

## Article

# Development of Herbal Tea Bags using Gotu Kola Leaves (*Centella asiatica* L. Urban) and Siamese Orange Peel (*Citrus nobilis*)

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Diana Sylvi<sup>1\*</sup>, Cesar Welya Refdi<sup>1</sup>, Sesi Kumala Dewi<sup>1</sup>

<sup>1</sup>Faculty of Agricultural Technology, Universitas Andalas, Padang, Indonesia

**Abstract.** Herbal tea is a beverage made from dried leaves, flowers, seeds, nuts, bark, fruits, and other plant components, excluding *Camellia sinensis*. This study developed herbal tea bags using gotu kola leaves (*Centella asiatica*) and Siamese orange peel (*Citrus nobilis*) sweetened with stevia to enhance flavor and antioxidants. The research aimed to assess the chemical and organoleptic characteristics and identify the most preferred formula. Treatments involved varying ratios of gotu kola leaves to Siamese orange peel: (100%: 0%), (80%: 20%), (75%: 25%), (70%: 30%), and (65%: 35%). A Completely Randomized Design (CRD) with five treatments and three replications was used, and data were analyzed using ANOVA and DNMRT at a 5% significance level. Results showed significant effects of mixing ratios on DPPH antioxidant activity, total polyphenols, water-soluble content, pH, IC50 antioxidant activity, and organoleptic attributes (color and aroma), but not on moisture content, ash content, TPC, or taste. Treatment E (65%: 35%) was optimal, with moisture content 7.54%, ash content 6.65%, DPPH antioxidant activity 58.02%, total polyphenols 746.67 mg GAE/g, water-soluble content 35.33%, TPC  $2.8 \times 10^3$  CFU/g, pH 6.71, and IC50 67 ppm. Panelists preferred this formulation, scoring its color (3.96), aroma (3.72), and taste (3.72) as "like".

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#### Corresponding Author :

Diana Sylvi  
Faculty of Agricultural Technology, Andalas University, Padang, Indonesia  
Email : [dianasyvil@gmail.com](mailto:dianasyvil@gmail.com)

## 1. Introduction

Tea commonly consumed by the public comes from tea shoots (*Camellia sinensis*) which are non-herbal teas that are grouped into three groups, namely black tea, green tea, and oolong tea [1]. However, the rising consumer preference for natural, functional foods has driven a significant market shift towards herbal teas, which are beverages made from a diverse range of plant components, excluding *Camellia sinensis*. These teas are valued not only for their unique flavors but also for their potential health benefits, such as relaxation, rejuvenation, immune support, and improved digestion [2]. The global herbal tea market is projected to grow steadily, driven by increasing awareness of health and wellness, as well as demand for convenient, naturally derived products [3].

Herbal tea is a mixture of several natural ingredients from a combination of dry leaves, grains, grasses, nuts, bark, fruits, flowers, or other plant elements that provide a refreshing taste and health benefits [3]. Herbal tea is packaged in the form of tea bags and brewed so that it can be consumed practically and provides benefits such as relaxation, rejuvenation, strengthening the immune system, and overcoming digestive problems [4]. Amidst this market expansion, there is a growing trend of formulating innovative herbal teas that cater to health-conscious consumers. Products incorporating ingredients with antioxidant, anti-inflammatory, and other bioactive properties are particularly sought after. Gotu kola (*Centella asiatica*), known for its medicinal properties, is a promising candidate for herbal tea formulations. Its bioactive compounds, such as asiaticoside and madecassoside, offer numerous benefits, including enhanced collagen production, improved memory, and protection against oxidative damage [4]. However, the natural bitterness of gotu kola poses a sensory challenge that limits its widespread acceptance as a standalone tea ingredient.

Pegagan (*Centella asiatica*) is a medicinal plant widely used as traditional medicine to treat various diseases. The primary compounds in *Centella asiatica* include triterpenoids such as asiaticoside, madecassoside, brahmoside, and brahminoside (saponin glycosides), asiaticoic acid, centellic acid, centoic acid, and madecassic acid [5]. Asiaticoside, one of the key compounds, is known to increase collagen production, accelerate wound healing, and improve blood circulation to the brain, thereby enhancing memory and preventing dementia [6]. Additionally, the ethanol extract of *Centella asiatica* exhibits significant antioxidant activity with an IC<sub>50</sub> value of 78.20 ppm [7].

Moreover, *Centella asiatica* contains vallerin compounds found in its leaves and resin in its roots, both of which contribute to its bitter or astringent taste. To address this limitation, this study incorporates Siamese orange peel (*Citrus nobilis*), rich in phenolic compounds, flavonoids, and essential oils, into *Centella asiatica*-based herbal tea. This combination not only masks the bitterness but also enhances the tea's antioxidant content and provides a refreshing citrus aroma. While orange peel has been explored in various food applications, its potential in herbal tea blends remains underutilized. The addition of stevia as a natural sweetener further aligns this product with market demands for calorie-free, health-oriented beverages [6].

Orange peel contains phenolic compounds (phenolic acids, flavonoids, and polymethoxylated flavones), carotenoids, and ascorbic acid [8,9]. Phenolic compounds in orange peel act as antioxidants, antimicrobials, anticancer, anti-inflammatory, and antiallergic agents [10,11,12]. Orange peel contains essential oils, also known as etheric oils, which are widely used to add orange aroma to drinks and are beneficial for body health (Montero-Calderón et al., 2019). The components of Siamese orange peel essential oil include limonene (95.36%), myrcene (2.02%), linalool (1.14%),  $\beta$ -pinene (0.66%), and  $\alpha$ -pinene (0.41%) [13]. Additionally, orange peel demonstrates strong antioxidant activity, with DPPH and total polyphenol content reported at 66.41% and 2,656.48 mg GAE/g, respectively [14].

The purpose of this study was to determine the chemical and organoleptic characteristics of herbal tea bags and to identify the formula for herbal tea bags mixed with pegagan leaves and Siamese orange peel that exhibit the best chemical characteristics and are preferred by panelists. This study is novel in

its development of a herbal tea bag formulation that synergistically combines the bioactive properties of gotu kola and Siamese orange peel with stevia. Unlike existing products, which often focus on single-ingredient or generic blends, this formulation is tailored to optimize both chemical and sensory characteristics. By leveraging the health benefits of these specific ingredients, this research contributes to the growing body of knowledge on functional herbal teas and provides a commercially viable product that aligns with current consumer trends.

