ORIGINAL ARTICLE

TOXICOLOGICAL SCREENING OF *PIMENTA PSEUDOCARYOPHYLLUM* (GOMES) L.R. LANDRUM EXTRACTS USING *ARTEMIA SALINA* BIOASSAY

TRIAGEM TOXICOLÓGICA DE EXTRATOS DE PIMENTA PSEUDOCARYOPHYLLUM *(GOMES) L.R. LANDRUM FRENTE À* ARTEMIA SALINA *LEACH*

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ABSTRACT

Objectives: To evaluate and compare the acute toxicity of leaf (Ppf) and stem (Ppc) extracts of *Pimenta pseudocaryophyllum* in *Artemia salina*. **Methods:** The Ppf and Ppc extracts were tested at concentrations of 1, 10, 100, and 1000 µg/mL in acute toxicity assays using the microcrustacean Artemia salina. Nauplii were incubated for 24 and 48 hours, and all experiments were performed in triplicate. Nauplii mortality was recorded, and LC_{50} values were calculated using nonlinear regression analysis. **Results:** The Ppc extract exhibited moderate toxicity only after 48h (LC_{50} = 140.2 ± 76.7 μg/mL). In contrast, the Ppf extract showed moderate toxicity at 24 hour (LC_{50} = 372.0 ± 58.1 μg/mL) and high toxicity at 48 hours (LC_{50} = 0.8 ± 0.1 μg/mL). **Conclusion:** Both stem and leaves of P. pseudocaryophyllus contain bioactive metabolites that induce toxicity in A. salina, which are likely different compounds or present at higher concentrations in the leaves.

Keywords: Medicinal plant; Vegetal extract; Toxicity

RESUMO

Objetivo: verificar e comparar a toxicidade aguda de extratos das folhas (Ppf) e do caule (Ppc) de *Pimenta pseudocaryophyllum* em *Artemia salina*. **Métodos:** Os extratos Ppf e Ppc, nas concentrações de 1, 10, 100 e 1000 µg/ mL, foram utilizados nos ensaios de toxicidade aguda utilizando o microcrustáceo Artemia salina, incubados por um período de 24 e 48 horas, realizados em triplicata. O número de náuplios mortos foram quantificados e a CL_{50} foram calculadas por regressão não linear. **Resultados:** o extrato Ppc apresentou toxicidade apenas em 48hs (CL_{50} = 140,2 ± 76,7 μg/mL), considerada moderada. Já o extrato Ppf foi tóxico tanto na exposição por 24hs (CL_{50} = 372,0 ± 58,1 μg/mL), quanto por 48hs (CL_{50} = 0,8 ± 0,1 μg/mL), apresentando toxicidade moderada e alta, respectivamente. **Conclusão:** caule e folhas de P. pseudocaryophyllum possuem metabólitos ativos que levam toxicidade a Artemia salina, que provavelmente são substâncias diferentes ou estão mais concentrados nas folhas.

Palavras-chave: Planta medicinal; Extrato vegetal; Toxicidade

INTRODUCTION

In developing countries,traditional medicine and medicinal plants are commonly used for health maintenance[1].

According to the toxic-pharmacological information system, poisoning from medicinal plants ranks as the second leading cause of intoxication-related deaths in humans. Several contributing factors include lack of knowledge about cultivation, misidentification of plant species, adverse reactions, drug interactions, and inappropriate dosage or frequency of herbal medicine use[2].

In Brazil, the most recent regulatory frame-

work to guide and strengthen health initiatives is the National Policy on Integrative and Complementary Practices of the Unified Health System (SUS)[3], which initially encompassed medicinal plants and herbal medicine, homeopathy, traditional Chinese medicine/acupuncture, and anthroposophical medicine. Additionally, the National Policy on Medicinal Plants and Regulation of Herbal Medicines was established[4].

The Myrtaceae family includes 121 genera and between 3,800 and 5,800 species of woody shrubs and trees, predominantly found in tropical and subtropical regions worldwide[5]. In Brazil, this family comprises around 23 genera and 1,000 species[6]. Among them is *Pimenta pseudocaryophyllum* (Gomes) L.R. Landrum, commonly known as "*pau-cravo*," "*craveiro-do-mato*," "*louro-cravo*," or "*chá-de-bugre*." Traditionally, leaf infusions of this species are used as tranquilizers, digestive regulators, and to relieve flu symptoms[7]. Studies identified that leaves of *P. pseudocaryophyllum* contain polyphenolic compounds, such as tannins and flavonoids[8, 9], and an oil rich in phenylpropanoids[10, 11]. Many biological activities have been reported for this species, including antioxidant[12, 13], anxiolytic[14] antifungal[15, 16], antidepressant[9], anti-inflammatory and anti-hyperuricemic[17], insecticidal[18], and antimicrobial effects[19, 20]. Despite these properties, no reports of toxicity have been found.

The lack of studies on the toxicity of *P. pseudocaryophyllum* prompted the present investigation, which aimed to evaluate and compare the acute toxicity of leaf (Ppf) and stem (Ppc) extracts of P. pseudocaryophyllum in *Artemia salina*.

MATERIALS AND METHODS

Leaves and stems of *P. pseudocaryophyllum* were macerated in 95% ethanol, and the extracts were obtained after solvent removal using a rotary evaporator. The extracts were provided by the Institute of Chemistry at the University of São Paulo (USP). The extracts used in the assays were solubilized in 0.1% Cremophor and diluted in distilled water to a concentration of 2.5 mg/mL. During the experiments, serial dilutions were performed to achieve the desired concentrations.

