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Phytochemical profile of leaves extract of *Azadirachta indica* A. Juss and toxicity against *Drosophila melanogaster*

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Abstract: *Azadirachta indica* A. Juss, is a large tree, native to India and Meliaceae family, has in its phytochemical constitution many phenolic compounds, several of its parts have been used for many medicinal purposes as antifungal, antibacterial and antidiabetic. The objective was to define the phytochemical profile of the ethanolic extract of the leaves of *A. indica* (EEAi) and determine the toxicity in the *Drosophila melanogaster* model. The quantification of the chemical constituents was done by High Performance Liquid Chromatography (HPLC) and to evaluate the toxicity was used the model of *D. melanogaster*, where it was evaluated the survival of the flies and negative geotaxia (Damage to the locomotive apparatus of the insect). Among the compounds found, the quercetin of the class of flavonoids was found in a higher concentration (14.05 ± 0.01 mg / g). In relation to the survival test, it was seen that the EEAi did not have relevant toxicity; when negative geotaxia was evaluated, there was a difference in the control only from the 24 hour and 48 hour readings at 10 mg / mL and 20 mg / mL respectively. Considering these results, it is shown that the EEAi has no significant toxic action.

Keywords: Natural products; Phytochemistry; Alternative Methods.

1. Introduction

Azadirachta indica, commonly known as "Nim", has many bioativities already known in the literature as antimicrobial, antihelmintic, antidiabetic, among others [1, 2, 3].

Natural products, even with their various pharmacotherapeutic benefits, should be submitted to toxicity analysis [4]. On the other hand, the *Drosophila melanogaster* model, which is an alternative method for this type of test, has a short life cycle, easy handling and low maintenance cost [5].

Thus, the present work aims to determine the chemical constituents of the ethanolic extract of the leaves of A. indica and verify its toxicity in *D. melanogaster*.

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2. Results and Discussion

2.1 Profile Phytochemical

The quantification of the chemical constituents of the ethanolic extract of the leaves of *A. indica* (EEAi), determined by HPLC, is shown in Table 1 and Figure 1, where flavonoids are predominantly composed and quercetin as the major compound. The same has been identified in other studies using this same species and part used [6, 7]. Compounds of this class possess a range of biological actions as antimicrobial, antioxidant, anti-inflammatory, among others [8, 9.]

2.2 Mortality

Figure 2 shows the survival profile of *D. melanogaster* at concentrations of 5, 10 and 20 mg/mL of the ethanolic extract of *A. indica*, where the data obtained did not differ statistically in relation to the control, diverging with others studies such as that of Viana and Prates (2003)[10], which used the aqueous extract of the leaves against *Spodoptera frugiperda*, where a higher mortality and developmental mortality was obtained at doses higher than that of the present study, as well as the study of Mourão et al. (2004)[11] that obtained a mortality of 99% of *Oligonychus ilicis* as a dose of 10.9 mg / mL from the hydroalcoholic extract of *A. indica* leaves.

2.3 Damage to the musculoskeletal system

In relation to the negative geotaxia, we observed that locomotor damage occurred only at the concentration of 10 mg/mL at the 24-hour reading and at the concentrations of 10 and 20 mg / mL at the 48-hour reading, proving that the extract did not demonstrate toxicity relevant.

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Table 1. Composition of the ethanolic extract of *Azadirachta indica*

Compounds	*A. indica*	LOD	LOQ
	mg/g	µg/mL	µg/mL
Gallic acid	2.68 ± 0.02 a	0.009	0.096
Catechin	2.51 ± 0.01 a	0.025	0.083
Chlorogenic acid	1.93 ± 0.01 b	0.017	0.059
Coumarin	4.37 ± 0.03 c	0.030	0.099
Rutin	5.86 ± 0.02 d	0.008	0.028
Quercitrin	7.92 ± 0.01 e	0.016	0.051
Quercetin	14.05 ± 0.01 f	0.011	0.037
Kaempferol	9.86 ± 0.02 g	0.029	0.096
Luteolin	5.93 ± 0.03 d	0.023	0.075

