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Antibacterial effectivity of coffee bean extract and instant coffee (spray drying) against porphyromonas gingivalis

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Abstract
Objective: This study aims to determine effectiveness of antibacterial of Coffee Bean Extract and Instant Coffee (Spray Drying) against Porphyromonas gingivalis.
Material and Methods: Dilution of coffee bean extract and instant coffee (spray drying) using the serial dilution method into several predetermined concentrations. Antibacterial activity using the disc diffusion method. The zone of inhibition was measured using a caliper
Results: The results showed that the inhibition zone of coffee bean extract was greater than that of instant coffee (spray drying) against Porphyromonas gingivalis.
Conclusion: The antibacterial of the coffee bean extract is more effective than instant coffee (spray drying) against Porphyromonas gingivalis
Keywords: Antibacterial, coffee bean extract, instant coffee (spray drying), P. gingivalis

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Introduction

Two types of coffee are cultivated in Indonesia, namely arabica (coffea arabica) and robusta (coffea canephora). The species of coffee that is widely cultivated in Indonesia is robusta coffee (C. canephora) due to suitable soil and climatic conditions.[1] Coffee beans are generally used by the community as a beverage to have benefits for dental and oral health. Some of the content contained in robusta coffee beans, namely, chlorogenic acid, caffeine, trigonelline, various phenolic compounds. The content has pharmacological activities in the form of antioxidants, antibacterial, antiviral, antihypertensive, antidiabetic.[2]

The inhibition zone of robusta coffee extract was greater than the inhibition zone of arabica coffee extract at concentrations of 100% and 75% against Lactobacillus acidophilus.[3] Robusta coffee has antibacterial bioactive compounds that are higher than arabica coffee. Caffeine compounds contained in robusta coffee are 2g/100g compared to arabica coffee which has 1g/100g caffeine. In addition, the chlorogenic acid content of Robusta coffee is 9g/100g compared to Arabica coffee which is only 5g/100g.[4]

Robusta coffee bean extract has the ability to inhibit the growth of dental plaque bacterial isolates in vitro. This is because several components in robusta coffee beans have antibacterial activity. Coffee processing, apart from being processed by maceration extraction, can also be produced without leaving any dregs, namely instant coffee. Instant coffee is made through the stages of roasting, grinding, extraction, spray drying, and packaging. Heating or roasting in the spray drying process causes changes in chemical composition and biological activity due to the Maillard reaction.[4] The method of preparing coffee drinks determines the phenol content and antioxidant capacity.[5] Instant coffee has antibacterial power with concentrations of 20 g/L, 40 g/L, 60 g/L, and 80 g/L against Streptococcus pneumoniae.[6]

The taste, aroma, and composition of coffee depending on how the coffee is processed. The composition, type, and processing of coffee including roasting can affect the antibacterial activity of coffee. The antibacterial activity of roasted robusta coffee bean water extract with concentrations of 62.5 mg/ml, 125 mg/ml, 250 mg/ml, 500 mg/ml, 1000 mg/ml had an antibacterial effect against Streptococcus mutans.[7] In addition, the ethanol extract of robusta

green coffee beans with concentrations of 125 mg/ml, 250 mg/ml, 500 mg/ml, 1000 mg/ml has an antibacterial effect against Fusobacterium nucleatum.[8]

Robusta coffee can be used to treat diseases in the oral cavity. The most common oral disease is periodontal disease or periodontitis. Periodontitis is generally caused by plaque bacteria found on the teeth, one of which is Porphyromonas gingivalis.[9] P. gingivalis in dental plaque shows 5-20 times greater numbers than other bacteria.[10] P. gingivalis can cause chronic periodontitis.[11] P. gingivalis is a melanogenic bacterium, non-saccharolytic, and part of the colony of black-pigmented gram-negative anaerobes located in the periodontal tissues, especially sub-gingival.[12] These bacteria can express virulence factors through fimbriae, lipopolysaccharides (LPS), and proteinases.[13] LPS causes periodontal tissue damage because it can increase the secretion of proinflammatory cytokines that increase the number of macrophages and lymphocytes.[14]

Periodontitis treatment is generally done mechanically with scaling root planning (SRP). However, cleaning with SRP is sometimes not optimal because there are parts that cannot be reached by the SRP device, so that systemic and local administration of antimicrobials is recommended to improve therapeutic results. Systemic and local administration of antimicrobials has side effects.[15] Efforts are made to avoid these side effects, it is necessary to use antimicrobial alternatives that utilize natural resources that are beneficial to health, such as robusta coffee (coffea canephora) with maceration extraction and spray drying methods.

