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Water extracts of Brazilian leguminous seeds as rich sources of larvicidal compounds against *Aedes aegypti* L.

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ABSTRACT

This study assessed the toxicity of seed water extracts of 15 leguminous species upon *Aedes aegypti* larvae. A partial chemical and biochemical characterization of water extracts, as well as the assessment of their acute toxicity in mice, were performed. The extracts of *Amburana cearensis*, *Anadenanthera macrocarpa*, *Dioclea megacarpa*, *Enterolobium contortisiliquum* and *Piptadenia moniliformis* caused 100% of mortalit y after 1 to 3 h of exposure. They showed LC_{50} and LC_{90} values ranging from 0.43 $\pm$ 0.01 to 9.06 $\pm$ 0.12 mg/mL and from 0.71 $\pm$ 0.02 to 13.03 $\pm$ 0.15 mg/mL, respectively. Among the secondary metabolite constituents, the seed water extracts showed tannins, phenols, flavones, favonols, xanthones, saponins and alkaloids. The extracts also showed high soluble proteins content (0.98 to 7.71 mg/mL), lectin (32 to 256 HU/mL) and trypsin inhibitory activity (3.64 $\pm$ 0.43 to 26.19 $\pm$ 0.05 gIT/kg of flour) The electrophoretic profiles showed a great diversity of protein bands, many of which already described as insecticide proteins. The extracts showed low toxicity to mice (LD_{50} > 0.15 $\pm$ 0.01 g/kg body weight), but despite these promising results, further studies are necessary to understand the toxicity of these extracts and their constituents from primary and secondary metabolism upon *Ae. aegypti*.

Key words: *Aedes aegypti*, larvicidal compounds, leguminous seeds, water extracts.

INTRODUCTION

The mosquito *Aedes aegypti* is the vector for the arboviruses responsible for dengue and yellow fever, both of which endemic to Central and South America, Asia and Africa (Consoli and Oliveira 1994). Contrary to yellow fever, which has been reasonably brought under control with its vaccine, dengue is still a major public health problem in many countries around the world. The only effective approach to reduce the incidence of dengue fever is by attacking the breeding places of *Ae. aegypti* larvae (Gluber 1989, Corbel et al. 2004), since it is more difficult to control the adult mosquito population (Dharmagadda et al. 2005).

This control depends basically on the use of synthetic (organochlorides, organophosphates, and carbamates) and biological insecticides (*Bacillus thuringiensis* spores). In Brazil, the control of *Ae. aegypti* populations with synthetic insecticides has turned into a serious problem. These populations have become highly resistant to these products, especially to organophosphates such as temephos (Beserra et al. 2007). Besides, most synthetic insecticides are toxic to mammals and adversely affect the environment (Dharmagadda et al. 2005). On the other hand, the biological control of

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Ae. aegypti populations with *Bacillus thuringiensis* is an effective alternative on combating the larval stage of the mosquito, since it is more biodegradable, non-pollutant and shows selective toxicity towards invertebrates (Polanczyk et al. 2003). Nevertheless, it is more expensive than synthetic insecticides and its effectiveness decreases in high solar incidence regions, where mosquitoes usually develop. Furthermore, studies have pointed out to the development of resistance to *Bacillus* spp. in *Culex* sp. (vector of filarial disease) (Silva and Trgis 1997, Wirth and Georghiou 1997). Considering that insects in general, and *Ae. aegypti* in particular, develop resistance to a variety of insecticides, this phenomenon might be demonstrated for *Ae. aegypti* in the near future (Hemingway and Ranson 2000, Murugan et al. 2007).

