Buletin Kedokteran & Kesehatan Prima
Vol.3 No.1 (2024)
DOI: 10.34012/bkkp.v3i1.5710

ORIGINAL ARTICLE

Inhibitory ability of robusta coffee bean extract against *Staphylococcus epidermidis*

Clarissa Lister[1], Rapael Ginting[2*], Endah Ansylla Panggabean[3], Ermilia Laurensya Tarigan[3]

ABSTRACT

Background: Treatment of infections generally uses chemical antibiotics, but chemical antibiotics have effects such as increasing toxicity to the body. The solution to overcome this problem is to use antibiotics from natural ingredients, such as coffee. **Objective:** This study aims to determine the effectiveness of robusta coffee bean extract (*Coffea canephora*) in inhibit the growth of *Staphylococcus epidermidis* bacteria. **Methods:** This research is an experimental study with a post test only control group design. There were 10 groups in this study, namely 2 control groups and 8 treatment groups. The technique used is disc diffusion to see the role of robusta coffee bean ethanol extract in inhibiting the growth of *Staphylococcus epidermidis* bacteria. The data was analyzed using the Mann-Whitney test to determine the difference between treatments. **Results:** The results of the Mann-Whitney test showed that there were differences in the average inhibition zone of Robusta coffee bean extract and the inhibitory power at each concentration ($p<0.05$). The concentration that has the highest inhibition is the concentration of 500 mg/ml. **Conclusion:** It can be said that there is an effectiveness of robusta coffee bean extract in reducing *Staphylococcus epidermidis* bacteria because the specified concentration inhibition zone is obtained.

Keywords: breast tumor, cytology, histopathology, ca. mamae

Introduction

Nosocomial infection is an infection that occurs when a patient is receiving medical care for an infection in a hospital. In addition, nosocomial infections are associated with length of stay and increased costs of care, especially in low and middle-income countries.[1,2] The proportion of patients with the most nosocomial infections occurring in the ICU was 51%, followed by patients with burns and patients undergoing transplantation.[3] As many as 1.4 million deaths per day in the world are caused by nosocomial infections.[4] In developing countries including Indonesia, the average the prevalence of nosocomial infections is around 9.1% with a variation of 6.1%-16.0%.[5] The prevalence rate of nosocomial infections in North Sumatra is 5.6% of cases.[6]

There are 43 pathogens that cause nosocomial infections, Which one of it is *Staphylococcus epidermidis* (gram-positive bacteria).[7] Infections caused by *Staphylococcus epidermidis* are opportunistic infections (attacking individuals with a weak immune system).[8] Patients undergoing treatment with surgery,

Affiliation
[1]Department of Medical Education, Universitas Prima Indonesia, Medan, Indonesia
[2]Departement of Epidemiology, Universitas Prima Indonesia, Medan, Indonesia
[3]Undergraduate Programme in Public Health, Universitas Prima Indonesia, Medan, Indonesia

***Korespondensi:**
rafaelginting@unprimdn.ac.id

catheter insertion, intravascular or transplantation are a population that is susceptible to exposure to *Staphylococcus epidermidis* bacteria. However, some patients undergoing treatment develop antibiotic resistance.[9] Most antibiotic resistance occurs in patients who use catheters because of the formation of microbial biofilms. Biofilm formation increases antibiotic resistance and decreases drug sensitivity.[10] The use of chemical antibiotics is often resistant, therefore another alternative is used, namely using natural, plant-based antibiotics. One of the natural ingredients that have anti-bacterial activity is robusta coffee beans. Caffeine components of volatile, non-volatile acids, phenols and aromatic compounds in coffee bean extract have antimicrobial effects against gram-positive and negative bacteria.[11] Coffee contains antibacterial compounds such as caffeine, phenolic, trigonelline, and chlorogenic acid. Robusta coffee bean extract can reduce the growth of *Staphylococcus epidermidis* bacteria. At a concentration of 25% resulted in an average inhibition zone of 6.53 mm with a strong inhibition category. At a concentration of 50% resulted in an average inhibition zone of 7.53 mm with a strong inhibiting category. At a concentration of 100% it produces an average inhibition zone of 8.21 mm with a strong inhibition category.[12]