Material and Methods

Coffee bean extract

Coffee beans are dried and blended. Then filtered using a 65 mesh sieve, weighed 300 grams, and extracted by maceration method in 97% ethanol solution for 24 hours. The samples were filtered using paper Whattman # 1, and then concentrated using a rotary evaporator, and the concentrated extract obtained 100% .[12,16]

Instant coffee (spray drying)

Robusta coffee beans are roasted, blended until they become coffee grounds. Then weighed 300 grams, extracted using boiling water with a ratio of coffee and water 1:2. The filtering process uses a 65 mesh sieve. The filtering results are sprayed at a temperature of 140°C for 6 hours, so the water will evaporate and the filtrate will fall into the tube into instant coffee powder.[17]

Dilution of coffee bean extract and instant coffee (spray drying)

The dilution was carried out by the serial dilution method. Before dilution, it was filtered using a 0.2 m syringe filter. 1000 mg of coffee/instant coffee bean extract (spray drying) was put in the first tube containing 1 ml of aquadest or 1:1, then homogenized. Take a sample of 0.5 ml from the first tube transferred to the second dilution tube which has been filled with 0.5 ml of distilled water, then homogenized again.[18] The process is continued in the same way until a dilution with a concentration of 1000 mg/ml, 500 mg/ml is obtained , 250 mg/ml, 125 mg/ml, 62.5 and mg/ml, 31.25 mg/ml.

The antibacterial activity test used the disk diffusion method with 5 mm diameter paper discs.[19] The paper discs were placed on blood agar media that had been inoculated with P. gingivalis with a dilution according to the Mc Farland standard 0.5 absorbances 0.05 and a wavelength of 560 nm using a spectrophotometer.[20] The disc paper was dripped with a sample of coffee bean extract/instant coffee (spray drying) with a concentration of 1000 mg/ml, 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml, a positive control sample of 0.2% chlorhexidine gluconate and a negative control sample of 20 µl sterile distilled water using a micropipette. Then incubated for 1x24 hours at 37°C. Observations were made after the formation of an inhibition zone around the paper disc. The inhibition zone was measured using a caliper by measuring the overall diameter of the area. The oval-shaped inhibition zone was

measured by following the vertical diameter (Dv) and horizontal diameter (Dh), the length of the measurement results was added and divided by two.

Results

The results showed a killing zone (radical zone) with a clear zone around the paper disc, in this zone, no bacterial growth was found at all. The zone of inhibition (irradical zone) is indicated by areas that appear infertile or cloudier than the negative control.

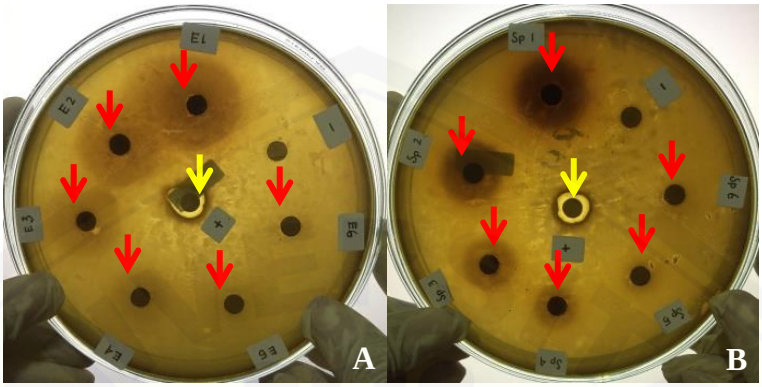

Figure 1. The results of the study of the inhibition zone of coffee bean extract. A. The inhibition zone of instant coffee (spray drying), B. The yellow arrow indicates the radical zone. Red arrows indicate the irradical zone with concentrations of 1000 mg/ml (E1 and Sp1), 500 mg/ml (E2 and Sp2), 250 mg/ml (E3 and Sp3), 125 mg/ml (E4 and Sp4), 62.5mg /ml (E5 and Sp5), 31.25 mg/ml (E6 and Sp6), K+: 0.2% chlorhexidine positive control, K-: sterile distilled water negative control.

