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Comprehensive evaluation of methylxanthines and phenolic compounds in Bahia's guarana (*Paullinia cupana*), Brazil: implications for variety selection and by-product utilization

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Abstract

The State of Bahia, Brazil, stands as the predominant global producer of guarana, which is recognized for its seeds' caffeine-rich composition. This study aimed to determine the concentrations of methylxanthines (theobromine, caffeine, and theophylline) and phenolic compounds (catechin and epicatechin) in guarana leaves, seeds, and pericarps collected from various producing cities in Bahia. High-performance liquid chromatography analysis was conducted, and the data set was subjected to principal component analysis (PCA) for evaluation. Theobromine and caffeine were found in all leaves and pericarps samples, where higher contents were quantified for theobromine. Caffeine was the major compound in seeds, followed by epicatechin and catechin. No statistical difference was observed for the average contents of caffeine in samples from different producing cities. PCA showed no clear guarana sample discrimination based on bioactive compound contents. This study holds substantial relevance for the valorization of guarana cultivation in Bahia, enabling the identification of cultivars harboring elevated levels of bioactive compounds, which can be selectively cloned to meet the demands of the consumer market. Furthermore, the investigation unveils the potential utilization of leaves and pericarps as sources for the extraction of theobromine and caffeine, exhibiting promising applications in the pharmaceutical industry.

Keywords: guarana cultivation; bioactive compounds; caffeine; HPLC analysis; principal component analysis.

Practical application: Identifying high methylxanthine and phenolic compound varieties in Bahia's guarana and by-product use.

1 INTRODUCTION

Guarana (*Paullinia cupana*), which is renowned for its stimulant properties, is a native species of the Amazon region (Marques et al., 2019; Schimpl et al., 2014; Tricaud et al., 2016). Initially prominent in Amazonas, the production expanded to other Brazilian states such as Acre, Rondônia, Mato Grosso, and Bahia, due to the great demand of guarana seeds for the production of energy and carbonated drinks. In the southern of Bahia state, weather and soil conditions benefited the guarana cultivation. Currently, Bahia is the largest national producer of guarana, accounting for about 70% of the total production vs. 23% in the Amazonas (Atroch & Nascimento Filho, 2018; IBGE, 2017; Marques et al., 2019; Schimpl et al., 2014; Suframa, 2003; Tricaud et al., 2016).

The stimulating action of guarana seeds is attributed to the presence of methylxanthines including theobromine, caffeine, and theophylline (Machado et al., 2018; Schimpl et al., 2014; Tricaud et al., 2016). These compounds have biological activities such as stimulation of the central nervous system, blood pressure control, muscle relaxant, and bronchoprotective effect. Caffeine is the major methylxanthine in guarana and may have a content of up to 6% in the seeds. In addition, guarana seeds present antioxidant activity due to phenolic compounds such as catechin and epicatechin (Carrageta et al., 2018; Oñatibia-Astibia et al., 2017; Peixoto et al., 2017; Schimpl et al., 2014; Silva et al., 2017). It has been reported that the content of these metabolites in guarana seeds may vary for the same species depending on the cultivation region, due to several factors including seasonality, temperature, water availability, nutrients, and induction by mechanical stimuli or attack by pathogens (Gobbo-Neto & Lopes, 2007; Salles et al., 2022).

Beverage industries also depend on the standardization of caffeine content in guarana seeds (2–6% range) in order to control the production process (Marques et al., 2019; Schimpl et al., 2013). This requirement represents a problem for farmers who see the devaluation of the product. In this sense, it is essential to offer support to the guarana producers for identifying and multiplying species/varieties with the highest caffeine content. However, few studies have focused on the content of micronutrients and bioactive compounds of guarana from the southern region of Bahia state (Santos et al., 2019; Silva et al., 2017). Such studies can aggregate value to the cultivation of guarana, and, consequently, generate employment and income for the region.

Another challenge to be overcome is to properly dispose of the large amount of waste generated in this agro-industrial activity. In addition to possible environmental issues, improper

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waste disposal from post-harvest practices can also represent a loss of bioactive compounds with potential applications in the pharmacological and food industries (Reguengo et al. 2022; Santana & Macedo, 2018). Aiming to improve the use of the guarana production chain in the Bahia state, it is important to carry out studies on the composition of different parts of guarana, contributing with essential information for the reuse of by-products.

