

Research Article

Antioxidant Potential of Avocado Leaf Kombucha (*Persea americana* Mill.) at Different Fermentation Times Using the DPPH Method

Muhammad Andry^{1*}, Rafifah Azzahra¹, Tiara Ramadaini¹, Cindy Marseli¹, Nurfitriyana¹,
Nia Novranda Pertiwi², Muhammad Amin Nasution³

¹Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Sriwijaya,
Indralaya, Sumatera Selatan, Indonesia

² Department of Pharmacy, Faculty of Pharmacy, Universitas Mulawarman, Samarinda, East
Kalimantan, Indonesia

³Department of Pharmacy, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda
Aceh, Indonesia

*Corresponding email: andry@unsri.ac.id

Abstract

Avocado leaves contain various bioactive compounds, including flavonoids, terpenoids, saponins, tannins, and polyphenols, which contribute to antioxidant activity. Antioxidants are crucial in safeguarding cells from oxidative stress and mitigating the risk of chronic illnesses. This study sought to assess the antioxidant capacity of avocado leaf kombucha across various fermentation durations. The experimental design entailed the preparation of kombucha tea, execution of antioxidant assays utilizing the DPPH method, and statistical analysis by One-Way ANOVA followed by Tukey HSD to ascertain significant differences. The IC₅₀ values recorded were 183.25 ppm (moderate) for unfermented tea, 67.99 ppm (moderate) at day 9, 49.91 ppm (very strong) at day 12, and 40.18 ppm (very strong) at day 14, whereas vitamin C, serving as a positive control, exhibited 9.16 ppm (very strong). These findings demonstrate that fermentation significantly enhances antioxidant activity, with the highest effect observed at 14 days of fermentation. In conclusion, avocado leaves represent a promising natural source for developing functional kombucha beverages with strong antioxidant activity.

Keywords: Avocado leaf, Kombucha, Fermentation, Antioxidant activity, DPPH

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1 Introduction

Free radicals are molecules with one or more unpaired electrons, rendering them highly reactive and capable of harming adjacent biomolecules, such as lipids, proteins, and DNA. Excessive free radical exposure, triggered by both endogenous processes and exogenous factors such as pollution, smoking, ultraviolet radiation, and stress, may result in oxidative stress, cellular malfunction, and the onset of chronic illnesses, such as cancer, cardiovascular problems, and diabetes [1]. Antioxidants are compounds that neutralize free radicals by donating electrons, thereby preventing chain reactions and reducing oxidative damage [2]. Despite the presence of endogenous antioxidant mechanisms in the human body, including superoxide dismutase, catalase, and glutathione peroxidase, additional supplementation of antioxidants from natural sources is frequently necessary [3].

Avocado (*Persea americana* Mill.), a species from the Lauraceae family, is widely cultivated in tropical regions and its leaves are traditionally used as herbal medicine. Phytochemical studies have shown that avocado leaves contain flavonoids, terpenoids, saponins, tannins, and polyphenols, which exhibit antioxidant, anti-inflammatory, hepatoprotective, and analgesic properties [4]. Previous studies reported that avocado leaf extract demonstrated strong antioxidant activity, with IC₅₀ values below 50 ppm, suggesting potential as a functional ingredient [5].

Kombucha is a fermented drink made from sweetened tea through a symbiotic culture of bacteria and yeast (SCOBY), has gained increasing popularity due to its health-promoting properties. During fermentation, microorganisms metabolize sugars to produce organic acids, vitamins, and bioactive metabolites, which contribute to its antioxidant capacity [6]. The quality and activity of kombucha are influenced by the type of substrate and fermentation duration [7]. Avocado leaves, when used as a tea base for kombucha, may provide additional antioxidant benefits.

The antioxidant activity of natural products can be measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, a rapid and sensitive method widely applied for evaluating radical scavenging activity [8]. Therefore, this study aimed to investigate the effect of different fermentation times (9, 12, and 14 days) on the antioxidant activity of avocado leaf kombucha using the DPPH method.

2 Method

Experimental Design

This study was conducted as an experimental laboratory research at the Pharmacy Laboratory, Faculty of Pharmacy and Health, Institut Kesehatan Helvetia, Medan, Indonesia. The objective was to evaluate the antioxidant activity of avocado leaf kombucha (*Persea americana* Mill.) at different fermentation times using the DPPH radical scavenging assay [9]

Materials

The raw material used was dried avocado leaves (*Persea americana* Mill.) collected from Medan, North Sumatra. Other materials included sucrose, distilled water, kombucha culture (SCOBY), methanol, aluminum foil, filter paper, and 1,1-diphenyl-2-picrylhydrazyl (DPPH, Sigma-Aldrich). Positive control was vitamin C (ascorbic acid).