The results of previous studies prove that coffee extract has antioxidant and antimicrobial effects using nano emulsion techniques. Coffee has bioactive compounds as antifungals and bacteria such as *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Escherichia coli*. After testing with formulations F7 and F16 and incubation at 37°C and found an inhibition zone with a range of 182 nm to 707 nm, with an average of 266 nm, and the conclusion is that each formula has antibacterial effectiveness.[13] With the addition of 70% ethanol, it was concluded that robusta coffee bean extract had antibacterial activity against *Staphylococcus epidermidis* and *Salmonella typhi* at concentrations of 3.125%, 6.25%, 12.5%, and 25%.[14] Extract robusta coffee bean ethanol is able to inhibit the growth of *Staphylococcus epidermidis* bacteria at concentrations of 50% and 100%.[15] In this study, the test used concentration groups, namely 500 mg/ml, 400 mg/ml, 300 mg/ml, 200 mg/ml, 100 mg/ml, 75 mg/ml, 50 mg/ml, and 25 mg/ml to determine the inhibition zone of robusta coffee bean extract. The organic compound used is 96% ethanol because it is a volatile polar compound so it is good to be used as an extracting solvent.

Method

Study Design

The type of research used is a true experimental design with a post test only control group design. Subjects were divided into two or more groups randomly. There were 10 groups in this study, namely 2 control groups and 8 treatment groups. The technique used is disk diffusion to see the role of robusta coffee bean ethanol extract in inhibiting the growth of *Staphylococcus epidermidis* bacteria. The research process lasts for one month and began on September 21, 2021.

Materials and Tools

The materials used include dry robusta coffee beans, nutrient browth, Mueller hinton agar, sterile distilled water, 96% ethanol, spritus, chloramphenicol antibiotics, chloramphenicol disk antibiotics, blank disks, *Staphylococcus epidermidis* bacteria, sterile distilled water, chloroform, ammonia, H_2SO_4, $FeCl_3$, HCl 1 N, NaCl solution and filter paper. The tools used include a shaker bath, rotatory evaporator, cotton swabs, sterile cotton sticks, petridish, ose needles, Bunsen, lighters, test tubes, tube racks, vials, scales/balances, Erlenmeyer tubes, glass beakers, glass spatulas, syringe, caliper, sterilizer, autoclave, micro pipette, micropipette tip, tweezers, incubator, laminar flow, thermolyne, desiccator, sterile driller (5mm diameter), vortex, aluminum foil, filter paper (whatman brand) and a timer.

Procedure

Tools preparation

All tools that will be used are washed first and then dried, for tools made of glass are sterilized using a sterilizer at 120^0C for 15 minutes and those made of plastic are wiped using sterile distilled water.

Making robusta coffee bean extract

Weighed 1.100 grams using a digital balance and then macerated with 8.5 liters of 96% ethanol solution in a vessel, macerated 3x24 hours, stirring occasionally (3 times a day). Filtered using a filter cloth to obtain maserat. Then it was concentrated using a rotary evaporator to obtain a thick extract.

