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Larvicidal properties of some Tanzanian plant species against *Anopheles gambiae s.s.* Gile (Diptera:Culicidae) mosquitoes

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ABSTRACT

Ethnobotanical survey was done among the Hehe community of central Tanzania to identify plants that can be used in the control of malaria transmitting mosquito vectors. Extracts from different plant parts were analysed for their larvicidal effects against *Anopheles gambiae s.s* mosquito by using the WHO (1996) protocols. The crude extracts that had highest larvicidal activity were the dichloromethane extract of the root barks of *S. araliacea* and *L. viburnoides* ssp. *viburnoides* var. *kisi* while the methanol extracts of the stem and root barks of *Kotschya uguenensis* showed low acute lethality but exhibited insect growth disruption activity. The extracts from *Synadenium glaucensen* were mildly active. Bisbenzocyclooctadiene lignans from *S. araliaceae* that had no lactone moiety exhibited higher larvicidal activity than the bisbenzocyclooctadiene lactone lignans. The high larvicidal potency of the three plant species *S. araliacea, L. viburnoides* ssp. *viburnoides* var. *kisi* and *K. uguenensis* indicates the importance of ethnobotanical criteria in selecting plant species having higher probability of possessing anti-mosquito compounds, a phenomenon that needs to be developed and incorporated in integrated mosquito vector management strategies.

Keywords: *Synadenium glaucenscen*; *Steganotenia araliacea*; *Kotschya uguenensis*; *Lantana viburnoides* ssp. *viburnoides* var. *kisi*; Larvicide; bisbenzocyclooctadiene lignans.

INTRODUCTION

Malaria is a disease of public and economic importance affecting about 40% of the world population, mainly in Asia, Latin America, Middle East, Eastern Europe and Africa (Kettle, 1994). Malaria occurs in about 100 countries, 90% of them being in Africa South of the Sahara (WHO, 1996, 2003). Malaria is caused by *Plasmodium* parasites that are specific to vertebrates as hosts and *Anopheles* mosquitoes as vectors. *Plasmodium falciparum* is the most deadly species, being responsible for 95% of the malaria deaths worldwide while *An. gambiae s.s.* is the vector associated with stable malaria in Africa because it is strongly anthropophilic transmitting malaria even when it is present in low density (Paskewitz et al., 1993; Kettle, 1994). To date, no single method has been successful in the eradication of malaria.

Larviciding mosquito breeding sites using 1,1,1-trichloro-2,2-*bis*(*p*chlorophenyl)

ethane (DDT) was one of the initial successful methods for malaria mosquito vector control in North America and Europe (Kettle, 1994; WHO, 1996, 2003). However, the use of DDT and other organochlorines such as, hexachlorocyclohexane (HCH), dieldrin and lindane was later banned (WHO, 1996, 2003). In recent year the use of these insecticides has been advocated as alternatives to indoor spray for large scale mosquito control interventions. Such interim measures not withstanding, environmental friendly insecticides commanding unrestricted use are still needed for the control of the malaria transmitting mosquitoes. In this case, plants that are used by communities in rural areas in the management of mosquitoes and other insects are interesting targets, since studies have shown a strong correlation between ethnopharmacological/ethnobotanical claims and associated biological activity of plants based materials (Gessler et al., 1995). As such enormous research efforts have been focused on evaluation of plant materials indigenously used for insect control for anti-mosquito agents and our studies formed part of such efforts.

MATERIALS AND METHODS

Plant materials

The investigated plant species were chosen based on their ethnobotanical use as insecticides (Table 1). All investigated plant species were identified in the field by a Botanist from the Department of Botany, University of Dar es Salaam and authenticated at the Herbarium of that Department where voucher specimens are kept. *Lantana viburnoides* ssp *viburnoides* var. *kisi* (A. Rich) Verdc (Verbenaceae) materials were collected from Lugaga village near the Mafinga Army Camp in Mufindi district, Tanzania (voucher specimen Ref. No. FMM 3290); Plant materials of *Steganotaenia araliacea* Hochst (Apiaceae) were collected from Ndolezi village near Mafinga in Mufindi District, Tanzania (voucher specimen Ref. No. FMM 3289); *Kotschya uguenensis* Verdc. (Fabaceae) materials were collected at Ngwazi dam in Mufindi district in Tanzania (voucher specimen Ref. No. 3292); while *Synadenium glaucenscen* Pax. (Euphorbiaceae) materials (voucher specimen Ref. No. LBM 10520) were collected from Ifunda in Iringa Region, Tanzania.