## 2. Method

### 2.1 Materials and Tools

The materials used in this study were pegagan leaves and orange peel obtained from around Lareh Sago Halaban District, 50 Kota Regency, West Sumatra and stevia leaves obtained from around Alahan Panjang, West Sumatra. The chemicals used in this study were distilled water, buffer solution, methanol, concentrated Hydrochloric Acid (HCL), ethanol, folin-ciocalteu reagent, 5% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>), gallic acid, and DPPH reagent (2,2-Diphenyl-1-1-Picrylhydrazyl), and PCA agar media.

The tools used in this study were analytical scales, aluminum cups, porcelain cups, furnaces, ovens, hot plates, pH meters, desiccators, centrifuges, food dehydrators, gegap, measuring cups, test tubes, test tube racks, vortexes, measuring flasks, aluminum foil, filter paper, 250 ml beakers, magnetic stirrers, droppers, micro pipettes, Erlenmeyer flasks, UV-Vis spectrophotometers, Gass Chromatography-Mass Spectrometry (GC-MS), ultrasonic baths (Elma-Elmasonic S 30H), petri dishes, spatulas, autoclaves, thermometers. The tools used for making tea were 20 mesh sieves, knives, spoons, blenders, plastic containers, strainers, tea bags, glasses, tissues, and pans.

### 2.2 Research Design

The research design used in this study was a Completely Randomized Design (CRD) with 5 treatments and 3 replications. The observation data were subjected to analysis of variance (ANOVA) if significantly different, followed by Duncan's New Multiple Range Test (DNMRT) at a significance level of 5%. The treatment in this study was a comparison of mixing pegagan leaves and siam orange peel. The treatment given to each group is as follows:

A = Gotu kola leaves 100%: Siamese orange peel 0%

B = Gotu kola leaves 80%: Siamese orange peel 20%

C = Gotu kola leaves 75%: Siamese orange peel 25%

D = Gotu kola leaves 70%: Siamese orange peel 30%

E = Gotu kola leaves 65%: Siamese orange peel 35%

### 2.3 Research Implementation

#### 2.3.1 Raw Material Preparation

In the preparation of herbal tea bags, the primary ingredients used in this study were gotu kola leaves (*Centella asiatica*) and Siamese orange peel (*Citrus nobilis*), which were obtained from Lareh Sago Halaban District, 50 Kota Regency, West Sumatra. Additionally, stevia leaves (*Stevia rebaudiana Bertoni*) were sourced from the Alahan Panjang area. The selection criteria for the gotu kola leaves focused on young, fresh leaves to ensure high-quality tea production. The detailed formulation of Siamese orange peel and gotu kola leaves for each treatment is presented in Table 1, which was modified based on a previous study [15].

**Table 1.** The herbal celup formulation

| Material                    | Treatment |      |     |      |      |
|-----------------------------|-----------|------|-----|------|------|
|                             | A         | B    | C   | D    | E    |
| Powder and pegagan (g)      | 1.6       | 1.28 | 1.2 | 1.12 | 1.04 |
| Orange peel powder Siam (g) | 0         | 0.32 | 0.4 | 0.48 | 0.56 |
| Powder stevia (g)           | 0.4       | 0.4  | 0.4 | 0.4  | 0.4  |

Note: the percentage of tangerine peel is taken from the weight. total pegagan powder control treatment.

### 2.3.2 Making Gotu Kola Tea

Picking fresh young and green pegagan leaves. Pegagan leaves are separated from the stalks and washed to remove dirt. The next process is wilting for 12-17 hours at room temperature 20-260C. Dried using a food dehydrator at a temperature of 600C for 5 hours until the water content of the leaves is approximately  $\pm 7\%$  like drying tea. After the pegagan leaves are dry, the size reduction process is carried out with a blender. Then sieved with a size of 20 mesh until it becomes powder.

### 2.3.3 Making Siamese Orange Peel Powder

Separation of orange peel from the flesh and sorting of good quality peel. Orange peel is washed to remove dirt that sticks. Orange peel is sliced 2-3 cm to facilitate the drying process. Orange peel is dried using a food dehydrator at a temperature of 600C for 12 hours until the water content is  $\pm 7\%$ . After the orange peel is dry, the size is reduced using a blender. Then sieved using a 20 mesh sieve until it becomes powder.

### 2.3.4 Making Stevia Leaf Powder

Stevia leaves are removed from the stems and washed to remove dirt. The wilting process takes 12-17 hours at room temperature 20-260C. Then dried with a food dehydrator at a temperature of 600C for 5 hours until the water content of the leaves is around  $\pm 7\%$ . After the stevia leaves are dry, the size reduction process is carried out using a blender. Then sieved using a 20 mesh sieve until it becomes powder.

### 2.3.5 Herbal Tea Bag Mixing Process

Siamese orange peel powder is mixed into pegagan tea powder according to the treatment of adding 0.4 grams of stevia leaves as a natural sweetener and as a fixed factor in herbal tea bags. Mixed until smooth and packed in a 5.5x7.5 cm tea bag.

### 2.3.6 Making Herbal Tea Bag Drinks

The packaged tea bags are brewed using 200 ml of hot water at a temperature of 70°C and dipped for 5 minutes. It produces good tea brewing water (tea drink).

### 2.3.7 Determination of Material Formulation

Formulation for making herbal tea bags from a mixture of pegagan leaves and Siamese orange peel with

### 2.3.8 Observation

Observations made on raw materials, namely pegagan leaves, Siamese orange peel, and stevia leaves are pH test, water content test, ash content test, antioxidant activity test, and total polyphenol test. Observations on herbal tea bag products include water content test, ash content test, DPPH antioxidant activity test, total polyphenol test, total water-soluble material test, and total plate count

test. Observations on brewed water include pH test, IC50 antioxidant test, and organoleptic test (color, aroma, and taste).

### 3. Results and Discussion

#### 3.1 Raw Material Analysis

Observations made on raw materials are pH value, water content test, ash content test, DPPH antioxidant activity test, and total polyphenol test. The results of raw material observations can be seen in table 2.

