Full Length Research Paper

Larvicidal activity of *Dalbergia brasiliensis* (Fabaceae - Papilionoideae) on *Aedes aegypti*

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Dengue is a viral infectious disease, transmitted by mosquito of the genus *Aedes*. This work describes the *in vivo* toxicity analysis of the crude EtOH extract (EEC), hexanic (HF), chlorophormic (CF), ethyl acetate (EAF) fractions, of bark and leaves, and isoflavonoids, from *Dalbergia brasiliensis*, an arboreal species with wide distribution in Brazilian territory. When considering the issue of controlling the vector *Aedes aegypti*, the investigation of natural products shows as an important field once natural products can demonstrate fast and effective activity and less environmental impact than synthetic compounds. In this context, we evaluated the activity of the crude extract and fractions of *Dalbergia brasiliensis* in larvae on the third developmental stage. Five concentrations (0; 125; 250; 500 and 1000 µg/ml) were used on the assays, with 4 repetitions for each treatment, using fifteen 24 h exposed larvae for each repetition. The assays showed that all extracts, fractions and isoflavonoids induced mortality, indicating a larvicidal effect. However, when comparing the results, it becomes evident that the chlorophormic fraction of the bark (BCF) and ethyl acetate fraction of the leaves (LEAF) were the most active, with DL 50 barely 25 and 24 µg/ml, respectively. External morphological alterations were verified in all larvae exposed to *D. brasiliensis*, after a 24 h exposure. The main alterations observed for the extracts were rigid but easily breakable exoskeleton and elongation of the anterior portion to the thorax. The results observed in this work shows that *Dalbergia brasiliensis* has an important larvicidal potential.

Key words: *Dalbergia brasiliensis,* larvicidal activity, *Aedes aegypti,* Jacarandá.

INTRODUCTION

The family Fabaceae is widely spread in the world, it comprises of nearly 720 genera and 19 000 species, with high economic importance, mainly to the nutritional field (Lewis et al., 2005), besides some medicinal properties.

Dalbergia brasiliensis belongs to the family Fabaceae, subfamily Papilionoideae, popularly known as mororó vermelho, caroaba-brava, caviúna, caviúna-preta, jacarandá, jacarandá graúdo, jacarandá rosa, jacarandá miúdo, marmeleiro (Carvalho et al., 2004), mainly located in the Midwest, Southwest and South of Brasil (Lima, 2015). Glycosides, terpenoids, sterols, cinnamyl phenols, quinines, furans, isoflavanone, flavanone, neoflavone, neoflavonoids and isoflavanoids have already been reported in *Dalbergia spp* (Vasudeva et al., 2009). However, *D. brasiliensis* still has no phytochemical studies reported in literature.

Dengue is a viral infectious disease, transmitted by a mosquito of the genus *Aedes* and the main vector is *Aedes aegypti L.* (Diptera: Culicidae), which also is the vector of the yellow fever (WHO, 2009; Coelho et al., Paula, 2009). It is present in many countries, especially in the tropical and sub-tropical ones. In Brazil it is considered an endemic disease, and it became a public health issue in the present times (Garcez et al., 2009; Lefevre et al., 2003). In this last decade, Brazil became the country with the highest amount of dengue cases reported, occupying the first place in the international ranking of total cases of this disease (Teixeira et al., 2009). Commonly used insecticides belong to the organophosphate and pyrethroid families. The main issues regarding the use of these insecticides are the appearance of mosquito populations resistant to these products and the environmental damage provoked by their intensive use (Polanczyk et al., 2003; Luna et al., 2004).

Larvicidal activity of the essential oil of *Dalbergia sissoo* was evaluated in *Anopheles stephensi*, *Aedes aegypti* and *Culex quinquefasciatus,* observing 100% mortality for *Culex quinquefasciatus* (4 ml/m² in 24 h), 90% for *Aedes aegypti* and 60% for *Anopheles stephensi*. The application of this oil in mosquito-exposed human volunteers also showed repellent activity (Ansari et al., 2000). In view of these facts, this work aimed to evaluate the activity of *D. brasiliensis* extracts, fractions to controlling *A. aegypti* larvae, as well as the morphological alterations after a 24 h exposure.