Table 1: Ethnobotanical uses of the plants tested for larvicidal activity using *Anopheles gambiae s.s.*

Plant species	Venacular name	Ethnobotanical uses	Scientific uses
Synadenium glaucenscen Pax. (Euphorbiaceae)	Manung'anunga	Poisonous* Poison to fish and humans (Weiss, 1979) Boils (Hedberg et al., 1983) Cough (Chhabra et al., 1984)	
Lantana viburnoides subsp *viburnoides* var *kisi* (A. Rich.) Verdc. (Verbenaceae)	Luhongole/mhongole	Leaves as mosquito repellent* Stomach relief and fruits as famine food (Watt and Breyer-Brandwijk, 1962).	
Kotschya uguenensis Verdc. (Fabaceae)	Msilahenge/Mtamba	Aerial parts used for repelling mites and stem for milk preservation*	
Steganataenia araliacea Hochst. (Apiaceae)	Mkalai	Repel snakes*, Treatment of malaria (Gessler et al., 1995; Hedberg et al., 1983), Snakebite, sore throat and lung diseases for animals like cattle, goats and calves (Watt and Breyer-Brandwijk, 1962).	Insecticidal activity against *Tribolium castaneum* and *Culex quinquefasciatus* (Abubaka et al., 2001)

*Ethnobatanical uses by the Hehe tribe of Iringa region in central Tanzania.

Extraction and bioassay guided fractionation

The plant materials were air-dried in the shade, pulverised and soaked sequencially in n-hexane, dichloromethane and methanol (2 x 72 h) and the extracts assayed for larvicidal activity. The most active *S. araliacea* dichloromethane root bark extract (Table 2) on vacuum liquid chromatography and repeated chromatography of the fourth and fifth fractions yielded steganoate A (**1**) and steganagin (**3**), and steganacin (**2**) respectively.

Larvicidal assay

Larvicidal assay was carried out by exposing 20 late 3rd or early 4th instar larvae of *An. gambiae s.s* mosquitoes (Mbita strain) that were obtained from a colony maintained at the International Center of Insect Physiology and Ecology (ICIPE) insectary. Various concentrations (50, 100, 250, 500, 750 and 1000 ppm for extracts and 1, 5 and 10 ppm for isolated compounds) were obtained by adding a known volume of stock solution in beakers to make up 100 ml of water-sample solution (WHO, 1996, 2003). Acetone was used for dissolving samples but in some cases ethanol or dimethyl sulphoxide (DMSO). In each case the solvent used in dissolving the sample was also used as blank in the control experiments. The test was done in triplicate from separately reared batches of larvae. The number of dead as well as deformed larvae was recorded every 24 h. During the experiment, water temperature was maintained at 26 ± 2 °C and larvae fed on Tetramin® fish food at 1 mg per beaker per day.

Study design and data analysis

Semi-structured questionnaires were used to explore the candidate mosquitocidal plant species. The four highly ranked plants were selected for this study. Block design was used during larvicidal experiments, in which various concentration levels and their control

Table 2: Mosquito larvicidal activity (LC_{50}) of extracts and some compounds from *Steganotaenia araliacea* against *An. gambiae* in 48 hours.

Plant/compound Name	Plant part extracted	Solvent used[#]	LC_{50} (μg/ml)	95% Confidence limit (μg/ml)	
				Lower	Upper
Synadenium glaucenscen Pax. (Euphorbiaceae)	Rootbark	Hexane	90.12	87.44	92.70
		Methanol	532.91	512.24	554.22
	Stembark	Hexane	209.02	198.34	213.65
		Dichloromethane	301.54	288.08	315.73
	Leaves	Hexane	98.11	95.11	101.42
		Dichloromethane	200.75	192.75	209.07
		Methanol	147.03	139.33	155.91
Lantana viburnoides ssp *viburnoides* var *kisi* (A. Rich.) Verdc. (Verbenaceae)	Rootbark	Hexane	81.84	73.31	90.77
		Dichloromethane	14.07	12.33	15.76
		Methanol	171.52	161.44	182.40
	Stembark	Hexane	63.89	61.11	66.69
		Dichloromethane	779.22	756.47	803.26
		Methanol	871.95	855.17	889.29
Kotschya uguenensis Verdc. (Fabaceae)	Rootbark	Dichloromethane	807.54	776.10	841.45
		Methanol	445.24	426.46	464.47
	Stembark	Methanol	1358.04	1257.11	1494.0
	Aerial parts	Hexane	68.66	65.80	71.69
		Dichloromethane	166.15	158.07	174.79
Steganataenia araliacea Hochst. (Apiaceae)	Rootbark	Dichloromethane	18.08	16.24	19.92
		Methanol	244.47	234.34	255.86
Steganoate A (**1**)			5.44	4.77	6.26
Steganacin (**2**)			9.10	7.96	10.73
Steganangin (**3**)			10.0	9.37	18.23

[#]All extracts obtained by sequential extraction, only those with high yields were tested; LC_{50} values are significant at 95% confidence level, lower and upper confidence limits which coincides are not significantly different.

