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Evaluation of Antioxidant and Antihyperglycemic Activities in Kombucha Made from Black Turmeric (*Curcuma aeruginosa* Roxb.) Rhizome Extract

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Abstract

Kombucha, a fermented beverage, has gained increasing attention because of its health benefits, particularly when it is combined with medicinal plants. One such plant with promising properties is black turmeric rhizome (*Curcuma aeruginosa* Roxb.), which is known for its various pharmacological effects. This study aimed to determine the total phenolic, flavonoid, and curcuminoid contents of kombucha produced from black turmeric rhizome and evaluate its *in vitro* antioxidant and antihyperglycemic activities. After kombucha fermentation at 25 - 30 °C for 10 days, the extract of black turmeric rhizome resulted in 85.538 mg GAE/L of phenolic content and 20.063 mg QE/L of flavonoid content, which was significantly higher ($p < 0.05$) than that of the unfermented black turmeric. UFLC spectrophotometry analysis showed an increase in curcumin (61.307 ppm), whereas bisdemethoxycurcumin and demethoxycurcumin contents were lower after kombucha fermentation of black turmeric rhizome. The IC_{50} values for DPPH antioxidant activity was 34.46 ± 0.34 µg/mL (using ascorbic acid as control) and for α-glucosidase inhibition was 9.41 ± 0.76 µg/mL (using acarbose as a control). Significant increases ($p < 0.05$) were observed in the antioxidant capacities of kombucha fermentation compared to that of unfermented black turmeric rhizome (ABTS: 167%, FRAP: 742%, and CUPRAC: 93%). The elevated total phenolic, flavonoid, and curcumin contents of kombucha were significantly correlated ($p < 0.01$) with the increase in antioxidant activity and capacity after the fermentation of black turmeric. These findings suggest that kombucha fermentation enhances the potential of black turmeric as a functional food *in vitro*. Further research should be conducted to optimize its efficacy, such as *in vivo* or bioavailability studies.

Keywords: Antihyperglycemic, Antioxidant, *Curcuma aeruginosa*, Curcuminoid, Kombucha

Introduction

The fermented beverage, kombucha, is primarily produced from black tea (*Camellia sinensis*) supplemented with sucrose. Kombucha made from oolong tea, green tea, as well as addition of other infused material can also be found [1]. A symbiotic culture of bacteria and yeast (SCOBY) is used for fermentation. The core drivers of kombucha fermentation are acetic acid bacteria (AAB) (*Komagateibacter*, *Acetobacter*, and *Gluconobacter*) and yeasts (*Zygosaccharomyces* sp., *Saccharomyces* sp., and *Brettanomyces* sp.), whereas lactic acid bacteria (LAB) (*Lactobacillus* and *Leuconostoc*) have been isolated from the beverage [2]. Kombucha fermentation is generally conducted at room temperature (22 - 30 °C) for 7 - 21 days [3].

In 2016 - 2025, publications related to kombucha mostly reported about its antioxidant activities, followed

by antimicrobial, anticancer, and anti-inflammatory activities. Overall, most studies on the role of kombucha in health were *in vitro* or animal-based [4]. An antioxidant activity assay using the DPPH method showed that black tea kombucha resulted in the highest inhibition, followed by green, white, and oolong tea kombucha [5]. Black tea kombucha and green tea kombucha exhibited higher antioxidant activity than tea that was not fermented [6]. The increase in antioxidant capacity is potentially due to the biotransformation of epigallocatechin-3-gallate (EGCG) into epicatechin gallate (ECG) and epicatechin (EC), as well as other complex polyphenols into simpler molecules through the enzymatic activity of microbes in SCOBY under acidic conditions [7,8].

In addition to its antioxidant effects, kombucha exhibits various bioactivities, including antidiabetic and antiobesity effects. Green and black tea kombucha administered to Wistar rats fed a high-fat high-fructose diet resulted in decreased systemic inflammation, total adipose tissue, and blood triglyceride levels, as well as observed modulation of adipogenesis and β-oxidation-related genes [9]. *In vivo* studies in diabetic rats showed a reduction in blood glucose levels, free radicals, and transaminases after daily administration of kombucha, encouraging kombucha potency for diabetes and hypercholesterolemia treatment [10]. The hypothetical mechanisms of hypoglycemic effects involve gut microbiota regulation to reduce dysbiosis, oxidative stress reduction, and enzymatic inhibition, such as the reduction of α-amylase activity [10]. A human *in vivo* study (four-week clinical trial of kombucha supplementation) in healthy individuals fed a low-fiber Western diet resulted in a detectable enrichment of ellagic acid bacteria, suggesting a potential interaction between polyphenol-rich kombucha consumption and the gut microbiota [11]. Dietary intervention in three studies using kombucha prepared from black or green tea resulted a shifting in the microbial composition and improvements in gastrointestinal symptoms [12]. The phenolic compounds and organic acids in kombucha may influence multiple molecular pathways across human organs owing to their antioxidant activities [12].