Acute toxicity was determined using the A. salina bioassay[21]. A total of 0.3 g of A. salina cysts was maintained in artificial seawater and incubated for 24 – 36 hours under artificial light at 22 °C. After hatching, ten nauplii were transferred to test tubes containing extract solutions (1, 10, 100, and 1000 μg/mL) and a saline control. After 24 and 48 hours of incubation, the number of surviving and dead nauplii was recorded. Nauplii were considered dead if they exhibited no active movement within approximately 20 seconds of observation. The median lethal concentration (LC50) for each extract was determined by nonlinear regression analysis of the number of viable nauplii at each concentration. All assays were performed in triplicate.

All analyses were performed using GraphPad Prism. Results were expressed as mean ± standard error of the mean (M ± SEM) and statistically analyzed with Student's t-test, with $p < 0.05$ considered as significant.

RESULTS AND DISCUSSION

To evaluate potential toxic activities of plant-derived products, bioassays using the microcrustacean A. salina are widely used for screening and therapeutic safety. Its ease of maintenance under laboratory conditions and broad geographic distribution make it a common organism in toxicity assays[22,23]. The lack of cytotoxicity in this model suggests that the plant extract is well tolerated by biological systems.

The Ppc extract did not reduce A. salina viability at any tested concentration after 24 hours of incubation, indicating no acute toxicity at this timepoint (Figure 1A). However, after 48 hours of incubation (Figure 1B), the Ppc extract was able to reduce viability, presenting an LC50 of 140.2 ± 76.7 μg/mL, which corresponds to moderate toxicity ($100 < LC50 \leq 500$ μg/mL)[21]. These findings suggest that stem-derived metabolites may not be efficiently metabolized by A. salina, leading to toxicity upon prolonged exposure.

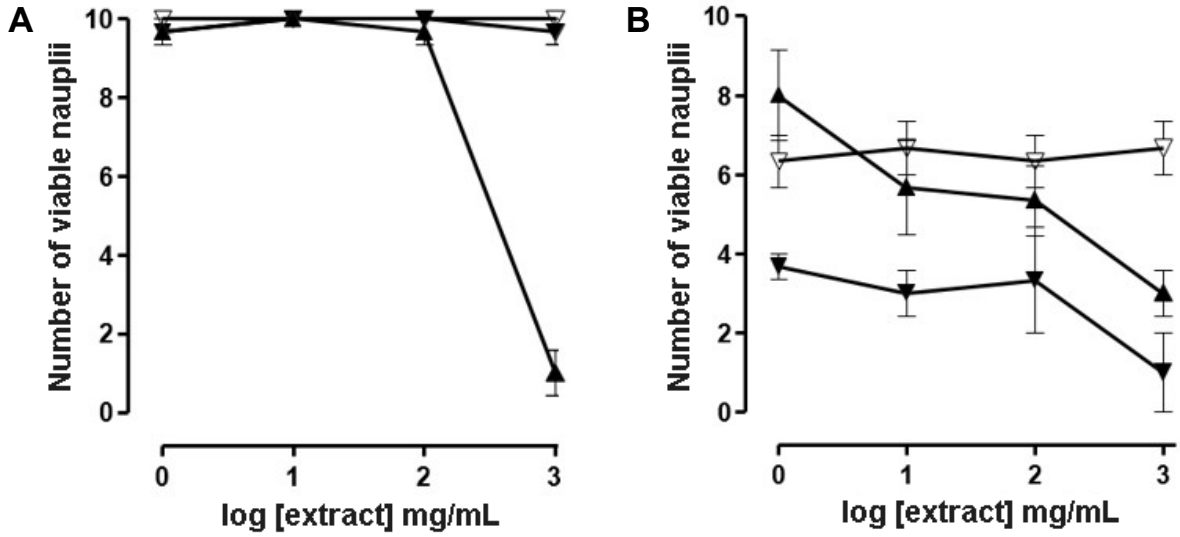

Figure 1. *A. salina* viability in the absence (▽) or presence of Ppc (▲) and Ppf (▲) extracts after 24 h (A) or 48 h (B) of incubation.

In contrast, the Ppf extract exhibited toxicity at both 24 and 48 hours (Figures 1A and 1B), with significantly greater lethality observed at 48h. This was confirmed by a significantly lower LC50 value ($p < 0.01$) at 48 hours compared to 24 hours (Table 1), indicating high toxicity (LC50 < 100 μg/mL). The observed cytotoxicity of the Ppf extract supports its potential for further investigation in cytotoxic studies[24]. These findings suggest that the leaves of *P. pseudocaryophyllus* also contain bioactive metabolites that are toxic to *A. salina*, which may differ from those found in the stem or may be present at higher concentrations in the leaves. This is consistent with previously reported insecticidal[18] and antimicrobial activities[19-20], reinforcing the biological relevance of its secondary metabolites.

Table 1. LC_{50} values of Ppc and Ppf extracts using *A. salina*.

Incubation period	LC_{50} (μg/mL)	
	Ppc	Ppf
24h	n.d.	372.0 ± 58.1
48h	140.2 ± 76.7*	0.8 ± 0.1**

n.d. (not determined). Student's t-test, *p < 0.05 (Ppc vs. Ppf); **p < 0.01 (Ppf 24 h vs. 48 h).

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