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Antimicrobial effect of alkaloids extract of *Areca catechu L* against *Methicillin Sensitive Staphylococcus aureus* Bacteria

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Abstract

Background: Infection caused by the bacterium *Metihcillin Sensitive Staphyloccous aureus* (MSSA) is still one of the infections that often occurs in humans. The emergence of various cases of resistance of MSSA bacteria to various antibiotics both in the community and in hospitals (nosocomial infections), has triggered the development of herbal medicines, one of which is the areca nut (*Areca catechu L*) which is thought to have antibacterial effects. The purpose of this research was to determine the antimicrobial effect of alkaloids in areca nut extract against MSSA bacteria.
Method: Areca nut was extracted with 96% ethanol using the maceration method and continued with areca nut seeds and fractionation of the alkaloid active substances with ethanol-water, n-hexane and ethyl acetate (1:1) solvents. The sensitivity test of bacteria using the Kirby Bauer disc diffusion method with duplex repetitions. Amoxcillin clavulanat used as standard antimicrobial of antimicrobial test.
Results: The extract components contained are alkaloids, flavonoids and tannins. Furthermore, the resistance test of MSSA bacteria to areca nut alkaloid extract and the amoxicillin antibiotic disc gave results of an average inhibitory zone diameter of 20.33 mm (strong activity) and 22.80 mm (strong activity).
Conclusion: MSSA bacteria showed sensitivity to areca nut alkaloid extract with strong activity.

Keywords: MSSA, alkaloids, areca nut, sensitivity.

INTRODUCTION

Methicillin Sensitive Staphylococcus Aureus (MSSA) is the main bacteria that causes infections in humans because this bacteria is a normal flora that is opportunistic in causing infections. MSSA bacteria is normally found in the nose and skin and has the potential for upper respiratory tract infections and skin diseases ranging from mild to severe infections such as bacteremia, endocarditis and osteomyelitis.(1,2)

MSSA infection is still a health problem both in the community and in hospitals (nosocomial infection).(3) This is related to bacterial resistance to certain antibiotics or mutations in bacterial genes due to errors in drug use or irrational use. To overcome this, many researchers have developed various new antibiotics, both synthetic and derived from herbal ingredients.(4–7)

Areca nut (*Areca catechu L)* contains polyphenols, alkaloids, flavonoids, steroids, saponins and tannins. This compound has various pharmacological activities such as antibacterial and antifungal effects and antiparasitic effects, even anti-depressive effects, anti-fatigue effects, antioxidant effects, antihypertensive effects, anti-inflammatory and analgesic effects, etc.(8–10) As an antibacterial, several studies have shown that Areca nut is effective against both gram-positive and gram-negative bacteria.(11–13) Several microbes that have been proven to be treated with Areca nuts include *Streptococcus mutans, Streptococcus*

salivarius, Klebsiella, Pseudomonas auroginosa, Candida albicans, and Fusiform nucleatum.(4,8,10,14–16)

Alkaloid is an active compound with a complex structure containing a heterocyclic ring, this compound comes from plants.(17) Based on their heterocyclic rings, alkaloids are classified into several groups, namely indole, purine, quinoline, isoquinoline, tropane, imidazole, among others. Alkaloids have antiproliferative, antibacterial, antioxidant potential, which can be used for the development of drugs.(18)

Jambi area is a producer of areca nut and several studies on areca nut seeds have shown that there is antimicrobial activity of areca nut extract against the bacteri. This research aims to see whether the alkaloid compounds contained in areca nut seeds can act as antimicrobials against MSSA bacteria.

METHOD

Good, fresh, not rotten, orange areca nuts are washed with soap to clean the areca nuts from dirt, and dried in the sun. Once dry, the areca nut is cut in half and the seeds are taken, the seeds are chopped and placed in the oven for 18 hours. Next, the areca nut powder is made by sifting it with a 30mesh sieve. The areca nut powder is stored in a damp place.

