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Enhanced antioxidant activity and flavonoid content in black tea kombucha via extended fermentation for anti-aging skincare applications

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Abstract: With the growing global demand for natural bioactive ingredients in cosmeceuticals, fermented plant extracts have gained increasing attention for their enhanced bioavailability and efficacy. This study investigates the effect of fermentation duration (17, 19, and 21 days) on the flavonoid content and antioxidant activity of black tea kombucha, aiming to explore its potential as a natural anti-aging agent. The research employed a post-test-only control group design and assessed secondary metabolite profiles, total flavonoid content, and antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. Phytochemical screening showed the consistent presence of flavonoids, phenols, saponins, and steroids/triterpenoids across all samples, while alkaloids were absent. A strong positive correlation was observed between fermentation duration and bioactive potential. Total flavonoid content increased from 67.23% (day 17) to 74.16% (day 21), while antioxidant activity improved significantly, with IC50 values decreasing from 23.23 µg/mL to 18.39 µg/mL, respectively. These findings indicate that extending fermentation up to 21 days enhances the antioxidant efficacy of black tea kombucha. This study provides preliminary evidence supporting the use of extended-fermentation kombucha as a promising active ingredient in anti-aging skincare formulations targeting oxidative stress.

Keywords: Kombucha; Flavonoid; Antioxidant; Anti-aging.

INTRODUCTION

Researchers have extensively studied natural products for skin care.[1–8] Natural products have exhibited biocompatibility and limited side effects compared to conventional medications.[9] Furthermore, Indonesia has diverse medicinal plants that are potentially used for herbal-based cosmetics.[10]

Recently, Kombucha as a skin care ingredient has emerged as a new trends in herbal-based cosmetics.[11,12] Kombucha is a probiotic drink derived from green or black tea fermented with symbiotic cultures of bacteria and fungi called SCOBY (Symbiotic Cultures of Bacteria and Yeasts).[13] The combination of SCOBY with sugar in tea initiates fermentation and forms a bioactive composition.[14] The bene fits obtained from Kombucha include antibacterial, antioxidant, and antidiabetic, able to reduce cholesterol concentrations, strengthen the immune system, and stimulate liver detoxification.[15,16]

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Kombucha is known as an anti-aging and anti-inflammatory, which is very important for skin conditions and preventing dermatological disorders.[17] Studies examining the effects of Kombucha as facial toner showed a positive effect on the metabolism and viability of keratinocyte and fibroblast cell cultures.[18] Kombucha tea is rich in antioxidants, especially polyphenols.[19] Black tea kombucha reached the highest polyphenol levels on day 14 of fermentation.[20] Kombucha also contains minerals (potassium, manganese, fluoride ions), vitamins (E, K, B), the amino acid theanine, which is a glutamine derivative, and compositions formed during fermentation such as catechins and flavonoids.[12]

Oxidative stress is a primary driver of extrinsic skin aging, leading to the search for potent natural antioxidants. Kombucha, a fermented tea beverage, produces organic acids, vitamins, and polyphenols with proven antioxidant properties. However, despite the growing interest in fermented skincare, few studies have systematically explored the impact of extended fermentation durations (>14 days) on the bioactive profile of black tea kombucha specifically for cosmetic applications. Most existing literature focuses on the 7-14 day window for beverage palatability, potentially missing the peak concentration of bioactive metabolites required for topical efficacy. Furthermore, there is limited scientific data correlating these longer fermentation periods with the specific enhancement of flavonoid content intended for anti-aging formulations. Therefore, this study addresses this gap by investigating the effect of 17, 19, and 21-day fermentation periods on the phytochemical profile and antioxidant activity of black tea kombucha, providing a scientific basis for its utilization in the developing herbal cosmeceutical industry.

Antioxidants are compounds that prevent or slow down oxidation by blocking chain reactions.[21] Testing antioxidant activity is important to assess the effectiveness of the sample and understand its mechanism.[22] Non-enzymatic antioxidants, such as polyphenols, are tested through free radical reduction reactions or chelation of free radical ions.[23] The DPPH (1,1-diphenyl-2-picrylhydrazyl) method is often used because it is simple, fast, cheap, and sensitive.[24] DPPH, a synthetic free radical soluble in ethanol and methanol, reacts with antioxidants through hydrogen and electron donor mechanisms.[25] Flavonoids in black tea kombucha play an anti-aging role due to antioxidant activity. Flavonoids increase collagen synthesis and DNA repair, protect against UVB radiation, and improve skin elasticity.[26]

This research aims to explore the phytochemicals, especially total flavonoid content and the antioxidant activity of fermented black tea kombucha extracts. This study is fundamental to the development of pharmaceutical products based on black tea kombucha extracts for anti-aging and anti-inflammatory in the future.

