Mosquito larvicidal constituents from *Lantana viburnoides* sp *viburnoides* var *kisi* (A. rich) Verdc (Verbenaceae)

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Abstract

**Background & objectives:** *Lantana viburnoides* sp *viburnoides* var *kisi* is used in Tanzania ethnobotanically to repel mosquitoes as well as in traditional medicine for stomach ache relief. Bioassay-guided fractionation and subtraction bioassays of the dichloromethane extract of the root barks were carried out in order to identify the bioactive components for controlling *Anopheles gambiae* s.s. mosquito larvae.

**Methods:** Twenty late III or early IV instar larvae of *An. gambiae* s.s. were exposed to various concentrations of the plant extracts, fractions, blends and pure compounds, and were assayed in the laboratory by using the protocol of WHO 1996. Mean mortalities were compared using Dunnett’s test (p<0.05) and lethal concentration calculated by Lackfit Inversel of the SAS programme.

**Results:** The crude extract (LC$_{50}$ = 7.70 ppm in 72 h) and fractions exhibited different level of mosquito larvicidal activity with subtraction of some fractions resulting in activity enhancement. The active fractions contained furanonaphthaquinones regio-isomers (LC$_{50}$ = 5.48–5.70 ppm in 72 h) and the lantadene triterpenoid camaric acid (LC$_{50}$ = 6.19 ppm in 72 h) as active principles while the lupane triterpenoid betulinic acid (LC$_{50}$ < 10 ppm in 72 h) was obtained from the least active fraction.

**Interpretation & conclusion:** Crude extracts and some fractions had higher or comparable larvicidal activity to the pure compounds. These results demonstrate that *L. viburnoides* sp *viburnoides* var *kisi* extracts may serve as larvicides for managing various mosquito habitats even in their semi-purified form. The isolated compounds can be used as distinct markers in the active extracts or plant materials belonging to the genus *Lantana*.

**Key words** *Anopheles gambiae* s.s. – bioassay-guided fractionation – *Lantana viburnoides* sp *viburnoides* var *kisi* – larvicide–subtraction bioassay

Introduction

*Lantana viburnoides* sp *viburnoides* var *kisi* (A. rich) Verdc belongs to the family Verbenaceae which comprises 100 genera and 2600 species that grow as herbs, shrubs or trees. The genus, *Lantana* consists of about 150 species occurring in tropical and sub-tropical countries$^{1-3}$. *L. viburnoides* sp *viburnoides* var *kisi* is indigenous to Tanzania. Ethnobotanically, the leaves are used as mosquito repellents and sometimes
chewed for stomach relief. The fruits are used as famine food by the Zulu in South Africa while the Luo of Northern Tanzania regard the plant as poisonous if eaten in large amount but non-poisonous to sheep and goats. There are no phytochemical and biological studies which have been carried out on *L. viburnoides* sp *viburnoides* var *kisi* except this. However, phytochemical studies of other plants of the genus *Lantana* have indicated presence of triterpenoids as main constituents. Also, presence of flavonoids and phenylpropanoid glycosides, volatile oils, furanonaphthaquinones and some hydrocarbons have been documented. In this communication, we discuss the bioassay-guided fractionation and subtraction bioassays of the dichloromethane root extract to isolate and identify the bioactive components. Also, we discuss the prospect of using purified and/or semi-purified compounds from *Lantana viburnoides* sp *viburnoides* var *kisi* in management of *Anopheles gambiae s.s.* (Diptera: Culicidae) mosquito that is the main malaria vector in Africa.

**Material & Methods**

**Plant materials and extraction:** The root barks of *Lantana viburnoides* sp *viburnoides* var *kisi* were collected from Iringa Region, Tanzania. The plant specimens were identified and deposited in the Herbarium at the Department of Botany, University of Dar es Salaam, Tanzania. The plant materials were air-dried in the shade, pulverised and soaked sequentially in *n*-hexane, dichloromethane and methanol for 72 h and then filtered. Soaking was done twice for every solvent. The crude filtrates were concentrated *in vacuo* using a rotary evaporator while maintaining the bath temperature at 40°C in order to avoid thermal decomposition of labile compounds. The crude fractions were stored at −4°C until use.

