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The Inhibiting Process of the Enterococcus Faecalis Growth using Coffea Arabica Extract

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Abstract. Arabica coffee seeds contain caffeine, chlorogenic acid, flavonoids, and trigonelline. This study aims to test the antibacterial of Arabica coffee seed extract against the bacterium *Enterococcus faecalis* at concentrations of 1,5625%, 3,125%, 6,25%, 12,5%, 25%, 50%, and 100%. The extraction method uses maceration with solvent 96%. The Kirby and Bauer diffusion test used the antibacterial activity test method. The results showed that mango seed extract could provide inhibition starting from 3,125% with an average diameter of inhibition zone of 1,16 mm to the largest concentration of 100% with an average zone diameter of inhibition of 14,6 mm. At the same time, the average diameter of the inhibitory zone of antibiotic ampicillin at a concentration of 1% as a control (+) is 24,6 mm. The results showed that the greater concentration, the greater the inhibitory zones are formed.

Keywords. Coffea arabica, Enterococcus faecalis, antibacterial, inhibition zone

Introduction

Coffee is a very famous drink in the world and has been consumed since the 9th century AD Coffee is the fruit seed of the genus Coffea tree. There are two well-known coffee types in the world: Arabica coffee (Coffea arabica) and Robusta coffee (Coffea robusta). One of the habits of Indonesian people is consuming coffee. Coffee as a soft drink has various health benefits, which have been proven from studies that have been conducted, including on dental and oral health. [1] Research conducted by Ferrazano et al. (2009) stated that coffee contains derivatives of hydroxy-dynamic acids, including caffeine, chlorogenic, coumarin, ferulin, sinapic acid, flavonoids, and polyphenols. [2] Caffeine in coffee is known to function as an antibacterial that can inhibit bacterial cell walls and DNA synthesis. [3]

Indonesia is a country rich in nutritious plants, including coffee. Indonesia is the third largest coffee producer in the world, after Brazil and Vietnam. Coffee, in general, has several benefits, such as stimulating the respiratory process, helping the assimilation and digestion of food, calming mental feelings when the body is tired, as a medicine for diarrhea, and preventing vomiting after surgery.

Research that is efficacious as a new antibacterial that has the potential to inhibit or kill bacteria is urgently needed and widely developed. One alternative that can be done is to utilize the active substance that kills bacteria contained in medicinal plants, namely coffee. [1]

Enterococcus faecalis has a major role in the etiology of root canal infections. It is generally found in many root canals as a single organism that can survive after treatment. These bacteria rely on their ability to survive as pathogens by displaying antibiotic-resistance genes or spontaneous mutations. The prevalence of endodontic infections caused by Enterococcus faecalis ranges from 24-77%. The present invention can be explained by variations in the resistance and virulence of the bacterium Enterococcus faecalis itself, including its ability to compete with other microorganisms for entry into the dentinal tubules and to survive under conditions of poor nutrition. Enterococcus faecalis bacteria left in the root canal can significantly reduce the success rate after root canal treatment. [4]

The use of root canal medication is highly recommended to prevent multiplication while killing the bacteria in it. The drug should have a broad spectrum of effects. Potassium hydroxide is a compound used in root canal medicines to kill germs. The alkaline atmosphere caused by calcium hydroxide causes root canal bacteria to be unable to survive in this environment, but this does not apply to Enterococcus faecalis bacteria. [5]

In recent years, enterococci have increased the rate of development of resistance to several antimicrobial drugs. Enterococcus expressed resistance to tetracyclines, erythromycin, trimethoprim, and high levels of clindamycin. Vancomycin-Resistant Enterococci (VRE) is the most serious challenge among microbial resistance and as a source of clinical infection in humans in the last decades. [6]

Antibiotic resistance is a current health problem that increases in cases of life-threatening infections. It is due to inappropriate doses, not following the disease and the period of use that is not right. Enterococcus faecalis bacteria are known to experience resistance to β-lactam class antibiotics, namely ampicillin, amoxicillin, and penicillin. [8]

