EVALUATION OF OVICIDAL AND LARVICIDAL EFFECTS OF LEAF EXTRACTS OF *Hyptis suaveolens* (L) POIT (*LAMIACEAE*) AGAINST *Anopheles gambiae* (DIPTERA: ANOPHELIDAE) COMPLEX

IVOKE, Njoku, OKAFOR, Fabian Chukwuemenam and OWOICHO, Laura Onyi
Parasitology Unit, Department of Zoology, University of Nigeria, Nsukka, Nigeria

Corresponding Author: Ivoke, N. Parasitology Unit, Department of Zoology, University of Nigeria, Nsukka, Enugu State. **Email:** njokuivoke@yahoo.com **Phone:** +234 8039524949

ABSTRACT

The female Anopheles gambiae s.l is the principal_intermediate host/vector of Plasmodium - the causative organism of malaria fever in many tropical countries. After a preliminary acute toxicity screening of leaf extracts of Hyptis suaveolens using descending series of concentrations (1000, 500, 100, 50 and 5 µg/ml), two extracts (aqueous and ethanolic) were assayed against freshly laid eggs and larval instars of An. gambiae following the standard World Health Organisation ovicide and insecticide susceptibility bioassay methodology. The results indicate that both ethanolic and aqueous extracts of the test plant significantly (F = 30.23, df = 5, p < 0.01; F = 45.28, df = 5, ρ < 0.01 respectively) reduced the viability of the An. gambiae ova exposed to different doses of the extracts. In the ovicidal assay the lethal inhibition doses of egg hatching (IH50) was 31.52 and 48.01/µg/ml respectively for the ethanolic and aqueous extracts. Also at a dose of 82.5 µg/ml the ethanolic extract completely inhibited An. gambiae hatching whereas the aqueous extract could inhibit only 70.42% egg hatching at the same dose. The results further indicate that both ethanolic and aqueous extracts also exhibited larval median lethal toxic (LC_{50}) values of 62.41 (range 61.22 – 67.04) and 80.02 (range 77.55 – 86.41) respectively thus making both extracts candidates for further fractionation and compound isolation studies to characterize the active phytochemical constituents.

Keywords: Malaria, *Hyptis suaveolens*, Leaf extracts, Bioassay, Ovicidal, Larvicidal, *Anopheles gambiae*

INTRODUCTION

Mosquitoes are the most important vectors of certain human infections and diseases. Mosquito-borne diseases such as malaria, yellow fever, filariasis and dengue contribute significantly to mortality in most tropical countries. Among these diseases malaria continues to dominate the public health spectrum especially in Africa continent where *Anopheles gambiae* sensu latu (Diptera: Anophelidae) complex occurs in endemic regions. This species complex is the vector of *Plasmodium*, the aetiologic organism of malaria fever which annually kills between 1.4 to 2.7 million of the estimated 300 – 500 million clinical cases (Snow *et al*., 1999; Wirth, 2003). Most of these deaths occur among African children and pregnant women living in endemic countries of sub-Saharan countries of stable malaria transmission.

The control of mosquito-borne diseases is however becoming increasingly difficult because the effectiveness of vector control has declined due to development of resistance by vectors against the currently used but toxic and environmentally persistent 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).

In the absence of effective prophylactic vaccine against most of the mosquito-borne diseases, it is desirable to find compounds that can effectively control mosquitoes with minimal damage to the environment. Naturally-occurring phytocompounds that are rich sources of bioactive chemicals appear to be the most likely candidates for the environmentally safe and degradable products targeted specifically against mosquitoes. So far research workers have identified a wide variety of plant species from various ecosystems that have produced a range of acute and chronic toxic effects against mosquitoes (Shaalam *et al*., 2005). Currently more than 2000 plant species have been identified as having insecticicidal properties and about 344 plant products are known to possess anti-mosquito characteristics (Sukumar *et*

al., 1991). Several researchers have also demonstrated ovicidal and larvicidal activities of various plant extracts (Lee, 2000; Kabaru and Gichia, 2001; Ivoke, 2005; Chansang *et al.*, 2005).

Hyptis suaveolens is a stocky tropical aromatic herb (common name = wild hops) of the mint family *Lamiaceae* (formerly *Labiatae*) possessing creeping underground stem and hairy opposite leaves (Oliver-Bever, 1986). The stem possesses flowers that are nearly always two-tipped, the petals being united to form a corolla tube bearing two or four stamens. The sepals form a tubular calyx which persists to surround the fruit which is usually a group of four nutlets. There are two carpels, the style arising from the base and dividing at the tip into two stigmas. In Nigeria *H. suaveolens* is of high economic and medicinal value and is employed widely for it's mosquito-repellent property. In addition the essential oil from the leaves have demonstrated antifertility and irritant properties (Oliver-Bever, 1986; Zebovitz, 1989).

