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Toxicological screening of *Lippia microphylla* extract on *Artemia salina*

Triagem toxicológica de extrato de *Lippia microphylla* frente à *Artemia salina*

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Abstract

Objective: to verify the acute toxicity of crude extracts from the leaves of *Lippia microphylla* on *Artemia salina*. **Methods:** The extracts of *L. microphylla* at 1, 10, 100, and 1,000 µg/mL were used in the acute toxicity tests in triplicates using the microcrustacean *A. salina* (n = 10), incubated for 24 h and 48 h. The number of dead nauplii larvae was quantified, and the mean lethal concentration (CL50) was calculated using nonlinear regression. **Results:** The extract of the *L. microphylla* during the 24-hour incubation produced toxicity ($p < 0.05$) only at the highest concentration of the extract (1,000 µg/mL). On the other hand, the median lethal concentration (LC50) was 246.7 ± 27.85 µg/mL after 48 h of exposure, indicating moderate toxicity. **Conclusion:** The leaves of *L. microphylla* have active ingredients that may not be fully metabolized by *A. salina* after 48 h, causing moderate toxicity on this microcrustacean.

Keywords: Medicinal plant; Plant extract; Toxicity.

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Resumo

Objetivo: Verificar a toxicidade aguda de extrato etanólico bruto das partes aéreas desta espécie vegetal frente a *Artemia salina*. **Métodos**: O extrato das partes aéreas da *Lippia microphylla*, nas concentrações de 1,0, 10,0, 100,0 e 1.000,0 µg/mL, foi utilizado nos ensaios de toxicidade aguda utilizando o microcrustáceo *Artemia salina* (n = 10), 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 da *Lippia microphylla* durante incubação por 24h promoveu toxicidade ($p < 0,05$) apenas na maior concentração do extrato (1.000 µg/mL). Já na exposição por 48h, mostrou CL_{50} de 246,7 ± 27,85 µg/mL, apresentando toxicidade moderada. **Conclusão**: As folhas de *Lippia microphylla* possuem princípios ativos, os quais, provavelmente, não conseguem ser totalmente eliminados pelo metabolismo da *Artemia salina* durante 48h, causando-lhes toxicidade moderada.

Palavras-chave: Planta medicinal; Extrato vegetal;Toxicidade.

INTRODUCTION

Scientifically tested plants in pharmaceutical forms have been widely used for treating and preventing diseases.[1,2,3] Medicinal plants have several biological activities (e.g., antifungal, antimicrobial, anti-inflammatory, antiallergic, antitumor, analgesic, and antioxidant).[4] In addition, medicinal plants are a natural, low-cost resource that is usually cultivated by users of public health services, making them accessible and contributing to treatment adherence and the health-disease process.[5]

Lippia species are widely distributed in Brazil and present relevant medicinal importance due to therapeutic activities; many are used to treat respiratory and gastrointestinal disorders. In addition, several pharmacological activities have been reported in studies with these species, including anticancer, antiradical, spasmolytic, acetylcholinesterase inhibition, antibacterial, and pathogenic microorganism elimination. However, reports on their toxicity are scarce.[6,7,8] On the other hand, the extract of the leaves of *Lippia microphylla in vitro* presented relaxing activity on isolated aorta and trachea from rats.[9]

Studies related to the efficacy of plant extracts of the genus *Lippia* showed good results on the chemical constitution and antioxidant properties of *Lippia* essential oil. This benefit is related to the presence of thymol and carvacrol, isomers considered promising in the study of therapeutic alternatives for infections.[10]

Despite the various benefits, some plants can cause adverse reactions, affecting the cardiovascular, respiratory, gastrointestinal, neurological, skin, and mucous membranes, and in some cases, death. Patients with risk factors (e.g., heart problems) may present severe poisoning after using low-toxicity plants.[11,12] These adverse effects occur during inappropriate, single, or chronic use or are associated with conventional medications, other plants, or herbal medicines, highlighting the need for more toxicological studies.[13]

Artemia salina is a small halophilic crustacean from a family that plays an important role in saltwater and marine ecosystems. They are highly valued for detecting toxicity and used in ecology, physiology, ecotoxicology, aquaculture, and genetics In addition, the lethality test with *Artemia* is fast, convenient, and low-cost.[14,15]

The absence of studies testing or reporting the toxicity of *L. microphylla* motivated the verification and comparison of acute toxicity of the leaves of this plant species on *A. salina*.

