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Drinking jelly from khlu (*Pluchea indica* less.) leaves tea with antioxidative and anti-inflammatory activities

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Abstract

This study aimed to develop a functional ready-to-drink jelly from *Pluchea indica* (L.) Less. leaves (“Khlu” in Thai), focusing on antioxidant and anti-inflammatory properties influenced by extraction method and sweetener concentration. Green and black tea preparation methods were compared, and the resulting infusions were used to formulate jelly products. Monk fruit sweetener was incorporated at four concentrations (0.5%, 0.75%, 1.00%, and 1.25% w/v) to evaluate its effect on taste and appearance. Antioxidant activity was assessed using the DPPH assay, while anti-inflammatory properties were measured via nitric oxide (NO) inhibition in macrophage cells. The black tea preparation showed significantly higher DPPH radical scavenging activity (423.36 mmol FAE/100 g) than green tea, indicating a stronger antioxidant capacity. Interestingly, although its total phenolic content (0.864 ± 0.006 mg GAE/g) was slightly lower than that of green tea, it exhibited greater NO inhibition at 10 mg/ml, suggesting the presence of other potent bioactive compounds generated during oxidation. Cytotoxicity testing revealed no toxicity at concentrations ≤ 10 mg/ml. Sensory evaluation using a 9-point hedonic scale identified 1.00% monk fruit as the most preferred level, balancing sweetness, color, and overall acceptability. Higher concentrations ($\geq 1.25\%$) led to darker jelly and lower scores for visual appeal. Nutritional analysis of the optimized product (per 100 g) showed 98.54 g of moisture, 1.13 g of carbohydrate, 0.15 g of protein, 0.18 g of ash, no detectable fat, and a low caloric value of 5.10 kcal. These findings suggest that jelly prepared with black tea extract and 1.00% monk fruit sweetener delivers desirable functional properties, safety, and consumer appeal. The product shows strong potential as a novel low-calorie, antioxidant-rich beverage for health-conscious consumers and could be further explored for commercial applications.

Keywords: Anti-inflammatory activity, Antioxidant activity, Drinking jelly, Monk fruit, *Pluchea indica* (L.) Less.

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Transparency: The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

Institutional Review Board Statement: The Ethical committee of Songkhla Rajabhat University, Thailand has granted approval for this study (COE No.002/2565).

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1. Introduction

Khlu is a plant that grows in areas with mangrove forests, such as seaside areas in southern Thailand, including Nakhon Si Thammarat, Phatthalung, and Songkhla. Khlu is a plant that grows naturally and does not require much treatment. The primary use of Khlu is currently for commercial tea production, and it is widely distributed, mainly in the Singhanakhon District, Songkhla Province, Thailand. Khlu leaves can be consumed fresh or used in traditional local cuisine.

Khlu leaves are well-known for their pain-relieving and anti-inflammatory properties. It is used in Thai traditional medicine for dysentery treatment to promote sweat relief. Besides, the calcium content in Khlu leaves is comparable to that of a glass of milk in terms of nutritional value. The leaves are rich in beta-carotene, carotenoids, phenolic compounds, antioxidants, and flavonoids [1], which are recognized for their anti-inflammatory and antioxidant properties. These compounds reduce the risk of non-communicable diseases (NCDs) and cancer [2].

The medicinal properties and nutritional value of Khlu make it an increasingly attractive option for value-added products. Thus, this research aims to utilize Khlu leaves for tea preparation and the production of ready-to-drink jelly. It is a distinctive beverage that combines fruit, vegetable, or herb extracts with gelling agents such as gelatin or carrageenan. This formulation creates a drinkable gel that is fluid enough to be consumed through a straw [3]. This product caters to health-conscious consumers and offers convenience in consumption.

Additionally, monk fruit is utilized as a sweetener in jelly. Monk fruit contains mogrosides, a type of glycoside from the triterpene group, which is approximately 300 times sweeter than sugar. Apart from its sweetness, monk fruit is rich in bioactive compounds, including triterpenoids, flavonoids, essential oils, amino acids, vitamins, minerals, and polysaccharides. Furthermore, monk fruit exhibits a range of health benefits, such as anti-hypoglycemic, anti-diabetic, anti-obesity, anti-fatigue, and anti-cancer properties [4].

