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Research Article

Antioxidant Activity and Toxicity of Stem Bark Extracts of *Hancornia speciosa* Gomes (Apocynaceae)

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Abstract

Hancornia speciosa Gomes, popularly known as mangabeira, is a species from the Apocynaceae family widely distributed in Brazil and used in traditional medicine due to its therapeutic properties. This study aimed to evaluate the antioxidant activity and toxicity of stem bark extracts from this species. The extracts were obtained through sequential extractions using n-hexane, ethyl ether, and methanol. Antioxidant activity was assessed using the DPPH radical scavenging assay, while toxicity was evaluated through the *Artemia salina* bioassay. The results showed that the extracts exhibited significant antioxidant capacity, with emphasis on the ethereal extract (EEHS), which presented an IC_{50} of 7.35 µg/mL, a value close to that of the positive control, ascorbic acid (IC_{50} of 4.31 µg/mL). In the toxicity assay, EEHS showed higher toxicity, with an LC_{50} of 67.35 µg/mL, comparable to potassium dichromate, while the methanolic extract (EMHS) exhibited an LC_{50} of 561.7 µg/mL, indicating lower toxicity. These findings highlight the potential of the species as a source of bioactive compounds with antioxidant properties, while also emphasizing the need for further studies to structurally characterize the involved metabolites, elucidate their mechanisms of action, and evaluate the safety of these extracts for possible therapeutic applications. Additional assays using more complex biological models are essential to determine their viability in the development of new antioxidant agents and natural product-based drugs.

Keywords: *Metabolites; Free Radicals; Artemia Salina; Flavonoids; Therapeutic Applications.*

1. Introduction

Hancornia speciosa Gomes, popularly known as mangabeira, is a species of the Apocynaceae family widely distributed across Brazil, occurring in the phytogeographic domains of the Amazon, Caatinga, Cerrado, and Atlantic Forest [1]. Traditionally, various parts of the plant, including fruits, latex, and bark, are used in folk medicine for the treatment of inflammation, infectious diseases, hypertension, and metabolic disorders [2-6].

Previous studies have shown that its extracts contain a rich phytochemical composition, including flavonoids, catechins, proanthocyanidins, and tannins—bioactive compounds associated with various pharmacological properties such as antioxidant, anti-inflammatory, antimicrobial, and wound-healing activities [3-11].

The Apocynaceae family is well known for its phytochemical richness and therapeutic potential. Several studies report that species from this family exhibit significant antioxidant activity, mainly attributed to the presence of phenolic compounds and flavonoids [12]. The antioxidant action of these compounds occurs through the neutralization of free radicals produced by endogenous and exogenous reactions, thereby reducing oxidative stress and preventing damage to essential biomolecules such as lipids, proteins, and DNA [13, 14]. These free radicals, including reactive oxygen species (ROS) and reactive nitrogen species (RNS), are highly unstable and can trigger chain reactions leading to cellular degeneration and the development of various chronic diseases, including cancer, diabetes, and neurodegenerative disorders—underscoring the importance of investigating new natural antioxidant sources [15, 16, 17].

In addition to antioxidant activity, the toxicity assessment of natural products, including plant extracts, is essential to ensure their safety and feasibility for therapeutic applications. Toxicological assays are crucial to determine potential adverse effects and to establish parameters for the safe use of bioactive compounds [18]. In this context, our hypothesis is that the extracts of *Hancornia speciosa* Gomes contain bioactive compounds with antioxidant activity and no significant toxicity in the *Artemia salina* model. The present study aimed to evaluate the antioxidant capacity and toxicity of stem bark extracts of *Hancornia speciosa* Gomes through the DPPH radical scavenging assay and the *Artemia salina* toxicity test, respectively.

2. Methodology

2.1 Botanical material collection and extract preparation

The stem bark of *H. speciosa* was collected in an Environmental Protection Area of the Chapada do Araripe, Jardim, Ceará, Brazil (7°29′02.4″S, 39°16′51.9″W, 920 m altitude), with proper authorizations from the Authorization and Information System on Biodiversity (SISBio), under registration number 80293–1, and the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen), under registration number A535238. A voucher specimen was deposited in the UFP Herbarium – Geraldo Mariz at the Federal University of Pernambuco (#88,947). Extracts were prepared following the methodology described by Silva et al. [10], in which the bark was dehydrated, ground, and subjected to sequential extraction with *n*-hexane, sulfuric ether, and methanol for 72 hours each. After filtration and solvent removal, the extracts were stored in amber vials until further analysis. The hexane extract was not used in this study.

