

## Evaluation of Processing Parameters Affecting Antioxidant Activity and Sensory Quality of Wild Bitter Melon Leaf Tea

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### ABSTRACT

Wild bitter melon (*Momordica charantia* var. *abbreviata*) leaves represent an underutilized resource rich in polyphenols and flavonoids. However, the processing parameters for producing a palatable and bioactive herbal tea from these leaves remain unclear. This study evaluated three critical factors—harvest age, vacuum-drying temperature, and stevia blending ratio—to maximize phenolic retention, antioxidant capacity, and sensory quality. Leaves of wild bitter melon (*Momordica charantia* var. *abbreviata*) cultivated at Kien Giang University (Vietnam) were harvested at 60, 80, 100, and 120 days of age. Processing was performed at the university’s Practice–Laboratory Center. The leaves were vacuum-dried at 45, 55, or 65 °C (70 kPa, 24 h; final moisture ~6–7%). Total polyphenols and flavonoids were determined by Folin-Ciocalteu and NaNO<sub>2</sub>–AlCl<sub>3</sub>–NaOH colorimetry, respectively, while antioxidant activity was evaluated by the DPPH assay with half-maximal inhibitory concentration expressed as mg dry weight per mL extract (mg/mL). Sensory evaluation of the teas brewed from leaf–stevia blends (9.5:0.5 to 7.5:2.5, w/w) followed TCVN 3218:2012 using a trained panel (n = 10). Harvesting at 80 days yielded the highest Total polyphenol content (29.36 mg gallic acid equivalents/g dry weight) and the lowest Half-maximal inhibitory concentration (1.34 mg/mL). Drying at 55 °C maximized total polyphenol content (47.67 mg gallic acid equivalents/g dry weight) and total flavonoid content (123.48 mg quercetin equivalents/g dry weight) while minimizing half-maximal inhibitory concentration (1.23 mg/mL). The 8.5:1.5 leaf–stevia blend achieved the highest sensory score (16.27; “Good”), balancing bitterness and sweetness. These integrated parameters provide a scientific basis for valorizing wild bitter melon leaves as a functional herbal tea with enhanced bioactivity and consumer acceptance.

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### 1. Introduction

Herbal teas are increasingly valued for their convenience and ability to deliver health-promoting bioactive compounds. Wild bitter melon (*Momordica charantia* var. *abbreviata*), widely distributed in Southeast Asia, is a promising source of polyphenols and flavonoids with antioxidant and health-supporting properties [1], [2], [3]. While most studies and applications have focused on the fruit, the leaves are often discarded despite their high bioactive content [4], [5]. Utilizing leaves not only adds value to agricultural by-products but also offers new opportunities for functional herbal tea development.

The timing of leaf harvest is critical, as early harvesting may reduce fruit yield, and phytochemical accumulation in leaves varies across growth stages [6], [7]. In addition, processing conditions, particularly drying methods and temperatures, strongly influence the retention of phenolics and antioxidant capacity [8], [9]. Vacuum drying at moderate temperatures has been reported to limit

oxidative degradation compared with conventional hot-air drying [10], but favorable conditions for wild bitter melon leaves have not been fully established.

Furthermore, the inherent bitterness of wild bitter melon leaves limits consumer acceptance. Stevia (*Stevia rebaudiana*), a natural non-caloric sweetener, has been widely applied to balance bitterness and improve taste [11], [12]. Preliminary Vietnamese studies have explored formulations combining wild bitter melon and stevia in tea bags [13], yet no comprehensive evaluation has been conducted that integrates phytochemical retention, antioxidant capacity, and sensory quality.

Therefore, this study aimed to identify the favorable harvest age, vacuum-drying temperature, and blending ratio of wild bitter melon leaves with stevia to maximize bioactive preservation and antioxidant activity while enhancing the sensory quality of the resulting herbal tea.

