Phytochemical Profile and Evaluation of Acute Toxicity of the Crude Extract from *Acanthospermum hispidum* Roots

Franciele Florencio Costa 1,2, Ewelyn Cintya Felipe dos Santos 1,2, Janaína Carla Barbosa Machado 1,2, Anderson Felipe Soares de Freitas 3, Alisson Macário de Oliveira 3, Thiago Henrique Napoleão 3, Magda Rhayanny Assunção Ferreira 1, Luiz Alberto Lira Soares 1,2,*

1 Postgraduate Program in Pharmaceutical Sciences, Federal University of Pernambuco (UFPE), Recife, PE, Brazil; franciele.florence94@hotmail.com (F.F.C.); ewelyn.cintya@ufpe.br (E.C.F.S.); janaina.machado@ufpe.br (J.C.B.M.); luiz.albertosoares@ufpe.br (L.A.L.S.);
2 Laboratory of Pharmacognosy (NUDATEF), Federal University of Pernambuco (UFPE), Recife, PE, Brazil; franciele.florence94@hotmail.com (F.F.C.); ewelyn.cintya@ufpe.br (E.C.F.S.); janaina.machado@ufpe.br (J.C.B.M.); magda.ferreira00@gmail.com (M.R.A.F.); luiz.albertosoares@ufpe.br (L.A.L.S.);
3 Department of Biochemistry, Federal University of Pernambuco (UFPE), Recife, PE, Brazil; afbiomedicine@gmail.com (A.F.S.F.); alissonmacario@hotmail.com (A.M.O.); thiago.napoleao@ufpe.br (T.H.N.);
* Correspondence: luiz.albertosoares@ufpe.br (L.A.L.S.);

Scopus Author ID 12763838000

Received: 11.11.2021; Accepted: 7.01.2022; Published: 24.03.2022

Abstract: *Acanthospermum hispidum* DC (Asteraceae), popularly known as "espinho-de-cigano", is a plant species used in folk medicine to treat gastrointestinal and respiratory disorders. These activities can be related to the presence of secondary metabolites, which can also play toxicity. Considering that, the objective of this work was to evaluate the toxicity of the crude extract from roots of *A. hispidum*. The herbal material was collected, ground, and the crude extract was obtained with acetone: water (7:3, v/v) at 10% (w/v) by turbo extraction. Phytochemical analysis by thin-layer chromatography (TLC) was performed, and the results were used to obtain the HPLC fingerprint. A single oral dose (2,000 mg/kg) was administered daily over 14 consecutive days to mice. Bodyweight, food and water intake, blood biochemical, and hematological parameters were measured. The results obtained in the TLC analysis suggested the presence of cinnamic derivatives, flavonoids, and terpenes and were confirmed by HPLC analysis. Additionally, the total polyphenol content was 10.1% ± 0.0093%, expressed as pyrogallol. According to the acute toxicity study, the data demonstrated the low toxicity of the crude extract of *A. hispidum* roots, considered very low acute toxicity or non-toxic, proved safe when administered orally.

Keywords: *A. hispidum*; acute toxicity; fingerprint analysis.

© 2022 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

*Acanthospermum hispidum* DC is a medicinal species that belongs to the Asteraceae family and the *Acanthospermum* genus [1]. In Brazil, the species is popularly known as "espinho-de-cigano", and occurs abundantly in the Northeast region [2, 3].

*A. hispidum* is used to treat gastrointestinal and respiratory disorders (such as cough, asthma, and bronchitis), mainly for its expectorant activity. In addition, in other countries, the species is indicated to treat diseases such as malaria, yellow fever, and stomach problems [1, 4, 5]. Based on the use of this species, studies have been developed to verify the biological activities attributed to the species, like antimicrobial and antiparasitic. As for its chemical
composition, some studies showed the presence of sesquiterpene lactones, terpenes, phenolic compounds, saponins, and alkaloids [1, 3, 4, 6].

In recent years, species' in vitro and in vivo studies have received special attention. In this sense, toxicity tests are important for evaluating possible effects that phytocompounds may develop before their use in clinical practice [7]. Considering several reasons justify the need to evaluate the biological activity of natural products, it is necessary to study acute toxicity in vivo, evaluating its possible effects on hematological, biochemical, and organ histology parameters.

Given the importance of A. hispidum and the absence of studies on the chemical composition of the roots, this work aimed to investigate the phytochemical profile and evaluate the toxicity to assess the toxic nature of bioactive compounds present in the crude extract.

2. Materials and Methods

2.1. Herbal material.

The roots of Acanthospermum hispidum DC. were collected in Passira, Pernambuco, Brazil (07°52’30” S 35°27’00” O). The exsiccate was deposited Herbário Dárdano de Andrade Lima of the Agronomic Institute of Pernambuco (IPA-PE), identified under number 93733. The access was registered in the SisGen (AA009E4). The material was dried in a circulating air oven at 45 °C - 7 days (Lucadema®; Brazil) and ground in forage.

2.2. Crude extract.

The roots were submitted to the turbo extraction process (Metvisa®; Brazil) for 20 min. For 10 g of roots, 100 mL of acetone: water (7:3, v/v) was used. The solution was filtered and concentrated (IKA®; Germany) and then lyophilized for 48 h (Liotop®; Brazil), giving the crude extract (CE) [8].

