Vol. 6 no. 2, pp. 50-53 (September 2009)

http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Larvicidal properties of three plants on *Anopheles gambiae*

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Received 22nd July 2009; Accepted 29th September 2009

Abstract
The larvicidal and growth inhibitory properties of methanol extracts of the leaves, stem and root barks of *Quassia africana* (Simaroubaceae), the leaves and stem bark of *Khaya senegalensis* (Meliaceae) and the leaves of *Lippia multiflora* (Verbanaceae), against *Anopheles gambiae* were evaluated in the laboratory. Extracts were applied at concentrations between 12.5 and 200 mg/ml in dechlorinated water. Larvicidal activity was concentration dependent. All extracts showed some larval toxicity after 24 hours of exposure, with the root and stem extracts of *Q. africana* producing 100% mortality at 50 mg/ml. Extracts also affected pupal development. No larvae exposed to *Q. africana* developed into pupae while development was also curtailed when exposed to *K. senegalensis* and *L. multiflora* extracts. Larvicidal activity was concentration dependent. These plants could be sources of botanical insecticides for malarial control.

Keywords: Larvicidal activity, *Anopheles gambiae*, Methanol extracts, *Quassia africana*, *Khaya senegalensis*, *Lippia multiflora*.

Introduction

Malaria is one of the most important vector-borne diseases, affecting millions of people mainly in the tropics. In spite of many efforts undertaken for its control, through chemotherapy treatment and vector control, an increase in malaria incidence has occurred in the last 30 years, primarily caused by socio-economic factors, underdevelopment and drug and insecticidal resistance. The female *Anopheles* mosquito is the vector for human malaria and over sixty species of *Anopheles* have been identified as vectors of *Plasmodium* (Phillips, 2001).

Vector control is facing a threat due to the emergence of resistance in vector mosquitoes to conventional synthetic insecticides, warranting either counter-measures or development of newer insecticides (Chandre *et al*., 1998). Natural insecticides are good alternatives to synthetic insecticides, as they are relatively safe, degradable and are readily available in many areas of the world. Though several plants from different families have been reported for insecticidal activity against mosquitoes, only a few have moved from the laboratory to field use, like neem-based insecticides (Shaalan *et al*., 2005). Limonoids have been isolated from

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ISSN 0189-8442

K. senegalensis and have shown antifeeding activities against *Spodaptera littoralis*, (Abdelgaleil & Nakatani, 2003). Plants of the Meliaceae family, especially neem (*Azadirachta indica*), are recognized for their toxic effects on insects (Sharma *et al.*, 1993; Dua *et al*., 1995). This was the basis for screening parts of this plant for larval toxicity against the malaria vector, and results have been promising.

Several plant extracts show larvicidal and insect-repellant properties on malaria vectors (Ansari *et al*., 2000; Chogo and Crank, 1981; Szafranski *et al.*, 1993). Phytochemicals may also have potential uses as growth and reproduction inhibitors. Plants of the family Simaroubaceae, Meliaceae, Verbanaceae, Rutaceae and Asteraceae appear to have potential for providing future mosquito control agents (Omolo *et al*., 2004; Tsao *et al*., 2002; Sukumar *et al.*, 1991). In this communication, we report the larvicidal and growth inhibitory properties of extracts from 3 plant species from different plant families in the West and Central African region against *An. gambiae*, the principal malaria vector in the region.

Experimental

Plant materials. The leaves, stem bark and roots of *Q. africana* were collected in January 2004 in Kribi, Cameroon and authenticated at the National Herbarium of Cameroon where voucher specimens were deposited. The plant voucher number is HNC 65074. The leaves and stem bark of *K. senegalensis* were collected in August 2004 at Ijesa, South West Nigeria and identified at Forestry research Institute of Nigeria, (FRIN) where voucher specimens were deposited. The leaves of *L. multiflora* were collected in February 2004 in Otu, Oyo State, Nigeria and authenticated in the Forestry Research Institute of Nigeria, (FRIN) herbarium. The voucher number was FHI 106562. Plant parts were dried under shade and later powdered using a blender. Powdered samples were extracted with methanol using a soxhlet extractor at 60 ^{0}C. The solvent was removed using a rotary evaporator under reduced pressure. The resulting extracts were stored at 4 ^{0}C until needed. Stock solutions of each extract were prepared at 200 mg/ml with ethanol. Test solutions of 12.5, 25, 50 and 100 mg/ml were then prepared by diluting the stock solution further with ethanol.

