East and Central African Journal of Pharmaceutical Sciences.
Vol. 4 (2001) 39 - 42

Mosquito Larvicidal Activity of Four Tanzanian Aromatic Plants

O. NGASSAPA[1*], S.M. MAREGESI[1], A.R. MAJALA[2]

[1]*Department of Pharmacognosy, Faculty of Pharmacy, Muhimbili University College of Health Sciences, P.O. Box 65013, Dar-es-Salaam, Tanzania.*
[2]*Department of Parasitology and Entomology, Institute of Public Health, Muhimbili University College of health Sciences, Dar-es-Salaam, Tanzania.*

As part of continuing efforts to search for pesticides from plants, four essential oil-containing plants were screened for mosquito larvicidal activity in the laboraroty. The plants included *Curcuma longa* L. (Zingiberaceae), *Hyptis suaveolens* Poit. (Labiatae), *Piper nigrum* L. (Piperaceae) and *Zingiber officinale* Roscoe (Zingiberaceae). Third to fourth instar larvae of Anopheles gambiae and *Culex quinquefasciatus* were exposed to various concentrations (0.32 – 200 ppm) of methanol extracts and essential oils from these plants. Mortality was assessed after a 24-hour exposure period and LC_{50} values were determined by probit analysis. Of all extracts and oils, the essential oil of *H. Suaveolens* was found to be the most active against both types of larvae, followed by *P. nigrum* extract. The methanol extract of *H. Suaveolens* was the least active. *Anopheles gambiae* larvae were, generally, more susceptible than *Cx. Quinquefasciatus* larvae. These preliminary findings have shown the potential of these plants for development as agents for the control of malaria and other mosquito-transmitted diseases.

Key Words: *Anopheles Gambiae*, aromatic plants, *Culex quinquefasciatus*, larvicidal activity

INTRODUCTION

Malaria and other mosquito-transmitted diseases, such as lymphatic filariasis, dengue hemorrhagic and yellow fevers, are major problems in many tropical countries. The vectors of such diseases are mosquitoes mainly of the genera *Anopheles*, *Aedes* and *Culex*. Mosquito control methods include the use of repellents for personal protection, insecticides and larvicides to interrupt their life cycle. In an effort to search for new, effective and environmentally friendly mosquito control agents, four aromatic Tanzanian plants were selected for larvicidal screening against mosquito larvae.

Previous studies have shown that a number of aromatic plants possess pesticidal properties. For example, *Piper decurrens* and *Piper guan-acastensis* were reported to have pesticidal activity against mosquito larvae and compounds responsible for activity were isolated from the two plants [1,2]. Some plants from the family Zingiberaceae were found to be insecticidal against food storage pests [3] and mosquito larvae [4]. Such studies formed the basis for inclusion, in the present study, of four aromatic plants, namely, *Piper nigrum* L. (Piperaceae), *Hyptis suaveolens* Poit. (Labiatae), *Curcuma longa* L. and *Zingiber officinale* Roscoe (Zingiberacea). These plants are distributed in various parts of Tanzania, where they are used medicinally or for culinary purposes.

MATERIALS AND METHODS

Plant Materials

Fresh rhizomes for *Curcuma longa* (turmeric) and *Zingiber officinale* (ginger) and dried fruits of *Piper nigrum* (black pepper) were purchased from Kariakoo Market, in Dar es Salaam, Tanzania. Leaves of *Hyptis suaveolens* were collected in Temeke district, Dar es Salaam. The plant materials were authenticated by the Institute of Traditional Medicine, Muhimbili University College of Health Sciences (MUCHS). A voucher specimen of *H. sauveolens* is deposited in the

herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, MUCHS.

Extraction of Plant Materials

For solvent extraction purpose, rhizomes of *C. longa* and *Z. officinale* were sliced, air-dried in the shade, and then in a hot-air oven, at 50° C, just before grinding. Leaves of *H. sauveolens* were air-dried, while fruits of *P. nigrum* were purchased in dry form. All dried plant materials were ground to a course powder, then 50 g of each was extracted with 200 ml of methanol, by maceration at room temperature for 24 hours, with occasional shaking. Extracts were decanted, filtered and the solvent was removed using a rotary evaporator, under reduced pressure at 50 °C. Extracts were kept in a refrigerator, at about 4 °C until when needed for larvidical testing.

