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The Effect of Dried Leaves Extract of *Hyptis suaveolens* on Various Stages of Mosquito Development in Benue State, Nigeria

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Abstract: *Malaria is transmitted by Anopheles mosquito which is usually controlled by chemical insecticides. These insecticides have resulted in environmental hazards and in resistance among mosquito species. Plant products are considered to be alternative means as they are environmentally safe and biodegradable. Previous works demonstrated effectiveness of the whole plant as mosquito repellent or the extract on selected developmental stages. The present study focused on the effect of leaves extract on developmental stages including the eggs. The leaves of Hyptis suaveolens were harvested and dried under shade. The dried leaves were then extracted in 80% ethanol for forty-eight hours. The different developmental stages of the mosquito (250 eggs, 80 larva, 80 pupae and 80adult mosquitoes were treated with different concentrations of the ethanol extract (50 mg/ml to 500 mg/ml)in plastic bowls. Results showed that H. suaveolens leaf extract contained tannins and cardiac glycosides in high amounts while steroids, terpenoids alkaloids, saponins and phlobatannins were in moderate amount. Flavonoids were not detected. The extract was more effective on the eggs than on the other developmental stages (larvae, pupae and adult). As the highest concentrations of the extract showed a higher mortality rate for eggs (99.2%) and adult mosquito (51.3%) ,its therefore shown that Hyptis suaveolens has great potential as alternative means of controlling mosquitoes, perhaps by targeting the eggs. Therefore, more research on isolation and characterization of bioactive components is definitely suggested.*
Keywords; *Mosquitoes, Developmental stages, Hyptis suaveolens, Mortality Rate.*

I. Introduction

Mosquitoes are the major vectors for many diseases such as dengue fever, yellow fever, malaria, filariasis, Japanese encephalitis and other fevers (Service, 1983). There are many types of mosquitoes living in the tropical and sub-tropical regions of the world - anopheles, aedes and culex. *Anopheles stephensi* is one of the most common mosquitoes in Nigeria and the primary vector of malaria (Donald, 2004).

The control of mosquitoes is however becoming increasingly difficult because the effectiveness of vector control has declined due to development of resistance by vectors against the synthetic organic insecticides and environmental hazards as a result of persistent use (WHO, 1986; Gratz, 1997; Chandre et al., 1998; Penilla et al., 1998; Dolianitis and Scinclair, 2002). In the absence of prophylactic vaccine, 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 effects against mosquitoes were used locally to control mosquitoes in communities (Shaalam *et al*, 2005). Currently more than 2000 plants species have been identified as having insecticidal, repellent properties and about 344 plant products are known to possess anti-mosquito characteristics (Sukumar *et al, 1991*).

Hyptis suaveolens is one among such plant species used locally in rural areas as repellent for biological control of mosquitoes (Hemen *et al.,* 2013). Considering the health challenges that the poor and less privileged people, mostly in tropical Africa and Nigeria, are facing, in particular, malaria, the control of this mosquito-borne disease is however becoming increasingly difficult. The effectiveness of vector control has declined due to development of resistance as earlier mentioned by vectors against the currently used organochlorine (DDT and Lindane), organophosphorus (malathion), carbamates (carboxyl) and pyrethroid insecticides (WHO, 1986; Gratz, 1997; Chandre *et al.,* 1998; Penilla *et al.,* 1998; Dolianitis and Scinclair, 2002). Based on these challenges, this study was designed to determine the effect of dried leaves extract *of Hyptis suaveolens* on various stages of mosquito development (ie. eggs, larvae, pupae and adults), with the hope that a new and more effective product may be developed.

II. Materials And Methods

Collection of Plant Materials and Preparation of Plant Extract

The fresh mature leaves of *Hyptis suaveolens* were collected around University of Mkar, Gboko Benue State, Nigeria, during the month of July. The harvested leaves were dried under shade and taken to the University chemical science laboratory where the dried leaves were pulverized into powder using electric blender. The ethanol extract was prepared in ratio of 1:5 by soaking 400g powdered sample into 2000ml of 80% ethanol in 4000ml beaker. The beaker was sealed with foil paper and placed in a freezer. Extraction was carried out for 48 hours. The extract was filtered through Whatman No. 1 filter paper and the ethanol (solvent) was evaporated using electric water bath at 50^0C. Each of 5g, 10g, 15g to 50g (at 5g intervals) were dissolved into 100ml of distilled water.

Source of Mosquito eggs, larvae, pupae and adults.

The mosquito ovipositing sites used for sourcing mosquito eggs, larvae, pupae and adults for this study were male hostels at the University of Mkar, Mkar. Seven black rubber buckets (6littres) each were half-filled with water and placed at different shady positions around male hostels for mosquito adults to lay eggs. The containers were observed daily and covered during the day time in order to protect it from the sun rays but uncovered at dusk. Some organic substances from the

top soil were supplied in the container as food for the hatched larvae in order to facilitate their development into adults. After a week, the mosquito eggs and larvae were harvested and used for the experiments. Then the pupae developed into the adults within 10 days of the setup. The adults were maintained by supplying them albino mice blood. After sourcing, each stage of mosquito development (eggs, larvae,pupae and adults) were treated with different concentrations of plant extract.

