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THE EFFECT OF SUGAR CONCENTRATION IN KOMBUCHA, KEJEK TEA AND ASAM KANDIS (Garciniaxanthochymus) ON ANTIOXIDANT ACTIVITY, TOTAL ALCOHOL AND TOTAL ACID

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Abstract

This study aims to determine the effect of sugar concentration and the addition of asamkandis on antioxidant activity, total alcohol and total acid in kombucha drinks. The test began with a test of the characteristics of the simplicia phytochemical screening. The antioxidant activity was then examined using the DPPH method, the total alcohol content was determined using gas chromatography, and the total acid test was performed using titration. The results of the simplicia characteristic test showed that the simplicia used had met the standards of the Indonesian herbal pharmacopoeia, and the results of the phytochemical screening test showed that only saponins did not show their presence, both kejek tea, asam kandis and a combination of both. The results of the DPPH test showed that the sample with optimal antioxidant activity was in Formula 4 with a percentage inhibition value of 82% ± 0.08, total alcohol of 0.09% ± 0.12 and total acid of 1.57% ± 0.17. This study also proves that the combination of kejek tea, kandis acid and higher sugar content can increase antioxidant activity, total alcohol and total acid, but the total alcohol and total acid are still below the safe limit.

Keywords: antioxidant activity, asam kandis (*Garciniaxanthochymus*), kejek tea, total acid, total alcohol

Introduction

Antioxidants can counteract or prevent oxidation reactions caused by free radicals. Antioxidants can inhibit free radicals and convert them into non-radical compounds. Antioxidants are found in the human body, but when there are too many free radicals, antioxidants from outside are needed.[1]

Additional antioxidants can be obtained from synthesis or nature. One of the natural compounds that can be used as antioxidants is flavonoids. Flavonoids work by donating hydrogen ions, are exogenous antioxidants that contain phenolic groups, and have been proven to prevent cell damage due to oxidative stress.[1] Phenolic compounds play a very active role as antioxidants. Phenolic compounds have a structure that can easily donate hydrogen or electrons to acceptors to reduce the activity of oxygen and peroxide radicals.[2]

Flavonoids are contained in various plants, one of which is tea. Tea processing is carried out in multiple ways, one of which is making kombucha drinks. Kombucha is a combination of tea and sugar that is then fermented with the help of bacterial and fungal

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symbiosis to form fermented tea.[3,4] The main ingredients are black tea, green tea or oolong tea. In addition, kombucha can also be made with infused water using fruits, mint leaves, jasmine flowers, and so on as the main ingredients.[4] The symbiotic culture used is kombu fungus, commonly called dipo mushroom or bull mushroom. This kombu mushroom is called SCOBY (Symbiotic Culture Of Bactery And Yeast). Kombucha is one of the drinks known to have antioxidant activity. The increase in antioxidant activity in kombucha can be caused by the presence of free phenolic compounds produced during the fermentation process and flavonoid metabolites derived from plants such as kandis acid, so the higher the phenolic and flavonoid levels, the higher the antioxidant activity. In addition, kombucha contains organic compounds that are beneficial to the body, so it has the potential as a health drink.[5] Kombucha shows increased antioxidant activity during fermentation. This indicates that fermentation time affects antioxidant activity.[5] From much literature, it is found that fermentation time dramatically affects the alcohol content and total acid, which can affect the quality and halalness of kombucha. In making kombucha, sugar is one of the main ingredients, leading researchers to explore the impact of different sugar content variations and the influence of incorporating kandis acid on antioxidant activity, total phenols, total flavonoids, total alcohol and total acid of kombucha. Kandis acid (Garciniaxanthohymus) is a plant included in the genus Garcinia. This genus is a tropical plant in the Clusiaceae family, with around 180 species. This plant is also commonly known as the mangosteen plant. Kandis acid, which is included in the Garcinia Genus, this genus is spread in Kalimantan as many as 25 species.[6] The Malay community usually flavours this tamarind fruit.[7]

