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Rhizophora apiculata Kombucha Fermented Beverage: Secondary Metabolite Content and Antioxidant Activity

Agustina Monalisa Tangapo[1*], Susan Marlein Mambu[1], Parluhutan Siahaan[1], Erika Kolompoy[1], Marsia Pasassa[1]

[1] Department of Biology, Faculty of Mathematics and Natural Sciences, Manado, Indonesia.

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Corresponding Author:
Agustina Monalisa Tangapo
agustina.tangapo@unsrat.ac.id

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Abstract: Kombucha is a functional beverage produced by the fermentation of bacteria and yeast (SCOBY). This fermented beverage contains various bioactive compounds with antioxidant potential. *Rhizophora apiculata* is a mangrove plant that contains bioactive compounds with rich health benefits. Based on this, modifying kombucha fermentation with a mangrove leaf tea substrate can increase kombucha's effectiveness as an antioxidant. This study aims to analyze the secondary metabolite content profile and antioxidant activity of R. *apiculata* kombucha. Kombucha fermentation was carried out for seven days with a 10% sugar concentration. Analysis of the secondary metabolite content profile of R. *apiculata* kombucha was carried out using GC–MS analysis. Antioxidant activity was evaluated using the DPPH assay. The results showed a decrease in pH during the fermentation period and an increase in total acidity. The results of GC–MS analysis of R. *apiculata* kombucha showed 23 secondary metabolite compounds with varying retention times, area sizes, and chemical structures. The composition of the identified compounds was dominated by acetic acid (26.9%), 5-hydroxymethylfurfural (HMF, 12.38%), N'-(diaminomethylidene) butanehydrazide (7.68%), 1,2,3-propanetriol, 1-acetate (6.91%), and methyl α-D-rhamnopyranoside (5.12%). R. *apiculata* kombucha has the potential to act as an antioxidant, with an IC_{50} value of 27.16 ppm, which is classified as a very strong antioxidant.

Keywords: Antioxidant; Fermentation; Kombucha; Secondary metabolite

Introduction

Various studies continue to be developed to obtain exogenous antioxidants from natural sources. Very high antioxidant potential can be obtained from kombucha fermentation (Hidayana et al., 2017; Puspaningrum et al., 2022a). Kombucha is a fermentation product of tea and sugar with SCOBY (Symbiotic Culture of Bacteria and Yeast) (Yuningtyas et al., 2021). The substrate used in kombucha fermentation is not only limited to black tea or green tea but has been developed using other substrates to enrich the flavor and increase the various potentials of kombucha.

The kombucha fermentation process can take up to 60 days, but the best results are obtained at a fermentation time of 7–10 days. Many studies have shown that kombucha is a fermented beverage that has many health benefits. Kombucha has proven to have potential as an antimicrobial, anticancer, antidiabetic, antihypercholesterol, and digestive aid and can enhance immunity (Hardoko & Garrido, 2020; Rezaldi et al., 2022; Sutyawan & Novidiyanto, 2022; Wistiana & Zubaidah, 2015). Very high antioxidant potential can be obtained because of kombucha fermentation (Hidayana & Kusuma, 2017; Puspaningrum et al., 2022). Polyphenols produced in kombucha fermentation have strong antioxidant activity that can inhibit the growth of cancer cells. Kombucha fermentation produces various product compounds such as ethanol, acetic acid, glucuronic acid, lactic acid, phenolic acid, enzymes, B vitamins, citric

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acid, tartaric acid, malonic acid, oxalic acid, succinic acid, pyruvic acid, ascorbic acid, amino acids, polyphenols, and antibiotics (Villarreal-Soto et al., 2018).

The results of the research that has been done show that kombucha has high antioxidant activity; apart from being the result of fermentation, it is also the contribution of substrates from various plant sources. Variations of kombucha fermentation substrates include the bark of faloak (*Sterculia quadrifa* R. Br.) (Lalong et al., 2022), bay leaves (*Syzygium polyanthum* (Wight) Walp.) (Yuningtyas et al., 2021), cacao leaf (*Theobroma cacao*) (Hidayana & Kusuma, 2017), telang flower (Rezaldi et al., 2022), guava leaf, betel leaf, soursop leaf, bay leaf, and coffee leaf (Suhardini & Zubaidah, 2016).