With ongoing research, interest in kombucha has expanded to explore alternative substrates beyond tea, such as plant leaves, fruit and juice, and herbaceous plants [8]. The exploration of kombucha from medicinal plants is growing because medicinal plants are believed to possess health effects based on empirical data. Studies on kombucha from Javanese turmeric (*Curcuma xanthorriza*) reported an increase in antioxidant activity by DPPH, as well as induced apoptosis in >22% of T47D cell population and lowered IC_{50} of cytotoxicity value in T47D cells, indicating its anti-cancer potential [13,14]. Both turmeric-based kombucha and turmeric-infused kombucha contain more chemical compounds [13,15], which is promising for their higher bioactivity. Turmeric kombucha has been reported to modulate the immune response and alter the gut microbiota composition in mice with lipopolysaccharide-induced sepsis [16]. Another study reported that kombucha from turmeric (*Curcuma domestica* Val.) administered to Wistar rats with a high-fat high-carbohydrate diet for 28 days, significantly improved the Lee index obese model [17], suggesting the potential of turmeric kombucha as an anti-obesity agent.

Other turmeric species, such as black turmeric or temu ireng (*Curcuma aeruginosa* Roxb.) is an underutilized Indonesian herb [18]. Black turmeric has a long history in Indonesian traditional medicine and is known to contain bioactive compounds such as saponins, flavonoids, polyphenols, triterpenoids, and curcumin. Other compounds, such as monoterpenes, monoterpenoids, terpenes, alkanes, sesquiterpenes, sesquiterpenoids, and ketones, have also been identified in black turmeric [18]. Its extract exhibited α-glucosidase inhibitory activity, which was attributed to the bioactivity of several compounds, including curcumin, demethoxycurcumin, and xanthorrhizol [19].

The potency of black turmeric in kombucha fermentation has not been extensively explored. The curcuminoid content of black turmeric and its correlation with the potential bioactivities of kombucha also require further investigation. Nonetheless, more in-depth studies are required to assess the total phenolic, total flavonoid, and curcuminoid contents of kombucha prepared from black turmeric, as well as its antioxidant and antihyperglycemic properties for functional foods with therapeutic benefits. This study aimed to determine the total phenolic and flavonoid contents, as well as the curcuminoid content of kombucha prepared from black turmeric rhizomes and evaluate its potential as an antioxidant and antihyperglycemic agent *in vitro*.

Materials and methods

Materials

Curcuma aeruginosa Roxb. rhizome powder was sourced from an e-commerce company in Bekasi, West Java, Indonesia. Kombucha starter culture was obtained from a supplier in Bogor, West Java, Indonesia, and sucrose was purchased from a local supermarket.

Moisture content analysis

By strictly adhering to the AOAC standards [20], the triplicate gravimetric method was used to assess the moisture content. Initially, an empty dish was weighed after drying in an oven at 105 ℃ for 15 min and then cooled in a desiccator for another 15 min. Next, 2.0 g of 60-mesh black turmeric rhizome powder was added to the pre-weighed dish, which was then dried at 105 ℃ for 4 h and cooled in a desiccator for 30 min before weighing. Two drying cycles were performed to obtain a constant weight of the sample. The moisture content was calculated using the following formula.

$$\text{Moisture content (\%)} = \frac{((A+B)-C)}{B} \times 100\% \quad (1)$$

where A = weight of the empty dish (g), B = weight of the test sample (g), C = weight of the dish containing the sample after drying (g).

Extraction and fermentation of kombucha

Black turmeric rhizome powder was extracted in duplicate using an infusion method [13]. The process began by weighing 1.0 g of rhizome powder and boiling it in 250 mL of H_2O at 90 °C for 15 min. The extract was then cooled and filtered through filter paper. The first filtrate (230 mL) was used for non-fermentation treatment and adjusted to reach a final volume of 250 mL with the addition of water. The second filtrate (235 mL) was prepared for fermentation by adding water to a final volume of 250 mL. Subsequently, 10% (w/v) sucrose, SCOBY culture sheet, and 10% (v/v) starter liquid were added to the mixture. Kombucha was fermented in a container covered with gauze at 25 - 30 °C for 10 days. After fermentation, the SCOBY culture was removed, and the kombucha filtrate was filtered to remove the residual culture and then subjected to further analysis. Fermentation was conducted in duplicate. In addition, freeze-dried filtrates of non-fermentation treatment (0.1 g of dry powder) and fermentation treatment (12.8 g of dry powder) were prepared for the analysis of total phenolic and flavonoid contents.