2.3. Phytochemical analysis.

2.3.1. Thin-Layer Chromatography (TLC).

The TLC was performed to analyze the presence/absence of secondary metabolites in the extract. The analysis used 60-F254 silica gel plates (Merck®, Germany), developed in a twin trough vertical glass chamber (10 x 10 cm; Camag®, Switzerland). Sample and standards were applied manually (15 µL) in bands of 1 cm. All information about standards, mobile phase, and reagents are described in table 1. For documentation, the plates were visualized in the UV light in the MultiDoc-It photo documenter (Model 125, USA) with the software UVP® and a camera (Canon® Rebel T3, EOS 1100 D) [9, 10].

| Metabolite              | Mobile phase* | Reagents# | Standards | Results |
|-------------------------|---------------|-----------|-----------|---------|
| Condensed tannins       | 90:5:5        | Chloridric vannilin | Catechin | Negative |
| Hydrolysable tannins    | 90:5:5        | FeCl3     | Gallic acid | Negative |
| Flavonoids              | 90:5:5        | NEU + PEG | Rutin     | Positive |
| Cinnamic derivatives    | 90:5:5        | NEU + PEG | Caffeic acid | Positive |
| Coumarins               | 50:50:50      | KOH       | Coumarin | Negative |
| Saponins                | 100:11:11:26  | LB + Δ    | Escin     | Negative |
| Terpenes and steroids   | 70:30         | LB + Δ    | β-sitosterol | Positive |
| Alkaloids               | 50:6.75:5     | Dragendorff | Pilocarpine nitrate | Negative |

Table 1. Mobile phases, developers, and standards used to analyze secondary metabolites in the crude extract of A. hispidum roots by thin-layer chromatography.
2.3.2. High-Performance Liquid Chromatography (HPLC).

The crude extract of *A. hispidum* roots (10 mg) was dissolved in 10 mL of HPLC grade methanol (Tedia®, USA) to obtain a solution of 1 mg/mL. The analysis was conducted on an Ultimate 3000 system (Thermo Fisher Scientific®, USA) coupled to a Diode Array Detector (Thermo Fisher Scientific®), a degasser, and an autosampler with a 20 mL loop (Thermo Fisher Scientific®). The wavelength was set at 330 nm. The chromatographic analysis was achieved on a C18 column (250 mm x 4.6 mm i.d., 5 µm; Dionex®, USA) equipped with a pre-column (C18, 4 mm x 3.9 µm; Dionex®), at a temperature of 25 ± 2 °C. The mobile phase was composed of purified water (A) and methanol (B), acidified with 0.05% trifluoroacetic acid, at a 0.8 mL/min flow rate. A gradient program was used as follow: 0–10 min at 25–40% B, 10–20 min at 40–75% B, 20–25 min at 75% B, 25–28 min at 75–25% B, 28–30 min at 25% B. For data analysis and processing was used the software Chromeleon® (Thermo Fisher Scientific®). The standards caffeic acid (98%) and rutin (98%) (Sigma-Aldrich®, USA) were used to calculate the total content of cinnamic acids and flavonoids, respectively.

2.4. Total Polyphenol Content (TPC).

The TPC was calculated by the Folin-Ciocalteu method and was expressed in grams of pyrogallol, and the results represent the mean of three determinations [11].

2.5. Toxicity evaluation.

2.5.1. *Artemia salina* lethality test.

*Artemia salina* cysts (San Francisco Bay Brand, Inc., USA) were incubated in natural seawater (25-30 °C), and the pH was adjusted to 9.0 with NaHCO₃. The assay was performed as described by Meyer et al. [12] with some modifications. The extract was diluted in seawater in Falcon tubes to obtain solutions with concentrations of 3.9 to 1000 µg/mL. Each trial contained 10 nauplii, and in the negative control, the nauplii were incubated in seawater. After 24 h, the survival was determined, and the concentration needed to kill 50% of nauplii was calculated. Three independent experiments were performed.

2.5.2 Animals.

Swiss mice (*Mus musculus*), with 30-40 g, were obtained from the Keizo Asami Immunopathology Laboratory of the Federal University of Pernambuco (Recife, Brazil). The mice were maintained at a temperature of 22 °C, with a 12 h: 12 h light-dark cycle and free access to food (Purina, Nestlé® Brazil) and water. The Animal Experimentation Ethics Committee of the Federal University of Pernambuco approved all experiments (003/2020).
2.5.3. Acute toxicity.

The acute toxicity of crude extract from *A. hispidum* roots was evaluated according to the instructions of the Organization for Economic Cooperation and Development (OECD, guideline 423) [13]. Female mice were separated into 3 groups (n = 3, for each group) that received a single dose of the treatment by gavage. The first group of mice received only saline solution (control). The extract was evaluated at a dose of 2000 mg/kg. Behavioral alterations were evaluated during the first 60 minutes after treatment administration, divided into four periods (15 min, until 60 min). The piloerection, stool appearance, sensitivity to sound and touch, mobility, and aggressive behavior were evaluated[14]. On the 15th day after the start of treatment, bodyweight variations and water and food consumption were determined. In addition, peripheral blood was collected. Next, the mice were euthanized, and the organs (livers, kidneys, lungs, spleens, and hearts) were removed, weighed, analyzed, and processed for histological evaluation.

