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Comparison of the effects of extracts from three *Vitex* plant species on *Anopheles gambiae* s.s. (Diptera: Culicidae) larvae

Mokua G. Nyamoita[a,b,c,*], Innocent Ester[a], Mbwambo H. Zakaria[a], Lwande Wilber[b], Ochola J. Bwire[b], Hassanali Ahmed[d]

[a] *Muhimbili University of Health and Allied Sciences, Institute of Traditional Medicine, P.O. Box 65001, Dar es Salaam, Tanzania*
[b] *International Centre of Insect Physiology and Ecology, Bio-Prospecting Unit, P.O. Box 30772-00100, Nairobi, Kenya*
[c] *Kenyatta University, Department of Pharmacy and Complementary/Alternative Medicine, P.O. Box 43844-00100, Nairobi, Kenya*
[d] *Kenyatta University, Department of Chemistry, P.O. Box 43844-00100, Nairobi, Kenya*

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ABSTRACT

Acetone and methanol extracts of different parts of three *Vitex* species (leaves and stem bark of *Vitex trifolia*, leaves, stem bark and root bark of *Vitex schiliebenii* and stem and root bark of *Vitex payos*) were evaluated for their potential to control *Anopheles gambiae* Giles s.s. larvae (Diptera: Culicidae). The extracts gave different levels and rate of mortality of the larvae. Some (methanol extract of *V. trifolia* leaves, acetone extracts of stem bark and leaves of *V. schiliebenii*, acetone extract of root bark of *V. payos*) caused 100% mortality at 100 ppm in 72 h, with those of *V. schiliebenii and V. payos* showing faster rate of mortality (LT_{50} = 8 h) than that of *V. trifolia* (LT_{50} = 14 h). At lower doses of these extracts (≤50 ppm), most of the larvae failed to transform to normal pupae but gave larval–pupal intermediates between 4 and 14 days of exposure. Some pupated normally but the adults that emerged appeared to be weak and died within 48 h. Extracts of the stem bark of *V. payos* showed interesting effects on the larvae. Initially, the larvae were relatively hyperactive compared to those in control treatments. Later, the ones that did not transform to larval–pupal intermediates became stretched and inactive and died and floated in clusters on the surface. These observations suggest some interesting growth-disrupting constituents in the plants, with possible application in the practical control of mosquito larvae in aquatic ecosystems.

1. Introduction

Plants have been recognized as rich sources of bioactive secondary metabolites with potential in the control of disease vectors and/or the diseases they transmit (e.g. de Omena et al., 2007; Githua et al., 2010; Khalid et al., 1989; Kihampa et al., 2009; Mackinnon et al., 1997; Singh et al., 2006; Sukumar et al., 1991). Given the high incidence of malaria in Africa and other tropical countries, the search for alternative tools and tactics for the control of mosquitoes has assumed special importance. Two types of plant products have been sought: volatile repellent blends for personal or space protection to reduce human-vector contacts (Birkett et al., 2011; Debboun et al., 2006; Omolo et al., 2005; Seyoum et al., 2002, 2003), and largely non-volatile plant constituents that are toxic or growth-disruptive to the larval or adult stages of mosquitoes (Govindarajan et al., 2008; Innocent et al., 2008; Kamaraj et al., 2009; Mwangi and Rembold, 1988; Ndung'u et al., 2004; Sharma et al., 2006). The use of plant repellents is widespread in different communities in the tropics and the performance of some has been evaluated experimentally (Curtis et al., 1991; Pålsson and Jaenson, 1999a,b; Seyoum et al., 2003). No similar traditional use of plant products targeting vector control has been documented. However, the possible deployment of natural products from readily accessible plants in community participation programmes to substantially reduce mosquito larval populations has been recognized, and plants belonging to the families Asteraceae, Verbenaceae, Meliaceae, and Rutaceae have been reported as potential sources of secondary metabolites for larval control (Innocent et al., 2008; Katade et al., 2006; Mwangi and Rembold, 1988; Ndung'u et al., 2004). *Vitex* species belonging to the family Lamiaceae (formally classified as Verbenaceae, Mabberley, 1997), have been reported to exhibit larvicidal activities against a number of mosquito species (Kannathasan et al., 2007; Karunamoorthi et al., 2008; Rahman and Talukder, 2006; Rodríguez-Lopéz et al., 2007; Yuan et al., 2006). Plants of this genus occur in both tropical and temperate regions of the world (Mabberley, 1997). In Kenya, different *Vitex* species are found growing naturally at different ecological settings, including the coast, the dry woodlands, Mount Kenya area, and across the Rift valley to the shores of Lake Victoria (Fig. 1; Beentje, 1994; Ruffo et al., 2002).