**Bioassay guided fractionation and subtraction bioassay of fractions (blends):** Bioassay guided fractionation to isolate bioactive compounds was done on silica gel (230–400 mesh size) or Sephadex® LH 20. Vacuum liquid chromatography (VLC) of the dichloromethane extract of the root barks (LRRD) was carried out on silica gel using a glass column (15 cm i.d x 25 cm) eluting with a mixture of *n*-hexane, ethyl acetate and methanol. The extract (LRRD, 23 g) yielded six VLC fractions namely LF1 (5% ethyl acetate/*n*-hexane, 0.5 g), LF2 (20% ethyl acetate/*n*-hexane, 0.5 g), LF3 (40% ethyl acetate/*n*-hexane, 1.5 g), LF4 (70% ethyl acetate/*n*-hexane, 12.5 g), LF5 (20% methanol/ethyl acetate, 6.4 g) and LF6 (100% methanol, 1.5 g). Percentage yield of each fraction was calculated and used in estimating the amount to be included in formation of blends. Subtraction bioassay was carried out by omitting one fraction at a time and its contribution compared with respect to the activity of the extract. Thus, Blend 1 (LB1) was prepared by omitting fraction 1 (LF1), Blend 2 (LB2) was prepared by omitting fraction 2 (LF2), likewise for LB3, LB4, LB5 and LB6.

**Mosquito larvae:** *An. gambiae s.s.* mosquitoes (Mbite strain), originating from the International Centre of Insect Physiology and Ecology (ICIPE) at Mbite Station in Kenya were used in this study. Larvae were allowed to emerge in plastic containers filled with distilled water. At the II instar stage, the larvae were transferred to large plastic pans (37 × 31 × 6 cm) at densities of 200–300. Larvae were fed on Tetramin® fish food [Tetra holding (U.S.) Inc., Blacksburg, VA, U.S.A.], and the water temperature was maintained at 26 ± 2ºC throughout larval development.

**Larvicidal assay:** Larvicidal assay was carried out by exposing 20 late III or early IV instar larvae of *An. gambiae s.s.* to various concentrations of the plant extracts, fractions, blends and pure compounds. Samples were dissolved with known volume of acetone to make-up a desired concentration of stock solutions. A known volume of pre-prepared stock solution was then added in beakers to make-up 100 ml of water-sample solution (water temperature 26 ± 2°C; concentrations 100, 50, 20, 10 and 5 ppm). The control experiment contained only acetone.
(blank). The test was triplicated from separately reared batches of larvae. The number of larvae died were recorded every 24 h. During the experiment, larvae were fed on Tetramin® fish food at 1 mg per beaker per day.

Data analysis: Data were subjected to analysis of variance (ANOVA) and mean percentage mortality were compared using Dunnett’s test of the SAS package. Probit analysis to compute LD₅₀ was done using the Lackfit Inversel procedure of the SAS programme.

Results

The cumulative mean percentage mortality due to the effect of exposure of the larvae to the extracts, fractions, blends and pure compounds were used to compare larvicidal activity among the fractions and hence identification of various interactions of compounds within the extract (Fig. 1 and Table 1). Larvicidal activity of fraction 4 (LF4) and fraction 5 (LF5) compared well with that of the parent extract (LRRD) after 72 h (Fig. 1). This observation suggested that, the active compounds were present in LF4 and LF5. High larvicidal activity was observed when fraction 3 (LF3) was subtracted to form blend 3 (LB3) after 24 h (Fig. 1). Thus, there was no significant difference between the extract (LRRD) and blend 3 while significant difference was observed with fraction 3 (Fig. 1). These observations suggested the presence of less active compounds in LF3. Both LF2 and LB2 had lower activity compared to the extract showing the potential contribution of the active compounds in LF2 which were present only in trace amounts. Similarly, there was no larvicidal activity for fraction 6 (LF6), probably because the fraction was obtained by washing the chromatographic column with methanol (residue). Consequentially, blend 6 (LB6) showed high larvicidal activity especially after a longer exposure time (72 h; Fig. 1). Also, low larvicidal activity was shown by fraction 1 (LF1).