Larval collection. A team of trained collectors carried out surveys in Oyo and Osun states of Nigeria. Larvae were collected from tire print and infiltration breeding sites. Collected larvae were washed in clean dechlorinated water, reared in plastic bowls and fed ground dog biscuit.

Larval toxicity assay. Concentrations of extracts consisting of 1% aqueous solution were placed in sterile disposable cups at room temperature. Twenty fourth instar larvae per 100 ml solution of each concentration were used; after 24 hrs the number of dead larvae in each cup was counted. Larvae were considered nonviable if unable to reach the water surface (Abubakar *et al*., 2001). Experiments showed no significant difference in mortality when the assay was extended to 48 hours. Controls consisting of 1% ethanol were run in parallel and mortality was always below 3%. All experiments were done in duplicate.

Results and Discussion

Six extracts from three plants belonging to three plant families were screened. Table I summarizes the mean percentage mortalities exhibited by each extract. In this assay, *Q. africana* root extract was the most toxic to the larvae. Generally, the three extracts of *Q. africana* tested gave the highest larvicidal activity. *K. senegalensis* leaf extract also had appreciable activity, while the methanol extract of *L. multiflora* leaf was the least toxic. Table 2 displays the percentage of

pupae developing from the larval stage, indicating the extent to which growth was inhibited on exposure to the extracts for 24 hours. None of the larvae that were exposed to *Q. africana* extracts developed into pupae.

Table 1: Larvicidal activity of methanol extracts from 3 plants against *Anopheles gambiae* mosquito larvae.

Concentration (mg/ml)	Mean % Mortality ±SEM					
	QAL	QAR	QAS	KSL	KSS	LML
12.5	34.4±10.9	87.3±10.4	67.1±19.5	5.0±7.1	4.4±5.6	31.4±10.7
25	68.4±2.4	94.4±7.9	92.7±3.2	5.0±7.1	6.3±8.8	35.0±9.8
50	71±10.3	100±0.0	100±0.0	12.5±3.5	17.0±8.5	45.0±10.0
100	100±0.0	100±0.0	100±0.0	40±0.0	17.5±10.6	82.0±5.6
200	100±0.0	100±0.0	100±0.0	92.5±3.5	32.5±10.6	97.0±3.5
100% Water	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
1% Ethanol	2.7±0.0	2.7±0.0	2.7±0.0	2.7±0.0	2.7±0.0	2.7±0.0

QAL- *Q. africana* leaf; QAR- *Q. africana* root; QAS- *Q. africana* stem; KSL- *K. senegalensis* leaf; KSS- *K. senegalensis* stem; LML- *L. multiflora* leaf

Table 2: Pupal development in the presence of plant extracts.*

Concentration of extracts (mg/ml)	% of larvae developing into pupae	
	L. multiflora leaf	*K. senegalensis* stem
200	2.5	0.0
100	6.9	0.0
50	12.8	0.0
25	2.8	18
12.5	19.5	39

*No pupae were obtained from larvae treated with extracts of QAL. QAR, QAS and KSL.

Q. africana Baill and Baill. is indigenous to West and Central Africa, Indonesia and Japan. It is a plant of high medicinal value (Iwu, 1993). The root and stem bark of this plant produced 100% mortality of *An. gambiae* larvae at 50 mg/ml after 24 hrs exposure. It is a promising candidate for further bioassay-guided fractionation to identify the most active larvicidal components. The findings in this study further identify the root and stem barks of this plant as sources of insecticides.

Plants of the Verbanaceae family have also displayed strong repellant effects against *An. gambiae* mosquitoes. The repellant action of the oils from *L. javanica* and *L. ukambensis* was comparable to the synthetic repellent DEET (Omolo *et al*., 2004). The foliar extract of *L. multiflora* in this study was less toxic than other extracts; however, it showed more than 50% mortality at 200 and 100 mg/ml.

Finally, *Q. africana* extracts totally prevented the development of mosquito pupae. *K. senegalensis* and *L. multiflora* also displayed growth inhibition to a considerable extent.

This is a preliminary study in our overall aim to study West and Central African medicinal plants for insecticidal properties, particularly against malaria vectors. Results of this study suggest that West/Central African biodiversity could lead to discovery of potential vector control agents for malaria.

Acknowledgements

Partial financial support was obtained from a WHO/TDR/MIM African grant, ID 980046.

We thank Dr. Tchokote Joseph, Mr. Courage and Mr. Dapo of the Departments of Economics, Computer Sciences and Zoology, UI, for assisting with mosquito larvae collection.

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