The blood was used to evaluate hematological and biochemical parameters. The hematologic parameters were analyzed using an automatic analyzer (Animal Blood Counter – ABC Vet, Montpellier, France) and optical microscopy: erythrocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and total and differentiated analysis of leukocytes. For biochemical analysis, the blood was evaluated for albumin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, γ-glutamyl transferase (GGT), total cholesterol, triglycerides, urea, and creatinine, using kits from Labtest Diagnóstica (Lagoa Santa, Brazil).

Histological analyses were performed, and the organs (liver, kidney, spleen, lung, and heart) of the rats in the treatment and control groups were observed by light microscopy. Pieces of the organs were fixed in formalin and then dehydrated in ethanol, diaphanized in xylene, and embedded in paraffin. Sections of 5 μm were stained with hematoxylin and eosin and mounted using coverslips with Entellan® resin (Merck®, Germany). The slides were observed with a Motic BA200 microscope coupled to a Moticam 1000 1.3 MP digital camera (Motic Incorporation Ltd., Hong Kong).

2.6. Statistical analyses.

The results are expressed as mean ± standard deviation (sd). One-way analyses of variance (ANOVA) followed by Bonferroni’s test were used to calculate statistical significance between groups (GraphPad® Software, La Jolla, CA, USA). A p-value < 0.05 was considered significant.

3. Results and Discussion

Although several herbal drugs have a well-established history of popular use, some of the species do not have reports of evidence of toxicity that should be considered for the safe use of derived products. In this context, the present study evaluated the toxicity of crude extract of *A. hispidum* roots.

General approaches allow the estimation of the total polyphenol content compounds in herbal materials, mainly by spectrophotometric methods. The calculated TPC for the acetonic extract of *A. hispidum* roots was 10.1 ± 0.0093% (2.18%) calculated as pyrogallol. Phenolic compounds are soluble in polar organic solvents, such as ethanol, methanol, and acetone, in
such a way that, recently, the use of acetone: water systems for polyphenol extraction has increased, including important results from our group for other species using the acetone: water 7:3 (v/v) [8, 15, 16, 17].

The TLC analysis made it possible to obtain a chemical profile of the crude extract from roots of *A. hispidum*, highlighting the classes of terpenes and steroids, flavonoids, and cinnamic derivatives. This result was confirmed by the HPLC analysis at 330 nm for the detection of flavonoids and cinnamic derivatives. A chromatogram was obtained and is shown in Figure 1. Several peaks were observed in the chromatogram, eight peaks were indicative of the presence of cinnamic derivatives [1, retention time (rt) = 8.76 min; 2, rt = 11.47 min; 3, rt = 13.00 min; 4, rt = 15.90 min; 5, rt = 16.10 min; 6, rt = 17.17 min; 7, rt = 17.51 min; 8, rt = 19.50 min], and one of flavonoid (9, rt = 20.25 min), as confirmed by the scan spectra at 330 nm and in accordance with the literature [18]. The total content of cinnamic derivatives was 0.54 ± 0.022% as caffeic acid, the total content of flavonoids was 0.03 ± 0.001% calculated as rutin.

![Figure 1](https://biointerfaceresearch.com/)  
*Figure 1*. Analysis of the chemical composition of crude extract of *A. hispidum* roots by high-performance liquid chromatography (HPLC). A representative chromatogram profile and the ultraviolet absorption spectra of the 9 peaks detected in the extract.
Among the most frequent causes of poisoning globally is the use of medicinal plants, generally associated with incorrect identification, supplements, or herbal medicines without evaluation of their toxicity [19]. Although the use of A. hispidum is common, studies on the toxicity of this species are scarce in the literature. Among the toxicity tests, the lethality test against the saltwater microcrustacean A. salina, usually used as food for fish, proved to be a method that presents simplicity, speed, and low cost that favors its routine use in preliminary analysis of general toxicity [20, 21].

The tests carried out in this study evaluated the crude extract of the roots of A. hispidum; the substance showed a moderate lethality against A. salina. At the maximum concentration tested (1,000 µg/mL), it caused the death of 50% of the nauplii. The A. salina test is used because it is a simple bioassay to identify the preliminary toxicity of natural products. Therefore, the preliminary test uses this technique in several studies [22]. Studies with aqueous, hydroalcoholic, and methanolic extracts, dichloromethane fraction, as well as compounds isolated from the leaves and aerial parts of A. hispidum have already been carried out and have shown no toxicity against A. salina with a value of LD₅₀ > 0.1 mg/mL [23-26]. According to the toxicity study by Hamidi et al. [27], crude extracts have high toxicity with LD₅₀ values less than 100 µg/mL; moderate toxicity has LD₅₀ between 100-500 µg/mL; those with LD₅₀ values between 500-1000 µg/mL, mildly toxic; and finally, LD₅₀ values above 1000 µg/mL are considered non-toxic.

Based on the previous results in A. salina, the acute toxicity in rats was then evaluated, as well as the possible effects on hematological and biochemical parameters and organ histology. Ethnomedicine has been reported for the different parts of A. hispidum, which indicates the use of roots against respiratory problems. In many cases consists of ingesting syrup containing the roots, which can result in doses equivalent to or higher than 2,000 mg/kg. In this study, the dose was administered in accordance with the OECD protocol.

