

PHYTOCHEMICAL AND ANTIOXIDANT ACTIVITY OF SOURSOP (*Annona muricata* L.) LEAVES KOMBUCHA ENRICHED WITH GALACTOMANNAN FROM ENDOSPERM OF *Arenga pinnata*

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ABSTRACT

Kombucha is a long-established healthy drink produced by the fermentation of tea leaves with sucrose, bacteria, and yeasts. The usage of different kinds of tea leaves and carbon sources can also be brewed to cultivate Kombucha. This research aimed to target the antioxidant and phytochemical properties of soursop leaves Kombucha with galactomannan as an alternative carbon source evaluated against different soursop leaves concentrations with a particular fermentation time. The earlier kombucha broth 20 % (v/v) was inoculated with 0.5 g of galactomannan and various concentrations of soursop leaves. Moreover, Kombucha was fermented and analyzed with an interval of 0, 7, and 14 days in triplicate, respectively. Bioactive compounds were analyzed using UV-spectrophotometer, and 1.5% (w/v) soursop leaves Kombucha accord the best results than the lower concentration. GC-MS analysis for kombucha cultivar after 14 days of fermentation contained longer chain organic acids such as pentanoic acid, butanoic acid, and propanoic acid.

Keywords: Kombucha, Galactomannan, Antioxidant, Soursop Leaves.

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INTRODUCTION

The oldest technology to preserve food is fermentation, which delays spoilage, preserves food, and increases health and other beneficial values.¹ Kombucha is a long-established beverage originating in Asia, and initially, this beverage is cloaked in a riddle.² Kombucha has earned an extraordinary reputation, notably in the West, since its practical consequences on individual health such as antioxidant, anticarcinogenic, antidiabetic, antimicrobial, and increasing body immunity feedback.³ Diversity in yeasts are found in the consortium of kombucha beverages such as *Brettanomyces*, *Pichia*, *Saccharomyces*, *Candida*, and *Zygosaccharomyces sp*) and *Acetobacter xylinum* has been spotted in Kombucha during fermentation.⁴ Precisely, Kombucha can obstruct a vast spectrum of Gram-negative bacteria (e.g., *Escherichia coli* and *Pseudomonas aeruginosa*) and Gram-positive (e.g., *Staphylococcus aureus* and *Bacillus cereus*).⁵ Furthermore, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activities, total flavonoid content, and total phenolic content of Kombucha escalate during fermentation.⁴ Traditional medicine uses various parts of soursop (bark, leaf, fruit, root, and seed) against certain diseases such as diabetes, gastrointestinal disorders, respiratory ailments, cancers, and hypertension. Moreover, the antioxidant activity of soursop leaves comes from their phenolic contents as phytochemical agents.⁶⁻⁷ The DPPH scavenging and phenolic content of *Annona muricata* L. leaves are considerably high and could be used as antioxidant sources.⁸ Galactomannans are a class of polysaccharides that have neutral characteristics that compose a (1→4)-β-D-mannan backbone with a single functional group of α-D galactopyranosyl linked at a position of O-6 of the D-mannopyranosyl residues.⁹ In recent scientific research, the measurements of Vitamin C content of soursop kombucha enriched with galactomannan using indophenol titration were studied, and the result was significantly increased during fermentation for two weeks.¹⁰ Furthermore,

soursop fruit juice kombucha was fermented using black tea was evaluated to determine the biological activities for 21 days during storage and exhibited tremendous antioxidant activity for the Kombucha that fermented for two weeks at room temperature and pressure in light.¹¹ In another study, In vivo studies for the antidiabetic activity of snake fruit kombucha were evaluated. It showed significant results in reducing diabetes in mice.¹² Storage during fermentation is a substantial impact on antioxidant activities and bioactive compounds in Kombucha. Therefore, escalation of time during fermentation is very crucial to yield Kombucha enriched with bioactive compounds.¹³ From the author's observation, no studies were performed using galactomannan as a carbon source in Kombucha to analyze the antimicrobial and antioxidant activities of producing soursop leaves in Kombucha. Moreover, this study aims to analyze bioactive metabolites, physicochemical study, and the composition of soursop leave Kombucha.

EXPERIMENTAL

Material and Methods

Soursop leaves (*A. muricata* L.) and endosperm of *Arenga pinnata* were obtained from Manaon Village, Padang Lawas Utara, North Sumatera, Indonesia. Ethanol, methanol, quercetin, gallic acid, DPPH, NaOH, Folin-Ciocalteu reagent, Na₂CO₃, and Phenolphthalein were purchased from Sigma-Aldrich.

