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*Research Article*

# Evaluation of Fermentation Duration Effect on the Antioxidant Activity of Pecut Kuda (*Stachytarpheta jamaicensis* L.) Leaf Kombucha

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## Abstract

Kombucha is a fermented beverage rich in various bioactive metabolites produced through the combined activity of yeast and bacteria. Pecut kuda (*Stachytarpheta jamaicensis* L.) leaves contain phenolic and flavonoids with potential antioxidant activity. This study aimed to evaluate the effect of fermentation duration on the antioxidant activity and physicochemical properties of pecut kuda leaf kombucha for 6, 10 and 14 days. The evaluation involved organoleptic assessment, pH measurement, total acetic acid determination, and antioxidant activity with DPPH assay. The antioxidant activity of pecut kuda leaf kombucha showed $IC_{50}$ of 63.08 ppm, 66.79 ppm, and 98.24 ppm for 6, 10, and 14 days of fermentation, respectively, which are classified as strong antioxidant activity. The fermentation duration has a significant effect on physicochemical parameters, with pH decreasing from 3.17 to 2.64 and 2.16, while total acetic acid increase from 0.25% to 0.54% and 0.86%. Organoleptic result showed a colour change from dark brown to brown of kombucha. These results indicated that pecut kuda leaf kombucha potential as functional beverage.

**Keywords**: Pecut kuda, *Stachytarpheta jamaicensis* L., Kombucha, Antioxidant.

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## 1 Introduction

Kombucha is fermented beverage traditionally produced from tea and sugar using a symbiotic culture of bacteria and yeast (SCOBY). It has been consumed for centuries in various regions such as China, Russia, and Germany. The SCOBY consists of *Acetobacter xylinum* and several yeast, such as *Brettanomyces* and *Saccharomyces*. During the fermentation process, SCOBY converts sugar into organic acids and bioactive metabolites that contribute to various bioactivities such as antioxidant, antimicrobial and metabolic regulation effects [1], [2]. Consuming kombucha regularly has been associated with a variety of health benefits, such as enhanced immune function, increase digestive health, and lower risk of cardiovascular disease and certain types of cancer [3]. In the kombucha fermentation process, black tea (*Camellia sinensis* L) is the most commonly used substrate. Currently, various plant leaves have been explored to improve the functional properties of kombucha [4]. Leaves rich in phenolic compounds are very suitable as substrate due to their potential as a source of natural antioxidants [1].

One of the plants rich in phenolic compounds is *Stachytarpheta jamaicensis* L. or known as pecut kuda. This plant is a traditional medicinal plant widely used by the local community. Its leaves are often boiled and consumed to treat inflammation, coughs, and hepatitis A [5]. This plant contains several bioactive compounds such as flavonoids, phenols, terpenoid, tannins and saponins. These compounds contributed to several bioactivities such as antibacterial, antifungal, antioxidant, and anti-inflammatory (Olivia & Teresita, 2015; Liew & Yong, 2016). Previous study reported that ethanol extract of *Stachytarpheta jamaicensis* L. leaves showed an $IC_{50}$ 74.32 ± 0.71 ppm, indicating strong antioxidant activity [6]. Considering its potential bioactive compounds, pecut kuda leaves show promise for fermented beverage such as kombucha. The fermentation process can increase the concentration of bioactive compounds and enhance the antioxidant activity of plant-based substrates.

Antioxidant activity in kombucha is affected by several factors, one of which is fermentation duration. It plays crucial role in determining the effectiveness of kombucha as a health-promoting beverage. Previous studies reported that the fermentation duration can increase antioxidant activity but prolonged fermentation beyond the optimum time can decrease its activity [1], [7]. Interest in developing kombucha from plant substrate continues to grow, but there is still limited study of *Stachytarpheta jamaicensis* L. leaves kombucha, particularly regarding the effect of fermentation duration on its antioxidant capacity and physicochemical characteristics. Therefore, in this study aimed to evaluation the effect of fermentation duration of *Stachytarpheta jamaicensis* L. leaves kombucha to its antioxidant activity and physicochemical properties.

## 2 Method

### 2.1 Material

Pecut kuda (*Stachytarpheta jamaicensis* L) leaves was determined by Laboratorium Ekologi and Konservasi Biodiversitas Hutan Tropis, Universitas Mulawarman, SCOBY was purchased from online market place, 2,2-diphenyl-1-picrylhydrazyl (DPPH), sugar, methanol, ascorbic acid, and tea as kombucha's medium.

