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TOXICITY ASSESSMENT OF A BEVERAGE MADE FROM AÇAÍ SEEDS USING BIOASSAYS

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ABSTRACT

This study evaluated the toxicity of a beverage prepared from roasted and ground açaí seeds, aiming to verify its safety for human consumption. Bioassays were conducted using Lactuca sativa (lettuce seeds) and Artemia salina (microcrustacean), chosen for their sensitivity and applicability in preliminary toxicity tests. The tested concentrations ranged from 1.0 to 10 mg/mL, with positive and negative controls. In the positive control, using 2% potassium dichromate, the tested organisms exhibited significant toxicity, confirming the efficacy of the assays. In the negative control, the artemia remained mobile, and the lettuce seeds germinated normally. At all tested product concentrations, no signs of toxicity were detected in the bioassays, reinforcing the beverage's toxicological safety. These findings indicate that açaí coffee is safe for human consumption, contributing to the sustainable reuse of açaí waste.

Keywords: Ethnobotany. Euterpe oleracea. Phytotherapy. Sustainability.

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INTRODUCTION

Phytotherapy, an ancient practice that uses plants and their derivatives for therapeutic purposes, has played a central role in various healthcare systems worldwide, being recognised by the World Health Organisation (WHO) as an important ally in expanding access to healthcare (WHO, 2004). In Brazil, this recognition led to the creation of the National Policy on Medicinal Plants and Phytotherapies (PNPMF) in 2006, aiming to promote the rational use of natural resources while balancing healthcare access and environmental conservation (BRASIL, 2006). Amazonian biodiversity, in particular, emerges as a strategic source for the development of phytopharmaceutical products, with emphasis on *Euterpe oleracea Mart.* (açaí palm), which possesses promising pharmacological properties, such as antioxidant, anti-inflammatory, and cardioprotective effects (PIMENTEL et al., 2005; DE BEM et al., 2020; MAGALHÃES, 2021).

While the pulp of açaí is widely consumed, its seed, often discarded as waste, has attracted increasing interest due to its bioactive and nutritional potential. Studies suggest that the seeds can be repurposed in the preparation of products such as "açaí coffee," a sustainable alternative that adds value to this by-product and has gained popularity in specialised markets in Brazil (COSTA et al., 2021). The repurposing of açaí seeds not only contributes to the circular economy but also promotes the sustainable development of the production chain. However, the use of new products derived from agro-industrial waste requires thorough safety assessments.

Despite its growing popularity, the consumption of açaí coffee lacks scientific validation, particularly regarding its potential toxicity. According to Fernandes et al. (2011), compounds present in the seeds may exhibit relevant pharmacological activities, but the absence of detailed toxicological studies limits their regulation and therapeutic application. In this context, the evaluation of the toxicity of natural products is a crucial step in the development of phytopharmaceuticals. Bioassays with biological models such as *Lactuca sativa* (lettuce) and *Artemia salina* (brine shrimp) are widely used to investigate toxicity, mutagenicity, and genotoxicity due to their high sensitivity, reproducibility, and low cost (LORENZI and MATOS, 2008).

This study is situated within the broader context of science aimed at the sustainable use of natural resources, with significant impacts on public health, the economy, and the environment. The repurposing of agro-industrial waste, such as açaí seeds, contributes to the promotion of sustainable practices and the circular economy. Amazonian biodiversity,

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rich in bioactive compounds, is a strategic resource for the development of new phytopharmaceutical products. However, the use of new derived products requires a rigorous evaluation of their safety to ensure consumer health. The toxicity of products like açaí coffee still lacks detailed studies to demonstrate their viability. Therefore, the objective of this study was to assess the toxicity of the beverage prepared from roasted and ground açaí seeds, using bioassays with *Lactuca sativa* and *Artemia salina*.

