



# The kombucha from *Rhizophora mucronata* Lam. herbal tea: Characteristics and the potential as an antidiabetic beverage

[Kombucha del té de hierbas de *Rhizophora mucronata*: Características y potencial como bebida antidiabética]

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## Abstract

**Context:** Kombucha from tea is reported to be beneficial for health. Moreover, it can be used as a hepatoprotective, antiproliferative, antimicrobial, antidiabetic, and antilipidemic agent and is capable of healing stomach ulcers. But kombucha from other herbal has not been studied, including kombucha from the mangrove (*Rhizophora mucronata*) fruit.

**Aims:** To evaluate the characteristics and the antidiabetic potential of kombucha herbal tea from *R. mucronata* fruit based on *in vitro*, chemistry, and physical analysis.

**Methods:** This study was conducted by an experimental method using *R. mucronata* herbal tea as a kombucha drink with different sugar concentrations (10, 20, 30%) and fermentation time (7, 14, 21 days) with three replications on each experiment. The analyzed parameters were the inhibition of the  $\alpha$ -glucosidase enzyme for antidiabetic activity, total phenolics, total acids, and organoleptic characteristics.

**Results:** The sugar concentration and fermentation time significantly affected the characteristics of the produced kombucha in inhibiting  $\alpha$ -glucosidase. The optimum treatment in inhibition was at 10% sugar concentration and 14 days of fermentation time with  $IC_{50}$  of 33.95 ppm. The kombucha from *R. mucronata* fruit had a pH of 3.11 and contained a total phenolics of 19,679.82 mg GAE/100g, 0.52% of total acids, and was quite preferred by panelists.

**Conclusions:** Kombucha herbal tea of *R. mucronata* fruit has the potential as an antidiabetic drink with a lower  $IC_{50}$  value than acarbose drug and commercial kombucha tea.

**Keywords:** antidiabetic; fruit;  $\alpha$ -glucosidase; herbal tea; kombucha; *Rhizophora mucronata*.

## Resumen

**Contexto:** Se informa que la kombucha del té es beneficiosa para la salud. Además, puede usarse como un agente hepatoprotector, antiproliferativo, antimicrobiano, antidiabético y antilipídico y es capaz de curar úlceras estomacales. Pero no se ha estudiado la kombucha de otras hierbas, incluida la kombucha de la fruta del mangle (*Rhizophora mucronata*).

**Objetivos:** Evaluar las características y el potencial antidiabético del té de hierbas kombucha de la fruta de *R. mucronata* con base en análisis *in vitro*, químicos y físicos.

**Métodos:** Este estudio se realizó mediante un método experimental utilizando té de hierbas de *R. mucronata* como bebida de kombucha con diferentes concentraciones de azúcar (10, 20, 30%) y tiempo de fermentación (7, 14, 21 días) con tres repeticiones en cada experimento. Los parámetros analizados fueron la inhibición de la enzima  $\alpha$ -glucosidasa para la actividad antidiabética, fenoles totales, ácidos totales y características organolépticas.

**Resultados:** La concentración de azúcar y el tiempo de fermentación afectaron significativamente las características de la kombucha producida en la inhibición de la  $\alpha$ -glucosidasa. El tratamiento óptimo en la inhibición fue a una concentración de azúcar del 10% y 14 días de tiempo de fermentación con  $IC_{50}$  de 33,95 ppm. La kombucha de la fruta de *R. mucronata* tuvo un pH de 3.11 y contenía un contenido de fenoles totales de 19.679,82 mg GAE/100g, 0,52% del ácido total, y fue muy preferida por los panelistas.

**Conclusiones:** La kombucha del té de hierbas de la fruta de *R. mucronata* tiene el potencial de ser una bebida antidiabética con un valor de  $CI_{50}$  más bajo que el fármaco de acarbose y la kombucha del té comercial.

**Palabras Clave:** antidiabético; fruta;  $\alpha$ -glucosidasa; té de hierbas; kombucha; *Rhizophora mucronata*.

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## INTRODUCTION

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Diabetes mellitus is a degenerative disease that has developed rapidly in the community. Diabetes can be treated with chemical drugs or natural medicines from plants or animals. Some people prefer natural medicine because it is considered to be easier and safer, and it can be consumed in the form of an herbal or functional food (Chan et al., 2018). Therefore, it is very important to explore the potential of natural sources to be used as medicine as well as functional food.