Acidity (pH) and Total Titratable Acidity (TTA) analyses

The pH and total titratable acidity (TTA) of the kombucha were analyzed in triplicate [21]. The pH of the kombucha was measured using a calibrated pH meter. For TTA determination, a titrimetric method was used, in which 5 mL of each fermented and unfermented sample was diluted with distilled water to a total volume of 50 mL and homogenized. Subsequently, 10 mL of the sample was pipetted into an Erlenmeyer flask, followed by the addition of 2 - 3 drops of a 1% phenolphthalein indicator. The titration endpoint was reached by slowly adding a 0.1 N NaOH solution until a stable pink color appeared. The total titratable acidity was calculated using the following formula.

$$\text{Total Titrable Acidity (\%)} = \frac{(V1 \times N \times B)}{(V2 \times \text{DF})} \times 100\% \quad (2)$$

where

V_1 = volume of NaOH (mL)
V_2 = volume of the sample (mL)
N = normality of NaOH (0.1 N)
B = equivalent weight of acetic acid (60)
DF = dilution factor.

Total Phenolic Content (TPC) analysis

To measure TPC, 20 µL of fermented and unfermented black turmeric (4,000 µg/mL) was added to 100 µL of 10% Folin-Ciocalteu reagent. After homogenization and incubation for 5 min at room temperature, 80 µL of 10% Na_2CO_3 solution was added, and the mixture was left to stand for 30 min. The absorbance of the samples was measured using a spectrophotometer at 750 nm. The TPC was calculated based on the gallic acid standard equivalents. TPC analysis was conducted on the filtrates and freeze-dried samples.

Total flavonoid content (TFC) analysis

For the determination of total phenolic content, fermented and unfermented black turmeric (4,000 µg/mL) were prepared as follows: 2 mL of the filtrate

was mixed with 2 mL of MeOH and 1 mL of DMSO to achieve a final ratio of MeOH:H_2O (2:2:1 v/v). To measure the total flavonoid content, 20 µL of the sample was combined with 20 µL of $AlCl_3$, 20 µL of 1 M potassium acetate, and 180 µL of MeOH:H_2O (2:2:1 v/v). The samples were then incubated for 30 min at room temperature. Absorbance was measured at 430 nm using a spectrophotometer. The total flavonoid content was expressed as quercetin equivalents. TFC analysis was conducted on the filtrates and freeze-dried samples.

Curcuminoid content analysis

The curcuminoid content in unfermented and fermented black turmeric was analyzed using Ultra-Fast Performance Liquid Chromatography (UFLC Shimadzu, Japan). The sample was diluted to 80 µg and filtered using a 0.45 µm microfilter (CHROMAFIL Nylon, Macherey-Nagel, Germany), and 20 µL was injected into the UFLC system. Separation was performed using a Shimadzu VP-ODS C-18 column (250×4.6 mm) with an isocratic mobile phase of 2% glacial acetic acid (A) and acetonitrile (B), with 65% of phase B for 15 min. The flow rate was set at 0.8 mL/min, with a column temperature of 40 °C. Curcuminoid standards were prepared at a concentration of 100 µg/mL and treated using the same procedure. Curcumin, bisdemethoxycurcumin, and demethoxycurcumin were detected at wavelengths of 190 - 800 nm using a diode array detector. The curcuminoid content was determined based on the calibration curve of each compound, with the coefficient of determination (R^2), limit of detection (LoD), and limit of quantification (LoQ) as follows: R^2 = 0.9963, LoD = 13.37 ppm, and LoQ = 44.55 ppm for curcumin; R^2 = 0.9932, LoD = 18.10 ppm, and LoQ = 60.32 ppm for bisdemethoxycurcumin; and R^2 = 0.9946, LoD = 16.05 ppm, and LoQ = 53.50 ppm for demethoxycurcumin.

Determination of antioxidant activity (IC_{50}) and antioxidant capacities

Antioxidant activity and capacity measurements were performed on unfermented and fermented black turmeric using ascorbic acid as a standard reference compound. To evaluate the antioxidant activity using the DPPH method, the samples were diluted with distilled water to obtain concentrations of 12.5, 25, 50, 100, 200, 400, and 800 µL/mL. Following the procedure outlined by Rale *et al*. [22], 100 µL of each sample was mixed with 100 µL of DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent. After incubation at room temperature in the dark for 30 min, the absorbance of each sample was measured using a nanospectrophotometer (BMG Labtech, Germany) at 523 nm. The absorbance values were used to calculate the percentage of inhibition and derive the regression equation, which was subsequently used to determine the IC_{50} value. The percentage inhibition was calculated, and the IC_{50} value was determined. The percentage of inhibition was calculated using the following formula.

$$\text{Inhibition (\%)} = \frac{AB-AS}{AB} \times 100\% \quad (3)$$

where

A_B = absorbance of blank

A_S = absorbance of the sample.

