Short Communication

Revista da Sociedade Brasileira de Medicina Tropical 47(5):646-648, Sep-Oct, 2014
http://dx.doi.org/10.1590/0037-8682-0010-2014

Larvicidal activity of the methanol extract and fractions of the green fruits of *Solanum lycocarpum* (Solanaceae) against the vector *Culex quinquefasciatus* (Diptera: Culicidae)

***Thamer Matias Pereira*[1], *Viviane de Cássia Bicalho Silva*[1], *José Antônio Ribeiro Neto*[1], *Stênio Nunes Alves*[1] and *Luciana Alves Rodrigues dos Santos Lima*[1]**

[1]. Campus Centro-Oeste Dona Lindu, Universidade Federal de São João Del-Rei, Divinópolis, MG.

ABSTRACT

Introduction: The larvicidal activity of *Solanum lycocarpum* against *Culex quinquefasciatus* is unknown. **Methods:** We evaluated the larvicidal activity of extracts of the green fruits of *Solanum lycocarpum* against third and fourth instar larvae of *C. quinquefasciatus*. **Results:** Dichloromethane and ethyl acetate fractions showed the greatest larvicidal effect at 200mg/L (83.3% and 86.7%, respectively). The methanol and dichloromethane, ethyl acetate, and hydromethanolic fractions demonstrated larvicidal effects against *C. quinquefasciatus,* with LC_{50} values of 126.24, 75.13, 83.15, and 207.05mg/L, respectively. **Conclusions:** Thus, when considering new drugs with larvicidal activity from natural products, *S. lycocarpum* fruits may be good candidate sources.

Keywords: Bioassay. Mosquito. Pesticide.

The species *Culex quinquefasciatus* (Diptera: Culicidae) is a mosquito inhabiting urban regions, with a wide geographical distribution. This species is involved in the transmission of lymphatic filariasis in the Americas, particularly in Brazil. *C. quinquefasciatus* is of great importance in public health as it creates administrative and public challenges for the control of mosquito vectors[1].

Synthetic insecticides have been widely used to control mosquito vectors of disease in various parts of the world; however, resistance to these insecticides has recently become problematic in vector control programs. Moreover, the continuous monitoring of mosquito populations may play an important role in attempting to develop management strategies that will prevent or minimize the development of resistance to pesticides, as well as to develop new products to combat insects[2]. Thus, the use of biological products is an alternative approach for preventing the development of resistance in mosquitoes.

Plants are a source of bioactive compounds that have insecticidal properties and therefore may be suitable for mosquito control[3]. Phytoextracts have previously been shown to be successful in various biocontrol programs, and essential oils of different plants are used as potential mosquito repellents. Different species of the genus *Solanum* have demonstrated larvicidal and pupicidal activities against *C. quinquefasciatus*[4,5]. The species *Solanum lycocarpum* A. St. Hil., belonging to the Solanaceae family, is popularly known as *lobeira* and is widely distributed in the Brazilian cerrado[6]. It is widely used in traditional medicine as a sedative, in the treatment of epilepsy, asthma, diabetes, obesity, for the reduction of cholesterol levels, and for abdominal and renal pain[7].

To our knowledge, for the first time, we show the larvicidal activities of the extracts and fractions obtained from the green fruits of *S. lycocarpum* against *C. quinquefasciatus*.

The green fruits of *S. lycocarpum* A. St. Hil. were collected in the City of São Sebastião do Oeste, State of Minas Gerais, Brazil, in August 2011. The plant material was identified by Dr. Alexandre Salino, and a voucher specimen (BHCB 159397) was deposited at the Instituto de Ciências Biológicas Herbarium, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil. Methanol was used as solvent to obtain the extract from 170.01g of dried and powdered green fruits, using a Soxhlet extractor. The extract was then concentrated in a rotary evaporator at 50°C under reduced pressure to yield the methanol extract (MET, 14.53g). Some of this extract (6.82g) was dissolved in MeOH/H_2O (1:1) and then partitioned successively with hexane, dichloromethane, and ethyl acetate (Vetec®, São Paulo, Brazil; 100 mL, twice with each solvent), yielding 0.09, 0.23, 0.34, and 5.50g of hexane (HEX), dichloromethane (DCM), ethyl acetate (AC), and hydromethanolic (HM) fractions, respectively. The extract and fractions were screened for the presence of different phytoconstituents such as saponins, tannins, alkaloids, steroids, triterpenes, coumarins, and flavonoids[8].