## 2. Materials and Methods

### 2.1. Study site

The study was conducted from April to August 2024 at the Practice–Laboratory Center, Kien Giang University (Vietnam). Wild bitter melon (*Momordica charantia* var. *abbreviata*) plants were cultivated in the experimental field of Kien Giang University, and their leaves were used as raw materials for the experiments.

### 2.2. Materials and equipment

Wild bitter melon leaves (*Momordica charantia* var. *abbreviata*) were supplied by VAPHACO (Vietnam; TCCS VP-BT08/16). Stevia leaves (*Stevia rebaudiana*) were used for blending. Analytical reagents included Folin–Ciocalteu reagent and  $\text{Na}_2\text{CO}_3$  (Merck, Germany). Other chemicals were  $\text{NaNO}_2$  (Merck),  $\text{AlCl}_3$  (Duchefa, Netherlands), DPPH (Alfa, UK), gallic acid (BioBasic, Canada) and quercetin (Fisher Scientific, USA) as standards, methanol, ethanol, and NaOH (Xilong, China). Deionized water (WASOL, Vietnam) was used throughout. Major instruments: vacuum oven VO101 (Mettler, Germany), analytical balance CPA224S (Sartorius, Germany), moisture analyzer MX50 (A&D, Japan), UV–Vis spectrophotometer UV-1800 (Shimadzu, Japan) with 1-cm quartz cuvettes, grinder RT08, thermostatic bath WNB7 (Mettler, Germany), volumetric glassware (Bomex), micropipettes (Eppendorf), and a  $-20\text{ }^\circ\text{C}$  freezer (Vestfrost Solutions, model VT406, Denmark).

### 2.3. Sample processing, storage, and experimental design

Fresh leaves of wild bitter melon (*Momordica charantia* var. *abbreviata*) were sorted to remove damaged and discolored parts, then washed in 1.5% brine for 30 seconds, rinsed twice with running water, and drained. The cleaned leaves were spread in a thin layer (~5 mm) on stainless steel trays and vacuum-dried in a VO101 vacuum oven (Mettler, Germany) under a pressure of 70 kPa for 24 hours until reaching a final moisture content of 6–7%, verified by a MX50 moisture analyzer (A&D, Japan).

Three drying temperatures (45, 55, and 65  $^\circ\text{C}$ ) were tested to evaluate the effects on total polyphenol content (TPC), total flavonoid content (TFC), and antioxidant activity ( $\text{IC}_{50}$ ). After drying, the leaves were ground using a RT08 grinder, sieved through a 60-mesh screen, vacuum-packed in polyethylene pouches, and stored in a  $-20\text{ }^\circ\text{C}$  freezer (Vestfrost Solutions VT406, Denmark) until analysis ( $\leq 90$  days).

For sensory evaluation, the dried wild bitter melon leaf powder was blended with stevia (*Stevia rebaudiana*) powder at five weight ratios (w/w): 9.5:0.5, 9:1, 8.5:1.5, 8:2, and 7.5:2.5, representing increasing sweetness levels. Each 1.5 g of the blended tea was brewed in 200 mL of boiling water for 5 minutes before sensory testing. All experiments were conducted following a completely randomized design (CRD) with three replications for each treatment. Results were expressed as mean  $\pm$  standard deviation ( $n = 3$ ).

### 2.4. Analytical methods

*Total polyphenols (TPC):*

TPC was determined using the Folin–Ciocalteu colorimetric method [14], [15] with minor modifications. Reaction: 1.0 mL extract + 1.0 mL water + 1.0 mL diluted Folin–Ciocalteu (1:4 v/v); add 1.0 mL Na<sub>2</sub>CO<sub>3</sub> 10%; incubate 30 minutes at 40 °C (dark); read at 765 nm. Calibration: gallic acid 0–70 µg/mL (0, 10, 20, 30, 40, 50, 60, 70), regression  $y = 0.0311x - 0.0637$  ( $R^2 = 0.992$ ). Results were expressed as mg gallic acid equivalents per gram dry weight (mg GAE/g DW).

