

FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF *Camellia sinensis* KOMBUCHA ETHYL ACETATE EXTRACT AND CREAM FORMULATION

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ABSTRACT

Kombucha, a fermented tea beverage made using a symbiotic culture of bacteria and yeast (SCOBY), is gaining interest due to its bioactive compounds, particularly flavonoids, which are known for their antioxidant properties. These natural antioxidants, derived from the tea leaves of *Camellia sinensis*, may offer functional benefits when applied in topical formulations such as creams. This study aimed to determine the flavonoid concentration in kombucha extracts after fermentation for 7 and 14 days and to evaluate the antioxidant activity of a cream formulation containing kombucha extract. Kombucha extract was obtained using a liquid-liquid extraction method with ethyl acetate as the solvent. Antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, where a lower IC₅₀ value indicates stronger antioxidant activity. The results showed that The total flavonoid content in the kombucha extract fermented for 7 days (H7) was 125.79 ± 0.192 mg QE/g, while the extract fermented for 14 days (H14) contained 35 ± 0.333 mg QE/g. Antioxidant activity testing revealed IC₅₀ values of 6.84 for the H7 extract and 7.48 for the H14 extract, indicating that the 7-day fermented kombucha had a higher antioxidant potential. These findings suggest that fermentation duration significantly affects the flavonoid content and antioxidant capacity of kombucha extracts, which may influence their efficacy when incorporated into topical formulations, such as creams. This study highlights the potential of kombucha extracts as natural antioxidant agents for cosmetic and pharmaceutical applications.

Keywords: antioxidant, *Camellia sinensis*, flavonoid, kombucha, SCOBY

INTRODUCTION

In recent years, there has been a growing awareness among the public regarding skin health, prompting the increased use of natural and synthetic skincare products. One natural product that has gained popularity is kombucha, a fermented tea beverage that originated in China. Kombucha is produced by fermenting tea using a symbiotic culture of bacteria and yeast, known as SCOBY (Symbiotic Cultures of Bacteria and Yeasts) (Purnami *et al.*, 2018). When SCOBY is combined with a sugared tea solution, fermentation begins, leading to the formation of various new bioactive compounds. In addition to organic acids and beneficial microorganisms, kombucha contains minerals derived from tea, such as potassium, manganese, and fluoride ions, vitamins including E, K, and B, and amino acids such as tannins and glutamine derivatives. The phenolic content in kombucha increases during fermentation due to the increasingly acidic environment and enzymatic stimulation by bacteria and yeast (Jakubczyk *et al.*, 2020).

The tea used in kombucha fermentation is derived from *Camellia sinensis* and serves as the main substrate in the process. Different manufacturing processes produce different types of tea, with green tea being one of the most notable types. Green tea is made from fresh leaves and retains a higher concentration of polyphenols, particularly catechins, resulting in a broader range of functional compounds than other types of tea (Miranda *et al.*, 2021). Catechins and tannins are among the most common polyphenolic compounds in tea, contributing to its bitterness, astringency, and subtle sweetness. Polyphenols are plant-based compounds that contain at least one phenolic ring in their structure and are known for their strong antioxidant properties. These compounds play a crucial role in protecting the body from damage caused by reactive oxygen species (ROS) and possess anti-inflammatory, antimicrobial, antihypertensive, and anticancer

activities (Feresin *et al.*, 2017). The semi-polar nature of kombucha polyphenols makes ethyl acetate an ideal solvent, as it selectively extracts compounds with high antioxidant potential while leaving behind highly polar or non-polar impurities (Bhattacharya *et al.*, 2016).

Antioxidants function as inhibitors of oxidation processes and serve as natural defense mechanisms in the body to neutralize free radicals. However, antioxidant levels in the body can decline due to factors such as bacterial or viral infections, chronic inflammation, and aging (Andarina and Djauhari, 2017). Exposure to free radicals is a primary contributor to the premature aging process. In Indonesia, approximately 57% of women report noticing signs of premature aging by the age of 25 years (Cahyani *et al.*, 2022). Free radicals are unstable molecules with unpaired electron. When these molecules actively interact with negatively charged electrons in the body, they can damage cell membranes, lipids, and DNA, leading to cellular dysfunction and homeostasis disruption (Haerani *et al.*, 2020).

