

SciForum MOL2NET

Phytochemical prospection of ethanolic extract of *Azadirachta indica* stem bark and its toxicity against *Drosophila melanogaster*

Paula Patrícia Marques Cordeiro^{1, 2}*, Dárcio Luiz de Sousa Júnior², Joycy Francely Sampaio dos Santos², Larissa da Silva², Maria Apoliana Costa dos Santos², Luiz Jardelino de Lacerda Neto², Henrique Douglas Melo Coutinho³, Francisco Assis Bezerra da Cunha⁴

¹ Master's Degree in Molecular Bioprospecting - URCA

- ² Semiarid Bioprospecting Laboratory, Department of Biological Chemistry, Regional University of Cariri - URCA
- ³ Laboratory of Microbiology and Molecular Biology, Department of Biological Chemistry-URCA
- ⁴ Coordinator of the Laboratory of Bioprospection of the Semiarid, Department of Biological Chemistry-URCA
- * paulabyos@gmail.com, (88) 999189423

Abstract: : Azadirachta indica is a plant of the family Meliaceae, originating in India, whose secondary metabolites are used for medicinal and insect purposes. The fruit fly - Drosophila *melanogater* is widely cited in the literature as a model for toxicity testing, especially since it presents rapid development and low maintenance cost. The aim of the present work was to carry out a phytochemical prospection of the ethanolic extract of A. indica stem bark (EECAi) and analyze its toxicity against D. melanogaster. The Colorimetric Method performed the chemical gait. The flies were submitted to different concentrations of the EECAi (5 mg/mL, 10 mg/mL and 20 mg/mL), being readings of 3 h, 6 h, 12 h, 24 h and 48 h, with light/dark cycles of 12 hours. Mortality rates and damage to the locomotor apparatus were analyzed by negative geotaxia test. Phytochemical prospecting of EECAi indicated the presence of tannins, flavones, flavonols, flavonones, flavonones, xanthones, chalcones, aurones and leocoantocyanidins. This extract did not present significant toxicity when compared to the control. The damage to the locomotor system was more significant at concentration of 10 mg/mL and 20 mg/mL, in the 48 h readings. The results of the mortality rate showed that the EECAi showed no significant toxicity at the concentrations and the model tested, diverging from other studies suggesting a bioinsecticity of this plant. Further studies need to test compounds isolated from this plant.

Keywords: Natural products; Phytochemistry; Alternative Methods.

1. Introduction

Azadirachta indica is a plant of the family Meliaceae, originating in India, whose secondary metabolites are used for medicinal purposes and insects¹. Its bioactive compounds, in addition to other applications, can be use as bioinsecticides against 430 different pests². Some plant compounds are toxic to living organisms³ and toxicity tests are required to ensure quality and

2. Results and Discussion

2.1 Phytochemical prospecting

Phytochemical prospecting of EECAi indicated the presence of tannins, flavones, flavonols, flavonones, flavononols, xanthones, chalcones, aurones and leucoanthocyanidins.

2.2 Toxicity

As shown in Figure 1, the mortality rate was small compared to the control group. On the other hand, the damage to the locomotor apparatus was more significant at the concentration of 20 mg/mL at the 48 hour reading, as shown in Figure 2.

3. Materials and Methods

3.1 Plant collection

The botanical material was obtained from exsicata identified with the geographical coordinates: 7°, 14 ', 17,7 "south latitude and 39°, 24' 52,6" west longitude of Greenwich and altitude of 449 m, is deposited in the Herbarium Caririense Dárdano de Andrade Lima under the number 10.787.3.2.

safety⁴. The fruit fly, *Drosophila melanogater* is widely cited in the literature as a model for toxicity testing, especially since it presents rapid development and low maintenance $costs^5$. The aim of the present work was to carry out a phytochemical prospection of the ethanolic extract of *A. indica* stem bark (EECAi) and analyze its toxicity against *D. melanogaster*.

Graph 1. Survival test with *D. melanogaster*.