Kandis acid is rich in nutrients containing primary metabolites such as carbohydrates, proteins and fats, as well as vitamins and minerals such as sodium, potassium, calcium, iron, phosphorus, magnesium, thiamine, riboflavin, niacin, ascorbic acid, and vitamin B12. Kandis acid also contains phenolic compounds of the flavonoid, xanthone, and benzophenone types. These compounds are known to have various biological activities, such as antioxidants, antimicrobials, and antimalarials.[8] Previous research has shown that the fruit of G. xanthochymus is a source of antioxidants. The use of kandis acid fruit as a combination in kombucha fermentation has never been reported.[3] Kombucha fermentation will produce alcohol resulting from sugar fermentation. However, because of the alcohol content, it has become an issue regarding the halalness of Kombucha. Halal status has become a mandatory requirement for food and beverages in Indonesia. According to ijtihad,[9] fermented beverage products containing alcohol/ethanol of less than 0.5% are legally halal if they are medically safe. To review and guarantee the halalness of kombucha, an analysis of the alcohol content in kombucha using gas chromatography is required. The process of making kombucha, sugar is also added in the appropriate amounts to produce kombucha that is safe for consumption.

Method

Tool

The tools used in this study were a heater (Omicko), stainless vessel, steamer, 120 mesh sieve, blender (Philips), beaker (Pyrex), measuring flask (Iwaki), the crucible, measuring cup (Iwaki), test tube (Pyrex), micropipette (Dragon Lab), desiccator (Iwaki), tweezers (Local), spectrophotometer (Thermo Scientific Genesys), oven (B-one Type Ov-30), aluminium foil and analytical balance (FUJITSU FS-AR), gas chromatography (Shimadzu®)InstrumenataualatditulisbersamaMerkalatdanasalnegaraalattersebut.

Material

The materials used in this study were kandis acid (Garciniaxanthohymus) and kejek tea. The chemicals used were 2,2-diphenyl-1-picryhidrazyl (sigma), methanol

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(Merck), aquadest (Brataco), chloroform (Merck), ethanol 96% (Merck), methanol 70% (Merck), Mg powder (Merck), HCl (Merck), amyl alcohol (Merck), FeCl3 (Merck), NaCl (Merck), anhydrous acetic acid (Merck), H2SO4 (Merck), NaOH (Merck).

Procedure

Collection of materials and preparation

This research was conducted experimentally in the laboratory of the Faculty of Mathematics and Natural Sciences, Garut University and the Indonesian Education University. This research began with preparing kejek tea leaves (Camellia sinensis (L) Kuntze) obtained from PT. Perkebunan Nusantara VIII, Cilawu, Garut, West Java and kandis acid fruit obtained from Sempadian Village, Sambas, West Kalimantan. Kombucha SCOBY starter was obtained from SCOBY livestock in the Bogor area, West Java, Indonesia. The determination was carried out at Tanjungpura University, Pontianak, West Kalimantan and supporting materials were prepared from several official chemical distributors. The manufacture of kejek tea simplicia was made in a traditional tea factory in Cigedug Village, Kaki Gunung Cikuray District, Garut, West Java, by collecting the ingredients then washing, sorting wet, drying, stepping on, drying them on hot coals and storing them. Kandis acid fruit simplicia was made by washing, sorting wet, drying, mashing and storing. The characteristic test of the simplicia was carried out on kejek tea and kandis acid, including water content, total ash content, acid-insoluble ash content, water-soluble extract content and ethanol extract content. Phytochemical screening was also carried out on both simplicia, which included screening alkaloids, flavonoids, phenols, tannins, quinones, saponins, and steroids/triterpenoids. Kejek tea and kandis acid were then made into Kombucha drinks with various sugar concentrations, namely 0%, 10%, 20%, and 30%, and then cooled until the temperature reached room temperature 25-28^{o}C. Kombucha starter was added as much as 15 g. The container was then covered with a clean cloth and fermented for 14 days at room temperature (25-28^{o}C) for the complete formula in Table 4.