Therefore, the properties of Khlu leaves and monk fruit were combined and made into a ready-to-drink jelly, aligning with the growing consumer demand for convenient and health-conscious beverages. In the experiment, the Khlu leaves were extracted by comparing the black tea and green tea methods. The extracts were then used in drinking jelly preparation with varying amounts of gelling agents and monk fruit sweeteners. The product was evaluated for its antioxidant activity using the DPPH scavenging assay and its anti-inflammatory properties by assessing the inhibition of lipopolysaccharide (LPS)-induced nitric oxide (NO) production in RAW 264.7 cell lines with the Griess reagent. This is to provide scientific evidence to support consumer confidence and encourage the use of local plants in various ways. Furthermore, it supports the use of Thai medicinal plants and adds value to Thailand's agricultural products.

2. Materials and Methods

The Khlu Leaves (*Pluchea indica* (L.) Less) were collected from a local area in Songkhla Province, Songkhla, Thailand. Monk fruit sweetener, carrageenan, and konjac were purchased commercially. The chemicals for the analysis of antioxidant activity and anti-inflammatory activity were of analytical grade and used as received.

2.1. Preparation of Khlu Leaves Tea

Tea from Khlu leaves was prepared using green and black tea methods [5].

2.1.1. Green Tea Preparation

The Khlu leaves are washed and drained at room temperature for 10 minutes. Then, they are cut to a width of 0.5 cm and subsequently blanched in hot water at 80°C for 30 seconds. After that, they are immediately dipped in cold water and drained for 10 minutes. The leaves are roasted at 50°C for 20 minutes and dried in a hot oven at 80°C for 1 hour.

2.1.2. Black Tea Preparation

Khlu leaves are washed and cut to a similar size to those in the green tea method. The leaves are directly roasted for 20 minutes at 50°C and then dried at 80°C for an hour in a hot air oven.

2.1.3. Tea Preparation

The prepared Khlu leaves from the individual method were mixed with boiling water in a ratio of boiling water to Khlu leaves of 10 to 1 by mass and then continuously stirred for 30 minutes. The extracted water is filtered and used as a biological activity agent in ready-to-drink jelly preparation.

2.2. Jelly Preparations

The ready-to-drink jelly preparation was performed according to Thummachart et al. [6]. The Khlu tea is heated to a temperature of 70–75°C. Then, the gelling agent, composed of 0.05% wt of carrageenan and 0.05% wt of konjac powder, is added and stirred until fully dissolved. Subsequently, the monk fruit sweetener is added at concentrations of 0.5%, 0.75%, 1.00%, and 1.25%, respectively. The mixture is then heated for 10 minutes, allowed to cool, and packaged in 150-mL stand-up, twist-cap pouches. The product is stored at a temperature of 4–10°C.

2.2.1. Physical Characterization of Jelly

The color of ready-to-drink jelly was determined using Hunter Lab (model color Flex EZ) equipped with a CIELab Scale D65/10° and a port size of 1.25 inches. For the sensory evaluation, the samples were subjected to a 9-point hedonic scale test of consumer sensory acceptance by 50 untrained consumers.

2.2.2. Proximate Analysis and Biological Activities

The moisture, protein, fat, and ash content of the Khlu jelly were measured using proximate analysis by AOAC International [7]. Calculations were carried out for total energy and carbohydrates.

2.3. Biological Activity Test

2.3.1. Determination of Antioxidant Activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical-scavenging activity was measured using the modified method described by Nimse and Pal [8]. The sample was prepared at 200 mg/mL in water and then diluted with ethanol to achieve concentrations of 0, 1, 10, 25, 50, 100, and 200 mg/mL, respectively. For the testing procedure, 20 µL of each concentration, including the standard and diluted sample solutions, was transferred into a 96-well plate with three replicates per concentration. Then, 40 µL of 10% Folin solution was added to each well, mixed thoroughly, and left to set for 6 minutes. Then, 125 µL of 7.5% sodium carbonate solution and 100 µL of distilled water were added to bring the final volume to 300 µL. After incubating in a dark place at room temperature for 90 minutes, the mixture was measured at an absorbance of 765 nm using a spectrophotometer to determine the total phenolic content, expressed as milligrams of gallic acid equivalents (GAE) per 100 grams of sample.

