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The Bioactive Components of Cascara Kombucha and Their Potential Use as a Functional Beverage

Syarifah Rohaya [1, 2], Sri Haryani Anwar [1], Amhar AB [3], Amalia Sutriana [4*]

[1] *Departement of Agricultural Product Technology, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia.*

[2] *Doctoral Program, Postgraduate School of Agricultural Science, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia.*

[3] *Departement of Animal Husbandry, Faculty of Agriculture, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia.*

[4] *Laboratory of Pharmacology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia.*

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Abstract

This study aims to analyze the antimicrobial activity of cascara kombucha and evaluate its potential as a functional beverage through in vivo experiments using male white rats (*Rattus norvegicus*). The antimicrobial activity test utilized the kombucha derived from three types of cascara: arabica (J1), robusta (J2), and liberica (J3), which fermented using 10% of sugar for 10 and 17 days. The antimicrobial activity was tested against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 in vitro using the disk diffusion method (*Kirby-Bauer* test). Black tea kombucha served as the control. The safety of cascara kombucha was evaluated by aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin levels in rats' blood. The data were analyzed using a T-test with RStudio software. The results indicated that cascara kombucha from arabica and liberica effectively inhibited the growth of *E. coli* and *S. aureus*. The administration of 1.8 ml of arabica cascara kombucha had no significant effect ($P > 0.05$) on the liver function based on the AST, ALT, and total bilirubin levels. The in vivo test demonstrated that cascara kombucha is safe for mammalian consumption. Overall, the findings pointed to the conclusion that cascara kombucha is an effective antimicrobial agent and has functional values without causing adverse effects on the liver function of male white rats.

Keywords: Antimicrobial; Bioactive Compounds; Cascara Kombucha; Functional Beverage; Liver Function.

1. Introduction

Functional beverages are types of beverages that contain bioactive compounds that provide benefits to human health [1]. One of the functional beverages that is increasingly popular is fermented tea, commonly known as kombucha. Kombucha is a fermented drink produced through the symbiotic mutualism between bacteria and yeast [2]. Over the years, kombucha has evolved to include a variety of raw materials, one of which is cascara. Cascara kombucha is made from cascara tea as the raw material, which is further fermented by the activity of symbiotic culture of bacteria and yeast, or SCOBY [3].

* Corresponding author: amalia_sutriana@usk.ac.id

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Cascara is a by-product of coffee bean processing that consists of the dried outer layer and pulp of a coffee cherry [4]. The use of cascara as raw material for kombucha will reduce the waste of coffee industries and at the same time will increase the added value of cascara by producing a functional beverage rich in bioactive compounds. Kombucha from cascara is characterized by its distinct sweet and sour flavor and rich aroma [3]. The production of cascara kombucha has been studied previously [3, 5, 6]; however, cascara kombucha made from various types of coffee pulp, such as arabica, robusta, and liberica, has not been thoroughly investigated. Different coffee by-products and processing methods will develop products with distinct characteristics. For example, the coffee cherry processed using the wet method produces the outer skin (pulp), while the one processed using the dry or natural method produces the husk.

Kombucha is known for its numerous health benefits, including antihyperlipidemic, antihyperglycemic, antimicrobial, antidiabetic, and anticarcinogenic properties. These functional traits are attributed to the presence of health-enhancing amino acids, polyphenols, organic acids, vitamins, microelements, and antibiotics in kombucha [7]. Kombucha also exhibits antimicrobial properties against pathogenic bacteria, attributed to its bioactive compounds [8]. Several studies examined the health benefits of tea-based kombucha, including its antimicrobial and hepatoprotective effects. Research done by Içen et al. [9] found the organic acid content in kombucha inhibits the growth of *Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes*. Another study by Battikh et al. [10] showed that green tea-based kombucha has antibacterial effects against *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

The effects of kombucha on rats' livers have also been investigated. Research by Pauline et al. [11] showed that black tea-based kombucha had a hepatoprotective effect on paracetamol-induced rats by significantly reducing ALT and AST levels. Another study underlined that kombucha consumption provided protection to rat livers due to the strong antioxidant mechanism of aflatoxin B1 [12]. Although some studies suggested that kombucha tea does not adversely affect liver tissue in animal models and shows comparable results to the control, several reports also indicate negative effects, such as cases of severe, occasionally lethal, lactic acidosis and liver failure that occur shortly after kombucha consumption [13]. In addition, excessive fermentation may lead to an increase in acetic acid concentration, which might also negatively affect liver function. Several organic acids in kombucha and their metabolites can impair liver and kidney function at high concentrations.

Cascara kombucha has a different composition compared to tea-based kombucha due to its unique bioactive compounds, including caffeine, chlorogenic acid, tannins, and anthocyanins [5]. These compounds are known to have antimicrobial and hepatoprotective activities, but their interactions during the fermentation process remain understudied. Further, the effect of the bioactive compounds contained in cascara kombucha on antimicrobial activity and liver function of rats has yet to be investigated. It is crucial to evaluate the safety of cascara kombucha consumption in rats before conducting clinical trials on humans. One analysis that can be performed to assess the safety of kombucha consumption is by analyzing the liver function impairment in male white rats. Liver dysfunction can be detected by measuring the increment of AST and ALT levels, which are commonly used as indicators of liver damage.

2. Material and Methods

2.1. Cascara Kombucha Pulp Production

The procedure of cascara kombucha production follows the method described by Muzaifa et al. [6] with slight modifications. In this study, three types of cascara were made by drying the dried outer layer and pulp of coffee cherries, where the cherries obtained from three districts in Aceh Province, Indonesia. They were arabica cascara (J1) from the Gayo region (Central Aceh), robusta cascara (J2) from the Lamno region (Aceh Jaya), and liberica cascara (J3) from the Tangse region (Pidie). The sugar concentration used was 10% (w/v). The kombucha starter used was derived from the cascara kombucha beverage. Kombucha made from black tea was used as a control (T).

The cascara kombucha was prepared as follows: in sterilized glass jars, 70 g of cascara was brewed with 2,500 ml of hot water at 90 °C. After 8 minutes, the cascara residue was separated by filtration. Next, 10% sucrose was added into the filtrate, and the mixture was then cooled at room temperature (25–28 °C). After cooling, a 10% (v/v) kombucha starter and a SCOBY sheet were added. The mixture was then fermented at room temperature at 20–30 °C for 10 days (L1) and 17 days (L2). The fermentation process was carried out aerobically, with the opening of the glass jar being covered with a sterile cloth and secured with a rubber band. After the fermentation period, the SCOBY was separated from the filtrate, and the cascara kombucha was ready to be analyzed. The same procedures were applied to the preparation of black tea kombucha, but with the omission of the cascara (control) [4].

2.2. Identification of Bioactive Compounds from Cascara Kombucha by LC-MS

Identification of bioactive compounds in cascara kombucha was carried out at the Forensic Laboratory Center, Sentul Bogor, Indonesia. The analysis was conducted using Liquid Chromatography Mass Spectrometry (LC-MS). The LC-MS/MS instruments used consisted of LC System with specifications of ACQUITY UPLC® H-Class System (Waters, USA), LC Column with specifications of ACQUITY UPLC® HSS C18 (Waters, USA), and Mass Spectrometer with specifications of Xevo G2-S QTof (Waters, USA). The cascara kombucha samples used were: arabica cascara kombucha (J_1G), robusta cascara kombucha (J_2G), and liberica cascara kombucha (J_3G), which fermented for 10 days. This analysis was performed in order to identify the best sample for in vivo administration to the white rats.

