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Evaluation of the Larvicidal Potential of the Leaf Extracts of *Hyptis suaveolens* Poit against *Anopheles* Mosquitoes

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluated the larvicidal potential of the ethanolic and aqueous leaf extracts of *Hyptis suaveolens* Poit on the 4th larval instar of laboratory-reared *Anopheles spp* at varying concentrations of 0.1ml, 0.2ml, 0.3ml, 0.4ml and 0.5ml for specified periods of 24hrs, 48hrs and 72hrs. Qualitative phytochemical screening of the leaf extracts identified bioactive components like alkaloid, saponin, phenol, anthraquinone and flavonoid. The LC_{50} and LC_{90} values obtained indicate that the ethanolic leaf extracts of *Hyptis suaveolens* Poit had the greatest toxicity on the test organisms within 24hrs of exposure at median LC_{50} value of 0.485ml compared to the LC_{50} value of 0.625ml by its aqueous extract. The relative median potency estimates indicate that within 24 hrs, the ethanolic *Hyptis suaveolens* Poit was 0.161 times more potent on the test organism than aqueous *Hyptis suaveolens* Poit. The result of this research, therefore, underscores the efficacy of *Hyptis suaveolens* Poit as an eco-friendly alternative in *Anopheles* 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 toxic phytochemicals with larvicidal potentiality.

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1. INTRODUCTION

Malaria is one of the world's deadliest diseases especially in the tropical and subtropical regions where its vector female *Anopheles* mosquitoes breed in abundance. In 2016, nearly half of the world's population was at risk of malaria, including regions of Africa, South-East Asia, Eastern Mediterranean, Western Pacific, and the Americas, resulting in 91 countries and territories having ongoing malaria transmission [1]. Africa carries a disproportionately high share of the global malaria burden. In 2016, the region was home to 90% of malaria cases and 91% of malaria deaths [2]. An estimated 216 million cases of malaria occurred in Africa and 445,000 people died, mostly children [3].

In recent years, mosquito resistance to synthetic insecticides has emerged in many countries [4]. This undermines malaria control programmes and poses a great risk to vulnerable populations [1]. 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 [5,6,7]. Leaves of *Hyptis suaveolens* Poit commonly referred in the Hausa language as 'Saurakwon sauro' is usually used by rural dwellers in Gwandu Emirate (Aliero, Bagudo, Birnin-Kebbi, Bunza, Gwandu, Jega, Kalgo, Koko/Besse, Maiyama and Suru LGAs) of Kebbi State to repel mosquitoes. This indicates it probably could possess bioactive components that are deleterious to mosquitoes. This paper, therefore, aims to assess and compare the potential of the ethanolic and aqueous leaf extracts of *Hyptis suaveolens* Poit on the *Anopheles spp* larvae.

2. MATERIALS AND METHODS

2.1 Study Area

The study area is the Gwandu Emirate (Aliero, Bagudo, Birnin-Kebbi, Bunza, Gwandu, Jega, Kalgo, Koko/Besse, Maiyama and Suru LGAs) of Kebbi State. Kebbi State, located in northwest of Nigeria, has a co-ordinate of 12º27'N and 4º12'E. It has an average annual temperature of 28ºC, and about 807mm of precipitation falling annually [8].

2.2 Collection and Preparation of Plant Materials

The plant leaves of *Hyptis suaveolens* Poit were collected from the bushes around Birnin-Kebbi area of Kebbi State, using sterilised 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.

2.3 Extraction of Plant Materials

The dried leaves were subjected to maceration extraction method as described by Odebiyi and Sofowora [9]. They were pulverised using an electric blender. Fifty gram (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 using Whatman filter paper, and the filtrates subjected to evaporation in a water bath set at 80°C 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 [10]. They were stored in the refrigerator until the dilute concentration was to be prepared for larvicidal assay.

2.4 Qualitative Phytochemical Screening

Crude ethanolic and aqueous extracts of the leaves of *Hyptis spp* were screened for phytochemicals using the methods described by [11,12].

2.5 Rearing of *Anopheles spp*

The larvae of *Anopheles spp* were collected from freshwater bodies like gutters in Gesse Phase 1 area of Birnin-Kebbi. The mosquito larvae were identified as belonging to *Anopheles spp* by having their body lie parallel to the water surface [13]. The test organisms were reared according to guidelines provided by WHO [9]. 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 homogenised 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 [10]. 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 [10].

2.6 Larvicidal Bioassay

Larvicidal bioassay was conducted according to the standard test methods adopted by WHO [10]. The dilute concentration (which was in ten fold) 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 (distinguished from other preceding instars by the possession of a wide and heavily sclerotised collar on the posterior border of the head) of *Anopheles spp* [13]. 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.