With the development of innovation in the food industry, other raw materials as alternatives for kombucha fermentation are being explored, one of which is *Rhizophora apiculata* mangrove leaf substrate. *R. apiculata*, one of the mangrove species that grows in coastal areas, contains rich bioactive compounds, such as flavonoids, tannins, and polyphenols, which have the potential to provide additional benefits when processed into kombucha (Dewanto et al., 2021; Wardina et al., 2023). *R. apiculata* is a mangrove plant that has bioactive compounds lyoniresinol-3α-O-β-arabinopyranoside, lyoniresinol-3α-O-β-rhamnoside, and afzelechin-3-O-L-rhamno-pyranoside (Pambudi & Haryoto, 2022; Seepana et al., 2016). This plant is one of the mangrove species that can be found along the coast of Indonesia. In addition to ecological benefits, *R. apiculata* mangroves have enormous potential in the health sector. This plant has the potential to act as a natural antioxidant with phenolic content, tannins, and flavonoids. Based on this, the modification of kombucha fermentation with mangrove leaf substrate can increase the effectiveness of kombucha as an antioxidant. However, there are no reports related to the potential of R. *apiculata* mangrove kombucha as a functional beverage with high antioxidant activity.

Metabolomics analysis on kombucha has undergone rapid development in recent years, driven by the increasing interest in the health benefits of kombucha and the need to understand the complexity of its chemical composition. In kombucha analysis, non-targeted metabolomics approaches have become one of the main methods used. Metabolomic approaches can identify various bioactive compounds in kombucha, such as gluconic acid, acetic acid, catechins, and other phenolic compounds, which play a role in providing positive health effects, including antioxidant properties (Belgis et al., 2023; Tran et al., 2022; Villarreal-Soto et al., 2020).

Kombucha fermentation with *R. apiculata* mangrove leaf substrate can be a source of functional beverages that can be accepted by the community with high antioxidant benefits. This study aims to analyze the secondary metabolite content of *R. apiculata* kombucha and to evaluate its antioxidant potential.

Method

Time and Place

The research was conducted at the Advanced Biology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Sam Ratulangi University, Manado. The research was conducted from October to November 2023. Mangrove samples were taken from mangrove forests in Meras Village, Bunaken District, Manado City, North Sulawesi.

Research Design

The sample used for kombucha fermentation was *R. apiculata* mangrove leaves. Kombucha fermentation was carried out in three replicates for chemical characterisation analysis. The SCOBY used was one produced from fermenting black tea kombucha for 14 days. The research began with the collection and drying of R. apiculata mangrove leaves (Figure 1).

Research Procedure

Kombucha Fermentation of Rhizophora apiculata Mangrove Leaves

R. apiculata leaves were washed and dried. The powder that was obtained was then prepared in tea bags with every two grams of powder per bag for further use. A total of 1000 mL of water was boiled, then 10% (b/v) sugar was added, and 8 g (0.8%; b/v) of *R. apiculata* mangrove leaves were added. Furthermore, SCOBY and kombucha starter were added to each treatment as much as 10% (v/v). Kombucha fermentation was carried out with repetition three times. Furthermore, it was incubated at 28°C for 7 days (Kolompoy et al., 2024).

Chemical Characterization of Kombucha

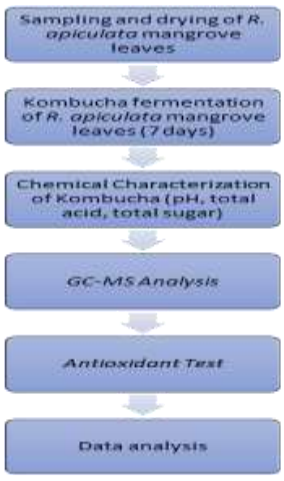


Figure 1. Research flow chart

Analysis of kombucha chemical characteristics was carried out by measuring pH using a pH meter (Hanna) calibrated at pH 4 and 7, measuring total sugar by a spectrophotometric method (AOAC, 1990) using a UV–VIS spectrophotometer (Shimadzu UV-1800), and measuring total acid by an acid–base titration method (AOAC (Association of Official Analytical Chemists), 1990). Total sugar analysis was performed using a UV-Vis spectrophotometer with λ=540 nm (Pasassa et al., 2025). The absorbance of standard glucose solution was calculated at various concentrations, and the regression equation y = 0.0046x + 0.0384 was obtained. The spectrophotometric results of the kombucha solution were entered in the regression equation as the y value, and the total sugar analysis data of kombucha were obtained.

