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Short Communication

Effect of Sugar Addition and Fermentation Process on Flavonoids, Phenols, and Antioxidant Activity of Telang (*Clitoria ternatea* L.) Kombucha

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ABSTRACT

Kombucha is a popular traditional tea prepared from different parts (leaves and flowers) of medicinal plants, including the leaves of telang (*Clitoria ternatea* L.). The fermented tea is usually flavoured with sugar. However, the concentration of added sugar affects the content of kombucha phytochemicals. Therefore, this study aimed to analyse the content of phenols and flavonoids and the antioxidant activity (DPPH) of telang flower kombucha sweetened with sugar. Three tea formulas were prepared with sugar concentrations of 20%, 30%, and 40%, respectively. The tea's total phenols and flavonoid contents and DPPH free radical scavenging activity were assessed using UV-Vis spectrophotometric analysis. The results showed an increase in total phenols and flavonoid contents after the fermentation process of telang flower tea into kombucha, from 87.2 to 110.8 ppm and 2.14 to 3.44 ppm, respectively. The highest phenolic content value of 129.6 ppm was obtained at 30% sugar (p<0.05). Results also show that adding sugar was directly proportional to the total flavonoid content (p<0.05). The antioxidant activity increased proportionally to 83.2 ppm at 30% sugar concentration, hence classified under the strong category (p>0.05). Based on the results, the fermentation process and adding sugar could increase the total phenols, flavonoids, and antioxidant activity in the formulation of telang flower kombucha.

Keywords: Telang flower, DPPH, Flavonoids, Kombucha, Phenols

Introduction

Kombucha tea has a long history of use, especially among the Chinese people over 2000 years ago, while in Indonesia, it is known as kombu tea, which began to be cultivated in the 1930s. One etymological theory states that the name 'Kombucha' means "seaweed-tea" or "kombu-cha" in Japanese.[1] Kombucha is a fermented drink with sugar media and uses a Symbiotic Culture of Bacteria and Yeast (SCOBY) starter.[2] SCOBY is a secondary metabolite that forms the cellulose structure of yeast and bacteria, making kombucha taste sour and sweet. Kombucha was originally made only from tea leaves (*Camelia sinensis*), such as black and green, but is currently being developed by brewing various leaves and even edible flowers.[3] Tea made from steeped leaves or edible flowers is believed to benefit health.[4] Flowers are attractive natural products with important nutritional content and phytochemical components.[5] Edible flowers can be consumed directly, brewed in tea, or served as a processed food mixture.[6] The content includes phytochemical compounds, namely anthocyanins, flavonoids, phenolics, and carotenoids, which are useful as antioxidants.[7] Indonesia is rich in biodiversity, with various types of plants grown, including telang (*Clitoria ternatea* L.), known as bunga telang.

Telang flower is a wild plant from the Fabaceae family,[8], and is reportedly native to Ternate, Indonesia.[9] The content includes anthocyanins, which can act as antioxidants and colour pigments.[10]

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Furthermore, the extract is known to have potential benefits as an antiproliferative agent against cancer cells *in vitro*.[11] Pre-clinical studies on experimental animals also proved that telang flower extract has hepatoprotective and neuroprotective activity.[12] Anthocyanin compounds are very stable when used as natural food or beverage dyes, underscoring the potential in the food industry. These compounds have an alkaline pH (4-7), low stability, more stable in acidic conditions, and are polar.[13] Given that Kombucha is acidic with a pH of 2.5-4.2[2], the anthocyanin content of telang flower tea will be more stable and provide optimal health benefits. While preparing kombucha, the added sugar acts as a substrate for bacteria and yeast to assist fermentation. Kombucha also has a specific sour taste, and the additional sugar increases consumer acceptance. The amount of sugar to be added and the potential impact on the phytochemical content are unclear. Therefore, this study aimed to formulate telang flower kombucha and analyse the content of phenols, flavonoids, and antioxidant activity based on differences in sugar concentrations.

Materials and Methods

Experimental Design

This study used an experimental approach and a completely randomised design (CRD) with one factor: adding sugar in variations of 20%, 30%, and 40%.

Materials

Plant collection and Identification

Fresh telang flower, used to prepare kombucha, was collected from a garden in Kulonprogo, Yogyakarta, Indonesia (-7°38'42.00"S 110°01'36.98"E). The plant was identified by United States Department of Agriculture where a nomen number 10942 was assigned.

