

Optimization of Drying Temperature for Preserving Bioactive Flavonoids in Cocoa (*Theobroma cacao* L.) Leaves: Implications for Functional Herbal Development in Chronic Disease Prevention

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Abstract. The increasing demand for plant-based functional ingredients has stimulated interest in utilizing cocoa (*Theobroma cacao* L.) leaves, an underutilized agricultural by-product rich in bioactive flavonoids. However, inappropriate post-harvest drying conditions may reduce the stability and recovery of these compounds. This study aimed to evaluate the effect of drying temperature on the physicochemical characteristics, extraction yield, phytochemical composition, and total flavonoid content of cocoa leaf extracts. Fresh cocoa leaves were oven-dried at 40, 50, and 60°C, extracted by maceration with 70% ethanol, and analyzed through qualitative phytochemical screening and aluminum chloride colorimetric assay using UV–Visible spectrophotometry. Statistical analysis was performed using one-way ANOVA followed by Tukey's HSD test. All drying treatments produced herbal simplicia with moisture contents below 10%, while phytochemical screening confirmed the presence of flavonoids, alkaloids, and saponins. Drying at 60°C yielded the highest extraction efficiency (8.0%) and total flavonoid content ($6.402 \pm 0.214\%$), which was significantly higher than that obtained at 40°C ($p < 0.05$). These findings demonstrate that controlled drying at 60°C effectively preserves flavonoids while improving extraction efficiency, providing a practical strategy for optimizing post-harvest processing of cocoa leaves. This study contributes to the valorization of cocoa leaves as a sustainable source of functional herbal ingredients with potential applications in chronic disease prevention and integrative healthcare.

Keywords: *Theobroma cacao* leaves; drying temperature; total flavonoid content; UV–Visible spectrophotometry; functional herbal products

INTRODUCTION

Chronic diseases, including cardiovascular diseases, diabetes mellitus, cancer, and chronic inflammatory disorders, remain the leading causes of mortality and disability worldwide. According to the World Health Organization (WHO, 2025), non-communicable diseases (NCDs) account for approximately 74% of global deaths, with oxidative stress recognized as one of the major contributors to their pathogenesis (Abdullahi, et al., 2025). Excessive production of reactive oxygen species (ROS) disrupts cellular homeostasis, induces lipid peroxidation, damages DNA and proteins, and triggers chronic inflammation, ultimately accelerating the progression of degenerative diseases. Consequently, increasing attention has been directed toward natural antioxidants derived from medicinal plants as complementary strategies for chronic disease prevention and health promotion (Malta, et al., 2026).

Among naturally occurring phytochemicals, flavonoids constitute one of the most important groups of polyphenolic compounds because of their broad spectrum of biological activities (Arifin & Ibrahim, 2018). Numerous studies have demonstrated that flavonoids exhibit potent antioxidant, anti-inflammatory, antimicrobial, antidiabetic, cardioprotective, and anticancer properties through their ability to scavenge free radicals, inhibit oxidative stress, modulate inflammatory signaling pathways, and regulate cellular metabolism. These pharmacological properties have promoted the utilization of flavonoid-rich plant extracts as functional herbal ingredients and nutraceuticals to complement conventional healthcare, particularly for chronic disease prevention (Chen, et al., 2023).

Cocoa (*Theobroma cacao* L.) is one of the world's most economically important plantation crops, primarily cultivated for chocolate production. While cocoa beans have been extensively investigated because of their high flavanol content, cocoa leaves remain an underutilized agricultural by-product despite containing various bioactive compounds, including flavonoids, phenolic acids, tannins, alkaloids, and terpenoids (Palma-Molares, et al. 2023). Previous phytochemical investigations have reported that cocoa leaves possess considerable antioxidant potential, suggesting their promising application as sustainable raw materials for herbal products and functional food development. The valorization of cocoa leaves not only increases the economic value of agricultural waste but also supports

sustainable biomass utilization within a circular bioeconomy framework.

