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Fitoterapia 79 (2008) 106–111

FITOTERAPIA

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Larvicidal and growth inhibition of the malaria vector *Anopheles stephensi* by triterpenes from *Dysoxylum malabaricum* and *Dysoxylum beddomei*

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Received 15 November 2006; accepted 27 July 2007
Available online 11 August 2007

Abstract

Secondary metabolites from *Dysoxylum malabaricum* and *Dysoxylum beddomei* were tested against mature and immature stage of the mosquito vector *Anopheles stephensi* under laboratory conditions. The triterpenes 3β,24,25-trihydroxycycloartane and beddomeilactone from *D. malabaricum* and *D. beddomei* showed strong larvicidal, pupicidal and adulticidal activity. They also affected the reproductive potential of adults by acting as oviposition deterrents. The highest concentration tested (10 ppm) of both compounds evoked more than 90% mortality and oviposition deterrence.

Keywords: *Dysoxylum malabaricum*; *Dysoxylum beddomei*; *Anopheles stephensi*; Larvicidal activity

1. Introduction

During the last decade, research on various natural plant products and botanicals against the mosquito vectors suggests that some may serve as possible alternatives to synthetic chemical insecticides [1–4]. Over two thousand plant species contain chemicals with pest control properties [1,5,6] and several have shown some degree of activity against mosquitoes [7,8]. The use of plant essential oils and phytochemicals against malaria vectors has been reviewed recently [2,3]. In recent years, a lot of work has been done on control of *Anopheles stephensi* by secondary plant metabolites [9–12].

For the past two decades a great deal of work has been undertaken on secondary metabolites from the Meliaceae [4,7,10,13]. Most of the *Dysoxylum* spp. are large sized trees with the leaves that contain several limonoids [14–17].

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doi:10.1016/j.fitote.2007.07.013

Leaf extracts of *Dysoxylum malabaricum* affect insects in a variety of ways, acting as an antifeedant and larvicide [12,18].

Mosquitoes of the *Anopheles* spp. transmit malaria parasites to humans. *Anopheles* spp. vary in their vector potential because of environmental conditions and factors affecting their abundance, blood-feeding behavior and survival [19]. *A. stephensi* predominantly breeds in wells, overhead or ground level water tanks, cisterns, coolers, roof gutters, and artificial containers [20,21]. The various malaria vectors exhibit a wide variety of life history strategies, thus there is no simple and universally applicable form of vector control. Crude extracts from *Dysoxylum* spp. leaves have shown excellent larvicidal and mosquitocidal properties against mosquito vectors [12], but the bioactivity of purified compounds or secondary metabolites against mosquitoes remains unexplored. The present investigation was undertaken to study the larvicidal effect of two secondary metabolites from *D. malabaricum* and *D. beddomei* spp., 3β,24,25-trihydroxycycloartane and beddomeilactone [15–17] against the larval and adult stages of *A. stephensi* mosquito.

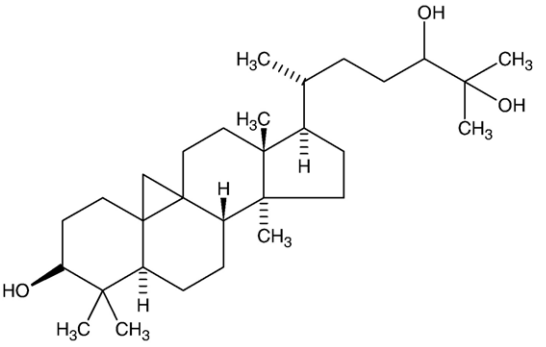
2. Experimental

2.1. Plant

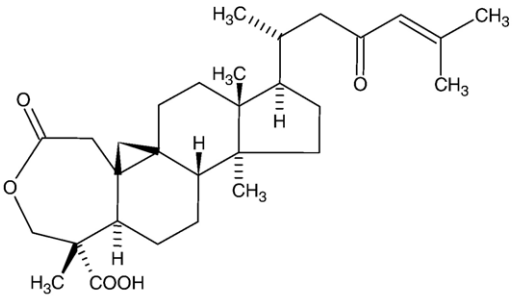
D. beddomei Hien (Meliaceae) and *D. malabaricum* Bedd. (Meliaceae) leaves were collected from the Herbal Garden of Kottakkal Arya Vaidyasala, Kottakkal, India and identified by Dr. Indira Balachandran, Research officer of the Herbal Garden where a voucher specimen (AH No. DL98) has been deposited.

