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Theobroma Genus: Exploring the Therapeutic Potential of *T. grandiflorum* and *T. bicolor* in biomedicine

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Abstract

The Amazon rainforest hosts a plethora of fruit-bearing plants, yet many remain untapped for commercial purposes. Among these, *Theobroma* genus stands out for its unique characteristics deeply rooted in culinary and traditional medicinal practices, significantly contributing to Amazonian biodiversity and cultural heritage. Particularly, *T. cacao*, the most renowned species, exhibits versatile applications owing to its health benefits, with distinct groups influencing cocoa quality. Similarly, *T. bicolor*, thriving in humid regions, has undergone domestication to yield pulp and seeds valuable in food and cosmetic industries. Meanwhile, *T. grandiflorum*, found across tropical regions of Central and South America, presents unique sensory profiles and fruit characteristics, making it a significant player in Amazonian agriculture. This review primarily aims to offer insights into the therapeutic potential of *T. grandiflorum* and *T. bicolor*, with comparisons to *T. cacao*, revealing a notable increase in publications concerning the physico-chemical and biological properties of these species in recent years. Specifically, the review examines their chemical composition, bioactive compounds, and methodologies for determination, with a focus on biological evaluations encompassing enzymatic, cellular, and animal tests, thereby shedding light on the medicinal properties of these species. Finally, future research perspectives, emphasising the utilisation of waste biomass and further exploration of these invaluable Amazonian resources, have been discussed.

Keywords: Amazonian fruits, *Theobroma* genus, phenolic compounds, antioxidant capacity, therapeutic applications.

Introduction

Within the diverse fruit production of the Amazon, numerous plants with potential for agro-industrial exploitation have been identified; however, they have not undergone widespread commercial development. Examples of such species include *E. oleracea*, *M. flexuosa*, *A. aculeatum, B. gasipaes, P. cupana, U. tomentosa,* etc.(Amorim et al., 2024; Souza et al., 2023). Among these promising species, the genus *Theobroma* is recognised for its distinctive characteristics, which are recognised and valued in culinary and traditional medicinal practices. Indeed, its species produce fruits with unique attributes and applications, playing a significant role in both the biodiversity and cultural heritage of the Amazon. Additionally, they hold substantial economic potential, particularly the main representative species, *Theobroma cacao*, due to its health benefits (González-Orozco et al., 2020), accounting 22 species native to the tropical forests of Central and South America (Huda-Shakirah et al., 2022). Certain species within this genus, such as *Theobroma grandiflorum* and *Theobroma bicolor*, are recognised as non-timber forest species that predominantly thrive in humid soils, native from the Amazon basin, sharing similarities with *T. cacao* (Abdullah et al., 2020). All these three species produce fruits with unique characteristics that make them special and appreciated in the gastronomy and traditional medicine of Amazonian culture. **Figure 1** shows the anatomical features of fruit and seed of *T. cacao*, *T. bicolor*, and *T. grandiflorum*. Particularly, *T. cacao* is native to South America and has successfully adapted to regions between 10° south and north of the equator worldwide. It is the smallest plant among these three species, and can grow up to 8 meters in height, with a trunk diameter of about 30 cm, featuring lanceolate leaves and white or pink flowers. Additionally, it is characterised by an elongated and winged fruit with a hard and rough shell, and it produces large, elongated seeds, wrapped in white and sweet pulp but not very abundant (**Figure 1a**). However, the bean is highly profitable due to its diverse applications in various industries, thanks to its fat profile, flavour, aroma, and antioxidant capacity derived from bioactive compounds such as polyphenols, which are linked to potential health benefits (Barrios-Rodríguez et al., 2022). There are three varieties of *T. cacao* (Criollo, Amazonian Forastero, and Trinitario) associated with specific fermentation processes, influencing the quality of cocoa derivatives: powder, liquor, and butter (Fanning et al., 2023).

Then, *T. bicolor* exhibits growth or cultivation proclivity within humid regions, not surpassing an elevation of 1000 meters above sea level, encompassing territories within Bolivia, Brazil, Ecuador, Colombia, and Peru, with some cultivation present in Mexico (Ponce-Sánchez et al., 2021). This plant has undergone domestication and technological refinement to produce pulp and seeds, presenting applications in the food and cosmetic industries (Mar et al., 2024). In its natural habitat, the tree can attain heights around 10 m, yet under cultivation, it undergoes pruning measures to sustain more manageable dimensions. The mature plants of *T. bicolor* can yield up to 40

fruits per harvest, occurring in February-March and September-October, necessitating daily harvesting to prevent fruit rot. The flowers are small and white; however, they are converted in the largest fruit within the genus *Theobroma*, characterised by an oval morphology, dimensions ranging from 25 to 35 cm in length, 12 to 15 cm in diameter, and a weight of up to ~3 kg. The fruit peel exhibits a woody and robust composition that falls to the ground when it reaches its optimum ripeness, it possesses a pod thickness of 12 mm, and features fissures (**Figure 1b**). Its colouration transitions to a yellow hue upon reaching maturity. The pulp presents as whitish or yellowish, with a strong odour and a sweet and sour taste. It is rich in proteins, both insoluble and soluble fibres, and fats, while its seeds produce butter with a fat content like cocoa, suggesting its potential use as a substitute or blend with cocoa butter, particularly in the chocolate industry (González et al., 2016).

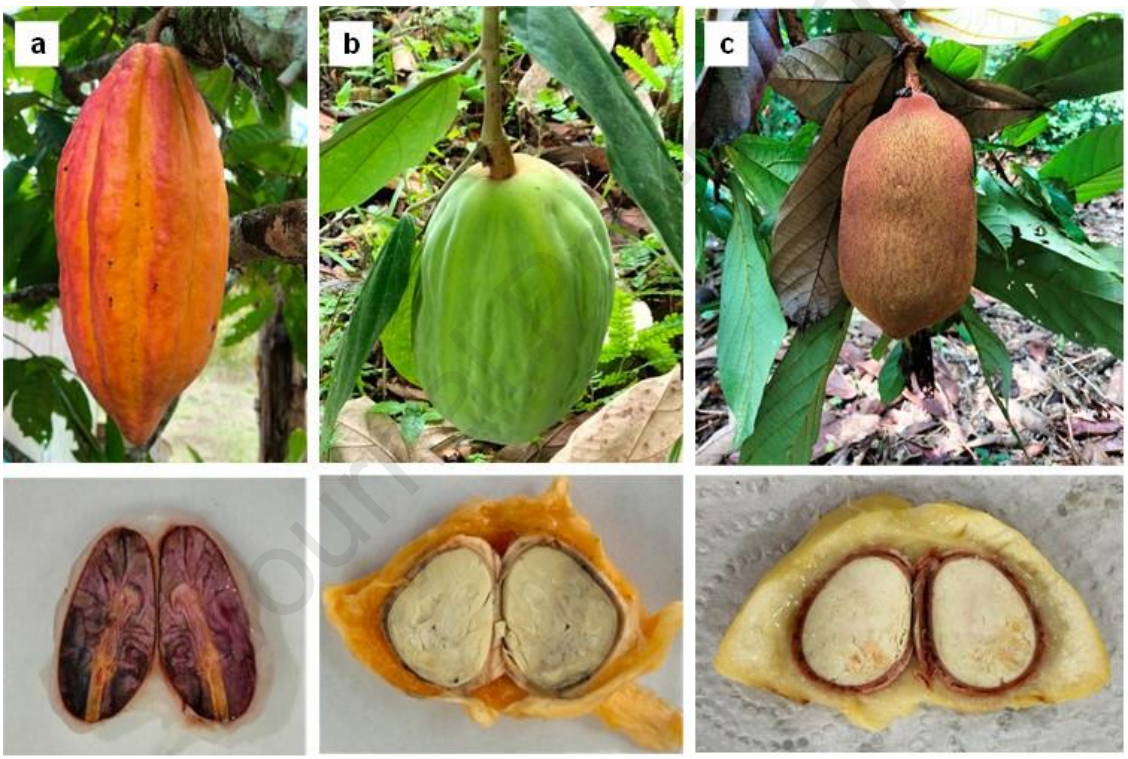

Figure 1. Images of fruit and seed of *T. cacao* (**a**), *T. bicolor* (**b**), and *T. grandiflorum* (**c**) taken in North-West area of the Amazon close to Florencia, Caquetá - Colombia.

Finally, *Theobroma grandiflorum* is grown in tropical regions of Central America (Costa Rica), South America (Ecuador, Peru, Venezuela, Colombia, Guyana, Suriname), and some Caribbean islands (Martinique, Trinidad and Tobago) (Duarte & Paull, 2015). This species has contributed to Amazonian agriculture, especially in Peru and Brazil, where most of the research mentioned in this document has been conducted. *T. grandiflorum* exhibits various varieties with differences in sensory profile, peel, and seed size (Febrianto & Zhu, 2022). It differs anatomically from *T. cacao* and *T. bicolor*, being notably larger, reaching heights of up to 15 m with a trunk diameter of up to 70 cm, and featuring a distinct internal structure and design of the kernel. Regarding its flowers,

they are the largest ones of the three species, presenting a white or pink colour. The fruit is an oval or elongated berry with a tough outer shell covered in hairs that become green when gently rubbed, signalling ripeness (**Figure 1c**). The pulp yield is approximately 40%, with a colour ranging from white to cream or creamy yellow and a sweet and sour taste. The seeds, located within the pulp, account for ~18% of the fresh fruit weight. Butter and liquor extracted from the seeds have a lipid content that is appealing to the cosmetic industry, while the highly aromatic pulp is of interest to the food industry (Pereira et al., 2018).