2.2 DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay

The DPPH free radical scavenging assay was employed to assess the antioxidant activity of *H. speciosa* extracts, as described by Kamdem et al. [19], with minor modifications. The assay was conducted using 96-well plates, where 100 µL of freshly prepared DPPH solution (0.3 mM in ethanol) was added along with 50 µL of ethanol and 50 µL of the extracts at final concentrations ranging from 2 to 1024 µg/mL. The plates were kept in the dark at room temperature for 30 minutes. The reduction of the DPPH radical was evaluated by measuring the decrease in absorbance at 517 nm using a microplate reader (SpectraMax, Sunnyvale, CA, USA). Ascorbic acid (Vitamin C) was used as a positive control. Results are expressed as the percentage of inhibition of DPPH absorbance relative to the control values. The inhibition percentage was calculated using the formula:% = 100 - [(Abs sample - Abs blank) × 100 / Abs control].

2.3 Artemia salina toxicity assay

The *Artemia salina* toxicity assay was conducted following the protocol established by Meyer [20], with adaptations. *A. salina* cysts were placed in artificially prepared seawater and kept under constant aeration for 24 hours. After this period, *H. speciosa* extracts were prepared at different concentrations (1–1000 µg/mL), and ten larvae were added to each concentration. Potassium dichromate ($K_2Cr_2O_7$), dissolved in DMSO, was used as the positive control. After 24 hours, sample readings were performed using a stereomicroscope. At the end of the experiment, the median lethal concentration (LC_{50}) was determined.

2.4 Statistical analysis

Statistical analyses for antioxidant activity and toxicity were performed using GraphPad Prism 6 software. Data were expressed as the mean of triplicate determinations (n = 3) ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey's test was used to compare groups, adopting a 95% confidence level ($p < 0.05$). For toxicity assays, Dunnett's test was also employed for multiple comparisons. The LC_{50} value was determined by linear regression, considering a compound active when $LC_{50} < 1000$ µg/mL.

3. Results

3.1 Antioxidant Activity

The results of the antioxidant activity showed that all tested concentrations of *Hancornia speciosa* extracts were capable of reducing the DPPH radical. The IC_{50} values for the ether and methanolic extracts were 7.35 and 11.45 µg/mL, respectively, while the IC_{50} of ascorbic acid, a potent antioxidant used as a positive control, was 4.31 µg/mL (Figures 1 and 2). These data suggest that ascorbic acid exhibited greater efficacy in neutralizing the DPPH radical. However, the extracts also demonstrated significant antioxidant effects, with the ether extract standing out particularly at concentrations of 128 and 256 µg/mL (Figures 1 and 2).

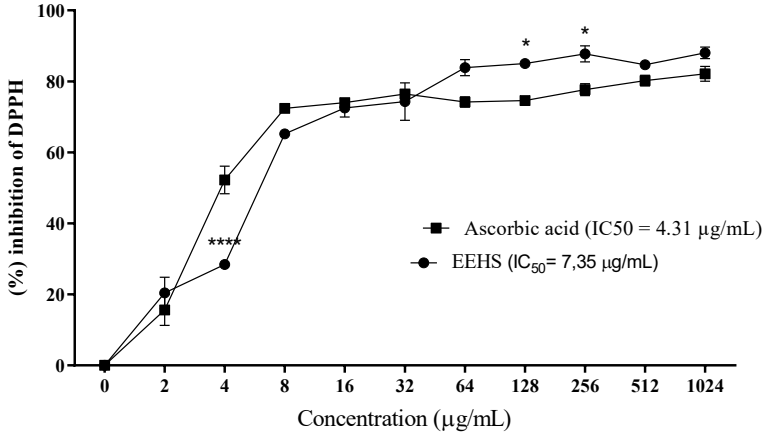

Figure 1. *Inhibitory capacity of DPPH radicals by the sulfuric ether extract of Hancornia speciosa Gomes (EEHS). Results are presented as mean ± standard error of the mean (SEM), with N = 3.*

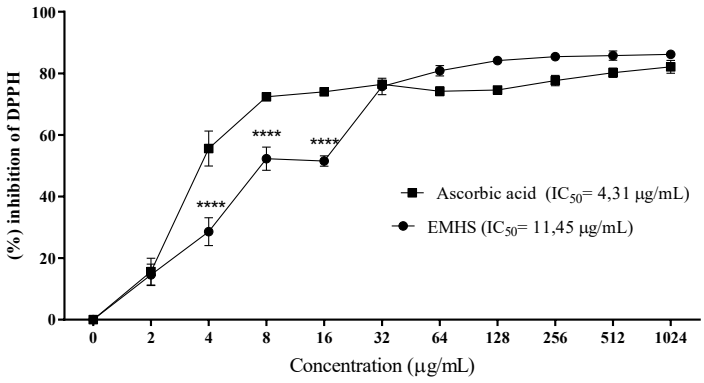

Figure 2. *Inhibitory capacity of DPPH radicals by the methanolic extract of Hancornia speciosa Gomes (EMHS). Results are presented as mean ± standard error of the mean (SEM), with N = 3.*

