Evaluation of repellent and larvicidal activity of *hyptis suaveolens* against filarial vector, *Culex quinquefasciatus*

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Abstract. Mosquito vector control is facing a threat due to the emergence of resistance to synthetic insecticides. Safe and eco-friendly insecticides of botanical origin may serve as suitable alternative biocontrol techniques in the future. *Hyptis sauveolens* (bush minth) belonging to the family, Lamiaceae is common and widely distributed in the south-eastern Nigeria. The repellent and larvicidal activity of the ethanol extract of this herbs against the filarial vector, *Culex quinquefasciatus* 3rd instar larvae and adults respectively *was* investigated or 48 hours. The extract was screened chemically for phytochemicals. The extracts showed larvicidal and repellent activity with Lethal Concentration ,$LC_{50,}$ LC_{90} and Effective Dose, ED_{50}, ED_{90} values 81.817mg/l, 166.704mg/g and 38.75mg/l , 258.925mg/l respectively. Alkaloids, flavonoids, Tannins, Saponins and Steroids were the phytochemicals present. Repellency and mortality were observed to significantly and positively correlated with the concentrations of the ethanol extracts ($p<0.05$). The present study revealed that an indigenous common weed, *Hyptis suaveolens* could be considered as potential natural larvicidal and repellent agent against mosqutoes.

Key words: Lamiaceae, *Culex quinquefasciatus, Hyptis suaveolens,* larvicidal. activity.mosquito

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INTRODUCTION

Mosquito-borne diseases such as malaria, filariasis, dengue, yellow fever and Japanese encephalitis are major public health problems in tropical and subtropical regions (Dua *et al.*, 2010). The tropical house mosquito *Culex quinquefasciatus* is the principal vector of lymphatic filariasis (Ramaiah *et al.*, 2000). The control of these diseases is largely dependent on spraying of chemical insecticides to kill mosquito adults or larvae. Larviciding is an effective method to reduce the mosquito densities before they emerge as adults and synthetic insecticides have been widely used for this purpose (TiwaryI *et al.*, 2007). Herbal products with proven potential as repellents can play an important role in the interruption of mosquito borne disease at both the individual and community level

Plants products have been used traditionally to repel or kill the mosquitoes in many parts of the world. The repellency of plant materials had been exploited for 100s of years by man in houses simply by hanging bruised plants in houses, a practice that is still in wide use throughout developing countries (Moore, *et al.*, 2006). Plants have also been used for centuries, in the form of crude fumigants where plants were

burnt, to drive away nuisance mosquitoes and later as oil formulations applied to the skin or clothes which was first recorded in writing by ancient Greek, Roman and Indian scholars (Moore *et al.*, 2007). Plant materials are still extensively used in this traditional way throughout rural communities in the tropics because for many of the poorest communities, they are the only means of protection from mosquito bites that are available. Certain Natural products have been investigated for repellent and larvicidal activities against mosquitoes. Although the primary function of these compounds is defence against phytophagous insects, many are also effective against mosquitoes and other biting Dipterans, especially volatile components released as a consequence of herbivory (Pichersky and Gevshenzon, 2002). Many plant volatiles are deterrents or repellents because they have high vapour toxicity to insects (Gershen and Dudareva, 2007). These volatiles are released when leaves are damaged in order to deter herbivores and several authors have shown strong responses of mosquito odour receptors to this class of volatiles including geranyl acetate and citronellal; 6-methyl-5-hepa-2-one (Carey *et al.*, 2010; Logan, *et al.*, 2010).