*Total flavonoids (TFC):*

TFC was measured by the NaNO<sub>2</sub>–AlCl<sub>3</sub>–NaOH colorimetry described by [16]. Quercetin standard (0–350 µg/mL: 0, 50, 100, 150, 200, 250, 300, 350) was used. Reaction: 800 µL extract (5,000 µg/mL) + 800 µL water + 160 µL NaNO<sub>2</sub> 5% (5 minutes) → 160 µL AlCl<sub>3</sub> 10% (6 minutes) → 1.6 mL NaOH 1 M + 480 µL water (final 4 mL); read at 510 nm. Regression:  $y = 0.0011x - 0.003$  ( $R^2 = 0.9946$ ). Results were expressed as mg quercetin equivalents per gram dry weight (mg QE/g DW).

*DPPH radical scavenging activity:*

The antioxidant capacity was determined by the DPPH radical scavenging assay following [17] with minor modifications. A DPPH stock solution (1,000 µg/mL in methanol) was prepared and aged for 24 h at 4 °C in the dark. For each measurement, 1.0 mL of sample extract (0.5–2.5 mg/mL) was mixed with 1.0 mL of DPPH solution, vortexed briefly, and incubated for 30 minutes in the dark at room temperature. Absorbance was recorded at 517 nm using a UV–Vis spectrophotometer (UV-1800, Shimadzu, Japan) with 1-cm quartz cuvettes.

The radical-scavenging activity was expressed as inhibition percentage and calculated as:

$$\text{Inhibition (\%)} = ((A_0 - A_s)/A_0) \times 100$$

Where  $A_0$  is the absorbance of the control (methanol + DPPH without extract) and  $A_s$  is the absorbance of the sample after reaction. The IC<sub>50</sub> value (extract concentration required to achieve 50% inhibition) was estimated by nonlinear regression fitted to inhibition (%) versus extract concentration. Results are reported as mean ± SD (n = 3), and differences were considered significant at  $p < 0.05$ .

## 2.5. Sensory evaluation and statistical analysis

Sensory quality evaluation was performed according to the Vietnamese National Standard TCVN 3218:2012 for tea products. Ten trained panelists (aged 20–35 years, balanced by gender) from the Faculty of Food Science and Health, Kien Giang University, participated in the evaluation. Each infusion was prepared from 1.5 g of sample brewed in 200 mL of boiling water for 5 minutes, then served at  $22 \pm 2$  °C under uniform white light in individual booths.

Panelists assessed four sensory attributes-Appearance/Clarity (25%), Color (15%), Odor (30%), and Taste (30%)-using a five-point hedonic scale, where 1 indicated “very poor,” 2 “poor,” 3 “average,” 4 “good,” and 5 “excellent.” The overall sensory quality was calculated as a weighted sum of the individual attributes and classified into five categories: Excellent (18.2–20.0), Good (15.2–18.1), Average (11.2–15.1), Poor (7.2–11.1), and Fail (<7.1).

Data were analyzed using the Friedman test followed by Nemenyi post hoc comparisons to determine significant differences among treatments. All analyses were conducted in Statgraphics Centurion XV, version 15, and results were expressed as mean ± standard deviation (n = 10) at a significance level of  $p < 0.05$ .