Other ingredients used were water, granulated sugar, starter solution (8% (v/v, % v/v = mL of solute/100 mL of solution),[14] and Symbiotic culture of bacteria and yeast (SCOBY) (10 grams; 9 cm in diameter and 0.6 cm thick). Other instruments include an Ovens (Memmert UM400,

English), stoves (Rinnai RI552C, Indonesia), pots (Maxim, Indonesia), thermometers (FRIO-Temp® digital thermometer, Germany), pH meters (IsteK P15, Seoul), incubator (Hipoint 1B-701, Taiwan), and spectrophotometer (Shimadzu UV 1800, Japan).

Making Telang Flower Kombucha

Fresh telang flower was harvested uniformly at 42 days of harvest,[15] followed by drying at 50°C for four hours.[16] The dried samples were placed into a tea bag, reaching 1 g/tea bag, and brewed with 250 mL of water at 75°C for 9 minutes. The mixture was filtered,[17] then sugar was added in variations of 20%, 30%, and 40%, then cooling to 20°C for 2 hours.[18] The infusion of telang flower that had reached room temperature was placed into a glass container[19] with a SCOBY and starter.[14] The sample was covered with gauze and stored out of direct sunlight for 10 days,[20] creating aerobic conditions in the fermentation process. After 10 days, the SCOBY was removed and filtered in the kombucha solution.

Total Phenol Analysis

The phenolic content of kombucha at concentrations of 20%, 30%, and 40% was determined using the Folin Ciocalteu method. Briefly, about 50 mg of kombucha extract was gradually dissolved to 25 mL in different volumetric flasks with a mixture of ethanol: dist. water (1:1). The extract solution obtained was pipetted into 300 µL, and then 1.5 mL of Folin Ciocalteu reagent was added and shaken. After incubation for 3 minutes, 1.2 mL of 7.5% Na_2CO_3 was added, and the solution was allowed to stand again for 60 minutes. The absorbance was measured at a wavelength of 760 nm using a UV-Vis spectrophotometer.[21] The determination was done in duplicates using gallic acid as standard. The total phenolic content of kombucha was expressed as gallic acid equivalent per gram of the extract concentration.[22]

Analysis of Total Flavonoids

The total flavonoid content of kombucha was determined using a previously established.[23] In this assay, 1500 ppm of the test samples was achieved by dissolving approximately 15 mg of each kombucha concentration in 10 mL of ethanol.[23] The standard quercetin solution was obtained by dissolving 25 mg in 25 mL of ethanol, resulting in a concentration of 100 ppm. Each concentration of the standard solution (1 ml) was combined with 1 mL of 10% $AlCl_3$ and 1 mL of 1 M potassium acetate to obtain concentrations of 1, 3, 5, 9, and 9 ppm. The mixtures were allowed to incubate for 30 minutes, and the absorbance was measured spectrophotometrically at 423 nm.[24] The extract (15 mg of kombucha) solution was dissolved in 10 mL of ethanol to give 1500 ppm used for the analysis.[23] The analysis was done in duplicates. The total flavonoid content was as quercetin equivalent per gram of the extract computed from the quercetin standard curve.

DPPH Analysis

The antioxidant potential of kombucha was determined using the 2,2 diphenyl-1 picrihydrazyl (DPPH) reduction method.[25] The stable DPPH free radical was dissolved in methanol to obtain 0.1 mM concentration, and 1 mL of this solution was mixed with 3.0 mL of different concentrations of the extract (20%, 30%, and 40%). The reaction mixture was left in the dark for 30 minutes before the absorbance was measured spectrophotometrically at 517 + 20 nm. The percentage of inhibition of the DPPH free radical was calculated from the plot of the percentage absorbance versus extract concentration.[26]

Statistical Analysis

Data processing and analysis were performed using Microsoft Excel and IBM SPSS Statistics Software (version 25). Analysis of Variance (ANOVA) test with $p \leq 0.05$ was used to analyse the difference in the effect of adding sugar on the content of kombucha's flavonoids, phenols, and antioxidant activity. Duncan's test was used when there was a significant difference.

