Indonesian Journal of Medical Laboratory Science and Technology

Open Access p-ISSN 2684-6748 | e-ISSN 2656-9825

Ability of Ethanol Extract from Ajwa and Sukkari Dates (*Phoenix dactylifera* L.) in Inhibiting the Growth of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

Putra Rahmadea Utami[1], Sri Indrayati[1], Nur Hayatang[1]

[1]Department of Medical Laboratory Technology, Faculty of Health Science, Universitas Perintis Indonesia, Padang, Indonesia

Correspondence:
Putra Rahmadea Utami,
Jl. Adinegoro KM 17, Simpang Kalumpang, Lubuk Buaya, Padang, West Sumatera, Indonesia
Zip Code: 25586

Email:
putrarahmadeautami123@gmail.com

Received: December 8th, 2020
Revised: March 12th, 2021
Accepted: March 15th, 2021
Published: April 28th, 2021

DOI: 10.33086/ijmlst.v3i1.1848

Abstract

Staphylococcus aureus is a pathogenic bacterium that spread throughout the world and still a problem that continues to increase both in hospitals and the community. Infections due to *S. aureus* usually treated with antibiotics, but in some cases, several strains of *S. aureus* found to be resistant to antibiotics, such as Methicillin-Resistant *Staphylococcus aureus* (MRSA). Based on the previous research, the ethanol extract from Ajwa and Sukkari dates formed an inhibitory zone against the MRSA bacteria growth. This study aims to determine the inhibition of the ethanol extract from Ajwa and Sukkari variety of dates (*Phoenix dactylifera* L.) on the *S. aureus* growth. The ethanol extract from Ajwa and Sukkari dates with a concentration of 5 mg/mL, 10 mg/mL, 15 mg/mL, and 20 mg/mL resulted in the same inhibition zone with a diameter of ≤ 6 mm which categorized as weak (resistant), whereas the positive control ciprofloxacin had a resistance zone with a diameter of 9 mm. This study results concluded that the ethanol extract of Ajwa and Sukkari dates only has a maximum concentration of 20 mg/mL, which is still classified as a low concentration and has not been able to inhibit MRSA bacteria growth.

Keywords

Dates Ajwa, Dates Sukkari, Extract Ethanol, MRSA.

INTRODUCTION

Staphylococcus aureus is treatable with antibiotics, but several strains of *S. aureus* found to be resistant to antibiotics, such as Methicillin-Resistant *Staphylococcus aureus* (MRSA). The spread of MRSA has been the subject of several studies because the danger of antibiotic resistance is a serious world health problem in both developed and developing countries. In 2010, the prevalence

of MRSA is 28% (Hong Kong and Indonesia) and 70% (Korea) among all clinical isolates of *S. aureus*, while the *S. aureus* infections found in related communities in Asian countries varied widely, from 5–35% (1).

The presence of MRSA found in the hospital environment. The study of Vysakh & Jeya (2) obtained 450 *S. aureus* isolates collected from patients from several hospitals in India, which 121 positives for MRSA (27%) and 329 as MSA (73%). The University of Alexandria, which collected 50 isolates of *S. aureus* strains from several hospitals, found 40% strains resistant to the antibiotics Oxacillin and cefoxitin, which indicates the presence of MRSA in these isolates (2). However, research conducted in Indonesia found that the prevalence of MRSA in RSUD Dr. Sutomo Surabaya, Indonesia was 8.2%. Despite the results, the study showed that with the low prevalence of MRSA in Indonesia, a coping strategy still needed to prevent further infection (1,3).

Dates are one of the plants in their role as medicine and food. Dates are a food ingredient that is rich in vitamins, minerals, fiber and sugar. In some varieties, the sugar content of dates can reach up to 88%. Many researchers researched twelve dates varieties to find out their chemical content, such as flavonoid compound (1,2). Aldaihan and Bhat examined the benefits of dates (*Phoenix dactylifera* L) in vitro and found that one of its benefits is that they have antibacterial properties. This research showed that flavonoids are the active substances in dates. Flavonoids can be used as immunomodulators to increase the work of macrophages on pathogenic microbial phagocytes that attack the body (4,5). Based on the previous research, the ethanol extract from Ajwa and Sukkari dates formed an inhibitory zone against the MRSA bacteria growth (2).

