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Effects of Peperomia Pellucida Leaf Tea Preparation on Phytochemical Content and Antioxidant Activity

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Abstract

Ka-sang trees (*Peperomia pellucida* Korth) are abundant in phytochemicals, such as polyphenols, tannins, and flavonoids. Folk literature indicate that it is employed to alleviate and address diseases such as inflammation. its recognized richness in bioactive compounds such as polyphenols, flavonoids, and tannins, there remains a lack of systematic scientific evidence regarding the optimal processing conditions for its utilization as a herbal tea. This research aimed to assess the impact of Ka-sang leaf tea processing on total flavonoid, tannin, phenolic content, and antioxidant activity levels. Samples were dried using a hot air oven at 40 and 60 degrees Celsius, as well as through sunlight for drying. The tea was prepared using a ratio of dried leaves to hot water at 1:50, steeping for durations of 3, 5, 7, 9, 11, and 15 minutes. The total phenolic, total flavonoid, and total tannin levels were assessed using colorimetric methods, while antioxidant activity was evaluated by the DPPH method. The findings indicated that tea leaves dried at 60 degrees Celsius and infused in hot water for 11 minutes exhibited elevated phytochemical levels and antioxidant efficacy. Consequently, the technique employed for producing dried Ka-sang leaves and Ka-sang leaf tea may be extended to future indigenous products.

1. Introduction

Health drinks, particularly herbal tea, are a preferred option among customers in Thailand. Thai customers like green tea and Japanese tea, followed by Chinese tea and Thai herbal tea, such as ginger tea, due to its palatability and health advantages [1]. Herbal tea is recognized for its cleansing properties, alleviating sore throats, and enhancing digestive health. Numerous herbal teas are low in calories and rich in nutrients, rendering them appropriate for health-conscious individuals [2]. Currently, customers are increasingly prioritizing health care. Previous studies in Thailand have demonstrated that herbal tea consumption is relatively widespread among Thai consumers. For instance, a recent study reported that approximately 89.3% of participants had consumed herbal tea at least once within the past two years, indicating a high level of exposure and acceptance [3]. Moreover, consumer behavior studies suggest that herbal tea is commonly consumed on a weekly basis, primarily for health promotion and general beverage purposes [4].

Ka-sang is the Thai designation for Peperomia pellucida. It belongs to the family Piperaceae and is located in South America and Asia, reaching a height of 20-40 cm [5]. This plant has been utilized to address several ailments, including fever, eczema, abdominal pain, and respiratory disorders, among others [6-8]. Furthermore, biological effects have been documented, encompassing antioxidant, anti-inflammatory, antibacterial, and anti-cancer properties, among others. A diversity of phytochemicals was discovered. Includes flavonoids, tannins, alkaloids, saponins, lignans, sesquiterpenes, phenylpropanoids, and polyphenols [7-9]. Ka-sang, owing to its biological activity and chemical composition, is a medicinal plant of significant relevance for research in herbal tea formulation.

Despite the widespread availability of *Peperomia pellucida* (Ka-sang) in Cho-airong District, Narathiwat Province, Thailand, and its recognized richness in bioactive compounds such as polyphenols, flavonoids, and tannins, there remains a lack of systematic scientific evidence regarding the optimal processing conditions for its utilization as herbal tea. In particular, the effects of drying methods and infusion time on the phytochemical content and antioxidant activity of Ka-sang leaf tea have not been clearly established. This knowledge gap limits the effective development of standardized processing techniques that could maximize the retention of beneficial compounds. Consequently, the potential of Ka-sang as a value-added functional beverage and its role in supporting local economic development through community-based product innovation remain underexplored. Therefore, it is essential to investigate how different processing conditions influence the phytochemical properties and antioxidant capacity of Ka-sang leaf tea to support its scientific validation and practical application.

2. Method

2.1 Sample Preparation

The leaves were collected from Cho-airong District, Narathiwat Province, Thailand in August 2023, and dehydrated under various settings using a hot air incubator (at 40 and 60 degrees Celsius) and sun-died (32-38 degrees Celsius) until the humidity dropped below 10%. The tea leaves were thereafter preserved in a bag in anticipation of the next infusion (Fig. 1).