An alternative for conventional chemical and biological control is the use of natural products from plants, which have been shown to be effective insecticides and to minimize environmental impact (Fatope et al. 1993, Consoli and Oliveira 1994). In the last years, several studies have focused on plant products for controlling *Ae. aegypti* as larvicides and adulticides, or repellents for personal protection (Jang et al. 2002, Carvalho et al. 2003, Ramos et al. 2006, Choochote et al. 2007, Silva et al. 2008). The Leguminosae (Fabaceae) family has a wide global distribution, being well represented in Brazilian ecosystems by over 2,000 native species congregated in 188 genera. Leguminosae species are especially recognized by the nutritional value of their seeds, which are rich in proteins, carbohydrates and oil. Nevertheless, seeds do not keep only storage material. They also need physical and chemical mechanisms for protection and/or defense of the developing embryo. The compounds involved in chemical defense include lectins, protease and amylase inhibitors, toxins and low molecular mass secondary metabolites (Xavier-Filho 1993). In spite of the diversity of defense compounds, few studies have been performed with seeds, especially those from Leguminosae family concerning toxicity upon larvae of Culicidae mosquitoes. In addition, works regarding the effectiveness of seed water extracts, which are rich in primary and secondary metabolites, against *Ae. aegypti* larvae, are even rarer. Thus, the aim of the present study was to evaluate the larvicidal activity of water extracts of 15 leguminous seeds from the Northeast Brazil against larvae of *Ae. aegypti*, to characterize chemical and biochemically the active compounds, as well as to assess their acute toxicity in mice.

MATERIALS AND METHODS

Plant Material

The fresh pods (at least 500 g) of each plant species (Table I) were collected in Ceará, Northeast Brazil. Plants were identified by the botanist Edson de Paula Nunes from the Universidade Federal do Ceará (UFC, Fortaleza, Brazil), and voucher specimens were deposited at Herbarium Prisco Bezerra (EAC) of the same institution.

Preparation of Seed Water Extracts

The seeds were separated from pods and transformed into fine flour (mesh size 1.0 mm) by using a blender and a coffee mill. The fine powder was placed in an oven at 45°C for three days to remove moisture and, then, stored in plastic bottles. The water extracts were prepared by suspending 1 g of seed flour into 10 mL of distilled water, mixing with a magnetic stirrer at 4°C for 4 h. The mixture was filtered through nylon cloth and centrifuged at 20,000 x g, for 30 min. The supernatant was used in the bioassays (larvicidal test and acute toxicity test in mice), in the quantification of soluble proteins, in the determination of protein profile and in phytochemical tests. The extracts were tested at different concentrations according to specific solubility of protein and non-protein constituents. We did not standardize the concentration to avoid extra manipulations, such as freeze-drying and other procedures, which could cause loss of the native structure of proteins and subsequent loss of activity.

Larvicidal Activity Assay

Larvae of *Ae. aegypti* were collected from a mosquito colony kept at NUVET/SESA (Núcleo de Controle de Endemias Transmissíveis por Vetores – Secretaria de Saúde do Estado do Ceará). Tests were run according to the methodology described by World Health Organization (WHO 2005) with some modifications. Third instar larvae of *Ae. aegypti* were collected with a Pasteur pipette, placed on filter paper for removal of excess water and transferred (20 *per* test) with a tiny brush

TABLE I
Leguminous species (Fabaceae) employed in this study, soluble solids concentration, protein content and toxic activities of seeds water extract against *Aedes aegypti* larvae and mice.

Subfamily Botanical name Voucher number	Extract soluble solids (mg/mL)	Protein content (mg/mL)	Larvicidal activity (% 3rd larvae mortality)	Acute toxicity (LD_{50} g/kg)[1]
Caesalpinoideae				
Caesalpinia bracteosa Tul. EAC 39616	28.11 ± 0.08	2.17 ± 0.02	ND	ND
Caesalpinia ferrea Mart. EAC 39616	28.14 ± 0.54	1.40 ± 0.01	85.04 ± 3.84	ND
Dimorphandra gardneriana EAC 39617	26.17 ± 0.31	2.33 ± 0.10	65.08 ± 2.56	ND
Hymenaea courbaril L. EAC 38108	11.80 ± 0.10	1.13 ± 0.07	13.33 ± 0.54	ND
Senna obtusifolia (L.) H.S. Irwin & Barneby EAC 39320	25.22 ± 0.95	1.47 ± 0.02	41.67 ± 1.97	ND
Senna rugosa (L.) H.S. Irwin & Barneby EAC 38112	54.71 ± 2.02	2.40 ± 0.11	16.67 ± 0.78	ND
Faboideae				
Amburana cearensis (Fr. Allem.) A.C.Sm. EAC 39618	24.75 ± 1.31	0.98 ± 0.01	100.0 ± 0.0	ND
Dioclea megacarpa Rolfe EAC 38110	26.33 ± 1.09	7.46 ± 0.73	100.0 ± 0.0	0.77 ± 0.04
Erythrina velutina Willd EAC 35979	32.25 ± 1.37	7.71 ± 0.66	75.12 ± 1.89	1.00 ± 0.01
Lonchocarpus sericeus (Poiret) Kunth EAC 39615	33.29 ± 1.22	7.70 ± 0.81	70.09 ± 2.34	ND
Mimosoideae				
Anadenanthera macrocarpa (Benth.) Brenan EAC 38697	42.64 ± 2.45	1.56 ± 0.21	100.0 ± 0.0	0.15 ± 0.008
Enterolobium contortisiliquum (Vell.) Morong EAC 38115	35.23 ± 1.54	3.13 ± 0.15	100.0 ± 0.0	1.1 ± 0.01
Parkia platycephala Benth. EAC 38109	28.50 ± 0.82	1.32 ± 0.08	7.89 ± 0.27	ND
Piptadenia moniliformis Benth. EAC 35974	36.70 ± 1.36	4.30 ± 0.21	100.0 ± 0.0	ND
Plathymenia reticulata Benth. EAC 38114	22.31 ± 0.75	1.22 ± 0.06	81.67 ± 3.29	ND
Distilled water	—	—	ND	ND

