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Research Article

Antibacterial Potention and pH Analysis of Kombucha with Anna Apple Peel as Its Substrate

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Abstract

This study aimed to determine the antibacterial abilities of Kombucha with Anna apple peel as the substrate. This study was conducted on the antibacterial assay of Kombucha with the formulation of 10g (formulation A), 15g (formulation B), and 20g (formulation C) apple peels. Each formulation then made three different test concentrations of an antibacterial test by the Kirby Bauer method. There was a decrease in the pH value measured before and after the fermentation of kombucha apple peel due to the production of acetic acid formed from metabolic activity between bacteria and yeast as a culture of kombucha beverage. The results of the antibacterial test showed that all of the test concentrations could inhibit the growth of the test bacteria until they were in the category of medium and high inhibition. The largest diameter of the inhibition zone was shown by formula C with a concentration of 100% (17 mm). The diameter of the inhibition zone is directly proportional to the amount of apple peel contained in the formula and the concentration of the sample being tested.

Keywords: Kombucha , apple Anna peel, antibacterial activity.

1. Introduction

Kombucha is the fermented tea used Kombucha starter culture called Scoby (Suhardini & Zubaidah, 2016). Scoby was a symbiotic interaction between mostly lactic acid bacteria and yeast (Zubaidah et al., 2018). Kombucha has several health effects, including antibacterial (Surahmaida & Lestari, 2019), improving intestinal microflora, increasing body resistance and lowering blood pressure (Wistianah & Zubaidah, 2015). One of the benefits was due to the content of phenolic compounds that have antioxidant activity. Higher phenolic compounds contained in the substrate of Kombucha , higher the antioxidant activity (Khaerah & Akbar, 2019). This increase in total phenol is thought to be influenced by the total phenol possessed by tea, which was a common substrate ingredient to make the Kombucha (Wistianah & Zubaidah, 2015).

The tea that has been widely carried out such as green tea (Lestari & Sadiyah, 2020) and black tea (Lestari et al., 2019; aufizan et al., 2019; sadiyah & Lestari, 2020; Cardoso et al., 2019). Research about substrate for making Kombucha continues to be carried out, including Kombucha with various leaves such as bay leaves (Wistianah & Zubaidah, 2018) (Suhardini & Zubaidah, 2016), *Annona* leaves (Wistianah & Zubaidah, 2018; Suhardini & Zubaidah, 2016; Falahuddin et al., 2017; Yanti et al., 2020), *Piper bettle* leaves (Wistianah & Zubaidah 2015; Suhardini & Zubaidah, 2016), *Guava* leaves (Wistianah & Zubaidah

2015; Suhardini & Zubaidah, 2016), Coffee leaves (Wistianah & Zubaidah 2015; Suhardini & Zubaidah, 2016), Cacao leaves (Nur et al., 2018), and Gaharu leaves (Nurmiati & Wijayanti, 2018). Kombucha is made from various plants such as the Apu-apu plant (Simanjuntak et al., 2018), coffee powder (Nur et al., 2018; Surahmaida & Lestari, 2019; Lestari et al., 2019), Cocoa powder (Nur et al., 2018; Surahmaida & Lestari, 2019; Lestari et al., 2019), Mangosteen peel (Nofiyanto et al., 2015), Coffee peel (Nurhayati et al., 2020) and Apple fruit (Zubaidah et al., 2018). Those research aims to get the highest polyphenol yield (Wistianah & Zubaidah, 2015). Phenolic compounds are known to function as antimicrobials. One of the ingredients that can be used for making Kombucha is apple.

Apples' phytochemical antioxidant compounds include *flavonoids*, *tocopherols*, *phenolic compounds*, *coumarins*, *cinnamic acid derivatives*, and *polyfunctional acids* (Taufiq & Ismail, 2020). The most famous Indonesian apple varieties, especially among West Java people, are Anna apples, Manalagi apples, and Rome beauty apples (Zubaidah et al., 2018). The total phenol of Kombucha from Anna apple, Manalagi, and Red Delicious as the substrate was 250 – 350 mg/ml GAE (Zubaidah et al., 2018). Apple peel has higher phenolic, and flavonoid compounds than apple flesh (Taufiq & Ismail, 2020), and apple peel's antioxidant and antiproliferative properties are much higher than flesh's (Shahidi & Ambigaipalan, 2015). Research on the antibacterial activity of Kombucha has been carried out for a long time (Lestari, 2019), but research on Kombucha with Anna apple peel as the substrate and its antibacterial ability has never been done. Based on this explanation, this study was conducted on the antibacterial test of Kombucha with the formulation of 10g (formulation A), 15g (formulation B), and 20g (formulation C) apple skin, which then made several different samples tested.