Based on the description above, researchers are interested in researching the antibacterial effect of Arabica coffee bean extract in inhibiting the growth of Enterococcus faecalis bacteria. So that this research can be used as an application in the medical field, based on the background of the existing problems, a problem arises, including: a) Can arabica coffee bean extract (Coffea arabica) inhibit the growth of Enterococcus faecalis? and b) What is the minimum inhibitory concentration of arabica coffee bean extract (Coffea arabica) on the growth of Enterococcus faecalis? The research aimed to see the effect of arabica coffee bean extract (Coffea arabica) as an antibacterial, especially on the growth of Enterococcus faecalis bacteria.

Literature Review

Arabica coffee was first discovered in the highlands of Ethiopia and popularized by the Arabs. Coffee beans from Ethiopia were brought by Arab traders to Yemen and traded. Entering the 17th century, Europeans began to develop their coffee plantations, but the climate was not suitable in the 19th century. The Dutch brought coffee to the island of Java and cultivated it. [8]

Arabica coffee has requirements in optimum climatic and soil conditions for its growth. Arabica coffee is very suitable for planting in the highlands with an altitude of 700 – 1400 meters above sea level, relatively low air temperature of 15 - 24oC, average rainfall of 2000-4000 mm/year, effective soil depth > 100 cm, and soil pH 5.3 – 6.0. [8] Arabica coffee is widespread and tastes differently depending on the soil and climatic conditions where the coffee plant is grown. [1] In Indonesia, the name Arabica coffee is based on the place of cultivation.

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The following are types of Arabica coffee that are well known and where they are grown: a) Garut Arabica Coffee (West Java), b) Arjuno Arabica Coffee (East Java), c) Mandailing Arabica Coffee (North Sumatra); d) Aceh Gayo Arabica Coffee (Aceh); d) Arabica Toraja Coffee Kalosi (Toraja); e) Kintamani Arabica Coffee (Bali); and f) Papua Wamena Arabica Coffee (Papua) [9]

Arabica coffee plant (Coffea arabica) is a shrub with two parts (dicot), so it has a taproot. This coffee plant has a total length of 5 m to 6 m and a diameter of 7 cm at the stem. The bark of the stems is light gray, thin, and, when old, becomes cracked and rough. While the wood is hard, heavy, and tough. [10]

Simple arabica coffee leaves (Coffea arabica) with short petioles are dark green, leathery, glossy, 4-8 inches long. On the underside of the leaves, there are small cavities called domatia which protrude onto the leaf surface. The life span of Arabica coffee leaves is less than one year. Location, shape, size, and the absence or presence of domatia on Arabica coffee leaves have been used to differentiate coffee species and varieties. The fruit on the Arabica coffee plant (Coffea arabica) is red in color, and the seeds have a slightly elongated shape, slightly convex, light brown, and a slit in the middle of the flat part is curved. [10]

Coffee has many ingredients that have health benefits. Arabica coffee beans (Coffea Arabica) contain caffeine, chlorogenic acid, flavonoids, and trigonelline. [11; 12; 13]. Coffee is a popular drink throughout the country. With a bitter taste, coffee has a unique taste image. In making coffee drinks, the part used is the coffee beans. Based on research, coffee has many benefits, including a) Coffee can stimulate the central nervous system, heart muscle, and smooth muscle relaxation, especially in the muscles in the bronchi. The resulting stimulant can raise one's spirits when the body feels tired from working or at night can make the body alert; [14] b) Coffee has an antioxidant activity which acts as a protector from liver damage caused by the side effects of paracetamol drugs, regulates fat and glucose metabolism by inhibiting G6Pase expression; [15] c) Coffee has an antiviral activity which can inhibit the replication of the Hepatitis B virus; [16] d) Extract from coffee beans has an antibacterial effect against E. coli bacteria; [17] and e) Extract from coffee beans also has an antibacterial effect against Staphylococcus aureus by damaging the cell wall structure and causing lysis. The minimum concentration of coffee bean extract is 12.5% [1]