In this review, we proposed an overview on the therapeutic potential of *Theobroma grandiflorum* and *Theobroma bicolor*, by including studies of *T. cacao* (although reviews on this species are present in literature) to allows comparisons with the other two species. Specifically, this review presents information gathered from several documents in scientific literature. By a simple key-word search on ISI Web of Science database of topic terms which includes the scientific names of the three species, alongside terms such as "proximal composition," "biomedicine," "therapeutic," "antioxidant capacity," "biological test", it is possible to appreciate that the number of publications on the physico-chemical and biological properties of these species increased about 57% since 2020. In this information collection, works focused on the characterisation of volatile compounds of the three species are excluded, as our focus primarily lies on their chemical composition and bioactive compounds profile, with a focus on the main methodologies for their determination, with the undertaking of a unit conversion task for both the proximal characterisation and antioxidant profile of the three species to facilitate their comparison. Furthermore, the review describes the biological evaluation of the *Theobroma grandiflorum* and *Theobroma bicolor*, including enzymatic, cellular, and animal tests. Finally, the review provides in the last section a visionary perspective on possible areas for further exploration of the analysed species, with the aim of leveraging these raw materials, including the use of their waste biomass.

Chemical constituents of *T. grandiflorum*, *T. cacao* and *T. bicolor*

Literature reports several works on the investigation of the chemical composition of *T. grandiflorum*, *T. cacao* and *T. bicolor*, reporting mainly records of their proximal composition as well as information on proteomic analysis. Additionally, different studies are focused on the antioxidant activity of polyphenols and flavonoids, contained in these *Theobroma* species. Interestingly, these antioxidants have been linked to various health benefits, including digestive health, anti-inflammatory properties and potential anticancer effects. Thus, studying the chemical composition of these bioactive compounds cab allow to design and manufacture products that can be used for biomedical applications, contributing to our well-being.

Table 1 shows the average values of the proximal composition of the three *Theobroma* species. Concerning *T. cacao*, the reviewed articles do not assess moisture in the pulp due to its close adherence to the seed and limited fleshy characteristics, while when evaluating moisture content in the pulp of other species, it was found that *T. bicolor* exhibits a higher content compared to *T. grandiflorum*. Moreover, the seeds, constituting the organ with the highest economic value in these species owing to their fat content, exhibit values within a range of 52 to 61%.

Table 1. Proximal composition of *Theobroma* species. Some original values have been converted to percentages to facilitate comparison.

Sample	Moisture (%)	Ashes (%)	Lipids (%)	Protein (%)	Carbs (%)	Fibre (%)	References
Theobroma bicolor							
Pulp fresh	92.1	0.7	0.29	1.3	4.8	0.8	(Sotelo & Alvarez, 1991)
Pulp + seeds	35.6	0.8	33	13.3	5.1	12.2	(Torres et al., 2002)
Seeds fresh	52.3	1.7	17	11.4	8.8	8.8	(Sotelo & Alvarez, 1991)
Seeds fermented-dried	4.4	3.5	38-41.3	22-23.8			(Sotelo & Alvarez, 1991), (Jee, 1984), (Vázquez-Ovando et al., 2015)
Shell	50.6	1.1	0.37	3.25	12.6	32	(Sotelo & Alvarez, 1991)
Theobroma cacao							
Seeds fresh	52.5-61	1.4-2.1	19.6-23.9	6.3-7.9	3.6-10.5	3.1-3.2	(Sotelo & Alvarez, 1991)
Seeds fermented-dried	5.41	5.23	46.1	13.6			(Vázquez-Ovando et al., 2015)
Liquor	26.4	3	54	10.5		2.7	(de Oliveira & Genovese, 2013)
Shell	90.1-94.7	0.5-1	0.01-0.1	0.4-1	2.8-4.4	1.5-3.4	(Sotelo & Alvarez, 1991)
Theobroma grandiflorum							
Pulp fresh or frozen	83-89.7	0.5-3.3	0.4-2.1	0.7-2.5	5.6-10.6		(Pugliese et al., 2013), (Zagmignan et al., 2023)
Seeds fresh	52.7-54.9	1.5	21.7	4.3	15.8		(Pugliese et al., 2013), (De Souza Schmidt Goncalves et al., 2010)
Liquor	22.6	7.3	52	15.2		3.2	(de Oliveira & Genovese, 2013)

To be noted, ash contents were higher in the seeds than in the pulps of *Theobroma* species. Indeed, the endosperm's mineral storage supports vital chlorophyll formation for cotyledon-initiated photosynthesis, impacting overall ash content (Sciammaro et al., 2016), which, upon incineration, leads to an elevation of this value. However, in the case of *T. grandiflorum*, observed ranges include slightly higher ash values in the pulp.

Moreover, a study employing inductively coupled plasma-atomic emission spectroscopy (de Oliveira & Genovese, 2013) analysed the mineral composition of *T. cocoa* and *T. grandiflorum* liquors, revealing the following results: nitrogen predominates in both species at 23.3 and 25.9 g/kg dry weight (DW), with potassium (K) following at 7.5 and 6.8 g/kg DW, phosphorus (P) at 2 and 3.6 g/kg DW, and calcium (Ca) at 0.3 and 0.5 g/kg DW respectively.

Regarding the presence of lipids in fresh seeds, all the reviewed documents adhered to the methodology proposed by the AOAC, where the highest content is reported for *T. cacao* (19.6-23.9%) with comparable values for *T. grandiflorum* (21.7%) (Sotelo & Alvarez, 1991), while the lowest content is observed for *T. bicolor* (17%) (Pugliese et al., 2013). Moreover, several authors have assessed the chemical composition of the fats in the three species (Barbalho et al., 2022), (Torres et al., 2002), (Gilabert-Escrivá et al., 2002), (Jee, 1984), (Vázquez-Ovando et al., 2015), primarily aiming to identify materials that can function as substitutes or be blended with cocoa fat. The prevailing methodology involves the initial extraction of fats using an organic solvent, such as hexane, diethyl ether, or petroleum ether, over a time range of 2 to 8 hours. Subsequently, the lipid profile has been determined using gas chromatography coupled with different detectors: specifically, an ionisation detector for identifying the sample composition through fatty acid mixture standards (Torres et al., 2002) (Gilabert-Escrivá et al., 2002), or a quadrupole mass spectrometer detector for identification based on comparing retention indices and mass spectra (Barbalho et al., 2022) (Vázquez-Ovando et al., 2015). In both methodologies, silica capillary columns are employed. **Figure 2** presents the relative abundance of major fatty acids reported in three species. Stearic acid 18:0 is notably higher in *T. bicolor* (~47%), surpassing 10% in comparison to the other reviewed species. In contrast, oleic acid (18:1, n-9), known for its anti-inflammatory properties through the modulation of immune responses and reduction of inflammatory markers (Santamarina et al., 2021), showed similar content between *T. grandiflorum* and *T. bicolor*, being superior to that found in *T. cacao*. Palmitic acid 16:0 has also been identified as a prominent component, especially in *T. cacao*, contributing to the distinctive hardness of cocoa butter. Particularly, some authors have studied the fatty acids in *T. bicolor*, reporting that its high melting point (~36°C) offers potential for applications in food and could contribute to mitigating the overexploitation of *T. cacao*, whose high demand and cultivation costs impact market prices (Gilabert-Escrivá et al., 2002). However, other studies have presented opposing arguments, suggesting that mixing cocoa butter with fat from *T. bicolor* could result in an undesirably soft product (Jee, 1984).

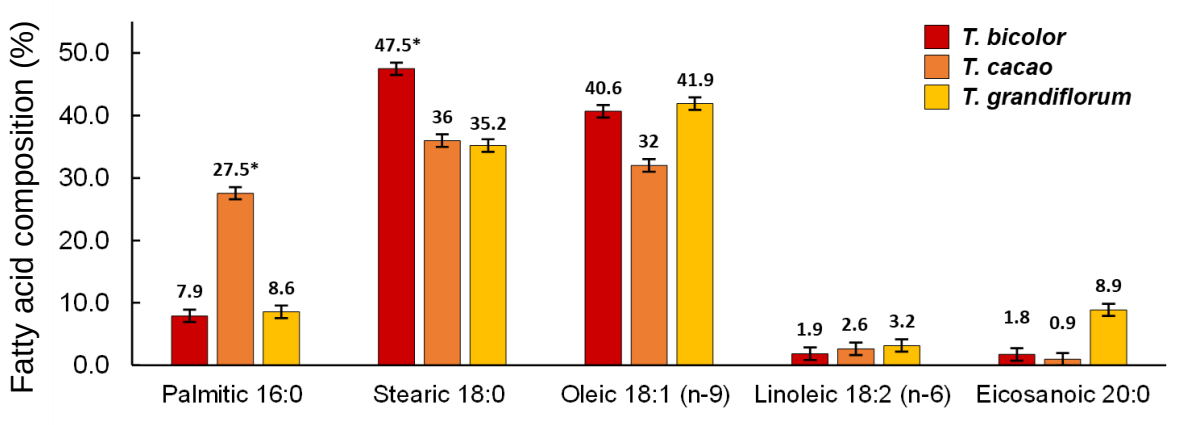

Figure 2. Fatty acid composition (%) of *T. bicolor*, *T. cacao*, and *T. grandiflorum*. Determined via Gas Chromatography analysis, *Significant differences reported by the authors. Data for construction sourced from (Barbalho et al., 2022), (Torres et al., 2002), (Gilabert-Escrivá et al., 2002), (Jee, 1984), (Vázquez-Ovando et al., 2015).