[1]Acute toxicity to mice ($n = 6$) was verified by intraperitonial injection (30 mL/Kg body weight) of diluted and crude water extract of each seed (Vasconcelos et al. 1994). ND = Not Detected. Values are means ± standard deviation ($n = 3$).

into 150-mL disposable plastic cups containing 100 mL of water extract of each seed. Larval mortality/survival was monitored during the first three hours of exposure and registered after 24 h at room temperature (25°C). For each extract, three independent experiments were run in triplicate and distilled water was used as negative control. Larvae were considered dead when no movement could be detected after they had been touched with a tiny brush. The results were expressed as mean ± standard deviation of per cent mortality for all experiments. The water extracts that showed 100% of larval mortality were diluted and evaluated again to determine the lethal concentration for 50% and 90% of larvae (LC_{50} and LC_{90}, respectively).

Assay for Acute Toxicity in Mice

Acute toxicity in mice ($n = 6$) was verified by intraperitonial injection (30 mL/Kg body weight) of diluted and crude water extract of each seed according to Vasconcelos et al. (1994). This procedure was approved by the

Animal Experimentation Ethics Committee of Universidade Federal do Ceará (CEPA), Protocol No. 34/09, which adopts the guidelines of Colégio Brasileiro de Experimentação Animal (COBEA).

Characterization of Seed Water Extracts

Soluble protein content

The total soluble protein content was determined by the colorimetric method of Coomassie Brilliant Blue according to Bradford (1976).

Content of some bioactive proteins

Lectin activity was assessed by serial two-fold dilution of samples (Moreira and Perrone 1977). The water extracts were diluted with 0.15 M NaCl and mixed with erythrocytes from rabbit blood (20 mg/mL suspension prepared in 0.15 M NaCl) treated with proteases from *Aspergillus oryzae* (EC 232-642-4, Sigma-Aldrich Co. USA), in a concentration of 1 mg/mL. The degree of agglutination was visually monitored after the tubes had been left to stand at 37°C for 30 min, and at room temperature (22 ± 3°C) for an additional 30 min. The results are reported as hemagglutination title (HU), which is the reciprocal of the highest dilution giving visible agglutination. Trypsin inhibitory activity was determined by a slight modification of the method originally described by Kakade et al. (1969), using trypsin enzyme and L-BAPNA as substrate. Activity was expressed as the amount of trypsin inhibited, calculated from a calibration curve using soybean trypsin inhibitor.

Electrophoresis

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli (1970). SDS-PAGE was carried out in a 2-mm vertical slab gel (10×8 cm) containing stacking gel mix, 5% total acrylamide, and main running gel mix, 17.5% acrylamide, prepared in 3.0 M Tris-HCl, pH 8.8. Samples (20 μl of each water extract) were dissolved in Tris-HCl 0.0625 M, pH 6.8, containing 1% SDS and 1% β-mercaptoethanol (1:1, v/v) and incubated at 100°C for 10 min. Treated samples containing approximately 50 μg of protein content were inoculated in wells, and electrophoresis was carried out at 20 mA constant current for 2 h. Bands were visualized by staining with 0.05% Coomassie Brilliant Blue R-250. Molecular mass markers employed were phosphorilase B (97.4 KDa), bovine serum albumin (66.2 KDa), ovalbumine (45.0 KDa), carbonic anhydrase (31.0 KDa), soybean trypsin inhibitor (20.1 KDa) and lysozyme (14.2 KDa) (AMRESCO Inc., Ohio, USA).

