ISSN 2548-6462 (online), ISSN 2088-8740 (print)

DOI: 10.30644/rik.v12i1.730

Antimicrobial effect of areca nut ethanol extract against methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *Staphylococcus aureus* (MSSA)

Humaryanto[1], Hanina[2]*, Lipinwati[2]
[1] Department of Surgery, Faculty of Medicine and Health Sciences, University of Jambi
[2] Department of Microbiology-Parasitology, Faculty of Medicine and Health Sciences, University of Jambi
*Correspondence email: (hanina_fkik@unja.ac.id)

Accepted: 2 January 2023; revision: 1 June 2023; published: 30 June 2023

Abstract

Background: *Developing herbal medicines for antibacterial activity has been challenging in Indonesia. Of the alternative antimicrobials, areca nut (Areca catechu L) has antimicrobial activity since it contains flavonoids, tannins, phenols, alkaloids, saponins, terpenoids, and glycosides. The use of antibiotics in underdeveloped and developing countries is still irrational, and bacterial infection in Indonesia is still a problem to resolve, so searching for new herbal medicines is very important. Staphylococcus aureus is the most common normal flora that causes infection. The two types of Staphylococcus aureus are Methicillin-resistant Staphylococcus aureus (MRSA) and Methicillin-sensitive Staphylococcus aureus (MSSA). The study aimed to determine the efficacy of the antibacterial activity of areca nut ethanol extract against MRSA and MSSA isolates.*
Methods: *The research was conducted at the Biomedical Laboratory, Faculty of Medicine and Health Sciences, University of Jambi. Young and old areca nut ethanol extracts were macerated using 96% ethanol. The antibacterial activity test of the ethanol extract was carried out using the Kirby-Bauer disc diffusion method against the isolates of MRSA (clinical isolates) and MSSA (SA 25923/K+, SA 29213/K-).*
Results: *The results showed that the phytochemicals of young areca nut extracts contained alkaloids, flavonoids, phenols, and steroids, and old areca nut extracts contained alkaloids, flavonoids, and phenols. The MRSA isolates did not show the activity of the two areca nut ethanol extracts and were also resistant to Ceftadizime, Gentamicin, Ceftriaxone, and Cefoxitin. The MSSA isolates showed strong activity against old areca nut ethanol extract, moderate activity against young areca nut ethanol extract, and resistance to gentamicin.*
Conclusion*: MRSA bacteria are resistant to the ethanol extract of young and old areca nuts, while the ethanol extract of old areca nuts has strong antibacterial activity against MSSA.*

Keywords: *antibacterial, areca seed ethanol extract, MRSA, MSSA*

Abstrak

Latar Belakang: Pengembangan obat herbal untuk aktivitas antibakteri masih menjadi tantangan di Indonesia. Salah satu antimikroba alternatif adalah buah pinang (*Areca catechu L*) yang memiliki aktivitas antimikroba karena mengandung flavonoid, tanin, fenol, alkaloid, saponin, terpenoid, dan glikosida. Penggunaan antibiotik di negara-negara tertinggal dan berkembang masih belum rasional, sehingga mencari obat herbal baru sangat penting. Infeksi bakteri di Indonesia masih menjadi masalah yang harus diselesaikan. *Staphylococcus aureus* merupakan flora normal terbanyak yang menyebabkan infeksi. Terdapat dua jenis *Staphylococcus aureus* yaitu Methicillin-resistant *Staphylococcus aureus* (MRSA) dan Methicillin-sensitive *Staphylococcus aureus* (MSSA). Penelitian ini bertujuan untuk mengetahui efikasi aktivitas antibakteri ekstrak etanolik buah pinang terhadap isolat MRSA dan MSSA.