Additionally, the presence of eicosanoic acid is significantly more pronounced in *T. grandiflorum,* reducing its melting point (~32 °C) (Gilabert-Escrivá et al., 2002). Lastly, the occurrence of other fatty acids, such as palmitoleic (16:1) <0.35% and docosanoic (22:0) <2%, has been reported in modest proportions across all the three species.

Works in literature report as well the protein content, primarily utilising the Kjeldahl technique, observing that fresh seeds of *T. bicolor* exhibit the highest values (Sotelo & Alvarez, 1991), (Pugliese et al., 2013), (De Souza Schmidt Goncalves et al., 2010). This trend is confirmed by experiments conducted by (Vázquez-Ovando et al., 2015), who identified a similar pattern in fermented-dehydrated seeds. In contrast, *T. grandiflorum* liquor demonstrates higher protein values compared to *T. cacao* liquor in the study conducted by (de Oliveira & Genovese, 2013). However, (Pérez-Mora et al., 2018) et al. reported average protein values of *T. bicolor* (31.3), *T. grandiflorum* (20.6), and *T. cacao* (25.1) in seeds expressed on a DW (mg/g). These values were determined using the Bradford assay, which is based on total protein content and selectively quantifies solubilised proteins. The same authors conducted a proteomic analysis of seeds and pulp juice of the three species, employing two separation techniques: First Dimension - Isoelectric Focusing and Second Dimension (sodium dodecyl sulphate polyacrylamide Gel Electrophoresis). This combined approach provided a high level of resolution to separate and visualise proteins in the samples. Following separation, the proteins underwent further analysis by mass spectrometry. The results indicated that *T. grandiflorum* presents the greatest number of protein species (122) in seeds, followed by *T. bicolor* (94) and *T. cacao* (62). Additionally, the three Theobroma species exhibit common

species-specific spots, with 4 in their pulps and 10 in seeds. Some of the identified proteins include vicilin, a protease inhibitor, and a flavonol synthase/flavanone 3-hydroxylase.

Finally, regarding carbohydrate content, higher levels are reported in the pulp of *T. grandiflorum* compared to *T. bicolor* (Pugliese et al., 2013), (Zagmignan et al., 2023), and (Sotelo & Alvarez, 1991). The latter also presents a higher fibre content (8.8%) in fresh seeds compared to *T. cacao* (3.2%) (Sotelo & Alvarez, 1991). When evaluating the content of simple sugars, expressed in g/100g DW, sucrose is the predominant sugar in *T. bicolor* (7.7) and *T. grandiflorum* (10.8), while fructose is prevalent in *T. cacao* (7.3). In a study conducted by (Pereira et al., 2017), it was observed that after a fermentation process with the addition of *L. casei* in *T. grandiflorum* juice, aiming to generate a probiotic beverage, the initial sugars content in the juice reduced as follows: glucose from 4 to 2.5, fructose from 10.8 to 2.9, and sucrose from 5.3 to 3.8 (in g/100 mL of beverage).

Bioactive compounds profile and their role in antioxidant activities

After describing the chemical composition of these Theobroma species, the presence of bioactive compounds represents a fascinating add-value for the exploitation of these raw materials in different fields, including the biomedical one. **Table 2** provides an overview of the different antioxidant compounds and values, extracted from the three Theobroma species using diffident processing parameters. For the characterisation and quantification of these antioxidant compounds, the assessment has predominantly focused on the overall evaluation of phenolic compounds, that could include phenolic acids, flavonoids, using the Folin–Ciocalteu's reagent (FCR). This is a colorimetric method based on an electron-transfer reaction, where the antioxidant species, rich in phenolic functional groups, serves as the electron donor, and the FCR reagent functions as the oxidant (Pérez et al., 2023). This assay is widely accepted by the scientific community and applied to different plant materials and foods(Chun & Kim, 2004). Moreover, numerous studies quantify flavonoids using the aluminium chloride ($AlCl_3$) method, where the flavonoids, with their abundant oxo and hydroxyl groups, exhibit a strong tendency to bind metal ions like Al(III), typically in a 1:1 ratio. This affinity is subject to experimental variables, notably pH (Matić et al., 2017) (Shraim et al., 2021), it has not been compared with *T. cacao*. Then, proanthocyanidins, a specific group of condensed tannins such as catechin and epicatechin, have been evaluated using various methods (Pugliese et al., 2013), (Pinent et al., 2016), (de Oliveira & Genovese, 2013). One method is represented by the Butanol-HCl assay, where proanthocyanidins react with butanol-HCl under acidic conditions to produce a pink-coloured complex, which is then measured spectrophotometrically at around 550 nm (Yu et al., 2023). Another method involves the use of 4-dimethylaminocinnamaldehyde (DMACA), which results in the formation of a blue-coloured complex under acidic conditions and is measured in the range of 640-650 nm (Chowdhury et al., 2023). This

200 determination has been reported for *T. grandiflorum* and *T. cacao*. However, comparing results from different
201 methodologies can be challenging due to specific aspects employed in the determinations, such as variations in
202 the type of the sample, the conducted extraction procedure, or the chemical used as equivalents, among other
203 factors.
204 Based on the data found in literature (**Table 2**) the phenolic content was higher in seeds than in pulp. Specifically,
205 in the study conducted by (Vázquez-Ovando et al., 2015), using a two-stage aqueous extraction for 24 hours,
206 higher content of phenolics are observed for *T. cacao*, ~40 mg GAE/g, compared to *T. bicolor* (~7 mg GAE/g).
207 Similar values are reported for *T. grandiflorum* (~8 mg GAE/g), when conducting an aqueous extraction and ~3
208 mg GAE/g in methanol extraction The optimization of ultrasonic-assisted extraction of Centaurea sp. antioxidative
209 phenolic compounds using response surface methodology. When comparing the phenolic content of fresh pulp
210 between *T. bicolor* and *T. grandiflorum*, the former exhibits higher content of 1.62 mg/g FW in water extraction
211 (González et al., 2016) compared to 0.66 mg/g FW in acetone extraction for the latter (Carmona-Hernandez et al.,
212 2021).

213 **Table 2**. Antioxidant profile, compound extraction parameters, values, and assessment methods for *T. bicolor*, *T. cacao*, and *T. grandiflorum*.

Sample	Compound Extraction parameters	Method	Value (mg/g)	Ref
		Theobroma bicolor		
Pulp lyophilised	Phenols. Acetone 80%, ratio 1:6.6 (w/v), T 0.5 h	FCR (6 min), Na_2CO_3 7%, React. T 90 min Abs 760 nm	0.17 TAE DW	(Pérez-Mora et al., 2018)
Pulp lyophilised Aril + seeds	Phenols. H_2O, concentration of 16-80µg/mL	FCR (10 min), Na_2CO_3 12%, React. T 120 min Abs 760 nm	0.10 GAE ext 0.10 GAE ext	(Tauchen et al., 2016)
Seeds fermented defatted	Phenols. MetOH 50%, pH 2, ratio 1:20 (w/v), T 48h	FCR (15 min), Na_2CO_3 1M, React. T 15 min Abs 760	6.81 GAE DW	(Vázquez-Ovando et al., 2015)
		***Theobroma** cacao*		
Pulp lyophilised	Phenols. MetOH 70%, ratio 1:20 (w/v), T 2 h	FCR (6 min), Na_2CO_3 7%, React. T 90 min Abs 760 nm	0.34 TAE DW	(Pérez-Mora et al., 2018)
Pulp lyophilised Aril + seeds	Phenols. H_2O, concentration of 16-80µg/mL	FCR (10 min), Na_2CO_3 12%, React. T 120 min Abs 760 nm	0.05 GAE ext 0.22 GAE ext	(Tauchen et al., 2016)
Seeds	Phenols. MetOH 50%, pH 2, ratio 1:20 (w/v), T 48h	FCR (15 min), Na_2CO_3 1M, React. T 15 min Abs 760	41.41 GAE DW	(Vázquez-Ovando et al., 2015)
Liquor	Phenols. MetOH 70%, ratio 1:20 (w/v), T 2 h	FCR. Abs 750nm	28.45 CE DW	(de Oliveira & Genovese, 2013)
	Proanthocyanidins. MetOH	$NH_4Fe(SO_4)_2 \cdot 12H_2O$ 2% (w/v), BuOH-HCl 95:5 (v/v) React. T 40 min (95 °C). Abs 520 & 580 nm	10.59 QTE DW	
	Proanthocyanidins, Acetone 70% + acetic acid 0.5%, ratio 1:10 (w/v), T 10 min (USB)	4-dimethylamin cinnamaldehyde-HCl (alcoholic solution). Abs 640 nm	22.01 QTE DW	
Leaves	Phenols. H_2O, concentration of 16-80µg/mL	FCR (10 min), Na_2CO_3 12%, React. T 120 min. Abs 760 nm	0.15 GAE ext	(Tauchen et al., 2016)
		Theobroma grandiflorum		
Pulp lyophilised	Phenols. Acetone 80%, ratio 1:6.6 (w/v), T 0.5 h	FCR (6 min), Na_2CO_3 7%, React. T 90 min Abs 760 nm	0.23 TAE DW	(Pérez-Mora et al., 2018)
	Phenols. H_2O, concentration of (16-80µg/mL)	FCR (10 min), Na_2CO_3 12%, React. T 120 min Abs 760 nm	0.16 GAE ext	(Tauchen et al., 2016)