The samples were diluted to 1,500 µL/mL for antioxidant capacity evaluation using the ABTS, FRAP, and CUPRAC methods. The determination of inhibition by the ABTS and FRAP methods was conducted by pipetting 20 µL of each sample and adding 180 µL of ABTS or FRAP reagent. The samples were then incubated for 6 min in the dark at room temperature (18 - 20 °C). Prior to the measurements, the samples were homogenized for 15 s. The absorbance of each sample was measured at 734 or 593 nm for the ABTS or FRAP method, respectively. Antioxidant capacity was measured using the CUPRAC method by pipetting 50 µL of the sample into a 96-well microplate and adding 50 µL of $CuCl_2.6H_2O$ 10^{-2} M, 50 µL of neocuproine 7.5×10^{-3} M, and 50 µL of ammonium acetate buffer pH 7. The samples were then incubated for 30 min in the dark at room temperature (18 - 20 °C). The absorbance of the samples was measured at 450 nm. Each experiment was conducted in triplicate. The antioxidant capacity was calculated from the absorbance values of the samples using the following formula.

$$\text{Antioxidant capacity (µmol AAEAC/L)} = \frac{\text{\% of inhibition} - \text{a}}{\text{b}} \quad (4)$$

where a and b are the coefficients of the standard curve equation ($y = ax + b$).

The antioxidant capacity was determined based on the standard curve, with the linear equation and coefficient of determination (R^2) as follows: ABTS: $y = 0.0063x + 0.0008$, $R^2 = 0.9967$; FRAP: $y = 0.0147x + 0.0444$, $R^2 = 0.9931$; CUPRAC: $y = 0.0007x + 0.0646$, $R^2 = 0.9941$.

Determination of *α*-glucosidase inhibitory activity

Unfermented and fermented black turmeric were dissolved in distilled water to obtain concentrations of 12.5, 25, 125, 250, and 1,000 µL/mL. Each concentration was added to a 96-wells microplate containing phosphate buffer and *p*-NPG substrate, followed by the addition of 25 µL of the enzyme solution (containing 0.32 U/mL enzyme), as specified in **Table 1**.

After incubation for 30 min at 37 °C, 100 µL of 0.2 M Na_2CO_3 was added to each well. Absorbance was measured at 410 nm using a spectrophotometer [23]. Each concentration was tested in triplicate. Acarbose was used as a positive control for standard inhibitors. The enzymatic reaction was indicated by the formation of yellow products. Inhibitory activity was calculated using the following equation.

$$\text{Inhibitory activity (\%)} = \frac{ANC - ASC}{ANC} \times 100\% \quad (5)$$

where

A_{NC} = corrected absorbance of negative control (N-BN)

A_{SC} = corrected absorbance of sample (S-BS).

Table 1 Composition of the reagent solutions for *α*-glucosidase inhibitory activity assay.

	Volume of reagent (in µL)			
Type of reagent	**BN**	**N**	**BS**	**S**
Phosphate buffer 0.1 M, pH 7	75	50	50	50
p-NPG	25	25	25	25
Sample	-	-	10	10
H_2O	10	10	-	-
Enzyme	-	25	-	25
Incubation for 30 min at 37 °C				
Na_2CO_3 0.2 M	100	100	100	100
Total	210	210	210	210

Note: BN = Negative control blank (system without enzyme and extract); N = Negative control (mixture with enzyme, without extract); BS = Sample blank (mixture without enzyme, with extract); S = Sample (mixture with both enzyme and extract).

Data analysis

The mean values of the measurements, calculations, and statistical analyses were conducted using Microsoft ® Excel ® for Microsoft 365 MSO (Version 2512) and IBM SPSS Statistics 27. A paired *t*-test at a 95% significance level was used to compare the analytical results between the unfermented and fermented black turmeric rhizomes. Correlation analysis was conducted between total phenolic content, total flavonoid content, and curcumin content and the IC_{50} of DPPH antioxidant activity and antioxidant capacities (ABTS, FRAP, and CUPRAC) at a 99% significance level.

Results and discussion

Moisture content and yield of extract

The average moisture content of black turmeric rhizome simplicia was 9.10 ± 0.12% (w/w), meeting the standard of <10% for good quality of the simplicia [24]. This low moisture level inhibits microbial growth and prevents the degradation of active compounds by microbial enzymes. Moisture content is also a correction factor for determining the extract yield. The yield from

the unfermented black turmeric aqueous extract was 15.36% (w/w), which aligns with the Indonesian Pharmacopoeia standard of ≥13.9% [24].

pH and Total Titratable Acidity (TTA) of kombucha

The most important parameter for the microbiological safety of kombucha is pH, which must be in the range of 2.5 - 4.2 [3]. The 10-days fermentation of kombucha made from black turmeric significantly lowered pH ($p < 0.05$) (**Figure 1(a)**).

