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Phytochemical composition of yerba mate (*Ilex paraguariensis*) leaves: influence of seasons and clonal variability

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Abstract

Ilex paraguariensis A. St.-Hil. is widely valued for its phytochemical composition, yet the combined influence of clonal variability and seasonality on its bioactive profile remains insufficiently characterized. This study quantified total phenolic compounds, flavonoids, caffeine, theobromine, and six caffeoylquinic acid (CQA) derivatives in four yerba mate clones (C26, C27, C33, and Aupaba) grown under controlled greenhouse conditions across the four seasons of the year. A strong clone × season interaction governed the accumulation of all compounds evaluated. Caffeine showed the greatest seasonal amplitude: clone C27 reached 2.41 g·100 g^{-1} in summer, a twofold increase relative to winter, whereas clone C33 maintained consistently low levels (0.25–0.39 g·100 g^{-1}) alongside progressive theobromine accumulation (up to 0.57 g·100 g^{-1} in autumn), suggesting a metabolic bottleneck at the final methylation step. Total phenolics peaked in summer for C27 (6.01 g·100 g^{-1}), whereas flavonoids were highest in winter, particularly in C26 (0.88 g·100 g^{-1}). Among chlorogenic acids, 3,5-di-CQA predominated, reaching 4.81 g·100 g^{-1} in C27 during spring. 3,4-di-CQA was the only derivative showing a consistent seasonal pattern across all genotypes, peaking in summer regardless of clone. The phytochemical composition of yerba mate is strongly modulated by the genotype × season interaction rather than by either factor alone. Clone C27 is the most suitable for applications requiring high caffeine and phenolic contents, whereas C33 shows potential for low-caffeine products. These findings support targeted clonal selection and strategic harvest scheduling to standardize raw material quality in the yerba mate production chain.

Keywords: caffeine; chlorogenic acids; phenolic compounds; HPLC-DAD; methylxanthines; seasonality.

1 Introduction

Ilex paraguariensis A. St.-Hil., commonly known as yerba mate, is a perennial tree species native to the subtropical region of South America, occurring naturally between latitudes 21°S and 30°S, with its distribution concentrated in Brazil, Argentina, Paraguay, and Uruguay

[1, 2]. In Brazil, the state of Paraná is the leading national producer, accounting for approximately 87% of extractive production and 49.8% of agricultural cultivation [3, 4], which establishes the species as a strategic raw material for a production chain that ranges from smallholder farmers to processing industries. The species can be propagated either from seed-derived progenies, which give rise to populations with greater genetic variability, or by vegetative cloning of previously selected elite mother plants, a strategy that ensures greater uniformity in yield, chemical quality, and stress tolerance in commercial plantations [5, 6]. It is consumed mainly as infusions such as *chimarrão* and *tereré*, and its presence in international markets has been growing, driven by the recognition of its functional properties [7].

Scientific and industrial interest in yerba mate has intensified owing to its rich phytochemical composition, particularly in methylxanthines, such as caffeine and theobromine, and in phenolic compounds, including flavonoids and chlorogenic acids, to which antioxidant, anti-inflammatory, neuroprotective, and central nervous system stimulant properties have been attributed [1, 7–9]. Flavonoids such as quercetin and rutin, in particular, have been associated with cardioprotective and anti-inflammatory effects in clinical studies [10], whereas chlorogenic acid derivatives are directly involved in the high antioxidant capacity of the species and contribute to its characteristic bitterness [11, 12]. The plant is estimated to contain more than 200 bioactive compounds [13], which has driven its use in the formulation of dietary supplements, cosmetics, and herbal medicines [14], expanding its added value beyond traditional consumption.

The concentration of these secondary metabolites results from the interplay of genetic, environmental, and technological factors [1, 15]. From a genetic standpoint, clones and progenies of *I. paraguariensis* exhibit distinct phytochemical profiles, reflecting differences in the expression of secondary metabolism enzymes, especially *N*-methyltransferases and enzymes of the phenylpropanoid pathway, which result in wide variability in caffeine, theobromine, and phenolic compound contents among genetic materials [7, 16, 17]. Caffeine biosynthesis in the species proceeds through a sequence of methylation steps catalyzed by *N*-methyltransferases (xanthosine methyltransferase, theobromine synthase, and caffeine synthase), whereas chlorogenic acid derivatives are synthesized via the phenylpropanoid pathway, involving key enzymes such as hydroxycinnamoyl transferase (HCT) and coumaroylquinate and caffeoylquinate transferases [18–23]. From an environmental standpoint, temperature, solar radiation, and photoperiod directly influence enzymatic activity

and metabolite accumulation throughout the year, conferring on seasonality a relevant modulatory role in the chemical profile of the species [16, 24–27].

Despite recent advances, most studies have assessed the effects of clonal variability or seasonality in isolation, without considering the interaction between these two factors [28]. This gap limits the understanding of the actual phytochemical behavior of the species under controlled cultivation conditions and hinders the establishment of harvest and clonal selection strategies aimed at raw material standardization. High-performance liquid chromatography with diode array detection (HPLC-DAD) has become established as a reference tool for the simultaneous identification and quantification of caffeine, theobromine, and chlorogenic acid derivatives in yerba mate [14, 17, 29], complemented by UV-Vis spectrophotometry for the determination of total phenolics and flavonoids [30].

In this context, the present study quantified and compared the contents of total phenolic compounds, flavonoids, caffeine, theobromine, and six caffeoylquinic acid derivatives in four *Ilex paraguariensis* clones (C26, C27, C33, and Aupaba) grown in a greenhouse under controlled irrigation and temperature conditions across the four seasons of the year. The aim was to evaluate the combined influence of clonal variability and seasonality on the phytochemical profile of the species and to provide support for clonal selection and harvest management strategies in the yerba mate production chain.

2 Materials and methods

2.1 Plant material and growing conditions

Leaves and thin branches from four yerba mate genotypes (*Ilex paraguariensis* A. St.-Hil.) developed by Embrapa were used: the experimental clones C26, C27, and C33, and the registered cultivar Aupaba. These materials were selected for their contrasting caffeine profiles (low, medium, and high content). The adult clonal mother plants (2 to 5 years old) were maintained at the Golden Tree nursery (Guarapuava, PR, Brazil; 25.567° S, 51.510° W; altitude 1,100 m), a region with a humid subtropical climate and well-defined seasonal temperature variations.

The plants were grown in a protected environment, in an arched metal-frame agricultural greenhouse fitted with a translucent plastic cover and an internal shading system (Figure 1). They were arranged in raised troughs under a hydroponic system, with fine gravel as an inert substrate. Nutrient management was carried out by drip fertigation twice daily with a balanced macro- and micronutrient solution (MAP, K and Ca nitrates, Mg, Mn, and Zn sulfates, Na

molybdate, boron, and iron). Supplementary sprinkler irrigation was adjusted according to ambient temperature to maintain adequate water conditions across clones.

Thermal and light conditions inside the greenhouse were controlled by the internal shading system, consisting of a metal frame with shade netting. The netting was kept closed during the hottest periods of the day and during rainfall in order to reduce the incidence of direct solar radiation and prevent excessive changes in growing conditions.

Air temperature was monitored with an analog chapel-type maximum–minimum thermometer (Incoterm, ref. 5201.03.0.00), with a range of −38 to 50 °C, 1 °C divisions, and an accuracy of ±1.0 °C. The instrument was installed at the center of the greenhouse, 1.5 m above the ground, protected by a perforated metal shield. Temperature readings were taken hourly between 08:00 and 17:00, allowing the diurnal temperature range to be monitored and the shading system to be adjusted according to the observed conditions.



