TOXICOLOGICAL SCREENING OF EXTRACTS FROM *CINNAMOMUM STENOPHYLLUM* ON *ARTEMIA SALINA LEACH*

TRIAGEM TOXICOLÓGICA DE EXTRATOS DE CINNAMOMUM STENOPHYLLUM FRENTE À ARTEMIA SALINA LEACH

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ABSTRACT

Objective: To observe and compare the acute toxicity of the leaf (Csf) and stem extracts (Csc) from *Cinnamomum stenophyllum* against the brine shrimp *Artemia salina* Leach. **Methods:** Csf and Csc at concentrations of 1, 10, 100, and 1000 μg/mL were evaluated in acute toxicity assays on *A. salina* for 24 and 48 h, in triplicate. The number of dead nauplii was quantified, and the median lethal concentration (LC_{50}) values were calculated using non-linear regression. **Results:** Csf showed toxicity only at 1000 g/mL after 48 h ($p < 0.05$); therefore, the LC50 was not calculated. Csc exhibited toxicity only at 48 h of exposure (LC_{50} = 8.7 ± 0.7 g/mL), indicating high toxicity (LC50 < 100 μg/mL). **Conclusion:** Csf and Csc from *C. stenophyllum* presented active metabolites that induced toxicity in *A. salina* under high exposure conditions. These metabolites are possibly different substances or are more concentrated in the stem. This is the first report of such findings in the literature.

Keywords: Medicinal plant; Toxicity; Vegetal extract

RESUMO

Objetivo: Observar e comparar a toxicidade aguda de extratos das folhas (Csf) e do caule (Csc) de *Cinnamomum stenophyllum* em *Artemia salina* Leach. **Métodos:** Os extratos Csf e Csc, nas concentrações de 1, 10, 100 e 1000 μg/mL, foram utilizados nos ensaios de toxicidade aguda utilizando o microcrustáceo *A. salina*, sob exposição de 24 e 48 horas, realizados em triplicata. O número de náuplios mortos foram quantificados e a CL50 foram calculadas por regressão não-linear. **Resultados:** Os extrato Csf apresentou toxicidade apenas com a concentração de 1000 g/mL em 48hs ($p < 0,05$), sendo assim, a CL50 não foi determinada. Já o extrato Csc foi tóxico apenas na maior exposição, de 48hs, mostrando CL50 de 8,7 0,7 g/mL, considerada uma alta toxicidade (CL50 < 100 g/mL). **Conclusão:** As folhas e caule de *C. stenophyllum* possuem metabólitos ativos que levam toxicidade a *A. salina* quando em alta exposição, os quais, provavelmente são substâncias diferentes ou estão mais concentradas no caule. Estes resultados são os primeiros na literatura para a espécie estudada.

Palavras-chave: Extrato vegetal; planta medicinal; toxicidade

INTRODUCTION

Traditional medicine and medicinal plants have been extensively used in developing countries, according to the norms for health maintenance[1].

According to the National Poison Information System, intoxication caused by medicinal plants is the second leading cause of death by poisoning in humans. Several factors may contribute to this outcome, including limited knowledge regarding cultivation practices, incorrect plant identification, adverse reactions, drug interactions, dosage, and frequency of herbal medicine use[2].

The national guideline developed to support

and strengthen public health initiatives in Brazil is the National Policy for Integrative and Complementary Practices in the Unified Health System[3]. This policy initially encompassed the areas related to medicinal plants and phytotherapy, homeopathy, traditional Chinese medicine, acupuncture, and anthroposophic medicine. A more specific policy, the National Policy for Medicinal Plants and Herbal Medicines, was also implemented[4].

The Lauraceae family is distributed across tropical and subtropical regions and comprises approximately 52 genera (between 2,500 and 3,500 species)[5]. In Brazil, this family is important and includes 23 genera and 420 species[6], with many species producing aromatic oils and alkaloids used in perfumery and the pharmaceutical industry, such as *Cinnamomum camphora* (camphor) and *Aniba roseadora* (*pau-rosa*); the latter is a source of linalool, which is widely used in the cosmetics industry. Other species also produce edible fruits and culinary condiments, such as *Persea americana* (avocado), *Laurus nobilis* (laurel leaf), and *C. verum* (glycosylate)[9].

As no studies reported the toxicological screening for *C. stenophyllum*, we aimed to evaluate and compare the acute toxicity of crude ethanolic extracts from the leaf (Csf) and stem (Csc) of this specimen on the microcrustacean *Artemia salina* Leach.