METHODS

The leaves of the *L. microphylla* were macerated in ethanol (95%), and the crude extract was obtained after solvent elimination in a rotary evaporator. This extract was provided by the *Programa de Pós-Graduação em Produtos Naturais e Sintéticos Bioativos da Universidade Federal da Paraíba*, Brazil. The crude extract was obtained by solubilization with cremophor (0.1%) and was diluted in distilled water to obtain a stock concentration (10 mg/mL). At the time of the experiment, they were serially diluted to obtain adequate concentrations for the tests. The *A. salina* method was used to determine acute toxicity.[16]

Cysts of *A. salina* (0.3 g) were kept in synthetic marine water (neutral pH) and incubated for 24 and 48 hours under artificial lighting, a temperature of 22°C, and no feeding. After hatching, ten nauplii were collected for each group: four test tubes containing the extract solution (1, 10, 100, and 1,000 µg/mL) and control (saline solution). The preparations were made in triplicates. After 24 and 48 hours, survivors and deaths were counted. They were considered dead when no active movement was observed for about 20 seconds. The median lethal concentration (LC_{50}) of the extract was obtained by nonlinear regression of the number of viable nauplii for each concentration; the test was performed in triplicates for each concentration. All results were expressed as mean ± standard error of the mean and analyzed using the t-test; $p < 0.05$ was considered significant. Analyses were performed in the GraphPad Prism.

RESULTS

The toxicity of *L. microphylla* in different concentrations is presented in Figure 1 for 24 h (Figure 1A) and 48 h (Figure 1B). Only the highest concentration of the extract (1,000 µg/mL) significantly induced death ($p < 0.05$) after 24 h incubation compared with the control. Therefore, LC_{50} was not determined since it did not have a concentration-response ratio in at least two different concentrations (the higher the concentration, the higher the effect). At 48 h incubation (Figure 1B), 10 and 1,000 µg/mL concentrations were toxic to *A. Salina* compared with the control group. Thus, LC_{50} was calculated and presented a 246.7 ± 27.85 µg/mL value, representing moderate toxicity ($100 < LC_{50} \leq 500$ µg/mL).