One potential source of natural materials to be used as a medicine is mangrove plants, which commonly grow in the tidal beach ecosystem (Salini, 2015). Traditionally, some people use mangroves as a medicine for diarrhea, coughing, ulcers, hepatitis, bleeding, and infections. The use of mangroves as traditional medicine indicates the various active components in the mangrove plants to cure disease (Ananthavalli and Karpagam, 2017). Mangrove plants contain steroids, triterpenes, saponins, flavonoids, alkaloids, and tannins (Pintoi et al., 2017). The bioactive compound is reported to have an antibacterial (Mishra and Sree, 2007; Sivaperumal et al., 2010; Ravikumar and Gnanadesigan, 2012; Saheb et al., 2016; Ananthavalli and Karpagam, 2017; Chan et al., 2018; Kamalambigeswari et al., 2020), antifungal (Heidari-Sureshjani et al., 2015; Pintoi et al., 2017; Rastegar and Gozari 2017) antioxidant (Ramde-Tiendrebeogo et al., 2012; Sudirman et al., 2014; Podungge et al., 2015; Dia et al., 2015; Fanga et al., 2019; Kaewkod et al., 2019), anticancer (Prabhu and Guruvayoorappan, 2012), and antidiabetic effect (Kannan et al., 2012; Hardoko et al., 2015a;b; Yamada et al., 2017).

Tannin is the most dominant compound in mangrove plants that are used as a natural dye for textiles and tanners (Hernawan and Setyawan, 2003; Arumungam et al., 2014). It also plays as a bioactive compound to cure diabetes disease (Hernawan and Setyawan, 2003; Subramoniam, 2016; Yamada et al., 2017). Hardoko et al. (2015b) reported that the tannin levels of *Rhizophora mucronata* Lam. (*Rhizophoraceae*) fruits reached 849

ppm. Besides, *R. mucronata* fruit also contains phenolic, flavonoid, and triterpenoid compounds. However, it was reported by Sadeer et al. (2019) that the total condensed tannin of *R. mucronata* was lower than its total phenolic content.

Tannin compounds in mangrove plants have the potential to be used as a beverage, such as tea, which relies on tannin to give its typical flavoring. Tea products that are not derived from tea leaves are often referred to as herbal teas. Herbal teas provide many health benefits, such as reducing obesity (Purwanto et al., 2014; Wulandari and Rahmanisa, 2016), antidiabetic (Rohdiana et al., 2012; Ray et al., 2014; Striegel et al., 2015) such as black tea (Deswati and Maryam, 2016; Rosalia et al., 2016) and white tea (Trinovian et al., 2016), decreasing cholesterol, and as an anticancer agent. According to Malanggi et al. (2012), there is a positive correlation between tannin content with the antioxidant and antidiabetic effect. Those benefits led to the production of various types of herbal teas with antidiabetic properties, such as stevia leaf herbal tea (Trinovian et al., 2016), Mulberry leaf tea (Banu et al., 2015), guava leaf tea (Musdja et al., 2017), *R. mucronata* herbal tea (Hardoko et al., 2015a), pandanus tea (Prameswari and Widjanarko et al., 2014), and olive leaf tea (pomace tea) (Oh et al., 2015).

The role of tea is quite a lot for health benefits, and this raises a variety of products derived from tea, like kombucha. Kombucha is a beverage with a slightly sweet and sour taste from the fermentation of steeping tea and sugar by the “tea mushroom” starter (Jayabalan et al., 2014; Leala et al., 2018). This drink was firstly originated from North China and later developed worldwide. Its functional properties induce the worldwide spread of kombucha; it can be used to lower blood pressure, reduce arthritis, increase immunity, cure cancer, as an antioxidant, and have anti-inflammatory effects (Leala et al., 2018).

The fermentation process of steeping tea and sugar using the “tea mushroom” starter results in the formation of a springy layer on the surface called “tea mushrooms” or “nata,” and tea liquid

at the bottom. The tea mushroom layer can be used as a starter culture, and its liquid is the kombucha drink (Jayabalan et al., 2014; Leala et al., 2018). The tea mushroom starter consists of bacteria and fungi in symbiosis. The types of bacteria and fungi found in the starter cultures vary so they can affect the characteristics of the fermented product. In general, the bacteria present are the genera of *Acetobacter* and *Gluconobacter*, whereas the yeast content in kombucha is *Saccharomyces*, *Saccharomyces*, *Schizosaccharomyces*, *Zygosaccharomyces*, *Brettanomyces*, *Candida*, *Torulospira*, *Kloeckera*, *Pichia*, *Mycotorula*, and *Mycoderma* (Jayabalan et al., 2014). The symbiosis of bacteria and fungi has been reported to cause no disease, side effects, or even toxicity (Villarreal-Soto et al., 2018). The *Acetobacter* species that are already identified are *Acetobacter nitrogenifigens* (Neera and Batra, 2015) and *Komagatabaeiacter rhaeticus* strain P 1463, both reported to produce a high level of cellulose (Semjonovs et al., 2017).

