Songklanakarin J. Sci. Technol.
47 (3), 185–191, May – Jun. 2025

http://www.sjst.psu.ac.th

Original Article

Validation of a capillary zone electrophoresis method for the quantification of catechins in gambir leaves (*Uncaria gambir* (Hunter) Roxb.)

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Received: 16 September 2024; Revised: 31 January 2025; Accepted: 8 April 2025

Abstract

Uncaria gambir (Hunter) Roxb. (Gambir) is a medicinal plant of significant economic importance, widely recognized for the high catechin content in its leaves. Catechin is a bioactive compound with numerous health benefits and substantial potential for development into standardized products. This study aimed to validate the Capillary Zone Electrophoresis (CZE) method for catechin quantification and to determine the optimal maturity stage of gambir leaves (apical, middle, or basal) and drying treatments to maximize catechin content. The quality of gambir leaves was assessed based on catechin content, measured using the CZE method. This method employed a silica capillary (75 µm diameter, total length 56 cm, effective length 47.5 cm), a borate buffer (20 mM, pH 8.4), and an applied voltage of 10 kV. Validation was conducted following the guidelines of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH), evaluating parameters such as linearity, accuracy, precision, and sensitivity, including the limit of detection (LOD) and limit of quantification (LOQ). Data on the effects of drying type and leaf maturity were analyzed using a two-way ANOVA, followed by Duncan's Multiple Range Test at a 95% confidence level. The validation results confirmed that the CZE method was linear, accurate, precise, and sensitive. Additionally, the findings revealed that apical leaves subjected to steaming at 85°C for three minutes followed by oven drying at 50°C for 24 hours yielded the highest catechin levels. These results underscore the applicability of the validated CZE method for catechin analysis and emphasize the importance of standardized harvesting and drying methods in determining catechin quality in gambir leaves.

Keywords: capillary zone electrophoresis, catechin, *Uncaria gambir* (Hunter) Roxb.

1. Introduction

Gambir (Figure 1) is a medicinal plant of significant economic importance. Gambir extract is a key product and export commodity of West Sumatra, highly sought after by various countries. Traditionally, gambir has been utilized in diverse applications, including as a dye in the batik industry, a leather tanning agent, a component in betel chewing mixtures, and a remedy for burns, diarrhea, dysentery, and canker sores. Additionally, it has been used as an ingredient in candy production (Fauza, 2014). The monographs of gambir simplicia and its extract have been standardized based on their

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catechin content and are officially included in the Indonesian Herbal Pharmacopoeia (Kementerian Kesehatan RI, 2017).

Gambir leaves are a promising raw material for developing herbal tea preparations due to their high catechin content, which is comparable to, but more abundant than, that found in *Camellia sinensis* tea leaves (Ferdinal, 2014; Meyer, White, McCormack, & Niemeyer, 2023). Studies have identified approximately 50 compounds in gambir, including flavan monomers, flavan dimers, and alkaloids. The tannin and condensed tannin content in gambir has been associated with its potent antioxidant properties, which contribute to various pharmacological activities. Catechins, the primary bioactive compounds in gambir, exhibit a range of beneficial effects, including antioxidant, antibacterial, antiviral, antidiabetic, and antitumor activities, as well as immune system enhancement (Munggari, Kurnia, Deawati, & Julaeha, 2022).

Gambir tea, made from dried gambir leaves packaged in tea bags, is primarily valued for its catechin content (Budaraga & Putra, 2023). However, the quality of gambir leaf tea products available on the market varies considerably due to the absence of standardized guidelines for leaf selection and post-harvest processing. Key factors such as drying techniques and leaf maturity significantly impact the phenolic content and antioxidant properties of the tea. Moreover, the drying process plays a crucial role in shaping the overall characteristics of herbal tea products (Tavita *et al*., 2023).

Advances in analytical technology, particularly the development and application of electrophoresis in the pharmaceutical field, have significantly enhanced natural product analysis (Gackowski *et al*., 2021). Among these, capillary electrophoresis (CE) has gained prominence for its versatility in analyzing active pharmaceutical ingredients (APIs), including cationic, anionic, and neutral compounds. CE offers multiple separation modes and detection techniques, distinguishing itself with rapid analysis times, high resolution, excellent separation efficiency, low reagent consumption, and minimal sample requirements, making it a cost-effective and efficient choice (Masár, Hradski, Schmid, & Szucs, 2020).