2.2. Extraction and isolation of compounds **1** and **2**

D. malabaricum and *D. beddomei* leaves shade dried and powdered were extracted with EtOAc at r.t. *D. malabaricum* and *D. beddomei* extracts were chromatographed over an alumina column eluting with solvents of



Compound **1**



Compound **2**.

Fig. 1. Compounds **1** and **2**.

increasing polarity to yield 3β,24,25-trihydroxycycloartane **1** (3β,24,25 THCL) and beddomeilactone **2** (BL), respectively (Fig. 1). THCL and BL were identified on the basis of H- and C-NMR spectral data [16–18].

2.3. Mosquito culture

A. stephensi Liston (Diptera), larvae were reared in plastic and enamel trays in tap water. They were maintained and all the experiments were carried out at 27±2 °C, and 75–85% relative humidity under a 14:10 light:dark photoperiod. *A. stephensi* cultures were maintained as described previously [11].

2.4. Bioassays and larval mortality

Fourth instar larvae and pupa were exposed to test concentrations of 1, 2.5, 5 and 10 ppm of **1** and **2** in distilled water for 24 h according to standard WHO procedure [22]. A minimum of 20 larvae/concentration were used. The dead larvae were counted every hour and percentage mortality is reported as the average of five replicates. Lethal concentrations (LC_{50} and LC_{90}) were calculated according probit analysis [23]. The percentage mortality was calculated as described previously [11] and corrections for mortality when necessary were done by using Abbott's [24] formula.

For adulticidal assay 10 fresh *A. stephensi* adults were exposed to filter paper (90 mm, Advantec Toyo, Japan) treated with concentrations of 1, 2.5, 5 and 10 ppm. The paper was kept inside the beaker. Muslin cloth covering the beaker also was treated. Control insects were exposed only to isopropanol-treated paper and muslin cloth. A mortality count was taken after 24 h. The experiment was repeated five times [11]. For oviposition assay 10 gravid females were

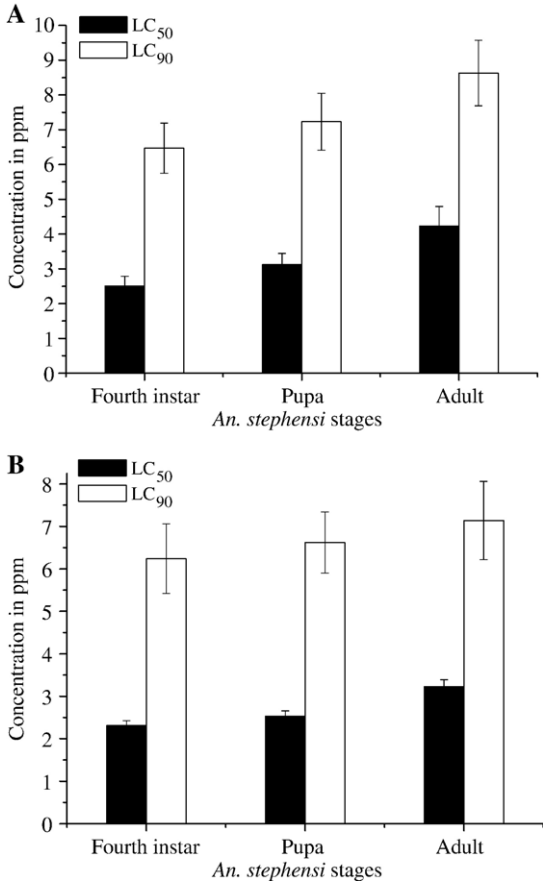


Fig. 2. Lethal concentrations (LC_{50} and LC_{50}) of compounds **1** (A) and **2** (B) against fourth instar larvae, pupae and adults of *A. stephensi* (values are mean of five replicates±standard error).