Figure 1. Interior view of the greenhouse: hydroponic cultivation system of *I. paraguariensis* clonal mother plants in raised troughs with fertigation.

2.2 Seasonal sampling

Sampling was standardized between 10:00 and 11:00 to minimize circadian variations in secondary metabolite contents and covered the four seasons: winter (August 2024), spring (November 2024), summer (February 2025), and autumn (May 2025). For each genotype, 200

g of plant material was collected along the entire length of the trough to reduce intra-plant variability.

2.3 Sample processing and extract preparation

Leaves were cleaned with distilled water, dried in a forced-air oven (45 ± 1 °C, 24 h), and ground in a knife mill, with particle size standardized using a 20-mesh (Tyler) sieve, according to a methodology adapted from [31]. Residual moisture was determined according to the Brazilian Pharmacopoeia [32]. The material was vacuum-packed and stored at −20 °C until analysis.

Extracts were prepared in triplicate by maceration of 1.000 g of dried sample in 10 mL of ethanol:water (70:30, v/v) under orbital shaking (175 rpm, 25 ± 1 °C, 24 h, protected from light); this solvent system has shown high efficiency in extracting phenolic compounds and alkaloids from *I. paraguariensis* [31, 33, 34]. After filtration through qualitative filter paper, the extracts were stored at −20 °C until analysis.

2.4 Total phenolic compounds and flavonoids

Total phenolic content (TPC) was determined by the Folin–Ciocalteu colorimetric method [30]. Aliquots of 250 µL of extract (diluted 1:100 in 70% ethanol) were reacted with Folin–Ciocalteu reagent, saturated sodium carbonate solution, and distilled water, incubated for 25 min at room temperature in the dark, and read at 725 nm. Results were expressed as g per 100 g of dry sample, using gallic acid as the external standard (0–200 mg L^{-1}).

Flavonoid content (FL) was quantified by the aluminum chloride method [30]. Extracts diluted 1:10 in methanol were reacted with 5% $AlCl_3$ solution (v/v in methanol) and incubated for 30 min, and absorbance was read at 425 nm. Quantification was performed using a quercetin calibration curve (0–100 mg L^{-1}), and results were expressed as g per 100 g of dry sample.

2.5 Determination of caffeine, theobromine, and chlorogenic acids by HPLC-DAD

Methylxanthines and caffeoylquinic acids (CQAs) were determined by HPLC-DAD (UltiMate 3000, Thermo Fisher Scientific) on a C18 reversed-phase column (ZORBAX Eclipse XDB, 250 mm × 4.6 mm, 5 µm) at 30 °C, following the method described in [14] with modifications. Extracts were diluted 1:10 in ultrapure water:methanol (85:15, v/v) and filtered

through a 0.45 μm nylon membrane prior to injection (20 μL). The mobile phase consisted of water acidified with 0.3% acetic acid (A) and HPLC-grade methanol (B), under gradient elution at 1.0 mL min^{-1}. Detection was performed at 265 nm for caffeine and theobromine and at 325 nm for the CQAs (3-CQA, 5-CQA, 4-CQA, 3,4-di-CQA, 3,5-di-CQA, and 4,5-di-CQA). Compounds were identified by comparing retention times and UV-Vis spectra with those of analytical standards (≥99%, Sigma-Aldrich/Supelco). Derivatives lacking an individual commercial standard were identified by relative retention times and spectral profiles [14, 35] and quantified using the 5-CQA calibration curve, applying a correction factor based on their molar mass ratio relative to 5-CQA (354.31 g mol^{-1}) [35].

2.6 Method validation

Selectivity and linearity ($R^2 > 0.99$) were evaluated according to the ICH guidelines [36] and Snyder et al. [37]. The limits of detection (LOD) and quantification (LOQ) were calculated from the regression residuals using the IUPAC statistical approach ($\alpha = \beta = 0.05$), as proposed by Olivieri [38], which accounts for the risks of type I and type II errors and provides a more rigorous estimate than the classical definition based on $3\sigma_{blank}$.

2.7 Statistical analysis

Data were analyzed under a 4 × 4 factorial design (four clones × four seasons), allowing the main effects and the clone × season interaction on bioactive compound contents to be assessed. Normality of residuals and homogeneity of variances were verified by the Shapiro–Wilk and Levene tests, respectively ($\alpha = 0.05$). Once these assumptions were met, factorial ANOVA was applied and, when significant effects were detected ($p < 0.05$), means were compared by Tukey's test. The univariate analyses were performed in R [39], version 4.4.3 (RStudio [40]). To explore the multivariate structure of the data and summarize the relationships between bioactive compounds and the experimental factors (clone and season), principal component analysis (PCA) was performed on the correlation matrix of all quantified chemical variables (total phenolics, flavonoids, methylxanthines, and six chlorogenic acid derivatives). The suitability of the data for PCA was verified by Bartlett's test of sphericity ($p < 0.01$) and the Kaiser–Meyer–Olkin measure of sampling adequacy (KMO > 0.70), indicating

adequate correlation among variables for component extraction. Sample scores were projected onto the first two principal components and plotted with 95% confidence ellipses for each level of the qualitative factors (clone and season). The PCA was conducted entirely in Python, using the scikit-learn library for multivariate decomposition, SciPy for statistical tests, and Matplotlib with Seaborn for plotting. Component interpretation was based on factor loadings with absolute values greater than 0.50, and the cumulative explained variance was used to define the number of retained components. This multivariate approach complemented the factorial ANOVA, enabling visualization of clustering and discrimination patterns among clones and seasons and identification of the compounds that contributed most to the observed separation.

3 Results and discussion

3.1 Environmental conditions and experimental context

Thermal conditions inside the greenhouse showed well-defined seasonal variation throughout the experimental period (Figure 2). Mean and maximum temperatures increased progressively from winter to summer and decreased in autumn, reflecting the expected climatic pattern for the growing region.

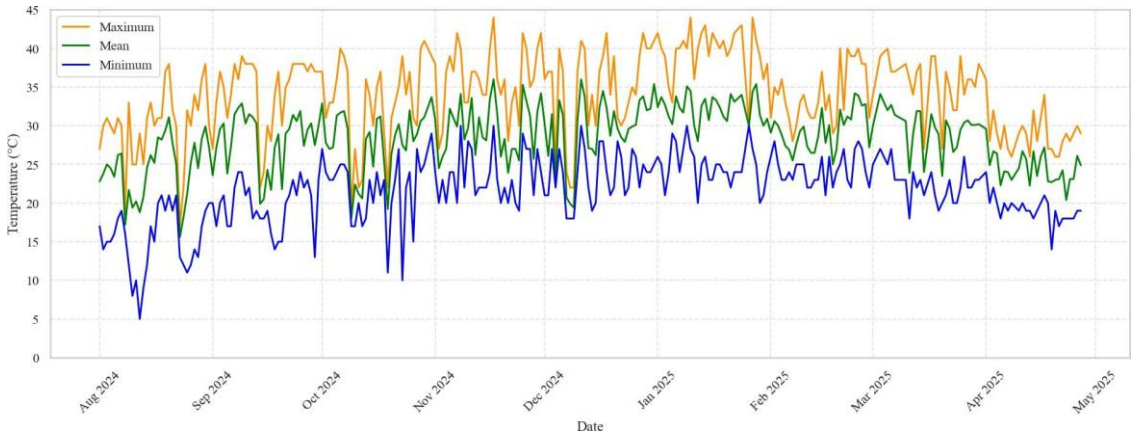


Figure 2. Daily maximum, mean, and minimum temperatures recorded in the greenhouse during the experimental period (August 2024 to May 2025).

The distribution of temperature ranges (Figure 3) allows a more detailed interpretation of this behavior.