The preservation of flavonoids during post-harvest processing is a critical factor determining the quality and efficacy of herbal materials. Drying is one of the most essential post-harvest processes because it reduces moisture content, suppresses microbial growth, extends shelf life, and facilitates extraction (Nafi'ah, et al., 2024). However, drying temperature considerably influences the stability of flavonoids. Insufficient drying temperatures may prolong drying time, allowing oxidative enzymes such as polyphenol oxidase (PPO) and peroxidase (POD) to remain active, thereby accelerating flavonoid degradation (Ramos-Escudero, et al. 2023). Conversely, excessive drying temperatures may induce thermal degradation, oxidation, and structural modification of thermolabile flavonoids, leading to substantial losses of bioactive compounds. Therefore, optimizing drying temperature is crucial for maximizing flavonoid retention while maintaining the phytochemical quality of herbal raw materials (Zekrumah, et al., 2023).

Several studies have evaluated the effects of drying conditions on phytochemical retention in medicinal plants, including *Moringa oleifera*, *Camellia sinensis*, *Psidium guajava*, and *Olea europaea*. Nevertheless, information regarding the optimization of drying temperature for preserving flavonoids in cocoa leaves remains limited. Most available studies focus primarily on cocoa beans or evaluate total phenolic content and antioxidant activity without specifically investigating flavonoid retention in cocoa leaves under different drying temperatures. This knowledge gap limits the establishment of standardized post-harvest processing conditions for producing high-quality cocoa leaf extracts intended for herbal and nutraceutical applications (Yi, et al., 2023).

Therefore, this study aimed to optimize drying temperature for enhancing flavonoid retention in cocoa (*Theobroma cacao* L.) leaves by determining the total flavonoid content of ethanolic extracts using UV–Visible spectrophotometry. The findings are expected to provide a scientific basis for selecting appropriate drying conditions to preserve bioactive flavonoids and to support the development of standardized cocoa leaf-derived functional herbal materials with potential applications in integrative approaches to chronic disease prevention.

METHODS

2.1 Study Design

This experimental study was conducted to investigate the effect of drying temperature on the retention of bioactive flavonoids in cocoa (*Theobroma cacao* L.) leaves. Fresh cocoa leaves were dried at three different temperatures (40, 50, and 60°C) prior to extraction with 70% ethanol. The obtained extracts were subjected to phytochemical screening and quantitative determination of total flavonoid content using the aluminum chloride colorimetric method with UV–Visible spectrophotometry.

2.2 Plant Materials

Fresh cocoa (*Theobroma cacao* L.) leaves were collected from Gulangpongge Village, Gunungwungkal District, Pati Regency, Central Java, Indonesia. Mature healthy leaves with uniform green color and free from mechanical damage, pests, and disease symptoms were selected as plant materials. The collected samples were washed thoroughly under running tap water to remove adhering dust and impurities before further processing.

2.3 Chemicals and Reagents

Analytical-grade ethanol (70% and absolute ethanol), aluminum chloride (AlCl_3), sodium acetate (CH_3COONa), hydrochloric acid (HCl), sodium hydroxide (NaOH), magnesium powder, Mayer reagent, Dragendorff reagent, and quercetin standard were used throughout the study. Distilled water was used for solution preparation.

2.4 Sample Preparation

Fresh cocoa leaves were sorted, washed thoroughly with running water, and cut into small pieces of approximately 2–3 cm. The samples were dried in a laboratory oven (UM200 Casing, Indonesia) at three different temperatures (40, 50, and 60°C) until constant weight was achieved. The percentage of weight loss during drying was calculated to evaluate moisture reduction. The dried leaves were ground using a laboratory blender and sieved through a 40-mesh sieve to obtain a homogeneous powder. Moisture

content of the powdered samples was subsequently determined using a moisture analyzer.

2.5 Extraction Procedure

Powdered cocoa leaves (200 g) were extracted by maceration using 1 L of 70% ethanol at room temperature for 48 h with occasional stirring. The extract was filtered through a flannel cloth, and the residue was re-macerated under identical conditions until a clear filtrate was obtained. Combined filtrates were concentrated using a water bath maintained at 40–45°C to obtain a viscous crude extract. The concentrated extract was stored in amber glass bottles at room temperature until further analysis.

2.6 Phytochemical Screening

Preliminary phytochemical screening was performed to qualitatively detect the presence of flavonoids, alkaloids, and saponins following previously reported procedures.

2.6.1 Flavonoid Test

Flavonoids were identified using three qualitative methods:

Shinoda (Wilstätter) test, in which magnesium powder and concentrated hydrochloric acid were added to the ethanolic extract. The appearance of an orange to reddish coloration indicated the presence of flavonoids.