Extraction is a process of separating a substance based on differences in its solubility in two different insoluble liquids, usually water and other organic solvents such as nutritious substances or active substances from a part of medicinal plants, animals, and several types of fish, including marine biota (3). Due to the different thickness of plant and animal cells, an extraction method with a particular solvent needed. This extraction method is based on the principle of mass transfer agent component in a solvent, wherein the displacement began in the interface layer and then diffuses into the solvent (6). This study aims to determine the inhibition of the ethanol extract from Ajwa and Sukkari variety of dates (*Phoenix dactylifera* L.) on the *S. aureus* growth.

MATERIALS AND METHODS

This research used an experimental research design and conducted in July-August 2020 at the Microbiology Laboratory of Andalas University, Padang, Indonesia.

This study performed to determine the inhibition zone of the ethanol extract from Ajwa and Sukkari dates against the MRSA.

This study used the ethanol extract from Ajwa and Sukkari dates. Extraction was carried out using 96% ethanol solvent for 3x24 hours and evaporated using a rotary evaporator to obtain a thick extract of ± 150 g. The extract concentrations used were 5 mg/mL, 10 mg/mL, 15 mg/mL and 20 mg/mL. The study used MRSA bacteria, namely *S. aureus*. Bacterial isolates obtained from RSUP Dr. M. Djamil Padang, Indonesia. Bacteria isolates were resistant to penicillin-derived antibiotics. The positive control used ciprofloxacin, while the negative control used sterile aquadest for the treatment.

RESULTS

Characteristics of Ethanol Extract of Sukkari and Ajwa Dates

The pulp of Ajwa and Sukkari dates each ±500 g separated from the seeds. Furthermore, from the ethanol extract, each obtained ±150 g of thick extract. The thick extracts from Ajwa dates and Sukkari dates shown in Figure 1. The color of Sukkari dates is darker than Ajwa dates.

Characteristics of Methicillin-Resistant *Staphylococcus aureus* (MRSA)

The bacteria used were MRSA bacteria obtained from the Microbiology Laboratory of Andalas University, Padang. The MRSA bacterial suspension is already present in the media (Figure 2).

Inhibition Test of Ethanol Extract of Ajwa Dates and Sukkari on the Growth of Methicillin-Resistant *Staphylococcus aureus*

The test results of the inhibition of the ethanol extract of Ajwa and Sukkari dates with various concentrations of 5 mg/mL, 10 mg/mL, 15 mg/mL, and 20 mg/mL can be seen in Figure 3. This study result showed that the ethanol extract compounds from Sukkari and Ajwa dates could not inhibit MRSA bacteria growth. After that, it observed that there was no clear zone or zone of inhibition around the disc.

In this study, the positive control were used ciprofloxacin 50 mg, while the negative control were used sterile distilled water. Table 1 showed that the extract from Ajwa and Sukkari dates could not inhibit MRSA bacteria growth. A seen in Figure 2, the zone of clearance appears from disk diameter paper discs. Repetition performed three times to show the inhibition zone diameter ≤ 6 mm (diameter paper discs).

