Analysis of Antioxidants Butterfly Pea Flower Herbal Syrup as a Functional Beverage

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Abstract. This study was aimed at analyzing the antioxidant capacity of butterfly pea flower herbal syrup with the addition of cardamom, cloves, and ginger. This research was an experimental study with a completely randomized design (CRD). The herbal syrup was made into three different treatments with different ratios of cardamom and cloves in each formula, namely 3:1, 5:3, and 1:1. Antioxidant capacity was analyzed using the DPPH method. The levels of flavonoid, tannin, saponin, kaempferol, quercetin, and gallic acid were also investigated in this study. The results showed that buttefly pea flower herbal syrup with the ratio of cardamom and cloves (1:1) has the highest level of flavonoid (16426.09 mg/L), tannin (3382.50 mg/L), saponin (1354.50 mg/L), kaempferol (1147.82 g/L), quercetin (1658.03 g/L), and gallic acid (4108.75 g/L). These results were in line with the antioxidant capacity of herbal syrup with a ratio of 1:1. The formula has the highest level of antioxidant capacity compared to the other formulas, as indicated by the IC 50 value of 40.777 ppm. Therefore, the more cloves used, the higher the antioxidant capacity of butterfly pea flower herbal syrup.

Keywords: antioxidant, antioxidant capacity, butterfly pea flower, syrup, herbal drink

1 Introduction

Functional food is a natural or processed food that contains more than one compound with various specific physiological functions that are beneficial to the human body. Functional foods are consumed to obtain health benefits, not only as a source of energy for the body. Functional food can consist of food or drink or be called functional drinks.

Functional drinks that are commonly found in Indonesia are spice drinks [1]. Spice drinks are herbal drinks derived from plant parts such as leaves, bark, fruit, and roots that contain health benefits if processed properly. Consuming spiced drinks can increase endurance and body fitness. The traditional method of making spice drinks is still used, which is pounding or cutting the ingredients into small pieces.Spice drinks are generally made from ingredients such as turmeric, ginger, temulawak, ginger rice, cardamom, and other spices.

The use of different basic ingredient types in the processing of spiced drinks produces different properties according to the basic ingredient [2]. However, some previous research

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results stated that spiced drinks contain good biological activity for the body due to the presence of bioactive compounds, namely antioxidant compounds.

Antioxidant compounds are compounds in low concentrations that can inhibit or prevent the substrate oxidation process in chain reactions [3]. Antioxidants can protect body cells from damage caused by free radicals from the oxidation process. Antioxidants are able to prevent the formation of free radicals so that the oxidation process in the body will be inhibited [4]. For example, previous studies have shown that cardamom contains antioxidant compounds such as alkaloids, tannins, flavonoids, polyphenols, and beta-carotene. Not only cardamom, but several ingredients commonly used in making spice drinks contain bioactive compounds that have high health benefits and are safe and effective.

The main characteristic of antioxidant compounds is their ability to scavenge free radicals [4]. One of the uses of spice drinks in functional food products is syrup processing. Syrup is a thick solution that contains a high sugar content and has a tendency to precipitate sugar crystals [5]. Before consumption, the syrup must be diluted with water due to its thick consistency, unlike fruit juice. Dilution is also necessary because the syrup contains a high sugar content.

Spice drinks are generally brown in color. The color comes from the pigments produced by the spices. Natural color pigments can be found in plants. One of the natural pigments comes from the butterfly pea flower (Clitoria ternatea L.). The butterfly pea flower produces a blue color. The addition of butterfly pea flowers is also able to increase the antioxidant content. Apriani's research [6] stated that the butterfly pea flower contains antioxidant compounds including alkaloids, flavonoids, saponins, tannins, and steroids.

The advantages of natural spice syrup are that it is easy to obtain, environmentally friendly, inexpensive, and has relatively few side effects [7]. Making spice drinks out of syrup makes them easier to consume. Utilization of herbal plants as raw materials in the manufacture of spice syrup makes it easier to serve and is also expected to contain good properties for the body. This study looked into the production of spice syrup made from cardamom, cinnamon, cloves, ginger, and butterfly pea flowers. The antioxidant content and antioxidant capacity of these raw materials were also analyzed. Compounds that are reliable in reducing free radicals are flavonoids, tannins, saponins, quercetin, gallic acid, and kaempferol. The combination of these ingredients was expected to attract people to be more aware of health and treat diseases by consuming spice syrup.

2 Materials and Methods

2.2 Materials

The materials used in this study included cardamom, cloves, cinnamon, ginger, dried butterfly pea flowers, water, and sugar. Materials preparation included collecting, cleaning, mashing with a mash tool, boiling, filtering, and sterilizing. The tools used in this research were tools for making herbal syrup, namely mash tools, scales, sieves, spatulas, pans, bowls, brix refractometers, and packaging bottles.