## 2.6. Ethics statement

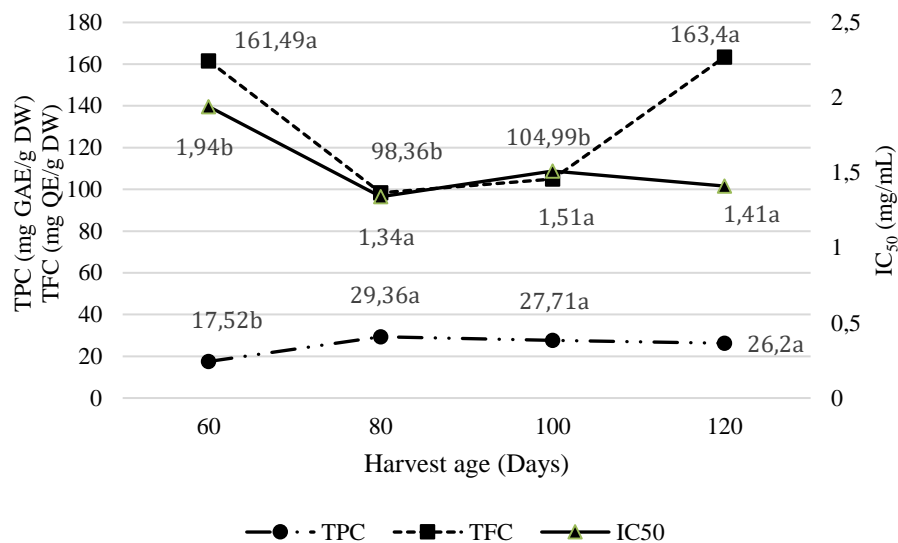
The study complied with the Declaration of Helsinki and was approved by the Kien Giang University Science & Health Committee (Approval No. 09-QĐ/KHTP&SK-ĐHKG; 12 Aug 2024). Written informed consent was obtained from all panelists.

## 3. Results and Discussion

### 3.1. Effect of harvest age

The harvest age of wild bitter melon leaves markedly influenced their phytochemical composition and antioxidant capacity. Figure 1 presents the changes in total polyphenols (TPC), total flavonoids

(TFC), and DPPH radical scavenging activity expressed as  $IC_{50}$  (mg/mL). Leaves harvested at 80 days showed the highest phenolic content (29.36 mg GAE/g DW) and the lowest  $IC_{50}$  value (1.34 mg/mL), demonstrating superior antioxidant activity compared with leaves at 60 days ( $p < 0.05$ ).



**Figure 1.** Effects of harvest age on TPC, TFC, and  $IC_{50}$  of wild bitter melon leaves.

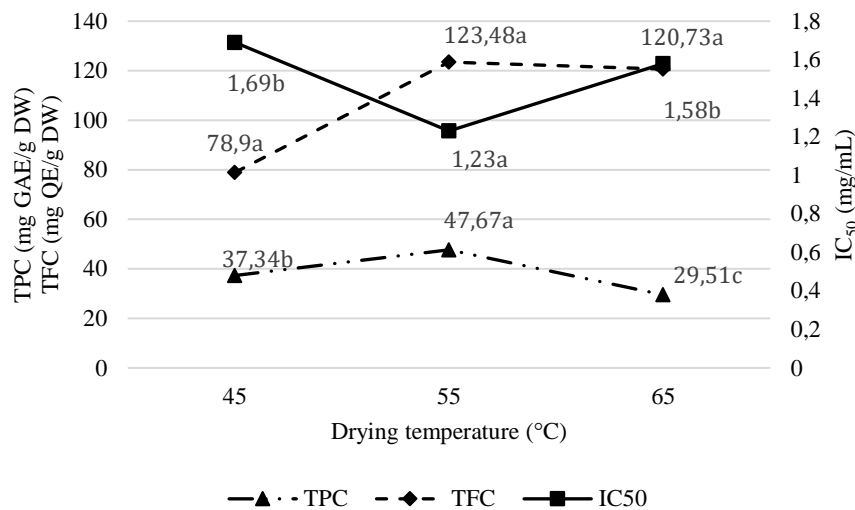
The observed trends can be explained by physiological changes in leaf development. Younger leaves (60 days) had lower phenolic content, likely due to incomplete secondary metabolite biosynthesis. At 80 days, phenolic and flavonoid pathways are more active, resulting in higher accumulation and stronger antioxidant activity. With further aging (100–120 days), TPC remained high, but  $IC_{50}$  values did not improve, suggesting possible onset of oxidative degradation or redistribution of metabolites. Similar age-dependent accumulation of bioactives has been reported in *Cistus incanus* leaves, where antioxidant defenses peaked at mid-maturity and declined thereafter [6], and in *Centella asiatica*, where polyphenol content correlated with growth stage [18].