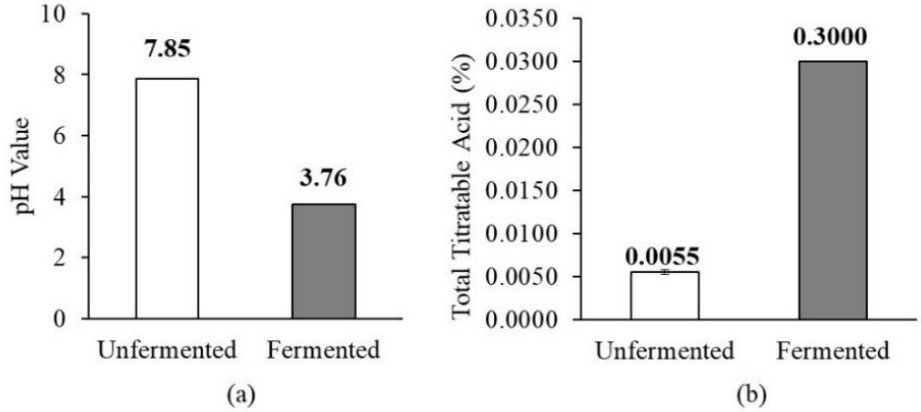

Figure 1 (a) pH values, and (b) total titratable acidity of unfermented and fermented black turmeric. Values are presented as mean ± standard deviation.

A significant increase in total acidity was observed during fermentation ($p < 0.05$) (**Figure 1(b)**). The total acidity was assessed as the total titratable acidity (TTA), which captures both the dissociated and undissociated acids. An increase was also reported in turmeric kombucha, where the TTA of the unfermented one was not detected, and that of the fermented one was 0.21% [13]. A study on the microbial identification of Indonesian SCOBY using Next-Generation Sequencing reported that the most abundant bacteria were *Komagataeibacter*, a known genus of AAB. AAB in SCOBY are more dominant than LAB [27]. During kombucha fermentation, the growth of acetic acid and lactic acid bacteria increases the organic acid content, which lowers the pH and increases the TTA.

Total phenolic and flavonoid contents (TPC and TFC)

As shown in **Figure 2(a)**, kombucha fermentation increased the TPC of black turmeric, which was significantly higher ($p < 0.05$) than that of unfermented black turmeric. The increase in phenolic content due to kombucha fermentation is closely associated with the enzymatic activity of yeast and bacteria in the SCOBY. Enzymes in SCOBY, such as phenol oxidase from LAB, convert complex phenolic compounds into simple free phenolic compounds. Moreover, LAB have been reported to exhibit β-glucosidase activity, which hydrolyzes phenolics and flavonoids by cleaving sugar groups, thereby generating active aglycones [28]. A similar increase in TPC due to fermentation has been observed in kombucha prepared from turmeric rhizome extract [13,14].

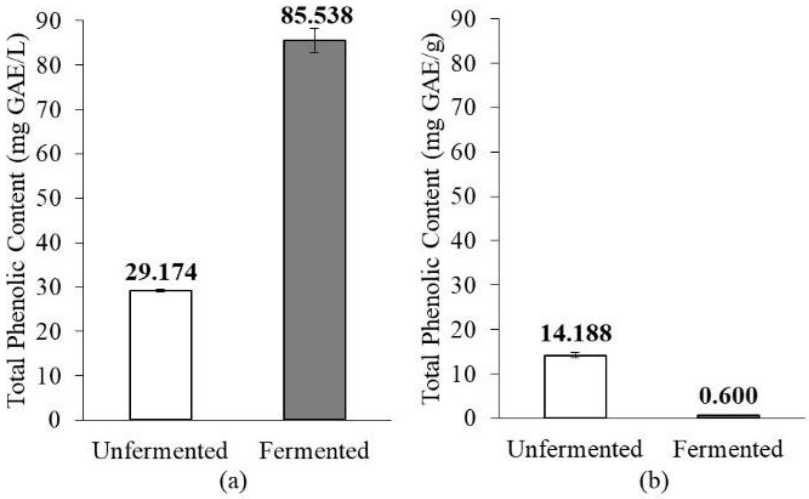

Figure 2 Total phenolic content of black turmeric rhizome extract and black turmeric kombucha. (a) Liquid and (b) Freeze-dried. Values are presented as mean ± standard deviation.