### 2.2 Sample Preparation

Pecut kuda leaves were collected and wet sorted to separate them from impurities. Then, the leaves were washed with tap water, weighed, and dried naturally in the air. After drying, the leaves were finely ground to obtain the powder.

### 2.3 Pecut Kuda Leaves Kombucha

Kombucha tea was prepared using 20 g of pecut kuda leaf powder. The infusion method was applied by heating the sample at 90 °C for 15 minutes to optimize the extraction of soluble compounds. The infusion was then filtered and transferred into a sterile glass container. Then, sugar was added at a concentration of 10%. Afterward, the infusion was allowed to cool to approximately 25 °C before added 10% (w/v) SCOBY culture. Then, the kombucha was fermented for 6, 10, and 14 days.

### 2.4 DPPH Assay

Pecut kuda leaf kombucha was prepared at several concentration 20, 40, 60, 80 and 100 ppm. Then, a 2 mL aliquot of each concentration was mixed with 2 mL of 0.1 mM DPPH solution. The mixture was homogenized and incubated for 30 minutes. After incubation, the absorbance was measured using a spectrophotometer UV-Vis at 516 nm with triplicate. The positive control was using an ascorbic acid. The measurement was conducted on kombucha samples fermented for 6, 10 and 14 days. The $IC_{50}$ was calculated using linear regression analysis [8].

### 2.5 Organoleptic Evaluation

Organoleptic evaluation is conducted visually and sensorily by observing changes in colour, aroma, and taste in kombucha samples. Observations were conducted at each fermentation interval to track qualitative changes that indicated the fermentation process.

### 2.6 pH Analysis

A total of 25 mL of kombucha samples were obtained from each fermentation period (6, 10, and 14 days). Pre-measurement, the pH meter was calibrated using standard buffer solution with pH 7.00 and pH 4.00. After calibration, the electrode was immersed in each kombucha sample. All measurement were performed at room temperature.

### 2.7 Determination of total acetic acid content (%)

The percentage of total acetic acid was determined using an alkalimetric titration method. A 25 mL aliquot of the kombucha sample was transferred into an Erlenmeyer flask, followed by addition of 2-3 drops of 1% phenolphthalein indicator. The sample was then titrated with 0.1 N NaOH. The total acetic acid content (%) was calculated using the following formula [9] :

$$\text{Total acetic acid (\%)} = \frac{V_{NaOH} \times N_{NaOH} \times MW_{acetic\ acid}}{V_{sample}} \quad (1)$$

## 3 Result and Discussion

The fermentation of pecut kuda (*Stachytarpheta jamaicensis* L.) leaf kombucha exhibited significant physicochemical, organoleptic, and functional changes during the fermentation duration (days 6, 10, and 14 days). The results of the physicochemical assays, including pH measurement, total acetic acid content, and organoleptic were presented in Table 1.

Table 1 Physicochemical characteristic of pecut kuda (*Stachytarpheta jamaicensis* L.) leaf kombucha.

| Parameter | Fermentation duration (days) | | |
|---|---|---|---|
| | 6 | 10 | 14 |
| pH | 3.17 ±0.058 | 2.64±0.034 | 2.16±0.017 |
| Total acetic acid (%) | 0.025%±0.00693 | 0.54%±0.00693 | 0.86%±0.01387 |
| Colour | Dark brown | Light brown (kombucha's colour) | Light brown (kombucha's colour) |
| Aroma | acidic and vinegar-like scent | acidic and vinegar-like scent | acidic and vinegar-like scent |
| Taste | sour | sour | sour |

The pH was gradually decreased from 3.1 to 2.6 and 2.16 for days 6, 10, and 14, respectively. The pH obtained remains within the safe consumption range 2.5-4.2. The reduction aligned with the significant increase in total acetic acid content, which increased from 0.25% to 0.54% and 0.86% during the fermentation period days 6, 10 and 14, respectively. This confirmed an increase in the activity of acetic acid producing bacteria as fermentation progressed.

The fermentation of pecut kuda (*Stachytarpheta jamaicensis* L.) leaf kombucha showed significant physicochemical and functional changes that were closely associated with microbial metabolism during days 6, 10 and 14 of fermentation. The pH values exhibited a continues decrease over time, indicating an increase in organic acid production by the symbiotic culture of bacteria and yeast (SCOBY). This finding was consistent with titration results, which showed a gradual increase in total acid content. The accumulation of acetic acid is expected during kombucha fermentation where yeast first convert sugars into ethanol, followed by the oxidation of ethanol to acetic acid by acetic acid bacteria [7], [10]. As the concentration of these organic acid increases, the pH decreases proportionally. A pH below 4.2 is widely recognized as microbiologically safe for kombucha, effectively inhibiting the growth of most pathogenic microorganism [11]. Therefore, the decreasing pH observed in this study not only reflects the metabolic activity of SCOBY but also contribute to the safety stability of the beverage.