Soursop Leaves Kombucha Preparation

Galactomannan extracted from the endosperm of *Arenga pinnata* (0.5 g) was added to distilled water, boiled, and poured into a beaker glass. Soursop leaves of 0.5% (w/v), 1.0% (w/v) and 1.5% (w/v) were added respectively to the beaker glass. Kombucha tea was cooled down at room temperature, combined with 20 mL of earlier kombucha broth, and allowed to ferment at an interval of 7 days for two weeks.¹⁰

Physicochemical Study

A calibrated electric pH meter was used to determine the pH (Hanna, Thermo Fischer Scientific, USA). A refractometer was used to determine the total alcohol content (Atago N1, Tokyo, Japan). Total acidity was determined using the method of Ranggana, which involved mixing 10 mL of soursop leaves Kombucha with different concentrations and diluting until 100 mL, then titrating with 0.1 NaOH and phenolphthalein indicator.¹⁴

Bioactive Compound Analysis

The reagent of Folin-Ciocalteu (FC) was applied to evaluate the total phenolic content (TPC) of soursop kombucha with gallic acid as standard developed by Chandra *et al.* 0.2 mL of soursop kombucha was added with 0.2 mL of FC then let on 25°C for 5 minutes before adding 1 mL of 0.075 M of Na₂CO₃ solution and 3 mL of ultra-pure water and incubating in the dark condition for half an hour. After incubation, the soursop kombucha was analyzed at 765 nm using a spectrophotometer UV-Visible (Shimadzu-1240, Tokyo, Japan). The TPC was declared as mg GAE/L.¹⁵ The Total flavonoid content (TFC) of soursop kombucha was evaluated in line with Chang *et al.*, whereas the standard was quercetin. 0.5 mL of soursop kombucha was added with 0.1 mL of 1% AlCl₃, 0.1 mL CH₃COONa 1 M, and 1.5 mL methanol, followed by the addition of 2.8 mL distilled water, then allowed to stand for half an hour at 25°C and evaluated at 415 nm using spectrophotometer UV-Visible (Shimadzu-1240, Tokyo, Japan). TFC content was declared as mg QE/L.¹⁶

GC-MS Analysis for Soursop Kombucha

GC-MS analysis for soursop kombucha with different concentrations was evaluated with the HP-5MS instrument (Hewlett-Packard, Palo Alto, CA, USA) coupled with a scrupulous mass detector (GCMS-QP2010 Ultra Shimadzu, Japan).

Antioxidant Scavenging Activity (IC₅₀) using DPPH

The DPPH test was known to estimate the free radical scavenging activity of soursop kombucha using Molyneux's method with a little modification. 1 ml of soursop kombucha was infused with 1 ml 0.5 mM DPPH (200 mg/L) in a 5 ml volumetric flask and let in the dark condition at 25°C for half hour before evaluating absorbance at 516 nm with a spectrophotometer (Shimadzu, UV-mini-1240, Tokyo Japan). Based on the plot of absorbance versus extract concentrations, the IC₅₀ value (half-maximal effective concentration) was calculated.¹⁷

RESULTS AND DISCUSSION

Physicochemical Study

Galactomannan is a polysaccharide that contains a linear mannan backbone and a single galactose unit. Full hydrolysis of galactomannan needs several enzymes described by Moriera *et al.*¹⁸ The endo- β -1,4-mannanase sunders the β -1,4-mannopyranosyl linkages in the mannan backbone, generating different lengths of oligosaccharides, and the removal of galactose is activated by α -galactosidases from the mannan chain is described by Stoll *et al.*¹⁹ Malherbe *et al.* using *S. cerevisiae* to hydrolyze lotus bean gum, mannose, and hexose equivalent to produce bioethanol and small composition of ethanol and short-chain organic acid is produced such as acetic acid.²⁰

In this research, galactomannan used in kombucha tea produces different organic acid products with longer chain carbon such as pentanoic acid, butanoic acid, and propanoic acid compared to using sucrose as a carbon source which has acetic acid, malic acid, and gluconic acid as the main product due to *Acetobacter* bacteria.¹³ The three significant compounds analyzed in GC-MS are compiled in Table-1.

Table-1: Three Major Compounds in Soursop Kombucha Tea Using GC-MS Analysis

Day 14, 0.5%(w/v)		Day 14, 1.0%(w/v)		Day 14, 1.5%(w/v)	
Compound	Area (%)	Compound	Area (%)	Compound	Area (%)
Pentanoic acid	11.63	hydroquinone	11.45	Butanoic acid	2.83
2-butanone	12.24	4-methylvaleric acid	13.37	hydroquinone	10.72
1,2-benzene dicarboxylic acid	16.09	Pentanoic acid	15.82	Pentanoic acid	40.51

The long-chain organic acids produced are closely related to pH, the longer carbon chain of the organic acids will increase the pH value and decrease the total acidity from day 0 to day 14 with different concentrations, as shown in Table-2.

