Helium: Journal of Science and Applied Chemistry
https://journal.unpak.ac.id/index.php/he_jsac
Volume 03, Number 01, June 2023, Page 01 - 05
e-ISSN: 2776-4508; p-ISSN: 2776-1711
Publisher: Universitas Pakuan

ANTIMICROBIAL ACTIVITY OF THE ETHANOL EXTRACT OF *Coffea canephora* L. SEEDS AGAINST *Staphylococcus aureus* and *Propionibacterium acnes*

Siska Elisahbet Sinaga[a,*)], Matius Paundanan[a)], Maharani Farah Dhifa Dg Masikki [b)], Diana Widiastuti[c)]

[a)] *Department of Nutritional Science, Universitas Widya Nusantara, Palu, 94148, Indonesia*
[b)] *Department of Medical Nursing, Universitas Widya Nusantara, Palu, 94148, Indonesia*
[c)] *Department of Chemistry, Faculty of Mathematics and Natural Science, Universitas Pakuan, Bogor 16163, Indonesia*
[*)] *Corresponding Author: siskaelisahbetsinaga@gmail.com*

Article history: *received: 15-04-2023; revised: 17-05-2023; accepted: 06-06-2023; published: 27-06-2023.*

ABSTRACT

Acne is a skin disease that causes humans to lack confidence in carrying out activities. Besides being sick, acne worsens the beauty of human skin. The cause of acne is gram-positive bacteria, namely *Staphylococcus aureus* and *Propionibacterium acnes*. Many studies reported that *Coffea canephora* L can inhibit the growth of bacteria. The objective of this study is to establish the minimum inhibitory concentration (MIC) of the ethanol extract of robusta coffee seeds in inhibiting the growth of *Staphylococcus aureus* and *Escherichia coli*. The ethanol extract of *C. canephora* L seed was obtained by maceration method using 96% ethanol. The extract obtained was evaluated for its antibacterial activity using the agar diffusion method with perforation techniques. The extract was divided into five various concentrations (50%, 25%, 12.5%, 6.25%, and 3.125%). The results of this study indicated that the ethanol extract of coffee seeds with a concentration of 50% resulted in a wider diameter of the zone of inhibition of bacterial growth in *S. aureus*, 30.00 mm compared to *P. acnes*, 26.22 mm. The MIC of ethanol extract for *S. aureus at 3.00% while P. acnes* was 6.00%. In conclusion, the antibacterial effect of the ethanol extract of coffee seeds at 50% concentration was stronger against *S. aureus* bacteria than *P. acnes*. The greater the concentration of the extract, the wider the resulting inhibition zone.

Keywords: antimicrobial, *Coffea canephora* L., *P. acnes, S. aureus*

1. INTRODUCTION

One of the skin diseases that humans often experience from adolescence to adulthood is acne. Acne is a fairly serious problem. Apart from giving a bad impression to the sufferer, this disease also creates scars that are difficult to heal [1–3]. The main factors involved in acne formation are increased sebum production, shedding of keratinocytes, bacterial growth, and inflammation. Inflammation can be triggered by *Propionibacterium acnes* bacteria, *Staphylococcus epidermis* and *Staphylococcus aureus* [4,5]. *Staphylococcus epidermidis* is a common cause of infection in humans that can cause skin infections, often associated with external contamination. *Propionibacterium acnes* coexist on human skin, and this species can be isolated from normal skin and skin affected by acne vulgaris [6,7].

Some acne lesions develop due to a major inflammatory process and usually require medical treatment. So now, many people are looking for acne-healing concoctions that are antimicrobial. Plant extracts can be used as an antimicrobial source [8–10]. One of the well-known plants in Indonesia and a portion of functional food is coffee. Coffee has several species, one of which is *Coffea canephora* L [11]. Coffee is a plant or tree that is efficacious for anti-cellulite, exfoliating dead skin cells, anti-aging, anti-inflammatory, antioxidant, and anti-acne [12–14]. The previous research reported that *C. canephora* leaves have antibacterial activity but there has been no research on antimicrobial tests against *S. aureus* and *P. acnes* from robusta coffee seeds [15]. Based on the reported literature, coffee contains volatile and non-volatile compounds. Non-volatile compounds that affect coffee quality include aliphatic hydrocarbons, acids, alcohols, quinones, phenols,

Volume 03, Number 01, June 2023, Page 01 - 05
e-ISSN: 2776-4508; p-ISSN: 2776-1711
Publisher: Universitas Pakuan

aromatic amines, caffeine and chlorogenic acid. [16]. Chlorogenic acid and caffeine are non-volatile compounds that can prevent the growth of gram-positive and gram-negative bacteria, including *Staphylococcus aureus* and *Propionibacterium acnes*, which cause infectious diseases, one of which is acne. [17,18].