Our study (**Figure 3(a)**) indicates that fermentation can significantly enhance TFC ($p < 0.05$). A positive impact of fermentation on flavonoid content has also been reported in kombucha from Telang flower tea, which increased from 0.045% to 0.072% [27]. The increase in flavonoid levels is likely attributable to biotransformation processes mediated by microbial enzymes in kombucha, which can enhance the biological activity of flavonoids [8].

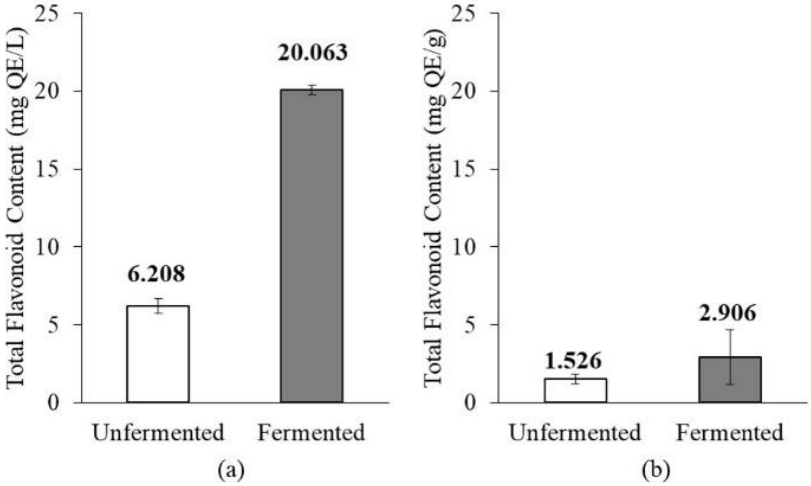

Figure 3 Total flavonoid content of black turmeric rhizome extract and black turmeric kombucha. (a) Liquid and (b) Freeze-dried. Values are presented as mean ± standard deviation.

Figures 2(b) - 3(b) show that freeze-drying treatment decreased the TPC and TFC of both unfermented and fermented black turmeric. This reduction suggests the degradation or loss of certain phenolic compounds due to freeze-drying, which allegedly resulted from the rapid pressure changes involved in the process [29]. Degraded phenolic compounds may be the polar compounds that dissolve in the water. However, there was no control for phenolic or flavonoid compounds used in the freeze-drying process, which is a limitation of this study.

Due to the low TPC and TFC of freeze-dried samples, further analysis of curcuminoid content, antioxidant activity and capacities, and α-glucosidase inhibitory activity was only conducted in liquid samples.

Curcuminoid content

Fermentation using medicinal plants as a medium has been shown to be an effective method for increasing the diversity, abundance, and novelty of active compounds [30,31]. Although curcuminoids are specific to *Curcuma* sp., previous research has identified that the curcuminoid content in 20 accessions of Indonesian black turmeric was relatively low, only 0.01% - 2% [32]. In our study, curcuminoid content analysis showed that curcumin increased after kombucha fermentation (61.307 ppm) compared to unfermented black turmeric (52.184 ppm). Our results were higher than the curcumin content in kombucha fermentation of turmeric, which was 0.73 ± 0.04 mg/L [17]. Conversely, the bisdemethoxycurcumin and demethoxycurcumin contents in kombucha prepared from black turmeric (3.823 and 14.869 ppm, respectively) were lower than those in the unfermented black turmeric (8.685 and 19.131 ppm, respectively).

In our study, kombucha fermentation of black turmeric increased curcumin content, while bisdemethoxycurcumin and demethoxycurcumin content decreased. The changes in curcuminoid content resulting from turmeric (*Curcuma longa* L.) fermentation were summarized by Salve *et al*. [33]. Fermentation of turmeric by *Lactobacillus plantarum*, *L. fermentum*, and *L. johnsonii* increases curcumin, bisdemethoxycurcumin, and demethoxycurcumin content, whereas fermentation by *Saccharomyces cerevisiae* increases curcumin content [33]. Metagenomic analysis of SCOBY and kombucha identified *Lactobacillus* sp. and *S. cerevisiae* [34]. Those two studies hypothetically explained the increase in curcumin content in black turmeric kombucha due to the activity of bacteria and yeast in this study. In contrast, another study reported that kombucha fermentation of *Curcuma longa* resulted in a significant decrease in curcumin, bisdemethoxycurcumin, and demethoxycurcumin [13]. Conjugated curcuminoids, such as curcumin monoacetate, curcumin monoglucoside, and curcumin glucuronide, were detected in Javanese turmeric kombucha, whereas these compounds were not detected in the unfermented one [13]. The microorganisms in SCOBY may metabolize curcuminoids and cause a decrease in curcuminoid content [35]. In this study, the decrease in bisdemethoxycurcumin and demethoxycurcumin content in black turmeric kombucha might be because of the biotransformation by yeast and bacteria; however, further study is needed to prove this hypothesis.