The transformation of tea to kombucha indicates the formation of new compounds and/or increased activity of compounds with health benefits. Jayabalan et al. (2015) reported the changes in the organic acids and polyphenols content during kombucha fermentation. Some of the metabolites are ethanol, lactic acid, acetic acid, gluconic acid, and glucuronic acid (Jayabalan et al., 2014; Mukadam et al., 2016), which are thought to play a vital role in providing health benefits. It has been reported that kombucha has a hepatoprotective effect (Kabiri et al., 2014; Watawana et al., 2015), antiproliferative effect against cancer cells, and antimicrobial effect against *Escherichia coli*, *Salmonella enterica*, *Micrococcus luteus*, and *Staphylococcus epidermidis* (Abou-Taleb et al., 2017), while also has hypoglycemic and antilipidemic (Zubaidah et al., 2018), and provides a better healing process to stomach ulcer than omeprazole and black tea (Kaewkod et al., 2019).

Tea is beneficial for health, and fermenting tea into kombucha may produce several compounds that contribute to health benefits, including antidiabetic. Meanwhile, *R. mucronata* leaf and fruit (Hardoko et al., 2017) have an antidiabetic function (Hardoko et al., 2015a; Hardoko et al., 2015b;

Hardoko et al., 2017; Hardoko et al., 2018). However, the characteristics and antidiabetic potentials of fermented mangrove fruit herbal tea are still unknown. Therefore, this study is necessary to determine the characteristics and the antidiabetic potential of kombucha herbal tea from *R. mucronata* mangrove fruit based on *in vitro*, chemical, and physical analyses.

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## MATERIAL AND METHODS

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### Materials and equipment

The materials for mangrove fruit kombucha tea preparation were mangrove fruit tea, water, granulated sugar (Gulaku, Lampung), 'nata', and liquid kombucha starter (Indokombucha, Bandung). Mangrove fruit herbal tea was made from ripe *R. mucronata* fruit, which was collected from the Nguiling region, Pasuruan Regency, East Java (7°42'13.4"S 113°05'35.7"E). This material was identified based on its physical and biological characteristic as *R. mucronata* considering previous studies (Hardoko et al., 2015a;b; 2017; 2018). The materials for experiment were filter paper, concentrated ammonia (Smartlab, Indonesia), Mayer's reagents (Local), Wagner's reagents (Local), anhydrous acetate (Merck, Germany), concentrated sulfuric acid (Merck, Germany), magnesium powder (Mg Merck, Germany), concentrated HCl (Merck, Germany), methanol (Merck, Germany), FeCl<sub>3</sub> (Merck, Germany), aquadest (Hydrobatt), gallic acid (Sigma-Aldrich, Singapore), Folin-Ciocalteu reagents (Merck, Germany), Na<sub>2</sub>CO<sub>3</sub> 7.5% (Merck, Germany), NaNO<sub>2</sub> (China), AlCl<sub>3</sub> 10% (Merck, Germany), quercetin (Sigma-Aldrich, Singapore), NaOH 10% (Sigma-Aldrich, Singapore), PVPP (polyvinylpyrrolidone), butanol-HCl (Smartlab, Indonesia), ferric reagents (Merck, Germany), aluminum foil, glucose (Merck, Germany), 5% phenol solution (Merck, Germany), concentrated H<sub>2</sub>SO<sub>4</sub> (Merck, Germany), phenolphthalein (PP) (Merck, Germany), 0.1 N NaOH (Merck, Germany), agar plate count (PCA) medium (Merck, Germany), butterfield's phosphate-buffered dilution water (BPB) (Merck, Germany), potato dextrose agar medium (Merck, Germany), α-glucosidase enzyme (Megazyme, UK), p-nitrophenyl α-D-glucopyrano-

side (PNPG) substrate (Megazime, India), dimethyl sulfoxide (DMSO) (Merck, Germany), pH buffer, Na<sub>2</sub>CO<sub>3</sub> (Merck, Germany), bovine serum albumin (Merck, Germany), and acarbose (Glucobay, Jakarta).