The long use of synthetic insecticides has created a number of ecological and medicinal problems, such as the development of resistant insect strains, ecological imbalance and high toxicity to mammals (Ranaweera, 1996). Plants are known to contain compounds of insecticidal, insect-repelling, and anti- juvenile properties. Biologically active materials derived from plants sources can act as larvicides, insect growth regulators, repellents and oviposition attractants and have deterrent activities (Kalu *et al.*, 2010; Kumar and Maneemegalai, 2008; Tandon and Sirohi, 2010). Most of these compounds are biodegradable and less harmful to mammals than synthetic insecticides. Concern about the deleterious effects associated with Synthetic chemicals has revived interests to explore plants as a source of natural insecticides, acaricides, and repellents for medical, veterinary and crop protection use (Jaenson *et al.*, 2006). Therefore, this study was undertaken to evaluate the repellent and larvicidal activity of *Hyptis suaveolens* against filarial vector, *Culex quinquefasciatus.*

MATERIALS AND METHODS

Collection, Identification and Preparation of plant materials.

The leaves of *Hyptis suaveolens* (Bush mint) were collected, identified by plant taxonomist, washed with distilled water and dried indoor for two weeks. The dried leaves were ground into fine powder using electric grinder and sieved. The fine powder was wrapped in extraction thimbles and put in soxhlet apparatus and extracted using absolute ethanol. The extract was concentrated in water bath at 100^0C to evaporate the ethanol which yielded dark residue that was further reduced to pastes by heating. Stock solutions were prepared according to WHO (2005) guidelines by dissolving 200mg each of the extracts in 20mls of water to make 1% stock solution from which series of concentrations were prepared. Three drops of acetone were added to dissolve the oil. The stock solution was kept in a screw cap vial, with aluminium foil over the mouth of the vial.

Rearing of mosquito larvae

Eggs of *Culex quinquefasciatus* were collected from breeding sites and identified at Arbovirus Vector Research Centre, Federal Ministry of health Enugu. After hatching larvae were reared in plastic buckets half filled with tap water and temperature maintained at 25-27^0C, 75-85% relative humidity under 12:12 (light and dark) photo period cycle. The larvae were fed with food consisting of Quaker oats and brewer's yeast in the ratio (3:1), once a day initially and twice a day during the later stages of development. Water in the rearing container was refreshed

everyday by removing a little quantity of water from the rearing buckets and replacing with fresh water. This was aimed at preventing scum from forming on the water surface. Adult mosquitoes that emerged were fed with 10% sucrose solution.

Repellency test

The plant leaves were kept inside the room with unscreened windows and doors from 18.30 - 6.30 hours during the experimental night, and the period of protection from mosquito bites was recorded. Outside the room, plant leaves were bruised with the hand to enhance the release of repellent volatiles. The bruised leaves were rubbed on one of the legs of the volunteers, while the unrubbed leg of the volunteers served as control. All other parts of the body (except the legs) were covered. Repellency percentage was calculated as recommended by WHO, (2009),by subtracting the number of mosquitoes landing on the rubbed leg from the number of mosquitoes landing on the unrubbed leg and dividing it by the number of mosquitoes landing on unrubbed legand multiplying by 100. The period of active repellency (protection time) was recorded. The plant extract was tested for repellency in the Laboratory. The hands of two volunteers were exposed to adult *Culex quinquefasciatus* mosquitoes in a cages (30cm X 30cm X 30cm) for one hour. Before the application of the plant extracts, the hands were washed and cleaned thoroughly with 70% ethanol. Aliquot of 0.3ml of the test solution was smeared on the dorsal side of one hand (wrist to finger tips) of the volunteer. The repellent chamber contained between 100 and 150 3-day old and 4-hour starved mosquitoes. After 30 minutes of application, the hand was placed inside the repellent chamber for 10 minutes through a hole up to the wrist and plugged with cotton wool to prevent escape of mosquitoes and to encourage the female mosquitoes to bite on. The test was repeated at 30 minutes interval. The interval between the application of plant extract and the first two consecutive bites occurring within 30 minutes was considered as protection time against the mosquito bites (Das *et al*., 2003; WHO, 2009). The test was repeated five times for each of the concentrations. Control readings were obtained by placing untreated hand in repellant chamber. All tests were conducted at room temperature of 27 ± 2^0C and relative humidity of 75 - 80%. The dose of plant extracts providing at least 6-8hours of protection in mosquito cage was considered to be an ideal compound for use as repellents as recommended by WHO(2009). Repellency percentage was calculated using the formula (WHO, 2009) given below:

$$\frac{\text{No. landing on negative control - No. landing on treated with repellent}}{\text{No. landing on negative control}} \times 100$$