* Corresponding author at: 15666-00503, Mbagathi, Kenya.
Mobile: +254 734 341 651/713 106 760.
E-mail address: mokuag@yahoo.com (M.G. Nyamoita).

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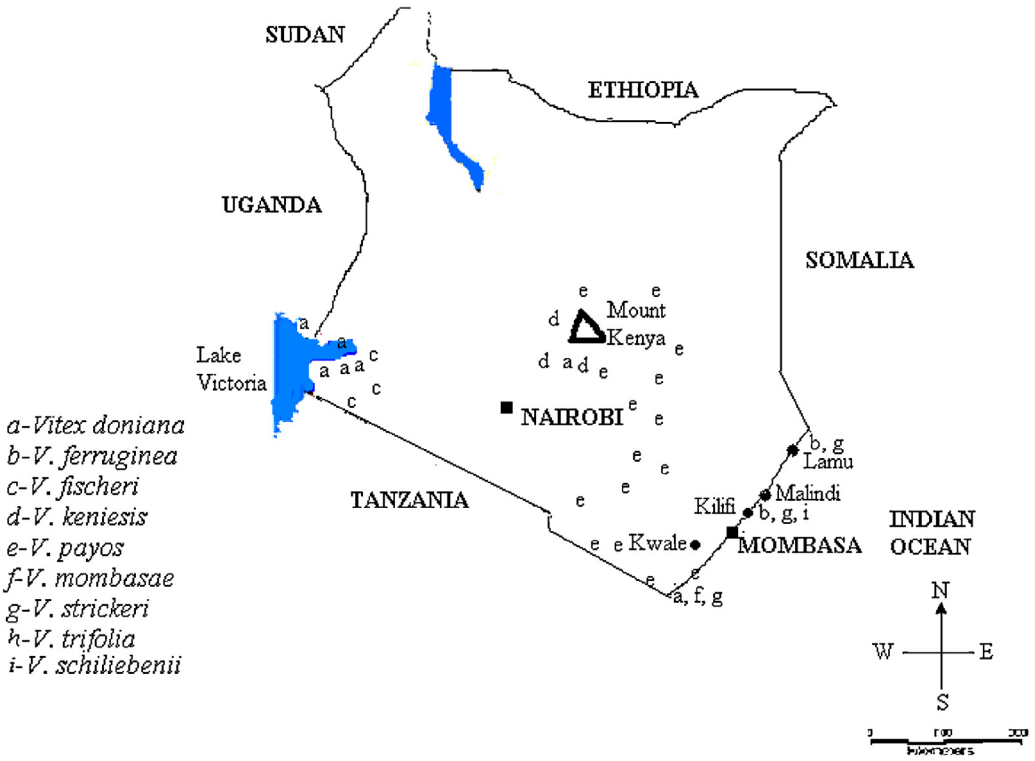

Fig. 1. Distribution of *Vitex* species in Kenya.

They are used in the local system of folk medicine by different communities for the treatment of a range of diseases (Kimondo et al., 2010).

In the present study, the effects of different doses of polar extracts of three *Vitex* species (*V. payos, V. schiliebenii* and *V. trifolia*) on *Anopheles gambiae* Giles s.s. were investigated with the overall aim of evaluating their potential as sources of anti-larval agents for community-based control of malaria vectors. *V. payos* (Louri) Merr. (commonly known as black plum in English, Mfudu in Kiswahili, Kimuu in Kikamba, Muburu in Embu, and Mfudu in Giriama) grows in semi-arid parts of eastern, coastal and central Kenya. It has round leathery leaves (Beentje, 1994; Ruffo et al., 2002). *V. schiliebenii* is a scrambling shrub that grows in the north coast around Watamu. The leaves are five foliolate. Its use in traditional medicine, if any, has not been documented. *V. trifolia* L. is commonly known as chaste tree (English). It is an exotic from Asia occasionally grown wild in shore vegetations. In Kenya, it is found in Kilifi, Mombasa, Diani and Shimoni, near the banks of Indian Ocean (Beentje, 1994; Ruffo et al., 2002). It is a shrub of 1–9 m with 3–5 foliolate leaves.