The rising awareness of premature aging has led to the increased use of cosmetic products designed to combat free radical damage. Creams are one of the most widely used topical formulations. Creams are semi-solid emulsions that contain no less than 60% water and are intended for external use (Kementerian Kesehatan Republik Indonesia 1979). In this study, a water-in-oil (W/O) cream formulation was selected because of its suitability for skin application and its ability to enhance the concentration gradient of active ingredients, thereby improving percutaneous absorption and optimizing the therapeutic effect (Isrul *et al.*, 2023). The selection of cream over gel or emulgel preparations is based on its superior moisturizing and occlusive properties, which help maintain skin hydration, an important factor in preventing premature aging. In addition, W/O creams are more effective in delivering lipophilic active compounds than gels or emulgels, which are predominantly aqueous and tend to evaporate more quickly. The rich and emollient texture of creams also provides a more pleasant sensory experience and longer skin contact time, allowing for better absorption and enhanced efficacy of active ingredients.

This study explored the potential use of green tea kombucha (*Camellia sinensis* L.) extract as a natural antioxidant in cream formulations. The study specifically aimed to determine the flavonoid concentration in kombucha extracts after 7 and 14 days of fermentation and to evaluate the antioxidant activity of cream formulations containing the extract. The outcomes of this study are expected to provide beneficial information for academic purposes, contribute to the author's knowledge of the antioxidant benefits of kombucha, and offer insight to readers regarding the application of green tea kombucha extract in topical skincare products.

RESEARCH METHODS

Equipment and Materials

The equipment used in this study included stirring rods (Iwaki, Japan), beaker glasses, glass funnels, separatory funnels, measuring glasses, hot plates (Jouan Lab, China), volumetric flasks, refrigerators, magnetic stirrers (Jouan Lab, China), ovens (Mettler, Germany), pH meters (Ametek, USA), dropper pipettes, rotary evaporators (IKA RV 10, Germany), spatulas, stands and clamps, UV-Vis spectrophotometers (Shimadzu, Japan), analytical balances (Shimadzu, Japan), glass jars, and Brookfield viscometers (Dahomey Meter, China).

The materials used were distilled water, aluminum foil, 10% AlCl₃, stearic acid, sugar, ethyl acetate, glycerin, quercetin, kombucha starter culture (Live SCOBY, Indonesia), methanol, methylparaben, 20% anhydrous sodium acetate, propylparaben, rose oil, DPPH powder, triethanolamine, green tea leaves (*Camellia sinensis* L.), and vitamin E (Jakubczyk *et al.*, 2020; Angelia *et al.*, 2022; Kambel *et al.*, 2022).

Research Procedure

This study was conducted at the Pharmaceutical Laboratory of Universitas Muhammadiyah Bandung, West Java, Indonesia. The procedures mainly consist of kombucha extraction, cream formulation, and determination of flavonoid content and antioxidant activity.

1. Preparation and Extraction of Kombucha

Green tea leaves (*Camellia sinensis* L.) were sourced from the Pangalengan tea garden and authenticated at the Plant Taxonomy Laboratory in the Department of Biology, Faculty of

Mathematics and Natural Sciences, Universitas Padjadjaran, Indonesia, with the registration number No. 45/HB/07/2024. . The kombucha starter culture (SCOBY) containing *Acetobacter xylinum*, *Gluconobacter* sp., and *Saccharomyces cerevisiae* was obtained online and refrigerated (Jakubczyk *et al.*, 2020). Kombucha tea was prepared by dissolving 150 g sugar in 1 L hot water, adding 50 g powdered green tea leaves, heating for 10 minutes, cooling, filtering, and fermenting with SCOBY at room temperature for 7 and 14 days (Purnami *et al.*, 2018). Ethyl acetate extraction of kombucha samples from days 7 and 14 involved liquid-liquid extraction twice with equal volumes of ethyl acetate, followed by evaporation under reduced pressure at 62°C and 50 rpm. The concentrated extract was stored in a refrigerator for at least 24 hours (Rodríguez *et al.*, 2022).