MATERIAL AND METHODS

The study was conducted at the Laboratory of Basic and Health Sciences (LaCiBS) at the Federal University of Tocantins (UFT), aiming to evaluate the toxicity of the beverage prepared from the roasted and ground seeds of the açaí palm (*Euterpe oleracea Mart.*). For this purpose, laboratory bioassays were employed using widely recognized methodologies for the toxicological screening of natural products, following the protocols described by Lorenzi and Matos (2008). The tests performed were essential to ensure the safety of the beverage consumption, providing a preliminary assessment of its potential toxic effects. All experimental stages strictly adhered to the established methodological principles, ensuring the validity and reliability of the results, in accordance with the relevant ethical and scientific standards.

This is an experimental laboratory study focused on acute toxicity testing, using bioindicators for the preliminary evaluation of the safety of açaí coffee for human consumption. The experiments were conducted at the LaCiBS facilities, located at UFT, in Palmas-TO, from December 2022 to July 2023. The açaí coffee powder samples were obtained directly from a local manufacturer. The infusion was prepared by dissolving 10 grams of the powder in 1 liter of distilled water, previously heated to the boiling point. After extraction, the solution was cooled to room temperature and diluted into five distinct concentrations: 1.0; 2.5; 5.0; 7.5; and 10 mg/mL. These dilutions were used in toxicity bioassays, performed with two biological models: *Lactuca sativa* (lettuce seeds) and *Artemia salina* (brine shrimp nauplii).

TOXICITY TEST WITH LACTUCA SATIVA

Organic lettuce seeds, free of agricultural pesticides, were used as toxicity bioindicators. The tests were conducted in sterile containers, with 10 seeds distributed on two sheets of filter paper, moistened with 5 mL of the different concentrations of the

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prepared beverage. As controls, distilled water (negative control) and a 2% potassium dichromate solution (positive control) were used. The containers were kept in a germination chamber at 25°C for five days. During this period, the seeds were rehydrated on the third day with an additional 5 mL of the corresponding solutions. At the end of the experiment, germination and root growth were evaluated using a digital caliper. The data were recorded in spreadsheets for subsequent quantitative analysis.

TOXICITY TEST WITH ARTEMIA SALINA

The bioassays with *Artemia salina* nauplii followed the guidelines of Mirzaei and Mirzaei (2013) and the Organisation for Economic Co-operation and Development (OECD, 2004). The *Artemia* eggs were incubated in artificial seawater prepared with 30 g/L of marine salt dissolved in distilled water, adjusted to pH 9.0, and maintained under continuous illumination for 72 hours at temperatures ranging from 27°C to 29°C. After hatching, the nauplii were transferred to test tubes containing 0.5 mL of each concentration of the prepared beverage, and the final volume was adjusted to 5 mL with artificial seawater. Negative (artificial seawater) and positive (2% potassium dichromate solution) controls were used. After 24 hours of exposure, nauplii mortality was recorded, with immobile nauplii, unable to move for more than 10 seconds, considered dead. The assays were performed in triplicate, and the data were recorded in spreadsheets to calculate the mortality rate.

The obtained results were analyzed using descriptive and inferential statistics, including the dose-response relationship analysis. Toxicity was interpreted through the comparison of the seed germination and root growth indices and the nauplii mortality rate, relative to the controls. The research did not involve direct intervention with human subjects, being conducted exclusively with model organisms widely used in the scientific literature. Therefore, prior approval by an Ethics Committee in Research was not required.

RESULTS

The bioassays conducted with *Lactuca sativa* and *Artemia salina* produced consistent results that demonstrate the toxicological safety of the açaí coffee, prepared from roasted and ground seeds, at the concentrations tested (1.0; 2.5; 5.0; 7.5; 10 mg/mL). The seeds exposed to the beverage exhibited germination rates and root development comparable to the negative control, which used distilled water. Analyses of mean growth

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and maximum root length (Figures 1 and 2) indicated that the açaí coffee did not exert any adverse effect on the biological processes essential for germination, even at the highest concentrations. In contrast, the positive control with potassium dichromate at 2% showed complete inhibition of seed germination, confirming the effectiveness of the experimental protocol in identifying toxic compounds.