The cascara kombucha sample was accurately weighed to 10.00 mg, dissolved in 10.00 ml of methanol, and injected into a 5 µL micro-syringe. The sample was eluted using a gradient elution system with a mobile phase of water/formic acid mixture (99.9/0.1 [v/v]) and acetonitrile/formic acid mixture (99.9/0.1 [v/v]). The resulting chromatogram from the LC-MS separation was processed using the MassLynx Version 4.1 software to obtain data in the form of peak areas and m/z spectra for each detected peak. Consequently, the predicted compounds were interpreted with the help of the ChemSpider and PubChem websites [14].

2.3. Antimicrobial Activity Analysis

The antimicrobial activity of cascara kombucha was tested against *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC29213 in vitro using the disk diffusion method (*Kirby-Bauer* test). *E. coli* and *S. aureus* bacteria were isolated on *Brain Heart Infusion* (BHI) agar medium and incubated at 37 °C for 24 hours. After incubation, several colonies were selected and transferred to *Mueller Hinton Broth* (MHB) medium, followed by incubation at 37 °C for two hours. The turbidity of the suspension was adjusted to the 0.5 McFarland standard (10^8 CFU mL^{-1}). One colony was selected and inoculated onto *Mueller Hinton Agar* (MHA) plates for disk diffusion susceptibility testing. Using a sterile cotton swab, the inoculum was evenly spread in three directions, rotating the plate 60° between each swab application to ensure uniform bacterial lawn formation.

Each blank disc (Oxoid Blank Disc 6 mm) was immersed in the kombucha samples, which included cascara kombucha fermented for 10 (L_1) and 17 (L_2) days and soaked for 30 minutes. Black tea kombucha was used as the control. After 30 minutes, each disc was retrieved using sterile forceps and placed onto a petri dish inoculated with *E. coli* and *S. aureus*. The discs were gently pressed to ensure firm contact with the agar surface. The petri dishes were incubated at 37 °C for 18 - 24 hours. Antimicrobial activity was considered positive when a clear zone formed around the disc. The diameter of the clear zone was measured to the nearest 0.01 mm using a digital caliper and recorded as the inhibition zone (colony-free area) against *E. coli* or *S. aureus* [15].

2.4. AST, ALT and Total Bilirubin Analysis

The sample selected for administration to the rats was arabica cascara kombucha, as it contained the largest number of bioactive compounds (38 compounds) as compared to robusta and liberica cascara kombucha. This test aimed to evaluate the safety of cascara kombucha for animal subjects. A total of 30 male white rats (*Rattus norvegicus*), aged 2 months with body weights ranging from 250 to 300 grams, were used in this study. A Completely Randomized Design (CRD) was employed, with two treatment groups, each consisting of 15 rats: T1 served as the control group receiving only drinking water, while T2 received drinking water supplemented with 1.8 mL of cascara kombucha administered twice daily for one month.

The cascara kombucha used in the in vivo test was derived from the optimized fermentation process of cascara kombucha. Feed and water were provided ad libitum. Blood samples were collected at the fourth week after the rats were assigned to their respective treatment groups. The research variables included independent and dependent variables as follows: the independent variable was the oral treatment with the optimized cascara kombucha, while the dependent variables included liver function markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin levels. The data obtained was processed using a T-test with RStudio software. This research has obtained ethical approval from the ethics committee of the Faculty of Veterinary Medicine, Universitas Syiah Kuala, with no. 337/KEPH/IX/2024. The flowchart of the methodology can be seen in Figure 1.