This study focuses on validating the Capillary Zone Electrophoresis (CZE) method for catechin quantification. Additionally, it aims to identify the optimal leaf maturity stages (apical, middle, or basal) and drying treatments to establish standardized production practices for gambir tea products.

2. Materials and Methods

2.1 Equipment

The experiment utilized an Agilent© 7100 capillary electrophoresis system, equipped with a diode-array detector (DAD) for data acquisition. The system was operated using Agilent™ software for data processing. The capillary had a total length of 56 cm, an effective length of 47.5 cm, and an internal diameter of 75 µm.

2.2 Materials and reagents

The reagents used in this study were of analytical grade and included 20 mM borax buffer solution (pH 8.4, Merck, Supelco, Darmstadt, Germany), double-distilled water (IKA Pharmindo, Jakarta, Indonesia), 1.0 N sodium hydroxide (Merck, Supelco, Darmstadt, Germany), methanol for HPLC (Merck, Supelco, Darmstadt, Germany), and catechin standard (Sigma-Aldrich, St. Louis, MO, USA).

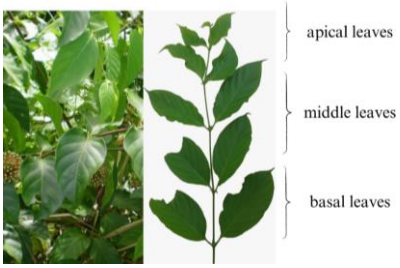

Figure 1. Gambir plant and leaf parts (*Uncaria gambir* Roxb.)

2.3 Sample collection and preparation of simplicia

The samples used in this study were *U. gambir* leaves, collected from the Medicinal Plant Garden of Universitas Andalas, Limau Manih, West Sumatra, Indonesia. The plant species was identified by a taxonomist from the Herbarium ANDA at Universitas Andalas and recorded under voucher specimen number 096/K-ID/ANDA/III/2020. Gambir leaves were harvested from three different parts of the plant: apical, middle, and basal (Figure 2). The samples were divided into four groups, each subjected to a distinct drying method: (1) steaming at 85°C for three minutes followed by oven drying at 50°C for 24 hours, (2) oven drying at 50°C for 24 hours without prior steaming, (3) air drying at room temperature for seven days, and (4) solar drying for three days. The final moisture content of all dried samples was controlled to remain below 10%. After drying, the leaf samples were ground using a blender and sieved through a 60-mesh sieve to achieve a uniform particle size.

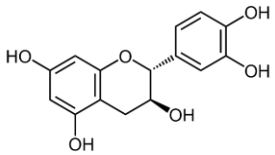

Figure 2. Structure of catechin

2.4 Extract preparation of simplicia

Weigh 100 mg of each *U. gambir* leaf sample. Extract the sample with 5 mL of methanol and sonicate at room temperature for 5 minutes. Subsequently, heat the mixture in a water bath at 55°C for 15 minutes. Filter the solution using Whatman no. 1 filter paper. Pipette 100 µL of the extract into a 5 mL volumetric flask and dilute to the mark with 20 mM borax buffer (pH 8.4) to obtain a 400 µg/mL sample solution. Further dilute this solution to 8 ppm by transferring 100 µL of the 400 µg/mL solution into a 5 mL volumetric flask and filling the flask to the mark with buffer. Filter the final sample solution through a 0.22 µm filter, discarding the first 1 to 2 drops of the filtrate, and analyze the solution by capillary electrophoresis.

2.5 Instrument conditions

The capillary electrophoresis system utilized in this study was an Agilent© 7100, employing the capillary zone electrophoresis (CZE) analysis method. The system was equipped with a 56 cm capillary (75 µm inner diameter) and a diode array detector. A 20 mM borate buffer (pH 8.4) was used as the separation medium, with detection conducted at a wavelength of 212.4 nm and a temperature of 20°C. Capillary conditioning was performed by sequential flushing with 1.0 N NaOH for 180 seconds, distilled water for 300 seconds, and the borate buffer for 180 seconds. Sample injection was carried out at an applied pressure of 50 mbar for 5 seconds, after which the buffer vial was reattached to the capillary inlet. The separation process was optimized by applying a voltage of 10 kV, 300 µA, and 6 W for 15 minutes. Post-separation, the capillary was cleaned by flushing with the borate buffer for 300 seconds, followed by flushing with distilled water for 180 seconds.