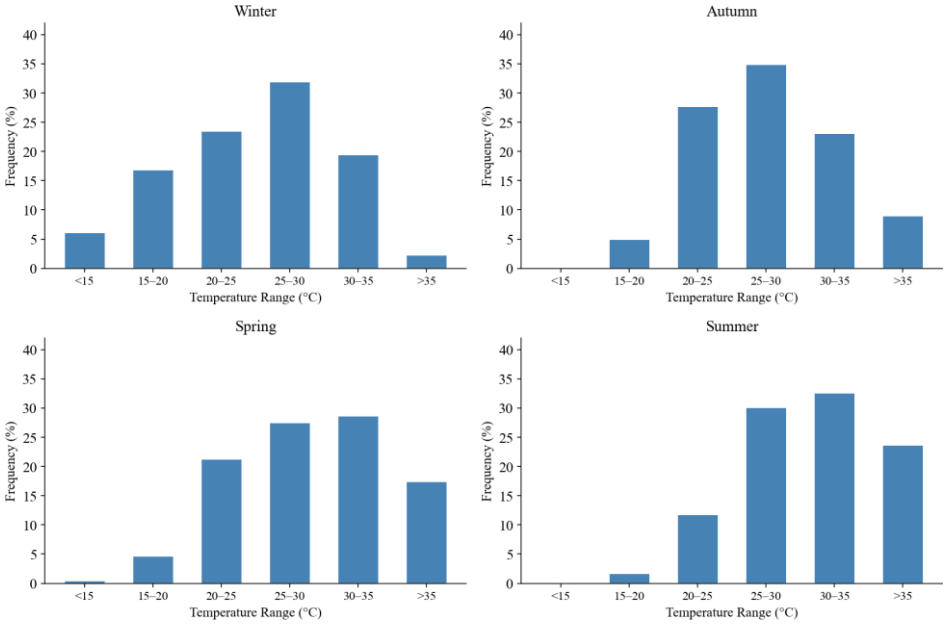


Figure 3. Percentage distribution of temperature ranges recorded in the greenhouse across the seasons.

During winter, the 20–25 °C range predominated, followed by the 25–30 °C range, with a low occurrence of temperatures above 30 °C, characterizing milder thermal conditions and lower plant exposure to heat stress. In autumn, a more balanced distribution among these ranges was observed, with a slight increase in the frequency of temperatures above 30 °C, indicating a transitional thermal condition in the growing environment. Spring was marked by a progressive increase in the frequency of the 25–30 °C and 30–35 °C ranges, evidencing the onset of thermal intensification. This pattern became more pronounced in summer, when the highest frequency of elevated temperatures was recorded, with substantial occurrence in the 30–35 °C and >35 °C ranges.

This scenario indicates that, during summer, the plants were subjected to a greater thermal load and higher solar radiation intensity, conditions known to induce physiological and metabolic responses, including enhanced biosynthesis of secondary metabolites [41–43]. According to Gobbo-Neto and Lopes [24], temperature, radiation, and water availability are among the main abiotic factors modulating plant secondary metabolism, acting on both the gene expression of key enzymes and enzymatic activity itself. The milder winter conditions, in turn, may favor metabolic stability and stimulate the production of compounds associated with cold tolerance, such as flavonoids [25]. Therefore, the thermal gradient observed across the seasons is a determining factor in interpreting the variations in the bioactive compounds analyzed in this study, consistent with the scenario reported for other perennial woody species grown under subtropical conditions [27].

3.2 Residual moisture and sample stability

Residual moisture contents remained low across all clones and seasons, ranging from 4.11 to 5.68%. These values are below the maximum limit of 10% established by Brazilian legislation (ANVISA RDC No. 277/2005), ensuring sample stability and the reliability of the chemical analyses. Similar results were reported by Tomasi et al. [44] and Frizon et al. [45], who observed contents between 5 and 7% for yerba mate leaves dried in a forced-air oven. The low moisture variability among clones and seasons in this study (range below 1.6 percentage points) indicates that the standardized drying protocol (45 ± 1 °C, 24 h) effectively neutralized seasonal differences in leaf moisture, reducing potential interference of this parameter in the quantification of bioactive compounds and allowing the observed variations to be attributed predominantly to genuine genotypic and environmental effects rather than to analytical artifacts.

3.3 Analytical performance of the HPLC-DAD method

Prior to discussing the phytochemical variations among clones and seasons, the performance of the chromatographic method used to quantify theobromine, caffeine, and 5-*O*-caffeoylquinic acid (5-CQA) was evaluated; 5-CQA was used as the reference compound for quantifying the remaining caffeoylquinic derivatives lacking individual commercial standards. Method selectivity was confirmed by the absence of relevant matrix interferences at the retention times and in the UV-Vis spectra of the analytes, and linearity was adequate across all concentration ranges evaluated, with coefficients of determination (R^2) of 0.9996 or higher (Table 1). Homoscedasticity of residuals was confirmed by the Breusch–Pagan test ($p > 0.05$ for all curves), which justified the use of an unweighted simple linear regression model instead of weighted models ($1/x$ and $1/x^2$), as the latter yielded no statistically relevant improvement.

Table 1. Summary of the analytical parameters of the HPLC-DAD calibration curves for theobromine, caffeine, and 5-CQA.

Parameter	Theobromine	Caffeine	5-CQA
R^2 (low/high range)	0.9998 / –	0.9999 / 0.9998	0.9997 / 0.9996
LOD (mg L^{-1})	1.80	1.49	3.24
LOQ (mg L^{-1})	5.46	4.51	9.83

The limits of detection and quantification, calculated using the IUPAC statistical approach [38], were within the order of magnitude expected for HPLC-DAD methods applied to complex plant matrices, in accordance with the ICH guidelines [36] and Snyder et al. [37]. Taken together, these parameters confirm the suitability of the method for the quantifications

performed, allowing the differences observed among clones and seasons to be confidently attributed to genuine phytochemical variations rather than to analytical limitations.

3.4 Influence of seasonality on total phenolic compounds and flavonoids

The heat maps of total phenolic and flavonoid contents (Figure 4) reveal a strong genotype × season interaction for both groups of compounds. Total phenolics tended to accumulate under higher-temperature conditions, especially in summer, most notably in clone C27, which reached 6.01 g·100 g^{-1}, the highest value observed among all clone × season combinations, followed by Aupaba (5.32 g·100 g^{-1}) and C33 (4.89 g·100 g^{-1}) in the same period. This behavior is consistent with the findings of Cao et al. [41] and Gaude and Jalmi [43], according to whom increased solar radiation and heat stress stimulate the phenylpropanoid pathway as an antioxidant defense mechanism, thereby enhancing the accumulation of total phenolic compounds. The total phenolic values reported here lie in the upper range of those described by Filip et al. [11] for other species of the genus *Ilex*, reinforcing the distinctive potential of yerba mate as a source of phenolic compounds, as also discussed by Croge et al. [7].