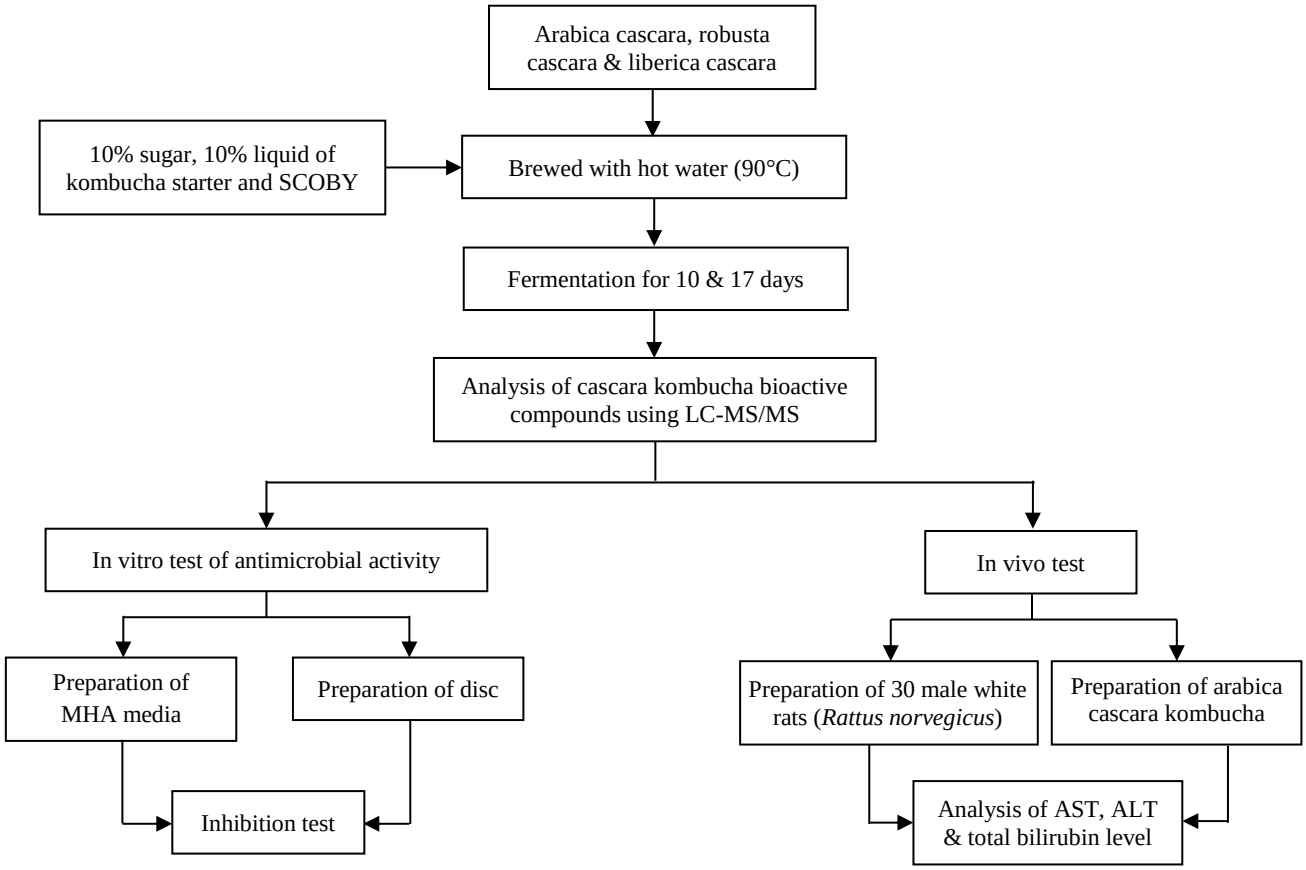


Figure 1. Flowchart of the research methodology

3. Result

3.1. Bioactive Compounds in Cascara Kombucha

The LC-MS results indicated that arabica cascara kombucha (J1G) contains a higher number of bioactive compounds compared to the robusta cascara kombucha (J2G) and liberica cascara kombucha (J3G). In arabica cascara kombucha (J1G), 38 chemical compounds were identified, including merulinic acid A (28.8%), glycerol pyruvate succinate (19.92%), caffeine (18.13%), dimethyl 4-(3-methoxy-3-oxopropyl)-4-nitroheptanedioate and kojic acid (7.39%), 2-amino-6,7-dimethoxy-3-quinoline carboxamide, carnosic acid (4.13%), and chlorogenic acid (1.6%) (Table 1). Less number (32 bioactive compounds) were identified in robusta cascara kombucha (J2G). As can be seen in Table 2, the bioactive compounds were 9-hydroxy-7-oxo-7H-furo[3,2-g]chromen-4-yl β-D-glucopyranoside and galactose galactosamine1 (36.91%), merulinic acid A (17.88%), caffeine (12.96%), scopoletin; 7-hydroxy-5-methoxycoumarin (6.68%), kojic acid (4.18%), and chlorogenic acid (3.47%). The liberica cascara kombucha (J3G) contained only 22 bioactive compounds (Table 3). These compounds were caffeine (43.16%), 9-hydroxy-7-oxo-7H-furo[3,2-g]chromen-4-yl β-D-glucopyranoside and aminobenzoic acids (37.2%), mitoxantrone (2.86%), merulinic acid A (2.5%), and α-linolenic acid (1.76%). All the bioactive compounds identified in arabica, robusta, and liberica cascara kombucha are presented in Tables 1 to 3.

Based on Tables 1 to 3, it can be observed that arabica cascara kombucha has a varied composition with the largest number of bioactive compounds. Therefore, arabica cascara kombucha was chosen for the liver function analysis on male white rats.

3.2. Antimicrobial Activity

The antimicrobial activity of cascara kombucha was higher compared to black tea kombucha (control), even though both have the same sugar concentration (10%) (G). The results of the study indicated that arabica (J_1) and liberica (J_3) cascara kombucha, with a fermentation time of 17 days (L_2), exhibited inhibition zones against *E. coli* and *S. aureus* bacteria. In contrast, robusta cascara kombucha (J_2), with a fermentation time of 17 days (L_2), only showed inhibition against *S. aureus* bacteria. While black tea kombucha (T_1) did not exhibit any inhibition zones, either with a fermentation time of 10 days (L_1) or 17 days (L_2). These findings are shown in Table 4 and Figure 2.

Table 1. LC-MS results of arabica cascara kombucha (J_1G) sample

Peak	Retention Time (RT)	% Area	Compound
1	1.417	19.92	Glycerol pyruvate succinate
2	2.139	7.39	Dimethyl 4-(3-methoxy-3-oxopropyl)-4-nitroheptanedioate
3	2.139		Kojic acid
4	2.884	2.45	L-Methyldopate; 3-Hydroxy-a-methyl-L-tyrosine Ethyl Ester
5	2.884		Mesalazine; 5-Amino-2-hydroxybenzoic acid
6	3.341	2.74	Theophylline; 1,3-Dimethylxanthine
7	3.386	0.25	Bifuhalol
8	4.073	1.6	Pterosin B glucoside
9	4.073		Kojic acid
10	4.199	1.6	Umbelliferone; 7-Hydroxycoumarin
11	4.199		Chlorogenic acid
12	4.466	18.13	Caffeine; 1,3,7-Trimethylxanthine ; 3,7-Dihydro-1,3,7-trimethyl-1H-purine-2,6-dion
13	4.86	0.3	N-Benzoyl-L-glutamic acid; Bz-Glu-OH
14	4.86		3-O-Caffeoyl-1-O-methyl quinic acid
15	5.387	3.56	Zingerone
16	5.387		Gibberellin A37 glucosyl ester
17	5.605	1.56	Scopoletin
18	6.182	1.14	(5E,13E,15S)-15-Hydroxy-9-oxoprosta-5,10,13-trien-1-oic acid
19	6.069		Gibberellin A110
20	6.659	0.22	Arborinine
21	7.186	0.18	N-(1-Deoxy-1-fructosyl)leucine
22	7.186		3,4-Methyleneazelaic acid
23	7.496	4.13	2-Amino-6,7-dimethoxy-3-quinoline carboxamide
24	7.496		Carnosic acid
25	8.002	0.05	Ginsenoyne G
26	8.262	0.05	Herniarin
27	8.262		2,3,4,5-Tetra-O-acetylhexonic acid
28	8.664	0.29	Mivazerol
29	8.902	0.1	Prostaglandin D2
30	9.45	0.68	Diethyl phenyl malonate
31	9.823	0.88	N-{2,2-Dimethyl-1-[3-(1H-1,2,4-triazole-3-yl)-1,2,4-oxadiazol-5-yl]propyl}-3-(4-nitro-1H-pyrazole-1-yl)propenamide
32	9.823		1-(4-Amino-1,2,5-oxadiazol-3-yl)-N'-cyclohexylidene-5-(3-nitrophenyl)-1H-1,2,3-triazole-4-carbohydrazide
33	10.287	2.08	myxochelin B
34	10.287		Diethyl(2-{4-[(3,7-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purine-1-yl) methyl]-1H-1,2,3-triazol-1-yl}ethyl)phosphonate
35	10.794	1.12	Diethyl(3-{4-[(3,7-dimethyl-2,6-dioxo-2,3,6,7-tetrahydro-1H-purine-1-yl) methyl]-1H-1,2,3-triazol-1-yl}propyl)phosphonate
36	10.794		Casimiroedine
37	11.145	0.38	1,1'-(1,4-Phenylene)bis{3-[2-(dimethylamino)ethyl]urea}
38	17.318	28.8	Merulinic acid A

Table 2. LC-MS results of robusta cascara kombucha (J_2G) sample

Peak	Retention Time (RT)	% Area	Compound
1	1.365	36.91	9-Hydroxy-7-oxo-7H-furo[3,2-g]chromen-4-yl β-D-glucopyranoside
2	1.365		Galactose.galactosamine1)
3	2.027	4.18	Kojic acid; 5-Hydroxy-2-(hydroxymethyl)-4-pyrone
4	2.027		Galactose.galactosamine1)
5	2.027		L-3-(3-Hydroxy-4-pivaloyloxyphenyl)alanine
6	2.027		2,3-dimethylidenepentanedioylcarnitine