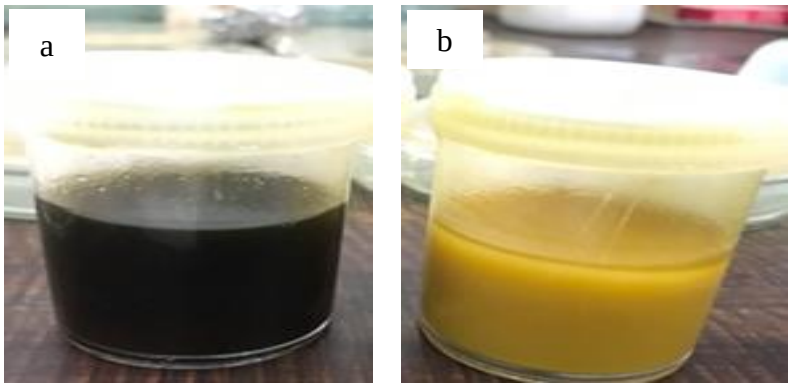

Figure 1. Dates extract using ethanol solvent. (a) Sukkari, (b) Ajwa.

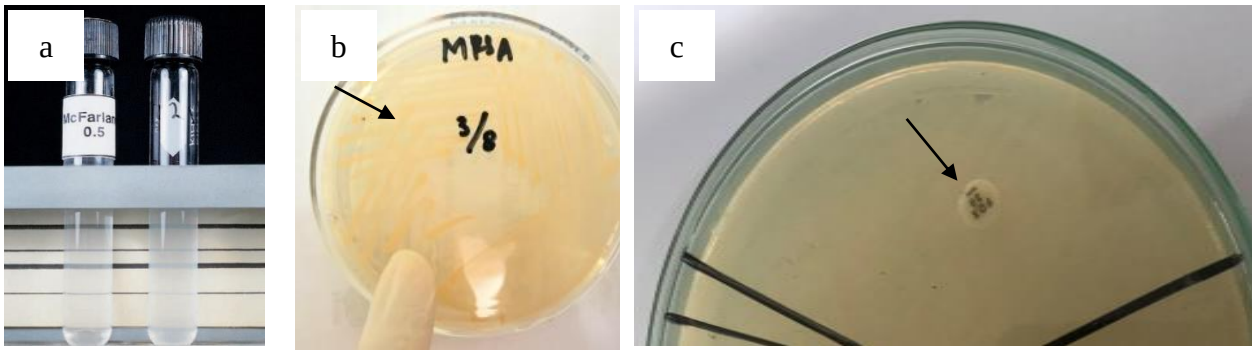

Figure 2. Characteristics of MRSA. (a) MRSA turbidity compared to McFarland standard of 0.5 (left) and bacterial suspense (right), (b) The arrow in figure B shows the MRSA colony of bacteria, (c) The arrow in Figure C shows cefoxitin resistance.

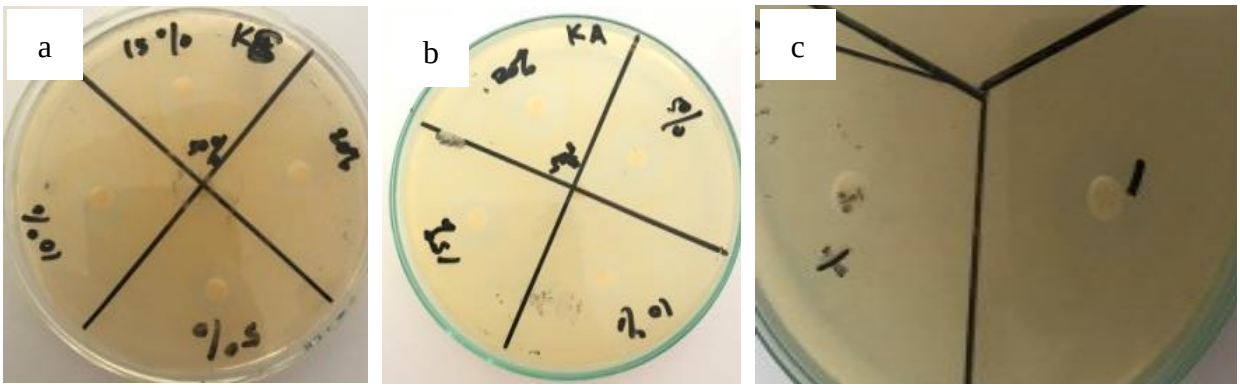

Figure 3. Inhibition test. (a) Sukkari dates ethanol extract concentrations of 5 mg/mL, 10 mg/mL, 15 mg/mL, and 20 mg/mL, (b) Ajwa date ethanol extract concentrations of 5 mg/mL, 10 mg/mL, 15 mg/mL, and 20 mg/mL, (c) and Ciprofloxacin.