3.2 Toxic Activity in Artemia salina

The ether (EEHS) and methanolic (EMHS) extracts of *H. speciosa* exhibited toxicity against *A. salina* starting at concentrations of 25 µg/mL and 10 µg/mL, respectively (Figures 3 and 4). This toxic effect progressively increased, reaching a lethality plateau at concentrations of 250 µg/mL (EMHS) and 1,000 µg/mL (EEHS). EEHS showed a toxic effect comparable to that of the positive control, with an LC_{50} of 67.35 µg/mL, while the positive control ($K_2Cr_2O_7$) exhibited an LC_{50} of 59.68 µg/mL. In contrast, EMHS presented an LC_{50} of 561.7 µg/mL, indicating it was less toxic than potassium dichromate ($K_2Cr_2O_7$) at concentrations below 1,000 µg/mL.

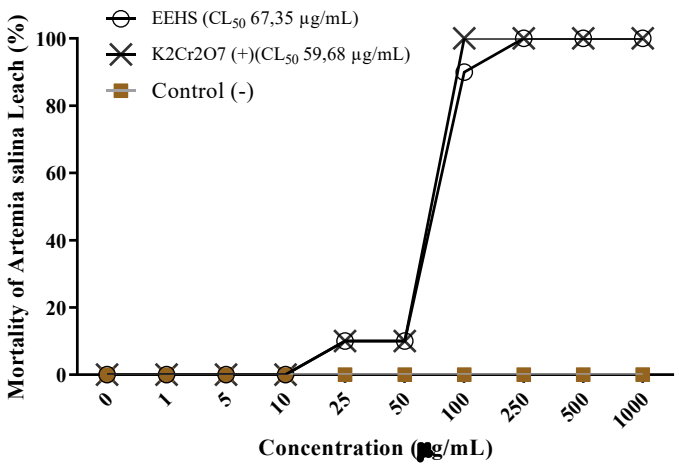

Figure 3. *Mortality percentage of Artemia salina exposed to the sulfuric ether extract of Hancornia speciosa Gomes (EEHS). Values were determined as the relative percentage of non-surviving organisms, with N = 3.*

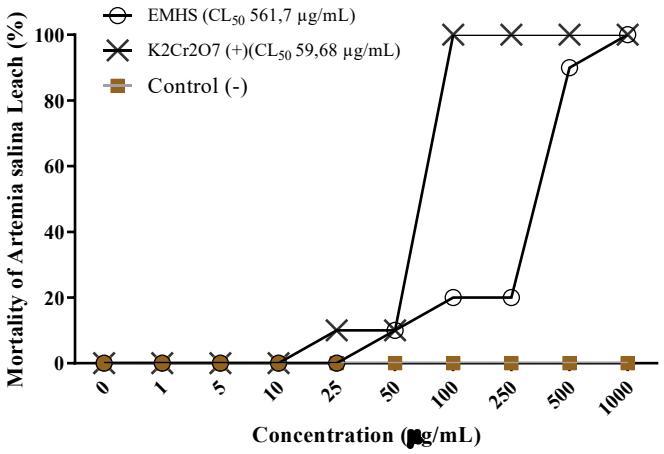

Figure 4. *Mortality percentage of Artemia salina exposed to the methanolic extract of Hancornia speciosa Gomes (EMHS). Values were determined as the relative percentage of non-surviving organisms, with N = 3.*

4. Discussion

Plant extracts are natural substances with complex chemical compositions. The compounds present in these extracts are responsible for their biological activity and may act either individually or synergistically [21]. Previous studies analyzed the chemical composition of the ether and methanolic extracts from the bark of *Hancornia speciosa* using different analytical techniques. Characterization through liquid chromatography (UPLC–QTOF–MS/MS) enabled the identification of compounds such as catechin, chlorogenic acid, epicatechin, procyanidin B dimer and trimer, vanillic acid, quinic acid, phloretin, cinchonain IIb, hexoside isomers of lariciresinol, cinchonain Ib isomers, and gluconic acid [11].

Meanwhile, analysis by gas chromatography coupled with mass spectrometry (GC–MS) revealed the presence of fatty alcohols, carbohydrates, fatty acids, phenolic acids, phytosterols, and triterpenoids in the same extracts [10].

