Short Communication

Evaluation of the larvicidal potential of root and leaf extracts of *Saussurea costus* (Falc.) Lipsch. against three mosquito vectors: *Anopheles stephensi*, *Aedes aegypti*, and *Culex quinquefasciatus*

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**Abstract**

**Introduction**: The larvicidal potential of *Saussurea costus* (Falc.) Lipsch. was studied against the early 4th instar larvae of *Anopheles stephensi* Liston., *Aedes aegypti* Linn., and *Culex quinquefasciatus* Say. because of the emergence of mosquito resistance to conventional synthetic insecticides. **Methods**: At concentrations of 12.5-200 ppm, larvicidal activities were studied under laboratory conditions. **Results**: After 24 h of exposure, the methanol extract of the roots recorded the highest larvicidal activity against *An. stephensi*, with LC$_{50}$ and LC$_{90}$ values of 7.96 and 34.39 ppm, respectively. **Conclusions**: We are developing potent larvicidal compound(s) from *S. costus* for controlling the mosquito larval population.

**Keywords**: *Anopheles stephensi*. *Aedes aegypti*. *Culex quinquefasciatus*. *Saussurea costus*. Vectors. Larvicidal activity.

The Culicidae family is comprised of approximately 3500 mosquito species. The genera *Anopheles*, *Aedes*, and *Culex* act as vectors of various diseases, such as encephalitis, chikungunya, dengue fever, filariasis, and malaria[1], which compromise human health[2]. The important vector of malaria in the urban districts of India and other West Asian countries is *Anopheles stephensi*[3], which afflicts 36% of people situated in tropical and subtropical regions[4]. The female mosquitoes of the genus *Aedes* transmit the viruses of dengue, zika, and chikungunya fever in the tropical and subtropical urban regions of the world. At present, approximately 2500 million people are facing the threat of dengue fever and nearly 50 million cases are recorded every year[5]. The parasitic filarial nematodes (roundworms – Family Filarioidea) *Wuchereria bancrofti* (90% of infections), *Brugia malayi* (9% of infections), and *Brugia timori* (1% infections) cause lymphatic filariasis, for which the vector is *Culex quinquefasciatus*. There are approximately 120 million prevalent infections that are caused by these filarial worms, most of which are due to *W. bancrofti*[6].

Mosquito control is facing timely challenges due to the inadequate success of bio-control programs and emergence of resistance to the conventional synthetic insecticides, which have necessitated the need to investigate and develop unconventional strategies by means of eco-friendly, environmentally safe, and biodegradable products as mosquito larvicides[7]. Natural products from plants have been evaluated as prototypes for new insecticidal agents, as they comprise a rich source of bioactive compounds that are potentially suitable for utilization in integrated management programs[8]. Consequently, the present study was undertaken to investigate the larvicidal potential of root and leaf extracts of *S. costus* against the early 4th instar larvae of *An. stephensi*, *Ae. aegypti*, and *Cx. quinquefasciatus*, as possible control measures to prevent the incidence of vector-borne diseases. This is the first study of its kind, reporting the larvicidal activities of root and leaf extracts of *S. costus* against the tested mosquito vectors.

The roots and leaves of *Saussurea costus* (Falc.,) Lipsch. were gathered in the month of August, 2016, from Jahama (34.198°N 74.364°E), the Baramulla district, Jammu and Kashmir, India. Then, 500 g of powdered plant material that was packed inside a Soxhlet apparatus was subjected to 72 h of successive extraction using threefold of solvent systems, like petroleum ether, chloroform, ethyl acetate, and methanol. The pooled extracts were evaporated under reduced pressure at 40 °C by a rotary evaporator (Heidolf-Germany) and stored at 4 °C until further assay. The voucher
specimen (AUBOT#347) is deposited at the herbarium, Department of Botany, Annamalai University.

The eggs of Anopheles stephensi Liston. and Aedes aegypti Linn., and the egg rafts of Culex quinquefasciatus Say. were procured from the Center for Research in Medical Entomology (ICMR-Government of India), Madurai, and reared in the laboratory (29±3 °C, 75 to 85% RH) by feeding with Brewer’s yeast/dog biscuits (1:3). The eggs/egg rafts were used for a larvicidal bioassay at the early 4th instar larval stage, as per the standard procedures recommended by the WHO. The mortality of the larvae was also checked using control groups (water and DMSO). Probit analyses (SPSS, version 21.0) were used for calculating the lethal concentrations, LC50 and LC90, and their 95% confidence limit of upper and lower confidence levels.