In evaluating behavioral changes, the administration of the crude extract showed agitation in the evaluation of the parameters in the first 60 min, and irritability could be noted in animals submitted to non-spontaneous ingestion of the vehicle used. However, after the first one hour, all signs had already ceased and were maintained during the 14 days of evaluation. During the 14 days, the animals did not show abnormal signs, not even differences in feed and water intake, and body weight (Table 2), indicating that these results are in accordance with OECD [13], in which the animal should not present a change in body mass greater than 20% of the initial average weight, suggesting a lethal dose above 2,000 mg/Kg as low toxicity.

**Table 2.** Evaluation of food and water consumption and weight gain of animals from the control group and treated orally with crude extract of A. hispidum roots for 14 days.

| Parameter            | Control               | Crude Extract (2,000 mg/kg) |
|----------------------|-----------------------|----------------------------|
| Water consumed (mL)  | 41.01 ± 1.05          | 41.05 ± 1.26               |
| Food consumed (g)    | 6.22 ± 0.12           | 6.19 ± 0.30                |
| Weight gain (g)      | 10.01 ± 0.55          | 10.12 ± 0.50               |
|                      | Data are the mean ± sd|                           |

In our study, although some behavioral reactions were detected in the Hippocratic screening, there were no deaths after the 14-day period, so the LD₅₀ > 2,000 mg/kg was considered, and the extract of A. hispidum can be classified as low acute oral toxicity and considering the criteria of the Globally Harmonized Classification System (GHS) the extract belongs to class 5, those that present very low or non-toxic acute toxicity.
The hematological analysis did not show significant changes \((p < 0.05)\) in the parameters in animals that received *A. hispidum* crude extract at 2,000 mg/kg compared to the control group (Table 3).

**Table 3.** Hematological parameters of animals in control (oral saline) and treated groups with crude extract of *A. hispidum* roots for 14 days.

| Parameters                  | Control                  | Treatment Crude extract (2,000 mg/kg) |
|-----------------------------|--------------------------|--------------------------------------|
| Erythrocytes \((10^6/mm^3)\) | 7.00 ± 0.21              | 6.98 ± 0.32                          |
| Hematocrit (%)              | 39.95 ± 0.21             | 40.27 ± 0.41                         |
| Hemoglobin (g/dL)           | 15.32 ± 0.22             | 15.55 ± 0.44                         |
| MVC (DL)                    | 65.00 ± 1.01             | 66.33 ± 0.03                         |
| MCH (pg)                    | 25.17 ± 0.82             | 24.91 ± 0.85                         |
| MCHC (%)                    | 38.20 ± 0.91             | 37.67 ± 0.85                         |
| Leukocytes \((10^9/mm^3)\)  | 6.56 ± 0.43              | 6.46 ± 0.46                          |
| Segmented (%)               | 53.79 ± 1.16             | 53.90 ± 1.38                         |
| Lymphocytes (%)             | 29.22 ± 1.70             | 28.28 ± 1.57                         |
| Monocytes (%)               | 16.94 ± 1.55             | 17.82 ± 1.22                         |

*Significantly different \((p < 0.05)\) from control. Data are the mean ± sd. Statistical analysis was performed by analysis of variance (ANOVA), followed by Bonferroni’s test. MVC: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.

In the biochemical analysis, no significant changes \((p < 0.05)\) were observed in the levels of ALT and AST (liver function markers), urea and creatinine (renal function markers) enzymes, albumin, total proteins, and alkaline phosphatase in animals treated with 2,000 mg/kg, compared to the control (Table 4). Corroborating with the OECD, the next dose to be tested would be 5,000 mg/Kg. However, a study with this dose is only recommended in cases that justify its need.

**Table 4.** Biochemical parameters of the blood of animals in control (oral saline) and treated groups with crude extract of *A. hispidum* roots for 14 days.

| Parameters                  | Control                  | Treatment Crude extract (2,000 mg/kg) |
|-----------------------------|--------------------------|--------------------------------------|
| Albumin (g/dL)              | 1.99 ± 0.09              | 2.01 ± 0.13                          |
| ALT (U/L)                   | 88.40 ± 1.12             | 87.3 ± 0.52                          |
| AST (U/L)                   | 122.09 ± 3.72            | 121.97 ± 2.01                        |
| Total protein (g/dL)        | 7.12 ± 0.20              | 7.09 ± 0.23                          |
| Alkaline phosphatase (IU/L) | 14.00 ± 1.0              | 13.33 ± 2.52                         |
| GGT (U/L)                   | 10.70 ± 0.90             | 11.07 ± 0.26                         |
| Urea (mg/dL)                | 54.02 ± 2.23             | 54.39 ± 2.53                         |
| Creatinine (mg/dL)          | 0.23 ± 0.08              | 0.20 ± 0.09                          |

*Significantly different \((p < 0.05)\) from control. Data are the mean ± sd. Statistical analysis was performed by ANOVA, followed by Bonferroni’s test. ALT: alanine aminotransferase; AST: aspartate aminotransferase; GGT: gamma-glutamyl transferase.