Enterococci are commensal bacteria in humans that normally live in the oral cavity, digestive tract, and vagina. These bacteria can cause various diseases that infect the urinary tract, blood vessels, endocardium, digestive tract, and oral cavity. Enterococci are ranked in the top three as pathogenic bacteria that cause nosocomial infections resistant to various antibiotics, causing problems in treatment. Enterococcus faecalis is involved in endodontic infections. These bacteria can still be found in the root canals after treatment. It is due to Enterococcus faecalis experiencing antibiotic resistance, making it difficult to treat infections in that area. [1] Enterococcus faecalis contaminates the root canals and forms colonies on the dentin surface with the help of lipoteichoic acid, while aggregate substance and surface adhesion play a role in other virulence factors. [18]

Enterococcus faecalis bacteria are coccobacillus-shaped, Gram-positive, facultative anaerobes and 0.5 - 1 µm in diameter. These bacteria have paired, short-chain, and single arrangements. Most of the strains are non-hemolytic and non-motile. Surface colonies on blood agar are round, smooth, and intact. [19] Enterococcus faecalis can live in environmental conditions at 10 ° C - 45 ° C, pH 9.6, in 6.5% NaCl content, and die at 60 ° C for 30 minutes. Enterococcus faecalis is a facultative anaerobe that can reproduce with or without oxygen. With a lack of oxygen, these bacteria will produce energy through fermentation. These bacteria

catabolize various energy sources, including carbohydrates, glycerol, lactate, citrate, arginine, argmantine, and other keto acids. This bacterium causes 80-90% of root canal infections, 63% of which are caused by failed root canal treatment of teeth that experience recurrent infections because the bacteria are resistant to antibiotics. [19]

Enterococcus faecalis can be found in cases of primary endodontic infection and is frequently encountered when endodontic therapy fails. These bacteria are well adapted to survive in various environments that are detrimental to these bacteria. Enterococcus faecalis is resistant to the antimicrobial effects of calcium hydroxide, which has an effective proton pump mechanism in bacteria to maintain optimal cytoplasmic pH levels. [20]

These organisms can naturally encode virulence properties that help colonize, compete with other bacteria, fight host defense mechanisms, and produce pathological changes directly by producing toxins that cause inflammation. With bacterial colonization, attachment also occurs to the walls of the root canal assisted by an aggregation agent. [21] Adhesion surface is a protein localized on the surface of bacterial cells that is useful for mediating plasmid exchange between recipient and donor strains. This way, genetic material such as antibiotic resistance can be transferred to other E. faecalis. Fibrionectin aggregation agents or binding groups facilitate organisms to accommodate type 1 collagen and extracellular matrix proteins in dentin. Aggregating agents can function as virulence determinants of E. faecalis in at least four ways. First, it plays a role in the dissemination encoded by virulence factor plasmids, such as enterococcal cytolysin and antibiotic resistance determinants. Second, such coding can occur in renal and intestinal epithelial cells. Third, these aggregating agents can protect bacteria from polymorphonuclear leukocytes (PMN) or macrophage-mediated cell destruction by phagocytosis of bacteria. Fourth, the aggregating agent and cytolysin have a synergistic action that enhances virulence by activating the quorum-sensing mode of cytolysin regulation. It will result in tissue damage and deeper tissue invasion. [1]

The main function of bacterial proteases is to provide peptide nutrients to organisms. However, it is possible that proteases cause direct or indirect damage to host tissues, and then they can be classified as virulence factors. Enterococcus faecalis has two secreted proteases, namely gelatinase and serine protease. Gelatinase is a non-plasmid-encoded metalloendopeptidase, a strongly hydrophobic protein with a pH of 6 to 8. Gelatinase can hydrolyze gelatin, casein, insulin, fibrinogen, and small peptides. [1] There are also toxins such as cytolysin, which can cause tissue damage, and bacteriocin, which can inhibit the growth of other organisms. Cytolysin is a toxin produced by beta-hemolytic Enterococcus faecalis. Lysing erythrocytes, neutrophils, and macrophages can cause a decrease in phagocytosis so that bacteria can still live. [1]

Meanwhile, lipoteichoic acid and superoxide compounds can modulate local inflammatory processes by stimulating leukocytes to release several mediators, such as tumor necrosis factor, interleukins, and prostaglandins which contribute to periradicular damage. The hyaluronidase enzyme also plays a role by interfering with the formation of connective tissue in dentine [1].