2. Materials and Method

2.1 Materials

2.1.1 Preparation of infusion and Kombucha

Apple Anna (*Malus domestica)* was collected from apple farmers in Malang city. The apple then peeled without hitting the flesh. The apples were dried for 2 weeks, ground into powder, and sieved. Apple peel powder was prepared for three formulas, 10g for formula A, 15g for formula B, and 20g for formula C. Kombucha was collected from the Akademi Farmasi Surabaya microbiology laboratory. The culture consists of cellulose culture, known as Scoby, and liquid culture. Each apple peel powder was brewed with 1000 ml of water at 70°C to 80°C for 15 minutes and filtered into a glass jar. Each solution was Added with 50 grams of sugar and stirred well. The solution at 40°C was added with 50 grams of SCOBY and 60 ml of liquid culture. Each solution in glass was covered using two layers of cloth and then incubated for one week at room temperature.

2.1.2 Bacterial culture and suspension

The bacteria tested in this study is *Bacillus cereus* ATCC 1178 was collected from Balai Besar Laboratorium Kesehatan (BBLK) Surabaya city. The bacteria were maintained by cultivation on Nutrient agar medium (Merck, Germany) for 24 hours at 30°C. The bacterial suspension was maintained by cultivation on Nutrient broth medium (Merck, Germany) for 24 hours at 30°C

2.2 Method

2.2.1 pH measurement

the pH of Kombucha was measured using a universal pH indicator. PH was measured on each formula before fermentation (day 0) and after fermentation (day 7).

2.2.2 Antibacterial activity test

The antibacterial activity test was in vitro using the Kirby Bauer method with 4 replications. The bacterial suspension (1 mL) was put in a sterile petri dish, added with sterile Nutrient agar media (20 mL), and then homogenized. Sterile disc paper was immersed in each sample for 15 minutes. The paper discs were placed on media containing bacterial suspension and then incubated in an incubator for 24 hours at 30°C.

3. Results and Discussion

3.1. Results

The results of measuring the pH of Anna apple peel Kombucha with various concentrations before and after the fermentation period can be seen in Table 1.

Table 1. Antibacterial activity test of Anna apple peel Kombucha

Kombucha	Cons. 0%	Cons. 50%	Cons. 75%	Cons. 100%
Formulasi A (10%)	0	4	5,3	7,3
Formulasi B (15%)	0	4	7,3	9
Formulasi C (20%)	0	4,3	8	17

Table 1 shows that each Kombucha formulation in this study was further divided into four different concentration samples. It is intended that researchers get information about the combination of the best formulation and concentration of antibacterial compounds from apple peel Kombucha.

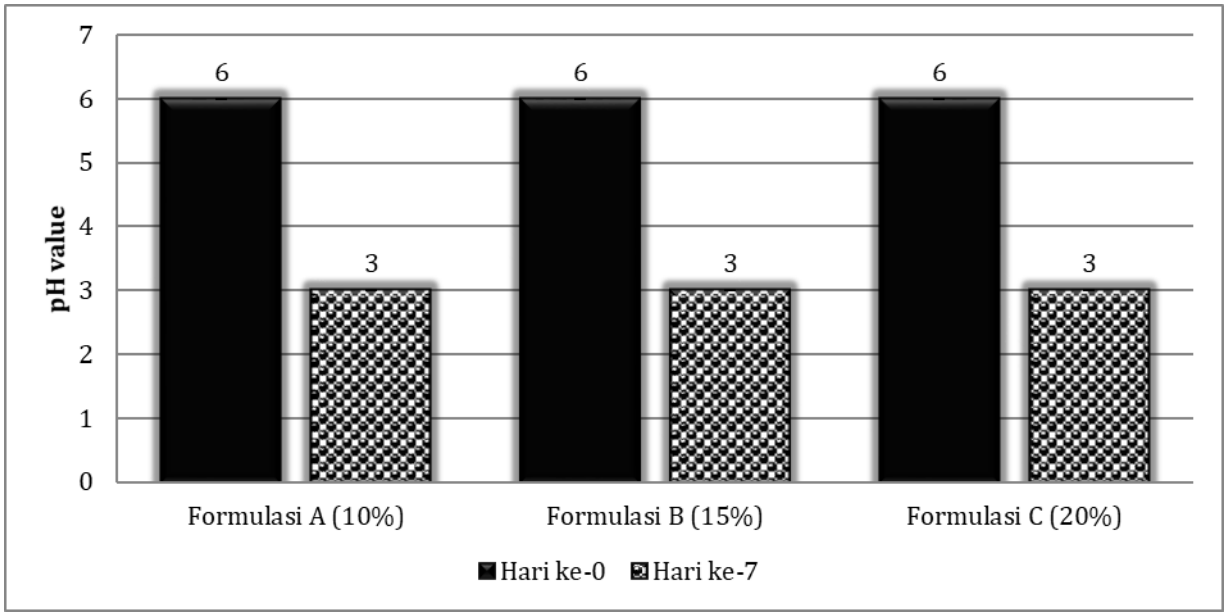

Figure 1. The results of measuring the pH of apple peel Kombucha with various Formula