This study was designed specifically to investigate the effects of aqueous and ethanolic leaf extracts of *H. suavoelens* on the eggs and larvae of *An. gambiae* complex vector of *Plasmodium* in Nsukka, Nigeria.

MATERIALS AND METHODS

Preparation of Plants Extracts: *Hyptis suaveolens* plants were collected around the Botanical, Zoological Gardens and Faculty of Veterinary Medicine premises all within the Nsukka campus of University of Nigeria. The identity of the herb was confirmed by Professor O.C. Nwankiti of the Department of Botany, University of Nigeria, Nsukka. The *Hyptis* plants were harvested, taken to the laboratory where the mature leaves were plucked, dried under shade and ground into powder.

Aqueous extract of the powdered leaves was prepared by blending 100g of the leaf powder with 500ml distilled water in a food blender. After 24 hours, the mixture was filtered and the filtrate collected and stored in an all-glass reagent bottle as stock solution for subsequent use in preparation of other aqueous dilutions.

Ethanolic extract was prepared by blending 100g of crushed and powdered leaves of *H. suaveolens* with an aliquot 100ml of ethanol (analytical). After 24 hours, ethanol was decanted and the residue extracted again with 2 aliquots of 100ml of ethanol. The combined filtrate was concentrated to dryness using a rotary evaporator. The dried extract was then weighed and reconstituted in ethanol. The reconstituted ethanolic extract was stored in an all-glass bottle and kept as stock solution.

Source of Mosquito Eggs and Larvae: Mosquito ovipositing sites used for the study consisted of 10 dark-coloured disposable plastic containers each measuring 60 × 30 × 20 cm. Each of the containers was filled with tap water and placed at different shady positions within human living quarters. The containers were observed daily and covered during the daylight hours for protection but uncovered at dusk. Water in the rearing containers was changed at 2-day intervals. The ova of the *Anopheles gambiae* were obtained from the naturally occurring wild stains reared in these plastic containers. The larvae of *An. gambiae* were similarly harvested and bred in plastic containers filled with tap water and fed *ad libitum* at 5 % yeast suspension (larval food). Before experimentation, normal hatching of the mosquito eggs was observed by placing some eggs in a plastic pan (20 cm) containing 1.0 litre of tap water and pellets of 5 % yeast suspension to serve as food for the larvae. It was observed that normal egg hatching occurred within two days.

Bioassay: In preliminary acute toxicity tests crude aqueous and ethanolic solutions were first screened in a descending series of concentrations (1000, 500, 100, 50 and 5 mg/ml) to identify the lowest dose that inhibited 100 % of the mosquito eggs from hatching. Concentrations that caused 100 % inhibition in 500 mg/ml or less were selected for further testing to calculate the median lethal inhibition dose (IH_{50}) of egg hatching. Standard methodology was followed in the determination of the lethal inhibition doses of egg hatching and larval mortality (LC_{50}) (WHO, 1986).

For bioassay, the methods of Rui-de Xue *et al.* (2000) were followed. All bioassays were conducted at ambient temperature of 29 ± 2 ºC, 80 ± 5 % relative humidity under a photoperiod of 13:11 hour light: dark cycles. Controls were prepared with 100ml of distilled water only. The eggs and larvae belonging to the peridomestic populations of *An. gambiae* were carefully recovered from the rearing containers with fine soft brush each morning, identified and counted under the low power of a binocular microscope.

Twenty (20) freshly laid *An. gambiae* eggs were placed into separate 200 ml disposable plastic cups each containing 100 ml of distilled water. Eggs in both the experimental and control cups were sourced from different rearing plastic containers to avoid the effect of differently reared batches of eggs and larvae (WHO, 1996). Five serial dilutions (10, 20, 30, 40 and 50 mg/ml of each of the aqueous and

ethanolic extracts) were made from the stock solutions. For treatment, 1ml of each concentration (serial dilutions) of each extract was added to a series of five cups, one cup maintained as control received 1ml of distilled water. Treatments were replicated 10 times. The content of each test cup was stirred gently with a glass rod to ensure homogeneity. Percentage of egg viability and larval mortality was calculated by dividing the number of larvae that emerged from the eggs and / or died 24 and 48-h after treatment by the total number of eggs laid. Abbot (1925) formula was employed to correct percentage viability of eggs if control inhibition of egg hatching was between 5 % and 20 %.

