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Nutritional and bioactive potential of *Theobroma subincanum* Mart.: a promising Amazonian fruit for the food industry

Edson Queiroz da FONSECA JUNIOR[1,2], Edinilze Souza Coelho OLIVEIRA[2], Daniel de Queiroz ROCHA[2], Samuel Oliveira da SILVA[1,2], Andreza Cavalcante de OLIVEIRA[1,2], Francisca das Chagas do Amaral SOUZA[3], Gabriela FURLANETO[4], Julia Carvalho Cardoso CONSENTINI[4], Luciana AZEVEDO[4], Pedro Henrique CAMPELO[5], Edgar Aparecido SANCHES[6], Railma Pereira MORAES[7], Jaqueline de Araújo BEZERRA[1,8*]

Abstract

Theobroma subincanum (cupuí) is an Amazonian species with recognized nutritional and bioactive potential, although its chemical composition and biological properties remain scarcely explored. This study characterized the chemical constituents and evaluated the cytotoxic effects of hydroalcoholic extracts obtained from the peel, pulp, and seeds of T. subincanum fruits. The extracts were analyzed by ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry and nuclear magnetic resonance, and their cytotoxic and antiproliferative activities were assessed against cancer (A549 and HCT8) and normal (human umbilical vein endothelial cells) cell lines. The seeds exhibited the highest levels of proteins, carbohydrates, and energy content, while the pulp and peel were rich in fatty acids such as palmitic and oleic acids. The compounds theacrine, epicatechin, clovamide, and luteolin were identified in the extracts. Cytotoxicity assays indicated moderate activity of the pulp extract against all tested cell lines (IC_{50} [inhibitory concentration that reduces cell viability by 50%]: 12.29–22.80 μg/mL), with higher selectivity toward cancer cells (selectivity index > 1). Overall, the findings highlight the nutritional relevance of the seeds and the bioactive potential of the pulp, suggesting promising applications in the food industry.

Keywords: Malvaceae; cytotoxicity; phenolic fractions; NMR; nutritional analysis.

Practical Application: The cupuí fruit shows potential for use in the food industry due to its nutritional and bioactive properties.

1 INTRODUCTION

The Amazon rainforest represents an invaluable reservoir of biological diversity, remarkable for its wide variety of edible fruits with distinctive flavors, as well as numerous unconventional food and medicinal plants. Although traditionally consumed by riverine and local populations, many of these species remain underexplored by both science and industry (Cruz et al., 2025). Within this rich biodiversity, species of the *Theobroma* genus (Malvaceae), such as cacao (*Theobroma cacao* L.) and cupuaçu (*Theobroma grandiflorum* (Willd. ex Spreng.) K. Schum.), stand out for their recognized applications in the food, cosmetic, and pharmaceutical sectors. In cacao processing, for instance, the beans produce a honey-like byproduct rich in reducing sugars, organic acids, and phenolic compounds, which contribute to its antioxidant potential and distinctive nutritional and physicochemical profile, highlighting its applicability in the food industry (Rocha et al., 2025; Silva et al., 2014). Conversely, cupuaçu seeds, owing to their high lipid content, are commercially exploited for butter and liqueur production, reinforcing their potential as a substitute for cacao derivatives in confectionery and chocolate industries (Benlloch-Tinoco et al., 2024; Bezerra et al., 2024).

Unconventional food plants (UFPs) have emerged as promising resources for the 21st-century food industry, offering alternative sources of nutrients, bioactive compounds, and natural

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[1] *Universidade Federal do Amazonas, Programa de Pós-Graduação em Química, Manaus, Amazonas, Brazil.*
[2] *Instituto Federal de Educação, Ciência e Tecnologia do Amazonas, Núcleo de Estudos em Ciência e Tecnologia da Amazônia, Manaus, Amazonas, Brazil.*
[3] *Instituto Nacional de Pesquisa da Amazônia, Laboratório de Análises Funcionais e Química dos Alimentos, Manaus, Amazonas, Brazil.*
[4] *Universidade Federal de Alfenas, Laboratory of Nutritional and Toxicological Analyses In Vitro and In Vivo, Alfenas, Minas Gerais, Brazil.*
[5] *Universidade Federal de Viçosa, Department of Food Technology, Viçosa, Minas Gerais, Brazil.*
[6] *Universidade Federal do Amazonas, Laboratory of Nanostructured Polymers, Manaus, Amazonas, Brazil.*
[7] *Instituto Federal de Educação, Ciência e Tecnologia do Amazonas, Presidente Figueiredo, Amazonas, Brazil.*
[8] *Instituto Nacional de Pesquisa da Amazônia, Coordenação de Tecnologia e Inovação, Manaus, Amazonas, Brazil.*
*Corresponding author: jaqueline.bezerra@inpa.gov.br

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ingredients with potential applications in food, pharmaceutical, and cosmetic formulations (Ferreira et al., 2024). These plants possess one or more edible parts, such as leaves, roots, fruits, or flowers, and may be wild or cultivated, native or exotic. They are typically absent from conventional diets, often due to limited consumer awareness or cultural habits (Milião et al., 2022). Among the lesser-known *Theobroma* species, *Theobroma sylvestre* has recently gained attention for its nutritional and pharmacological potential and is currently classified as a UFP (Kinupp & Lorenzi, 2014; Mar et al., 2024). Studies have revealed the presence of organic acids, amino acids, purine alkaloids, proanthocyanidins, flavonoids, and glycosylated terpenoids in this species, as well as antiproliferative activity associated with these metabolites (Fonseca Júnior et al., 2025).

Similarly, another underexplored Amazonian species is *Theobroma subincanum* Mart., commonly known as "cupuí" or "macambillo." This species is native to South America and is distributed in countries such as Peru, Colombia, Venezuela, and the northern regions of Brazil (Amazonas, Acre, and Rondônia). The fruits (Figure 1) are ellipsoid in shape, measuring approximately 7–11 cm in length and 5–6 cm in diameter. They have a thick and resistant pericarp covered with an indumentum similar to that of true cupuaçu. The numerous seeds are oblong, about 2–2.5 cm long, and are surrounded by a whitish-yellow pulp that is sweet and odorless (Lira et al., 2020). Although less commercially exploited than cacao and cupuaçu, this fruit is highly appreciated by local communities and consumed fresh or used in juices, refreshments, ice creams, liqueurs, and wines, owing to its edible yellowish pulp (Figure 1A) with a distinctive flavor and pronounced acidity (Lim, 2014; Lira et al., 2020; Martini et al., 2008). The seeds of *T. subincanum* contain tocopherols, fatty acids, and sterols distributed in their parts (embryo, tegument, and endosperm), highlighting their potential as an alternative to *T. cacao* in the production of chocolates and confectionery products (Bruni et al., 2002). Beyond its nutritional value, the species has attracted scientific attention due to its potential as a source of bioactive compounds (Febrianto & Zhu, 2022). However, its physicochemical characteristics, proximate composition, chemical profile, and biological activities remain poorly characterized.