2.3.2. Total Phenolic Content

The Folin-Ciocalteu method was applied to measure the phenolic content in tea and jelly, with gallic acid as a standard. The method was modified from Yingngam et al. [9]. The sample was prepared with a concentration of 200 mg/mL in water, then diluted with ethanol to 0, 1, 10, 25, 50, 100, and 200 mg/mL, respectively. For testing, 20 µL of each standard and sample solution was transferred into a 96-well plate (three wells per concentration) and then mixed with 40 µL of 10% Folin solution and let sit for 6 minutes. After that, 125 µL of 7.5% sodium carbonate was added, followed by 100 µL of distilled water to obtain a final volume of 300 µL. The mixed solution was incubated at room temperature for 90 minutes and then measured at an absorbance of 765 nm using a spectrophotometer to calculate total phenolic content as mg GAE per 100 g of sample.

2.3.3. Anti-Inflammatory Activity

The anti-inflammatory activity assay was modified from the protocols of Owolabi et al. [10] and Chakree et al. [11] to assess the efficacy of the sample in alleviating inflammation.

2.3.3.1. Growth and Activation of Cells

Murine macrophage RAW264.7 cells obtained from the American Type Culture Collection (Manassas, VA, USA) were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. All cultures were incubated at 37°C in a humidified atmosphere with 5% CO₂. During the experiment, the cells were maintained and washed with phosphate buffer (PBS), controlled at pH 7.2.

2.3.3.2. Nitric Oxide (NO) Assay

RAW 264.7 cells with a density of 1x10⁶ cells/ml were seeded in 96-well flat-bottomed plates and incubated at 37°C to allow macrophage adherence. After two hours, the non-adherent cells and medium were removed, and the adherent cells were cultured in fresh medium containing 1 µg/ml of lipopolysaccharide (LPS) from *E. coli* to stimulate the cells to produce NO. The 100 µl extracts at different concentrations were added and incubated for 24 hours. The amount of NO production in each well was assessed by measuring nitrite accumulation (NO₂⁻) in the culture medium using Griess reagent. Then, 100 µl of the supernatants were transferred to a 96-well plate with 100 µl Griess reagent added. The mixtures were read at 570 nm using a microplate reader (Thermo Scientific, Model VARIOSKAN LUX). The percentage of NO production inhibition was calculated from Equation 1.

$$\text{NO Inhibition (\%)} = \frac{(\text{Control} - \text{Blank of control}) - (\text{Sample} - \text{Blank of the sample})}{(\text{Control} - \text{Blank of control})} \times 100 \quad (1)$$

2.3.3.3. Cytotoxicity Assay

Cytotoxicity was determined by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. Briefly, after 24 hours of incubation with the extracts, MTT solution (10 µl at 5 mg/ml in PBS) was added to the wells. After 2 hours in culture, the solution was removed, and dimethyl sulfoxide solution (DMSO) was added to dissolve the formazan crystals in the cells. The optical density (O.D.) of the formazan solution was measured with a microplate reader at 570 nm. The cell viability percentage was calculated as Equation 2. As the O.D. of the extract-treated group was reduced to below 80% of the O.D. of the control group, the test compound was considered to exhibit a cytotoxic effect.

$$\text{Cell viability (\%)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100 \quad (2)$$

2.4. Statistical Analysis

Experimental results are reported in the form of mean and standard deviation. The variance of the experimental data was analyzed using one-way ANOVA. The difference in means was examined using Duncan's New Multiple Range Test at a confidence level of 95 percent.

3. Results and Discussion

3.1. Effects of Tea Preparation Methods on the Antioxidant and Anti-Inflammatory Activities of Khlu Leaf Tea

The experiment evaluated the antioxidant and anti-inflammatory properties of Khlu leaves tea prepared by comparing two different methods black tea (KBT) and green tea (KGT) to signify the bioactivity of Khlu leaf tea. The antioxidant ability of Khlu leaves tea to scavenge free radicals was assessed using the DPPH method, and the total phenolic contents (TPC) of each obtained tea were also examined. The results in Table 1 show that KBT exhibited significantly greater DPPH radical scavenging activity than that obtained from the KGT, despite having a lower total phenolic content (34.31 ± 0.27 mg GAE/ml for KBT vs. 40.26 ± 2.82 mg GAE/ml for KGT). The DPPH scavenging activity indicates the ability of tea to neutralize free radicals, signifying the ability of tea to reduce oxidative stress and could contribute to the prevention of chronic diseases associated with oxidative damage, such as cardiovascular diseases and cancer [12]. The results demonstrate that KBT has greater antioxidant ability compared to KGT, despite its lower total phenolic content.