2. Flavonoid Content Analysis

A standard quercetin solution (1000 ppm) was prepared by dissolving 25 mg of quercetin in 25 mL of 96% ethanol and then diluted to 50–800 ppm to generate a calibration curve. Each standard was reacted with AlCl_3 , sodium acetate, methanol, and distilled water, incubated for 30 minutes, and measured at maximum absorbance between 400 and 500 nm using UV-Vis spectrophotometry (Lindawati and Ma'ruf, 2020). The analysis was performed in triplicate, and the absorbance and concentration values were consistent across the three replicates for each sample. Samples (5 mg in 10 mL 96% ethanol) were treated similarly, and flavonoid concentration was calculated using the following linear calibration curve:

$$y = a + bx.$$

y = Absorbance

a = constant

x = Concentration (ppm)

b = Slope

After the concentration was determined, the percentage of flavonoid content was calculated using the following formula (Gaissman, 1962; Ramonah *et al.*, 2020):

$$\%flavonoid = \frac{C \times V \times Fp}{W}$$

C = Concentration of flavonoid in sample solution (g/mL)

V = Sample volume (mL)

Fp = Dilution Factor

W = the weight of sample (g)

3. Cream Formulation of Kombucha Extract

The cream formulations contained 3% kombucha extract (on days 7 or 14) with paraffin liquid (5%), cetyl alcohol (2%), vitamin E (0.01%), triethanolamine (4%), glycerin (25%), stearic acid (13%), rose oil (0.05%), methylparaben (0.1%), propylparaben (0.1%), and distilled water (to 100 %). The oil phase (paraffin liquid, cetyl alcohol, rose oil, stearic acid, vitamin E, and propylparaben) was heated, and the aqueous phase (triethanolamine, methylparaben, glycerin, and distilled water) was separately heated to 80°C. The aqueous phase was gradually added to the oil phase with homogenization at 1000 rpm, followed by the incorporation of the extract (Kambel *et al.*, 2022).

4. Antioxidant Activity Test Using DPPH Method

DPPH stock solution (100 ppm) was prepared by dissolving 10 mg of DPPH in 100 mL of methanol and diluting it to 40 ppm. Blank and sample solutions were incubated with DPPH in the dark for 30 minutes and then measured at 515 nm using UV-Vis spectrophotometry. Vitamin C standards and extract solutions were prepared at varying concentrations for calibration and activity testing. The percentage inhibition of DPPH radical scavenging was calculated, and IC_{50} values were determined from the inhibition curves (Angelia *et al.*, 2022).

5. Evaluation of Cream Formulation

The organoleptic properties (color, odor, and texture) were assessed visually (Nurjanah *et al.*, 2019). Homogeneity was checked microscopically for the particle distribution (Tungadi *et al.*, 2023). The pH was measured using a pH meter and was expected to range between 4.5–8 to suit the skin pH (Liony, 2014). Viscosity was measured using a Brookfield viscometer (2500–5000 cPs is ideal for creams) (Erwiyani *et al.*, 2018). Spreadability was evaluated by measuring the diameter of a 0.5 g cream sample spread under a 125 g weight, with 5–7 cm considered acceptable (Tungadi *et al.*, 2023). Adhesion was tested by timing how long the cream adhered to the skin (>4 seconds is good) (Parwanto *et al.*, 2016). Stability was assessed using freeze–thaw cycles (4°C, room temperature, and 45°C) to observe physical changes after three cycles (Lamsari *et al.*, 2023; Kementerian Kesehatan Republik Indonesia, 2020).

Data Analysis

Data were analyzed descriptively and by paired t-test using SPSS software to compare means and test hypotheses about the paired samples.