Phytochemical screening

Phytochemical screening refers to Harborne's method (1987) with seven secondary metabolite tests including alkaloid, flavonoid, tannin, saponin, quinone, steroid, and triterpenoid. In the alkaloid test, extracts containing alkaloids will produce a precipitate. A total of 1-2 ml of extract was added to 2 ml chloroform ($CHCl_3$) and 2 ml of ammonia (NH_3), shaken, and filtered. The filtrate was added 3-5 drops of concentrated H^2SO_4, then shaken until two layers were formed. The acid layer was taken and tested with 4-5 drops of Mayer, Wagner and Dragendorf reagents. If a precipitate is formed, it indicates alkaloids, with Mayer's reagent giving a white precipitate, Wagner's reagent the precipitate is brown and the Dragendroff's reagent the precipitate is orange-red. The flavonoid test was carried out by adding a few drops of lead acetate solution to the extract. The formation of a yellow precipitate indicates flavonoids. In the phenolic test, as much as 1-2 ml of the extract was added to 10 drops of 1% $FeCl_3$. The formation of a green, red, purple, blue, or solid black precipitate indicates phenol. While in the tannin test, 1-2 ml of the extract was added 2-3 drops of 10% $FeCl_3$. The formation of a greenish color indicates tannins. A total of 1-2 ml of the extract was added to 10 ml of distilled water and shaken for 1 minute, then 2 drops of HCl 1 N were added and shaken vigorously to form a foam after being allowed to stand for 10 minutes, indicating saponins. The triterpenoid-steroid test was carried out by adding 10 drops of glacial acetic acid and 2 drops of concentrated sulfuric acid in 1-2 ml. The solution was shaken gently and left for a few minutes. Steroids give a blue or green color, while triterpenoids give it a red or purple color.

Mueller Hinton media creation

Dissolve 6.46 grams of Mueller Hinton agar into 170 ml of distilled water in an Erlenmeyer tube. Stirred using a stirrer until evenly distributed and heated until boiling until all dissolved into one. After that, it was sterilized in an autoclave at 121°C for 15 minutes. Then poured into a Petri dish as much as 15 ml and level. Then put it in an incubator with a temperature of 37°C. And when it is finished, it is stored at 8-15°C if it is not used immediately.

Giving treatment

All treatments were carried out in laminar flow to prevent contamination with the external environment. At the bottom of each Petri dish, labeled paper according to the given concentration, positive control with chloramphenicol suspension, negative control with 10 liters sterile distilled water. After the dry disc is inserted into the Petri dish containing the Staphylococcus epidermidis bacteria culture according to the labeled place. Then it was incubated for 24 hours at 37°C.

Data Analysis

The data was analyzed first using the normality test to determine the normality of the data. If the data is normally distributed and homogeneous, then the One Way ANOVA test can be performed. If the data is not normal, then the Kruskal-Wallis test is carried out followed by the Mann-Whitney test to determine the difference between treatments.

Results

Researchers conducted a phytochemical test to determine the secondary metabolite compounds contained in robusta coffee beans. The test results show that the secondary metabolites contained in robusta coffee beans are phenols, flavonoids, alkaloids, saponins, tannins, and steroids. The test results showed a concentration of 500 mg/ml had the highest inhibition zone of (16 mm) in treatment 1, (15 mm) in treatment 2, (14 mm) in treatment 3, and 12 mm in treatment 4, while the inhibition zone was the most. The small size was at a concentration of 50 mg/ml (6 mm) in the second treatment.

The average value of the effectiveness of robusta coffee bean extract in reducing *Staphylococcus epidermidis* bacteria at a concentration of 500 mg/ml is 14.25, a concentration of 400 mg/ml is 11.00, a concentration of 300 mg/ml is 8.00, a concentration of 200 mg/ml is 5 .25, the concentration of 100 mg/ml was 1.50, the concentration of 75 mg/ml was 1.25, the concentration of 50 mg/ml was 1.50, the concentration of 25 mg/ml was 0.00, the control + (Chloramphenicol) was 17 .00 and control – (Aquades) of 0.00 (see Table 1).

In table 2, it can be seen that only at a concentration of 500 mg/ml was normally distributed, while at other concentrations the distribution was not normal. So it can be concluded that the data is not normally distributed and therefore for the bivariate test using the Kruskal-Wallis test.

