International Journal of Research and Review
Volume 11; Issue: 5; May 2024
Website: www.ijrrjournal.com
Original Research Article *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Antibacterial Activity by Diffusion and Dilution Methods of Liquid Smoke *Cocos nucifera* L.

Lusi Mardika Ariyanti[3], Masfria[1,2], Muchlisyam[1]

[1]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
[2]Nanomedicine Center of Innovation, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia
[3]Undergraduate Program, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Indonesia

Corresponding Author: Masfria

DOI: https://doi.org/10.52403/ijrr.20240511

ABSTRACT

Introduction: Liquid smoke is a product that obtained industrially from coconut shell through pyrolysis process at 400°C for 2,5 hours and redestillation to obtain grade 1 liquid smoke. Coconut shell liquid smoke contains chemical compounds, namely phenol, which has been researched to act as a disinfectant that has broad spectrum antibacterial activity against gram positive and gram negative bacteria.
Method: This study aims to determine the potential of *cocos nucifera* L. liquid smoke as an antibacterial against *Staphylococcus aureus* and *Methicillin-resistant staphylococcus aureus* (MRSA) bacteria using diffusion and dilution methods. The concentrations used were 100%, 80%, 60%, 40%, 35%, 30%, 15%, 10%, 7%, 5%. As positive control was used Tetracycline 25 ppm.
Results: Liquid smoke has the potential to inhibit the growth of both bacteria. The effective concentration to inhibit the growth of *S. aureus* at 40% concentration and MRSA at 80% concentration. The MIC value of liquid smoke against *S. aureus* is 0,78%, while on MRSA 3,12%. The MBC of *S. aureus* and MRSA bacteria at concentrations 1,56% and 6,25%. *Staphylococcus aureus* has higher sensitivity to *Cocos nucifera* L. grade 1 liquid smoke than MRSA bacteria.
Conclusion: *Cocos nucifera* L. liquid smoke has antibacterial activity against *Staphylococcus aureus* and MRSA at effective concentrations of 40% and 80%. The MIC value of *S. aureus* is 0,78%, while MRSA is 3,12%. The MBC of *S. aureus* and MRSA bacteria at concentrations 1,56% and 6,25%.

Keywords: antibacterial, liquid smoke, phenol, *Cocos nucifera* L.

INTRODUCTION

Antimicrobial resistance is one of the biggest challenges in public health. Infections caused by resistant bacteria can lead to more severe clinical infections, treatment failure, extended hospitalization and higher healthcare costs[(1)]. Antimicrobial resistance occurs when bacteria, viruses, fungi and parasites evolve to make antibiotics and other antimicrobial drugs ineffective, making infections more difficult or impossible to treat.
Staphylococcus aureus is a major bacterium in humans and animals that causes a wide variety of infections, ranging from superficial skin infections to life-threatening systematic diseases[(2)].
Methicillin-resistant staphylococcus aureus (MRSA) has emerged leading to limited therapeutic options. MRSA shows different levels of resistance to methicillin, penicillin, oxacillin, cloxacillin and cefoxitin. MRSA can cause a variety of serious infections

such as pneumonia, sepsis and endocarditis[2]. According to WHO data, *methicillin-resistant Staphylococcus aureus* bacteria show that individuals infected with MRSA have a 64% higher chance of death than those infected with other bacteria[3].

Herbal treatments need to be developed because of the increasing cases of antibiotic resistance made from chemical substances. Many studies have been conducted to find drugs with natural antibacterial properties that have a lower risk of side effects than synthetic antibiotics[4].

Coconut shell is a side product of coconut meat processing. Cellulose, hemicellulose and lignin are the main components contained in coconut shell. The lignin compound content of 27% in coconut shell is a good source of phenolics to produce liquid smoke[5].

Previous research states that coconut shell liquid smoke contains chemical compounds including phenols, flavonoids, triterpenoids/steroids and saponins[6]. Phenol compounds in liquid smoke derived from coconut shells are antioxidants that can bound free radicals such as the formation of ROS[7]. Phenol has been studied to act as a disinfectant that has broad spectrum antibacterial activity against gram positive and gram negative bacteria. In high concentrations phenol content can break through bacterial cell walls and precipitate proteins in bacterial cells, in low concentrations phenol activates important enzyme systems in bacterial cells. The results of previous studies show that coconut shell liquid smoke has antibacterial activity against *Staphylococcus aureus* bacteria in the very strong category with 40 mm inhibition zone at a concentration of 40% liquid smoke[8].