RESULTS AND DISCUSSION

Ethyl acetate, a semi-polar solvent, effectively extracts flavonoid compounds while maintaining their stability. A total of 1 liter of kombucha was used, with 2 liters of ethyl acetate solvent. The obtained ethyl acetate fractions were 1,750 mL for H7 and 1,880 mL for H14. The fractions were then concentrated using a rotary evaporator and evaporated using a water bath to remove the residual solvent. The concentrated extracts were brownish in color. The yields of the concentrated extracts were 5.01 g for H7 and 13.42 g for H14.

The total flavonoid content of the kombucha extracts was determined using UV-visible spectrophotometry, which is suitable for compounds with chromophores and conjugated aromatic systems, such as flavonoids (Ni'ma and Lindawati, 2022). The colorimetric method used AlCl_3 as a reagent and quercetin as the standard, forming a yellow complex with a maximum absorbance at 427 nm and 0.994 absorbance units. A quercetin calibration curve was constructed using concentrations of 50, 200, 400, and 600 ppm. The regression equation obtained was $y = 0.0006x + 0.0703$, with a correlation coefficient (r) of 0.9934, indicating a strong linear relationship between the two variables. The H7 extract showed an average flavonoid content of 125.79 ± 0.192 mg QE/g, whereas the H14 extract contained significantly lower flavonoid levels, averaging 35 ± 0.333 mg QE/g. These results indicate that the H7 extract had a higher flavonoid content than H14. These results indicate that the flavonoid content in H7 was significantly higher than that in H14. This contrasts with the findings of Jakubczyk *et al.* (2020), who reported that the polyphenol content, including flavonoids, typically increases during fermentation. The decline in H14 may be due to acid degradation, high temperatures, prolonged microbial activity, or other environmental factors during fermentation (Gaggia *et al.*, 2019; Wardaniati and Taibah, 2019). Previous studies have shown that organic acid and monosaccharide concentrations increase during fermentation, which is affected by temperature, fermentation duration, SCOBY, and other factors. Differences in concentration can result from the number of acid-producing strains, such as glucuronic acid, whose concentrations increase during fermentation. Glucuronic acid enhances polyphenol bioavailability, antioxidant activity, and catechin levels (Gaggia *et al.*, 2019).

Generally, antioxidant activity increases during fermentation as microorganisms break down phenolic and flavonoid compound bonds, releasing these compounds to act as antioxidants. However, prolonged fermentation allows microorganisms to use the available compounds as substrates, resulting in continued fermentation that increases non-flavonoid phenolic compounds more than total flavonoid compounds. During the early stages of fermentation, microbial enzymes enhance the biotransformation of polyphenols and flavonoids, increasing the release of smaller and more active antioxidant molecules. However, prolonged fermentation and continued microbial metabolism can lead to the degradation or polymerization of these phenolic and flavonoid compounds, thereby reducing their antioxidant capacity. In addition, extended fermentation increases the concentration of organic acids, such as acetic and gluconic acids, which lower the pH of the medium. This acidic environment may cause oxidative degradation of sensitive antioxidant compounds, such as catechins and other flavonoids, naturally present in *Camellia sinensis*.

Moreover, microorganisms may utilize some phenolic compounds as carbon sources during prolonged fermentation, further contributing to the decline in antioxidant activity (Jayabalan *et al.*, 2014). Furthermore, monosaccharides are utilized as energy sources by yeast and bacteria. Their presence stimulates vigorous microbial activity, which can accelerate the conversion, transformation, or degradation of polyphenols by microbial enzymes.

Thus, the decrease in antioxidant activity in H14 compared to H7 is primarily due to the excessive fermentation time, which results in the loss or transformation of key phenolic antioxidants that are most abundant and active at the optimal 7-day fermentation stage. Therefore, the fermentation period is crucial to ensure optimal compound bond breakdown without allowing the compounds to become substrates for microbial growth (Karimi *et al.*, 2017), meaning the balance and interaction between organic acids, monosaccharides, and phenolic compounds play a crucial role in determining the antioxidant activity of fermented products

