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

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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).

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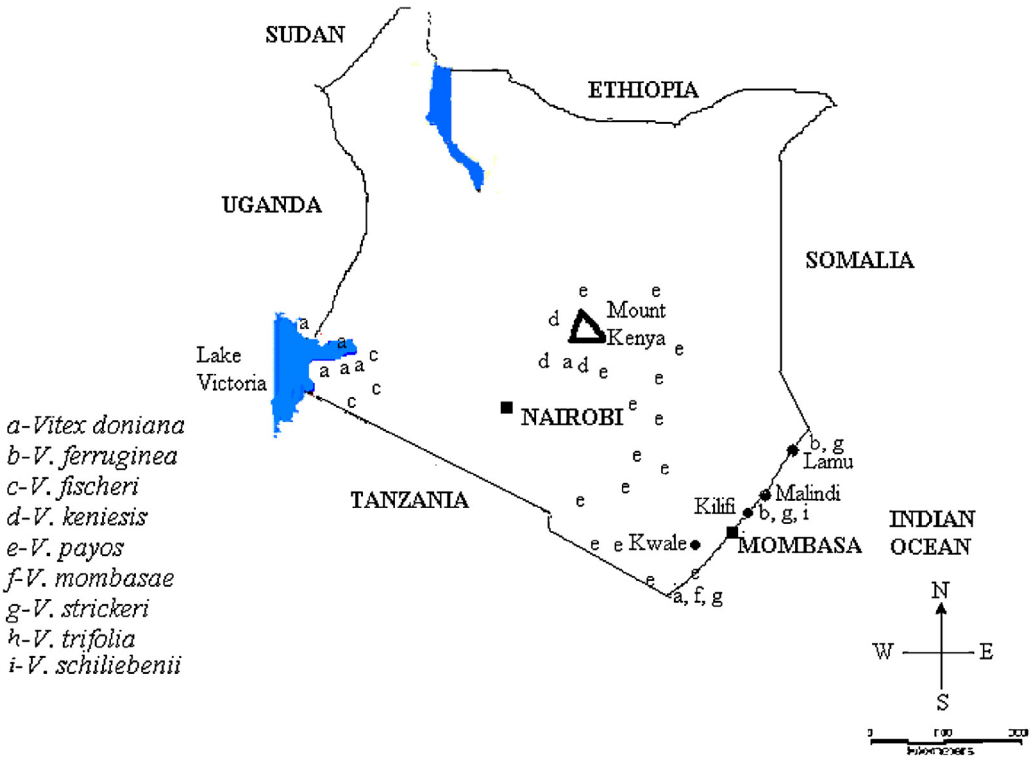

Fig. 1. Distribution of *Vitex* species in Kenya.

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