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

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Abstract

The purpose of this study was 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 antibacterial test is then performed by Kirby Bauer method. there was a decrease in the pH value measured before and after fermentation of kombucha apple peel due to the production of acetic acid which is 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 were able to 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 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 was 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. More higher phenolic compounds contained in the substrate of kombucha, more 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 common substrate ingredient to made 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 were 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 was made from various plants such as

seperti 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). The purpose of those researchs were 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..

Some of the phytochemical as antioxidants compounds present in apples such as flavonoids, tocopherols, phenolic compounds, coumarins, cinnamic acid derivatives, and polyfunctional acids (Taufiq & Ismail, 2020). The most famous apple varieties in Indonesian especially West Java people are Anna apples, Manalagi apples, and Rome beauty apples (Zubaidah et al, 2018). Total phenol of kombucha from apel Anna, Manalagi dan Red Delicious as the substrate were 250 – 350 mg/ml GAE (Zubaidah et al., 2018). Apple peels was has higher phenolic and flavonoid compounds than apple flesh (Taufiq & Ismail, 2020) and antioxidant and antiproliferative properties of apple peel are much higher than it flesh (Shahidi & Ambigaipalan, 2015). The research of 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 test.

2. Material and Method

2.1. Preparation of infusion and kombucha

Apple Anna (*Malus domestica)* were collected from apple farmer in Malang city. The apple then peeled without hitting the flesh. The apples then dried for 2 weeks, ground into powder and sieved. Apple peel powder were prepared for three formulas, 10g for formula A, 15g formula B and 20g for formula C. Kombucha was collected from microbiology laboratory of Akademi Farmasi Surabaya. The culture used consists of cellulose like culture or 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 solutions was Added with 50 grams of sugar and stir well. The solution which had 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 then incubated for 1 week at room temperature.

2.2. 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 were maintained by cultivation on Nutrient broth medium (Merck, Germany) for 24 hours at 30°C

2.3. pH measurement

pH of th Kombucha were measurement uses a universal pH indicator. Measurement of pH was carried out on each formulas before fermentation (day 0) and after fermentation (day 7).

2.4. Antibacterial activity test was in vitro using Kirby bauer method with 4 replications. The bacterial suspension (1 mL) was put in a sterile petri dish and added with sterile Nutrient agar media (20 mL) then homogenized. Sterile disc paper was immersed in each samples for 15 minutes. The paper discs were placed on media which already contained 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 fermentation period, can be seen in **Table 1:**

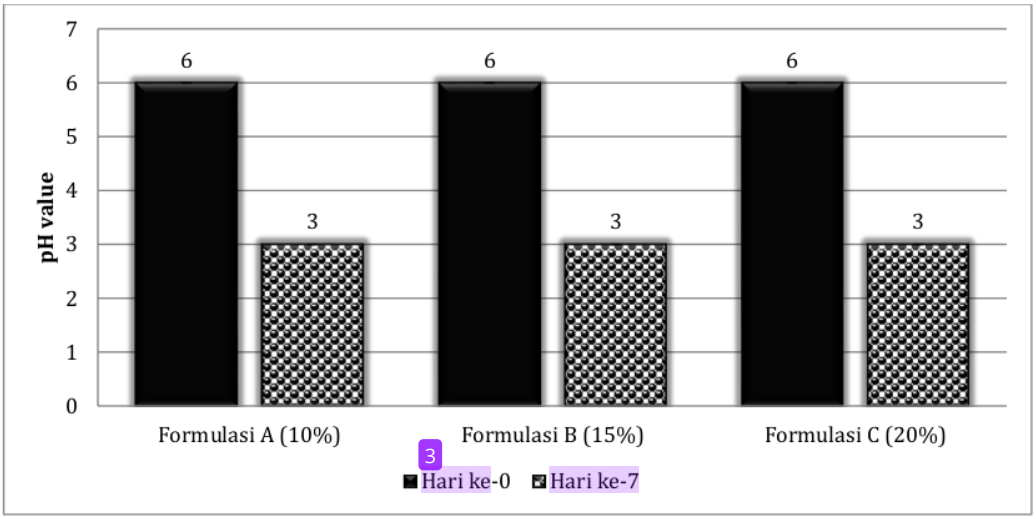

Figure 1. The results of measuring the pH of apple peel kombucha with various Formula

Figure 1 shown the data of pH measurement from each formulation before and after fermentation. This data shown that the pH of the three formulation of kombucha both before and after has a same pH value.

The result of antibacterial activity test of Anna apple peel kombucha, can be seen in **Table 1**:

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 4 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. **Table 1** and **Figure 2** shown that the inhibition zone of all samples concentrations are varies. 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.