Figure 1 Mean growth (in cm) of *Lactuca sativa* seedlings exposed to different concentrations of açaí coffee beverage.

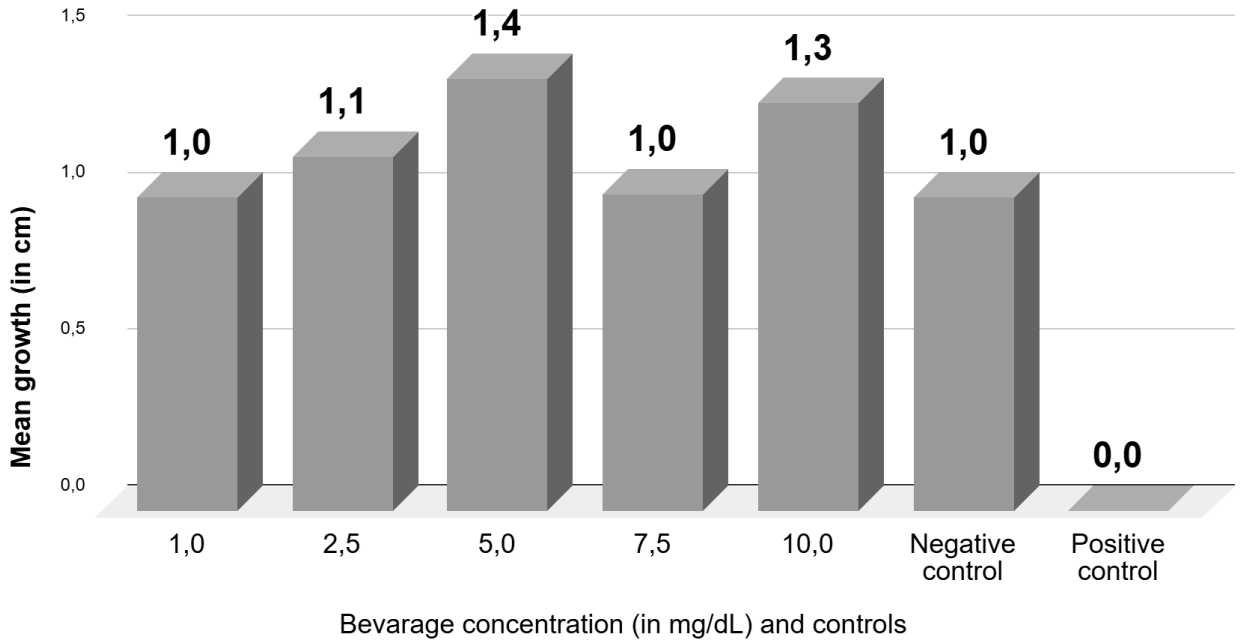
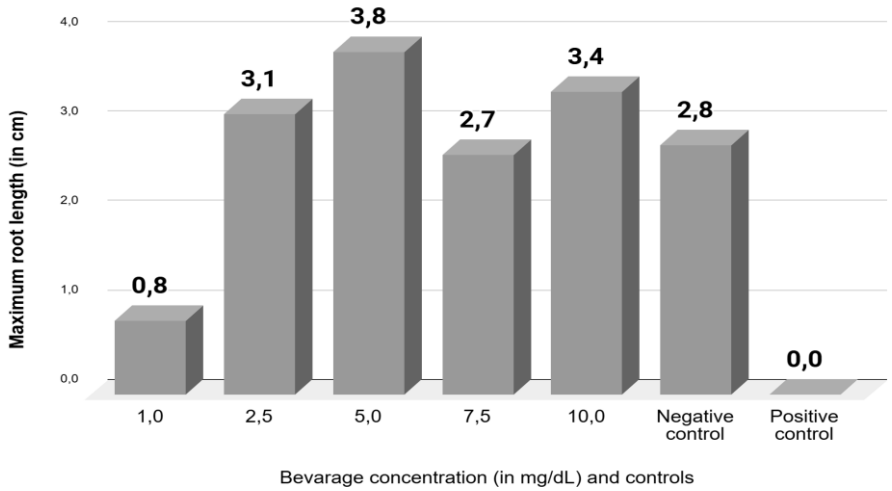


Figure 2 Maximum root length (in cm) of *Lactuca sativa* seedlings exposed to different concentrations of açaí coffee beverage.