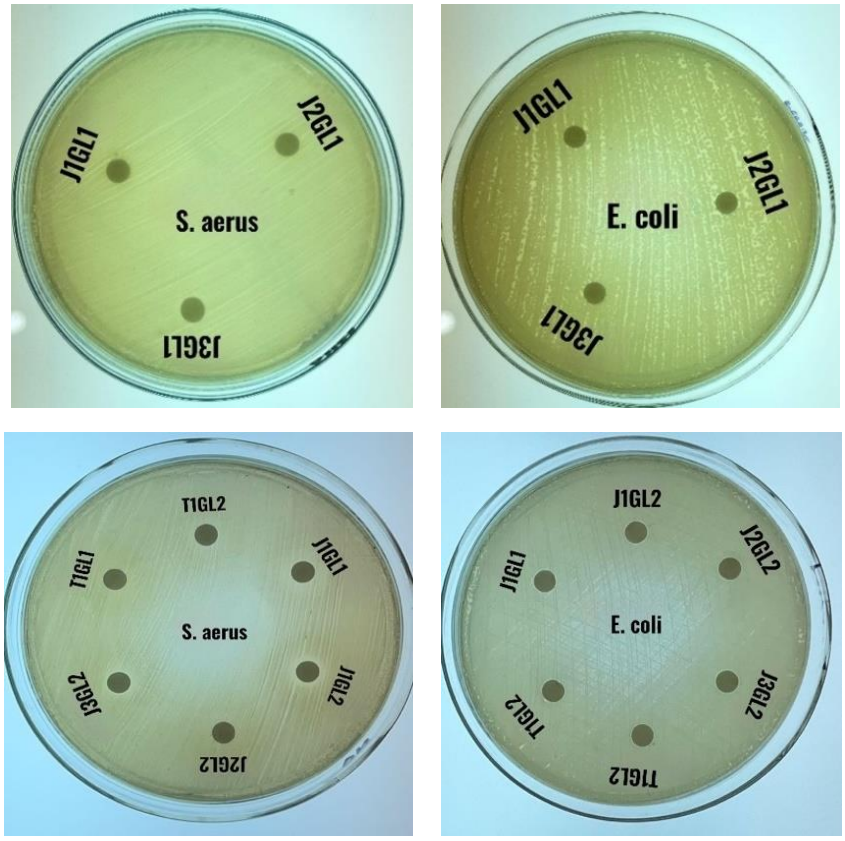
7	2.792	1.12	Mesalazine; 5-Amino-2-hydroxybenzoic acid
8	2.792		Sarmentosin
9	3.215	1.26	Theophylline; 2,6-Dihydroxy-1,3-dimethylpurine
10	3.587	0.17	Umbelliferone
11	3.587		Chlorogenic acid
12	3.805	0.21	(+)-Procyanidin B2
13	4.094	3.3	Umbelliferone
14	4.094		Chlorogenic acid
16	4.374	12.96	Caffeine; 1,3,7-Trimethylxanthine
17	4.839	1.67	Fraxetin ; 7,8-Dihydroxy-6-methoxycoumarin
18	5.479	6.68	Scopoletin; 7-Hydroxy-5-methoxycoumarin
19	6.069	1.07	(5E,13E,15S)-15-Hydroxy-9-oxoprosta-5,10,13-trien-1-oic acid
20	6.069		Isofraxidin; 7-Hydroxy-6,8-dimethoxy coumarin
21	7.341	1.33	Carnosic acid
22	9.429	2.43	2,3,4,5-Tetra-O-acetylhexonic acid
23	10.35	1.49	2,3,4,5-Tetra-O-acetylhexonic acid
24	10.948	4.41	1,1'-(1,4-Phenylene)bis{3-[2-(dimethylamino)ethyl]urea}
25	10.948		2,2'-(Tridecylimino)diethanol
26	13.409	0.27	Diisobutyl phthalate
27	13.978	0.12	2,3,4,5-Tetra-O-acetylhexonic acid
28	14.527	0.01	2,3,4,5-Tetra-O-acetylhexonic acid
29	14.836	0.09	2-[(2E)-2-(2,4-Dimethylbenzylidene)hydrazino]-4,6-di(4-morpholinyl)-1,3,5-triazine
30	15.405	0.07	2,3,4,5-Tetra-O-acetylhexonic acid
31	17.284	17.88	Merulinic acid A
32	20.967	2.36	2,3,4,5-Tetra-O-acetylhexonic acid

Table 3. LC-MS results of liberica cascara kombucha (J_3G) sample

Peak	Retention Time (RT)	% Area	Compound
1	1.436	37.2	9-Hydroxy-7-oxo-7H-furo[3,2-g]chromen-4-yl β-D-glucopyranoside
2	1.436		aminobenzoic acids
3	2.989		Isopropyl bis(3-methyl-1H-pyrazol-1-yl)phosphinate
4	2.89	0.47	Mesalazine; 5-Amino-2-hydroxybenzoic acid
5	2.989		Theophylline; 2,6-Dihydroxy-1,3-dimethylpurine
6	3.868	1.1	Quinic acid
7	4.55	43.16	Caffeine; 1,3,7-Trimethylxanthine ; 3,7-Dihydro-1,3,7-trimethyl-1H-purine-2,6-dion
8	5.626	2.22	Scopoletin; 7-Hydroxy-5-methoxycoumarin
9	6.203	0.33	(5E,13E,15S)-15-Hydroxy-9-oxoprosta-5,10,13-trien-1-oic acid
10	6.203		Gibberellin A110
11	7.559	0.56	Carnosic acid
12	9.296	0.21	BOC-β-CYCLOHEXYL-ALA-OH N-(tert-Butoxycarbonyl)-3-cyclohexyl-L-alanine
13	9.844	1.61	2,2,6,6-Tetraphenylcyclohexanone
14	10.4	0.51	Nona-4,6-dienoylcarnitine
15	10.949	2.86	Mitoxantrone
16	12.334	1.76	α-Linolenic acid
17	13.008	0.04	13-Oxo-9Z,11E-octadecadienoic acid
18	13.451	0.45	Diisobutyl phthalate
19	14.154	0.08	Methyl Linolenate
20	15.757	0.39	2,4-Methylene cholesterol
21	17.163	2.5	Merulinic acid A
22	17.163		N-Stearoyl Lysine

Table 4. Inhibition zone diameters of cascara kombucha against *S. aureus and E. coli*

Sample	Inhibition Zone	
	***S. aureus* ATCC 29213**	***E. coli* ATCC 25922**
T_1GL_1	0	0
T_1GL_2	0	0
J_1GL_1	0	0
J_1GL_2	7.46 mm	6.26 mm
J_2GL_1	0	0
J_2GL_2	7.36 mm	0
J_3GL_1	0	0
J_3GL_2	6.29 mm	6.12 mm



Legend:

T1GL1 = Black tea kombucha with 10% sugar concentration and 10 days fermentation time.

T1GL2 = Black tea kombucha with 10% sugar concentration and 17 days fermentation time.

J1GL1 = Arabica cascara kombucha with 10% sugar concentration and 10 days fermentation time.

J1GL2 = Arabica cascara kombucha with 10% sugar concentration and 17 days fermentation time.

J2GL1 = Robusta cascara kombucha with 10% sugar concentration and 10 days fermentation time.

J2GL2 = Robusta cascara kombucha with 10% sugar concentration and 17 days fermentation time.

J3GL1 = Liberica cascara kombucha with 10% sugar concentration and 10 days fermentation time.

J3GL2 = Liberica cascara kombucha with 10% sugar concentration and 17 days fermentation time.

Figure 2. Antimicrobial activity of cascara kombucha against *S. aureus and E. coli*

3.3. Liver Function Examination

AST and ALT values are widely recognized as indicators of liver injury. The liver function values, including AST, ALT, and total bilirubin of control and arabica cascara kombucha groups are presented in Figures 3 to 5.