The variation between TPC and TFC at different harvest ages can be explained by the differential biosynthesis and oxidation of phenolic subclasses during leaf maturation. While total phenolic content (TPC) represents the overall pool of phenolic compounds, flavonoids (TFC) are a specific subgroup whose synthesis is highly responsive to developmental and environmental factors. At early growth stages (60 days), the elevated flavonoid concentration likely reflects the rapid production of flavonols and flavones functioning as photoprotective compounds. As leaves reach mid-maturity (around 80 days), enhanced secondary metabolism promotes the accumulation of non-flavonoid phenolics such as phenolic acids and tannins, resulting in the observed TPC peak.

At later stages (100–120 days), partial oxidative degradation and polymerization of flavonoids may occur, reducing TFC even though total phenolic levels remain stable. Similar age-dependent variations in phenolic and flavonoid accumulation have been reported in *Cistus incanus* and *Centella asiatica*, where mid-maturity stages exhibited the highest total phenolics but not necessarily the highest flavonoid contents [6], [18]. These results suggest that both biosynthetic activity and oxidative turnover jointly determine the phenolic profile of wild bitter melon leaves during ontogeny.

### 3.2. Effect of vacuum-drying temperature

Drying temperature significantly influenced the retention of phenolic compounds and antioxidant activity of wild bitter melon leaves. As shown in Figure 2, samples dried at 55 °C contained the highest TPC (47.67 mg GAE/g DW) and TFC (123.48 mg QE/g DW), along with the lowest  $IC_{50}$  value (1.23 mg/mL). In contrast, drying at 65 °C markedly reduced TPC to 29.51 mg GAE/g DW, while antioxidant capacity also declined ( $IC_{50} = 1.58$  mg/mL).



**Figure 2.** Effects of vacuum-drying temperature on TPC, TFC, and IC<sub>50</sub> of wild bitter melon leaves.

These results highlight that moderate drying temperature (55 °C) under vacuum preserved phenolic compounds more effectively than either lower (45 °C) or higher (65 °C) conditions. At 45 °C, the incomplete inactivation of oxidative enzymes such as polyphenol oxidase may have contributed to phenolic losses under relatively mild conditions [19]. At 65 °C, thermal degradation and structural changes in leaf tissues likely accelerated the breakdown of sensitive polyphenols [20]. Comparable findings were reported for citrus seeds [10] and apricots [21], where intermediate drying temperatures evaluated antioxidant retention. Thus, vacuum drying at 55 °C can be considered the favorable condition to balance enzymatic inactivation and thermal stability in wild bitter melon leaves.

### 3.3. Sensory acceptance of leaf–stevia blends

The blending ratio of wild bitter melon leaves with stevia strongly influenced sensory quality (Table 1). Among the tested ratios, the 8.5:1.5 blend achieved the highest total sensory score (16.27), classified as “Good.” In contrast, low stevia addition (9.5:0.5, 9:1) and high stevia addition (7.5:2.5) both resulted in lower scores, rated as “Average.”

**Table 1.** Sensory evaluation of wild bitter melon leaf–stevia blends.

Blend ratio (leaf:stevia, w/w)	Total sensory score	Rank
9.5:0.5	11.93 <sup>d</sup> ± 0.06	Average
9:1	12.4 <sup>d</sup> ± 0.35	Average
8.5:1.5	16.27 <sup>a</sup> ± 0.60	Good
8:2	15.3 <sup>b</sup> ± 0.50	Good
7.5:2.5	13.73 <sup>c</sup> ± 0.11	Average

\*Note: Values are mean ± SD (n = 10). Different superscript letters within a column indicate significant differences (p < 0.05) by Friedman test followed by Nemenyi post hoc.