2.6 Method validation

Validation is a systematic process used to confirm the performance characteristics of an analytical method, ensuring its suitability for the intended application. In this study, the capillary electrophoresis method was validated in accordance with the guidelines established by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (International Council for Harmonization (ICH), 2023).

2.6.1 Linearity

Linearity was evaluated using standard catechin solutions at concentrations of 2.5 µg/mL, 5 µg/mL, 10 µg/mL, 15 µg/mL, and 20 µg/mL. The solutions were analyzed under the specified instrument conditions, and a calibration curve was generated by plotting the peak area against catechin concentration (µg/mL). The linearity (R^2 value) was assessed through regression analysis of the calibration curve.

2.6.2 Accuracy

Accuracy was evaluated by determining the percentage of recovery of the catechin from *U. gambir* leaf extract. Recovery tests were conducted at three different concentration levels to assess the method's accuracy. A fixed volume (0.1 mL) of the gambir leaf extract, with an initial concentration of 8 µg/mL, was spiked with standard catechin solutions at concentrations of 5.02 µg/mL, 9.43 µg/mL, and 16.55 µg/mL. The recovery was calculated based on the total concentration of the sample and the spiked catechin. The recovery percentages were found to range from 90.65% to 106.64% (Table 2), which is within the acceptable range of 80–120% according to ICH guidelines, indicating that the method is accurate.

2.6.3 Precision

Samples were prepared at three concentration levels-5 µg/mL, 10 µg/mL, and 16 µg/mL-with three replicates per concentration. Precision was evaluated by assessing intraday and interday variability. Intraday variability was measured through repeated analyses conducted within the same day, while interday variability was assessed over three consecutive days. The precision of the method was determined by calculating the relative standard deviation percentage (%RSD) for each parameter.

2.6.4 Limit of detection (LOD) and Limit of quantification (LOQ)

Sensitivity was evaluated by determining the limit of detection (LOD) and limit of quantification (LOQ) based on the standard deviation (SD) of the catechin standard solution. The LOD was calculated as LOD=3xSD/slope (µg/mL), while the LOQ was calculated as LOQ=10xSD/slope (µg/mL).

2.7 Catechin analysis from gambir leaves (*Uncaria gambir* Roxb.)

To quantify catechin concentration in gambir leaves subjected to different drying treatments (apical, middle, and basal leaves), an 8 µg/mL sample solution was prepared and filtered through a 0.22 µm membrane filter. The filtered sample was analyzed under the capillary electrophoresis conditions established for the calibration curve. The development and scanning procedures were performed as previously described. All analyses were conducted in triplicate to ensure accuracy and reproducibility.

2.8 Data analysis

Extraction and analyses were performed in triplicate, with results presented as the mean along with the standard deviation (SD) and relative standard deviation (%RSD). Data processing was conducted using Microsoft Excel (Microsoft Corp., Redmond, USA). Statistical analysis was carried out using SPSS Version 24.0, employing a two-way ANOVA followed by Duncan's Multiple Range Test (DMRT) to assess the relationship between catechin content and the different drying treatments applied to various parts of gambir leaves. Statistical significance was defined as $p < 0.05$.

3. Results

3.1 Optimization of analysis conditions and system suitability

Capillary zone electrophoresis (CZE) was employed in this study for the analysis of catechin. The CZE method was optimized to ensure a stable and reliable analytical procedure. Various analysis conditions were systematically modified and evaluated, with the optimal conditions established as follows: a detection wavelength of 212.4 nm, a 20 mM borate buffer at pH 8.4 as the mobile phase, an applied voltage of 10 kV/300 µA/6 W, and an injection pressure of 50 mbar. Under these conditions, catechin was detected at approximately 11 minutes, with a total analysis time of 15 minutes. The separation of standard catechin and test samples is presented in Figure 3. The system suitability test confirmed that the method met the acceptance criteria. Specifically, the relative standard deviation (%RSD) for migration time and

peak area was below 2%, the tailing factor was less than 2, the number of theoretical plates (N) exceeded 2,000, and the resolution (Rs) was greater than 2. These results are summarized in Table 1.