Antioxidant Activity Test

After the sample was available, the next step was to test the antioxidant activity using the DPPH method. The parameter tested was the % inhibition value by not forgetting to verify the technique first. The test was carried out by mixing the test sample with a 50 ppm DPPH solution and, incubating it at room temperature for 30 minutes and, measuring it at a wavelength of 518 nm, then calculating it using the following formula:

$$\%\ Inhibition = \frac{DPPH\ absorbance - Sample\ Absorbance}{DPPH\ absorbance} x\ 100\%$$

Alcohol Concentration Test

Alcohol concentration measurement using gas chromatography (GC) begins with a 10 mL kombucha sample diluted with aquabidest in a measuring flask. Then, it is distilled to obtain 75% distillate, and the distillate is transferred quantitatively into a 10 mL measuring flask and aquabidest is added to the limit mark. A little solution is taken and poured into an Evendoff tube to be injected into the GC injector using a micro syringe as a syringe into the GC injector, then compared between the sample solution and the standard solution using a 10% ethanol standard.

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Total Acid Measurement

Measurement of total acid in kombucha drink samples using the alkalimetric titration method, namely, 20 mL of kombucha sample was put into an Erlenmeyer flask, and 2 times the volume of sample was added with distilled water, then 0.1 mL of 1% phenolphthalein indicator was added, then titrated with 0.1 N NaOH until a pink colour was formed, and could last for 30 seconds then calculated using the following formula

$$\%\ total\ acid = \frac{V\ NaoH\ x\ Concentration\ of\ NaOH\ x\ Dilution\ factor}{sample\ volume}\ x\ 100\%$$

Result

This study used kejek tea and kandis acid fruit simplicia with kombucha fermentation as an alternative functional drink.

Figure 1. Fermented drinks kombucha and SCOBY

Table 1. Sample Characteristic Test Results

No	Testing	Level (%)		Literature[1] (%)
		Kejek tea	Kandis acid	
1	Water Content	4,15	4,62	<10,0
2	Total Ash Content	5,65	5,985	<10,0
3	Acid insoluble ash content	0,65	0,291	<2,0
4	Water soluble extract content	21,44	20,71	>10,0
5	Ethanol soluble extract content	14,12	17,45	>10,8

Table 2. Phytochemical Screening Results

No	Secondary Metabolites	Observation result		
		Kejek tea	Kandis acid	A combination of Kejek tea and Kandis acid
1	Flavonoid	+	+	+

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Table 2.(Extension)

No	Secondary Metabolites	Observation result		
		Kejek tea	Kandis acid	A combination of Kejek tea and Kandis acid
2	Fenol	+	+	+
3	Alkaloid	+	+	+
4	Tanin	+	+	+
5	Kuinon	+	+	+
6	Saponin	-	-	-
7	Steroid/Terpenoid	+	+	+

The + sign indicates the presence of secondary metabolites being analyzed.

Table 3. Method Verification Test Results

No	Types of Testing	Observation Result	
		Result	Reference[2,3]
1	Maximum wavelength	518 nm	515-520 nm
2	Linearity	0,9966	1
3	Precision	1.6%	<2%
4	Accuracy	98%-103%	95%-105%

Table 4. Formula of Kombucha Kejek Tea Combined with Kandis Acid

	Formula 0	Formula 1	Formula 2	Formula 3	Formula 4
Aquades	500 mL	500 mL	500 mL	500 mL	500 mL
Sugar	30%	0	10%	20%	30%
Kejek tea	2,5 g	2,5 g	2,5 g	2,5 g	2,5 g
Kandis acid	0 g	2,5 g	2,5 g	2,5 g	2,5 g
SCOBY	15 g	15 g	15 g	15 g	15 g
Starter	50 mL	50 mL	50 mL	50 mL	50 mL

Table 5. Antioxidant, Total Alcohol and Total Acid Test Results on the Kombucha Kejek Tea Combined with Kandis Acid

Formula	% Inhibition	Total Alcohol	Total Acid
0	40%± 0,09	0.08%± 0,17	1.40%± 0,18
1	46%± 0,03	0.03%± 0,12	1.25%± 0,16
2	57%± 0,01	0.05%± 0,11	1.32%± 0,12
3	70%± 0,05	0.07%± 0,12	1.44%± 0,15
4	82%± 0,08	0.09%± 0,12	1.57%± 0,17
ANOVA test (sig)	0,042	0,034	0,023

