The Potential of Robusta Coffee Bean Gel as a Non-Pharmacological Material to Inhibit the Growth of Porphyromonas gingivalis Bacteria That Cause Periodontitis (laboratory study)

Ardi Mohamad Bukhari, Supriyana, Lanny Sunarjo, Suharyo Hadisaputro, Endah Aryati Ekoningtyas

Posgraduate Program of Dental and Oral Therapist, Semarang Health Polytechnic, Indonesia

Abstract: **Chronic periodontitis is a multifactorial infectious disease of the dental support tissue caused by microorganisms. *Porphyromonas gingivalis* is a bacterium that has an important role in the initiation, growth, and severity of chronic periodontitis. So far, periodontitis treatment is carried out with scaling therapy or using chemical topical antibacterial agents such as aloclair gel. This topical antibacterial ingredient has side effects. For this reason, it is necessary to conduct research to prove herbal ingredients as a potential to inhibit the bacteria *Porphyromonas gingivalis*, the cause of periodontitis which is expected to contribute to dental therapy services. The purpose of this study was to prove robusta coffee bean gel alkaloid and flavonoids compounds of various concentrations in inhibiting the growth of *porphyromonas gingivalis*.The study used *True Experiment post test only control group design*. The subjects used the bacteria *porphyromonas gingivalis* ATCC 33277 with Suspense *Mc Farland* 0.5. Inhibitory power test using disc diffusion method. The Results of Robusta coffee bean gel alkaloid compounds all concentrations of 0.39%, 0.78%, 1.56% have inhibitory power with a weak category (<5mm) and are less effective in inhibiting the growth of *porphyromonas gingivalis* bacteria and robusta coffee bean gel flavonoid compounds concentrations of 0.39% and 0.78% have inhibitory power with a weak category (<5 mm), while a concentration of 1.56% has an inhibitory power with a moderate category (5-10 mm). This study Conclude Robusta coffee alkaloid and flavonoid compounds concentrations of 0.39%, 0.78%, 1.56% have the potential to inhibit the growth of porphyromonas gingivalis bacteria statistically meaningfully (ANOVA, p<0.05).**

Keyword: **Robusta Coffee, Porphyromonas gingivalis, periodontitis, Gel, Bacteria**

I. INTRODUCTION

According to the Regulation of the Minister of Health Number 89 of 2015 concerning dental and oral health efforts, dental and oral health is a healthy state of hard tissue and soft tissues of teeth.[1] *By The Global Burden of Disease Study 2016* health problems experienced by almost half of the psyche population. periodontal disease affects approximately 20-50% of the population worldwide.[2]

Periodontal disease ranks eleventh most common in the world [3]. The results of Basic Health Research (Riskesdas) in 2018 showed that Indonesians who have dental and oral health problems are 57.6% of the total 267 million people, the prevalence of periodontitis in Indonesia is still fairly high at 74.1%, in Central Java Province is a province with quite high dental and oral problems of 25.4%, only 31.0% of people get treatment and treatment from the medical team, and the largest proportion of dental health problems in Indonesia are cavities while the majority of dental and oral health problems experienced by the Indonesian population are swollen gums [4]

Periodontal disease initially occurs with symptoms of gingivitis that are not treated will be periodontitis.[5] Periodontitis is an inflammatory condition that affects 10%-14% of the adult population that causes chronic pain and loss of teeth and dental support tissue Plaque is one of the etiologies that functions in causing periodontal disease.[3] The bacteria in the dental plaque will spread and develop over time, so the toxin produced by the bacteria will irritate the gingiva and damage other dental support tissues

Periodontitis treatment so far is carried out with scaling therapy or using chemical topical antibacterial agents. This topical antibacterial ingredient has side effects such as burning sensation and is relatively expensive. Therefore, it is necessary to provide alternative anti-inflammatory ingredients using natural ingredients that are commonly found in Indonesia such as robusta coffee. Active substances that accelerate the wound healing process include flavonoids, polyphenols, and proantisianidin.