Results and Discussion

This study investigated the effects of fermentation and the addition of sugar on the total phenolic and flavonoid contents of kombuchas and the antioxidant potential using established procedures. Results of the total flavonoids, phenolic content, and antioxidant potential of kombucha are shown in Figures 1-3, respectively. Figure 1 showed that the total flavonoids were in the range of 2.14-3.44 ppm, increasing linearly with increasing concentration of sugar added. The fermentation process caused an increase in the content from 2.14 ppm (BPT) to 2.48 (BPK20). Analysis of this result showed that adding sugar significantly affected the total flavonoid content of telang kombucha ($p<0.05$). As reported in a previous study, adding sugar influences the inhibition mechanism of gram-positive bacteria. Furthermore, the total flavonoid in the kombucha tea correlates with increased inhibitory action of bacteria. Flavonoids, which include flavonics, tastes, isophones, flavanones, anthocyanidins, and flavonoids, are the most prevalent group of polyphenols in common diets. These groups are naturally occurring chemicals with a phenolic structure in various foods, including fruits, vegetables, cereals, flowers, tea, and wine.[28] In numerous nutraceutical, pharmacological, therapeutic, and cosmetic applications, flavonoids are now considered essential due to their ability to alter enzyme activity and anti-oxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic effects.

Kombucha was fermented by lactic acid bacteria and yeast found in SCOBY, producing valuable substances for the body. The fermentation lasted for 7 - 12 days to obtain optimal results, and in the process, sugar served as a food substrate for energy utilisation by the bacteria and yeast cells. The added sugar was degraded by yeast on SCOBY into alcohol and CO_2, then broken down by lactic acid bacteria to produce probiotic bacteria. In general, fermentation can cause changes in physical and chemical properties, including starch and alcohol content, pH, antioxidant levels, and total acid. According to a previous study, the residue from this process is converted into organic acids such as acetic, glucuronic, gluconic, folic, and amino acids, along with vitamin B complex, riboflavin, and enzymes.[29] Raw materials, ingredient concentration, sugar concentration, fermentation duration, and temperature can affect kombucha content.[28]

Similarly, Figure 2 showed that the phenol content increased linearly up to the addition of 30% sugar (BPK30) at 129.64 ppm but decreased to 95.64 ppm at 40% sugar (BPK40). In general, the addition of sugar had a significant effect ($p<0.05$) on the phenol content of the telang kombucha tea. The fermentation process also affected the total phenols, as shown in the elevated level from 87.24 ppm for telang flower tea (BPT) to 95.64-110.84 ppm after becoming kombucha. The fermentation effect caused the total phenolic content of kombucha to increase, primarily due to the action of microorganisms that influenced metabolism through enzymatic reactions.

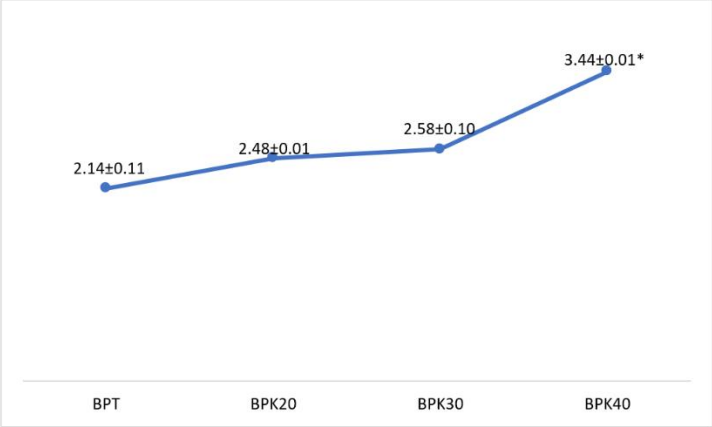

Figure 1: Flavonoid content of Telang tea and kombucha at various sugar concentrations. Data presented in average±SD, BPT= Telang tea, BPK20= Telang kombucha with 20% sugar, BPK30= Telang kombucha with 30% sugar, BPK40= Telang kombucha with 40% sugar, * Significant at p<0.05 level

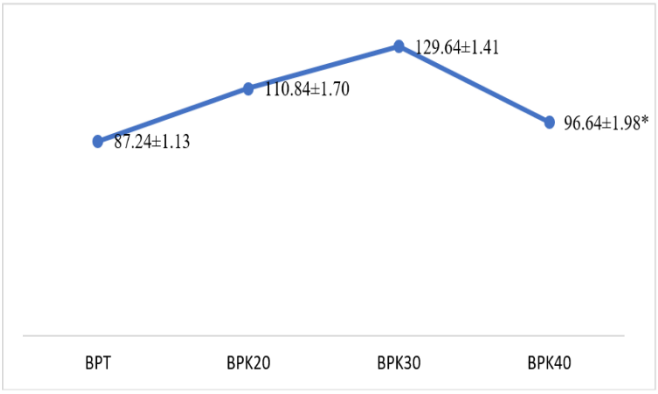

Figure 2: Phenol content of Telang tea and kombucha at various sugar concentrations. Data presented in average±SD, BPT= Telang tea, BPK20= Telang kombucha with 20% sugar, BPK30= Telang kombucha with 30% sugar, BPK40= Telang kombucha with 40% sugar, *Significant at p<0.05 level

