# ENZYME-ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM GUARANA LEAVES (*Paullinia cupana*)

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## ABSTRACT

The Guarana tree (*Paullinia cupana*) is a plant native to the Amazon region, recognized for its fruits rich in caffeine, theobromine, tannins, and flavonoids. Preliminary studies have highlighted the benefits of these compounds in the treatment of various diseases. However, so far, no attention has been given to the plant's leaves. For this reason, this study aimed to quantify the total phenolic compound content and determine the percentage reduction of antioxidant activity using the DPPH free radical method. The results revealed that the concentrations of the enzyme Pectinase (*Aspergillus aculeatus*) did not have a significant effect on the antioxidant activity and the content of phenolic compounds in the guarana tree leaves. However, bioactive compounds were extracted similarly, regardless of the presence of the enzyme, and demonstrated antioxidant potentials ranging from 30,73 mL/100mL ± 2,13 to 82,10 mL/100mL ± 1,93, as measured by the DPPH method, and total phenolics ranged from 200,00 mg GAE 100 $^{-1}$ ± 7.55 to 830,10 mg GAE 100 $^{-1}$ ± 67,96. These results drive promising applications of these compounds in sectors such as the food bioindustry, cosmetics, and nutritional supplements, contributing to the development of the bioeconomy.

**Keywords:** Guarana Tree 1. Leaf 2. Bioactive Compounds 3. Sustainable Technologies 4. Enzymes 5.

## 1 INTRODUCTION

Guarana (*Paullinia cupana*) plays a significant role in the culture and economy of the Amazon region, acting both as a traditional medicinal plant and as a source of commercial products. Its traditional applications include the treatment of fatigue, headaches, and as a general stimulant.[1]. Additionally, it represents an important source of income for many local communities due to its commercialization in various forms, including beverages, supplements, and cosmetic products.[2]. With the growing demand in the food, pharmaceutical, and nutraceutical sectors, there is an intensification and need to extract bioactives using a variety of methods, aiming to obtain pure compounds and increase yields.[2]. Among the available alternatives, the use of enzymes stands out. Enzymes are essential biological catalysts in accelerating biochemical reactions in living organisms. They have the potential to facilitate hydrolysis, oxidation, and synthesis reactions. Their commercial application is extensive and crucial, providing catalytic efficiency in a variety of processes.[2].

When it comes to extracting bioactive compounds from plant materials, enzymes often play a crucial role in releasing these compounds, ensuring an optimized, efficient, sustainable, and high-quality extraction procedure.[3]. However, in other cases, the extraction process is not efficient due to a series of factors involving the chemical structure of the compounds, their interactions with other components present in the plant, temperature, and pH during the extraction process.[3]. In view of this, the aim of this work was to investigate the presence of bioactive compounds in guarana leaves by quantifying the total phenolic compound content and determining the percentage of reduction in antioxidant activity using the DPPH free radical method, using enzyme-assisted extraction

## 2 MATERIAL & METHODS

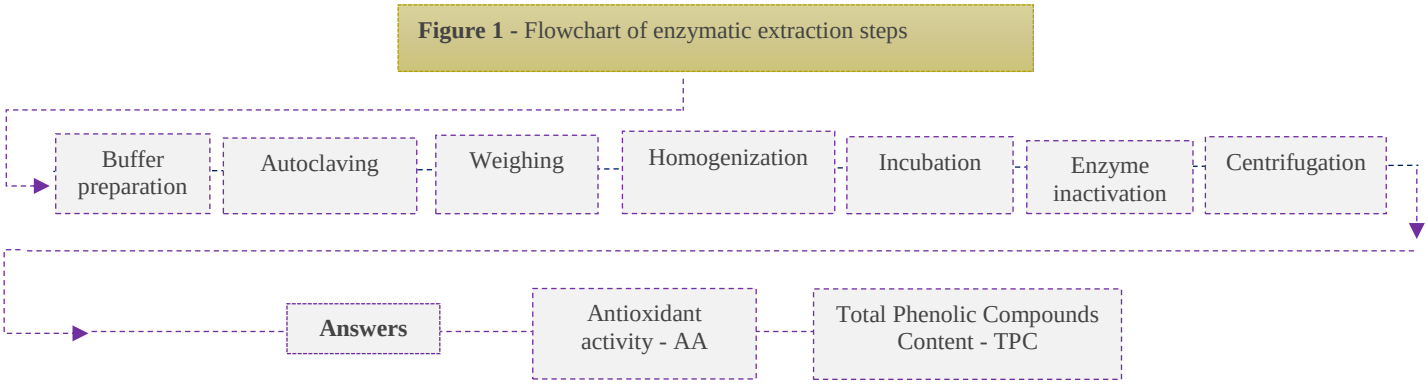
The collection of plant material from guarana leaves was carried out in August 2023 in the municipality of Maués, located 258km from the city of Manaus, Amazonas, Brazil. The leaves were sanitized with sodium hypochlorite (40mg/L) for 20 minutes, rinsed under running water, and then subjected to a 40°C oven for 24 hours until completely dried. The samples were ground using the Lucadema 220V Knife Mill - Luca-226/2 and subsequently stored in a refrigerator at (-18°C) in dark plastic containers until manipulation.