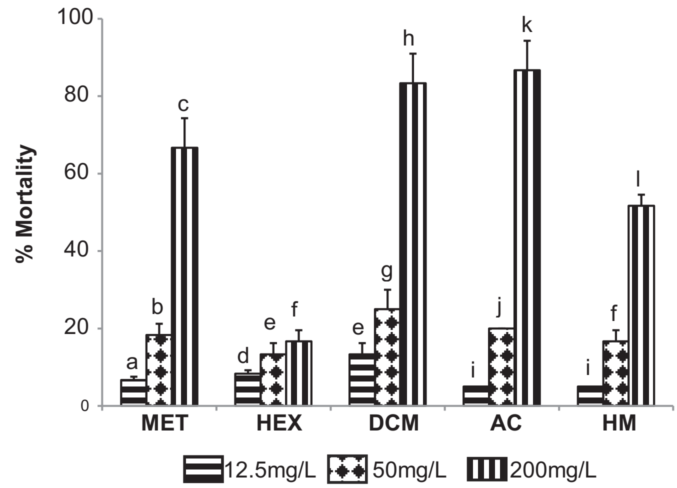
Address to: Drª Luciana Alves Rodrigues dos Santos Lima. Campus Centro-Oeste Dona Lindu/UFSJ. Rua Sebastião Gonçalves Coelho 400, Chanadour, 35501-296 Divinópolis, MG, Brasil.
Phone: 55 37 3071-1878; **Fax**: 55 37 3221-1614
e-mail: luarsantos@ufsj.edu.br
Received 22 January 2014
Accepted 29 May 2014

Culex quinquefasciatus larvae were obtained from a laboratory culture, as previously described by Gerberg[9]. Both the third and fourth instar larvae of *C. quinquefasciatus* were exposed to different concentrations (12.5, 50, and 200mg/L; dissolved in 1% dimethylsulfoxide) of the methanol extract and fractions up to the emergence of adults, to determine the optimal sub-lethal concentration. For each sample tested, the larvae were divided into test and control groups, consisting of 60 specimens each, with three replications for each treatment. The control group larvae were exposed to water dissolved in 1% dimethylsulfoxide. The temperature was maintained at 26 ± 1°C throughout all of the tests. The larvicidal bioassay was performed according to the World Health Organization standard protocols[10]. The larvae were exposed to these solutions, and mortality was recorded every 24h over a total period of 144h. Three replications of each treatment were performed and the larvae used were all of the first generation. Larvae were considered dead when they did not respond to the stimulus or when they did not rise to the surface of the solution. LC_{50} and LC_{90} values were calculated by Probit regression[11].

In this study, phytochemical tests revealed the presence of coumarins, flavonoids, and tannins in the methanol extract. Saponins were present in the dichloromethane fraction, while alkaloids and coumarins were found in the hexane fraction. On the other hand, flavonoids, coumarins, and tannins were found to be present in the dichloromethane, ethyl acetate, and hydromethanolic fractions, and terpenes/sterols were found in the dichloromethane and hydromethanolic fractions.

Larval mortality rates in each group, expressed as percentages, are shown in **Figure 1**. Analysis of variance tests, comparing the percentage of mortality between the various treatments, showed significant differences, depending on the concentrations and extracts or fractions used. Furthermore, a positive correlation was observed between the concentration of the methanol extract and fractions and the mortality rate **(Figure 1)**, with the mortality rate being directly proportional to concentration. The dichloromethane and ethyl acetate fractions showed the greatest larvicidal effect at 200mg/L (83.3% and 86.7%, respectively), and was significantly different compared to the other treatments ($p < 0.05$). The greatest mortality was observed at 200mg/L for the methanol extract (66.7%) as well as for the hydromethanolic fraction (51.7%). The hexane fraction did not show larvicidal activity, yielding values lower than 20% (**Figure 1**). No significant differences in mortality were observed for the ethyl acetate and hydromethanolic fractions at 12.5mg/L, the dichloromethane fraction at 12.5mg/L, the hexane fraction at 50mg/L, the hydromethanolic fraction at 50mg/L, and the hexane fraction at 200mg/L. There was a complete absence of larval mortality in the dimethylsulfoxide controls.

