

**PUBLICATION
FIRST ABSTRACT**

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

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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.

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PUBLICATION

SECOND ABSTRACT

Time-course effects of *Vitex schiliebenii* (Verbenaceae) solvent extracts on *Anopheles gambiae* giles s.s. larvae under simulated semi-field conditions

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Objectives: To determine the growth-disrupting effects of polar phyto-extracts of *V. schiliebenii* on *Anopheles gambiae* larvae in a simulated semi-field condition and to undertake phytochemical screening of constituents present.

Materials & Method: 3rd and early 4th instars of *An. gambiae* larvae were exposed to acetone and methanol extracts of stem bark and leaves of *V. schiliebenii* and their effects on larval, pupal and adult stages recorded. Phytochemical screening of the extracts was undertaken using standard methods.

Results: The results revealed that *An. gambiae* larvae were susceptible to *V. schiliebenii* extracts with less than 20 % adult emergence at concentrations ≥ 25 ppm except for methanol extract of stem bark. About 11 % pupae emerged in *V. schiliebenii* acetone leaf extract (VSL 1) between day 6 and 10 but they did not transform into viable adults. Phytochemical screening revealed the presence of flavonoids, terpenoids, steroids, alkaloids, saponins and tannins in the extracts.

Conclusion: Eco-friendly polar extracts of *V. schiliebenii* show potential for mosquito control in small breeding habitats, which may be a useful component in integrated control of malaria vectors. Characterization of the active constituents of the extracts of the plant is in progress.

KEYWORDS: *Anopheles gambiae* s.s., *Vitex schiliebenii*, Verbenaceae, larvicidal effects, Photochemical

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THIRD ABSTRACT

Larvicidal and Brine Shrimp Activities of *Vitex Schiliebenii* Extracts and Isolated Phytoecdysteroids on *Anopheles gambiae* Giles S.S Larvae

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Acetone, methanol and aqueous extracts of the leaves, stem bark and root bark of *Vitex schiliebenii* belonging to the family Verbenaceae were evaluated for their larvicidal activity against late 3rd/early 4th *Anopheles gambiae* Giles s.s. larvae (Diptera: Culicidae). The extracts of the acetone leaves and stem bark were active with LC₅₀ values of 14.6 and 17.4 ppm respectively at 24 hrs. These extracts exhibited low toxicity to brine shrimps with LC₅₀ values of 180.9 and 154.4 ppm respectively. The constituents in these extracts were isolated and evaluated and the phytoecdysteroids 20-hydroxyecdysone (**1**) and stigmasterol (**2**) were identified as the active principles in the acetone stem bark while γ -sitosterol (**3**) was the active principle of the acetone leaf extract. The methanol leaf extract, the stem bark aqueous extract and the acetone root bark also showed potency against the mosquito species.

PUBLICATION
FOURTH ABSTRACT

Chemical composition and evaluation of mosquito larvicidal activity of *Vitex payos* extracts against *Anopheles gambiae* Giles S.S larvae

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SUMMARY

AIM: The aim of this study was to evaluate the acetone and methanol root bark extracts of *Vitex payos* and different fractions thereof, following acetone extraction and column chromatographic separation, for their immediate toxicity and long term effects on *Anopheles gambiae* Giles sensu stricto larvae under simulated semi-field conditions.

METHODS: In the present study, acetone and methanol extracts and acetone chromatographic fractions were investigated against late third/early fourth-instar larvae of laboratory reared *A. gambiae*. The tests were conducted according to the method of the World Health Organization (WHO) on guidelines for laboratory and field testing of mosquito larvicides. In addition, chemical compositions of the extracts were carried out using gas chromatography-mass spectrometry (GC-MS). The study was conducted in 2012.

RESULTS: Acetone chromatographic fractions showed significant larvicidal activity against *A. gambiae* larvae with LC₅₀ values ranging between 0.7 - 0.9 ppm. Dose-response relationships were established with the highest dose of 500 ppm causing over 85% mortality in all the test extracts. Long-term (11-14 days) post-exposure observations at lower doses showed that the extracts achieved over 90% adult growth inhibition with morphogenetic variations and behavioral changes. In acetone and methanol extracts, carbonyl compounds were determined as the main constituents with percentage compositions of 34.26% and 23.53% respectively. The other class of compounds determined was the phytoecdysteroids where gamma sitosterol (6.43%) was the major sterol in acetone extract while stigmasterol (2.83%) was major in methanol extract.

CONCLUSION: Extracts and chromatographic fractions from *V. payos* possess larvicidal and/or insect growth regulatory (IGR) principles which can be used in aquatic ecosystems by the local communities where most of the people cannot afford synthetic larvicides.

Key words: *Anopheles gambiae*, *Vitex payos*, larvicidal activity, phytoecdysteroids, saponins, growth inhibition.

**PUBLICATION
FIFTH ABSTRACT**

**Toxicity of Individual and Blends of Pure Phytoecdysteroids Isolated from
Vitex Schliebenii and *Vitex Payos* against *Anopheles Gambiae* S.S. Larvae**

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Four phytoecdysteroids: (20-hydroxyecdysone-20, 22-monacetone (1), 20-hydroxyecdysone (2), stigmaterol (3), gamma-sitosterol (4), were investigated for toxic effect against 3rd /4th instar larvae of *Anopheles gambiae* under laboratory conditions as individual compounds and in blends. The test larvae were treated with solutions containing the phytoecdysteroids of concentrations 1, 5 and 10ppm. The blends were prepared in the ratio of 1:1. Compounds 1 and 2, isolated from acetone extracts of *Vitex payos* caused 100% mortality at 10ppm. Compound 3 isolated from acetone leaves of *V. schliebenii* and compound 4 isolated from acetone stem bark of *V. schliebenii* also showed potent activity against the larvae at 10ppm. At the lower concentrations, abnormal mobility and impaired development was observed. Phytoecdysteroids (20-hydroxyecdysone-20, 22-monacetone (1) and 20-hydroxyecdysone (2) are larvicidal against *An. gambiae*. stigmaterol (3), gamma-sitosterol (4) also show potent IGR activities against *An. gambiae*. Also addition of compounds 1 and 2 to stigmaterol (3) and gamma-sitosterol (4) separately improved the activity of the two compounds.

Keywords: *anopheles gambiae*, *phytoecdysteroids vitex schliebenii*, *V. payos*