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 Durodola (1977) and Odebiyi and Sofowora (1978).

Experimental design of the study
Experiment 1: Effect of *Hyptis suaveolens* leaf extract on mosquito eggs, larvae and pupae

The strips of filter papers containing mosquito eggs were soaked in 10mg/ml concentration of *Hyptis suaveolens* extract in Petri dishes for 24 hours. The filter papers were transferred into fresh-clean water in 100ml plastic containers. The hatching ability of mosquito eggs was observed within 4 days and the result was recorded. The harvested larvae and pupae samples were treated with different concentrations of *Hyptis suaveolens* leaf extract ranging from 50 to 500 mg/ml. 50mg/ ml of each concentration was measured and poured into 100ml of plastic containers containing the larvae and pupae and their mortality rates were recorded after 48 hours.

Experiment 2: Effect of *Hyptis suaveolens* leaf extract on adult mosquitoes.

Adult mosquitoes were reared and confined in the mosquito cage (net cage), 5mg/ ml of each concentration were measured and sprayed around each compartment using sprayer bottles. The same dose was repeated after 12 hours and their mortality rates were noted after 48hours.

III. Results

The result of phytochemical screening of the ethanolic extract of dried leaves of *Hyptis suaveolens* is presented in Table 1. The analysis revealed the presence of tannins, steroids, terpenoids, saponins, phlobatannins and cardiac glycosides while flovanoids were absent. The result also showed that the presence of tannins and cardiac glycosides were in significant higher amount as compared to other bioactive components which were in moderate amount.

Effect of the extract on the different stages in the development of mosquito:
Egg Stage;

Table 2 shows the results of the ovicidal effect of *Hyptis suaveolens* ethanolic leaf extract.. The result recorded a total number of 250 eggs (a batch of mosquito eggs) which were treated with different concentration of the extract. Results consistently showed higher percentage efficacy values at all concentrations of the extraction with higher percentage efficacy (95.6%) at 50mg/ml and (99.2%) 50mg/ml. This showed that the higher the concentration, the higher the percentage of efficacy.

Larval Stage:

The results of the larvicidal effect of *Hyptis suaveolens* of leaf extract are shown in Table 3. It was observed that 500mg/ml concentration of the extract killed 51.3%. At lower concentration (50mg/ml), the extract exhibited percentage mortality rate of 15.0%. This also showed that the higher the concentration of the extract (mg/ml), the higher the percentage mortality rate. (Table 3.)

Pupal stage:

The results of pupacidal effect of dried leaf extract of *Hyptis suaveolens* is presented in Table 4 below. It can be seen from these results that 12.5% mortality rate was recorded when the pupae were treated with 50mg/ml extract while at 500 mg/ml, the rate was 43.8%. This showed that the higher the concentration of the extract, the higher the mortality rate.

Adult stage:

Table 5 shows the results of the effect of dried lea extract *of Hyptis suaveolens* on adult mosquitoes. At lower concentration (50 mg/ml), of the extract, much lower percentage mortality rate (5.0 %) was recorded, compared with larval and pupal stages. At 500 mg/ml concentration, 51.3 % was recorded. This was similar to the above two stages, larval and pupal (51.3% and 43.8% respectively). The patterns of the effect were similar throughout the treatments, that is, effect was directly proportional to concentration, as previously stated. It can be seen, however, that at lower concentration, the degree of the extract effect on adult mosquitoes is lower when compared to the larva or pupa or even the eggs at high concentrations.

The Phytochemical Analysis of the dried leaf extract of *Hyptis suaveolens* .

The phytochemical properties in *Hyptis suaveolens* plant extract showed the presence of tannins, steroids, terpenoids, saponins, pholbatannins and cardiac glycosides. Flavonoids were not detected. Steroids, terpenoids, saponins and phlobatannins were in moderate amounts while tannins and cardiac glycosides were in high amounts. These results agree with those reported by Egunyomi *et al.,* (2010). They, however, used different solvent systems (hexane and methanol) and obtained some bioactive ingredients which were not detected in this present study. This study also confirmed the research work of Lawal *et.al.* (2012), who used fresh leaf extract with different solvents such as ethanol, polyethylene glycol and water for extraction.

IV. Discussion

Ethnobotanical Effect of the Extract on the Developmental Stages of Mosquitoes.