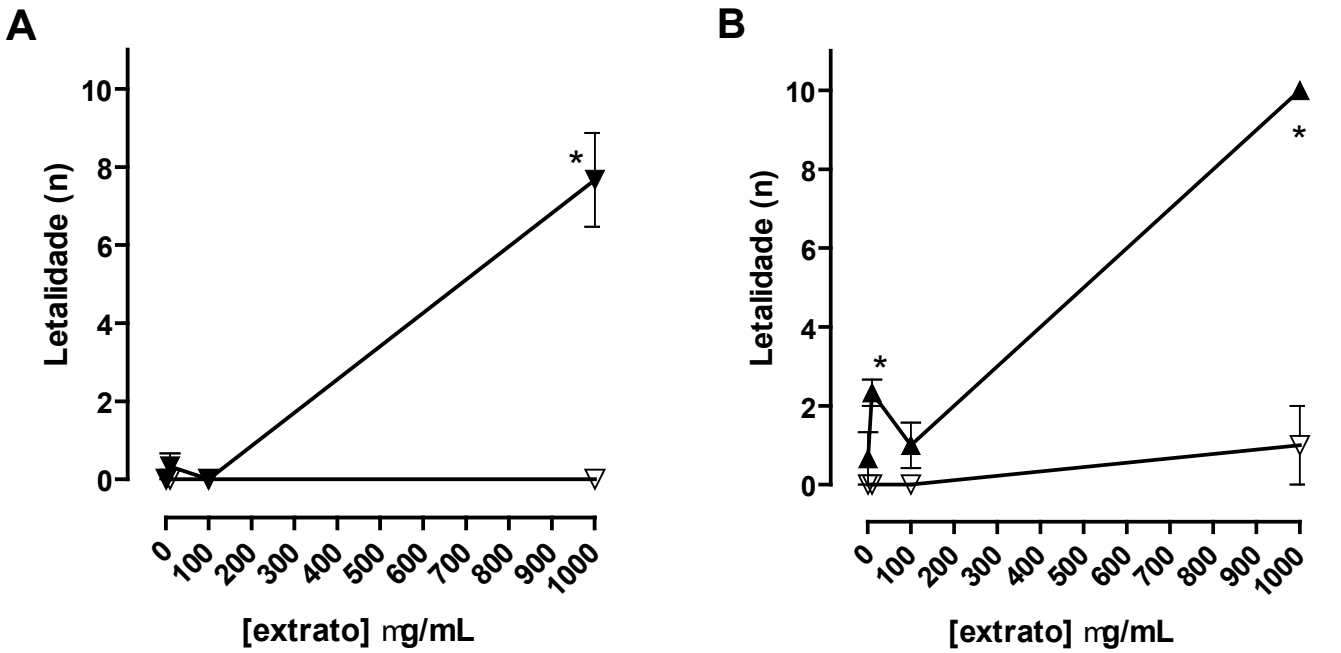

Figure 1 – Effect of the presence (q) and absence (s) of the crude extract of *Lippia microphylla* on *Artemia salina* after 24 h (A) and 48 h (B), n = 10. * The tracings correspond to the concentration-response curves, and the vertical bars represent the standard error of the mean. **p < 0.05; t-test (extract vs. control).

DISCUSSION

The scientific community uses this bioassay because it is quick, easy, and low-cost. Considering the dose per unit of body surface area, the toxic effects in humans are considerably within the same limits as those observed in laboratory animals, and possible human risks can be discovered.[17]

The species of *Lippia* present many bioactive substances with economic potential for local communities. Specifically, *L. microphylla* is an endemic species from the Brazilian vegetation that is little explored; however, the species has medicinal properties that are gaining attention from the scientific community. Despite the interest from scientists, few studies analyzed its toxicity. Natural products, especially medicinal plants, may be an important source of new agents against infectious diseases, cardiovascular diseases, cancer, and immunomodulation,[18] highlighting the importance of toxicological studies.

In the present study, the *Lippia* species presented moderate toxicity, revealing that the microcrustacean could not metabolize (i.e., detoxify) potentially toxic active ingredients in two concentrations after 48 h, and in 1,000 µg/mL in 24 h. The literature indicates several factors interfering with toxicity, including the concentration of the sample tested.[19] Similar results were obtained with the stem extract of *Pepper pseudocaryophyllus*, which showed toxicity on *A. salina*

after 48 h incubation.[18] However, an *in vivo* toxicity study with the same extract of *L. microphylla* did not show toxicity in mice.[20]

The same method was used in a toxicological study of the leaves of *Myosotis sylvatica*, extracts from leaves (Csf), and stem (Csc) of *Cinnamomum stenophyllum* on *A. salina*, which obtained LC_{50} = 38.1 µg/mL and Csf with indeterminate LC_{50}, and Csc with LC_{50} = 8.7 ± 0.7 µg/mL, respectively. As a result, *M. sylvatica* was classified as potentially toxic and Csc as highly toxic (CL < 100 µg/mL).[21-22]

The results obtained in this study also corroborate with a study conducted to evaluate the toxicity of *Lippia alba, Cymbopogon citratus,* and *Rosmarinus officinalis* on *A. salina* at different concentrations (100 ppm, 500 ppm, and 1,000 ppm). All three extracts showed biological activity, concluding that these plants were toxic if used in high concentrations.[23]

Similar results were also found in a study that evaluated the possible toxic, cytotoxic, genotoxic, and mutagenic effects of the leaves of the *Lippia sidoides* at different concentrations on the cell cycle of the *Allium cepa.*The cytotoxicity was verified by the decreased cell division using optical microscope analysis after tissue staining and fixation.[24]