Organ weight is a parameter frequently used to assess toxicity, check if there has been any change in organs, and confirm the findings of biochemical and hematological analyses [28]. There were no relevant changes in mice treated with the crude extract of *A. hispidum* roots compared to the control group (Table 5). The macroscopic analysis of the organs of animals treated with extract did not show significant changes in color or texture.

The acidified aqueous extract of the aerial parts of the species has already been evaluated for acute toxicity in mice. The extract administered at a dose of 2,000 mg/kg did not show lethal or toxic effects in animals [29]. Another study carried out with the aqueous extract of the whole plant in Swiss albino chicks and mice revealed that the oral administration of the
extract at a dose of 2,000 mg/kg did not produce clinical symptoms of toxicity and mortality in the animals tested [30]. The aqueous extract of A. hispidum leaves was also tested for acute toxicity in Wistar rats, the mean lethal dose was above 5,000 mg/kg, and no signs of toxicity were observed [31]. These results corroborate the results of our study.

Table 5. Effect of crude extract of A. hispidum roots on organ weight in control (oral saline) and treated groups after 14 days of single-dose treatment.

| Parameters      | Control          | Treatment Crude extract (2,000 mg/kg) |
|-----------------|------------------|-------------------------------------|
| Heart (g)       | 0.18 ± 0.00      | 0.18 ± 0.01                         |
| Lung (g)        | 0.24 ± 0.00      | 0.26 ± 0.02                         |
| Liver (g)       | 2.11 ± 0.10      | 2.05 ± 0.05                         |
| Kidney (g)      | 0.23 ± 0.01      | 0.25 ± 0.02                         |
| Spleen (g)      | 0.33 ± 0.03      | 0.29 ± 0.041                        |
| Stomach (g)     | 0.28 ± 0.02      | 0.29 ± 0.02                         |
| Brain (g)       | 0.45 ± 0.01      | 0.46 ± 0.02                         |

*Significantly different (p < 0.05) from control. Data are the mean ± sd. Statistical analysis was performed by ANOVA, followed by Bonferroni’s test.

Figure 2 shows photomicrographs of the organs (liver, kidney, and spleen) of mice in the control (oral saline) and treated groups (2,000 mg/kg of crude extract from A. hispidum roots). The liver of animals treated with the crude extract showed well-delimited hepatocytes, nuclei with visible chromatin, central lobular veins of various sizes with preserved characteristics, and absence of connective tissue fibrosis showed developed parenchyma. The kidneys showed well-defined structures with a fibrous capsule, cortical and medullary region. The capsular space and renal tubules are well delimited without infiltration, both in control animals and in those treated with the extract, indicating that the acute treatment with the crude extract did not interfere with the balance of the renal tubule reabsorption process. The spleen from treated mice did not show histological changes compared to controls showed preserved architecture with white and red pulps normal in morphology and number and absence of hyperactivation in both groups. The parenchyma and interalveolar septa were observed in the lungs of the groups treated with mice without alterations.

Figure 2. Representative photomicrographs liver, kidney, and spleen, of mice from control and treatment with 2,000 mg/kg of crude extract from A. hispidum roots. Cv: centrilobular vein; Rt: renal tubule; Gr: glomerulus.
With the current increase in demand for treatments that use medicinal plants and their derivatives, several studies have been carried out on the toxicity of the species to obtain extracts that are safe to use. Considering data from the literature, several extracts have been evaluated for their toxicity, such as an aqueous extract from *Hibiscus sabdariffa* [32], a methanolic extract from *Geophila obvallata* [33], and an ethyl acetate extract from saline extract, a lectin-rich fraction of *Microgramma vacciniifolia* rhizome [34].

Data obtained by Gomes et al. [35] with medicinal plants about acute toxicity carried out for the first time in *Drimys angustifolia*, and *D. brasiliensis*, that have the same indications in folk medicine and have secondary metabolites like those present in *A. hispidum*, including flavonoids and terpenes, and also studies in *Pyrethrum pulchrum* [36], *Rheum turkestanicum* [37], *Rotula aquatica* [38] and *Croton blanchetianus* [39] did not present observed changes in body weight, organ, decreased food and water intake, factors that suggest systemic toxicity. As with *A. hispidum* roots, the estimated LD$_{50}$ was not calculated because, in this study, no deaths were observed during the experiment.

4. Conclusions

The results obtained in the TLC analysis suggested the presence of secondary metabolites, mainly cinnamic derivatives, flavonoids, and terpenes, which play an important role in plant survival and several therapeutic applications. The results were confirmed by HPLC analysis, and it was possible to establish a fingerprint for *A. hispidum* roots. Furthermore, according to the study of acute toxicity by the oral administration in mice, the data demonstrated the low toxicity of the crude extract of the roots of *A. hispidum*, being considered of very low acute toxicity or non-toxic. Therefore, the crude extract of *A. hispidum* roots proved safe in mice when administered orally, without modifications on hematological, biochemical, and histological evaluations.

Funding

This research was funded by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (405297/2018-1) and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE) (APQ-0493-4.03/14).

Acknowledgments

The authors would like to thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco (FACEPE).

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Adepiti, A.O.; Adewunmi, C.O.; Agbedahunsi, J.M. Antitrichomonal activity of *Acanthospermum hispidum* DC (Asteraceae). *Afr. J. Biotechnol.* 2014, 13, 1303-1307, https://doi.org/10.5897/AJB2013.13064.