For larval bioassay aqueous and ethanolic extracts of *H. suaveolens* were evaluated at the levels of 20.0, 40.0, 60.0, 80.0, 100.0 and 120.0 mg/ml in distilled water. Distilled water only was similarly used as control as in the ovicidal bioassay using the WHO (1996) test procedures. Twenty larvae were put into each of 10 cups containing 100ml of the test solution of each concentration.

Larval mortalities were counted at 24 hours after treatment. Mortality served as the end point for the tests and the results were used to determine the LC_{50} value for the extract. The LC_{50} is defined as the lethal concentration of the bioactive extract that kills 50 % of the test species. Larvae were considered either alive (clearly moving normally) or dead (no movement and no response to gentle probing with a fine glass rod three times, 10 seconds each).

During the experimental period food was not available to the larvae. Toxicity and activity were reported as LC_{50} and LC_{90} representing the concentrations in microgram per microlitre that caused 50 and 90 % larval mortality after 24 hours.

Data Analysis: Probit regression program in SPSS version (SPSS Inc. Chicago, IL) was used to evaluate the lethal inhibition dose of mosquito egg hatching (IH_{50}) and the corresponding 95% fiducial limit. A 2 × 5 × 1 factorial split-plot design (Steel and Torrie, 1980) was employed for analysis of the larval data. Factor 1 consisted of two treatment materials (aqueous and ethanolic extracts), Factor 2 was five application concentrations (0.1, 0.05, 0.01, 0.005 and 0.001%) of each extract, and factor 3 was one exposure period (24 hours) of the *An. gambiae* larvae to the test extracts. A computer-based probit analysis (Finney, 1971) was used to analyse dosage response of the larvae to the test materials. ANOVA (Analysis of variance) was used to determine whether there was a significant difference between the LC_{50} values obtained for the aqueous and ethanolic solvents.

RESULTS AND DISCUSSIONS

The primary acute toxicity tests of the two extracts of *H. suaveolens* to the eggs of *An. gambiaese* is summarized in Figure 1. The freshly laid eggs of the insect showed a somewhat similar susceptibility response to the two test materials with an inhibition to egg hatching (IH_{90}) range of 0.040 % (ethanolic extract) to 0.073 % (aqueous extract) although the ethanolic extract appeared to be more toxic to the eggs as indicated by the corresponding IH_{50} and IH_{90} values. Probit linear regression analysis performed on the data showed that ethanolic and aqueous extracts of the test plant significantly ($F= 30.23$, $df= 5$, $\rho < 0.01$; $F = 45.28$, $df= 6$, $\rho < 0.01$, respectively) reduced the viability of the eggs of *An. gambiae* exposed to the different doses of the extracts (Figure 1). Ethanolic extracts completely inhibited egg hatching at a dose of 82.5/µg/ml whereas the aqueous extract of the same dose could inhibit only 70.42 % eggs hatching (Figure 1). The lethal inhibition dose of egg hatching (IH_{50}) was 31.52 and 48.01 µg/ml for the ethanolic and aqueous extracts respectively.

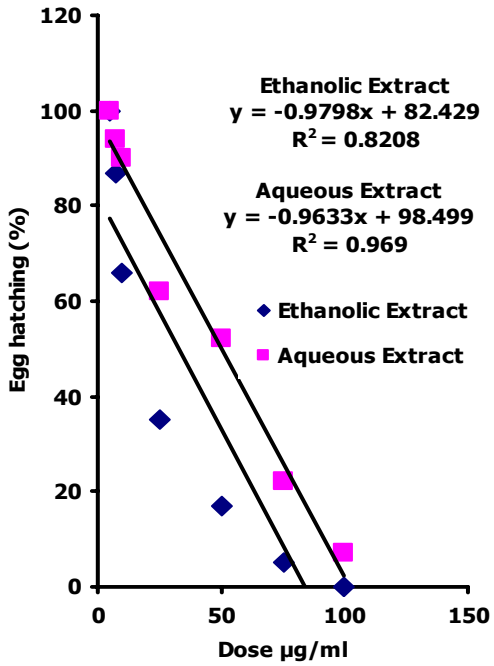

Figure 1: Relationship of percentage of egg hatching of *Anopheles gambiae* eggs to doses of aqueous and ethanolic extracts of *Hyptis suaveolens*

Thus although the aqueous extract appeared to exhibit similar ovicidal efficacy against *An. gambiae*, ethanolic extracts appeared to outperform the aqueous extract as indicated by the slope (0.13 : 0.3)

making both extracts candidates for further investigation.