Fig. 1 *Samples of dried leaves packed into tea leaf wrapping bags*

2.2 Ka-sang Leaf Tea Preparation

Around 2-gram sample of dried Ka-sang leaves and load it in a tea packet. The tea packet was then immersed in 100 ml of hot water at 90-95 degrees Celsius for durations of 3, 5, 7, 9, 11, and 15 minutes (Fig.2).

Fig. 2 *Dried Ka-sang leaves are soaked in hot water*

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2.3 Determination of Moisture Content

Moisture content assessment in accordance with [10]. Heat the container for moisture analysis to 105 degrees Celsius. Place in the desiccant jar for three hours. Allow to cool and measure the weight of the container. Continue the baking process until the weight variation is consistently between 1 and 3 milligrams. Incubate at 105 degrees Celsius. Place it in the desiccant jar for 5 to 6 hours. Allow to cool and measure the mass of the container with the sample. Determine the moisture content using Equation (1).

$$\text{Calculate Moisture conten (\%)} = (W1-W2) / W1 \times 100 \quad (1)$$

W1 is the weight of the pre-dried sample (g)

W2 is the weight of the post-drying sample (g)

2.4 Determination of Total Phenolic Content

Phenolic quantification was conducted using Folin-Ciocalteu's method using a microplate reader to quantify absorbance, employing gallic acid as a standard solution, in accordance with the methodologies of [11] and [12]. Transfer 12.5 µL of extract into a 96-well plate, include 50 µL of deionized water (DI) and 50% V/V of Folin-Ciocalteu reagent, incubate in darkness at ambient temperature for 10 minutes, introduce 125 µL of 7% Na2CO3, add 100 µL of deionized water, and heat to 45°C. The absorption value was subsequently measured at a wavelength of 765 nm. Determine the total phenolic content using the calibration curve of the gallic acid standard solution, prepared at concentrations of 10, 50, 100, 150, 200, and 250 µg/mL. The data is shown in micrograms of gallic equivalents (GAE) per milliliter of extract (µg GAE/mL extract).

2.5 Determination of Total Flavonoid Content

Colorimetric measurement of aluminum employing a microplate reader to assess absorption values, utilizing quercetin as the standard solution in accordance with the methodologies of [11] and [12]. Allow it to equilibrate at room temperature for 15 minutes, then assess the light absorption at a wavelength of 435 nm. Determine the total concentration of flavonoids from the curve graph of the quercetin standard solution. Standard solutions of quercetin were produced at concentrations of 2.5, 5, 10, 20, and 30 µg/mL (QE) per 1 mL of extract (µg QE/mL extract).

2.6 Determination of Total Tannin Content

Analysis of total tannins adjusted in accordance with the methodology of [13] and [14]. Introduce 18.2 µL of Folin-Ciocalteu reagent, agitate, and allow to stand for 6 minutes, followed by the addition of 72.7 µL of 7% Na2CO3. Agitate in darkness for 90 minutes, then assess the absorption at a wavelength of 700 nm. The total tannin content in the extract was quantified by correlating the observed results with a standard curve derived from a tannic acid solution at five concentrations: 50, 150, 200, 500, and 1000 µg/mL. The data is shown in micrograms of Tannic per milliliter of extract.

2.7 Antioxidant Activity Assay

According to [11] and [12], antioxidant activity is measured using the DPPH (2,2-diphenyl-1-picrydrayl) technique with vitamin C as a reference solution. Transfer 100 µL of extract into a 96-well plate, introduce 100 µL of DPPH radical, incubate at ambient temperature in a dark environment for 15 minutes, and measure the absorbance at a wavelength of 517 nm. Ascorbic acid served as the reference compound by creating a standard solution of vitamin C at concentrations of 2.5, 5, 10, 15, 20, and 25 µg per mL. Determine the percentage of antioxidant activity using equation (2) and compute the antioxidant capacity of the extract, expressed as the equivalent antioxidant capacity of vitamin C (µg Vit. CE/mL extract).

$$\text{Percentage of Antioxidant Activity} = [(A_{control} - A_{sample})/A_{control}] \times 100 \quad (2)$$

$A_{control}$ is the absorption value of the control unit

A_{sample} is the absorption value of a sample or standard

2.8 Statistical Analysis

The statistical analysis comprises three repetitions of the test and the standard deviation (SD) value for the total phenolic content assessment. The total flavonoids, tannins, and antioxidant activity were assessed by conducting three trials on the same sample, calculating the average values, determining the standard deviation (SD), and assessing variance and statistical significance using the One-way ANOVA approach at a 95% confidence level.