Bioassay

Standard methods and guidelines for laboratory and field testing of mosquito larvicides as stipulated by WHO, (2005) were adopted. The bioassay were performed at room temperature of 25-27^{0}C and Relative humidity of between 70 and 85%, photoperiod of 12:12 (light: dark) and pH 7.0 of distilled water. The larvae were exposed to test concentrations of 25, 50,100,200,300 mg of extracts in 1000ml of water. 100mls of tap water was taken in a series of 500ml glass beaker. The measured amount of extract was dissolved in 1ml of the solvent (ethanol) and added into water in the beakers. A control was also maintained by adding 1ml of solvent ethanol to 100 ml of tap water. The total of 25, 3rd or 4th instar larvae were selected by means of strainer and rubber pipette and introduced into the 500ml beakers containing different concentrations of the plant extracts .Thus each concentration contains 25 larvae. The treatments were replicated four (4) times and each replicate set contained one control. The larvae in all the beakers were fed with

equal amount of Quaker oat and yeast powder in the ratio, 3:1 every 24 hours and this was spread evenly across the water surface. Larval mortality was recorded at intervals of 24 and 48 hours exposure. The moribund and dead larval in the four (4) replicates were combined and expressed as percentage mortality for each concentration. Larvae were declared dead when they failed to move after probing with a needle. Moribund larvae were those unable to rise to the surface within a reasonable period of time.

Phytochemical analysis

Glycosides

The presence of glycosides was determined using the method described by Evans (2002) . Two drops of the plant extracts were put in small beaker. 15ml of distilled water and 3mls of 10% sulphuric acid were added and mixture boiled for 15minutes.The boiled mixture was then made alkaline by adding 10ml solution of 5% potassium hydroxide. 10% of Fehling's solution was added and the beaker boiled for three (3) minutes .The occurrence of a brick precipitate indicated the presence of glycosides.

Tannins

Method described by Evans (2002) was adopted. The filtrate obtained from boiling 2g of the samples with 20ml of 45% ethanol for 5 minutes was used for these tests.

Ferric chloride test: 1ml of filtrate was diluted with 2ml of distilled water and 2 drops of ferric chloride solution added and observed for transient greenish to black colour.

Lead acetate test: To 1ml of the filtrate, 3 drops of 5% lead acetate solution was added and observed for gelatinous precipitate.

Alkaloids

Method described by Evans (2002) was also adopted.

Dragendorff's Test: Two drops of the extract were dissolved in 1% dilute sulphuric acid and boiled. The mixture was filtered hot and a drop of freshly prepared Dragendroff's reagent was added. Formation of pink or red precipitate was taken as a positive test.

Mayer's Test: Two (2) drops of the extract were dissolved in 10% dilute H_2SO_4 and boiled. The mixture was filtered hot and 1-3drops of Mayer's reagent were added. A white or yellow precipitate was taken as a positive test.

Saponin

The frothing test was used. 0.1g of the powdered sample was boiled with some distilled water for 5 minutes and decanted while hot. 1ml of the filtrate was diluted with 4ml of distilled water and the mixture shaken vigorously and observed for stable frothing on standing (Evans, 2002).

Flavonoids

The Shinoda test was used. 0.5g of the powdered sample was extracted in ethanol by boiling in a water bath for 5 minutes; this was filtered and cooled. To the filtrate was added 4 pieces of magnesium fillings followed by 1-3 drops of concentrated

hydrochloric acid. A pink or red colour indicated the presence of flavonoids (Harborne, 1984).