Pulp	Phenols. EtOH 80%, ratio 1:5 (w/v), T 0.5 h (mixer)	FCR (2 min), Na_2CO_3 3.5%, React. T 90 min Abs 655 nm	0.52 GAE FW	(Carmona-Hernandez et al., 2021)
	Phenols. MetOH 70%, ratio 1:5 (w/v), T 0.5 h (mixer)		0.47 GAE FW	
	Phenols. Acetone 70%, ratio 1:5 (w/v), T 0.5 h (mixer)		0.66 GAE FW	
	Phenols (EC50). MetOH 70%. T 24 h. From a concentrated extract, dilutions 1:40 (v/v)	FCR React. T 30 min Abs 760 nm	683 GAE ext	(Curimbaba et al., 2020)
Pulp lyophilised	Phenols. H_2O/ pulp, ratio 2:5 (w/v), T 2 min (mixer), filtration	FCR (1 min), Na_2CO_3 15%, React. T 120 min Abs 750 nm	0.02 GAE DW	(Costa et al., 2022)
Pulp dried 40 °C	Phenols. H_2O, ratio 1:10 (w/v), T 1 h (60 °C, USB)	FCR (8 min), Na_2CO_3 20%, React. T 5 h Abs 765 nm	2.01 GAE DW	(Andrade et al., 2022)
	Phenols. EtOH 12%, ratio 1:10 (w/v), T 1 h (USB)		2.64 GAE DW	
Pulp	Phenols. H_2O, diluted 34%, supernatant filtered,	FCR Na_2CO_3 20%, React. T 30 min Abs 765 nm	0.11 GAE DW	(Pereira et al., 2017)
Pulp Pulp frozen	Phenols. MetOH 70%, ratio 1:40 (w/v), T 1 min (mixer)	FCR. Abs 750nm	0.06 CE DW 0.04 CE DW	(Pugliese et al., 2013)
Aril	Phenols. H_2O, concentration of 16-80µg/mL	FCR (10 min), Na_2CO_3 12%, React. T 120 min Abs 760 nm	0.06 GAE ext	(Tauchen et al., 2016)
Pulp dried 40 °C	Flavonoids. H_2O, ratio 1:10 (w/v), T 1 h (60 °C, USB)	$NaNO_2$ 5% (5 min), $AlCl_3$ 10% (1 min), NaOH (1 M). Abs 415 nm	2.05 QE DW	(Andrade et al., 2022)
	Flavonoids. EtOH 12%, ratio 1:10 (w/v). T 1 h (USB)		2.47 QE DW	
Pulp fresh	Flavonoids. EtOH 80%, ratio 1:10 (w/v), T 0.5 h (USB)	$NaNO_2$ 5% (5 min), $AlCl_3$ 10% (1 M), NaOH (1 min). Abs 415 nm (excitation filter), 520 (reads)	0.04 QE FW	(Carmona-Hernandez et al., 2021)
	Flavonoids. MetOH 70%, ratio 1:10 (w/v), T 0.5 h (USB)		0.01 QE FW	
	Flavonoids. Acetone 70%, ratio 1:10 (w/v), T 0.5 h (USB)		0.63 QE FW	
Pulp Pulp frozen	Proanthocyanidins. MetOH: acetic acid (99%).	$FeSO_4 \cdot 7H_2O$ 154 mg /L of BuOH-HCl (3:2) React. T 15 min (90 °C), Abs 540 nm	5.71 QTE DW 4.72 QTE DW	(Pugliese et al., 2013)
Seeds dried 40 °C	Phenols. H_2O, ratio 1:10 (w/v),	FCR (8 min), Na_2CO_3 20%,	7.89	(Andrade et al., 2022)

	T 1 h (60 °C, USB) Phenols. EtOH 12%, ratio 1:10 (w/v), T 1 h (USB)	React. T 5 h. Abs 765 nm	GAE DW 2.62 GAE DW	
Seeds	Phenols. MetOH 70%, ratio 1:40 (w/v), T 1 min (mixer)	FCR. Abs 750nm	0.22 CE DW	(Pugliese et al., 2013)
Seeds	Phenols	Not reported	7.64 GAE DW	(Pinent et al., 2016)
Seeds lyophilised	Phenols. MetOH 70%, acetic acid 5%. Filtered	FCR (3 min), Na_2CO_3 saturated, React. T 30 min (37 °C). Abs 750 nm	0.05 CE DW	(De Souza Schmidt Goncalves et al., 2010)
Seeds dried 40 °C	Flavonoids. H_2O, ratio 1:10 (w/v), T 1 h (60 °C, USB)	$NaNO_2$ 5% (5 min), $AlCl_3$ 10% (1 min), NaOH (1 M). Abs 415 nm	5.39 QE DW	(Andrade et al., 2022)
	Flavonoids. EtOH 12%, ratio 1:10 (w/v), T 1 h (USB)		5.06 QE DW	
Seeds	Proanthocyanidins. MetOH: acetic acid (99%)	$FeSO_4 \cdot 7H_2O$ 154 mg /L of BuOH-HCl (3:2) React. T 15 min (90 °C). Abs 540 nm	0.32 QTE DW	(Pugliese et al., 2013)
	Proanthocyanidins	Not reported	1.98 QTE DW	(Pinent et al., 2016)
Liquor	Phenols. MetOH 70%, ratio 1:20 (w/v), T 2 h	FCR. Abs 750nm	7.84 CE DW	(de Oliveira & Genovese, 2013)
	Proanthocyanidins. MetOH.	$NH_4Fe(SO_4)_2 \cdot 12H_2O$ 2% (w/v), BuOH-HCl 95:5 (v/v). React. T 40 min (95 °C). Abs 520 & 580 nm	5.85 QTE DW	
	Proanthocyanidins. Acetone 70% + acetic acid 0.5%, ratio 1:10 (w/v), T 10 min (USB)	4-dimethylamin cinnamaldehyde-HCl (alcoholic solution). Abs 640 nm	2.18 QTE DW	
Leaves	Phenols. H_2O, concentration of 16-80µg/mL	FCR (10 min), Na_2CO_3 12%, React. T: 120 min. Abs 760 nm	0.40 GAE ext	(Tauchen et al., 2016)

EtOH: ethanol, MetOH: methanol, BuOH: butanol, Folin-Ciocalteu reagent: FCR, TAE: tannic acid equivalents, GAE: gallic acid equivalents, CE: catechin equivalents, QTE: quebracho tannin equivalents, QE: quercetin equivalents, DW: Dry weight, FW: Fresh weight, T: time, USB: ultrasonic bath

Regarding the phenolic compound content in pulps, *T. grandiflorum* presents values in the range of 0.02 to 2.64 mg/g, where lower values are observed using aqueous extractions; however, assisting the extraction with ultrasounds helps to get the highest value. Indeed, ultrasound technique is effective in recovering bioactive compounds from plant materials, thereby enhancing the processing (Bouafia et al., 2021). This effectiveness extends not only to the extraction of secondary metabolites but also to the extraction of pectin from *T. cacao* pod husk, which presents a rich content of phenolics when compared with commercial pectin (Girón-Hernández et al., 2024). Furthermore, the influence of the solvent has also been assessed in *T. grandiflorum*, revealing that a 12% ethanol concentration in the extraction solution increased the flavonoid and phenolic content when conducting extractions on dehydrated pulp (Andrade et al., 2022). However, the same study demonstrated that water represents the most effective solvent when evaluating these compounds in seeds. Furthermore, an investigation of different solvents (acetone, methanol and ethanol) on the *T. grandiflorum* has indicated that acetone is capable to enhance the yield of phenolic compounds, while ethanol has been proved to be the optimal solvent for quantifying flavonoids from fresh samples (Carmona-Hernandez et al., 2021). Certainly, an increase in ethanol concentration up to 40% in the extraction solution resulted in improved recovery of both phenolic and flavonoid contents, as evaluated in powdered leaf samples of *O. stamineus* by Chew (Chew et al., 2011). The quantification of the total flavonoids in both the pulp and seeds of *T. grandiflorum* has revealed values for the pulp ranging from 0.01 to 0.63 mg QE/g on a fresh weight, with higher results obtained from extractions using acetone (Carmona-Hernandez et al., 2021). Ethanol also exhibited higher values compared to aqueous extraction, with 2.05 and 2.47 mg QE/g on a dry weight basis respectively (Andrade et al., 2022). Indeed, flavonoid solubility is relatively low in water, prompting some authors to recommend the use of water-organic solvent mixtures to enhance the extraction of these compounds (Chebil et al., 2007). However, in studies conducted by (Andrade et al., 2022), the flavonoid content doubled in aqueous extraction from the seeds compared to the pulp of *T. grandiflorum*, particularly yielding higher amounts compared to extraction with a water-ethanol mixture. Additionally, the total flavonoid values reported in *T. grandiflorum* seeds are lower compared to those reported for defatted *T. cacao* seeds (~8 mg epicatechin equivalents/g) using comparable methodologies (Zzaman et al., 2014). Proanthocyanidins, another family of compounds, are also assessed, demonstrating similar values (~6 mg QTE/g DW) in the pulp and liquor of *T. grandiflorum*, while the evaluation of these compounds in *T. cacao* liquor is ~11 mg QTE/g DW when using methanol extractions (Pugliese et al., 2013) (de Oliveira & Genovese, 2013). The total proanthocyanidins content in *T. grandiflorum* seeds ranges from 0.3 to 2 mg QTE/g DW with comparable methodologies (Pugliese et al., 2013), (Pinent et al., 2016).