Based on the description above, it is necessary to determine the grade 1 liquid smoke of coconut shell from pyrolysis and purification using the distillation method on the antibacterial activity of *Staphylococcus aureus* and *Methicillin-resistant staphylococcus aureus* (MRSA).

MATERIALS & METHODS

Tools and Materials

The tools used are autoclave (*Portable*), desiccator, a set of distillation tools, a set of UV-VIS spectrophotometer tools (*Orion AquaMate 8000*), a set of AAS tools (*Atomic Absorption Spectrometer*). petri dish, test tube, ose needle, incubator (*Memmert*), vernier caliper, refrigerator (*Toshiba*), electric balance (*Mettler Toledo*), hot plate, electric oven (*Fisher scientific*), and paper disk.

The materials used in this study were coconut shell, Mueller Hinton Agar (*Merk*), Nutrient agar (*Merk*), Nutrient Broth solution (*Merk*), aquadest, bacterial culture of *Staphylococcus aureus*, MRSA, methanol and grade 1 liquid smoke.

Liquid Smoke Preparation

The manufacture of liquid smoke is carried out using a set of pyrolysis reactors with burning for 2.5 hours at a temperature of 400°C, the liquid smoke produced from the pyrolysis process is called crude smoke liquid (grade 3 liquid smoke), distilled at 150°C (grade 2 liquid smoke) and then redistilled at 100°C to produce grade 1 liquid smoke.

Antibacterial Activity Test

Bacterial cultures of *S. aureus* and MRSA were suspended in sterile distilled water and the turbidity was measured in spectrophotometry with a wavelength of 580 nm until a turbidity with a transmittance of 25% (number of bacteria 10^6 CFU/mL) was obtained.

0,1 ml of test bacterial suspension on agar media, placed sterile paper discs that have been immersed in grade 1 liquid smoke with concentrations of 100%, 80%, 60%, 40%, 35%, 30%, 20%, 15%, 10%, 7%, 5%, as well as positive and negative controls, namely tetracycline 25 ppm and aquadest. Incubated at 370°C for 18-24 hours. The clear zone around the disc paper was calculated in millimeters (mm)[9].

Antibacterial Activity of Cocos nucifera L. Liquid Smoke by Microdilution and Minimum Inhibitory Concentration (MIC) Test

The variation of liquid smoke concentration was carried out by serial dilution on a microplate so the concentration variation was 100; 50; 25; 12.5; 6.25; 3.12; 1.56; 0.78; and 0.36% v/v. 100 µL of NB media was entered and each column and liquid smoke in column 1, 100 µL was taken to fill column 2 carried out in a row until the last column. In each column, 50 µL of bacterial suspension was filled. negative control and positive control using aquadest and 25 ppm tetracycline. Incubation was carried out at 37°C for 18-24 hours then observed the presence or absence of bacterial growth in each well by looking at the culture clarity compared to the control[10,11].

Minimum Bactericidal Concentration (MBC) Test

Columns of microplates that showed clarity were scratched using an ose on sterile MHA media. Furthermore, incubation was carried out for 18-24 hours at 37°C. The part of the agar media that showed no bacterial growth with clear visual results was determined as the MBC value[12].

RESULT

Antibacterial Activity

Table 1. Antibacterial activity test results of *Cocos nucifera* L. grade 1 liquid smoke

No.	Concentration (%) v/v	Diameter Inhibition (mm)	
		Staphylococcus aureus	MRSA
1.	100%	21,53±2,57	20,66±1,46
2.	80%	18,83±4,11	16,58±2,54
3.	60%	16,93±4,16	13,18±0,28
4.	40%	16,3±3,85	11,28±1,28
5.	35%	15,38±3,50	10,41±1,05
6.	30%	14,46±3,35	8,75±0,36
7.	20%	10,1±1,05	-
8.	15%	8,66±0,93	-
9.	10%	8,19±0,68	-
10.	7%	7,00±0,18	-
11.	5%	-	-
12.	Tetrasiklin 25 ppm	17,4±0.1	16,5±0,01
13.	Aquades	0	0

Table 1. the results obtained by liquid smoke which is effective for inhibiting the growth of *S. aureus* bacteria is at a concentration of 40%, while for MRSA bacteria is at a concentration of 80%. The positive control of tetracycline 25 ppm gave an inhibition zone diameter of 17.4±0.1 mm against *S. aureus* bacteria and 16.5±0.01 mm against MRSA bacteria.

