Cytothological effects of the aqueous extract of *Lippia sidoides* Cham.

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1 INTRODUCTION

Despite the advancement of modern medicine, much of the population still uses plants for medicinal purposes (BADKE et al., 2016). The medicinal action of plants is related to the compounds produced by their secondary metabolism, substances that are recognized for being selective and play an important role in the evolution and adaptation of plants to the environment (BORGES; AMORIM, 2020).

The perception by the population that the natural product does not present toxic effects can induce the misuse of these products and consequently cause undesirable effects (BEDNARCZUK et al., 2010). Ingestion, inhalation and contact are the main ways to trigger an undesirable reaction from the misuse of natural products, thus, the use of plants in therapeutics should be conducted from a prior knowledge of the characteristics of the plant, preparation and mode of use. (JESUS; SUCHARA, 2013).

In this context, it is important to develop toxicological studies with plant species to ensure their proper use, as well as, to seek in these species the proof of their use, through the investigation and isolation of active substances for the future development of drugs.

Research aimed at determining the toxicological profile of a given species seeks to conduct *in vivo* and *in vitro* tests. These tests are the basis for the development of drugs, since from them it is possible to determine the risks that substances can cause to humans (SILVA et al., 2021). Among the most performed preliminary tests is the lethality test in *Artemia salina*, which allows to have previous recognition of the toxicity of the sample (FERREIRA et al, 2017).

Another widely investigated assay is the acute toxicity in animals such as the zebrafish , which aims to establish a certain concentration of the plant extract that can kill the animal in a certain time, delimiting a lethal dose (ASSESSMENT et al., 2015). The result obtained in this toxicological test

varies according to the method used, route of administration, dose, frequency of administration and time of exposure of the animal to the product (ALMEIDA et al., 2010).

Several studies have demonstrated the pharmacological potential of products extracted from *L. sidoides*. This species has antimicrobial activity (VERAS et al., 2012; FARIAS et al., 2012), acaricidal (CAVALCANTI et al., 2010), anti-inflammatory, antioxidant and gastroprotective (MONTEIRO et al., 2007). Despite this, there are still few reports on its toxic potential, requiring further research on this topic of study.

2 OBJECTIVE

To evaluate the cytotoxic activity of the aqueous extract of *Lippia sidoides* in the models of toxicity in *Artemia salina* and acute toxicity in zebrafish.

3 METHODOLOGY

Obtaining plant material and preparing liquid extract

The leaves of *Lippia sidoides* were collected from the medicinal plant garden of the Regional University of Cariri-URCA, with latitudinal coordinates 7º14'20.1" S and longitudinal coordinates 39º24'53.1 W. The collection was carried out in the month of March 2019 at 9 am. A sample containing the reproductive part (flowers) of the species was deposited in the Herbário Caririense Dárdano de Andrade Lima, of the aforementioned University, for identification and registration, receiving registration number 3038.

To obtain the liquid extract, initially the leaves were selected (150g) and submitted to fat removal with the solvent hexane, then they were submitted to agitation with water in an incubator shaker refrigeration following the following parameters: Temperature: 50 ºC; Rotation: 180 rpm; Agitation time: 4 hours daily. A total of 4 liters of sample extract was obtained.

Obtaining the powdered extract

The aqueous extract (5 liters) was dried using the *spray drying* technique (spray drying) with the *Mini-spray dryer* MSDi 1.0 Mini-spray dryer (Labmaq do Brasil), using a 1.2 mm nozzle, under the following operational conditions: a) flow control: 200 mL/h; b) inlet temperature: 120±2º C; c) outlet temperature: 88±2º C; d) atomization air flow: 45 L/min; e) blower flow: 1.80 m^3 /min. The atomization drying process consists in the change of a product that is in the fluid state to the solid state in powder form, through its passage in a heated medium, in a continuous operation.

