
Assessment of the Larvicidal Potential of the Leaf Extracts of *Hyptis suaveolens* Poit against *Culex* Mosquitoes

By

Amaka, John I.¹, Abubakar, U.² and Attah, D. Daniel¹

¹Department of Biological Sciences,
Kebbi State University of Science and Technology, Aliero, Nigeria

²Department of Biological Sciences,
Usmanu Danfodiyo University, Sokoto, Nigeria

ABSTRACT

The larvicidal potential of the ethanolic and aqueous leaf extracts of Hyptis suaveolens Poit on the 4th larval instar of laboratory-reared Culex spp at varying concentrations of 0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml for specified periods of 24hrs, 48hrs and 72hrs was evaluated in this study. Qualitative phytochemical screening of the leaf extracts identified bioactive components like alkaloid, saponin, phenol, anthraquinone and flavonoid. The LC₅₀ and LC₉₀ values obtained indicate that the ethanolic leaf extracts of Hyptis spp had the greatest toxicity on the test organisms within 24hrs of exposure at median LC₅₀ value of 0.497 as against the LC₅₀ value of 0.637 by its aqueous extract. The relative median potency estimates indicate that within 24 hrs, the ethanolic Hyptis spp was 0.185 times more potent on the test organism than aqueous Hyptis spp. The result of this research, therefore, gives credence to the efficacy of botanicals like Hyptis suaveolens Poit as an eco-friendly alternative in Culex mosquito control. It is, therefore, recommended that quantitative phytochemical screening, application of column chromatography as well as thin layer chromatography be carried out on the extracts to isolate and purify phytochemicals with larvicidal potentiality.

Keywords: *Culex spp*, Larvicidal Potential, Lethal Concentration, Phytochemicals, Potency Ratio, Vector Control

INTRODUCTION

Lymphatic filariasis, commonly known as elephantiasis, is a neglected tropical disease (NTD) causing disabling infections in rural communities. Infection occurs when filarial parasites are transmitted to humans through mosquitoes, mainly of *Culex spp* [29]. Currently, 856 million people in 52 countries are living in areas that are vulnerable to the spread of the infection. The global baseline estimate of persons affected by lymphatic filariasis is 25 million men with hydrocele and over 15 million people with lymphoedema. At least 36 million persons remain with these chronic disease manifestations [29].

In recent years, mosquito resistance to synthetic insecticides has emerged in many countries [30]. This undermines control programmes for mosquito-borne diseases, and poses a great risk to vulnerable populations [26]. Due to the concern over the quality and safety of life and the environment, the emphasis on controlling mosquitoes has shifted steadily from the use of conventional synthetic insecticides towards alternative phytochemical products that are target-specific, biodegradable, affordable, efficacious and environmentally safe.

Several research works have documented the efficacy of the extracts of many plant materials as possible alternatives in vector control programmes [23, 24, 27]. This paper aims to assess and compare the potential of the ethanolic and aqueous leaf extracts of *Hyptis suaveolens* Poit on the larvae *Anopheles spp.*

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

The leaves of *Hyptis suaveolens* Poit were collected from the bushes around Birnin-Kebbi area of Kebbi State, Nigeria, using sterilized specimen bags. The leaves were identified by a plant taxonomist in the Botany Unit of the Department of Biological Sciences, Kebbi State University of Science and Technology, Aliero, with voucher number 289A. Afterwards, the leaves were rinsed with distilled water to remove dirt and spread out on a clean surface, and allowed enough time to air-dry under shade at room temperature.