3. Results and Discussion

This study examines the preparation of tea from Ka-sang leaves. Samples of dried leaves in various conditions were subjected to a hot air oven (40 and 60 degrees Celsius) and sun drying, accompanied by a moisture analysis of the dried leaves (Table 3.1). The duration for steeping the tea leaves is 3, 5, 7, 9, 11, and 15 minutes. The samples dried at 40 degrees Celsius for 48 hours and at 60 degrees Celsius for 16 hours, as well as those sun-exposed for 3 days, exhibited moisture contents of 7.62±0.02%, 5.08±0.32%, and 8.59±0.49% of the sample weight, all conforming to the standard range for dried herbs as specified in the Community Product Standard (480/2547).

Table 1 *Sample codes of Ka-sang leaf tea and sample preparation conditions*

Sample codes	Sample Preparation Conditions
KL-1	Samples were dried at 40 ºC
KL-2	Samples were dried at 60 ºC
KL-3	Samples were dried with Sun-dried

3.1 Total Flavonoids Content of Ka-sang Leaf Tea

The analysis indicates that sample KL-2 possesses the greatest total flavonoid content. Upon initiating the infusion of the leaves for 3 minutes, the concentration of active compounds escalated until reaching 11 minutes, at which point it measured 6.45±0.05 µg QE/100 ml extract. Following a 15-minute immersion, the concentration of chemicals diminished, resulting in samples KL-1 and KL-3 exhibiting maximum dosages of 4.09±0.20 and 2.93±0.01 µg QE/100 ml extract, respectively, after 11 minutes (Table 3.2).

The results indicated that sample KL-2 (dried at 60°C) exhibited the highest total flavonoid content, particularly at an infusion time of 11 minutes. This suggests that drying at moderate temperatures enhances the retention and extractability of flavonoid compounds. The higher flavonoid content observed at 60°C may be attributed to the inactivation of degradative enzymes such as polyphenol oxidase, which are known to accelerate the oxidation of phenolic compounds under suboptimal drying conditions [15].

The increase in flavonoid content with increasing infusion time from 3 to 11 minutes can be explained by enhanced diffusion and solubilization of bioactive compounds into the aqueous medium, as mass transfer plays a key role in the extraction of phenolic compounds from plant matrices [16]. However, the decline observed at 15 minutes suggests that prolonged exposure to heat may lead to thermal degradation or oxidation of flavonoids, which has been widely reported in studies on herbal infusions [18].

Table 2 *The total flavonoid content of Ka-sang leaf tea*

Sample code	Total Flavonoid Content (µg QE/100 ml extract)					
	Sample Soaking Time (minute)					
	3	5	7	9	11	15
KL-1	2.65±0.05b	2.91±0.14b	3.46±0.02b	3.41±0.08b	4.09±0.20b	3.27±0.21b
KL-2	3.13±0.03c	4.37±0.06c	4.83±0.05c	5.45±0.10c	6.45±0.05c	6.37±0.09c
KL-3	1.20±0.01a	1.77±0.03a	1.94±0.01a	2.23±0.06a	2.93±0.01a	2.47±0.02a

Note: The same column with different English letters (a, b, c) represents a significant difference in value at 95 percent confidence (P-Value > 0.05).

3.2 Total Phenolic Content of Ka-sang Leaf Tea

The KL-2 sample had the greatest total phenolic concentration of 17.91±0.32 µg GAE/100 ml extract after an 11-minute infusion. The dosage of the medication escalates from 3 to 11 minutes, culminating at 15 minutes. The samples KL-3 and KL-1 contained 13.95±0.15 and 12.74±0.68 µg GAE/100 ml extract, respectively (Table 3.3).