METHODS

Leaves and stems of *C. stenophyllum* were macerated in ethanol (95%). Extracts were obtained by solvent removal using a rotary evaporator at 60° C. The extracts were provided by the Instituto de Química at Universidade de São Paulo (USP). To prepare the solutions, extracts were solubilized in Cremophor (0.1%) and diluted in distilled water to a concentration of 2.5 mg/mL. Serial dilutions were performed to obtain the desired concentrations[7]. *C. stenophyllum* (Meisn.) Vattimo-Gil, known as "*canela-vassoura*", is native to the Brazilian states of Minas Gerais, São Paulo, and Paraná[6,8]. Extracts from species of the genus *Cinnamomum* have been reported to possess astringent, carminative, blood-purifying, digestive, antiseptic, antifungal, antiviral, antibacterial, antioxidant, anti-inflammatory, immunomodulatory, hypolipidemic, and hypoglycemic properties[9]. Several chemical compounds have been reported in these species, including aldehydes, acetates, alcohols, terpenes, flavonoids, alkaloids, anthraquinones, coumarins, phenols, saponins, tannins, carboxylic acids, hydrocarbons, spathulenol, fatty acids, butanolides, lignans, steroids, propenoids, and kaempferol.

The *A. salina* assay was used to determine the acute toxicity[10]. A quantity of 0.3 g of *A. salina* cysts was incubated in synthetic seawater for 24 to 36 h under artificial lighting at 22° C. After hatching, ten nauplii were transferred to test tubes containing the extract solutions (1, 10, 100, and 1000 µg/mL) or the control (saline). After 24 and 48 h, the number of live and dead larvae was quantified. Larvae were considered dead when no active movement was observed for approximately 20 seconds. The median lethal concentration (LC_{50}) for each extract was determined using non-linear regression analysis based on the number of viable nauplii at each concentration. The assay was conducted in triplicate for each tested concentration.

Results were expressed as mean ± standard error of the mean (X ± s.e.m.) and analyzed using the t-test; a p-value < 0.05 was considered statistically significant. Analyses were performed using GraphPad Prism software.

RESULTS AND DISCUSSION

Tests using *A. salina* are commonly applied to investigate the potential toxic activity of plant-derived products and for the preliminary screening and assessment of therapeutic safety. Due to its ease of maintenance in laboratory environments, *A. salina* has become a standard model for toxicity testing[11,12]. The absence of cytotoxicity from the tested extracts in *A. salina* indicated that the plant material is well tolerated by this biological system.

Exposure to the Csf at different concentrations for 24 h did not affect *A. salina* viability compared with the control, indicating a lack of toxicity (Figure 1A). At 48 h of exposure, only the highest concentration (1000 g/mL) produced a statistically significant reduction in viability (Figure 1B). These results suggest that the stem of *C. stenophyllum* may contain active compounds that are not fully eliminated by the metabolism of *A. salina* when exposed to high concentrations, resulting in toxic effects.

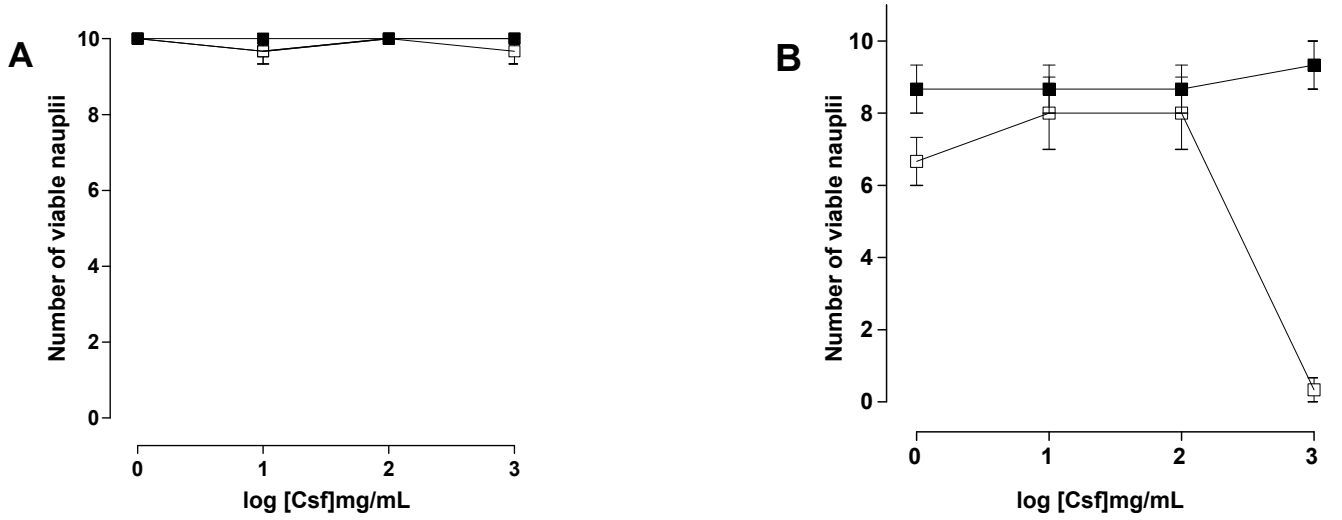

Figure 1. Viability of *Artemia salina* in the absence (■) or presence (□) of *leaf* extract (Csf) after 24 h (A) or 48 h (B) of exposure.

