



## In vitro toxic activity of *Croton heliotropiifolius* Kunth (Euphorbiaceae)

### Atividade tóxica in vitro de *Croton heliotropiifolius* Kunth (Euphorbiaceae)

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**Abstract:** Often found in some Brazilian biomas as caatinga, brejo, restinga and cerrado, the velame, *Croton heliotropiifolius* Kunth, is popularly known for its medicinal properties used in stomach ache, gastric reflux, vomiting, bloody diarrhea and to reduce fever. In addition to the pharmacological properties, its essential oil has larvicidal activity against *Aedes aegypti*, as well as the ethanol extract showed significant insecticidal activity against *Sitophilus zeamais*. This study aimed at evaluating the toxicity of preliminary methanolic extract of *Croton heliotropiifolius* Kunth on *Artemia salina* larvae. The bioassay was carried out at concentrations of 50 µg/ml, 100 µg/ml, 250 µg/ml, 500 µg/ml, 750 µg/ml, 1000 µg/ml of methanolic extract of leaves. The extract studied showed a moderate toxicity to *Artemia salina*, with LC<sub>50</sub> values in the range of 637,29 µg/mL.

**Key words:** Toxicity; Methanolic extract; *Artemia salina*.

**Resumo:** Frequentemente encontrado na caatinga, brejo, restinga e cerrado brasileiro, o velame, *Croton heliotropiifolius* Kunth é popularmente conhecido por suas propriedades medicinais utilizada na dor de estômago, mal estar gástrico, vômitos, diarreia com sangue e para baixar a febre. Além das propriedades farmacológicas, seu óleo essencial apresenta atividade larvicida contra *Aedes aegypti*, assim como o extrato etanólico apresentou atividade inseticida considerável contra *Sitophilus zeamais*. O presente trabalho teve como objetivo avaliar a toxicidade preliminar do extrato metanólico de folhas de *Croton heliotropiifolius* Kunth frente à *Artemia salina*. O bioensaio foi realizado nas concentrações de 50 µg/ml, 100 µg/ml, 250 µg/ml, 500 µg/ml, 750 µg/ml, 1000 µg/ml de extrato metanólico de folhas. O extrato em estudo apresentou uma moderada toxicidade para a *Artemia salina*, apresentando valores de CL<sub>50</sub> na faixa de 637,29 µg/mL.

**Palavras-chave:** Toxicidade; Extrato metanólico; *Artemia salina*.

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## INTRODUCTION

For a long time the use of medicinal herbs is based on knowledge of the intuitive man, who in the course of history, has learned to differentiate the beneficial action of toxic action, thus enabling the therapeutic use (LEITE, 2008).

Popular wisdom about the medicinal properties of plants has accumulated for centuries and this knowledge, sometimes, it was the only way of treatment used by many communities and ethnic groups. The effectiveness of these methods is assigned to the observations that collaborate in an intense way, to the spread of therapeutic qualities of plants (MACIEL et al., 2002).

The investigation of the toxicity is possible to determine the potential of new substances and products causing damage. In this way, tests that evaluate the toxic action are used to classify substances according to their potential for mortality or toxicity, in addition to the toxicokinetics and on dose response (PURCHASE et al., 1998; BLAAUBOER, 2003).

Many tests involved with the analysis of toxic agents use laboratory animals. However, there is a growing search for in vitro methods that do not use animals in their implementation (HARBELL et al., 1997).

The bioactive compounds generally have toxicity, especially in high doses. Thus, the evaluation of mortality in a less complex animal body can be used for a simple and rapid monitoring extracts (MACIEL et al., 2002).

The *A. salina* Leach (Artemiidae) microcrustacean is an invertebrate from saline and marine aquatic ecosystem used in laboratory tests for toxicity and estimating lethal doses (AYO et al., 2007). They are used to evaluate plant extracts, being considered a fast, efficient, and inexpensive test that requires a small amount of sample (PIMENTA et al., 2003).

Ethnobotanical studies focus on the Euphorbiaceae family due to its different uses (ALBUQUERQUE et al., 2007; NASCIMENTO et al., 2009; MELO et al., 2009; CARTAXO et al., 2010), including several species used in different situations. Particularly, the medicinal uses have been deemed the most significant for the genus *Croton* (CREPALDI et al., 2016).

*Croton heliotropiifolius* Kunth, popularly known in Brazil as "valame", "velaminho" and "velame-de-cheiro" due to its tiny bristles, is endemic in Brazilian Northeast and can be found frequently in the vegetation of caatinga, brejo, restinga and cerrado (RANDAU et al., 2004).

The species blossoms in May, June, July and November. The fruiting occurs in May and June (SILVA et al., 2010). It differs from *C. campestris* by its concolorous leaves, inflorescences, glandular trichomes, smooth seeds and branches with dendritic trichomes (SÁTIRO et al., 2008). The nomenclature of this species was *C. rhamnifolius*. Its name was changed to *C. heliotropiifolius* Kunth (GOVAERT et al., 2000).

Studies about *C. heliotropiifolius* reveal the predominant presence of alkaloids, polyphenols and reducing compounds, being referred to as helpful in relieving stomach ache, stomach discomfort, vomiting, dysentery and antipyretic (RANDAU, 2004).

Thus, this study aimed at evaluating the toxicity of methanol extract of *Croton heliotropiifolius* Kunth leaves by the larvae of *Artemia salina*, in order to investigate the safety and efficacy of its medicinal use.