Furthermore, different compounds have been identified in the pulp and seeds of *Theobroma* species, employing chromatographic techniques based on their UV spectra or mass spectrometry (MS). Particularly, UV has been utilised to quantify the content of hypolaetin, isoscutellarein, catechol, isoscutellarein, quinic acid, chlorogenic acid, and theobromine based on their standards (Andrade et al., 2022; Carmona-Hernandez et al., 2021; de Moraes Barros et al., 2016; de Oliveira et al., 2015; Pugliese et al., 2013; Sotelo & Alvarez, 1991; Tauchen et al., 2016). In these studies, compounds such as catechin and kaempferol are either traced or not detected. The MS technique has revealed a greater number of compounds based on their mass-to-charge ratio, facilitating the identification of Theograndins, which have shown lower antioxidant capacity compared to other known polyphenols. However, their bioavailability might be better due to their water solubility, thus hydrolysing to their corresponding flavonoids (Yang et al., 2003).

Regarding comparative studies between the three species, Tauchen et al. (2016) conducted a metabolomic analysis on ethanolic extracts (70%) of edible and medicinal Amazonian plants, including *T. bicolor*, *T. cacao*, and *T. grandiflorum*, using Ultra-High Performance Liquid Chromatography and Tandem Mass Spectrometry (UPLC-MS/MS). **Figure 3** illustrates the major components in the pulp of these species, derived from data obtained from Tauchen et al., 2016. Three big groups of compounds have been reported: flavonoids, flavonoids derivatives and phenolic acids. Based on the reviewed data, *T. bicolor* shows greater similarity to *T. cacao*, presenting similar proportion and species of compounds on the most abundant within the groups. In the case of *T. bicolor*, it is particularly interesting due to its usable pulp proportion of ~26% (González et al., 2016), rendering this fruit a valuable raw material for flavonoids. Additionally, unlike the other two species, this fruit also contains a notable proportion of chlorogenic acids and rutin. *T. cacao* is characterised by the presence of luteolin, and *T. grandiflorum* by showing the presence of kaempferol in low amounts.

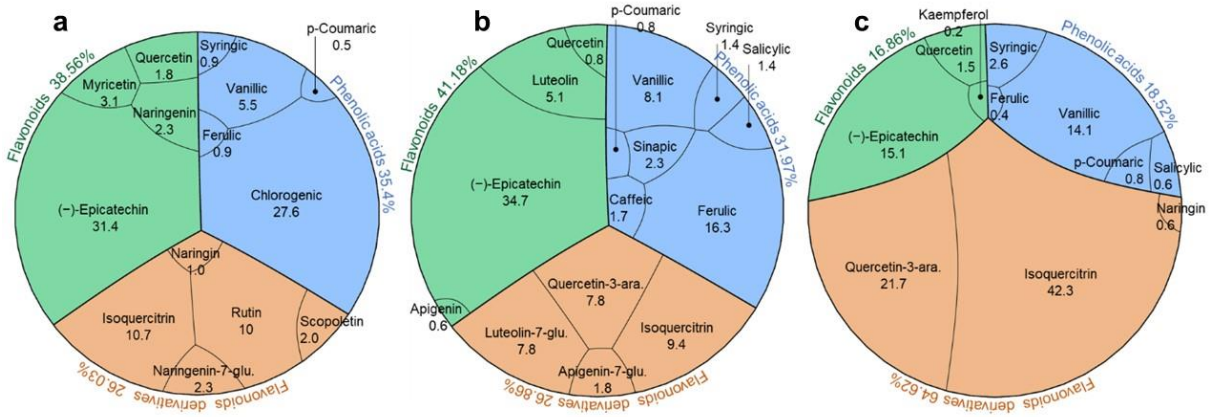

Figure 3. Treemaps of the relative abundance (%) of major metabolites in the pericarps (pulp) of (**a**) *T. bicolor*, (**b**) *T. cacao*, and (**c**) *T. grandiflorum*, determined via UHPLC–MS/MS analysis. Figure created using data sourced from (Tauchen et al., 2016)

Moreover, the antioxidant capacity has been assessed mainly on various components (pulp, seed, liquor) of fruits from the *Theobroma* species using analyses such as 2,2-diphenyl-1-picrylhydrazyl (DPPH), Oxygen Radical Absorbance Capacity (ORAC), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) ($ABTS^{\bullet+}$), Ferric Reducing Ability of Plasma (FRAP), or testing the antioxidant activity of Butylated hydroxytoluene (BHT). After *T. cacao*, *T. grandiflorum* presents more available information from literature. However, comparing the reported values is not straightforward due to the diversity of the experiments, but it can generally be expected that the antioxidant capacity tends to be higher in the materials extracted from the seeds compared to those ones from the pulp (Pugliese et al., 2013), (Andrade et al., 2022), (Tauchen et al., 2016), and (González et al., 2016). Particularly, the most used method for evaluating antioxidant activity is DPPH. In this regard, a comparative study on pulp, showed higher values for *T. grandiflorum* (~188 µg Trolox Equivalent (TE)/mg), followed by *T. bicolor* (~107 µg TE/mg), while the pulp of *T. cacao* presented significant differences with a value of approximately 52 µg TE/mg extract (Tauchen et al., 2016). However, other studies evaluated DPPH in the liquor of *T. grandiflorum* and *T. cacao*, presenting values of ~79.6 and 19.1 µg TE/mg per gram of dry weight, respectively (de Oliveira & Genovese, 2013), showing lower antioxidant capacity compared with the corresponding pulp.

Moreover, ither studies have reported their finding as IC50 value, that indicates the concentration of a sample required to scavenge 50% of DPPH free radicals (Martinez-Morales et al., 2020). For instance, (Yang et al., 2003) reported values ranging from 40 to 90 µM for the specific fraction of Theograndin I and 120 µM for Theograndin II. Then, the authors have also analysed the influence of the solvent used for extracting the compounds, revealing that acetone is the most favourable one, presenting a DPPH IC50 values of Log ascorbic acid equivalent 2.6 µg/mL, calculated from their reported 44.0 mg AAE/100 g (fresh weight).

Furthermore, ORAC was the second most popular method, (Tauchen et al., 2016) evaluated fresh pulps and observed that *T. grandiflorum* showed higher values (~435 µg TE/mg extract) than *T. bicolor* (243 µg TE/mg extract) and *T. cacao* (180 µg TE/mg extract). Similarly, it has been observed that the ORAC presents higher values when measured in fresh pulp in wet basis (~217 µmol TE/g DW) compared to frozen (~109 µmol TE/g DW) (Pugliese et al., 2013). Values decreased significantly (~17 µmol TE/g DW) when the pulp was dehydrated using forced air at 40 °C; however, in vitro digestion and fermentation of the dehydrated pulp increased antioxidant capacity (~94 and ~108 µmol TE/g DW) respectively. Additionally, studies conducted in liquor showed higher values for *T. cacao* (~451 µmol TE/ g DW) compared to *T. grandiflorum* (~136 µmol TE/ g DW).

Moreover, $ABTS^{\bullet+}$ values in fresh pulp and seeds of *T. grandiflorum* were similar, ~29 µmol TE/ g of sample, and increased after in vitro digestion to ~46 µmol TE/ g. However, after fermentation with *Lactobacillus* bacteria

(rhamnosus, longum, delbrueckii) this value decreased for the pulp down to ~19 µmol TE/ g but not for the seed (~37 µmol TE/ g) after fermentation (Andrade et al., 2022). A similar trend was observed after fermenting the pulp in a ratio 34% (w/v) for 18 hours with L. casei, resulting in increased $ABTS^{\bullet+}$ values for the treated pulp from 0.52 to 0.67 µmol TE/ mL (Pereira et al., 2017). Regarding the determination of antioxidant capacity using the FRAP method, no reports were found for samples of *T. bicolor*, while results ranging between 15.26 and 18.69 µmol TE/g have been reported for non-fermented seeds of *T. grandiflorum* (Pinent et al., 2016), (Andrade et al., 2022). The antioxidant capacity evaluated by this method was reduced after both in vitro digestion and fermentation of the seeds (Andrade et al., 2022). Furthermore, a study examining flowers of the Theobroma genus evaluated their aqueous extracts, demonstrating antioxidant capacities assessed using DPPH ranging from 199 to 1634 µmol TE/g and from 361 to 1991 µmol TE/g for $ABTS^{\bullet+}$. However, the study did not specifically report the related species (Mar et al., 2021). Finally, information related to leaves showed that *T. grandiflorum* presents a higher antioxidant capacity compared to *T. cacao* based on DPPH and ORAC assays (**Table 2**).

Therapeutic potential

The content of secondary metabolites, particularly the fraction of phenolic extracts from the *Theobroma* species evaluated in this review, has prompted the develop of various experiments to assess their potential in therapeutic and biomedical applications. To date, research has primarily focused on the biological evaluation of both seed and pulp of *T. grandiflorum*, with some studies comparing this species with *T. cacao*. The following information comprises *in vitro*, including cellular tests, and *in vivo* animal studies. No clinical studies have been reported; however, both pulp, butter, and liquor extracted from these fruits are frequently consumed without any reported adverse effects.