Robusta coffee beans contain active compounds such as caffeine (alkaloids), phenolic compounds (flavonoids), trigenoline and chlorogenic acid which are antibacterial, in addition to being antibacterial, the compounds in Robusta coffee beans also have antioxidant and anti-inflammatory effects

II. RESEARCH METHODE

The type of research used in this study is in the form of *true experiment with post test only control* group using Complete Randomized Design (RAL). This design was chosen because it was grouped into treatment and control groups and samples were not pretested before intervention. The study was conducted in the Microbiology laboratory of the Faculty of Medicine of Unissula in June-August. In this study, repetition

was carried out to avoid human errors, followed by the Federer Formula formula $(t - 1)(r - 1) \geq 15$. The research procedure begins after the bacteria are suspended to the agar medium (SDA). Each petri dish at the bottom is written using a marker.

A39 is (robusta coffee bean alkaloid gel with a concentration of 0.39%), A78 is (robusta coffee bean alkaloid gel with a concentration of 0.78%) and A56 is (robusta coffee bean alkaloid gel with a concentration of 1.56%), FL39 is (robusta coffee bean flavonoid gel with a concentration of 0.39%), FL78 Is (robusta coffee bean flavonoid gel with a concentration of 0.78%) and FL56 (robusta coffee bean flavonoid gel with a concentration of 1.56%), K+ (Positive Control) Aloclair Gel and K- (Negative control) Aquabides Each treatment and control was carried out for 3 repetitions.

The results of measuring the diameter of the resistance zone were compared between the treatment group, the control group (aquabides). To obtain reports on each group which is said to be sensitive (high inhibiting), intermediate (medium inhibiting), and resistant (low inhibiting, sensitive when an organism responds antibacterial at a set concentration. Intermediates are used as zones or medium sensitivity. Resistant, it is considered that the organism is thought not to respond to antibacterials

Data analysis using the SPSS (Statistical Package for the Social Science) program version 20. Perform a data normality test with the Shapiro-Wilk test. If the meaningfulness value ($p<0.05$) then the data is normally distributed. The data obtained were normally distributed to the One Way ANOVA test to see whether or not there was signification of differences between the treatment groups. Furthermore, a follow-up test was carried out in the form of a Post Hoc test to find out which treatment groups had different effects

III. RESULTS

Inhibitory Power of Robusta Coffee Bean Gel Concentration Variations (Alkaloids and Flavonoids) Against Porphyromonas Gingivalis

Table 1. Average Inhibitory Power of Variants of Concentration of Alkaloid Gel and Flavonoid Gel of robusta Coffee Beans against Porphyromonas gingivalis

Treatment	Observation of inhibition-free areas (once every 24 hours in mm units)			Average
	I	II	III	
Aquades (-)	0	0	0	0
Gel Aloclair (+)	13,66	14,5	17,5	15,22
Gel Alkaloid Konsentrasi 0.39%	1.25	1,75	1,5	1,625
Gel Alkaloid Konsentrasi 0.78%	2,25	2,58	2,83	2,55
Gel Alkaloid Konsentrasi 1.56%	2,83	3,16	4,16	3,38
Gel Flavonoid Konsentrasi 0.39%	3,91	4	5,09	4,33
Gel Flavonoid Konsentrasi 0.78%	4,25	4,5	4,75	4,5
Gel Flavonoid Konsentrasi 1.56%	5,75	7	6,75	6,5

Tabel 1 the above shows that the average inhibitory zone of alkaloid gel and flavonoid gel robusta coffee beans has inhibitory power in the concentration variant against the growth of Porphyromonas gingivalis within 24 hours for 3 repetitions. At the lowest gel concentration of 0.39% to a high concentration of 1.56% showed an increase in inhibitory power against Porphyromonas gingivalis

Inhibitory Power of Gingival Porphyroomonas between treatment groups

Table 2. Normality Test Results Data Inhibition Zone Growth of Porphyromonas gingivalis with Alkaloid Gel treatment

Inhibitory Power	Treatment	Shapiro-Wilk (Sig.(p))	Sig.(p)
Porphyromonas gingivalis	Gel Aloclair (+)	.400	0.004
	Gel Alkaloid Konsentrasi 0.39%	.215	
	Gel Alkaloid Konsentrasi 0.78%	.200	
	Gel Alkaloid Konsentrasi 1.56%	.339	

Table 2 above shows the sig values. in the Shapiro-Wilk test, > 0.05 (α) in all treatment groups, so that it can be concluded that all data are normally distributed and homogeneous data variants are seen from Levene statistical sig values.= .226 > 0.05 (α), which means that the data variants between groups of treatment of porphyromonas gingivalis inhibitory are inhomogeneous, so that they can be continued to alternative trials