Additionally, the anti-inflammatory activity of KGT and KBT is indicated by the IC_{50} values. The lower IC_{50} value indicates a higher anti-inflammatory capacity. Thus, KBT demonstrates a lower IC_{50} value compared to that of KGT, indicating a slightly higher anti-inflammatory effect. Furthermore, the cytotoxicity IC_{50} values for both teas are greater than 23 mg/ml, suggesting that neither KGT nor KBT exhibits significant cytotoxic effects at the concentrations tested. It can be seen that both tea preparation methods are safe for consumption at these levels.

The results revealed that KBT demonstrated stronger potential for antioxidant and anti-inflammatory properties than those of KGT, though the total phenolic content was lower. Oxidation during black tea preparation is possibly responsible for the transformation of polyphenolic compounds during the process, leading to its lower content. The findings align with prior studies suggesting that some polyphenols observed in black tea preparation, such as theaflavins, exhibit stronger anti-inflammatory and antioxidant activities than the catechins predominantly found in green tea preparation [13]. Thus, black tea could possess antioxidant and anti-inflammatory properties greater than those of green tea with lower total polyphenolic compounds. The superior antioxidant and anti-inflammatory activities of KBT and its non-toxic nature led to the selection of the black tea method for further study.

Table 1.

The anti-inflammatory and antioxidant properties of tea leaves obtained via different methods.

Biological activities	Tea preparation methods	
	Green Tea	Black Tea
DPPH (mmol FAE/100g)	927.01	948.91
Total Phenolic (mg GAE/ml)	40.26 ± 2.82	34.31 ± 0.27
Anti-inflammatory activity, IC_{50} (mg/ml)	9.43 ± 0.19	8.62 ± 0.66
Cytotoxicity IC_{50} (mg/ml)	$>25.46 \pm 4.06$	$> 23.43 \pm 0.73$

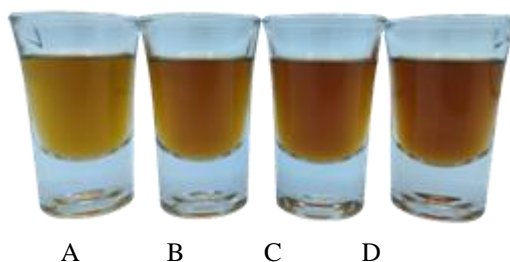
3.2. The Influence of Monk Fruit on Color and Sensory Taste of Ready-to-Drink KBT Jelly

The drinking jelly from KBT was prepared using carrageenan and konjac powder at a ratio of 1:1 (0.05% each as a gelling agent). The monk fruit was used as a sweetener and varied at the levels of 0.5, 0.75, 1.00, and 1.25 % by weight. Typically, the Monk fruit contains mogrosides, often has a slight brown hue, which can impact the color of beverages to which it's added. It can also cause a slight increase in turbidity or cloudiness in beverages, which can further influence color perception in tea or similar liquids [14]. Thus, the color of KBT with different amounts of Monk fruit was measured and reported in Table 2. The results reveal that the addition of Monk fruit decreases the values of red (a^*) and yellow (b^*), indicating the darker color of the KBT (see Figure 1).

Table 2.

The color of ready-to-drink Khlu black tea jelly at various concentrations of monk fruit.

Monk fruit (%)	Color		
	L^*	a^*	b^*
0.50	29.43 ± 1.11^a	14.11 ± 0.06^a	39.76 ± 1.59^a
0.75	24.98 ± 0.33^b	16.35 ± 0.13^b	34.23 ± 0.57^b
1.00	22.79 ± 0.54^c	17.55 ± 0.22^c	31.76 ± 1.05^c
1.25	20.50 ± 0.78^d	17.84 ± 0.23^d	28.57 ± 1.15^d

**Figure 1.**

Ready-to-drink Khlu black tea jelly containing Monk fruit at a 0.5% (A), 0.75% (B), 1.0% (C), and 1.25% (D), respectively.

The studies on the sensory properties of ready-to-drink KBT jellies at different monk fruit contents are shown in Table 3. The study found that the tendency of the acceptance scores decreased with increasing monk fruit content. No statistically significant difference was observed in the tasting score ($p \geq 0.05$). The product may taste slightly bitter and astringent as the amount of monk fruit increases, pairing with the bitter taste of tea, which contributes to the lower consumer acceptability scores. Suri et al. [15] suggested that monk fruit's intense sweetness could be accompanied by subtle bitterness, which might affect overall flavor perception, especially in products like tea that already have bitter or astringent notes. Notably, the optimum formulation for drinking jelly from KBT was chosen at a sweetness level of 1%, offering a balance between sweetness, color changes, and the compromise of flavor for consumer acceptance, though future KBT developments may explore other forms of Khlu leaves tea products, such as hot beverages, to improve flavor perceptions.