GC-MS Analysis

R. apiculata kombucha samples were prepared for metabolomics analysis using the GC-MS Agilent 8890 GC System. The column used was HP-5MS. A total of three grams of sample was dissolved in methanol before being injected into the system. Operating conditions included an injector temperature of 290°C with a split ratio of 50:1, helium carrier gas (flow rate of 1 mL/min), and a column temperature program starting at 40°C then increasing 15°C/min until it reached 290°C and was maintained for 10 minutes. An MSD detector was used with a solvent delay of 2.6 minutes. Data analysis was performed through MassHunter Workstation Quantitative Analysis software (version 10.0.707.0) (Sanggor et al., 2024).

Antioxidant Test

Antioxidants were analyzed using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. The test sample solution in the form of mangrove kombucha *R. apiculata* (KMR) was made as a 500ppm master solution by weighing the sample as much as 0.005 g and then dissolving it in 10 ml of methanol to obtain a sample of 500 ppm. Furthermore, each kombucha mother solution was diluted to 20, 40, 60, 80, and 100 ppm. In the antioxidant test carried out, vitamin C (ascorbic acid) control was made as a comparison by diluting 2, 4, 6, 8, and 10 ppm for vitamin C in the antioxidant test. Measurement of antioxidant activity was carried out by taking 2 ml of each sample (kombucha and control) and adding 2 ml of DPPH, then homogenizing and allowing it to stand for 30 minutes in the dark. The absorbance was then measured with a spectrophotometer at a wavelength of 517 nm (Yuningtyas et al., 2021).

Data Analysis

The antioxidant activity measurement was calculated using the following equation:

$$\text{Antioxidant activity} = (A1\text{-}A2)/A1 \text{ X } 100\% \quad (1)$$

Information:
A1 = Absorbance not containing sample
A2 = Sample Absorbance

The equation was used to determine the IC_{50} value of each sample. The IC_{50} value is the concentration of the sample that can reduce the DPPH radical by 50% of its initial concentration. Percent (%) antioxidant activity that has been obtained is plotted as a graph between the concentration and the average % antioxidant activity until the value y = bx + a is obtained, after which the IC_{50} value (x) is calculated using the equation:

$$\text{IC50 (x)} = \frac{50-a}{b} \quad (2)$$

The antioxidant properties of each sample will be determined based on the IC50 value rubric in Table 1.

Table 1. Antioxidant Properties based on IC50 Value (Molyneux, 2004)

IC50 value	Antioxidant Properties
50 ppm <	Very Strong
50 – 100 ppm	Strong
100 – 150 ppm	Medium
150 – 200 ppm	Weak
200 ppm >	Very weak

Results and Discussion

Characterization of Rhizophora apiculata Kombucha

In this study, fermentation of R. *apiculata* mangrove kombucha was carried out for seven days. Kombucha fermentation also involves the formation of a thin layer or membrane on the surface, which thickens with increasing fermentation time. This thickened layer is known as SCOBY. In the process of polysaccharide fermentation, cellulose is formed; the cellulose forms fibre threads that continue to thicken, forming a strong network called nata follicles, known as SCOBY (Laavanya et al., 2021). This happens because the yeast contained in the kombucha symbiotic culture breaks down sugar into alcohol, while the alcohol that has been formed is oxidized by lactic acid bacteria into acetic acid. After SCOBY is formed, it functions to support the fermentation process of making kombucha (Kitwetcharoen et al., 2023; Laavanya et al., 2021). The success of the fermentation process is characterized by the formation of colonies of bacteria and yeast that float on the surface of the kombucha solution, called SCOBY, and the absence of contamination (Figure 2). Contaminated kombucha can be identified by the growth of mold on the surface of the nata, abnormal

texture, and uncharacteristic odors. This can be caused by less-sterile environmental conditions.