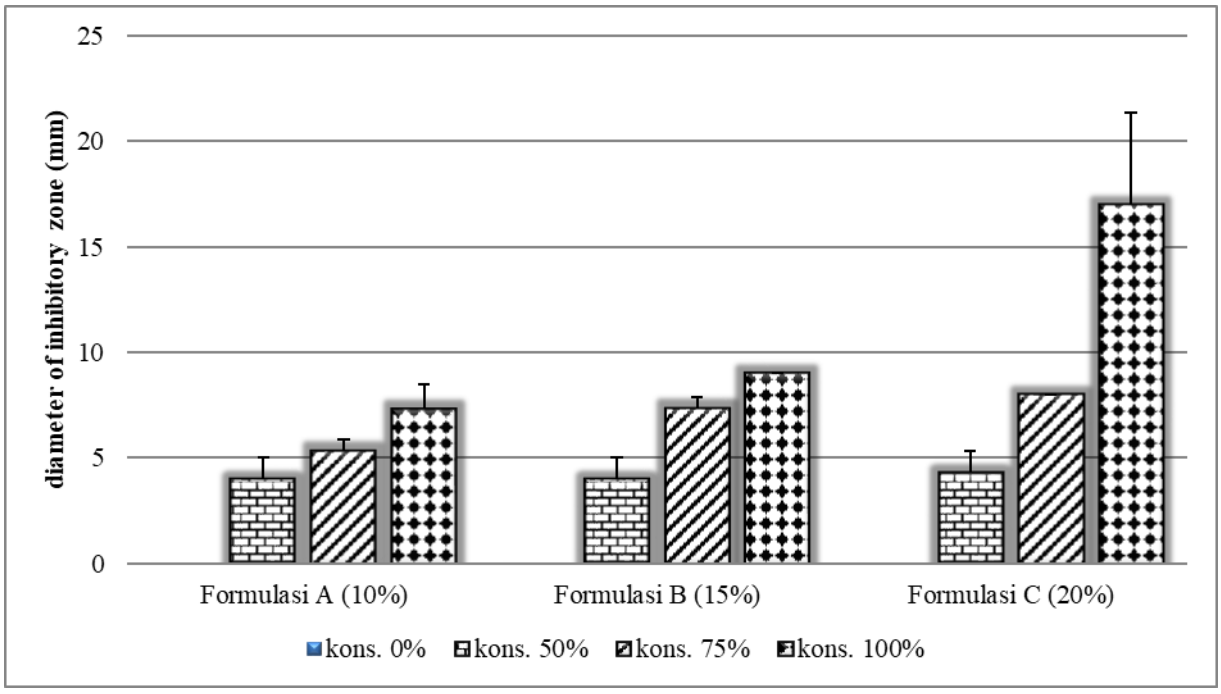

Figure 2. The results of antibacterial activity of apple peel Kombucha with various Formula and concentration

Figure 1 shows the pH measurement data from each formulation before and after fermentation. This data shows that the pH of the three formulations of Kombucha, both before and after has the same pH value. Table 1 and Figure 2 show that all sample concentrations' inhibition zones vary. The inhibition zone of Formula A in concentration

50% is 4 mm, concentration 75% is 5.3 mm, and concentration 100% is 7.3 mm. The inhibition zone of Formula B in concentration 50% is 4 mm, concentration 75% is 7.3 mm, and concentration 100% is 9 mm. The inhibition zone of Formula C in concentration 50% is 4.3 mm, concentration 75% is 8 mm, and concentration 100% is 17 mm.

3.2.Discussion

Initial pH measurements were carried out when the Kombucha was on day 0 using a universal pH indicator. The initial pH of each formulation (formulations A, B and C) was 6. However, after 7 days of fermentation with the Kombucha consortium, all formulations experienced a decrease in pH to 3 (acidic) (Figure 1). The minimum standard pH for food or drink consumption 3, so that Kombucha with formulations A, B and C is still safe to consume. Beverages with a pH less than 3 must be diluted with water before being consumed by humans.