The equipment for preparing mangrove fruit herbal tea were knives and basins. The tools for preparing mangrove herbal kombucha tea were glass jars, spoons, filters, digital scales (AND EK-6T0i), glass tools (Pyrex), gauze cloth, rubber, wood stirrer, and water bath (Memmert type W 350). The tools for the experiments were vortex mixer (VM-2000), glass equipment (Pyrex), stopwatch, UV-Vis spectrophotometer (Spectroquant Pharo 300), hot plate (Ikamag Ret), centrifuge (Heraeus), thermometer, burette (Pyrex), Petri dish (Pyrex), autoclave (Hirayama HL-36Ae), incubator (Memmert), colony counter (Gallenkamp), micrometer screw (NSK), glass, spoon, and micropipette (Accumax Pro).

### Experimental design

The study used an experimental method for preparing kombucha from *R. mucronata* herbal tea with different sugar concentrations (S) (10, 20, 30%) and fermentation time (FT) (7 days, 14 days, and 21 days) with three replications of each treatment. The analyzed parameters were enzyme  $\alpha$ -glucosidase inhibition as an *in vitro* antidiabetic activity analysis, total phenol, total acid, and organoleptic characters.

### Mangrove (*R. mucronata*) herbal tea preparation

The process of making mangrove fruit tea (*R. mucronata*) was based on Shofiati's method (Shofiati et al., 2014). Old *R. mucronata* mangroves were used in this study. The fruit was washed with flowing water until clean to remove any impurities, then thinly sliced about 2 mm thick, and sun-dried until the water content was around 8% (SNI, 2013, about tea products). After the desired water content was reached, then the mangrove fruit herbal tea was obtained.

### Preparation of kombucha of mangrove *R. mucronata* fruit herbal tea

Mangrove (*R. mucronata*) herbal tea conversion to kombucha was based on Wistiana and Zubaidah (2015). Dried mangrove fruit herbal tea was weighed for 20 g and then brewed with 1 L of water at 90°C and allowed to sit for 20 min. Subsequently, it was filtered to obtain a steeping mangrove herbal tea. The steeping herbal tea was put into a glass jar and cooled until 25°C with cooling time did not exceed 4 hours. After that, it was added with sugar with various concentrations, 10, 20, and 30% (w/v), stirred, and then added with the 10% liquid kombucha starter (v/v). Next, the glass jar was covered with a gauze cloth and tied with a rubber. Then, the jar was stored at room temperature for 7, 14, and 21 days to do the fermentation process. After the fermentation was complete, the kombucha (nata) starter was separated, and the fermented liquid was filtered to produce mangrove kombucha tea. This experiment was repeated three times.

### Antidiabetic activity assay through inhibition of $\alpha$ -glucosidase

The antidiabetic activity was measured based on the inhibition of the  $\alpha$ -glucosidase enzyme *in vitro*, according to Sugiwati et al. (2009). The principle was to make various concentrations of kombucha herbal tea samples (6.25, 12.5, 25, 50 ppm) in the DMSO (w/v) solution and add 250  $\mu$ L of p-nitrophenyl- $\alpha$ -D-glucopyranoside (PNPG) and phosphate buffer pH 7. The mixture was incubated at 37°C for 5 min, and then the enzyme  $\alpha$ -glucosidase was added and incubated for another 15 min at 37°C in a water bath. Finally, the mixture was added with 200 mM Na<sub>2</sub>CO<sub>3</sub> to form p-nitrophenol. The mixture was then measured using a UV-Vis spectrophotometer on the wavelength of 400 nm. Its inhibitory activity was determined using the following formula [1]:

$$\% \text{ Inhibition} = \frac{\text{Control} - \text{Sample}}{\text{Control}} \times 100\% \quad [1]$$

Acarbose was used as a positive control, and all treatment was conducted in triplicate (n=3). The concentration of the samples required to inhibit 50% of  $\alpha$ -glucosidase activity under the assay conditions was defined as the IC<sub>50</sub> value. A linear regression was made between % inhibition (Y) and sample concentration (X) to obtain a linear equation. IC<sub>50</sub> values of samples were calculated based on the equation obtained.