Extraction of Plant Materials

The dried leaves were subjected to maceration extraction method as described by [22]. They were pulverized using an electric blender. Fifty gramme (50g) of each powdered leaf sample was soaked in 500ml of each solvent (ethanol and water). The contents were stoppered with aluminium foil in a 1000ml beaker capacity, and kept in a cool dry place. The contents were stirred at intervals to avoid fungal growth. The aqueous extracts were due in 24 hours while the ethanolic extracts were due in 72 hours. In each case, they were filtered, and the filtrates subjected to evaporation, in open air (for ethanolic extract) in the laboratory, and using a water bath set at 80°C (for aqueous extract) until the solvents evaporated. The crude ethanolic and aqueous extracts obtained were weighed and recorded. Approximately 1000mg (1g) of the crude extract was re-dissolved in 100cm of the appropriate solvents (ethanol and water) to obtain the stock solution in line with World Health Organization guidelines [28]. They were stored in the refrigerator until the dilute concentration was to be prepared for larvicidal assay.

Qualitative Phytochemical Screening

Crude ethanolic and aqueous extracts of the leaves of *Hyptis spp* were screened for phytochemical components using the methods described by [13, 16].

Rearing of Culex spp

The larvae of *Culex spp* were collected from freshwater bodies like gutters in Gesse Phase 2 area of Birnin-Kebbi, Kebbi State. The mosquito larvae were identified as belonging to *Culex spp* by having their body lie at an angle to the water surface [17]. The test organisms were reared according to guidelines provided by WHO [28]. They were transferred to a transparent plastic container enclosed in a cage measuring about 26 x 10 x 6 cm built with nets. They were maintained at room temperature and normal photoperiod. The larvae were fed on a diet of yeast until pupae emerged. After adult emergence, they were fed on a glucose meal (sugar solution). Restrained and de-feathered pigeons were used to provide blood meal for the females. Eggs were laid in the larval container usually at night. In the morning, the eggs were collected and kept in a beaker lined with tissue paper, and maintained at room temperature to allow eggs to accumulate, giving room for a homogenized hatching.

When enough eggs had been accumulated, they were immersed in distilled water in a transparent plastic container. To promote hatching, a little quantity of larval food (yeast) was added to the water 24hrs before adding the eggs. The bacterial growth would de-oxygenate the water and this would trigger egg-hatching. This process usually induced the first instars to hatch within 12hrs of hydration [28]. Larval food consisting of yeast was used. The amount of yeast was kept low to avoid strong bacterial growth (which kills the larvae), and to prevent turbidity and scum. The larvae were observed on daily basis for about 5-6 days until characteristic 4th instars emerged.

Larvicidal Bioassay

Larvicidal bioassay was conducted according to the standard test methods adopted by WHO [28]. The dilute concentration (which was in tenfold) of the stock was prepared by dissolving 5ml of each stock solution in 45ml of water. The varying concentrations of 0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml of the individual extracts of *Hyptis spp* were tested against twenty (20) healthy 4th instar larvae. The larvae were distinguished from other preceding instars by the possession of a wide and heavily sclerotized collar on the posterior border of the head [17]. The tests were conducted in 150ml plastic containers containing 100ml of water. Three replicates per concentration and a control were run simultaneously during each trial. For positive control, 1ml of ethanol in 100ml of water was used while 100ml of water with no treatment was used for negative control. The experimental set-up was maintained at room temperature, and mortality was observed at 24, 48 and 72hrs after treatment.

Larvicidal activity of each extract was determined by counting the number of dead and moribund larvae on daily basis (24hrs interval) for three days. The moribund and dead larvae in the three replicates were combined as mean and expressed as percentage mortality for each concentration. Dead larvae were determined and recorded when they failed to move after probing with a sterile needle for 10 seconds. The percentage mortality, as described by WHO [28], was calculated as follows:

$$\% \text{ Mortality} = \frac{\text{Number of dead/moribund larvae}}{\text{Number of larvae introduced}} \times \frac{100}{1}$$

The corrected percentage mortality, as described by WHO [28], was used where a minimal proportion of the larvae (between 5% and 20%) in the control batches died during the experiment. To correct for this, the Abbott's formula was used:

$$P = \frac{P_t - P_c}{100 - P_c} \times 100 \quad \text{or} \quad P = \frac{X - Y}{X} \times 100$$

Where P is the corrected mortality, P_t is the observed mortality in the test sample, and P_c is the mortality in the control, X = percentage survival in the untreated control and Y = percentage survival in the treated sample [1].