Therefore, this study aimed to investigate the chemical composition and cytotoxic effects of extracts obtained from the peel, pulp, and seeds of *T. subincanum* fruits. The extracts were analyzed by ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QToF/MS) and nuclear magnetic resonance (NMR) spectroscopy. The cytotoxic and antiproliferative effects of the extracts were assessed in cancer cell lines as well as in healthy cells to evaluate selectivity.

1.1 Relevance of the work

This study performed the chemical characterization of phenolic fractions from different parts of *Theobroma subincanum* (cupuí) fruit, together with the cytotoxicity assessment against tumor and normal cells. The analysis of proximate composition, fatty acid profile, and phenolic compounds identified potential sources of nutrients and bioactive compounds for the food industry. These findings contribute to understanding the health potential and safety of this unconventional food plant, promoting the valorization of Brazilian biodiversity and encouraging the development of new applications for native fruits.

2 MATERIALS AND METHODS

2.1 Standards, reagents, and solvents

Methanol, acetonitrile, and formic acid of HPLC grade were obtained from Merck (Darmstadt, Germany). Distilled water was purified using a Milli-Q water purification system (Millipore, Bedford, MA, USA). The leucine enkephalin reference solution was acquired from Waters Co. (Manchester, UK). Analytical grade reference standards were obtained from Sigma-Aldrich (St. Louis, MO, USA) and used to prepare the

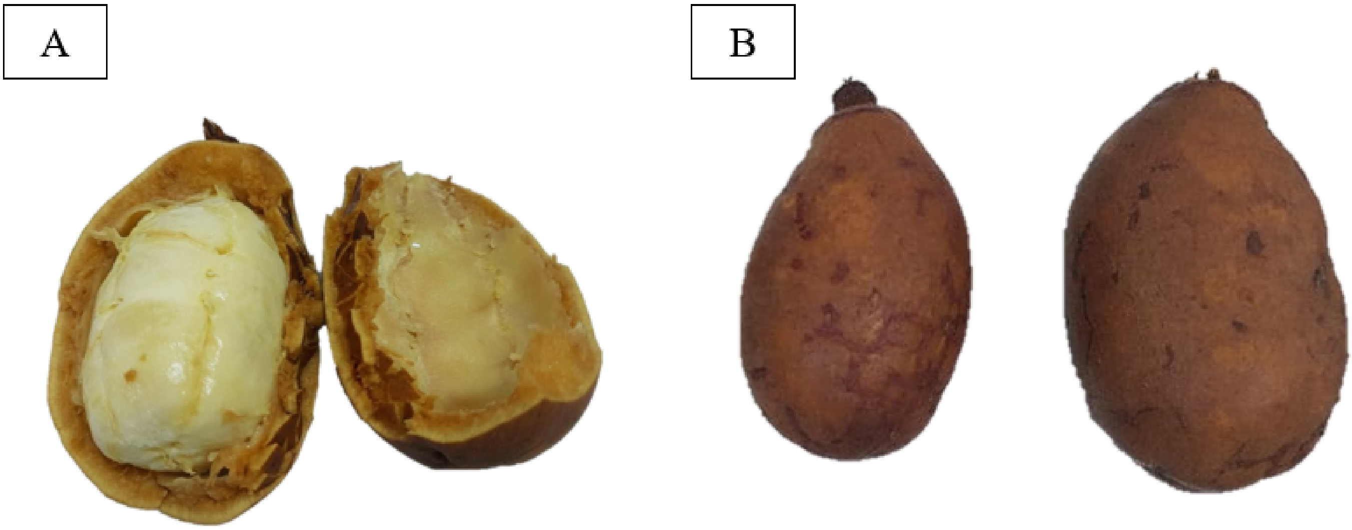

Figure 1. *Theobroma subincanum* Mart.: (A) Fruit pericarp; (B) Ripe cupuí fruits, highlighting their morphological and phenotypic characteristics. Images taken in Manaus, Amazonas, Brazil.

external calibration curves. All other reagents and solvents were of analytical grade and commercially available.

2.2 Collection and preparation of fruit samples

Fruits of *T. subincanum* Mart. were collected in February 2023 near Manaus, Amazonas, Brazil (Ramal da Boa Esperança, BR-174; 2°45′14.93″S, 60°2′22.47″W). Peel, pulp, and seeds were freeze-dried and extracted with ethanol/water (8:2, v/v) using an ultrasonic bath at room temperature for 30 min, repeated three times. Extracts were concentrated under ambient conditions, yielding 14.14, 46.34, and 8.72% for peel, pulp, and seed, respectively. Samples were defatted with hexane and further fractionated on Strata X SPE cartridges (Phenomenex, CA, USA), eluting sequentially with water and 100% methanol, resulting in phenolic compound-enriched extracts from peel (ECC), pulp (EPC), and seed (ESC). These extracts were used for UPLC-QToF/MS and NMR analyses.

2.3 Proximate composition

The proximate composition of *T. subincanum* fruit parts (peel, pulp, and seeds) was assessed using standard analytical methods. Moisture content was determined by oven drying at 105 °C, ash by incineration at 550 °C, lipids by Soxhlet extraction with hexane for 8 h, and proteins by the classical Kjeldahl method (Lutz, 2008). Carbohydrate content was estimated following the procedure of Ogunlaja et al. (2020). The total energy value was calculated using the Equation 1 (Palmeira et al., 2019):

$$\text{Energy (kcal)} = 4 \times (\text{g of proteins} + \text{g of carbohydrates}) + 9 \times (\text{g of lipids}) \quad (1)$$

2.4 Gas chromatography coupled with mass spectrometry (GC-MS) analysis

Approximately 10 mg of lipid fractions (hexane extracts) were dissolved in 0.25 mL of chloroform:methanol (2:1, v/v) and mixed with 500 μL of 0.1 M NaOH in methanol. The mixture was heated at 60 °C for 30 min to form fatty acid methyl esters (FAMEs), and the reaction was stopped with 0.2 mL of distilled water. FAMEs were extracted three times with 1 mL of GC-grade hexane, and the pooled hexane layer was allowed to stand for 30 min. An aliquot of 1 mL was analyzed by GC-MS (Shimadzu Nexis GC2030—GCMS-QP2020 NX) with a split–splitless injector. Separation was achieved on an SH-RTx-5Sil-MS column (30 m × 0.25 mm, 0.25 μm) using helium as a carrier gas (initial pressure 51.2 kPa). The temperature program started at 40 °C, increased at 3 °C/min to 210 °C, and was held for 5 min, for a total run time of 60:09 min. Fatty acids were identified using the WILEY 275 and NIST 3.0 libraries. Analyses were performed in triplicate, and results are reported as mean relative peak area ± standard deviation (Oliveira et al., 2025).