The higher percentage mortality rate of larvae and pupae as compare to adult stage could be because, mosquitoes in their developmental stages are good target for pesticides. As mosquito vectors generally breed in water, it could be easy to target their different stages of development. (Arivoli, 2012). Both the present and past studies inferred that the solvent types selected for the extraction processes may also influence the efficacy of the extract (Shaalam *et al.,* 2005). Similarly, Mulla and Su (1999) had also reported that ethanolic extract of both *Ruta graveolens* and *Haplophyllum tuberculatum* were more toxic against *culex pipiens* than petroleum ether extracts. Apart from the varying volatility, it has been shown that the polarity of the solvents may also affect the effectiveness of the extract (Mulla and Su, 1999 and Shaalam *et al*., 2005). From the results of this present study, it can be seen that, the effect of this extract at 50mg/ml effectively destroyed the eggs and prevented them from hatching (96.6%), it had much lower effect on the larvae (15.0%), pupae (12.5%) and adult (5.0%). The results of the effect on adult mosquitoes were unexpected. This is because the plant is known and has been reported to be mosquitocidal as similarly reported by Hemen*etal*.,(2013).

One explanation for these results could be sample type. *Hyptis suaveolens* has very strong aromatic property which is found to be due to the presence of certain essential oils. Some of the secondary metabolites that are lipid-like are found in the essential oils. Much of these are lost usually with drying especially if exposed to sunlight. It is possible that some of the active constituents were lost during drying. Though the leaves were shade dried, it is also possible that some immature leaves were included, thereby lessening its effectiveness on the Laval, pupal and even the adult mosquitoes. Mature leaves have a stronger aroma than young leaves. A second possibility may be due to the parts of the plants used. Perhaps, the whole plant might have been more effective since the constituents that contribute to strong aroma found, for example, in the stem or root, may be lost at a slower rate during drying. Yet another reason could be attributed to the nature of the sample used for extraction, that is, fresh versus dried leaves.

This factor, however, may not generally apply, due to differences between the active ingredients in the plants. The Mosquitocidal effect of the *H. suaveolens* leaf extract at higher concentration on the adult mosquitoes was significant ($p<0.05$) compared with lower concentrations. The resultant efficacy of the extract against the developmental stages most especially the adult stage of mosquito could be as the result of the bioactive ingredients in the plant extract as it was reported to have an extensive repellent activity on Mosquitoes as vectors (Cavalcantic *et al*., 2004; Hemen *et al*., 2013).

Table 1 Phytochemical Constituents of the Leaves of *Hyptis suaveolens*.

Phytochemical Constutient	Status
Flavonoids	-
Tannins	+++
Steroids	++
Terpenoids	++
Saponins	++
Phlobatannins	++
Cardiac glycosides	+++

Note:
- = not present
++ = present in moderate concentration (amount)
+++ = present in high concentration (amount)

Table2 Ovicidal Effect of Dry Leaves Extract of *Hyptis suaveolens*

Concentration (Mg/ml)	Number of Eggs in batches			% Efficacy
	Observed	Destroyed	Hatched	
0 (control)	250	5	245	2.0
50	250	239	11	95.6
100	250	240	10	96.0
150	250	242	8	96.8
200	250	243	7	97.2
250	250	244	6	97.6
300	250	245	5	98.0
350	250	245	5	98.0
400	250	247	3	98.8
450	250	248	2	99.2
500	250	242	-	99.2
Total	**2750**	**2446**	**304**	

Table 3: Larvicidal Effect of Dried leaves Extract of *Hyptis suaveolens*.

Concentration (mg/ml)	Number of larvae			% Mortality rate
	Observed	Survived	Destroyed	
0 (control)	80	80	-	-
50	80	68	12	15.0
100	80	66	14	17.5
150	80	63	17	21.3
200	80	61	19	23.8
250	80	58	22	27.5
300	80	54	26	32.5
350	80	51	29	36.3
400	80	48	32	40.0
450	80	45	35	43.8
500	80	39	41	51.3
Total	**880**	**633**	**247**	

Table 4: Pupacidal effect of Dried leaves Extract *of H. suaveolens*.

Concentration of extract (mg/ml)	Number of pupae		Mortality rate	% mortal
	observed	survived		
0 (control)	80	80	-	-
50	80	70	10	12.5
100	80	68	12	15.0
150	80	64	16	20.0
200	80	62	18	22.5
250	80	60	20	25.0
300	80	57	23	28.8
350	80	53	27	33.8
400	80	51	29	36.3
450	80	48	32	40.0
500	80	45	35	43.8
Total	**880**	**658**	**222**	

Table 5: Effect of *H. suaveolens* Dried Leaves Extract on Mosquito Adults

Concentration observed	Number of adult mosquitoes			% Mortality rate	(mg/ml)
	survived	Dead			
0 (control)	80	80	-	-	
50	80	76	4	5.0	
100	80	74	6	7.5	
150	80	73	7	8.8	
200	80	68	9	15.0	
250	80	68	12	15.0	
300	80	62	12	22.5	
350	80	59	18	26.3	
400	80	56	21	30.0	
450	80	52	24	35.0	
500	80	39	26	51.3	
Total	**880**	**635**	**245**		

Note: (Control) = Distilled water; = Nil

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