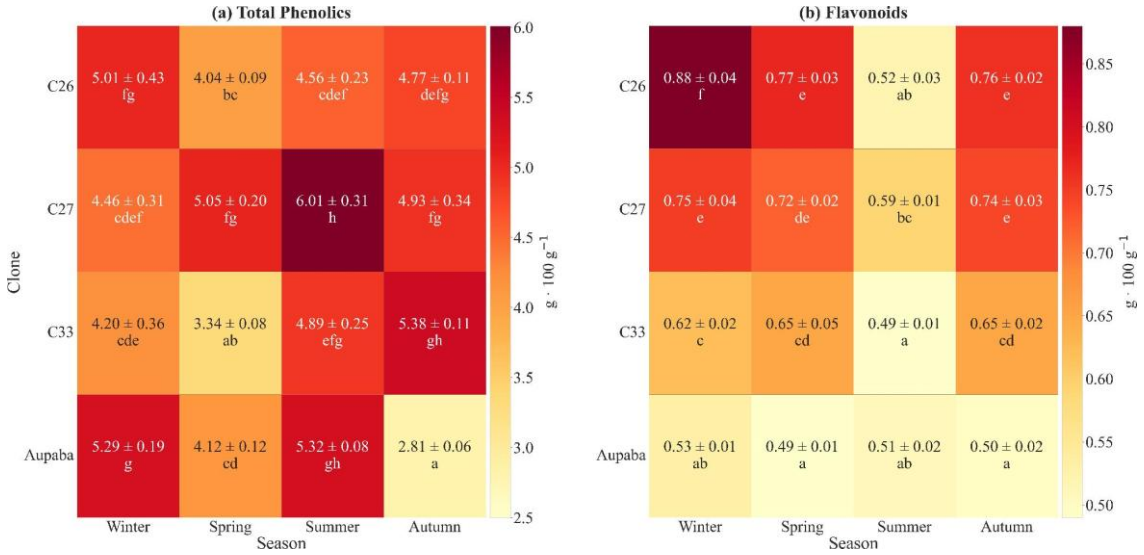


Figure 4. Heat map of the mean contents of total phenolic compounds (a) and flavonoids (b) in yerba mate clones, expressed as g·100 g^{-1} (dry basis), as a function of season.

Conversely, flavonoids showed the opposite trend, with higher concentrations in winter and lower levels in the warmer seasons. This pattern was particularly evident in clone C26, which reached 0.88 g·100 g^{-1} in winter compared with only 0.52 g·100 g^{-1} in summer, a reduction of approximately 41%. This behavior suggests that flavonoids play a relevant role in protection against cold-related stresses, contributing to the maintenance of membrane integrity and to the scavenging of reactive oxygen species generated at low temperatures, in line with

the mechanism proposed by Dabeek and Marra [10] for flavonoids such as quercetin and rutin, the major constituents of the flavonoid fraction of yerba mate [7]. The variation observed among clones, with C26 consistently exceeding the other genotypes in all seasons, reinforces the role of the genetic factor in modulating the phenylpropanoid pathway [7, 46] and suggests that this clone may represent genetic material of interest for applications aimed at obtaining flavonoid-rich extracts.

3.5 Methylxanthines: caffeine and theobromine

The methylxanthine contents (Figure 5) demonstrate that seasonality significantly influenced the production of these compounds, with the greatest impact observed for caffeine. Caffeine contents increased markedly during summer, particularly in clone C27, which reached 2.41 g·100 g^{-1}, nearly twice the value observed in winter (1.19 g·100 g^{-1}), indicating greater *N*-methyltransferase activity under high temperature and light conditions [25–27]. This value exceeds the upper limit of the range historically reported for commercial yerba mate (0.7 to 2.0 g·100 g^{-1}), according to the review by Heck and de Mejia [1], showing that C27 is a genotype with high caffeine potential even when compared with commercial progenies. This behavior suggests an adaptive mechanism associated with environmental stress, consistent with the hypothesis that caffeine acts as a chemical defense compound against herbivory and abiotic stress in *Ilex paraguariensis* [18, 20].

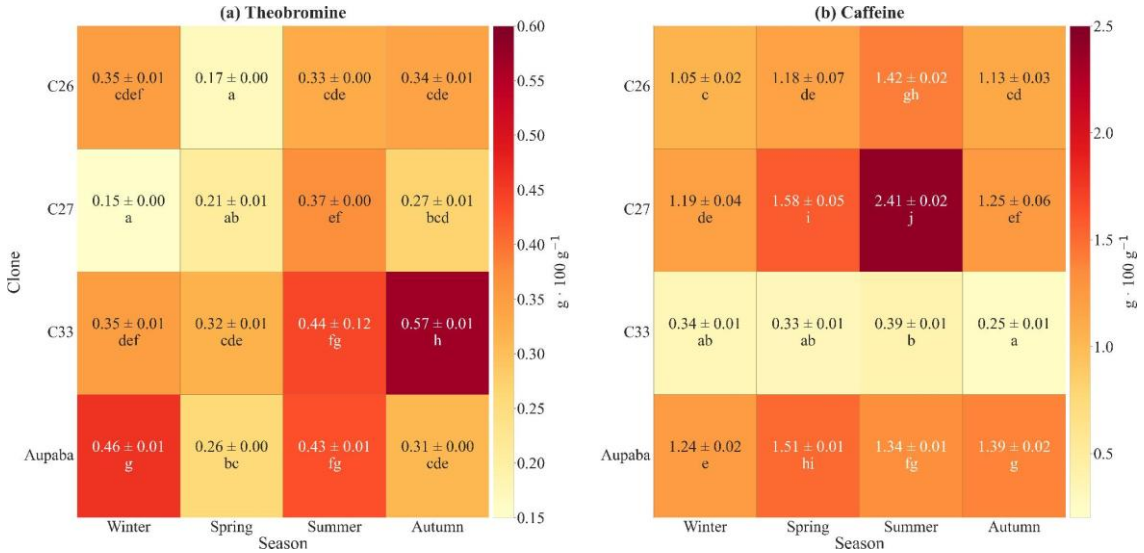


Figure 5. Heat map of the mean contents of theobromine (a) and caffeine (b) in yerba mate, expressed as g·100 g^{-1} (dry basis), as a function of season.

Clone C33 exhibited low caffeine contents across all seasons (0.25–0.39 g·100 g^{-1}), concomitantly with a progressive accumulation of theobromine, which peaked in autumn (0.57

g·100 g^{-1}), the highest theobromine content recorded among all clone × season combinations in this study. This pattern indicates a possible limitation at the final methylation step of the biosynthetic pathway, namely the conversion of theobromine into caffeine by caffeine synthase, as described in the *N*-methylation sequence proposed by Ashihara and Crozier [19] and detailed by Yin et al. [18] for yerba mate leaves. This uncoupling between precursor and end product characterizes C33 as a potential model for studies on caffeine synthase regulation and as genetic material of interest for the development of low-caffeine products, a growing market niche, as noted by Gebara et al. [14].

In contrast, the Aupaba cultivar showed greater stability in caffeine content throughout the year (a range of only 0.27 g·100 g^{-1} between the seasons with the lowest and highest contents), indicating lower sensitivity to seasonal variations and possibly constitutive expression of the enzymes involved in the methylxanthine biosynthetic pathway. This stability is particularly relevant from an agro-industrial perspective, since commercial cultivars with a more predictable chemical composition throughout the year tend to facilitate raw material standardization in the production chain [17, 28]. Overall, the results confirm that methylxanthine biosynthesis is regulated by both environmental and genetic factors [18, 20], with the clone × season interaction exceeding the isolated effects of each factor in magnitude, a finding that directly addresses the gap identified by Tomasi et al. [28] regarding the scarcity of studies integrating clonal variability and seasonality.

3.6 Chlorogenic acids and derivatives

The heat maps of the individual chlorogenic acids (Figure 6) reveal complex, strongly genotype-dependent patterns of seasonal variation. Overall, 3,5-di-CQA was the predominant derivative in nearly all clone × season combinations, reaching its maximum in clone C27 during spring (4.81 g·100 g^{-1}), followed by Aupaba in the same season (4.05 g·100 g^{-1}). This predominance of dicaffeoylquinic over monocaffeoylquinic derivatives is consistent with the phytochemical profile previously described for the species by Clifford [12] and quantitatively detailed by Gebara et al. [35], who identified 3,5-di-CQA as the major chlorogenic acid in traditional yerba mate beverages.