Enterococcus faecalis causes 80% of all infections caused by enterococci, whereas E. faecium causes the remaining 20%. Enterococci are responsible for 8-15% of endocarditis and have a high affinity for heart valve tissue like streptococci and staphylococci. Enterococcal endocarditis is difficult to treat due to antibiotic resistance, such as β-lactams, aminoglycosides, clindamycin, lincomycin, and fluoroquinolone. E. faecalis causes endocarditis more often than E. faecium. [1] Enterococci cause 5–15% of noscomial urinary tract infections reported in the

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US. Urinary tract infections caused by enterococci are most likely to be acquired in a hospital while on long-term treatment. [1]

Enterococcus is isolated in the oral cavity and is most commonly found in E. faecalis. These bacteria are commensalism suitable to survive in the intestine, vaginal canal, and oral cavity. According to Williams et al., the research found enterococci in the saliva of 21.8% of the 206 people studied. Meanwhile, Sedgley et al. studied the prevalence of oral enterococcal phenotype and genotype. Enterococci were detected in oral rinse samples from 11% of 100 patients receiving endodontic treatment and 1% of 100 teeth of students with no history of endodontic treatment. All enterococcal isolates were identified as E. faecalis. [22; 23] Antibiotics are chemical compounds produced by microorganisms to kill or inhibit the growth of bacteria. Antibiotics are divided into two types, namely those that kill germs (bactericidal) and inhibit the growth of germs (bacteriostatic). Bactericidal antibiotics, for example, include penicillins, cephalosporins, aminoglycosides, rifampicin, isoniazid, and others. While bacteriostatic antibiotics are sulfonamides, tetracyclines, erythromycin, and others. [24] The mechanism of anti-bacterial action is inhibiting bacterial cell wall synthesis, modifying or inhibiting protein synthesis, damaging the cell wall membrane, and affecting the synthesis or metabolism of nucleic acids.

Table 1. Criteria for Activity of the Inhibitory Zone according to Greenwood

Inhibition Zone Diameter (mm)	Growth Inhibition Response
>20	Strong
16-20	Moderate
10-15	Weak
<10	None

According to Greenwood, the effectiveness of antibacterials is determined based on the zone of inhibition that occurs during the antibacterial sensitivity test. The effectiveness of an antibacterial substance can be grouped as follows: [28] Extracts are preparations obtained by extracting the active compounds from vegetable or animal simplicia using a suitable solvent. The solvent is evaporated, and the remaining mass or powder is treated in such a way as to comply with predetermined standards. [1] There are several types of extracts, namely: liquid extracts, thick extracts, and dry extracts. Liquid extract, if the extraction results can still be poured, usually the water content is more than 30%. The extract is thick with a 5 – 30% moisture content. Dry extract if it contains less than 5% moisture content. [1] Extraction is separating the material from the mixture using a suitable solvent. Factors affecting extraction include raw materials, solvent selection, processing time, and extraction temperature. The choice of solvent will be affected by the temperature and time of the extraction process. The choice of solvent is an important factor in the extraction process. [25]