Table 1. The average value of the inhibition zone of coffee bean extract and instant coffee (spray drying) on the growth of P. gingivalis

Treatment Group	Concentration (mg/ml)							
	1000	500	250	125	62.5	31.25	K+	K-
Coffee bean extract	28.3 ^{A}	22.6 ^{A}	18.8 ^{A}	15.4 ^{A}	10.1 ^{A}	6.5 ^{A}	12.1^{B}	0
Instant coffee (spray drying)	21.6 ^{A}	15.3 ^{A}	12.4 ^{A}	9.8 ^{A}	6.9 ^{A}	4.8 ^{A}	12.1 ^{B}	0

$\bar{x}$:Average diameter of inhibition zone
K+ :Positive control chlorhexidine gluconate 0.2%
K- :Sterile aquadest negative control
^{A} :The zone that appears is an irradical zone
^{B} :The zone that appears is a radical zone

Based on the results of observations showed that the greater the concentration of coffee bean extract and instant coffee (spray drying), the greater the diameter of the inhibition zone. The average diameter of the inhibition zone of the extract on the growth of P. gingivalis showed that it was greater than that of instant coffee (spray drying) with an incubation period of 24 hours presented in the form of a histogram in figure 2.

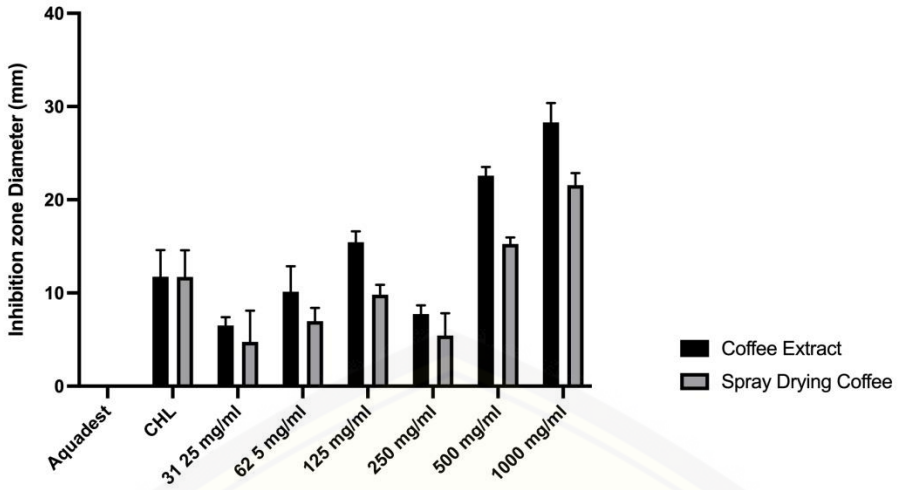

Figure 2. Histogram of the average diameter of the inhibition zone of coffee bean extract and instant coffee (spray drying) in each study group with an incubation period of 24 hours

Data analysis used was non-parametric statistical test Kruskall Wallis which is then continued with Mann-Whitney U test to know the differences among the research groups, p-value used for Kruskall Wallis and Mann-Whitney test in this study was 0.05. The result of data analysis using Kruskall Wallis test returned a significance value of data p = 0.000 ($p<0.05$). This data showed that coffee bean extract and instant coffee (spray drying) had antibacterial activity against P. gingivalis.

Discussion

Coffee is a drink that has been consumed since the time of our ancestors and is currently one of the world's favorite drinks. Raw coffee beans are rich in bioactive compounds such as chlorogenic acid, trigonelline, caffeine, and flavonoid compounds.[21] The benefits contained in coffee beans are anti-inflammatory, antioxidant, antifungal, and antibacterial.[22] Robusta coffee bean ethanol extract can inhibit the growth of dental plaque bacteria isolates in vitro.[4] The solvent extract uses ethanol because it is non-toxic, selective, miscible with water in all ratios, economical and universal, which is suitable for extracting all classes of secondary metabolites.

The antibacterial activity of the coffee bean extract was indicated by the presence of an inhibitory zone on the petridish around the paper disc. Table 1. shows that coffee bean extract concentrations of 1000 mg/ml, 500, mg/ml, 250 mg/ml, 125 mg/ml, 62.5 mg/ml, 31.25 mg/ml had antibacterial activity against P. gingivalis. Observations were made visually showing (irradicular zone) areas that looked infertile or more cloudy, which means that bacterial growth was not completely inhibited, so there were still some bacterial colonies. Coffee bean extract is bacteriostatic because it shows an irradical zone.[23]

Caffeine in robusta coffee bean extract acts as a DNA interchelator that causes cells to undergo mutations or genetic damage. This is because caffeine contains very high quaternary aromatic compounds.[24] Trigonelline acts as an anti-adsorption agent by inhibiting the adsorption of bacteria in saliva and reducing the adhesion of bacteria to the tooth surface. Trigonelline can inhibit bacterial growth by inhibiting the synthesis of bacterial enzymes and proteins.[25]