A study evaluating the acute toxicity of essential oils on *A. salina* showed that all essential oils tested manifested high acute toxicity at low concentrations. Some of these oils that showed high lethality included species of *Lippia*, which were *C. citratus* (CL_{50} = 1,212 µg/ml) > *Lippia rotundifolia* (CL_{50} = 1,256 µg/ml) > *Lippia origanoides* (CL_{50} = 1,267 µg/ml) > *C. citratus* lemongrass (CL_{50} = 1,284 µg/ml).[25]

CONCLUSION

The leaves of *L. microphylla* have active ingredients that *A. salina* did not fully metabolize after 48 h and at the highest concentration (1,000 µg/ml) in 24 h, causing moderate toxicity.

Therefore, the toxicity observed for this plant extract should be considered a relevant use characteristic in cytotoxicity studies, providing more robust evidence of properties and effects.

Different environmental conditions can influence the effects of toxic agents on microcrustaceans. Therefore, these findings highlight the need for new tests for safety in the use of this species in developing new studies, and specialized clinical trials and consumption by the population.

CONFLICT OF INTERESTS

Nothing to declare.

AUTHOR CONTRIBUTIONS

GGA, **NSF,** and **AJSVS**: preparation and writing of the manuscript. **TKBO**: conception, elaboration, and writing of the article. **JLVS**: data analysis and final writing of the manuscript. All

authors proofread and approved the final version of the manuscript.

REFERENCES

1. Teixeira, JPS, Macedo, APV, Cândido GS, Magalhães JKA, Silva MW, Nunes HML, Lima VS, Silva GC. Perfil epidemiológico dos casos de intoxicação por plantas medicinais no Brasil de 2012 a 2016. Brazilian Journal of Development 2020;6(10):82199–82209.
2. Silva MG, Furtado MM, Osório AT, Morais ICP, Amaral MPM, Coêlho AG, Arcanjo DDR. The importance of toxicity tests for development and phytotherapy registration, Research, Society and Development 2021;10(12).
3. Melo DB, Macedo LM, Almeida IO, Pereira TRS, Silva TM, Leal MMT, Melo GA, Santana LLB. Intoxicação por plantas no Brasil: uma abordagem cienciométrica. Brazilian Journal of Development 2021;7(4):40919–40937.
4. Rocha LPB, Alves JV, Aguiar IF, Silva FH, Silva RL, Arruda LG, Nascimento EJ, Barbosa BVD, Amorim LC, Silva PM, Silva MV. Use of medicinal plants: History and relevance. Research, Society and Development 2021;10(10).
5. Rodrigues TA, Neto JL, Carvalho TAR, Barbosa ME, Guedes JC, Carvalho AV. A valorização das plantas medicinais como alternativa à saúde: um estudo etnobotânico. Revista Ibero-Americana de Ciências Ambientais 2020;11(1).
6. Jesus CO de, Pereira SLA. Potencial de *Lippia microphylla* cham. (verbenaceae) como tratamento alternativo para acantocefalose no tambaqui (Colossoma macropomum). Brazilian Journal of Development 2020;6(2):6293–6305.
7. Silva MCC, Souza ILL, Vasconcelos LHC, Ferreira PB, Araujo LCC, Sampaio RS, Tavares JF, Silva BA, Cavalcante FA. Essential oil from *Lippia microphylla* Cham. modulates nitric oxide pathway and calcium influx to exert a tocolytic effect in rat uterus. Natural Product Research 2021;35(6):1046-1051.
8. Oliveira GA, Pereira JC, Martins IRR, Correia AC, Travassos R, Silva M, Souza ILL, Tavares JF, Paredes-Gamero EJ, Silva BA. Spasmolytic activity of essential oil from *Lippia microphylla* Cham. (Verbenaceae) is mediated by modulation of Ca2+ signaling on animal and cellular models. Research, Society and Development 2021;10(7).
9. Antunes ROG, Silva APS, Silva ACS, Oliveira PR, Tavares JF, Nouailhetas VLA, Silva JLV. Relaxant effects of *Lippia microphylla* Cham. (Verbenaceae) on isolated rat aorta and trachea. Pharmacologyonline 2012; 2:82-86.
10. Silva MM. Estudo da composição Química do óleo essencial de *Lippia microphylla* Cham. em três anos diferentes e atividade antioxidante. 2015. 69p. Dissertação (Mestrado) - Programa de Pós-Graduação em Química, Universidade Federal de Roraima, Roraima, 2014.
11. Góes ACC, Silva LSL, Castro NJC. Uso de plantas medicinais e fitoterápicos: saberes e atos na atenção primária à saúde. Revista de Atenção à Saúde 2019;27(59).
12. Gonçalves JQ, Morais ICO. Uso terapêutico de plantas medicinais e efeitos adversos. Mostra Científi-