Statistical Analysis

Probit Analysis as described by Finney [14] was employed using SPSS version 20. The 24, 48 and 72hrs lethal concentration values (LC₅₀ and LC₉₀) as well as the relative potency

ratio of the extracts were determined using Probit Analysis. Comparisons of the larvicidal efficacies of the extracts were also estimated.

RESULTS

Qualitative Phytochemical Screening of Leaf Sample Extracts

Qualitative phytochemical screening of the leaf sample extracts of *Hyptis suaveolens* Poit revealed the presence of bioactive compounds such as alkaloid, saponin, tannin, phenol, anthraquinone, flavonoid and steroid (Table 1).

Median Lethal Concentration (LC_{50} and LC_{90})

The larvae of *Culex spp* were tested for resistance by assessing their mortality after exposure to varying concentrations of the extracts over a range of time. At 24hrs, the LC_{50} values for the ethanolic and aqueous extracts are 0.497ml and 0.637ml respectively (Table 2).

Table 1: Phytochemical Screening Result of the Leaf Sample Extracts of *Hyptis suaveolens* Poit

Phytochemical	<i>Hyptis suaveolens</i> Poit	
	Ethanolic	Aqueous
Alkaloid	+	+
Saponin	+	+
Phenol	+	+
Tannin	+	+
Anthraquinone	+	+
Glycoside	-	-
Flavonoid	+	+
Steroid	-	+
Terpenoid	-	-

Key: + present, - absent

Table 2: LC_{50} and LC_{90} Values of *Hyptis spp* Extracts on *Culex spp* at Varying Periods at 95% C.L.

Time (hrs)	Extract	Lethality	Conc (ml)	Lower bound	Upper bound	Log conc	Lower bound	Upper bound
24	ET	LC_{50}	0.497	-0.203	1.197	-0.303	-0.403	-0.203
		LC_{90}	0.994	0.294	1.694	-0.002	-0.102	0.097
	AQ	LC_{50}	0.637	-0.163	1.237	-0.195	-0.370	-0.170
		LC_{90}	1.275	0.375	1.775	0.105	-0.069	0.131
48	ET	LC_{50}	1.398	0.462	1.862	0.145	-0.035	0.165
		LC_{90}	2.825	1.625	3.025	0.451	0.266	0.466
	AQ	LC_{50}	0.869	0.169	1.569	-0.060	-0.160	0.040
		LC_{90}	1.739	1.039	2.439	0.240	0.140	0.340
72	ET	LC_{50}	1.153	0.453	1.853	0.061	-0.039	0.161
		LC_{90}	2.307	1.607	3.007	0.363	0.263	0.463
	AQ	LC_{50}	2.307	1.607	3.007	0.363	0.263	0.463

LC₉₀ 4.615 3.915 5.315 0.664 0.564 0.764

Key: ET – Ethanolic, AQ – Aqueous, LC – Lethal Concentration

Larvicidal Potential of the Leaf Extracts

The larvicidal potential of the leaf extracts of *Hyptis spp* was evaluated against the 4th instar stage of *Culex spp*. Though there were differences in mortality recorded at varying concentrations and periods of exposure, both the ethanolic and aqueous extracts tested exhibited larvicidal potential against the test organisms. Mortality increased with increase in concentration (Table 3). Ethanolic extract had the highest cumulative mortality of 94.5% while aqueous extract had a cumulative mortality of 63% using the highest dose of 0.5ml. The lethality pattern of the ethanolic leaf extract of *Hyptis spp* indicates that the test organism showed the highest mortality of 51.5% within the first 24hrs of exposure to the extract at the highest concentration of 0.5ml of the ethanolic extract (Figs 1, 2 and 3).

