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Insecticidal Activity of Crude Extracts of *Hyptis suaveolens* (Bush Mint) on *Anopheles* Mosquitoes Collected from Lafia, Nasarawa State, Nigeria

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ABSTRACT

Anopheles gambiae is a vector that is responsible for the transmission of malaria parasites which causes high morbidity and mortality in Nigeria and the world at large. Human-vector contact can be reduced by the use of conventional repellents being sold in the market, though some of these repellent are not environmentally friendly and *An. gambiae* have developed resistance to some of these repellents. To this end, the phytochemical constituents and insecticidal activity of crude extracts of *Hyptis suaveolens* (bush mint) was determined on adult *An. gambiae* mosquitoes collected from Lafia, Nasarawa State, Nigeria to evaluate its effect in controlling them. Here, 70% ethanolic and diethyl ether fat crude extracts were made from *H. suaveolens* dried leaves and used to carry out the experiment. The phytochemical screening of the ethanol extract revealed the presence of alkaloids, flavonoid, saponins, tannins, steroids and reducing sugar. Glycoside was not detected in the ethanol extract. Only steroids was detected in diethyl ether extract the rest of the phytochemical tested were absent. *An. gambiae* larvae were collected from the field and were raised to F₁ progeny adults that were used for the study. WHO protocol for carrying out human bait repellency cage test was used. Human hand treated with the extracts was exposed to a cage containing 30 female mosquitoes for each of the extracts respectively. The ethanolic crude extract treatment proved to be more effective in repelling mosquitoes with 0% (0/30) mosquito landing than diethyl ether extract which had 40% (12/30) mosquitoes landing and the control hand (untreated left hand) had the highest landing rate 63.3% (19/30) which showed very high significant difference ($\chi^2 = 27.2619$, df = 2, P < 0.00001) in relation to the treatments. After 24 hours holding period, the mortality rate of exposed mosquitoes was observed to be highest in the ethanolic treatment 73.3% (22/30). In conclusion, *H. suaveolens* extracts have repellency potential in controlling adult *An. gambiae*.

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1. Introduction

Malaria is one of the world's most important mosquito borne disease because of its great morbidity and mortality rate. It was estimated that about 300 million new cases of malaria occur every year worldwide that results in 1-2 million deaths^[1]. Mosquitoes are responsible for transmitting malaria and other diseases such as dengue fever, yellow fever, filariasis and other fevers^[2]. There are different types of mosquito species living in tropics and sub tropics region of the world such as *Anopheles*, *Aedes* and *Culex*. *Anopheles* is one of the most common mosquitoes in Nigeria and it is the primary vector that transmit malaria parasite^[3].

The control of mosquito is becoming increasingly difficult because of the development of resistance by the vector against the synthetic inorganic insecticide and environmental hazard caused by the insecticide as a result of persistent use^[4] in the absence of DDT and other insecticides for effective control of mosquitoes, wide variety of plant species from various ecosystems that have a range of acute and chronic toxic effect against mosquitoes were used locally to control mosquitoes in communities^[5]. Currently more than 2000 plant species have been identified as having insecticidal and repellent properties. About 344 plants are known to possess anti-mosquito characteristics and this includes *Hyptis suaveolens*^[6].

Hyptis suaveolens (L. Poit) also known as bush mint, bush tea, pignut, or chan is a very common plant found along roadsides and farmsteads in different parts of the world mainly in the tropics and subtropics, the leaves are opposite and ovate, about 2.5 cm to 10 cm long^[7].

Hyptis suaveolens is used in rural areas as repellent for biological control of mosquitoes^[8]. Abok *et al.*^[9] opined that *Hyptis suaveolens* exhibited larvicidal activity against *Anopheles gambiae* larvae. In Kenya an ethnobotanical studies carried out on *H. suaveolens* showed that the plant was able to repel mosquitoes effectively after burning overnight in a room^[10]. Similarly, fieldworks carried out with *H. suaveolens* crude extract showed that the effect of a solution containing 8% of the plant oil persisted and repelled up to 97% of mosquitoes by 5 hours after application^[11]. The development of such alternative repellent will go a long way considering the health challenges that the poor and less privileged individuals, mostly in tropical Africa and Nigeria in particular are facing to control mosquitoes. The effectiveness of the currently used organochlorine (DDT and Lindane), organophosphorus (malathion), carbamates (carboxyl) and pyrethroid insecticides against the vector is reducing everyday^[4,12-14].