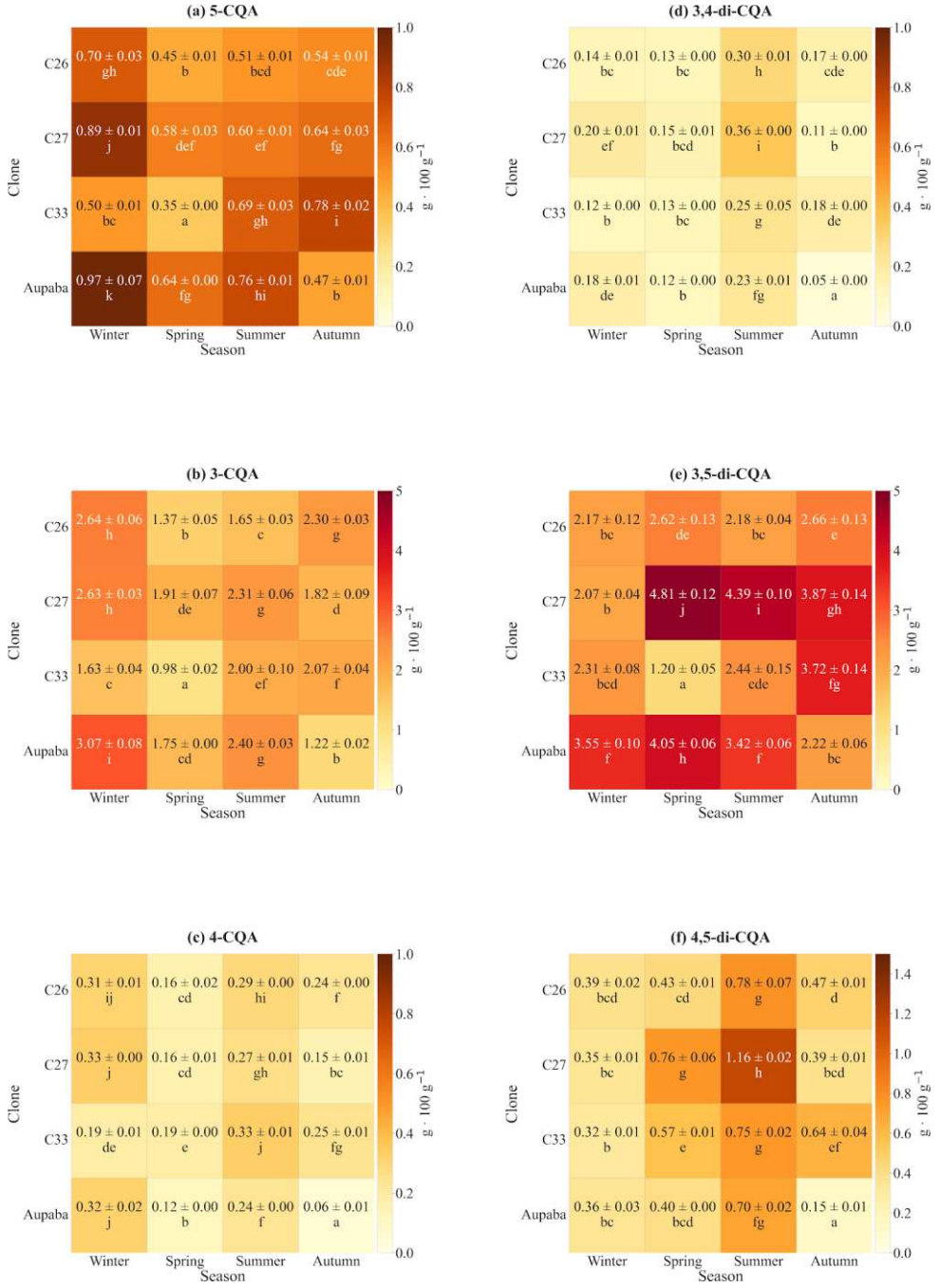


Figure 6. Heat maps of the mean CQA contents: 5-CQA (a), 3-CQA (b), 4-CQA (c), 3,4-di-CQA (d), 3,5-di-CQA (e), and 4,5-di-CQA (f) in different yerba mate clones, expressed as g·100 g^{-1} (dry basis), according to the sampling season.

Among the compounds evaluated, 3,4-di-CQA was the only one to exhibit consistent behavior across all clones, with greater accumulation in summer in all genotypes (e.g., C26 increased from 0.14 g·100 g^{-1} in winter to 0.30 g·100 g^{-1} in summer, and C27 from 0.20 to 0.36 g·100 g^{-1}). This pattern suggests a conserved, largely genotype-independent physiological response associated with high irradiance and temperature, supporting the hypothesis that this specific derivative is under tighter regulatory control within the phenylpropanoid pathway,

possibly related to the activity of hydroxycinnamoyl transferases (HCT) that are more conserved among the genotypes studied [22, 23].

Conversely, compounds such as 5-CQA, 3-CQA, and 3,5-di-CQA showed a strong clone × season interaction, with the dominant clone changing according to the time of year; for instance, 3-CQA peaked in Aupaba in winter (3.07 g·100 g^{-1}) but in C26 in autumn (2.30 g·100 g^{-1}). This behavior shows that the regulation of the phenylpropanoid pathway is highly genotype-dependent, supporting the model proposed by Clifford et al. [21] of multiple acylation and isomerization routes competing for the same precursors (quinic acid and hydroxycinnamic derivatives), whose relative predominance may be modulated by both environmental factors and differences in the expression of specific transferases among genotypes [47].

The dicaffeoylquinic derivatives (3,4-, 3,5-, and 4,5-di-CQA) generally exhibited a greater relative amplitude of seasonal variation than the monocaffeoylquinic derivatives (3-, 4-, and 5-CQA), indicating a higher sensitivity of this class to environmental conditions. This pattern is illustrated by 4,5-di-CQA in clone C27, which ranged from 0.35 g·100 g^{-1} in winter to 1.16 g·100 g^{-1} in summer (an increase of more than 200%). These results corroborate studies highlighting the joint influence of environmental and genetic factors on the regulation of key enzymes of the phenylpropanoid pathway, such as HCT and caffeoylquinate synthases (CQS) [23, 47], and suggest that di-CQAs may be particularly useful as phytochemical markers of heat stress in future seasonal monitoring programs of raw material quality.

3.7 Integrated synthesis of results

Principal component analysis (Figure 7) summarizes the patterns discussed in the previous sections. The first two components jointly explained 64.7% of the total variance (41.6% for PC1 and 23.1% for PC2). The separation of the confidence ellipses by season (Figure 7b) is clearer than that by clone (Figure 7a), particularly between summer samples and those from the other seasons. This indicates that seasonality is the main axis of phytochemical variation in this dataset, whereas the genotype effect acts mainly by modulating the magnitude and direction of the seasonal responses, that is, an interaction effect rather than a purely additive one. This pattern is consistent with the observation that virtually all compounds evaluated showed a statistically significant clone × season interaction, supporting the limitation pointed out by Tomasi et al. [28] regarding the inadequacy of approaches that treat these factors in isolation.