MATERIALS AND METHOD

Materials used in this research are; Ethanol 70% (Merck), Kombucha culture obtained from ®Mambucha, Black tea, Sugar, Distilled water, Gallic acid (Merck), DPPH (Merck), DMSO (Merck), Ethyl acetate (Merck), Methanol (Merck), n-Hexane (Merck), Chloroform (Merck), Ammonia (Merck), HCl (Merck), $FeCl_3$ (Merck), H_2SO_4 (Merck), $AlCl_3$ (Merck), CH_3COOH glacial (Merck), Mayer reagent (Merck), Wagner reagent (Merck), Dragendorff reagent (Merck), Quercetin (Merck).

The following process made kombucha tea: 1 L distilled water and 150 g sugar were boiled, then added by 50 g Black tea was stirred for 10 min, then put at room temperature. The tea dregs were removed by filtering the solvent, resulting in 750 ml of tea extract. Then, pour the tea extract into glass jars and add 10% kombucha starter (5% solids, 5% liquid). Then, ferment the tea extract at room

temperature for 21 days, resulting in three solutions based on fermentation days: 17, 19, and 21 days.

The common preliminary screening of phytochemistry has been conducted to detect the presence of alkaloids, flavonoids, tannins, saponins, steroids, and triterpenoids. To evaluate the total flavonoid content, we employed Quercetin as a standard solution at 200-600 nm wavelength under UV-vis spectrophotometer. Determination of flavonoid content in 1 mL of kombucha extract sample was done by adding 0.2 mL of 10% $AlCl_3$, 0.2 mL of 10% acetic acid, 3 mL of 96% ethanol, and 5.6 mL of distilled water. The absorbance was measured at the maximum wavelength. Out of three samples of kombucha extract fermented for 17, 19, and 21 days, three samples with the highest flavonoid content were selected.

Antioxidant activity was evaluated by using the DPPH method. About 10 mg DPPH was dissolved in 100 mL methanol in an aluminum foil-lined volumetric flask. The solution was diluted by dripping 15 mL of DPPH stock into a 50 mL flask and fully added methanol. The maximum wavelength of DPPH was determined by pipetting 3.8 mL of aqueous DPPH solution, putting it into an aluminum foil-lined vial, adding 0.2 mL of methanol, homogenizing, and incubating in a dark room for 30 min. Using a UV-vis spectrophotometer, the maximum wavelength was determined at 400-800 nm. The gallic acid solution was prepared by dissolving 10 mg of gallic acid in methanol to 100 mL (100 µg/mL). Make concentration series of 4, 6, 8, 10, and 12 µg/mL. Pipette 0.4, 0.6, 0.8, 1, and 1.2 mL of each test solution to a 10 mL volumetric flask, and add methanol to the mark. For the antioxidant test, pipette 0.2 mL of sample, put into the aluminum foil-lined vial, add 3.8 mL of 30 µg/mL DPPH, homogenize, incubate 30 minutes in a dark place, then measure absorbance at maximum wavelength of DPPH. Determination of antioxidant activity (IC_{50}) was conducted by pipetting 0.2 mL of sample solution into an aluminum foil-lined vial, then adding 3.8 mL of 30 µg/mL DPPH, homogenizing, incubating 30 minutes in a dark place, measuring the absorbance with a UV-Visible spectrophotometer at the maximum wavelength of DPPH. The IC_{50} value is calculated from a linear regression equation using data on percent inhibition and solution concentration. Microsoft Excel was used for linear regression analysis.

Data obtained from the Total Flavonoid and DPPH assays were analyzed statistically. A One-Way ANOVA followed by a Post-Hoc test (if applicable) was conducted to determine significant differences between fermentation durations (Day 17, 19, 21), with a significance level of $p < 0.05$.