Antioxidant activity and capacities

Antioxidant activity was measured using the DPPH method, and the results were expressed as IC_{50} values. Lower IC_{50} values indicate stronger antioxidant activity. As shown in **Table 2**, the IC_{50} of black turmeric after fermentation significantly decreased ($p < 0.05$), indicating enhanced antioxidant activity. The IC_{50} of ascorbic acid was used as a benchmark and was not potentially equivalent to that of the samples. These findings demonstrate that kombucha fermentation effectively boosts antioxidant activity. Other studies on the fermentation of Javanese turmeric using kombucha also reported a lower IC_{50} than that without fermentation [13,14].

Table 2 Antioxidant activity of fermented and unfermented black turmeric.

Sample	IC_{50} (µg/mL)
Unfermented black turmeric	34.46 ± 0.34
Fermented black turmeric	5.98 ± 0.22
Ascorbic acid	13.91 ± 1.50

The antioxidant capacities of unfermented and fermented black turmeric at a concentration of 76.8 µg/mL, determined using the ABTS, FRAP, and CUPRAC methods, are shown in **Figure 4**. Kombucha fermentation of black turmeric rhizome extract significantly increased antioxidant capacities evaluated by all three methods ($p < 0.05$). Kombucha from black tea with table sugar showed a significant increase in antioxidant capacity by ABTS from days 7 to 14 of fermentation, while the antioxidant capacity of FRAP was higher on day 7 than on day 14 of fermentation [6]. In our study, the increase in antioxidant activity and

capacity after fermentation of black turmeric extract was significantly correlated ($p < 0.01$) with the elevated total phenolic and flavonoid content of kombucha. This correlation is in line with a previous finding that the total phenolic and flavonoid contents of black and green tea kombucha fermented for 7 days were correlated with DPPH and FRAP activities [6]. Different antioxidant mechanisms, such as the scavenging activity of hydrophilic and lipophilic antioxidant compounds in ABTS and CUPRAC compared to only hydrophilic antioxidant compounds in FRAP [36], might affect the quantification of activities.

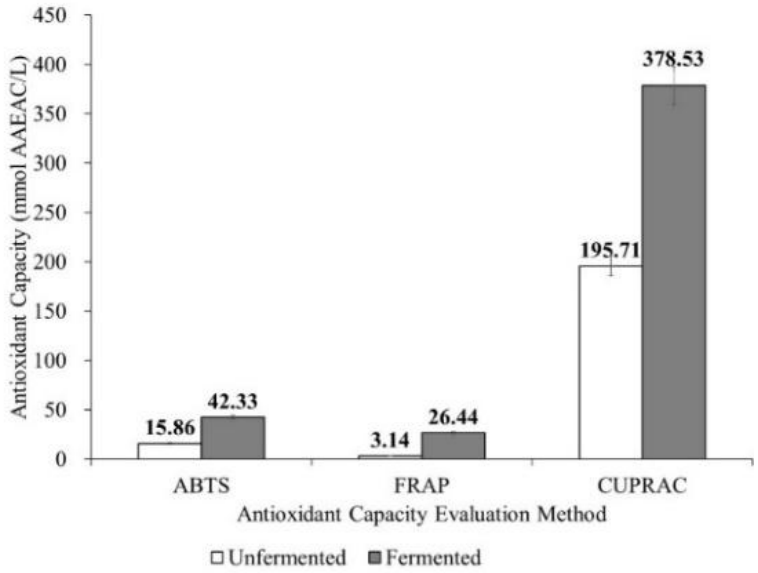

Figure 4 Antioxidant capacities of black turmeric rhizome extract and black turmeric kombucha evaluated by ABTS, FRAP, and CUPRAC methods.

Several phenolic and flavonoid compounds play critical roles in antioxidant activity. This role is largely attributed to the presence of functional hydroxyl groups in the phenolics of black turmeric kombucha, which can donate electrons or hydrogen atoms to neutralize free radicals and produce more stable compounds. Curcumin is also known to exhibit antioxidant activity [37]. The observed increase in curcumin content in black turmeric kombucha, which was significantly correlated ($p < 0.01$), may be contributed to the increase in antioxidant activity and capacity. In a previous study, kombucha prepared from a combination of black tea and black turmeric demonstrated antioxidant potential with an IC_{50} value of 13.17 ppm [38]. This difference in potency may be attributed to the different base ingredients used in each type of kombucha. When two types of herbs, each containing unique bioactive compounds, are combined, the interactions between these compounds may produce a synergistic effect, enhancing their overall bioactivity [15]. Increasing the turmeric concentration in kombucha results in higher antioxidant activity [38].