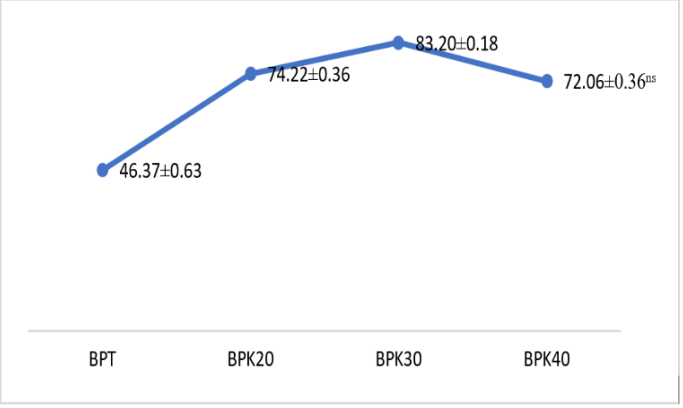

Figure 3: Antioxidant activity of Telang tea and kombucha at various sugar concentrations. Data presented in average±SD, BPT= Telang tea, BPK20= Telang kombucha with 20% sugar, BPK30= Telang kombucha with 30% sugar, BPK40= Telang kombucha with 40% sugar, (^{ns} = not significant).

The increase in total phenolic levels may be due to enzymes secreted by bacteria and yeast in kombucha, which degraded complex polyphenolic compounds into simple ones.[30] Phenols refer to a group of polyphenols,[31] naturally occurring compounds in plants. Polyphenols, present in many medicines and vegetables, are considered the most active components for improving metabolic syndrome *in vitro* and *in vivo* studies. Enzymes in the intestine hydrolyse these compounds before absorption. Most polyphenic compounds contain several hydroxyl groups, which enzymes convert by methylation, glucuronidation, or sulfation. However, only 5-10% can be absorbed in the small intestine; the remaining is collected in the large intestine or excreted in the faeces.[32]

The antioxidant activity result of kombucha is shown in Figure 3. There was a linear increase of the antioxidant activity (72.06-83.20 ppm) up to the addition of 30% sugar (BPK30). The addition of sugar did not significantly (p>0.05) affect the antioxidant activity. The increase in antioxidant activity may have been due to fermentation, compared to the initial value of 46.37 ppm in telang flower tea. Antioxidant activities are categorised into very strong (<50 ppm), strong (50-100 ppm), moderate (100-150 ppm), weak (150-200 ppm), and very weak (≥ 200 ppm).[33,34] Based on the results, kombucha had antioxidant activity in the strong category. The DPPH free radical determination kombucha extract solution showed that the telang flower had strong antioxidant activity.[25] Assessment of antioxidant activity can be influenced by the maximum wavelength used and the storage temperature of a plant extract.[35]

Polyphenols are chemical components with hydroxyl groups that can contribute electrons to stabilise reactive free radicals.[36] This explains the relationship between phenolic compounds and antioxidant activity. The results of the study revealed that measurements of DPPH free radical activity showed that kombucha had powerful antioxidant activity.[25]

The sugar added as a substrate to the production of kombucha affected bacteria functions, leading to an increase in antioxidant activity. Studies on preparing kombucha from green tea with various sweeteners also revealed that using sugar as substrate yielded a very strong antioxidant activity with a value of 35.01 ppm.[37] Antioxidant activity refers to a sample concentration required to inhibit 50% of the oxidation process of free radicals.[38] Lower values indicate a greater ability to repel free radicals, and the antioxidant potential of the telang flower may have a positive role in preventing diabetes mellitus.[39]

Conclusion

In conclusion, the fermentation process and addition of sugar significantly increased total phenols, flavonoids, and antioxidant activity in telang kombucha formulation. Although the amount of sugar added was in line with the antioxidant content, restrictions must be ensured during the preparation of kombucha, considering the potential of sugar in various non-communicable diseases, particularly diabetes mellitus.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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