Figure 2. SCOBY formation on the surface

The kombucha microbial community can be classified into two parts: those found in the floating cellulose biofilm and those present in the liquid broth. Sucrose is hydrolyzed by yeast cells into fructose and glucose, which are metabolized by yeast to produce ethanol and carbon dioxide. Glycerol can also be produced by yeast due to high osmotic pressure and is further oxidized by bacterial acetic acid (BAA) to dihydroxyacetone (DHA). Some esters are also produced during this process, which contribute to the development of kombucha aroma. Fructose is preferentially used as a substrate over glucose, with the resulting ethanol being further metabolized by BAA to produce acetic acid, thus reducing the ethanol content in kombucha. The low ethanol concentration facilitates the formation of cellulose pellicles. Glucose is metabolized by BAA into gluconic and glucuronic acids. Yeast autolysis provides vitamins and other nutrients to support the growth of AAB. Yeast autolysis is usually detected during the maturation of alcoholic beverages, which may affect the aroma and flavor of kombucha products (Wang et al., 2022; Yang et al., 2022).

Table 2. Chemical Characterization of *Rhizophora apiculata* Kombucha

Measured Value	Day of Fermentation (days)		
	0	4	7
pH	3.5 ± 0.1	3.13 ± 0.06	2.93 ± 0.06
Total acid (%)	0.54 ± 0.058	0.94 ± 0.058	1.34 ± 0.58
Total sugar (%)	0.069 ± 0.001	0.068 ± 0.002	0.021 ± 0.008

Kombucha fermented using *R. apiculata* mangrove leaf substrate showed a significant decrease in pH over seven days of fermentation. On day 0 (after SCOBY and starter inoculation), the pH value was 3.5 ± 0.1 (Table 2). This low pH value indicates weak acidic conditions derived from the starter inoculated into the mangrove tea solution. As reported by Taufik et al. (2024), the higher the concentration of starter added at the beginning of fermentation, the lower the initial pH of fermentation. On day 4, the pH dropped to 3.13 ± 0.06, and on day 7 it reached 2.93 ± 0.06, indicating an increase in acidity with the length of fermentation time. Kombucha fermentation produces organic acids (mainly acetic, gluconic, and lactic acids) by the dominant microbes (*Acetobacter*, *Komagataeibacter*, and *Saccharomyces* yeast) that lower the pH of the medium (Selvaraj & Gurumurthy, 2023).

The pH decreases in R. *apiculata* kombucha in this study was faster than that observed in several other studies that used black or green tea substrates (usually the initial pH is around 4.5–5.0, decreasing to 2.5–3.5 in 7–14 days). This may be due to the nutrient composition of the mangrove substrate, which is rich in phenolic compounds and fermentable sugars, thereby accelerating microbial metabolism and stimulating acid production. The interaction between mangrove tannins and microbes may modify the fermentation pathway.

Studies by Chakravorty et al. (2016) reported that alternative substrates such as fruits or herbs can affect pH dynamics depending on their chemical composition. The final pH value on day 7 of *R. apiculata* kombucha fermentation (2.93 ± 0.06) falls within the optimal range of commercial kombucha (pH 2.5-3.5), which ensures microbiological safety by inhibiting pathogen growth. The pH value of *R. apiculata* kombucha in this study, which decreased with fermentation time, was consistent with the increase in total acid value, which showed an increase with fermentation time. Kombucha consists of several organic acids, such as acetic, gluconic, glucuronic, citric, L-lactic, malic, tartaric, malonic, oxalic, succinic, and pyruvic (Bishop et al., 2022). The composition and concentration of metabolites in kombucha can vary greatly due to the starter culture used, sugar and tea concentration, fermentation time, and fermentation temperature (Ahmed et al., 2020; Barbosa et al., 2021; Bishop et al., 2022; Chakravorty et al., 2016; Suhardini & Zubaidah, 2016). Yeast and bacteria hydrolyze sucrose into glucose and fructose using the enzyme invertase. The yeast in the matrix then produces ethanol through glycolysis, using fructose as the main substrate. Acetic acid bacteria use glucose to make gluconic acid and utilize the ethanol produced by the yeast and convert it into acetic acid.

Acetic acid is the organic compound responsible for the vinegar flavor and aroma commonly associated with kombucha (Bishop et al., 2022). The total sugar of kombucha in this study showed a decreasing trend with the length of fermentation (Table 2). At the beginning of the fermentation the total sugar reached 0.069%, which decreased to 0.021% at the end of day 7 of fermentation. The longer the fermentation, the more the total sugar will decrease; this is because sugar is used as a substrate by kombucha culture so that at the end of fermentation alcohol, organic acids, and other metabolites are produced. The decrease in sugar during fermentation is