Based on a literature review conducted by Rodrigues et al. in 2023, it was explained that coffee grounds are widely used as skincare formulas. In addition, Gupta et al. researched coffee extract and its antimicrobial test against *Streptococcus mutans* in 2023. The results of their research indicated that coffee extract had good activity against bacteria in reducing the growth of *S. mutans* [19–21]. So, research on antimicrobial tests on other bacterial species is interesting to do. Therefore, researchers conducted an antimicrobial test of the ethanol extract of *Coffea canephora* L. seeds against *Staphylococcus aureus* and *Propionibacterium acnes*, both types of bacteria that cause acne.

2. METHODS

The ethanol extract of *C. canephora* L. (Robusta coffee) seed was obtained by maceration method using 96% ethanol. The powder of Robusta coffee seeds as much as 500 grams was macerated with ethanol for 3x24 hours. The ethanol extract was concentrated using a rotary evaporator to produce 11 grams of coffee seed ethanol extract. The method used to test the antibacterial activity of the ethanol extract of *Coffea canephora* L. seed was the agar diffusion method with the perforation technique. The samples used were *Coffea canephora* L seed extract and glassware in the laboratory. The solvents used were DMSO, Mueller Hilton Agar (MHA), and organic solvents.

2.1. Preparation of Test Media and Bacterial Suspension.

Mueller Hilton Agar (MHA) of as much as 3.8 grams was dissolved in 100 mL of sterile distilled water. Then the medium was heated and sterilized by autoclaving at 121 ^{o}C for 15 minutes. The bacteria to be used must be regenerated first. Bacteria originating from the primary culture were cultured into test tubes containing slanted agar media. One loop of bacteria was streaked onto the agar slant and then incubated at 37 ^{o}C for 18-24 hours. The test bacteria that have been rejuvenated are streaked with 3-4 streaks, then put into a test tube containing 0.9% NaCl and incubated for 24 hours at 37 ^{o}C. The growth of the test bacterial cells was indicated by the formation of turbidity, which was measured using Uv-Vis spectrophotometry with a wavelength of 580 nm which showed a transmittance of 25%.

2.2. Determination of the Activity of *Coffea canephora* L. Seed Ethanol Extract

The coffee ethanol extract was dissolved in DMSO to obtain extract solutions with concentrations of 50%, 25%, 12.5 %, 6.250%, and 3.125% (v/v). As much as 200 µL of the tested bacterial suspensions, *Staphylococcus aureus* ATCC 25923, and *Propionibacterium acne* ATCC 11827, were put into a sterile petri dish, and then 20 mL of MHA medium was added, which was still liquid. After shaking gently until homogeneous, the media is allowed to solidify. The medium was perforated using a perforator, and then as much as 50 µL of each variation of the concentration of the extract solution was put into a different hole. The plates were incubated at 37^{o}C for 18-24 hours. The clear zone shows the antibacterial activity of the extract around the reserved hole, which is caused by the inhibition of bacterial growth by the extract.

2.3. Determination of Minimum Inhibitory Concentration of Ethanol Extract of *Coffea canephora* L. Seeds

MIC determination was carried out by the solid dilution method on *Staphylococcus aureus* bacteria with concentrations of 3.125% (v/v), 3.000% (v/v), 2.875% (v/v), 2.750% (v/v) and 2.625% (v/v), while for *Propionibacterium acnes* bacteria with concentrations of 6.250% (v/v), 6.125% (v/v), 6.000% (v/v), 5.875% (v/v), and 5.750 % (v/v). The ethanol extract as much as 1000 µL dissolved in DMSO solvent to obtain various concentrations then added to 19 mL of agar media diluted into a sterile petri dish. The mixture is homogenized and cooled until it becomes solid. Bacterial suspension is taken one ose inoculated on the surface to make it solid,

Volume 03, Number 01, June 2023, Page 01 - 05
e-ISSN: 2776-4508; p-ISSN: 2776-1711
Publisher: Universitas Pakuan

then incubated at 37 ^{o}C for 18-24 hours. The expected data from determining the minimum inhibitory concentration is the sensitivity of the bacteria to the test substance in the minimum concentration, which can still inhibit microbial growth.

