Antibacterial Activity of Pineapple Infused Arak Bali Against *Escherichia coli*

Nur Habibah, Gusti Ayu Made Ratih*

Medical Laboratory Technology of Poltekkes Kemenkes Denpasar, Indonesia
Corresponding author: iga_ratihkurada@yahoo.com

Abstract. Arak Bali is one of the gastronomic drinks that has the potential to be developed to attract tourists. The use of pineapple as an infusion ingredient in Arak Bali has been proven to be able to increase the type and concentration of bioactive compounds in Pineapple Infused Arak Bali. The bioactive compounds play an important role in the bioactivity of Pineapple Infused Arak Bali, one of which is an antibacterial activity. This research is a true experimental type with a Post-test Only Control Group Design. The purpose of this study was to determine the antibacterial activity of Pineapple Infused Arak Bali (FU and FD formulas) against *Escherichia coli*. The antibacterial activity was determined by dilution and diffusion method to find out the Minimum Inhibitory Concentration (MIC) and the antibacterial activity. The results showed that Pineapple Infused Arak Bali had an antibacterial activity with MIC at a concentration of 60%, whereas in Arak Bali no MIC was found. The results of this study prove that Pineapple Infused Arak Bali has better antibacterial activity against *Escherichia coli* than the original formula, Arak Bali.

Keywords: Antibacterial Activity, Pineapple Infused Arak Bali, *Escherichia coli*

1 BACKGROUND

Bali is a famous international tourist destination because of its beauty of nature, diversity of arts and culture, and also religious traditions (Pratiwi, D.P.E.; Ayomi, P.N.; Candra, 2017; Jaya et al., 2019; Rosilawati and Ariyati, 2021). The competition between tourist destinations was increased due to the development of the tourism industry. Several commodities continue to be developed in order to attract tourists, one of which is introducing the local culture in the form of activities and products (Sucitawathi P, I.G.A.AG D; Dewi, N.L.Y.; Joniarta, 2019). One of the local cultures that have the potential to be developed as a gastronomic drink is Arak Bali (Ratih and Habibah, 2022). Arak Bali is a traditional drink made from glutinous rice and coconut water

which is simply processed, through fermentation and distillation processes. Arak Bali is included in Category C with an alcohol content of more than 25%. This type of drink has the characteristics of a clear liquid, colorless with a strong alcoholic taste (Sukadana, 2009; Sukadana and Tenaya, 2016).

One of the potential local fruits for the formation and diversification of Arak Bali is pineapple. The use of pineapple as the additive ingredient to Arak Bali was done to enrich the taste and increase its benefits. The pineapple is a bush plant with the scientific name Ananas comosus (L.) Merr. This plant is easy to grow in areas with tropical climates. Various studies have proven that pineapple contains secondary metabolites such as alkaloids, flavonoids, steroids, terpenoids, and tannins (Gunwantrao et al., 2016; Nurdalilah et al., 2018; Yusliana et al., 2019; Hikal et al., 2021; Saleh et al., 2021; Sayago-Ayerdi et al., 2021; Susanti et al., 2021; Owoeye et al., 2022; Rivera et al., 2022). Various parts of the pineapple plant, such as fruit and tubers, and peels are proven to have antibacterial activity against Escherichia coli, Salmonella typhi, Klebsiella pneumonia K2044, Pseudomonas aeruginosa MTCC4676, Bacillus subtillis Py79 and Xanthomonas axonopodis pv. malvacearum LMG859 (Gunwantrao et al., 2016; Yusliana et al., 2019; Juariah and Wati, 2021).

Pineapple Infused Arak Bali is made by soaking 400 grams of sliced pineapple in Arak Bali for 7 days at room temperature. This immersion process imitates the extraction process with the maceration method. The pineapple-infused Arak Bali has an ethanol content of 18.08% and is included in group B in accordance with government regulations. Pineapple fruit as an infusion ingredient of Arak Bali is reported to be able to increase the taste and consumer preference for the resulting product. Organoleptic tests on Pineapple Infused Arak Bali showed higher scores on color, taste, and smell parameters compared to the original product. The optimum formulation is organoleptically acceptable to consumers (Ratih and Habibah, 2022).