A similar trend was observed for total phenolic content, where the concentration increased with infusion time up to 11 minutes and decreased thereafter. Phenolic compounds are highly soluble in hot water, and their extraction is strongly influenced by both temperature and time (Naczk & Shahidi, 2004). The initial increase in

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phenolic content can be attributed to enhanced mass transfer and disruption of plant cell structures during the extraction process [16].

The reduction in phenolic content at 15 minutes may be associated with oxidative degradation and polymerization reactions occurring under prolonged heating conditions [15]. Additionally, some phenolic compounds are thermolabile and may undergo structural degradation when exposed to elevated temperatures for extended periods [17]. These findings are consistent with previous studies demonstrating that an optimal extraction time exists for maximizing phenolic yield, beyond which degradation processes become dominant [18]; [19].

Furthermore, the higher phenolic content observed in samples dried at 60°C supports the importance of appropriate drying conditions in preserving phytochemical integrity, as controlled thermal processing can reduce enzymatic degradation while maintaining compound stability (15).

Table 3 *The total phenolic content of Ka-sang leaf tea*

Sample code	Total phenolic content (µg GAE/100 ml extract)					
	Sample Soaking Time (minute)					
	3	5	7	9	11	15
KL-1	10.38±0.24b	10.97±0.65b	11.09±0.44b	12.29±0.51b	13.95±0.15b	13.31±0.46b
KL-2	12.52±0.10c	14.59±0.38c	13.96±0.33c	13.96±0.33c	17.91±0.32c	15.66±0.16c
KL-3	8.01±0.16a	8.19±0.12a	8.95±0.15a	10.76±0.26a	12.74±0.68a	11.01±0.44a

Note: The same column with different English letters (a, b, c) represents a significant difference in value at 95 percent confidence (P-Value > 0.05).

3.3 Total Tannin Content of Ka-sang Leaf Tea

The analysis of total tannin content in the tea samples revealed a consistent pattern of increase and decrease, with a maximum total tannin content observed at 11 minutes. Notably, the KL-2 sample exhibited the highest total tannin content, measuring 20.42±0.82 µg TE/100 ml extract. KL-1 and KL-3 produced 16.46±0.75 and 11.31±0.50 µg TE/100 ml extract, respectively (Table 3.4).

The analysis of total tannin content revealed a similar pattern of increase and decrease, with the highest concentration observed at 11 minutes of infusion. Tannins, as water-soluble polyphenolic compounds, are readily extracted during infusion, and their extraction efficiency increases with time due to diffusion processes [20]. However, the decline at longer infusion times may be attributed to the instability of tannins under prolonged heating conditions. Tannins can undergo hydrolysis, oxidation, and complex formation with proteins or other macromolecules, leading to a reduction in measurable tannin content (Dai & Mumper, 2010). This behavior is consistent with general observations in plant-based infusions, where excessive extraction time can negatively affect phytochemical stability [18].

In addition, variations in tannin content among different samples may be influenced by drying conditions. Samples dried at higher temperatures tend to exhibit better preservation of tannins due to reduced enzymatic degradation, whereas less controlled drying conditions may lead to inconsistent phytochemical retention [17].

Table 4 *The total tannin content of Ka-sang leaf tea*

Sample code	Total tannin content (µg TE/100 ml extract)					
	Sample Soaking Time (minute)					
	3	5	7	9	11	15
KL-1	6.28±0.34b	10.39±0.46b	13.28±0.08b	10.45±0.52b	16.46±0.75b	15.22±0.30b
KL-2	15.68±0.82c	16.27±0.34c	16.04±1.15c	22.67±2.22c	20.42±0.82c	20.45±0.02c
KL-3	5.53±0.07a	7.04±0.35a	8.12±0.28a	8.19±0.62a	11.31±0.50a	10.91±0.79a

Note: The same column with different English letters (a, b, c) represents a significant difference in value at 95 percent confidence (P-Value > 0.05).