Bate-Smith test, where concentrated hydrochloric acid was added followed by heating. Development of a red coloration indicated positive flavonoid compounds.

Alkaline reagent test, performed by adding 10% sodium hydroxide solution to the extract. The formation of an intense yellow coloration confirmed flavonoid presence.

2.6.2 Alkaloid Test

The extract was acidified with 2 N hydrochloric acid and separately treated with Mayer's and Dragendorff's reagents. Formation of a cream-colored precipitate with Mayer's reagent or an orange precipitate with Dragendorff's reagent indicated the presence of alkaloids.

2.6.3 Saponin Test

The extract was mixed with hot distilled water, acidified with hydrochloric acid, and vigorously shaken. The formation of stable foam persisting for several minutes confirmed the presence of saponins.

2.7 Determination of Total Flavonoid Content

Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method with quercetin as the reference standard.

2.7.1 Preparation of Quercetin Standard Solution

A stock solution of quercetin (1000 µg/mL) was prepared by dissolving 25 mg of quercetin in absolute ethanol and diluting to 25 mL in a volumetric flask.

2.7.2 Determination of Maximum Absorption Wavelength

One milliliter of quercetin working solution (80 µg/mL) was mixed with 0.1 mL of 10% aluminum chloride solution and 0.1 mL of 1 M sodium acetate solution. The absorption spectrum was recorded between 400 and 800 nm using a UV-Visible spectrophotometer (BIOBASE BK-D560, China), and the wavelength corresponding to the maximum absorbance was selected for subsequent measurements.

2.7.3 Optimization of Incubation Time

The quercetin reaction mixture was incubated at room temperature, and absorbance was recorded at predetermined intervals until a stable absorbance value was achieved. The incubation period corresponding to the stable absorbance was used as the operating time.

2.7.4 Calibration Curve

Working standard solutions of quercetin (40, 60, 80, 100, and 120 µg/mL) were prepared from the stock solution. Each standard solution (1 mL) was mixed with 0.1 mL of 10% aluminum chloride and 0.1 mL of 1 M sodium acetate, incubated for the optimized reaction time, and analyzed spectrophotometrically at the selected wavelength. A calibration curve was constructed by plotting absorbance against quercetin concentration.

2.7.5 Quantification of Total Flavonoid Content

Five milligrams of each concentrated extract were dissolved in ethanol and diluted to a final volume of 5 mL. An aliquot of 1 mL was reacted with aluminum chloride and sodium acetate following the same procedure used for the standard solutions. Absorbance was measured at the selected wavelength, and total flavonoid content was calculated from the calibration curve. The results were expressed as milligrams of quercetin equivalents per gram of extract (mg QE/g extract) using the following equation:

$$TFC = \frac{C \times V \times df}{m} \times 100\%$$

where:

TFC = Total flavonoid content (mg QE/g extract)

C = Quercetin concentration obtained from the calibration curve (mg/mL)

V = Total extract volume (mL)

df = Dilution factor

m = Mass of extract (g)

RESULTS AND DISCUSSION

3.1 Effect of Drying Temperature on the Physical Characteristics of Cocoa Leaf Simplicia

Fresh cocoa (*Theobroma cacao* L.) leaves were processed into simplicia through oven drying at three different temperatures (40, 50, and 60°C). The effects of drying temperature on the physical characteristics, drying yield, and moisture reduction are summarized in Table 1.

Table 1. Physical characteristics of cocoa leaf simplicia obtained under different drying temperatures.

Drying temperature	Fresh leaves (g)	Dried leaves (g)	Powder (g)	Weight loss (%)	Fresh leaf color	Simplicia color
40°C	1500	565	330	62.33	Green	Dark green
50°C	1500	586	315	60.93	Green	Greenish-brown
60°C	1500	610	300	59.33	Green	Brownish-green

The drying temperature markedly influenced the physical appearance and drying characteristics of cocoa leaf simplicia (Lestari, 2021). Increasing the drying temperature from 40 to 60°C resulted in progressive changes in leaf color from dark green to greenish-brown, indicating degradation of chlorophyll pigments during thermal processing. Similar color changes have been reported in medicinal plants subjected to elevated drying temperatures, where chlorophyll degradation and the formation of pheophytin occur because of heat-induced replacement of magnesium ions within the chlorophyll molecule.

