



Bio-Potency of *Hyptis suaveolens* Leaf on the Larvicidal Stages of Development of *Anopheles* Mosquitoes

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ABSTRACT

Malaria is one of the most severe public health concerns worldwide. It is highly endemic particularly in Africa, where Nigeria has the greatest burden. *Hyptis suaveolens* is one of the most important traditional medicinal plants used to repel insects. The study was conducted with the aim of determining the bio-potent larvicidal ability of *Hyptis suaveolens* fresh leaves on the various developmental stages of Larva of *Anopheles* mosquitoes, using ethanol extract and crushed leaf paste. The result of the ethanol crude extract against the instar larvae at 0.5 mg and 1.0mg concentration showed significant difference in mortality rates between the two samples ($p < 0.005$). At 0.1 mg and 0.2 mg the larvae were resistant at 1hr and at 3hrs respectively but became susceptible at 6hrs with a mortality rate of 75 %. The fresh leaves showed significant increase in susceptibility of the larvae at 5.0mg concentration with a mortality rate of 0.50 % at 12hr. At 0.5 mg, 1.0 mg, 2.0 mg, the mortality rate of the fresh leaf aqueous solution was 0.50 % at 24 hr. From the results obtained, *Hyptis suaveolens* ethanolic extract and fresh leaves could be applied as potential measures to control malaria vector right from the larvae stage of development. Further research should be carried out on the application and utilization of fresh leaves using improved technique.

Keywords: Bio-potency, Larvicidal activities, *Hyptis suaveolens*, *Anopheles* Mosquitoes, fresh leaves

INTRODUCTION

Malaria is one of the most prevalent vector-borne diseases that man has contend with for ages. It is a leading cause of death and disease in many developing countries, where young children and pregnant women are the groups most affected (Terseer and Ali, 2019). According to the World Health Organization's World Malaria Report, nearly half the world's population lives in areas at risk of malaria transmission in 87 countries and territories. They maintained that in 2019, malaria caused an estimated 229 million clinical episodes, and 409,000 deaths. 94% of which were estimated to be from the WHO African Region (W.H.O. 2020).

There are hundreds of different species of mosquitoes, so many that scientist doesn't agree they should be classified (Mosquito Review, 2019). However, the predominant parasite species is *Plasmodium falciparum*, which is the species that is most likely to cause severe malaria and death (WHO, 2017; Biology of Mosquitoes, 2018). Malaria imposes substantial

costs on both individuals and governments, thereby increasing the Social and Economic Toll. Direct costs implication, for example on illness, treatment and premature death has been estimated to be at US\$ 12 billion per year (WHO, 2020). These costs of malaria to individuals, families, communities, and nations is indeed enormous.

Aside scarce resources and socio-economic instability which have hindered efficient malaria control strategies, the control of mosquitoes is however becoming increasingly demanding due to the sharp decline in the effectiveness of vector control measures put in place as a result of resistance developed by vectors against the synthetic organic insecticides and environmental risk, or effect on non-target organisms and human health due to repeated application (Ayange-kaa *et al.*, 2015;

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Desiyamani *et al.*, 2018). These inherent challenges have provoked the need for alternate approach in search of plant products with new active ingredients for the control of mosquito parasite.

In the past, a wide variety of plant species from various plants with potentials against mosquitoes were used locally to control mosquitoes in communities (Shaalam *et al.*, 2005) amongst this is *Hyptis suaveolens* of the family Lamiaceae (Hemen *et al.*, 2013; Singh *et al.*, 2021).

The leaves of the plant are the source of pharmacologically important secondary metabolites having antispasmodic, anti-colic, anti-rheumatic, and anti-fertility properties (Pratibha *et al.*, 2021). Furthermore, the pharmacological significance of *Hyptis suaveolens* can be attributed to its sedative, diuretic, anti-spasmodic, aromatic, anti-inflammatory, anti-catarhal, anti-cutaneous, anti-pyretic, anti-rheumatic, anti-soporific, and anti-cancer properties (Ghaffari *et al.*, 2014; Saha *et al.*, 2017; Choy *et al.*, 2019). Equally, the presence of essential oils contained in the leaves have potential antimicrobial and antifungal properties (Dubey *et al.*, 2014; Oumarou *et al.*, 2018). The root extract contains anti-retroviral compound called urosolic acid, a triterpenoid that may target retroviral integrases and proteases by blocking the replication of retroviruses such as HIV (Tohme *et al.*, 2019). Most of these bioactive compounds found in *Hyptis suaveolens* are used as therapeutic agents and sometime as precursors of useful drugs (Hemen *et al.*, 2013). The mature leaves of *Hyptis suaveolens* contain alkaloids as the major secondary metabolite followed by tannins and saponins respectively (Ulhe and Narkhede, 2013; Oscar *et al.*, 2020).

METHODOLOGY

Study Area

This study was conducted in Keffi – Nigeria in the Middle Belt Geo-political zone of Nigeria with an area of 138 km² and a population of 92,664 at the (Akwa *et al.*, 2007). Keffi is on longitude 7°49'38.10"E and latitude 8°50'16.62"N.