Bioassay-guided fractionation lead to the isolation

![Graph showing larvicidal efficacy of fractions and blends from L. viburnoides var. kisi](image)
of camaric acid from fractions 4 and 5 as the active principle, exhibiting an LC\textsubscript{50} of 6.19 ppm after 72 h of exposure (Table 1). Camaric acid is the lantadene-type triterpenoid, that was previously isolated from many Lantana sp. Other compounds isolated from fraction 4 were inseparable mixtures of regio-isomeric prenylated naphthaquinones that were inactive. However, compounds isolated from fraction 2 as inseparable mixtures of regio-isomeric furanonaphthaquinones were more active compared to the mother fraction (Table 1). The LC\textsubscript{50} of the furanonaphthaquinone mixtures were 5.48–5.70 ppm after 72 h exposure (Table 1). The compounds occurred in small quantities and this may have contributed to the observed low activity of the fraction (LF2). The larvicidal activity of fraction 2 may also have been obscured by a lupanoid triterpenoid, betulinic acid.

**Table 1. Larvicidal activity (LC\textsubscript{50}) of extract LRRD, fractions, blends and pure compounds against Anopheles gambiae s.s. in 72 h**

<table>
<thead>
<tr>
<th>Sample code</th>
<th>LC\textsubscript{50} (CI) in ppm</th>
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<tbody>
<tr>
<td>LF1</td>
<td>73.61 (61.03–106.18)</td>
</tr>
<tr>
<td>LF2</td>
<td>60.97 (54.04–75.46)</td>
</tr>
<tr>
<td>LF3</td>
<td>60.73 (50.03–80.72)</td>
</tr>
<tr>
<td>LF4</td>
<td>15.14 (13.98–16.36)</td>
</tr>
<tr>
<td>LF5</td>
<td>9.11 (8.21–10.11)</td>
</tr>
<tr>
<td>LRRD</td>
<td>7.7 (7.09–8.29)</td>
</tr>
<tr>
<td>LB1</td>
<td>22.62 (19.95–25.76)</td>
</tr>
<tr>
<td>LB2</td>
<td>20.21 (17.77–23.35)</td>
</tr>
<tr>
<td>LB3</td>
<td>7.72 (6.7–8.98)</td>
</tr>
<tr>
<td>LB4</td>
<td>10.6 (9.52–11.88)</td>
</tr>
<tr>
<td>LB5</td>
<td>10.55 (9.75–11.84)</td>
</tr>
<tr>
<td>LB6</td>
<td>10.01 (9.11–11.23)</td>
</tr>
<tr>
<td>Furanonaphthaquinones</td>
<td>5.48–5.7</td>
</tr>
<tr>
<td>Lantadene triterpenoid, camaric acid</td>
<td>6.19 (5.5–6.99)</td>
</tr>
<tr>
<td>Lupane triterpenoid, betulinic acid</td>
<td>10.43 (9.21–12.11)</td>
</tr>
</tbody>
</table>

Values in parentheses represent lower and upper confidence limit; Values are significant at p < 0.05 by Lackfit Inversel.

Betulinic acid was the major constituent in fraction 3 and exhibited only mild activity (LC\textsubscript{50} >10 ppm in 72 h) (Table 1). Due to the inadequate amount of active compounds obtained from fraction 2 (LF2), synergistic studies could not be carried out with the active pure compounds obtained from fraction 4 and 5.

**Discussion**

Eco-friendly chemicals are recommended in larviciding mosquito breeding sites. In this case, a plant species that is used by the communities in rural areas in management of mosquitoes and other insects was analyzed for its larvicidal properties. Plants being a natural source of compounds, are known to contain larvicidal agents, which may act in combination or independently\textsuperscript{19–21}, hence necessitating to carry out studies of the compounds interactions. In this study, the crude extracts and some fractions had higher or comparable larvicidal activity than that for the pure compounds. These results demonstrated that L. viburnoides sp viburnoides var kisi extracts could serve as a source of a larvicide for managing various mosquito habitats in the field even in their semipurified form. Similarly, the presence of lantadene triterpenoids and furanonaphthaquinones in Lantana sp may serve as an indicator for the plants’ mosquito larvicidal properties. Previously, lantadene A and lantadene B were found to exhibit insecticidal activity\textsuperscript{22}. However, in the present study, neither lantadene A nor lantadene B was isolated. Camaric acid was obtained instead, which indicates the diverse chemical composition from various species of Lantana\textsuperscript{3}.

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