Thus, this study aimed to determine the concentrations of methylxanthines (theobromine, caffeine, and theophylline) and phenolic compounds (catechin and epicatechin) in guarana leaves, seeds, and pericarps collected from various producing cities in Bahia.

2 MATERIALS AND METHODS

2.1 Samples

Guarana samples were randomly collected from small-scale producers in the southern region of the State of Bahia, Brazil, in April 2018. Except for Valença, the samples were collected in two different farms for each producing city. Details on the origin and type of guarana are shown in Table 1. The fruits and leaves were manually collected using pruning shears and stored in a freezer at 2°C. Then, the samples were submitted to pulping (*i.e.*, removal of pericarp from fruits), washing, drying, freeze-drying, and grinding.

2.2 Reagents and solutions

Ultrapure water of resistivity 18.2 MΩ cm^{-1} was obtained from a Milli-Q® purification system (Millipore, Merck KGaA, Darmstadt, Germany). Methanol and 2-propanol (both HPLC grade 99.9%) used for preparation of eluent and/or extracts, and standard compounds of caffeine (1,3,7-trimethylxanthine), theobromine (3,7-dimethylxanthine), theophylline (1,3-dimethylxanthine), (+)-catechin, and (–)-epicatechin were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Glacial acetic acid 99.7% was purchased from Dinamica (São Paulo, Brazil).

2.3 Extraction procedure

The samples were lyophilized in a model FreeZone freeze-dryer (Labconco, Kansas City, MO, USA), ground, and sifted to 80 mesh. An extraction procedure proposed by Antunes (2011) was used. Briefly, a sample mass of 500 mg was submitted to two extraction cycles using an extractor mixture composed of methanol:water (80:20 v v^{-1}). The samples were suspended in the mixture by magnetic stirring for 1 h at room temperature, followed by centrifugation at 3,000 rpm for 15 min. The supernatants were collected at each cycle and transferred to a 50-mL flask. Then, the extracts were filtered through a 0.45-µm PTFE syringe filter (Merck KGaA, Darmstadt, Germany), diluted to 1:100 (seeds and leaves) or 1:200 (pericarp), and injected into the HPLC.

2.4 HPLC analysis

The analyses were performed using a model Prominence LC-20A HPLC (Shimadzu, Kyoto, Japan) with a UV-vis detector set at 274 nm and column over at 45°C. A Kinetex® C18 column (4.6 mm ID, 100 mm length, 100 Å pore size, and 2.6 µm particle size) from Phenomenex (Torrance, CA, USA) was used for the separation of compounds. The mobile phase consisted of 5.2% methanol, 2.4% 2-propanol, and 92.4% acetic acid solution (0.1% v v^{-1}) (Barreto, 2018). The samples were injected in a volume of 25 µL and isocratically eluted at a flow rate of 0.2000 mL min^{-1} and a pressure of 16–18 MPa. Bioactive compounds were quantified using external calibration at concentration ranges of 0.03–0.93 mg L^{-1} for theobromine, 0.01–0.31 mg L^{-1} for theophylline, and 0.40–12.4 mg L^{-1} for caffeine, catechin, and epicatechin.

For data treatment, PCA was applied using the Statistica 10 software (Statsoft, Tulsa, OK, USA).

3 RESULTS AND DISCUSSION

3.1 Content of bioactive compounds in guarana leaves

Theobromine was the major methylxanthine in guarana leaves, followed by caffeine. The contents of theophylline and

Table 1. Guarana sampling points in the southern region of Bahia state.

City	Code	District	Guarana type	Geographic coordinates
Taperoá	T1–T2	–	Organic	Lat: -13°36’37.34636” Long: -39°11’4.55273”
Valença	V	–	Traditional	Lat: -13°19’52.00309” Long: -39°16’0.28006”
Nilo Peçanha	NP1–NP2	Cavaquinho	Traditional	Lat: -13°36’37.00753” Long: -39°8’52.17889”
Ituberá	IT1–IT2	Colônia Santa Luzia	Traditional	Lat: -13°47’50.69594” Long: -39°12’36.25373”
Camamu	C1–C2	Assentamento Zumbi dos Palmares	Traditional	Lat: -14°2’6.71629” Long: -39°10’18.57918”

Lat: latitude; Long: longitude.