Results are expressed as mean ± standard deviation (SD) of three determinations. Means followed by different letters differ by the Tukey test with $p < 0.05$

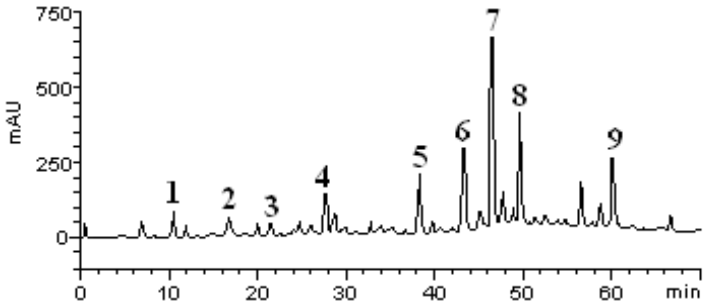

Figure 1. Representative of high-performance liquid chromatography of the ethanolic extract of *Azadirachta indica*. (Peak 8), catechin (peak 2), chlorogenic acid (peak 3), coumarin (peak 4), rutin (peak 5), quercitrin (peak 6), quercetin (peak 7), and kaempferol luteolin (peak 9).

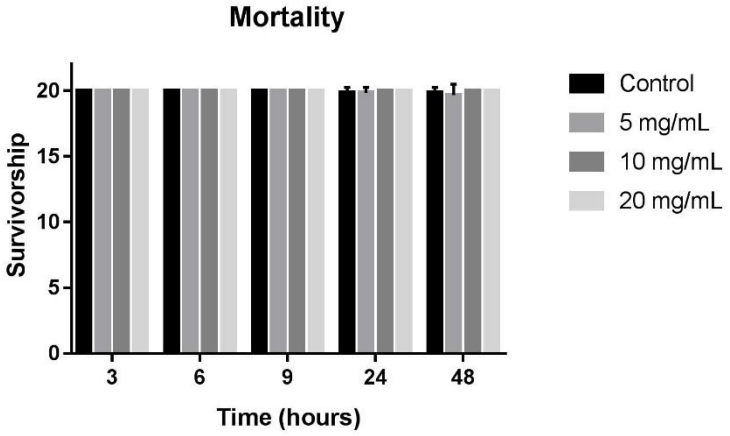

Figure 2. Survival test with model *D. melanogaster*.

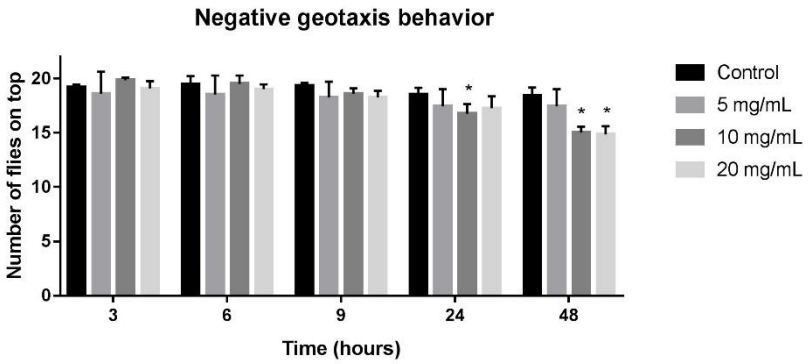

Figure 3. Negative geotaxia test with model *D. melanogaster*.

3. Materials and Methods

3.1 Plant Material

Uma exsicata da planta com as coordenadas geográficas: 7º, 14', 17,7" de latitude Sul e 39º, 24' 52,6" de longitude Oeste de Greenwich e altitude de 449 m, encontra-se depositada no Herbário Caririense Dárdano de Andrade-Lima sob o número 10.787.

