Laboratory Evaluation of Natural Saponin as a Bioactive Agent against *Aedes aegypti* and *Culex pipiens*

by

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Abstract

Quillaja saponaria is a South American saponin-producing plant and the *Balanites aegyptiaca* is an African - Asian saponin-producing plant. The efficacy of these two plant sources of natural saponins for the control of *Aedes aegypti* and *Culex pipiens* was studied in laboratory.

The data showed that extracts of these two natural saponin plant sources can be used for an efficient bioactive preparation in *Aedes aegypti and Culex pipiens* mosquito control.

Due to the fact that saponins are efficient in mosquito control, safe to mammals and available in high concentrations in many plant species all over the world, the cost of intensive use of saponin plant extract preparations should be relatively low and affordable to poor communities in the world.

Keywords: *Aedes aegypti*, *Culex pipiens*, dengue fever, West Nile virus, *Balanites aegyptiaca*, *Quillaja saponaria*, Saponin.

Introduction

Aedes aegypti is the principal vector of dengue fever and dengue haemorrhagic fever. It infects more than 100 million people every year in more than 110 countries in the tropics[1]. The regions of the Americas, Africa and South-East Asia are the most affected areas. Children are the most vulnerable to dengue infection and 95% of all dengue cases are those under 15 years of age.

Culex pipiens (northern house mosquito) is the vector of the West Nile virus (WNV) that causes encephalitis or meningitis. Generally, people over the age of 50 years are at greater risk to serious illness when infected with WNV. The disease affects the brain tissue and the most serious of the cases can result in permanent neurological damage and even be fatal[2]. WNV is distributed throughout Africa, the Middle East, and the southern temperate and tropical Eurasia. It was recently introduced into North America as well[3].

Since there are no vaccines for these two arbovirus diseases, vector control is the only option available for reducing the morbidity. The most widely-used vector intervention methods are insecticide-based. Insecticides are generally chemical agents,

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which are expensive and harmful to the environment as well as to humans. This study reflects a broad international interest in finding eco-friendly natural systems for vector control. Our research focuses on insect control using saponins.

Saponins are high molecular weight glycosylated plant secondary metabolites, consisting of a sugar moiety linked to a triterpene or steroid aglycone[4]. Detergent properties are the typical characteristics of saponins. They produce stable foam when dissolved in water, which is why some saponin-containing plants have been used as soaps for hundreds of years. Saponin-containing plants are used as folk medicines, especially in Asia, and are intensively used in food, veterinary and medical industries[5]. The pesticidal activity of saponins has long been reported[6]. Saponin-glycosides are very toxic to cold-blooded organisms, but apparently not to mammals[5,7]. Plant extracts containing a high percentage of saponins are commonly used in Africa to treat water supplies and wells contaminated with disease vectors; after treatment, the water is safe for human drinking[7]. The following saponin-containing plants have been exploited for the industrial production of saponins: horse chestnuts (*Aesculus*), climbing ivy (*Hedera*), peas (*Pisum*), cowslip (*Primula*), soapbark (*Quillaja*), soapwort (*Saponaria*), sugar beet (*Beta*) and balanites (*Balanites aegyptiaca*)[5,7].

In this study, we demonstrate the efficacy of two plant sources (*Quillaja saponaria* and *Balanites aegyptiaca*) of natural saponins for the control of *Aedes aegypti* and *Culex pipiens*. *Quillaja saponaria* is a large evergreen tree with shiny, leathery leaves and a thick bark which is native to Peru and the arid region of Chile[8] while *Balanites aegyptiaca* is a spiny evergreen tree about 6-10 metres in height which is found in the drier regions of Africa, the Arabian peninsula, India and Myanmar[7]. Our aim is to find an efficient, safe and low-cost mosquito control system that might be suitable for a wide range of communities most affected by these disease vectors.

Materials and methods

Saponin sources

We tested a commercial saponin mixture extracted from quillaja bark (S7900, Sigma, USA) containing quillaic acid, a triterpene, as its main sapogenin. We also used a methanol extract of balanites fruit mesocarp, which contains steroid saponins. Both extracts were tested against larvae of *Aedes aegypti* and *Culex pipiens.* The sapogenin content was not less than 10% as quoted by the suppliers for quillaja and about 4% for balanites extract[9].

Larvicidal bioassay

Eggs of laboratory-grown *Aedes aegypti* and *Culex pipiens* were hatched in tap water in a growth chamber with a 16-hour photoperiod, at a temperature of 25 ± 3°C. Twenty to twenty-five larvae at late third or early fourth instars were placed into 150-ml disposable plastic cups containing 100 ml of tap water together with a water-soluble saponin solution (1,000, 500, and 0 mg/L extract in the quillaja saponin experiment and 1000 and 0 mg/L extract for the balanites saponin experiment) in three replicates. During the experiments, treated and control (0 mg/L extract) cups were examined every 2, 3, 6, 7, 11 and 14 days and the number of live larvae or adults counted. The data are presented in both percentage and absolute numbers, together with an analysis of variance using the Tukey-Kramer HSD test (SAS, JMP software) at 0.01 space level of significance. Since each treatment was conducted twice with similar results, data are presented only for a single independent experiment.