3. RESULTS AND DISCUSSION

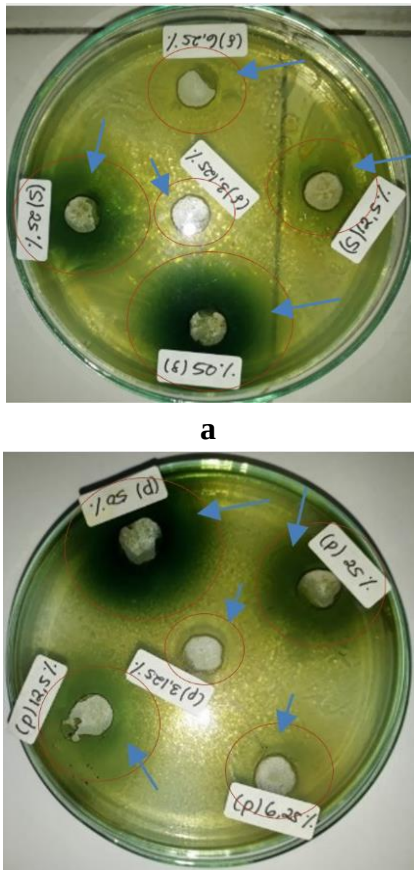
The seed extract of *Coffea canephora* L. was obtained by extraction by maceration method using 96% ethanol solvent. The filtrate from the extraction results was concentrated with a rotary evaporator obtained from 500 grams of coffee seed simplicial as much as 11 grams with an extract yield value of 2.2%.

Screening for antimicrobial activity of the ethanol extract of coffee seeds was carried out using Gram-positive bacteria, namely *S. aureus* a`nd *P. acnes*. The results of this coffee extract antimicrobial screening are shown in Table 1.

Table 1. Results of antibacterial activity test of ethanol seed extract of *Coffea canephora* L.

Inhibition zone (mm)					
Bacteria	Concentration				
	50%	**25%**	**12.5%**	**6.25%**	**3.125%**
***S. aureus* (mm)**	30.00	25.01	15.41	13.89	11.51
***P. acnes* (mm)**	26.22	21.46	14.87	10.33	9.01

The results showed that the ethanol extract of coffee seeds had antibacterial activity for *S. aureus* with an inhibition zone diameter of 30.00 mm for a concentration of 50%; 25.01 mm for a concentration of 25%; 15.41 mm for a concentration of 12.5%; 13.89 mm for a concentration of 6.25% and 11.51 mm for a concentration of 3.125% while the results of the antibacterial for *P. acnes* bacteria with an inhibition zone diameter of 26.22 mm for a concentration of 50%; 21.46 mm for a concentration of 25%; 14.87 mm for a concentration of 12.5%; 10.33 mm for a concentration of 6.25% and 9.01 mm for a concentration of 3.125%. The greater the concentration of the extract, the wider the inhibition zone. The inhibitory effect was stronger at higher concentrations, namely at concentrations of 50% and 25%. This proved that the ethanol extract from robusta coffee seeds has an antimicrobial effect against *S. aureus* and *P. acnes* bacteria. The results of the coffee extract activity test can be seen in Figure 1.



a

b

Figure 1. Activity test results of the ethanol extract of *Coffea canephora* L. seeds (a: *S. aureus* and b: *P. acnes*)

Testing the minimum inhibitory concentration (MIC) results on *Staphylococcus aureus* using various concentrations of 3.125%, 3.000%, 2.875%, 2.750% and 2.625%, and *Propionibacterium acnes* bacteria with concentrations of 6.250%, 6.125%, 6.000%, 5.875% and 5.750% can be seen in Table 2.

Volume 03, Number 01, June 2023, Page 01 - 05
e-ISSN: 2776-4508; p-ISSN: 2776-1711
Publisher: Universitas Pakuan

Table 2. Minimum Inhibitory Concentration of Ethanol Extract of *Coffea canephora* L. Seeds

Bacteria Name	**Concentration**				
	3.125%	**3.00%**	**2.875 %**	**2.75%**	**2.625%**
S.aureus	-	-	+	+	+
	6.25%	**6.125%**	**6.000%**	**5.875%**	**5.75%**
P. acnes	-	-	-	+	+