Table-2: Physicochemical Study of Soursop Leaves Kombucha Enriched with Galactomannan

Fermentation, Concentration of Soursop Leaves	pH	Total Acidity (%)	Ethanol (%v)
Day 0, 0.5%(w/v)	3.772 \pm 0.0279	0.068 \pm 0.0011	0.2 \pm 0
Day 0, 1.0%(w/v)	3.804 \pm 0.0429	0.063 \pm 0.0010	0.233 \pm 0.0384
Day 0, 1.5%(w/v)	3.863 \pm 0.0236	0.061 \pm 0.0015	0.267 \pm 0.0384
Day 7, 0.5%(w/v)	3.963 \pm 0.0066	0.053 \pm 0.0013	0.2 \pm 0
Day 7, 1.0%(w/v)	3.971 \pm 0.0059	0.053 \pm 0.0010	0.267 \pm 0.0384
Day 7, 1.5%(w/v)	4.044 \pm 0.0360	0.052 \pm 0.0011	0.3 \pm 0
Day 14, 0.5%(w/v)	4.251 \pm 0.0124	0.049 \pm 0.0021	0.167 \pm 0.0384
Day 14, 1.0%(w/v)	4.267 \pm 0.0076	0.046 \pm 0.0007	0.167 \pm 0.0384
Day 14, 1.5%(w/v)	4.298 \pm 0.0099	0.041 \pm 0	0.2 \pm 0

Ethanol produced from this Kombucha is relatively low because the carbon substrate is not sucrose but galactomannan. If the sucrose is the substrate, it will undergo a glycolysis pathway to produce ethanol with a higher concentration. A higher concentration of ethanol is non-Halal for Moslem as ethanol indicated limited to less than 1% described by Alzer *et al.*²¹ Moreover, this indicates that soursop kombucha enriched with galactomannan can be the alternative probiotic drink for Moslem, especially in Indonesia.

TPC and TFC of Soursop Kombucha

In SCOBY, yeasts and bacteria create an enzyme that converts polyphenolic complexes into simple phenolic compounds. The heightened phenol concentration might be attributed to bioconversion, which involves enzymes that are utilized to catalyze specific biological activity. Antioxidant activity is substantially related to phenolic compounds, the more significant the amounts of phenolic compounds, the stronger the antioxidant activity. Moreover, they are known for lowering oxidative force and free radical activity.²² The different bioactive chemicals (phenolics and flavonoids) naturally present in the cultivar are assumed to be responsible for the antioxidant activity observed (Table-3).

Table-3: TPC and TFC of Soursop Kombucha Enriched with Galactomannan

Fermentation, Concentration of Soursop Leaves	TPC (mg GAE/L)	TFC (mg QE/L)
Day 0, 0.5%(w/v)	128.876 ± 24.656	248.241 ± 3.0601
Day 0, 1.0%(w/v)	224.933 ± 13.9644	524.096 ± 14.5390
Day 0, 1.5%(w/v)	376.031 ± 19.9242	644.470 ± 3.7719
Day 7, 0.5%(w/v)	164.211 ± 21.0544	308.636 ± 8.2617
Day 7, 1.0%(w/v)	287.762 ± 18.2737	565.777 ± 1.4049
Day 7, 1.5%(w/v)	425.032 ± 3.4777	739.877 ± 17.1992
Day 14, 0.5%(w/v)	260.467 ± 32.5997	325.234 ± 9.4346
Day 14, 1.0%(w/v)	301.637 ± 13.4780	737.867 ± 3.3699
Day 14, 1.5%(w/v)	499.315 ± 15.0769	808.431 ± 2.8851

The fermentation technique was advantageous to discharge these bioactive chemicals, presumably because their complicated states were split into simple states.³⁻⁴ Compared to snake fruit kombucha fermented by Zubaidah *et al.*²³ the TFC is also higher than its TPC. In this research, the most increased antioxidant activity (TPC and TFC) is soursop Kombucha which is fermented for 14 days with 1.5% (w/v) of the soursop leaves.

DPPH Scavenging Activity (IC₅₀) of Soursop Kombucha

In Vitro DPPH Scavenging activity through IC₅₀ measurement (the concentration required to inhibit free radicals by 50%) is summarized in Table-4.

Table-4; IC₅₀ (ppm) of Soursop Kombucha Enriched with Galactomannan

Soursop Kombucha	IC ₅₀ value (ppm)		
	Day 0	Day 7	Day 14
1.5 % (w/v)	119.595 ± 9.6556	95.354 ± 20.3877	78.145 ± 6.4813

The IC₅₀ value is 50 ppm, 50-100 ppm, and 101-250 ppm indicating that the antioxidant activity is classified as extremely powerful, strong, and moderate, respectively.²⁴ Based on this factor, the antioxidant activity of soursop kombucha enriched with galactomannan after fermentation for 14 days is categorized as a strong antioxidant. Soursop kombucha stored for 14 days in the presence of lights indicated the highest DPPH scavenging activity described by Tan *et al.*¹¹

CONCLUSION

Soursop leaves Kombucha, which enriched with galactomannan can be potentially be used as a probiotic beverage. This Kombucha consortium boosts the antioxidant activity after fermentation for 14 days, the pH, total acidity, and ethanol concentration of the soursop kombucha is relatively halal for consumption.

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