Research article

Insecticidal efficacy of *Rubus steudneri* and *Rubus apetalus* against *Aedes* (Diptera: Culicidae) and *Culex* (Diptera: Culicidae) mosquitoesRaghavendra H. L.^{1,2}, Prashith Kekuda T. R.³, Chaithra R. Shetty⁴, Manjula Shantaram⁵¹Faculty of Medicine (FMB), São Paulo State University (UNESP), Botucatu -18618687, São Paulo State, Brazil²College of Medical and Health Sciences, Wollega University, PO Box: 395, Nekemte, Oromia, Ethiopia³Dept. of Microbiology, S.R.N.M.N College of Applied Sciences, Balraj Urs Road, Shimoga-577201, Karnataka, India⁴Dept. of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, Nitte (Deemed to be University), Paneer, Deralakatte, Mangaluru –575018, Karnataka, India⁵Dept. of Studies and Research in Biochemistry, Mangalore University, Jnana Kaveri Post Graduate Centre, Chikka Aluvvara571 232, Kodagu, Karnataka, India

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ABSTRACT

Introduction and Aim: *Rubus steudneri* Schweinf. 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 gunya, 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* Schweinf. 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 antidiabetic 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 $\pm$ SD. The Median lethal concentrations, LC₅₀ (dose that kill 50% of the exposed larvae) and LC₉₀ (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 *Aedes* and *Culex* species. The extract was effective in causing mortality of larvae stages of both the mosquitoes in dose dependent manner. The 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* as well as *Aedes* 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.

Fig. 2: Mortality (%) of *Culex* larvae in presence of extract of *R. steudneri*

Fig. 3: Mortality (%) of *Aedes* larvae in presence of extract of *R. steudneri*

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. 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: Mortality (%) of *Culex* larvae in presence of extract of *R. apetalus*

Fig. 5: Mortality (%) of *Aedes* larvae in presence of extract of *R. apetalus*

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)
<i>R. steudneri</i>	<i>Culex</i> species	II instar	0.144	0.408
		III instar	0.296	0.789
	<i>Aedes</i> species	II instar	0.291	0.801
		III instar	0.416	0.858
<i>R. apetalus</i>	<i>Culex</i> species	II instar	0.157	0.409
		III instar	0.378	0.847
	<i>Aedes</i> species	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 have been documented (25-27). In our previous study, the butyl isobutyl phthalate and 2-pyrrolidinone 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 4th 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.

REFERENCES

- Mullai, K., Jebanesan, A. Larvicidal, ovicidal and repellent activities of the leaf extract of two cucurbitaceous plants against filarial vector *Culex quinquefasciatus* (Say) (Diptera: Culicidae). Trop Biomed. 2007; 24(1): 1-6.
- Vinayachandra, Shwetha, R., Chandrashekar, K. R. Larvicidal activities of *Knema attenuata* (Hook. f. & Thomson) Warb. (Myristicaceae) extracts against *Aedes albopictus* Skuse and *Anopheles stephensi* Liston. Parasitol Res. 2011; 109(6): 1671-1676.
- Kamaraj, C., Bagavan, A., Elango, G., Zahir, A.A., Rajakumar, G., Marimuthu, S., et al., Larvicidal activity of medicinal plant extracts against *Anopheles subpictus* & *Culex tritaeniorhynchus*. Indian J Med Res. 2011; 134(1): 101-106.
- Senbeta, F., Gole, T. W., Denich, M., Kellbessa, E. Diversity of useful plants in the coffee forests of Ethiopia. Ethnobotany Research & Applications 2013; 11: 49-69.
- Yineger, H., Kelbessa, E., Bekele, T., Lulekal, E. Plants used in traditional management of human ailments at Bale