3.2 Evaluation of method linearity

The method was validated following the guidelines established by the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH, 2023). Validation encompassed assessments of key analytical parameters, including linearity, accuracy, precision, and sensitivity, determined through the limits of detection (LOD) and quantification (LOQ). Linearity was evaluated using five standard catechin solution concentrations, yielding a regression equation of $Y = 6{,}106.858x + 6{,}724.628$ with a correlation coefficient (R^2) of 0.9996, indicating excellent linearity (Table 2). The corresponding standard catechin calibration curve is depicted in Figure 4. Sensitivity analysis, based on the calibration curve, established LOD and LOQ values at 0.44 µg/mL and 1.49 µg/mL, respectively, confirming the method's suitability for detecting and quantifying catechin (Table 2).

3.3 Assessment of accuracy

The method's accuracy was assessed by calculating the percent recovery of catechin from an 8 µg/mL *U. gambir* leaf extract spiked with standard catechin solutions at concentrations of 5.02 µg/mL, 9.43 µg/mL, and 16.55 µg/mL. The total calculated concentrations, comprising both the extract and the spiked standard catechin, were 10.69 µg/mL, 14.8 µg/mL, and 21.56 µg/mL, respectively. The corresponding measured catechin concentrations in these mixtures were 9.69 µg/mL, 15.78 µg/mL, and 22.32 µg/mL. Consequently, the percent recovery ranged from 90.65% to 106.64% (Table 2), adhering to the acceptable range of 80–120% specified by the ICH guidelines (ICH, 2023).

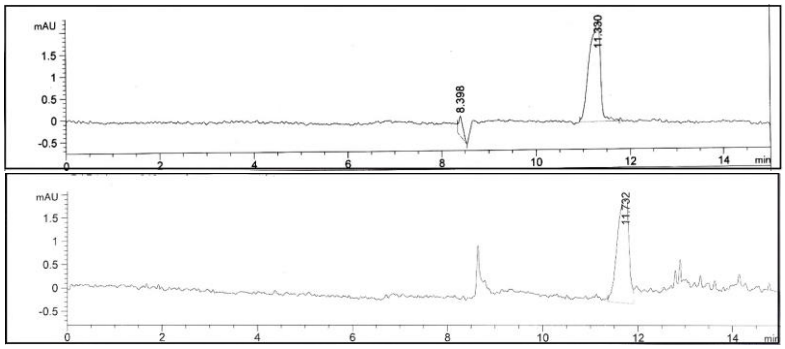

Figure 3. Electropherogram of catechin standard and gambir leaf sample

Table 1. Results of the suitability test of catechin by capillary electrophoresis method (n=3)

Parameter	Average ± SD	RSD (%)
Precision of migration time (minutes)	11.224 ± 0.136	1.211
Resolution	12.653 ± 0.08	0.685
Number of theoretical plates (N)	44,570 ± 405	0.908
Tailing factor (T)	1.01 ± 0.009	0.936
Precision of peak area (mUA*s)	38,472.57 ± 195.805	0.508

Table 2. Validity results for CZE method in catechin quantification

Parameter	Catechin
Linearity range (µg/mL)	2.5-20
Regression equation (y = ax + b)	y = 6,106.858x + 6,724.628
Slope (a)	6,106.858
Intercept (b)	6,724.628
Correlation Coefficient (R^2)	0.9996
Limit of Detection (LOD) (µg/mL)	0.44
Limit of Quantification (LOQ) (µg/mL)	1.49
Precision[a]	
1. Range % RSD of Intra-Day	0.62-1.13
2. Range % RSD of Inter-Day	4.15-10.61
Accuracy[b]	
Recovery (%)	90.65±1.98-106.64±0.35

[a]Intra-Day and Inter-Day (n=3)
[b]Three concentration levels:10.69, 14.8, and 21.56 µg/mL (n=3)