Enzymatic extraction was conducted using Pectinase enzyme (*Aspergillus aculeatus*) under different conditions, varying the solid-to-enzyme volume (m/v) ratio between 2.5%, 5%, 7.5%, and 10% solids, along with enzyme concentrations of 0.5%, 1%, 1.5%, and 2% for each percentage of solids. The enzyme volume was determined relative to the sample mass (guarana leaf), while the solid mass was calculated based on the reaction volume, corresponding to the volume of buffer used in the Erlenmeyer flask. Samples were weighed in Erlenmeyer flasks, and 25mL of sodium acetate buffer ($NaO_2CCH_3$ at 50 mM) with pH 4.5 (adjusted with 1M HCl) was added. This extraction condition was applied for each percentage of solids, performed in duplicate. Subsequently, the extraction was carried out in a Shaker Incubator Lucadema- Luca 223 at 150 rpm and 50°C for 2 hours, followed by vacuum filtration using qualitative filter paper 11.0 cm in diameter.

To determine antioxidant activity, the method of Rufino (2007)[4] of percentage reduction of the DPPH radical was adapted, as described in Technical Bulletin 127 of Embrapa Agroindústria Tropical. The UV-M51-BEL PHOTONICS spectrophotometer was

set to 517 nm, and the samples were kept in the dark. Readings were taken after thirty minutes. To determine the total phenolic content, the procedure of the Follin-Ciocalteau method was adapted from both George (2005)[5] and Singleton and Rossi (1965)[6]. Readings were made on the UV-M51-BEL PHOTONICS spectrophotometer at 760 nm. Statistical analysis was conducted using the Tukey test with a confidence level of 95%. The overall flowchart of the enzymatic extraction steps of bioactives is presented in Figure 1.

**Figure 1 -** Flowchart of enzymatic extraction steps




## 3 RESULTS & DISCUSSION

Table 1 displays the results of analyses of antioxidant activity (AA) by DPPH radical reduction and total phenolic compounds (TPC) for enzyme-assisted extraction processes. Tukey's statistical analysis, with 95% significance, revealed that enzymatic extracts showed no significant difference in antioxidant activity. In other words, the variation in enzyme concentration had no significant effect, and in some cases, lower enzyme concentrations resulted in higher levels of bioactive compounds.

The results obtained in this study corroborate with the findings of Scafi and Castro (2019)[7], who investigated the extraction of bioactive compounds from banana peel (*Musa acuminata*). It was observed that a concentration of 0.5% enzyme with higher solid concentrations of 7.5% and 10% resulted in a decrease in the recovery of bioactive compounds, totaling 21,86 ± 0,31 µmoL TE/g and 16,42 ± 0,30 µmoL TE/g, respectively, as described by the DPPH method. On the other hand, lower solid concentrations of 5% yielded superior results, reaching 33,01 ± 0,32 µmoL TE/g. This can be explained by the greater susceptibility to degradation of some antioxidant compounds, as prolonged extraction periods increase the likelihood of oxidation of these compounds.[8]. Additionally, the efficiency of vacuum filtration is crucial to prevent the loss of bioactives. Enzymes are sensitive and require specific conditions for optimal performance; any deviation from these conditions can compromise the quality and activity of bioactive compounds.[9,10].

