

Evaluation of Vitamin C Content and Antioxidant Activities in Kombucha Fermented with Diverse Herbal Substrates

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ABSTRACT

Kombucha is a prebiotic of non-dairy products. It is made from fermented plant-based infusion using symbiotic culture of bacteria and yeast (SCOBY). During the fermentation process, yeast metabolize glucose in the substrate transforms into organic acid, vitamins, and microminerals. This transformation effect increases concentration and pharmacological activity. This research aimed to evaluate the antioxidant activity, vitamin concentration, and correlation between the kombucha process and increasing in antioxidants of kombucha. The antioxidant activity was analyzed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and vitamin content was analyzed using spectroscopy UV Vis. Kombucha was prepared using five different substrates such as *Etlingera elatior* flowers, *Senna alexandrina* leaves, *Syzygium malaccense* pericarp, *Sargassum crassifolium*, and *Kappaphycus alvarezii*. The antioxidant activity, expressed as IC₅₀, was 27.930, 8.769, 25.132, 22.238, and 118.874 µg/mL, respectively, while the vitamin C contents were 7.97%, 6.92%, 1.568%, 1.51%, and 0.364%, respectively. Among the substrates evaluated, *S. alexandrina* and *E. elatior* kombucha exhibited the strongest antioxidant activity and the high vitamin C content. Correlation analysis revealed a strong positive correlation between the antioxidant activity of the infusions and that of the resulting kombucha ($r = 0.9656$), indicating that substrates with higher initial antioxidant activity tended to produce kombucha with greater antioxidant activity. These findings demonstrate that substrate selection plays an important role in determining the antioxidant properties of kombucha and that *S. alexandrina* represents a promising substrate for producing kombucha with high antioxidant potential.

Keywords: Kombucha, Antioxidant, Vitamin C, DPPH Methode, Spectroscopy UV Vis

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DOI: 10.14203/ann.bogor.2022.v30.n1.24-31

Received: August 13, 2026; Revised: September 16, 2026; Accepted: September 16, 2026

Available online: September 30, 2026; Published: September 30, 2026

ISSN 0517-8452 (print) | 2407-7518 (online)

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INTRODUCTION

Kombucha is a prebiotic drink by fermentation using Symbiotic Culture of Bacteria and Yeast (SCOBY). In the kombucha fermentation process, bacteria and yeast convert organic compounds into organic acids (Júnior et al. 2022). Kombucha consists of the following compounds such as organic acids, minerals, polyphenols, water soluble vitamin (B1, B12, B6 and C), amino acids, protein, hydrolitic enzymes, and metaboic product of bacteria and yeast (Jakubczyk et al. 2020) (Geraris Kartelias et al. 2023). Some research explain pharmacologically kombucha is known to have antioxidant (Saimaiti et al. 2022), anti-cancer (Rasouli et al. 2021), anti-inflammatory (Sales et al. 2023), anti-bacterial, detoxification effects and regulates glucose metabolism (Hooi et al. 2023). Furthermore, kumbucha as prebiotic corellated with mental health by modulated intestine microbiom that effect to brain respon such neurotransmitter biosynthesis (Batista et al. 2023). In cancer, kombucha inhibit growth of colorectal cancer cell line. Some research show kombuca as anti bacterial agains *Staphylococcus aureus* and *Eschericia coli* (Rasouli et al. 2021).

Currently, kombucha drinks are very popular because it have a positive impact on health. Kombucha is an alternative for lactos tolerance, vegans and non-dairy prebiotic (Selvaraj and Gurumurthy 2022). Kombucha offers advantages such as high antioxidant and polyphenol content, lower calorie and fat levels, and greater microbial stability, leading to increased consumption compared to dairy-based probiotic products. Some people are turning tea as a fermentation medium for kombucha into extracts or infusions of flowers and plants (Zubaidah et al. 2021). The selection of plant-based substrates in kombucha production is important because botanical materials may vary considerably in their phytochemical composition, including phenolic compounds, flavonoids, pigments, organic acids, and antioxidant activity as well as.