3.4 Antioxidant Activity of Ka-sang Leaf Tea

The antioxidant activity of the three samples, KL-1, KL-2, and KL-3, was measured, yielding values of 1.85-3.06, 2.05-3.17, and 1.74-2.27 µg Vit.CE /100 ml extract, respectively. KL-2 samples exhibited significant antioxidant activity throughout the 11-minute soaking duration. (Table 3.5).

The antioxidant activity of Ka-sang leaf tea followed a trend similar to that of total phenolic, flavonoid, and tannin contents, with the highest activity observed at an infusion time of 11 minutes. This finding indicates a strong relationship between phytochemical content and antioxidant capacity, as phenolic compounds, including flavonoids and tannins, play a major role in free radical scavenging activity [15]; [21].

The decline in antioxidant activity at 15 minutes may be explained by the degradation of phenolic compounds under prolonged heating conditions, resulting in reduced radical scavenging ability [17];[18]. This highlights the importance of optimizing extraction conditions to preserve bioactive compounds and maintain functional properties.

Additionally, the higher antioxidant activity observed in samples dried at 60°C further supports the role of controlled drying conditions in maintaining phytochemical stability and enhancing the functional quality of plant-based beverages [15].

Table 5 *Antioxidant activity of Ka-sang leaf tea*

Sample code	Antioxidant activity (µg Vit.CE /100 ml extract)					
	Sample Soaking Time (minute)					
	3	5	7	9	11	15
KL-1	1.85±0.04c	2.05±0.65b	2.59±0.12b	2.89±0.23b	3.06±0.02b	2.78±0.03b
KL-2	2.05±0.03b	2.20±0.05c	3.02±0.03c	3.04±0.04c	3.17±0.03c	2.93±0.02c
KL-3	1.74±0.30a	1.99±0.02a	2.00±0.06a	2.09±0.06a	2.27±0.05a	2.12±0.04a

Note: The same column with different English letters (a, b, c) represents a significant difference in value at 95 percent confidence (P-Value > 0.05).

Recent research has examined the effects of extraction using the Maceration and Reflux methods. The extraction process utilizing solvents includes methanol, butanol, and ethyl acetate, applied to samples that have been sun-dried. The reflux method utilizing ethyl acetate as a solute resulted in a higher total phenolic content compared to the maceration method [22]. This aligns with the findings of the research, indicating that soaking dried leaves for 3-11 minutes retains sufficient heat to facilitate the extraction of essential compounds. After approximately 15 minutes of reduced heat, the levels of key substances, including total phenolic content, total flavonoid content, and total tannin content, start to stabilize or decline. This aligns with the research conducted by [23], which examined the impact of infusion time on red rang leaf tea, specifically brewing durations of up to 10 minutes. The quantity of significant compounds derived from Ka-sang leaf tea preparation may be lower than that reported in prior studies on compound extraction. This discrepancy could be attributed to the solubility of these compounds in the solvent utilized for extraction [24]; [25]. The antioxidant activity of the Ka-sang leaf tea sample ranges from 1.74 to 3.17 µg Vit.CE per 100 ml extract. This activity may be associated with the structural characteristics of the compounds involved in the reaction and the concentration of active components [11]; [12]. Brewing hot herbal tea for an extended period can result in a bitter and astringent flavor; the optimal brewing time is typically 3-5 minutes [23].

4. Conclusion

This study examined the optimal conditions for preparing leaf tea with respect to flavonoid content. The processing of dried leaves was conducted using a hot air dryer at a temperature of 60 degrees Celsius. The moisture level is 5.08±0.32 of the sample weight, conforming to the criteria for dried herbs and Community Standard Products (480/2547). The infusion of dried tea leaves to get a high concentration of phytochemicals and antioxidant activity should occur within 9-11 minutes. This study may necessitate an examination of the healing age of dried coconut leaves, along with additional research on taste testing and market demand for herbal goods to promote indigenous plants in the future.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and design:*** *Surannee Teng, Imron Meechai;* ***data collection:*** *Surannee Teng, Imron Meechai;* ***analysis and interpretation of results:*** *Surannee Teng, Romalee Cheadoloh, Imron Meechai;* ***draft manuscript preparation:*** *Imron Meechai. All authors reviewed the results and approved the final version of the manuscript.*

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