3.2 Preparation of Extract

The leaves of the plant were collected at 9:00 am. ± 30 min. This material was then sprayed and immersed in ethanol PA for 72 h. After this time, the extract was filtered and the liquid was concentrated on a rotary evaporator (Fisatom). After this procedure, the material was placed in a water bath (Quimis) at 60ºC for water evaporation. After concentration, the extract was packed in an amber cup and stored in a freezer. The extract obtained from leaves showed yield of 4.5%.

3.3 Phytochemical Prospecting

The identification of its phytochemicals was done by High Performance Liquid Chromatography (HPLC). The extract was injected onto a reverse phase (4.6 mm x 250 mm) PhenomenexC18 column filled with 5 | the day. Mobile phases A and B were acidified with Milli-Q water to pH 3.0 with 2% formic acid and acetonitrile, respectively. The corresponding solvent gradients were used as follows: 0 min, 5% B; 0 to 5 min, 15% B; 5 to 10 min, 15% B; 10 to 30 min, 40%; 30 to 45 min at 70% B; 45-60 min, 100% B. The extract from *A. indica* and the mobile phase were filtered through of a 0.45 'membrane filter (Millipore) and then ultrasonically degassed prior to use. Chromatographic peaks were confirmed by comparing their retention time with the reference standards and by the DAD spectrum (200 to 500 nm). Galic acid calibration curve: Y = 12573x + 1329,6 (r = 0,9998); catechin: Y = 11845x + 1173,9 (r = 0,9997); ï¿½ido clorogï¿½ico: Y = 11948 x + 1205,7 (r = 0,9995); coumarin: Y = 12685x + 1156,3 (r = 0,9999); routine: Y = 13476 x + 1279,8 (r = 0,9997); quercetin: Y = 11672x + 1249,5 (r = 0,9998); quercitrin: Y = 12408x + 1347,9 (r = 0,9999); luteolin: Y = 13508x + 1351,3 (r = 0,9996) and kaempferol: Y = 12834x + 1367,2 (r = 0,9997). All chromatography operations were performed at room temperature and in triplicates. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope was determined using three independent analytical curves as defined by Boligon et al. (2012)[12].

3.4 Strain and Creation

Drosophila melanogaster (Harwich strain) was obtained from the National Species Stock Center, Bowling Green, OH.

3.5 Mortality Testing

With this model, the mortality test was performed, following the one proposed by Cunha et al. (2015) [13], where adult flies (males and females) were placed in 130 ml glass bottles (6 cm high and 6.5 cm in diameter) containing filter paper. For the control was added on this paper 1000 µL of sacarose a 20 % in distilled water.

For the other groups, 1000 µL of the EEAI diluted in sucrose at 20% at the concentrations of 5 mg / mL, 10 mg / mL and 20 mg / mL were added. Throughout the procedure, the 12 hour light / dark cycle and controlled temperature at 25 ° C and relative air humidity of 60% were maintained. The experiment was performed in triplicate where each "n" was the average of two experiments, and in each, 20 flies were used. The readings for the mortality check were performed every 3, 6, 12, 24 and 48 hours.

3.5 Locomotor Damage Test

In the negative geotaxia test, the locomotor damage was identified through the test described by Coulom and Birman (2004) [14]. Each group of live flies exposed to the EEA at reading times of 3, 6, 12, 24 hours were conducted to the bottom of the containers and after one minute the number of flies reaching 4 cm in height of the containers was counted. Assays were repeated twice at one minute intervals..

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4. Conclusions

In this study, it is concluded that the ethanolic extract of the leaves of *Azadirachta indica*, has in phytochemical composition many compounds of the flavonoid class, and quercetin is its major compound. The same did not demonstrate relevant toxicity in the mortality test with *D. melanogaster*, however, locomotor damage was verified only in the last readings and at the highest concentrations used when administered acutely, demonstrating the need to evaluate the exposure of the extract for long periods in order to determine its action in a chronic period.

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Conflicts of Interest

The authors declare no conflict of interest in this research.

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