Steroids and Terpenes

5 grams of the powdered sample was extracted by maceration with 50ml of ethyl alcohol (95%), filtered and the filtrate evaporated to dryness and used for the Liberman acid test. A portion of the organic extract was treated with drops of acetic an hydrate, and then concentrated H_2SO_4 acid was carefully added by the side of the test tube. The presence of a brown colour at the boundary of the mixture was taken as positive result (Evans, 2002).

Ethical consideration

All the volunteers used were properly educated on the nature of the work to be done. All health matters of the volunteers were taken care of properly during the study period.

Statistical analysis

Data from all the replicates were pooled for analysis. Percentage mortality was calculated and corrections of mortality if needed were done by using the formula:

$$\text{Mortality (\%)} = \left(\frac{X-Y}{X}\right) 100$$

Where X = % survival in the untreated control; Y= % survival in the treated sample. Analysis of data was done using Probit analysis (Finney, 1971). Lethal Concentrations, LC_{50} , LC_{90} and Effective Doses, ED_{50} and ED_{90} values were calculated from log dosage-probit mortality regression line using computer soft ware SSPS. Standard deviation or confidence intervals of the means of $LC_{50,}$ $LC_{90,}$ ED_{50} and ED_{90} values were calculated. Student's t-test was used to compare the toxicity effects of the extract on larvae.

RESULTS

The highest concentrations of ethanol extract of *Hyptis suaveolens*, 300mg/l, caused 100% mortality while the lowest concentration, 25mg/l caused the least mortality, 4.00% (Table 1.). Mortalities increased with increasing concentrations of the plant extract. There was no mortality in the controls. The mortalities significantly and positively correlated with the concentrations of *Hyptis suaveolens* extracts ($P<0.05$). When treated against the 3^{rd}-4^{th}instar larvae of *Culex quinquefasciatus,* the lethal concentration, LC_{50} and $LC_{90,}$ were 81.817mg/l and 166.704mg/l respectively.

Table 1: Percent mortality of 3^{rd}-4^{th} instar larvae of *Culex quinquesfasciatus* treated with different concentrations of ethanol extracts of *Hyptis suaveolens* (Bush mint) for 48 hours

Plants	Concentra-tion (mg/l)	Mortality percentage								
		3hrs	%	6hrs	%	12hrs	%	24hrs	%	48hrs
Hyptis suaveolens	25	0	0.00	0	0.00	0	0.00	0	0.00	1
	50	0	0.00	0	0.00	1	4.00	1	4.00	2
	100	2	8.00	3	12.00	2	8.00	4	16.00	4
	200	3	12.00	4	16.00	4	16.00	6	24.00	7
	300	3	12.00	5	20.22	7	28.00	10	40.00	0
	Control	0	0.00	0	0.00	0	0.00	0	0.00	0

NB: Values are means of four replicates

The highest mean protection time and repellency percentages was recorded against the highest concentration, 300mg/l of the plant extract (Table 2).,. This includes 386 minutes and 97.74% respectively. Protection time and repellency percentage increased with concentration. There was no significant difference in the mean protection time and repellency percentage ($P>0.05$). There was very strong and significant positive correlation between the concentrations of the plant extract and mean protection time and repellency ($P<0.05$). The Effective Dose, ED_{50} and ED_{90} for *Hyptis suaveolens*, were 38.715mg/l and 258.925mg/l respectively.

Table 2: Relative repellency and protection time of the three (3) herbs against *Culex quinquefascintus* in the laboratory

Herb	Concentration in mg/L	Mean of the protection time (in minutes)	Means of the number of bites	Repellency percentage
Hyptis suaveolens	25	12.6	24.2	45.25
	50	33.2	22	50.23
	100	64.4	12.8	71.04
	200	116	7.8	82.35
	300	386	1.0	97.74
	Control	---	44.2	-----

The indoor and outdoor repellent activities of the herb against the mosquitoes are shown in Table 3. Protection time, 5.28 hours, was recorded for *Hyptis suaveolens* indoors more than outdoors 3.48 hours ; but the percent repellency was greater outdoors (82.22%) than indoors (61.86%). There was no significant difference in the mean protection time of the herb against indoor and outdoor man-biting mosquitoes ($P>0.05$).