2.5 UPLC-QToF/MS analysis

Metabolite profiling of the extracts was conducted using a Xevo® G2-XS QTof mass spectrometer (Waters Corp., Manchester, UK) coupled to an Acquity H-Class UPLC system, operated with MassLynx® software (v4.1). Chromatographic separation was performed on an Acquity UPLC BEH C18 reversed-phase column (100 mm × 2.1 mm i.d., 1.7 *m*m particle size) maintained at 40 ± 2 °C. The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), delivered at a flow rate of 0.3 mL/min. The gradient elution program was as follows (A:B, %): 0–15 min (98:2), 15–20 min (80:20), 20–25 min (60:40), 25–27 min (2:98), 27–27.1 min (98:2), and 27.1–30 min (98:2). The injection volume was 10 *m*L. Mass spectrometric detection was performed in both negative and positive electrospray ionization (ESI^- and ESI^+) modes, using a mass scan range of *m/z* 100–1500 and a scan time of 0.2 s. The ESI source parameters were set as follows: capillary voltage, 3.0 kV; source temperature, 100 °C; desolvation temperature, 250 °C; cone voltage, 30 V; cone gas flow, 50 L/h; and desolvation gas flow, 700 L/h. Data were acquired in continuous Mass Spectrometry at Elevated Energy continuum mode, with low-energy scans at 6 eV and high-energy scans ramped from 20 to 35 eV over the 30-min run. Leucine enkephalin (*m/z* 554.2615 $[M-H]^-$ and *m/z* 556.2771 $[M+H]^+$) was used as a lock mass reference through the LockSpray™ system.

2.6 NMR analysis

Extracts of *T. subincanum* (peel, pulp, and seeds) were analyzed by NMR (^1H, $^1H-^{13}C$ HSQC, and $^1H-^{13}C$ HMBC) on an 11.7 T spectrometer (Bruker® Avance III HD 500, 13 MHz for 1H and 125.8 MHz for 13C, BBFO Plus SmartProbe™, New York, NY, USA) at 298.0 K. Samples were dissolved in 550 mL of CD_3OD containing trimethylsilylpropanoic acid (purity ≥ 99.0%) as an internal reference (0 ppm). Spectra were processed and analyzed using TopSpin™ software version 4.1.3 (Bruker®).

2.7 Cytotoxicity assay

The cytotoxic potential of the different fruit parts of *T. subincanum* was assessed using the colorimetric MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay, following the experimental conditions and methodological procedures described by de Lima et al. (2024). Human cell lines A549 (lung adenocarcinoma), HCT-8 (colorectal adenocarcinoma), and human umbilical vein endothelial cells (HUVEC) (umbilical vein endothelial) were obtained from the Banco de Células do Rio de Janeiro (BCRJ, RJ, Brazil) and used in the *in vitro* assays. The MTT reagent (3-[4,5-dimethylthiazol-2-yl]--2,5-diphenyltetrazolium bromide) is a yellow compound that is enzymatically reduced by mitochondrial dehydrogenases to form insoluble blue formazan crystals in metabolically active cells (Geirnaert et al., 2017). Cells were cultured in Dulbecco's Modified Eagle Medium Ham-F12 medium supplemented with 10% (v/v) fetal bovine serum and 100 mg/mL penicillin, and seeded into 96-well plates (100 *m*L per well) at densities of 1×10^4 cells/well for HCT-8 and 6×10^3 cells/well for HUVEC and A549. After cell attachment, cultures were exposed for 48 h to serial concentrations (5, 10, 25, 50, and 100 *m*g/mL) of *T. subincanum* fruit extracts. Subsequently, MTT solution (0.5 mg/mL) was added to each well, followed by incubation for an additional 4 h at 37 °C. The resulting formazan crystals were solubilized in 100 *m*L of dimethyl sulfoxide, and absorbance was measured at 570 nm. The IC_{50} (inhibitory concentration that reduces cell viability by 50%), GI$_{50}$ (growth inhibition concentration that suppresses proliferation by 50%), and LC$_{50}$ (lethal concentration resulting in 50% cell death) values were determined according to the procedures of Carmo et al. (2018; 2019). The selectivity index (SI) was calculated as the ratio between the IC$_{50}$ value for the non-tumor HUVEC line and the IC$_{50}$ for each cancer cell line. According to Carmo et al. (2019), SI values greater than three are indicative of a high degree of selectivity toward tumor cells.

2.8 Statistical analysis

Data are presented as mean ± standard deviation. Statistical differences were evaluated by one-way ANOVA followed by Tukey's test ($p < 0.05$). Analyses were performed using Minitab® version 18.1 (State College, PA, USA).

3 RESULTS AND DISCUSSION

3.1 Physicochemical parameters and proximate composition

Table 1 presents the physicochemical parameters of the freeze-dried pulp, peel, and seeds of *T. subincanum*, highlighting their nutritional potential. The high moisture content of the pulp (83.61 g/100 g) contributes to the fruit's succulent texture, similar to that observed in mango, cacao, and blue cacao fruits (Fonseca Júnior et al., 2025; Nguyen et al., 2025). The peel and seeds exhibited the highest ash levels, suggesting a greater concentration of inorganic minerals. In food matrices, ash primarily represents the inorganic residue remaining after complete combustion, consisting mainly of elements such as calcium, phosphorus, potassium, and trace minerals. These elements play essential physiological roles, including bone formation, muscle contraction, and oxygen transport (Alzahrani et al., 2017). These findings indicate that the peel and seeds could serve as potential sources of dietary minerals, contributing to nutritional value and functional food applications (Edo et al., 2023).