Instruments

The instruments used were glass jars (fermentation vessels), beakers, volumetric flasks, analytical balance, hot plate, pH meter, micropipettes, vortex mixer, water bath, and UV-Vis spectrophotometer (Shimadzu, Japan).

Preparation of Avocado Leaf Kombucha

Dried avocado leaves were powdered into simplisia and used as the main substrate. A total of 5 g of avocado leaf powder was infused in 1000 mL of boiling distilled water for 15 minutes, then filtered to obtain a tea base. The filtrate was supplemented with 200 g sucrose (10% w/v) to provide a carbon source for microbial growth. Upon reaching room temperature (25–28 °C), the solution was infected with 100 mL of kombucha starting culture (SCOBY), consisting of symbiotic bacteria (e.g., *Acetobacter xylinum*) and yeasts (*Saccharomyces* spp.) [9]

The fermentation process was conducted in sterile glass jars covered with clean cloth to allow aeration while preventing contamination. Samples were incubated at ambient room temperature (20–30 °C) for 9, 12, and 14 days. These fermentation times were selected based on previous reports and preliminary trials, representing early, intermediate, and optimal stages of kombucha fermentation [10]. After the fermentation period, the kombucha tea was filtered and stored in sterile containers for further analysis. Unfermented avocado leaf tea (day 0) served as the control. To minimize variability, all preparations were performed in triplicate batches under identical conditions [10].

The fermented products were then subjected to organoleptic evaluation (color, aroma, taste), hedonic testing, pH measurement, phytochemical screening, and antioxidant activity determination using the DPPH method [11].

Antioxidant Activity Assay (DPPH Method)

The antioxidant activity of avocado leaf kombucha was determined using the DPPH radical scavenging method, which is widely applied due to its simplicity, sensitivity, and reliability in evaluating hydrogen-donating ability of antioxidants [12].

A stock solution of DPPH (100 ppm, equivalent to 0.4 mM) was prepared in methanol and further diluted to obtain a working solution of 40 ppm. Kombucha samples obtained from different fermentation durations (0, 9, 12, and 14 days) were prepared at five concentrations: 100, 200, 300, 400, and 500 ppm [12].

Each test involved mixing 1 mL of kombucha with 1 mL of 40 ppm DPPH solution in methanol. The mixtures were vortexed, covered with aluminum foil, and incubated in the dark at room temperature for 30 minutes to prevent photodegradation of the DPPH radical. Absorbance values were measured at 516 nm using a UV-Vis spectrophotometer, with methanol as the blank [13].

The percentage of radical scavenging activity was calculated using the formula:

$$\% \text{ Inhibition} = (A_{(0)} - A_S) / A_{(0)} \times 100$$

where:

A_0 = absorbance of the control

A_S = absorbance of the sample

A calibration curve was constructed by plotting inhibition percentage against sample concentration, and the IC_{50} value (concentration required to reduce 50% of DPPH radicals) was determined by linear regression [13].

Positive control was vitamin C (ascorbic acid) due to its well-established antioxidant potency. All experiments were performed in triplicate, and results were expressed as mean $\pm$ SD [14].

Evaluation Parameters

The quality and functional properties of the avocado leaf kombucha preparations were evaluated through several parameters. Organoleptic assessment was conducted to observe sensory characteristics, including color, aroma, and taste, at different fermentation stages. A hedonic test involving 15 untrained panelists was carried out using a five-point hedonic scale ranging from “dislike” to “very like,” in order to determine consumer acceptance based on preference for taste, aroma, and overall acceptability. The pH values of the kombucha samples were measured in triplicate using a calibrated digital pH meter to monitor acidity changes during fermentation. In addition, preliminary phytochemical screening and pharmacognostic evaluation of the avocado leaf simplisia were performed to confirm the presence of bioactive compounds and to ensure raw material quality. The antioxidant activity of the fermented products was assessed using the DPPH radical scavenging assay, and the results were expressed as IC_{50} values, representing the concentration required to inhibit 50% of free radicals. All evaluations were performed in triplicate, and data were reported as mean $\pm$ standard deviation (SD) [10], [15].

Data Analysis

Data were presented as mean $\pm$ standard deviation (SD). One-Way ANOVA was utilized for statistical analysis to assess the impact of fermentation duration on antioxidant activity. A post-hoc analysis utilizing Tukey HSD was performed upon the observation of significant differences ($p < 0.05$).

