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Research Article

# Effect of Arabica Coffee Bean Extract (Coffea arabica) as a Growth Inhibitor of Enterococcus faecalis ATCC 29212

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Abstract

Arabica coffee seeds contain a composition of Caffeine, Chlorogenic acid, Flavonoids, and Trigonelline. The purpose of this study is to test the antibacterial of Arabica coffee seed extract against bacterium *Enterococcus faecalis* at concentrations of 1,5625%, 3,125%, 6,25%, 12,5%, 25%, 50% and 100%. The extraction method using maceration with solvent 96%. Antibacterial activity test was done by using the Kirby and Bauer diffusion test method. The results showed that Arabica coffee 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 Coffea genus tree. There are two well-known types of coffee in the world, namely arabica coffee (Coffea arabica) and robusta coffee (Coffea robusta). One of the habits of Indonesian society is consuming coffee. Coffee as a soft drink has various health benefits, and this has been proven from previous studies, including oral health.[1] The coffee contains derivatives of hydroxycinnamic acids, including caffeine, chlorogenic, coumarin, ferulic, sinapic acid, flavonoids, and polyphenols.[2] Caffeine in coffee is known to function as an antibacterial which can inhibit the cell wall and bacterial DNA synthesis.[1]

Indonesia is a country that is rich in nutritious plants, one of which is coffee. Indonesia is the number 3 coffee producer in the world after Brazil and Vietnam. Coffee, in general, has several benefits such as stimulating the respiratory process, helping assimilation and digestion of food, calming mental feelings when the body is tired, as a medicine for diarrhoea, preventing vomiting after surgery. Research that has the power to act as a new antibacterial that can inhibit or kill bacteria is needed and developed. One alternative that can be done is to utilize the active substances that kill bacteria in medicinal plants, namely coffee.[3] Enterococcus faecalis has a significant role in the aetiology of infection in the root canals of teeth. It is generally found in a high percentage of root canals as a single organism surviving after treatment. These bacteria rely on their ability to survive as pathogens by exhibiting antibiotic resistance genes or spontaneous mutations. The prevalence of endodontic infections caused by the bacteria Enterococcus faecalis ranges from 24-77%. This discovery can be explained by the variety of resistance and virulence of the bacterium Enterococcus faecalis itself, including its ability to compete with other microorganisms to enter the dentinal tubules and survive poorly nourished conditions. The bacteria Enterococcus faecalis remaining in the canal can significantly reduce the success rate after root canal treatment.[4]

The use of root canal drugs is highly recommended to prevent multiplication and at the same time kill the bacteria in it. The drug must have a broad-spectrum effect. Potassium hydroxide is a compound that is present in root canal medicines to kill germs. The alkaline atmosphere caused by calcium hydroxide makes root canal bacteria unable to survive in this environment, but this is not the case for Enterococcus faecalis.[5] In recent years, enterococci have increased rates of development of resistance to several antimicrobial drugs. Enterococcus expressed resistance to tetracyclines, erythromycin, trimethoprim, and high levels of clindamycin. Vancomycin-Resistant Enterococci (VRE) represent the most severe challenge among microbial resistance and as a source of clinical infection in humans in the last decade.[1]

Antibiotic resistance is a current health problem increasing the incidence of life-threatening infections. It is due to improper use in dosage, incompatible with the disease and inaccurate duration of use. Enterococcus faecalis

bacteria are known to have resistance to β-lactam antibiotics, namely ampicillin, amoxicillin, and penicillin.[6] Therefore, researchers are interested in researching the antibacterial effect of arabica coffee bean extract in inhibiting the bacterium Enterococcus faecalis. 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, among others: a) Can the extract of Arabica coffee beans (Coffea arabica) inhibit the growth of Enterococcus faecalis? Furthermore, b) What is the minimum inhibitory concentration of arabica coffee bean extract (Coffea arabica) on the growth of Enterococcus faecalis? With the aim of a) To determine the antibacterial activity seen from the diameter of the growth inhibition zone Enterococcus faecalis based on concentrations of 1.5625%, 3.125%, 6.25%, 12.5%, 25%, 50% and 100% extract of arabica coffee beans (Coffea arabica) and b) To determine the zone of minimal inhibition of extract of Arabica coffee beans (Coffea arabica) in inhibiting the growth of Enterococcus faecalis.