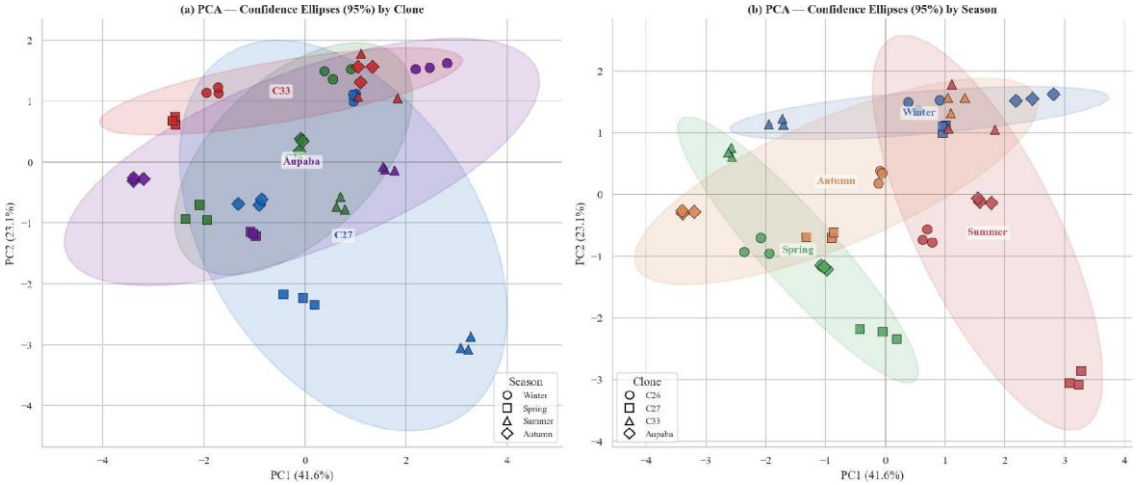


Figure 7. Principal component analysis (PCA) of the phytochemical profiles of yerba mate clones, with 95% confidence ellipses grouped by clone (a) and by season (b).

Overall, the results demonstrate that the chemical composition of yerba mate is strongly influenced by the interaction between seasonal and genetic factors rather than by either factor alone. Summer favored the accumulation of total phenolic compounds, caffeine, and certain chlorogenic acid derivatives (notably 3,4-di-CQA), whereas winter promoted higher flavonoid contents. Furthermore, some compounds behaved consistently across clones, as was the case for 3,4-di-CQA, whereas others were highly genotype-dependent, as was the case for caffeine, 3,5-di-CQA, and 3-CQA. From an applied perspective, clone C27 emerges as the most suitable genetic material for applications requiring high caffeine and total phenolic contents, especially when harvested in summer, whereas clone C33 shows potential for the development of low-caffeine products, particularly when harvested in milder seasons. These findings reinforce the importance of combining clonal selection with the definition of the harvest period as complementary, rather than substitutive, strategies to optimize and standardize the production of bioactive compounds in the *Ilex paraguariensis* production chain [7, 28].

4 Conclusion

The results demonstrate that the phytochemical composition of *Ilex paraguariensis* cannot be satisfactorily explained by models that treat genotype and seasonality as independent factors: for virtually all compounds evaluated, the clone × season interaction was the main determinant of the observed variation, exceeding the isolated effects of each factor in magnitude. This finding reframes the discussion on raw material quality in the yerba mate production chain, shifting the focus from generic management or clonal selection recommendations toward combined strategies that simultaneously consider the cultivated genetic material and the timing of harvest.

From a practical standpoint, the four genotypes evaluated showed distinct and complementary aptitudes. Clone C27 stood out as the most suitable option for obtaining raw material rich in caffeine and total phenolic compounds, provided that it is harvested in summer, when these compounds reached their maximum values. Clone C33, owing to its consistently low caffeine contents associated with progressive theobromine accumulation, represents material of interest both for the development of low-caffeine products and as a biological model for investigating limitations at the final step of the methylxanthine methylation pathway. The Aupaba cultivar, in turn, showed greater compositional stability throughout the year, a desirable trait for industrial processes that depend on predictable raw material. Clone C26 exhibited the highest flavonoid contents in winter, suggesting suitability for applications that value this class of compounds. Among the chlorogenic acid derivatives, the conserved behavior of 3,4-di-CQA across all genotypes, in contrast with the strong genotypic dependence of compounds such as 3,5-di-CQA and 3-CQA, indicates that different steps of the phenylpropanoid pathway are subject to distinct levels of regulatory control, some more closely associated with general environmental responses and others more sensitive to genetic variability.

In applied terms, these results support the adoption of clone-specific staggered harvest strategies, rather than a single harvest calendar for the entire plantation, as a means of maximizing the yield of specific bioactive compounds of interest and standardizing the raw material intended for different industry segments, from traditional infusion consumption to the production of standardized extracts for supplements and cosmetics. Clonal selection guided by seasonal phytochemical profiles thus emerges as a tool complementary to current agronomic management practices for the crop.

Regarding the limitations of the present study, it should be noted that the four genotypes were evaluated under controlled greenhouse conditions over a single annual cycle. Although this design allowed the effect of climatic seasonality on each genetic material to be isolated with greater precision, it does not capture interannual variations or the behavior of these clones under open-field conditions, where factors such as water availability, direct radiation, and management practices tend to interact with the genotype in a more complex manner. Future studies should replicate the clone × season design over multiple years and in different growing environments in order to distinguish recurrent seasonal effects from occasional climatic variations, and should incorporate gene expression analyses of key enzymes in the methylxanthine and chlorogenic acid biosynthetic pathways (*N*-methyltransferases, hydroxycinnamoyl transferase, and caffeoylquinate synthases), which would allow the

phenotypic patterns described here to be directly linked to the underlying molecular mechanisms. Finally, bioactivity and sensory acceptance studies of extracts obtained from the different clones and seasons could consolidate the use recommendations proposed for each genetic material, bringing the findings of this work closer to practical applications in the food, dietary supplement, and cosmetic industries.

Declarations

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Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Kellen Nascimento dos Santos, Jucelina Oliveira Cardoso dos Santos. The first draft of the manuscript was written by Kellen Nascimento dos Santos and Ricardo Fiori Zara, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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References

[1] Claudio Inácio Heck and Elvira Gonzalez de Mejia. Yerba mate tea (*Ilex paraguariensis*): a comprehensive review on chemistry, health implications, and technological considerations. *Journal of Food Science*, 72(9):R138–R151, November 2007. doi: 10.1111/j.1750-3841.2007.00535.x.

[2] Gilcimar Adriano Vogt, Gilberto Neppel, and Adriano Martinho de Souza. A atividade ervateira no planalto norte catarinense: a indicação geográfica como alternativa para a (re)valorização do produto erva-mate. *Desenvolvimento Regional em Debate*, 6(2):64–87, July 2016. ISSN 2237-9029. doi: 10.24302/drd.v6i2.1205.

[3] IBGE. Erva-mate (cultivo agrícola) – Instituto Brasileiro de Geografia e Estatística, 2023. Available at: https://www.ibge.gov.br/explica/producao-agropecuaria/erva-mate-cultivo/br. Accessed: May 2025.

[4] IBGE. Produção da extração vegetal e da silvicultura (PEVS 2023) – Instituto Brasileiro de Geografia e Estatística, 2023. Available at: https://www.ibge.gov.br/estatisticas/economicas/agricultura-e-pecuaria/9105-producao-da-extracao-vegetal-e-da-silvicultura.html. Accessed: May 2025.

[5] Ivar Wendling. *Propagação vegetativa de erva-mate (Ilex paraguariensis Saint Hilaire): estado da arte e tendências futuras*. Number 91 in Documentos. Embrapa Florestas, Colombo, PR, 2004. URL https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/308921/1/Doc91.pdf.

[6] Ivar Wendling and Delmar Santin. *Propagação e nutrição de erva-mate*. Embrapa, Brasília, DF, 2015. ISBN 978-85-7035-365-8. URL https://www.infoteca.cnptia.embrapa.br/infoteca/bitstream/doc/1013131/1/EmbrapaFlorestas-2015-PropagacaoNutricaoErvaMate.pdf.