A solvent liquid is a substance to dissolve solutes by separating active compounds from other ingredients. Solvents are grouped into non-polar solvents (hexane, benzene, chloroform, toluene), polar aprotic solvents (acetone, dichloromethane, dimethyl sulfoxide), and polar protic solvents (ethanol, methanol, water, acetic acid, etc.). However, the government limits liquid solvents: water, ethanol, methanol, hexane, toluene, chloroform, and acetone. [26] Ethanol is a versatile solvent with high polarity, extracting more compounds than other organic solvents. Ethanol has a boiling point of 79°C and is harmless. Commonly used as a solvent, antiseptic, dye, or ingredient in the cosmetic and pharmaceutical industries. Ethanol has a lower toxin content than methanol, making it a good solvent for coffee bean extraction. Ethanol is an

efficient solvent for extracting antioxidants in phenolic acid compounds, which play an important role as antimicrobials. [1] Ethanol solvent has selective properties, can mix with water, is economical, and can extract most chemical compounds in simplicia such as tannins, polyphenols, alkaloids, essential oils, glycosides, curcumin, chlorophyll, steroids, and flavonoids. [1] Hartati et al.'s research showed that coffee leaf extract using 70% ethanol inhibited S. aureus and E. coli bacteria more than ethyl acetate solvent. [27] Tanauma et al. proved that extraction of robusta coffee beans using 96% ethanol solvent inhibited the growth of E. coli bacteria, producing an inhibition zone of 22.5 mm at a 10% concentration of robusta coffee bean extraction. [28] Extraction methods are divided into two types based on the process: cold extraction (maceration and percolation) and hot extraction (reflux and soxhletation).

Research Method

This type of research design is the Post Test Control Group Design giving two control groups. The negative control used sterile distilled water, and the positive control used was ampicillin antibiotic discs against the growth of Enterococcus faecalis bacteria, as well as the inhibition test using the agar diffusion test method (Kirby and Bauer method). The research was conducted from February to March 2021 at the Microbiology Laboratory, FK UKI, and the arabica coffee bean extraction process was carried out at the Bogor Spice and Medicinal Plants Research Laboratory. Arabica Coffee Beans (Coffea arabica) were purchased at the Senen market in Central Jakarta. The extraction process was carried out at the Bogor Spice and Medicinal Plant Research Agency Laboratory. The bacterial sample used was Enterococcus faecalis ATCC 29212 from the Food and Drug Supervisory Agency. The samples used in this study were arabica coffee bean extract in various concentrations of 1.5625%, 3.125%, 6.25%, 12.5%, 25%, 50%, and 100%, and the antibiotic ampicillin as a positive control and sterile aquadest as a negative control. This treatment was repeated in 3 trials. The research instruments were racks and test tubes, ose, beaker glass, dropper and micropipette, alcohol cotton, sterile cotton swabs, bunsen and matches, spatulas, Petri dishes, Erlenmeyer tubes, calipers, scales and balances, autoclaves, paper Whatman filters, trays, incubators, vortexes, laminar outflows, wipes, empty test discs, and vacuum rotary evaporators. The research materials used were arabica coffee bean extract (Coffea arabica), Enterococcus faecalis ATCC 29212 bacteria, Mueller Hinton Agar media, sterile aquadest, 0.5 McFarland standard solution, 96% ethanol solvent (absolute), and ampicillin antibiotic discs. The data were obtained by looking at and measuring the results of the inhibition zone on the Mueller Hinton Agar medium using a vernier caliper. The research was carried out through several procedures: tool sterilization, preparation of arabica coffee bean extract, dilution of arabica coffee bean extract, and media preparation. Data were obtained descriptively by recording the results of the inhibition zones of Gram-positive bacteria Enterococcus faecalis after being treated with Arabica coffee bean extract at various concentrations, negative control (sterile aquadest), and positive control (ampicillin). The data will be presented as statistical tables and processed using SPSS with editing, coding, and tabulating data processing. Data were analyzed using the SPSS application program using the Shapiro-Wilk normality test to determine whether the data in each group was normally distributed ($p>0.05$). Furthermore, the Kruskal Wallis test was conducted to determine statistically significant differences in administering arabica coffee bean extract (Coffea arabica) against Enterococcus faecalis bacteria.