Extraction of Essential Oils

Powdered dried fruits of *P. nigrum,* fresh leaves of *H. suaveolens* and fresh, sliced rhizomes *of C. longa* and *Z. officinale* were utilized for the extraction of essential oils. The oils were obtained by hydro-distillation, using a clevenger-type apparatus. The oils, were collected over distilled water, separated, dried over anhydrous sodium sulphate and stored at 4°C.

Preparation of Test Solutions

Each extract or essential oil was initially dissolved in ethanol to make a stock solution at a concentration of 40 mg/ml of test substance. Dilutions were made with an appropriate volume of distilled water to make the required test concentrations. Initially, extracts were tested at 200 ppm (ug/ml), and for the determination of concentrations which caused 50 % larvae mortality (LC_{50}), two- of five-fold serial dilutions were prepared for extracts/oils exhibiting at least 50% larval mortality at 200 ppm. The final test concentrations ranged from 0.32 ppm to 200 ppm.

Larvicidal Assay

Third and fourth instar *Anopheles gambiae* and *Culex quinquefasciatus* larvae were used in this study. *Anopheles gambiae* larvae were obtained from a laboratory-reared strain of the mosquito, maintained in the insectary unit of the Department of Parasitology and Entomology, Institute of Public Health (IPH), MUCHS. *Culex quinquefasciatus* larvae were collected from water pools within the Muhimbili Medical Centre, in Dar es Salaam and were identified by the staff of the Department of Parasitology and Entomology, IPH. They were allowed to aclimatize to laboratory conditions for 24 hours prior to performing larvicidal tests. For preliminary screening at 200 ppm, *Cx. quinquefasciatus* larvae were used, due to their ready availability, whereas, for tests at serial dilutions, both species of larvae were utilized. Tests were performed at a temperature of 26 – 29° C and a relative humidity of about 70 %.

The larvicidal assay involved exposure of at least ten larvae to 100 ml extract solution in a 250 ml beaker. Mortality was assessed after an exposure period of 24 hours. A beaker containing an equal volume of 0.5 % v/v aqueous ethanol served as a control. Tests were performed in duplicate and were exposed to each concentration. Larvae mortality was determined after correction for control using the Abbott's formula [5]. Tests in which control mortality exceeded 10 % were discarded. The LC_{50} values (24 hours) were determined by probit analysis, using a log-probit paper.

RESULTS AND DISCUSSION

This study has revealed that the essential oils and extracts of all four aromatic Tanzanian plants possess larvicidal activity against both *Culex quinquefasciatus* and *Anopheles gambiae* larvae. The activity varied among the plant species, between oils and extracts from same plants and between the two species, between oils and extracts from same plants and between the two species of test organisms. Table 1 gives a summary of the results.

The essential oil from *Hyptis suaveolens* oil was the most active of all, against both mosquito species, while the extract from the same plant was the least active. In general, volatile oils were more active than the corresponding extracts, except for *Piper nigrum*, for which the extract was

about fourteen times more active than the essential oil against *Cx. quinquefasciatus* larvae. These results suggest that the chemical constituents present in the oils and their proportions may be different from those present in extracts obtained from the same plants. In addition, the methanol extracts usually are mixtures of polar and non-polar, volatile substances. Compounds in such complex mixtures could exert either synergistic or antagonistic effects, resulting into higher or lower percent mortality, respectively, than that observed for volatile oils.