Sample Collection and Screening

Fresh matured leaves of *Hyptis suaveolens* were collected from their natural habitat along combatant Road, Phase II in Keffi, Nasarawa State. The plants were taken to the Department of Plant Science and Biotechnology, Nasarawa State University, Keffi for authentication. The sample preparation was done following the (WHO standard, 2005) with minor modification. The harvested fresh leaves of *Hyptis suaveolens* were set up as follows:

Ethanol extract and processing

The plant leaves were dried under shade for 7 days at a room temperature; the dried leaves were mechanically powdered, weighed and stored in an airtight container. The powdered material was used for further phytochemical analysis. From the collection of plant powder, 200g of *Hyptis suaveolens* was extracted for 72hr by cold maceration at room Temperature in 1 Litre (1000 ml) of ethanol (Sigma Aldrich), in the Biochemistry and Molecular Biology Laboratory, Nasarawa State University, Keffi. The extract was filtered using a Whatman No.1 filter paper. The residue of maceration was rinsed and filtered thrice with ethanol until a clear phase was obtained. The solvent was evaporated off to give absolute dryness using a water Bath controlled at 75 °c to obtain a crude extract. The crude ethanol extract was put in an air tight container and stored in a refrigerator at 4 °c until when needed for bioassay.

Fresh leaves processing

Fresh matured leaves of *Hyptis suaveolens* were plucked and mechanically crushed into paste, weighed and sieved using an Adventurer scale. The fresh leaves extracts were applied directly for use immediately for bioassay respectively.

Test Organism

First, second, Third and fourth instar Anopheles mosquito larvae were collected from different ovipositing site within Nasarawa State University community, Keffi. The larvae were identified by the Insectary units, Department of Zoology, Nasarawa State University, Keffi. They were kept in larva bowls with a netty lid opening. The larvae were left to acclimatize for 24 hours using the same water fetched from their breeding site. Cabin biscuit was used for larvae feed. A total of 1,620 larvae were collected.

Phytochemical Analysis

Phytochemical Screening: The extract of the dried leaves was tested for the presence of alkaloids, tannins, saponins, anthraquinones, steroids and phenols, using the methods of Harborne (1973), Durodola (1977), Odebiyi and Sofowora (1978) and Trease and Evans (1983).

Larvicidal Test

All the Bioassay were carried out according to the standard protocol of WHO (2005) with minor modification.

Bioassay (Ethanol Crude Extract)

Fifteen *Anopheles* larvae (1st, 2nd, 3rd, and 4th) each with their control were selected by means of rubber pipette and placed in vessels containing 250ml of water (fresh water from their habitat) and then exposed to different concentrations of 0.1mg, 0.2mg, 0.5mg, 1.0mg ethanolic crude extract diluted with 2.0ml of acetone.

Bioassay (Aqueous solution)

Fifteen *Anopheles* larvae (1st, 2nd, 3rd, and 4th) each having their control were selected by means of rubber pipette and placed in vessels containing 250ml water (fresh water from their habitat) and then exposed to different concentration of 0.1mg, 0.2mg, 0.5mg, 1.0mg of the fresh leave. Larvae were considered either alive, if they were clearly moving normally, or dead when there is no movement when probed with a fine glass rod. All Bioassays were repeated three times.

RESULTS

The extraction yield of *Hyptis suaveolens* is presented in Table 1: From 200 g of the plant powdered used, the ethanolic extract yielded (20.25 %).

Table 1: Extraction yield (%) of ethanol leaf extracts of *Hyptis suaveolens*

Extraction	Powdered used (g)	Yield (%)
Cold maceration (ethanolic extract)	200g	20.25

The qualitative phytochemical analysis of *Hyptis suaveolens* using the ethanolic extraction investigated showed the presence of Tannins, Saponins, Flavonoids, Steroids, Phenolics, Cardiac Glycosides, Terpenoids and Alkaloids respectively as presented in Table 2.

The quantitative estimation of the phytochemical analysis of *Hyptis suaveolens* indicates the presence of 0.149 of Tannins, 0.135 of Saponins, 0.203 of Flavonoids, 0.456 of Phenols, and 0.149 of Alkaloids, as presented in Table 3. Cardiac Glycosides, Terpenoids and Steroids were however not determined.

Table 2: Qualitative analysis of ethanol leaf extracts of *Hyptis suaveolens*

Phytoconstituents	Leaf Extract
Tannins	+
Saponins	+
Flavanoids	+
Steroids	+
Phenolic acid	+
Cardiac Glycosides	+
Terpenoids	+
Alkaloids	+

+ = Presence of constituent.