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 or moribund larvae were determined and recorded following a 10 seconds observation when they failed to move after probing with a sterile needle. The percentage mortality, as described by WHO [10], was calculated as follows:

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

The Abbott's formula [14] was used to correct for percentage mortality where a minimal proportion of the larvae (between 5% and 20%) in the control batches died during the experiment [10].

$$P = \frac{Pt - Pc}{100 - Pc} \times 100 \quad \text{or} \quad P = \frac{X - Y}{X} \times 100$$

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

2.7 Statistical Analysis

Probit Analysis as described by Finney [15] was employed using SPSS version 20. The 24, 48 and 72hrs lethal concentration values (LC_{50} and LC_{90}) 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.

3. RESULTS

3.1 Qualitative Phytochemical Screening of the Leaf 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).

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

The larvae of *Anopheles 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 0.485 and 0.625 respectively (Table 2).

3.3 Larvicidal Potential of the Leaf Extracts

The larvicidal potential of the leaf of *Hyptis spp* was evaluated against the 4th instar stage of *Anopheles spp*. All the extracts tested exhibited larvicidal potential against the test organism The result indicates that increase in concentration or dose leads to increase in mortality. At 0.5ml, being the highest dose, ethanolic extract had a total mortality of 88% while aqueous extract had a total mortality of 54.5% (Table 3).

The lethality pattern of the ethanolic leaf extract of *Hyptis suaveolens* Poit on *Anopheles spp* indicates that the test organism showed the highest mortality of 55% within the first 24hrs of exposure to the extract at the highest concentration of 0.5ml of the ethanolic extract. Mortalities in the 48hrs and 72 hrs hovered around 10-20% (Figs. 1, 2 and 3).

3.4 Relative Potency Ratio

The toxicities of two or more larvicides against an organism are compared on the basis of potency or the reciprocal of an equitoxic dose or concentration. The relative potency of the extracts was compared using Probit Analysis. The result indicates that the ethanolic extract was 0.161 times more efficacious at 24hrs, 0.49 times more efficacious at 48hrs and 0.4 times more efficacious at 72hrs than aqueous extract (Table 4).

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

Phytochemical	*Hyptis suaveolens* Poit	
	Ethanolic	Aqueous
Alkaloid	+	+
Saponin	+	+
Phenol	+	+
Tannin	+	+
Anthraquinone	+	+
Glycoside	–	–
Flavonoid	+	+
Steroid	–	+
Terpenoid	–	–

Key: + present, – absent

4. DISCUSSION

In this study, qualitative phytochemical screening revealed the presence of the following bioactive compounds such as alkaloid, saponin, flavonoid, tannin, phenol, anthraquinone and steroid (Table 1). This agrees with the works of Ohimain et al. [5] 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. [16] which also recorded the presence of the phytochemicals in *Hyptis suaveolens* Poit extracts which had high

Table 2. LC_{50} and LC_{90} values of *Hyptis spp* extracts on *Anopheles spp* at varying periods at 95% confidence level (C. L.)

Time (hrs)	Extract	Lethality	Conc (ml)	Lower bound	Upper bound	Log conc	Lower bound	Upper bound
24	ET	LC_{50}	0.485	-0.215	1.185	-0.314	-0.414	-0.214
		LC_{90}	0.970	0.270	1.670	-0.013	-0.113	0.087
	AQ	LC_{50}	0.625	-0.075	1.325	-0.204	-0.304	-0.104
		LC_{90}	1.250	0.550	1.950	0.096	-0.004	0.196
48	ET	LC_{50}	1.388	0.688	2.088	0.142	0.042	0.242
		LC_{90}	2.777	2.077	3.477	0.443	0.343	0.543
	AQ	LC_{50}	0.765	0.065	1.465	-0.116	-0.216	-0.016
		LC_{90}	1.534	0.834	2.234	0.185	0.085	0.285
72	ET	LC_{50}	1.000	0.300	1.700	0.000	-0.100	0.100
		LC_{90}	2.000	1.300	2.700	0.301	0.201	0.401
	AQ	LC_{50}	1.923	1.223	2.623	0.283	0.183	0.383
		LC_{90}	3.846	3.146	4.546	0.585	0.485	0.685

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

Table 3. Larvicidal effect of the leaf extracts of *Hyptis suaveolens* poit on *Anopheles spp*