Lestari et al. (2020) in her research stated that the pH of Kombucha Beverages decreased along with the length of fermentation time. The decrease in pH is also in line with the formation of organic acids during the fermentation process in Kombucha. There was no difference in pH in the three formulations made possible because the concentration of Anna apple peel as the substrate did not differ much (10g, 15g and 20g). The decrease in pH was seen in Kombucha drinks during the fermentation period. In addition, the Kombucha drink's color is also lighter compared to the first day of fermentation. This change occurs due to the metabolic activity of microbes in Kombucha, which produce enzymes during the fermentation process to form a complex formation of phenolics (Liu et al., 1996). The decrease in pH during the fermentation process is also influenced by the formation of acids, especially acetic acid, in Kombucha beverages (Cardoso et al., 2019). This acetic acid results from sugar metabolism by acetic acid bacteria in the Kombucha culture.

The fermentation period was carried out for seven days. Due to the fermentation process, bacteria and yeast produce alcohol and acid by breaking down sugar. Yeast cells will hydrolyze sucrose into glucose and fructose as the essential ingredients for ethanol production, while bacteria will convert glucose into gluconic acid and fructose to form acetic acid. *Acetobacter* sp in Kombucha culture oxidizes ethanol to acetaldehyde and becomes acetic acid. The presence and acid levels are influenced by the various substrates used in Kombucha (Velicanski et al., 2013). Adding Kombucha substrate and its metabolites compound will form glucoronic acid, lactic acid, vitamins, amino acids, and antimicrobial agents. (Puspita et al., 2017).

Previous studies have shown that apple Kombucha has 290 g/ml GAE of total phenol (Zubaidah et al., 2018). The total phenol affects the potency of Kombucha beverages as an antibacterial agent. Antibacterial activity was performed on the seventh day of the fermentation period. The results show that apple peel Kombucha inhibits the growth of bacteria tested. The results obtained inhibition zone of test formulations in the

medium to high barriers category, according to Rahim et al. (2008) and Trisunawati (2017). The minimum yield was shown by Formulations A and B at a concentration of 50%, while the maximum yield was shown by Formulation C at 100%. The 0% concentration of this study was distilled water which was used as a negative control compared to the results of the tests.

Kombucha is an antibacterial agent (Cardoso et al., 2019). Figure 2 shows the concentration of the test sample is directly proportional to the inhibition zone. During fermentation, the content of the active ingredient that acts as an antimicrobial agent is increasing. Literature shows the antibacterial ability of Kombucha beverages can be attributed to their low pH, indicating an increase in acetic acid in its beverage. Acetic acid in Kombucha is one of the antibacterial agents formed in Kombucha beverages during fermentation. The lipophilic state of acetic acid makes it easier for this compound to enter bacterial cells through its membrane (Zubaidah et al., 2018). The entry of acetic acid into bacterial cells interferes with the work of the cell membrane, thereby disrupting cell permeability, causing protein denaturation in cells and reducing the work of bacterial cells (Naidu & Clemens, 2000).

Antibacterial activities in Kombucha beverages are not only due to the presence of acetic acid or other organic acids. Literature shows that the antibacterial potential of Kombucha is also influenced by other biologically active compounds with antibacterial activity produced during the fermentation process, such as bacteriocins, proteins and enzymes (Battikh et al., 2020; Bhattacharya et al., 2016). meanwhile, various substrate as an ingredient of Kombucha beverage also affects its antibacterial potential. This study used apple peels known to have high polyphenol compounds (Zubaidah et al., 2018). Previous research uses Anna apple (*Malus domestica*) as a Kombucha substrate, but research about Anna apple peel (*Malus domestica*) as the substrate has never been done. Apple skin contains more polyphenols than the apple itself (Budiyati & Utami, 2013). The polyphenolic compounds in anna apple peels include flavonoids, terpenoids, alkaloids, saponins, tannins, and phenolic compounds. These secondary metabolites are reported to have many biological and therapeutic potentials, including antimicrobial activities (Anand et al., 2019). Flavonoids can cause damage to the permeability of bacterial cell walls. Alkaloids have antibacterial activity by disrupting the peptidoglycan components in bacterial cells, so the cell wall layer is not formed intact and causes cell death (Lestari et al., 2020).

4. Conclusion

Overall, there was a decrease in the pH value measured before and after the fermentation of Kombucha apple peel. There is no difference in the value of each Kombucha formula before and after fermentation. The decrease in Kombucha pH is due to the production of acetic acid formed from metabolic activity between bacteria and yeast as a culture of Kombucha beverage. The acetic acid in Kombucha beverages

increases during the fermentation process. This increase has an impact on the antibacterial ability of Kombucha apple peel. The results of the antibacterial test showed that all of the test concentrations could inhibit the growth of the test bacteria until they were in the category of medium and high inhibition. The diameter of the inhibition zone is directly proportional to the amount of apple peel contained in the formula and the concentration of the sample being tested.

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