During the tests with the Csc (Figure 2), the mortality of *A. salina* was observed only after 48 h of exposure (Figure 2B); Csc produced an LC_{50} of 8.7 ± 0.7 g/mL, classified as highly toxic (LC_{50} < 100 g/mL)[10]. This toxicity was greater than that observed in the leaves and bark of *C. travancoricum*, *C. walaiwarense*, *C. wightii*, *C. verum*, *C. sulphuratum*, *C. riparium*, and *C. perrottetii*, and was performed in similar assays with *A. salina*[13]. The significant toxicity observed in the Csc suggests a potential for future cytotoxicity screening[14]. The greater toxicity of the Csc compared with the Csf of *C. stenophyllum* suggests that the active metabolites responsible for this effect may differ between plant parts or may occur at higher concentrations in the stem. Similar findings have been reported for bark extracts, which exhibited greater activity than the Csf of *C. travancoricum*, *C. walaiwarense*, *C. wightii*, *C. verum*, *C. sulphuratum*, *C. riparium,* and *C. perrottetii*[13].

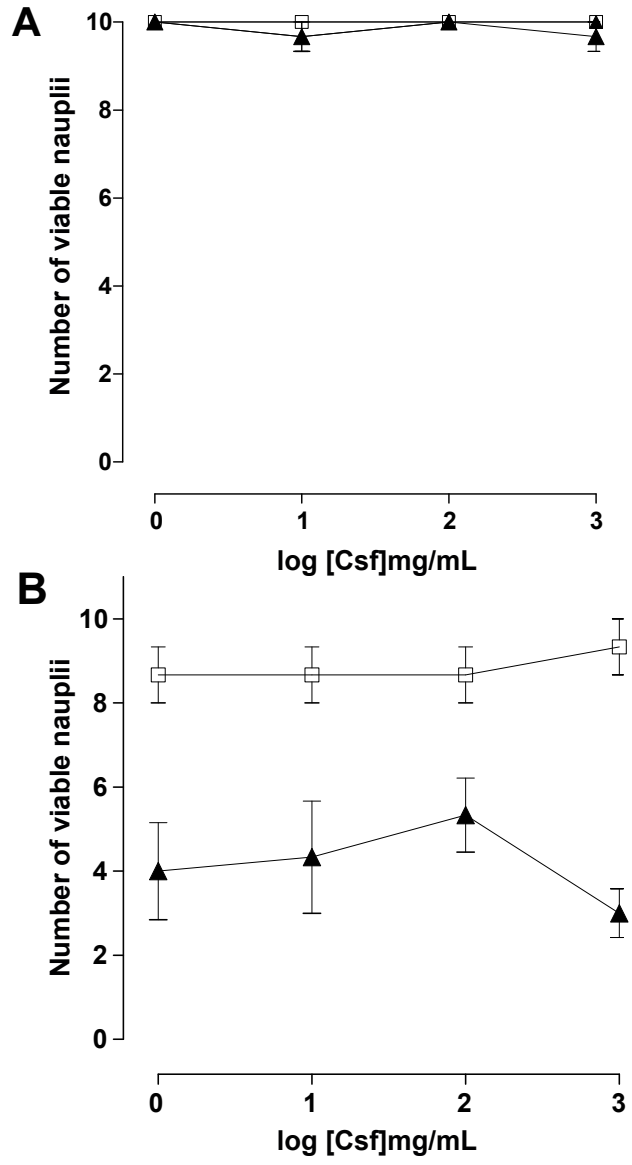

Figure 2. Viability of *Artemia salina* in the absence (□) or presence (▲) of stem extract (Csc) after 24 h (A) or 48 h (B) of exposure.

CONCLUSION

The leaves and stems of the *C. stenophyllum* contain active metabolites that cause toxicity to *A. salina* under prolonged exposure. The compounds responsible for this effect are probably different from those found in the leaves or are more concentrated in the stem. These are the first data reported for this plant specimen.

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