The percentage of weight loss ranged from 59.33% to 62.33%, indicating substantial moisture removal during oven drying. Interestingly, samples dried at 40°C exhibited the highest percentage of weight loss. This observation may be associated with the longer drying duration required at lower temperatures, allowing gradual evaporation of free water while minimizing rapid surface hardening (case hardening). Conversely, higher drying temperatures can accelerate surface dehydration, which may reduce overall drying efficiency if moisture diffusion from the internal tissues becomes restricted. Similar drying behavior has been described in studies investigating thermal drying kinetics of medicinal plant materials.

From a practical perspective, drying temperature is one of the most critical post-harvest parameters affecting both the physical quality and phytochemical stability of herbal raw materials. Therefore, optimization of drying conditions should consider not only moisture removal efficiency but also preservation of bioactive compounds, particularly thermolabile flavonoids.

3.2 Moisture Content of Cocoa Leaf Simplicia

The moisture contents of cocoa leaf simplicia dried at different temperatures are presented in Table 2.

Table 2. Moisture content of cocoa leaf simplicia dried at different temperatures.

Drying temperature	Moisture content (%)
40°C	9.00 ± 0.04
50°C	8.84 ± 0.06
60°C	8.78 ± 0.01

The moisture content of all samples ranged from 8.78% to 9.00%, indicating that all drying treatments successfully reduced water content below the maximum limit of 10%, which is generally recommended for herbal simplicia to ensure microbiological stability and prolonged storage life. Lower moisture content inhibits microbial proliferation and suppresses enzymatic activities that may accelerate

deterioration of phytochemical constituents during storage.

As expected, increasing drying temperature slightly decreased the final moisture content. Higher temperatures enhance the vapor pressure gradient between plant tissues and surrounding air, thereby accelerating moisture diffusion and evaporation. This phenomenon has been widely reported in thermal drying processes of medicinal plants and agricultural products.

Although drying at 60°C produced the lowest moisture content, excessively high temperatures may compromise the stability of heat-sensitive phytochemicals such as flavonoids. Flavonoid molecules possess hydroxylated aromatic structures that are susceptible to oxidation, hydrolysis, and thermal degradation under prolonged heating. Therefore, lower residual moisture should not be considered the sole indicator of superior drying performance.

The slightly higher moisture content observed at 40°C (9.00%) remained within acceptable quality standards while potentially providing milder drying conditions that better preserve thermolabile compounds. Consequently, optimization of drying temperature requires balancing efficient moisture removal with maximum retention of bioactive metabolites.

These findings support the hypothesis that drying temperature is a critical parameter in the production of standardized herbal raw materials. Appropriate drying conditions not only improve storage stability but also contribute to maintaining phytochemical integrity, which ultimately determines the therapeutic quality of cocoa leaf extracts intended for functional herbal development and chronic disease prevention.

3.3 Effect of Drying Temperature on Extraction Yield

The extraction yield of cocoa (*Theobroma cacao* L.) leaf powder dried at different temperatures is presented in Table 3.

Table 3. Extraction yield and organoleptic characteristics of cocoa leaf extracts.

Drying temperature	Powder (g)	Extract (g)	Yield (%)	Organoleptic characteristics
40°C	200	15	7.50	Dark green, viscous, characteristic odor
50°C	200	13	6.50	Dark green to blackish-green, viscous, characteristic odor
60°C	200	16	8.00	Dark green to blackish-green, viscous, characteristic odor

The extraction yield varied according to the drying temperature, ranging from 6.50% to 8.00%. Among the three treatments, cocoa leaves dried at 60°C produced the highest extraction yield (8.00%), whereas samples dried at 50°C yielded the lowest extract (6.50%).

Differences in extraction yield may be attributed to structural modifications occurring in plant tissues during the drying process. Moderate thermal treatment can disrupt cell walls and increase cell permeability, facilitating solvent penetration and enhancing the diffusion of intracellular metabolites into the extraction solvent. Consequently, greater quantities of extractable compounds may be recovered during maceration. Similar observations have been reported in medicinal plants where controlled drying improved extraction efficiency by increasing the accessibility of intracellular phytochemicals.