Based on the above stated challenges and burden

caused by mosquitoes, this study was designed to determine the phytochemical constituent of *Hyptis suaveolens* dried leaves crude extracts and the effect of the plant extract in controlling adult *An. gambiae* mosquitoes with the hope that a novel and more effective product may be developed from the plant for controlling mosquitoes in the tropics where the disease is more endemic.

2. Materials and Methods

2.1 Study Area

Nasarawa State is located in the middle belt region of Nigeria. It lies between latitude: 8°34'13.8544" N and longitude: 8°18'31.8388"E (using DMS) the state shares boundary with Benue and Kogi States at the South. It shares the north boundary with Kaduna State. The west boundary it shares with the Federal Capital Territory, Abuja. And the east boundary it shares with Taraba State and Plateau State. The plant sample and mosquito larvae used for this study were collected from Mararraba-Akunza area, Lafia, Nasarawa State.

2.2 Sample Collection

H. suaveolens sample was collected from Mararraba-Akunza area, Lafia, Nasarawa State. The plant was transported to the Botany laboratory of Federal University of Lafia for identification after which it was air-dried under shade at room temperature.

The *Anopheles* mosquito larvae that was raised to adults and used for the study were collected from Mararraba-Akunza area, Lafia, Nasarawa State, thereafter transported to Zoology laboratory of Federal University of Lafia for sorting and identification then raised to adult F1 progeny for the study.

2.2.1 Plant Sample Preparation and Extraction

The leaves of the test plant were rinsed with water to remove dirt and was spread out on a clean surface and allowed enough time to air-dry under shade at room temperature. The extraction was carried out in the department of Chemistry laboratory of Federal University of Lafia.

The cold organic method of extraction by Harborne^[15] was employed for the plant extraction. The ratio of plant to solvent is 1:5 i.e. for every 1 gram of plant sample it was diluted with 5 milliliters of the organic solvent.

Firstly to defat, the 200 gram of the pulverized plant sample was weighed into a Winchester bottle and 1000 milliliter of diethyl ether was poured into the bottle. The solution was kept for 24 hours and was shaken periodically to enable thorough mixture of the solution. After 24

hours, the solution was filtered, the plant extract was kept in a fume cabinet to allow the diethyl ether to evaporate and the residue was shade dried.

Secondly, to obtain the 70% ethanol extracts. The dried residue was weighed into a clean Winchester bottle after which 1000 milliliter of 70% ethanol was introduced into the bottle. 700 milliliter of ethanol was diluted with 300 milliliter of distilled water to obtain the 1000 milliliter of 70% ethanol. The solution was kept for 24 hours and was shaken periodically to enable thorough mixture of the solution. After 24 hours the solution was filtered and the plant extract was kept in a fume cabinet for evaporation.

Furthermore after the evaporation of the solvents, the fat extract and the 70% ethanol extract was kept in a refrigerator to maintain the potency of the extract before the experiment was carried out.

Qualitative Phytochemical Screening

Phytochemical screening of the plant extracts was carried out employing standard procedures and tests^[16-18]. The procedures used for the screening process are briefly described below.

Test for Alkaloids

Alkaloids content of the extracts was determined using techniques by Trease and Evans^[18], 5 mL of the extract was added to a test tube and 2 mL of 1% HCl was added and boiled for 10 minutes on a steam bath. The mixture was cooled and filtered. After which, the filtrate was treated with Mayer's, Wagner's and Dragendroff's reagents. The turbidity of the resulting precipitate was an indication of the presence of alkaloids.

Test for Flavonoids

Flavonoids content of the extract was determined using techniques by Trease and Evans^[18], 0.5 g of the extract was added into a test tube and 10 mL of ethylacetate was added then boiled in a water bath for 5 minutes. To five (5) mL of the extract, 1 mL of diluted aqueous ammonia was added and shaken vigorously. The layers were allowed to separate and the colour of the ammonia layer observed for a yellow colouration in the aqueous ammonia layer which indicates the presence of Flavonoids.

Test for Saponins

Saponins content of the extract was determined using the techniques by Vishnoi^[16] and Sofowora^[17], here 2 mL of the extract was added into a test tube and a few volume of distilled water added. The solution was shaken vigorously. The presence of a stable froth (Foaming) indicates the presence of Saponins.

