TOXICITY IN *ARTEMIA SALINA* OF FRACTIONS DERIVED FROM DICHLOROMETHANE EXTRACT OBTAINED FROM LEAVES OF *MONTRICHARDIA LINIFERA* (ARRUDA) SCHOTT, ARACEAE

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ABSTRACT

Records of medicinal uses involving the *Montrichardia linifera* exist for more than a century, but very little is known about the biological activity this plant that has ethnopharmacological wide use in the Amazon. A previous study showed that the dichloromethane extract obtained from the leaves showed high toxicity on *Artemia salina*, and this may be pointing to the presence of bioactive substances in this plant. In this work was made the fractionation of dichloromethane extract in hexane, dichloromethane, ethyl acetate and methanol. The resulting fractions were evaluated for their toxicity against *Artemia salina* in order to verify in which one (s) of them there are concentrated such toxicity and therefore the greater probability of finding bioactive substances. All samples tested showed high toxicity against brine shrimp (LD_{50} < 100 μg mL^{-1}). The results indicate that the samples are promising for futures phytochemical studies, so that we can isolate and purify these substances with potential biological activity.

KEYWORDS*: Montrichardia linifera*, Araceae*,* Amazon traditional medicine, toxicity against *Artemia salina*

TOXICIDADE EM *ARTEMIA SALINA* DE FRAÇÕES DERIVADAS DO EXTRATO DICLOROMETÂNICO OBTIDO DAS FOLHAS DE *MONTRICHARDIA LINIFERA* (ARRUDA) SCHOTT, ARACEAE:

RESUMO

Registros de usos tradicionais medicinais envolvendo a *Montrichardia linifera* já existem há mais de um século, porém ainda muito pouco se conhece sobre a atividade biológica desta planta de ampla utilização etnofarmacológica na Amazônia. Estudo anterior demonstrou que o extrato diclorometânico obtido das folhas apresentou alta toxicidade frente à *Artemia salina*, o que pode estar apontando para a presença de substâncias bioativas nesta planta. Neste trabalho foi realizado o fracionamento do extrato diclorometânico em hexano, diclorometano, acetato de etila

e metanol e as frações resultantes foram também avaliadas quanto à sua toxicidade contra a *Artemia salina* a fim de se verificar em qual(is) delas se concentra(m) tal toxicidade e, consequentemente, a maior probabilidade de se encontrarem substâncias bioativas. Todas as amostras testadas apresentaram alta toxicidade contra *Artemia salina* (DL_{50} < 100 μg mL^{-1}). Os resultados indicam que as amostras são promissoras para estudos fitoquímicos posteriores, a fim de que se possa isolar e purificar tais substâncias com potencial atividade biológica.

PALAVRAS-CHAVE: *Montrichardia linifera*, Araceae, medicina tradicional amazônica, toxicidade contra *Artemia salina*

INTRODUCTION

Brazil possesses the largest genetic diversity of plant species in the world, but less than 10% have been evaluated with respect to their biological characteristics, and fewer than 5% have been subjected to detailed phytochemical studies. Despite a recent increase in research activity in this area, plants still constitute a relatively under-utilised, and potentially very valuable, source for the further discovery of biologically active substances (LUNA et al., 2005). Studies on the toxicity of medicinal plants are important because, still today, many people make use of herbs to treat their ailments without the prior knowledge, only by indication of others and there may be health risks (PESSOA & CARTÁGENES, 2010).

Artemia salina, commonly known as the brine shrimp, is a small crustacean, which has been the subject of many physiological studies. The brine shrimp lethality assay is considered to be one of the most useful tools for the preliminary assessment of general toxicity, and the bioassay has shown good correlation with cytotoxic activity against some human solid tumours (MCLAUGHLIN et al., 1991), antibacterial (BRASILEIRO et al., 2006; NIÑO et al., 2006; MAGALHÃES et al., 2003) and antifungal activities (NIÑO et al., 2006; MAGALHÃES et al., 2003), and parasiticide activity (COWAN, 1999), such as against *Trypanosoma cruzi* (DOLABELA, 1997). In general, plant extracts and derivatives with high toxicity against *A. salina* have high potential for these biological activities.

Montrichardia linifera (Arruda) Schott, popularly known as aninga, belongs to the Araceae family and is distributed in the tropics (MAYO et al., 1997), occurring widely on the banks of rivers and streams of the Amazon. It is an amphibious macrophyte that is characterized by having wide ecological amplitude, ie, can be found emerging from the land of soil saturated with water. As a pioneer species, has considerable ecological importance in the formation of banks of rivers and streams of white water (muddy), it is the first vegetation in the formation of alluvial islands forming extensive clonal populations by sprouting from underground stems and submerged. *M. linifera* is an herbaceous with 4 - 6 meters of tall, leaf blades about 45 - 66 cm of length and 35 - 63 cm of width, with frequent crystals of calcium oxalate (MACEDO et al., 2005).