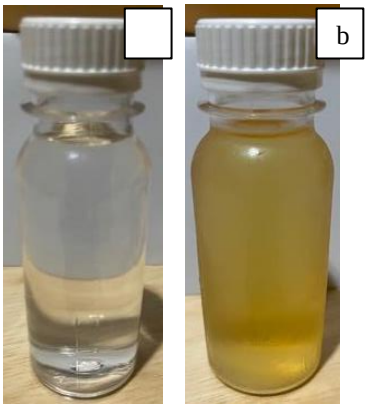

Fig. 1. a. Basic formula (FD) of sample Arak Bali, b. Test formula (FU) of sample Pineapple Infused Arak Bali

In addition, the pineapple infusion process in the Pineapple Infused Arak Bali product can increase the type and amounts of bioactive compounds compared to the

initial formulation, Arak Bali. The results of a previous study proved that pineapple-infused Arak Bali contains phytochemical compounds, such as alkaloids, tannins, flavonoids, phenols, and terpenoids. The quantitative analysis results showed that the pineapple-infused Arak Bali had tannin, total phenol, and flavonoid level of 52.9545, 42.005, and 6.8995 mg/100g (Habibah and Ratih, 2023). The various contents of these secondary metabolites are important factors for the antibacterial activity of pineapple-infused Arak Bali.

Diarrhea is one of the most common diseases that occur among tourists (Gandamayu, Agustini and Kusuma, 2016). Diarrhea can be caused by consuming food or drink contaminated with bacteria, viruses, or parasites. One of the causes of diarrheal disease is the presence of Escherichia coli bacteria in water or food. Escherichia coli is one of the microbiological indicators of water or food contamination that causes diarrheal disease. Based on this description, the authors are interested to examine the antibacterial activity of the gastronomic drink Pineapple Infused Arak Bali on the growth of Escherichia coli as the main bacteria that cause diarrhea. This research was conducted to explore the potential of Pineapple Infused Arak Bali as a gastronomic drink that has health benefits, especially its antibacterial ability against Escherichia coli.

2 RESEARCH METHODS

The type of research used is true experimental with Post-test Only Control Group Design which aims to measure the effect of treatment (intervention) on the experimental group by comparing the group with the control group. The samples used were Arak Bali obtained from Merita Village-Karangasem and fresh local pineapple. The basic formula (FD) is the initial formula for Arak Bali which is obtained without the addition of pineapple fruit, while the test formula (FU) is Pineapple Infused Arak Bali which is made by soaking 400 grams of pineapple fruit in 500 mL of Arak Bali for 7 days at room temperature in a closed container and protected from the direct sunlight. The data obtained in this study are the antibacterial activity which is expressed by the Minimum Inhibitory Concentration (MIC, %) and the diameter of the inhibition zone on the growth of Escherichia coli.

Antibacterial tests on FU and FD samples in this study were carried out using dilution and diffusion methods. The dilution method was used to obtain qualitative data, which showed the antibacterial ability of Pineapple Infused Arak Bali against Escherichia coli bacteria expressed in Minimum Inhibitory Concentration (MIC) (Soelama, Kepel and Siagian, 2015; Rahmawati, Sudjarwo and Widodo, 2014). While the disk diffusion and well diffusion methods were used to obtain quantitative data in the form of the diameter of the inhibition zone for bacterial growth on agar media. In the disk diffusion method, the bacterial suspension was streaked with a sterile cotton swab over the test medium. Paper disks with a diameter of 6 mm were soaked in a positive control of 10µg Ampicillin, negative control of distilled water, and Pineapple Infused Arak Bali sample for 15 minutes. Then the disk paper is placed on the surface of the media according to the desired position. Whereas in the antibacterial activity test using the well-diffusion method, 20µL of the positive control of 10 µg Ampicillin, distilled water as the negative control, and the Pineapple Infused Arak Bali sample were put into the wells