α-Glucosidase inhibitory activity

The initial screening for α-glucosidase inhibition was performed using two sample concentrations. As shown in **Table 3**, kombucha prepared from black turmeric exhibited higher α-glucosidase inhibitory activity than unfermented black turmeric (**Table 4**). The IC_{50} for unfermented black turmeric could not be calculated because its maximum tested concentration (51.20 µg/mL) achieved only 33.81% inhibition, falling short of the 50% threshold required for IC_{50} determination. The IC_{50} value of the positive control, acarbose, was 0.09 ± 0.01 µg/mL.

Table 3 Preliminary screening of *α*-glucosidase inhibitory activity of fermented and unfermented black turmeric.

Sample	Concentration (µg/mL)	*α*-Glucosidase inhibitory activity (%)
Negative control (-)	0	0.00
Unfermented black turmeric	1.28	8.54 ± 3.72
	12.80	19.38 ± 1.81
Fermented black turmeric	1.28	12.60 ± 3.20
	12.80	47.67 ± 3.52

Table 4 IC_{50} of the *α*-glucosidase inhibitory activity of fermented and unfermented black turmeric.

Sample	IC_{50} (µg/mL)
Unfermented black turmeric	N/A
Fermented black turmeric	9.41 ± 0.76
Acarbose	0.09 ± 0.01

The results of this study indicated that the bioactive components of non-fermented black turmeric showed weaker inhibition of *α*-glucosidase. This might be attributed to the low concentration or efficacy of the active compounds in the extracts. Furthermore, the tested extract remained in the form of a crude solution, suggesting that it may contain additional components without any inhibitory effect on α-glucosidase. Fermentation enhanced *α*-glucosidase inhibitory activity, as evidenced by the lower IC_{50} value of the fermented extract. In this study, undiluted black turmeric kombucha exhibited the maximum inhibition of *α*-glucosidase (84.76%), although this value was still lower than that of undiluted sea grape (*Caulerpa racemosa*) kombucha filtrate, which showed 90.42% inhibition [39].

The increase in the total phenolic, total flavonoid, and curcumin content of black turmeric kombucha was hypothetically contributed to the increase in *α*-glucosidase inhibitory activity. Correlation analysis could not be performed because the IC_{50} of unfermented black turmeric could not be calculated. A review reported that curcumin may reduce fasting blood glucose levels, improve insulin sensitivity, and regulate lipid profiles in humans and animals with diabetes [37]. Fermentation of turmeric effectively improved insulin sensitivity and reduced blood glucose levels in diabetic rats compared to non-fermented turmeric [33]. Another study on the bioactive compound analysis of kombucha from black tea and black turmeric using LC-HRMS identified sesquiterpenoids as the most abundant components [38], which may exert potential antidiabetic effects that were not analyzed in this study.

The *in vitro* results of TPC, TFC, curcumin content, antioxidant activity and capacities, as well as the *in vitro α*-glucosidase inhibition, suggest the potency of black turmeric kombucha as a functional food. However, the present study has several limitations, such as the fermentation conditions and curcuminoid content analysis. Kombucha fermentation in this study was only conducted under one condition, at 25 - 30 °C for 10 days. Hence, the results of this study can be further explored by optimizing fermentation conditions, such as temperature and incubation duration. Curcuminoid content analysis, which showed a decrease in bisdemethoxycurcumin and demethoxycurcumin content after fermentation, can be confirmed with more specific analysis, such as LC-MS/MS, for more information on the results of compound biotransformation.

Conclusions

Curcuma aeruginosa rhizome extract fermented by SCOBY into kombucha showed significantly higher total phenolic, flavonoid, and curcumin contents than the unfermented extract. This increase was correlated with improved antioxidant activity as well as the antioxidant capacities by ABTS, FRAP, and CUPRAC. This increase was also in line with the increase in the *in vitro α*-glucosidase inhibitory activity of black turmeric kombucha. After fermentation, the bisdemethoxycurcumin and demethoxycurcumin

contents decreased, but the possibility of biotransformation needs to be confirmed. These results indicate that kombucha fermentation enhances the antioxidant and *in vitro* antihyperglycemic effects of black turmeric. Further *in vivo* validation is required to ascertain the potential of black turmeric kombucha as a functional food.

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Declaration of Generative AI in Scientific Writing

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CRediT Author Statement

Uswatun Hasanah: Formal Analysis; Visualization; Writing – Original draft preparation; Writing - Reviewing and Editing. **Lelly Aulia Damarhati:** Investigation; Formal Analysis; Methodology; Visualization. **Sulistiyani:** Data curation; Supervision. **Sera Budi Verinda:** Data curation; Supervision. **Syaefudin Suminto:** Conceptualization; Methodology; Writing - Original draft preparation, Writing - Reviewing and Editing; Supervision.

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