3 Result and Discussion

Organoleptic Evaluation

The avocado leaf kombucha preparations exhibited progressive changes in sensory characteristics during fermentation. The unfermented sample showed a light greenish-brown color with a mild aroma and slightly bitter taste typical of avocado leaves. After 9 days of fermentation, the beverage developed a darker brown color, a characteristic sweet–sour aroma, and a more balanced taste that was generally preferred by the sensory panel. By day 12 and 14, the color further deepened, the aroma became stronger and more acidic, and the taste shifted toward pronounced sourness. Overall, panelists indicated that kombucha fermented for 9 days was the most acceptable in terms of color, aroma, and taste, whereas longer fermentation times reduced consumer preference due to excessive acidity. The detailed organoleptic observations are presented in Table 1[16].

Table 1 Organoleptic characteristics of avocado leaf kombucha during fermentation

Fermentation Time	Color Description	Aroma Description	Taste Description	Panelist Preference
0 days (unfermented)	Light greenish-brown	Mild, leafy	Slightly bitter, herbal	Low
9 days	Darker brown	Sweet–sour, pleasant	Balanced sour–sweet	High (most preferred)
12 days	Deep brown	Stronger, acidic	Noticeably sour	Moderate
14 days	Very dark brown	Strong, acidic	Pronounced sour, sharp taste	Low

The observed changes in organoleptic properties are consistent with the biochemical transformations that occur during kombucha fermentation. Yeasts present in the symbiotic culture of bacteria and yeast (SCOBY) convert sucrose into ethanol and carbon dioxide, while acetic acid bacteria oxidize ethanol into organic acids such as acetic, lactic, and gluconic acid. [17] These metabolic activities explain the darker color, stronger aroma, and more acidic taste observed with longer fermentation periods. Similar findings have been reported in previous studies of herbal kombucha, where prolonged fermentation enhanced acidity and altered sensory quality. Importantly, although extended fermentation (12–14 days) improved antioxidant activity, it reduced consumer acceptability due to excessive sourness. Thus, from a functional beverage perspective, fermentation around 9 days may represent the optimal balance between bioactivity and sensory preference [18]

Hedonic Test

The hedonic evaluation involving 15 untrained panelists assessed the acceptability of kombucha samples fermented for 9, 12, and 14 days. The results demonstrated that fermentation time significantly influenced consumer preference. The 9-day fermented kombucha received the highest scores for color, aroma, and taste, with 86.7% of panelists indicating “liked” or “very liked” ratings for taste, 80.0% for aroma, and 86.7% for color. In contrast, the 12-day and 14-day samples received progressively lower acceptance, particularly in taste, where only 60.0% and 53.3% of panelists, respectively, expressed favorable ratings. These data confirm that increasing fermentation time decreases consumer preference, primarily due to increased acidity and sourness. The detailed hedonic responses are summarized in Table 2 [19].

Table 2 Hedonic evaluation of avocado leaf kombucha

Attribute	9 days (% liked/very liked)	12 days (% liked/very liked)	14 days (% liked/very liked)
Color	86.7%	73.3%	66.7%
Aroma	80.0%	66.7%	60.0%
Taste	86.7%	60.0%	53.3%

The decline in hedonic scores with prolonged fermentation reflects the biochemical changes in kombucha. Longer fermentation increases the concentration of organic acids (especially acetic acid), lowering the pH and intensifying sourness, which many panelists perceived as less palatable. Although antioxidant activity was higher in the 12- and 14-day fermented samples, the sharp acidic taste reduced consumer acceptability. These findings are consistent with previous reports on herbal kombucha, where optimal fermentation times balance functional properties with sensory qualities. From a product development perspective, 9-day fermentation may represent the most suitable duration, as it achieves good antioxidant activity while maintaining high consumer preference [20].

pH Measurement

The acidity of the kombucha preparations decreased progressively with fermentation time. The average pH value of the 9-day fermented sample was 3.38 ± 0.02 , which further declined to 3.14 ± 0.01 on day 12 and 3.03 ± 0.01 on day 14. These findings clearly indicate a consistent reduction in pH as fermentation proceeds, reflecting the accumulation of organic acids by microbial metabolism. The detailed results are presented in Table 3 [21].

Table 3 pH values of avocado leaf kombucha during fermentation

Fermentation Time	Mean pH $\pm$ SD
9 days	3.38 ± 0.02
12 days	3.14 ± 0.01
14 days	3.03 ± 0.01

The observed decrease in pH is a hallmark of kombucha fermentation. Yeasts initially convert sucrose into ethanol, which is subsequently oxidized by acetic acid bacteria into acetic acid, gluconic acid, and other organic acids. This continuous production of acids explains the gradual pH decline from day 9 to day 14. A lower pH not only contributes to the increasingly sour taste, as reflected in the hedonic test, but also enhances the microbial stability of the beverage by inhibiting the growth of spoilage microorganisms. However, excessive acidity may reduce consumer acceptability despite improving safety and stability. Therefore, balancing pH reduction with sensory quality is essential in determining the optimal fermentation duration [22].