Test for Tannins

Tannins content of the extract was determined using

techniques by Trease and Evans^[18], here 2 mL of the extract was added into a test tube and 1-2 drops of diluted ferric chloride solution was added. A blue-black green or blue-green precipitate indicates the presence of tannins.

Test for Steroids

Steroids content of the extract was determined using the technique of Sofowora^[17], here 5 mL of the extract was added into a test tube and 2 mL of chloroform was added. After which 2 mL of concentrated sulphuric acid was carefully added to form a lower layer. A red-brown colour of the interphase indicates the presence of Steroidal ring.

Test for Reducing Sugar

Reducing sugar content of the extract was determined using techniques by Trease and Evans^[18], here 5 mL of the extract and 3-4 drops of Fehling reagents (I and II) was added and the mixture heated on a water bath to boil. A red precipitate indicates the presence of reducing sugars.

Test for Glycosides

Glycosides content of the extract was determined using the techniques by Vishnoi^[16] and Sofowora^[17], here 2 mL of the extract was added into a test tube and 5 mL of Fehling's reagents (I and II) added into the test tube the mixture was boiled in a water bath for 5 minutes (this is to remove any reducing sugar present in the sample). After boiling it was allowed to cool and filtered. To the filtrate, 2 mL of diluted sulphuric acid was added. The mixture was reheated, cooled and neutralized with an equal volume of sodium hydroxide. To this, another 5 mL of Fehling's reagents (I and II) was added and the mixture reheated on a water bath for 10 minutes. A brick red precipitate indicates the presence of glycosides.

2.2.2 Rearing of the *Anopheles* Larvae to Adult

The field caught larvae of *Anopheles* mosquito used for this research, were reared to adults in cages in the Department of Zoology Laboratory of Federal University of Lafia, Nasarawa State. The adult mosquitoes were fed on sugar solution for continuous maintenance of mosquito colony.

Test for Repellency

The repellency test was carried out using the cage test of World Health Organization (WHO)^[19]. The cage test is the most common way of testing the effectiveness of mosquito repellency. Three cages with a slot for inserting an arm were used as described by WHO^[19].

After inserting the various arms into different cages, the data on the number of landing on the hands were collected. According to the WHO protocol, if within 3 minutes there is no mosquito landing on the untreated hand then the test will stop, because the volunteer might be naturally repellent to mosquitoes^[19].

Three cages were used, each containing 30 mosquitoes, one cage for the control arm, second cage for the arm having diethyl ether extract and the last cage arm having the ethanol crude extract was inserted. The mosquitoes were starved for 48 hours before the test ^[19].

During the experiment the volunteer who most refrain from smoking and use of scented product 12 hours before the experiment, inserted his arm without the plant extract in the control cage ^[20]. Numbers of mosquitoes landing was recorded within the 30 minute test time. After 24 hours the mortality rate was recorded ^[19].

Two tests were carried out. The first test was for the 70% ethanol extract and the second test was for the diethyl ether extract. This was done in other to compare the two *H. suaveolens* extracts which is more effective on repelling mosquito.

2.3 Statistical Analysis

Data obtained was analyzed using R Console software version 3.2.2. Pearson Chi-square test was used to compare the proportion of *An. gambiae* that landed on the hand in relation to extracts of *H. suaveolens* treatments. The P-value < 0.05 was considered statistically significant.

3. Results

Qualitative Phytochemical Constituents of Crude Extracts of *H. suaveolens* Leaves

The qualitative phytochemical result (Table 1) depicts the presence of alkaloids, flavonoid, saponins, tannins, steroids, reducing sugar in the 70% ethanolic crude extract of *H. suaveolens*. Only steroids was detected in diethyl ether extract the rest of the phytochemical tested were absent. Glycosides was not detected in the plant extracts.

Table 1. Qualitative Phytochemical Constituents of Crude Extracts of *H. suaveolens* Leaves

Secondary metabolite	<i>H. suaveolens</i> Ethanolic extract	<i>H. suaveolens</i> Diethyl ether extract
Alkaloids	+	-
Flavonoid	+	-
Saponins	+	-
Tannins	+	-
Steroids	+	+
Reducing Sugar	+	-
Glycosides	-	-

Key: + = Present
- = Not detected

Repellency Activity of *H. suaveolens* Ethanol and Diethyl Ether Extracts on Treated Baited Arm against Adult *Anopheles gambiae* Landing

The total number of *Anopheles gambiae* that landed on the hand was highest in the control 19 (63.3%) followed by those exposed to diethyl ether extract 12 (40%) while

none 0 (0%) landed on crude ethanol extract treatment as shown in (Table 2). Therefore, the number of *An. gambiae* that landed in relation to different extracts of *H. suaveolens* showed a very high significant difference ($\chi^2 = 27.2619$, df = 2, P = 0.000001202).