### Analysis of total phenolics using Folin-Ciocalteu method

Total phenolics determination was using the Folin-Ciocalteu method, according to Baba and Malik (2015). Samples, with a concentration of 1000 ppm, were taken (0.5 mL) and added in 2.5 mL of Folin-Ciocalteu reagent, which was diluted with distilled water (1:10). The mixture was left for 4 min in the dark and then added with 2 mL of Na<sub>2</sub>CO<sub>3</sub> (7.5%). The solution mixture was incubated at room temperature for 2 h. The absorbance was read using a UV-Vis spectrophotometer at 760 nm wavelength. Standard concentrations for calibration curves were made at concentrations of 25–200  $\mu$ g/mL. Gallic acid standard curves were made by a linear regression equation, which states the relationship between the concentration of gallic acid in the X-axis and the amount of absorbance resulting from the reaction of gallic acid with the Folin-Ciocalteu recorded in the Y-axis. Total phenol was expressed gallic acid amount (mg GAE/100 g) that was obtained from the standard curve equation:  $Y = aX + b$ . This experiment was repeated three times (n=3).

### Total acid analysis

The total acid test was determined by the titration method by the method of Prastujati (2018) with modification. In the first step, the 10 mL sample was titrated using the burette apparatus, graduated to 0.1 mL, and with an accuracy of 0.05 mL. Before titration, two drops of phenolphthalein (PP) were added. Subsequently, the sample was

titrated with 0.1 N NaOH until a consistent pink color appeared. The total acids can be calculated using the formula [2] below:

$$\text{Total acids} = \frac{V1 \cdot N1 \cdot B}{V2 \cdot 1000} \times 100\% \quad [2]$$

Where V1 is the volume of NaOH (mL); V2 is the volume of sample (mL); N is the normality of NaOH (0,1 N); B is the molecular weight of lactic acid (90). This analysis was repeated three times (n=3).

### Organoleptic test

Several attributes were organoleptically tested using the hedonic test, which was taste, aroma, color, and overall acceptance. There were 25 semi-trained panelists who participated in this test. Those panelists were chosen based on their interest in kombucha. The organoleptic test was done based on the method by Koch et al. (2012) with modification. Before the test, the panelists were informed about the study background, study objectives, and was given guidelines about how to perform the test. Those panelists were instructed to open the plastic lid, rotate the glass several times, and evaluate the color of kombucha by direct observation, then evaluate the aroma by sniffing on the given samples. Furthermore, the panelists were asked to evaluate taste by drinking the samples slowly using a tablespoon, then evaluate the overall acceptance of the samples. The panelists were asked to give a hedonic score with a scale of 1-7, in which 1 means extremely dislike and 7 means extremely like on the questionnaire paper. The panelists were also asked to rest and rinse their mouths regularly using mineral water to prevent any sensory fatigue and the accumulation of astringency.

### Statistical analysis

Data were analyzed by analysis of variance (ANOVA) and Tukey's LSD test using the SPSS 16.0 program (IBM, USA), using the p-value of 0.05 to detect statistically significant differences.

**Table 1.** IC<sub>50</sub> of *R. mucronata* fruit herbal tea kombucha.

Group	Fermentation period (days)		
	7	14	21
<b>α-Glucosidase inhibition (IC<sub>50</sub>) (ppm)</b>			
Sucrose 10%	106.58 ± 1.67 <sup>d</sup>	33.95 ± 0.70 <sup>a</sup>	174.48 ± 2.01 <sup>g</sup>
Sucrose 20%	121.07 ± 1.07 <sup>e</sup>	57.44 ± 2.57 <sup>b</sup>	201.33 ± 1.70 <sup>h</sup>
Sucrose 30%	141.34 ± 5.28 <sup>f</sup>	80.51 ± 8.32 <sup>c</sup>	236.96 ± 1.98 <sup>i</sup>
Acarbose	85.61 ± 1.95		
Kombucha tea (commercial)	67.78 ± 0.61		

A different letter indicating statistically significant differences (p<0.05), n=3.

## RESULTS AND DISCUSSION

### Inhibition power of kombucha mangrove fruit against α-glucosidase

The inhibition of α-glucosidase is a basis to assess the potential of antidiabetic products by comparing with other antidiabetic drugs or similar products, like commercial kombucha tea. The amount of inhibition of the α-glucosidase enzyme can be shown by the IC<sub>50</sub> value, which describes the concentration of samples that can inhibit 50% of α-glucosidase enzyme activity. The IC<sub>50</sub> value of kombucha herbal tea from *R. mucronata* based on sugar concentration and fermentation time can be seen in Table 1.