phenolic compounds were below the limit of detection (LOD) (Supplementary Table S1). As shown in Figure 1, the content of bioactive compounds in 45 samples from different Bahia-producing cities ranged from 0.6 to 13.7 and 0.6 to 15.9 mg g^{-1} for theobromine and caffeine, respectively. The highest theobromine contents were found for guarana samples from Valença (2.9 a 13.7 mg g^{-1}), whereas the caffeine contents presented the lowest values (0.6–1.0 mg g^{-1} range) among all evaluated samples. The lowest contents of theobromine (0.9–5.7 mg g^{-1}) were found in samples from point 2 of Nilo Peçanha (NP2). In turn, guarana leaves from NP1 presented the highest caffeine contents (0.6–15.8 mg g^{-1}) and were the only samples with considerable contents of epicatechin, ranging from 5.7 to 8.2 mg g^{-1}.

This study was performed with guarana leaves at the ripening stage, where older leaves were analyzed. A previous study reported that younger leaves presented higher contents of theobromine, but caffeine was found only on partially or fully ripened guarana leaves (Schimpl et al., 2014). A similar trend was reported for coffee leaves (*Coffee arabica* L.), where theobromine content in mature leaves was also lower than in leaves at the intermediate ripening stage where the growth of leaves leads to caffeine biosynthesis via theobromine (Fujimori & Ashihara, 1994). The influence of leaves ripening on the theobromine and caffeine contents was also observed for cacao (*Theobroma cacao*) because the content of both compounds decreased by almost 75% during the development of leaves (Koyama et al., 2003).

Compared with mature guarana leaves from Amazonas (Oliveira, 2010; Schimpl et al., 2014), lower theobromine contents were found in samples of the southern region of Bahia (1.3% *vs.* 1.7–3.0%). However, despite the low contents of caffeine in leaves, guarana samples from Bahia presented higher contents (up to 1.6%) than those found in samples of the Amazonas state, which ranged from 0.1 to 1% (Oliveira, 2010; Schimpl et al., 2014).

3.2 Content of bioactive compounds in guarana pericarps

Similar to that found in leaves, theobromine was also the major methylxanthine in pericarps of guarana, followed by low contents of caffeine. Theophylline, catechin, and epicatechin were not detected in any sample (values below the LOQs). Figure 2 shows that theobromine and caffeine contents in pericarps ranged from 0.8 to 16.1 and 0.04 to 9.6 mg g^{-1}, respectively. The highest average contents of theobromine were found in samples from point 2 of Ituberá (IT2), ranging from 2.8 to 13.9 mg g^{-1}. Pericarps of guarana collected at point 1 of Camamu (C1) presented the

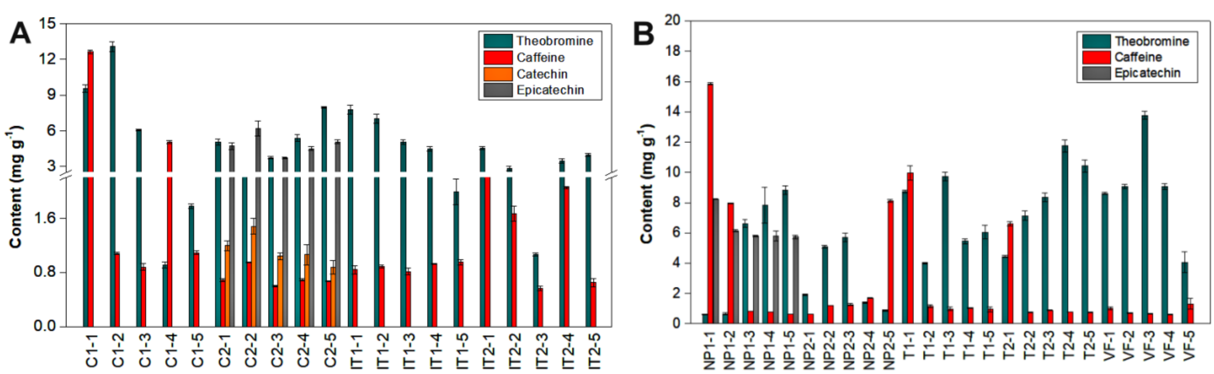

Figure 1. Theobromine, caffeine, and epicatechin contents in guarana leaves from the southern region of Bahia state. (A) Camamu and Ituberá. (B) Nilo Peçanha, Taperoá, and Valença.) Error bars indicate confidence interval at a 95% level.