In this research, *Etlingera elatior* flower, *Senna alexandrina* leaves, *Syzygium malaccense* pericarpium, *Sargassum crassifolium* and *Kappaphycus alvarezii* were used as tea substitutes. This simplicia is widely known to have pharmacological effects, such as *Etlingera elatior* flower and *Syzygium malaccense* for balancing oxidative stress (Naufalin, Sutrisna,

and Wicaksono 2021), *senna alexandrina* leaves and *sargasum* have an effect on the intestines (Omer et al. 2022). The aim of this reseach to evaluate antioxidant activity of kombucha with different substrate.

MATERIALS AND METHODS

Materials

Materials used in this study include, *Etlingera elatior* flower, *Senna alexandrina* leaves, *Syzygium malaccense* pericarpium, *Sargassum crassifolium*, *Kappaphycus alvarezii*, Aquadest, glucose, SCOBY, peptone, 96% ethanol, vitamin C, 2,2 diphenyl-1-picrylhydrazyl (DPPH).

Methods

Infusion of herbs

To prepare the infusion, 2 g of simplicia was extracted with 100 mL of distilled water maintained at a temperature of 80–90 °C

Kombucha Fermentation

The first step is to make a SCOBY starter with 250 grams of glucose, 2.5 grams of peptone, then all compounds are dissolved in 1 liter of distilled water and incubated for 14 days. The next step is to make the kombucha fermentation medium such glucose and herbs infusion, then the mixture is added with 50% SCOBY starter. Kombuca was incubated for 8 days.

pH Analysis

The pH analysis in this test uses a calibrated pH meter. A total of 100 ml of kombucha was put into a container and then measured directly using a pH meter.

Analysis of Antioxidant Activity

Before the antioxidant analysis was carried out, the harvested kombuca was measured for the BJ value for the volume to mass conversion process. A total of 2 ml of the sample solution and the Vitamin C comparison solution were put into a test tube, added 2 ml of 40 ppm DPPH solution until homogeneous, then vortexed and incubated for 30 minutes in a dark room then the absorbance was measured at a different wavelength. optimally and carried out 3 times.

IC value 50 was determined using the linear regression equation with the equation $Y=bx+a$, sample concentration (ppm) as the axis (X), and the percentage inhibition value as the axis (Y) with a value of 50 (Kholifah, Nurazizah, and Noviyanto 2023).

Analysis of Vitamin C

Ascorbic acid compounds as standard raw materials were diluted in 1, 2, 4, 8, 16 ppm and then analyzed using UV Vis spectroscopy. Next, the 100 ppm sample was read and the absorbance value was plotted in a linear regression curve $Y=bx+a$ (Kholifah, Nurazizah, and Noviyanto 2023).

RESULTS

The kombucha is a fermentation process using Symbiotic Culture of Bacteria and Yeast (SCOBY). Yeast produces the enzyme invertase, which breaks down sucrose into glucose and fructose, increasing the availability of these reducing sugars to both yeast and acetic acid bacteria. These sugars are metabolized by the yeast and acetic acid bacteria, resulting in the production of ethanol and organic acids. Additionally, glucose, a key building block for bacterial cellulose (BC) biosynthesis, is utilized by acetic acid bacteria to produce BC, which appears as a floating pellicle on the surface of Kombucha tea. This process effect for pH, concentration chemical compound which depend on the type of tea or SCOBY growing medium(Devanthi et al. 2021).



(a)



(b)

Figure 1. (a) SCOBY, (b) Kombucha

pH analysis

Fermentation is natural reaction process of microorganisms to convert some compound become new compound. Kombucha is fermented process by SCOBY to convert glucose become organic acid, this process has an effect on pH(Cohen, Sela, and Nolden 2023). Figure 1

shows the pH value of infusion and kombucha that all samples decreased in pH value. *Sargassum crassifolium* and *Kappaphycus alvarezii* have the highest decrease at pH 2.25 and 2.07 respectively. Research shows that the decrease in pH during kombucha fermentation is caused by the formation of organic acids and other compounds (Figure 2).

Table 1. pH value of infusion and kombucha.