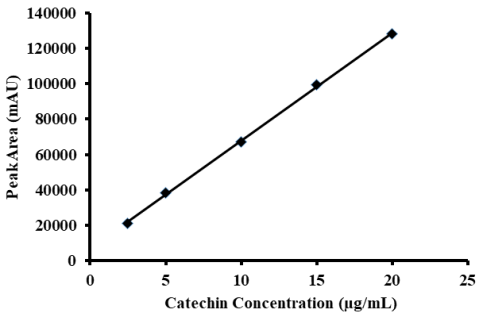

Figure 4. Standard curve of catechin

3.4 Precision evaluation

Precision was evaluated through repeatability, intermediate precision, and reproducibility. Intra-day precision was assessed by analyzing three concentrations of catechin, each with three replicates, within the same day. Inter-day precision was evaluated by analyzing the same concentrations over three consecutive days. The intra-day precision, expressed as the relative standard deviation (RSD) of three repetitions at each concentration, was consistently below 2.0%, indicating good repeatability. However, inter-day precision exhibited RSD values exceeding 2.0% (Table 2), suggesting reduced accuracy in intermediate precision for standard catechin measurements.

The observed reduction in catechin levels over time may be attributed to catechin's instability in alkaline environments, where degradation gradually occurs (Chiang, Shiue, Pai, & Liu, 2002). Previous studies have highlighted that catechin stability is influenced by factors such as temperature, pH, and humidity (Li, Taylor, Ferruzzi, & Mauer, 2012; Li, Taylor, & Mauer, 2011; Ortiz, Ferruzzi, Taylor, & Mauer, 2008). To maintain accuracy, it is recommended that catechin sample preparation and analysis be performed on the same day.

3.5 The impact of drying techniques and leaf maturity on catechin contents

The capillary zone electrophoresis (CZE) analysis demonstrated that catechin levels vary significantly with different drying methods and leaf maturity stages. The two-way ANOVA results confirmed a significant effect of both drying type and leaf maturity stage (apical, middle, and basal) on catechin content, with p-values of 0.000 ($p < 0.05$) indicating strong statistical significance. This suggests that both the drying method and the maturity stage of the leaves play crucial roles in determining the final catechin content of gambir leaves.

Duncan's post hoc test further revealed that among the evaluated drying methods-steaming and oven drying, oven drying, air drying (at room temperature), and solar drying-steaming and oven drying consistently resulted in the highest catechin concentrations. Similarly, the post hoc analysis for leaf maturity showed that the apical leaves consistently had the highest catechin content when compared to the middle and basal leaves. These results are presented in Table 3 and Figure 5. This finding underscores the effectiveness of steaming plus oven drying in preserving catechins, likely due to its ability to rapidly inactivate polyphenol oxidase (PPO) and minimize the enzymatic degradation of catechins (Caffin, Arcy, Yao, & Rintoul, 2004; Wang *et al*., 2022). Additionally, it highlights the importance of selecting the appropriate maturity stage for gambir leaves to optimize catechin content.

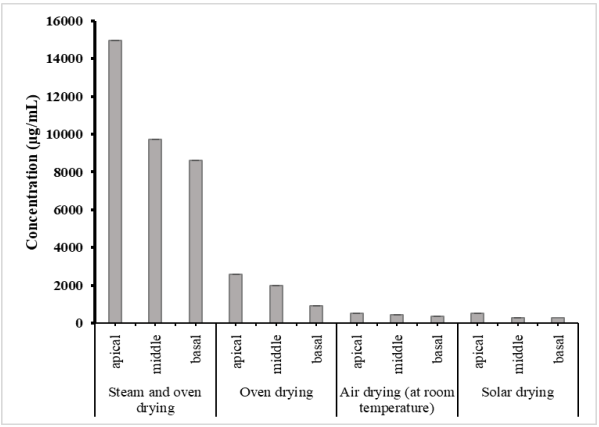

Figure 5. Catechin content in leaf samples

4. Discussion

Gambir leaves (*Uncaria gambir* Roxb.) hold significant potential as a raw material for producing gambir and its derivatives, such as herbal tea. Despite this, the standardization of catechin content in gambir leaves-considering factors like leaf maturity and drying techniques-remains insufficiently explored. This study aimed to address this gap by evaluating the effects of different drying methods and maturity stages of catechin levels, intending to establish a standardized approach to optimize catechin content in gambir leaves.