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In the bioassay with *Artemia salina*, the nauplii mortality was assessed after 24 hours of exposure. At all tested concentrations, the nauplii remained active and mobile, with no signs of mortality (Table 1), suggesting the absence of acute toxic effects associated with the beverage. In the negative control, composed of artificial seawater, the nauplii also remained viable, supporting the reliability of the results. In contrast, the positive control, using a 2% potassium dichromate solution, resulted in complete mortality, confirming the sensitivity of the model to detect toxicity.

Table 1: Mortality rate of *Artemia salina* nauplii after 24 hours of exposure to different concentrations of açaí coffee.

Sample	01	02	03	Mortality (%)
Positive control	0	0	0	100
Negative control	10	10	10	0
Concentration 10mg/mL	10	10	10	0
Concentration 7,5 mg/mL	10	10	10	0
Concentration 5,0 mg/mL	10	10	10	0
Concentration 2,5 mg/mL	10	10	10	0
Concentration 1,0 mg/mL	10	10	10	0

A comparative analysis of the two bioassays demonstrates that the açaí coffee does not exert adverse effects on the tested organisms, both in plant and aquatic models. This consistency in the results strengthens the robustness of the methodological approach used and indicates the absence of significant toxicity of the product, suggesting its safety under the tested conditions. Furthermore, the data obtained from the replicates conducted in both assays enhance the reliability of the findings, positioning açaí coffee as a safe and promising product, both for human consumption and sustainable development.

DISCUSSION

The bioassays conducted with *Lactuca sativa* confirmed the absence of phytotoxicity associated with açaí coffee. The seeds exposed to the beverage showed normal germination and root development at all tested concentrations, with results consistent with the negative control (distilled water). These findings suggest that the compounds present in açaí coffee do not interfere with the biological processes essential for the initial growth of plants, reinforcing the safety of the product.

The positive control, consisting of 2% potassium dichromate, resulted in complete inhibition of seed germination, thus validating the sensitivity and efficacy of the experimental

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protocol. The reliability of the *Lactuca sativa* model for preliminary screening of environmental and food toxicity is widely recognized, as highlighted by Apolleti et al. (2017), and is effective in identifying potentially harmful substances. Moreover, *Lactuca sativa*, both raw and processed, has other uses beyond toxicity evaluation, such as in electricity generation and efficient organic fertilization (Lima, 2015). These data expand the applicability of the experimental model, offering new perspectives for its use in different sustainability contexts and the utilization of natural resources.

Similarly, tests with *Artemia salina* revealed the toxicological safety of açaí coffee in simple aquatic systems. The absence of nauplius mortality at all tested concentrations suggests that the product does not present acute toxicity, even at high exposure levels. The positive control with potassium dichromate caused total mortality, confirming the method's efficacy in detecting toxic compounds. These results align with the findings of Meyer et al. (1982), who validate *Artemia salina* as a sensitive model for evaluating acute toxicity in natural products. The convergence of the bioassay results in two distinct experimental models strengthens the robustness of the conclusions of this study. The observed absence of toxicity in both plant models and simple aquatic organisms suggests that açaí coffee is safe for human consumption and environmentally compatible. This consistency is particularly relevant in the context of natural product toxicology, as it demonstrates that the beverage does not negatively interfere with representative biological systems. The safety profile obtained positions açaí coffee as a viable and sustainable alternative, supporting its potential as an innovative product derived from Amazonian biodiversity.