Cupuí seeds showed the highest protein (10.88 ± 0.59 g) and carbohydrate (75.01 ± 0.68 g) contents compared with the pulp and peel ($p < 0.05$). This nutritional pattern aligns with that of other *Theobroma* species, including *T. cacao* (cocoa), *T. bicolor* (macambo), *T. grandiflorum* (cupuaçu), and *T. sylvestre* (blue cacao), whose seeds are typically rich in lipids and carbohydrates, whereas the pulp contains higher levels of soluble sugars and fibers (Benlloch-Tinoco et al., 2024; Fonseca Júnior et al., 2025).

The high energy content of the seeds (360.69 ± 0.36 kcal) reinforces their potential as ingredients in energy-dense foods or functional formulations. Previous studies have shown that *Theobroma* seeds are traditionally recognized for their high energy and protein content, supporting the present findings (Araujo et al., 2021). It is important to note that fruit composition can vary depending on seasonality, region of collection, and processing methods, suggesting that further studies on vitamins, fiber fractions, and detailed lipid profiles are warranted to fully characterize the nutritional and functional potential of *T. subincanum.*

3.2 Fatty acid profile of lipid fractions

Esterified fatty acid profiles of the pulp, seed, and peel of *T. subincanum* were analyzed by gas chromatography coupled with mass spectrometry (GC-MS), allowing the identification of five major compounds. Results are expressed as relative peak areas (%).

The pulp showed the highest proportion of total FAMEs (91.68%), followed by the peel (71.23%) and the seeds (47.58%). In all parts of the fruit, palmitic, linoleic, and stearic acids were detected, with a predominance of palmitic acid in the peel (24.43%) and pulp (15.34%). This finding suggests that these tissues may serve as potential sources of saturated fatty acids. Similar profiles have been reported for *T. subincanum* from Ecuador (Bruni et al., 2000).

Linoleic acid, an essential polyunsaturated fatty acid known for its anti-inflammatory and cardioprotective effects, was also identified in the peel, further supporting its nutritional and medicinal relevance. Elaidic acid (a trans fatty acid) was detected in the pulp (46.75%) and peel (12.44%) but was absent in the seeds, indicating that the latter may be more suitable for direct consumption. Comparable results were reported by Bruni et al. (2002). Given that high intake of trans fatty acids is associated with cardiovascular risk (Mozaffarian et al., 2006), their presence in the pulp should be carefully considered when developing food products. In contrast, the seeds, free of elaidic

Table 1. Physicochemical parameters, proximate composition (g/100 g of dry matter) and energy (kcal/100 g of dry matter) of pulp, seed, and peel of *T. subincanum* (cupuí)*.

Parameters	Pulp	Seed	Peel
pH	3.47 ± 0.06	—	—
°Brix	14.43 ± 0.40	—	—
Moisture	83.61 ± 0.20^{a}	8.76 ± 0.13^{c}	76.37 ± 0.52^{b}
Ash	0.55 ± 0.01^{c}	3.45 ± 0.04^{a}	1.16 ± 0.04^{b}
Lipids	0.05 ± 0.00^{b}	1.90 ± 0.00^{a}	0.04 ± 0.00^{c}
Proteins	1.20 ± 0.31^{c}	10.88 ± 0.59^{a}	4.47 ± 0.22^{b}
Carbohydrates	14.58 ± 0.20^{c}	75.01 ± 0.68^{a}	17.96 ± 0.69^{b}
Energy	63.59 ± 0.79^{c}	360.69 ± 0.36^{a}	90.06 ± 1.92^{b}

*Mean ± standard deviation. Identical letters indicate that there is no statistically significant difference, according to the Tukey test.

acid, appear more suitable for direct consumption or use in food formulations. These results emphasize the importance of a detailed lipid profile analysis for *T. subincanum* fruit extracts, both to highlight their nutritional and bioactive potential and to ensure safety in food and nutraceutical applications.

3.3 Chemical description

The chemical profile of *T. subincanum* fruit extracts was characterized by LC-HRMS and one- and two-dimensional NMR spectroscopy (^{1}H, HSQC, and HMBC), allowing the identification of six constituents (Table 2) supported by comparison with literature data.

Tryptophan (1) was detected exclusively in the pulp extract (EPC), with characteristic signals at d_H 7.18 (s, H-8) and d_H 7.35 (sl, H-6). In the HSQC spectrum, these protons correlated with carbons at d_C 108.3 (C-7) and d_C 111.2 (C-6), respectively. The occurrence of this amino acid, both in free form and protein-bound, has previously been reported in cocoa beans from different origins (Bertazzo et al., 2011) and in the fruits of *T. sylvestre* (Fonseca Júnior et al., 2025). In addition, Theacrine (2), a purine alkaloid reported in *Theobroma* and *Herrania* species (Fonseca Júnior et al., 2025; Hammerstone Jr. et al., 1994), was identified in both peel (ECC) and pulp (EPC) extracts. The molecular ion at *m/z* 225.0980 $[M+H]^+$ and fragments at *m/z* 210.0756 and 168.0771 are typical of methylated xanthines. Methyl hydrogen signals at d_H 3.23–d_H 3.66, correlating with carbons at d_C 28.2–d_C 31.7 ppm, together with HMBC correlations to C-2 (d_C 152.2) and C-4 (d_C 136.5), confirmed the structure.

Table 2. Compounds in *T. subincanum* extracts identified by UPLC-QToF/MS and NMR. ECC: peel, EPC: pulp, ESC: seed.