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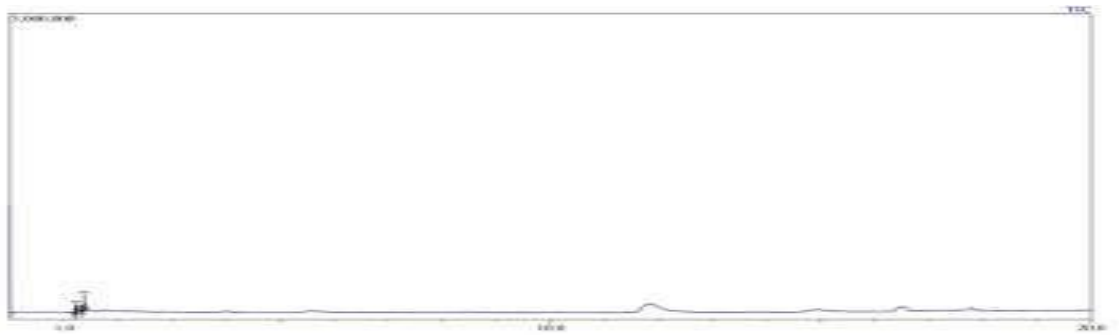

Figure 2. Chromatogram results of kombucha with gas chromatography (GC)

Table 6. Chromatogram Description

No	No Peak	R.Time	Area	Area%	Height	A/H	SI%	Name
1	3	1.370	176629	48.21	163650	1.08	97	Ethanol(CAS)Ethyl alcohol
2	2	1.276	104171	28.44	36198	2.88	88	Tetradeuteromethane
3	1	1.213	85542	23.35	81569	1.05	98	Carbon dioxide(CAS)Dryice

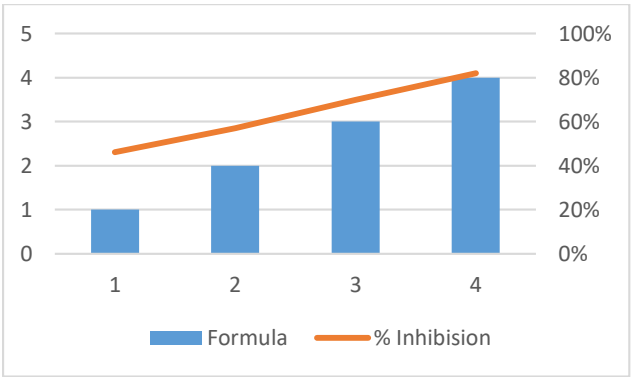

Figure 3. Antioxidant test results on kombucha kejek tea combined with kandis acid

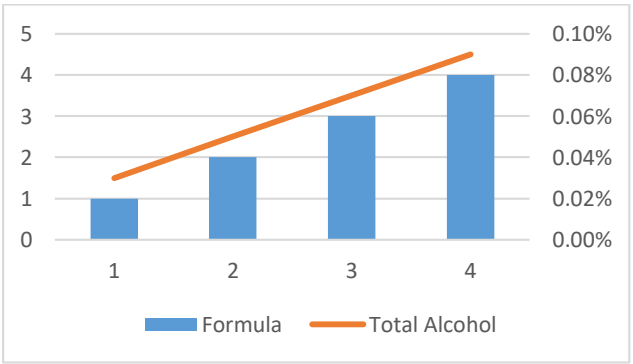

Figure 4. Total alcohol test results on kombucha kejek tea combined with kandis acid

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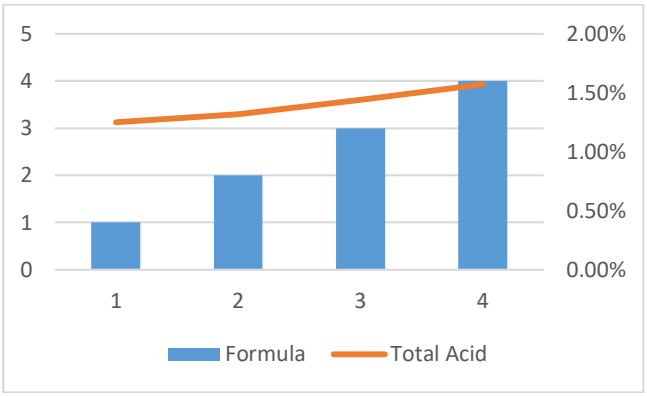

