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Brine Shrimp Bioassay of Plants of the Brazilian Amazon Rainforest

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Abstract

This article describes the evaluation of the toxic potential of extracts of plant species Amazon using the brine shrimp lethality bioassay method. Many studies in the literature on phytochecial do not describe the biological activities of any kind. The test for the assessment of the toxicity was performed using larvae of *Artemia salina* with 48 h of hatching, the type nauplii, in concentrations of 1000, 100 and 10 $\mu g.mL^{-1}$. Was observed that, of the species that have been assessed, the vast majority has the potential cytotoxic activity, being that few extracts showed inactivity. Currently searching for substances with pharmacological activities promising is the first step in the development of new drugs from plants.

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1. Introduction

The plants are the main sources of natural products biologically active. A plant produces thousands of different substances, being only a small part responsible for their pharmacological activity. Many natural products are characterized and published without its biological activity has been tested. Many times this is due to the cost or complexity of the test.

The search for substances with potential biological activities, in their vast majority, it is guided by information in respect of the use of certain plants in folk medicine, chemotaxonomic information or even for biomonitoring, but with the small number of plants studied scientifically, it is very likely that new substances with promising potential to be discovered so completely random, due to research in plants little known or difficult to access.

In recent years, has been a great interest in alternative therapies and therapeutic use of natural products, especially those that are derived from plants. This interest in drugs originating in plants has several reasons, you can quote among the main ones: a) conventional medicine can often be inefficient (low effects on cure or therapy ineffective); b) high side effects of conventional drugs; (c) misuse and/or incorrect synthetic drugs, resulting in resistance to drugs (Peet, 2010); (d) a large percentage of the world's population does not have access to conventional pharmacological treatment; e) the popular medicine suggests that natural products are dressings (Zomlefer, 1994).

The test of lethality against the microcrustacean *Artemia salina* is a simple test at low cost, and the results from this test are correlated with the cytotoxicity of some types of cancer cells (Grayer & Kokubun, 2001), with insecticides (Kraft *et al.,* 2000) in addition to cytotoxic activities (Meyer *et al.* , 1982).

In this work we investigated the cytotoxic potential of some Amazonian species, using the brine shrimp lethality bioassay method with *Artemia salina* Leach. The bioassay was chosen because it is easy to perform and the microcrustacean of easy reproduction. Were employed different dosages of extracts, quantifying the number of individuals live and dead individuals (Raven *et al.,* 1996).

2. Material and Methods

The test for the assessment of the toxicity was performed according to the methodology described by McLaughlin and collaborators (1995). The plant material was dried, pulverized and through extraction from cold with solvents of different polarities (dichloromethane, methanol and water) were obtained the plant extracts that were subjected to the test of lethality front to *Artemia salina*.

Eggs of *Artemia salina* were placed to erupt in a solution of sea salt (38 $g.L^{-1}$), a small container partially covered, because the larvae have positive phototropism (are attracted by the light). This system was left at rest for 48 h so that the eggs should repent in larvae type nauplii.

The obtained extracts of plant materials studied were weighed (20 mg) and diluted in 2,0 mL of methanol, DMSO or water, depending on the type of extract. From this stock solution (10 mg.mL^{-1}), two other dilutions were carried out in order to obtain solutions with concentrations of 1 and 0,1 mg.mL^{-1}. Each extract was tested in triplicate, and each well was added 500 mL of the stock solution of extract, 10 larvae of *Artemia salina* and the solution of sea salt to adjust the volume to 5 mL, resulting in solutions of concentrations of 1000, 100 and 10 kg.mL^{-1}. The nine bottles tests and a bottle of white control were rested and discovered and, after 24 hours was the count of the number of surviving larvae (Figure 1). The data obtained were statistically analyzed and was calculated based on the LC_{50} of the extracts. LC_{50} is the concentration lethal to 50% of the organisms. Positive result of this test indicates potential toxic activity of bioactive compounds in plant extracts.