that had been made aseptically on agar media. Furthermore, the media was incubated at 37°C for 24 hours, then the diameter of the inhibition zone was measured with a vernier caliper and expressed in millimeters of the inhibition zone (Arirahmayanti, Artini and Ernawati, 2019; Nurhayati, Yahdiyani and Hidayatulloh, 2020; Puspita Sari, Susanah Rita and Puspawati, 2015). The obtained data is recorded, processed, and presented in the form of figures and tables, then narrated and compared with related literature.

This research has received Ethical Approval with the number of No.: LB.02.03/EA/KEPK/0410/2022 by the Health Research Ethics Commission of the Health Research Polytechnic of the Poltekkes Kemenkes Denpasar.

3 RESULTS AND DISCUSSION

3.1 Results

Antibacterial activity test on FU and FD samples was carried out by dilution and diffusion methods. The dilution method was used to obtain qualitative data, which showed the antibacterial ability of Pineapple Infused Arak Bali against Escherichia coli bacteria expressed in Minimum Inhibitory Concentration (MIC). The results of the antibacterial activity test with the dilution method are presented in Table 1.

Based on the data in Table 1, it is known that MIC of Pineapple Infused Arak Bali against Escherichia coli bacteria was obtained at a concentration of 60%. There was a decrease in turbidity in the test tube which indicated the inhibition of bacterial growth at these concentrations, as shown in Figure 2.

Furthermore, the antibacterial test was carried out using disk diffusion and well diffusion methods to obtain quantitative data in the form of the diameter of the inhibition zone for bacterial growth on agar media. The results of the antibacterial test of FU and FD samples against Escherichia coli bacteria are presented in Table 2.

Table 1. Antibacterial Activity Test Results of Pineapple Infused Arak Bali with the Dilution Method

No.	Conc. (%)	Results
1	10	+
	20	+
	30	+
	40	+
	50	+
	60	-
	70	-
	80	-
	90	-
	100	-

No.	Conc. (%)	Results
2	Negative control (FD)	-
3	Positive control	+

Notes:

The (+) sign of the liquid in the tube looks turbid, showed that the bacteria are still growing

The sign (-) of the liquid in the tube begins to decrease in turbidity, showed that the growth of bacteria is inhibited

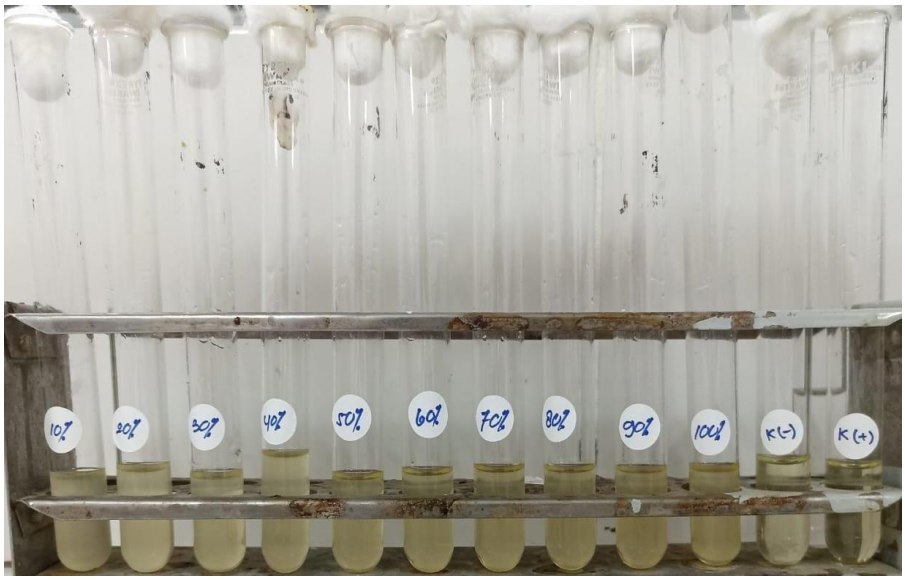

Fig. 2. Antibacterial Activity Test Results of Pineapple Infused Arak Bali with the Dilution Method

