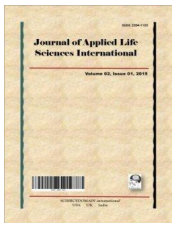

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Larvicidal Activities of *Hyptis suaveolens and Ocimum sanctum* against *Anopheles gambiae*

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Authors' contributions

This work was carried out in collaboration between all authors. Authors EIO and TCNA designed the study and wrote the protocol. Authors EIO and TCNA wrote the first draft of the manuscript, performed the statistical analysis. Authors EIO and JFB revised the manuscript. All authors managed the literature searches of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine the larvicidal activities of *Hyptis suaveolens and Ocimum sanctum* against *Anopheles gambiae*.
Study Design: A 24 h LC_{50} concentration-mortality static non-renewal bioassay was carried out.
Place and Duration of Study: The study was carried out at Rohi Biotechnologies Ltd's Toxicity Laboratory, Port-Harcourt, Rivers State Nigeria, between January and March 2015.
Methodology: The solvent extracts were assessed against the mosquito larva at varying concentrations in a 2-phased rapid and final screening test.
Results: Results show that the methanol extract of *H. suaveolens* induced the highest mortality with LC_{50} values of 73.25 ppm, while the chloroform and hexane extracts induced mortalities with LC_{50} values of 76.25 and 97.25 ppm respectively. On the other hand, the *O. sanctum* extracts induced mortalities of 125.00, 150.00 and 194.08 ppm for methanol, chloroform and hexane extracts respectively.
Conclusion: Based on our findings, we recommend solvent extracts of *H. suaveolens and O. sanctum* as prospective larvicides in the integrated management of malaria.

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Keywords: Malaria; solvent extracts; biopesticides; disease vectors.

1. INTRODUCTION

In the past decades, the morbidity burden and incident rate of malaria have become of global concern. Statistics in literature reveal that malaria is the most prevalent insect-borne disease, globally affecting about 3.5 billion persons per annum [1]. Report of the World Health Organization also stated that malaria is the most devastating parasitic disease [2,3]. Malaria is endemic in many developing countries (especially in Africa). For instance, there are over 100 countries, with an annual global incident rate of 700 million [4]. There are several species of mosquitoes of which only about 30-40 species transmit malaria [1,4]. However, in Africa the predominant transmitter is *Anopheles gambiae* [1,3].

Hyptis suaveolens is a medium aromatic weedy shrub found in the tropics and subtropical region [5]. In Nigeria, it is abundantly distributed as aggressive annual weedy species (especially in the northern and western Nigeria). The genus *Hyptis* is well known for its traditional application as an anticancer [5,6], antibacterial [7], antifungal [8,9], and anticonvulsant agent [10].

Ocimum sanctum is an annual spice plant believed to have originated from Iran, Afghanistan and India [11,12]. This plant has found several traditional therapeutic applications in the treatment of ailment like; headaches, cough, diarrhea, constipation, warts, kidney malfunctions, nasal polyps and ulcers [5,11,12]. Furthermore, its insectidal, nematicidal, fungicidal and antimicrobial activities of *O. sanctum* have been reported in literature by several authors [5,13-15].

Plants generally have curative application due to the metabolites and secondary compounds they possess. *Ocimum* species has been widely reported to possess repellent properties against mosquito [16], elephantiasis [17] and some parasitic pathogen [18]. Multifaceted combat against malaria has witnessed global remarkable success. Some challenges associated with malaria control include; rapid mosquito prolificacy and disease re-emergence as chemotherapy only proves to be abatement [19,20]. Furthermore, the toxicity of synthetic pesticides to non-targeted species, high cost and abatement of morbidity burden observed in chemotherapeutic techniques have become sources of concern to indigent people in endemic areas [19]. A result, integrated exploration of eco-friendly pesticides, plant-derived pesticides have emerged as basic panacea in the control of most insect-borne public health diseases. The objective of the research is to investigate the larvicidal activities of *H. suaveolens* and *O. sanctum* against *Anopheles gambiae* mosquito vectors.