Organoleptic evaluation confirmed these physicochemical changes. The visual observation showed a progression of colour change (from dark brown to light brown kombucha's colour) and increased turbidity of the liquid, consistent with the formation of cell biomass and accumulation of metabolites [12]. The aroma changed from light tea-like scent to a more acidic and vinegar-like scent as fermentation continues, in line with the increase in acetic acid concentration. The taste also changed from slightly sweet to distinctly sour with longer fermentation. These characteristics were typically indicators of active fermentation and play a role in determining product acceptance.

The antioxidant activity of kombucha was evaluated using the DPPH method. The $IC_{50}$ value obtained for fermentation at days 6, 10 and 14 were 63.08 ppm, 66.79 ppm, and 98.24 ppm, respectively (Table 2). According to defined classification, $IC_{50}$ values below 100 ppm indicate strong activity [13]. This antioxidant activity was higher than the non-fermented of pecut kuda (*Stachytarpheta jamaicensis* L.) leaf, indicating that the fermentation process increased the antioxidant potential of the raw material.

Table 2 Antioxidant activity ($IC_{50}$) of Pecut Kuda (*Stachytarpheta jamaicensis* L.) Leaf Kombucha at Different Fermentation Duration.

| **Fermentation Duration (days)/Sample** | **Linear Regression** | **$IC_{50}$ (ppm)** |
|---|---|---|
| 6 | y = 0.664x + 7.5654 | 63.91 |
| 10 | y = 0.5653x + 12.246 | 66.79 |
| 14 | y = 0.3787x + 8.7094 | 98.25 |
| Ascorbic acid (positive control) | y = 6.6265x + 9.5783 | 6.10 |
| Ethanol extract of *Stachytarpheta jamaicensis* L.) Leaf [a] | - | 74.32 |

[a] based on reference [6]

Thus, all samples exhibited strong radical-scavenging potential, although a decrease in activity was observed in longer fermentation. This decrease maybe attributed to degradation or structural transformation of phenolic and flavonoid compounds overtime. These compounds which are abundant in pecut kuda (*Stachytarpheta jamaicensis* L.) leaf and they are sensitive to acidic condition and microbe enzyme activity. Previous studies have also reported that although total phenolic content may increase at later stage, the antioxidant activity may decrease due to changes in reactivity or loss of active functional group [14]. In comparison with Ascorbic acid ($IC_{50}$ 6.10 ppm), which is used as positive control, kombucha was showed lower antioxidant activity but remained within the strong activity category. This indicated that kombucha of pecut kuda (*Stachytarpheta jamaicensis* L.) leaf retains significant biological activity derived from plant extracts and fermentation metabolites.

Overall, these results indicated that pecut kuda (*Stachytarpheta jamaicensis* L.) leaf kombucha not only complies with physicochemical safety criteria but also offers higher antioxidant activity compare to raw leaf extract, supporting the developments of this kombucha as a healthy functional beverage.

## 4. Conclusion

Kombucha produced from pecut kuda (*Stachytarpheta jamaicensis* L.) leaf exhibited strong antioxidant activity throughout all fermentation periods with $IC_{50}$ value obtained for fermentation at days 6, 10 and 14 were 63.08 ppm, 66.79 ppm, and 98.24 ppm, respectively. Significant physicochemical changes occurred during fermentation, marked by a colour shift from dark brown to light brown, a stronger acidic aroma, and an increasingly sour-sweet taste. The pH decreased from 3.17 to 2.64 and 2.16, while total acetic acid increased from 0.25% to 0.54% and 0.86%, indicating active microbial fermentation and organic acid production. These findings show that pecut kuda leaf kombucha has strong antioxidant activity and good physicochemical qualities, supporting its potential as a functional beverage with health benefits.

## 5. Declarations

### 5.1 Acknowledgements

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### 5.2 Author contributions

Conceptualization P.N.A, V.W, H.I.F; Methodology P.N.A, V.W, H.I.F; Data collection and analysis P.N.A; Writing original draft: P.N.A; Review and editing V.W, H.I.F; Supervision V.W, H.I.F.

### 5.3 Conflict of Interest

There is no conflict of interest

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