The different concentrations used for *S. aureus* and *P. acnes* were different because the inhibition zone results shown for *S. aereus* have a significant difference of 6.25% (13.89 mm) and 3.125% (11.51 mm), so the concentration of 3.125% was chosen for diluted to 3.125%, 3.000%, 2.875%, 2.750% and 2.625%, then look again at the minimum inhibitory concentration that can inhibit the growth of *S. aureus*, whereas in *P. acnes* with a concentration of 6.25% (10.33 mm) and 3.125% (9.01 mm) there was no significant difference in the inhibition zone, so the concentration of 3.125% which was chosen to be diluted became 6.250%, 6.125%, 6.000%, 5.875%, and 5.750 %, then look again at what is the minimum inhibitory concentration that can inhibit the growth of *P. acnes.*

The results showed that there was no bacterial growth at concentrations of *Staphylococcus aureus* bacteria at concentrations of 3.125% and 3.000%, while for *Propionibacterium acnes* at concentrations of 6.25%, 6.125% and 6.000%, there was no bacterial growth. This data showed that the higher the concentration of coffee extract, the higher the inhibition of bacterial growth against *S. aureus* and *P. acnes*.

4. CONCLUSION

These data indicate that *Coffea canephora* L. coffee seeds can be an anti-acne because they can inhibit acne-causing bacteria. The average minimum inhibitory concentrations of these two cancer-causing gram-positive bacteria were 3% for *S. aureus* and 6% for *P. acnes*. These results provide clues for future researchers to make anti-acne drug formulas from coffee seed extract. The development needs this data and can explore more about the test.

REFERENCES

[1] Gamil, H.D.; Mustafa, S.A.G.; Amer, R.M.M.; Khashaba, S.A.E. Implications of Antimicrobial Resistance during Acne Treatment: Review Article. *Egypt. J. Hosp. Med.* **2023**, *90*, 821–823, doi:10.21608/ejhm.2023.279944.

[2] Chaudhari, D.; Vohra, R.R.; Abdefatah Ali, M.; Nadeem, H.; Tarimci, B.; Garg, T.; Sharari, R.A.; Joseph, A.; Khan, A. A Rare Phenomenon of Lithium-Associated Acne Inversa: A Case Series and Literature Review. *Cureus* **2023**, *15*, doi:10.7759/cureus.36051.

[3] Zhou, C.; Vempati, A.; Tam, C.; Khong, J.; Vasilev, R.; Tam, K.; Hazany, S.; Hazany, S.; Zhou, C.; Vempati, A.; et al. Beyond the Surface : A Deeper Look at the Psychosocial Impacts of Acne Scarring Beyond the Surface : A Deeper Look at the Psychosocial Impacts of Acne Scarring. *Clin. Cosmet. Investig. Dermatol.* **2023**, doi:10.2147/CCID.S406235.

[4] Christensen, G.J.M.; Scholz, C.F.P.; Enghild, J.; Rohde, H.; Kilian, M.; Thürmer, A.; Brzuszkiewicz, E.; Lomholt, H.B.; Brüggemann, H. Antagonism between Staphylococcus epidermidis and *Propionibacterium acnes* and its genomic basis. *BMC Genomics* **2016**, *17*, 1–14, doi:10.1186/s12864-016-2489-5.

[5] Alston, M.; Hobbs, G.; Nakouti, I. Acne vulgaris : the skin microbiome , antibiotics and whether natural products could be considered a suitable alternative treatment ? *J. Nat. Prod. Discov.* **2022**, *1*, 1–11, doi:10.24377/jnpd.article652.

[6] Sinaga, S.E.; Fajar, M.; Mayanti, T. Bioactivities Screening and Elucidation of Terpenoid from the Stembark Extracts of Lansium domesticum Corr . cv . Kokosan (Meliaceae). *Sustain.* **2023**.

[7] Mohsin, N.; Hernandez, L.E.; Martin, M.R.; Does, A. Vander; Nouri, K. Acne treatment review and future perspectives. *Dermatol. Ther.* **2022**, *35*, 2022–2023, doi:10.1111/dth.15719.

[8] Sinaga, S.E.; Mayanti, T.; Naini, A.A.; Harneti, D.; Nurlelasari, N.; Maharani, R.; Farabi, K.; Supratman, U.; Fajriah, S.; Azmi, M.N. Sesquiterpenoids from the Stem Bark of Lansium domesticum Corr . Cv . Kokossan and Their Cytotoxic Activity against MCF-7 Breast Cancer Cell Lines. *Indones. J. Chem.* **2022**, *22*, 1035–1042, doi:10.22146/ijc.72742.

[9] Miazga-karska, M.; Michalak, K.; Ginalska, G. Anti-acne action of peptides isolated from burdock root. *Molecules* **2020**, 1–17.