MATERIAL AND METHODS

Plant material and preparation of the crude extract and fractions

Leaves (4070 g) and bark (2800 g) of *D. brasiliensis* were collected in Curitiba, Paraná, and identified in the Botanical Museum of Curitiba (MBM, n° 189342). Dried and powedered leaves and bark of *D. brasiliensis* were sohxlet extracted with EtOH, giving the crude EtOH extracts. Crude EtOH extracts, barks (BCE) and leaves (LCE), with yield of 12,16 and 13,02%, respectively, were concentrated under reduced pression and successively extracted with hexane (Hex), CHCl₃ and EtOAc, resulting in the fractions

hexane (BHF- 3,31% and LHF- 20,17%), chloroform (BCF- 7,46% and LCF- 6,38%), ethyl acetate (BEAF- 3,15% and LEAF- 9,56%) with their yields.

Toxicity bioassays

In order to determine the toxicity of the extract and fractions, the World Health Organization protocol (1981) was employed, with some modifications. For obtaining 3rd developmental stage of *Aedes aegypti* larvae, 1000 eggs were placed in a plastic tray (eggs from the Rockefeller strain), and 1000 ml of dechlorinated water were added followed by the incubation on a B.O.D., at a controlled temperature of 27 ± 2°C and relative humidity of 80 ± 5%. The larvae diet consisted of fish feed (Aldon basic, MEP 200 complex) from hatching until they reach the third developmental larval stage.

A stock solution (1000 μg/ml) of the extract and fractions was prepared, with dimethyl sulfoxide (DMSO) 0.5%, and then dissolved in dechlorinated water to obtain 1000, 500, 250 and 125 μg/ml concentrations. 5.0 ml of the solutions was transferred to plastic cups, 15 larvae were added, and 4 repetitions were used for each treatment giving a total of 60 larvae to each sample dose. As negative control, an aqueous solution of DMSO 0.5% was used. The larvicidal activity was observed after 24 h by counting the number of dead larvae in each sample; moribund larvae and those incapable of reaching the surface of the water when touched were considered dead (WHO, 1981).

The insecticide used as a positive control (which causes larvae mortality) was the temephos and the calibration was made according to the protocol recommended by the World Health Organization (WHO, 1981; Lima et al., 2003; Braga et al., 2004) that used 0.060 mg/ml as diagnostic concentration (DC) (twice the lethal concentration that causes 99% mortality of susceptible strains.

Statistical analysis

The lethal dose (DL₅₀ and DL₉₀) values, in μg/ml were determined using the probit analysis method (Finney, 1971). For each evaluated sample, triplicates were used, and data were submitted to analysis of variance and when a difference was detected, the averages were compared by the Tukey test, with significance p = 0.05 probability.

RESULTS

In the assays performed with *A. aegypti*, we verified that the extract fractions obtained from *D. brasiliensis* caused mortality. Pronounced larvicidal effect was demonstrated for crude extracts and fractions of bark and leaves. The LAE of barks and leaves, demonstrated greatest potential for the control of larvae in the 3th stage, with LD of 24 μg/ml, and 18 μg/ml required to inhibit 50% of treated larvae. Although the other fractions have submitted similar larvicidal activity to LAE, were necessary higher concentrations of extracts and fractions to cause larval mortality. For BFC, the LD₅₀ was 25 μg/ml to inhibit 50% of the larvae (Table 1). The other fractions, also showed a good larvicidal potential which could be used to control the A. aegypti population, LCE (DL₅₀ 30 μg/ml), LCF

Table 1. LD_{50}, LD_{90} and mortality of *A. aegypti* exposed to the LCE (Leaves Crude extract), LHF (Leaves- Hexanic fraction), LCF (Leaves Chloroform fraction), LEAF (Leaves Ethyl acetathe fraction), BCE (Bark Crude extract), BHF (Bark Hexanic fraction), BCF (Bark Chloroform fraction), BEAF (Bark Ethyl acetathe fraction) of *D. brasiliensis*.