## LITERATURE REVIEW

Arabica Coffee Beans (Coffea arabica) - Arabica coffee was first discovered in the highlands of Ethiopia and was popularized by the Arabs. Coffee beans from Ethiopia were brought by Arab traders to Yemen and traded. Entering the 17th century, the Europeans started to develop their coffee plantations, but the climate was not suitable and in the 19th century. The Dutch brought coffee to the island of Java and cultivated it.[7]

Arabica coffee has conditions in climate and soil conditions that are optimum for its growth. Arabica coffee is very suitable to be planted in the highlands with an altitude of 700 - 1400 meters above sea level, relatively low air temperature, namely 15 - 24oC, average rainfall 2,000-4,000 mm / year, effective soil depth> 100 cm and soil pH. 5,3 - 6,0.[8] Arabica coffee is widespread and has different taste images depending on the soil and climate conditions in which the coffee plant is grown. In Indonesia, the name Arabica coffee is based on where it is planted. The following are types of Arabica coffee that are well known and in the area of origin:[9]

1. Garut Arabica Coffee (West Java)
2. Arjuno Arabica Coffee (East Java)
3. Mandailing Arabica Coffee (North Sumatra)
4. Aceh Gayo Arabica Coffee (Aceh)
5. Toraja Kalosi (Toraja) Arabica Coffee
6. Kintamani Arabica Coffee (Bali)
7. Papua Wamena (Papua) Arabica Coffee [9]

Figure 1: *Coffea arabica* (Source: https://www.filosofikopi.com/2019/04/perbedaan-kopi-robusta-dan-arabika.html)

Figure 2: *Coffea arabica* (Source: https://id.wikipedia.org/wiki/Berkas:Starr_070308-5472_Coffea_arabica.jpg )

Morphology and Efficacy of Arabica Coffee Beans (Coffea arabica) - The Arabica coffee plant (Coffea arabica) is a shrub that is divided into two pieces (dicots) 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 from the trunk is light grey, thin, and when it gets old, it becomes cracked and rough. At the same time, the wood is hard, heavy and tough.[9]

Simple leaf arabica coffee (Coffea arabica) with short petioles has dark green, leathery, shiny, oval 4 - 8 inches in colour. On the underside of the leaf, there is a small cavity called domatia and protrudes outward into the leaf surface. The life span of Arabica coffee leaves is less than one year. The location, shape, size, and absence of domatia on arabica coffee leaves have been used to differentiate coffee species and varieties. The fruit of the Arabica coffee plant (Coffea arabica) is red, and the seeds have a slightly elongated shape, slightly convex, light brown, and the middle gap is curved flat.[10]

Chemical Content of Arabica Coffee Beans (Coffea arabica) - Coffee has many ingredients that have health benefits. The content of Arabica coffee beans (Coffea Arabica) is caffeine,[10,29] Chlorogenic Acid,[11,12] Flavonoids,[13] and Trigonelin.[14]

Benefits of Arabica Coffee - Coffee is a popular drink throughout the country. With a bitter taste but coffee has a unique taste image. In making coffee drinks, the part used is 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;[10] b) Coffee has antioxidant activity that acts as a protector against liver damage caused by the side effects of paracetamol, regulates fat and glucose

metabolism by inhibiting G6Pase expression;[11] c) Coffee has an antiviral activity that can inhibit the replication of the Hepatitis B virus;[11] d) coffee beans have antibacterial effects against E. coli bacteria,[15] 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%.[16]