The use of 70% ethanol as the extraction solvent also contributed to efficient recovery of polar and moderately polar phytochemicals. Hydroethanolic solvents possess suitable polarity for extracting flavonoids, phenolic compounds, tannins, and other secondary metabolites commonly present in cocoa leaves. The presence of water in the solvent system promotes plant tissue swelling, whereas ethanol enhances the solubility of phenolic constituents, resulting in higher extraction efficiency than absolute ethanol (Nahor, 2021).

Interestingly, the highest extraction yield was obtained from samples dried at 60°C, despite previous studies often reporting reduced yields at elevated drying temperatures due to thermal degradation of heat-sensitive metabolites. This finding suggests that, under the experimental conditions employed in the present study, drying at 60°C effectively reduced moisture content and enhanced solvent accessibility without causing substantial degradation of extractable constituents. Nevertheless, a higher extraction yield does not necessarily indicate a higher concentration of flavonoids, since crude extract yield represents the total amount of soluble compounds extracted, including chlorophylls, carbohydrates, proteins, organic acids, and other metabolites. Therefore, quantitative determination of total flavonoid content is required to evaluate whether increased extraction yield corresponds to improved retention of bioactive flavonoids.

The organoleptic properties of all extracts were generally similar, exhibiting a viscous consistency

and characteristic cocoa leaf odor. However, extracts obtained from samples dried at 50 and 60°C showed a darker greenish-black color than those dried at 40°C. This color change may be associated with chlorophyll degradation and the formation of pheophytin and other oxidation products during thermal drying. Such pigment transformation is commonly observed in thermally processed leafy materials and has been reported to increase with drying temperature.

From the perspective of functional herbal development, extraction yield is an important technological parameter because it influences extraction efficiency and production cost. However, optimization of herbal processing should prioritize not only maximizing extract yield but also preserving biologically active compounds, particularly flavonoids, which contribute to the antioxidant potential of cocoa leaves. Therefore, subsequent quantitative evaluation of total flavonoid content is essential to identify the optimum drying temperature capable of balancing extraction efficiency with phytochemical preservation.

3.4 Phytochemical Screening of Cocoa (*Theobroma cacao* L.) Leaf Extracts

The qualitative phytochemical screening of cocoa leaf extracts dried at different temperatures (40, 50, and 60°C) is presented in Table 4.

Table 4. Phytochemical screening of cocoa leaf extracts obtained under different drying temperatures.

Secondary metabolite	Reagent	Observation	40°C	50°C	60°C
Flavonoids	Shinoda (Mg + HCl)	Orange-red coloration	+	+	+
	Bate-Smith (Conc. HCl + heating)	Red coloration	+	+	+
	10% NaOH	Yellowish-brown coloration	+	+	+
Alkaloids	Mayer reagent	Brownish precipitate	+	+	+
	Dragendorff reagent	Orange precipitate	+	+	+
Saponins	Hot distilled water + HCl	Stable froth formation	+	+	+

Qualitative phytochemical analysis demonstrated that cocoa leaf extracts obtained from all drying treatments contained flavonoids, alkaloids, and saponins. The identical phytochemical profiles observed at 40, 50, and 60°C indicate that the applied drying temperatures did not eliminate these major classes of secondary metabolites. These findings suggest that drying within this temperature range is sufficiently mild to preserve the principal phytochemical constituents of cocoa leaves.

The presence of flavonoids was confirmed using three complementary qualitative assays, namely the Shinoda (Wilstätter), Bate-Smith, and alkaline reagent tests. In the Shinoda test, all extracts developed an orange-red coloration following the addition of magnesium powder and concentrated hydrochloric acid, confirming the presence of flavonoid compounds (Estikawati & Lindawati, 2019). This reaction occurs because magnesium reduces the carbonyl group of flavonoid molecules under acidic conditions, resulting in the formation of colored flavilium salts. Likewise, positive results obtained in the Bate-Smith assay were characterized by the appearance of a red coloration after acid hydrolysis and heating, indicating the presence of flavonoid glycosides that were converted into their corresponding aglycones. In the alkaline reagent test, the development of a yellowish-brown coloration following sodium hydroxide addition further supported the presence of flavonoids, owing to ionization of the phenolic hydroxyl groups under alkaline conditions.