Metode: Penelitian dilakukan di Laboratorium Biomedik Fakultas Kedokteran dan Ilmu Kesehatan Universitas Jambi. Ekstrak etanol buah pinang muda dan tua diproses maserasi dengan menggunakan etanol 96%. Uji aktivitas antibakteri ekstrak etanol dilakukan menggunakan metode difusi cakram *Kirby-Bauer* terhadap isolat MRSA (isolat klinik) dan MSSA (SA 25923/K+, SA 29213/K-).
Hasil: Hasil penelitian menunjukkan fitokimia ekstrak buah pinang muda mengandung alkaloid, flavonoid, fenol, dan steroid dan ekstrak buah pinang tua mengandung alkaloid, flavonoid, fenol. Isolat MRSA tidak menunjukkan aktivitas dari kedua ekstrak etanol buah pinang, dan juga resisten terhadap Ceftazidime, Gentamisin, Ceftriaxone, dan Cefoxitin. Isolat MSSA menunjukkan aktivitas yang kuat terhadap ekstrak etanol buah pinang tua, aktivitas sedang terhadap ekstrak etanol buah pinang muda, dan resisten terhadap gentamisin.
Kesimpulan: Bakteri MRSA resisten terhadap ekstrak etanol buah pinang muda dan pinang tua, sedangkan ekstrak etanol buah pinang tua memiliki aktivitas antibakteri yang kuat terhadap MSSA.

Kata kunci: antibakteri, ekstrak etanol biji pinang, MRSA, MSSA

INTRODUCTION

Staphylococcus aureus bacteria are pathogenic bacteria in humans and one of the main causes of healthcare-associated infections (HAIs). Invasive *S. aureus* infection is a public/community health problem directly related to nosocomial infection. S. aureus colonizes the nose and skin of healthy individuals (30-50%) and is at risk for causing infections, such as bacteremia, endocarditis, osteomyelitis, pneumonia, and skin diseases(1)(2).

Staphylococcus aureus bacteria resistant to Methicillin have the mecA gene related to the production of protein binding penicillin which is a global problem for health facilities(2)(3)(4).

In America, the incidence of MRSA infection reaches 94,000 cases, and the morbidity rate from MRSA infection is 18,650 cases. The prevalence of MRSA infection reached 70% in Asia and 23.5% in Indonesia in 2006. In Jambi Province, the prevalence of MRSA was 45.83% of all S. aureus isolates with the discovery of the mecA gene through a conventional molecular technique, namely Polymerase Chain Reaction (PCR)(5). The incidence of MRSA infection, according to WHO, is estimated to be more than 64% in cases of MSSA infections(6).

Misuse and irrational use of antibiotics, such as long-term use, can cause gene mutations and recombination of gene structures in bacterial cells resulting in bacteria resistance to antibiotics(7). Poor drug quality and unhygienic conditions can also cause bacteria to become resistant to certain antibiotics.

The development and discovery of new antibiotics do not follow the increasing bacteria resistance to antibiotics. This condition urges the need for the development of herbal medicines that have antimicrobial effects to become new alternatives to antibiotics.

Indonesia is one of the areca-nut-producing countries. Areca nut is an alternative herbal medicine with an antimicrobial effect that can be used to treat infections. Areca nut contains polyphenols, alkaloids, flavonoids, steroids, saponins, and tannins that have antioxidant effects, hypoglycemic activity, anti-hypertensive, aphrodisiac activity, antidepressant, antifungal, anti-bacterial, antiviral, anticarcinogenic, anti-inflammatory, anti-malarial, anti-HIV, anti-nociceptive, analgesic, dental caries prevention, hypoallergenic, anti-migraine, and immunosuppression(8)(9). Areca nut is used as an alternative medicine for treating infections due to its antimicrobial effect against gram-positive and gram-negative bacteria(10)(11)(12). This effect has been tested on microbes, such as *Streptococcus mutans, Streptococcus salivarius, Candida albicans, and Fusiform*

nucleatum(8)(13)(14)(15). However, the study on the antimicrobial effects of areca nuts against Staphylococcus aureus is still controversial.

This research aimed to determine the efficacy of the antibacterial activity of areca nut (Areca catechu L) ethanol extract against MRSA and MSSA isolates.

METHOD

Areca nut ethanol extract and bacterial samples

The identification of areca plant specimens was carried out at Celebense Herbarium, Tadulako University, Sulawesi. Furthermore, the ethanol extract of young and old areca nuts was macerated using 96% ethanol.