Enterococcus faecalis - Enterococcus is a commensal bacteria in normal humans that 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 in the top three ranks of pathogenic bacteria that cause nosocomial infections resistant to various antibiotics, causing problems in treatment. Enterococcus faecalis is involved in edondotic infections. These bacteria can still be found in the root canals of teeth after treatment; this is because Enterococcus faecalis has antibiotic resistance, making it difficult to treat infections in the area.[17]

Enterococcus faecalis contaminates root canals and forms colonies on the dentin surface with the help of lipoteichoic acid, while the aggregate substance and surface adhesion play a role in other virulence factors.[18]

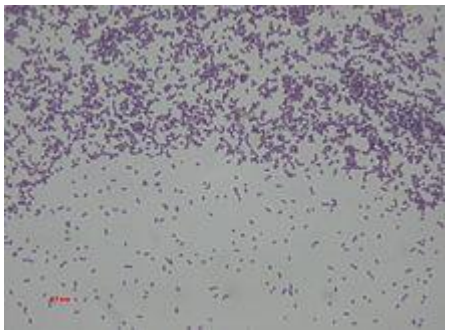

Figure 3: *Enterococcus faecalis* (Source: https://upload.wikimedia.org/wikipedia/commons/thumb/6/65/Enterococcus_faecalis.jpg/220px-Enterococcus_faecalis.jpg)

Morphology and Identification of Enterococcus faecalis - The bacteria Enterococcus faecalis are cocobacil, Gram-positive, facultatively anaerobic, and 0.5 - 1 µm in diameter. These bacteria have pairs, short chains, and are single. Most of the strains are non-hemolytic and non-motile. The surface colonies on blood agar are round, smooth, and intact.[17] Enterococcus faecalis can live in environmental conditions at a temperature of 10 ° C - 45 ° C, pH 9.6, in a NaCl content of 6.5%, and die at 60 ° C for 30 minutes. Enterococcus faecalis is facultatively anaerobic, which means that it 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, argmantin and other keto acids. These bacteria cause 80-90% of root canal infections, 63% of which result from failure of root canal treatment of teeth with recurrent infections because the bacteria are resistant to antibiotics.[17]

Pathogenesis of Enterococcus faecalis - Enterococcus faecalis can be found in cases of primary endodontic infection and is often found when endodontic therapy has failed. These bacteria are well adapted to survive in a variety of environments that are detrimental to these bacteria. Enterococcus faecalis is resistant to the antimicrobial effect of calcium hydroxide, which has an effective proton pump mechanism in bacteria to maintain optimal cytoplasmic pH levels.[5] These organisms have the natural ability to encode virulence properties that help colonize, compete with other bacteria, fight host defence mechanisms and produce pathological changes directly through the production of toxins that cause inflammation. With the presence of bacterial colonization, there is adhesion to the walls of the tooth root canals assisted by an aggregation agent.[18]

The adhesion surface is a protein localized on the surface of bacterial cells, which helps mediate the exchange of plasmids between recipient and donor strains. In this way, genetic material such as antibiotic resistance can be transferred to another E. faecalis. Aggregating agents or fibronectin-binding groups facilitate the organism to accommodate collagen type 1 and extracellular matrix proteins present in dentin. Aggregation agents can serve as determinants of the virulence of E. faecalis in at least four different ways. First, it plays a role in spreading plasmid-coded virulence factors, such as enterococcal cytolysin and determinants of antibiotic resistance. Second, the coding can occur in the epithelial cells of the kidneys and intestines, and third, the aggregated substance can protect bacteria from polymorphonuclear leukocytes (PMN) or macrophage-mediated cell destruction by bacterial phagocytosis. Fourth, aggregating agents and cytolysin have a synergistic action that increases virulence by activating the quorum-sensing mode of cytolysin regulation. It will result in more profound tissue damage and tissue invasion.[17,18]