**Table 2.** Results of raw material analysis

| Parameter Analysis                  | Gotu Kola Powder<br>(Value±SD) | Siamese Orange<br>Peel (Value±SD) | Stevia powder<br>(Value±SD) |
|-------------------------------------|--------------------------------|-----------------------------------|-----------------------------|
| pH value                            | 6.58±0.32                      | 5.76±0.07                         | 6.19±0.04                   |
| Moisture content (%)                | 7.08±0.22                      | 7.82±0.33                         | 7.21±0.50                   |
| Ash content (%)                     | 7.08±0.52                      | 5.11±0.76                         | 6.44±0.51                   |
| Antioxidant activity (% inhibition) | 55.06±0.71                     | 65.97±0.59                        | 47.51±0.82                  |
| Total Polyphenols (mg GAE/g)        | 680.58±0.66                    | 971.01±0.90                       | 593.33±0.66                 |

Description: Average result value with 3 repetitions

Based on the analysis results in Table 2, the average pH value of the gotu kola leaf powder infusion was 6.58, the pH value of the Siamese orange peel was 5.76, and the pH value of the stevia powder was 6.19. This can be attributed to the fact that the samples were brewed using drinking water, which typically has a pH of 6–7, as supported by research conducted by [16]. Siamese orange peel exhibited a lower pH compared to gotu kola and stevia leaves due to its citric acid content. In the study conducted by [17], orange peel extract had a pH value of 6.62, while in another study by [18], dried Siamese orange peel was reported to have a pH value of 5.15. Furthermore, the pH value of food products can be influenced by the concentration of dissolved hydrogen ions ( $H^+$ ), where a higher concentration of hydrogen ions results in a more acidic (lower) pH value [13],[19],[20].

The results of the analysis of the average water content in pegagan powder were 7.08%, Siamese orange peel powder 7.82%, and stevia powder 7.21%. According to SNI 03-3836-2013 concerning dry tea in packaging, the maximum water content quality requirement is 8% [21]. Pegagan leaf powder, Siamese orange peel powder, and stevia powder have met orange peel 971.01 mg GAE/g, and stevia powder 593.33 mg GAE/g in a concentration of 10,000 ppm. In a recent study, the total polyphenol content of sweet orange peel extract was reported to be 2,656.48 mg GAE/g, indicating its significant antioxidant potential [22]. Testing of phenol compounds is known to have antioxidant activity. Phenolic compounds, such as flavonoids, are secondary metabolites that play a critical role in neutralizing free radicals and protecting the human body from oxidative stress [23].

#### 3.2 Observations on Herbal Tea Bags

The analysis conducted on herbal tea bags from a mixture of pegagan leaves and Siamese orange peel with stevia powder sweetener was water content, ash content, DPPH antioxidant activity, total polyphenols, water-soluble substances, and total plate count.

##### 3.2.1 Water Content

The results of the analysis of the water content of herbal tea bags mixed with pegagan and Siamese orange peel can be seen in table 3.

**Table 3.** Results of analysis of water content of herbal tea bags

| Gotu Kola Powder: Siamese Orange Peel Powder Ratio | Moisture Content (%) (Mean $\pm$ SD) |
|--|--------------------------------------|
| A (100%: 0%)                                       | 7.08 $\pm$ 0.16                      |
| B (80%: 20%)                                       | 7.21 $\pm$ 0.20                      |
| C (75%: 25%)                                       | 7.32 $\pm$ 0.32                      |
| D (70%: 30%)                                       | 7.44 $\pm$ 0.38                      |
| E (65%: 35%)                                       | 7.54 $\pm$ 0.20                      |

**KK: 0.72%**

Notes: Values followed by different lowercase letters indicate significant differences at the 5% level according to DNMRT.

Based on the research results, the average moisture content ranged from 7.08% to 7.54%. The highest moisture content was observed in treatment E (65%: 35%) with an average value of 7.54%, while the lowest was in treatment A (100%: 0%) with an average value of 7.08%. Increasing the proportion of Siamese orange peel relative to Gotu Kola leaves resulted in a slight increase in moisture content. However, variance analysis indicated that the moisture content differences among the herbal tea bag formulations were not statistically significant at the  $\alpha = 5\%$  level.

This was attributed to the relatively similar moisture levels of the raw materials: Siamese orange peel (7.82%), Gotu Kola leaves (7.08%), and stevia leaves (7.21%). Compared to the SNI 3836:2013 standard for packaged dry tea, which sets a maximum moisture content of 8.2%, the moisture content of the herbal tea bags met the standard [21].

Moisture content is also influenced by drying methods and the type of material being dried. Proper drying can prevent bacterial growth, extend shelf life, and reduce enzymatic activity that may degrade the material [24-25]. Effective drying methods, such as tray and vacuum drying, are crucial in preserving the nutritional and organoleptic qualities of herbal tea formulations while maintaining acceptable moisture content levels.

### 3.2.2 Ash Content

The results of the ash content analysis of herbal tea bags made from a mixture of gotu kola (*Centella asiatica*) and Siamese orange peel can be seen in Table 4.

**Table 4.** Ash content analysis results of herbal tea bags

| Mixture (Gotu Kola Powder: Siamese Orange Peel Powder) | Ash Content (%) (Average $\pm$ SD) |
|--|------------------------------------|
| E (65%: 35%)   | 6.65 $\pm$ 0.34                    |
| D (70%: 30%)   | 6.85 $\pm$ 0.49                    |
| C (75%: 25%)   | 6.98 $\pm$ 0.35                    |
| B (80%: 20%)   | 7.17 $\pm$ 0.21                    |
| A (100%: 0%)   | 7.53 $\pm$ 0.50                    |

**KK: 1.12%**

Note: Different letters following the numbers indicate significant differences at the 5% level according to DNMRT.