2. Alva, M.; Popich, S.; Borkosky, S.; Cartagena, E.; Bardón, A. Bioactivity of the essential oil of an Argentine collection of *Acanthospermum hispidum* (Asteraceae). *Nat. Prod. Commun.* 2012, 7, 245-248.
3. Araújo, E.L.; Randau, K.P.; Sena-Filho, J.G.; Mendonça Pimentel, R.M.; Xavier, H.S. *Acanthospermum hispidum* DC (Asteraceae): Perspectives for a phytotherapeutic product. *Braz. J. Pharmacogn.* 2008, 18, 777-784, https://doi.org/10.1590/S0102-695X2008000500024.

4. Tirloni, C.A.S.; Lívero, F.A.R.; Palozzi, R.A.C.; Silveira, R.C.A.; Vasconcelos, P.C.P.; Souza, R.I.C.; dos Santos, A.C.; de Almeida, V.P.; Budel, J.M.; de Souza, L.M.; Gasparotto Junior, A. Ethnopharmacological investigations of the cardio-renal properties of a native species from the region of Pantanal, state of Mato Grosso do Sul, Brazil. *J. Ethnopharmacol.* 2017, 206, 125-134, https://doi.org/10.1016/j.jep.2017.05.027.

5. Vivekanandhan, P.; Senthil-Nathan, S.; Shivakumar, M.S. Larvicidal, pupicidal and adult smoke toxic effects of *Acanthospermum hispidum* (DC) leaf crude extracts against mosquito vectors. *Physiol. Mol. Plant Path.* 2018, 101, 156-162, https://doi.org/10.1016/j.pmpp.2017.05.005.

6. Araújo, L.B.D.C.; Silva, S.L.; Galvão, M.A.M.; Ferreira, M.R.A.; Araújo, E.L.; Randau, K.P.; Soares, L.A.L. Total phytosterol content in drug materials and extracts from roots of *Acanthospermum hispidum* by UV-VIS spectrophotometry. *Braz. J. Pharmacogn.* 2013, 23, 736-742, https://doi.org/10.1590/S0102-695X2013000500004.

7. Lekshmi, V.S.; Rauf, A.A.; Kurup, G.M. Toxicological Evaluation of Bioactive Sulfated Polysaccharides from the Edible Marine Algae *Padina tetrastromatica* and *Ulva fasciata* in Sprague-Dawley Rats. *J. Biol. Act. Prod. Nat.* 2019, 9, 79-96, https://doi.org/10.1080/22311866.2019.1599730.

8. Ramos, R.T.M.; Bezerra, I.C.F.; Ferreira, M.R.A.; Soares, L.A.L. Spectrophotometric Quantification of Flavanoids in Herbal Material, Crude Extract, and Fractions from Leaves of *Eugenia uniflora* Linn. *Pharmacogn. Res.* 2017, 9, 253-260, https://doi.org/10.4103/pr.pr_14_16.

9. Ferreira, E.C.B.; Nova, I.C.V.; Almeida, W.A.; Albuquerque, F.M.S.; Cruz, G.S.; da Costa, H.N.; Procópio, T.F.; da Silva, W.A.V.; Ferreira, M.R.A.; Paiva, P.M.G.; Soares, L.A.L.; Teixeira, A.A.C.; Teixeira, V.W.; Napoleão, T.H.; Barros, R.; Pontual, E.V. *Opuntia ficus-indica* cladode extract is an embryotoxic, larvicidal, and oviposition-deterrent agent for the diamond back moth, *Plutella xylostella*. *Crop Prot.* 2021, 39, 105351, https://doi.org/10.1016/j.cropro.2020.105351.

10. Almeida, W.A.; Nova, I. C. V.; Nascimento, J.D.; Moura, M.C.; Agra-Neto, A.C.; Costa, H.N.; Teixeira, A.A.C.; Wanderley-Teixeira, V.; Ferreira, M.R.A.; Soares, L.A.L.; Coelho, L.C.B.B.; Navarro, D.M.A.F.; Paiva, P.M.G.; Napoleao, T.H.; Pontual, E.V. Effects of *Plectranthus barbatus* leaf extract on survival, digestive proteases, midgut morphophysiology and gut microbiota homeostasis of *Aedes aegypti* larvae. *S. Afr. J. Bot.* 2021, 141, 116-125, http://dx.doi.org/10.1016/j.sajb.2021.04.023.

11. Ferreira, M.R.A.; Souza, P.A.; Machado, J.C.B.; Soares, L.A.L. Extraction of monomers of hydrolysable tannins from pods of *Libidibia ferrea* (Mart. ex Tul.) L. P. Queiroz: effects of solvent and amount of drug using response-surface methodology and desirability profile. *Quim. Nova.* 2020, 43, 738-746, http://dx.doi.org/10.21577/0100-4042.20170536.

12. Meyer, B.N.; Ferrigni, N.R.; Putnam, J.E.; Jacobsen, L.B.; Nichols, D.E.; McLaughlin, J.L. Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Med.* 1982, 45, 31-34, https://doi.org/10.1055/s-2007-971236.