2. MATERIALS AND METHODS

2.1 Mosquito Larvae Culture and Identification

The larvae were cultured *in-vivo* in the wild, close to a bush in a fenced compound with plantain vegetation, located at Uyo street in Port Harcourt, Rivers State, Nigeria (Latitude 4° 49$^{!}$ 45.2$^{!!}$ N and Longitude 007° 01$^{!}$ 27.9$^{!!}$ E). The larva culture was carried out using standard methods as described by Ohimain et al. [1] and Angaye et al. [20]. The emerging larvae were identified using the identification protocol of Ahmed and Ahmed [21].

2.2 Plant Collection and Extraction

The leaf of *H. Suaveolens* and *O. Sanctum* were collected from Edo State, Nigeria in August 2014. The taxonomic identification of both plants was carried out using identification keys as described by Ogunkunle [22]. The leaves were shade-dried for 7 days at ambient environmental temperatures (31±2°C). About 300 g of the leaves were powdered and macerated in 500 ml of the respective solvents (hexane, Fisher Scientific international Company; chloroform and methanol, BHD Chemical Ltd. Poole England) for 72 hours. The filtrates of the concoctions were respectively extracted in a rotary evaporator (60°C). The obtained residues (i.e. extracted active ingredients) were preserved at 4°C.

2.3 Experimental Setup

The experimental setup of this bioassay to verify the larvicidal activity of the solvent extracts against the larvae was carried out following standard protocol [23,24], with slight modifications incorporating rapid and final screening as described by several authors [1,2,20]. The positive control was adjusted with 1 ppm pyrethrum pesticide, while the negative

control was set up with water from the breeding site.

2.4 Rapid and Final Screening

The rapid screening was set up (in replicates), in order to determine the range of activity as per the solvent extracts. A wide range of concentrations (ranging from 500-1000 ppm) were first used to deselect extract(s) whose minimal total mortality rate (*MTMrt*) exceeds 500 ppm after 24 hours. This implies that, only extract(s) with *MTMrt* at 500 ppm within 24 hours were selected for the final screening.

2.5 Statistical Analysis

The mean mortality and standard deviation of data from the bioassay were calculated, furthermore, in order to estimate the median lethal concentration; the primary mean mortality data were statistically subjected to concentration-mortality assessment using the 2013 version of Microsoft Excel package with 5% error.

3. RESULTS AND DISCUSSION

The result of the rapid screening (not presented) indicated that all extracts of *H. suaveolens* and *O. sanctum* scaled the final screening phase (i.e. *MTMrt*≤500 ppm). Results of the final screening (Table 1), shows that the solvent extracts of *H. suaveolens* had minimal total mortality rates (*MTMrt*), at the following concentrations; 200-250 ppm for chloroform extract, 150-200 ppm for methanol extract and 250-300 ppm for hexane extract. On the other hand, the solvent extracts of *O. sanctum* were toxic to the larvae with *MTMrt* of 250-300 ppm for both chloroform and methanolic extracts, while the hexane extract had *MTMrt* at a range of 300-350 ppm. Furthermore, it was worthy of note that while the positive control was lethal at 1 ppm, the negative control induced no mortality throughout the bioassay.

Result of the median lethal dose (LC_{50}) as presented in Fig. 1, shows that the methanolic extracts of *H. suaveolens* induced the highest activity with LC_{50} value of 73.25 ppm, followed by the chloroform and hexane extracts of the same plant with LC_{50} values of 76.25 and 97.25 ppm respectively. On the other hand, *O. sanctum* extract induced moderate activities with higher LC_{50} values of 125.00, 150.00 and 194.50 ppm for methanol, chloroform, and hexane extracts respectively. The positive control induced total mortality at 1 ppm in less than 24 hours, while the negative control induced no mortality throughout the experiment.