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Result and Discussion

Arabica coffee beans that have been cleaned and then dried and ground to a powder (dried simplicia of Arabica coffee beans) are then weighed using a balance until they reach a weight of 1000 grams. One thousand grams of Arabica coffee bean simplicia was dissolved in 4000 ml of 96% ethanol; then, the solution was filtered using filter paper to produce a filtrate. The filtrate still contains solvent and must be removed using a Vacuum Rotatory Evaporator to produce the extract. Thus, Arabica coffee bean extract was obtained in liquid form with a very thick brown consistency weighing 43.4 grams.

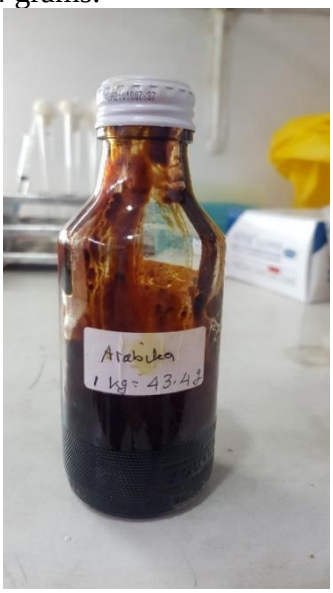

Figure 1. Results of Arabica Coffee Bean Extract (Source: Personal Documents)

Gram staining is used to distinguish Gram-negative bacteria from Gram-positive and determine the morphology of bacteria. Gram staining was carried out using a solution of crystal violet, Lugol, alcohol, and fuchsin, then viewed under a microscope with a magnification of 1000x so that you could see purple coccobacillus-shaped bacteria. It is a characteristic of Gram-positive bacteria that corresponds to Enterococcus faecalis.

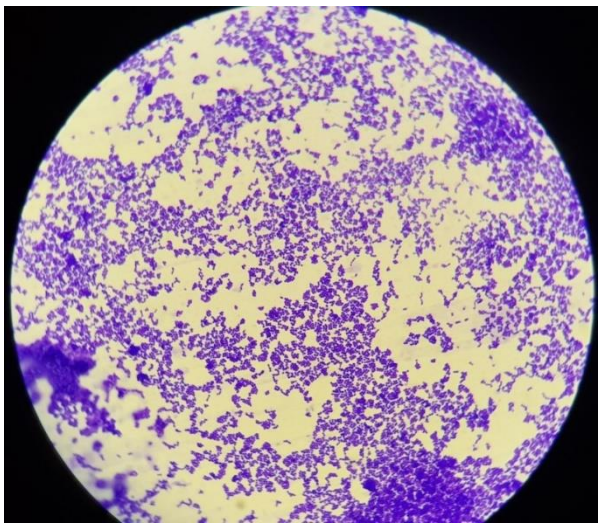

Figure 2. Enterococcus Faecalis Gram Staining Results (Source: Personal document)

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Test the inhibition of arabica coffee bean extract on the growth of Enterococcus faecalis using the Kirby Bauer Disc Diffusion method at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, 3.125%, 1.5625%, and positive controls in the form of ampicillin antibiotics and negative control using sterile aquadest. Enterococcus faecalis was inoculated on Mueller Hinton agar, given test discs that had been soaked in Arabica coffee bean extract with various concentrations, positive control, and negative control, then incubated at 370C for 24 hours. If there is a zone or halo around the bacterial colony, the inhibition zone is formed, then it is measured with a caliper. The inhibition zone formed after administration of Arabica coffee bean extract with various concentrations and positive and negative controls on Enterococcus faecalis bacteria can be seen in Table 2 below.