[7] Camila Pereira Croge, Francine Lorena Cuquel, and Paula Toshimi Matumoto Pintro. Yerba mate: cultivation systems, processing and chemical composition. A review. *Scientia Agricola*, 78(5), 2021. doi: 10.1590/1678-992x-2019-0259.

[8] Julie A. Ross and Christine M. Kasum. Dietary flavonoids: bioavailability, metabolic effects, and safety. *Annual Review of Nutrition*, 22(1):19–34, 2002. doi: 10.1146/annurev.nutr.22.111401.144957.

[9] Bent H. Havsteen. The biochemistry and medical significance of the flavonoids. *Pharmacology & Therapeutics*, 96(2–3):67–202, November 2002. ISSN 0163-7258. doi: 10.1016/S0163-7258(02)00298-X.

[10] Wijdan M. Dabeek and Melissa Ventura Marra. Dietary quercetin and kaempferol: bioavailability and potential cardiovascular-related bioactivity in humans. *Nutrients*, 11(10):2288, 2019. doi: 10.3390/nu11102288.

[11] R. Filip, P. López, G. Giberti, J. Coussio, and G. Ferraro. Phenolic compounds in seven South American *Ilex* species. *Fitoterapia*, 72(7):774–778, November 2001. ISSN 0367-326X. doi: 10.1016/S0367-326X(01)00331-8.

[12] Michael N. Clifford. Chlorogenic acids and other cinnamates – nature, occurrence, dietary burden, absorption and metabolism. *Journal of the Science of Food and Agriculture*,

80(7):1033–1043, May 2000. ISSN 1097-0010. doi: 10.1002/(SICI)1097-0010(20000515)80:7<1033::AID-JSFA595>3.0.CO;2-T.

[13] Yiting Song, Jun Cao, Fuliang Cao, and Erzheng Su. A systematic review on the yerba mate (*Ilex paraguariensis* A. St. Hil.). *Journal of Food Composition and Analysis*, 142:107466, June 2025. ISSN 0889-1575. doi: 10.1016/j.jfca.2025.107466.

[14] Karimi S. Gebara, Arquimedes Gasparotto Junior, Rhanany A. C. Palozi, Christine Morand, Carla I. Bonetti, Paula T. Gozzi, Martha R. F. de Mello, Telma A. Costa, and Euclides L. Cardozo Junior. A randomized crossover intervention study on the effect a standardized maté extract (*Ilex paraguariensis* A. St.-Hil.) in men predisposed to cardiovascular risk. *Nutrients*, 13(1):14, 2020. doi: 10.3390/nu13010014.

[15] Deborah Helena Markowicz Bastos, Ana Claudia Fornari, Yara Severino de Queiroz, Rosana Aparecida Manólio Soares, and Elizabeth Aparecida Ferraz da Silva Torres. The chlorogenic acid and caffeine content of yerba maté (*Ilex paraguariensis*) beverages. *Acta Farmacéutica Bonaerense*, 24(3):191–197, 2005.

[16] Natalia Saudade de Aguiar, Mônica Moreno Gabira, Manoela Mendes Duarte, Jéssica de Cássia Tomasi, Fabricio Augusto Hansel, Osmir José Lavoranti, Cicero Deschamps, Cristiane Vieira Helm, and Ivar Wendling. How shading levels affect bioactive compounds in leaves of yerba mate clones. *Biochemical Systematics and Ecology*, 113:104796, April 2024. ISSN 0305-1978. doi: 10.1016/j.bse.2024.104796.

[17] Euclides Lara Cardozo, Carmen Maria Donaduzzi, Osvaldo Ferrarese-Filho, Juliana Cristhina Friedrich, Adriana Gonela, and José Alfredo Sturion. Quantitative genetic analysis of methylxanthines and phenolic compounds in mate progenies. *Pesquisa Agropecuária Brasileira*, 45(2):171–177, February 2010. ISSN 0100-204X. doi: 10.1590/S0100-204X2010000200008.

[18] Yuling Yin, Riko Katahira, and Hiroshi Ashihara. Metabolism of purine alkaloids and xanthine in leaves of maté (*Ilex paraguariensis*). *Natural Product Communications*, 10(5), May 2015. ISSN 1555-9475. doi: 10.1177/1934578X1501000503.

[19] Hiroshi Ashihara and Alan Crozier. Caffeine: a well known but little mentioned compound in plant science. *Trends in Plant Science*, 6(9):407–413, September 2001. ISSN 1360-1385. doi: 10.1016/S1360-1385(01)02055-6.

[20] Federico A. Vignale, Andrea Hernandez Garcia, Carlos P. Modenutti, Ezequiel J. Sosa, Lucas A. Defelipe, Renato Oliveira, Gisele L. Nunes, Raúl M. Acevedo, German F. Burguener, Sebastian M. Rossi, Pedro D. Zapata, Dardo A. Marti, Pedro Sansberro, Guilherme Oliveira, Emily M. Catania, Madeline N. Smith, Nicole M. Dubs, Satish Nair, Todd J. Barkman, and Adrian G. Turjanski. Yerba mate (*Ilex paraguariensis*) genome provides new insights into convergent evolution of caffeine biosynthesis. *eLife*, 14, January 2025. ISSN 2050-084X. doi: 10.7554/eLife.104759.

[21] Michael N. Clifford, Indu B. Jaganath, Iziar A. Ludwig, and Alan Crozier. Chlorogenic acids and the acyl-quinic acids: discovery, biosynthesis, bioavailability and bioactivity. *Natural Product Reports*, 34(12):1391–1421, 2017. doi: 10.1039/C7NP00030H.

[22] Fabio D'Orso, Laura Hill, Ingo Appelhagen, Tom Lawrenson, Marco Possenti, Jiesi Li, Wendy Harwood, Giorgio Morelli, and Cathie Martin. Exploring the metabolic and physiological roles of HQT in *S. lycopersicum* by gene editing. *Frontiers in Plant Science*, 14:1124959, 2023. doi: 10.3389/fpls.2023.1124959.

[23] Anna Kołton, Olga Długosz-Grochowska, Renata Wojciechowska, and Monika Czaja. Biosynthesis regulation of folates and phenols in plants. *Scientia Horticulturae*, 291:110561, January 2022. ISSN 0304-4238. doi: 10.1016/j.scienta.2021.110561.

[24] Leonardo Gobbo-Neto and Norberto P. Lopes. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. *Química Nova*, 30(2):374–381, April 2007. ISSN 0100-4042. doi: 10.1590/S0100-40422007000200026.

[25] Anket Sharma, Babar Shahzad, Abdul Rehman, Renu Bhardwaj, Marco Landi, and Bingsong Zheng. Response of phenylpropanoid pathway and the role of polyphenols in plants under abiotic stress. *Molecules*, 24(13):2452, July 2019. ISSN 1420-3049. doi: 10.3390/molecules24132452.

[26] Carlos H. Blum-Silva, Vitor C. Chaves, Eloir P. Schenkel, Geraldo C. Coelho, and Flávio H. Reginatto. The influence of leaf age on methylxanthines, total phenolic content, and free radical scavenging capacity of *Ilex paraguariensis* aqueous extracts. *Revista Brasileira de Farmacognosia*, 25(1):1–6, January 2015. ISSN 0102-695X. doi: 10.1016/j.bjp.2015.01.002.

[27] Miroslava Rakocevic, Aline de Holanda Nunes Maia, Marcus Vinicius de Liz, Rafaela Imoski, Cristiane Vieira Helm, Euclides Lara Cardozo Junior, and Ivar Wendling. Stability of leaf yerba mate (*Ilex paraguariensis*) metabolite concentrations over the time from the prism of secondary sexual dimorphism. *Plants*, 12(11):2199, June 2023. ISSN 2223-7747. doi: 10.3390/plants12112199.