Table 3: Repellent activities of *Cymbopogon citratus* against indoor and outdoor man-biting mosquitoes

Herbs	Indoor Biting Mosquitoes				Outdoor Biting Mosquitoes	
	Means no. of mosquito bite Test	Control	Mean protection time in hours	Repellency percentage	Means no. of mosquito bite Test	Control
Hyptis suaveolens	18	47.20	5.28	61.86	3.20	18

The phytochemcial compound, glycosides, was not present whereas alkaloids flavionoids, tannins, saponins and steroids were present in *Hyptis suaveolens* (Table 4).

Table 4: Qualitative analysis of phytochemicals of ethanol extracts *Hyptis suaveolens*.

Phytochemcials compounds	*Hyptis suaveolens*
Alkaloids	+
Flavonoids	+
Glycosides	-
Tannins	+
Saponin	+
Steroids	+

KEY: +: Indicate Present - : Indicate Absent

DISCUSSION

The repellency of plant materials had been exploited for hundreds of years by man in houses simply by hanging bruised plants in houses; a practice that is still in wide use throughout developing countries (Moore *et al*., 2006). Plant materials are still extensively used in the tropics because they are the only means of protection from mosquito bites that are available for many of the poorest communities (Moore *et al*., 2007). Many plant volatile components released by these plants are deterrent or repellents because they have high vapour toxicity to insects (Gershen and Dudareva, 2007); and also have shown strong responses to mosquito odour receptors (Carey *et al*., 2010). Data obtained in this study indicated that *Hyptis suaveolens* had larvicidal and repellent activity against the larvae of *Culex quinquefasciatus* . The larvicidal and repellent activities varied with the concentrations of the plant extract.. This study confirms larvicidal and repellent activities of natural products of plants origin, with insecticidal properties against mosquitoes as reported by different researchers. Equally proven is the fact that *Hyptis suaveolens* has insecticidal/mosquitocidal properties and can be used as useful, cost effective, inexpensive, safe and environmentally friendly control measure, an alternative to chemical insecticides. Also revealed in this study were the presence of some

phytochemical substances in the herb. These phytochemicals may be associated with the larvicidal and repellent activities recorded in this herb.

Results of this study showed that as the concentration of extract increased, mortality of *Culex quinquefasicatus* larvae also increased. The highest mortality of 100% was recorded in the highest concentration of 300mg/l, of the plant extract. Mortality with the various concentrations of plant extracts was significantly higher than that in the control ($P<0.05$). These could have been bioactive compounds responsible for larval mortality.

Normally, as LC_{50} and LC_{90} values decrease toxicity increase. *Hyptis suaveolens* was toxic to *Culex quinquefaciatus* with LC_{50} of 81.817mg/l. Data obtained were in consonance with the findings of several researchers who worked on the effect of same plants, and other plants against *Culex quinquefaciatus*. Amusan *et al*., (2005) recorded that ethanolic extracts of *Hyptis suaveolens* had toxic effect on larvae of *Aedes aegypti*, with the highest concentration of 0.9ppm causing 80 percent larval mortality within 36 hours. Kalu *et al*., (2010) reported that ethanol extract from garlic bulb exhibited effective larvicidal properties, with LC_{50} of 184.18±0.8ppm, against *Culex quinquefasciatus* 4th Instar larvae.