Cytotoxic activity against Artemia salina

A. salina eggs were incubated in artificial seawater under light at 28°C. After 24 h of incubation, larvae were collected with a Pasteur pipette and kept for another 24 h under the same conditions to reach the largest stage. The sample was dissolved in Tween 80 and serially diluted (1000, 250, 125, 100, 75 µg / mL) in seawater. Then 10 larvae were added to each set of tubes containing the samples. A control was performed with potassium dichromate. 24 hours later, the number of survivors was counted (MEYER et al., 1982)

Zebrafish toxicity test

Zebrafish

Adult zebrafish (ZFa) animals aged 60 to 90 days (0.4 ± 0.1 g), wild, of both sexes, were obtained from a commercial supplier (Fortaleza, CE). The animals were kept in glass aquarium (n=5/L), at a temperature of 25 ± 2°C, in light-dark cycles for 24 h. Water was treated with antichlorine. The bioassays performed are in accordance with the Ethical Principles of Animal Experimentation and were approved by the Ethics Committee for Animal Use (CEUA) of Ceará State University (04983945/2021). After the experiments, the animals were sacrificed by freezing, immersed in ice water (2-4ºC), for 10 min until loss of opercular movements (CONCEA , 2018).

Acute toxicity 96h

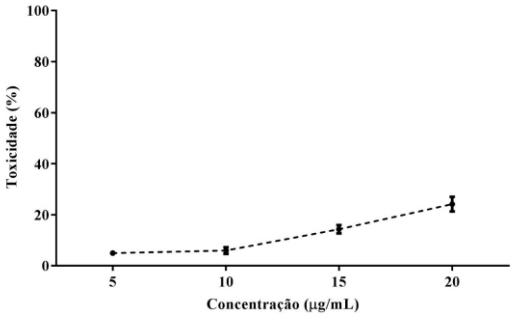
The acute toxicity study was conducted against adult zebrafish according to the Organization for Economic Cooperation and Development Standard Method (OECD, 1992) to determine the LD_{50} -96h. Mortality was monitored every 12 h after the start of testing. Animals (n=6/group) were treated, intraperitoneally, with 20 µL of the extracts (40;200 and 400mg/kg; 20 µL; i.p), vehicle (DMSO 3%). After 96 h, the number of dead fish in each group was counted and the lethal dose capable of killing 50 % of animals (LD_{50}) was determined using the Trimmed Spearman-Karber method with 95% confidence interval (ARELLANO-AGUILAR et al., 2015).

4 RESULTS AND DISCUSSION

The *spray-dried* extract showed a brownish color, with a total mass after drying of 77.60g and a yield of 43.11%.

The toxicity analysis against the microcustacean *A. salina* showed low toxicity at the tested concentrations. According to figure 1 there is a relationship between concentration and toxic activity, since increasing the concentration the percentage of larval death also increases. Although a dose-dependent relationship was verified, the results obtained did not allow the calculation of CI_{50} , showing that very high concentrations would be necessary to eliminate 50% of the artemia population.

Figure 1. Percentage of deaths of *A. salina* larvae by aqueous extract of *L. sidoides.*



Different results were observed in the study with methanol/water extract of L. multiflora where high toxicity was verified, presenting LC_{50} result of 1.1 µg/mL (AJAIYEOBA et al., 2006). The analysis of methanolic extracts prepared with different parts (stem, leaf and flowers) of *L. citriodora* showed that all extracts present significant lethality against A. salina, being its composition rich in tannins, polyphenols, triterpenes, catechins and alkaloids (VÉLEZ et al., 2019).

Regarding the acute toxicity test, the extract was not toxic against *zebrafish* up to 96h of analysis, as shown in Table 1. This test allows establishing a safe dose range and characterizing adverse effects for the use of extracts, such as *L. sidoides* (RASHID et al., 2022). As in the previous analysis, the toxicity of *L. sidoides* extract could only be observed from high concentrations, demonstrating that the use of this extract is suitable for the development of studies aiming the elaboration of phytochemicals.

Table 1. Results of acute toxicity tests (96h)

Sample	Mortality				96h LD_{50} (mg/kg) / IV
	C	I	D	I	
	N	1	2	3	
L. sidoides extract	0	0	0	0	> 400

CN- Negative control group: DMSO 3%. D1 - Dose 1 (40 mg/kg). D2 - Dose 2 (200 mg/kg). D3 - Dose 3 (400 mg/kg). DL50-Lethal Dose to kill 50% of adult Zebrafish; IV - confidence interval;

5 CONCLUDING REMARKS

In general, the extract did not show cytotoxic activity by the methods developed. These findings may demonstrate the safety in the use of the species for the development of more specific studies for other biological activities, as well as for use by populations in traditional medicine.

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