Concentration (ml)	Mortality (%)			Total
	Ethanolic			
	24hrs	48hrs	72hrs	
Control	0	2.5	0	2.5
0.1	10.00	11.65	11.65	33.30
0.2	28.30	11.65	13.30	53.25
0.3	51.50	13.30	13.30	78.10
0.4	50.00	16.50	10.00	76.50
0.5	55.00	21.50	11.50	88.00
		Aqueous		
Control	5	0	0	5
0.1	6.50	6.50	6.50	19.50
0.2	15.00	11.50	6.50	33.00
0.3	30.00	10.00	5.00	45.00
0.4	35.00	10.00	10.00	55.00
0.5	46.50	5.00	3.00	54.50

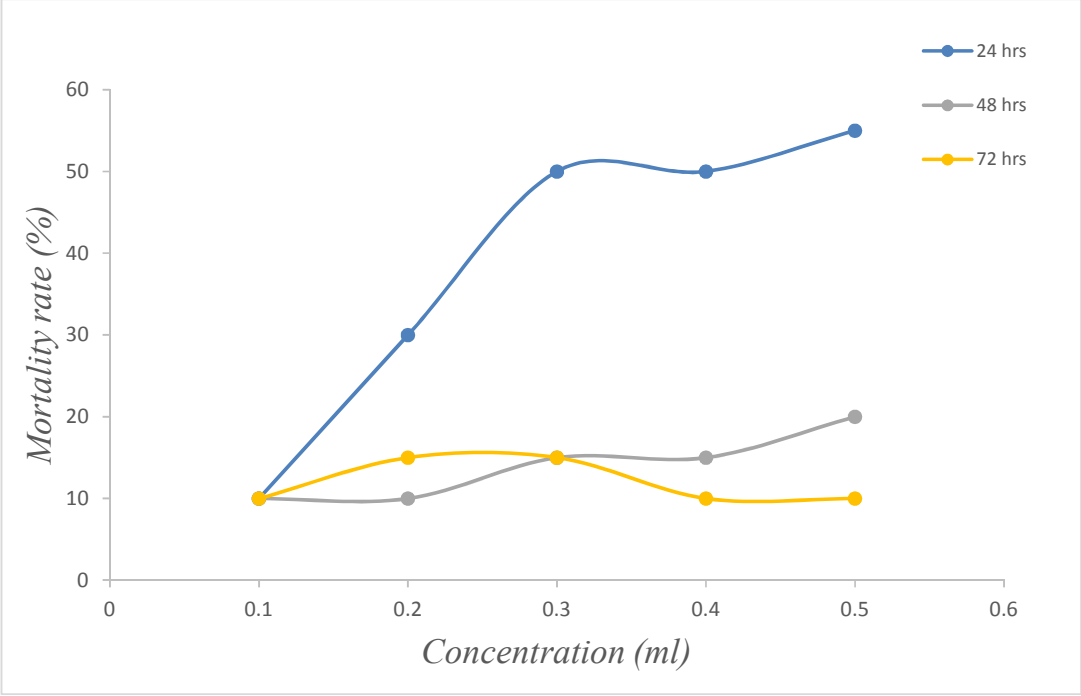

Fig. 1. Larvicidal effect of ethanolic leaf extract of *Hyptis suaveolens* poit on *Anopheles spp*

toxicity on the larvae of the *Culex spp*. Anupam et al. [17] reported that phytochemicals such as alkaloid, saponin, tannin, anthraquinone and steroid and so on, have shown larvicidal activities on mosquitoes. The phytochemicals of the leaves of the Lamiaceae family (of which *Hyptis suaveolens* Poit is a member) are reported to possess larvicidal properties on mosquitoes [5]. This agrees with the report that phytochemicals such as alkaloid, flavonoid, saponin and tannin possess mosquito larvicidal activity [18,19,20].

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 [21]. The result of this study 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. This agrees with the result obtained by Babatunde *et al*. [22] which reported that increase in the concentration of the extracts led to higher mortality. In the bioassay for both *Anopheles spp* and *Culex spp* of this study, 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*. [23] and Sakthivadivel *et al*. [16] which reported highest mortality at 72hrs.