Based on the research results, the average ash content of the herbal tea bags made from a mixture of gotu kola leaves and Siamese orange peel ranged from 6.65% to 7.53%. The highest ash content

was found in treatment A (100%: 0%) with an average value of 7.53%, while the lowest ash content was in treatment E (65%: 35%) with an average value of 6.65%. The higher the proportion of Siamese orange peel in relation to gotu kola leaves in the herbal tea bags, the ash content slightly decreased. However, variance analysis showed no significant differences in ash content at  $\alpha = 5\%$ . This is due to the relatively similar ash content of the raw materials, where gotu kola had the highest ash content at 7.85%, Siamese orange peel at 5.11%, and stevia leaves at 6.44% [26].

Compared to the Indonesian National Standard (SNI) 3836:2013, which sets a maximum total ash content of 8% for dry tea in packaging, the ash content of the produced herbal tea bags meets the standard [21]. High ash content indicates a significant mineral content in the ingredients; however, excessively high mineral content is not recommended. Therefore, a maximum limit is set for ash content in food products. Excessive mineral content may result from improper processing [27]. Gotu kola contains mineral salts such as potassium, sodium, magnesium, calcium, iron, and phosphorus [28]. Orange peel contains minerals like selenium, calcium, zinc, and manganese [29-30]. Minerals in stevia leaves include sodium, iron, calcium, phosphorus, magnesium, zinc, and chloride [31-32].

### 3.2.3 DPPH Antioxidant Activity

The average DPPH antioxidant activity of herbal tea made from a mixture of gotu kola (*Centella asiatica*) and Siamese orange peel, based on the results of the DNMRT post hoc test, is shown in Table 5.

**Table 5.** Analysis results of DPPH antioxidant activity in herbal tea bags

| Mixture (Gotu Kola Powder: Siamese Orange Peel Powder) | Antioxidant Activity (% inhibition) (Average $\pm$ SD) |
|--|--|
| A (100%: 0%)   | 53.43 $\pm$ 0.82a                                      |
| B (80%: 20%)   | 55.68 $\pm$ 0.48b                                      |
| C (75%: 25%)   | 56.54 $\pm$ 0.84bc                                     |
| D (70%: 30%)   | 57.08 $\pm$ 0.94bc                                     |
| E (65%: 35%)   | 58.02 $\pm$ 1.05c                                      |
| <b>KK: 0.30%</b>                                       |  |

Note: Different letters following the numbers indicate significant differences at the 5% level according to DNMRT.

The DPPH antioxidant activity of herbal tea bags made from a mixture of gotu kola leaves and Siamese orange peel showed a significant increase with higher proportions of Siamese orange peel. The highest antioxidant activity was observed in treatment E (65% gotu kola: 35% Siamese orange peel) with an inhibition value of 58.02%, compared to the lowest in treatment A (100% gotu kola: 0% Siamese orange peel) at 53.43%. This is attributed to the higher antioxidant activity of Siamese orange peel (65.97%) compared to gotu kola leaves (55.06%) and stevia leaves (47.51%) as raw materials [33].

The DPPH antioxidant activity of the herbal tea showed a clear trend, with increasing proportions of Siamese orange peel in the mixture resulting in higher antioxidant activity. This trend can be attributed to the higher concentration of phenolic compounds and flavonoids in Siamese orange peel compared to gotu kola leaves and stevia. Phenolic compounds are known for their ability to donate hydrogen atoms to free radicals, thereby stabilizing them and reducing oxidative stress. In this study, the antioxidant activity increased from 53.43% (100% gotu kola) to 58.02% (65% gotu kola: 35% Siamese orange peel).

Antioxidant activity is expressed as the percentage of inhibition, representing the ability of antioxidants to neutralize free radicals. Higher antioxidant activity indicates a greater ability of the substance to combat free radicals in the body [34,35].

Siamese orange peel contains compounds such as limonene, linalool, flavonoids, carotenoids, terpenoids, and phenolic acids, which act as antioxidants [36,37]. The chemical components in gotu kola responsible for antioxidant activity include polyphenols, flavonoids, tannins, and terpenoids. One of the triterpenoid glycosides found in gotu kola, asiaticoside, helps improve blood flow to the brain, enhances memory, and protects brain cells from oxidative damage caused by free radicals [28,38]. In addition, stevia leaves contain phenolic compounds, diterpene glycosides, triterpenes, tannins, and steroids, which also function as antioxidants [39,40].

### 3.2.4 Total Polyphenols

The average total polyphenol content of herbal tea made from a mixture of gotu kola (*Centella asiatica*) and Siamese orange peel, based on the results of the DNMRT post hoc test, is shown in Table 6.

**Table 6.** Analysis results of total polyphenol content in herbal tea bags

| Mixture (Gotu Kola Powder: Siamese Orange Peel Powder) | Total Polyphenol Content (mg GAE/g)<br>(Average $\pm$ SD) |
|--|---|
| A (100%: 0%)   | 665.51 $\pm$ 5.87a  |
| B (80%: 20%)   | 705.22 $\pm$ 10.28b                                       |
| C (75%: 25%)   | 722.61 $\pm$ 5.88c  |
| D (70%: 30%)   | 737.10 $\pm$ 7.20d  |
| E (65%: 35%)   | 746.67 $\pm$ 7.46d  |
| <b>KK: 0.21%</b>                                       |   |

Note: Different letters following the numbers indicate significant differences at the 5% level according to DNMRT.

The variance analysis of total polyphenols in herbal tea bags made from a mixture of gotu kola leaves and Siamese orange peel showed a significant effect at  $\alpha = 5\%$ . Based on the research results, the total polyphenol content in herbal tea bags ranged from 665.51 mg GAE/g to 746.67 mg GAE/g at a concentration of 10,000 ppm. The highest total polyphenol content was found in treatment E (65%: 35%) with an average value of 746.67 mg GAE/g, while the lowest was in treatment A (100%: 0%) with an average value of 665.51 mg GAE/g. The study indicated that increasing the ratio of gotu kola leaves to Siamese orange peel resulted in higher total polyphenol content. This is attributed to the higher total polyphenol content in Siamese orange peel (971.01 mg GAE/g) compared to gotu kola leaves (680.58 mg GAE/g) and stevia leaves (593.33 mg GAE/g) [23].

