Research article

Insecticidal efficacy of Rubus steudneri and Rubus apetalus against Aedes (Diptera: Culicidae) and Culex (Diptera: Culicidae) mosquitoes

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ABSTRACT

Introduction and Aim: Rubus steudneri Schwein. and Rubus apetalus Poir. belonging to the family Rosaceae is one of the ethnomedicinal plants used widely in Ethiopia as food, for construction and as medicine. To the best of our knowledge, insecticidal activity of R. steudneri and R. apetalus has not been investigated so far. In this study, we report insecticidal efficacy of R. steudneri and R. apetalus in terms of larvicidal effect against II and III instar larvae of Aedes and Culex mosquitoes.

Materials and Methods: The shade-dried and powdered leaves were extracted using methanol by maceration process. Insecticidal activity of leaf extract was determined by larvicidal assay against II and III instar larvae of Aedes and Culex species. LC50 and LC90 values were calculated.

Results: The leaf extract of both Rubus species exhibited concentration dependent larvicidal effect. II instar larvae were shown to be highly susceptible than III instar larvae. Among leaf extracts, extract of R. steudneri exhibited marked insecticidal activity when compared to extract of R. apetalus as revealed by lower LC50 and LC90 values. Culex larvae displayed marked susceptibility to leaf extracts when compared to Aedes larvae.

Conclusion: Marked larvicidal effect was observed against larvae of Culex species when compared to Aedes species as indicated by lower LC50 and LC90 values. It is evident that the leaf extracts of both Rubus contain insecticidal principles.

Keywords: Rubus steudneri; insecticidal; larvicidal; Aedes; Culex.

INTRODUCTION

Mosquitoes are one among the important vectors that transmit human diseases. Since development, chemical insecticides have shown to be promising with respect to control of insect vectors that transmit dreadful diseases such as dengue, malaria, chicken guya, Filariasis and Japanese encephalitis. However, their indiscriminate usage results in deleterious effects on environment, emergence of resistant races of insect vectors and toxic effects on humans. Botanical extracts are promising with respect to their potent activity against eggs, larvae, pupae, and adult stages of mosquito vectors (1-3). Rubus steudneri Schwein. is a scandent shrub belonging to the family Rosaceae. The plant is commonly known as Gora and is characterized by deeply furrowed greyish-tomentose stems that are covered with stellate hairs. The fruits of the plant are edible. The plant has got ethnobotanical significance (4-8) and the studies revealed that R. steudneri displayed pharmacological activities such as antimicrobial, antioxidant, antiproliferative and antidiabetic activities (9-12).

Rubus apetalus Poir. (Family: Rosaceae) is a scrambling shrub growing up to a height of 2.5m, characterized by hairy branches and scattered hooked spines on the stem. The plant is traditionally grown as living fences and as an ornamental plant. The fruits are edible and are eaten. R. apetalus possess immense therapeutic properties. It is shown that R. apetalus display biological activities such as antimicrobial, antioxidant, and anti diabetic activity (12-15). To the best of our knowledge, no work has been done on insecticidal properties of R. steudneri and R. apetalus. Hence, in this study, we report the insecticidal efficacy of extract of R. steudneri and R. apetalus leaves against larvae of Aedes and Culex mosquitoes.

MATERIALS AND METHODS

Collection of plant material

The leaves were collected at Nekemte, Oromia region, Ethiopia and identified by Dr. Tesfaye Awas and authenticated at the Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia (12).
Extraction
Maceration process was employed to extract the shade dried and powdered leaves of *R. steudneri* and *R. apetalus*. Methanol was used as menstruum. The leaf powder was left in methanol in stoppered container for 48 hours followed by filtration and evaporation of the filtrate at room temperature (16). The leaf extract thus obtained was used to assess larvicidal activity.

Larvicidal activity of *R. steudneri*
II and III instar larvae of *Aedes* and *Culex* species were subjected for screening their susceptibility to leaf extract of *R. steudneri* and *R. apetalus* (Fig. 1). The protocol employed by Kamaraj et al. (3) was used to determine larvicidal potential of leaf extract. The II and III instar larvae were exposed to different concentrations of extract (0.1 to 5.0mg extract/ml of water) for 24 hours. The number of dead larvae were counted after 24 hours of exposure. DDT (0.1% in water) was used as positive control. DMSO was used as negative control.

Figure 1: Experimental set up: Extracts in water (A), larvae in water containing extract (B)

Statistical analysis
The experiment was conducted in triplicates and the data presented as mean±SD. The Median lethal concentrations, LC$_{50}$ (dose that kill 50% of the exposed larvae) and LC$_{90}$ (dose that kill 90% of the exposed larvae) were determined by the regression equations (Y= a + bX; where, X=concentrations; Y=% mortality) using MS Excel 2010.

RESULTS
Fig. 2 and 3 show the result of larvicidal potential of leaf extract of *R. steudneri* against both the mosquito species. The extract revealed dose dependent mortality of both larval instars. Here also, the leaf extract was more effective against larvae of *Culex* species when compared to *Aedes* species. A mortality of 50% and higher against II and III instar larvae of *Culex* species was recorded at extract concentration 0.25mg/ml and 0.5mg/ml, respectively. In case of II and III instar larvae of *Aedes* species, a mortality of 50% and higher was recorded at extract concentration of 0.5mg/ml and higher. At extract concentration 1mg/ml and higher, 100% larval mortality was observed in case of *Culex* species while 100% mortality of II instar larvae of *Aedes* species was recorded at the highest concentration of leaf extract tested.