Table 1. Inhibition zone of robusta coffee extract in the treatment group and control group

Group (mg/ml)	Treatment				Mean±SD
	1	2	3	4	
	Inhibition Zone (mm)				
Treatment Group					
500 mg/ml	16 mm	15 mm	14 mm	12 mm	14,25 ± 1,708
400 mg/ml	11 mm	11 mm	11 mm	11 mm	11,00 ± 0,000
300 mg/ml	7 mm	9 mm	7 mm	9 mm	8,00 ± 1,155
200 mg/ml	5 mm	6 mm	5 mm	5 mm	5,25 ± 0,500
100 mg/ml	0 mm	6 mm	0 mm	0 mm	1,50 ± 3,000
75 mg/ml	0 mm	5 mm	0 mm	0 mm	1,25 ± 2,500
50 mg/ml	0 mm	6 mm	0 mm	0 mm	1,50 ± 3,000
25 mg/ml	0 mm	0 mm	0 mm	0 mm	0,00 ± 0,000
Control Group					
Control + (Cloramfenicol (3 mg/ml))	17 mm	17 mm	17 mm	17 mm	17,00 ± 0,000
Control – (Aquades (3 mg/ml))	0 mm	0 mm	0 mm	0 mm	0,00 ± 0,000

Table 2. Data Normality Test with Saphiro Wilk

Variable	Sig	Result
500 mg/ml	0.850	data is normally distributed
300 mg/ml	0.024	data is not normally distributed
200 mg/ml	0.001	data is not normally distributed
100 mg/ml	0.001	data is not normally distributed
75 mg/ml	0.001	data is not normally distributed
50 mg/ml	0.001	data is not normally distributed

In table 3 it can be seen that there is a significant difference in the robusta coffee bean extract between the two groups (p= <0.001) because it is worth <0.05. Furthermore, the Mann-Whitney test was carried out to determine the comparison of the inhibitory power at each concentration. The higher the concentration of robusta coffee bean extract, the greater the inhibition zone produced. From the results of the Mann-Whitney test, it was found that there were significant differences in inhibition at each concentration. The concentration that has the highest inhibition is the concentration of 500 mg/ml. However, when compared with control + (Chloramphenicol) then chloramphenicol is more effective.

Table 3. Kruskal-Wallis and Mann-Whitney test results

Robusta Coffee Bean Extract	*Kruskal-Wallis test*		*Mann-Whitney test*	
	n	p	Z	p
500 mg/ml	4		-2.460	0.014
400 mg/ml	4		-2.646	0.008
300 mg/ml	4		-2.494	0.013
200 mg/ml	4		-2.530	0.011
100 mg/ml	4	<0,001	-2.530	0.011
75 mg/ml	4		-2.530	0.011
50 mg/ml	4		-2.530	0.011
25 mg/ml	4		-2.646	0.008
Control + (Chloramphenicol)	4			

Discussion

This research was conducted to see the effectiveness of robusta coffee bean extract in reducing *Staphylococcus epidermidis* bacteria in various concentrations. The results showed that the higher the concentration, the higher the inhibition zone obtained by robusta coffee bean extract in reducing *Staphylococcus epidermidis* bacteria. In a previous study using the cylinder cup method, it was concluded that the highest inhibition zone was found at a concentration of 100% extract (8.2 mm) in the first treatment, 8.2 mm in treatment 2 and (8.25 mm) in treatment 3. The smallest inhibition was in the extract concentration of 25% (6.5mm) in the first treatment, 6.5 mm in the second treatment, 6.6 mm in the third treatment.[12] Study results showed the highest inhibition zone at 25% and the smallest inhibition zone was found at 3.125%.[14]

There is no inhibition zone in the control - (Aquades) while in the control + (Chloramphenicol) there is an inhibition zone of 17 mm in each treatment (see Table 1). This means that the use of chloramphenicol is still more effective than robusta coffee bean extract in inhibiting the growth of *Staphylococcus epidermidis* bacteria. Likewise with the results of previous studies, when compared with robusta coffee bean extract, chloramphenicol is more effective.[14] The inhibition zone on oxytetracycline is also more effective than robusta coffee bean extract.[12]