Table 2. The results of measuring the inhibition zone of Arabica coffee bean extract against bacteria

Repetition of Treatment	Obstacles zone (mm)								
	1,5625%	3,125%	6,25%	12,5%	25%	50%	100%	Control (+)	Control (-)
1	0	1	1,6	3,2	5,4	10,3	14,3	25,6	0
2	0	1	2	3,4	4,8	10,6	15,1	24	0
3	0	1,5	2,1	2,9	5	10,1	14,4	24,2	0
Average count	**0**	**1,16**	**1,9**	**3,16**	**5,06**	**10,3**	**14,6**	**24,6**	**0**
Repetition of Treatment	Obstacles zone (mm)								
	1,5625%	3,125%	6,25%	12,5%	25%	50%	100%	Control(+)	Control (-)
1	0	1	1,6	3,2	5,4	10,3	14,3	25,6	0
2	0	1	2	3,4	4,8	10,6	15,1	24	0
3	0	1,5	2,1	2,9	5	10,1	14,4	24,2	0
Average count	**0**	**1,16**	**1,9**	**3,16**	**5,06**	**10,3**	**14,6**	**24,6**	**0**

Table 2 above shows that the arabica coffee bean extract at a concentration of 1.5625% did not form an inhibition zone, and an inhibition zone was formed at a concentration of 3.125%, 6.25%, 12.5%, 25%, 50%, and 100%; this showed that the smaller the concentration of arabica coffee bean extract, the smaller the inhibition zone formed. The inhibition zone in the extract with a concentration of 3.125% obtained the largest diameter of 1.5 mm and the smallest 1 mm with an average inhibition zone value of 1.16 mm. At a concentration of 6.25%, the largest diameter was 2.1 mm, and the smallest was 1.6 mm, with an average inhibition zone value of 1.9 mm. At a concentration of 12.5%, the largest diameter was 3.4 mm, and the smallest was 2.9 mm, with an average inhibition zone value of 3.16 mm. At a concentration of 25%, the largest diameter was 5.4 mm, and the smallest was 4.8 mm, with an average inhibition zone value of 5.06 mm. At a concentration of 50%, the largest diameter was 10.6 mm, and the smallest was 10.1 mm, with an average inhibition zone value of 10.3 mm. At 100% concentration, the largest diameter was 15.1 mm, and the smallest was 14.3 mm, with an average inhibition zone value of 14.6 mm. In the positive control using the antibiotic ampicillin, the largest inhibition zone was 25.6 mm, and the smallest was 24 mm, with an average value of 24.6 mm. In the negative control using aquadest, no inhibition zone was found.

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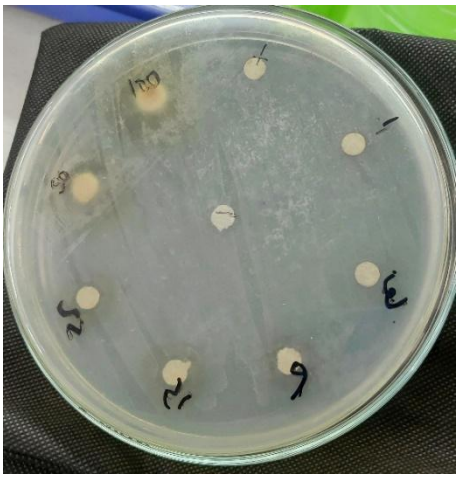

Gambar 3. Zona hambat ekstrak biji kopi arabika terhadap pertumbuhan bakteri *Enterococcus faecalis*. Sumber: Dokumen pribadi

Gambar 4. Zona hambar ekstrak biji kopi arabika terhadap pertumbuhan bakteri *Enterococcus faecalis*. Sumber: Dokumen pribadi

This study showed that arabica coffee bean extract at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125% formed an inhibition zone, while at a concentration of 1.5625%, no inhibition zone was formed. The largest diameter of the inhibition zone was found in arabica coffee bean extract at 100% and the smallest at a concentration of 3.125%. The emergence of the diameter of the inhibition zone is due to the presence of an active substance in Arabica coffee beans that has antibacterial properties. In this study, the resulting inhibition zone differed and increased according to the higher concentration. At higher concentrations, it contains more and more antibacterial compounds, and it can be proven that the inhibition zone is getting bigger with every increase in the concentration of arabica coffee bean extract. Based on the activity criteria of the inhibitory power zone according to Greenwood, it can be seen in Table 3 below.