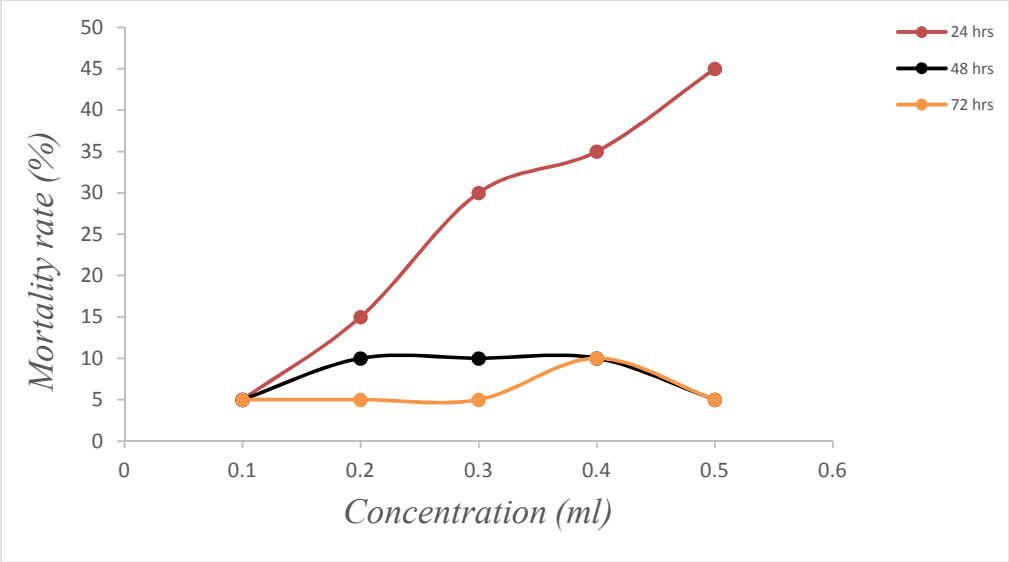

Fig. 2. Larvicidal effect of aqueous leaf extract of *Hyptis spp* on *Anopheles spp*

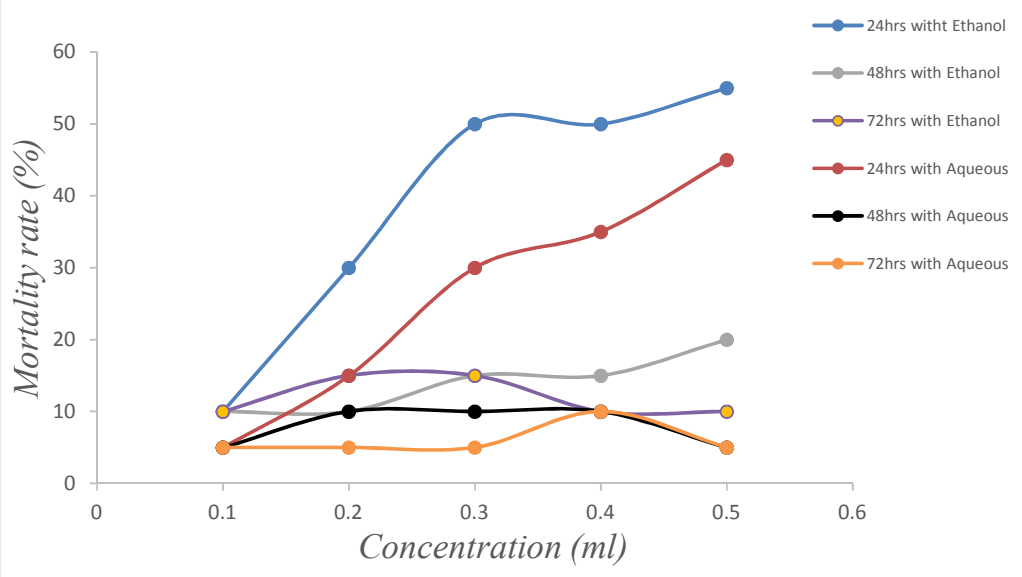

Fig. 3. Comparative larvicidal activity of ethanolic and aqueous leaf extract of *Hyptis spp* on *Anopheles spp* at 24, 48 and 72hrs

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	3.359	1.659	5.059	0.526	0.026	1.026
	HA	3.198	1.035	4.605	0.504	0.004	1.004
48hrs	HE	1.835	1.135	3.535	0.263	-0.237	0.763
	HA	1.345	-0.355	3.045	0.128	-0.372	0.628
72hrs	HE	1.756	0.056	3.456	0.756	0.256	1.256
	HA	1.356	-0.344	3.056	0.132	-0.365	0.635

Key: HE – Hyptis Ethanolic, HA – Hyptis Aqueous

LC_{50} and LC_{90} values of the ethanolic leaf extracts of *Hyptis suaveolens* Poit are 0.485ml and 0.970ml respectively while its aqueous extracts have LC_{50} and LC_{90} values of 0.625ml and 1.250ml 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.

Even though 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 (ethanolic and aqueous) 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%.

The relative potency ratio in this study indicates that at 24hrs, ethanolic *Hyptis suaveolens* Poit leaf extract was 0.161 times more potent on the test organism than aqueous *Hyptis suaveolens* Poit leaf extract. This might be due to the tendency of the phytochemicals which are organic in nature to dissolve better in ethanol than water [10].

5. 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 [7]. 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 [7].

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 standardisation 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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