There were significant differences in inhibition at each concentration from the results of the Mann-Whitney test. The concentration with the highest inhibition is 500 mg/ml. However, chloramphenicol is more effective when compared to control + (chloramphenicol). Similar studies also concluded that the inhibitory zone formed will increase when the concentration of ethanol extract of robusta coffee beans is getting higher. The minimum inhibitory concentration in this study was at a concentration of 25 mg/ml.[16] Likewise with the results of the Yaqin & Nurmilawati[17] study which concluded that the higher the concentration of the solution, the higher the inhibitory zone power of robusta coffee bean extract for reduce the staphylococcal bacterial colony. The inhibition test used was the disc paper method which was dripped with robusta coffee bean extract. Robusta coffee bean extract has antibacterial and antioxidant compounds, namely caffeine, chlorogenic acid and flavonoids, thereby inhibiting the growth of *Staphylococcus epidermidis* ATCC 12228 at a concentration of 50 % and 100% with an average inhibition zone diameter of 6.8 mm to 9 mm.[15]

In another study it was proven that the concentration of robusta coffee beans maintains salivary pH and determines the minimum inhibitory and minimum killing levels of robusta coffee bean extract against *Staphylococcus aureus* bacteria. The best concentration of robusta coffee extract in maintaining salivary pH and useful as an antibacterial is a concentration of 12.5%.[18] Robusta coffee bean extract is also able to inhibit the growth of *Porphyromonas gingivalis* bacteria with an inhibition zone (14.68 mm) then at a concentration of 1.25% (17.51 mm) at a concentration of 1.5% (18.43 mm), and at a 3% concentration of (19.28 mm).[19] Zone diameter inhibitors were categorized into: weak <5 mm, moderate 5-10 mm, strong 10-20 mm, and very strong >20 mm. The concentration of the extract affects the rate of diffusion of the efficacious substances, the greater the concentration, the wider the diameter of the inhibition zone formed.[20]

Conclusion

It can be concluded that the ethanolic extract of robusta coffee beans is effective in reducing the growth of *Staphylococcus epidermidis* bacteria. The difference in the average inhibition zone of robusta coffee bean extract was significant at each concentration. Robusta coffee bean extract at a concentration of 500 mg/ml has the highest inhibition, which is 16 mm.