The lethal toxicity doses of both aqueous and ethanolic extracts to the larvae of the test mosquito are shown in Figure 2. Both the ethanolic and aqueous extracts exhibited LC_{50} values of 62.58 (range 61.22 — 67.04) and 80.02 (range 77.55 — 86.41) respectively. The dose-response curves for the two extracts were similar but differed by a magnitude of approximately 10. This suggests that the two extracts either contain similar active ingredients or possess a similar mode of action, but with different ovicidal and larvicidal intensity. In addition, the dose-response results indicated that the two botanical extracts had good performance when compared with *Azadirachta indica* and *A. Juss*. (LC_{50} = 57.1 mg/litre) both which are considered to be the most potent phytochemical pesticide when tested against larvae of *An. gambiae* s.l. with synthetic tetranortriterpenoids as control (Ndungu *et al*., 2004).

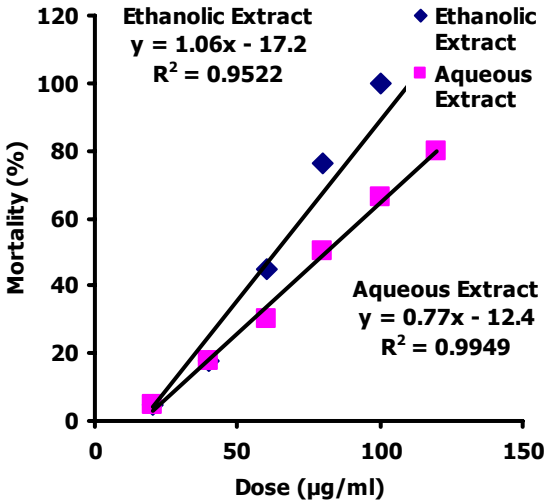

Figure 2: Dose-response relationship of the larval mortrality of *Anopheles gambiae* exposed to aqueous and ethanolic extracts of *Hyptis suaveolens*

Generally, the results of the study further confirmed that the efficacy of botanical extracts against developmental stages of mosquitoes may be influenced by factors such as extraction solvent (Shalaby *et al*., 1998). It may therefore be inferred from the results of the present study that the solvent types (ethanol and water) selected for the extraction processes affected the efficacy of the extract because it has been demonstrated that different phytochemicals of varying volatility usually constitute the final extract (Shaalam *et al*., 2005). Earlier, Mulla and Su (1999) had reported that ethanol extracts of both *Ruta graveolens* and *Haplophyllum tuberculatum* (LC_{50} = 230 and 20 ppm respectively) were more toxic against *Culex pipiens* than petroleum ether extract (LC_{50} = 380 and 87 ppm respectively). Apart from the varying volatility, it has been shown that the polarity of the solvents can also affect the effectiveness of the extract (Mulla and Su, 1999; Shaalam *et al.,* 2005) although this factor generally does not apply due to differences between the active ingredients in the plants. In this study it would appear that the ethanol solvent extracted more of the efficacious phytochemicals possessing ovicidal and larvicidal potentials from *H. suaveolens* than did the aqueous solvent. In addition, the extraction process with specific but different solvents may exert a great influence on the resultant bioactivity due to the polarity range of the solvents (Sukumar *et al*., 1991; Napolitano *et al.,* 2005). Finally it could be inferred from our results that aqueous and ethanolic extracts of *H. suaveolens* besides possessing potent ovicidal qualities may equally be utilized as larvicides for purposes of controlling instars of *An. gambiae* and possibly other dipterous arthropod vector populations. However, further fractionation and compound isolation studies are needed to identify the active phytochemical components of the extracts. The plant extracts thus offer the promise of the creation of another effective and affordable approach to the control of insect vectors.

REFERENCES

ABBOT, W. S. (1925). A method of computing the effectiveness of an insecticide. *Journal of Economic Entomology,* 18: 256 – 257.

CHANDRE, F., DARDER, M., CAUNY, A., DUANNIO, J. M. C., PASTEUR, N. and GUILLET P. (1998). Pyrethroid resistance in *Culex quinquifasciatus* from West Africa. *Medical Veterinary Entomology,* 12: 359 – 366.

CHANGSANG, U., ZHAIRI, N. S., BANSIDDHI, J., BOONRUAD, T., THONGSRIRAK, P., MINGMUANG, J., BENJAPONG, N. and MULLA, M. S (2005). Mosquito larvicidal activity of extracts of long pepper (*Piper retrofractum* Vab) from Thailand. *Journal of Vector Ecology,* 30: 195 – 200.

DOLIANITIS, C. and SINCLAIR, R. (2002). Optimal treatment of head lice: is a no-nit policy justified? *Clinical Dermatology,* 20: 94 – 96.