In vitro studies

The phenolic content in *T. grandiflorum* has sparked interest in its potential for anti-diabetic effects thanks to its capacity for α-amylase inhibition, the enzyme responsible for breaking down carbohydrates, specifically starches, into smaller sugars such as maltose and glucose (Andrade et al., 2022). In this context, a rich fraction of flavonoids was obtained through Solid Phase Extraction using a polyamide column and evaluated for its ability to reduce α-amylase activity, exhibited a half-maximal inhibitory concentration (IC50) at 1.1 mg of dried *T. grandiflorum* pulp/mL of reaction, expressed in terms of catechin equivalents, totalling 0.3 mg/mL (De Souza Schmidt Goncalves et al., 2010). Furthermore, another study examined the interaction of ethanolic and aqueous extracts from pulp and seeds of *T. grandiflorum* with the α-amylase enzyme, following simulated gastrointestinal digestion and probiotic fermentation (using *L. delbrueckii*, *L. jhonsoni*, *L. rhamus*, and *B. longum*). As result, the aqueous

extract from *T. grandiflorum* seeds exhibited a higher percentage of α-amylase inhibition (~97.4%) compared to the ethanol extracts from the pulp and seeds, which showed moderate inhibition ranging between 57 and 75% (Andrade et al., 2022). Another finding to consider from this research was that, after simulating gastrointestinal digestion, the aqueous extract of seeds and pulp exhibited the presence of flavonoids such as epicatechin, rhamnetin and daidzein with contents of ~112.2, 7.5, and 0.5 μg/g for pulp respectively (Andrade et al., 2022). Other interesting application is represented by the skincare, where fat extracted from *T. grandiflorum* seeds has been utilised in the production of emulgels, a combination of emulsions and gels (Erum et al., 2024). Various fat proportions (5, 10, and 20%), combined with polysorbate 80, dehydroacetic acid benzyl alcohol and water, where heated to produce an emulsion, and then incorporated into a carbomer gel. The resulting emulgel was studied in vitro for its UV light filtering ability. Samples at 5, 10, and 20% concentrations, diluted with 0.2 mg/mL ethanol, were measured for UV absorption (290-320nm). Sun protection factor was calculated using Mansur's equation. The resulting that the formulation with the highest fat concentration (20%) exhibited the best sun protection factor (~12), making it suitable for sunscreen product development (Aparicio-Álvarez et al., 2023).

Finally, the in vitro inhibitory effect of Amazonian fruit juices on CYP3A4, a crucial enzyme in the metabolism of many medications, was assessed by incubating liver microsomes containing the CYP3A4 enzyme with midazolam and the juice extracts. Following the reaction period, samples were analysed using high-performance liquid chromatography with a diode array detector. *T. grandiflorum* juice presented interesting results, where the pulps not only reduced the enzyme activity but also exhibited residual activity on 44.3% compared to the positive control star fruit (*Averrhoa carambola*) (Costa et al., 2022). Therefore, the fruit juice of *T. grandiflorum* could potentially enhance the assimilation of certain drugs, thereby reducing the amount of drug consumed. Based on the authors' observation, this activity could be attributed to the proportion of flavonoids contained in the fruit juice.

Certainly, *in vitro* cell tests are considered crucial to proceed with the validation of the efficacy of biocompounds in animal experimentation. Among the scrutinised *Theobroma* species, attention is once again directed towards *Theobroma grandiflorum*. Assessments have mainly focused on its cytotoxicity and interaction with intestinal cells (e.g., Caco-2), oxidative stress with renal mesangial cells, regenerative ability with skin fibroblasts, and antitumoral capacity against alveolar epithelial cells, which can be the origin of lung cancer. Particularly, some authors studied the fractionation of a seed extract, leading to the identification of two compounds labelled as Theograndins I and II (Yang et al., 2003). Despite demonstrating a reduced antioxidant capacity compared to other flavonoid compounds found in *T. grandiflorum* seeds, such as epicatechin, kaempferol, and quercetin,

Theograndins exhibit heightened water solubility owing to their sulphated structure, thereby enhancing their bioavailability in contrast to other flavonoids. Theograndin I displayed weak cytotoxicity in the HCT-116 and SW-480 human colon cancer cell lines, with IC50 values of 205 μM and 164 μM, respectively. Meanwhile, Theograndin II exhibited cytotoxic effects in the HCT-116 and SW-480 human colon cancer cell lines, with IC50 values of 143 μM and 125 μM, respectively (Yang et al., 2003). Interestingly, the unfermented *T. grandiflorum* seed extract has been compared with grape seed extract (rich in proanthocyanidins) for its antioxidant effect on Caco-2 and STC-1 cells (Pinent et al., 2016). It was found that *T. grandiflorum* extract prevented tert-butylhydroperoxide-induced oxidative stress in both cell lines for one hour after application at a lower concentration than grape seeds. However, no effect was observed for either extract after 20 hours. Regarding to oxidative stress, Purano et al. (Punaro et al., 2019) assessed the impact of *T. grandiflorum* pulp on nitrosative stress and inflammatory mediators. The authors cultured mouse immortalised mesangial cells under high glucose concentrations with and without the extract, at concentrations of up to 500 mg/mL, for a maximum of 72 hours. Following this period, the cells underwent analysis for proliferation, nitric oxide levels, and reactive oxygen or nitrogen species (**Figure 4a**). The findings revealed significant antioxidant properties, as the extract was able to reduce oxidative and nitrosative stress in the presence of high glucose, while also maintaining normal viability and showing no cytotoxic effects for up to 72 hours on the cells.

Furthermore, cytocompatibility of the extracts has been evaluated using fibroblasts cells. Specially, in vitro tests with L929 cells, derived from the subcutaneous connective tissue of male mice, showed that the aqueous extracts of the peel and seeds exhibited very high cell viability ranging from 81 to 109% (evaluated by MTT assay), at concentrations from 10 to 1000 μg/mL (Andrade et al., 2022). Additionally, Barbalho et al (Barbalho et al., 2022) studied the potential of *T. grandiflorum* seed extract in expediting the wound healing process was evaluated by incubating primary human dermal fibroblasts cells with the seed extract at a concentration of 100 μg/mL. After 24 hours, the fibroblasts presented high viability (assessed using calcein assay), but they also showed an increase in their metabolic activity via MTT, particularly evident after 4 and 7 days of incubation. Similar results were reported by Sano et al. (Sano et al., 2018), who observed that *T. grandiflorum* butter induced fibroblast cell confluence 18 hours post-wound formation, with more evident results when using 1 μg/ml of *T. grandiflorum* butter compared with the concentration of 0.1 μg/ml as well as with the control represented by only cells cultured on tissue culture plate (**Figure 4b**).

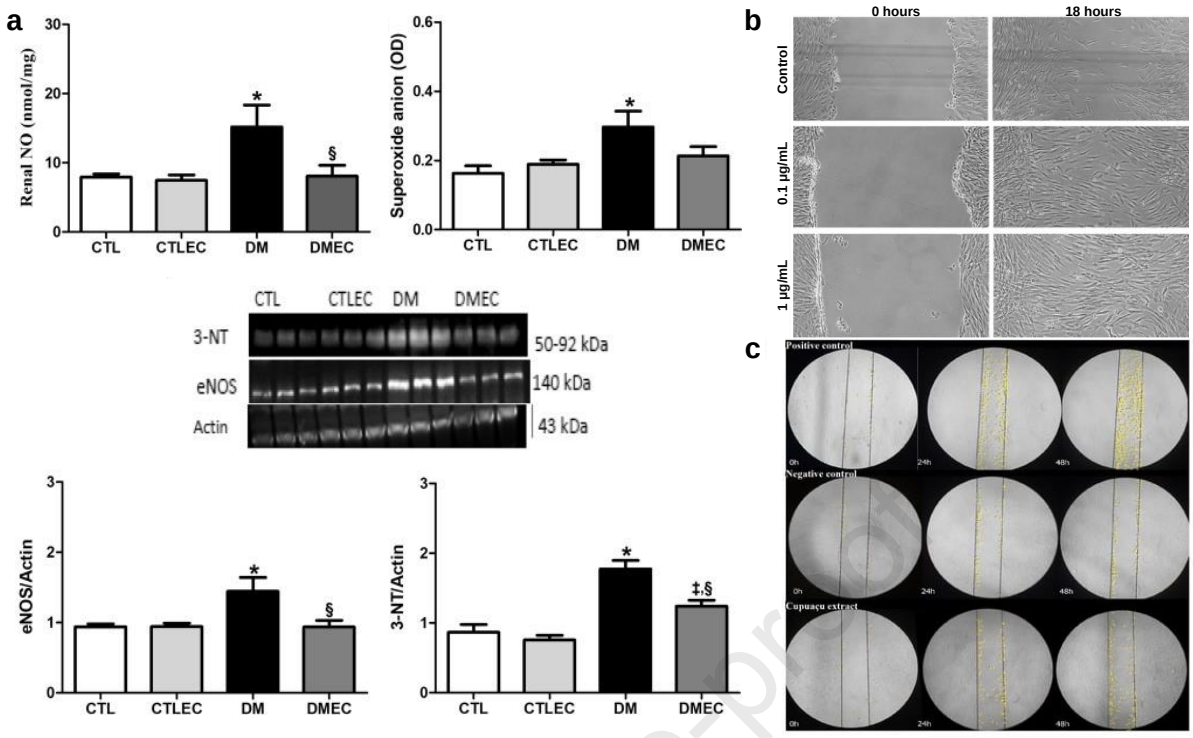

Figure 4. *In vitro* biological assessment of *T. grandiflorum* (**a**) against nitrosative stress: NO, superoxide anion, 3-NT and eNOS in the kidneys after treatment (NO: nitric oxide; 3-NT: 3-nitrotyrosine; eNOS: endothelial nitric oxide synthase; EC: extract of cupuaçu. Control (CTL); control plus EC (CTLEC); diabetic (DM); diabetic plus EC (DMEC) (Punaro et al., 2019)) and on improving wound healing after scratch tests as demonstrated by (Sano et al., 2018) (**b**) and (Barbalho et al., 2022) (**c**).