The results demonstrated that the bark extracts of *H. speciosa* exhibited antioxidant activity. Previous studies have also reported antioxidant activity in extracts from *H. speciosa* leaves [9] and fruits [22]. In our study, this activity can be attributed to the presence of phenolic compounds and flavonoids previously identified in the species. Chlorogenic acid, for example, is widely recognized for its potent antioxidant properties, stemming from its ability to protect against oxidative stress and play an important role in preventing cellular damage [23]. Additionally, phloretin, identified in the extracts, may also contribute to the observed antioxidant activity. Studies indicate that phloretin is an effective antioxidant in scavenging reactive oxygen species, including peroxynitrite, and inhibiting lipid peroxidation, due to the presence of aromatic hydroxyl groups and a carbonyl group in its structure. These groups play a key role in stabilizing free radicals, preventing oxidative damage in biological systems [24, 25].

Flavonoids such as catechin and epicatechin also play a significant role in neutralizing reactive oxygen species (ROS), either by directly donating electrons or by regulating endogenous antioxidant enzymes. Catechin contains phenolic hydroxyl groups in its structure, allowing it to donate electrons to neutralize free radicals and interrupt the propagation of oxidative stress. Furthermore, these molecules may modulate cell signaling pathways, promoting the expression of antioxidant enzymes like superoxide dismutase and catalase, which are essential in cellular defense against oxidative damage [26].

A previous study observed that the EEHS and MEHS extracts showed significant concentrations of phenolic compounds, with 346.4 and 340.0 mg of GAE/g, respectively. In contrast, flavonoids were found in lower amounts, with 7.6 and 6.9 mg of QE/g, respectively [10]. Phenolic compounds and flavonoids are among the main contributors to the antioxidant properties of medicinal plants due to their effectiveness in combating oxidative stress [9]. Phenolic compounds are capable of donating hydrogen atoms, allowing them to directly neutralize free radicals and minimize the damage caused by oxidative stress. This characteristic gives these compounds strong antioxidant activity, making them essential in protecting cells against damage related to aging and various degenerative diseases [14, 27].

Previous studies also suggest that phenolic compounds and flavonoids can modulate the inflammatory response, promoting additional therapeutic effects such as reducing the risk of cardiovascular disease and cancer by protecting biological systems from inflammatory damage caused by excess free radicals [28-30]. The presence of these compounds in the bark of *H. speciosa* highlights the plant's therapeutic potential, emphasizing its relevance in traditional medicine and its promise as a source of new natural antioxidant agents.

In our study, the toxic effect of EEHS was similar to that of potassium dichromate ($K_2Cr_2O_7$), while MEHS showed lower toxicity, being less lethal at concentrations below 1,000 µg/mL. On the other hand, when tested in *Drosophila melanogaster*, the extracts showed no toxicity at clinically relevant concentrations. EEHS caused no toxic effects up to 10 mg/g, and MEHS showed no toxicity at any of the concentrations tested [10].

The toxicity observed in this study may be related to the presence of phenolic compounds and flavonoids in the extracts. Although these secondary metabolites are recognized for their antioxidant and therapeutic properties, they may exhibit cytotoxic effects depending on the dose and the type of cells involved. These findings underscore the importance of assessing the toxicity of plant extracts in different biological systems to determine their safety and suitability for therapeutic applications [29, 31, 32].

Further studies are needed to better understand the mechanisms of action of the compounds present in the extracts, as well as their safety and therapeutic potential. Additional tests, including those involving more complex animal models, may provide more detailed information on the toxicity and feasibility of using these products as potential antioxidant or therapeutic agents. Moreover, pharmacokinetic and pharmacodynamic studies are essential to understand the absorption, distribution, metabolism, and excretion of these compounds in the body, as well as their biological effects and possible interactions with cellular targets. Evaluating these parameters can help determine the bioavailability, safe dosage, and therapeutic efficacy of the extracts, enabling their safer and more effective use in the development of new drugs.

5. Conclusion

The stem bark extracts of *Hancornia speciosa* demonstrated significant antioxidant activity, as evidenced by their DPPH radical scavenging capacity. These findings reinforce the potential of this species as a source of bioactive compounds with antioxidant properties, supporting previous studies that highlight the presence of flavonoids, tannins, and other secondary metabolites with protective effects against oxidative stress.

Both extracts showed toxic effects in *Artemia salina*, with the ethereal extract being more potent, presenting an LC_{50} close to the positive control. These results support further investigations aimed at structurally characterizing the compounds responsible for antioxidant activity and conducting broader biological assays to validate their efficacy and safety in more complex models and their potential therapeutic applications.

Conflict of Interest

The authors declare no conflict of interest.

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