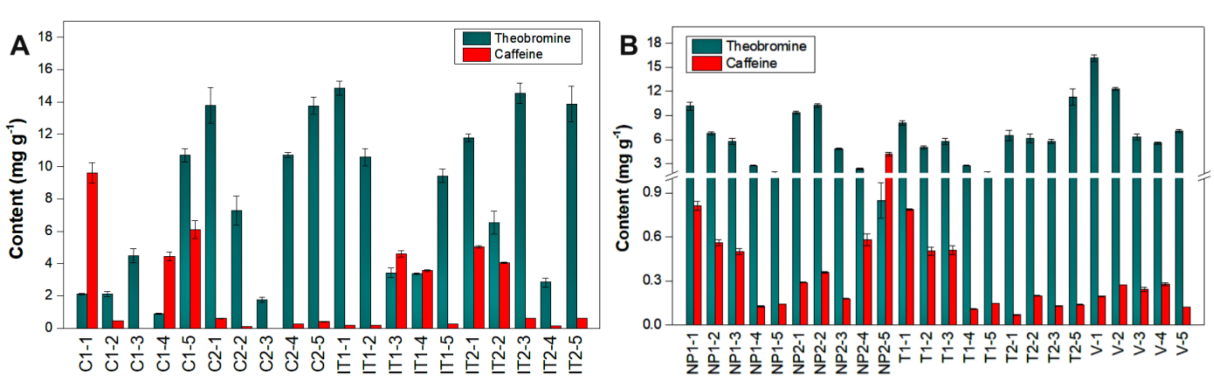

Figure 2. Theobromine and caffeine contents in pericarps of guarana from the southern region of Bahia state. (A) Camamu and Ituberá. (B) Nilo Peçanha, Taperoá, and Valença.) Error bars indicate confidence interval at a 95% level.

highest caffeine contents (0.1–5.0 mg g^{-1}) and the lowest contents of theobromine (0.9–10.7 mg g^{-1}). The lowest caffeine contents were found in pericarps of guarana from Taperoá (T2), whose values were in the range of 0.07–0.2 mg g^{-1}.

A trend of decreasing alkaloid content according to the progress of fruit ripening has already been reported for pericarps of guarana (Schimpl et al., 2014) and *coffee (Coffea arábica* and *Coffea canéfora*) (Koshiro et al., 2006). In our study using mature fruits, maximum contents of 0.9% and 1.5% were found, respectively, for caffeine and theobromine in pericarps of guarana from Bahia. These values were slightly higher than those reported by Oliveira (2010) and Schimpl et al. (2014) in guarana samples from Amazonas, ranging from 0.5 to 0.9% for caffeine and 0.7 to 1.2% for theobromine.

As shown in Figure 2, theobromine contents higher than caffeine were found for most guarana pericarp samples. Similar results were previously reported for guarana from Amazonas (Oliveira, 2010; Schimpl et al., 2014), indicating a general trend of higher levels of theobromine in pericarps of guarana.

3.3 Content of bioactive compounds in guarana seeds

As expected, caffeine was the major compound in guarana seeds. Figure 3 shows that caffeine content ranged from 15.5 to 47.9 mg g^{-1} or ≈ 1.5–4.8%. Unlike the leaves and pericarps, low contents of theobromine (maximum of 3.91 mg g^{-1}) and theophylline (maximum of 0.28 mg g^{-1}) were found in most guarana seeds (data not shown). The highest average contents of caffeine were found in guarana seeds NP1, whereas samples from NP2 presented the lowest average values.

As shown in Figure 3, guarana samples from Nilo Peçanha also presented the highest (16.0 mg g^{-1} for NP1) and lowest (1.3 mg g^{-1} for NP2) average contents of catechin in seeds. In turn, the highest epicatechin contents were found in guarana seeds from Ituberá (IT2), with an average value of approximately 15 mg g^{-1}. As observed for catechin, guarana seeds from NP2 presented the lowest average contents of epicatechin (3.8 mg g^{-1}).

As for leaves and pericarps, alkaloid contents in guarana seeds have decreased as a result of the ripening progress (Schimpl et al., 2014). However, despite the discrepancies between methylxanthine content in leaves and pericarps of guarana from the same location/city, most seed samples presented similar average contents of bioactive compounds. Guarana seeds analyzed in this study presented caffeine contents slightly lower than those obtained by Oliveira (2010) and Schimpl et al. (2014) for samples from Amazonas (5–6%). In addition, lower contents of theobromine and theophylline were also found in guarana samples from the southern region of Bahia state. It is likely that such differences in methylxanthine content are associated with post-harvest processing because the guarana seeds were submitted to different drying methods.

