EVALUATION OF COMPOSITION, ANTIOXIDANT ACTIVITY AND CYTOTOXICITY OF GUARANA PEEL (*Paullinia cupana*) LIPID FRACTION

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ABSTRACT

This study aimed to evaluate guarana peel oil's composition, cytotoxicity, and antioxidant activity. The extraction was carried out in a Soxhlet apparatus using hexane. Composition, acid value, and gas chromatography-mass spectroscopy (GC-MS) analyses were carried out on the extracted oil, in addition to the evaluation of antioxidant activity and cytotoxicity. The extractive composition in guarana peel (10.81 %) revealed possibilities for studies with the lipid fraction. The extracted oil showed characteristics of an oleoresin. The acid number was high (27.81 mg KOH g^{-1}), which is associated with the hot extraction process. The chromatogram of the extracted oil identified sesquiterpene hydrocarbons alpha-copaene, caryophyllene, and delta cadinene, which demonstrates the potential of this bioproduct, as sesquiterpenes are bioactive and associated with anti-inflammatory, antimicrobial, antitumor and other properties. The compound 8-Androsten-3-ol was predominant in the chromatogram (33.87 %), however, more studies are needed to elucidate its properties and functions. The oil had an IC_{50} of 70.22 mg/mL and a cytotoxic concentration of 750 µg/mL.

Keywords: Byproduct. Lipid fraction. Potential assessment.

1 INTRODUCTION

Guarana (*Paullinia cupana*) is a fruit of the guarana tree, a plant native to the Amazon region, widely cultivated and economically important in Brazil. The seed is the only part of fruit commercialized, with production focused heavily on the formulation of carbonated and energy drinks.[1] In the guarana production chain, by-products such as peels, husks, and depleted seeds are formed. After harvesting the fruit, the peels are removed, then the seeds are roasted and finally, the husk is removed.[2] In the beverage industry, roasted seeds are subjected to hydroalcoholic extraction to obtain extracts, which generates depleted seeds.[3]

Residual biomass from the guarana production chain has not yet been widely studied regarding the investigation and extraction of natural bioactive. The use of by-products from agroindustry is directly linked to the concept of circular bioeconomy and alternative sources for the production of bioproducts are currently being researched. The composition of guarana peel includes insoluble fibers, macro (Ca, K, and Mg) and micro (Cu, Fe, Mn, and Zn) minerals, caffeine, theobromine, phenolic compounds, and carotenoids. Trials indicated that guarana peel extract has valuable potential for application as an antioxidant in functional food products.[4,5] Based on this, the objective of this study was to investigate the extraction and composition of fixed oil from guarana peel, in addition to evaluating its antioxidant activity and cytotoxicity, to present the potential of this bioproduct.

2 MATERIAL & METHODS

The guarana peels were manually removed from the fresh fruits, sourced from Urucará, a city in Amazonas. The peels were dried at 50 °C for 24 h, and then the dried sample was ground. The composition of the guarana peel was determined according to NREL (National Renewable Energy Laboratory) methods[6,7] and the AOAC (Association of Official Analytical Chemists) method for protein content.[8] The acidity index of the extracted oil was determined according to AOCS (American Oil Chemist's Society).[9] In gas chromatography coupled to mass spectroscopy (GC-MS), the esterification step was carried out according to O'Fallon et al.[10] Methyl esters were analyzed on an Agilent 7890A GC-MS (Agilent Technologies) with an HP-5MS column. The evaluation of antioxidant activity was carried out using the DPPH radical scavenging method, with analysis carried out on a methanolic sample extract. The results were expressed as an IC_{50} (mg/mL) value, as described by Purkait et al.[11] with modifications. In the cytotoxicity assay, samples were resuspended in DMSO (dimethyl sulfoxide), filtered (0.22 µm), and diluted in a medium at different concentrations quadruplicate (0-1000 µg/mL). The cytotoxicity after direct contact with extracts was evaluated with a murine cell line of fibroblasts (L929, Thermo Scientific, Brazil) seeded in 96-well cell culture plates (Corning Life Sciences), following the ISO 10993-5 (2009).[12]

3 RESULTS & DISCUSSION

The guarana dry peel (50 °C) composition presented 12.39 ± 0.03 % moisture, 1.30 ± 0.02 % ash, 10.81 ± 1.89 % extractives, 8.04 ± 0.39 % proteins, and 67.46 % carbohydrates. The extractive content present in dried guarana peel demonstrates that the lipid fraction can be explored. The extracted oil did not present a liquid characteristic, it presented a viscous appearance. Based on RDC Resolution N° 270 of 2005[13], the extracted lipid presented characteristics of a vegetable fat at room temperature, or can even be defined as an oleoresin, as it appeared semi-solid. The oil presented an acidity index of 27.81 ± 0.03 mg KOH g^{-1}, above that observed in vegetable oils, which is associated with the process of hot extraction, which causes thermal degradation and greater release of free fatty acids. The acidity index presented values relatively close to oleoresins extracted from panca pepper

(*Capsicum chinense*) (39.17 mg KOH g^{-1})[14] and copaíba (*Copaifera reticulata*) (35.80 mg KOH g^{-1})[15]. Table 1 shows the identification of compounds related to the peaks highlighted in the chromatogram.

Table 1 Compounds identified in the chromatogram.