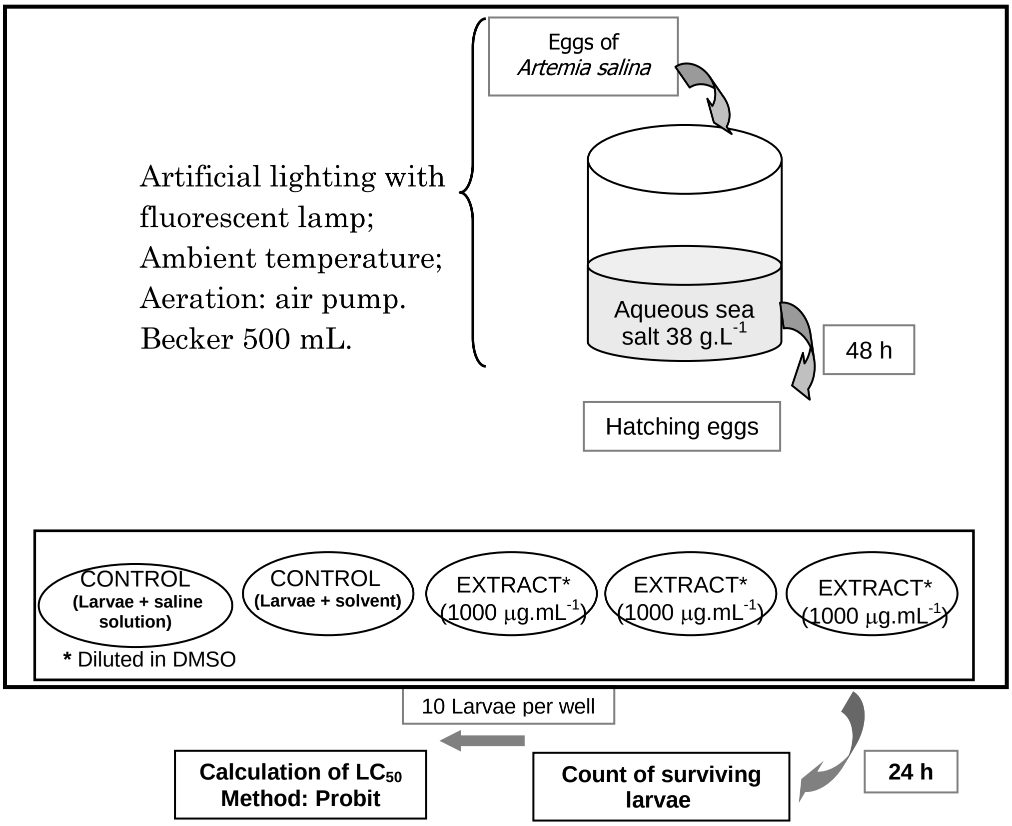

Figure 1. Scheme of the brine shrimp lethality bioassay method (from Mesquita *et al.*, 2015).

Figure 2. Assay being performed.

3. Results and Discussion

The bioassay front to the larva of microcrustacean *Artemia salina* has been used as an indicator of toxicity for both screening for substances with activity anti-tumor, as for substances with pesticide activity and cytotoxic (McLaughlin *et al.,* 1995). This test is considered to be a quick test, cheap and very easy to be implemented. Also called "*Brine Shrimp Letality Test*" (BST), the test has the advantage of using individuals living well small and in small quantity. LC_{50} values < 1000 μg.mL^{-1} are considered significant for crude extracts (Parra *et al.,* 2001). This bioassay shows a positive correlation with tests on cells 9 kb (nasopharyngeal carcinoma human p = 0.036 and kappa = 0.56), where the values of ED_{50} for cytotoxicity are generally close to a tenth part of LC_{50} values found in bioassays with *Artemia salina*, as observed by McLaughlin and collaborators (1995) and cytotoxicity on cancerous cells P-388, observed by Meyer and collaborators (1982).

Table 1 illustrates the species used in the test as well as the CL_{50} obtained and their respective activities. Was observed that, of the species that have been assessed, the vast majority has the potential cytotoxic activity, being that few extracts showed inactivity. The results obtained have been a sign of departure for future phytochemical studies and other biological activities.

Table 1. Results of the species tested front of lethality against *Artemia salina*.

PLANT FAMILY Scientific Name	Part used	CL_{50} (μg.mL^{-1})		
		DCM	MeOH	H_2O
CAPPARACEAE *Crateva benthamii*	Peel	410	520	660
CECROPIACEAE *Cecropia purpurascens,*	Leaves	230	280	490
FABACEAE *Campsiandra laurifolia*	Leaves	290	330	560
	Branches	450	760	990 (-)
FABACEAE *Deguelia duckeana*	Roots	230	590	870
OLACACEAE *Minquartia guianenis*	Leaves	250	320	790
RUTACEAE *Zanthoxylum* sp	Leaves	250	480	720
SALICACEAE *Salix martiana*	Seeds	670	790	980 (-)
VERBENACEAE *Vitex cymosa*	Roots	250	420	610

* Legend: (-) = considered inactive; DCM = dichloromethane extract; MeOH = methanol extract ; H_2O = aqueous extract.

4. Conclusion

The tests performed with the various crude extracts of the various species of the Amazon region have shown promising activity in their great majority, encouraging the phytochemical studies of the same in search for substances that will introduce the activity observed. It is important to emphasize that the lethal test front to *Artemia salina*, in addition to simple and easy to be implemented is pretty accurate.

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