Phytochemical Study of Seed Water Extracts

Phytochemical tests to detect the presence of secondary metabolites as phenols, tannins, leucoantocianidins, flavonoids, esteroids, triterpens, and alkaloids were performed according to Matos (1988). These tests are based on visual observation of color modification or precipitates formation after the addition of specific reagents.

Statistical Analysis

Mortality data were subjected to probit analysis (Finney 1971) to estimate lethal concentration that kills 50% and 90% of *Ae. aegypti* larvae (LC_{50} and LC_{90}, respectively).

RESULTS AND DISCUSSION

Many studies have described the larvicidal activity against *Ae. aegypti* of extracts from leaves, stems, and roots of leguminous species prepared with organic solvents (Pohlit et al. 2004, Luna et al. 2005, Mendonça et al. 2005, Omena et al. 2007). Nevertheless, little attention has been addressed to the potential of seeds as sources of secondary compounds with larvicidal properties (Jang et al. 2002, Luna et al. 2005). Much scarcer are the studies that use water extracts for obtaining primary compounds admittedly toxic to *Ae. aegypti* larvae (Carlini et al. 1997).

The results of the water extract toxicity assays against larvae of *Ae. aegypti* (Table I) showed that, among fifteen extracts, only four have larval mortality rates lower than 50%. The other extracts caused larval mortality rates over 60%, being noteworthy the water extracts activity of *Amburana cearensis*, *Dioclea megacarpa*, *Anadenanthera macrocarpa*, *Enterolobium contortisiliquum* and *Piptadenia moniliformis*, which caused 100% of mortality after 1 to 3 h of exposure. Luna et al. (2005) did not detect *Ae. aegypti* larval mortality in ethanolic extract of *A. macrocarpa* wood bark. These

authors have described only 5% of larval mortality after the exposure to ethanolic extract of *Dioclea virgata* leaves, which is much smaller than that shown for *D. megacarpa* seed water extract (100%) observed in the present work. In spite of the water extracts present high concentrations of total soluble solids (>20 mg/mL), it seems that these are not directly associated with the mortality rate. One extract (*Senna rugosa*) with a concentration above 40 mg/mL showed a relatively low larval mortality rate, whereas other extracts with half this concentration caused 100% of mortality after a short exposure time.

Can these promising results be attributed only to secondary metabolites present in the extracts? According to Table II, among secondary metabolites, the seed water extracts of these five plant species contain mainly tannins, phenols, flavones, favonols, xanthones, saponins and alkaloids. These secondary compounds were similar to those shown by Luna et al. (2005) in leaves, roots and wood bark alcoholic extracts of several plant species. Specifically, the leguminous species studied by Luna et al. (2005) with strong larvicidal activity were shown to contain flavones, phenols and alkaloids, the same compounds detected in the present work. Besides, the role of phenol compounds, such as thymol (an alkyl-derivative of phenol) and tannins as larvicidal compounds against *Ae. aegypti*, is well known (Carvalho et al. 2003, Silva et al. 2004). However, it is evident that these compounds are also present in the water extracts of species with the lowest activities, *Senna rugosa* and *S. obtusifolia*. Thus, it is questionable whether this could be explained only by the low levels of these larvicidal compounds in the less active extracts, or whether other compounds, such as proteins with insecticide activity, are present in the extracts. It is possible that proteins with insecticidal activity such as lectins, toxins, arcelins and protease inhibitors (Carlini and Grossi-de-Sá 2002, Whetstone and Hammock 2007) may be present in the water extracts, considering the extraction conditions and the common occurrence of these compounds in species of the Leguminosae family (Vasconcelos and Oliveira 2004). Thus, it is likely that these compounds contribute for larvicidal activity of the five most potent seed extracts aforementioned.