Peak	Area (%)	Compound	CAS number	Molecular formula	Molar mass (g/mol)
1	1.7353	Alpha-Copaene	1000360-33-0	$C_{15}H_{24}$	204.35
2	1.0328	Caryophyllene	000087-44-5	$C_{15}H_{24}$	204.35
3	2.5032	Delta-Cadinene	000483-76-1	$C_{15}H_{24}$	204.35
4	7.5758	9,19-Cyclocholest-24-en-3-ol, 14-methyl-, (3.beta.,5.alpha.)-	068654-82-0	$C_{28}H_{46}O$	398.70
5	16.1428	(E)-14.alpha.-Methyl-5.alpha.-ergosta-8,23-dien-3.beta.-ol	090195-44-1	$C_{29}H_{48}O$	412.00
6	33.869	8-Androsten-3-ol, 17-(2-methylallyl) -4,4,14-trimethyl-	1000197-34-3	$C_{28}H_{44}O_2$	412.60
7	17.4761	Cycloertano	000469-38-5	$C_{30}H_{50}O$	426.70

Alpha-copaene is a tricyclic sesquiterpene with related properties of antioxidant, antimicrobial, antimutagenic[16], analgesic, anti-inflammatory[17], etc. Caryophyllene is a natural bicyclic sesquiterpene that exerts anti-inflammatory action[18]. Delta-cadinene is a bicyclic sesquiterpene, with reports of antimicrobial[19], antioxidant[20], and anticancer actions[21]. Compounds of peaks 4, 5, and 6 have scarce information in the literature. The compound 8-Androsten-3-ol presented the largest area (33.87 %), and its predominant presence in other biomasses was not found in the literature, more studies must be carried out to elucidate its properties. Cycloertanol is a phytosterol compound, a precursor to several sterol compounds. It has anti-inflammatory, antitumor, antioxidant, antibiosis, and anti-Alzheimer properties. Furthermore, it acts in the process of plant growth and development.[22]

It is reported in the literature that the guarana peel has valuable potential for application as an antioxidant[4]. However, in the present study, the antioxidant evaluation of the oil extracted from the peel revealed an IC_{50} of 70.22 mg/mL, a relatively high concentration, which does not indicate strong antioxidant activity. Factors such as extraction methods, solvent, plant origin, and climatic and environmental aspects directly affect IC_{50} values[23]. The hot extraction method used in the study may be related, as thermal degradation of antioxidant bioactive is possible.

The results of the cytotoxicity assay are presented in Figure 1. The dotted line in the graph corresponds to 70 % cell viability. According to ISO 10993-5:2009[12], an L929 cell viability above 70 % does not present cytotoxicity, and below 70 % the extract is considered cytotoxic. Extracts with concentrations of 0 to 500 µg/mL did not show cytotoxicity, while higher concentrations did, with 750 µg/mL being the cytotoxic concentration. The results presented are important to verify the concentration at which the extracts can be harmful in their application[24]. The revealed cytotoxic concentration can be used to adapt the qualitative and quantitative composition of the oil into final products, and assays with other cell types based on other cellular functions can further elucidate its cytotoxic characteristic[25].

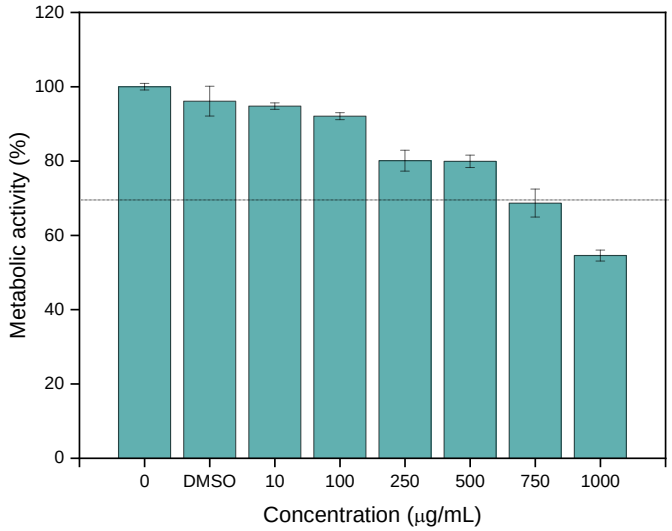

Figure 1 Cytotoxicity of L929 cells treated with extracts.

4 CONCLUSION

The present study obtained important results, as there is no data in the literature about the lipid fraction (10.81 % of extractives) of guarana peel. The extracted lipid showed characteristics of vegetable fat and oleoresin. The chromatogram identified sesquiterpene hydrocarbons, which demonstrates the potential of this bioproduct, as sesquiterpenes are bioactive associated with anti-inflammatory, antimicrobial, antitumor, and other important properties for the food, pharmaceutical, and cosmetics. 8-Androsten-3-ol was predominant in the chromatogram (33.87 %), but more studies must be carried out to elucidate its properties and actions. Cytotoxicity demonstrated a possible harmful effect of the oil only in high concentrations, with a cytotoxic value of 750 µg/mL. The acid value showed a high value (27.81 mg KOH g^{-1}), and the IC_{50} value (70.22 mg/mL) indicates weak/medium antioxidant activity. The results obtained in this study demonstrate that the lipid fraction of guarana peel can be better explored through extractive technologies with minimal thermal degradation, to favor the bioactive function of this bioproduct, making it more suitable for application in different segments and adding value to the chain guarana production.

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ACKNOWLEDGEMENTS

To financing entities: Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) e Universidade Federal do Amazonas (UFAM).