TABLE 1: Larvicidal activity of extracts and essential oils

Test material	C. quinquefasciatus	A. gambiae
	LC_{50} ppm	LC_{50} ppm
C. longa		
Extract	33.5	53.2
Oil	12.3	38.9
H. suaveolens		
Extract	>200.0	>200.0
Oil	0.9	0.9
P. nigrum		
Extract	1.1	0.9
Oil	15.0	-
Z. officinale		
Extract	17.5	22.4
Oil	46.8	33.8

- Not tested

The observed larvicidal activity, further supports previous studies, which revealed that volatile oils, such as those from citrus peels [6-8] and *Ocimum* species [9] were active against mosquitoes. Previous studies have also shown that some Piper species had pesticidal effect on mosquito larvae, which was linked to neolignans [1] and butenyl benzoate compounds [2]. *Hyptis suaveolens* is a promising pest control agent, since the oil was very active in this study. Previously, the smoke resulting from burning the leaves of this plant was found to be repellent to mosquitoes [1], indicating that the plant could also be used as a repellent. Other studies on related species, demonstrated that diterpenoids were responsible for their insecticidal properties, against animal and crop pests [11,12]. However, in this study the extract of *H. suaveolens*, was inactive. This could be due to the fact that the test organisms used in the two studies were different, and that the active compounds in those plants may be completely different form those present in *H. suaveolens*.

Members of the Zingiberaceae were reported to have insecticidal activity against food storage pest-insects [3]. The active constituents of *Curcuma* species, which are members of this family, were found to be sesquiterpenoids and the diaryheptanoid, curcumin. Similar types of compounds could be responsible for the larvicidal effect of *C. longa* extract. Recently, leaves and tubers of a related species, *Curcuma raktakanda* were found to be larvicidal against mosquito larvae [4]. The activity was found to be associated with the non-polar extract of the plant. Thus, the findings in the present study further imply that the genus *Curcuma* is of potential value in the control of mosquito populations.

Regarding the susceptibility of the test organisms, A. *gambiae* larvae were, generally, slightly more susceptible to substances under test, when compared with *C. quinquefasciatus*, except for *curcuma longa* oil and extract, to which it was, vise versa. The difference is susceptibility could be due to differences in the genetic make-up of the organisms, as well as environmental factors. *A. gambiae* larvae were obtained from a laboratory-reared strain, while *C. Quinque-fasciatus* larvae was field-collected. The latter might have been adapted to the harsh environmental conditions, as a result of frequent pesticide application. This study has shown that the four aromatic plants under study have the potential for development of mosquito control agents.

ACKNOWLEGEMENTS

The authors acknowledge the technical assistance of Ms. E. Kategere and Ms. E. Salaba of the Department of Pharmacognosy, Faculty of Pharmacy, MUCHS and Mr. C. Membi of the Insectary Unit, Department of Parasitology and Entomology, IPH, MUCHS.

REFERENCES

[1] D.C. Chauret, C.B. Bernard, J.T. arnason, T. Durst, H.G. Krishnamurty, P. Sanches-Vindas, N. Moreno, L. San Roman and L. Poveda, J. Nat. Prod. 59 (1996) 152-155.

[2] R. Pereda-Miranda, C.B. Bernard, T. Durst, J.T. Arnason, P. Snches-Vindas, L. Poveda and L. San Roman, J. Nat. Prod. 60 (1997) 282-284.

[3] C. Pandji, P. Grimm, V. Wray, L. Witte and P. Proksch, Phytochemistry 34 (1993) 415-419.

[4] C. Latha and J. Ammini, Pharm. Biol. 38 (2000) 167-170.

[5] O. Ngassapa, D.J. David and A.R. Majala. East Cent. Afri. J. Pharm. Sci., 2 (1999) 60-63.

[6] G.L. Mwaiko,. East Afri. Med. J. 69 (1992) 223-226.

[7] G.L. Mwaiko and Z.X. Savaeli. East Afri. Med. J. 71(1994) 97-99.

[8] A.A. Shalaby, K.A. Allam, A.A. Mostafaand S.M. Fahmy. J. Egypt. Soc. Parasitol. 28 (1998) 595-606.

[9] N. Lukwa, East Afri. J. Med 40 (1994) 306-309.

[10] K. Palsson and T.G. Jaenson. J. Med. Entomol, 36 (1999) 144-148.

[11] R.B. Porter, P.B. Reese, L.A. Williams and D.J. Williams. Phytochemistry 40 (1995) 735-738.

[12] M. Fragoso-Serrano, E. Gonzalez-Chimeo and R. Pereda-Miranda. J. Nat. Prod. 62 (1999) 45-50.