RESULTS AND DISCUSSION

Phytochemistry of Secondary Metabolites

Phytochemical screening of kombucha tea extract fermented for 17, 19, and 21 days was aimed to identify secondary metabolites, including alkaloids, flavonoids, phenols, saponins, and terpenoids/steroids. The test results are presented in Table 1. The data showed that fermented kombucha tea extract on days 17, 19, and 21 contained secondary metabolites such as flavonoids, phenols, saponins, and steroids/triterpenoids. However, kombucha tea extract has no alkaloid content, in line with previous research that the extracts tested negative for alkaloids, consistent with previous findings.[27] The flavonoid compound test showed positive results in all three fermentations. After being given concentrated Mg and HCl, the color changes to reddish-orange. Flavonoids are polyphenolic structured substances and are found in many plants.[28] Flavonoids are classified into flavone, flavanone, catechins, and anthocyanin. The most common types of flavonoids found in tea extracts are catechins and flavones.[29]

Table 1. Secondary Metabolites in Kombucha Tea Extract

Secondary Metabolites	Reagents	Observation Results (day)		
		17	19	21
Alkaloids	Mayer	White precipitate (-)	White precipitate (-)	White precipitate (-)
	Bouchard	Chocolate precipitate (+)	Chocolate precipitate (+)	Chocolate precipitate (+)
	Dragendroff	Red/orange deposits (-)	Red/orange deposits (-)	Red/orange deposits (-)
Flavonoids	Concentrated Mg and HCl	Reddish orange (+)	Reddish orange (+)	Reddish orange (+)
Phenol	$FeCl_3$ 1%	Blackish green (+)	Blackish green (+)	Blackish green (+)
Saponins	Aquadest	Foaming (+)	Foaming (+)	Foaming (+)
Steroids/triterpenoids	Acetylenhydric Acid and Concentrated Sulfuric Acid	Brownish-red (+)	Brownish-red (+)	Brownish-red (+)

Total Flavonoid Content

In this study, the maximum wavelength of Quercetin was found to be 371.50 nm, as shown in Figure 1.

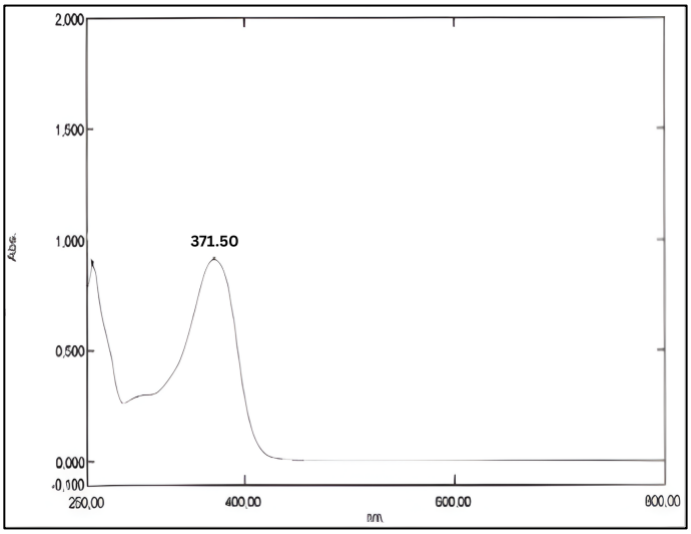

Figure 1. Maximum wavelength of Quercetin

Examination of the standard curve using quercetin standard solution with concentrations of 30 ppm, 40 ppm, 50 ppm, 60 ppm, and 70 ppm showed absorbances at 0.354, 0.391, 0.428, 0.461, and 0.501, respectively. The regression equation for Quercetin was found to be y = 0.0036x + 0.2452 with a correlation coefficient (r) of 0.99951. Since the value (r) is close to 1, the linear relationship between absorbance and concentration is very strong, where the higher the concentration of Quercetin standard solution, the higher the absorbance value.[30]

Furthermore, the total flavonoid content of kombucha extract based on the day of fermentation was calculated with the regression equation, as presented in Table 2 and Figure 2. The data showed that the highest content was found in the 21 days of fermentation extract, and the lowest content was in the 17 days of fermentation extract. This data was supported by another study that the concentration of flavonoids increases with the length of fermentation due to the presence of enzymes that convert polyphenolic substances into flavonoids.[31]