The research samples were clinical isolates of MRSA bacteria, MSSA ATCC 25923 as a positive control, and MSSA ATCC 29213 as a negative control. Confirmation of the MRSA and MSSA phenotypes was performed using the disc diffusion method with 30 µg cefoxitin. Genotype confirmation was conducted using MecA gene PCR(5)(16).

Antibacterial Activity Test

The ethanol extracts of young and old areca nuts were carried out using the *Kirby-Bauer* disc diffusion method against the isolates of MRSA (clinical isolates) and MSSA (SA 25923/K+, SA 29213/K-). Antibacterial activity test was done in Mueller Hinton Agar with other commonly used antimicrobials, namely Ceftadizime/CAZ (30 g), Gentamicin/CN (10 g), Amoxycillin/ AMC (30 g), Ceftriaxone/CRO (30 g), Vancomycin/ VA (30 g), Cefazolin/KZ (30 g), and Cefoxitin/FOX (30 g). Bacterial culture in Mueller Hinton agar plates was given standard antibiotic discs and areca nut aseptically (1 petri dish consists of 5 discs). The petri dish was left for 1 hour at 25°C then incubated aerobically at 37°C for 24 hours for bacterial growth(16). The diameter of the disc inhibition zone was measured using a caliper(17). The sensitivity criteria for areca nut extract used were 0 mm: no activity/TA; 7-11 mm: weak activity/AL; 11-16 mm: moderate activity/AS; >16 mm: strong activity/AK.

Numerical data was recorded, and the average calculation of each bacterial strain for each variable was carried out.

RESULTS

This research showed that the phytochemicals of young areca nut extracts contained alkaloids, flavonoids, phenols, and steroids and those of old areca nut extracts contained alkaloids, flavonoids, and phenols (Table 1).

Table 1. Phytochemical test results for young and old areca nut extracts

Identification of secondary metabolite compounds	Library	Young Areca nut	Old Areca Nut
1.Alkaloids			
a. Mayer	Deposits (+)	+	+
b. Dragendorff	Deposits (+)	+	+
2.Flavonoids	Coating of Orange yellow or alcohol amyl red	+	+
3.Phenol	Solutions of green, red, purple, blue, solid black	+	+
4.Steroid	Solutions of red, blue, green	+	-

The MRSA and MSSA phenotypes were confirmed using the disc diffusion method with 30 µg cefoxitin (Table 2). Meanwhile, genotypic confirmation was done by MecA gene PCR (Figure 1).

Table 2. Confirmation table for MRSA and MSSA phenotypes based on disc diffusion with 30 µg cefoxitin antibiotic

Bacterial		Diameter (cm)	Interpretation
MRSA		15,25	MRSA
MSSA	ATCC	32,49	MSSA (K+)
25923			
MSSA	ATCC	28,62	MSSA (K-)
29213			

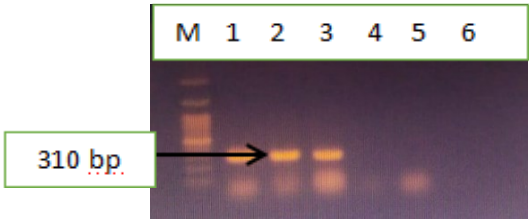

Figure 1. Results of gel electrophoresis PCR MecA.M:Marker 100 bp;1-3:Clinical isolates;4:MSSA ATCC 25923;5:MSSA ATCC 29213;6:K-.

The MRSA isolates did not show the activity of the two areca nut ethanol extracts and were also resistant to Ceftadizime, Gentamicin, Ceftriaxone, and Cefoxitin. MSSA isolates showed strong activity against ripe areca nut ethanol extract, moderate activity against unripe areca nut ethanol extract, and resistance to gentamicin (Table 3).