Simplicia	Infusion	Kombucha
<i>Etlingera elatior</i> flower	4.72	2.60
<i>Senna alexandrina</i> leaves	6.29	3.97
<i>Syzygium malaccense</i> pericarpium	3.44	2.64
<i>Sargassum crassifolium</i>	7.51	2.25
<i>Kappaphycus alvarezii</i>	7.31	2.07

Analysis of Vitamin C

Table 2 show vitamin C contain of infusion and kombucha of *etlingera elatior* flower, *Senna alexandrina* leaves, *Syzygium malaccense* pericarpium, *Sargassum crassifolium*, *Kappaphycus alvarezii*. In this research, there was an increase of vitamin C Infusion and kombucha levels. The infusion of *Senna alexandrina* leaves and *Syzygium malaccense* pericarpium detect to containing highest vitamin C at 1.778% and 1.911% respectively. However, the kombucha of *etlingera elatior* flower show the highest vitamin C at 7.97%. In addition,

kombucha is fermented process involving symbiotic culture of bacteria and yeast (SCOBY). Yeast cells convert glucose to alcohol, while bacteria convert alcohol to organic acid such vitamin c. glucose in the kombucha medium is reduced to D-arabinose via D-arabino-1,4 lactone and converted into L-ascorbic acid, where it is known that LAA is the main precursor of ascorbic acid (M. Zhou et al. 2021). On the other hand, secondary metabolite of *etlingera elatior* flower support biosynthesis of vitamin c, such gallic acid, tannic acid, chlorogenic acid, caffeic acid, quercetin, apigenin, kaemferol, luteolin, and myricetin (Hamad and Hartanti 2023) (Hamed et al. 2022).

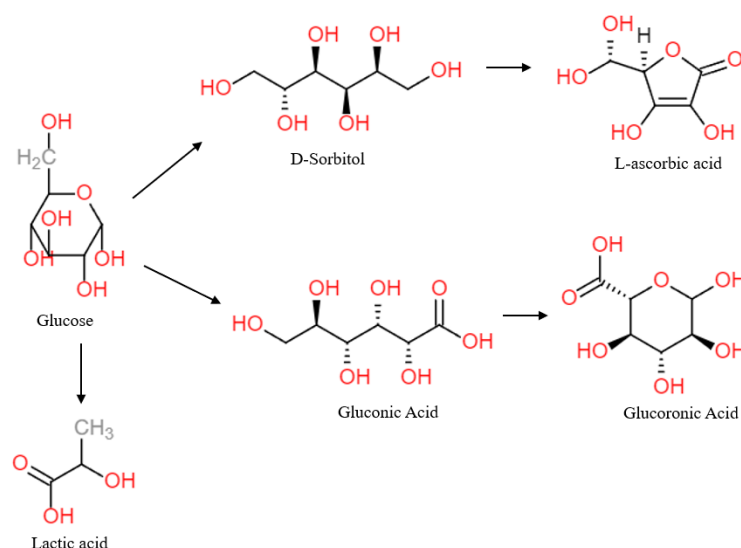
**Figure 2.** Reaction of synthesis organic acid from glucose.

Table 2. Vitamin C concentration of infusion and kombucha.

Simplicia	Vitamin C Concentration (%)	
	Infusion	Kombucha
<i>Etlingera elatior</i> flower	0,043	7,97
<i>Senna alexandrina</i> leaves	1,778	6,92
<i>Syzygium malaccense</i> pericarpium	1,911	1,568
<i>Sargassum crassifolium</i>	0,46	1,51
<i>Kappaphycus alvarezii</i>	0,275	0,364