Table 2. Antibacterial Activity Test Results of Pineapple Infused Arak Bali with the Diffusion Method

No.	Sample	Zone of inhibition (mm)	
		Disk Diffusion Method	Well Diffusion Method
1	FD	0	0
2	FU	0	0
3	Negative control	0	0
4	Positive control (amphicillin)	18.415±0.542	18.026±0.9595

The results of the antibacterial test with the diffusion method presented in Table 2 showed that no inhibition zones for bacterial growth were formed in the FU, FD, and negative control. This was indicated by the absence of clear zones around the paper disks and wells as shown in Figure 3. Whereas, in the positive control, inhibition of bacterial growth occurred as indicated by the formation of inhibition zone diameters of 18.415 mm ± 0.542 and 18.026 mm ± 0.9595.

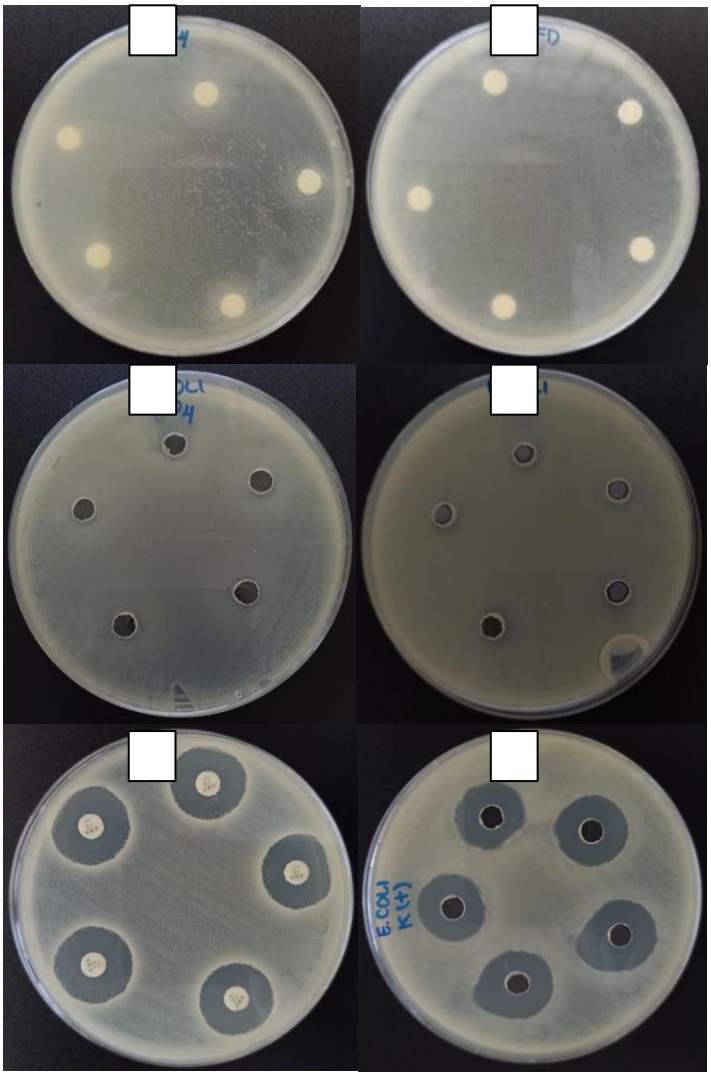

Fig. 3. a. Disk-diffusion test results of Pineapple Infused Arak Bali (FU), b. Disk-diffusion test results of Arak Bali (FD), c. Well-diffusion test results of Pineapple Infused Arak Bali (FU), d. Well-diffusion test results of Pineapple Infused Arak Bali (FU), e. Disk-diffusion test results of positive control, f. Well-diffusion test results of positive control