Fig. 4 and 5 show the result of larvicidal potential of leaf extract of *R. apetalus* against both the mosquito species. The extract revealed dose dependent mortality of both larval instars. Here also, the leaf extract was more effective against larvae of *Culex* species when compared to *Aedes* species. A mortality of 50% and higher against II and III instar larvae of *Culex* species was recorded at extract concentration 0.25mg/ml and 0.5mg/ml, respectively. At extract concentration 1mg/ml and higher, 100% larval mortality was observed in case of both mosquito species.
The *R. steudneri* and *R. apetalus* leaf extract exhibited larvicidal effect against II and III instar larvae of *Culex* and *Aedes* species. The LC$_{50}$ and LC$_{90}$ values were determined by the regression analysis and the results are depicted in the table 1. The LC$_{50}$ for *R. steudneri* leaf extract are 0.144mg/ml and 0.296mg/ml against II and III instar larvae of *Culex* species and 0.291mg/ml and 0.416mg/ml against II and III instar larvae of *Aedes* species, respectively. In the case of *R. apetalus*, LC$_{50}$ values were found to be 0.157mg/ml and 0.378mg/ml against II and III instar larvae of *Culex* species and 0.362mg/ml and 0.488mg/ml against II and III instar larvae of *Aedes* species, respectively. Furthermore, LC$_{90}$ values are shown in table 1. The order of activity observed was *R. steudneri* > *R. apetalus* against both mosquito species.

Table 1: LC$_{50}$ and LC$_{90}$ values of leaf extracts

| Extract       | Mosquito  | Larval stage | LC$_{50}$ (mg/ml) | LC$_{90}$ (mg/ml) |
|---------------|-----------|--------------|-------------------|-------------------|
| *R. steudneri* | *Culex*   | II instar    | 0.144             | 0.408             |
|               |           | III instar   | 0.296             | 0.789             |
|               | *Aedes*   | II instar    | 0.291             | 0.801             |
|               |           | III instar   | 0.416             | 0.858             |
| *R. apetalus*  | *Culex*   | II instar    | 0.157             | 0.409             |
|               |           | III instar   | 0.378             | 0.847             |
|               | *Aedes*   | II instar    | 0.362             | 0.857             |
|               |           | III instar   | 0.488             | 4.034             |

**DISCUSSION**

Interest in botanicals with insecticidal activities arose as a consequence of several drawbacks that are associated with the use of synthetic insecticides. It has been shown that crude extracts, as well as purified compounds from higher plants, display marked insecticidal activity in terms of their ovicidal, larvicidal, pupicidal and repellent activities (1, 17-20). In our study, the leaf extract of *R. steudneri* and *R. apetalus* were effective in causing mortality of *Aedes* and *Culex* larvae in a dose dependent manner. It is clear from the results that the larvicidal potential observed is influenced by the larval developmental stage as II instar larvae were susceptible to extract to high extent than III instar larvae. In similar studies, the larvicidal effect of extracts of *Aloe vera* (21), *Ficus benghalensis* (22), and *Couroupita guianensis* (23) was marked against lower instar stages than higher instar stages. The positive control i.e., DDT, at tested concentration, was effective in causing 100% mortality of both the larval instars of test mosquitoes. DMSO showed no larvicidal property. Among leaf extracts, the extract of *R. steudneri* displayed higher insecticidal potential than the leaf extract of *R. apetalus* as indicated by lower LC$_{50}$ and LC$_{90}$ values (Table 1). Our earlier studies have shown the presence of various phytochemicals viz. saponins, alkaloids, flavonoids, and tannins in *R. steudneri* and *R. apetalus* (14,24). Insecticidal activities of plant secondary metabolites such as alkaloids, flavonoids and tannins has been documented (25-27). In our previous study, the butyl isobutyl phthalate and 2-pyrolidinone 5-(cyclohexylmethyl) were identified in chloroform fraction of *R. steudneri* using GC-MS (28). The dibutyl phthalate isolated from ethyl acetate extract of *Ipomoea carnea* showed larvicidal activity against 4$^{th}$ instar larvae of *Aedes aegypti* and *Culex quinquefasciatus* (29).

**CONCLUSION**

The leaf extract of *R. steudneri* and *R. apetalus* displayed marked larvicidal property in a dose-dependent manner indicating the utilization of the plant species for developing novel insecticidal formulations that can be used to manage mosquito-borne diseases such as malaria, dengue, chikungunya and filariasis. The presence of butyl isobutyl phthalate in *R. steudneri* and other metabolites such as flavonoids, alkaloids, and tannins in both the plants might be responsible for the observed insecticidal activity. Further studies are warranted to identify the insecticidal principles present in the *Rubus* species selected in this study.

**CONFLICT OF INTEREST**

Authors declare no conflict of interest.

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