Analysis of Antioxidant Activity

Table 3 presents overview of comparison infusion and kombucha antioxidant activity of *Etlingera elatior* flower, *Senna alexandrina* leaves, *Syzygium malaccense* pericarpium, *Sargassum crassifolium*, *Kappaphycus alvarezii*. Antioxidant activity value is definition in inhibition concentration 50% (IC₅₀), The lower concentration of IC₅₀ that inhibit free radicals, the better the antioxidant activity (Flieger et al. 2021). In this research, all antioxidant activity increase of infusion to kombucha. The higher antioxidant activity of infusion and kombucha is *Senna alexandrina* leaves of value 13.239 µg/ml to 8.769 µg/ml. *Senna alexandrina* have secondary metabolite such rutin, hyperoside, kaempferol, isoquercetin, and Trans-cinnamic acid, this compound impact to antioxidant activity (Omer et al. 2022). Rutin and hyperoside structure contain hydroxyl group that donating to free radical and stabilizing the radical. Moreover, carbon-carbon bond contribute to stabilizing the radical compound (Chaudhary et al. 2023). However Figure 4 show *Etlingera elatior* flower is the better improving antioxidant activity value of 7 times than other samples. The high antioxidant increase of *Etlingera elatior* flower is possible due to secondary metabolites that influence the biosynthesis of organic acids in kombucha such as acetic acid, citric acid, malic acid, gluconic acid, glucuronic acid (Saimaiti et al. 2022). This result related with Zhou research that there is enhance antioxidant activity in black and green tea (D.-D. Zhou et al. 2022).

Table 3. Antioxidant activity of infusion and kombucha.

Sample	Antioxidant Activity Value (IC ₅₀)	
	Infusion	Kombucha
<i>Etlingera elatior</i> flower	199,6	27,93
<i>Senna alexandrina</i> leaves	13,239	8,769
<i>Syzygium malaccense</i> pericarpium	110,829	25,132
<i>Sargassum crassifolium</i>	112,827	22,238
<i>Kappaphycus alvarezii</i>	446,390	118,874

The antioxidant activity of the initial infusion exhibited a strong positive correlation with that of the resulting kombucha ($r = 0.9656$, $p < 0.05$), indicating that substrates with higher initial antioxidant activity tended to produce kombucha with correspondingly higher antioxidant activity. This significant correlation suggests that the antioxidant properties of the initial substrates may substantially contribute to the antioxidant capacity of the fermented beverages.

In this study, there was an increase antioxidant activity in kombucha. Figure 4a displays correlation analysis of increasing antioxidant activity of infusion and kombucha, this correlation allows relating to biosynthesis of organic compound, such glucosa converts to organic acid (Figure 2). Furthermore, the tea contains polyphenols which are responsible for antioxidant activity, during the fermentation process there is an increase in polyphenols and flavonoids (D.-D. Zhou et al. 2022). Moreover, the secondary metabolites present in tea, catechins, are broken down into small molecule by bacteria and fungi thereby increasing their antioxidative activity (Wang et al. 2022). Some research explain there is correlation between antioxidant increasing and kombucha fermentation process. However, increasing vitamin c concentration does not show correlation (Figure 4b), this analysis shows that there are other coefficient that affect the vitamin C biosynthesis process in kombucha fermentation.