4 Discussion

The antibacterial activity test of FU and FD samples in this study was carried out by dilution and diffusion methods. The dilution method was used to obtain qualitative data, which showed the antibacterial ability of pineapple-infused Arak Bali against Escherichia coli bacteria expressed in Minimum Inhibitory Concentration (MIC). While the disk diffusion and well diffusion methods were used to obtain quantitative data in the form of the diameter of the inhibition zone for bacterial growth on agar media.

In this study, the antibacterial activity test by dilution method was carried out by serial dilution with a ratio of 1:2 (w/v). The dilution process was followed by the incubation and visual observation of turbidity (Rahmawati, Sudjarwo and Widodo, 2014; Soelama, Kepel and Siagian, 2015). The results of turbidity observations showed that MIC of Pineapple Infused Arak Bali against Escherichia coli bacteria was obtained at a concentration of 60% as presented in Table 1. In the test tube, there was a decrease in turbidity when compared to the K(+) tube containing Escherichia coli bacteria suspension equivalent to McFarland 1. This shows that the growth of Escherichia coli bacteria in the 60% Pineapple Infused Arak Bali tube began to be inhibited. Based on the data obtained, it is known that the greater the concentration of Pineapple Infused Arak Bali, the more turbidity also decreases. This shows that the growth of Escherichia coli bacteria is also increasingly inhibited.

The antibacterial ability of Pineapple Infused Arak Bali against Escherichia coli bacteria is closely related to the secondary metabolites contained in the sample. The results of the phytochemical screening proved that Pineapple Infused Arak Bali contains various secondary metabolites, including alkaloids, tannins, flavonoids, phenols, and terpenoids. Flavonoids act as antibacterials through their mechanism of forming complex compounds with extracellular and dissolved proteins so that they can damage the bacterial cell membrane and be followed by the release of intracellular compounds. The mechanism of action of tannins as an antibacterial is related to their ability to inhibit reverse transcriptase enzymes and DNA topoisomerase so that bacterial cells cannot form and the ability of tannins to shrink the cell wall or cell membrane thereby disrupting the permeability of the bacterial cell wall (Nuria, Maulita Cut; Faizatun, 2009; Alamsyah, Widowati and Sabdono, 2014; Julianto, Eko; Susilowati, Eka; Johan, 2019).

The antibacterial ability of alkaloid compounds is due to the fact that alkaloids are able to interfere with the constituent components of peptidoglycan in bacterial cells so that the bacterial cell layer is not completely formed and causes cell death in these bacteria (Alamsyah, Widowati and Sabdono, 2014). Phenol compounds and their derivatives can denature bacterial cell proteins so that they can cause cell death (Prayoga, 2013). The mechanism of terpenoids as antibacterial occurs through the reaction between terpenoid compounds and porins (transmembrane proteins) on the outer membrane of the bacterial cell wall, forming strong polymeric bonds that result in damage to the porins. Damage to the porin which is the entry and exit point for compounds will reduce the permeability of the bacterial cell wall which will result in a lack of nutrients in the bacterial cell so that the growth of the bacteria is inhibited or dies (Rahmawati, Sudjarwo and Widodo, 2014).

Based on the results of the dilution test obtained in this study, it is known that the higher the concentration of Pineapple Infused Arak Bali, the greater the ability of the sample to inhibit the growth of Escherichia coli bacteria. This is because, at a higher concentration of the test sample, the content of secondary metabolites is also greater, so the antibacterial ability increases. This can be seen in Figure 3, wherein in the test tube with a concentration of 60% (MIC) the turbidity that was observed visually decreased when compared to the K(+) tube.