Similarly, Claire and Amanda (1999) studied the use of garlic and lemon peel extracts as *Culex pipens* larvicides. *Hyptis suaveolens* is used for some ethnobotanicals applications in rural communities in African countries Kossou *et al*., 2001; Edeoga *et al.*, 2006) and the plant is readily available close to villages, along roadsides, on farmsteads, etc. *Hyptis suaveolens* has been reported to be biologically effective against lepidopteran pests (Prakash *et al*., 2008) and may have caused insects feeding on the maize plants to reduce their food intake.
Extracts of *H. suaveolens* have been successfully used not only against devastating insects, but have also contributed to the reduction of *Sclerotium* wilt of tomato caused by *Sclerotium rolfsii* by exerting some antifungal protective action on the tomato plants (Okereke *et al*., 2007). *H. suaveolens* extract compares favorably with the insecticide Furadan in reducing *S. calamistis* densities on maize. In a previous study, *H. suaveolens* leaf extract also gave similar effect in comparison with the fungicide Captan on *Sclerotium* wilt of tomato (Okereke *et al*., 2007).

Larval mortalities increased with concentration and confirms the report of Shadia *et al*., (2007), that there is a positive correlation between concentration and the percentage of larval mortality.

The toxicity of the three plant extracts could be due to the presence of to a wide range of chemicals including alkaloids, glycosides, tannins, quinines, terpenoids, non-metabolized amino acids which are part of the chemical resistance mechanisms of various plant groups against degradation of herbivorous insects (Hedin, 1983).. Presence of the phytochemicals recorded in this study confirms the reports by Edeoga *et al*., (2006) Mgbemena (2010) and Gopieshkhanna and Kannabiran (2007) who recorded presence of Alkaloids, Tannins, Phenols, Saponins, flavonoids, Steroids, carbohydrates, phytosterols in various plant species including *Hyptis suaveolen.* This study has revealed that *Hyptis suaveolens* showed significant repellent activity against *Culex quinquefasciatus,* with ED_{50} and ED_{90} of 38.715mg/l and 258.925mg/l respectively The repellency and protection time increased with increasing concentrations, due to increased phytochemical contents against *Culex quinquefaciatus.*

H. suaveolens recorded the highest repellency and protection time when placed indoors. This is similar to other reports. Abagali *et al*., (2002) recorded that 6% of *H. suaveolens* essential oil repelled 97% and 100% of tested mosquitoes when tested in the laboratory and field conditions for at least 15 minutes post application.

Abagali and Alavo (2011) reported that 10% *H. suaveolens* essential oil is as effective as 30% DEET for personal protection against mosquito bites, providing protection time to at least 5 hours. This is in agreement with the present study where *H. suaveolen* provided 5.28 hours of protection against mosquito bites when placed indoors. Fradin and Day (2002) also reported that effects of a solution containing 8% of the oil persisted and repelled up to 97.56% of mosquitoes by 5 hours post application.

The repellent activities of the herbs studied is attributed to their phytochemical content. The mode of action of these photochemicals can not be unconnected with the suggestions by Ansari and Razdam (1995). They are of the opinion that the active ingredients (alkaloids, flavonoids, saponins, phenolics, and tannins) present in phytochemical extracts from the herb might have exerted some inhibitory effects on lactic acid receptor cells by masking or changing the lactic acid that normally attracts mosquitoes thereby confusing or distracting the mostquitoes. Thus, the blood-feeding contact or response is prevented. Consequently, with the application of the phytochemicals extracts on the skins, the mosquitoes could not bite because the active ingredients did not let them smell the attractant (Lactic acid) and could not therefore identify the human as their source of meal. This implies that the active ingredients confused the olfactory receptors and the mosquito simply could not smell the host.

CONCLUSION

This study has provided information on *Hyptis suaveolens* as indigenous plant with mosquitocidal properties and as such may offer potential tools that could supplement currently available control techniques for mosquito vectors. Therefore, in the context of integrated pest management, the ethanol extract of *H. suaveolens* may play a significant role as revealed this study. This calls for innovative methods and more appropriate formulation, perhaps in the form of spray/aerosol as well as quantitative analysis of the phytochemicals.

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