Table 1. The results of the inhibition test of MRSA

Ethanol Extract Dates	Concentration (mg/mL)	Extract Repetition (mm)			X
		1	2	3	
Ajwa dates	5	≤ 6	≤ 6	≤ 6	≤ 6
	10	≤ 6	≤ 6	≤ 6	≤ 6
	15	≤ 6	≤ 6	≤ 6	≤ 6
	20	≤ 6	≤ 6	≤ 6	≤ 6
Sukkari dates	5	≤ 6	≤ 6	≤ 6	≤ 6
	10	≤ 6	≤ 6	≤ 6	≤ 6
	15	≤ 6	≤ 6	≤ 6	≤ 6
	20	≤ 6	≤ 6	≤ 6	≤ 6
Control positive	Ciprofloxacin 50 mg			9	
Negative control	Aquadest			≤ 6	

Note: Description of disc paper diameter = 6 mm

DISCUSSION

The study results showed no inhibition zone formed from the ethanol extract of Ajwa and Sukkari dates against the growth of MRSA bacteria. Ajwa and Sukkari dates ethanol extract with concentrations of 5 mg/mL, 10 mg/mL, 15 mg/mL and 20 mg/mL formed weak (resistant) inhibition zone diameter of ≤6 mm. Meanwhile, the positive control ciprofloxacin formed an inhibition zone with a diameter of 9 mm (7).

This condition happened because the bacteria used for this study was MRSA, where these bacteria are a group of bacteria that are already resistant to antibiotics. MRSA is a *S. aureus* that immune to methicillin-type antibiotics. MRSA experiences resistance due to genetic changes caused by irrational exposure to antibiotic therapy (8). Besides, the research results also prove that MRSA is also resistant to other antibiotics. This methicillin resistance will be followed by the simultaneous emergence of resistance to a large number of other antibiotic classes through the acquisition of additional receptor determinants, or as a result of mutations. All of which will cause the receptors at the target site to become resistant (9,10).

One of the factors of differences in sensitivity patterns or determinants of bacterial resistance to antimicrobials carries by genetic information outside the chromosomes, namely plasmid (11). *S. aureus* is a bacterium that has small plasmids and large plasmids that have more than one resistance gene. Based on the study result, it can be assumed that there are large plasmids that carry more than one antibiotic-resistant gene in MRSA. Additionally, the methicillin-

resistant gene may be linked to other antibiotic-resistant genes. MRSA bacteria, which are Gram-positive bacteria, have a peptidoglycan layer that is 20–80 nm thick. Gram-positive bacteria also have a cell wall containing teichoic acid, which is a water-soluble polymer that functions as a transport for positive ions in and out (11,12,13).

Other studies conducted on the use of plant extracts to inhibit MRSA growth. One of the studies showed that the clove flower extract has an antimicrobial effect, which containing eugenol, flavonoids, tannins, saponins, alkaloids and phenols that can damage the structure of bacterial cells. The results showed that the Minimum Bactericidal Concentration (MBC) was 0.39%. One Way ANOVA test showed a significant difference ($p < 0.05$) between clove flower extract concentration and the number of MRSA colonies (1). Clove flower extract can kill MRSA by damaging the structure of bacterial cells. Another study was to test the inhibition of the ethanol extract of bitter melon (*Momordica charantia*) against the growth of MRSA. The ethanol extract of bitter melon fruit used as a test solution with a concentration (w/v) of 20%, 40%, 60%, and 80%. The results showed that the average diameter of the inhibition zone of the ethanol extract of bitter melon against the growth of MRSA with concentrations of 20%, 40%, 60%, and 80% was 6.16 mm, 9.5 mm, 10.83 mm, 12.3 mm respectively. The higher the ethanol extract concentration of bitter melon fruit, the higher the inhibition power of the growth of MRSA (14,15,16).