The pertinent values for the methanol extract and different fractions, estimated by the LC_{50}-LC_{90} regression equation and chi-square tests, are presented in **Table 1**. The methanol extract and dichloromethane, ethyl acetate, and hydromethanolic fractions had larvicidal effects against *C. quinquefasciatus,* with LC_{50} values between 75.13 and 207.05mg/L; this represents higher larvicidal activity than that of other plant species of the



Different letters between columns indicate significant differences ($p < 0.05$) between treatments.

FIGURE 1 - Percentage larval mortality of *Culex quinquefasciatus* after exposure to different concentrations of the methanol extract and fractions of the green fruits of *Solanum lycocarpum*. MET: methanol extract; **HEX:** hexane fraction; **DCM:** dichloromethane fraction; **AC:** ethyl acetate fraction; **HM:** hydromethanolic fraction.

Solanum genus. For instance, ethanolic extracts of the leaves of *S. xanthocarpum* showed activity against the third and fourth instar larvae of *C. quinquefasciatus*, with LC_{50} values of 271.12 and 377.40mg/L, respectively[4]. However, the larvicidal activity of the methanol extract of *S. lycocarpum* reported in the present study are comparable to those achieved by Sakthivadivel and Daniel[5], who demonstrated the larvicidal effect of the ethanolic extract of leaves of the *S. trilobatum* on the fourth instar larvae of *C. quinquefasciatus*, with $LC_{50} > 200$mg/L.

In this study, the dichloromethane and ethyl acetate fractions showed the lowest LC_{90} values (387.18 and 271.57mg/L, respectively), making them the most toxic fractions against *C. quinquefasciatus*. The methanol extract and hydromethanolic fraction showed LC_{90} values of 709.53 and 1,743.57mg/L. Ethanolic extracts of the leaves of *S. xanthocarpum* also showed activity against the third and fourth instar larvae of *C. quinquefasciatus,* with LC_{90} values of 1,011.89 and 1,058.85mg/L, respectively[4]. Changbunjong et al.[12] also showed larvicidal activity of the ethanolic extract of green fruits of *S. xanthocarpum* against *C. quinquefasciatus,* with LC_{50} and LC_{90} values of 573.20 and 1,066.93mg/L, respectively, which were greater than those found in our study.

The effectiveness of the derived secondary compounds of a plant, such as saponins, steroids, isoflavonoids, and tannins have been well documented for their larvicidal activity[13]. Chowdhury et al.[14] also evaluated the green berries of *S. villosum*, and suggested that a steroid compound was responsible for its observed larval toxicity. Thus, the higher larvicidal activity found in the dichloromethane fraction can be attributed, at least partially, to the action of saponins, steroids and tannins.

In conclusion, our preliminary results indicate that the methanol extract and dichloromethane, ethyl acetate, and

TABLE 1 - Lethal concentrations of the methanol extract and fractions of the green fruits of *Solanum lycocarpum* against *Culex quinquefasciatus*.

Samples	LC_{50} (95% CI) mg/L	LC_{90} (95% CI) mg/L	Regression equations	χ^2	P value
MET	126.24 (89.90–177.27)	709.53 (346.17–1,454.31)	$y = 1.568x + 1.629$	2.77	0.76
DCM	75.13 (49.79–113.35)	387.18 (188.73–794.29)	$y = 1.748x + 1.757$	7.93	0.57
AC	83.15 (60.69–113.91)	271.57 (166.72–442.35)	$y = 0.714x + 2.280$	8.22	0.62
HM	207.05 (125.85–340.62)	1,743.57 (575.56–5,281.86)	$y = 2.008x + 1.283$	0.74	0.96

LC_{50}: median lethal concentration; **CI:** confidence interval; **LC_{90}:** 90% lethal concentration; **χ^2:** chi-square value. **MET:** methanol extract; **DCM:** dichloromethane fraction; **AC:** ethyl acetate fraction; **HM:** hydromethanolic fraction; ***y*:** probit value**;** ***x*:** log concentration of the methanol extract and fractions of the green fruits.

hydromethanolic fractions of the green fruits of *S. lycocarpum* showed larvicidal activity against *C. quinquefasciatus*. These results form the basis for additional studies for evaluating the possibilities of using the extracts and fractions of green fruits of *S. lycocarpum* as natural, plant-based sources of larvicides.