FINNEY, D. J. (1971). *Human lice: their prevalence, control and resistance to insecticides: a review 1985 – 1997.* World Health Organization, Geneva, Switzerland. WHO/CTP/WHOPES/97.8

GRATZ, N. G. (1997). *Human lice: their prevalence, control and resistance to insecticides: a review 1985 – 1987.* World Health

Organization, Geneva, Switzerland. WHO/CTP/WHOPES/97.8

IVOKE, N. (2005). Preliminary studies on the efficacy of *Aloe vera* (*Aloe barbadensis*) extracts in experimental *Trypanosoma brucei bucei* infection of mice. *Bio-Research,* 3(1): 21 – 25.

KABARU, J. M. and GICHIA, L. (2001). Insecticidal activity of extracts derived from different parts of the mangrove tree (*Rhizophora mucronata* (Rhizophoraceae) Lam against three arthropods. *African Journal of Science and Technology,* 2: 44 – 49.

LEE, S. E. (2000). Mosquito larvicidal activity of pipernonaline, a piperidine alkaloid derived from long pepper, *Piper longum*. *Journal of American Mosquito Control Association,* 16: 245 – 247.

MULLA, M. S. and SU, T. (1999). Activity and biological effects of neem products against arthropods of medical and veterinary importance. *Journal of American Mosquito Control Association,* 15: 133 – 152.

NAPOLITANO, D. R., MINCO, J. R., SOUZA ,M. A., DE PAULO ,J. E., ESPINDOLA, L. S. and ESPINDOLA, F. S. (2005). Down modulation of nitric oxide production in marine macrophages treated with crude plant extracts from the Brazilian Cerrado. *Journal of Ethnopharmacology,* 99: 37 – 41.

NDUNG'U, M. W., KAONEKA, B., HASSANLI, A., LWANDE, W., HOOPER, A. M., TAYMAN, F., ZERBE, O. and TORTO, B. (2004). New mosquito larvicidal tetranortriterpenoids from *Terraea wakefieldii* and *Terraea floribunda. Journal of Agriculture and Food Chemistry,* 52: 5027 – 5031.

OLIVER-BEVER, B. E. P. (1986). *Medicinal plants of tropical Africa.* Cambridge University Press, London.

PENILLA, P. R., RODRIGUEZ, A. D., HEMMINGWAY, TORRES, J. L., ARREDONDU-JIMENEZ, J. I. and RODRIGUEZ, M. H. (1998). Resistance management strategies in malaria vector mosquito control: baseline data for a large-scale field trial against *Anopheles albimanus* in Mexico. *Medical Veterinary Entomology,* 12: 217 – 233.

SHAALAM, E., CANYON, D. V., YOUNES, M., ABDEL-WAHAB, M. and MANSOUR, A. (2005). A review of botanical phytochemicals with mosquitocidal potential. *Environment and International Health,* 15: 1149 – 1166.

SHALABY, A. A., ALLAM, K. A., MOSTAFA, A. A. and FAHMY, S. M. (1998). Insecticidal properties of citrus oils against *Culex pipiens* and *Musca domesticus. Journal of Egyptian Science and Parasitology,* 28: 595 – 606.

SNOW, R. W., CRAIG, M., DEICHMANN, U. and MARSH, K. (1999). Estimating mortality, morbidity and disability due to malaria among Africa's non-pregnant population. *Bulletin of World Health Organisation,* 77: 624 – 640.

STEEL, R. D. and TORRIE, J. H. (1980). *Principles and procedure of statistics. A biometrical approach.* Second Edition, McGraw-Hill, New York.

SUKUMAR, K., PERICH, M. J. and BOOBAR, L. R. (1991). Botanical derivatives in mosquito control: a review. *Journal of American Mosquito Control Association,* 7(2): 210 – 237.

WIRTH, D. (2003). *Plasmodium falciparium:* A genome revealed. *In:* Report of the WHO Scientific Working Group Meeting on Malaria. Geneva, Switzerland

WHO (1986). *Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides.* World Health Organization, Mimeograph Document, WHO/ VBC / 81.807, Geneva, Switzerland.

WHO (1992). *Vector resistance to pesticides. Fifteenth Report of the World Health Organization Expert Committee on Vector Biology and Control.* Geneva, WHO Technical Report Series No 818

WHO (1996). *Report of the World Health Organization informal consultation on the evaluation and testing of insecticides*. CTD/ WHOPES/IC/96, World Health Organization, Geneva, Switzerland.

ZEBORITZ, T. C. (1989). Flavour and fragrance substances. In: KEITH L. H. and WALTERS D. B. (Eds.) *Compendium of safety data sheets for research and industrial chemicals.* VCH Publishers, New York.