Table 3: Larvicidal Effect of the Leaf Extracts of *Hyptis spp* on *Culex spp*

Concentration (ml)	Mortality (%)			
	Ethanolic			
	24hrs	48hrs	72hrs	Total
Control	5	0	0	5
0.1	8.00	15.00	8.00	31.00
0.2	20.00	8.00	16.50	44.50
0.3	36.50	8.00	5.00	49.50
0.4	45.00	6.50	16.50	68.00
0.5	51.50	18.00	25.00	94.50
	Aqueous			
Control	0	0	0	0
0.1	3.00	6.50	8.00	17.50
0.2	11.50	1.50	8.00	21.00
0.3	21.50	8.00	5.00	34.50
0.4	26.50	6.50	6.50	39.50
0.5	40.00	10.00	13.00	63.00

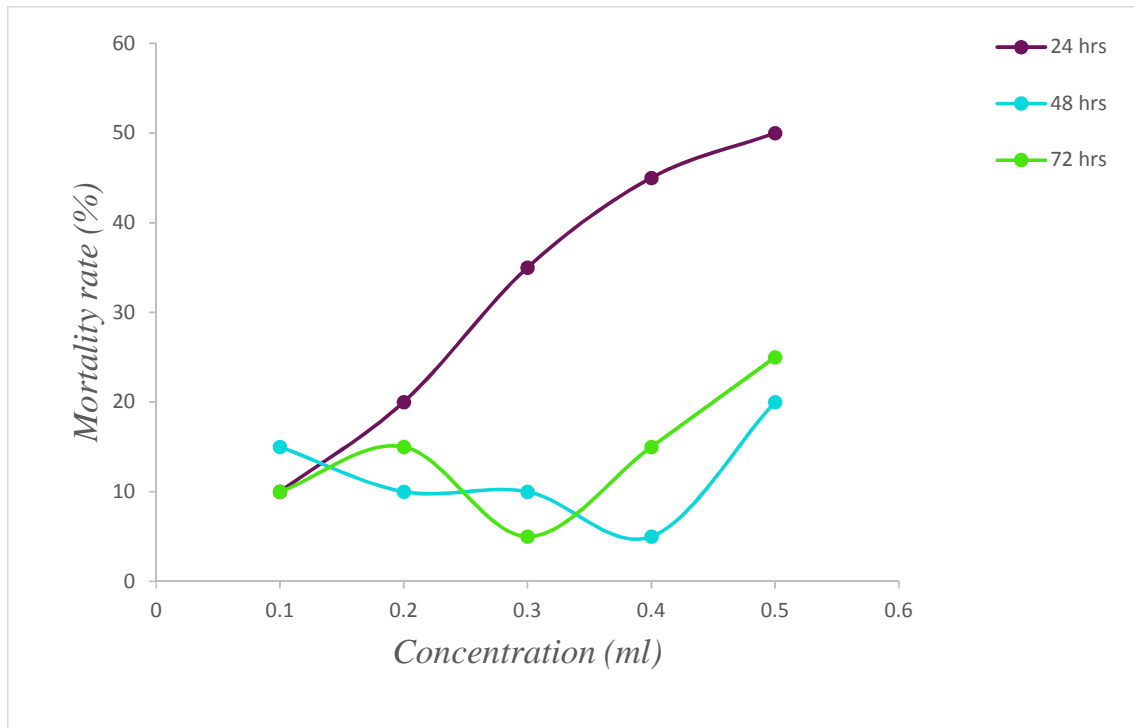


Fig 1: Larvicidal Effect of Ethanolic Leaf Extract of *Hyptis spp* on *Culex spp*