Table 3: Quantitative analysis of ethanol leaf extracts of *Hyptis suaveolens*

Phytoconstituents	% Composition
Tannins	0.149
Saponins	0.135
Flavanoids	0.203
Phenolics	0.456
Alkaloids	0.149
Cardiac Glycosides	ND
Terpenoids	ND
Steroids	ND

Table 4: Larvicidal activities of ethanolic leaf extract of *H. suaveolens* against the instars larvae of *Anopheles gambiae*

Extract Conc. (mg/L)	Period of exposure							
	5Mins	10mins	30mins	1hr	3hrs	6hrs	12hrs	24hrs
0.1	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.75±0.48 ^a	1.50±0.50 ^a	2.00±0.41 ^a
0.2	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	1.50±0.50 ^a	2.25±0.25 ^a	2.75±0.48 ^a
0.5	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	1.00±0.00 ^a	2.00±0.00 ^a	3.00±0.00 ^b	4.00±0.00 ^b	4.50±0.29 ^b
1.0	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	1.00±0.00 ^a	2.00±0.00 ^a	3.00±0.00 ^b	4.00±0.00 ^b	4.50±0.29 ^b
Control	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

*Values are presented in mean ± standard error of 4 replicates

Values with the same superscript alphabet on the same column are not significantly different at (p<0.05)

Table 5: Larvicidal activities of aqueous extract of *H. suaveolens* against the instars larvae of *Anopheles gambiae*

Extract Conc. (mg/L)	Period of exposure							
	5Mins	10mins	30mins	1hr	3hrs	6hrs	12hrs	24hrs
0.1	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.50±0.29 ^a
0.2	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.50±0.29 ^a
0.5	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.50±0.29 ^a
1	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.50±0.29 ^b	0.50±0.29 ^a
Control	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a	0.00±0.00 ^a

*Values are presented in mean ± standard error of 4 replicates

Values with the same superscript alphabet on the same column are not significantly different at (p<0.05)

DISCUSSION

Hyptis suaveolens has gained increasing attention over the years due to its insecticidal properties, feeding deterrent and insect repellent activities. The use of plant extracts in mosquito control is indeed a potential alternative measure for minimizing the noxious effects of some larvicidal compounds on the environment. In this study, the phytochemical analysis revealed the presence of biologically active constituents such as Tannins, Saponins, Alkaloids, Flavonoids, Cardiac glycoside, Steroids, Terpenoids and Phenols using the ethanolic extract. Similarly, the quantitative estimation of the phytochemical components revealed that the plant was rich in phenols, but alkaloids, tannins, Saponins, and Flavonoids were in moderate quantity. Cardiac glycosides, Steroids, and Terpenoids were not determined due to the absence of reagents. The larvicidal activity of the plant extract could be attributed to the various Phytoconstituents including Phenols, Steroids, Flavonoids, Saponins, Alkaloids,

Tannins and Terpenoids. The presence therefore of these constituents in our findings supports the outcome of the previous work of Egunyomi *et al.*, (2010), Lawal *et al.*, (2012); Sekyere *et al.*, (2018).

Phytochemicals derived from plants are known to provide protection against insect attacks and plant diseases. Many species of the lamiaceae such as *ocimum* and *Hyptis* are strongly aromatic and toxic to insects (Dubey *et al.*, 2014; Desiyamani *et al.*, 2018). The presence of these constituents in our findings is in line with the reports of so many researchers (Syed and Noor, 2011; Chore *et al* 2014; Goyal *et al.*, 2019) who recorded the presence of almost all the bioactive constituents, where exceptions occurred, they were associated with the species, different solvents or methods used.

The results of the ethanol crude extract against the instar larvae in Table 4 revealed that at 0.5mg and 1.0mg concentration, significant

differences in mortality rates between the two samples were observed ($p < 0.005$). At 0.1mg and 0.2mg, the larvae were resistant at 1hr and at 3hrs but became susceptible at 6hrs with a mortality rate of 75%. For all extracts used during this study, the rate of mortality increased with increase in the concentration of extract. This outcome is similar to those reported by Rawani *et al.* 2009; Arivoli, 2012; Nwafor, 2014; Murugesan *et al.*, 2015; Terseer and Ali, 2019. They postulated that higher mortality rate was recorded with increase in concentration against mosquito larva at various stages of developments.

The result of the crushed fresh leaves in Table 5 showed significant increase in susceptibility at 1.0mg concentrations with a mortality rate of 0.50% at 12hrs. At 0.1mg, 0.2mg, 0.5mg the mortality rate of the larva on fresh leave aqueous solution was 0.50% at 24hr, this result agrees with the findings of (Murugesan *et al.*, 2015; Sekyere *et al.*, (2018) and Terseer and Ali, (2019). Terseer *et al.*, (2019) posited that their findings recorded a gradual overall mortality rate increase in every experimental unit as the quantity (concentration) of the test plant (*Hyptis suaveolens*) introduced into the units increased. They added that a resultant highest cumulative death rate of 119 was recorded in the evening. They asserted comparatively that; fresh portion of the test plant was more effective for killing the adult mosquitoes during the study. Statistically, it also showed a strong correlative relationship ($r = 0.9832$) between the concentration of the test plant (*H. suaveolens*) and the death rate of the vectors.

The finding in this current study opens another perspective to the use of a simple, less rigorous, less toxic and environmentally friendly strategy for the effective control of mosquito development and spread in our communities. Further research using improved technique on the processing, application and utilization of the fresh leaves will ascertain further bio-potent larvicidal properties of *Hyptis suaveolens*.

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