The soluble protein contents (Table I) ranged from 4 to 28% of total soluble solids. Ramos et al. (2006) have detected high mortality rates of *Ae. aegypti* larvae working with fractions of *Calotropis procera* latex, which are rich in cysteinic proteases (5.2 mg/mL). Recently, Sá et al. (2008) showed the first report of larvicidal activity against *Ae. aegypti* of a plant purified protein, the lectins from *Myracrodruon urundeuva*. It has been reported that lectins and toxins possess insecticidal activity against Diptera, but little is known about the toxic activity of serine and cysteine protease inhibitors, alfa-amylase inhibitors and arcelins, in spite of the reported action of these compounds against other insect orders (Carlini and Grossi-de-Sá 2002). The five most potent plant species described in the present work contain potentially active proteins against insects (Table III). Lectin activity was detected in the seed water extracts from *D. megacarpa* (256 HU/mL) and *E. contortisiliqüum* (32 HU/mL). Besides, all the studied seed water extracts showed trypsin inhibitory activity, ranging from 3.64 ± 0.43 to 26.19 ± 0.05 g of inhibited trypsin *per* kg of flour. Some compounds responsible for these activities in the water extracts were previously purified. The *A. cearensis* and *E. contortisiliqüum* seeds possess trypsin inhibitors with molecular mass of 13.6 KDa (Tanaka et al. 1989) and 23.0 KDa (Oliva et al. 1987), respectively. *D. megacarpa* seeds possess a three subunits lectin with molecular masses of 25-26, 13-14, and 8-9 KDa (Moreira et al. 1983). Recently, a chitin-binding vicilin from *E. contortisiliqüum* seeds was purified and shown to be toxic against bean bruchid pests (*Callosobruchus maculatus* and *Zabrotes subfasciatus*) (Moura et al. 2007). These protein compounds could be observed in the electrophoretic profiles of the water extracts (Fig. 1). Therefore, it is reasonable to consider that the proteins naturally involved in plant defense can contribute somehow to the larvicidal activity of the seeds water extract.

The LC_{50} and LC_{90} values of the most active seed water extracts (*A. macrocarpa*, *A. cearensis*, *D. megacarpa*, *E. contortisiliquum*, and *P. moniliformis*) against *Ae. aegypti* larvae (Table IV) ranged from 0.43 ± 0.01 to 9.06 ± 0.12 mg/mL and from 0.71 ± 0.02 to 13.03 ± 0.15 mg/mL, respectively. The *A. macrocarpa* water extract was the most powerful with LC_{50} of 0.43 ± 0.01 mg/mL and LC_{90} of 0.71 ± 0.02 mg/mL. These values are much higher than those detected in samples

TABLE II
Classes of compounds detected in the seed water extracts employed in this study.

Species	Tannins	Phenols	Saponnins	Anthocyanins Anthocyanidins	Flavones, flavonol, xanthones	Chalcones Aurones	Flavonols	Leucoanthocyanidins	Catechins (catechin tannins)	Flavanones	Alkaloids	Steroids	Triterpenoids
Caesalpinia bracteosa	+[1]	–[1]	–	–	+	–	–	–	–	–	+	–	–
Caesalpinia ferrea	+	+	+	–	+	–	–	–	+	–	–	–	–
Dimorphandra gardneriana	–	–	+	–	–	–	–	–	+	–	+	–	–
Hymenaea courbaril	–	–	+	–	–	–	+	–	–	–	–	–	–
Senna obtusifolia	–	+	+	–	–	–	+	–	–	+	+	–	–
Senna rugosa	–	+	+	–	–	+	–	–	–	–	–	–	+
Amburana cearensis	+	+	–	–	+	–	–	–	–	–	–	–	–
Dioclea megacarpa	–	–	+	–	+	–	–	–	–	–	+	–	–
Erythrina velutina	–	–	+	–	+	–	–	–	–	–	+	–	+
Lonchocarpus sericeus	–	–	+	–	+	–	–	–	–	–	+	–	+
Anadenanthera macrocarpa	+	+	–	–	+	–	–	–	–	–	+	–	–
Enterolobium contortisiliquum	–	–	+	–	–	–	–	–	–	–	–	–	–
Parkia platycephala	–	–	+	–	+	–	–	–	–	–	–	–	–
Piptadenia moniliformis	–	+	–	–	+	–	–	–	–	–	–	–	–
Platymenia reticulata	–	–	+	–	+	–	–	–	–	–	+	–	+

[1] +: detected; –: not detected.