not only caused by the activity of yeast in metabolizing sugar into alcohol but also by the activity of *Acetobacter*, which metabolizes glucose into gluconic acid. In addition, there is also the activity of *Acetobacter xylinum*, which synthesizes cellulose. In the analysis, it was found that there was a decrease in total sugar due to the addition of the kombucha starter to the tea brew. The process of increasing and decreasing total sugar is influenced by A. *xylinum* bacteria and yeast contained in the media. The decrease in total sugar was caused by the hydrolysis of sucrose into glucose by the enzyme invertase. Hydrolysis occurs because the pH of the media is very low; under these pH conditions, sucrose is very easily hydrolyzed by the enzyme invertase (de Miranda et al., 2022; Hassmy et al., 2017; Júnior et al., 2022; Leal et al., 2018).

Secondary Metabolite Content of Rhizophora apiculata Kombucha

GC–MS results showed 23 base peak chromatograms (Figure 3). GC–MS analysis of R. *apiculata* kombucha showed 23 secondary metabolite compounds with variations in retention time, peak area, and chemical structure (Table 3).

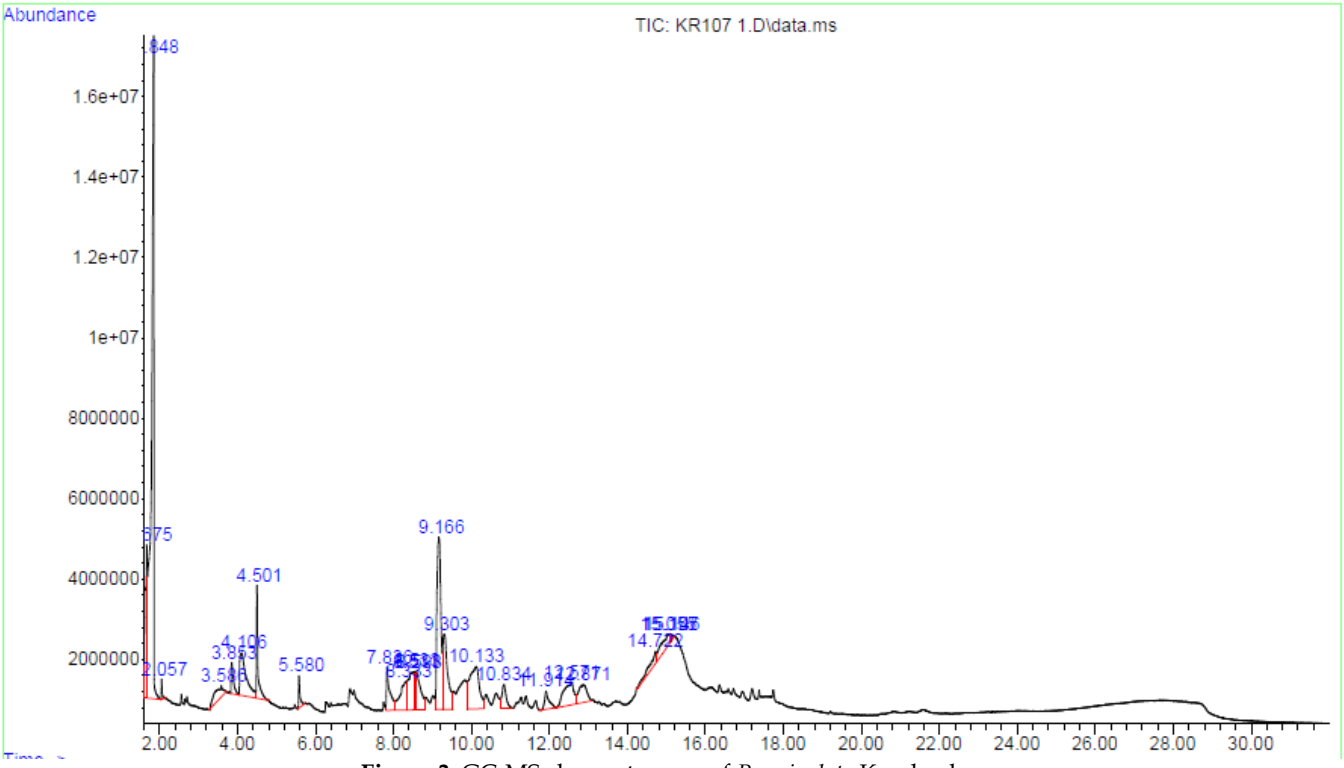
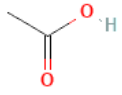
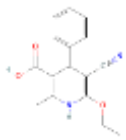
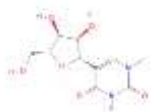
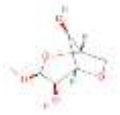
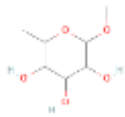
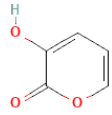
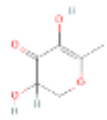
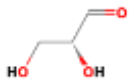
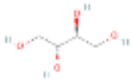
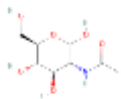
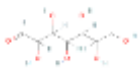
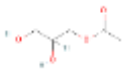
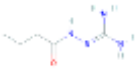
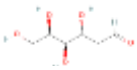
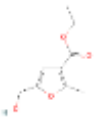
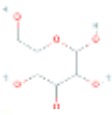
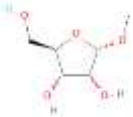
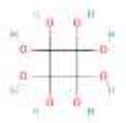
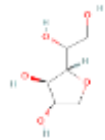
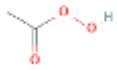