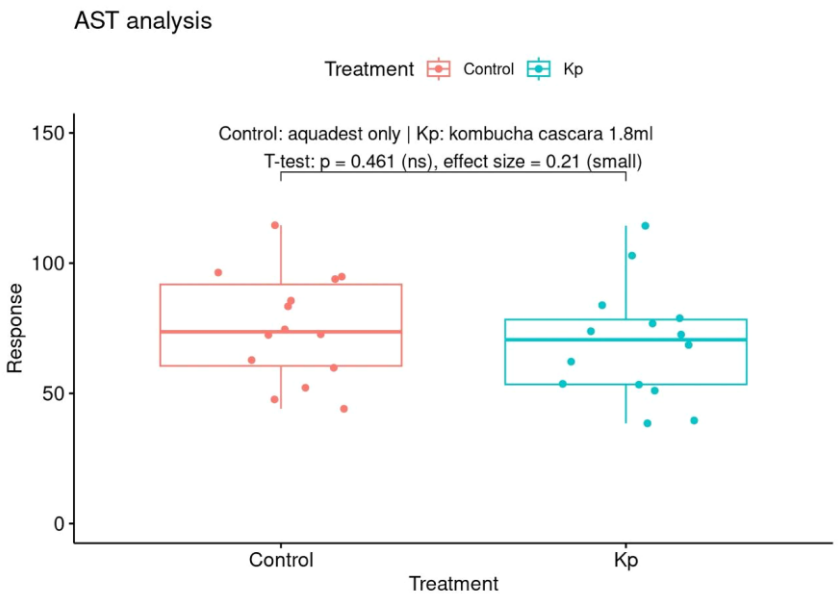


Figure 3. Aspartate aminotransferase (AST) levels in control and arabica cascara kombucha (Kp) treatments

Figure 3 shows the result of the T-test analysis, where the p-value was 0.461 (p> 0.05), indicating no significant difference between the control and arabica cascara kombucha (Kp) treatments. The average value of AST control was 82.75 ± 9.01 IU/L, while for the Kp treatments was 77.91 ± 10.18 IU/L. The effect size was 0.21, indicating only a small effect from the treatment. Additionally, the box plot visualization showed that the data distribution was predominantly centered within the inner quartile range, with no outliers.

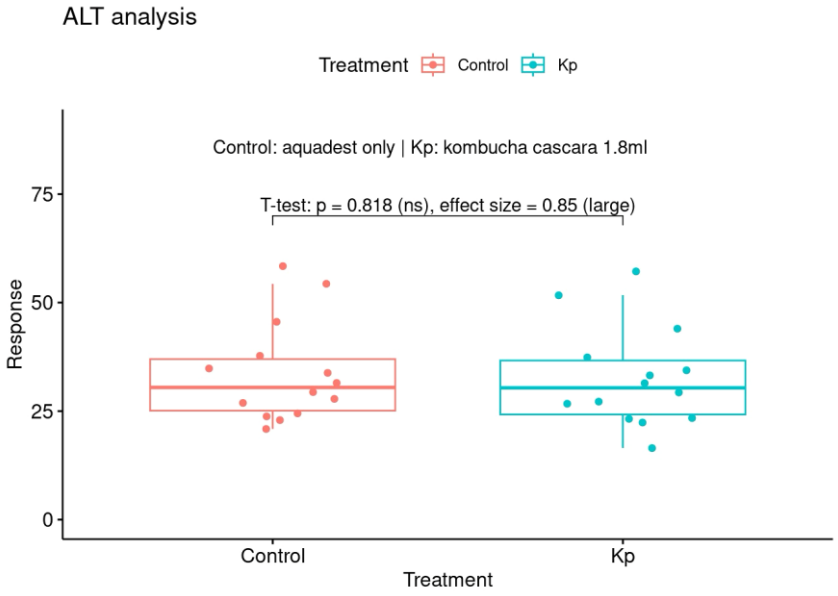


Figure 4. Alanine aminotransferase (ALT) levels in control and arabica cascara kombucha (Kp) treatments

As shown in Figure 4, the average value of ALT in control was 36.94 ± 4.32 IU/L, while in Kp treatments was 35.50 ± 4.01 IU/L. The T-test analysis showed a p-value of 0.818, also indicating no significant difference between the control and Kp treatments. However, a large effect size was observed between the control and Kp, as evidenced by Cohen's d value of 0.85. The box plot visualization clearly showed that the data distribution was concentrated within the inner quartile range, though one outlier was present in both the control and Kp treatments.

Figure 5 demonstrates that the average values of bilirubin observed in control group was 0.48 ± 0.11, while the total bilirubin in Kp treatments was 0.42 ± 0.11. T-test analysis results in a p-value of 0.443, indicating no significant difference between the control and Kp treatments. Nevertheless, a large effect size was still observed, as indicated by Cohen's d-value of 1.34. The box plot visualization shows some data points extending beyond the inner quartile range, but the majority of the data remains within the inner quartile range. Additionally, an extreme outlier was visible in the control treatment.

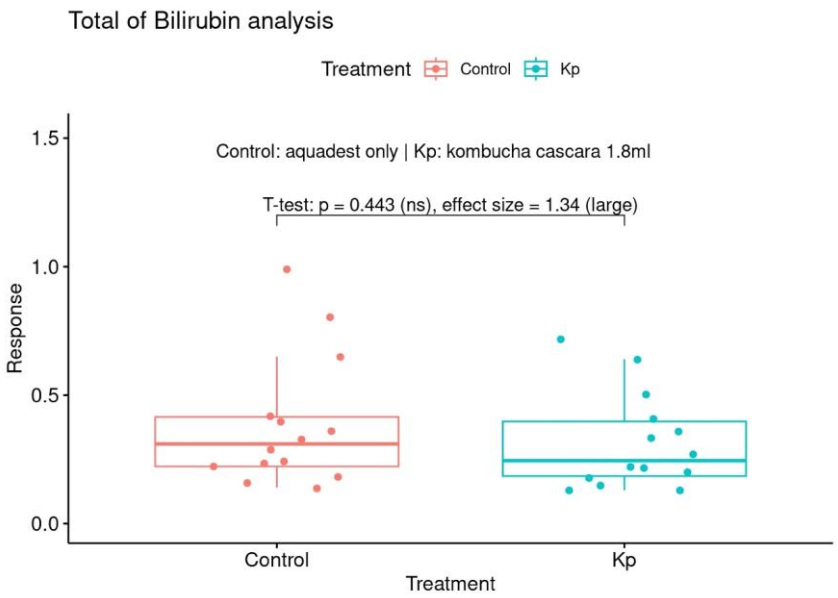


Figure 5. Total bilirubin levels in control and arabica cascara kombucha (Kp) treatments

4. Discussion

4.1. Bioactive Compounds of Cascara Kombucha

The selection of cascara kombucha samples for in vivo analysis was based on the bioactive components produced by the three samples using the LC-MS/MS method. Arabica cascara kombucha (J_1G) was found to contain a more complex bioactive compounds, with diverse organic acids, phenolic compounds, fatty acids, and amino acids. Additionally, arabica kombucha was shown to contain lower amounts of saccharides compared to robusta (J_2G) and liberica (J_3G) cascara kombucha. One of these compounds was merulinic acid A (an organic acid belonging to the hydroxybenzoate acid group) which constituted 28.8% of the total composition. Hydroxybenzoate acids are compounds that can act as oxidant cleansers, such as free radicals, through their hydroxyl groups. Thus, this compound can serve as an antioxidant and free radical scavenger [16]. According to Le et al. [17], the bioactive compounds particularly polyphenol and flavonoid in cascara kombucha will increase significantly during fermentation thus increasing the antioxidant properties as well as increasing the reduction of free radicals.

In cascara kombucha, several compounds classified as saccharides were found in both significant and small percentages. For example, in robusta cascara kombucha (J_2G), 36.91% of saccharides were identified in the form of 9-hydroxy-7-oxo-7H-furo[3,2-g] chrome-4-yl β-D-glucopyranoside and galactose galactosamine. Saccharides are compounds that belong to the carbohydrate group. The main function of carbohydrates is to provide energy but they also add sweetness to food, spare protein, and assist in fat metabolism. A deficiency in carbohydrates can lead to negative impacts, causing weakness as the body resorts to using protein and fat for energy. The breakdown of fats for energy can lead to ketone accumulation in the blood. If left unaddressed, this can lead to ketosis, which may cause dizziness, weakness, nausea, and dehydration. While carbohydrate deficiency can increase the risk of deficiencies of essential nutrients [18], excessive consumption of carbohydrates in the form of sugars may contribute to hepatic dysfunction and hyperglycemia [19].