2. Materials and methods

2.1. Plant collection and treatment

The plant materials were collected from different parts of the Kenyan coastal region. They were authenticated by Simon Mathenge of the National Museum of Kenya (NMK). Preliminary screening of extracts of *V. payos* leaves showed no significant anti-larval activities. Accordingly, in the present study the following plant parts were used in the study: leaves and stem bark of *Vitex trifolia*, leaves, stem bark and root bark of *Vitex schiliebenii* and stem and root bark of *Vitex payos*. The materials were air-dried at room temperature in shade for three weeks and ground into powder in an electric miller. Each powdered material was extracted three times in acetone (5-fold volume) for 24 h with occasional stirring. The extracts were filtered and concentrated to dryness using a rotary evaporator at 40 °C and the combined extract stored at 4 °C. This procedure was repeated with methanol in the same proportion and for the same periods.

2.2. Mosquito rearing

Larvae of *A. gambiae* Giles s.s. used in bioassays were obtained from a colony maintained at the international Centre of Insect Physiology and Ecology (ICIPE) Insect Mass Rearing Unit. This strain of mosquitoes originates from Njage village, 70 km from Ifakara, south eastern Tanzania and has been reared under laboratory conditions since April 1996. Larvae were allowed to emerge from eggs in plastic containers filled with distilled water and were transferred to larger pans (37 × 31 × 6) at densities of 200–300 at 2nd instar stage. Larvae were fed on Tetramin fish food and the water temperature was maintained at 28 ± 2 °C throughout larval development.

2.3. Biossays exposing larvae to phytoextracts

Laboratory bioassays were conducted in accordance to the World Health Organization method (WHO, 1996). Crude extracts of each plant material (5 mg) was dissolved in 1 ml distilled water (*V. schiliebenii* leaves) containing 5% dimethyl sulphoxide (DMSO) or in 1 ml absolute ethanol (all other *Vitex* extracts) to obtain stock solutions each of 5000 ppm. For each extract, 100 ml of different doses (25, 50, 100, 250 and 500 ppm) were prepared by serial dilutions. Three replicates of twenty freshly moulted late 3rd and early 4th instar larvae of *A. gambiae* s.s. were exposed to each dose of each extract with two negative controls (treated with absolute ethanol or DMSO-distilled water). Larval mortality (at higher doses), abnormal behavior and morphological deformations (at lower doses) were recorded at 24 h intervals until the death of the last larva or emergence to adult (WHO, 1996). The bioassay room was kept at a temperature of 30 °C, an average humidity of 78% and a photo

Table 1
Mean percentage mortality induced by the phytoextracts of *Vitex* species against 3rd/4th instar larvae of *Anopheles gambiae* Giles s.s. after 24 h exposure.

Extract code	Mean mortality (% ± SE)/concentration (ppm)					Lethal (ppm)	Concentration values
	500	250	100	50	25	LC_{50}	95% CL
VSR-1	96.7 ± 1.6a	43.3 ± 3.3c	3.3 ± 1.6e	0.0 ± 0.0e	0.0 ± 0.0e	252.1	225.0–281.8
VSR-2	56.7 ± 1.6bc	15.0 ± 2.9de	5.0 ± 2.0e	0.0 ± 0.0e	0.0 ± 0.0e	444.0	392.0–505.0
VSS-1	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	78.3 ± 6.0b	17.4	14.6–20.3
VSS-2	43.3 ± 1.7b	6.7 ± 1.7d	0.0 ± 0.0e	0.0 ± 0.0e	0.0 ± 0.0e	522.6	462.8–594.9
VSL-1	100.0 ± 0.0a	96.7 ± 3.3a	100.0 ± 0.0a	87.0 ± 4.4b	83.3 ± 0.3b	14.6	11.9–17.6
VSL-2	100.0 ± 0.0a	100.0 ± 0.0a	31.6 ± 3.3d	10.0 ± 2.9e	6.7 ± 1.7e	136.3	120.8–154.8
VPR-1	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	100.0 ± 0.0a	85.0 ± 1.7b	15.6	12.9–18.6
VPR-2	55.0 ± 1.7cd	36.7 ± 4.4d	0.0 ± 0.0e	0.0 ± 0.0e	0.0 ± 0.0e	377.8	334.7–427.1
VPS-1	45.0 ± 1.6b	27.5 ± 1.6d	15 ± 3.3de	0.0 ± 0.0e	0.0 ± 0.0e	511.6	451.2–581.0
VPS-2	40.0 ± 1.7b	25.3 ± 1.6d	0.0 ± 0.0e	0.0 ± 0.0e	0.0 ± 0.0e	587.5	478.5–597.6
VTL-1	45.0 ± 1.6c	43.3 ± 1.7c	0.0 ± 0.0e	0.0 ± 0.0e	0.0 ± 0.0e	513.2	455.0–583.2
VTL-2	100.0 ± 0.0a	100.0 ± 0.0a	91.7 ± 4.4b	0.0 ± 0.0e	0.0 ± 0.0e	76.6	68.2–86.0
VTS-1	8.3 ± 3.3e	0.0 ± 0.0e	0.0 ± 0.0e	0.0 ± 0.0e	0.0 ± 0.0e	925.9	753.4–1164.6
Control	2.3 ± 3.3e						