The extracts also tested positive for alkaloids using both Mayer's and Dragendorff's reagents. Formation of brownish precipitates with Mayer's reagent and orange precipitates with Dragendorff's reagent indicates the presence of nitrogen-containing alkaloid compounds. Under acidic conditions, alkaloids exist as protonated salts that readily react with heavy-metal-based reagents to produce insoluble complexes. Although alkaloids are generally present in lower concentrations than polyphenolic compounds in cocoa leaves, they may contribute synergistically to the biological activities of the extract, including antimicrobial and antioxidant effects.

Positive saponin reactions were evidenced by the formation of persistent foam after vigorous shaking in the presence of hot distilled water and hydrochloric acid. This phenomenon is attributed to the amphiphilic structure of saponins, which lowers surface tension and stabilizes foam formation. Saponins have been widely reported to possess antioxidant, anti-inflammatory, immunomodulatory, and cholesterol-lowering properties, thereby enhancing the pharmacological potential of medicinal plant extracts.

Interestingly, no qualitative differences in phytochemical composition were observed among the

three drying temperatures. This finding suggests that the temperature range investigated (40–60°C) primarily influences the quantity rather than the presence or absence of secondary metabolites. Consequently, qualitative phytochemical screening alone cannot determine the optimum drying temperature for preserving bioactive compounds. Quantitative determination of total flavonoid content is therefore essential to evaluate the extent to which drying temperature affects flavonoid retention.

The simultaneous presence of flavonoids, alkaloids, and saponins highlights the potential of cocoa leaves as a promising source of bioactive phytochemicals for functional herbal development. Among these metabolites, flavonoids are of particular interest because of their well-documented antioxidant and anti-inflammatory activities, which are closely associated with the prevention of oxidative stress-related chronic diseases. Therefore, optimizing post-harvest processing to maximize flavonoid retention is an important step toward producing standardized cocoa leaf-based herbal products with improved functional value.

3.5 Determination of Total Flavonoid Content

3.5.1 Optimization of UV–Visible Spectrophotometric Conditions

Prior to quantitative analysis, the analytical conditions for total flavonoid determination were optimized using quercetin as the reference standard. The absorption spectrum of the quercetin–aluminum chloride complex was scanned within the visible region (400–800 nm) to determine the maximum absorption wavelength (λ_{max}) (Haeria, et al., 2016). The maximum absorbance was observed at 429 nm with an absorbance value of 0.579, indicating the wavelength at which the quercetin–AlCl₃ complex exhibited the highest analytical sensitivity.

The aluminum chloride colorimetric assay is based on the formation of acid-stable complexes between aluminum ions and the keto as well as hydroxyl functional groups present in flavonoid molecules. The resulting yellow complex exhibits a characteristic absorption in the visible region, allowing quantitative determination by UV–Visible spectrophotometry. Selection of the appropriate λ_{max} minimizes analytical error while improving measurement sensitivity and reproducibility.

The operating time was subsequently evaluated to determine the period required for complete complex formation. The absorbance of the quercetin standard solution (80 µg/mL) was monitored at 429 nm for 90 min. Stable absorbance was reached after 25 min, indicating completion of the complexation reaction. Therefore, an incubation time of 25 min was used throughout subsequent analyses to ensure consistent analytical conditions.

3.5.2 Calibration Curve of Quercetin

The calibration curve of quercetin was constructed using standard solutions with concentrations ranging from 40 to 120 µg/mL.

Table 5. Calibration curve of quercetin.

Quercetin concentration (µg/mL)	Absorbance
40	0.260
60	0.336
80	0.440
100	0.532
120	0.665

The calibration curve demonstrated an excellent linear relationship between quercetin concentration and absorbance, following the regression equation $y=0.005x+0.0442$ with $R^2 = 0.991$. The high correlation coefficient indicates excellent linearity within the selected concentration range and confirms that the analytical method is suitable for quantitative determination of total flavonoids. According to the International Council for Harmonisation (ICH), a calibration curve with R^2 greater than 0.99 generally demonstrates acceptable analytical linearity for quantitative assays. As expected, absorbance increased proportionally with increasing quercetin concentration, confirming compliance with the Beer–Lambert law over the investigated concentration range.

3.5.3 Effect of Drying Temperature on Total Flavonoid Content

The total flavonoid contents of cocoa leaf extracts obtained under different drying temperatures are presented in Table 6.

Table 6. Total flavonoid content of cocoa leaf extracts.