The presented results are even more significant when considered in the current context of valorizing natural and regional products. The açaí seed, previously discarded as agricultural waste, has gained relevance as raw material for high-value-added products, such as açaí coffee. This transformation aligns with the principles of circular economy, promoting the reuse of by-products and waste reduction. Dias et al. (2020) highlight the positive economic and environmental impact of reusing açaí seeds, contributing to sustainable agricultural practices and income generation in local communities.

It is important, however, to acknowledge the limitations of the bioassays conducted. Although *Lactuca sativa* and *Artemia salina* are widely validated models, they provide only a preliminary and qualitative assessment of the product's toxicity. To confirm the safety of açaí coffee at more complex levels of biological organization, further studies are needed, such as analyses in cellular systems, pre-clinical trials, and clinical trials in humans.

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Additionally, the characterization of the bioactive compounds in the beverage, such as polyphenols and flavonoids, could provide more detailed information on the mechanisms underlying its safety and potential health benefits.

Finally, the introduction of açaí coffee into the herbal medicine and functional beverage markets presents a significant opportunity for the valorization of Amazonian biodiversity and for the economic diversification of extractivist communities. By adding value to a previously discarded by-product, açaí coffee contributes to the development of more inclusive and sustainable production chains. Pacheco et al. (2021) emphasize that sustainable natural resource management practices are essential for regional development. However, challenges such as the standardization and regulation of natural products must be addressed, considering the chemical variability of these products and their impact on safety and efficacy.

This study not only validates the toxicological safety of açaí coffee but also opens new perspectives for its application and commercialization. With further investigations into its functional benefits and the development of methods for standardization and quality control, açaí coffee can consolidate itself as a safe, innovative, and sustainable alternative in the functional food and herbal medicine market.

The results obtained from the bioassays with *Artemia salina* and *Lactuca sativa* consistently demonstrated the absence of significant toxicity of açaí coffee, even at the highest tested concentrations, ranging from 1.0 to 10 mg/mL. These findings indicate that the beverage prepared from roasted and ground açaí seeds is safe for human consumption under the evaluated conditions, reinforcing its potential as a herbal and nutritional alternative. The absence of toxic effects observed in widely recognized model organisms for toxicity screening further corroborates the product's safety profile and its viability as an innovation in the functional food market.

In addition to validating the safety of açaí coffee, this study highlights its environmental and economic relevance, promoting the reuse of agricultural by-products. The transformation of açaí seeds into a high-value product reflects an approach aligned with circular economy principles, contributing to the sustainable management of natural resources. This initiative not only reduces waste but also has the potential to generate positive impacts on the socio-economic development of producing communities.

The contributions of this research include preliminary data that establish a solid foundation for the toxicological characterization of açaí coffee, raising important questions

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for future studies. Further investigations, including cellular models, pre-clinical trials, and clinical trials in humans, are essential to confirm the product's safety at higher levels of biological organization. An in-depth analysis of the bioactive compounds in the beverage, such as polyphenols and flavonoids, could provide evidence on the mechanisms associated with its safety, as well as its potential functional properties, such as antioxidant and anti-inflammatory effects.

Therefore, we can mention that açaí coffee represents a safe, sustainable, and innovative alternative, with the potential to contribute to the diversification of food products and the valorization of regional by-products. By integrating science, sustainability, and innovation, this study establishes a promising initial foundation for expanding the use of açaí coffee in the herbal medicine and functional food market, while also reinforcing the importance of integrated practices that promote the preservation of Amazonian biodiversity and strengthen local production chains, contributing to a more sustainable and inclusive future.

CONCLUSION

The bioassays with *Lactuca sativa* and *Artemia salina* demonstrated that the beverage made from roasted and ground açaí seeds is not toxic, indicating its safety for human consumption. These results suggest that the beverage does not present significant adverse effects on basic biological processes, supporting its potential as a safe and viable product for human use.

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