The plant extracts used also contain content that is almost the same as other plants, namely eugenol, flavonoids, tannins, saponins, alkaloids and phenols, which can damage the structure of bacterial cells. However, in this study, the ethanol extract of dates did not have a significant ability to inhibit the growth of MRSA (2). Whereas in research, a combination of extracts with different plants can inhibit the growth of other bacteria. In the research of Utami *et al.* (13), the results of testing the combination of Chinese petai extract and *Aloe vera* showed significant differences ($p < 0.05$) at the concentrations of 25 g, 50 g, 75 g, and 100 g. The ethanol extract of Chinese petai and *Aloe vera* can inhibit the growth of E. coli. The results showed the most effective concentration of 100 g/mL resulted in an interaction between the ethanol extract of *Aloe vera* and china petai in inhibiting the growth of *E. coli* (12,17). In contrast, this Ajwa and Sukkari date research, only have 20 mg/mL as the maximum concentration, which still classified as a low concentration. As a result, the active compound content is the only low level and unable to inhibit the growth of MRSA bacteria (4,18,19).

CONCLUSIONS

The ethanol extract of Ajwa and Sukkari dates with a concentration of 5 mg/mL, 10 mg/mL, 15 mg/mL, and 20 mg/mL formed the same inhibition zone with a diameter of ≤6 mm. It conclusion, this study indicate that the ethanol extract of Ajwa and Sukkari dates only has a maximum concentration of 20 mg/mL, which is still classified as a low concentration and has not been able to inhibit MRSA bacteria growth by damaging the bacterial cell structure.

AUTHOR CONTRIBUTIONS

Putra Rahmadea Utami: conceptualization, methodology, writing-reviewing and editing. Sri Indrayati: data curation, writing-original draft preparation, supervision. Nur Hayatang: visualization, investigation.

ACKNOWLADGEMENTS

This study was supported by the Universitas Perintis Indonesia.

CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Setiawan HG, Kaligis AHM, Assa YA. Description of serum apolipoprotein B (APO-B) levels in lacto-ovo vegetarians. Ebiomedik. 2017;5(1):2–5.
2. Kuntaman K, Hadi U, Setiawan F, Koendori EB, Rusli M, Santosaningsih D, et al. Prevalence of methicillin resistant *Staphylococcus aureus* from nose and throat of patients on admission to medical wards of Dr Soetomo hospital, Surabaya, Indonesia. Southeast Asian J Trop Med Public Health. 2016;47(1):66–70.
3. Kader OA, El-Batouti GA, Ghazal AA, Baraka KM. Hospital-acquired methicillin resistant *Staphylococcus aureus*: analysis of mec A gene and taphylococcal cassette chromosome. Int J Curr Microbiol Appl Sci. 2015;4(9):805–15.
4. Nismawati, Rizalinda S, Agus R. Deteksi Methicillin Resistant *Staphylococcus aureus* (MRSA) pada pasien Rumah Sakit Universitas Hasanuddin dengan metode kultur [Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA) in Hasanuddin University Hospital patients by culture method]. Pros Semin Nas Biol. 2018;4(1):978–602.
5. Hamad I, Abdelgawad H, Al Jaouni S, Zinta G, Asard H, Hassan S, et al. Metabolic analysis of various date palm fruit (*Phoenix dactylifera* L.) cultivars from Saudi Arabia to assess their nutritional quality. Molecules. 2015;20(8):13620–41.
6. Hariadi B, Widodo A. The Effect of Dates (*Phoenix Dactylifera* L.) Extract of Ajwa varieties on no levels in Balb/C mice infected with *Salmonella typhimurium*. Diponegoro Med J (Jurnal Kedokteran Diponegoro). 2018;7(2):751–61.
7. Syarif RA, Sari F, Ahmad AR, Indonesia UM, Urip J, Km S. Rimpang kecombrang (*Etlingera elator* Jack.) as a phenolic source. J Feto Ina. 2000;2(2):102–6.
8. Fiţ NI, Chirilă F, Nadăş G, Pall E, Mureşan R. Comparative testing of antimicrobial activity of aqueous extracts of *Aloe vera* and *Lycium barbarium*. Bull Univ Agric Sci Vet Med Cluj-Napoca - Vet Med. 2013;70(1):72–6.
9. Hu W, Li C, Dai J, Cui H, Lin L. Antibacterial activity and mechanism of *Litsea cubeba* essential oil against methicillin-resistant *Staphylococcus aureus* (MRSA). Ind Crops Prod. 2019;130(November 2018):34–41.
10. Bussmann RW, Glenn A, Sharon D. Antibacterial activity of medicinal plants of Northern Peru - can traditional applications provide leads for modern science?. Indian J Tradit Knowl. 2010;9(4):742–53.
11. Craft KM, Nguyen JM, Berg LJ, Townsend SD. Methicillin-resistant: *Staphylococcus aureus* (MRSA): Antibiotic-resistance and the biofilm