Table 1 showed that the higher the sugar level in the fermentation process, the higher the IC<sub>50</sub> value. Interestingly, the longer the fermentation process did not make the lower IC<sub>50</sub> value. The lowest IC<sub>50</sub> value was at 14 days of fermentation. This phenomenon indicates that the sugar level and a certain amount of fermentation are required to produce kombucha that able to inhibit the enzyme activity optimally. In the case of mangrove kombucha, it needs a 10% sugar addition and 14 days fermentation time to produce a kombucha that is capable to significantly inhibit α-glucosidase or producing the lowest IC<sub>50</sub> value.

The different sugar concentrations and fermentation times were assumed to be related to the

types of microbes present in the kombucha starter and the compounds variety in the raw material. This assumption was based on the statement of Jayabalan et al. (2014), which suggests that the type of bacteria and fungi contained in the starter culture varies so that it can affect the characteristics of the produced product, including the IC<sub>50</sub> values. The functional characteristics of kombucha are related to the produced metabolites during fermentation. Jayabalan et al. (2015) reported that there was a change in the organic acids and polyphenols content during fermentation. Some of the kombucha tea metabolites are identified as ethanol, lactic acid, acetic acid, gluconic acid, and glucuronic acid (Jayabalan et al., 2014; 2015). In mangrove fruit kombucha, the metabolites are still unknown, especially those that play a vital role in inhibiting α-glucosidase.

The IC<sub>50</sub> value of acarbose and commercial kombucha tea is higher than *R. mucronata* herbal tea kombucha. It can be said that kombucha from *R. mucronata* herbal tea is more effective in inhibiting α-glucosidase activity than the acarbose and the commercial kombucha tea. This finding indicates that the mangrove herbal kombucha has the potential to be a better antidiabetic functional drink than the acarbose drug and kombucha tea. Holiday et al. (2018) who stated obtained IC<sub>50</sub> values of 54.86 μg/mL from black tea extract, 44.79 μg/mL from green tea, 55.46 μg/mL from oolong tea, and 43.42 μg/mL from white tea.

## Total phenolics

Phenol is a compound commonly found in plants and characterized by an aromatic ring with a series of a hydroxyl group and has many variations. The group of phenolic compounds, or often referred to as total phenolics, are related to the antidiabetic activity (Kunyanga et al., 2012; Adefe-gha et al., 2015; Adekola et al., 2017; Rohaeti et al., 2017; Sarian et al., 2017). It was stated that the higher the total phenol, the higher inhibitory activity against  $\alpha$ -glucosidase will be. Phenol compounds are found to exist naturally and can also form or break down during the fermentation process. The total content of herbal kombucha phenols from mangroves based on sugar concentration and fermentation time can be seen in Table 2.

Table 2 showed the higher the concentration of sugar used during fermentation, the lower the total phenol produced in the product, and the total phenols fluctuated based on the fermentation period. The increase in total phenol with the increase of sugar concentration during fermentation is related to the microbial activity during fermentation, namely yeast and acetic acid bacteria. Ivanisova et al. (2019) stated that bacterial and yeast enzymes produced during kombucha fermentation initiate a degradation of complex polyphenols to small molecules. It is also due to the acidic environment of kombucha that complex phenolic compounds could be degraded into smaller compounds, which then increase the total phenolic content.

Cardoso et al. (2020) also stated that kombucha prepared from black tea contained higher diversity of phenolic compounds due to degradation of dimeric and polymeric phenolic compounds, forming lower molecular weight. The decrease in phenol compounds after 14 days of fermentation is thought to be related to the changes in the pH of kombucha that can damage the phenol compounds.

The total phenol changes during the fermentation were similar to that of  $\alpha$ -glucosidase inhibition (Table 1), the higher the total phenol, the higher the inhibitory activity to the  $\alpha$ -glucosidase. The highest total phenol was found at 14 days fermentation time and 10% of sugar addition (Table 2), whereas  $IC_{50}$  was the lowest at 14 days fermentation time and 10% sugar addition. However, the type of phenolic compounds that can be found in mangroves herbal kombucha is still unknown, but according to Sarian et al. (2017), the phenolic compounds that play a role in antidiabetic activity are those that have double bonds in C-2-C-3 and C-4 ketonic group.

## pH and total acids

pH indicates the concentration of hydrogen ions from a solution, and there is a general relationship between pH and total acid. The higher the total acid, the lower the pH value. The average pH and the total acids of *R. mucronata* kombucha herbal tea can be seen in Table 3.