The primary function of bacterial proteases is to provide peptide nutrients to organisms. However, it is possible that the proteases cause direct or indirect damage to the host tissue and then they can be classified as virulence factors. Enterococcus faecalis has two proteases that are secreted, namely gelatinase and serine protease. Gelatinase is a non-plasmid-encoded metalloendopeptidase with a potent hydrophobic protein and a pH of 6 to 8. Gelatinase can hydrolyze gelatin, casein, insulin, fibrinogen and small peptides.[17,18,19] There are also toxins such as cytolysin, which can cause tissue damage and bacteriocins, which can inhibit other organisms' growth. Cytolysin is a toxin produced by Enterococcus faecalis beta-hemolytic. By lysing erythrocytes, neutrophils and macrophages can cause decreased phagocytosis so that bacteria can survive.[20] Meanwhile, lipoteichoic acid and superoxide compounds can modulate the local inflammatory process by stimulating leukocytes to release several mediators such as tumour necrosis factor, interleukins, and prostaglandins to periradicular damage. Hyaluronidase enzymes also play a role by interfering with the formation of connective tissue in dentin.[20]

Enterococcus faecalis infection - Enterococcus faecalis causes 80% of all infections caused by enterococci, whereas E. faecium causes the remaining 20% of infections. Enterococci are responsible for 8-15% of endocarditis and have a high affinity for heart valve tissues such as streptococci and staphylococci. Enterococcal endocarditis is challenging to treat due to resistance to antibiotics such as β-lactams, aminoglycosides, clindamycin, lincomycin, and fluoroquinolones. E. faecalis causes endocarditis more frequently than E. Faecium.[20]

Enterococci cause 5–15% of reported nosocomial urinary tract infections in the US. Urinary tract infections caused by enterococci are most likely to be acquired in the hospital while undergoing long-term care.[20] Enterococcus isolated in the oral cavity. The most common is E. faecalis. These bacteria are commensalism suitable for survival in the intestine, vaginal tract and oral cavity. Enterococci in saliva as much as 21.8% of the 206 people studied. At the same

time, the prevalence of the oral enterococcal phenotype and genotype were examined. Enterococci were detected in oral rinses of 11% of 100 patients receiving endodontic treatment and 1% of 100 student teeth without a history of endodontic treatment. All enterococcal isolates were identified as E. Faecalis.[20,21]

Enterococcus spp was not a typical isolate for primary apical periodontitis when sampling started before the treatment procedure. Enterococcus faecalis is the bacteria most resistant to calcium hydroxide both in vivo and in vitro. Usually, Candida sp oral is also found after administration of a saturated calcium hydroxide solution. Enterococcus faecalis was more common in teeth with chronic infections than in acute infections. Although these bacteria are the dominant species in the root canal of teeth with antibacterial resistance, there is no evidence that E. faecalis is responsible for acute infection.[22]

Extraction - Extract is a preparation obtained by extracting the active compound from vegetable or animal simplicia using a suitable solvent. The solvent is evaporated, and the remaining mass is treated to meet the predetermined standards.[23] There are several types of extracts, namely: liquid extract, thick extract, and dry extract. Liquid extract if the extract can still be poured, usually the water content is more than 30%. Thick extract if it has a moisture content between 5 - 30%. Dry extract if it contains less than 5% moisture content.[23] Extraction is the process of separating material from its mixture using a suitable solvent. Factors that influence extraction include raw materials, solvent selection, processing time, and extraction temperature. The choice of solvent will be influenced by temperature and extraction process time.[24]

Solvent selection is an essential factor in the extraction process. The solvents' requirements in the extraction process are;[25] a) Has high solubility and solute selectivity; b) The solvent does not cause chemical changes in the components of the extracted material; c) Does not cause emulsion formation; d) Non-corrosive; e) Non-toxic; f) Non-flammable; g) Chemically and thermally stable; h) Harmless to the environment; i) Has a low viscosity, and j) Has a low enough boiling point for easy evaporation.