Antioxidant Activity

The antioxidant activity of avocado leaf kombucha was evaluated using the DPPH radical scavenging assay at 516 nm. All samples showed a concentration-dependent increase in radical scavenging, with longer fermentation resulting in higher inhibition values compared with the unfermented sample [23]. The IC₅₀ values, summarized in Table 4, confirmed this trend. The unfermented kombucha (F0) showed the weakest activity with an IC₅₀ of 106.40 ppm (moderate), while fermentation markedly improved antioxidant potential to 67.99 ppm (F9), 49.91 ppm (F12), and 40.18 ppm (F14). Vitamin C as the positive control exhibited the strongest activity with an IC₅₀ of 9.16 ppm [24]

Table 4 IC₅₀ values of avocado leaf kombucha and vitamin C

Sample	Regression equation ($y = ax + b$)	R ²	IC ₅₀ (ppm)	Category
F0 (Unfermented)	$y = 0.01x + 23.76$	0.995	106.40	Moderate
F9 (9 days)	$y = 0.01x + 23.93$	0.978	67.99	Strong
F12 (12 days)	$y = 0.01x + 26.74$	0.987	49.91	Very strong
F14 (14 days)	$y = 0.01x + 27.26$	0.939	40.18	Very strong
Vitamin C (control)	$y = 0.09x + 25.66$	0.999	9.16	Very strong

These results demonstrate that fermentation enhances the antioxidant potency of avocado leaf kombucha, reflected in progressively lower IC₅₀ values. The improvement is attributed to microbial metabolism, which breaks down complex polyphenols into simpler phenolic derivatives and produces additional bioactive metabolites such as organic acids and flavonoids, contributing to radical scavenging

activity. Similar findings have been reported for other herbal kombuchas, where prolonged fermentation enhanced phenolic content and antioxidant activity [24]

From a functional perspective, 14-day fermentation produced the strongest antioxidant effect; however, its excessive acidity reduced consumer acceptability. Therefore, 9–12 days of fermentation may represent the optimal balance between bioactivity and sensory preference [16].

Data Analysis

Data are expressed as mean $\pm$ SD from triplicate measurements. One-Way ANOVA revealed that fermentation time significantly affected antioxidant activity (IC_{50} , $p < 0.05$). Tukey's HSD post-hoc test showed that unfermented kombucha (F0) differed from all fermented samples (F9, F12, F14). Among fermented groups, IC_{50} decreased progressively from F9 (67.99 ppm) to F12 (49.91 ppm) and F14 (40.18 ppm), with no significant difference between F12 and F14 ($p > 0.05$), indicating a plateau in antioxidant enhancement after 12 days. Vitamin C (positive control) had the lowest IC_{50} (9.16 ppm), significantly different from all kombucha samples ($p < 0.05$). These results suggest that fermentation improves antioxidant potential via breakdown of complex phenolics and formation of new metabolites, with 9–12 days representing an optimal balance between antioxidant efficacy and sensory acceptability [25].

4. Conclusion

Persea americana Mill. leaves can be utilized as raw material for kombucha tea preparation. Fermentation time significantly influenced the antioxidant activity of avocado leaf kombucha, as reflected by the decreasing IC_{50} values. The unfermented sample exhibited an IC_{50} of 106.40 ppm (moderate), while fermentation for 9 days reduced the IC_{50} to 67.99 ppm (strong). Further fermentation to 12 and 14 days enhanced antioxidant activity with IC_{50} values of 49.91 ppm and 40.18 ppm, respectively, both classified as very strong antioxidants. The positive control, vitamin C, demonstrated the highest action with an IC_{50} of 9.16 ppm (extremely strong). Based on these findings, 14 days of fermentation provided the best antioxidant potential, as indicated by $IC_{50} < 50$ ppm, although sensory acceptability must also be considered in determining optimal fermentation duration.

5. Declarations

5.1 Author contributions

S.O.T. designed the study, conducted the experiments, and drafted the initial manuscript. M.A. supervised the methodology design, data analysis, and refinement of the discussion. A. contributed to data validation, critical review of the manuscript content, and revision of the final version. All authors read and approved the final manuscript.

5.2 Ethics

This study received Ethical Approval for Health Research from the Animal Research Ethics Committees (AREC), Department of Mathematics and Natural Sciences, Universitas Sumatera Utara, with approval number No. 0306/KEPH-FMIPA/2024, issued on August 23, 2024.

5.3 Conflict of Interest

The authors declare that there is no conflict of interest regarding the research, authorship, or publication of this article.

5.4 Funding Statement

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