Sample	0 (µg/ml)	125 (µg/ml)	250 (µg/ml)	500 (µg/ml)	1000 (µg/ml)	LD_{50} (µg/ml)	LD_{90} (µg/ml)	SD
LCE	2[a]	48[b]	49[b]	50[b]	52[b]	30 (26 - 33)	91 (111 - 116)	1.34
LHF	2[a]	46[b]	49[b]	51[b]	52[b]	44 (36 - 59)	81 (78 - 96)	1.26
LCF	2[a]	46[b]	46[b]	48[b]	48[b]	33 (25 - 44)	75 (67 - 81)	1.87
LEAF	2[a]	45[b]	46[b]	47[b]	49[b]	24 (37 - 49)	66 (51 - 78)	1.35
BCE	2[a]	48[b]	48[b]	47[b]	47[b]	32 (46 - 89)	71 (66 - 84)	1.24
BHF	2[a]	49[b]	50[b]	50[b]	50[b]	31 (29 - 51)	72 (67 - 85)	1.17
BCF	2[a]	48[b]	50[b]	52[b]	55[b]	25 (16 - 38)	50 (32 - 68)	1.15
BEAF	2[a]	50[b]	50[b]	49[b]	49[b]	28 (19 - 33)	93 (115 - 121)	1.21

Note: Means followed by the same letters do not differ significantly (p < 0.05) by Tukey test.
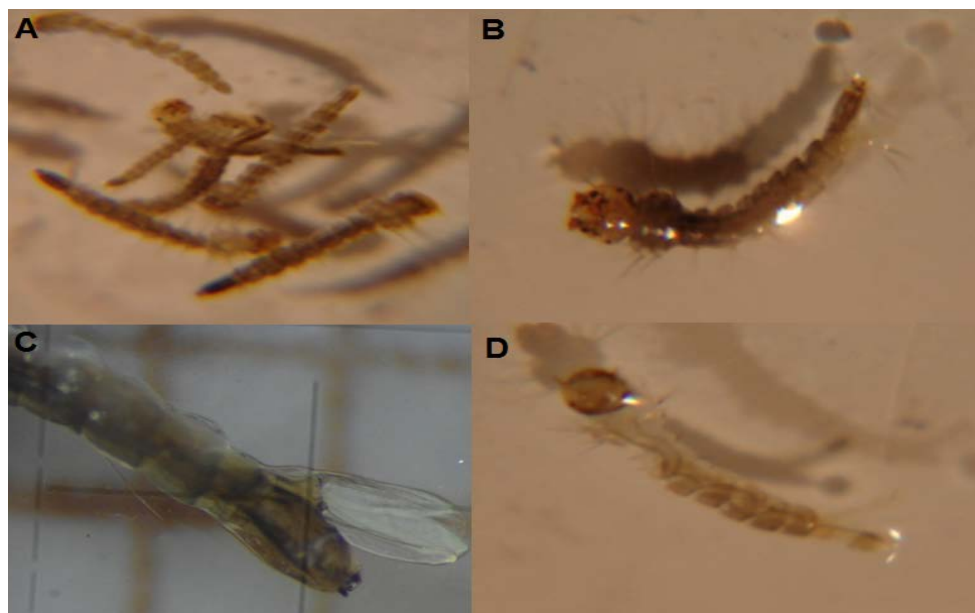

Figure 1. *Aedes aegypti* larvae with morphological alterations provoked by *D. brasiliensis* extracts and fractions. Note; A: Overall view of the larvae; B and C: Rigid and easily breakable exoskeleton, D: Elongation of the anterior portion to the thorax.

(DL_{50} 33 µg/ml), BCE (DL_{50} 32 µg/ml), BHF (DL_{50} 31 µg/ml) e BEAF (DL_{50} 28 µg/ml). Several species of plants improve their defenses against insects and some microbes as a response to an attack pathogenic. The produced substances are natural sources of substances that may have insecticidal activities, larvicide, antimicrobial and antitumoral.