In general, a large variation in the contents of the bioactive compounds was observed (especially for leaves and pericarps) even for samples collected at the same producing farm/city. These results may be explained by the genetic variability, as the guarana of the Bahia's southern region is cultivated from seeds and does not use cloned species. Due to different genetic characteristics, the same species present different chemical compositions (Bianchi et al., 2016; Lima & Farah, 2019; Salles et al., 2022; Silva et al., 2017). Thus, it is important to characterize the plants and standardize the cultivation in order to produce guarana with higher uniformity for metabolite content and high caffeine levels. The selection of varieties or use of clones can be an efficient strategy to improve the quality of guarana from Bahia state.

3.4 Principal component analysis

Exploratory analysis using PCA was applied to verify separation trends based on the composition of guarana seeds from different producing cities. A data matrix consisting of 44 rows (guarana samples) and 5 columns (variables theobromine, caffeine, theophylline, catechin, and epicatechin) was obtained. As all studied variables have the same importance for sample discrimination, the original values were initially pre-treated by auto-scaling (Ferreira, 2022). Based on the Kaiser criterion, *i.e.*, eigenvalues > 1, two principal components (PCs) were evaluated (Granato et al., 2018). The first two PCs explained 75.8% of the cumulative variance. As shown in Figure 4A, PC1 showed positive loading for theophylline content (Teof) and higher negative loadings for caffeine, catechin, and epicatechin contents (Caf, Cat, and Epic, respectively). PC2 presented a high positive loading

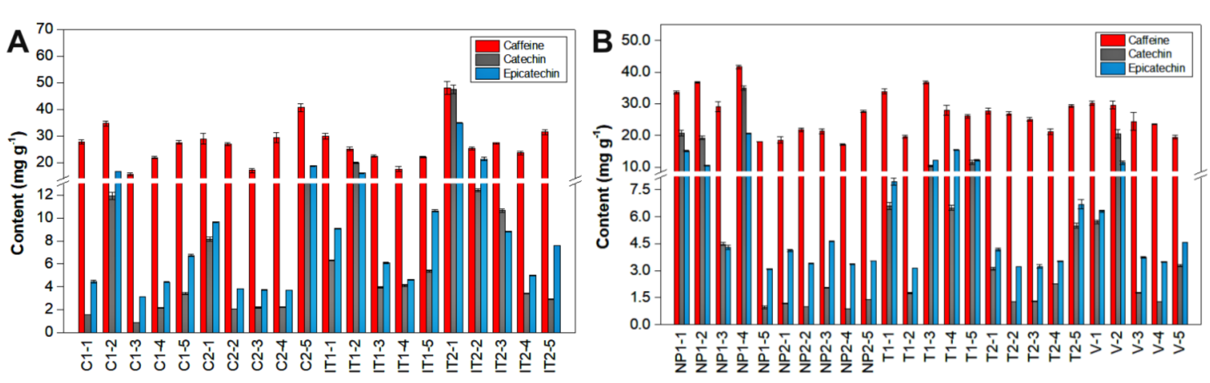

Figure 3. Caffeine, catechin, and epicatechin contents in guarana seeds from the southern region of Bahia state. (A) Camamu and Ituberá. (B) Nilo Peçanha, Taperoá, and Valença.) Error bars indicate confidence interval at a 95% level.