The solvent is a substance to dissolve the solute by separating the active compound from other ingredients. Solvents are classified into non-polar solvents (hexane, benzene, chloroform, toluene), aprotic polar solvents (acetone, dichloromethane, dimethyl sulfoxide), and protic polar solvents (ethanol, methanol, water, acetic acid, etc.). However, the government limits what solvents are allowed, namely water, ethanol, methanol, hexane, toluene, chloroform, and acetone [30]. Ethanol is a multipurpose solvent with a high polarity to extract more compounds than other types of organic solvents. Ethanol has a boiling point of 79 ° C and is harmless. Commonly used as solvents, antiseptics, dyes, ingredients in the cosmetic and pharmaceutical industries.[26]

Ethanol has lower levels of toxins than methanol, making it a suitable solvent for the extraction of coffee beans. Ethanol is an efficient solvent for the antioxidant extraction of phenolic acid compounds, which plays an essential role in antimicrobials.[27] Ethanol solvent has particular properties, can mix with water, is economical, can extract most of the chemical compounds in simplicia such as tannins, polyphenols, alkaloids, essential oils, glycosides, curcumin, chlorophyll, steroids, and flavonoids.[14]

The coffee leaf extract using 70% ethanol had a higher inhibitory power against S. aureus and E. coli bacteria than using ethyl acetate solvent.[28] The extraction of robusta coffee beans using 96% ethanol solvent could inhibit the growth of E. coli bacteria which produces an inhibition zone of 22.5 mm in 10% concentration from the extraction of robusta coffee beans.

## RESEARCH METHOD

This type of research design is the Post Test Control Group Design by giving two control groups. The negative control used sterile distilled water, and the positive control used was ampicillin antibiotic disc against the growth of Enterococcus faecalis bacteria. As well as the inhibition test using the agar diffusion test method (Kirby and Bauer methods). The research was conducted from February to March 2021 at the Microbiology Laboratory of FK UKI, and the extraction process of Arabica coffee beans was carried out at the Bogor Herbs and Medicinal Plants Research Laboratory. The materials used in this study were Arabica coffee beans (Coffea arabica) purchased at the Senen market in Central Jakarta. The extraction process is carried out at the Laboratory of the Bogor Medicinal and Spice Crops Research Institute. The bacterial sample used was Enterococcus faecalis ATCC 29212 from the Food and Drug Administration. 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 ampicillin antibiotics as a positive control and sterile aqua dest as a control. This treatment was repeated three times the experiment. The repetition determination is determined by Federer's formula, as follows:

| | | |
|---|---|---|
| | Federer formula: (n-1)(k-1) | ≥ 15 |
| | (n-1)(9-1) | ≥ 15 |
| | (n-1)(8) | ≥ 15 |
| | 8n-8 | ≥ 15 |
| | 8n | ≥ 23 |
| | n | ≥ 2,87 |
| Note | n = number of repetitions | |
| | k = number of groups = 6 | |

Data obtained descriptively by recording the inhibition zone results of the Gram-positive bacteria Enterococcus faecalis after being given the treatment of Arabica coffee bean extract at various concentrations. Negative control (sterile aquadest), and positive control (ampicillin). . Data will be presented in statistical tables that are processed using SPSS with data processing steps Editing, Coding and Tabulation. 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) or not. Furthermore, the Kruskal Wallis test was carried out, aiming to determine statistically significant differences in the administration of Arabica coffee bean extract (Coffea arabica) to Enterococcus faecalis bacteria. The interpretation of the test, i.e.:

1. Sig> 0.05 = no difference between samples (treatment)

2. Sig <0.05 = there is a difference between the samples (treatment)

## RESULT AND DISCUSSION

Arabica Coffee Bean Extract (Coffea arabica) - Arabica coffee beans cleaned and then dried and pulverized to become a powder (dry simplicia of Arabica coffee beans) are then weighed using a balance until they reach 1000 grams in weight. Simplicia 1000 grams of arabica coffee beans

dissolved with 4000 ml 96% ethanol, then the solution is filtered using filter paper to produce a filtrate. The filtrate still contains solvent and must be removed using a Vacuum Rotatory Evaporator to produce an extract. Thus, the extract of Arabica coffee beans is obtained in liquid form with a very thick consistency, brown, weighing 43.4 grams.