Results and discussion

Effect of quillaja saponin preparation

Two days after the application of the quillaja saponin preparation (1,000 mg/L treatment rate) the mean number of live *Aedes aegypti* larvae was reduced from 22 to 0.3 (1.5%) in comparison to 97.4% live larvae in the control (0 mg/L) treatment at the same duration (Table 1a). Seven days after treatment, no live larvae were found in 1,000 mg/L, while in the control treatment there was a mean of 79.7% live larvae. Quillaja extract preparation at half concentration (500 mg/L) was also found active in *Aedes aegypti* larvae control. This concentration reduced the number of live larvae from 23.3 to 9.3 (39.9%) at two days after treatment as compared with 97.4% in the control. After seven days, there were 8.1% larvae versus 79.7% in the control. The results of the 1,000 mg/L and 500 mg/L quillaja extract treatments showed a clear pattern of dose dependent effects on reducing the live *Aedes aegypti* larvae numbers in function of time. Seven days after the initiation of the experiment, 5.46% of the larvae successfully emerged as adults in the control treatment. This number increased to 74.4% after 14 days (Table 1A). At the 500mg/L quillaja extract rate, we observed 2.7% emergence of *Aedes* adults after 14 days. No adult *Aedes* mosquitoes emerged from the 1,000 mg/L quillaja extract treatment. This suggests an absolute *Aedes aegypti* mosquito control by quillaja saponin preparation. In order to understand the difference between the number of live larvae and live adult mosquitoes, one must remember that between these two stages, there is a pupal stage and the saponin treatment has also some effect on the time of pupation and vigour of pupa in the beginning of the experiment, but at the later stages, the effect was very low (unpublished data).

Table 1A. *Effect of quillaja saponin on Aedes aegypti control*

Concentration	Live larvae				Live adults			
	Day 0	Day 2	Day 7	Day 14	Day 0	Day 2	Day 7	Day 14
Percentage								
0 mg/L	100 ± 0 a	97.4 ± 1.3 a	79.7 ± 3.9 a	0	0	0	5.46 ± 2.7 a	74.3 ± 71.4 a
500 mg/L	100 ± 0 a	39.9 ± 4.7 b	8.1 ± 3.3 b	0	0	0	0.0 ± 0 a	2.7 ± 1.3 b
1,000 mg/L	100 ± 0 a	1.5 ± 1.3 c	0.0 ± 0.0 c	0	0	0	0.0 ± 0 a	0.0± 0 b
Absolute numbers								
0 mg/L	24.7 ± 0.8 a	24.0 ± 0.6 a	19.7 ± 1.2 a	0	0	0	1.33 ± 0.7 a	18.3 ± 0.9 a
500 mg/L	23.3 ± 2.0 a	9.3 ± 1.5 b	2.0 ± 1.0 b	0	0	0	0.0 ± 0 a	0.7 ± 0.3 b
1,000 mg/L	22.0 ± 0.6 a	0.3 ± 0.3 c	0.0 ± 0.0 c	0	0	0	0.0 ± 0 a	0.0 ± 0 b

Means in each column followed by different letters are significantly different at $P = 0.01$ (n=3)

A similar effect of quillaja saponin preparation on *Culex pipiens* mosquito control was also obtained (Table 1B). The higher concentration (1,000 mg/L) of quillaja saponin extract provided an absolute control of adult *Culex pipiens* emergence during the 14 days of the experiment. The lower concentration (500 mg/L) reduced the mean number of live larvae from 20 to 15 at two days. After seven days, the mean number of live larvae dropped to 6.3. In this treatment, the number of emerged adults averaged to one out of 20 (5%) after 14 days. In the control treatment at seven days, 50% live larvae were recorded and after 14 days, the emerged adults reached 13.3 (66.5%) out of 20.

Table 1B. *Effect of quillaja saponin on Culex pipiens control*

Concentration	Live larvae				Live adults			
	Day 0	**Day 2**	**Day 7**	**Day 14**	**Day 0**	**Day 2**	**Day 7**	**Day 14**
Percentage								
0 mg/L	100 ± 0 a	100 ± 1.3 a	50.0 ± 7.5 a	0	0	0	6.6 ± 3.5 a	66.5 ± 5.2 a
500 mg/L	100 ± 0 a	75 ± 4.7 b	31.7 ± 1.7 a	0	0	0	1.5 ± 1.5 a	5.0 ± 0.4 b
1,000 mg/L	100 ± 0 a	1.7 ± 1.7 c	0.0 ± 0.0 b	0	0	0	0.0 ± 0 a	0.0 ± 0 b
Absolute numbers								
0 mg/L	20 ± 0 a	20.0 ± 0 a	10 ± 1.5 a	0	0	0	1.33 ± 0.9 a	13.3 ± 1.3 a
500 mg/L	20 ± 0 a	15 ± 0 b	6.3 ± 0.3 a	0	0	0	0.3 ± 0.3 a	1.0 ± 0.0 b
1,000 mg/L	20 ± 0 a	0.3 ± 0.3 c	0.0 ± 0.0 b	0	0	0	0.0 ± 0 a	0.0 ± 0 b

Means in each column followed by different letters are significantly different at $P = 0.01$ ($n = 3$).