Figure 5. Total acid test results on kombucha kejek tea combined with kandis acid

Discussion

In this study, fermented kombucha drinks were made with the main ingredient of kejek tea, which has various sugar content and a combination of kandis acid. This study aims to determine the effect of sugar content and the combination of kejek tea-kandis acid kombucha on the antioxidant activity, total alcohol and total acid contained. Antioxidant activity was tested using the DPPH method, total alcohol content was tested with gas chromatography, and total acid content was tested using titration. This study began with manufacturing kejek tea kombucha with various sugar content variants, which were then fermented for 14 days. Before testing, a method verification was carried out, which included determining the maximum wavelength and measuring linearity, precision, and accuracy. The maximum wavelength obtained from this experiment was at λ max 518. The wavelength of 518 nm is a wavelength that is under the literature, around 515-520 nm.2 Furthermore, a linearity test was carried out, which obtained a standard curve of vit.c with the equation y = 0.228x - 0.016. The relation coefficient of the curve is 0.9966. The coefficient value approaching 1 indicates a linear relationship between concentration and absorption. The precision value created from the research results obtained %RSD of 1.6%, while the acceptance of the RSD value is <2%, while for the accuracy test, it is produced in the range of 98% -103%, which can be seen in Table 3.[10,11] The main ingredients used, namely kejek tea and kandis acid simplicia have met the quality requirements, where these requirements are based on the results of the simplicia characteristic test that meets the requirements of the Indonesian Herbal Pharmacopoeia. Phytochemical screening tests were also carried out to determine secondary metabolites in simplicia, including qualitative tests of alkaloids, flavonoids, phenols, tannins, quinones, saponins and steroids/triterpenoids. From the screening test results, only one gave a negative value, namely saponin, which can be seen in Table 2.

Testing of the test samples began on kombucha with various variations of sugar not combined with kandis acid, including antioxidant activity as seen by inhibition presentation, total alcohol test and total acid test. The same test was also conducted on kombucha combined with kandis acid. Table 5 shows an increase in antioxidant activity in kombucha combined with kejek tea and kandis acid due to the synergistic activity of kejek tea and kandis acid. These results are also supported by phytochemical screening data, where Table 2 shows the same secondary metabolites so that antioxidant activity increases. Antioxidant activity testing is carried out to obtain information on whether kombucha drinks can benefit users, including to ward off free radicals. Total alcohol content testing is carried out to determine the safety of users of this drink. The gas chromatography method is used to determine the total alcohol content.

The gas chromatography method was used in this study because it is more efficient, sensitive, has high resolution, can detect in ppm (parts per million) and even

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ppb (parts per billion), performs quantitative analysis with high accuracy, requires small samples (usually in µL), is dependable, reasonably easy, and cost-effective. This method is also capable of dynamic separation identification of all volatile organic compounds, has high sensitivity, and can perform qualitative and quantitative analysis of compounds in a mixture using relatively few samples. Suppose the alcohol content is more than the standard limit. In that case, the alcohol content can be controlled by various methods, such as dilution, pasteurization, distillation, and filtration of bacteria or yeast that produce alcohol. This is done to ensure that the alcohol content produced remains below the standard limit of 0.5%.[2,9,12] Sugar variations play a crucial role in increasing the total alcohol content. However, the total alcohol content of kombucha in each sugar variation, as seen in Table 5, is still within a safe concentration, below 0.5%. The last step is testing the total acid content using the titration method. Table 5 shows that the total acid content is obtained in a safe range, in the range of 1-15%.[13,14,15]

Conclusion

Based on the results of research that have been conducted on the antioxidant activity of kombucha kejek tea and a combination with kandis acid (*Garcinia xantohymus*) with various sugar variations, it can be concluded that the DPPH test results showed samples with optimal antioxidant activity in Formula 4 with an inhibition percent value of 82% ± 0.08, total alcohol of 0.09% ± 0.12 and total acid of 1.57% ± 0.17. This study also shows that combining kejek tea and kandis acid and the higher sugar content can increase antioxidant activity, total alcohol and total acid, but total alcohol and total acid are still below the safe limit.