Table 3. Diameter of Inhibition Zone of Honey and Areca Nut Ethanol Extract against MRSA and MSSA

Diameter	MRSA		MSSA (SA25923)		MSSA (SA29213)	
	Diameter (cm)	Note	Diameter (cm)	Note	Diameter (cm)	Note
Old Areca Nut Extract	6	TA**	11.345	AS**	12.25	AS**
Young Areca Nut Extract	6	TA**	17.68	AK**	16.705	AK**
Ceftazidime (CAZ-30)	11.89	R	24.105	S	21.255	S
Ceftriaxone (CRO-30)	13.02	R	27.75	S	29.38	S
Amox-Clav (AMC-30)	10.60	R	34.855	S	32.245	S
Vancomycin (VA-30)	18.075	S	18.71	S	17.41	S
Cefazolin (KZ-30)	20.66	S	35.27	S	30.99	S
Cefoxitin (FOX-30)	15.83	R	31.88	S	28.535	S
Gentamicin (CN10)	15.75	R	24.73	R	21.865	R
Ethanol (K-)	0	R	0	R	0	R

Note: **0 mm (no activity/TA); 7-11 mm (weak activity/AL); 11-16 mm (moderate activity/US); >16 mm (strong activity/AK);

DISCUSSION

MRSA bacteria showed resistance to ceftazidime, ceftriaxone, cefoxitin, and gentamicin antibiotics and did not show an inhibition zone against both young and old areca ethanol extracts. MSSA bacteria show resistance to gentamicin antibiotics and are sensitive to Ceftazidime, Ceftriaxone, Amoxycillin, Vancomycin, Cefazolin, and Cefoxitin antibiotics. MSSA on young areca nut ethanol extract showed moderate activity results and on old areca nut ethanol extract showed strong activity results (inhibition zone >16 mm). The ethanol extracts of young and old areca nuts can be used as antimicrobials for MRSA.(18) The antimicrobial effect occurs due to the combined effect of the ethanol extract, and the microorganism has not been exposed to the extract before(19)(20).

The ethanol extracts of young and old areca nuts did not provide an inhibition zone for MRSA bacteria. The ethanol extracts of young and old areca nuts provide inhibition

zones on MSSA bacteria with moderate and strong activity, respectively.

The old areca nut contains a compound of secondary alkaloid metabolites, flavonoids, and phenols. Alkaloids have antibacterial activity by interfering with the formation of peptidoglycan cross-bridge components in bacterial cells so that the cell wall layer is not completely formed, and the cell will lyse. Flavonoids are phenolic compounds that tend to cause changes in the composition of membrane phospholipids, followed by swelling and cell wall lysis(21). Different results were found in Lozada's study (2021) that the ethanol extract of areca nuts did not have antibacterial activity against S. aureus bacteria(14).

Antibiotic resistance mechanisms include target changes, antibacterial inactivation, decreased bacterial cell permeability, blocking of antibacterial pathways, and bacterial metabolic changes(22).

CONCLUSION

MRSA bacteria are resistant to the ethanol extract of young and old areca nuts, while the ethanol extract of old areca nuts has strong antibacterial activity against MSSA.