To determine whether S. costus possess a larvicidal effect against the early 4th instar larvae of the selected mosquito species, the larvae were exposed to different root and leaf extracts of S. costus in a concentration dependent manner during 12 and 24 h of exposure. Varied levels of larvicidal activities were observed for all tested extracts, while there were no recorded larval mortalities during the control treatments (DMSO and water). Among the different extracts of S. costus roots, the methanol extract recorded the highest larval mortality. After 12 h of exposure, the root methanol extract had LC50 and LC90 values of 10.70 and 53.91 ppm for An. stephensi, 14.97 and 108.15 ppm for Ae. aegypti, and 23.90 and 185.03 ppm for Cx. quinquefasciatus, respectively. After 24 h of exposure, the root methanol extract had LC50 and LC90 values of 7.96 and 34.39 ppm for An. stephensi, 10.79 and 60.71 ppm for Ae. aegypti, and 15.31 and 105.63 ppm for Cx. quinquefasciatus, respectively. The larvicidal activity of the methanol extract was followed by that of petroleum ether >chloroform and ethyl acetate extracts of S. costus after 24 h of exposure (Figure 1).

The larvicidal activity of the petroleum ether leaf extract of S. costus was higher than that of the other leaf extracts tested against An. stephensi, Ae. Aegypti, and Cx. quinquefasciatus. After 12 h of exposure, the petroleum ether leaf extract had LC50 and LC90 values of 27.83 and 184.82 ppm for An. stephensi, 62.73 and 249.03 ppm for Ae. aegypti, and 87.56 and 269.59 ppm for Cx. quinquefasciatus, respectively. After 24 h of exposure, the petroleum ether leaf extract had LC50 and LC90 values of 17.72 and 138.32 ppm for An. stephensi, 23.49 and 172.91 ppm for Ae. aegypti, and 50.12 and 165.77 ppm for Cx. quinquefasciatus, respectively. The larvicidal activity of the petroleum ether leaf extract was followed by that of methanol >chloroform and ethyl acetate extracts after 24 h of exposure (Figure 2).

![FIGURE 1: Larvicidal potential of Saussurea costus root extracts against An. stephensi, Ae. aegypti, and Cx. quinquefasciatus.](image-url)
Dehydrocostus lactone and costunolide, isolated from the essential oils of the roots of S. costus, strongly support the theory that S. costus could be an effective larvicidal plant, as they exhibit strong larvicidal activity against Ae. albopictus with LC₅₀ values of 2.34 and 3.26 µg/mL, respectively. In addition, An. stephensi and Ae. aegypti larvae were found to be more susceptible to plant extracts than other mosquito species, since the methanol extract of Terminalia chebula was more effective against An. stephensi (LC₅₀ = 87.13 ppm) and Ae. Aegypti (LC₅₀ = 93.24 ppm) than Cx. Quinquefasciatus (LC₅₀ = 111.98 ppm). Additionally, Ramya et al. observed the highest larval mortality from the ethyl acetate fraction of a leaf extract of Catharanthus roseus, followed by the methanol fraction against the I, II, III, IV, V, and VI instar larvae of Helicoverpa armigera. Similar to our results, among the different extracts tested, the methanol extract of the roots of R. cordifolia was more potent against Cx. quinquefasciatus, with LC₅₀ and LC₉₀ values of 95.69 and 347.96 mg/L, respectively. Moreover, the methanol extract of Andrographis echioides had a higher toxicity against Ae. Aegypti (LC₅₀ = 93.00 and LC₉₀ = 83.06 ppm) than against Cx. quinquefasciatus (LC₅₀ = 171.81 and LC₉₀ = 171.76 ppm). Likewise, the petroleum ether extract of the leaves of Ruta graveolens showed the highest larvicidal activity against An. stephensi, having an LC₅₀ value of 31.89 µg/mL and LC₉₀ value of 66.96 µg/mL, while it had an LC₅₀ value of 66.96 µg/mL against Ae. aegypti after 24 h of exposure. In another study, the larvicidal efficacy of the ethanol, acetone, and petroleum ether extracts of the leaves of Tribulus terrestris were studied against 3rd instar larvae of Ae. aegypti. Among the other tested extracts, the petroleum ether extract was found to be the most effective, with an LC₅₀ value of 64.6 ppm.

The present work demonstrates that S. costus could be considered as a novel and effective source for use in vector control programs because of its biocidic effect against the larval stages of An. stephensi, Ae. aegypti, and Cx. quinquefasciatus at low concentrations. The compound(s) responsible for the larvicidal activity should be isolated from the methanol extract of the roots of S. costus through bioassay-guided fractionation, which is under way in our laboratory.
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AUTHOR’S CONTRIBUTION

Both authors equally contributed to the paper.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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