No	TR (min)	Compounds (Chemical formula)	Precursor ion (m/z) $[M-H]^-$/$[M+H]^+$	Fragmentations *(m/z)*	d ^{1}H in ppm (*J*, Hz)	d ^{13}C in ppm	Samples	References
1	4.48	Tryptophan ($C_{11}H_{12}N_2O_2$)	205.0961 $[M+H]^+$	146.0588, 188.0700 118.0628	7.18 (s, H-8), 7.35 (sl, H-6)	108.3 (C-7), 111.2 (C-6), 127.8 (C-2).	EPC	(Fonseca Júnior et al., 2025)
2	5.93	Theacrine ($C_9H_{12}N_4O_3$)	225.0980 $[M+H]^+$	210.0756 168.0771 153.0536	3.44 (s, 3H), 3.56 (s, 3H,), 3.23 (s, 3H), 3.66 (s, 3H).	152.2 (C-2), 136.5 (C-4), 99.0 (C-5), 153.5 (C-6), 150.5 (C-8), 29.2 (CH_3-1), 30.6 (CH_3-3), 28.2 (CH_3-7), 31.7 (CH_3-9).	ECC EPC	(Hammerstone Jr. et al., 1994; Wang et al., 2010)
3	7.84	Epicatechin ($C_{15}H_{14}O_6$)	289.0731 $[M-H]^-$	245.0157	5.92 (d, *J* = 2.3 Hz, H-6), 5.89 (d, *J* = 2.3 Hz, H-8), 6.80 (d, *J* = 8.0 Hz, H-5′).	95.2 (C-6), 94.7 (C-8), 115.5 (C-5′),	ESC	(Febrianto & Zhu, 2022; Huang et al., 2021)
4	9.57	Clovamide ($C_{18}H_{17}NO_7$)	358.0969 $[M-H]^-$	254.9837 222.0416 178.0534 135.0459	—	—	ESC EPC	(Barros et al., 2016; Febrianto & Zhu, 2022)
5	11.51	Hypolaetin-8-*O*-b-D-glucoronide 3″-*O*-sulfate ($C_{21}H_{18}O_{16}S$)	557.0275 $[M-H]^-$	301.0389 254.9837 175.0255	—	—	ESC EPC	(Febrianto & Zhu, 2022; Moita, 2019)
6	17.36	Luteolin ($C_{15}H_{10}O_6$)	285.0462 $[M-H]^-$	254.9837 175.0285 133.0315	—	—	ESC EPC	(Sánchez-Rabaneda et al., 2003)

The MS spectrum of epicatechin (*m/z* 289.0731 $[M-H]^-$) exhibited typical fragment ions at *m/z* 245.0157, confirming retro-Diels–Alder cleavage of the flavan-3-ol skeleton. In the ^1H NMR spectrum, doublets at d_H 5.92 ($J = 2.3$ Hz) and d_H 5.84 ($J = 2.3$ Hz) of ring A and at d_H 6.80 ($J = 8.0$ Hz) of ring B supported its identification (Huang et al., 2021). Epicatechin (3) was previously reported in the beans of *T. grandiflorum*, *T. bicolor*, and *T. subincanum* (Febrianto & Zhu, 2022). Similarly, clovamide (4) and hypolaetin-8-*O*-β-D-glucuronide 3″-*O*-sulfate (5) were detected in the seed and pulp extracts of *T. subincanum* and have also been reported in the beans of *T. grandiflorum*, *T. bicolor*, and *T. subincanum* (Febrianto & Zhu, 2022), reinforcing the chemical similarity among members of this genus. The flavone luteolin (6) was identified by its molecular ion at *m/z* 285.0462 $[M-H]^-$ (Sánchez-Rabaneda et al., 2003). *T. cacao* is also characterized by the presence of luteolin (Benlloch-Tinoco et al., 2024), which has been associated with antioxidant and anti-inflammatory activities (Tian et al., 2021). Taken together, these findings highlight that *T. subincanum* is a promising source of antioxidant phenolic compounds, supporting its potential use in the development of functional foods and nutraceutical formulations.

3.4 Cytotoxic and antiproliferative effects

The extracts obtained from the pulp, seeds, and peel of *T. subincanum* fruits were evaluated for cytotoxicity against lung adenocarcinoma epithelial cells (A549), human colon carcinoma cells (HCT8), and HUVEC. According to the cytotoxicity classification proposed by Anywar et al. (2022) and the National Cancer Institute (NCI), the seed and peel extracts showed no cytotoxic activity toward any of the tested cell lines, including both healthy and cancerous cells, with IC_{50} values above 100 *mg*/mL (Figure 2). This absence of cytotoxicity indicates that these fractions are safe and suitable for potential food or nutraceutical applications. In contrast, the pulp extract exhibited moderate cytotoxicity toward both cancerous (A549 and HCT8) and healthy (HUVEC) cells, with IC_{50} values ranging from 12.29 to 22.80 *mg*/mL. Although the SI values were below the threshold of three, indicating low selectivity for tumor cells, the pulp extract showed slightly higher activity against cancer cell lines compared to normal endothelial cells ($SI > 1$) (Granato et al., 2022) (Table 3). These findings suggest that, while the pulp exhibits moderate bioactivity, the seed and peel extracts demonstrate promising profiles of biocompatibility and safety, reinforcing their potential as functional ingredients or sources of bioactive compounds in food formulations.

Compared to the peel and seeds, the cupuí pulp exhibited the highest capacity to inhibit cell growth, with GI_{50} values ranging from 10.32 to 11.66 *mg*/mL, an effect also reported for another species of the genus, *T. sylvestre* (Fonseca Júnior et al., 2025). Notably, it was the only fruit fraction to exert a lethal effect at a concentration of 23.46 mg/mL in both healthy and cancerous cell lines. In the present study, clovamide was identified in the pulp extract of *T. subincanum*, a compound widely associated with antioxidant, anti-inflammatory, neuroprotective, antiplatelet, and anticancer activities (Kolodziejczyk-Czepas, 2024). Luteolin, also detected, has been shown to inhibit the