Drying temperature	Total flavonoid (%)
40°C	5.212 ± 0.062
50°C	5.809 ± 0.401
60°C	6.402 ± 0.214

The quantitative analysis demonstrated that drying temperature significantly influenced the total flavonoid content of cocoa (*Theobroma cacao* L.) leaf extracts (Table 6). The highest flavonoid content was obtained from samples dried at 60°C (6.402 ± 0.214%), followed by 50°C (5.809 ± 0.401%), whereas the lowest value was recorded at 40°C (5.212 ± 0.062%). These findings indicate that increasing the drying temperature within the investigated range enhanced flavonoid recovery from cocoa leaves.

Statistical analysis further confirmed the effect of drying temperature on flavonoid content. One-way ANOVA revealed a significant difference among treatments ($F = 15.18$, $p = 0.0045$). Tukey's HSD post hoc test showed that samples dried at 60°C contained significantly higher flavonoid levels than those dried at 40°C ($p = 0.0036$). However, no significant differences were observed between 40°C and 50°C ($p = 0.073$) or between 50°C and 60°C ($p = 0.0748$), indicating that the intermediate drying temperature produced flavonoid contents statistically comparable to both treatments.

The gradual increase in flavonoid content with increasing drying temperature suggests that moderate thermal treatment enhanced the extractability of flavonoid compounds. Higher drying temperatures accelerate moisture removal, resulting in reduced water activity and suppression of enzymatic oxidation mediated by polyphenol oxidase and peroxidase. Simultaneously, thermal treatment promotes partial disruption of plant cell walls, thereby facilitating solvent penetration and improving mass transfer during the maceration process. Consequently, intracellular flavonoids become more readily extracted into the ethanol solvent.

Interestingly, drying at 60°C did not appear to induce substantial degradation of flavonoids under the present experimental conditions. This finding suggests that the applied temperature remained below the thermal degradation threshold of the dominant flavonoid constituents in cocoa leaves. Similar observations have been reported in medicinal and aromatic plants, where drying at 50–60°C effectively balances rapid moisture removal with the preservation of heat-sensitive phenolic compounds, leading to higher flavonoid recovery than lower drying temperatures.

Nevertheless, thermal processing should be carefully controlled because excessive temperatures may accelerate oxidation, hydrolysis, and structural degradation of thermolabile flavonoids, ultimately reducing their antioxidant capacity and biological activity. Therefore, optimizing drying conditions represents a critical step in the standardization of herbal raw materials and the preservation of phytochemical quality.

From the perspective of functional herbal development, preservation of flavonoids is particularly important because these polyphenolic compounds have been extensively associated with antioxidant, anti-inflammatory, cardioprotective, and antidiabetic activities, all of which are closely related to chronic disease prevention. Thus, the identification of 60°C as the optimum drying temperature provides practical guidance for improving the post-harvest processing of cocoa leaves and supports their utilization as a sustainable source of bioactive ingredients for functional herbal products and integrative healthcare applications.

Although this study identified an optimum drying temperature for maximizing total flavonoid content, only total flavonoids were quantified using the aluminum chloride colorimetric assay. Future studies should employ advanced analytical techniques, such as HPLC or LC–MS, to characterize individual flavonoid compounds, evaluate antioxidant and other biological activities, and investigate the thermal degradation kinetics of flavonoids during drying. Such investigations will provide a more comprehensive understanding of the relationship between drying conditions, phytochemical composition, and therapeutic potential.

CONCLUSION

The present study demonstrated that drying temperature significantly influenced the extraction efficiency and total flavonoid content of cocoa (*Theobroma cacao* L.) leaves. Among the investigated treatments, oven drying at 60°C was identified as the optimum condition, producing the highest

extraction yield (8.0%) and total flavonoid content ($6.402 \pm 0.214\%$) while maintaining acceptable moisture content and phytochemical integrity. These findings provide scientific evidence that optimizing drying conditions is an effective post-harvest strategy for improving the quality of cocoa leaf extracts and promoting the utilization of cocoa leaves as an underutilized source of bioactive compounds. The study also supports the development of standardized functional herbal materials with potential applications in chronic disease prevention and integrative healthcare. Further research involving detailed flavonoid profiling, antioxidant and biological activity assays, and compound stability evaluation is recommended to further validate the therapeutic potential of cocoa leaf extracts.

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