References

1. Davoudi AR, Najafi N, Hoseini Shirazi M, Ahangarkani F. Frequency of bacterial agents isolated from patients with nosocomial infection in teaching hospitals of Mazandaran University of Medical Sciences in 2012. Casp J Intern Med [Internet]. 2014;5(4):227–31. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25489435
2. Mohammed D, El Seifi OS. Bacterial nosocomial infections in neonatal intensive care unit, Zagazig University Hospital, Egypt. Egypt Pediatr Assoc Gaz. 2014;62(3–4):72–9.
3. Khan HA, Baig FK, Mehboob R. Nosocomial infections: Epidemiology, prevention, control and surveillance. Vol. 7, Asian Pacific Journal of Tropical Biomedicine. Elsevier B.V.; 2017. p. 478–82.
4. Rafi'ah R, Hariyanto D. Analysis of nurse's efforts to control nosocomial infections in the COVID-19 pandemic period in hospital. J Keperawatan Respati Yogyakarta [Internet]. 2021;8(1):1–5. Available from: http://nursingjurnal.respati.ac.id/index.php/JKRY/article/view/583
5. Irdan. Factors related to nosocomial infection (INOS) by nurses at IRNA Surgery at Kayu Agung Hospital, OKI Regency in 2017. In: Seminar Nasional dan Diseminasi Penelitian Kesehatan STIKes Bakti Tunas Husada Tasikmalaya. 2018.
6. Siregar IS. Description of nursing student knowledge about transmission of nosocomial infections at PTPN II Bangkatan Binjai Hospital. J Ris Hesti Medan. 2017;2(1):54–9.
7. He Y, Li W, Wang Z, Chen H, Tian L, Liu D. Nosocomial infection among patients with COVID-19: A retrospective data analysis of 918 cases from a single center in Wuhan, China. Vol. 41, Infection Control and Hospital Epidemiology. 2020. p. 982–3.
8. Warmasari NWM, Ernawati DK, Indrayani AW, Dewi NWS, Jawi IM. Antibacterial Activity From Temulawak Extract (Curcuma Xanthorrhiza Roxb) on Growth Inhibition of Staphylococcus Epidermidis in Vitro. J Epidemiol Kesehat Komunitas. 2020;5(1):1–7.
9. Nguyen TH, Park MD, Otto M. Host response to Staphylococcus epidermidis colonization and infections. Vol. 7, Frontiers in Cellular and Infection Microbiology. 2017. p. 1–7.
10. Nouri F, Karami P, Zarei O, Kosari F, Alikhani MY, Zandkarimi E, et al. Prevalence of common nosocomial infections and evaluation of antibiotic resistance patterns in patients with secondary infections in Hamadan, Iran. Infect Drug Resist. 2020;13:2365–74.
11. Yi T, Shah M, Raulji D, Dave D. Comparative Evaluation of Antimicrobial Efficacy of Coffee Extract and 0.2% Chlorhexidine Mouthwash on the Periodontal Pathogens *Porphyromonas Gingivalis*, *Prevotella Intermedia*, *Fusobacterium Nucleatum* and <i>Aggregatibacter Actinomyc. Adv Hum Biol [Internet]. 2016 May 1;6(2):99–103. Available from: https://www.aihbonline.com/article.asp?issn=2321-8568
12. Setiawan MA, Tee SA. Inhibition test of robusta coffee bean extract (Coffea robusta) against Staphylococcus epidermidis. War Farm. 2017;6(1):12–8.
13. Buzanello EB, Pinheiro Machado GTB, Kuhnen S, Mazzarino L, Maraschin M. Nanoemulsions containing oil and aqueous extract of green coffee beans with antioxidant and antimicrobial activities. Nano Express. 2020;1(1):010058.
14. Ranasatri AA, Mahmudah N, Aisyah R, Sintowati R. Antibacterial activity of 70% ethanol extract of robusta coffee beans (Coffea canephora) against Staphylococcus epidermidis and Salmonella typhi. Biomedika. 2021;13(2):101–10.
15. Widyasari PAM, Aman I, Mahendra AN. Aktivitas antibakteri ekstrak etanol biji kopi robusta (Coffea canephora) terhadap bakteri Staphylococcus epidermidis ATCC 12228 penyebab infeksi nosokomial. E-Jurnal Med Udayana. 2021;10(6):74–8.

16. Prasetya RC, Agustina D, Firdaus J. Effectiveness of robusta coffee bean (Coffea cannephora) ethanol extract in inhibiting the growth of Salmonella typhi in vitro. Universitas Jember; 2015.
17. Yaqin MA, Nurmilawati M. Effect of robusta coffee extract (Coffea robusta) as a growth inhibitor of Staphylococcus aureus. In: Seminar Nasional XII Pendidikan Biologi FKIP UNS 2015. 2015. p. 867–72.
18. Lubis MRF, Lindawati Y. Effect of robusta coffee bean extract (Coffea canephora) on salivary pH and the growth of Staphylococcus aureus (ATCC® 29213™) (in vitro). J Ilm PANNMED (Pharmacist, Anal Nurse, Nutr Midwivery, Environ Dent. 2019;12(3):309–12.
19. Dianastri RNT, Astuti P, Prasetya RC. Inhibitory Power of Robusta Coffee Bean Extract (Coffea Canephora) against Porphyromonas gingivalis Bacteria (in vitro). STOMATOGNATIC - J Kedokt Gigi. 2021;18(2):69.
20. Suhayat CK, Bahar M, Thadeus MS. Comparison of antibacterial sensitivity test results of robusta coffee bean (Coffea canephora) ethanol extract before and after roasting against bacterial isolates of dental plaque at STAN Polyclinic, South Tangerang. Bina Widya. 2015;26(3):135–44.