The biocidal activities of several solvent extracts of *H. suaveolens* and *O. sanctum* had been reported in literature [25-29]. All solvent extracts of *H. suaveolens* and *O. sanctum* in our study induced varying degrees of toxicity. The toxicological disparities between the plants and amongst the solvent extracts are largely attributed to the genetic makeup of the plants and/or the chemistry of the applied solvents. Comparatively, the LC_{50} values of this study (using *An. gambiae*) was higher compared to the previous studies of Okigbo et al. [29] using the *Culex* mosquito and obtained LC_{50} values of 14.3, 11.40 and 66.83 ppm with extracts of *A. indica*, *O. gratissimum and H. suaveoleus* respectively. Ivoke et al. [28] reported the larvicidal activities of both aqueous and ethanol extracts of *H. Suaveolens* with LC_{50} values of 80.02 and 62.4 ppm respectively. Using *H. suaveolens*, Amusan et al. [25] even recorded a more active LC_{50} value of 1.45 ppm against larvae of *Aedes aegypti* (yellow fever vector).

In another study, a lower LC_{50} value (88.78 ppm) was obtained using *O. sanctum* [30]. Also, the ethyl acetate leaf extract of *Ocimum tenuiflorum* induced LC_{50} value of 44 ppm against anopheles mosquito [26]. The termiticidal activities of crude and some solvent extracts of *O. sanctum* was also reported showing varying toxic activities between 1-11 days, however the minimum and maximum mortalities were recorded as 43.89±39.97% for the stem water extract and 84.45±27.21% for the ethyl acetate leaves extract [31]. Plants belonging to the genus, *Ocimum,* have demonstrated varying degrees of insecticidal properties against mosquitoes [13,16,27], and elephantiasis vector [17].

The phytochemical properties of the leaves, stems, and root of *Hyptis suaveolens* have been established in literatures from the foregoing by several authors [32-34].Furthermore, their results indicated the absence of saponin in the root [34], compared to the stem (10.50±0.79%), and the leaves (6.10±0.42%) [32,33]. Alkaloids in the leaves was 2.80±0.28%, flavonoids was 1.90±0.14%, and tannins 5.50±0.074%; alkaloids was 1.60±0.00%, flavonoids in the leaves and tannins was 0.30±0.14 and 0.23±0.07% in the stem [32]. Also the biocidal activities of essential oil from both *H. suaveolens* and *O. sanctum* have been reported for repellence against malaria and filariasis vectors [7-9,11,17].

Table 1. Results of final screening for solvent extracts of *H. suaveolens* and *O. Sanctum*

		Mortality rates								
		0-50 (ppm)	**50-100 (ppm)**	**100-150 (ppm)**	**150-200 (ppm)**	**200-250 (ppm)**	**250-300 (ppm)**	**300-350 (ppm)**	**350-400 (ppm)**	**400-450 (ppm)**
H. suaveolens	Chloroform	56.2±0.943%	67.4±0.403%	77.3±3.030%	88.1±1.09%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%
	Methanol	69.2±2.200%	81.4±2.142%	89.2±1.410%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%
	Hexane	47.4±1.11%	56.3±1.003%	63.1±1.043%	75.0±1.13%	83.2±1.303%	100±0.00%	100±0.00%	100±0.00%	100±0.00%
O .sanctum	Chloroform	35.2±0.101%	47.0±1.013%	55.4±1.330%	63.3±2.03%	78.0±1.003%	100±0.00%	100±0.00%	100±0.00%	100±0.00%
	Methanol	43.3±0.443%	53.3±2.413%	61.2±0.424%	70.0±2.02%	81.1±1.410%	100±0.00%	100±0.00%	100±0.00%	100±0.00%
	Hexane	31.1±0.41%	42.3±1.021%	48.4±3.043%	57.0±0.931%	70.2±1.132%	89.4±1.04%	100±0.00%	100±0.00%	100±0.00%
Positive control		100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%	100±0.00%
Negative control		0±0.00%	0±0.00%	0±0.00%	0±0.00%	0±0.00%	0±0.00%	0±0.00%	0±0.00%	0±0.00%