Table 3. Normality Test Results Data on Growth Inhibition Zone of Porphyromonas gingivalis with Flavonoid Gel treatment

Inhibitory Power	Treatment	Shapiro-Wilk (Sig.(p))	Sig.(p)
Porphyromonas gingivalis	Gel Aloclair (+)	.400	0.006
	Gel Flavonoid Konsentrasi 0.39%	.215	
	Gel Flavonoid Konsentrasi 0.78%	.200	
	Gel Flavonoid Konsentrasi 1.56%	.339	

Table 3 above shows the sig values. in the Shapiro-Wilk test, > 0.05 (α) in all treatment groups, so that it can be concluded that all data are normally distributed and homogeneous data variants are seen from Levene statistical sig values.= .226 > 0.05 (α), which means that the data variants between groups of treatment on the inhibitory power of Porphyromonas gingivalis are homogeneous, so that they can be continued to alternative bonferroni tests.

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Table 4. Anova Test Results of the Growth Inhibition Zone of Porphyromonas gingivalis

Variable	Treatment	N	*Sig**
Inhibitory Power	Gel Alkaloid 0.39%	3	0,000
	Gel Alkaloid 0,78%	3	
	Gel Alkaloid 1,56%	3	
	Gel Aloclair	3	
	Gel Flavonoid 0.39%	3	0,000
	Gel Flavonoid 0.78%	3	
	Gel Flavonoid 1,56%	3	
	Gel Aloclair	3	

***** Tested with Anova (sig.p < 0.05)

Table 4 shows that all compounds have significant differences in the growth inhibitory power of porphyromonas gingivalis with ($p<0.05$)

IV. DISCUSSION

Mechanism of alkaloid compounds in Robusta Coffee Bean Gel (Coffea canepora) against the growth of Porphyromonas Gingavlis bacteria that cause periodontitis

The results showed that after exposure to robusta coffee bean gel alkaloid compounds for 24 hours which were divided into 3 concentrations The higher the inhibitory power ability which is characterized by a clear zone around the disk on the SDA media. Figure 1 shows that robusta coffee bean gel can inhibit porphyromonas gingivalis within 24 hours with an average inhibitory zone diameter on the robusta coffee bean alkaloid gel concentration of 0.39% = 1.6 mm, concentration of 0.78% = 2.55 mm and concentration of 1.56% = 3.38 mm

Statistically alkaloid compounds (1.359%) robusta coffee bean extract There is no significant difference between alkaloid compounds 0.39%, with alkaloids 0.78%, and alkaloids 1.56%. As well as alkaloids of 0.78% with 1.56% indicating that there is no significant difference ($p > 0.05$) which means that alkaloid compounds have weak inhibitory power. Statistically, robusta coffee beans have inhibitory power with a weak category, this is in accordance with previous studies alkaloids in robusta coffee beans have moderate to weak antibacterials. It was concluded that the alkaloid compounds of robusta coffee bean gel at concentrations of 0.39%, 0.78%, and 1.56% provide inhibitory power through a mechanism of action able to inhibit protein ploriferation and respiation of porphyromonas gingivalis bacteria.

Alkaloid compounds in robusta coffee beans can inhibit bacteria, according to the results of previous studies said alkaloid compounds are one of the ingredients in coffee beans that can inhibit bacterial growth, where robusta coffee beans have a content of 1.6%-2.4%.[6] Alkaloids are alkaline nitrogen compounds present in plants. Alkaloids are physiologically active basic compounds of plant origin where there is one nitrogen atom in their cyclic structure.[7] Free alkaloids in organic solvents such as chloroform, relatively nonpolar solvents, mixed solvents, and low alcohol and how alkaloids work in inhibiting gingival porphyromonas by disrupting the peptidoglycan component of cells so as to damage the cell wall in bacteria.[8]

The antibacterial activity of alkaloids is related to many factors, such as the content of the active substance in the extract, which is influenced by internal and external factors. Internal factors are influenced by plant genetics, while external factors are more influenced by plant physiology and ecology. Alkaloids or caffeine are crystalline substances that are white in color. Alkaloids in the content of robusta coffee beans are able to anchor the growth of bacteria. The ability of alkaloid compounds is greatly influenced by the biological activity of such compounds, which is due to the presence of nitrogen-containing alkaline groups. The existence of this alkaline group will react When it comes into contact with bacteria that have amino acid compounds that make up the cell wall and bacterial DNA which is the main constituent of the cell nucleus, with the damage to the DNA, the bacterial cell nucleus will be damaged. Cell damage to these bacteria over time will make bacterial cells unable to metabolize so that they will undergo lysis[9]. Thus inhibiting the growth of porphyromonas gingivalis bacteria that cause periodontitis