Table 1. Butterfly Pea Syrup Formula

No	Material	Formula A	Formula B	Formula C
1.	Cinnamon	40 g	40 g	40 g
2.	Cardamom	30 g	25 g	20 g
3.	Clove	10 g	15 g	20 g
4.	Butterfly pea flower	8 g	8 g	8 g
5	Ginger	20 g	20 g	20 g

6.	Water	250 g	250 g	250 g
7.	Sugar	100 g	100 g	100 g

2.2 Methods

This study used two research designs, namely explorative, descriptive, and experimental. The explorative descriptive research included the process of making spice syrup with two replications, while the experimental research was conducted through antioxidant analysis, which included flavonoid content, tannin content, saponin content, kaempferol content, gallic acid content, quercetin content, and antioxidant capacity. This study used three formulas as samples with different ratios of cardamom and cloves, namely 3:1, 5:3, and 1:1. Meanwhile, other spices such as cinnamon, ginger, and butterfly pea flower have the same ratio in each formula. Data were analyzed using one-way ANOVA with a significance level of 5%. If the significance level is $p<0.05$, it shows a significant difference between the formulas. If $p>0.05$, there is no significant difference between the formulas.

3 Results and Discussion

This research was conducted on butterfly pea flower herbal syrup to determine the antioxidant content of flavonoids, tannins, saponins, kaemferol, quercetin, and gallic acid, as well as their antioxidant capacity. The results of the antioxidant content and antioxidant capacity of the butterfly pea flower herbal syrup are shown in Table 2.

Table 2. Average Content of Flavonoid, Tannin, Saponin, Kaempferol, Quercetin, Gallic Acid, and Antioxidant Capacity

Formula	Flavonoid (mg/L)	Tannin (mg/L)	Saponin (mg/L)	Kaempferol (µg/L)	Quercetin (µg/mL)	Gallic Acid (µg/mL)	Antioxidant Capacity (IC_{50} ppm)
A	12643.48	2295.00	926.58	887.22	1285.70	3159.76	52.574
B	14377.17	2793.44	1120.83	1006.80	1456.54	3595.61	45.581
C	16426.09	3382.50	1354.50	1147.82	1658.03	4108.75	40.777

3.1 Flavonid Content

Based on the results in Table 2, the flavonoid content of butterfly pea flower herbal syrup in Formulas A, B, and C showed a significant difference ($p = 0.003$). Formula C has the highest flavonoid content, which was 16426.09 mg/L. Meanwhile, formula A and formula B were 12643.48 mg/L and 14377.17 mg/L, respectively.

Flavonoids are one of the compounds that are important for the body. Flavonoids are known to have antioxidant, anticarcinogenic, and antimutagenic functions [8]. The most abundant flavonoid compounds in nature are flavonols, flavones, flavones 3-0l, isoflavones, flavanones, proanthocyanidins, and anthocyanidins [9]. In humans, flavonoids act as antioxidants in fat, heart stimulants, hydroxylated flavones act as diuretics, and hispederin affects capillary blood flow [9]. The addition of foodstuffs containing flavonoids to instant drinks can increase flavonoid activity.

Flavonoids are antioxidant compounds that capture various free radicals (such as superoxide anions and peroxynitrite). The use of a butterfly pea flower herbal syrup formula

with different ratios of cardamom and cloves resulted in varying flavonoid content. According to the USDA, the flavonoids contained in cardamom were 0.28% [10]. In the study [11], the flavonoid content in cloves was 337.29 10.59 mg QE/100 g. Clove flowers contain flavonoids of 126.50 mg QE/100 g, higher than the flavonoid content found in clove stems. A similar study stated that the total flavonoid content in cloves was 318.67 0.88 mg QE/g [12]. Table 2 showed that the highest flavonoid content was found in formula C, with an average of 16426.09 mg/L. Formula C used more cloves and cardamom than other formulas, which was 20 g for cardamom and cloves. According to the results, the use of cloves contributed to the difference in each formula of butterfly pea flower herbal syrup.

The highest flavonoid content was found in cloves, followed by cinnamon and cardamom [11]. However, cloves were not the only source of the flavonoid content in the butterfly pea flower syrup. Cardamom has a high flavonoid content of 24.8 mg QE/g, according to previous research [13]. The effect of adding an herbal ingredient is able to increase the flavonoid content; this was evidenced by the addition of dried Artocarpus heterophyllus in herbal drinks [14]. These confirm that the more clove and cardamom ingredients used in the processing of this syrup, the higher the flavonoid content will be.

3.2 Tannin Content

Tannins are active compounds of secondary metabolites that have several properties, such as antioxidant, antibacterial, and anti-diarrheal [15]. Tannins are polar compounds and are resistant to heating. The content of tannins, which are secondary metabolites, can work as antibacterial, anticancer, and antioxidant derivatives.