phenotype. Medchemcomm. 2019;10(8):1231–41.

12. Suryati N, Bahar E, Ilmiawati I. Antibacterial effectiveness test of *Aloe vera* extract against *Escherichia coli* growth in vitro. J Kesehat Andalas. 2018;6(3):518.
13. Rahmadea UP, Chairani, Yudha H. Combination test of chinese leaf extract (*Leucaena leucocephala* folium) and *Aloe Vera* inhibiting growth *Escherichia coli*. Indones J Med Lab Sci Technol. 2020;2(2):60–7.
14. Utami PR, Chairani C, Ilhamdi I. Interaksi ekstrak etanol daun petai cina (*Leucaena leucocephala* folium) dan lidah buaya (*Aloe vera* L.) menghambat pertumbuhan *Staphylococus aureus* secara invitro [The Interaction of ethanol extract of chinese petai leaves (*Leucaena leucocephala* folium) and *Aloe vera* (*Aloe vera* L.) inhibiting the growth of *Staphylococus aureus* by invitro]. J Kesehat PERINTIS (Perintis's Heal Journal). 2019;6(2):186–92.
15. Azizah A, Suswati I, Agustin SM. Anti-microbial effect of clove flower extract (*Syzygium Aromaticum*) against Methicillin-Resistant *Staphylococcus Aureus* (MRSA) in vitro. Saintika Med. 2018;13(1):31.
16. Suhaimi S, Indrawati T, Kumala S. Combination test of crude fruit extracts (*Aloe vera*. (L) brum. F.) and blue leaves (*Piper crocatum* ruiz & pav) extracts for purple leaf (*Piper crocatum* ruiz & pav) antibacteries for acne. JIFFK J Ilmu Farm dan Farm Klin. 2018;15(01):12.
17. Cuny C, Friedrich A, Kozytska S, Layer F, Nübel U, Ohlsen K, et al. Emergence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in different animal species. Int J Med Microbiol. 2010;300(2–3):109–17.
18. Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H, et al. Meticillin-resistant *Staphylococcus aureus* (MRSA): Global epidemiology and harmonisation of typing methods. Int J Antimicrob Agents. 2012;39(4):273–82.
19. Pratama P, Purwanta M, Qurnianingsih E. The effectiveness of ethanol extract of Egyptian date palm seeds (*Phoenix dactylifera* L.) as an antibacterial agent against *Streptococcus pyogenes* in vitro. 2019;19(3):135–40.
20. Vysakh PR, Jeya M. A comparative analysis of community acquired and hospital acquired methicillin resistant *Staphylococcus aureus*. J Clin Diagnostic Res. 2013;7(7):1339–42.