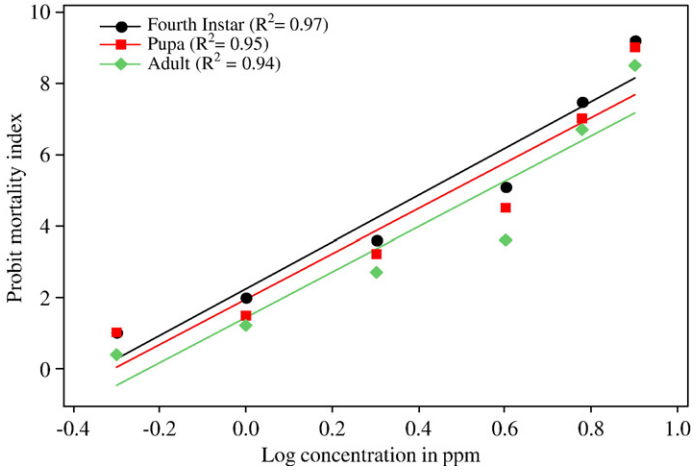


Fig. 3. Probit mortality regression index of fourth instar larvae, pupae and adults of *A. stephensi* after treatment with compound **1**. (The data were fitted by linear regression).

given a choice between treated and control jars. Dilutions of isopropanol served as a control (five replicates). During the tests, the groups of ten females (ten numbers) were kept separate for 48 h in cages measuring 25 × 25 × 30 cm. After the eggs were counted, the oviposition deterrence index was calculated [25].

2.5. Statistical analysis

Data from mortality and oviposition deterrence were fitted with linear regression using Minitab^R statistical software.

3. Results and discussion

In the search for an eco-friendly pesticide, researchers have considered pesticides of biological origin, and the replacement of chemical pesticides with biopesticides as a generally acceptable one. Plant derived products have

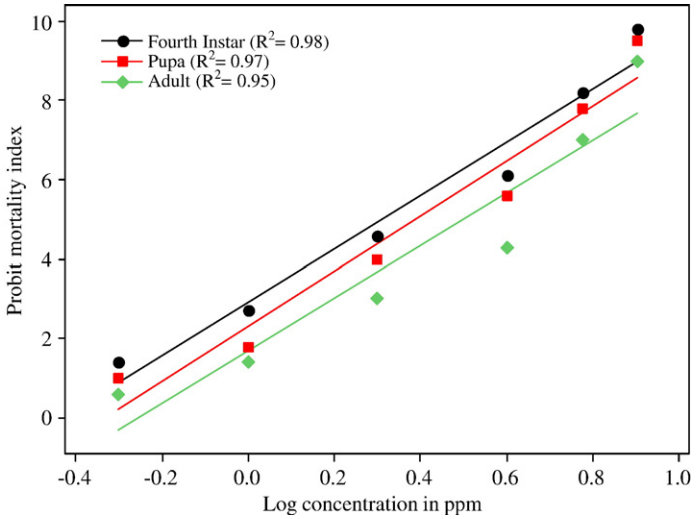


Fig. 4. Probit mortality regression index of fourth instar larvae, pupae and adult of *A. stephensi* after treatment with compound **2**. (The data were fitted by linear regression).

received increased attention from scientists and more than two thousand plant species are already known to have insecticidal properties [1,5,6,26]. The LC_{50} and LC_{90} values for *A. stephensi* exposed to compounds **1** and **2** are shown in Fig. 2. Fourth instars were more susceptible to both compounds (**1**, Fig. 2A; **2**, Fig. 2B) when compared with pupae and adults.