Cascara kombucha also contains alkaloid compounds, notably caffeine. In the J_3G sample, alkaloid compounds were found in the form of caffeine; 1,3,7-trimethylxanthine; and 3,7-dihydro-1,3,7-trimethyl-1H-purin-2,6-dion, accounting for a total of 43.16%. The J_1G sample contained 18.13% alkaloids in the form of caffeine, 1,3,7-trimethylxanthine; 3,7-dihydro-1,3,7-trimethyl-1H-purin-2,6-dion, while the J_2G sample contained 12.96% alkaloids in the form of caffeine; 1,3,7-Trimethylxanthine. Caffeine is an alkaloid with pharmacological effects that are clinically beneficial, such as stimulating the central nervous system, relaxing muscles, particularly bronchial muscles, and stimulating the heart muscles [20]. According to Al-Mozie'l et al. [21], caffeine works by stimulating the central nervous system, providing positive effects when consumed at the prescribed doses. However, daily consumption of 100 mg caffeine may lead to dependence in some individuals. Moreover, high-dose caffeine consumption has side effects on the biochemical and histological functions of male rats. In a study involving 24 white rats divided into four groups and administered varying doses of caffeine (25 mg/kg, 50 mg/kg, and 100 mg/kg), histological changes were observed, including proliferation of bile ducts, central vein dilation, hepatocyte vacuolization, and portal vein congestion.

In cascara kombucha, alkaloids were not only found in the form of caffeine but also other compounds with smaller percentages. Alkaloids are bioactive plant-derived compounds that function as both medicinal agents and potent

immunostimulants, activating immune cells to combat bacterial, viral, and fungal pathogens, as well as cancer cells. These compounds demonstrate antimicrobial activity through multiple mechanisms, including inhibition of esterase, DNA/RNA polymerase, and cellular respiration, along with DNA intercalation capacity [22].

Another abundant group of compounds in cascara kombucha was coumarin. This compound was found in the forms of herniarin, scopoletin, mbelliferone, 7-hydroxycoumarin, umbelliferone, fraxetin, 7,8-dihydroxy-6-methoxycoumarin, Scopoletin, 7-hydroxy-5-methoxycoumarin, isofraxidin, and 7-hydroxy-6,8-dimethoxy coumarin. Coumarins have been shown to possess various bioactive properties, including anti-inflammatory, antimicrobial, anti-HIV, anti-tuberculosis, anticancer, and antioxidant activities [23].

4.2. Antimicrobial Activity

As shown in Table 4, no inhibition zone was observed in the cascara kombucha samples fermented for 10 days, but it appeared in the samples fermented for 17 days. This result indicated that the longer the fermentation process, the larger the inhibition zone produced. The fermentation time significantly affected the decrease in pH values, which in turn impacted the formation of inhibition zones. Bacteria and yeast in SCOBY affect the sucrose metabolism and produce organic acids including acetic acid, citric acid, glucoronic acid hence reducing pH to become 2.5 - 3.0 [24]. Microbial activity during kombucha fermentation produces enzymes that help convert sugars into various types of acids [24]. Le et al. [17] reported that the antimicrobial activity of kombucha is influenced by the production of acetic acid which is dominated during fermentation. In addition, conversion of sugar also produces acetic acid, gluconic acid, and glucuronic acid [25]. The absence of a clear zone in the antimicrobial activity test may also be due to different proportions of ingredients used, such as sugar and kombucha media, during preparation [26].

Table 4 also showed that while robusta cascara kombucha and black tea kombucha (control) did not produce clear zones, arabica cascara kombucha and liberica cascara kombucha displayed clear zones when fermented for 17 days. This indicated that the type of tea used in kombucha fermentation also influences the antimicrobial activity related to the active compounds that may be present in the tea. The variation types of tea have resulted in distinct physicochemical properties of kombucha [27].

Essentially, kombucha has good antimicrobial properties, which vary depending on the ingredients used in its brewing. Recent studies have shown that kombucha made from torch ginger flower (*Etlingera elatior*) had significant antimicrobial activity, successfully inhibiting the growth of pathogenic and commensal bacteria such as *E. coli* and *S. aureus* [28]. Similarly, kombucha also showed antibacterial activity against *E. coli*, *S. typhi*, *V. cholera*, and *S. dysenteriae*. However, unfermented (broth culture contained only 1% of tea leaves) and neutralized kombucha (kombucha tea neutralized with NaOH (1 M) at pH 7.0) did not show antimicrobial activity [29].

Kombucha's antibacterial properties were derived from the symbiotic actions between bacteria and yeast during the fermentation process [17]. Active compounds that contribute to its antimicrobial activity include usnic acid in kombucha cultures, acetic acid [10], and other compounds such as bacteriocins, enzymes, phenolic compounds from tea, and tannins [30, 31]. This is supported by numerous studies demonstrating kombucha's effectiveness as an antimicrobial agent. Recent research reported that kombucha's antimicrobial activity against pathogenic bacteria is largely attributed to its low pH, particularly due to the presence of organic acids like acetic acid and lactic acid [32].

4.3. Liver Function Examination

In this study, applying of 1.8 ml of cascara kombucha to rats over a specified period did not show any liver dysfunction. The AST, ALT, and bilirubin values in this study was within the normal value for rats. The normal range of AST, ALT and total bilirubin in rats were 13-56 U/L, 34-109 U/L, and 0.2-0.7 mg/dl, respectively [33]. Based on the results of the T-test, the treatment of administering cascara kombucha to rats did not show any significant difference in AST, ALT and total bilirubin levels as compared to the control. This indicated that the kombucha cascara consumed by the rats was not harmful and could be considered safe for consumption, similar to the condition of rats administered with distilled water. These results corroborate previous findings that kombucha is safe for mammalian consumption, as no hepatotoxic effects were observed in liver tissue [34].

This finding is supported by previous research indicating that administering of 1.8 ml of 50% kombucha tea for 35 days did not affect the hepatocyte diameter of the liver [35]. Furthermore, administering of 1.8 ml of 75% kombucha tea for 35 days also resulted in no significant changes in the liver diameter, body weight, water and feed consumption [36]. This suggested that kombucha did not have an impact on liver functions or hepatocyte diameter. Jayabalan also explained that kombucha tea is not categorized as a toxic substance [37]. On the contrary, Sannapaneni et al. [38] has reported the severe acute liver injury in woman associated with ingestion of kombucha tea. Adverse effects that arise from drinking kombucha might be due to the probiotic, acid, alcohol, or sugar content, as well as potential contamination if not properly produced or stored, consumption of excessive amounts of kombucha, unsafe sources of kombucha, and health conditions [39].

Kombucha is fundamentally beneficial as a hepatoprotector, with its hepatoprotective abilities have been studied in various animal models. Additionally, studies on the hepatoprotective effects of black tea kombucha against paracetamol [11] and aflatoxin B1 [12] have shown that kombucha tea did not negatively affect liver tissue in animal models. The bioactive compounds found in kombucha such as flavonoids, tannins, and saponins are believed to play roles in repairing liver damage [35, 40]. Given the fact that these compounds were also observed in cascara kombucha in this study, further studies are needed to evaluate the hepatoprotective effect of cascara kombucha in animal models of drug-induced liver damage, as well as the damage that occurs in other organs.