Table I. Cream Formulation Evaluation

a. Organoleptic and Homogeneity Results			
Evaluation	Before Stability Test		After Stability Test
Color	Cream		Orange
Odor	Rose		Rose
Form	Cream		Cream
Texture	Thick		Thick
Homogeneity	Homogeneous		Homogeneous
b. pH Test Results			
Sample	pH Before (Average)	pH After (Average)	Significance (p)
H7 Cream	7.4 ± 0.056	7.28 ± 0.023	0.026 ^s
H14 Cream	7.65 ± 0.191	7.34 ± 0.056	0.060 ^{ns}
c. Viscosity Test Results (in mPa·s)			
Sample	Before Stability	After Stability	Significance (p)
H7 Cream	2396.3 ± 12.7	4554.3 ± 37.1	0.000 ^s
H14 Cream	2447.0 ± 13	3243.7 ± 18.8	0.001 ^s
d. Adhesion Results (seconds)			
Sample	Before Stability	After Stability	Significance (p)
H7 Cream	4.61 ± 0.155	5.77 ± 0.115	0.001 ^s
H14 Cream	5.73 ± 0.230	6.14 ± 0.237	0.000 ^s
e. Spreadability Test Results (cm)			
Sample	Before Stability	After Stability	Significance (p)
H7 Cream	5.5 ± 0.058	5.6 ± 0.058	0.402 ^{ns}
H14 Cream	6.1 ± 0.231	5.9 ± 0.252	0.402 ^{ns}

^s significant, ^{ns} not significant

The stability testing results are summarized in **Table I**. After the stability testing, the cream revealed a noticeable color change in the cream, shifting from cream to orange after incubation, which suggests oxidation likely caused by exposure to high temperatures (Sahlia *et al.*, 2020). Additionally, ingredient incompatibility—such as interactions between parabens and vitamin E—may also contribute to this discoloration. To prevent such changes, the cream should be stored in airtight, dark containers. Although the pH of both creams slightly decreased following the stability test, they remained within the safe range of 4.5–8. However, the significant pH change observed in H7 cream indicates that it may be more sensitive to temperature or storage conditions (Putra *et al.*,

2016). Viscosity measurements, conducted using a Brookfield Viscometer (Spindle No.4, 60 rpm), showed significant changes in both creams after stability testing, suggesting that they are unstable under extreme temperature conditions. In contrast, the adhesion test demonstrated that both creams maintained good adhesion, with values above 4 s, and even showed significant increases after stability testing, an outcome favorable for prolonged skin contact (Tungadi *et al.*, 2023). Lastly, both creams met the standard for good spreadability, ranging between 5 and 7 cm, with no significant differences observed after stability tests.

Antioxidant activity testing on kombucha extract samples was carried out using the DPPH method with vitamin C as a positive control, as shown in **Table II**. Absorbance measurements of the vitamin C sample and kombucha extract were performed using a UV-vis spectrophotometer at a wavelength of 517 nm. The determination of the maximum wavelength was conducted before measuring absorbance on the samples, with 517 nm providing maximum absorption. Antioxidant activity measurement of the samples was performed at various concentrations. Kombucha extract and vitamin C were prepared in a concentration series of 2, 4, 6, and 8 µg/mL. Based on the results obtained, the antioxidant activity test conducted shows that the IC₅₀ value for vitamin C was 7.89 µg/mL, the IC₅₀ value for kombucha extract H7 was 6.84 µg/mL, and for kombucha extract H14 was 7.48 µg/mL. The IC₅₀ values obtained from the samples indicate that the kombucha extract samples H7 and H14 and vitamin C have strong antioxidant activity, as shown by IC₅₀ values <50 µg/mL (Jumina *et al.*, 2019).