13. OECD. *Guideline 423: acute Oral Toxicity- Acute Toxic Class Method*. OECD, 2001, https://doi.org/10.1787/20745788.

14. Malone, M.H. The pharmacological evaluation of natural products - general and specific approaches to screening ethnopharmaceuticals. *J. Ethnopharmacol.* 1983, 8, 127–147, https://doi.org/10.1016/0378-8741(83)90050-8.

15. Bezerra, I.C.F.; Ramos, R.T.M.; Ferreira, M.R.A.; Soares, L.A.L. Chromatographic Profiles of Extractives from leaves of *Eugenia uniflora* L. *Braz. J. Pharmacogn.* 2018, 28, 92-101, https://doi.org/10.1016/j.bjp.2017.11.002.

16. Lima, L.B.; Viturino, W.A.; Silva, S.L.; Santos, E.C.F.; Machado, J.C.B.; Procopio, T.F.; Moura, M.C.; Napoleao, T.H.; Ferreira, M.R.A.; Soares, L.A.L. Chemical and antibacterial analysis of *Cinnamomum verum* leaves extract and fractions against multidrug resistant bacteria. *Nat. Prod. Res.* 2021, 1-6, https://doi.org/10.1080/14786419.2021.1902323.

17. Lima, L.B.; Viturino, W.A.; Silva, S.L.; Santos, E.C.F.; Machado, J.C.B.; Procopio, T.F.; Moura, M.C.; Napoleao, T.H.; Ferreira, M.R.A.; Soares, L.A.L. Evaluation of Antioxidant, Antibacterial and Enhancement of Antibiotic Action by *Punica granatum* Leaves Crude Extract and Enriched Fraction against Multidrug-Resistant Bacteria. *Chem. Biodivers.* 2021, e2100538, https://doi.org/10.1002/cvbd.202100538.
extract from *Croton blanchetianus* (Euphorbiaceae) leaves. *S. Afr. J. Bot.* **2020**, *133*, 30-39, https://doi.org/10.1016/j.sajb.2020.06.015.

19. Bandaranayake, W.M. Quality Control, Screening, Toxicity, and Regulation of Herbal Drugs. In *Modern Phytochemistry - Turning Medicinal Plants into Drugs*, 1st ed.; Ahmad, I., Aql, F., Owais, M., Eds.; Verlag GmbH & Co. KGaA: Weinheim, **2006**, 1, 25-53.

20. Shokry, A.; Khalil, M.; Ibrahim, H.; Soliman, M.; Ebrahim, S. Acute toxicity assessment of polyaniline/Ag nanoparticles/graphene oxide quantum dots on *Cypripedium vidae* and *Artemia salina*. *Sci Rep.* **2021**, *11*, 5336, https://doi.org/10.1038/s41598-021-84903-5.

21. Okumu, M.O.; Mbaru, J.M.; Gikunju, J.K.; Mbuthia, P.G.; Madati, V.O.; Ochola, F.O.; Jepkorir, M.S. *Artemia salina* as an animal model for the preliminary evaluation of snake venom-induced toxicity. *Toxicon* **2021**, *19*, 100082, https://doi.org/10.1016/j.toxicon.2021.100082.

22. Rachman, S.R.; Maryam, F.; Limbong, B.A. The Toxicity Test of Ethanol Extract of *Averhoa bilimbi* L. Using Brine Shrimp Lethality Test (BSLT) Method on shrimp larvae (*Artemia salina* Leach). *J. Pharm. Med. Sci.* **2020**, *4*, http://dx.doi.org/10.32814/jpms.v4i1.82.

23. Houngbémè, A.G.; Gomes, M.R.F.; Souza, I.A.; Paiva, P.M.G.; Napoleão, T.H. Assessment of Sub-Acute Toxicity of Saline Extract and Lectin D. brasi for its use in folkloric management of malaria. *Adv. Biotechnol.* **2019**, *6*, 1, https://doi.org/10.4103/0976-1290.121508.

24. Ogbole, O.O.; Aliu, L.O.; Abiodun, O.O.; Ajaiyeoba, E.O. Alpha-amylase Inhibition and Brine Shrimp Lethality Activities of Nine Medicinal Plant Extracts from South-West Nigerian Ethnomedicine. *J. Herbs Spices Med. Plants*. **2016**, *22*, 319-326, https://doi.org/10.1080/10496475.2016.1214941.

25. Ogbole, O.O.; Segun, P.A.; Adeniji, A.J. *In vitro* cytotoxic activity of medicinal plants from Nigeria ethnomedicine on Rhabdomyosarcoma cancer cell line and HPLC analysis of active extracts. *BMC Complement. Altern. Med.* **2017**, *17*, 1, https://doi.org/10.1186/s12906-017-2005-8.

26. Olawumi, O.; Ibrahim, O.; Christianah, F. Tetrahydrodronantharine Derivative: Anti-microbial Isolate from *Acanthospermum hispidum* DC. *Int. J. Adv. Chem.* **2014**, *2*, 182-184, https://doi.org/10.14419/ijac.v2i2.3519.

27. Hamidi, M.R.; Jovanova, B.; Panovska, T.K. Toxicological evaluation of the plant products using Brine Shrimp (*Artemia salina* L.) model. *Macedonian Pharm. Bull.* **2014**, *60*, 9-18, https://doi.org/10.33320/maced.pharm.bull.2014.60.01.002.