In addition to the dilution method to determine the MIC of the sample, the antibacterial activity of Pineapple Infused Arak Bali against Escherichia coli bacteria was also carried out using the diffusion method, both disk diffusion and well diffusion. Both diffusion methods are used in order to obtain more accurate data. The working principle of the two methods is the same, by observing the formation of a clear zone around the disk or well which indicates a zone of inhibition of bacterial growth. The antibacterial test using the disk diffusion method has advantages because it is relatively more practical and easy to perform, whereas, in the well diffusion method, the observation of clear zones around the wells is easier to do and usually gives greater results (Zada, 2021).

In the disk diffusion method, paper disks that had been saturated with Pineapple Infused Arak Bali (FU) samples, ampicillin as the positive control, and FD as the negative control were aseptically placed on the surface of the agar media. Then, during the incubation process of the active compound from the test sample (FU), positive control and FD will diffuse from the paper disk to the media for bacterial growth. From the research results presented in Table 2, it is known that the test sample (FU) and negative control did not form a clear zone around the disk. Whereas in the positive control, a clear zone was formed which indicated the inhibition of the growth of Escherichia coli bacteria with an average diameter of 18.415 mm ± 0.542. Similar results were also obtained in the antibacterial activity test of Pineapple Infused Arak Bali using the well diffusion method.

In the well-diffusion method, samples of Pineapple Infused Arak Bali (FU), ampicillin as the positive control, and FD as the negative control were put into the wells made on the growth medium for Escherichia coli bacteria. During the incubation process, there will be an osmolarity process from a higher compound concentration resulting in a process of inhibiting bacterial growth. Based on the research data presented in Table 8, it is known that the test sample (FU) and the negative control did not form a clear zone around the wells. Whereas in the positive control, a clear zone was formed which indicated the inhibition of the growth of Escherichia coli bacteria with an average diameter of 18.026 mm ± 0.9595.

The absence of an inhibition zone for the growth of bacteria in the FU test sample using both the disk diffusion and well diffusion methods could be caused by the Gram type of the test bacteria used. Escherichia coli bacteria are gram-negative bacteria that are relatively more difficult to inhibit their growth when compared to gram-positive bacteria. This is because the composition of the cell wall of gram-negative bacteria is more complex. Most gram-negative bacteria have lipopolysaccharide complexes in their cell walls. These substances are endotoxins, lipopolysaccharide endotoxins array in the cell wall i.e., specific O-polysaccharides which are somatic antigens of smooth colonies which induce specific immunity, general polysaccharide cores (coarse colony antigens) which induce some nonspecific resistance against gram-negative sepsis,

lipids A with acid-2–keto–3–deoxy–octanoic which is responsible for the primary poisoning (Jawetz, et al., 1986). This lipopolysaccharide layer strengthens the rigidity of the cell wall of gram-negative bacteria through intermolecular cationic cross-linking (Nurhayati, Yahdiyani, and Hidayatulloh, 2020). Theoretically, gram-positive bacteria have more permeable peptidoglycan, the better the interaction between antibacterials and bacteria, so that Gram-positive bacteria are more susceptible to antibacterials. Whereas Gram-negative bacteria have a peptidoglycan layer that is less permeable so that the effect of an antibacterial becomes more minimal (Adu et al., 2011). This causes Escherichia coli bacteria to become stronger and more difficult to penetrate by antibacterial compounds from the Pineapple Infused Arak Bali sample. The results of this study are in line with several previous studies that showed that the ethanol extract of Jatropha leaves was not able to inhibit the growth of Escherichia coli bacteria and the antibacterial ability of yogurt starter against Escherichia coli bacteria was relatively lower when compared to its antibacterial ability against S. aureus bacteria (Nuria and Faizatun, 2009; Nurhayati et al., 2020).

5 CONCLUSION AND RECOMMENDATION

Pineapple Infused Arak Bali has antibacterial activity against Escherichia coli with MIC at a concentration of 60%. Pineapple Infused Arak Bali has better antibacterial activity against Escherichia coli than the original formula, Arak Bali.

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