Therefore, studies that demonstrate the larvicidal potential of plant extracts have the advantage of being less aggressive to the environment than synthetic larvicides found in commercial networks, which can be toxic to the environment. External morphological alterations were verified in all larvae that had been exposed to *D. brasiliensis* extracts for 24 h (Figure 1A). The main observed alterations for the extracts were rigid (Figure 1B and 1C) and easily breakable exoskeleton and elongation of the anterior portion to the thorax (Figure 1D). Changes in the morphology of the larvae, which occur by the use of fitolarvicidas has the advantage of preventing the larvae pass to the next stage preventing them from becoming mosquito. The changes observed in the outer anterior larvae can lead to changes in the gut of

the larvae; can stop the passage of food necessary for survival and development to the later stages. The changes caused by the extract and fractions of *D. brasiliensis* also reached the posterior region, affecting the anal papillae and respiratory siphon, which can affect the flow of oxygen and swim of these larvae. These changes are the result of *D. brasiliensis* extracts of action, demonstrating its *in vivo* toxicity and its potential as a larvicide.

DISCUSSION

The resistance of *Aedes aegypti* population to insecticides, the use of conventional insecticides that presents environmental hazards and dangers to living organisms, and the ineffectiveness in fighting the vector has aroused the need to develop new effective compounds to fight dengue vectors, to minimize or to avoid the current problem (Garcez et al., 2009). Current strategies based on the elimination of breeding sites and applications of chemical insecticides for larval and adult mosquito control have resulted in development of resistance without eliminating the constant risk of dengue epidemics (Lima et al., 2011). Thus new approaches are urgently needed. Interest on possible use of environment friendly natural products such as oils of plants or plant parts increased for vector control. Plant derived products have received increased attention from scientists and more than 2000 plant species are already known to have insecticide properties (Pankaj and Anita, 2010; Kamaraj et al., 2011).

The use of plant extracts in the control of *Aedes* has increased, once these are effective in the control of larvae in various developmental stages. Natural products research also led to the identification of various active substances, such as azadirachtin, plumbagin, and β-sitosterol, with demonstrated toxic activity against these insects (Kamaraj et al., 2009; Evergetis et al., 2009; Maniafu et al., 2009). As shown in the present work, the *D. brasiliensis*, BCF and LEAF may be used to control *Aedes aegypti* larvae, due to the observed mortality in 3rd stage larvae, which are considered more resistant (Silva and Silva, 1999). Vegetable extracts and pure substances may manifest their toxic effects upon mosquitoes in various ways; by reproduction and fertility suppression, by mortality or by means of intoxication with trypsin inhibitors, toxicity, mortality and growth inhibition (Jbilou et al., 2006).

When the larvicidal effect was compared by the Tukey test, we observed that the obtained mortality between the different samples did not present important differences among themselves; the differences appeared only when tested samples were compared to those of the controls. Controlling *Aedes* larvae may be a more effective way to

control dengue, as in this immature stage the larvae are relatively immobile, remaining in more concentrated areas than adult mosquitoes, which can move to other areas (Rutledge et al., 2003). Morphological alterations were observed in *A. aegypti* larvae which had been exposed to ethanolic extracts of three piperaceae species, observed cuticle destruction in the anal papillae (gills), caused by the extrusion of the peritrophic matrix of the larvae as a way to eliminate the toxic substances from its interior and thus avoid further tissue damage that could lead to morphological deformation (Chaithong et al., 2006).

The insect development alterations range from the epithelial cell morphology to malformations in the exoskeleton leading to severe deformations and death (Chaithong et al., 2006). The observed effects in the external morphology of *A. aegypti* larvae may have taken place because of alterations in the ecdysone hormone, preventing physiological changes and growth (Martinez, 2006). Secondary metabolites have been reported as active components of vegetal extracts which exhibit insecticide activity. Detected substances such as quercetin may be involved in the larvicide activity because they are able to kill the mosquito larvae in all its development stages (Rahuman et al., 2008; Nikkon et al., 2010; Ochieng et al., 2010). Toxic substances interact specifically with apical membrane receptors of the median gut, causing serious damage to the epithelium that culminate with larvae death (Charles, 1981; Gill et al., 1992).

Based on these results, it can be inferred that all fractions have substances that act in the *Aedes aegypti* larvae, especially the BCF and LEAF showing a more effective result, leading to external morphological alterations due to toxic effects, and that it may be used in the larvae control, since this activity was demonstrated to the most resistant larval stage.

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Conflicts of interest

Authors have none to declare.

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