins	-	++
9	Flavonoids	++	+++
10	Steroids	+	+

Key: +++: Strongly positive,
++: Positive,
+: Trace
- : Not detected

Table 2. Mean mortality rate of *Anopheles species* larvae across concentrations of aqueous extract of *H. suaveolens* in relation to time

Concentration (Mg/L)	Log concentration	Mean No. of larvae exposed	Mean mortality rate		% Mortality rate	Probit mortality
			24hours	48hours		
0.0	0.0	20	0.00	0.00	0.0	0.0
200	2.30	20	1.00	5.00	25.0	4.32
400	2.60	20	3.67	11.0	55.0	5.12
600	2.77	20	5.00	12.7	63.5	5.40
800	2.90	20	6.67	16.0	80.0	5.84
1000	3.00	20	7.67	19.0	95.0	6.70

Table 3. Mean mortality rate of *Anopheles species* larvae across concentrations of methanol extract of *H. suaveolens* in relation to time

Concentration (Mg/L)	Log concentration	Mean No. of larvae exposed	Mean mortality rate		% Mortality rate	Probit mortality
			24hours	48hours		
0.0	0.0	20	0.0	0.0	0.0	0.0
200	2.30	20	2.33.	7.33	36.7	4.66
400	2.60	20	2.67	8.67	43.4	4.83
600	2.77	20	5.00	13.00	65.0	5.38
800	2.90	20	6.33	17.33	86.7	6.11
1000	3.00	20	6.67	20.00	100	8.71

Table 4. Mortality rate in relation to extracts of *H. suaveolens* at the end of 48 hours exposure time

Concentration (mg/L)	% Mortality rate		χ^2	Df	P value
	Aqueous extract	Methanol extract			
200	25.0	36.7	2.2186	1	0.1364
400	55.0	43.4	1.3675	1	0.2422
600	63.5	65.0	0.01751	1	0.8947
800	80.0	86.7	0.26929	1	0.6038
1000	95.0	100	0.12821	1	0.7203

Table 5. Compound identified from the methanol leaf extract of *H. suaveolens*

Peak No	RT (min)	Compound Name	Area (%)	Molecular Formula	Molecular Weight (g/mol)
1	6.469	2-Hexanone, 3-methyl-4-methylene	3.57	$C_8H_{14}O$	126
2	7.203	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	6.06	$C_6H_8O_4$,	144
3	8.636	4-Hepten-3-one, 4-methyl	7.0	$C_8H_{14}O$	126
4	12.395	1,3-Cyclopentanediol, trans	9.60	$C_5H_{10}O_2$	102
5	18.630	n-Hexadecanoic acid	9.01	$C_{16}H_{32}O_2$	256
6	20.570	Octadecanoic acid, methyl ester	1.39	$C_{19}H_{38\ O2}$	298
7	21.378	Oleic Acid	33.33	$C_{18}H_{34}O_2$	282
8	21.602	Octadecanoic acid	13.52	$C_{18}H_{36}O_2$	284
9	23.461	Methoxyacetic acid, 2-methylphenyl ester	4.31	$C_{10}H_{12}O_3$	180
10	24.616	9-Octadecenal	1.17	$C_{18}H_{34}O$	266
11	24.810	Decyl fluoride	0.56	$C_{10}H_{21}F$	160
12	25.703	1,3-Cyclohexadiene-1-methanol, .alpha.,2,6,6-	10.42	$C_{11}H_{18}O$	166

4. DISCUSSION

The result of the Phytochemical screening (as shown in Table 1) revealed that Tannins, Terpenoids, Flavonoids, Steroids and Carbohydrates were present in both methanolic and aqueous extracts of *H. suaveolens*. Cardiac glycoside was detected only in methanolic extract while resins were positive in aqueous extract only. There was absence of saponins and alkaloids in both methanolic and aqueous extracts. It is in accordance to the work of [30] who recorded absence of saponins in *H. suaveolens* in a study carried out on the larvae of *Culex quinquefasciatus* to test the larvicidal activity of the plant, but detected strong presence of alkaloids using ethanol as the solvent for extraction. Also, [31] reported that *H. suaveolens* was devoid of saponins but tannins (0.520%) and phenols (0.050%) were present. Researchers suchas [32] identified the above mentioned components except for saponins and alkaloids in eucalyptus and tamarind using methanol as solvent when tested against *Anopheles gambiae*.