VS: *V. schiliebebenii*; VP: *V. payos*; VT: *V. trifolia*; R: root bark; S: stem bark; L: leaves; 1: acetone; 2: methanol; means with the same letters within a column are not significantly different at 5% level.

period of 12 h of light and 12 h of darkness. The larvae were fed on Tetramin® fish food (Terta GmbH, Germany) at about 1 mg per beaker every 24 h.

2.4. Analyses of results

The average larval mortality (±SE) resulting from each dose of each extract was calculated and the data was subjected to probit analysis for calculating the lethal concentrations of the extracts at LC_{50} at 95% confidence limit of upper and lower levels. The values were calculated using GenStat Teaching edition version. Results with $p < 0.05$ were considered to be statistically significant.

3. Results

3.1. Larval mortality at higher doses

Table 1 presents a summary of the mean mortality ± SE of *A. gambiae* larvae, LD_{50} of the extracts after 24 h exposure. In all the experiments, mortality in treated tests was significantly higher than in control treatments. The highest larval mortality was obtained with the acetone leaf extract of *V. schiliebeniii* (LC_{50} 14.6 ppm), followed by acetone root extract of *V. payos* (LC_{50} 15.6 ppm), acetone stem bark extract of *V. schiliebenii* (LC_{50} 17.4 ppm) and methanol leaf extract of *V. trifolia* (LC_{50} 76.6 ppm). At 100 ppm, the first three caused 100% mortality of the larvae within 24 h (Table 1) while that of *V. trifolia* caused this level of mortality in 72 h (result not included in Table 1).

3.2. Growth disruption effects at lower doses

Generally, at lower doses the larval stage was prolonged (7–10 days post-treatment) (In press) compared to that in control (4–5 days). It was observed that at lower doses, viz. 50 ppm and below, extracts of *V. payos* caused interesting physiological and neurophysiological effects to the exposed larvae. The larvae were smaller in size compared with those in control treatments. Gentle introduction of a pipette into the beaker triggered a series of abnormal behaviors, including initial coiling followed by stretching and loss of mobility. In 8–10 days, about 50% of the larvae died and floated on the surface of the solution in clusters, some of which were black (Fig. 2A). The other 50% either transformed into lighter-coloured larval–pupal intermediates or emerged as weak adults. Interesting observations were also made with extracts of *V. schiliebenii* root bark extracts at 100 ppm. Although mortality was <10% in 24 h, the surviving larvae appeared smaller in size compared with those in the control. Moreover, they were sluggish and failed to move toward deeper sections of the solution. Morphological abnormalities (Fig. 2B and C) were observed 48 h post-treatment. About 30% of the larvae failed to transform to normal pupae, and instead produced larval–pupal intermediates. Approximately 60% successfully pupated, but the resulting pupae either failed to emerge as adults or emerged as weak adults that died within 48 h. Lower doses (<50 ppm) of *V. trifolia* leaf extracts did not appear to cause any morphological deformities, but led to retardation and 100% mortality in 7–8 days.