Table 3. Catechin contents in leaf samples

Drying method	Concentration of catechin (mean ± SD µg/mL)			Mean ± SE (µg/mL)
	Apical leaves	Middle leaves	Basal leaves	
Steaming and oven drying	14,983.18±0.77	9,729.55±1.39	8,602.30±0.10	11,105.00±0.377^{a}
Oven drying	2,587.69±1.41	1,985.07±0.78	918.04±0.73	1,830.27±0.377^{b}
Air drying (at room temperature)	533.76±0.64	421.11±1.19	347.44±0.64	434.10±0.377^{c}
Solar drying	522.92±0.62	293.05±1.22	277.74±0.72	364.57±0.377^{d}
Mean ± SE (µg/mL)	4,656.88±0.327^{p}	3,107.20±0.327^{q}	2,536.38±0.327^{r}	

Significant values were obtained statistically from two-way ANOVA and Duncan's Multiple Range T Test.
Values having different superscrips in the same column are statistically significantly different ($p < 0.05$)

Polyphenol oxidase (PPO), a widely occurring enzyme in plants, plays a critical role in the oxidative transformation of catechins. It catalyzes the oxidation of catechins into quinones, which subsequently polymerize into brown pigments. This enzymatic activity leads to the degradation of catechins, negatively impacting the quality and stability of plant-derived products (Vámos-Vigyázó, 1981). The transformation of catechins into various metabolites often reduces their beneficial properties. To preserve the bioactivity and quality of catechins during post-harvest processing, it is crucial to inactivate PPO (Queiroz, Mendes-Lopes, Fialho, & Valente-Mesquita, 2008).

The steaming process is particularly effective in inactivating polyphenol oxidase (PPO). During steaming, the high temperature and moisture content rapidly denature the enzyme, preventing it from catalyzing catechin oxidation (Gupta, Mamtani, Guttapadu, & Venkatesh, 2014). This explains why steaming and steam oven drying methods are superior in preserving catechin content compared to other drying techniques (Roslan, Ismail, Ando, & Azlan, 2020). By inhibiting PPO activity, these methods maintain higher concentrations of catechins and minimize the formation of undesirable metabolites, thereby preserving the nutritional and functional qualities of the final product (Iqbal *et al*., 2019).

In addition to drying methods, leaf maturity plays a significant role in catechin content. The results of this study demonstrate that apical leaves contain substantially higher levels of catechins compared to middle and basal leaves. This observation aligns with the understanding that the comparatively younger apical leaves are more metabolically active and thus accumulate higher catechin concentrations (Caffin *et al*., 2004; Turkmen, Sarı, & Sedat Velioglu, 2009).

The variation in catechin distribution within the leaf structure is influenced by leaf maturity. Younger apical leaves exhibit higher rates of photosynthesis, resulting in the production of primary metabolites that serve as precursors for catechin biosynthesis (Singh, Kumar, Rani, Gulati, & Ahuja, 2009). As leaves mature, their biosynthetic pathways shift towards alternative physiological processes, leading to a reduction in catechin accumulation. Middle and basal leaves, being older, have transitioned to these pathways, resulting in lower catechin concentrations (Guo, Guo, Wang, Wang, & Ni, 2017; Wang *et al*., 2018).

These findings underscore the importance of selecting the appropriate leaf maturity stage during harvesting to maximize catechin content in plant-derived products. Specifically, using apical leaves combined with steam oven drying has emerged as the optimal approach for producing high-quality gambir tea. This method effectively preserves catechin levels, enhancing the tea's antioxidant properties.

Given the significant variation in catechin content influenced by drying method and leaf maturity, it is crucial for producers to adopt standardized conditions to ensure consistency and quality in gambir-based products. The results of this study provide a foundation for establishing quality control parameters and guiding future research aimed at refining gambir tea production and standardization. Future research could investigate additional factors that influence catechin stability and content, such as varying drying temperatures, storage conditions, and different processing methods. Long-term studies on the effects of these factors on catechin degradation and overall tea quality would further contribute to the optimization of gambir tea production practices.

5. Conclusions

This study successfully validated the Capillary Zone Electrophoresis (CZE) method for quantifying catechins in gambir leaves, demonstrating its linearity, accuracy, precision, and sensitivity in accordance with ICH guidelines. Additionally, the findings revealed that drying methods and leaf maturity stages significantly influence catechin content. Among the treatments, apical leaves subjected to steaming at 85°C for three minutes followed by oven drying at 50°C for 24 hours yielded the highest catechin levels. Proper standardization of leaf maturity selection and drying techniques is critical to optimizing catechin quality in gambir leaves, underscoring their potential for development into high-value, standardized gambir tea products.

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