Identification of Enterococcus faecalis - Gram stain is used to distinguish Gram-negative from Gram-positive bacteria and determine the morphology of the bacteria. Gram staining was carried out using a solution of crystal violet, lugol, alcohol, and fukhsin and then viewed under a microscope with a magnification of 1000x so that purple cocobacil-shaped bacteria could be seen. It is a characteristic of Gram-positive bacteria which is compatible with Enterococcus faecalis.

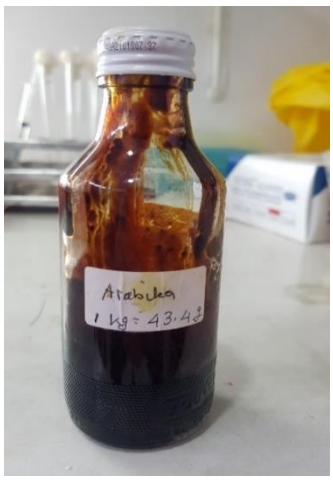

Figure 4: Arabica coffee bean extract results (Source: Personal documents).

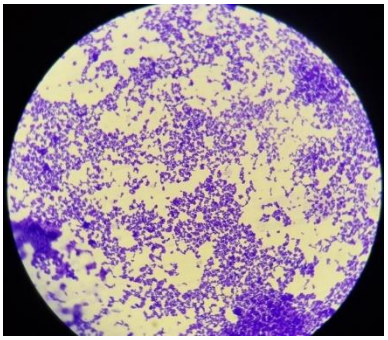

Figure 5: The results of Gram stain Enterococcus faecalis (Source: Personal documents).

Results of Arabica Coffee Bean Extract Inhibition Test on Enterococcus faecalis Growth - Inhibition test of Arabica coffee bean extract against the growth of Enterococcus faecalis bacteria using the Kirby Bauer Disc Diffusion method at a concentration of 100%, 50%, 25%, 12.5%, 6.25 %, 3.125%, 1.5625% and positive control in the form of antibiotic ampicillin and negative control using sterile aqua dest. Enterococcus faecalis was inoculated on Mueller Hinton agar, then given a test disc soaked with various Arabica coffee bean extract concentrations, positive control and negative control, then incubated at 37$^{o}$C for 24 hours. The inhibition zone is formed if there is a zone or halo around the bacterial colony, then it is measured by a calliper. The inhibition zone formed after giving arabica coffee bean extract with various concentrations and positive and negative controls on the Enterococcus faecalis bacteria can be seen in table 1 below.

Table 1: Measurement results of the inhibition zone of arabica coffee bean extract against bacteria

| Treatment Repetition | Zone of Obstacle (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 1 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%. It shows that the smaller the concentration of Arabica coffee bean extract, the smaller the inhibition zone. The inhibition zone in the extract with a concentration of 3.125% obtained the largest diameter of 1.5 mm and the slightest 1 mm with an average value of the inhibition zone 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 ampicillin antibiotics, the largest inhibition zone was obtained 25.6 mm, and the smallest was 24 mm with an average value of 24.6 mm. In the negative control using aqua dest, no inhibition zone was found.

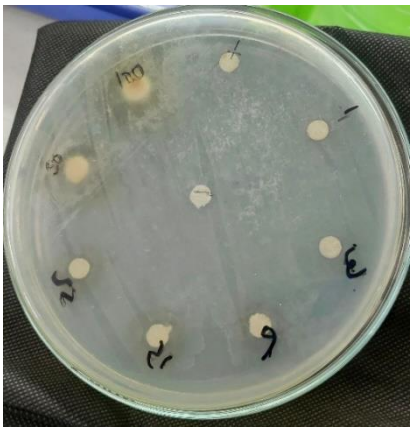

Figure 6: Inhibition zone of arabica coffee bean extract against the growth of Enterococcus faecalis bacteria (Source: Personal documents).