Furthermore, Barbalho et al. observed the regenerative potential of *T. grandiflorum* in supporting wound closure immediately after scratch, 24 and 48 h later (**Figure 4c**) in addition to a significant induction in mRNA expression of MKI67 (a cellular proliferation marker) and elastin, without promoting excessive expression of HAS2, which is associated with abnormal keratinocyte migration (Barbalho et al., 2022). Additionally, there was a lack of expression of matrix metalloproteinases, which are associated with poor lesion healing. Therefore, *T. grandiflorum* seed extract can represent suitable biocompatible compounds with regenerative tissues functionalities.

Finally, a preliminary study is reported in literature on the additional e.g., antitumoral ability of *T. bicolor* extracts. Indeed, a profiling study on the bioactivity of 280 plants in Panama reported that the leaves of *T. bicolor* exhibited outstanding antioxidant capacity as evaluated by a non-enzymatic test (Roy et al., 2019). However, the extract from *T. bicolor* leaves was not found to preferentially inhibit the growth of the lung cancer cell line A549 alveolar epithelial cells (EC50 < 10 µM).

In vivo animal studies

Regarding the *in vivo* validation of *T. bicolor* and *T. grandiflorum* to assess their therapeutic properties, it is noted that the available information focuses exclusively on *T. grandiflorum*, leaving a gap for research on *T. bicolor*. Among the gathered literature, studies related to the processing within the intestinal transit have reported the potential benefits of *T. grandiflorum* extracts to mitigate reactive nitrogen and oxygen species, and its association with diabetes and inflammatory processes are highlighted. Particularly, a study with C57BL/6 mice provided insights into how *T. grandiflorum* pulp extracts are processed in the gastrointestinal tract, revealing their distribution and microbial metabolic conversion (de Moraes Barros et al., 2016). The resulting compounds were mainly found in the stomach and small intestine, with lesser amounts in the caecum and colon due to microbial activity. Furthermore, flavonoids bound to sugars and epicatechin metabolites were detected in the caecum, colon and faeces. Interestingly, glucuronide flavones were hydrolysed into aglycones (the flavonoid itself, devoid of any attached sugar molecules), while procyanidins were transformed into different metabolites. Finally, this study mentioned that *T. grandiflorum* flavonoids can influence the intestinal microbiota, affecting the profile of microbial metabolite.

Other works have investigated the impact of *T. grandiflorum* liquor (de Oliveira & Genovese, 2013), (de Oliveira et al., 2015) and *T. grandiflorum* pulp (Punaro et al., 2019) on oxidative stress and nitrosative stress, respectively, in diabetic-mimicking conditions, revealing differences in their mechanisms and pathways. Particularly, Belchor and Genovese (de Oliveira & Genovese, 2013) examined the effects of the daily consumption of *T. grandiflorum* and *T. cacao* liquors (3.6 and 7.2 g/kg body weight respectively) on oxidative stress and lipid profile in induced diabetic rats. Both liquors improved body weight gain and liver weight/body weight ratio, while also improving the lipid profile by reducing triacylglycerol levels and increasing HDL-cholesterol levels, particularly with higher liquor doses. Additionally, extracts enhanced plasma antioxidant capacity and reduced lipid peroxidation in a dose-dependent manner. The synergy between fatty acids and phenolic compounds in these liquors may contribute to mitigating hypertriglyceridemia linked to insulin deficiency. Furthermore, the researchers found that despite *T. grandiflorum* liquor containing fewer phenolic compounds than *T. cacao*, its lower palmitic acid content can improve lipid profile and antioxidant status in the diabetic rats, underscoring the benefit of *T. grandiflorum*'s specific fatty acid and phenolic profile over *T. cacao*. This investigation on the liquors of *T. cacao* and *T. grandiflorum* continued when Prof. Genovese's research group decided to explore the impact of their phenolic content on oxidative stress, potentially associated with metabolic changes induced by a high-fat diet (de Oliveira et al., 2015). Upon identifying flavonoids unique to *T. grandiflorum*, absent in cocoa liquor, the animals were divided into groups receiving either a standard or high-fat diet for 16 weeks, with some rats receiving one of the

liquor extracts during the final 4 weeks. The results showed that both extracts effectively reduced liver damage from the high-fat diet by lowering lipid peroxidation and boosting antioxidant capacities in plasma and tissues. Importantly, only phenolics from *T. grandiflorum* liquor improved glucose tolerance by enhancing insulin sensitivity, attributed to its unique flavonoids.

Moreover, concerning specifically the effect of *T. grandiflorum* on oxidative stress, Curimbaba et al. reported that its antioxidant compounds, released by intestinal bacteria, can offer protection. *T. grandiflorum* pulp flour reduced diarrhoea by 75% and colon adherence by 50% in animals with induced intestinal damage (Curimbaba et al., 2020). Authors found that these effects underscored its intestinal antioxidant and anti-inflammatory properties and its ability to enhance mucin production for barrier function. However, the severity of intestinal damage diminished this protective effect (**Figure 5a**). Indeed, enzymatically, myeloperoxidase and alkaline phosphatase activities decreased, but glutathione depletion in colon tissue persisted. Additionally, dietary *T. grandiflorum* lowered IL-6 and IL-1β levels in the colon but had no effect on short-chain fatty acid production, associated with TNF-α reduction. On the other hand, regarding nitrosative stress, Punaro et al. investigated the impact of *T. grandiflorum* pulp extract on renal complications in induced diabetic rats (Punaro et al., 2019). They administered 1 mL/day of *T. grandiflorum* (1 g/mL) to adult male rats for 8 weeks, resulting in reduced food intake and nitrosative stress, as evidenced by decreased levels of O_2^-, NO, and 3-NT (a marker of nitrosative stress). Additionally, they observed inhibition of NF-kB, which plays a crucial role in inflammation-related renal damage, and reduced cytokine production, particularly IL-6. These findings suggest that *T. grandiflorum* extract may help mitigate nitrosative stress and inflammation, potentially delaying renal complications in diabetic patients.

As mentioned before, another area with potential use of the *T. grandiflorum* products is the skincare, where in their *in vivo* evaluation has been assessed by Aparicio-Alvarez et al. by using photoprotective formulations containing up to 20% of *T. grandiflorum* butter (producing an emulgel). in Balb/c male mice (Aparicio-Álvarez et al., 2023). The animals were shaved and topically treated with the emulgel three times over 24 hours. Subsequently, the mice were irradiated from a UVB lamp (5,000 J m^{-2}) for 15 consecutive sessions of 30 minutes each. Mice treated with the formulation containing *T. grandiflorum* butter at 20%, displayed either minor damage or reduced severity of the damage, comparable to a commercial standard photoprotector (**Figure 5b**).

Finally, investigations on the potential immunomodulatory effect of *T. grandiflorum* pulp have been proposed in literature, mainly when interacting with *Lactobacillus*. For instance, Zagmignan et al. examined the impact of fermenting the *T. grandiflorum* juice with *Lactobacillus rhamnosus* on the immunomodulatory response during induced short-term endotoxemia in C57BL/6 mice (Zagmignan et al., 2023). The study revealed notable

enhancements in various facets of the endotoxemic condition, including reductions in weight loss (observed in the spleen, liver, intestine, and kidneys), hypothermia, severity index, and cell migration (**Figure 5c**). These improvements were notably superior to those observed in control endotoxemic groups, which were administered either juice alone or saline solution. Furthermore, another study (Barros-Santos et al., 2020) focused on the isolation of *Lactobacillus plantarum* strains from fermented *T. cacao* and *T. grandiflorum* pulps. Subsequently, the cognitive, anxiety, and depressive-like behaviours in male swiss mice were evaluated after supplementation with *Theobroma* extracts separately. The findings indicated that *Lactobacillus plantarum* 286, isolated from *T. cacao*, exhibited notable effects. Conversely, strain 81 isolated from *T. grandiflorum* did not elicit any discernible effects on mouse models of anxiety and depressive-like behaviours. These insights underscore the potential significance of *Lactobacillus* species isolated from fermented *Theobroma* pulps in modulating physiological responses, particularly in the context of mental health.