Table 2. Repellency Activity of *H. suaveolens* Ethanol and Diethyl Ether Extracts on Treated Baited Hands against Adult *Anopheles gambiae* Landing

Treatment	No. of <i>An. gambiae</i> Exposed	No. of <i>An. gambiae</i> that landed (%)
Control arm (untreated arm)	30	19 (63.3)
Ethanol extract arm	30	0 (0.0)
Diethyl ether extract arm	30	12 (40.0)

Mortality of Adult *Anopheles gambiae* in Relation to 24 Hours Holding Period

Mortality was highest in the crude ethanol extract 22 (73.3%) while only 19 (63.3%) died in diethyl ether extract and control 19 (63.3%) respectively (Table 3). However, there was no significant difference ($\chi^2 = 0.9$, df = 2, P = 0.6376) in mortality rate across extracts.

Table 3. Mortality of Adult *Anopheles gambiae* in Relation to 24 Hours Holding Period

Arms Exposed	No. <i>An. gambiae</i> Exposed	No. Dead (%)
Control Arm	30	19 (63.3)
Ethanol extract Arm	30	22(73.3)
Diethyl ether extract Arm	30	19 (63.3)

4. Discussion

The result obtained clearly showed that ethanolic crude extract of *H. suaveolens* was made up of 6 phytochemical constituents with the exception of glycosides. Also, only steroids were present in screened diethyl ether extract. The phytochemicals recorded in both crude extracts is in agreement with study done by Dakum *et al.* ^[21] who documented the presence of same phytochemicals in the phytochemical analysis of *H. suaveolens* methanolic and aqueous extracts. Also, the presence of steroid in the two crude extracts in this study is in accordance with the finding of Shenoy *et al.* ^[22] who also reported the presence of steroids in phytochemical screening of *H. suaveolens* diethyl ether extract.

During the bioassay, the control group had the highest number of mosquitos landing 19 (63.3%) and there were more than 2 mosquitoes that landed on the volunteer con-

trol arm within 3 minutes, which indicated that the volunteer was not naturally repellent to mosquitoes as reported by WHO^[19] and Anuar and Yusof^[20].

The repellency exhibited by 70% ethanol extract which yield 0% landing on the treated arm as against the diethyl ether extract in which 40% (12/30) *Anopheles gambiae* landed possibly suggest that ethanolic solvent tends to be effective in extracting high number of active ingredients present in plant products that can serve as repellants. This agrees with Shaalam *et al.*^[5] in a review of botanical phytochemicals with mosquitocidal potential reported that ethanolic extract had the highest efficacy on mosquito repellency compared to diethyl ether (fat) extract.

The mortality rate was very high for those exposed to the 70% crude ethanol extract 22 (73.3%) and low in those exposed to fat extract. This might be as a result of the phytochemicals present in each of the extract making the ethanol extract very effective and having very high repellency and mortality ability compare to the diethyl ether fat extract as reported by Shaalam *et al.*^[5] and Hemen *et al.*^[8]. The 63.3% (19/30) mortality recorded in the control might be as a result of the 48 hours starvation observed in order for the test to be conducted, although sugar solution was placed immediately after the test on top of the cage for the 24 hours holding period before mortality rate was recorded.

From the result obtained in this bioassay, *H. suaveolens* crude extracts have proven to be as effective as DEET (N, N-dimethyl-3-methylbenzamide) for personal protection against adult *An. gambiae* mosquitoes bite as reported by Hemen *et al.*^[8] and as reported by Abgali and Alavo^[11] that the plant is having mosquitocidal ability.

5. Conclusions

Results from this study have proven that *H. suaveolens* crude extracts can serve as alternative repellent to synthetic insecticides in the control of *An. gambiae*, the causative agent of malaria in Nigeria and this will also help reduce the burden caused by the vector to humans. The plant is abundantly available and the processing of it to obtain crude extract is very easy and cost effective, hence could serve as a more favorable option in the control of mosquitoes in our environment since it is biodegradable, environmentally friendly and affordable.

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Conflict of Interest

There is no conflict of interest.

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