Table II. IC₅₀ Value of Extract and cream

Samples	IC ₅₀ (µg/mL)	Activity
Vitamin C	7.89	Strong
Kombucha extract H7 ^{ab}	6.84 µg/mL	Strong
Kombucha extract H14 ^{ab}	7.48 µg/mL	Strong
Cream H7 ^{ac}	184.16 µg/mL	Moderate
Cream H14 ^{ac}	192.50 µg/mL	Moderate

^aSignificant compared to Vitamin C, ^bSignificant difference compared to other extract (p-value <0.001), ^cSignificant difference compared to other Cream (p-value = 0.003)

Based on the analysis of the ability of kombucha tea to inhibit free radicals, it decreased as the fermentation time increased. The IC₅₀ result for extract H7 increased by 0.64 µg/mL in extract H14, where a higher IC₅₀ value corresponds to lower antioxidant activity. This research aligns with Jakubczyk *et al.* (2020), who stated that among tea types, kombucha made from green tea has the highest antioxidant potential, reaching its peak on the first day of fermentation, and its ability to neutralize free radicals decreases with longer fermentation times. The highest antioxidant activity of kombucha from green tea leaves occurred on the 7th day of fermentation and decreased by the 14th day. This is influenced by the phenolic and flavonoid content, which increases with longer fermentation in a weak acidic solution. The sample solution becomes more acidic owing to bacterial activity, which converts alcohol into various acids, such as citric, gluconic, malic, and lactic acids. Phenolic and flavonoid compounds become stable in a weakly acidic environment, making it difficult for protons that can bind with DPPH to be released. This causes a decrease in antioxidant activity (Gaggia *et al.*, 2019).

Antioxidant testing of kombucha cream preparations showed promising results. Kombucha extract creams were prepared in a concentration series of 50, 100, 150, and 200 µg/mL and measured using a UV-vis spectrophotometer at a wavelength of 517 nm. The antioxidant activity measurements indicated IC₅₀ values of 184.16 µg/mL for kombucha cream extract H7 and 192.50 µg/mL for kombucha cream extract H14. Based on the IC₅₀ values obtained, kombucha cream extract H7 had higher antioxidant activity than kombucha cream extract H14; however, both creams fell into the moderate category with IC₅₀ values >151.

Based on the IC₅₀ results, the difference in the extracts used can influence the antioxidant activity. Kombucha cream extract H7 had a higher IC₅₀ than extract H14 because the extract used

to prepare cream H7 had a higher IC_{50} than that used to prepare cream H14. The antioxidant activity of the kombucha cream extracts was lower than that of the kombucha extracts. This may be caused by the stability of the active compounds within the preparation. Storage factors can affect the release of active compounds from the base during the reaction with DPPH, incubation time during antioxidant testing, and other environmental factors such as light, which can cause oxidation processes leading to decreased antioxidant activity in the preparation (Suhery *et al.*, 2016). This is supported by Septiani *et al.* (2020), who stated that storage factors can influence antioxidant activity, and environmental factors, such as light, can cause oxidation, reducing antioxidant activity in preparations. Additionally, packaging methods can affect the decrease in the antioxidant activity. Poor packaging increases the preparation's contact with the environment, thus lowering the antioxidant levels. The stability of antioxidant activity in cream preparations should be assessed by comparing IC_{50} values during the stability test process using freeze-thaw cycles. The decline in antioxidant activity during storage may be due to exposure to high temperatures. The higher the heating temperature, the more antioxidant compounds are damaged during the process. Similarly, the longer the heating duration, the more antioxidant compounds degrade (Prihantini *et al.* 2021).

CONCLUSION

This study demonstrated that kombucha extract derived from green tea leaves (*Camellia sinensis* L.) exhibits notable flavonoid content and antioxidant activity, which varies significantly with fermentation time. The higher flavonoid concentration and stronger antioxidant capacity observed on day 7 suggest that earlier fermentation yields a more potent extract. However, by day 14, a substantial decline in flavonoid content and antioxidant efficacy was evident, indicating that prolonged fermentation may reduce the levels of bioactive compounds. These findings highlight the importance of optimizing fermentation duration to maximize the therapeutic potential of kombucha green tea extracts. Moreover, the moderate antioxidant activity observed in the cream formulations suggests their potential for topical applications, although formulation adjustments, such as increasing the extract concentration, may be necessary to enhance efficacy. Future research should focus on refining extraction and measurement methods and evaluating the stability of antioxidant activity over time to ensure product effectiveness during storage. Overall, this study provides valuable insights into the bioactive properties of kombucha green tea extracts and offers practical recommendations for their use in antioxidant-rich formulations.

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