- Mountains National Park, Southeastern Ethiopia. J Med Plants Res. 2008; 2(6): 132-153.
6. Luizza, M. W., Young, H., Kuroiwa, C., Evangelista, P., Worede, A., Bussmann, R. W., Weimer, A. Local Knowledge of Plants and their uses among Women in the Bale Mountains, Ethiopia. Ethnobotany Research & Applications. 2013; 11: 315-339.
7. Yassin, S., Abera, B., Kelbessa, E. Ethnobotanical study of indigenous knowledge of plant-material culture in Masha and Yeki districts, Southwest Ethiopia. Afr J Plant Sci. 2015; 9(2): 25-49.
8. Kefalew, A., Asfaw, Z., Kelbessa, E. Ethnobotany of medicinal plants in Ada'a District, East Shewa Zone of Oromia Regional State, Ethiopia. J Ethnobiol Ethnomed. 2015; 11: 25.
9. Lulekal, E., Rondevaldova, J., Bernaskova, E., Cepkova, J., Asfaw, Z., Kelbessa, E., et al., Antimicrobial activity of traditional medicinal plants from Ankober District, North Shewa Zone, Amhara Region, Ethiopia. Pharm Biol. 2014; 52(5): 614-620.
10. Tauchen, J., Daskocil, I., Caffi, C., Lulekal, E., Marsik, P., Havlik, J., et al., In vitro antioxidant and anti-proliferative activity of Ethiopian medicinal plant extracts. Ind Crops Prod. 2015; 74: 671-679.
11. Raghavendra, H. L., Kekuda, P. T. R. Antiradical and lipid peroxidation inhibitory activity of ripe and unripe fruit of *Rubus steudneri* Schweinf. (Rosaceae). Pharmacogn J. 2018; 10(4): 818-822.
12. Raghavendra, H. L., Upashe, S. P., Reyes, D. R. A., Floriano, J. F. Antidiabetic and antioxidant activity of *Rubus apetalus* Poir. and *Rubus steudneri* Schweinf. leaf extract on alloxan induced diabetes mellitus. J Bioanal Biomed. 2019; 11: 149-154.
13. Seshathri, K. Antimicrobial properties of Ethiopian chewing sticks against *Candida albicans*. J Appl Pharm Sci. 2012; 2(1): 45-50.
14. Raghavendra, H. L., Kekuda, P. T. R., Chethan, D. M. Phytochemical analysis and in vitro antioxidant activity of *Rubus apetalus* Poir. (Rosaceae). Pharmacology OnLine 2018; 1: 187-195.
15. Worku, A., Bayu, N., Teshager, A. The first 31 medicinal plant species prioritized for conservation and sustainable utilization at Fedis and Midega districts of Oromiya regional state, Ethiopia. Advanced Journal of Medicinal Plants Research 2020; 5(1): 27-32.
16. Sukthankar, J. H., Kumar, H., Godinho, M.H.S., Kumar, A. Larvicidal activity of methanolic leaf extracts of plant, *Chromolaena odorata* L. (Asteraceae) against vector mosquitoes. International Journal of Mosquito Research 2014; 1(3): 33-38.
17. Pushpanathan, T., Jebanesan, A., Govindarajan, M. Larvicidal, ovicidal and repellent activities of *Cymbopogon citratus* Stapf (Graminae) essential oil against the filarial mosquito *Culex quinquefasciatus* (Say) (Diptera: Culicidae). Trop Biomed. 2006; 23(2): 208-212.
18. Krishnappa, K., Dhanasekaran, S., Elumalai, K. Larvicidal, ovicidal and pupicidal activities of *Gliricidia sepium* (Jacq.) (Leguminosae) against the malarial vector, *Anopheles stephensi* Liston (Culicidae: Diptera). Asian Pac J Trop Med. 2012; 5(8): 598-604.
19. Fotakis, E. A., Chaskopoulou, A., Grigoraki, L., Tsiamantas, A., Kounadi, S., Georgiou, L., et al., Analysis of population structure and insecticide resistance in mosquitoes of the genus *Culex*, *Anopheles* and *Aedes* from different environments of Greece with a history of mosquito borne disease transmission. Acta Trop. 2017; 174: 29-37.
20. Dufour, I., Vontas, J., David, J.P., Weetman, D., Fonseca, D.M., Corbel, V., et al., Management of insecticide resistance in the major *Aedes* vectors of arboviruses: Advances and challenges. PLoS Negl Trop Dis. 2019; 13(10): e0007615.
21. Subramaniam, J., Kovendan, K., Kumar, M. P., Murugan, K., Walton, W. Mosquito larvicidal activity of *Aloe vera* (Family: Liliaceae) leaf extract and *Bacillus sphaericus*, against Chikungunya vector, *Aedes aegypti*. Saudi J Biol Sci. 2012; 19(4): 503-509.
22. Govindarajan, M. Larvicidal efficacy of *Ficus benghalensis* L. plant leaf extracts against *Culex quinquefasciatus* Say, *Aedes aegypti* L. and *Anopheles stephensi* L. (Diptera: Culicidae). Eur Rev Med Pharmacol Sci. 2010; 14: 107-111.
23. Raghavendra, H. L., Kekuda, P.T.R., Pushpavathi, D., Shilpa, M., Petkar, T., Siddiqua, A. Antimicrobial, radical scavenging, and insecticidal activity of leaf and flower extracts of *Couroupita guianensis* Aubl. Int J Green Pharm. 2017; 11(3): 171-179.
24. Raghavendra, H. L., Kekuda, P.T.R. Preliminary phytochemical analysis, antiradical and lipid peroxidation inhibitory activity of *Rubus steudneri* Schweinf. (Rosaceae). Int J Green Pharm. 2018; 12(1): 49-55.
25. François, G., Van Looveren, M., Timperman, G., Chimanku, B., Aké Assi L., Holenz, J., et al., Larvicidal activity of the naphthylisoquinoline alkaloid dioncophylline A against the malaria vector *Anopheles stephensi*. J Ethnopharmacol. 1996; 54(2-3): 125-130.
26. da Silva, H. H. G., da Silva, I. G., dos Santos, R. M. G., Filho, E. R., Elias, C. N. Larvicidal activity of tannins isolated of *Magonia pubescens* St. Hil. (Sapindaceae) against *Aedes aegypti* (Diptera, Culicidae). Rev Soc Bras Med Trop. 2004; 37(5): 396-399.
27. Gautam, K., Kumar, P., Poonia, S. Larvicidal activity and GC-MS analysis of flavonoids of *Vitex negundo* and *Andrographis paniculata* against two vector mosquitoes *Anopheles stephensi* and *Aedes aegypti*. J Vector Borne Dis. 2013; 50(3): 171-178.
28. Raghavendra, H. L., Chaitanya, V.N.L.M., Kekuda, P.T.R., Murthy, C. D., Patil, R.H.S., Vijayananda, B. N. Gas chromatography-mass spectrometry fingerprint and in vitro cytotoxic studies of *Rubus steudneri* leaf fractions against Michigan cancer foundation-7 breast cancer cell line. Phcog Mag. 2021; 17: S54-S62.
29. Khatiwora, E., Adsul, V. B., Pawar, P., Joseph, M., Deshpande, N. R., Karhalkar, R. V. Larvicidal activity of *Ipomoea carneastem* extracts and its active ingredients dibutyl phthalate against *Aedes aegypti* and *Culex quinquefasciatus*. Der Pharma Chemica. 2014; 6: 155-161.