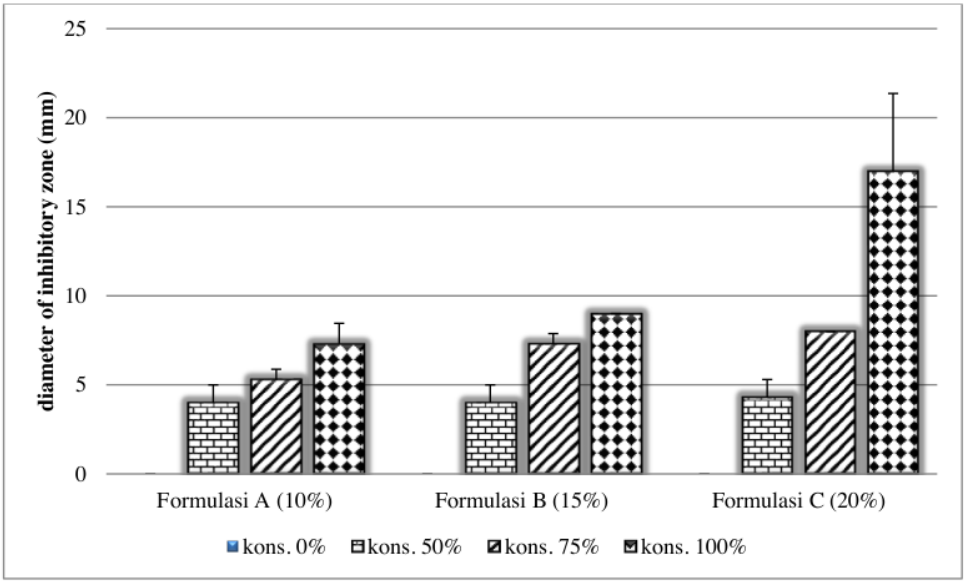

Figure 2. The results of antibacterial activity of apple peel kombucha with various Formula and concentration

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) (**Fig. 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. Beverage 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 Beverage 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 color of the kombucha drink also lighter when 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 that occurs during the fermentation process is also influenced by the formation of acids, especially acetic acid in kombucha beverage (Cardoso et al., 2019). This acetic acid is the result of sugar metabolism by acetic acid bacteria in kombucha culture.

The fermentation period was carried out for seven days. Due to the fermentation process, bacteria and yeast will produce alcohol and acid by breaking down sugar. Yeast cells will hydrolyze sucrose into glucose and fructose as the basic 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 become acetic acid. The presence and levels of acid are influenced by the variety of substrates used in kombucha (Velicanski et al., 2013). The addition of 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 beverage as an antibacterial agent. Antibacterial activity performed on the seventh day of fermentation period. the results shows that apple peel Kombucha inhibits growth of bacterial tested. The results obtained inhibition zone of test formulations in the category of medium to high barriers, according to Rahim et al. (2008) and Trisunawati (2017). The minimum yield was shown by Formulation A and B at a concentration of 50%, while the maximum yield was shown by Formulation C at a concentration of 100%. The 0% concentration of this study was distilled water which was used as a negative control as a comparison from the results of the tests carried out.

Kombucha is known as 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

beverage can be an attribute to its low pH which indicates an increase in acetic acid in its beverage. Acetic acid in kombucha is known to be one of the antibacterial agents formed in kombucha beverages during the fermentation period. 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 beverage is 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 are produce during the fermentation process such as bacteriocins, proteins and enzymes (Battikh et al., 2020; Bhattacharya et al., 2016). meanwhile, various of substrat as the ingredient of kombucha beverage also also affects its antibacterial potention. This study used apple peels which are known to have high polyphenol compounds (Zubaidah et al., 2018). Previous reseach use Anna apple (*Malus domestica*) as 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 group of polyphenolic compounds contained in anna apple peels include flavonoids, terpenoids, alkaloids, saponins, tannins, and phenolic compounds. These secondary metabolites are reported to have many biological and therapeutic potential 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 components of peptidoglycan in bacterial cells, so that the cell wall layer is not formed intact and causes cell death (Lestari et al., 2020).

Conclusion

Overall, there was a decrease in the pH value measured before and after fermentation of kombucha apple peel. There is no difference in the value of each kombucha formula made both before fermentation state and after fermentation state. The decrease in kombucha pH is due to the production of acetic acid which is formed from metabolic activity between bacteria and yeast as a culture of kombucha beverage. The acetic acid in kombucha beverage 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 were able to inhibit the growth of the test bacteria until they were in the category of medium and high inhibition. Where 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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