Based on the results in Table 2, the tannin content of butterfly pea flower herbal syrup in Formulas A, B, and C showed a significant difference ($p = 0.003$). Formula C has the highest tannin content, which is 3382.50 mg/L. while the tannin content of formula A was 2295 mg/L and that of formula B was 2793.44 mg/L.

Previous studies had shown that the tannin content of cloves was 14.06 0.49 mg GAE/g. Cloves were not the only tannin source in the butterfly pea flower herbal syrup [11].In this study, cinnamon, cloves, and cardamom had the highest tannin content of all the spices. The tannin content of cardamom was 12.88 0.54 mg GAE/g.One of the factors influencing the tannin content was the amount of cloves used in the herbal syrup.The formula with the highest tannin content was Formula C, which contained 3382.50 mg/L and included 20 g of cloves and cardamom.Herbal syrup formula C used more cardamom and cloves than formulas A and B. Similar studies [14] demonstrated the effect of a large number of ingredients in increasing the tannin content of a drink.This study investigated the effect of the total ratio of dryArtocarpus heterophylluson functional drinks. Therefore, similar results showed that formula C had a higher tannin content than formulas A and B.

3.3 Saponin Content

Saponins can be found in various types of food ingredients, such as cloves [16] and cardamom [17]. Similar research has found that the higher the proportion of clove ingredients used in functional drinks, the higher the saponin content [7]. Table 2 showed that the saponin content of butterfly pea flower herbal syrup in Formulas A, B, and C had a significant difference at the 5% significance level ($p = 0.004$). The highest saponin content in herbal syrup was formula C, 1354.50 mg/L. Meanwhile, the saponin contents of formulas A and B were 926.578 mg/L and 1120.83 mg/L, respectively. The amount of cardamom and cloves used influenced the difference in the increase in saponin content of herbal syrup. Cardamom

and cloves used in formula C were 20 g each, more than in formula A and formula B. The number of cloves used in the herbal syrup contributed to the saponin content of the butterfly pea flower herbal syrup. Cloves have a saponin content of 3.7% [18]. This confirms that as more cloves and cardamom ingredients were used in the process of making this syrup, the saponin content increased as well.

3.4 Kaempferol Content

Kaempferol is one of the polyphenolic compounds that can be found in fruits and vegetables [19]. Kaempferol is one of the six subclasses of flavonoid compounds that have antioxidant activity. The number of herbal ingredients used in the herbal drinks process, according to Devi et al. (2021), could increase kaempferol content [14].Kaempferol has biological activities such as being an antioxidant, preventing the formation of cancer cells, and preventing atherosclerosis.

Based on the results in Table 2, the kaempferol content of the butterfly pea flower herbal syrup in Formulas A, B, and C showed a significant difference, $p = 0.003$. The herbal syrup in formula A had an average concentration of 887.22 g/mL, the kaempferol content in formula B was 1006.80 g/mL, and the kaempferol content in formula C was 1147.82 g/mL.

The use of different ratios of cardamom and cloves resulted in varying levels of kaempferol. The use of cloves and cardamom in formula C was known to be greater than in formulas A and B.As a result, cloves and cardamom contribute the most kaempferol to formula C's herbal syrup.This was due to the high content of kaemferol in cloves, i.e., 23.8 mg/100 g [20]. In the previous study that compared the amount of kaempferol in five herbal plants, cloves had the highest kaempferol content of 41.0 ppm, followed by thyme with a kaempferol content of 28.7 ppm [21].

The use of cloves and cardamom in formula C was more than that of formulas A and B, which was 20 g each. These were one of the factors causing the increase in the amount of kaempferol content in formula C. Similar results were obtained through the addition of dryArtocarpus heterophyllusin functional drinks, which were able to increase the content of kaempferol [14].

3.5 Quercetin Content

Quercetin is a compound that belongs to the flavonoid group. Quercetin is classified as an active ingredient with biological abilities including anticancer, antibacterial, antiinflammatory, antigonadotropic, and antihepatotoxic [22]. Quercetin is able to bind free radicals in large quantities and is classified as a strong antioxidant [23].

Based on the results in Table 2, the quercetin content in butterfly pea flower herbal syrup in formula A, formula B, and formula C showed that there was a significant difference ($p=0.003$). Formula C had the highest quercetin content, at 1658.03 g/mL. Meanwhile, the quercetin content of formula A was 1285.70 g/mL and 1456.54 g/mL for formula B.This could be influenced by the amount of clove and cardamom used. Moreover, the use of cloves in formula C was more than formulas A and B, which was 20 g. Cloves were known to have a quercetin content of 28.4 mg/100 g [20]. Not only cloves, but cardamom also plays a role in increasing the quercetin content in the herbal syrup. Cardamom contains quercetin, kaempferol, and luteolin [24]. Clove is one of the herbal ingredients that has the highest quercetin content of the 26 types of herbal plants [20]. A similar study compared quercetin in five herbal ingredients and found a high quercetin content in cloves, namely 14.8 ppm, below thyme with the highest quercetin content at 29.1 ppm [21].