Table 3 shows that the average pH value was  $2.08 \pm 0.07 - 3.88 \pm 0.04$ . The longer the fermentation process, and the higher the sugar concentration, the lower the pH produced in the kombucha. The pH phenomenon is inversely proportional to the total acids produced. The decrease in pH in the sugar fermentation process is related to the activity of bacteria and yeast, which convert sucrose into organic acids, thereby increasing the amount of the produced acid (Junior et al., 2009). Besides, the increase in total acids during fermentation was due to yeast and bacteria metabolizing sucrose and producing several organic acids, such as acetic acid, gluconic acid, and glucuronic acid (Sreeramulu et al., 2000). Microbes will use the sugar as nutrients that will be converted into alcohol and  $CO_2$ .  $CO_2$  then reacts with water vapor and forms carbonic acid (Jayabalan et al., 2014; Leala et al., 2018).

**Table 2.** Total phenolics of *R. mucronata* fruit herbal kombucha tea using the Folin-Ciocalteu method.

Group	Fermentation period (days)		
	7	14	21
	Total phenolics (mg GAE/100 g)		
Sucrose 10%	19679.82 ± 649.06 <sup>ef</sup>	21302.63 ± 876.10 <sup>f</sup>	14460.37 ± 531.77 <sup>c</sup>
Sucrose 20%	17267.54 ± 748.83 <sup>d</sup>	18802.63 ± 862.82 <sup>e</sup>	12092.11 ± 748.19 <sup>b</sup>
Sucrose 30%	10469.30 ± 648.77 <sup>b</sup>	12311.40 ± 821.71 <sup>bc</sup>	8013.16 ± 558.24 <sup>a</sup>

A different letter in the same protocol indicating statistically significant differences ( $p < 0.05$ ),  $n = 3$ .

**Table 3.** pH and total acids of mangrove kombucha herbal tea based on the sugar addition and fermentation time.

Treatment (FT <sub>days</sub> - S <sub>%</sub> )	pH	Total acids (%)
FT <sub>7</sub> - S <sub>10</sub>	3.88 ± 0.04 <sup>h</sup>	0.02 ± 0.01 <sup>a</sup>
FT <sub>14</sub> - S <sub>10</sub>	3.11 ± 0.06 <sup>e</sup>	0.52 ± 0.04 <sup>c</sup>
FT <sub>21</sub> - S <sub>10</sub>	2.34 ± 0.05 <sup>b</sup>	0.95 ± 0.05 <sup>e</sup>
FT <sub>7</sub> - S <sub>20</sub>	3.63 ± 0.10 <sup>g</sup>	0.34 ± 0.02 <sup>ab</sup>
FT <sub>14</sub> - S <sub>20</sub>	2.86 ± 0.04 <sup>d</sup>	0.74 ± 0.03 <sup>d</sup>
FT <sub>21</sub> - S <sub>20</sub>	2.20 ± 0.03 <sup>ab</sup>	1.28 ± 0.09 <sup>f</sup>
FT <sub>7</sub> - S <sub>30</sub>	3.37 ± 0.13 <sup>f</sup>	0.43 ± 0.03 <sup>bc</sup>
FT <sub>14</sub> - S <sub>30</sub>	2.60 ± 0.04 <sup>c</sup>	0.87 ± 0.05 <sup>de</sup>
FT <sub>21</sub> - S <sub>30</sub>	2.08 ± 0.07 <sup>a</sup>	1.74 ± 0.07 <sup>g</sup>

FT = fermentation time; S = sugar percentage. The different superscript letter indicating statistically significant differences ( $p < 0.05$ ),  $n = 3$ .