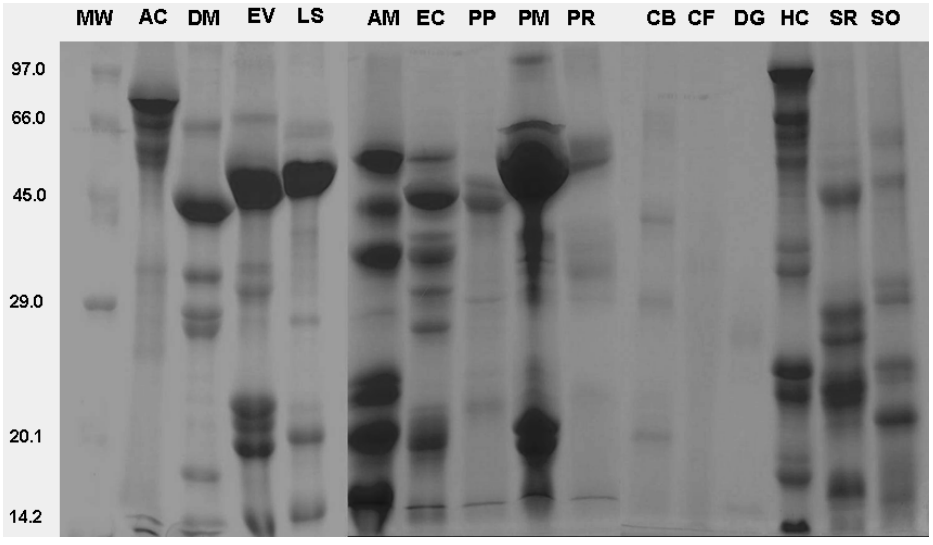

Fig. 1 – Electrophoresis of water extract proteins of *Amburana cearensis* (AC), *Dioclea megacarpa* (DM), *Erythrina velutina* (EV), *Lonchocarpus sericeus* (LS), *Anadenanthera macrocarpa* (AM), *Enterolobium contortisiliquum* (EC), *Parkia platycephala* (PP), *Piptadenia moniliformis* (PM), *Plathymenia reticulata* (PR), *Caesalpinia bracteosa* (CB), *Caesalpinia ferrea* (CF), *Dimorphandra gardneriana* (DG), *Hymenaea courbaril* (HC), *Senna rugosa* (SR), *Senna obtusifolia* (SO). A total of 50 μg of protein was applied in the gel.

TABLE III
Content of some bioactive proteins (lectins and trypsin inhibitors) in leguminous seed water extracts with larvicidal activity against 3rd instars of *Aedes aegypti*.

Species	Lectin activity[1]	Trypsin inhibitor activity
Faboideae		
Amburana cearensis	ND	12.23 ± 0.29
Dioclea megacarpa	256	10.78 ± 0.10
Mimosoideae		
Anadenanthera macrocarpa	ND	3.64 ± 0.43
Enterolobium contortisiliquum	32	26.19 ± 0.05
Piptadenia monoliformis	ND	8.85 ± 0.47

[1] Lectin activity is expressed as hemagglutination unit (HU) *per* mL of water extract. One HU represents the reciprocal of the highest dilution giving visible agglutination of the treated rabbit erythrocytes with protease solution (1mg/mL; 1:100, v/v). [2] Trypsin inhibitory activity is expressed as g of trypsin inhibited *per* kg of flour. ND = Not Detected. The values are mean ± standard deviation ($n = 3$).

obtained from more selective extractions with apolar solvents such as ethanol, methanol etc. Jang et al. (2002), working with methanolic extracts of leguminous seeds, showed approximately 50% of *Ae. aegypti* larvae mortality at the concentration of 0.04 mg/mL. However, the LC_{50} value of *A. macrocarpa* water extract is much lower than that found by Sá et al. (2008) for bark and heartwood crude water extracts of *Myracrodruon urundeuva* (LC_{50} 8.81 and 14.86 mg/mL, respectively). The lectins purified from these extracts showed low values of LC_{50}, 0.125 mg/mL for bark lectin, and 0.04 mg/mL for heartwood lectin (Sá et al. 2008). Thus, if the compound responsible for the larvicidal activity in the most promising water extracts is of protein nature, its activity during the purification process will tend to increase, being comparable to extracts rich in secondary compounds.