REFERENCES

1. Sakr A, Brégeon F, Mège JL, Rolain JM, Blin O. Staphylococcus aureus nasal colonization: An update on mechanisms, epidemiology, risk factors, and subsequent infections. Front Microbiol. 2018;9(OCT):1–15.
2. Hassoun A, Linden PK, Friedman B. Incidence, prevalence, and management of MRSA bacteremia across patient populations-a review of recent developments in MRSA management and treatment. Crit Care. 2017;21(1):211.
3. Lee AS, De Lencastre H, Garau J, Kluytmans J, Malhotra-Kumar S, Peschel A, et al. Methicillin-resistant Staphylococcus aureus. Nat Rev Dis Prim [Internet]. 2018;4(May):1–23.
4. Kale P, Dhawan B. The changing face of community-acquired methicillin-resistant Staphylococcus aureus. Indian J Med Microbiol [Internet]. 2016;34(3):275–85.
5. Humaryanto, Simanjuntak CA, Hanina, Lipinwati. Identification of methicillin resistant staphylococcus aureus (mrsa) using cefoxitin disc diffusion test and dupleks polymerase chain reaction in Jambi city hospitals. J Phys Conf Ser. 2019;1246(1).
6. Naimi T, Ringwald P, Besser R, Thompson S, Bell D. Antimicrobial resistance. Vol. 7, Emerging Infectious Diseases. 2001. p. 548.
7. Peterson E, Kaur P. Antibiotic resistance mechanisms in bacteria: Relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. Front Microbiol. 2018;9(NOV):1–21.
8. Jam N, Hajimohammadi R, Gharbani P, Mehrizad A. Antibacterial activity of Punica granatum L. and Areca nut (P.A) combined extracts against some food born pathogenic bacteria: Antibacterial activity of Punica granatum L. and Areca nut (P.A). Saudi J Biol Sci [Internet]. 2022;29(3):1730–6.
9. Ansari A, Mahmood T, Bagga P, Ahsan F, Shamim A, Ahmad S, et al. Areca catechu: A phytopharmacological legwork. Food Front. 2021;2(2):163–83.
10. Perumal Samy R, Gopalakrishnakone P. Therapeutic potential of plants as anti-microbials for drug discovery. Evidence-based Complement Altern Med. 2010;7(3):283–94.
11. Anupama M, Puspita D, Rajesh K. Studies on antimicrobial properties of areca nut Areca catechu. J Pharmacogn Phytochem [Internet]. 2021;10(1):961–3.
12. Nelson Anthikat R, Michael A. Study on the areca nut for its antimicrobial properties. J Young Pharm. 2009;1(1):42.
13. Chin AA, Clariza DF, Renalyn BS, Marie SSB, Regine FT, Frederick RM. Antimicrobial performance of ethanolic extract of areca catechu L seeds against mixed-oral flora from tooth scum and gram negative laboratory isolates. Int J Res Ayurveda Pharm. 2013;4(6):876–80.
14. Lozada A, Damasin G, Perez A, Villasan

A, Tomboc A, Pescador A, et al. Antibacterial Activity of Santan (Ixora coccinea) Leaf, Cacao (Theobroma cacao) Pod Husk and Betel Palm (Areca catechu) Seed Extracts Against Staphylococcus aureus. IMCC J Sci [Internet]. 2021;2021:20–31.

15. Baiti Mi, Elfrida S, Lipinwati L. Pengaruh Pemberian Ekstrak Ethanol Biji Buah Pinang (Areca Catechu L.) Terhadap Pertumbuhan Staphylococus Aureus Secara in Vitro. JAMBI Med J "Jurnal Kedokt dan Kesehatan." 2018;6(1):10–9.
16. Nursidika P, Saptarini O, Rafiqua N. Aktivitas Antimikroba Fraksi Ekstrak Etanol Buah Pinang (Areca catechu L) pada Bakteri Methicillin Resistant Staphylococcus aureus. Maj Kedokt Bandung. 2014;46(2):94–9.
17. Palavecino EL. Chapter 2 : Rapid Methods for Detection of MRSA in Clinical Specimens. In: Methicillin-Resistant Staphylococcus Aureus (MRSA) Protocols Yinduo Ji Editor Cutting-Edge Technologies and Advancements Methods in Molecular Biology. 2020. p. 29–45.
18. Sepehri Z, Hassanshahian M, Shahi Z, Kiani Z, Nasiri AA, Ali M, et al. Antibacterial Effect of Ethanol Extract of Camellia Sinensis L against Escherichia Co li. 2014;2(1):6–8.
19. Koike Y, Nishiura H. Recovery of antimicrobial susceptibility in methicillin-resistant Staphylococcus aureus (MRSA): A retrospective, epidemiological analysis in a secondary care hospital, Sapporo, Japan. PeerJ. 2021;
20. Wang D, özen C, Abu-Reidah IM, Chigurupati S, Patra JK, Horbanczuk JO, et al. Vasculoprotective effects of pomegranate (Punica granatum L.). Front Pharmacol. 2018;9(MAY):1–15.
21. Guerrero-Solano JA, Jaramillo-Morales OA, Jiménez-Cabrera T, Urrutia-Hernández TA, Chehue-Romero A, Olvera-Hernández EG, et al. Punica protopunica balf., the forgotten sister of the common pomegranate (punica granatum L.): Features and medicinal properties—a review. Plants. 2020;9(9):1–15.
22. Köves B, Magyar A, Tenke P. Spectrum and antibiotic resistance of catheter-associated urinary tract infections. GMS Infect Dis [Internet]. 2017;5:Doc06.