28. Parasuraman, S. Toxicological screening. *J. Pharmacol. Pharmacother.* **2011**, *2*, 74-79, https://doi.org/10.4103/0976-500X.81895.

29. Ganfon, H.; Bero, J.; Tchinda, A.T.; Gbaguidi, F.; Gbenou, J.; Moudachirou, M.; Frédérich, M.; Quentin-Leclercq, J. Antiparasitic activities of two sesqui-terpenic lactones isolated from *Acanthospermum hispidum* D.C. *J. Ethnopharmacol.* **2012**, *141*, 411-417, https://doi.org/10.1016/j.jep.2012.03.002.

30. Yahaya, T.; Sadiq, A.; Ifeoluwa, A.; Ikebeke, N.; Okahale, S.E.; Adeola, S.O. Behavioural effect of standardized aqueous whole plant extract of *Acanthospermum hispidum*: Ethnopharmacological justification for its use in folkloric management of malaria. *Pharmacologyonline*. **2013**, *3*, 45-55.

31. Chika, A.; Onyebueme, D.C.; Bello, S.O. Phytochemical analysis and evaluation of antidiabetic effects in alloxan-induced diabetic rats treated with aqueous leaf extract of *Acanthospermum hispidum*. *Afr. J. Biomed. Res.* **2018**, *21*, 81-85.

32. Njinga, N.S.; Kola-Mustapha, A.T.; Quadri, A.L.; Atolani, O.; Ayanni, R.O.; Buhari, M.O.; Amusa, T.O.; Ajani, E.O.; Folaranmi, O.O.; Bakare-Odunola, M.T.; Kambizi, L.; Oladiji, A.T.; Ebong, P. Toxicity Assessment of Sub-Acute and Sub-Chronic Oral Administration and Diuretic Potential of Aqueous Extract of *Hibiscus sabdariffa* Calyces. *Heliyon*. **2020**, *6*, https://doi.org/10.1016/j.heliyon.2020.e04853.

33. Isfernriehn, O.L.; Okolie, P.N. Acute and Sub-Acute Toxicity Profile of Methanol Leaf Extract of *Geophila obvallata* on Renal and Hepatic Indices in Wistar Rats. *Cogent Food Agric.* **2020**, *6*, https://doi.org/10.1080/23311932.2020.1794240.

34. Da Silva, G.C.; De Oliveira, A.M.; Machado, J.C.B.; Ferreira, M.R.A.; Medeiros, P.L.; Soares, L.A.L.; De Souza, I.A.; Paiva, P.M.G.; Napoleão, T.H. Toxicity assessment of saline extract and lectin-rich fraction from *Microgramma vaccinifolia* rhizome. *Toxicon*. **2020**, *187*, 65-74, https://doi.org/10.1016/j.toxicon.2020.08.021.

35. Gomes, M.R.F.; Schuh, R.S.; Jacques, A.L.B.; Dorneles, G.G.; Montanha, J.; Roche, P.M.; Bordignon, S.; Dallegrave, E.; Leal, M.B.; Limberger, R.P. Biological assessment (antiviral and antioxidant) and acute toxicity of essential oils from *Drimys angustifolia* and *D. brasiliensis*. *Braz. J. Pharmacogn.* **2013**, *23*, 284-290, https://doi.org/10.1590/S0102-695X20120005000142.
36. Erdenetsogt, U.; Nadmid, S.; Paulus, C.; Chanagsuren, G.; Dolgor, E.; Gotov, C.; Dahse, H.-M.; Luzhetskyy, A.; Dagvadorj, E. Bioactive flavonoids from plant extract of *Pyrethrum pulchrum* and its acute toxicity. *Nat. Prod. Res.* **2020**, *17*, 1-4, https://doi.org/10.1080/14786419.2020.1806271.

37. Jahani Yazdi, A.; Javanshir, S.; Soukhtanloo, M.; Jalili-Nik, M.; Jafarian, A.H.; Iranshahi, M.; Hasanpour, M.; Khatami, S.-M.; Hosseini, A.; Amiri, M.S.; Ghorbani, A. Acute and sub-acute toxicity evaluation of the root extract of *Rheum turkestanicum* Janisch. *Drug Chem. Toxicol.* **2020**, *4*, 609-615, https://doi.org/10.1080/01480545.2018.1561713.

38. Vysakh, A.; Jayesh, K.; Helen, L.R.; Jyothis, M.; Latha, M.S. Acute oral toxicity and anti-inflammatory evaluation of methanolic extract of *Rotula aquatica* roots in Wistar rats. *J. Ayurveda Integr. Med.* **2020**, *11*, 45-52, https://doi.org/10.1016/j.jaim.2017.09.007.

39. De Oliveira, A.M.; De Freitas, A.F.S.; Costa, W.K.; Machado, J.C.B.; Bezerra, I.C.F.; Ferreira, M.R.A.; Paiva, P.M.G.; Napelão, T.H.; Soares, L.A.L. Flavonoid-rich fraction of *Croton blanchetianus* Baill. (Euphorbiaceae) leaves: Chemical profile, acute and subacute toxicities, genotoxicity and antioxidant potential. *S. Afr. J. Bot.* **2022**, *144*, 238-249, https://doi.org/10.1016/j.sajb.2021.08.040.