Polyphenol compounds in gotu kola leaves include flavonols such as quercetin, kaempferol, and myricetin, which function as antioxidants [40]. Polyphenol compounds in Siamese orange peel include flavonoids, limonoids, phenolic acids, coumarins, tannins, terpenoids, stilbenes, lignans, and carotenoids [42]. Phenolic compounds in stevia leaves include vanillic acid 4-HAI- $\beta$ -D-glucopyranoside, caffeic acid, protocatechuic acid, chlorogenic acid, and cryptochlorogenic acid [43].

Phenolic compounds are prone to damage due to oxidation and degradation during processing and storage. High temperatures used in processing can break down cell walls, facilitating the release and extraction of phenolic compounds during drying. Storing materials at high temperatures increases the likelihood of phenolic compound degradation due to a greater chance of oxidation. Storage at temperatures around  $\pm 25^\circ\text{C}$  has been shown to maintain the stability of phenolic compounds [44,45].



### 3.2.5 Total Water-Soluble Substances

The average water-soluble substances in herbal tea made from a mixture of gotu kola (*Centella asiatica*) and Siamese orange peel, based on the DNMRT post hoc test results, are shown in Table 7.

**Table 7.** Analysis results of total water-soluble substances in herbal tea bags

| Mixture (Gotu Kola Powder: Siamese Orange Peel Powder) | Total Water-Soluble Substances (%) (Average $\pm$ SD) |
|--|---|
| A (100%: 0%)   | 26.00 $\pm$ 2.00a                                     |
| B (80%: 20%)   | 28.67 $\pm$ 1.15a                                     |
| C (75%: 25%)   | 32.00 $\pm$ 2.00b                                     |
| D (70%: 30%)   | 33.33 $\pm$ 1.15bc                                    |
| E (65%: 35%)   | 35.33 $\pm$ 1.15c                                     |
| <b>KK:</b> 1.00%                                       |   |

Note: Different letters following the numbers indicate significant differences at the 5% level according to DNMRT.

The variance analysis of water-soluble substances in herbal tea bags made from a mixture of gotu kola leaves and Siamese orange peel showed a significant effect at  $\alpha = 5\%$ . Based on the research results, the total water-soluble substances in herbal tea bags ranged from 26% to 35.33%. The highest water-soluble content was found in treatment E (65%: 35%) with an average of 35.33%, while the lowest was in treatment A (100%: 0%) with an average of 26%. The study indicated that increasing the ratio of gotu kola leaves and Siamese orange peel led to an increase in total water-soluble substances. This is due to the higher content of water-soluble compounds in Siamese orange peel, such as flavonoids, saponins, tannins, and alkaloids [36]. Water-soluble compounds in gotu kola leaves include flavonoids, saponins, and tannins [46].

The total water-soluble substances produced in the herbal tea bags meet the standards of SNI 3836:2013, which require a minimum water extract content of 32% [21]. Flavonoid compounds are abundant in the material and are polar in nature, requiring polar solvents for dissolution. This aligns with the principle "like dissolves like," where compounds dissolve in solvents of similar polarity. Polar solvents include methanol, ethanol, acetone, and water [47].

The higher the measurable water-soluble substances, the better the quality of the extract. The fineness of the particles also plays a significant role in influencing the total water-soluble content, as smaller particle sizes dissolve more efficiently. This finding is consistent with the study by [48], which reported that the degree of fineness directly affects the solubility of materials in water.

### 3.2.6 Total Plate Count

The results of the Total Plate Count (TPC) analysis for herbal tea bags can be seen in Table 8.

**Table 8.** Analysis results of total plate count in herbal tea bags

| Mixture (Gotu Kola Powder: Siamese Orange Peel Powder) | Total Plate Count (CFU/g) |
|--|---------------------------|
| E (65%: 35%)   | $2.8 \times 10^3$         |
| D (70%: 30%)   | $3.1 \times 10^3$         |
| C (75%: 25%)   | $3.6 \times 10^3$         |
| B (80%: 20%)   | $4.0 \times 10^3$         |
| A (100%: 0%)   | $4.5 \times 10^3$         |

Based on the research results, the total plate count (TPC) of the herbal tea bags made from a mixture of gotu kola leaves and Siamese orange peel ranged from  $2.8 \times 10^3$  CFU/g to  $4.5 \times 10^3$  CFU/g. The lowest colony count was found in treatment E (65%: 35%) at  $2.8 \times 10^3$  CFU/g, while the highest colony count was observed in treatment A (100%: 0%) at  $4.5 \times 10^3$  CFU/g. The higher the proportion of Siamese orange peel in relation to gotu kola leaves, the lower the total plate count. According to the Indonesian National Standard (SNI) 3836:2013, the maximum allowable total plate count for packaged dry tea is  $3 \times 10^3$  CFU/g [21]. The treatment that met the TPC standard was treatment E (65%: 35%) with a colony count of  $2.8 \times 10^3$  CFU/g.

The decrease in colony count in the TPC test is attributed to the antibacterial compounds found in Siamese orange peel, particularly essential oils composed of limonene (95.36%), myrcene (2.02%), and linalool (1.14%), which are effective in inhibiting various microorganisms such as *E. coli*, *S. aureus*, *Saccharomyces cerevisiae*, and *Aspergillus niger*. Recent studies confirm that citrus peel essential oils exhibit significant antibacterial activity, with *Citrus reticulata* essential oil showing strong inhibition against *E. coli* and *Staphylococcus aureus* [49]. Furthermore, Siamese orange peel produces essential oils rich in phenolic compounds, carotenoids, tocopherols, and phytosterols. Phenolic compounds inhibit the formation of cell walls, while flavonoids can disrupt cell walls, and terpenoid compounds are capable of damaging bacterial membranes [50].

### 3.3 Observations on Brewed Water

The analysis conducted on the brewed water of herbal tea bags made from a mixture of gotu kola leaves, Siamese orange peel, and stevia sweetener included pH value, IC50, and organoleptic properties.

#### 3.3.1 pH Value

The analysis of variance results showed that the addition of beetroot puree had a significant effect at the 5% significance level on the color of the dried noodles produced. The results of the color analysis of the dried noodles can be seen in Table 9.