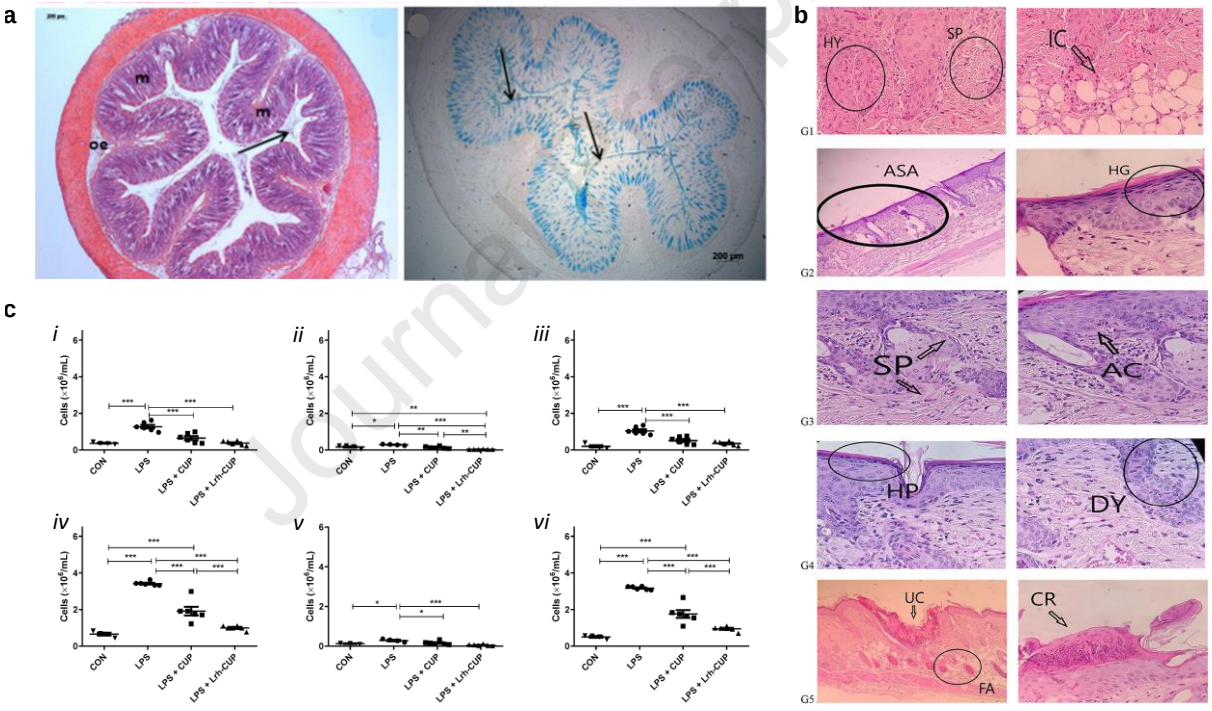

Figure 5. *In vivo* biological assessment of *T. grandiflorum.* Photomicrographs of rat colons treated with *T. grandiflorum* diets using hematoxylin and eosin (Left) and PAS/Alcian Blue (Right) (Curimbaba et al., 2020) (**a**); Histopathological evaluation of the skin of mice after 2 weeks. G1: mice exposed to UVB light without any treatment (negative control group): (Left) HY, hypergranulosis; SP, spongiosis; (Right) IC, increased collagen; G2: mice exposed to UVB light treated with commercial sunscreen product (positive control): (Left) ASA, absence of skin attachments; (Right) HG, hypergranulosis; G3, mice exposed to UVB light treated with *T. grandiflorum* seed butter emulgel at 5%: (Left) SP, spongiosis; (Right) AC, acanthosis; G4, mice exposed to UVB light treated with *T. grandiflorum* seed butter emulgel at 10%: (Left) HP, hyperplasia; (Right) DY, dysplasia; G5, mice exposed to UVB light treated with *T. grandiflorum* seed butter emulgel at 20%: (Left) UC, ulcer; FA, focal atrophy of hair

follicles; (Right) CR, crust. (Aparicio-Álvarez et al., 2023)(**b**); Effects of fermented and unfermented *T. grandiflorum* juice in the migration of cells to the peritoneal cavity in mice submitted to LPS-mediated endotoxemia. (i) Total leukocytes in the peritoneal cavity after 6 h of LPS-mediated endotoxemia; (ii) Polymorphonuclear leukocytes in the peritoneal cavity after 6 h of LPS-mediated endotoxemia; (iii) Mononuclear cells in the peritoneal cavity after 6 h of LPS-mediated endotoxemia; (iv) Total leukocytes in the peritoneal cavity after 120 h of LPS-mediated endotoxemia; (v) Polymorphonuclear leukocytes in the peritoneal cavity after 120 h of LPS-mediated endotoxemia; (vi) Mononuclear cells in the peritoneal cavity after 120 h of LPS-mediated endotoxemia (Zagmignan et al., 2023) (**c**).

Current Research and Future Prospects

Undoubtedly, *T. bicolor* presents an intriguing subject for comprehensive investigation, given the compelling bioactive profile of its fruits, enriched with phenolic compounds. Employing mass spectrometry techniques, *T. bicolor* has been linked with the presence of noteworthy compounds such as naringenin, scopoletin, myricetin, alongside a substantial quantity of chlorogenic acids. -

Consequently, despite the time and resource-intensive nature of characterising the chemical composition of both species, undertaking such an endeavour would yield substantial benefits. For instance, identifying and quantifying their secondary metabolites using chemical standards would furnish more precise insights valuable in tailoring therapeutic applications. For *T. bicolor* specifically, while existing reports focus on total polyphenol content, discerning the specific concentrations of flavonoids and proanthocyanidins warrants attention. Further exploration into its antioxidant capacity, assessed through the FRAP, alongside expanding information on other reported determinations like ORAC, DPPH, and $ABTS^{\bullet+}$, holds promise. By augmenting data on this species across these tests, seasonal effects on the chemical composition of its fruits could potentially be elucidated.

In terms of biological analyses, a constructive approach would involve mirroring the precedents set by studies on *T. grandiflorum*. This entails investigating the potential of *T. bicolor,* concerning blood sugar regulation, due to its chlorogenic acid content. Extracts from this species may exert effects on glucose absorption and production in the liver, holding implications for managing diabetes. Furthermore, the notable presence of phenolic compounds in extracts from both *T. bicolor* and *T. grandiflorum* warrants evaluation for their interaction with Caco-2 cells. Existing research points towards a protective effect of phenolic compounds on the digestive system. Exploring additional components such as liquor and butter from the fruits, noted for their heightened antioxidant capacity, could yield further insights.

Furthermore, the polyphenols found in *Theobroma* species, primarily *in T. cacao*, such as flavonoids and phenolic acids, have garnered significant research interest for their potential applications in promoting mental health. These compounds have shown promise in alleviating symptoms of depression, managing stress, and enhancing

resilience. However, further research, including not only *T. cacao* but other *Theobroma* species mentioned in this review, is necessary to fully elucidate the mechanisms and efficacy of *Theobroma* secondary metabolites in promoting mental health. Preliminary animal studies suggest promising potential for their use as adjunctive or complementary therapies in the management of depression, stress, and resilience enhancement. Additionally, exploring deeper into the interaction with friendly microorganisms such as *Lactobacillus* could provide insights into this symbiotic association with mental health. Building upon recommendations from studies reviewed here that present evidence from animal models, future investigations could prolong the assessment period for interactions of fruit extracts. Additionally, ethical considerations suggest an expansion in the number of animals to enhance result variability. Given the absence of reported adverse effects from consuming fruits of the studied *Theobroma* species, coupled with evidence supporting cytocompatibility, clinical interventions employing extracts from these fruits could offer a more nuanced evaluation of their therapeutic potential.

In this regard, nanotechnology has been recognised as a vital tool for creating promising nanostructures in potential nutraceutical delivery systems (Martínez-Ballesta et al., 2018). These nanocarrier systems can encapsulate, protect, and/or regulate the release of the polyphenols, thereby enhancing their biostability and improving absorption and oral bioavailability (Nsairat et al., 2023). Furthermore, a variety of these nano-delivery systems are classified as biodegradable and biocompatible, and are reported to employ minimal nondrug additives to enhance the pharmacokinetics of their polyphenols payloads (Charoo et al., 2019). Additionally, nanotechnology can augment the biological and antioxidant activity of many polyphenols and prolong their half-lives by shielding them from physical, biological, and chemical destructive factors (Dini, 2022). Moreover, nanotechnology can be engineered to enable encapsulated polyphenols to evade first-pass metabolism by the gastrointestinal tract and liver enzymes, thereby increasing their bioavailability. However, encapsulating polyphenols into a novel delivery system may incur expenses and result in an unusual appearance and unpleasant odour, potentially impacting consumer demand (Puri et al., 2022).

Among all the available nanotechnologies, Layer-by-Layer assembly, an environmentally friendly process based on the electrostatic interactions between polyelectrolytes (where *Theobroma*-extracted pectin can act as polyanion), allows to create functionalised multilayered nanocoated carriers that can timely release in a controlled manner the bioactive compounds (Desmond et al., 2024). Due to the high versatility of the Layer-by-Layer assembly, the polyphenols can be loaded into the core as well as within the multilayered coating forming the shell of the nanocarrier.

An area ready for exploration lies in valorising the biomass resulting from fruit processing. Notably, the shells of *T. bicolor* and *T. grandiflorum* represent over 40% of the fresh fruit weight. Drawing from existing literature on *T. cacao* utilisation, embracing circular economy principles could facilitate the sustainable utilisation of other *Theobroma* species that normally, after the extraction of beans and pulps, produce a large volume of biowaste rich in bioactive compounds. Thus, this can serve as a new source suitable for therapeutic applications. Furthermore, the intensification of the production of promising *Theobroma* species, particularly *T. grandiflorum* and *T. bicolor*, presents various advantages, including economic benefits. Diversification of income for farmers is a significant positive outcome, reducing their dependency on traditional crops such as cocoa. In terms of agricultural benefits, integrating these species into agroforestry systems promotes biodiversity and sustainable land use. However, there are also environmental limitations associated with this intensification, including the risk of land use changes, such as deforestation, if not managed sustainably. Finally, social and cultural factors play a crucial role. Smallholder farmers may be hesitant to adopt new crops due to unfamiliarity and risk aversion. Effective knowledge transfer regarding cultivation techniques and the benefits of these crops to local communities is essential and can present a significant barrier.

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CRediT authorship contribution statement

Maria Benlloch-Tinoco: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualisation. **Jose Manuel Nuñez Ramírez**: Writing – review & editing. **Paola García**: Writing – review & editing. **Piergiorgio Gentile**: Writing – review & editing, Supervision, Resources. **Joel Girón-Hernández**: Writing – review & editing, Supervision, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) utilised ChatGPT 3.5 for proof-editing purposes. Following the use of this tool/service, the author(s) thoroughly reviewed and edited the content as necessary and take full responsibility for the content of the publication.

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Declaration of interests

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