5. Conclusion

The study results indicate that arabica cascara kombucha contains complex bioactive compounds, including organic acids, phenolic compounds, fatty acids, amino acids, with lower amounts of saccharide when compared to robusta and liberica cascara kombucha. Arabica cascara kombucha has 38 bioactive compounds, including merulinic acid A (28.8%), glycerol pyruvate succinate (19.92%), caffeine (18.13%), dimethyl 4-(3-methoxy-3-oxopropyl)-4-nitroheptanedioate and kojic acid (7.39%), 2-Amino-6,7-dimethoxy-3-quinoline carboxamide, carnosic acid (4.13%) and chlorogenic acid (1.6%). Meanwhile, robusta and liberica cascara kombucha contain only 32 and 22 bioactive compounds, respectively. In vitro test indicates that arabica cascara kombucha and liberica cascara kombucha exhibit functional properties as an effective antimicrobial agent after 17 days of fermentation time. Both arabica cascara kombucha and liberica cascara kombucha demonstrated the ability to inhibit the growth of *S. aureus* ATCC 29213 and *E. coli* ATCC 25922. Robusta cascara kombucha only showed inhibition against *S. aureus* bacteria after fermented for 17 days. The fermentation period affected the decrease in pH values, which in turn has impacted the formation of inhibition zones. The longer the fermentation time, the larger the inhibition zone produced. In vivo test reveal that the administration of 1.8 ml of arabica cascara kombucha did not adversely affect the liver function parameters. The levels of AST, ALT, and total bilirubin fall within the normal value both in control group and treatment group. This result indicates that kombucha can be safely consumed by mammals because it does not cause liver damage in animal model studied.

6. Declarations

6.1. Author Contributions

Conceptualization, S.R. and S.H.A.; methodology, S.H.A., A.S., and S.R.; software, A.AB.; validation, S.H.A. and A.S.; formal analysis, A.S. and S.R.; investigation, S.R. and A.S.; resources, S.R.; data curation, S.R., S.H.A., and A.S.; writing—original draft preparation, S.R., S.H.A., A.AB., and A.S.; writing—review and editing, S.H.A., A.S., and A.AB.; visualization, S.H.A. and A.AB.; supervision, S.H.A., A.AB., and A.S.; project administration, S.R. and A.S. All authors have read and agreed to the published version of the manuscript.

6.2. Data Availability Statement

The data presented in this study are available in the article.

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6.5. Institutional Review Board Statement

Not applicable.

6.6. Informed Consent Statement

Not applicable.

6.7. Declaration of Competing Interest

The authors declare that there are no conflicts of interest concerning the publication of this manuscript. Furthermore, all ethical considerations, including plagiarism, informed consent, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancies have been completely observed by the authors.

7. References

[1] Jankovic, I., Sybesma, W., Phothirath, P., Ananta, E., & Mercenier, A. (2010). Application of probiotics in food products-challenges and new approaches. Current Opinion in Biotechnology, 21(2), 175–181. doi:10.1016/j.copbio.2010.03.009.

[2] Coelho, R. M. D., Almeida, A. L. de, Amaral, R. Q. G. do, Mota, R. N. da, & Sousa, P. H. M. d. (2020). Kombucha: Review. International Journal of Gastronomy and Food Science, 22(October), 100272. doi:10.1016/j.ijgfs.2020.100272.

[3] Nurhayati, Yuwanti, A. & Urbahillah, A. (2020) Physicochemical and Sensory Characteristics of the Cascara (Dried Cherries Coffee Peels) Kombucha. Jurnal Teknologi dan Industri Pangan. 31(1), 38-49. doi:10.6066/jtip.2020.31.1.38.

[4] Heeger, A., Kosińska-Cagnazzo, A., Cantergiani, E., & Andlauer, W. (2017). Bioactives of coffee cherry pulp and its utilisation for production of Cascara beverage. Food chemistry, 221, 969-975. doi:10.1016/j.foodchem.2016.11.067.

[5] Rohaya, S., Multahadi, & Sulaiman, I. (2022). Improving the quality of kombucha cascara with different varieties and fermentation time in diverse arabica coffee (Coffea arabica L) cultivars. Coffee Science, 17, 172056– 172056. doi:10.25186/.v17i.2056.

[6] Muzaifa, M., Rohaya, S., Nilda, C., & Harahap, K. R. (2022). Kombucha Fermentation from Cascara with Addition of Red Dragon Fruit (Hylocereus polyrhizus): Analysis of Alcohol Content and Total Soluble Solid. Proceedings of the International Conference on Tropical Agrifood, Feed and Fuel (ICTAFF 2021), Vol. 17, 125–129. doi:10.2991/absr.k.220102.020.

[7] Massoud, R., Jafari, R., & Khosravi-Darani, K. (2024). Kombucha as a Health-Beneficial Drink for Human Health. Plant Foods for Human Nutrition, 79(2), 251–259. doi:10.1007/s11130-024-01169-8.

[8] Battikh, H., Bakhrouf, A., & Ammar, E. (2012). Antimicrobial effect of Kombucha analogues. LWT-Food Science and Technology, 47(1), 71-77. doi:10.1016/j.lwt.2011.12.033.

[9] Içen, H., Corbo, M. R., Sinigaglia, M., Korkmaz, B. I. O., & Bevilacqua, A. (2023). Microbiology and antimicrobial effects of kombucha, a short overview. Food Bioscience, 56, 103270. doi:10.1016/j.fbio.2023.103270.

[10] Battikh, H., Chaieb, K., Bakhrouf, A., & Ammar, E. (2013). Antibacterial and antifungal activities of black and green kombucha teas. Journal of Food Biochemistry, 37(2), 231–236. doi:10.1111/j.1745-4514.2011.00629.x.

[11] Pauline, T., Dipti, P., Anju, B., Kavimani, S., Sharma, S. K., Kain, A. K., Sarada, S. K. S., Sairam, M., Ilavazhagan, G., Devendra, K., & Selvamurthy, W. (2001). Studies on Toxicity, Anti-stress and Hepato-protective Properties of Kombucha Tea. Biomedical and Environmental Sciences, 14(3), 207–213.

[12] Jayabalan, R., Baskaran, S., Marimuthu, S., Swaminathan, K., & Yun, S. E. (2010). Effect of kombucha tea on aflatoxin B1 induced acute hepatotoxicity in albino rats-prophylactic and curative studies. Journal of Applied Biological Chemistry, 53(4), 407–416. doi:10.3839/jksabc.2010.063.

[13] Kole, A. S., Jones, H. D., Christensen, R., & Gladstein, J. (2009). A case of Kombucha tea toxicity. Journal of Intensive Care Medicine, 24(3), 205–207. doi:10.1177/0885066609332963.

[14] Mutiah, R., Rachmawati, E., Fitrianingsih, A. A., & Zahiro, S. R. (2023). Metabolite profiling of anticancer compounds in Saussure lappa based on UPLC-QToFMS/MS. Pharmacy Education, 23(4), 37–42. doi:10.46542/pe.2023.234.3742.

[15] Al-Mohammadi, A. R., Ismaiel, A. A., Ibrahim, R. A., Moustafa, A. H., Abou Zeid, A., & Enan, G. (2021). Chemical constitution and antimicrobial activity of kombucha fermented beverage. Molecules, 26(16), 5026. doi:10.3390/molecules26165026.