Mechanism of flavonoid compounds in Robusta Coffee Bean Gel (Coffea canepora) against the growth of Porphyromonas Gingavlis bacteria that cause periodontitis

The results showed that after exposure to robusta coffee bean gel alkaloid compounds for 24 hours which were divided into 3 concentrations The higher the inhibitory power ability which is characterized by a clear zone around the disk on the SDA media. Figure 1 shows that robusta coffee bean gel can inhibit porphyromonas gingivalis within 24 hours with an average diameter of the inhibitory zone in flavonoid gel obtained results with a concentration of 0.39% = 4.33 mm, a concentration of 0.78% = 4.5 mm and a concentration of 1.56% = 6.5%, Statistically confirmed flavonoid compounds (71.65%) concentrations of 0.39%, 0.78%, 1.56% indicate that there is no significant difference ($p > 0.05$) which means that flavonoid compounds have weak inhibitory power. Statistically robusta coffee beans at 3 significant concentrations this is due to a high range of average values. Statistically, robsuta coffee bean gel begins to provide inhibitory power at concentrations of 0.39%, 0.78%, 1.56% through the mechanism of action of inhibiting the function of cytoplasmic membranes and energy metabolism of bacterial cells porphyromonas gingivalis

Previous research has shown that flavonoid compounds in robusta coffee beans function as antibacterials.[10] Whose research results show robusta coffee bean extract has inhibitory power against bacteria. Flavonoids have a broad spectrum in inhibiting the growth of porphyromonas gingivalis bacteria.[6] Although the inhibitory power is not so strong, flavonoid compounds in robusta coffee beans have antibacterial potential because they can interfere with cell metabolism by inhibiting the transport of nutrients. This is in accordance with Pelzer and Chan in their research, that the higher the concentration of a bacterial material, the stronger its antibacterial activity will be

Forming complex compounds against extracellular protein compounds that interfere with the integrity of membranes and cell walls with concentrations and are in accordance with the workings of flavonoid compounds which are a group of compounds that have a basic framework structure of C6-C3-C6.[11] Each part of C6 is a benzona ring used with C3 atoms. Flavonoids are found in plants and products related to propolis and honey. In the leaves, flavonoids are useful as a physiological function of plants, namely guarding against bacteria and radiation. Flavonoids are useful in photosynthesis, energy transfer, growth hormone performance, control respiration and morphogenesis. Flavonoids are divided into 14 classes that are distinguished by their basic forms, such as flavones, isoflavones, and flavonols. Potential flavonoids are

thought to be the treatment of infections and bacteria, toxics, and diuretics.[12] Flavonoids are the largest group of phenols that can denature proteins and function as antibacterial genes. Flavonoids can cause damage to the cytoplasmic membrane by reducing the fluidity of the membrane, causing leakage, and producing hydrogen peroxide. The working system of flavonoids in inhibiting the synthesis of nucleic acids is by inhibiting topoisomerase. Antibacterial mechanisms are inhibited by the synthesis of ATP. Flavonoids with bacterial cell walls can cause damage to hydrogen bonds in cell wall proteins, if the metabolism is inhibited then the bacterial molecules do not develop into complex molecules, thus making the growth of porphyromonas gingivalis bacteria disturbed because it inhibits the function of cell membranes and can change the host's response in dealing with bacterial invasion which can prevent periodontitis from occurring

V. CONCLUSION

Robusta coffee bean gel alkaloid compounds of all concentrations of 0.39%, 0.78%, 1.56% have inhibitory power with a weak category (<5mm) and are less effective in inhibiting the growth of porphyromonas gingivalis bacteria that cause periodontitis, because the concentration used is so low that it cannot damage the DNA of bacterial cells.

Flavonoid compounds gel robusta coffee beans concentrations of 0.39% and 0.78% have inhibitory power with a weak category (<5 mm), because the concentration used is very low so that it cannot damage the bacterial cell wall while the concentration of 1.56% has an inhibitory power with a moderate category (5-10 mm), due to the biological activity of flavonoid compounds in damaging the bacterial cell wall, thus making the growth of porphyromonas gingivalis bacteria disturbed because it inhibits the function of cell membranes and can change the host's response in dealing with bacterial invasion which can prevent periodontitis from occurring.