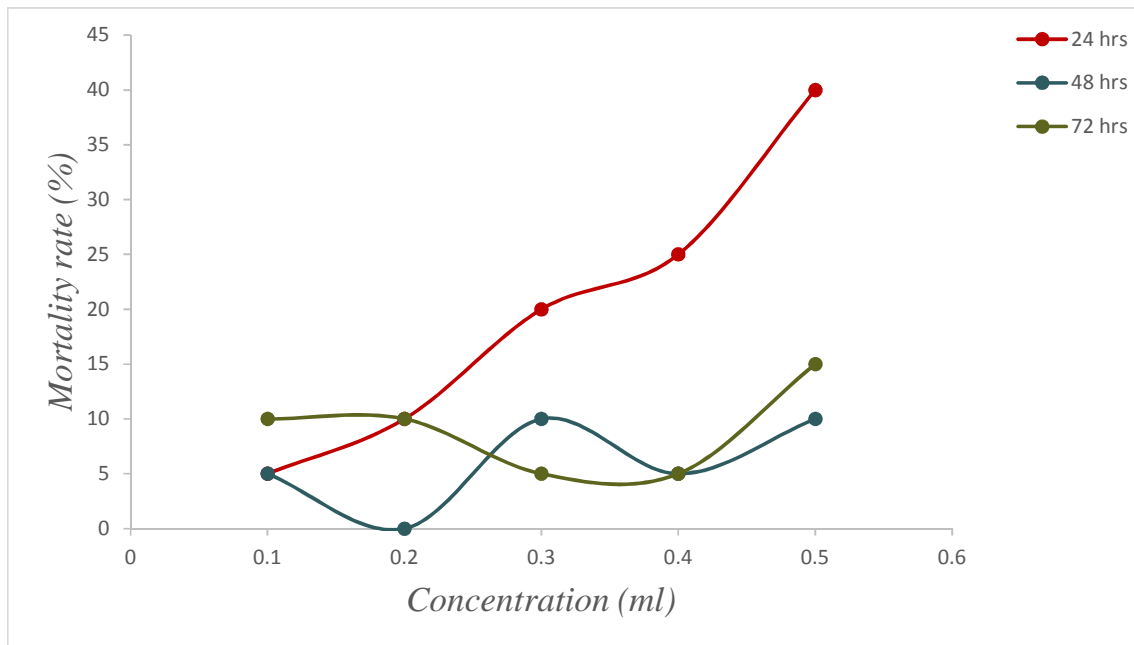


Fig 2: Larvicidal Effect of Aqueous Leaf Extract of *Hyptis spp* on *Culex spp*

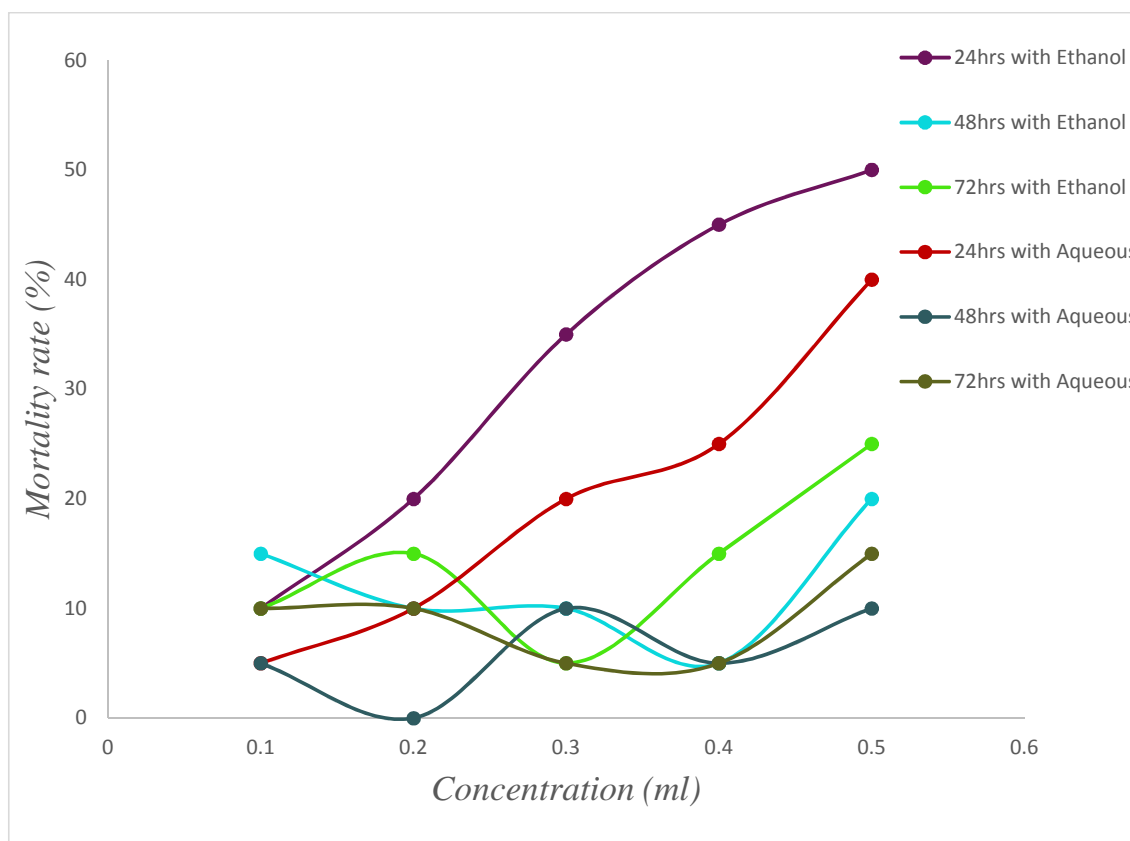


Fig 3: Comparative Larvicidal Activity of Ethanolic and Aqueous Leaf Extract of *Hyptis* spp on *Culex* spp at 24, 48 and 72 hrs

Relative Potency Ratio

The toxicities of two or more larvicides to the test organisms are compared on the basis of potency or the reciprocal of an equitoxic dose or concentration. The relative potency of the extracts estimated using Probit Analysis indicates that the ethanolic extract was 0.185 times more efficacious at 24hrs, 0.528 times more efficacious at 48hrs and 0.399 times more efficacious at 72hrs than aqueous extract (Table 4).

Table 4: Relative Median Potency Ratio

Extract	Relative Median Potency at 95% C.L			LOG Transform			
	Potency	Lower Bound	Upper Bound	Log Potency	Lower Bound	Upper Bound	
24hrs	HE	3.298	1.598	4.998	0.518	0.018	1.018
	HA	3.113	1.413	4.813	0.493	-0.007	0.993
48hrs	HE	1.785	0.085	3.485	0.251	-0.249	0.751
	HA	1.257	-0.443	2.975	0.099	-0.401	0.599
72hrs	HE	2.002	0.302	3.702	0.301	-0.199	0.801
	HA	1.603	-0.097	3.303	0.204	-0.296	0.704

Key: HE - *Hyptis* Ethanolic, HA - *Hyptis* Aqueous

DISCUSSION

Qualitative phytochemical screening revealed the presence of some bioactive compounds such as alkaloid, saponin, tannin, phenol, anthraquinone, flavonoid and steroid (Table 1). This agrees with the works of Ohimain *et al.* [23] which reported the presence of these phytochemicals in *Hyptis spp* that had high toxicity on the larvae of *Anopheles gambiae* as well as Sakthivadivel *et al.* [25] which also recorded the presence of the phytochemicals in *Hyptis suaveolens* extracts which had high toxicity on the larvae of the *Culex spp*. Anupam *et al.* [2] reported that phytochemicals such as alkaloid, saponin, tannin, phenol, anthraquinone, flavonoid, steroid and so on, have shown larvicidal activities on mosquitoes. This agrees with the report that phytochemicals such as alkaloid, flavonoid, saponin and tannin possess mosquito larvicidal activity [32].