Table 2. Total Flavonoid Contents in Kombucha Tea Extracts

Day	Absorbance	Total Flavonoids (%)
17	0.387	67.23
19	0.504	71.95
21	0.512	74.16

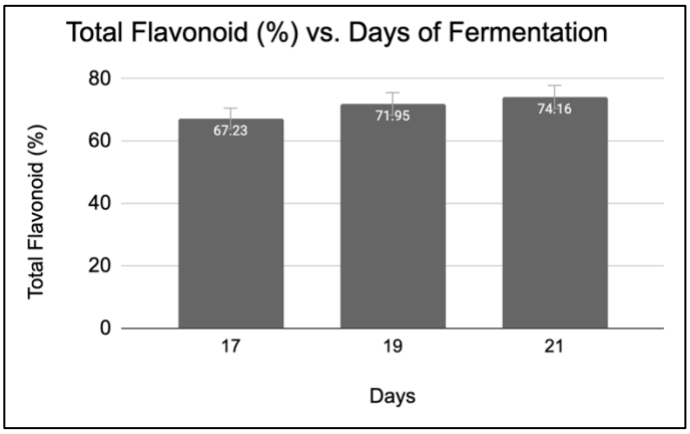

Figure 2. Flavonoid content vs Fermentation days

Antioxidants Activity

The measurement result of the maximum wavelength using a UV-vis Spectrophotometer is 515 nm with an absorbance value of 0.654, as shown in Figure 3.

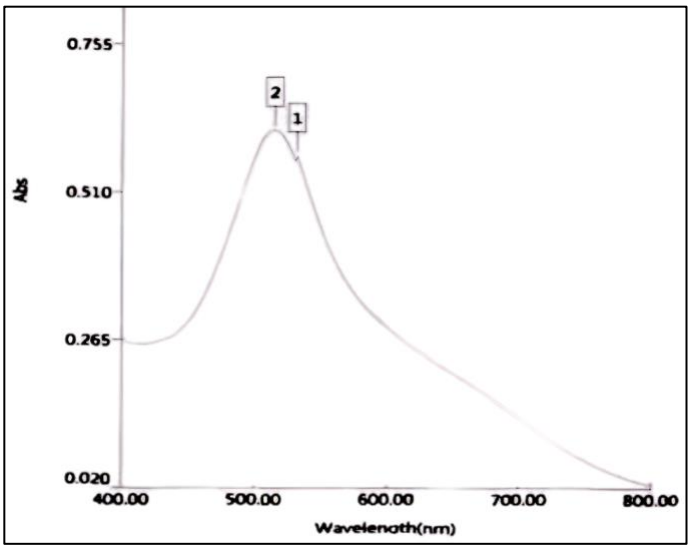

Figure 3. Maximum wavelength of DPPH

Furthermore, the antioxidant activity of the gallic acid comparison solution was tested, where the absorbance value of the gallic acid solution was determined by UV-Vis spectrophotometry at the maximum wavelength of DPPH. Examination of the standard curve using a gallic acid standard solution with concentrations of 4, 6, 8, 10, and 12 (µg/mL) showed absorbances at 0.498, 0.436, 0.370, 0.315, and 0.249, respectively. The regression equation for gallic acid was found to be y = 5.363x – 7.662. The IC_{50} value of gallic acid as of 10.628 was obtained from the linear regression equation of gallic acid solution from the relationship between percent inhibition as ordinate and concentration as abscissa.[32]

Furthermore, the antioxidant activity of kombucha tea extract was calculated with the regression equation based on the day of fermentation as presented in Table 3.

Table 3. Antioxidant Activity of Kombucha Black Tea Extract

Days of Fermentation	Concentration (µg/mL)	Abs. of Gallic acid	Abs. of Kombucha	% Inhibition	Regression	IC_{50} (µg/mL)
17	4	0.645	0.575	10.852	y = 1.634 + 2.008x R^2 = 0.991	23.237
	8		0.552	14.418		
	12		0.487	24.496		
	16		0.400	37.981		
	20		0.389	39.670		
19	4	0.645	0.585	9.302	y = 2.628x – 2.574 R^2 = 0.995	20.006
	8		0.535	17.054		
	12		0.465	27.906		
	16		0.385	40.310		
	20		0.321	50.232		
21	4	0.645	0.536	16.899	Y = 8.294 + 2.268 x R = 0.997	18.393
	8		0.475	26.356		
	12		0.412	36.124		
	16		0.352	45.426		
	20		0.305	52.713		