**Table 9.** Analysis results of pH values of herbal tea bags

| Mixture (Gotu Kola Powder:<br>Siamese Orange Peel Powder) | pH Value<br>(Average $\pm$ SD) |
|---|--------------------------------|
| E (65%: 35%)  | 6.71 $\pm$ 0.21a               |
| D (70%: 30%)  | 6.99 $\pm$ 0.27ab              |
| C (75%: 25%)  | 7.20 $\pm$ 0.20bc              |
| B (80%: 20%)  | 7.47 $\pm$ 0.27cd              |
| A (100%: 0%)  | 7.77 $\pm$ 0.20d               |
| <b>KK : 0.64%</b>   |                                |

Note: Different letters following the numbers indicate significant differences at the 5% level according to DNMR.

The variance analysis of the pH values of the brewed water of herbal tea bags made from a mixture of gotu kola leaves and Siamese orange peel showed a significant effect at  $\alpha = 5\%$ . Based on the research results, the average pH of the herbal tea bags ranged from 6.71 to 7.77. The highest pH was found in treatment A (100%: 0%) at 7.77, while the lowest pH was in treatment E (65%: 35%) at 6.71. The study indicated that increasing the proportion of Siamese orange peel relative to gotu kola leaves resulted in a decrease in pH. This is due to the lower pH of Siamese orange peel (5.76) compared to gotu kola leaves (6.58) and stevia leaves (6.19), as well as the brewing water used, which had a pH

of 7.00. This aligns with Sukmawati et al. (2023), who stated that the pH of drinking water typically ranges from 6 to 7.

The low pH of Siamese orange peel is attributed to its citric acid content, which makes the resulting pH more acidic. A recent study on *Citrus sinensis* peel confirmed high citric acid content, contributing to its acidic nature [51]. The pH values produced by the brewed herbal tea bags in all formulations meet the requirements for food product pH, which generally falls between 3.0 and 8.0 according to Buckle et al. (1985) as cited by [52].

The element responsible for the sour taste in a product is the concentration of H<sup>+</sup> ions; the higher the H<sup>+</sup> ion concentration in a solution, the more acidic it becomes, resulting in a lower pH [53]. Products with lower pH values are generally more shelf-stable [52,54].

### 3.3.2 Antioxidant Activity (IC<sup>50</sup>)

The average IC<sub>50</sub> values of the brewed herbal tea made from a mixture of gotu kola (*Centella asiatica*) and Siamese orange peel, based on the DNMRT post hoc test, are shown in Table 10.

**Table 10.** IC<sup>50</sup> antioxidant activity analysis results for herbal tea bags

| Mixture (Gotu Kola Powder:<br>Siamese Orange Peel Powder) | IC <sup>50</sup> (ppm) (Average ± SD) |
|---|---------------------------------------|
| E (65%: 35%)  | 67 ± 6.43a                            |
| D (70%: 30%)  | 83 ± 5.13b                            |
| C (75%: 25%)  | 98 ± 5.57c                            |
| B (80%: 20%)  | 126 ± 4.58d                           |
| A (100%: 0%)  | 145 ± 6.03e                           |
| <b>KK : 1.08%</b>   |                                       |

Note: Numbers followed by different lowercase letters indicate significant differences at the 5% level according to DNMRT.

The IC<sub>50</sub> values of the herbal tea formulations ranged from 145 ppm (100% gotu kola) to 67 ppm (65% gotu kola: 35% Siamese orange peel). IC<sub>50</sub> values represent the concentration of a substance required to inhibit 50% of free radical activity. Lower IC<sub>50</sub> values indicate stronger antioxidant activity. Based on IC<sub>50</sub> classification, the formulation with 65% gotu kola and 35% Siamese orange peel demonstrated strong antioxidant activity (IC<sub>50</sub> = 67 ppm), whereas formulations with higher proportions of gotu kola exhibited moderate antioxidant activity.

This improvement in antioxidant strength with higher orange peel content highlights the contribution of bioactive compounds like limonene, flavonoids, and phenolic acids, which are known to reduce oxidative stress effectively. Additionally, the synergy between the phenolic compounds in gotu kola and orange peel likely enhances the overall antioxidant capacity of the tea blends. A recent study confirmed that *Centella asiatica* extracts have significant antioxidant potential, with IC<sub>50</sub> values as low as 20.43 µg/mL, depending on extraction methods [55].

The observed trends indicate that the combination of gotu kola and Siamese orange peel in the formulation significantly enhances antioxidant properties. These findings suggest that the herbal tea bags may provide health benefits associated with antioxidant activity, such as reducing oxidative damage and supporting cellular function. The enhanced antioxidant activity and lower IC<sub>50</sub> values in formulations with higher proportions of Siamese orange peel suggest that the tea blend could serve as a functional beverage with potential health benefits, such as reducing oxidative damage and improving cellular function. The results also imply that incorporating orange peel not only improves

the sensory characteristics (aroma and flavor) but also significantly enhances the functional properties of the tea, making it a competitive product in the growing market for health-oriented beverages.

The higher the proportion of Siamese orange peel compared to gotu kola leaves in the brewed tea, the lower the IC50 value, indicating stronger antioxidants in counteracting free radicals. This is because Siamese orange peel has higher antioxidant activity than other raw materials, as shown in Table 2. In line with this, *Citrus amblycarpa* peel extracts have demonstrated exceptional phenolic content and antioxidant activity, confirming their potential in functional beverages [37].

In the brewing process with water, water-soluble compounds such as flavonoids dissolve. Flavonoids are polar compounds, and they dissolve in polar solvents such as water [35]. Antioxidant activity can also be influenced by the temperature and time used in the brewing process. If tea is brewed for too long and at too high a temperature, the antioxidant compounds can degrade. Research by Mutmainnah et al. (2018) on determining the optimal temperature and time for brewing green tea stems to optimize the content of antioxidants, caffeine, tannins, and catechins found the best brewing treatment to be at a temperature of 70°C for 5 minutes.

### 3.3.3 Organoleptic

The organoleptic test on the brewed herbal tea bags made from a mixture of gotu kola leaves and Siamese orange peel was conducted with 25 panelists, focusing on preferences for color, aroma, and taste. The scoring system for organoleptic evaluation ranged from 1 to 5: Score 1: Strongly dislike, Score 2: Dislike, Score 3: Neutral, Score 4: Like, Score 5: Strongly like.