Antibacterial Activity of Liquid Smoke by Microdilution for MIC and MBC Determination

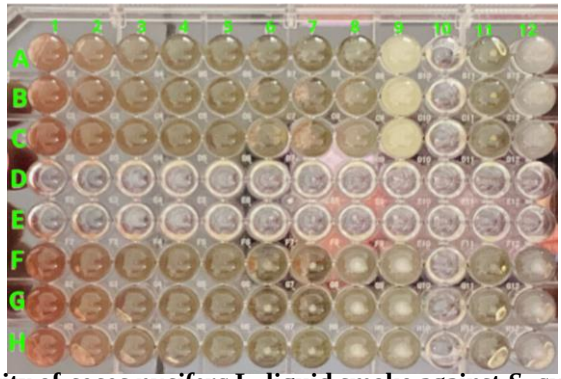

Figure 1. Antibacterial activity of *cocos nucifera* L. liquid smoke against *S. aureus* and MRSA bacteria by microdilution method.

Liquid smoke columns 1-8 rows A, B and C can inhibit the growth of *S. aureus* bacteria, in columns 1-6 rows F, G and H can inhibit the growth of MRSA bacteria this is indicated by the clear color of the wells, while in columns that change color or turbid and there is sediment, it can be concluded that sample tests with certain concentrations cannot inhibit bacterial growth.

Table 2. Minimum inhibitory concentration of liquid smoke against *S. aureus* and MRSA

Bacteria Test	Columns										
	1	2	3	4	5	6	7	8	9	K(-)	K(+)
S. aureus	-	-	-	-	-	-	-	-	+	-	+
S. aureus	-	-	-	-	-	-	-	-	+	-	+
S. aureus	-	-	-	-	-	-	-	-	+	-	+
MRSA	-	-	-	-	-	-	+	+	+	-	+
MRSA	-	-	-	-	-	-	+	+	+	-	+
MRSA	-	-	-	-	-	-	+	+	+	-	+

Description:

a) Columns 1, 2, 3, 4, 5, 6, 7, 8, 9: Liquid smoke 100%; 50%; 25%; 12,5%; 6,25%; 3,12%; 1,56%; 0,78%; 0,39%, **b)** K (+): positife control, **c)** K (-): negatife control, **d)** (+): there is bacterial growth (turbit), (-): there is no bacterial growth (clear)

Table 2. that liquid smoke has antibacterial activity against *S. aureus* and MRSA tested by microdilution method with the occurrence of growth inhibition in columns 1 (100%); 2 (50%); 3 (25%); 4 (12.5%); 5 (6.25%), 6 (3.12%); 7 (1.56%); 8 (0.78%). The MIC value of liquid smoke against *S. aureus* is 0,78%. While in MRSA the occurrence of growth inhibition in columns 1 (100%); 2 (50%); 3 (25%); 4 (12,5%); 5 (6,25%), 6 (3,12%) and the MIC value was determined at a concentration of 3,12%.

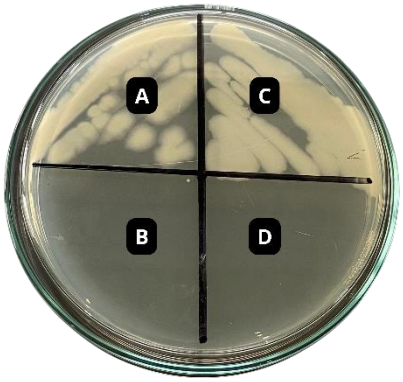

Figure 2. Results of MBC testing of liquid smoke A) *S. aureus* (0.78%); B) *S. aureus* (1,56%); C) MRSA (3,12%; D) MRSA (6,25%)

Figure 2. It can be observed that at concentrations 0,78% and 3,12% liquid smoke there is still growth of *S. aureus* and MRSA bacteria, while at concentrations 1,56% on *S. aureus* and 6,25% on MRSA there is no growth of *S. aureus* or MRSA bacteria with visual results that look clear.