Volume 03, Number 01, June 2023, Page 01 - 05
e-ISSN: 2776-4508; p-ISSN: 2776-1711
Publisher: Universitas Pakuan

[10] Gholais, N.S.; Shi, C. Review of the Therapeutic Advance of Acne Treatment. *J. Biosci. Med.* **2022**, *10*, 66–78, doi:10.4236/jbm.2022.106006.
[11] Zaidan, I.R.; Ferreira, A.; Noia, L.R.; Santos, J.G.; de Arruda, V.C.; do Couto, D.P.; Braz, R.A.; de Brites Senra, J.F.; Partelli, F.L.; Azevedo, C.F.; et al. Diversity and structure of *Coffea canephora* from old seminal crops in Espírito Santo, Brazil: genetic resources for coffee breeding. *Tree Genet. Genomes* **2023**, *19*, doi:10.1007/s11295-023-01594-x.
[12] Agustini, S.; Agustina, S.; Dharmaputera, D. The Determination of Quality and Sensory Profile of Arabica and Robusta Coffee Originated from Semendo Indonesia. *AIP Conf. Proc.* **2022**, *2638*, doi:10.1063/5.0104045.
[13] Ferreira, I.A.; Fávaris, N.A.B.; da Rosa, S.D.V.F.; Coelho, S.V.B.; Ricaldoni, M.A.; Costa, M.C. Trolox equivalent antioxidant capacity of Coffea arabica L. seeds. *Cienc. e Agrotecnologia* **2022**, *46*, doi:10.1590/1413-7054202246000522.
[14] Babova, O.; Occhipinti, A.; Maffei, M.E. Chemical partitioning and antioxidant capacity of green coffee (Coffea arabica and *Coffea canephora*) of different geographical origin. *Phytochemistry* **2016**, *123*, 33–39, doi:10.1016/j.phytochem.2016.01.016.
[15] ZAMHARIRA MUSLIM; YONANIKO DEPHINTO Antibacterial Activity of Robusta Coffee (*Coffea canephora* L.) Leaves To *Staphylococcus aureus* and Escherichia Coli. *Asian J. Pharm. Clin. Res.* **2019**, *12*, 113–115, doi:10.22159/ajpcr.2019.v12i12.35589.
[16] Lestari, C.R.; Ayu, R.; Pustika, E.; Nurjanah, S.; Sabarani, L.C. The Effect of Green Coffee Seed (*Coffea canephora*) and Yellow Turmeric (Curcuma Domestica Val .) Extract on TNF-α Level in Acute Respiratory Distress Syndrome. *Int. J. Nutr. Sci.* **2022**, *7*, 210–216, doi:10.30476/ijns.2022.97406.1209.Introduction.
[17] Mediani, A.; Kamal, N.; Lee, S.Y.; Abas, F.; Farag, M.A. Green Extraction Methods for Isolation of Bioactive Substances from Coffee Seed and Spent. *Sep. Purif. Rev.* **2023**, *52*, 24–42, doi:10.1080/15422119.2022.2027444.
[18] Kaban, V.E.; Nasri, N.; Syahputra, H.D.; Lubis, M.F. Uji Aktivitas Antibakteri Ekstrak Daun Karenda (Carissa carandas Linn .) Terhadap Bakteri Propionibacterium acne dan Staphylococcus epidermidis. *J. Pharm. Heal. Res.* **2023**, *4*, 91–96, doi:10.47065/jharma.v4i1.3181.
[19] Gupta, S.A.; Potdar, G.V.; Jain, K.D.; Jethwa, K.P.; Thakkar, V.P.; Ram, S.M.; Pachpute, S.R. Antimicrobial Effects of Green and Roasted Beans of Coffee Robusta and Coffee Arabica on Streptococcus mutans – An in vitro Comparative Study. *J. Indian Assoc. Public Heal. Dent.* **2023**, *21*, 27–33, doi:10.4103/jiaphd.jiaphd.
[20] Rodrigues, R.; Oliveira, M.B.P.P.; Alves, R.C. Chlorogenic Acids and Caffeine from Coffee By-Products: A Review on Skincare Applications. *Cosmetics* **2023**, *10*, doi:10.3390/cosmetics10010012.
[21] Tran, T.M.K.; Akanbi, T.O.; Kirkman, T.; Nguyen, M.H.; Vuong, Q. Van Recovery of Phenolic Compounds and Antioxidants from Coffee Pulp (*Coffea canephora*) Waste Using Ultrasound and Microwave-Assisted Extraction. *Processes* **2022**, *10*, doi:10.3390/pr10051011.