#### 3.3.3.1 Color

The variance analysis showed that the average panelist preferences for the color of the brewed herbal tea bags made from a mixture of gotu kola leaves and Siamese orange peel differed significantly at the  $\alpha = 5\%$  level. Based on Table 11, the average panelist scores for color preference ranged from 3.28 to 3.96. The treatment with the most preferred color was treatment E (65%: 35%) with an average score of 3.96, while the least preferred was treatment C (75%: 25%) with an average score of 3.28. The level of preference for color can be seen in Table 11.

**Table 11.** Average panelist scores for color preference of herbal tea bags

| Mixture (Gotu Kola Powder: Siamese Orange Peel Powder) | Color (Average $\pm$ SD) |
|--|--------------------------|
| C (75%: 25%)   | 3.28 $\pm$ 0.61a         |
| D (70%: 30%)   | 3.36 $\pm$ 0.64a         |
| B (80%: 20%)   | 3.52 $\pm$ 0.65a         |
| A (100%: 0%)   | 3.72 $\pm$ 0.84ab        |
| E (65%: 35%)   | 3.96 $\pm$ 0.84b         |

**KK: 20%**

Note: Scores: 1 = strongly dislike, 2 = dislike, 3 = neutral, 4 = like, 5 = strongly like. Numbers followed by different lowercase letters indicate significant differences at the 5% level according to DNMR T.

Increasing the proportion of the herbal tea mixture of gotu kola leaves and Siamese orange peel produced a slightly darker or yellow-brown color in the herbal tea. This is due to the increase in dissolved compounds in the tea, which affects the color of the brewed tea water. The color differences in the brewed herbal tea bags can be seen in Figure 1.



**Figure 1.** Brewed Herbal Tea Bags, A (100% gotu kola powder: 0% Siamese orange peel powder), B (80% gotu kola powder: 20% Siamese orange peel powder), C (75% gotu kola powder: 25% Siamese orange peel powder), D (70% gotu kola powder: 30% Siamese orange peel powder), E (65% gotu kola powder: 35% Siamese orange peel powder)

From the figure, it can be observed that the color becomes progressively darker as the proportion of Siamese orange peel increases relative to gotu kola leaves. The resulting color is a combination of the ingredients used, namely gotu kola leaves, Siamese orange peel, and stevia leaves. Gotu kola and stevia leaves contain chlorophyll, which produces a green color, but when dried, the compounds oxidize to yellow-brown. The fading of the green color in gotu kola and stevia leaves occurs because of the degradation of pigments, especially chlorophyll, which degrades into pheophytin, causing a brown color, and flavonoid pigments that produce a yellow color [56].

Siamese orange peel contains carotenoid compounds that result in a yellow color [57]. Carotenoids are more stable under certain drying conditions, although they also undergo degradation depending on temperature and oxygen exposure [58].

### 3.3.3.2 Aroma

The variance analysis showed that the average panelist preferences for aroma of the herbal tea bags made from a mixture of gotu kola leaves and Siamese orange peel differed significantly at the  $\alpha = 5\%$  level. The level of preference for aroma can be seen in Table 12.

**Table 12.** Average panelist scores for aroma preference of herbal tea bags

| Mixture (Gotu Kola Powder: Siamese Orange Peel Powder) | Aroma (Average $\pm$ SD) |
|--|--------------------------|
| B (80%: 20%)   | 3.08 $\pm$ 0.49a         |
| C (75%: 25%)   | 3.20 $\pm$ 0.41a         |
| D (70%: 30%)   | 3.20 $\pm$ 0.50a         |
| A (100%: 0%)   | 3.40 $\pm$ 0.58a         |
| E (65%: 35%)   | 3.72 $\pm$ 0.74b         |

**KK : 17%**

Note: Scores: 1 = strongly dislike, 2 = dislike, 3 = neutral, 4 = like, 5 = strongly like. Numbers followed by different lowercase letters indicate significant differences at the 5% level according to DNMRT.

Based on Table 12, the average panelist scores for aroma preference ranged from 3.08 to 3.72. The treatment with the most preferred aroma was treatment E (65%: 35%) with an average score of 3.72, while the least preferred aroma was in treatment B (80%: 20%) with an average score of 3.08. Differences in aroma preference were due to the varying ingredient concentrations in each treatment. The higher the proportion of Siamese orange peel compared to gotu kola leaves, the more pronounced the citrus aroma in the brewed tea water.

Each ingredient contains different chemical compositions. The aroma released from the brewed water originates from the essential oils present in the ingredients. Gotu kola contains various

terpenoids, including  $\beta$ -caryophyllene, trans- $\beta$ -farnesene, and germacrene (sesquiterpenes), which are the main components, as well as  $\alpha$ -pinene and  $\beta$ -pinene. Additionally, gotu kola contains alkaloids such as hydrocotylin and vallerin [59].

On the other hand, Siamese orange peel contains a higher amount of essential oils composed of limonene, linalool,  $\alpha$ -pinene,  $\beta$ -pinene, myrcene, octanal, and decanal. The dominant components are limonene and linalool, which are widely used as fragrance chemicals and to enhance the aroma in food and beverage products [11].

### 3.3.3.3 Taste

The variance analysis showed that the average panelist preferences for taste of the herbal tea bags made from a mixture of gotu kola leaves and Siamese orange peel did not differ significantly at the  $\alpha = 5\%$  level. Based on Table 13, the average panelist scores for taste ranged from 3.24 to 3.72. The treatment with the most preferred taste was treatment E (65%: 35%) with an average score of 3.72, while the least preferred taste was in treatment C (75%: 25%) with an average score of 3.24. The level of preference for taste can be seen in Table 13.