**Table 1:** Antioxidant Activity (AA) and Total Phenolic Compounds (TPC) obtained from Enzyme-Assisted Extraction (EAE)

| % of solid (m/v) | % of enzyme (m/v) | % Reduction of DPPH (mL/100mL | Total plenolics (mg GAE $100^{-1}$) |
|---|---|---|---|
| **2,5** | 0,5 | 35,17±1,45$^{ab}$ | 224,27±66,59$^{a}$ |
| | 1 | 38, 11±0,58$^{b}$ | 200,00±7,55$^{a}$ |
| | 1,5 | 33,66±1,26$^{ab}$ | 219,90±24,71$^{a}$ |
| | 2 | 30,73±2,13$^{a}$ | 204,85±14,42$^{a}$ |
| **5** | 0,5 | 44,1,6±4,79$^{a}$ | 378,64±14,42$^{a}$ |
| | 1 | 47,49±4,79$^{a}$ | 386,89±4,12$^{a}$ |
| | 1,5 | 43,69±1,73$^{a}$ | 347,57±10,30$^{a}$ |
| | 2 | 54,08±2,56$^{a}$ | 482,04±6,87$^{b}$ |
| **7,5** | 0,5 | 75,96±3,96$^{a}$ | 729,13±24,03$^{a}$ |
| | 1 | 78,82±2,89$^{a}$ | 736,41±83,38$^{a}$ |
| | 1,5 | 74,67±4,79$^{a}$ | 703,88±21,28$^{a}$ |
| | 2 | 80,45±0,41$^{a}$ | 666,02±51,49$^{a}$ |
| **10** | 0,5 | 78,34±4,73$^{a}$ | 802,91±33,64$^{a}$ |
| | 1 | 82,10±1,93$^{a}$ | 830,10±67,96$^{a}$ |
| | 1,5 | 72,33±1,06$^{a}$ | 688,35±47,37$^{a}$ |
| | 2 | 72,19±3,57$^{a}$ | 735,92±17,16$^{a}$ |

*The results presented on a dry basis (mean ± standard deviation) for antioxidant activity (AA) and phenolic compound content(TPC). Tukey's test with 95% confidence was performed for each solid concentration of 2.5, 5, 7.5, and 10% individually. Values followed by identical lowercase letters in the columns are numerically different, but do not differ statistically from each other, and different letters indicate significant difference. Source: Author, 2024.

Although enzyme-assisted extraction of natural plant products leads to a reduction in extraction time and organic solvent, as well as an increase in system transparency, yield, and product quality, its effectiveness may depend on various factors, including the chemical structure of the compounds, interaction between compounds and plant matrix, extraction temperature and pH, among others.[9]. The enzyme cannot completely break down plant cell walls.[9]. The primary target of the pectinase enzyme is the breakdown of pectin, present in the leaf cell wall, not necessarily the bioactive compounds.[9].

Regarding total phenolic content (TPC), it was also observed that the enzyme's effect did not make a significant difference in phenolic extraction. This suggests that varying enzyme concentration did not substantially influence the amount of total phenolics extracted from guarana leaves. There was a significant increase in bioactive compounds from a solids concentration of 7.5%. According to Vasco, Ruales, and Kamal-Eldin's classification (2008)[11], values above 500 mg GAE/100 g are considered high. Phenols possess antioxidant, anti-inflammatory, antimicrobial, and anticancer properties, helping neutralize free radicals in the body, reducing oxidative stress, and potentially protecting against chronic diseases such as heart disease, cancer, and neurodegenerative diseases.[12].

## 4 CONCLUSION

Based on the results obtained from the extraction of bioactive compounds from guarana leaf, it was possible to conclude that the use of the enzyme pectinase for the extraction of bioactive compounds, under the assay conditions performed, did not show a significant impact on the antioxidant properties and total phenolics of the produced extracts. However, it is worth noting that guarana leaf is a natural source of compounds with significant antioxidant activity and phenolic content. These results suggest that the leaf of the species *Paullinia cupana* is potentially beneficial for health, as antioxidant compounds may help combat oxidative stress in the body, reducing the chances of developing diseases. Furthermore, the production of bioproducts obtained from enzymatic extraction has a wide range of applications and can be directed towards the pharmaceutical industry, as therapeutic agents or ingredients in medicines, among others.

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