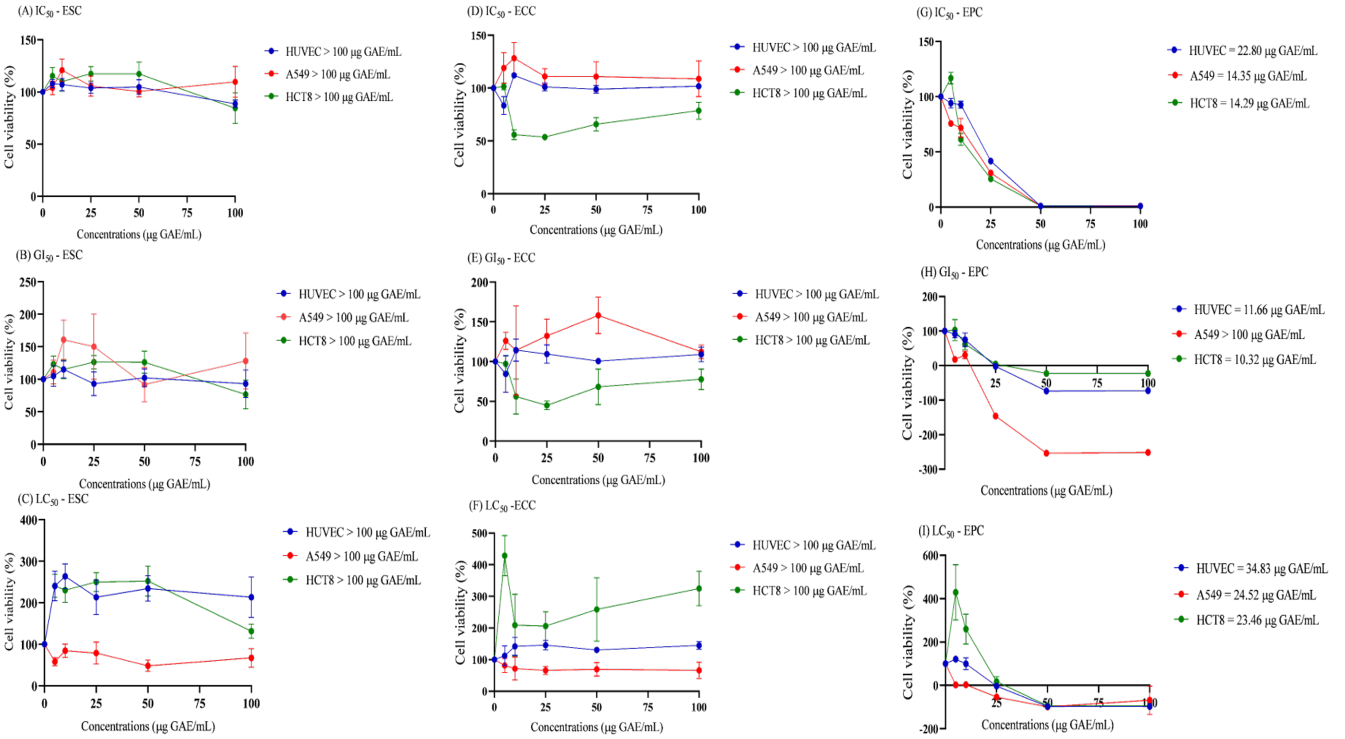

Figure 2. Cytotoxicity assessment of ESC (seed extract of Cupuí), ECC (peel extract of Cupuí), and EPC (pulp extract of Cupuí) was performed on A549, HCT8, and HUVEC cell lines. (A, D, and G) IC_{50}: the concentration of the agent that reduces cell growth by 50%. (B, E, and H) GI_{50}: the agent concentration that decreases growth by 50% compared to untreated cells. (C, F, and I) LC_{50}: the agent concentration that causes a net reduction of 50% of the cells relative to the initial cell count at the beginning of treatment.

Table 3. IC_{50} and selectivity index (SI) of the extracts ESC (seed extract of Cupuí), ECC (peel extract of Cupuí), and EPC (pulp extract of Cupuí)*.

Samples	IC_{50} (mg GAE/mL)			SI	
	HUVEC	A549	HCT-8	HUVEC/A549	HUVEC/HCT-8
ESC	> 100	> 100	>1 00	1	1
ECC	> 100	> 100	> 100	1	1
EPC	22.80	14.35	14.29	1.59	1.60

*IC_{50} represents the concentration of an agent needed to decrease cell growth by 50%, determined when (T/C) × 100 = 50. Here, T is the cell count at the time of treatment, and C is the cell count in the control group at the same moment. The selectivity index (SI) is calculated by comparing the IC_{50} in healthy HUVEC cells to the IC_{50} in cancer cells, offering insight into the agent's potential to selectively target cancer cells over healthy ones.

growth of human prostate tumors (Pratheeshkumar et al., 2012). These findings indicate the potential of *T. subincanum* pulp as a source of bioactive compounds for food applications.

4 CONCLUSION

The study concludes that the different parts of the *T. subincanum* fruit present distinct chemical and biological profiles. The seeds stand out for their high nutritional value, being rich in proteins and carbohydrates, which makes them a promising ingredient for the food industry. The peel and pulp are sources of fatty acids and phenolic compounds, such as theacrine and epicatechin. From a biological perspective, the pulp was the only part that showed significant cytotoxic activity, albeit with low selectivity between cancerous and normal cells, indicating the need for further studies to ensure its safe application. The presence of elaidic acid in the pulp also warrants attention in future research. Overall, the results highlight the potential of *T. subincanum* as a source of nutrients and bioactive compounds, reinforcing the importance of exploring Amazonian biodiversity for food and technological applications.

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REFERENCES

Alzahrani, H. R., Kumakli, H., Ampiah, E., Mehari, T., Thornton, A. J., Babyak, C. M., & Fakayode, S. O. (2017). Determination of macro, essential trace elements, toxic heavy metal concentrations, crude oil extracts and ash composition from Saudi Arabian fruits and vegetables having medicinal values. *Arabian Journal of Chemistry, 10(7)*, 906–913. https://doi.org/10.1016/j.arabjc.2016.09.012

Anywar, G. U., Kakudidi, E., Oryem-Origa, H., Schubert, A., & Jassoy, C. (2022). Cytotoxicity of medicinal plant species used by traditional healers in treating people suffering from HIV/AIDS in Uganda. *Frontiers in Toxicology, 4*. https://doi.org/10.3389/ftox.2022.832780

Araujo, N. M. P., Arruda, H. S., Marques, D. R. P., Oliveira, W. Q., Pereira, G. A., & Pastore, G. M. (2021). Functional and nutritional properties of selected Amazon fruits: A review. *Food Research International, 147*, 110520. https://doi.org/10.1016/j.foodres.2021.110520

Barros, H. R. M., García-Villalba, R., Tomás-Barberán, F. A., & Genovese, M. I. (2016). Evaluation of the distribution and metabolism of polyphenols derived from cupuassu (*Theobroma grandiflorum*) in mice gastrointestinal tract. *Journal of Functional Foods, 22*, 477–489. https://doi.org/10.1016/j.jff.2016.02.009

Benlloch-Tinoco, M., Nuñez Ramírez, J. M., García, P., Gentile, P., & Girón-Hernández, J. (2024). *Theobroma* genus: Exploring the therapeutic potential of *T. grandiflorum* and *T. bicolor* in biomedicine. *Food Bioscience, 61*, 104755. https://doi.org/10.1016/j.fbio.2024.104755

Bertazzo, A., Comai, S., Brunato, I., Zancato, M., & Costa, C. V. L. (2011). The content of protein and non-protein (free and protein-bound) tryptophan in *Theobroma cacao* beans. *Food Chemistry, 124(1)*, 93–96. https://doi.org/10.1016/j.foodchem.2010.05.110