[28] Jéssica de Cássia Tomasi, Renata de A. Maggioni, Mônica Moreno Gabira, Natália Saudade de Aguiar, Manoela Mendes Duarte, Leandro Marcolino Vieira, Cristiane Vieira Helm, and Ivar Wendling. Influence of agronomic and processing factors on bioactive compounds of yerba mate (*Ilex paraguariensis* A. St.-Hil.): a systematic review. *Journal of Applied Research on Medicinal and Aromatic Plants*, 48:100652, September 2025. ISSN 2214-7861. doi: 10.1016/j.jarmap.2025.100652.

[29] Adriana Dillenburg Meinhart, Carolina Schaper Bizzotto, Cristiano Augusto Ballus, Ana Cecília Poloni Rybka, Merenice Roberto Sobrinho, Romina Sofia Cerro-Quintana, José Teixeira-Filho, and Helena Teixeira Godoy. Methylxanthines and phenolics content extracted during the consumption of mate (*Ilex paraguariensis* St. Hil) beverages. *Journal of Agricultural and Food Chemistry*, 58(4):2188–2193, January 2010. doi: 10.1021/jf903781w.

[30] Marcela Boroski, J. S. Visentaner, S. M. Cottica, and D. M. Morais. *Antioxidantes: Princípios e Métodos Analíticos*. Appris, Curitiba, 2015. ISBN 8581927300.

[31] Silvie Balzan, Aline Hernandes, Cristiane Loiva Reichert, Carmen Donaduzzi, Vanessa Aranega Pires, Arquimedes Gasparotto, and Euclides Lara Cardozo. Lipid-lowering

effects of standardized extracts of *Ilex paraguariensis* in high-fat-diet rats. *Fitoterapia*, 86:115–122, April 2013. ISSN 0367-326X. doi: 10.1016/j.fitote.2013.02.008.

[32] BRASIL. *Farmacopeia Brasileira*, volume 1. Agência Nacional de Vigilância Sanitária (ANVISA), Brasília, DF, 6th edition, 2019.

[33] Aneta Krakowska-Sieprawska, Katarzyna Rafińska, Justyna Walczak-Skierska, and Bogusław Buszewski. The influence of plant material enzymatic hydrolysis and extraction conditions on the polyphenolic profiles and antioxidant activity of extracts: a green and efficient approach. *Molecules*, 25(9):2074, April 2020. ISSN 1420-3049. doi: 10.3390/molecules25092074.

[34] Juliana Andriolli Ribeiro, Ederlan Magri, Itamar Luís Gonçalves, Karina Paese, Juliana Roman, and Alice Teresa Valduga. Photoprotector effect of emulsions with yerba-mate (*Ilex paraguariensis*) extract. *Scientia Pharmaceutica*, 91(2):22, April 2023. ISSN 2218-0532. doi: 10.3390/scipharm91020022.

[35] Karimi Sater Gebara, Arquimedes Gasparotto-Junior, Patricia Gonçalves Santiago, Claudia Andrea Lima Cardoso, Lauro Mera de Souza, Christine Morand, Telma Aparecida Costa, and Euclides Lara Cardozo-Junior. Daily intake of chlorogenic acids from consumption of maté (*Ilex paraguariensis* A. St.-Hil.) traditional beverages. *Journal of Agricultural and Food Chemistry*, 65(46):10093–10100, November 2017. ISSN 1520-5118. doi: 10.1021/acs.jafc.7b04093.

[36] ICH Q2(R1). Validation of analytical procedures: text and methodology, 2005. Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf. Accessed: 25 May 2025.

[37] Lloyd R. Snyder, Joseph J. Kirkland, and John W. Dolan. *Introduction to Modern Liquid Chromatography*. John Wiley & Sons, Hoboken, 3rd edition, 2010.

[38] Alejandro C. Olivieri. Practical guidelines for reporting results in single- and multi-component analytical calibration: a tutorial. *Analytica Chimica Acta*, 868:10–22, April 2015. ISSN 0003-2670. doi: 10.1016/j.aca.2015.01.017.

[39] R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, 2025. Available at: https://www.R-project.org/. Accessed: 26 Jan. 2026.

[40] Posit Team. *RStudio: Integrated Development Environment for R*. Posit Software, PBC, Boston, 2025. Available at: https://posit.co/products/open-source/rstudio/. Accessed: 26 Jan. 2026.

[41] Yanni Cao, Shengzuo Fang, Xiangxiang Fu, Xulan Shang, and Wanxia Yang. Seasonal variation in phenolic compounds and antioxidant activity in leaves of *Cyclocarya paliurus* (Batal.) Iljinskaja. *Forests*, 10(8):624, July 2019. ISSN 1999-4907. doi: 10.3390/f10080624.

[42] Raquel Oliveira Claro, Carla Letícia Gediel Rivero-Wendt, Ademir Kleber Morbeck de Oliveira, Denise Renata Pedrinho, José Antônio Maior Bono, and Rosemary Matias. Efeitos da sazonalidade e da adubação na composição química de *Genipa americana* L.

Ensaios e Ciência: Ciências Biológicas, Agrárias e da Saúde, 28(2):209–217, July 2024. ISSN 1415-6938. doi: 10.17921/1415-6938.2024v28n2p209-217.

[43] Aishwarya Ashok Gaude and Siddhi Kashinath Jalmi. Environmental stress induced biosynthesis of plant secondary metabolites – transcriptional regulation as a key. *Crop Design*, 4(2):100100, May 2025. ISSN 2772-8994. doi: 10.1016/j.cropd.2025.100100.

[44] Jéssica de Cássia Tomasi, Gabriel Goetten de Lima, Ivar Wendling, Cristiane Vieira Helm, Fabrício Augusto Hansel, Rossana Catie Bueno de Godoy, Renata Lúcia Grunennvaldt, Tamires Oliveira de Melo, Maíra Maciel Tomazzoli, and Cícero Deschamps. Effects of different drying methods on the chemical, nutritional and colour of yerba mate (*Ilex paraguariensis*) leaves. *International Journal of Food Engineering*, 17(1):51–60, 2021. doi: 10.1515/ijfe-2020-0312.

[45] Cláudia N. T. Frizon, Diego P. Lemos, Camila A. Zardo, Marcela T. Barcia, Mauro Salvador, and Gustavo A. Micke. Influence of drying methods on the physicochemical characteristics and antioxidant activity of yerba mate leaves (*Ilex paraguariensis*). *Brazilian Journal of Food Technology*, 20:e2016094, 2017. doi: 10.1590/1981-6723.9416.

[46] Gustavo Zambon Mendonça, Charles Windson Isidoro Haminiuk, Alessandra Cristina Pedro, Lucia Regina Rocha Martins, Fernando Molin, Gabriel Oales Kaminski Larsen, Cristiane Vieira Helm, Ivar Wendling, and Giselle Maria Maciel. Extraction of bioactive compounds from different yerba mate genotypes and development of an antimicrobial gel. *Fitoterapia*, 183:106582, June 2025. ISSN 0367-326X. doi: 10.1016/j.fitote.2025.106582.

[47] Raquel Mateos, Gema Baeza, Beatriz Sarriá, and Laura Bravo. Improved LC-MS^n characterization of hydroxycinnamic acid derivatives and flavonols in different commercial mate (*Ilex paraguariensis*) brands. Quantification of polyphenols, methylxanthines, and antioxidant activity. *Food Chemistry*, 241:232–241, February 2018. ISSN 0308-8146. doi: 10.1016/j.foodchem.2017.08.085.