

SHORT COMMUNICATION

A note on the larvicidal efficacy of saponin constituted crude extracts of plant and animal origin against *Aedes aegypti* L.

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Abstract: Natural products of plant and animal origin are preferred over synthetic insecticides due to their eco-friendly nature. The use of natural products and their derivatives are being advocated for the control of insect vectors of human diseases. The crude extracts of the fruits of *Sapindus emarginatus* (a medicinal plant) and the skin of *Holothuria atra* (a non-edible sea cucumber) were tested under laboratory conditions against *Aedes aegypti*, a vector of dengue and chikungunya, for their larvicidal properties. Bioassay experiments carried out with crude extracts of *S. emarginatus* and *H. atra* revealed LC₅₀ values of 92.9 and 68.82 ppm respectively. Both crude extracts showed positive result for the presence of saponin. This preliminary study suggests that not only plant but also animal sources can be effectively used to produce less expensive and safe compounds to control mosquito vectors in Sri Lanka.

Keywords: *Aedes aegypti*, *Holothuria atra*, larvicide, *Sapindus emarginatus*, vector control.

INTRODUCTION

Mosquito-borne diseases such as malaria, dengue and chikungunya are of public health importance to Sri Lanka¹⁻³. Even though there has been a drastic decline in reported malaria cases, severe epidemics of chikungunya and Dengue Fever (DF) / Dengue Hemorrhagic Fever (DHF) were reported from different parts of the country in recent times²⁻⁴. One option for management of insect vector-borne disease is to control the immature forms of vector mosquitoes. This method is more appropriate for the control of domestic and peri-domestic container breeding mosquitoes such as *Aedes aegypti* and *A. albopictus*, the vectors of dengue and chikungunya. At present, synthetic insecticides

are heavily used for this purposes. The uses of chemical constituents of plant origin as a part of integrated vector control programme is encouraging due to their eco-friendly nature.

Natural products of plant origin have been tested for insecticidal properties to control disease vectors⁵⁻⁸, but extracts from animal origin have been rarely tested or reported. Sukumar *et al.*⁹ reported plants belonging to 99 families, 276 genera and 346 species to have insecticidal properties. Among the families reported 3 species of family Sapindaceae, namely *Koelreuteria paniculata* (extracts of seeds and leaves), *Poullinia fuscescens* (extracts of seeds and fruits) and *Sapindus saponaria* (extracts of seeds and fruits) were found to be effective against mosquito larvae. The present study was carried out in a laboratory to assess the larvicidal efficacy of the fruit extract of *Sapindus emarginatus* Vahl (Sapindaceae), a medicinal plant widely distributed in the dry-zone of Sri Lanka, against *A. aegypti* larvae. In addition, an attempt was made to identify crude extracts of animal sources with mosquito larvicidal properties. As sea cucumbers are reported to have medicinal values¹⁰, extracts of dried skin of *Holothuria atra* Jaeger (Holothuroidea), a sea-cucumber widely present in the shallow waters of Sri Lanka with less consumer or economical demand were tested and the results are reported here.

METHODS AND MATERIALS

The fruits of *S. emarginatus* were collected in mid 2006 from Vavuniya (a locality in the northern dry zone of

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Sri Lanka) and *H. atra* was collected in early 2007 from the east coast of Jaffna peninsula of northern Sri Lanka. The collected *H. atra* was washed in running water and the skin was removed. The fruits of *S. emarginatus* and skin of *H. atra* were exposed to direct sunlight for 6-8 h a day for 14 days. The sun dried materials were chopped into small pieces with a knife and were powdered using a table model grinder. The powdered materials were stored in desiccators covered with aluminum foil. In the case of *H. atra*, initially 74 g of skin material was defatted using 250 mL dichloromethane at 40-45°C for 8 h. Five grams each of the stored materials of *S. emarginatus* and *H. atra* were used for extraction. The crude extraction of *S. emarginatus* was carried out in a Soxhlet apparatus using 200 mL of absolute ethanol at 78°C for 8 h. The residue fat-free *H. atra* was also extracted in Soxhlet apparatus in 250 mL of methanol at 60°C for 7 h. The crude extracts were concentrated and solvents were removed under reduced pressure using a rotary vacuum evaporator. A portion of both crude extracts was tested for the presence of saponin using a standard method¹¹. Stock solutions of desired concentrations (0.45 g/20 mL) of *S. emarginatus* crude extract and (1 g/20 mL) of *H. atra* were prepared using distilled water. Larval bioassays were carried out as per WHO techniques with slight modifications under laboratory conditions (29 ± 2°C). For each bioassay 15 healthy third instar larvae of laboratory reared *A. aegypti* were tested in plastic containers of 300 mL capacity containing 200 mL of dechlorinated water. In each case 5 different concentrations were prepared using the above stock solutions (ranging from 67.5 – 157.5 ppm for *S. emarginatus* and 62.5 – 175 ppm for *H. atra*) and bioassayed using 3 replicates of each concentrations. Controls were run simultaneously with each set of experiments. Mortality of larvae was recorded after 24 h. The dose-mortality response values were analysed using log-probit regression analysis using the statistical software package 'Statistical Package for the Social Sciences' (SPSS; SPSS 12.0.1 for Windows, Chicago, Illinois, USA) to determine lethal concentrations for the mortalities of 50% and 90% of the treated larvae along with 95% Fiducial Limits.

Table 1: Laboratory evaluation of crude extracts of *S. emarginatus* and *H. atra* against third instar larvae of *A. Aegypti*

Source	Part of source	Solvent used	LC ₅₀ (95% F.L.)* ppm	LC ₉₀ (95% F.L.)* ppm	χ ² (df=3)
<i>S. emarginatus</i>	fruit	ethanol	92.9 (81.96-101.46)	152.6 (139.66-174)	2.654
<i>H. atra</i>	skin	methanol	68.82 (39.12-89.22)	180.76 (154.53-227.25)	3.266

*Fiducial Limits

RESULTS AND DISCUSSION

Bioassays with crude extract of *S. emarginatus* and *H. atra* against *A. aegypti* revealed the LC₅₀ values of 92.9 and 68.82 ppm respectively (Table 1). Both extracts were positive for the presence of saponin.

S. emarginatus and *H. atra* could be used as sources to extract active compounds to produce effective mosquito larvicidal agents to control mosquito vectors. Preliminary studies are encouraging and studies on the larvicidal efficacy with other solvents and against other vectors are in progress.

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