The riverines consider this plant as poisonous to humans because its sap causes skin burns and eye contact can cause blindness (AMARANTE et al., 2009). However, paradoxically, it is widely used in traditional medicine in the Amazon,

especially the healing property of the sap and juice of the plant that has been mentioned in the literature since the nineteenth century for the treatment of wounds and ulcers (D'OLIVEIRA, 1854; MOREIRA, 1862; CASTRO, 1878 apud FENNER et al., 2006). In the twentieth century there are records of the use of 'goo' released by maceration of the leaves in case of snake bites and fucked stingray, to relieve pain (AMOROZO & GÉLY, 1988) and, more recently, it was reported that compresses and poultices of the leaves are used to treat abscesses and tumors (MATOS, 2002) and the mucilage removed the petiole is used to cure tinea (AMARANTE et al., 2009). Thus, these ethnomedicinal applications suggest that this plant has anti-inflammatory, analgesic, antitumoral and antimicrobial activities, respectively.

However, in spite of having gone 150 years since the first record of its use in traditional medicine, very little is known about its chemical composition and biological activity. Preliminary phytochemical studies using the ethanol extract of the leaves of *M. linifera* suggested the presence of alkaloids, flavonoids, tannins, triterpenes and steroids, however the extract showed low toxicity to brine shrimp (COSTA et al., 2009).

In this sense, the objective of this work was to continue the search for general toxicity towards *Artemia salina*, using fractions derived from dichloromethane extract obtained from the leaves of *Montrichardia linifera* (Arruda) Schott. and, this way, to contribute in the guiding of future studies in chemical, biological and pharmacological of this plant that, with more than a century of traditional use, possibly contain biologically active substances.

MATERIALS AND METHODS

Plant Material

Leaves of Montrichardia linifera were collected in the Universidade Federal do Pará, Belém-PA, on the bank of Guama River (01°28'41,3" S e 48°27'29,0" W), in July 2008 (dry period), the time between 10:00 and 11:00 hours during low tide. The plant species M. linifera was identified by Drª Alba Lúcia Ferreira de Almeida Lins (Coordenação de Botânica do Museu Paraense Emílio Goeldi) and a voucher specimen was deposited in the herbarium of this institutions (MG 188906).

Assays

Preparation of fractions

A sample of 62g of dichloromethane extract of the leaves was subjected to fractionation in open column chromatography and eluted with solvents of increasing polarity in the sequence: hexane, dichloromethane, ethyl acetate and methanol. The four fractions were concentrated in a rotary evaporator.

Toxicity against Artemia salina

The evaluation of the toxicity of fractions was performed using the bioassay with Artemia salina Leach, according to McLaughlin et al. (1993) and Dolabela (1997). For the artificial sea water were weighed 36 g of sodium chloride to 1000 mL, 15 g of magnesium sulphate and 5 g of sodium bicarbonate. After homogenization, filtered the solution and the pH was maintained around 9.0 and then were added 3 grams of brine shrimp cysts. The solution was stored in a tank with artificial illumination of approximately 28°C for 24 h. Samples of fractions were solubilized in

DMSO and tested in five different concentrations (500, 250, 125, 62.5 and 31.25 μg mL^{-1}) cointaning the brine shrimp (10-20 larvae). The tubes were kept for 24 hours in artificial illumination at 28ºC and then were made counting of dead and living animals. To calculate the LD_{50} method was used GraphPad Prism 5 program, and it was considered low toxicity when the 50% lethal dose (LD_{50}) was greater than 500 μg mL^{-1}, moderate to LD_{50} between 100 to 500 μg mL^{-1} and very toxic when the LD_{50} <100 μg mL^{-1}.

RESULTS AND DISCUSSION

Were obtained four fractions of dichloromethane extract from leaves. The data of the yields and toxicity of the samples, expressed in 50% lethal dose (LD_{50}), are shown in Table 1. Hexane, dichloromethane and ethyl acetate fractions showed LD_{50} < 31.75 μg mL^{-1}, therefore the lowest concentration tested, only the methanolic fraction showed LD_{50} = 58.50 μg mL^{-1}..

Thus, it is possible to conclude that all test samples showed highly toxic against *Artemia salina* (LD_{50}<100 μg mL^{-1}). In general, extracts and derivatives with high toxicity (LD_{50}<100μg mL^{-1}) have a high potential for other biological activities such as antitumor activity (MCLAUGHLIN, 1991; MCLAUGHLIN et al., 1991; 1993), antibacterial (BRASILEIRO et al., 2006; NIÑO et al., 2006; MAGALHÃES et al., 2003), antifungal (NIÑO et al., 2006; MAGALHÃES et al., 2003), among others. Soon these samples have a high potential to contain active substances. The methanolic fraction showed the highest yield, while the hexane extract, showed the smallest (Table 1).

TABLE 1. Yield and evaluation of toxicity in brine shrimp (LD_{50}) of fractions derived from dichloromethane extract obtained from leaves of *M. linifera*.

SAMPLE	***YIELD (%)***	***TAS LD_{50}* (μg mL^{-1})***
Hexane fraction	8,74	< 31.75
Dichloromethane fraction	4,84	< 31.75
Ethyl acetate fraction	16,43	< 31.75
Methanolic fraction	28,66	= 58.50

* LD_{50} = median lethal dose

It was concluded that all fractions derived from dichlorometane extract obtained from leaves of *M. linifera* are promising for detailed phytochemical studies and biological assays, aiming the possible isolation, purification and identification of the substances with potential biological activity of this plant.

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