Table 3.

Sensory scores of ready-to-drink Khlu black tea jelly at various concentrations of Monk fruit.

Attribute	Monk fruit (%)			
	0.5	0.75	1.00	1.25
Appearance	6.64±1.54 ^a	6.48±1.62 ^{ab}	7.00±1.57 ^a	5.76±1.73 ^b
color	6.64±1.64 ^a	6.28±1.76 ^a	6.61±1.78 ^a	6.30±1.97 ^a
Odor	6.12±1.83 ^a	6.25±1.58 ^a	6.38±1.63 ^a	6.07±1.81 ^a
Flavor	5.89±1.61 ^a	6.05±1.58 ^a	6.28±1.79 ^a	5.79±1.90 ^a
Taste	5.66±1.76 ^a	5.76±1.58 ^a	5.71±1.80 ^a	5.34±2.28 ^a
Texture	7.10±1.77 ^a	6.58±1.63 ^a	7.12±1.73 ^a	5.20±2.29 ^b
Overall liking	6.20±1.57 ^a	6.02±1.75 ^a	6.33±1.73 ^a	5.64±2.07 ^a

3.3. Nutritional Compositions and Biological Activities of Ready-to-Drink KBT Jelly

The nutritional values of ready-to-drink KBT jelly are shown in Table 4. The results reveal that each 100 grams of KBT jelly contains 1.13 grams of carbohydrates, 0.15 grams of protein, 98.54% moisture, and 0.18 grams of ash. No fat is present. The total energy from KBT jelly is observed to be around 5.10 kcal. Consuming KBT jelly is clearly considered a low-energy diet because it has less than 40 kcal per 100 g portion according to FDA guidelines. Additionally, the antioxidant and anti-inflammatory properties of the KBT jelly are tabulated in Table 5. The jelly had a high DPPH scavenging activity of 423.36 mmol FAE/100 g, indicating its ability to neutralize free radicals and reduce oxidative stress. The total phenolic content was observed at 0.864 mg GAE/g, which aligns with the known health benefits of plant-based foods high in polyphenols. These compounds are often associated with a reduced risk of chronic diseases, such as heart disease and cancer, due to their antioxidant capacity [2]. Furthermore, the KBT jelly reduced NO secretion by 29.34% at a concentration of 10 mg/ml. This implies the jelly's ability to mitigate inflammation. No toxicity was observed at concentrations up to 10 mg/ml, confirming the KBT jelly's safety for consumption at typical dietary levels.

Table 4.

Chemical compositions of ready-to-drink Khlu black tea jelly.

Parameters	Quantity (%)
Protein	0.15
Fat	-
Moisture	98.54
Ash	0.18
Carbohydrate	1.13
Energy (Kcal/100g)	5.10

Table 5.

Biological activities of ready-to-drink Khlu black tea jelly.

Activity	Biological activities
DPPH	423.36 mmol FAE/100g
Total phenolic content	0.864 ± 0.006 mg GAE/ g sample
Anti-inflammatory (Raw 264.7 cell line)	Inhibited nitric oxide release at 10 mg/ml: 29.34 ± 2.83 %
Cytotoxicity	No toxicity at concentrations up to 10 mg/ml

This finding reveals that the ready-to-drink KBT jelly is a low-calorie food item, making it an alternative to a low-energy diet. Apart from its nutritional benefits, KBT jelly exhibits antioxidant and anti-inflammatory properties and positions itself as a promising functional food. It offers a novel option for consumers seeking health-promoting snacks without compromising on taste or nutrition.

4. Conclusion

In comparison to the preparation method for green tea, the tea made from Khlu leaves prepared using the black tea method has superior antioxidant capabilities against DPPH and a greater ability to suppress NO release. However, compared to the process of black tea, producing Klue leaves tea using the green tea method yields a higher overall phenolic content. Customers regarded jelly produced with 1% sweetener as acceptable, according to a study on the quantity of monk fruit used as a sweetener. It can be demonstrated that consuming jelly is a low-energy food based on the analysis of the chemical composition of ready-to-drink jelly made from Khlu leaves. Khlu leaves jelly drinks have the potential to be developed further as health foods since they exhibit antioxidant qualities against DPPH and the ability to suppress NO release in cultured cells.

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