Figure 3. GC-MS chromatogram of *R. apiculata* Kombucha

Table 3. Secondary Metabolites in GC-MS Analysis of *R. apiculata* Kombucha

Retention Time (Min)	Area %	Compound	Molecular Formula	Molecular Weight	Chemical Structure
1.848	26.9	Acetic acid	$C_2H_4O_2$	60.05	
2.057	0.26	1,4-Dihydro-pyridine-3-carboxylic acid, 5-cyano-6-ethoxy-2-methyl-4-phenyl-	$C_{16}H_{16}N_2O_3$	284.31	
3.586	1.90	2,4 (1H,3H)-Pyrimidinedione, 5-.beta.-D-ribofuranosyl	$C_{10}H_{14}N_2O_6$	258.23	

Retention Time (Min)	Area %	Compound	Molecular Formula	Molecular Weight	Chemical Structure
3.853	1.31	alpha.-D-Glucopyranoside, methyl 3,6-anhydro-	$C_7H_{12}O_5$	176.17	
4.106	5.12	Methyl.alpha.d-rhamnopyranoside	$C_7H_{14}O_5$	178.18	
4.501	2.84	2-Cyclopenten-1-one, 2-hydroxy	C5H6O2	98.10	
5.580	0.78	3-Hydroxy-2H-pyran-2-one	$C_5H_4O_3$	112.08	
7.836	2.60	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	$C_6H_8O_4$	144.12	
8.333	3.44	Propanal, 2,3-dihyroxy-, (S)-	$C_3H_6O_3$	90.08	
8.511	3.68	Erythritol	$C_4H_{10}O_4$	122.12	
8.533	1.20	N-Acetyl-D-glucosamine	$C_8H_{15}NO_6$	221.21	
8.593	3.26	d-Glycero-d-galacto-heptose	$C_7H_{14}O_7$	210.18	
9.166	12.38	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.11	
9.303	6.91	1,2,3-Propanetriol, 1-acetate	$C_5H_{10}O_4$	134.13	
10.133	7.68	N'-(Diaminomethylidene) butanehydrazide	$C_5H_{12}N_4O$	144.18	
10.834	1.87	2-Deoxy-D-galactose	$C_6H_{12}O_5$	164.16	
11.914	1.43	Furancarboxylic acid, 5-(hydroxymethyl)-	$C_9H_{12}O_4$	184.19	
12.571	4.15	3-Methylmannoside	$C_7H_{14}O_6$	194.18	
12.871	2.67	Methyl-.alpha.-d-ribofuranoside	$C_6H_{12}O_5$	164.16	

Retention Time (Min)	Area %	Compound	Molecular Formula	Molecular Weight	Chemical Structure
14.722	1.52	Cyclobutaneoctol	$C_4H_8O_8$	184.1	
15.055	2.08	1,4-Anhydro-d-galactitol	$C_6H_{12}O_5$	164.16	
15.107	0.22	Dimetylamine	C_2H_7N	45.08	
15.146	0.14	Peracetic Acid	CH_3COOOH	76.05	

The composition of the identified compounds was dominated by acetic acid (26.9%), 5-hydroxymethylfurfural (HMF, 12.38%), N'-(Diaminomethylidene) butanehydrazide (7.68%), 1,2,3-Propanetriol, 1-acetate (6.91%), and Methyl.alpha.d-rhamnopyranoside (5.12%). Some of the compounds obtained in this study were also obtained in the profile of secondary metabolite compounds from kombucha langsat, namely 5-Hydroxymethylfurfural and 4H-Pyran-4-one,2,3-dihydro-3,5-dihydroxy-6-methyl (Isdaryanti et al., 2023).