All these considerations may provide evidences that seeds may be promising sources of larvicidal compounds, and that water may be an efficient solvent to extract bioactive substances.

Among all evaluated water extracts (15), only four of them (*A. macrocarpa*, *D. megacarpa*, *E. contortisiliquum* and *E. velutina*) presented acute toxicity on mice (Table I). The extract of the species *A. macrocarpa* showed the highest toxicity with a LD_{50} of 0.15 g/kg body weight, a value considered as slightly toxic according to Hodge and Sterner's criteria (1949), and as

TABLE IV
LC_{50} and LC_{90} of water extracts of leguminous seeds against 3rd instars of *Aedes aegypti* after 24h of exposure[1].

Species	LC_{50} (mg/mL)	LC_{90} (mg/mL)
Faboideae		
Amburana cearensis	8.10 ± 0.27	11.67 ± 0.32
Dioclea megacarpa	6.68 ± 0.11	9.75 ± 0.17
Mimosoideae		
Anadenanthera macrocarpa	0.43 ± 0.01	0.71 ± 0.02
Enterolobium contortisiliquum	2.39 ± 0.03	3.79 ± 0.05
Piptadenia monoliformis	9.06 ± 0.12	13.03 ± 0.15

[1] LC_{50} and LC_{90} are the lethal concentrations (mg/mL) at which 50% and 90% of the larvae showed mortality, respectively. Values are means ± standard deviation ($n = 3$).

toxic by WHO's criteria (1994). On the other hand, the extract of the other most active species, *A. cearensis*, *D. megacarpa*, *E. contortisiliquum* and *P. moniliformis*, were considered slightly toxic (Hodge and Sterner criteria) and noxious (WHO criteria). Thus, it seems that there is a trend of these bioactive compounds to show higher toxicity towards invertebrates and be safe to mammals.

In conclusion, leguminous seeds are promising sources of primary metabolites, especially proteins, and secondary metabolites with larvicidal activity against *Ae. aegypti* with low toxicity to mammals. Nevertheless, further studies must be dedicated to understand the high toxicity of the seed extracts upon larvae of *Ae. aegypti* and to identify the compounds from primary and secondary metabolism involved in the toxicity.

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RESUMO

Este trabalho objetivou avaliar a toxicidade dos extratos aquosos de sementes de 15 espécies de leguminosas contra larvas de *Aedes aegypti*. Foi realizada uma caracterização química e bioquímica parcial dos extratos aquosos e a avaliação da toxicidade aguda em camundongos. Os extratos de *Amburana cearensis*, *Anadenanthera macrocarpa*, *Dioclea megacarpa*, *Enterolobium contortisiliquum* e *Piptadenia moniliformis* causaram 100% de mortalidade depois de 1 a 3 h de exposição e mostraram valores de CL_{50} e CL_{90} entre 0,43 ± 0,01 e 9,06 ± 0,12 e entre 0,71 ± 0,02 e 13,03 ± 0,15 mg/mL, respectivamente. Dentre os constituintes do metabolismo secundário, os extratos das sementes apresentaram taninos, fenóis, flavonas, flavonóis, xantonas, saponinas e alcalóides. Os extratos apresentaram alto teor de proteínas solúveis (0,98 to 7,71 mg/mL), lectina (32 to 256 UH/mL) e inibidor de tripsina (3,64 ± 0,43 to 26,19 ± 0,05 gIT/kg de farinha). O perfil eletroforético mostrou uma grande diversidade de proteínas, muitas das quais já descritas como inseticidas. Os extratos mostraram baixa toxicidade ao camundongo ($DL_{50} > 0,15 \pm 0,01$ g/kg peso corporal), porém apesar desses resultados promissores, estudos posteriores são necessários para compreender a toxicidade desses extratos e de seus constituintes do metabolismo primário e secundário sobre *Ae. aegypti*.

Palavras-chave: *Aedes aegypti*, compostos larvicidas, sementes de leguminosas, extratos aquosos.

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