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Reference

1. Rafif SN, Hikmatiana BN, Mikdar NN, Febriyanti RM, Maisyarah IT. Antioxidant activity of extract and fractions of bebuas leaves (premna serratifolia l.) using dpph method. Indones J Biol Pharm. 2024;4(1):1–10.
2. Lubis N, Mubarok A, Junaedi C, Soni D. Definition of halal analysis of formulation of kombucha drink from simplicia of malay apple fruit (Syzygium malaccense). J Halal Prod Res [Internet]. 2024;7(1). Available from: https://dx.doi.org/10.
3. Aini FN, Budiandari RU. Kajian penambahan konsetrasi gula terhadap karakteristik sifat organoleptik kombucha rosella (hibiscus sadbariffa l). Universitas Muhammadiyah Sidoarjo; 2024.
4. Suhardini PN, Zubaidah E. Studi aktivitas antioksidan kombucha dari berbagai jenis daun selama fermentasi. J Pangan dan Agroindustri. 2016;4(1):221–9.
5. Oktavia S, Novi C, Handayani EE, Abdilah NA, Setiawan U, Rezaldi F. Pelatihan pembuatan immunomodulatory drink kombucha untuk meningkatkan perekonomian masa new normal pada masyarakat desa majau dan kadudampit kecamatan saketi kabupaten pandeglang, banten. J Pengabdi Pada Masy. 2021;6(3):716–24.
6. Anantachoke N, Duangrat R, Sutthiphatkul T, Ochaikul D, Mangmool S. Kombucha beverages produced from fruits, vegetables, and plants: a review on their pharmacological activities and health benefits. Foods. 2023;12(9):1818–35.
7. Berlian Z, Aini F, Ulandari R. Evaluation of the alcohol content of white sticky rice and cassava through fermentation with different yeast doses. J Biota. 2016;2(1):106–11.
8. Kamaluddin MJN, Handayani MN. Pengaruh perbedaan jenis hidrokoloid terhadap karakteristik fruit leather pepaya. Edufortech. 2018;3(1):24–32.

9. MUI. Fatwa MUI tahun 2018 tentang produk makanan dan minuman yang mengandung alkohol/etanol untuk bahan obat. Indonesia; 2018.
10. Wahyuni AM, Afthoni MH, Rollando R. Pengembangan dan validasi metode analisis spektrofotometri uv vis derivatif untuk deteksi kombinasi hidrokortison asetat dan nipagin pada sediaan krim. Sainsbertek J Ilm Sains Teknol. 2022;3(1):239–47.
11. Kamilla L, Ramadhanty K, Purwaningsih I. Verifikasi metode analisa albumin dengan metode bromcressol green. J Lab Khatulistiwa. 2020;3(2):40.
12. Setiawati L, Rizqiati H, Susanti S. Analisis rendemen, kadar alkohol, nilai ph dan total bal pada kefir whey susu kambing dengan lama fermentasi yang berbeda. J Teknol Pangan. 2019;3(1):142–6.
13. Mulyani S, Sunarko KMF, Setiani BE. Pengaruh lama fermentasi terhadap total asam, total bakteri asam laktat dan warna kefir belimbing manis (averrhoa carambola). J Ilm Sains. 2021;21(2):113.
14. Rachma YA, Darmanti S. Total asam, total padatan terlarut, dan rasio gula-asam buah pisang raja (musa paradisiaca l.) pada kondisi penyimpanan yang berbeda. Bul Anat dan Fisiol. 2023;8(1):36–41.
15. Nurul Aini A, Rina Bintari Y, Sri Damayanti D. Kadar total asam dan potensi penghambatan pertumbuhan escherichia coli dan salmonella typhi oleh isolat bakteri asam laktat dari kombucha daun sirsak (annona muricata linn). J Bio Komplementer Med. 2021;8(2):1–7.

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