ca da Farmácia 2018;5(1).

13. Pedroso RS, Andrade G, Pires RH. Medicinal plants: an approach to rational and safe use. Revista de Saúde Coletiva 2021;31(2).
14. Gonçalves RN, Gonçalves JRSN, Buffon MCM, Negrelle RRB, Rattmann YD. Plantas medicinais na Atenção Primária à Saúde: riscos, toxicidade e potencial para interação medicamentosa. Revista APS, 2022;25(1):120-153.
15. Ntungwe NE, Domínguez-Martín, Roberto A, Tavares J, Isca VMS, Pereira P, Cebola M-J, Rijo P. Artemia species: An Important Tool to Screen General Toxicity Samples. Current Pharmaceutical Design, 2020:26;2892-2908.
16. Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, Maclaughlin JL. Brine shrim: a convenient general bioassay for active plant constituents. Plantas Medicinais 1982;45:31-34.
17. Amaral, FMM. Avaliação da qualidade de drogas vegetais avaliadas em São Luiz/Maranhão. Revista Brasileira de Farmacognosia. Maringá.2007;13(1):27-30.
18. Menezes DA, Silva ADN, Dantas GS, Silva HCSL, Lyra RP, Lima BL, Silva FL, Moreno PRH, Oliveira TKB, Silva JLV. Triagem toxicológica de extratos de Pimenta pseudocaryophyllus (Gomes) L.R. Landrum frente à *Artemia salina* Leach. Anais da Faculdade de Medicina de Olinda, 2019;1(3):12-15.
19. Cazarin KCC, Corrêa LC, Zambroni FAD. Redução, refinamento e substituição do uso de animais em estudos toxicológicos: uma abordagem atual. Revista Brasileira de Ciências Farmacêuticas, 2004;40(3).
20. Silva JLV, Sá AND, Santos JRB, Tavares JF. Evaluation of the oral acute toxicity of *Lippia microphylla* Cham. (Verbenaceae) on mice. In: XXII Simpósio de Plantas Medicinais do Brasil, 2012.
21. Batista NY. Avaliação da toxicidade do extrato aquoso e óleo essencial Myrcia sylvatica (G.Mey.) DC. frente a *Artemia salina*. Reunião Anual Da Sociedade Brasileira Para O Progresso Da Ciência (SPBC), 2010;62.
22. Silva AD, Silva H, LYRA RP, Menezes DA, Dantas GS, Silva FL, Moreno PR, Oliveira TK, Silva JL. Triagem toxicológica de extratos de Cinnamomum stenophyllum frente à *Artemia salina* Leach. An Fac Med Olinda, Recife, 2019;2(2):11
23. Silva GA, Hidekazu BL. Avaliação da toxicidade de três plantas medicinais frente à *Artemia salina*. 2014. 8p. Trabalho de Conclusão de Curso – Graduação em Farmácia, Faculdade Evangélica de Ceres, Goiás, 2014.
24. Terceiro DAJM, Oliveira MAS. Avaliação da toxicidade, citotoxicidade, genotoxicidade e mutagenicidade do infuso das folhas de Lippia sidoides (Verbenaceae). Revista Ciência E Estudos Acadêmicos De Medicina, 2020;1(12):1-7.
25. Valdés NV, Martins ER, Fonseca FSA. Toxicological effect of essential oils of plants against Artemia salina. Revista Brasileira de Plantas Medicinais, 2019; 21:261-268.