## MATERIALS AND METHODS

### Collection and preparation of plant material

The leaves were obtained from *velame* tree (*C. heliotropiifolius* - Euphorbiaceae) in the urban area of the municipality of Garanhuns, Pernambuco State, Brazil. It was prepared a voucher specimen, which was deposited in the Dárdano de Andrade Lima Herbarium, in the Agronomic Research Institute (IPA), listing number of 90440, being identified by a botanist of the institution. The samples were collected in July, 2015.

### Obtainment of methanol extract

The dried crude extract was treated according to the maceration method described by Filho e Yunes (1998). The leaves (10g) were processed for 10 days in methanol (100ml) at room temperature and subjected to occasional agitation. After this period, the mixture was filtered and submitted to complete solvent evaporation by means of a rotary evaporator.

### Toxicological test

The performance of the test employed was based on the methodology described by Meyer et al. (1982) with adaptations. Encysted *A. Salina* were placed to hatch in filtered seawater for 48 hours and placed under the action of an aerator.

After hatching, the larvae were observed considering the viability (normal motility) and placed in groups of 10 larvae into tubes containing 5 ml of filtered seawater and the extracts in concentrations of 50 µg/ml, 100 µg/ml, 250 µg/ml, 500 µg/ml, 750 µg/ml, 1000 µg/ml. The negative control contained only seawater. Each concentration was performed in triplicate. Copies of *A. salina* were exposed to the extract in different concentrations for 24 h, then was observed the results. The vitality was defined considering the movement of microcrustaceans.

### Statistical analysis

The median lethal concentration (LC<sub>50</sub>) of the methanolic extract of *C. heliotropiifolius* leaves was determined using the OriginPro 8 program.

## RESULTS AND DISCUSSION

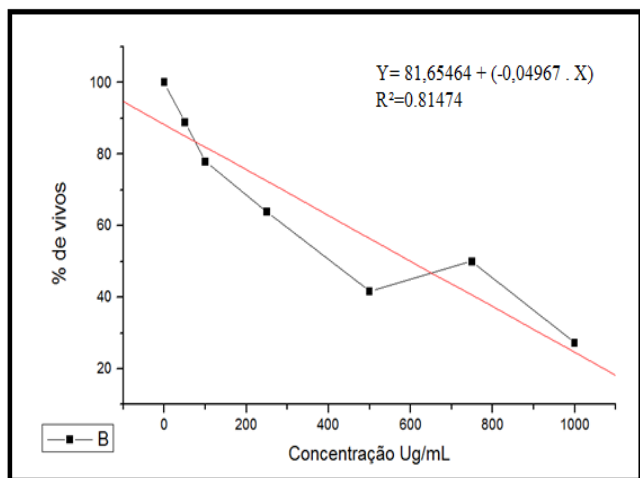
The methanolic extract of *C. heliotropiifolius* leaves showed a moderate toxicity to *Artemia salina* (Figure 1), with LC<sub>50</sub> values in the range of 637,29 µg/mL.

The number of dead larvae was proportional to the increase of the concentrations tested. During reading, *A. salina* species showed movement similar to the control test, except at concentrations of 750 µg/mL e 1000 µg/mL, which showed slow movement.

The extract showed a LC<sub>50</sub> of 637,298973 µg/mL, which qualifies as moderate toxicity for the microcrustacean. Whereas the toxic potential is numerically less than 1000 µg/mL (FERREIRA et al., 2000).

Other toxicity tests, as against the diamondback moth (*Plutella xylostella* L.) showed that the ethanol leaves extract of *C. heliotropiifolius* is the most toxic to larval stage, followed by the ethanol stem extract of the same species, with CL<sub>50</sub> 14,95 e 42,40µg/ml, respectively (SILVA, 2007).

**Figure 1.** Survival of *A. salina* exposed to the methanolic extract of *C. heliotropiifolius*. The row represents linear steadiness and  $R_2 = 0.81474$  shows the significant correlation between the survival of the lavas of *A. salina* and the concentration of the methanolic extract of *C. heliotropiifolius*.



In some *Croton* species was related a toxicity that appears to be due to the presence of diterpenoids (RODRIGUEZ et al., 2004), alkaloids even at low concentrations, are naturally toxic substances. The triterpenic saponins are natural compounds also associated with toxicity, due to their ability to produce hemolysis (DEWICK, 2002).

The essential oil of *C. heliotropiifolius* presented a relevant larvicidal effect against *Aedes aegypti*, which may represent a contribution to alternative methods of mosquito control (DÓRIA et al., 2010).

The acetylcholinesterase inhibitory activity of the *C. heliotropiifolius* ethanolic extract from the stem bark was described by Queiroz et al. (2014). In their study, the alkaloid taspine was identified, a compound reported as a strong acetylcholinesterase inhibitor (ROLLINGER et al., 2006). The identification of acetylcholinesterase inhibitor compounds may explain the insecticidal activity of the essential oil of this plant (MAGALHÃES et al., 2015).

Magalhães et al. (2015) also reported a repellent activity of the essential oil against *Tribolium castaneum*. This activity is an important property for pest control: the higher the repellency, the lower the infestations (COITINHO et al., 2006).

Thus, according to the moderate toxicity found out in this work, the authors propose the rational and moderate use of the species by the population, because they usually present as herbs or shrubs, which are used simply as drinks in the form of teas (HOEHNE et al., 1935). It is necessary to carry out different pre-clinical toxicological tests to confirm these obtained results.

## CONCLUSIONS

The methanolic of *croton heliotropiifolius* showed moderate toxicity to the microcrustacean *Artemia salina*.

In this way, our results may influence the performance of new toxicological tests and also the indiscriminate use of the species by the population.

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