Table 3. Criteria for inhibition zone activity according to Greenwood

Inhibition Zone Diameter (mm)	Growth inhibition response
>20	Strong
16-20	Moderate
10-15	Weak
<10	None

Based on the activity criteria of the inhibition zone according to Greenwood, it was found that the Arabica coffee bean extract concentration of 100% with an average inhibition zone of 14.65 mm and a concentration of 50% with an average inhibition zone of 10.3 mm is said to have a weak inhibition against Enterococcus bacteria. faecalis. Arabica coffee bean extract concentration of 25% with an average inhibition zone of 5.06 mm, a concentration of 12.5% with an average inhibition zone of 3.16 mm, a concentration of 6.25% with an average inhibition zone of 1.9 mm, and a concentration of 3.125% with an average inhibition zone of 1.16 mm

was declared to have no inhibition because the diameter of the inhibition zone formed was less than 10 mm.

Research by Willy Wijaya et al. proved that arabica coffee bean extract has anti-bacterial activity against Lactobacillus acidophilus with an average inhibition zone of 12.53 mm at a concentration of 100%, 10.66 mm at a concentration of 75%, 9.31 mm at a concentration of 50% and 8.14 mm at a concentration of 25%. 39. Caffeine and trigonelline are the biggest components in Arabica coffee beans with anti-bacterial activity. Caffeine has the activity of inhibiting DNA synthesis in bacteria, and chlorogenic acid can increase the permeability of the cell wall and disrupt the metabolism of bacterial cells. [11] The content of other compounds in Arabica coffee beans, such as flavonoids, has several mechanisms in inhibiting bacterial growth, namely by inhibiting DNA and RNA synthesis, disrupting the function of the cytoplasmic membrane and bacterial energy metabolism. Flavonoids cause damage to the permeability of bacterial cell walls and lysosomes. [29]

The existence of an inhibition zone depends on several factors, such as the higher the microbial concentration, the smaller the inhibition zone, whether or not contamination occurs in the media, the rate of diffusion, the stability of the antibacterial material, and the nature of the media used. [30] The positive control used was the antibiotic ampicillin which produced the largest inhibition zone compared to the inhibition zone produced by arabica coffee bean extract. Ampicillin works by inhibiting bacterial cell wall synthesis. Based on the Clinical & Laboratory Standard Institute, ampicillin is said to be sensitive if the inhibition zone is 17 mm in Enterococcus faecalis bacteria, and in this study, it was proven by an average ampicillin inhibition zone of 24.6 mm.

Table 4. Kruskal Wallis Statistical Test

Test Statistics[a,b]

	Diameter
Chi-Square	25.783
df	8
Asymp. Sig.	.001

a. Kruskal Wallis Test

b. Grouping Variable: Perlakuan

Based on the Kruskal Wallis statistical test, if P < 0.05, there is a significant difference in the concentration of Arabica coffee beans in inhibiting the growth of Enterococcus faecalis bacteria. If P > 0.05, there is no significant difference in the concentration of Arabica coffee beans in inhibiting the growth of Enterococcus faecalis. Because P = 0.001, there is a significant difference which means coffee bean extract (Coffea arabica) is effective in inhibiting the growth of Enterococcus faecalis.

Conclusion

Based on the results of this study, it can be concluded that: a) Arabica coffee bean extract in 96% ethanol solvent has effectiveness as an antibacterial for Enterococcus faecalis; b) The higher the concentration of arabica coffee bean extract, the greater the resulting inhibition zone; c) Arabica coffee bean extract (Coffea arabica) has antibacterial activity with a minimum inhibitory concentration of 3.125%; and d) In this study according to Greenwood's

criteria which had an inhibition zone of more than 10 mm was arabica coffee bean extract in concentrations of 50% and 100%. Thus it is necessary to do the following: a) good management and processing of Arabica coffee beans to obtain the desired compound to obtain the maximum inhibitory effect; b) further research on the bacterial inhibition of arabica coffee bean extract using different extraction methods; c) different inhibition test methods to obtain the maximum inhibition effect; and d) test the inhibition of arabica coffee bean extract against other pathogenic bacteria.

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