The mechanism of phenol of robusta coffee bean extract as an antibacterial is that the alcohol group on the phenol compound interacts with bacterial cells involving hydrogen bonds through the adsorption process and then damaging the cytoplasmic membrane which causes leakage of the cell nucleus in bacteria. In addition, phenols can poison cell protoplasm and

damage cell walls by precipitating microbial cell proteins. Phenol is a flavonoid compound that can damage bacterial cell walls through differences in polarity between the lipids that make up DNA and the alcohol groups in flavonoid compounds.[26]

Different coffee processing before consumption affects the taste, aroma, and composition. Coffee beans that are commonly consumed are coffee beans that have been roasted. In the current era, coffee is produced without leaving any residue when brewed, namely instant coffee. The stages of instant coffee processing that are often consumed by the public consist of roasting, grinding, extraction, drying (spray drying). Drying process (spray drying) by spraying aqueous coffee extract using high temperatures and drying the extract into instant coffee powder that is easily soluble in water.[27] During the green coffee bean roasting process, changes in coffee content both physically and chemically occur.

That instant coffee (spray drying) concentrations of 1000 mg/ml, 500 mg/ml, 250 mg/ml, 125 g/ml, 62.5 mg/ml, 31.25 mg/ml had antibacterial activity against P. gingivalis with values of 21.6 mm, 15.3 mm, 12.4 mm, 9.8 mm, 6.9 mm, 4.8 mm, respectively. The results of the research on the diameter of the inhibition zone are in line with another study by Rahman et al.[6] the higher the concentration of instant coffee, the fewer bacteria will grow. During the roasting process, melanoidins, glyoxal, methylglyoxal, diacetyl, and dicarbonyl compounds are formed which can inhibit the adsorption of streptococcus mutans in saliva which can reduce the adhesion of bacteria to the tooth surface through the Maillard reaction process.[28] The antibacterial activity of instant coffee (spray drying) can inhibit bacterial growth. The non-enzymatic browning reaction between reducing sugars and amino acids that occurs during coffee processing is called the Maillard reaction.

The thermolabile compounds of chlorogenic acid and trigonelline in coffee beans that have been roasted are degraded so that their levels are lower than in green coffee beans. Coffee becomes very dark and produces only a small fraction of its original trigonelline content due to roasting.[29]

The content of chlorogenic acid in robusta green coffee beans is 6.1-11.3 mg per gram of coffee beans, but when heating or roasting at a temperature of 180-200°C causes changes in chemical composition and biological activity as a result of the Maillard reaction.[30] Chlorogenic acid compounds it has the property of being easily hydrolyzed into compounds that are easily soluble in water at high temperatures. In addition, in the roasting process, the chlorogenic acid isomer is easily decomposed into free radical compounds at high temperatures.[31] Chlorogenic acid of green coffee was higher than that of roasted coffee. This is proven in his research that green coffee is stronger to increase the viability of PBMC and salivary leukocytes than roasted coffee.[32]

In the process of making instant coffee, there is a heat treatment that causes the breakdown of complex bonds of caffeine to take place more quickly. The breakdown of caffeine compounds becomes freer with a smaller size, easy to move, and diffuses through cell walls, and dissolves in solvents.[33]

The results showed that the irradical zone due to antibacterial activity can be influenced by the characteristics of the bacteria, including the type, age, concentration, and condition of the bacteria. P. gingivalis cell wall arrangement of lipoprotein, lipopolysaccharide, and peptidoglycan. The inner layer (peptidoglycan) in gram-negative is thinner, but has a more complex outer membrane layer so that the active compound is more difficult to penetrate the cell wall of gram-negative bacteria. The gram-negative peptidoglycan layer is adjacent to the cytoplasmic membrane and outer membrane. The gram-negative outer membrane is composed of phospholipids and lipopolysaccharides and forms a hydrophilic permeability barrier that provides protection against hydrophobic antibacterials.[34] The high lipid content of gram-negative bacteria 11-12% allows it to prevent antibacterial compounds from entering it.

Conclusion

Based on the research results of coffee bean extract and instant coffee (spray drying) robusta coffee (C. canephora) concentrations of 1000mg/ml, 500mg/ml, 250mg/ml, 125mg/ml, 62.5mg/ml, 31, 25mg/ml has the ability to inhibit P. gingivalis. The antibacterial power of coffee bean extract is more effective than instant coffee (spray drying). Antibacterial activity of coffee bean extract and instant coffee is indicated by the irradical zone which is a bacteriostatic indicator.

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Conflict of Interest
The authors report no conflict of interest.

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