Bezerra, J. A., Corrêa, R. F., Sanches, E. A., Lamarão, C. V., Stringheta, P. C., Martins, E., & Campelo, P. H. (2024). "Cupuaçu" (*Theobroma grandiflorum*): A brief review on chemical and technological potential of this Amazonian fruit. *Food Chemistry Advances, 5*, 100747. https://doi.org/10.1016/j.focha.2024.100747

Bruni, R., Bianchini, E., Bettarello, L., & Sacchetti, G. (2000). Lipid composition of wild Ecuadorian *Theobroma subincanum* Mart. seeds and comparison with two varieties of *Theobroma cacao* L. *Journal of Agricultural and Food Chemistry, 48(3)*, 691–694. https://doi.org/10.1021/jf991015n

Bruni, R., Medici, A., Guerrini, A., Scalia, S., Poli, F., Romagnoli, C., Muzzoli, M., & Sacchetti, G. (2002). Tocopherol, fatty acids and sterol distributions in wild Ecuadorian *Theobroma subincanum* (Sterculiaceae) seeds. *Food Chemistry, 77(3)*, 337–341. https://doi.org/10.1016/S0308-8146(01)00357-0

Carmo, M. A. V., Fidelis, M., Pressete, C. G., Marques, M. J., Castro-Gamero, A. M., Myoda, T., Granato, D., & Azevedo, L. (2019). Hydroalcoholic *Myrciaria dubia* (camu-camu) seed extracts prevent chromosome damage and act as antioxidant and cytotoxic agents. *Food Research International, 125*, 108551. https://doi.org/10.1016/j.foodres.2019.108551

Carmo, M. A. V., Pressete, C. G., Marques, M. J., Granato, D., & Azevedo, L. (2018). Polyphenols as potential antiproliferative agents: scientific trends. *Current Opinion in Food Science, 24*, 26–35. https://doi.org/10.1016/j.cofs.2018.10.013

Cruz, J. M. A., Corrêa, R. F., Kinupp, V. F., Pereira, M. F., Neves, K. O. G., Rodrigues, J., Ventura, B. M. L., Mendes, T. A. O., Sanches, E. A., Campelo, P. H., & Bezerra, J. de A. (2025). Amazon fig (*Ficus subapiculata*): A unconventional food plant with technological potential for the food industry. *Food Chemistry X, 29*, 102699. https://doi.org/10.1016/j.fochx.2025.102699

Edo, G. I., Samuel, P. O., Oloni, G. O., Ezekiel, G. O., Onoharigho, F. O., Oghenegueke, O., Nwachukwu, S. C., Rapheal, O. A., Ajokpaoghene, M. O., Okolie, M. C., Ajakaye, R. S., Ndudi, W., & Igbodo, P. C. (2023). Review on the biological and bioactive components of nutrition. *Natural Resources for Human Health, 3(4)*, 426–448. https://doi.org/10.53365/nrfhh/174302

Febrianto, N. A., & Zhu, F. (2022). Comparison of bioactive components and flavor volatiles of diverse cocoa genotypes of *Theobroma grandiflorum*, *Theobroma bicolor*, *Theobroma subincanum* and *Theobroma cacao*. *Food Research International, 161*, 111764. https://doi.org/10.1016/j.foodres.2022.111764

Ferreira, C. P., Lima, M. C., Silva, J. G., & Peixoto Araujo, N. M. (2024). Nutritional composition, phenolic compounds and biological activities of selected unconventional food plants. *Food Research International, 191*, 114643. https://doi.org/10.1016/j.foodres.2024.114643

Fonseca Júnior, E. Q., Rocha, D. Q., Oliveira, E. S. C., Silva, O. S., Souza, F. C. A., Furlaneto, G., Cruz, L. C., Azevedo, L., Corrêa, R. F., Sanches, E. A., Campelo, P. H. & Bezerra, J. A. (2025). Chemical characterization, nutritional profile, and cytotoxicity potential of Amazonian *Theobroma sylvestre* Mart. fruits. *Food Bioscience, 73*, 107673. https://doi.org/10.1016/j.fbio.2025.107673

Geirnaert, A., Calatayud, M., Grootaert, C., Laukens, D., Devriese, S., Smagghe, G., De Vos, M., Boon, N., & Van de Wiele, T. (2017). Butyrate-producing bacteria supplemented in vitro to Crohn's disease patient microbiota increased butyrate production and enhanced intestinal epithelial barrier integrity. *Scientific Reports, 7*, 11450. https://doi.org/10.1038/s41598-017-11734-8

Granato, D., Reshamwala, D., Korpinen, R., Azevedo, L., Carmo, M. A. V., Cruz, T. M., Marques, M. B., Wen, M., Zhang, L., Marjomäki, V., & Kilpeläinen, P. (2022). From the forest to the plate – Hemicelluloses, galactoglucomannan, glucuronoxylan, and phenolic-rich extracts from unconventional sources as functional food ingredients. *Food Chemistry, 381*, 132284. https://doi.org/10.1016/j.foodchem.2022.132284

Hammerstone Jr., J. R., Romanczyk, L. J., & Aitkent, W. M. (1994). Purine alkaloid distribution within *Herrania* and *Theobroma*. *Phytochemistry, 35(5)*, 1237–1240. https://doi.org/10.1016/S0031-9422(00)94827-1

Huang, H., Ho, C., Chang, G., Shia, W., & Lai, C. (2021). HPLC/ESI-MS and NMR analysis of chemical constitutes in bioactive extract from the root nodule of *Vaccinium emarginatum*. *Pharmaceuticals, 14(11)*, 1098. https://doi.org/10.3390/ph14111098

Kinupp, V. F., & Lorenzi, H. (2014). *Plantas alimentícias não convencionais (PANC) no Brasil: guia de identificação, aspectos nutricionais e receitas ilustradas*. Instituto Plantarum da Flora.