The results indicate that moderate addition of stevia successfully balanced the intrinsic bitterness of wild bitter melon leaves, enhancing overall acceptability. When stevia content was too low, bitterness remained dominant and suppressed consumer preference. Conversely, excessive stevia led to an overly sweet taste that masked the characteristic herbal flavor, reducing balance and harmony. Similar patterns were observed in other herbal teas where stevia improved sensory scores at best-performing levels but caused rejection at higher concentrations [11], [12]. Thus, the 8.5:1.5 leaf–stevia ratio represents an evaluated compromise between natural bitterness and palatability for consumer acceptance.

### 3.4. Integrated discussion

Integrating the three experimental parameters—harvest age, drying temperature, and blending ratio—reveals a coherent framework linking plant physiology, process engineering, and consumer perception. Mid-mature leaves harvested at 80 days provided the highest levels of extractable phenolics due to elevated secondary metabolism and enzyme activity supporting phenolic biosynthesis. However, the preservation of these bioactives depends strongly on post-harvest treatment. Vacuum drying at 55 °C proved optimal because moderate heat combined with reduced oxygen tension suppressed polyphenol oxidase-mediated degradation while preventing thermal destruction of heat-sensitive compounds. This agrees with recent studies demonstrating that vacuum drying minimizes oxidative stress, shortens drying time, and preserves volatile integrity compared with conventional convection drying [22], [23].

From a sensory standpoint, the interaction between bioactive content and flavor balance was evident. The blend ratio of 8.5:1.5 (leaf:stevia, w/w) achieved not only the best overall acceptability but also correlated with higher antioxidant capacity. This relationship supports the notion that consumers prefer teas with moderate bitterness and enhanced sweetness, where high phenolic retention contributes both to health benefits and to complex flavor perception. As observed in similar herbal matrices, stevia addition within a limited range can mask bitterness without diminishing desirable herbal aromas, aligning chemical quality with sensory preference.

Collectively, these findings emphasize that optimizing processing parameters in isolation is insufficient; rather, their interactions must be harmonized to achieve a product that is chemically stable, organoleptically appealing, and economically viable. The integration of agronomic maturity (80 days), vacuum drying at moderate temperature (55 °C), and controlled sweetening (8.5:1.5 ratio) forms a reproducible technological pathway for optimizing the balance between bioactive retention, sensory appeal, and production efficiency in functional tea development. This study primarily focused on biochemical indicators (TPC, TFC, IC<sub>50</sub>) reflecting the antioxidant aspect of product quality. Other physicochemical or microbiological indicators (e.g., saponine content, color stability, volatile profile, moisture migration, and microbial safety) were beyond the scope of this work and should be addressed in future studies.

### 4. Conclusion

This study demonstrated that harvesting wild bitter melon leaves at 80 days, vacuum-drying at 55 °C (70 kPa, 24 h), and blending with stevia at a ratio of 8.5:1.5 (w/w) produced an herbal tea with enhanced polyphenol and flavonoid contents, strong antioxidant activity, and improved sensory acceptance. These findings provide a scientific basis for the efficient use of bitter melon leaves, which are often discarded, thereby contributing to value-added utilization of agricultural by-products. The evaluated parameters can serve as practical guidelines for small-scale tea production and technology transfer to community-based enterprises. In line with the study's limitations noted in the Integrated Discussion, future research should include additional physicochemical and microbiological quality indicators to ensure comprehensive evaluation of product stability and safety for broader commercialization.

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### Conflict of Interest

The authors declare that they have no financial, commercial, academic, or personal conflicts of interest that could influence the research, analysis, or interpretation presented in this manuscript. All authors have read and approved this statement.

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