Using probit regression analysis, regression lines were calculated for dose-dependent mortality to the **1** and **2** treatment. Results on mortality and oviposition deterrence of *A. stephensi*, with increase in **1** and **2** concentration and with respect to different stages are presented in Figs. 3–5, respectively. At concentrations higher than 10 ppm the larvae showed irregular movement and died immediately after exposure to the treatment. No pupal or adult emergence was observed at 10 ppm and mortality occurred within 24 h, while control, larvae completed their life cycle. The mortality of fourth instar larvae was greater than other stages [$R^2=0.97$ for 3β,24,25 (**1**) and $R^2=0.98$ for BL (**2**)]. The 10 ppm concentration of **1** killed more than 90% of pupae ($R^2=0.95$) and 85% of adults ($R^2=0.94$) (Fig. 3). The same trend was also observed on percentage mortality of *A. stephensi* treated with **2**, with more than 95% of pupal and larval mortality ($R^2=0.98$ for fourth instar larvae and $R^2=0.97$ for pupae) and more than 90% mortality in case of adult *A. stephensi* ($R^2=0.95$) (Fig. 4). Thus, lethal effects on larvae appear to greatly reduce ultimate numbers of adult mosquitoes. The direct and indirect contributions of such effects to treatment efficacy through reduced larval feeding and fitness need to be properly understood in order to improve the use of botanical insecticides for management of *A. stephensi*. Exposure of *A. stephensi* larvae to sub-lethal and lethal doses of neem extract and seed and leaf extracts of *Melia azedarach* in the laboratory prolonged larval development, reduced pupal weight, caused high oviposition deterrence, and caused high mortality [10,11,27].

Fig. 5 demonstrates the efficacy concentration-dependent of **1** and **2** against reproduction potential of adult female *A. stephensi*. The highest concentration, 10 ppm of the **1** and **2**, produced more than 90% oviposition deterrence in adult female *A. stephensi*. There was a gradual decrease in eclosion from pupae when female *A. stephensi* were treated with **1** and **2** at lower dose treatments, but the effect was more pronounced for treatments greater then 5 ppm. Maximum ovipostion deterrence was observed at 10 ppm [$R^2=0.982$ for 3β,24,25 (**1**) and $R^2=0.995$ for BL (**2**), respectively]. In the present study, application of two *Dysoxylum* triterpenoids greatly affected the growth of *A. stephensi.* The lower dose treatments inhibited growth and caused mortality in a dose-dependent manner. Secondary metabolites extracted from many plant species show growth inhibiting effects on the various developmental stages of different mosquito species. A range of pre-emergent effects can occur such as delays in larval development and extended pupal durations, molting inhibition, morphological abnormalities, and mortality especially during molting and melanization processes [3].

The results of the present study showed that **1** and **2**, from two *Dysoxylum* spp. were effective against larvae, pupae and adult *A. stephensi*.

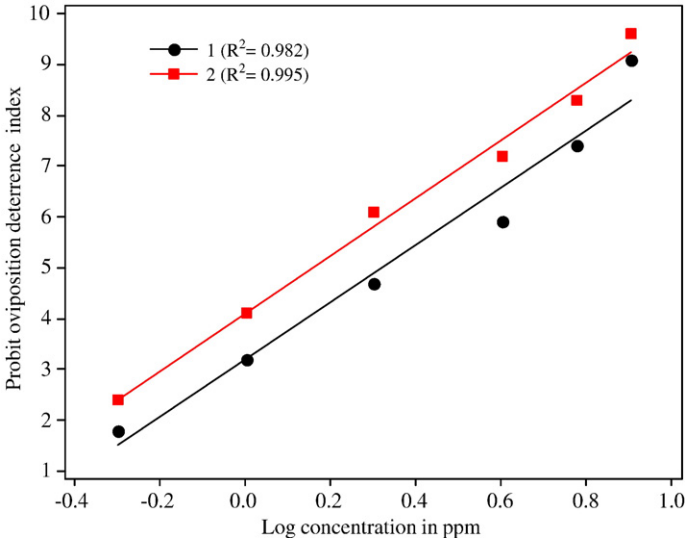


Fig. 5. Oviposition deterrence index (ODI) of *A. stephensi* after treatment with compounds **1** and **2.** (The data were fitted by linear regression).

Acknowledgments

The authors wish to thank Mr. Karthikeyan, Technician for his help during the research period. Special thanks are also given to Dr. Thomas W Sappington for his valuable comments on an earlier draft of the manuscript. Financial help to the first author from the HARI, RDA, NICS to conclude this work is gratefully acknowledged.

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