4. Discussion

No previous studies on bioactivities of phytochemical extracts of *V. payos* and *V. schiliebenii* have been reported. In the present study, acetone extracts of different parts of the two plants exhibited potent larvicidal effects at higher doses (≥100 ppm). Of particular interest, are the longer term growth-disrupting effects of the extracts at lower doses. Larvae exposed to the extracts exhibited structural deformation and dysfunction, which resulted either in

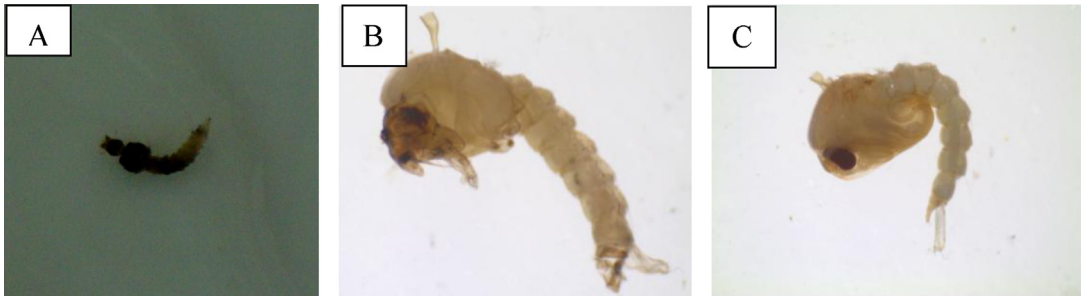

Fig. 2. Phytoextracts induced morphological deformities (A) black larva; (B) and (C) partially formed pupae (larval/pupal intermediates).

their death, or short-lived adults. Similar structural deformations were previously reported with *A. gambiae* larvae exposed to root bark extracts of Meliaceae species (Ndung'u et al., 2004). These deformities were associated with blends of several limonoids, which are structurally related to ecdysteroids and have been specifically considered as potential ecdysteroid agonists or antagonists. Indeed, two limonoids from *Turraea obtusifolia* have been shown to antagonize 20-hydoxyecdysone action in a *Drosophilia* cell line (Sarker et al., 1997). Limonoids have also been implicated as possible disruptants of the endocrine system of other insects by different authors (Jayaprakasha et al., 1997; Lopez-Olquin et al., 1997; Roel et al., 2002 and Rembold, 1995). Previous phytochemical screening of *V. agnuscastus* revealed the presence of different classes of constituents, including iridoid glycosides, flavonoids, alkaloids and terpenoids (Artz, 2007). Of particular interest is demonstration of an insect growth hormone pterosterone in *V. glabrata* (Suksamrarn et al., 1999) and related ecdysterioids, including (24R)-11 alpha, 20, 24-trihydroxyecdysone and 11 alpha, 20, 26-trihydoxyecdysone from *V. canescens* root bark (Suksamrarn et al., 2000). We are currently undertaking bioassay-guided studies to isolate and characterize the active compounds from the acetone extracts of *V. payos* and *V. schiliebenii* responsible for the observed activities, and it would be interesting to see if the growth-disrupting effects observed with the extracts of these plants are due to similar or different constituents.

In the present study, unlike the extracts of *V. payos* and *V. schiliebenii*, methanol leaf extract of *V. trifolia* did not show growth-disrupting effects on *A. gambiae* larvae, but physical retardation and delayed mortality occurred at lower doses. Previously, extracts of the plant were shown to have anti-feedant effects on the insect pest *Spodoptera frugiperda* (Hernández et al., 1999). The plant has also been reported to have pharmacological properties, including antipyretic (Ikram et al., 1987) and antibacterial (Hossain et al., 2001), as well as asthma and allergy relieving effects (Ikwati et al., 2001). Phytochemical studies have shown the presence of monoterpenes (Pan et al., 1989), halimane-type diterpenes and vitetrifolins (Ono et al., 2001). It would be interesting to see which of these or other phytoconstituents of the plant are responsible for the effects observed on *A. gambiae* in the present study.

In summation, results of this study show interesting larvicidal and/or growth-disrupting effects of *V. trifolia*, *V. payos* and *V. schiliebenii* extracts. The active constituents responsible for these effects remain to be characterized. Enriched extracts of the plants may have potential for controlling malaria vectors in breeding sites around human dwellings.

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