DISCUSSION

In the antibacterial testing of liquid smoke Cocos nucifera L. has a very active inhibitory response to the growth of *S. aureus* bacteria compared to MRSA bacteria, because the MRSA bacteria have experienced resistance due to the production of betalactamase so that it is different from *S. aureus* which causes the test compound to be difficult to penetrate the bacterial cell wall because it is resistant. An effective concentration for testing antibacterial activity is when the concentration can produce an inhibition zone between 14mm-

16mm. The difference inhibition zone formed in the antibacterial activity test can be caused by several factors, namely the variation in the concentration of the sample, the content of metabolite compounds, the diffusion rate of the test sample and the type of bacteria inhibited[13].

The negative control used in this study is aquadest which does not give an inhibition zone, this shows that antibacterial activity is not affected by solvent factors but the resulting antibacterial activity is the potential contained in liquid smoke. The reason for using aquadest as negative control because the compounds from aquadest are neutral which will not have an effect on bacterial growth or have no antibacterial activity[14]. Tetracycline is one of the common drug options used for clinical treatment of *S. aureus*. Tetracyclines have a broad antimicrobial spectrum and can inhibit both gram positive and gram negative bacteria. The mechanism of tetracycline is inhibit protein synthesis in bacterial cells, this prevents the elongation of polypeptide chains that are being shaped and results in the cessation of protein synthesis[15].

From the results of measuring diameter of inhibition zone, it shows that the results are linearly related to the increase in liquid smoke concentration, the higher concentration of liquid smoke makes the diameter of inhibition zone bigger[16]. The inhibition zone in each variation of extract concentration is thought to be due to the presence of active compounds in the form of secondary metabolite compounds contained in coconut shell liquid smoke including phenols, flavonoids, triterpenoids/steroids and saponins[6]. Phenol is a component contained in liquid smoke that acts as antimicrobial, Phenol has been researched to act as a disinfectant that has broad spectrum antibacterial activity against gram positive and gram negative bacteria. In high concentrations phenol content can penetrate the bacterial cell wall and precipitate proteins in bacterial cells, in low concentrations phenol activates important enzyme systems in bacterial cells[17,5].

Bacterial cells are arranged in cell membrane, cell wall, cytoplasm and nucleoplast. The cell membrane is an important medium for the exchange of material and energy between bacteria and the outside world. When the permeability of the cell membrane of pathogenic bacteria is damaged, the electrolytes in the bacteria will leak into the culture medium so the bacteria will die[18].

CONCLUSION

Based on the results of the research conducted, it can be concluded that *cocos nucifera* L. liquid smoke has antibacterial activity against *Staphylococcus aureus* and MRSA at effective concentrations of 40% and 80%. The MIC value of *S. aureus* is 0,78%, while that of MRSA is 3,12%. The MBC of *S. aureus* and MRSA bacteria at concentrations of 1,56% and 6,25%.

Declaration by Authors

Ethical Approval: None
Acknowledgement: None
Source of Funding: None
Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. Chandra Deb L, Jara M, Lanzas C. Early evaluation of the Food and Drug Administration (FDA) guidance on antimicrobial use in food animals on antimicrobial resistance trends reported by the National Antimicrobial Resistance Monitoring System (2012–2019). One Heal [Internet]. 2023;17(June):100580. Available from: https://doi.org/10.1016/j.onehlt.2023.100580
2. Parikh MP, Octaria R, Kainer MA. Methicillin-resistant staphylococcus aureus bloodstream infections and injection drug use, Tennessee, USA, 2015-2017. Emerg Infect Dis. 2020;26(3):446–53.
3. Gu S, Fan B, Wan F, Gao T, Qi Y, Zhou J, et al. Antibacterial Activity and Mechanism of Canagliflozin against Methicillin-