It is curious that both the ethanolic and aqueous leaf extracts of *Hyptis suaveolens* Poit used in this study almost possess the same classes of phytochemicals, but had different toxicity levels on the test organism. This might be due to difference in the amount of each phytochemical present in each leaf extract, as well as antagonistic effect of the interaction of any two or more phytochemicals. Reports of antagonistic effect of alkaloids and saponins on bioactivity in the quinine tree have been documented [19]. The result showed a strong correlation between the concentration of the extracts and the mortality rate of the larvae. This is evident in carefully examining the effect of concentration on the mortality rate: higher concentrations lead to higher mortalities (Table 3). This agrees with the result obtained by Babatunde *et al.* [3] which reported that increase in the concentration of the extracts led to higher mortality. In the bioassay for both *Anopheles spp* and *Culex spp*, it can be observed that the highest mortalities were achieved within the first 24hrs of exposure of the test organisms to the extracts. This disagrees with the results obtained by Mahmoud *et al.* [18] and Sakthivadivel *et al.* [25] which reported highest mortality at 72hrs.

This study revealed that increase in the concentration of the leaf extracts led to a corresponding increase in the mortality of *Anopheles spp*. However, increase in the duration of the exposure (time) of the test organism to the leaf extracts did not necessarily lead to mortality increase. The highest mortalities of the test organism by various extracts occurred in the first 24hrs, with ethanolic *Hyptis spp* being the most efficacious. At the end of 72hrs, ethanolic *Hyptis spp* induced the highest mortality of 88% in the test organism, followed closely by its aqueous extract which had a mortality effect of 54.5%.

LC₅₀ and LC₉₀ values of the ethanolic leaf extracts of *Hyptis spp* are 0.497ml and 0.994ml respectively while its aqueous extracts have LC₅₀ and LC₉₀ values of 0.637ml and 1.275ml respectively within 24hrs (Table 2). This clearly indicates that the highest mortality of the test organism was recorded in the treatment using ethanolic leaf extracts.

The relative potency ratio in this study indicates that at 24hrs, ethanolic *Hyptis spp* leaf extract was 0.161 times more potent on the test organism than aqueous *Hyptis spp* leaf extract. This might be due to the tendency of the phytochemicals which are organic in nature to dissolve better in ethanol than water [28].

CONCLUSION

Over the years, the use of synthetic insecticides in vector control programmes has created multifarious problems including environmental pollution, toxicity to humans and other non-target organisms, insecticide resistance and so on. This, therefore, places great emphasis on the assessment and development of alternative products that are cheaply

available, environmentally safe, biodegradable and efficacious against these disease vectors. Studies have shown that humanity is blessed with potent phytochemicals which meet these requirements [27]. The activity of plant extract is often attributed to the complex mixture of active organic compounds. Unlike conventional insecticides which are based on a single active ingredient, plant-derived insecticides comprise botanical blends of chemical compounds which act concertedly on both behavioural and physiological processes of target organisms. Thus there is very little chance of pests developing resistance to such substances [27].

The result of this study has important implications in the practical control of mosquitoes using botanical extracts. This is because mosquitoes must have their larval development in fresh water bodies, and so it is much better and cost-effective to arrest their development at this stage, than allowing them to develop into adults and constitute a menace to human health and comfort. These plant extracts are easy to prepare, inexpensive, and environmentally safe for mosquito control. They possess enough potential that can be used directly as larvicidal agents in small volume aquatic habitats in/around human dwellings. It is hoped that the result of this study will provoke further investigations into the evaluation, identification, bioassay-guided fractionation, isolation and purification of bioactive ingredients especially from the crude extracts of the leaves of *Hyptis suaveolens* Poit, and its systemic effects on target mosquitoes, which may enable the standardization and practical application of the extract as larvicide in small-volume aquatic habitats or breeding sites of limited size in/around human settlements for the effective control of mosquitoes.

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