Robusta coffee bean flavonoid gel has the potential as an alternative to prevent periodontitis caused by porphyromonas gingivalis bacteria because it has an inhibitory power with a moderate category and is able to damage cell walls, compared to alkaloid gels which are only able to have low inhibitory power, because they cannot damage DNA in bacterial cells.

REFERENCES

[1] Permenkes RI, "Peraturan Menteri Kesehatan Republik Indonesia Nomor 89 Tahun 2015 Tentang Upaya Kesehatan Gigi Dan Mulut," J. Teknosains, vol. 44, no. 8, p. 53, 2015.

[2] Y. Wang, "ePortfolios: A New Peer Assessment Technology in Educational Context," in 2008 International Symposiums on Information Processing, May 2008, vol. 1, no. 2, pp. 360–363, doi: 10.1109/ISIP.2008.139.

[3] N. J. Kassebaum et al., "Global, Regional, and National Prevalence, Incidence, and Disability-Adjusted Life Years for Oral Conditions for 195 Countries, 1990–2015: A Systematic Analysis for the Global Burden of Diseases, Injuries, and Risk Factors," J. Dent. Res., vol. 96, no. 4, pp. 380–387, Apr. 2017, doi: 10.1177/0022034517693566.

[4] Kemenkes RI, "Riskesdas 2018," Kemenkes RI. 2018.

[5] N. Nwizu, J. Wactawski-Wende, and R. J. Genco, "Periodontal disease and cancer: Epidemiologic studies and possible mechanisms," Periodontol. 2000, vol. 83, no. 1, pp. 213–233, Jun. 2020, doi: 10.1111/prd.12329.

[6] H. A. Tanauma, G. Citraningtyas, and W. A. Lolo, "AKTIVITAS ANTIBAKTERI EKSTRAK BIJI KOPI ROBUSTA (Coffea canephora) TERHADAP BAKTERI Escherichia coli," vol. 5, no. 4, pp. 243–251, 2016.

[7] R. F. Tjandra, . Fatimawali, and O. S. Datu, "Analisis Senyawa Alkaloid dan Uji Daya Hambat Ekstrak Buah Sirih (Piper betle L) terhadap Bakteri Staphylococcus epidermidis," J. e-Biomedik, vol. 8, no. 2, pp. 173–179, Aug. 2020, doi: 10.35790/ebm.v8i2.28963.

[8] dkk. 2020 A.R. Pratiwi, "Uji Bioaktivitas Minyak Cengkeh (Syzygium aromaticum) Terhadap Pertumbuhan Bakteri Streptococcus mutans Penyebab Karies Gigi," J. Biol. Makasar, vol. 5, no. 2, pp. 241–250, 2020.

[9] M. A. Setiawan, M. D. Retnoningrum, F. Yahya, R. R. Andika, and D. H. A. Sudarni, "AKTIVITAS ANTIBAKTERI EKSTRAK BIJI JERUK SIAM (Citrus reticulata) PADA BAKTERI Escherichia coli," J. Bioteknol. Biosains Indones., vol. 7, no. 2, 2020, doi: 10.29122/jbbi.v7i2.4194.

[10] T. Ermawati, R. C. Prasetya, N. Fatimatuzzahro, and A. G. N. Iffah, "Efek Gel Ekstrak Biji Kopi Robusta (Coffea canephora) terhadap Jumlah Sel Makrofag dan Limfosit Jaringan Gingiva Tikus Periodontitis," Insisiva Dent. J. Maj. Kedokt. Gigi Insisiva, vol. 9, no. 2, pp. 46–51, 2020, doi: 10.18196/di.9217.

[11] A. Paliling, J. Posangi, and P. S. Anindita, "Uji daya hambat ekstrak bunga cengkeh (Syzygium aromaticum) terhadap bakteri Porphyromonas gingivalis," e-GIGI, vol. 4, no. 2, Nov. 2016, doi: 10.35790/eg.4.2.2016.14159.

[12] V. Mierza and Sudewi, "Antibacterial Activity Mouthwash Preparation Ethanol Extract of Cardamom (Amomum compactum Sol. ex Maton) For Growth of Bacteria Staphylococcus aureus And Streptococcus mutan," J. Pharm. Sci., vol. 3, no. 1, pp. 51–57, 2020.