Graph 2. Toxicity test by the negative geotaxia test with *D. melanogaster*



3.2 Obtaining the statement

The plant shells were immerse in ethanol for 72 h. After this period, the liquid was processed in a rotary evaporator. Then, submitted to the water bath at 60° C for water evaporation. The extract was placed in amber glass and stored in the freezer.

3.3 Phytochemical prospecting

The chemical gait to identify the classes of secondary metabolites present in the extracts was

performed by the colorimetric method, described by MATOS $(2000)^6$.

3.4 Toxicity test

Drosophila melanogaster (Harwich strain) was obtain from the National Species Stock Center, Bowling Green, OH. The flies were created according to the methodology proposed by Cunha et al. (2015)⁷. The determination of damage to the locomotor apparatus was made through the Coulom and Birman (2004)⁸ tests.

4. Conclusions

The EECAi presented different classes of metabolites. Which corroborates for their different actions cited in other works, such as, for example, anti-inflammatory, antioxidant, antimicrobial, among others⁹. The results of the mortality rate showed that the extract had no significant toxicity at the concentrations and in the model tested, diverging from other studies, suggesting a bioinsecticity of this plant. Further studies are need to test compounds isolated from this plant.

References

- 1. MOSSINI, S.A.G.; KEMMELMEIER, C. A árvore Nim (Azadirachta indica A. Juss): múltiplos usos. Acta Farm. Bonaerense, v. 24, n. 1, p. 139-48, 2005.
- 2. MARTINEZ SS. O Nim Azadirachta Indica: Natureza, Usos múltiplos, Produção. IAPAR, Londrina, 2002.
- 3. PEREIRA, V, S.; SILVA, C. R. N.; ROCHA, J.E., et al. Estudo químico, toxicidade e atividade antibacteriana do óleo essencial de *Ocimum gratissimum*. Rev. Interfaces: Saúde, Humanas e Tecnologia, v.2, 2014.
- 4. DOS REIS TUROLLA, M.S., et al. Informações toxicológicas de alguns fitoterápicos utilizados no Brasil. **Braz. J. Pharma. Sci.,** v. 42, 2006.
- 5. DE FREITAS, M.V., et al. Influence of aqueous crude extracts of medicinal plants on the osmotic stability of human erythrocytes. **Toxicol. In Vitro,** v.22, p.219-224, 2008.
- 6. Matos FJA. Introdução à Fitoquímica Experimental. 3 ed: Fortaleza, UFC; 2009.
- CUNHA, F. A. B.; WALLAU, G. L.; PINHO, A. I.; NUNES, M. E. M.; LEITE, N. F.; TINTINO, S. R.; COSTA, G. M.; ATHAYDE, M. L.; BOLIGON, A. A.; COUTINHO, H. D. M.; PEREIRA, A. B.; POSSER, T.; FRANCO, J. L. *Eugenia uniflora* leaves essential oil induces toxicity in *Drosophila melanogaster*: involvement of oxidative stress mechanisms. **Toxicology Research**, v. 4, n. 3, p. 634-644, 2015.
- 8. COULOM, H.; BIRMAN, S. Chronic exposure to rotenone models sporadic Parkinson's disease in *Drosophila melanogaster*. Journal of Neuroscience, v. 24, n. 48, p. 10993-10998, 2004.
- CRISTO, J. S., MATIAS, E. F., FIGUEREDO, F. G., SANTOS, J. F., PEREIRA, N. L., JUNIOR, J. G. A. S.; AQUINO, P. E. A.; NOGUEIRA, M. N. F.; RIBEIRO-FILHO, J.; CUNHA, F. A. B.; COSTA, M. S.; CAMPINA, F. F.; TINTINO, S. R.; SALGUEIRO, C. C. M.; COUTINHO, H. D. M. HPLC profile and antibioticmodifying activity of *Azadirachta indica* A. Juss (Meliaceae). Industrial crops and products, v. 94, p. 903-908, 2016.