**Table 13.** Average panelist scores for taste preference of herbal tea bags

| Mixture (Gotu Kola Powder: Siamese Orange Peel Powder) | Taste (Average $\pm$ SD) |
|--|--------------------------|
| C (75%: 25%)   | 3.24 $\pm$ 0.52          |
| D (70%: 30%)   | 3.32 $\pm$ 0.69          |
| A (100%: 0%)   | 3.44 $\pm$ 0.71          |
| B (80%: 20%)   | 3.52 $\pm$ 0.71          |
| E (65%: 35%)   | 3.72 $\pm$ 0.74          |
| <b>KK : 20%</b>  |                          |

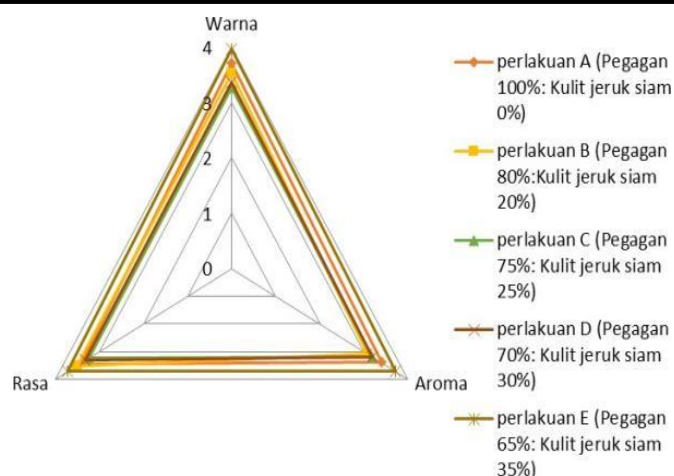
Note: Scores: 1 = strongly dislike, 2 = dislike, 3 = neutral, 4 = like, 5 = strongly like.

The similar average taste scores are attributed to the addition of 20% stevia in each treatment, which masked the bitterness of the herbal tea mixture of gotu kola leaves and Siamese orange peel. Stevia is a natural sweetener that is calorie-free and has a sweetness level 70–400 times that of sucrose. Stevia contains eight diterpene glycosides responsible for its sweetness: stevioside, steviolbioside, rebaudiosides A-E, and dulcoside A [39],[60].

Gotu kola leaves contain valerine, a compound that imparts bitterness (Sutardi, 2016). Meanwhile, Siamese orange peel is rich in phenolic compounds and flavonoids. Flavonoids, such as naringin and hesperidin, are colorless, water-soluble compounds that contribute to the bitter and astringent taste of the tea infusion. Recent studies suggest citrus flavonoids can inhibit bitterness and improve flavor perception in food and beverage formulations [61-63].

### 3.3.3.4 Organoleptic Radar Chart

The organoleptic radar chart is used to simplify the visualization of the average scores from the organoleptic test results, which include color, aroma, and taste. The radar chart for herbal tea can be seen in Figure 2.



**Figure 2.** Organoleptic Radar Chart of Brewed Herbal Tea Bags with Gotu Kola Leaves and Siamese Orange Peel

Based on the radar chart of the organoleptic test for the herbal tea bags made from a mixture of gotu kola leaves and Siamese orange peel with stevia as a natural sweetener, it can be concluded that the herbal tea bags were generally well-accepted by panelists in terms of color, aroma, and taste. On average, panelists preferred treatment E (65%: 35%) with organoleptic scores for color at 3.96, aroma at 3.72, and taste at 3.72.

### 3.4 Practical Implications

The herbal tea product developed using gotu kola and Siamese orange peel has significant potential for commercial application. As a functional beverage, this product offers a combination of health benefits from its ingredients, such as high antioxidant content and a refreshing flavor. However, its commercial feasibility also depends on effective marketing strategies and production efficiency.

From a market perspective, the herbal tea based on gotu kola and Siamese orange peel has great potential to meet the demands of consumers seeking natural and functional products. It can be positioned as a healthy beverage rich in antioxidants and low in calories, appealing to health-conscious consumers. However, potential market challenges include educating consumers about the benefits of the ingredients, differentiating the product in a competitive herbal tea market, and managing production costs to remain competitive.

In terms of shelf-life, the product demonstrates good stability. The moisture and ash content of the tea bags meet the SNI standards for packaged dry tea, ensuring the product is durable and safe for consumption. The shelf-life of this product is influenced by moisture content, phenolic content, and packaging methods. Since the moisture content of the tea bag formulations complies with SNI standards (maximum 8%), the product is stable against microbial growth. However, phenolic compounds may degrade during storage if not packaged properly. Therefore, using airtight packaging with protection against light and oxygen is highly recommended to preserve quality over the storage period [63].

Scaling up production is another critical aspect to consider for the commercial success of this product. Key processes such as raw material drying, mixing, and packaging can be optimized using automated equipment to enhance efficiency. However, quality control remains a priority, particularly to ensure consistency in phenolic content, antioxidant activity, and organoleptic characteristics across production batches. Investments in production technology and workforce training are essential strategies for achieving large-scale production success.

The organoleptic tests showed high panelist preferences for the color, aroma, and taste of formulation E (65% gotu kola: 35% Siamese orange peel), indicating good consumer acceptance. However, broader consumer acceptance studies are needed to understand preferences in larger markets. Additional research could also explore specific market segments, such as consumers with particular health needs or those seeking caffeine-free alternatives.

With its health benefits, good shelf-life, and positive consumer acceptance, this herbal tea shows promising potential as a natural health beverage. However, further efforts in consumer education, market development, and process optimization are required to ensure its commercial success.

Further research is recommended to explore the stability of phenolic compounds in tea bags during storage and brewing processes. Additionally, future studies could investigate the potential health benefits of this tea blend in clinical or consumer contexts.

From a commercial perspective, the findings support the use of Siamese orange peel as a valuable ingredient in herbal tea formulations. The observed trends suggest that balancing the ratio of gotu kola and orange peel is crucial to optimizing both antioxidant activity and sensory appeal. Future research could explore the long-term stability of phenolic compounds in the tea blends and investigate their effects on specific health outcomes [64].

#### 4. Conclusion

The study concluded that the concentration of gotu kola leaves and Siamese orange peel, with stevia as a sweetener, significantly affected pH, DPPH antioxidant activity, total polyphenols, water-soluble substances, IC50, and organoleptic color and aroma, but not moisture, ash content, total plate count, or taste. The best formulation was treatment E (65%: 35%), which achieved optimal physicochemical properties and high panelist preference scores for color (3.96), aroma (3.72), and taste (3.72). Based on the research conducted, the author suggests that future studies include additional parameters such as vitamin C analysis, flavonoid content, and GC-MS testing to identify the secondary metabolites present in the herbal tea bags.

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