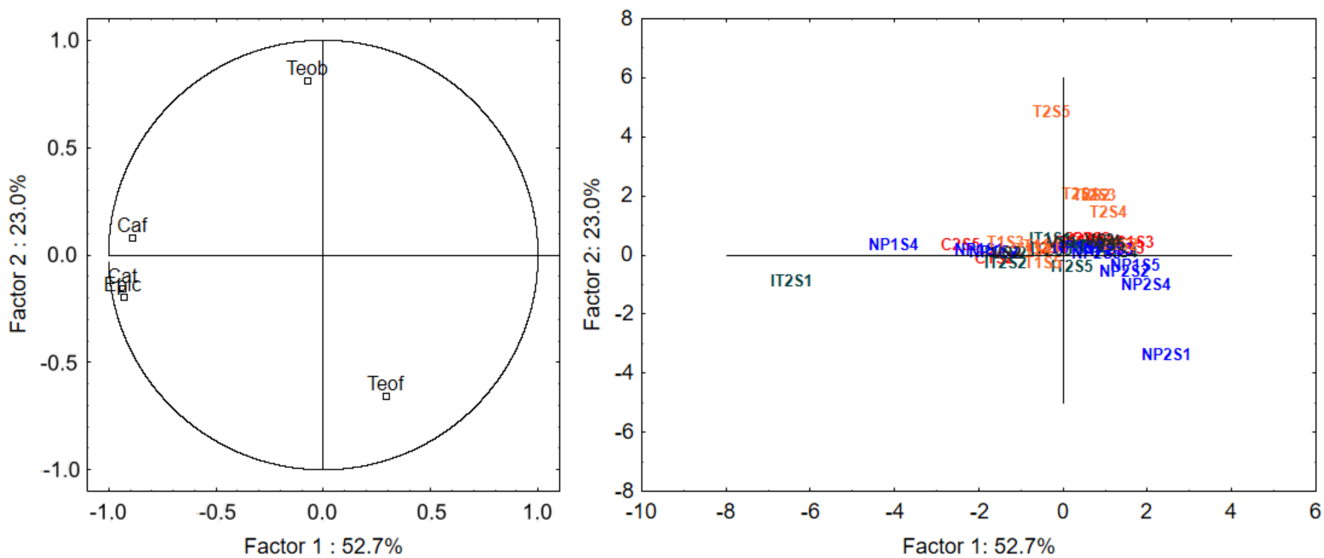

Figure 4. (A) Values for theobromine, theophylline, caffeine, catechin, and epicatechin. (B) Score graph of guarana seeds samples based on the bioactive compound content.

value for theobromine content (Teob) and negative loading for "Teof," whereas caffeine and phenolic compounds contents did not influence the sample discrimination in this PC (Figure 4A).

The score graph is shown in Figure 4B. In a general way, there was no clear separation between the samples, except for T2 and some samples from NP. Such seed samples formed two distinct groups in PC2 based on theobromine and theophylline content, respectively. PCA was also applied to verify separation trends based on the composition of leaves (theobromine and caffeine), pericarps (theobromine and caffeine), and seeds (theobromine, theophylline, caffeine, catechin, and epicatechin) of guarana samples, *i.e.*, data matrix composed of 44 rows and 9 columns/variables. An unclear separation trend was observed for PC1 × PC2 and PC2 × PC3 (data not shown). These results can be justified by the similar edaphoclimatic conditions, as the guarana samples analyzed in this study come from producing cities in the same region (Barreto, 2018). In addition, guarana from Bahia are cultivated from seeds and features high genetic variability.

3.5 Use of guarana by-products with potential application

The growth of agro-industrial activities has increased waste generation, which may be used as a by-product (Santana & Macedo, 2018). Different parts of guarana present antioxidant activity due to phenolic compounds in their composition and can be used by the food industry as natural antioxidants to replace synthetic ones. Such application contributes to food preservation, delaying losses by lipid oxidation and maintaining the sensory and nutritional quality of foods (Göktürk Baydar et al., 2007; Ramalho & Jorge, 2006).

It has been reported that guarana seed extract presents antibacterial (Basile et al., 2005; Majhenič et al., 2007) and antifungal actions (Majhenič et al., 2007). Such results indicate a potential application in the pharmaceutical and cosmetic industry. Leaves and pericarps of guarana analyzed in our study presented moderate/high contents of theobromine and caffeine. Thus, these by-products can be promising alternatives for application in the pharmaceutical industry in order to develop value-added products with potential stimulant action.

4 CONCLUSIONS

Methylxanthines and phenolic compounds were determined in leaves, pericarps, and seeds of guarana samples from Bahia's southern region. Theobromine and caffeine were found in all leaves and pericarps samples, where higher contents were quantified for theobromine. Caffeine was the major compound in seeds, followed by epicatechin and catechin. No statistical difference was observed for the average contents of caffeine in samples from different producing cities. PCA showed no clear guarana sample discrimination based on bioactive compound contents.

This study holds substantial relevance for the valorization of guarana cultivation in Bahia, enabling the identification of cultivars harboring elevated levels of bioactive compounds, which can be selectively cloned to meet the demands of the consumer market. Furthermore, the investigation unveils the potential utilization of leaves and pericarps as sources for the extraction of theobromine and caffeine, exhibiting promising applications in the pharmaceutical industry.

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