Effect of balanites saponin preparation

The second source of saponin we tested, a methanol extract of *Balanites aegyptiaca* fruit mesocarp, also showed an effective biocontrol of both *Aedes aegypti* and *Culex pipiens* mosquitoes (Table 2A and 2B). Three days after treatment, the number of live *Aedes* larvae was reduced in the balanites saponin preparation (1,000 mg/L) from 21 larvae to 2.3 (11%) in comparison to 96.7% live larvae in the control treatment (Table 2A). The percentage of adult mosquitoes emerging after six and 11 days was 0% and 5%, respectively. In the control treatment, 85% of the larvae emerged as adult *Aedes* mosquitoes after 11 days.

The effect of balanites saponin extract on *Culex pipiens* was more moderate than on *Aedes aegypti* (Table 2b). A mean of 23.3% of the larvae were alive after both three and six days in comparison to 90% and 43.3% in the control, respectively. At the end of the study, a mean of 20% of the initially treated *Culex* larvae had reached the adult stages as compared with 83.3% in the control.

Table 2A. *Effect of blanites saponin on Aedes aegypti control*

Concentration	Live larvae				Live adults			
	Day 0	Day 3	Day 6	Day 11	Day 0	Day 3	Day 6	Day 11
Percentage								
0 mg/L	100 ± 0 a	96.7 ± 1.7 a	36.7 ± 4.4 a	0	0	0	21.6 ± 6.0 a	85 ± 2.9 a
1,000 mg/L	100 ± 0 a	2.9 ± 1.6 b	0.0 ± 0.0 b	0	0	0	0.0 ± 0 a	5.0 ± 2.8 b
Absolute numbers								
0 mg/L	20 ± 0.8 a	19.3 ± 0.3 a	6.77 ± 0.8 a	0	0	0	4.3 ± 1.2 a	17.0 ± 0.6 a
1,000 mg/L	21 ± 0.6 a	2.3 ± 0.3 b	0.0 ± 0.0 b	0	0	0	0.0 ± 0 a	1.0 ± 0.6 b

Means in each column followed by different letters are significantly different at $P = 0.01$ (n=3).

Table 2B. *Effect of balanites saponin on Culex pipiens control*

Concentration	Live larvae				Live adults			
	Day 0	Day 3	Day 6	Day 11	Day 0	Day 3	Day 6	Day 11
Percentage								
0 mg/L	100 ± 0 a	90 ± 5.8 a	43.3 ± 0.3 a	0	0	0	23.3 ± 3.3 a	83.3 ± 3.3 a
1,000 mg/L	100 ± 0 a	23.3 ± 3.3 b	23.3 ± 2.3 b	0	0	0	16.7 ± 6.6 a	20.0 ± 5.7 b
Absolute numbers								
0 mg/L	10 ± 0 a	9.0 ± 0.6 a	4.3 ± 0.3 a	0	0	0	2.33 ±0.3 a	8.3 ± 0.3 a
1,000 mg/L	10 ± 0 a	2.3 ± 0.3 c	2.3 ± 0.3 b	0	0	0	1.7 ± 0.7 a	2.0 ± 0.6 b

Means in each column followed by different letters are significantly different at $P = 0.01$.

A moderate effect of mosquito control from balanites saponin preparation should be expected due to its lower content of saponin in comparison to the quillaja saponin preparation (as stated in Materials and methods). The result of 20% of the initial larvae emergence to the adult stage at the end of the experiment in *Culex pipiens* indicates that a higher concentration of balanites extract would be needed to make the control of *Culex* mosquitoes effective.

In a previous study, we demonstrated the effect of the quillaja saponin extract on larvae mortality(10). In this study, we focused on the effect on the survival of larvae and adult mosquitoes of two sources of saponin: *Quillaja saponaria* representing South American saponin-producing plants and *Balanites aegyptiaca,* representing African and Asian saponin-producing plants. In both the South American and Afro-Asian sources, the plant material used for the preparation of the saponin is low cost and affordable for many communities. The data previously reported about the safe use of saponin plant extracts for mammals(5), together with their larvicidal effects(10,11) and common ethno-medicinal uses in many areas of the world, make saponin plant preparations very good candidates for practical use in the control of dengue and WNV vectors.

Conclusions

The following conclusions can be drawn based on the experimental results demonstrated in the present study:

(1) Various saponin sources can be used for efficient bioactive preparation in *Aedes aegypti* and *Culex pipiens* mosquito control.

(2) Due to the fact that saponins are effective in mosquito control, safe to mammals and available in high concentrations in many plant species all over the world, the cost of intensive use of saponin plant extract preparations should be relatively low and affordable to poor communities in many parts of the world.

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