Acetic acid (26.9%) is the main compound produced by acetic acid bacteria during fermentation. This compound is known to have potential as a natural antimicrobial by inhibiting pathogen growth through a decrease in medium pH (Ibrahim et al., 2021), as a biological preservative that extends the shelf life of kombucha, and as a typical flavor constituent that imparts a distinctive sharp sour aroma (Shafira et al., 2022). In addition, the results of this study also detected peracetic acid (0.14%) and furancarboxylic acid (1.43%), which may be derived from the oxidation of mangrove phenolic compounds or the degradation of sugars. The presence of these acidic compounds indicates intensive metabolic activity of acetic acid bacteria during fermentation.

There are several bioactives that have pharmacological potential, namely 5-Hydroxymethylfurfural (HMF, 12.38%), N'-(Diaminomethylidene) butane-hydrazide (7.68%), 3-Hydroxy-2H-pyran-2-one (0.78%), and 2,3-dihydroxy-6-methyl-4H-pyran-4-one (2.60%). The compound 5-Hydroxymethylfurfural is a furan formed from sugar degradation. HMF is reported to have antioxidant and anticancer activities (Moghaddam et al., 2024). The compounds 3-Hydroxy-2H-pyran-2-one (0.78%) and 2,3-dihydroxy-6-methyl-4H-pyran-4-one (2.60%) are pyranone compounds known as natural antioxidants.

Several sugar-derived compounds and glycosides were detected as secondary metabolite compounds in *R. apiculata* kombucha, namely Methyl-α-d-rhamnopyranoside (5.12%), Erythritol (3.68%), 2-Deoxy-D-galactose (1.87%), and N-Acetyl-D-glucosamine (1.20%). Methyl-α-d-rhamnopyranoside is a glycoside with prebiotic and antimicrobial potential (Moghaddam et al., 2024). Erythritol and 2-deoxy-D-galactose include sugar alcohols that act as natural sweeteners and modulators of the gut microbiome (Moghaddam et al., 2024). N-Acetyl-D-glucosamine is a microbial cell wall constituent compound that exhibits immunomodulatory activity (Murphy et al., 2023). These compounds may originate from the degradation of mangrove polysaccharides by microbial enzymes during fermentation.

The GC-MS results have implications for the quality and potential of kombucha, as the dominance of acetic acid and HMF gives a sour-sweet flavor with a distinctive furan aroma. The combination of antimicrobial (acetic acid, hydrazide), antioxidant (pyranones), and immunomodulatory (glycoside) compounds supports the potential of kombucha as a functional beverage. The metabolite profile of *R. apiculata* kombucha showed a unique combination of common fermentation compounds (acetic acid, HMF) and mangrove-specific compounds (glycosides, pyridine derivatives). These findings open opportunities for the utilization of mangroves as innovative substrates for the development of kombucha with specific bioactivities. This approach confirms that the selection of *R. apiculata* mangrove substrate not only affects the metabolite composition but also expands the therapeutic potential of kombucha.

The composition of secondary metabolite compounds in kombucha is highly dependent on the type of substrate used during fermentation, although some common compounds remain consistently present due to microbial metabolic processes. Research shows that different substrates lead to the production of compounds that vary, both in type and concentration. Green tea-based kombucha tends to produce more epicatechin and gallic acid, while kombucha from black tea is richer in theaflavin and thearubigin (Jayabalan et al., 2014). Clove kombucha contains the compound eugenol, which is a compound contained in the clove plant itself (Sanggor et al., 2024).

Despite the variation in substrates, some compounds are almost always detected in kombucha, as they are the main metabolic products of the microbial consortium (SCOBY). These compounds include acetic acid—produced by Acetobacter bacteria through ethanol oxidation—and 5-Hydroxymethylfurfural—a furan-derived compound formed from sugar degradation, especially if fermentation is carried out at high temperatures. These compounds were also detected from kombucha with other substrates (Isdaryanti et al., 2023; Sanggor et al., 2024; Tangapo et al., 2025). 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl is a metabolite of Saccharomyces cerevisiae. 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl is known to have significant antimicrobial, anti-inflammatory, and antioxidant activities (Isdaryanti et al., 2023). Substrate variation affects not only the compound profile but also the biological activity of kombucha.