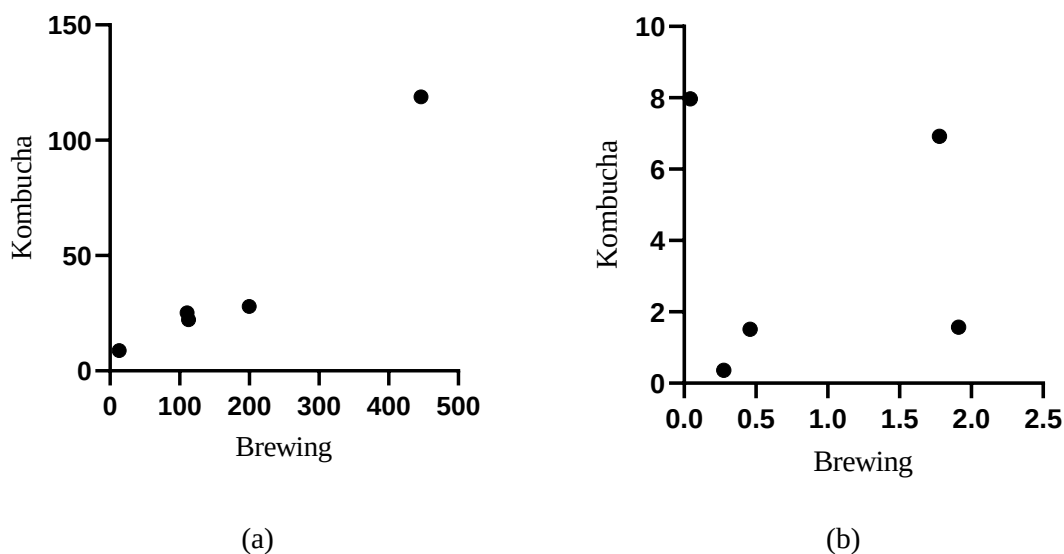


Figure 4. Correlation between kombucha and increasing of antioxidant activity and vitamin C concentration (a) Antioxidant activity ($r=0.9656$)^{**}, (b) Vitamin C ($r=0.0048$)^{ns}.

DISCUSSION

Kombucha is a fermented tea produced using a culture of bacteria and yeast (SCOBY). During the fermentation process, tea serves as the primary substrate, undergoing biotransformation into new compounds. The choice of substrate is crucial, as each one yields compounds with distinct characteristics and results in varying pharmacological effects (Su et al. 2023). In this study, conventional tea was replaced with five different plant-based substrates: *Etlingera elatior* flowers, *Senna alexandrina* leaves, *Syzygium malaccense* (L.) Merr. & L.M. Perry, *Sargassum polycystum*, and *Kappaphycus alvarezii*. The results demonstrated that substrate selection influenced the vitamin C content of the resulting kombucha. An increase in vitamin C content was observed when the initial infusion were compared with the corresponding fermented kombucha beverages. Among the substrates evaluated, kombucha produced from *Etlingera elatior* flowers exhibited the highest vitamin C content.

Furthermore, the change in substrate influenced antioxidant activity. The study showing increased antioxidant activity in the kombucha compared to the initial infusion. This enhancement is attributed to the biosynthesis of new compounds resulting from SCOBY fermentation. The added sugar is converted into organic acids, which contribute to antioxidant

activity, while secondary metabolites also play a role in boosting this activity (Chou et al. 2024). The study revealed that *Senna alexandrina* leaves exhibited the highest antioxidant activity. This research indicates that *Senna alexandrina* leaves contain secondary metabolites—such as flavonoids, tannins, and alkaloids—capable of stabilizing free radicals and acting as antioxidants. In this research *Senna alexandrina* leaves infusion have the highest antioxidant activity compare with other. This finding may partly explain the high antioxidant activity observed in the kombucha produced from *Senna alexandrina* leaves substrate. The correlation analysis revealed a strong association between the antioxidant activity of the initial infusion and that of the resulting kombucha, suggesting that substrate selection plays an important role in determining the antioxidant capacity of the fermented beverage. Thus, substrates with higher initial antioxidant activity tended to produce kombucha with greater antioxidant activity.

This research, we conclude that the type of infusion used to make kombucha affects the final product and its antioxidant activity. The findings obtained show differences in the vitamin content and antioxidant activity of kombucha drinks derived from *Etlingera elatior* flower, *Senna alexandrina* leaves, *Syzygium malaccense* pericarpium, *Sargassum crassifolium* and

Kappaphycus alvarezii. The results show that kombucha has higher antioxidant and vitamin activity compared to regular infusion, besides this research shows that there is a relationship between the kombucha process and increased antioxidant activity. Therefore, this type of research will be useful for the industry to promote probiotic products

CONCLUSIONS

Our study show fermentation process increasing antioxidant activity and vitamin C concentration. Increasing of antioxidant activity corellation with substrat selection. The best antioxidant activity is *Senna alexandrina* leaves kombucha at IC50 8,769 µg/ml. This resut indicated that kombucha has potency to developed as prebiotic non-dairy products.

ACKNOWLEDGEMENTS

We would like to thanks Instrument Laboratory of Sekolah Tinggi Ilmu Kesehatan Salsabila Serang for vitamin C and other chemicals to support this research.

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