Figure 7: Bland zone of arabica coffee bean extract against the growth of Enterococcus faecalis bacteria (Source: Personal documents).

This study showed that the 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 with a concentration of 100% with smallest at a concentration of 3.125%. The inhibition zone diameter is due to the active substance in Arabica coffee beans, which has antibacterial properties.

In this study, the resulting inhibition zone is different and increases according to the higher concentration. At greater concentrations, it contains more and more antibacterial compounds, and it can be proven that the inhibition zone is more significant with each increase in the concentration of the arabica coffee bean extract. Based on the activity criteria of the inhibition zone according to Greenwood, it can be seen in Table 2 below.

Table 2: Criteria for inhibition zone activity according to Greenwood

| Inhibition Zone Diameter (mm) | Inhibited growth response |
|---|---|
| >20 | Strong |
| 16-20 | Moderate |
| 10-15 | Weak |
| <10 | Nothing |

Based on the activity criteria of the inhibitory power zone according to Greenwood, it was found that the concentration of Arabica coffee bean extract was 100% with an average inhibition zone of 14.65 mm, and a concentration of 50% with an average inhibition zone of 10.3 mm, it was stated that the weak inhibition of Enterococcus bacteria faecalis. In the Arabica coffee bean extract concentration 25% with an average inhibition zone of 5.06 mm, a concentration of 12.5% with an average hambata 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 is stated to have no inhibitory power because the diameter of the formed zone is less than 10 mm.

The arabica coffee bean extract has antibacterial activity against Lactobacillus acidophilus with an average inhibition zone of 12.53 mm 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%.[29] Caffeine and trigonelline are the most significant components in arabica coffee beans which have antibacterial activity. Caffeine has the activity of inhibiting DNA synthesis in bacteria, and chlorogenic acid can increase cell wall permeability and interfere with bacterial cell metabolism.[30,31] 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.[32]

The presence of an inhibition zone depends on several factors such as the microbial concentration the higher the microbial concentration, the smaller the inhibition zone, the presence or absence of contamination that occurs in the media, diffusion speed, stability of the antibacterial material, and the properties of the media used.[33] The positive control used was ampicillin antibiotic which produced the largest inhibition zone compared to the inhibition zone produced by the arabica coffee bean extract. Ampicillin works by inhibiting bacterial cell wall synthesis. Based on the Clinical & Laboratory Standard Institute, ampicillin said to be sensitive if the inhibition zone is 17 mm in the bacterium Enterococcus faecalis. In this study, it was proven by an average inhibition zone of 24.6 mm of ampicillin.

Tabel 3: Kruskal Wallis Statistical Test

Statistical Test[a,b]

| | diameter |
|---|---|
| Chi-Square | 25.783 |
| Df | 8 |
| Asymp. Sig | .001 |

a. Kruskal Wallis Test
b. Grouping Variable: Treatment

Based on the Kruskal Wallis statistical test, if P <0.05, there was a significant difference in the concentration of Arabica coffee beans in inhibiting the growth of the bacterium Enterococcus faecalis. If P> 0.05, there was no significant difference in the concentration of Arabica coffee beans in inhibiting the growth of the bacterium Enterococcus faecalis. Because P = 0.001, there is a significant difference which means that the coffee bean extract (Coffea arabica) effectively inhibits 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 agent 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 with an inhibition zone of more than 10 mm, the extract of Arabica coffee beans in a concentration of 50% and 100%. From these results, it is advisable to a) Perform good management and processing of Arabica coffee beans in order to obtain the desired compound to obtain the maximum inhibitory effect; b) It is necessary to carry out further research on the inhibitory power of bacteria from the extract of Arabica coffee beans using different extraction methods; c) Perform different test methods of inhibition to get the maximum effect of the inhibition; and d) To test the inhibition of arabica coffee bean extract against other pathogenic bacteria.

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