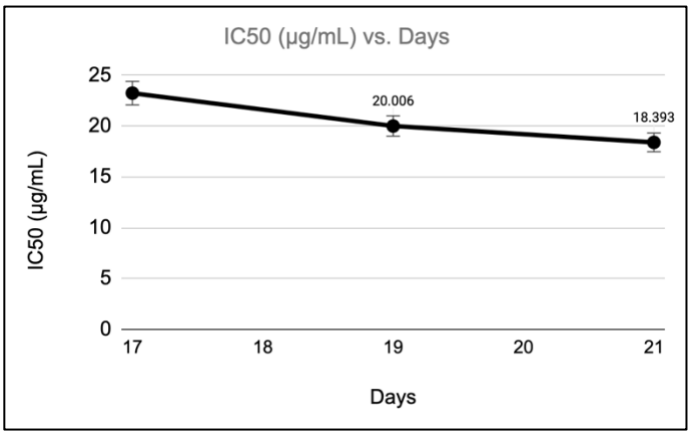

Figure 4. IC_{50} values vs Fermentation days

The results in the Table 3 demonstrated a dose-dependent relationship between fermentation time and antioxidant potency. Figure 4 also showed that the IC_{50} value on Day 21 (18.39 µg/mL) was significantly lower than on Day 17, indicating superior activity. This finding aligns with recent studies that extended fermentation enhances the release of low-molecular-weight polyphenols due to the enzymatic hydrolysis of complex compounds by the SCOBY[33]. Furthermore, while green tea kombucha antioxidant activity often plateaus after 14 days due to the rapid degradation of catechins, black tea kombucha appears to require a longer fermentation duration (21 days) to maximize the biotransformation of thearubigins and theaflavins into highly active antioxidant metabolites. This distinction underscores the importance of optimizing fermentation time specific to the tea substrate used. Similarly, a study on kombucha-based cosmetic serums indicated that flavonoid accumulation peaks at later fermentation stages, correlating with increased inhibition of skin-aging enzymes like collagenase. These results align with findings that antioxidant activity increases as well as the length of fermentation time.[34] The increased value of antioxidant activity in kombucha black tea occurs due to the metabolism of microorganisms during fermentation.[35] The biotransformation process by microorganisms increases phenol compounds, which utilize plant enzymes to enhance biological activity.[35,36] The tea leaves used are also rich in phenol compounds, which increased along with the length of fermentation.[35] Free phenolics produced during fermentation contribute to

antioxidant activity, so the higher the phenolic content, the greater the antioxidant activity.[32]

However, this contrasts with studies on green tea kombucha, where antioxidant activity often plateaus after 14 days. This suggests that the complex polyphenol profile of black tea requires a longer fermentation duration (21 days) to achieve optimal biotransformation into bioactive flavonoids compared to green tea variants. A limitation of this study is the use of a specific commercial starter culture; variability in SCOBY microbial composition could influence metabolite production in large-scale applications. Nevertheless, the consistent increase in flavonoid content observed here supports the viability of prolonged fermentation for extracting maximum bioactive yield for topical use.

CONCLUSION

The study concludes that the duration of fermentation significantly affects the phytochemical profile and antioxidant activity of black tea kombucha. The 21-day fermentation period yielded the highest total flavonoid content and the strongest antioxidant activity (lowest IC_{50}). These findings provide foundational evidence that extended fermentation enhances the bio-efficacy of black tea kombucha, making it a viable candidate for natural anti-aging skincare products. Future research should focus on formulating this extract into stable topical delivery systems and conducting in vivo dermatological safety tests.

AUTHORS' CONTRIBUTIONS

Merisca Gianthra Ryosa: Data curation, Investigation, Writing- Original draft preparation; Larastika Yovianda: Data curation, Investigation; Ully Chairunisa: Data curation, Investigation; Erny Tandanu: Supervision, Methodology, Validation; Refi Ikhtiari: Supervision, Conceptualization, Reviewing and Editing.

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DATA AVAILABILITY STATEMENT

The utilized data to contribute to this investigation are available from the corresponding author upon reasonable request.

DISCLOSURE STATEMENT

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors. The data is the result of the author's research and has never been published in other journals.

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