These secondary metabolites are known to be effective against a wide range of insect pests as well as mosquito vectors [33]. The most reported insecticidal group of compounds from plants with larvicidal activity against various species of mosquito are steroids, flavonoids, phenols, tannins, terpenoids, carbohydrates and saponins [34,35]. These phytochemicals may be responsible for their insecticidal properties [36]. The failure of the larvae to moult and then develop into perfect pupae may be due to the delayed lethal effects of the phytocompounds which cause disturbances in the endocrine mechanism regulating moulting and metamorphosis [37]. In the screening of the phytochemicals, methanolic extract detected more metabolites than the aqueous extract which possibly suggest that it is more sensitive in detection of active ingredients in the plant used. This is in agreement with the findings of [38] who worked on the efficacy of aqueous and methanol extracts of the plant *Desmodium triflorum* for potential antibacterial activity. Their work confirmed that methanol extract detected more metabolites than aqueous extract. Therefore, the potent larvicidal activity of aqueous and methanolic extracts of *H. suaveolens* could be attributed to chemical compounds detected in this study.

The bioassay investigation revealed that the aqueous and methanolic extracts of *H. suaveolens* showed promising larvicidal activity against *Anopheles species.* This is in agreement with the study by [39] who tested the larvicidal activity of *H. suaveolens* acetone aerial extracts against the larvae of filarial vector *Culex quinquefasciatus.* On the contrary, *H. suaveolens* petroleum leaf extracts did not show any effective larvicidal activity against *Culex* species mosquito larvae [40]. Possibly this could be as a result of inability of the solvent to extract the major active ingredients needed for the purpose.

In aqueous extract, mortality of *Anopheles* species larvae recorded after 24hours increased

as the concentrations of the extracts increased. This possibly suggest that the metabolites get to the target sites of *Anopheles* species larvae much faster with increase in concentration of *H. Suaveolens* aqueous extract. This is in agreement with the finding of [41] who assessed larvicidal activity of *H. suaveolens* and *Balanites aegyptica* leaves and root extracts against mosquito species. After 48hours, observed mortality increased as concentration increased, with 95% mortality at the highest concentration. This is in agreement with the studies by [42] who recorded 95% larval mortality of aqueous extracts of *Phyllanthus emblica* (dry seed) on *Aedes albopictus.* This is also in accordance with the result of [43] who recorded higher mortality rate with increase in concentration against mosquito species. The *Anopheles* species larvae were tolerant to concentrations of aqueous extract between 200 to 600 mg/L while they showed possible tolerance between 800 to 1000 mg/L concentrations.

At 24 hours larval exposure to methanol extracts, the result demonstrated progressive increase in percentage mortality as concentrations increased (Table 3). After 48 hours, the methanol extract demonstrated significant larval mortality progressively as the concentration increased as well. A mortality of 100% was recorded in the highest concentration of 1000mg/L. This result is in agreement with the finding of [44] who reported 100% larval mortality of acetone aerial extract of *Hyptis suaveolens* at the highest concentration of 1000 mg/L against the larvae of filarial vector *Culex quinquefasciatus* say after 48hours bioassay. Also, [45] recorded 100% mortality of stored product pests, *Sitophilus oryzae, S. zeamais and Callosobruchus maculatus* using methanol extract of *H. suaveolens.* A significant mortality of fourth instars larvae of *Culex quinquefasciatus* was also recorded with methanolic extract of *Cliona celata* with LC_{50}=95.63 ppm and LC_{90}=242.16ppm [46,47] reported also that methanol leaf extract of *Acalypha indica* was more lethal to the egg and larvae of *An. stephensi and Culex quinquefasciatus.* In this study, the *Anopheles* species larvae were tolerant to concentrations of methanol extract between 200 to 600 mg/L while they showed possible tolerance to 800 mg/L concentration, but were susceptible to concentration of 1000 mg/L.

Among the twelve compound detected in the GC-MS chromatogram of the methanol extract of *H. suaveolens*, the highest peak area is recorded in peak seven, having 33.33% with 21.378 seconds retention time (RT),and the compound name is Oleic Acid. Hence, [48] suggested that the compound oleic acid, eicosyl ester from *Thalictrum javanicum* are effective source of mosquito larvicides as they reduce the activity of chitinase and ecdysone 20- monooxygenase in both*A.aegypti* and *Culex quinquefasciatus* [49] also reported that oleic and linoleic acid were quite potent against the fourth instar larvae of *Aedes aegypti*, *Anopheles stephensi* and *Culex quinquefasciatus* say after 24h bioassay. In the contrary [50] reported that the bioassay of oleic acid against a Subterranean Termite, *Coptotermes gestroi wasmann* and a drywood Termite, *Crytotermes cynocephalus* light showed low termicidal activity as it delivered low mortality in both species and generated lower protection against *Crytotermes cynocephalus*.

5. CONCLUSION

The investigation established the potential of *H. suaveolens* methanol and aqueous leaf extracts against the fourth larval stage of *Anopheles* species. The types and levels of active components of the extracts may be responsible for their potential against the *Anopheles* species larvae. Therefore, it is evident from this present investigation that crude extracts from *H. suaveolens* have promising larvicidal efficacy. Since this present investigation was undertaken under Laboratory conditions, field application of this plant for mosquito vector control should be testedand further investigation should also be done on the effect of the extracts on non-target organisms.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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