ACKNOWLEDGMENTS

The authors are grateful to Prof. Dr. Alexandre Salino for botanical identification of this plant.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

FINANCIAL SUPPORT

The study was supported by *Fundação de Amparo a Pesquisa do Estado de Minas Gerais* (FAPEMIG) and the *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior* (CAPES).

REFERENCES

1. Campos J, Andrade CFS. Susceptibilidade larval de populações de *Aedes aegypti* e *Culex quinquefasciatus* a inseticidas químicos. Rev Saude Publica 2003; 37:523-527.
2. Alves SN, Tibúrcio JD, Melo AL. Susceptibility of *Culex quinquefasciatus* larvae to different insecticides. Rev Soc Bras Med Trop 2011; 44:486-489.
3. Prophiro JS, Rossi JCN, Pedroso MF, Kanis LA, Silva OS. Leaf extracts of *Melia azedarach* Linnaeus (Sapindales: Meliaceae) act as larvicide against *Aedes aegypti* (Linnaeus, 1762) (Diptera: Culicidae). Rev Soc Bras Med Trop 2008; 41:560-564.
4. Mahesh Kumar P, Murugan K, Kovendan K, Subramaniam J, Amaresan D. Mosquito larvicidal and pupicidal efficacy of *Solanum xanthocarpum* (Family: Solanaceae) leaf extract and bacterial insecticide, *Bacillus thuringiensis*, against *Culex quinquefasciatus* Say (Diptera: Culicidae). Parasitol Res 2012; 110:2541-2550.
5. Sakthivadivel M, Daniel T. Evaluation of certain insecticidal plants for the control of vector mosquitos viz. *Culex quinquefasciatus*, *Anopheles stephensi* and *Aedes aegypti*. Appl Entomol Zool 2008; 43:57-63.
6. Vieira Jr G, Ferreira PM, Matos LG, Ferreira EC, Rodovalho W, Ferri PH, et al. Anti-inflammatory effect of *Solanum lycocarpum* fruits. Phytother Res 2003; 17:892-896.
7. Munari CC, Oliveira PF, Lima IMS, Martins SPL, Costa JC, Bastos JK, et al. Evaluation of cytotoxic, genotoxic and antigenotoxic potential of *Solanum lycocarpum* fruits glicoalkaloid extract in V79 cells. J Food Chem Toxicol 2012; 50:3696-3701.
8. Wagner H, Bladt S, Zgainski EM. Plant drug analysis. A thin layer chromatography atlas. London: Springer; 2001.
9. Gerberg EJ. Manual for mosquito rearing and experimental techniques. Am Mosq Control Assoc 1979; 5:1-124.
10. World Health Organization. Instructions for determining the susceptibility or resistance of mosquito larvae to insecticides. Vector Biol Control 1981; 81.807:1-6.
11. Finney DJ. Probit analysis, a statistical treatment of the sigmoid response curve. Cambridge: Cambridge University Press; 1980.
12. Changbunjong T, Wongwit W, Leemingsawat S, Tongtokit Y, Deesin V. Effect of *Solanum xanthocarpum* against snails and mosquito larvae. Southeast Asian J Trop Med Public Health 2010; 41:320-325.
13. Singha S, Chandra G. Mosquito larvicidal activity of some common spices and vegetable waste on *Culex quinquefasciatus* and *Anopheles stephensi*. Asian Pac J Trop Med 2011; 4:288-293.
14. Chowdhury N, Ghosh A, Chandra G. Mosquito larvicidal activities of *Solanum villosum* berry extract against the dengue vector *Stegomyia aegypti*. BMC Complement Altern Med 2008; 8:1-8.