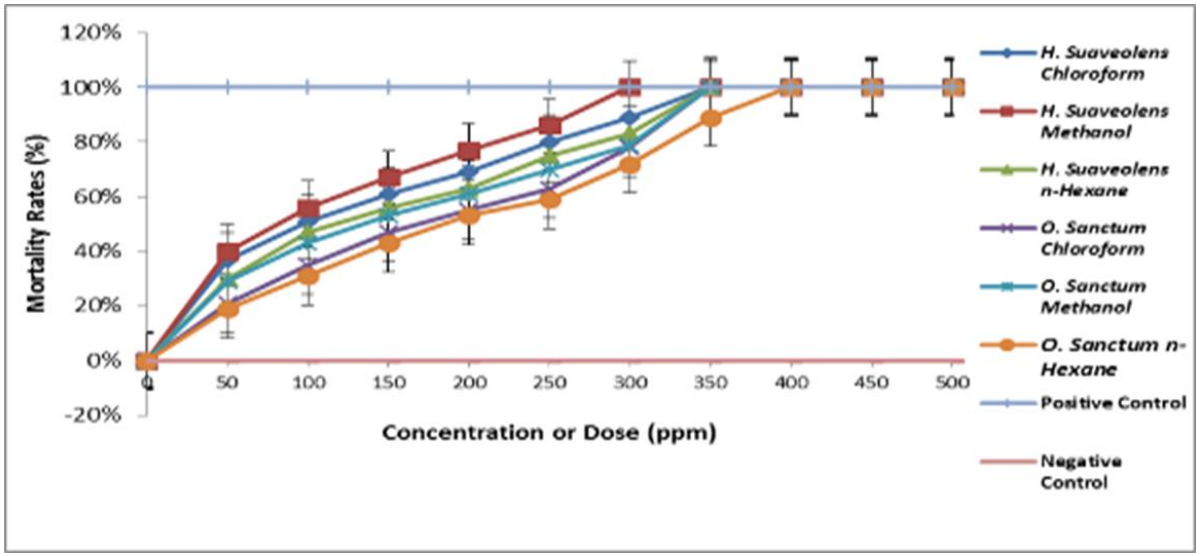

Fig. 1. Dose-mortality graph based on final screening for solvent extracts of *H. suaveolens* and *O. sanctum*

The results of several studies have indicated better insecticidal activities of *H. suaveolens* over *O. sanctum*. Furthermore, our most recent research [35], had reported the occurrence of several metabolites in both *H. suaveolens* and *O. sanctum*. In *H. suaveolens* we reported higher level of tannin compared to phenol and steroid. On the other hand, *O. sanctum* had lower level of flavonoid, but no trace of steroid. In addition, *H. suaveolens* have been found to contain high level of monoterpenes, whose toxicity mechanism (inhibition of metamorphosis), have been corroborated in literature by several authors [25,36-39]. Monoterpene has the ability to stimulate the cellular leakage of potassium [40], which results to membrane disruption and mortality [39]. Although, the metabolites of the plant were largely responsible for the mortality. However, the applied solvents (chloroform, methanol and hexane), being medium for extraction, are similar as alcoholic solvents, but dissimilar polarity wise. The dissimilarity in polarity of the solvents can affect the enrichment of envisaged metabolites of the plant [19] and possibly induce varying degree of toxicity as indicated in the results.

4. CONCLUSION

Malaria is a hyper-endemic vector-borne disease both in tropical and subtropical region. In malaria control, adoption of several techniques will best abate the morbidity burden. Targeting the stationary larvae phase with eco-friendly pesticides (especially plant-derived pesticides) is desirable as this will supress their fecundity and is environmentally friendly. Solvent extracts of both plants induced moderate activities against the larvae. This study justifies the application of *H. suaveolens* and *O. sanctum* in integrated pest management. We recommend the field application of these plants for the control of the vector.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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