(blank) were arranged in three replicates at ago (Goupy, 1993). The mean of larvae and pupa density in three replicate was calculated. Data was subjected to analysis of variance (ANOVA) and Probit analysis to compute LC_{50} using the Lackfit inversel procedure of the SAS program (SAS, 2000).

RESULTS AND DISCUSSION

In this study, we report the *An. gambiae s.s.* larvicidal activity of 20 crude extracts derived from the four plant species *S. araliacea*, *L. viburnoides* ssp *viburnoides* var. *kisi*, *S. glaucenscen* and *K. uguenensis* (Table 1). In additional, larvicidal activity results of some isolated compounds from the dichloromethane extract of the root barks of *Steganataenia araliacea* are reported (Table 2). The structures of the isolated compounds were established by correlation with spectral data previously reported for steganoate A (Wickramaratne et al., 1993), steganacin (Kupchan et al., 1973; Robin et al., 1986; Wickramaratne et al., 1993) and steganangin (Kupchan et al., 1973). High larvicidal effect was observed on post-exposure of larvae to dichloromethane extracts of the root bark of *S. araliacea.* The lethal concentration which gave 50% mortality of the tested larvae (LC_{50} at 95%) was 18.08 ppm in 48 h. The larvicidal activity of the compounds isolated from *S. araliacea* (Figure 1) indicated steganoate A (**1**) (LC_{50} 5.44 ppm after 48 h) to be more active than steganacin (**2**) (LC_{50} 9.10 ppm after 48 h) while steganangin (**3**) was not active. Previously, antitumour activity of steganacin (**2**) and steganangin (**3**) were shown to be more potent than steganoate A (**1**) and the C-5 substitution of ring B was established to be responsible for the observed activity (Okano et al., 1981). In the present study, larvicidal activity of bisbenzocyclooctadiene lignan 1 lacking a lactone moiety was higher than lactonyl bisbenzocyclooctadiene lignans 2 and 3.

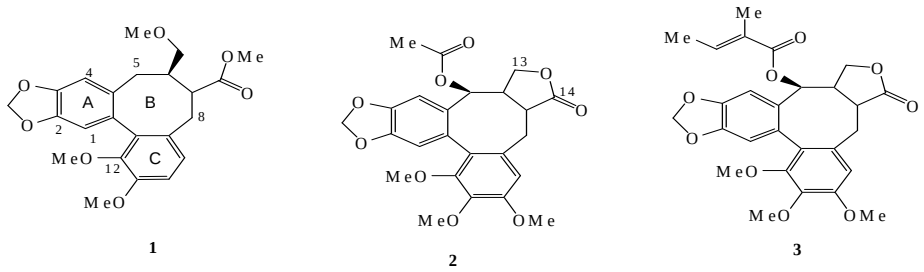
Larvae treated with *L. viburnoides* ssp *viburnoides* var. *kisi* dichloromethane extracts of the stem bark exhibited lower larvicidal activity than those treated with the root bark extracts (Table 2). Previously, furano-naphthaquinones and lantadene triterpenoids were reported to be the active compounds in *L. viburnoides* root barks (Innocent et al., 2008a).

Despite the toxicity exerted by a number of Euphorbiaceae plants, extracts from *S. glaucenscen* exhibited only mildly larvicidal activity with the n-hexane extracts being more potent than dichloromethane and methanol extracts (Table 2). It will be of interest to isolate and test the larvicidal property of the compounds from this plant.

Extracts from *K. uguenensis* showed mild larvicidal activity within 48 h exposure (Table 2), but similar study conducted by Innocent et al. (2008b) showed that, on long term exposure time beyond 48 h, the methanol extract of the stem and root barks exhibited growth disruption leading the larvae to form an elongated gut with eventual death (Innocent et al., 2008b).

Conclusion

In general long exposure time of *An. gambiae s.s.* to the extracts progressively resulted into better larvicidal activity. The high larvicidal potency of the three plant species *viz. S. araliacea, L. viburnoides* subsp. *viburnoides* var. *kisi* and *K. uguenensis* show that selecting plants by ethnobotanical criteria enhances the probability of finding species with anti-mosquito properties, which could be developed and incorporated into integrated mosquito vector management.



1 **2** **3**

Figure 1: Chemical structures of bisbenzocyclooctadiene lignans compounds isolated from the dichloromethane extract of the root barks of *Steganotaenia araliacea.*

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