**Table 4.** Organoleptic hedonic kombucha herbal from mangrove fruit.

Treatment (FT <sub>days</sub> - S <sub>%</sub> )	Organoleptic hedonic			
	Taste	Aroma	Color	Overall acceptance
FT <sub>7</sub> - S <sub>10</sub>	4.47 ± 0.18 <sup>f</sup>	4.47 ± 0.07 <sup>g</sup>	3.91 ± 0.20 <sup>e</sup>	4.18 ± 0.20 <sup>f</sup>
FT <sub>14</sub> - S <sub>10</sub>	5.95 ± 0.04 <sup>i</sup>	5.53 ± 0.24 <sup>i</sup>	5.84 ± 0.10 <sup>i</sup>	5.69 ± 0.14 <sup>i</sup>
FT <sub>21</sub> - S <sub>10</sub>	3.47 ± 0.29 <sup>d</sup>	1.91 ± 0.17 <sup>b</sup>	3.44 ± 0.14 <sup>d</sup>	3.20 ± 0.07 <sup>d</sup>
FT <sub>7</sub> - S <sub>20</sub>	3.95 ± 0.17 <sup>e</sup>	2.93 ± 0.17 <sup>d</sup>	4.38 ± 0.10 <sup>f</sup>	3.69 ± 0.20 <sup>e</sup>
FT <sub>14</sub> - S <sub>20</sub>	4.95 ± 0.17 <sup>g</sup>	4.98 ± 0.13 <sup>h</sup>	4.87 ± 0.07 <sup>g</sup>	4.67 ± 0.07 <sup>g</sup>
FT <sub>21</sub> - S <sub>20</sub>	2.49 ± 0.17 <sup>b</sup>	2.42 ± 0.04 <sup>c</sup>	2.98 ± 0.10 <sup>c</sup>	2.22 ± 0.10 <sup>b</sup>
FT <sub>7</sub> - S <sub>30</sub>	2.98 ± 0.10 <sup>c</sup>	3.44 ± 0.10 <sup>e</sup>	2.04 ± 0.08 <sup>a</sup>	2.71 ± 0.17 <sup>c</sup>
FT <sub>14</sub> - S <sub>30</sub>	5.47 ± 0.07 <sup>h</sup>	3.96 ± 0.10 <sup>f</sup>	5.36 ± 0.28 <sup>h</sup>	5.18 ± 0.14 <sup>h</sup>
FT <sub>21</sub> - S <sub>30</sub>	2.00 ± 0.18 <sup>a</sup>	1.38 ± 0.10 <sup>a</sup>	2.51 ± 0.17 <sup>b</sup>	1.73 ± 0.29 <sup>a</sup>

FT = fermentation time; S = sugar percentage. Different superscript letter indicating statistically significant differences ( $p < 0.05$ ),  $n = 25$ . Score hedonic: 1 = extremely dislike; 7 = extremely like.

When compared with the pH of kombucha tea, kombucha herbal mangrove fruit had a lower pH; i.e., 2.08, whereas that of kombucha green tea, kombucha black tea, and kombucha waste tea from 18 days fermentation ranges from 3.0 to 4.0 (Semjonovs et al., 2017).

### Organoleptic hedonic kombucha herbal from mangrove fruit

Table 4 presented the level of the panelists' preference for taste, aroma, color, and the overall acceptance of mangrove herbal kombucha. The hedonic organoleptic phenomenon showed that longer fermentation duration caused the decrease of preference for the kombucha, but the addition of sucrose produced varying degrees of preference. The decrease of the preference level from a longer fermentation duration is associated with the lower pH values and the higher total acidity (Table 3). The most preferred mangrove herbal kombucha product in terms of taste, aroma, color, and overall acceptance is that at 14 days fermentation and 10% sugar addition, with a preference level of 5.53 – 5.95 (likes). Chemically, this product has a pH value of 3.11 and a total acid of approximately 0.52%.

### CONCLUSIONS

Kombucha herbal tea from *R. mucronata* fruit has the potential to be used as an antidiabetic functional drink due to the lower IC<sub>50</sub> value than commercial kombucha tea and acarbose antidiabetic drug. Moreover, it is quite preferred by panelists. Kombucha herbal tea of *R. mucronata*, which has the most potential to be used as an antidiabetic functional drink is made with the addition of 10% sugar and 14 days fermentation time characterized by the IC<sub>50</sub> against  $\alpha$ -glucosidase of  $33.95 \pm 1.07$  ppm, total phenol of  $19679.82 \pm 649.06$  mg GAE/100 g, pH of  $3.11 \pm 0.06$ , and total acid of  $0.52 \pm 0.04\%$ . Further research is required to determine the compounds that play a role in the *R. mucronata* kombucha herbal tea and the appropriate dosage for human consumption.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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**AUTHOR CONTRIBUTION:**

Contribution	Hardoko	Harisman EK	Puspitasari YE
Concepts or ideas	x		
Design	x		x
Definition of intellectual content		x	
Literature search		x	x
Experimental studies	x		x
Data acquisition	x		
Data analysis	x	x	
Statistical analysis			x
Manuscript preparation	x		
Manuscript editing	x		
Manuscript review	x	x	x

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