Rhizophora apiculata Kombucha Antioxidant

The results of the antioxidant activity test of *R. apiculata* leaf kombucha using a spectrophotometer at concentrations of 20, 40, 60, 80, and 100 ppm obtained the percentage value of inhibition successively of 47.059%, 54.954%, 59.752%, 65.325%, and 75.542% (Table 4). From the value of the formula equation obtained, regression was then made, and the equation y = 16.16x-3.357 with (R^2 = 0.9141) was obtained. Based on the regression equation and % inhibition, the IC50 value of *R. apiculata* kombucha was 27.16 ppm.

Table 4. *Rhizophora apiculata* Kombucha Antioxidant

Concentration	Absorbance	% Inhibition	Regression Linear	IC_{50} (ppm)
100 ppm	0.158	75.542		
80 ppm	0.224	65.325	y=16.16x–3.357	27.16
60 ppm	0.260	59.752	R^2 = 0,9141	
40 ppm	0.291	54.954		
20 ppm	0.342	47.059		

Control Absorbance= 0.646

Percent inhibition is a description of the ability of antioxidant compounds in the sample to capture free radicals at the concentration of the test solution. The increase in inhibition is caused by a decrease in DPPH absorption produced by the sample. From the research conducted, the IC_{50} value of the *R. apiculata* kombucha test sample was 27.16 ppm, which is classified as a very strong antioxidant. This IC_{50} value indicates that the bioactive compounds in *R. apiculata* mangrove leaf kombucha are effective in neutralizing free radicals in low concentrations. This makes *R. apiculata*-based kombucha a potential functional beverage candidate in counteracting oxidative stress in biological systems.

According to the metabolomic profile, kombucha from *R. apiculata* contains bioactive compounds with natural antioxidant potential, such as 2-Cyclopenten-1-one, 2-hydroxy; 3-Hydroxy-2H-pyran-2-one; 5-Hydroxymethylfurfural; 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-; acetic acid; and erythritol. 2-Cyclopenten-1-one, 2-hydroxy, includes cyclic lactone compounds containing ketone and hydroxyl groups. 3-Hydroxy-2H-pyran-2-one (lactone) is known as DDMP—an alcohol derivative that is a flavor-forming volatile (lactone). DDMP-like derivatives exhibit galvinoxyl and DPPH radical-catching effects up to ~90% at 350 μM. The main signal is an enol at the C5 position that donates H^+ (Chen et al., 2021).

In addition to the influence of the substrate used, the content of secondary metabolite compounds in *R. apiculata* kombucha is also the result of metabolism by microbes. During the fermentation process, the activity of microorganisms in SCOBY cultures can break down complex compounds into more biologically active forms, increasing the bioavailability and effectiveness of antioxidants (Bishop et al., 2022; Leal et al., 2018). Fermentation can also produce organic acids and other secondary metabolites that contribute to the antioxidant effect. Overall, the results of this study indicate that *R. apiculata* leaf kombucha has potential as a functional beverage that is effective in providing protection against free radical damage. With its high antioxidant activity and low IC_{50} value, this *R. apiculata* kombucha drink can be developed as a healthy and natural food product.

Conclusion

The chemical characteristics of R. *apiculata* mangrove kombucha showed a decrease in pH during the fermentation period and an increase in total acid. The results of the analysis of secondary metabolite content of R. *apiculata* kombucha showed 23 secondary metabolite compounds with variations in retention time, area, and diverse chemical structures. The composition of the identified compounds was dominated by acetic acid, 5-hydroxymethylfurfural, N'-(diaminomethylidene) butanehydrazide, 1,2,3-propanetriol, 1-acetate, and methyl α-D-rhamnopyranoside. R. *apiculata* kombucha has potential as an antioxidant, with an IC50 value of 27.16 ppm, which is classified as a very strong antioxidant.

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Author Contributions
AMT and SMM were involved in concepting and planning the research, AMT, EK, dan MP performed the data acquisition, AMT and PS analyzed the experimental data, AMT drafted the manuscript. Each author contributed to the manuscript's critical editing.

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Conflicts of Interest
The authors declare no conflict of interest.

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