[16] Sroka, Z., & Cisowski, W. (2003). Hydrogen peroxide scavenging, antioxidant and anti-radical activity of some phenolic acids. Food and Chemical Toxicology, 41(6), 753–758. doi:10.1016/S0278-6915(02)00329-0.

[17] Nguyen Le, B. X., Van, T. P., Phan, Q. K., Pham, G. B., Quang, H. P., & Do, A. D. (2024). Coffee Husk By-Product as Novel Ingredients for Cascara Kombucha Production. Journal of Microbiology and Biotechnology, 34(3), 673–680. doi:10.4014/jmb.2310.10004.

[18] Panjaitan, R. S., Christian, Y. E., Sari, D. P., Luthfiana, F., Ardian, K. D., Nurulah, M., & Astiani, R. (2024). Education on Various Types and Benefits of Carbohydrates for Balanced Nutrition in Children. Pharmacy Action Journal. 12-18.

[19] Brouns, F. (2020). Saccharide Characteristics and Their Potential Health Effects in Perspective. Frontiers in Nutrition, 7. doi:10.3389/fnut.2020.00075.

[20] Nhan, P. P., & Phu, N. T. (2012). Effect of time and water temperature on caffeine extraction from coffee. Pakistan Journal of Nutrition, 11(2), 100–103. doi:10.3923/pjn.2012.100.103.

[21] Al-Mozie'l, M. S. G., Khudhair, A. A., & Zubairi, M. B. (2019). Effect of caffeine therapeutic dose on rat organs.PDF. International Journal of Sciences and Technology, 14(2), 15. doi:15-23.https://doi.org/10.116/j.lfs.2019.05.007.

[22] Maisarah, M., Chatri, M., Advinda, L., & Violita. (2023). Characteristics and Functions of Alkaloid Compounds as Antifungals in Plants. SERAMBI Biologi, 8(2), 231–236. doi:10.02/b.24i2.2947.

[23] Li, S., Jiang, S., Jia, W., Guo, T., Wang, F., Li, J., & Yao, Z. (2024). Natural antimicrobials from plants: Recent advances and future prospects. Food Chemistry, 432. doi:10.1016/j.foodchem.2023.137231.

[24] Ansari, F., Pourjafar, H., & Esmailpour, S. (2017). Study on citric acid production and antibacterial activity of Kombucha green tea beverage during production and storage. Annual Research and Review in Biology, 16(3), 1–8. doi:10.9734/ARRB/2017/35664.

[25] Ayu Puji Lestari, K. (2019). Artikel Penelitian Tea and Coffee Kombucha Activity Test as Antibacterial for Gram Positive Bacteria and Gram Negative Bacteria. Journal of Pharmacy and Science, 4(2), 61–65.

[26] Jakubczyk, K., Kałduńska, J., Kochman, J., & Janda, K. (2020). Chemical profile and antioxidant activity of the Kombucha beverage derived from white, green, black and red tea. Antioxidants 9(5), 447. doi:10.3390/antiox9050447.

[27] Cardoso, R. R., Neto, R. O., dos Santos D'Almeida, C. T., do Nascimento, T. P., Pressete, C. G., Azevedo, L., Martino, H. S. D., Cameron, L. C., Ferreira, M. S. L., & Barros, F. A. R. de. (2020). Kombuchas from green and black teas have different phenolic profile, which impacts their antioxidant capacities, antibacterial and antiproliferative activities. Food Research International, 128. doi:10.1016/j.foodres.2019.108782.

[28] Fadhilah, F. R., Pakpahan, S., Rezaldi, F., Kusmiran, E., Cantika, E., Julinda, O., & Muhammad, R. (2024). Antimicrobial Potential of Torch Ginger (Etlangia elatior) Flower Kombucha Tea. The Indonesian Journal of Infectious Diseases, 10(1), 24–35. doi:10.32667/ijid.v10i1.186.

[29] Kaewkod, T., Bovonsombut, S., & Tragoolpua, Y. (2019). Efficacy of Kombucha obtained from green, oolongand black teas on inhibition of pathogenic bacteria, antioxidation, and toxicity on colorectal cancer cell line. Microorganisms 7(12), 700. Microorganisms. doi:10.3390/microorganisms7120700.

[30] Vastrad, J. V., Badanayak, P., & Goudar, G. (2022). Phenolic compounds in tea: phytochemical, biological, and therapeutic applications. Phenolic compounds-Chemistry, synthesis, diversity, non-conventional industrial, pharmaceutical and therapeutic applications, 23, 452. doi:10.5772/intechopen.98715.

[31] Todorov, S. D., de Almeida, B. M., Lima, E. M. F., Fabi, J. P., Lajolo, F. M., & Hassimotto, N. M. A. (2025). Phenolic Compounds and Bacteriocins: Mechanisms, Interactions, and Applications in Food Preservation and Safety. Molecular Nutrition and Food Research, 69(2), 202400723. doi:10.1002/mnfr.202400723.

[32] Sreeramulu, G., Zhu, Y., & Knol, W. (2001). Characterization of antimicrobial activity in Kombucha fermentation. Acta Biotechnologica, 21(1), 49–56. doi:10.1002/1521-3846(200102)21:1<49::AID-ABIO49>3.0.CO;2-G.

[33] Kurtz, D. M., & Travlos, G. S. (2017). The Clinical Chemistry of Laboratory Animals. CRC Press, Florida, United States.

[34] Villarreal-Soto, S. A., Beaufort, S., Bouajila, J., Souchard, J. P., & Taillandier, P. (2018). Understanding Kombucha Tea Fermentation: A Review. Journal of Food Science, 83(3), 580–588. doi:10.1111/1750-3841.14068.

[35] Isdadiyanto, S., & Tana, S. (2021). Effect of Giving Kombucha Tea Concentration of 50% with Different Fermentation Time on Histologi Structure of Liver of Wistar Rats (Rattus norvegicus) Male. Bioma, 51-56. doi:10.23917/pharmacon.v18i2.15732.

[36] Isdadiyanto, S., & Tana, S. (2019). Histological Structure of Male Wistar Rat (Rattus norvegicus) Liver after Giving Kombucha Tea Concentration 75% with Different Fermentation Times. Bioma: Berkala Ilmiah Biologi, 21(2), 165–172. doi:10.14710/bioma.21.2.165-172.

[37] Jayabalan, R., Malbaša, R. V., Lončar, E. S., Vitas, J. S., & Sathishkumar, M. (2014). A review on Kombucha tea-microbiology, composition, fermentation, beneficial effects, toxicity, and tea fungus. Comprehensive Reviews in Food Science and Food Safety, 13(4), 538–550. doi:10.1111/1541-4337.12073.

[38] Sannapaneni, S., Philip, S., Desai, A., Mitchell, J., & Feldman, M. (2023). Kombucha-Induced Massive Hepatic Necrosis: A Case Report and a Review of Literature. Gastro Hep Advances, 2(2), 196–198. doi:10.1016/j.gastha.2022.09.014.

[39] de Oliveira, P. V., da Silva Júnior, A. H., de Oliveira, C. R. S., Assumpção, C. F., & Ogeda, C. H. (2023). Kombucha benefits, risks and regulatory frameworks: A review. Food Chemistry Advances, 2, 100288. doi:10.1016/j.focha.2023.100288.

[40] Özdemir, N. & Con, A. H. (2017). Kombucha and Health. Journal of Health Science, 244-250. doi:10.17265/2328-7136/2017.05.005.