The herbal syrup in formula C used more cardamom and cloves than formulas A and B. Therefore, formula C had the highest quercetin content. These statements were supported by another research study on increasing the amount of dryArtocarpus heterophylluscould increase the quercetin content in functional drinks [14]. Quercetin is the compound most commonly found in various types of foods such as apples, tea, onions, nuts, berries, and cabbage [25].

3.6 Gallic Acid Content

Gallic acid is one of the active compounds that have high benefits in the medical field. It has many benefits, including antibacterial, antiviral, analgesic, and antioxidant [26]. Gallic acid is able to provide protection against oxidative damage caused by reactive species that are often encountered in biological systems [25].

Table 2 showed that the gallic acid content in butterfly pea flower herbal syrup in formulas A, B, and C had a significant difference at the 5% significance level ($p = 0.003$). Formula C had the highest gallic acid content, 4108.75 g/mL. followed by formula B, 3595.61 g/mL, and formula A, 3159.76 g/mL.

The gallic acid content of formula C was high because it used more cloves and cardamom. It was in line with another research study on functional drinks containing more driedHeterophyllus artocarpuscould increase galic acid content [14]. Research on phenolic content in 26 types of spices also showed that the highest gallic acid found in cloves was 783.5 mg/100 g [20]. Similar research comparing the content of gallic acid in five different herbal ingredients finds that cloves rank first with the highest gallic acid content of 9764.0 ppm, followed by cumin, cinnamon, ginger, and thyme [21]. Cloves were found to be one of the most important ingredients in the gallic acid content of butterfly pea flower herbal syrup.

3.7 Antioxidant Capacity

Antioxidants are compounds with low concentrations that can prevent or inhibit the oxidation process and protect cells from damage caused by free radicals. Natural antioxidants are safer for consumption because they are easily absorbed by the human body [27]. The antioxidant capacity test is a parameter that illustrates the percentage of a food ingredient's ability to inhibit free radicals.

The results in Table 2 indicated that the antioxidant capacity in butterfly pea flower herbal syrup had a significant difference ($p = 0.008$). Formula A had the lowest antioxidant capacity, with an IC50 value of 52.574 ppm, while formula C had the highest antioxidant capacity, with an IC50 value of 40.777 ppm.In addition, a compound is considered to have very strong antioxidant activity if the IC50 value is less than 50 ppm, strong antioxidant activity if the IC50 value is between 51 and 100 ppm, moderate antioxidant activity if the IC50 value is between 101 and 150 ppm, and weak antioxidant activity if the IC50 value is between 151-200 ppm [12].

One study showed that cloves had the highest eugenol content, followed by kaempferol, quercetin, and gallic acid [20]. These compounds contribute to increasing antioxidant activity. [28]. The study uses IC50, which is a number that shows a sample concentration (PPM) that is able to inhibit 50% of free radicals [28]. The highest antioxidant capacity was shown in the herbal syrup of Formula C, with an average of 40.777 ppm. These were influenced by the use of cardamom and cloves in formula C, which was more than formula A and formula B. The use of more cloves in formula C, 20 g, plays a major role in increasing antioxidant activity in herbal syrups. These results were supported by another study that showed that increasing the number of herbal ingredients could increase antioxidant capacity [29].

The results of other studies related to antioxidant activity in several herbal ingredients using IC50showed that the highest antioxidant activity was found in cloves with a value of 4.16 g/ml, while cardamom antioxidant activity was 177.90 g/ml. Cloves had the highest antioxidant capacity among 30 different types of herbal plants in previous studies [30].The antioxidant activity contained in these ingredients synergizes to increase antioxidant capacity in butterfly pea flower herbal syrup. The use of various ingredients containing antioxidants can increase antioxidant activity when used separately [31].

4 Conclussions

Each butterfly pea flower syrup formula had a significant difference. Formula C had the highest content of flavonoids (16426.09 mg/L), tanin (3382.50 mg/L), saponins (1354.50 mg/L), kaempferol (1147.82 g/mL), gallic acid (4108.75 g/ml), and quercetin (1658.03 g/ml). The antioxidant capacity in formula C was also higher than that in formulas A and B, which was 40.777 ppm. Therefore, the use of more cloves and cardamom in formula C could increase the content of flavonoid compounds and the antioxidant capacity of the butterfly pea flower herbal syrup.

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