Kolodziejczyk-Czepas, J. (2024). Clovamide and its derivatives—Bioactive components of *Theobroma cacao* and other plants in the context of human health. *Foods, 13(7)*, 1118. https://doi.org/10.3390/foods13071118

Lim, T. K. (2014). *Malvaviscus arboreus*. In *Edible medicinal and non-medicinal plants* (pp. 405–408). Springer. https://doi.org/10.1007/978-94-017-8748-2_29

Lima, A. S., Maltarollo, V. G., Carmo, M. A. V., Pinheiro, L. C., Cruz, T. M., Barros, F. A. R., Pap, N., Granato, D., & Azevedo, L. (2024). Blackcurrant press cake by-product: Increased chemical bioaccessibility and reduced antioxidant protection after in vitro simulation of gastrointestinal digestion. *Food Research International, 182, 114099*. https://doi.org/10.1016/j.foodres.2024.114099

Lira, N. R., Santos, E. F., Santos, D. N., Bezerra, P. G. F., & Lima, D. E. S. (2020). Physical-chemical and centesimal characterization of cupuí (*Theobroma subincanum* Mart.). *Brazilian Journal of Development, 6*(3), 15798–15813. https://doi.org/10.34117/bjdv6n3-451

Lutz, I. A. (2008). *Métodos físico-químicos para análise de alimentos*. Instituto Adolfo Lutz.

Mar, J. M., Fonseca Júnior, E. Q., Corrêa, R. F., Campelo, P. H., Sanches, E. A., & Bezerra, J. A. (2024). *Theobroma spp.: a review of its chemical and innovation potential for the food industry. Food Chemistry Advances, 4*, 100683. https://doi.org/10.1016/j.focha.2024.100683

Martini, M. H., Lenci, C. G., Figueira, A., & Tavares, D. Q. (2008). Localization of the cotyledon reserves of *Theobroma grandiflorum* (Willd. ex Spreng.) K. Schum., *T. subincanum* Mart., *T. bicolor* Bonpl. and their analogies with *T. cacao* L. *Revista Brasileira de Botânica, 31(1), 147-154*. https://doi.org/10.1590/S0100-84042008000100013

Milião, G. L., Oliveira, A. P. H., Soares, L. S., Arruda, T. R., Vieira, É. N. R., & Leite Junior, B. R. C. (2022). Unconventional food plants: nutritional aspects and perspectives for industrial applications. *Future Foods, 5*, 100124. https://doi.org/10.1016/j.fufo.2022.100124

Moita, I. S. (2019). *Caracterização química das cascas das sementes de espécies do gênero Theobroma* (Master's Dissertation). Universidade Federal do Amazonas.

Mozaffarian, D., Katan, M. B., Ascherio, A., Stampfer, M. J., & Willett, in mango jam formulations: effects on jam qualities during storage and sensory discrimination. *Sustainable Food Technology, 3(1)*, 333–342. https://doi.org/10.1039/D4FB00331D

Nguyen, V.-L., Tran, M.-T., Nguyen-Thi, T.-D., Nguyen, M.-A., Le, M.-T., Nguyen, T.-M., & Nguyen, Q.-D. (2025). Valorization of cocoa (Theobroma cacao L.) pod husks as a fruit pulp substitute in mango jam formulations: effects on jam qualities during storage and sensory discrimination. Sustainable Food Technology, 3(1), 333–342. https://doi.org/10.1039/D4FB00331D

Ogunlaja, O. O., Moodley, R., Baijnath, H., & Jonnalagadda, S. B. (2020). Elemental distribution and health risk assessment of the edible fruits of two *Ficus* species, *Ficus sycomorus* L. and *Ficus burtt-davyi* Hutch. *Biological Trace Element Research, 198*, 303–314. https://doi.org/10.1007/s12011-020-02048-4

Oliveira, A. C., Cruz, J. M. A., Mar, J. M., Corrêa, R. F., Rodrigues, J. V. S., Mendes, T. A. O., Aguiar, J. P. L., Souza, F. C. A., Neves, K. O. G., Santos, A. D. C., Sanches, E. A., Campelo, P. H., & Bezerra, J. A. (2025). Abiu-do-Mato (*Pouteria caimito*, Sapotaceae): A promising Amazonian fruit with rich chemical composition, antioxidant potential, and cytotoxic safety. *Food Research International, 205, 115984*. https://doi.org/10.1016/j.foodres.2025.115984

Palmeira, L., Pereira, C., Dias, M. I., Abreu, R. M. V., Corrêa, R. C. G., Pires, T. C. S. P., Alves, M. J., Barros, L., & Ferreira, I. C. F. R. (2019). Nutritional, chemical and bioactive profiles of different parts of a Portuguese common fig (*Ficus carica* L.) variety. *Food Research International, 126*, 108572. https://doi.org/10.1016/j.foodres.2019.108572

Pratheeshkumar, P., Son, Y. O., Budhraja, A., Wang, X., Ding, S., Wang, L., Hitron, A., Lee, J. C., Kim, D., Divya, S. P., Chen, G., Zhang, Z., Luo, J., & Shi, X. (2012). Luteolin inhibits human prostate tumor growth by suppressing vascular endothelial growth factor receptor 2-mediated angiogenesis. *PLoS One, 7*(12), e52279. https://doi.org/10.1371/journal.pone.0052279

Rocha, G. H. A. M., Almeida, M. C., Silva, L. L. P., Flores, I. S., Castiglioni, G. L., Oliveira, T. F., & Pereira, J. (2025). Food-related properties and composition of cocoa honey (*Theobroma cacao* L.): An integrated investigation. *Food Research International, 202*, 115694. https://doi.org/10.1016/j.foodres.2025.115694

Sánchez-Rabaneda, F., Jáuregui, O., Casals, I., Andrés-Lacueva, C., Izquierdo-Pulido, M., & Lamuela-Raventós, R. M. (2003). Liquid chromatographic/electrospray ionization tandem mass spectrometric study of the phenolic composition of cocoa (*Theobroma cacao*). *Journal of Mass Spectrometry, 38(1)*, 35–42. https://doi.org/10.1002/jms.395

Silva, E. N., Ramos, D. C., Menezes, L. M., Souza, A. O., Lannes, S. C. S., & Silva, M. V. (2014). Nutritional value and antioxidant capacity of "cocoa honey" (*Theobroma cacao* L.). *Food Science and Technology, 34(4)*, 755–759. https://doi.org/10.1590/1678-457X.6447

Tian, C., Liu, X., Chang, Y., Wang, R., Lv, T., Cui, C., & Liu, M. (2021). Investigation of the anti-inflammatory and antioxidant activities of luteolin, kaempferol, apigenin and quercetin. *South African Journal of Botany, 137*, 257–264. https://doi.org/10.1016/j.sajb.2020.10.022

Wang, Y., Yang, X., Zheng, X., Li, J., & Ye, C. (2010). Theacrine, a purine alkaloid with anti-inflammatory and analgesic activities. *Fitoterapia, 81(6)*, 627–631. https://doi.org/10.1016/j.fitote.2010.03.008