Resistant Staphylococcus aureus. Molecules. 2023;28(15).
4. Ariyanti LM, Supomo S, Saâ€™adah H, Syamsul ES, Kintoko K, Witasari HA. Uji Aktivitas Antibakteri EkstrakEtanol dan Fraksi Batang Kuning (Fibraurea Tinctoria Lour) terhadap Bakteri Escherichia coli dan Staphylococcus aureus. JI-KES (Jurnal Ilmu Kesehatan). 2022;5(2):229–37.
5. Rahmasari Y, Yemiş GP. Characterization of ginger starch-based edible films incorporated with coconut shell liquid smoke by ultrasound treatment and application for ground beef. Meat Sci. 2022;188(October 2021).
6. Lumban Tobing RDDM, Defiani MR, Susun Parwanayoni NM. UJI DAYA HAMBAT ASAP CAIR TEMPURUNG KELAPA (Cocos nucifera L.) TERHADAP PERTUMBUHAN Escherichia coli SECARA In Vitro. Simbiosis. 2021;9(2):81.
7. Surboyo MDC, Arundina I, Rahayu RP, Mansur D, Bramantoro T. Potential of Distilled Liquid Smoke Derived from Coconut (Cocos nucifera L) Shell for Traumatic Ulcer Healing in Diabetic Rats. Eur J Dent. 2019;13(2):271–9.
8. Indriati A, Hidayat DD, Darmajana DA. Performance Test and Economic Evaluation of Pyrolysis Furnace Small Scale-Worth the Business for Producing Liquid Smoke. IOP Conf Ser Earth Environ Sci. 2019;251(1).
9. Maimunah S, Pratama HA, Mayasari U. Antibacterial Activity Assay From Sintrong Leaf Crassocephalum crepidiodies Against Staphylococcus aureus Bacteria. J Pembelajaran Dan Biol Nukl. 2020;6(1):103–11.
10. Tuesta-Chavez T, Monteza J, Silva Jaimes MI, Ruiz-Pacco GA, Changanaqui K, Espinoza – Suarez JB, et al. Characterization and evaluation of antioxidant and antimicrobial capacity of prepared liquid smoke-loaded chitosan nanoparticles. J Food Eng. 2022;319(June 2021).
11. Assauqi NF, Hafshah M, Latifah RN. Penentuan Nilai Konsentrasi Hambat Minimum (KHM) dan Konsentrasi Bunuh Minimum (KBM) Ekstrak Etanol Daun Pandan (Pandanus Amaryllifolius Roxb) Terhadap Bakteri Streptococcus Mutans. JC-T (Journal Cis-Trans) J Kim dan Ter. 2023;7(1).
12. Mulqie L, Suwendar S, Rajih MF, Mardliyani D, Yumniati I, Widiasari W, et al. AKTIVITAS ANTIBAKTERI EKSTRAK ETANOL DAUN JAMBU AIR [Eugenia aqueum (Burm. F) Alston] DENGAN MIKRODILUSI AGAR. J Ilm Farm Farmasyifa. 2022;5(1):1–8.
13. Sholihah NF, Saula LS, Sholih MG. Comparison of Antibacterial of Sambiloto (Andrographis paniculata) Leaf and Kemangi (Ocimum sanctum) Leaf Extract Against Staphylococcus aureus. J Pharm Sci. 2022;5(2):279–85.
14. Gerung WHP, Fatimawali, Antasionasti I. Uji Aktivitas Antibakteri Ekstrak Daun Belimbing Botol (Averrhoa bilimbi L.) terhadap Pertumbuhan Bakteri Propionibacterium acnes Penyebab Jerawat. Pharmacon. 2021;10(4):1087–93.
15. Panjaitan RS, Sarungngu' AR, Sitorus LL. Antibacterial Activity of Brown Macroalgae Lipid (Sargassum duplicatum) to The Methicillin Resistant Staphylococcus aureus (MRSA) And Shigella dysentriae Bacteria. EduChemia (Jurnal Kim dan Pendidikan). 2020;5(1):1.
16. Nasri N, Kaban VE, Satria D, Syahputra HD, Rani Z. Mekanisme Antibakteri Ekstrak Etanol Daun Kemangi (Ocimum basilicum L.) terhadap Salmonella typhi. J Pharm Heal Res. 2023;4(1):79–84.
17. Sari RM, Gea S, Wirjosentono B, Hendrana S, Torres FG. The effectiveness of coconut coir as tar adsorbent in liquid smoke integrated into the pyrolysis reactor. Case Stud Therm Eng [Internet]. 2021;25(March):100907. Available from: https://doi.org/10.1016/j.csite.2021.100907
18. Wei C, Cui P, Liu X. Antibacterial Activity and Mechanism of Madecassic Acid against Staphylococcus aureus. Molecules. 2023;28(4).

How to cite this article: Lusi Mardika Ariyanti, Masfria, Muchlisyam. Antibacterial activity by diffusion and dilution methods of liquid smoke *Cocos nucifera* L. *International Journal of Research and Review*. 2024; 11(5): 87-92. DOI: *https://doi.org/10.52403/ijrr.20240511*

******