Areca nut extract is obtained by adding 96% ethanol for 3 x 24 hours using a maceration method with a ratio of 1:10.(4) The maserate is evaporated using an evaporator, so that a thick areca nut extract is obtained. The extract was fractionated using a separating funnel using ethanol-water, n-hexane and ethyl acetate solvents in a 1:1 ratio.(19) The fractionation results were carried out by phytochemical testing.

The antimicrobial test used the Kirby-Bauer disk diffusion method. The bacterial culture suspension was incubated for 6 hours with an OD of 0.5 Mc-Farland which is equivalent to 1.5 x 108 CFU/ml in 5 ml of peptone water, spread on a Mueller-Hinton agar petri dish and allowed to dry for 0.5 hour in the incubator. Each bacteria was carried out in 2 petri dishes. On the empty disc, alkaloid extract was given which was left for 1 day. The same thing is done with areca nut extract. The standard antibiotic used is Amoxicillin clavulanate (30 µg).(4)

Bacterial cultures on Mueller Hinton agar petri plates were given standard antibiotic discs, and areca nut alkaloid extract aseptically (1 petri dish consists of 5 discs). The Petri dish was left for 1 hour at 25 °C and then incubated aerobically at 37 °C for 24 hours for bacterial growth.(4) Inhibition zone observations were measured and evaluated. The bacterial sensitivity test was repeated using duplex on MSSA bacteria and the zone of inhibition was measured in mm.

RESULTS

Ethanol extract was obtained by macerating areca nut seeds with the addition of 96% ethanol for three times 24 hours. The ethanol extract was then fractionated using a separating funnel using ethanol-water, n-hexane, and ethyl acetate solvents to obtain areca nut alkaloid extract. Fractionation is carried out in three phases, where phase 1 is the phase where the largest alkaloid compounds will be separated from the ethanol extract, then phases 2 and 3 function to filter again if there are still alkaloid compounds remaining in the extract. The characteristics of the fractionation results are presented in Table 1.

The alkaloid extract that has been obtained is subjected to qualitative phytochemical testing to prove what compounds are still contained in the extract. The results of the phytochemical tests are presented in Table 2. In table 2 it can be seen that the results of the fractionation of areca nut extract in this study still do not contain pure alkaloid compounds. It was proven that the flavonoid and tannin test results also showed positive results. However, the alkaloid content in phase 1 is greater than the alkaloid content in phase 2. Likewise, the alkaloid content in phase 2 is greater than in phase 3

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Table 1. Organoleptic Characteristics of Areca Seed Extract

Organoleptic	**Ethanol Extract**		
Colour	Blackish brown		
Smell	Typical of ethanol		
Texture	Not sticky		
pH	4,6		
Organoleptis	**Ethanol Extract Fractioation Results**		
	Phase 1	**Phase 2**	**Phase 3**
Shape	Liquid 150 ml	Liquid 250 ml	Liquid 200 ml
Smell	Typical acidic ethanol	Typical of ethanol	Typical of ethanol
Colour	Reddish brown	Clear brown	Clear yellow

Table 2. Phytochemical Test Results of Areca Seed Alkaloid Extract

Identification of secondary metabolite compounds	**References**	**Result**		
		Phase 1	**Phase 2**	**Phase 3**
1. Alkaloid				
a. Mayer	White precipitate	+++	++	+
b. Dragendorf	Orange red precipitate	+++	++	+
2. Flavonoid	Yellow orange or red layer of amyl alcohol	+	+	+
3. Tanin	The solution is dark blue or black	+	+	+
4. Saponin	There is foam that lasts for 5 minutes	-	-	-

The antimicrobial test used the Kirby-Bauer disk diffusion method. On the blank disc, various phases of alkaloid extract were given. The positive control used a standard antibiotic, namely Amoxicillin clavulanate (30 µg) which has been proven to be an antimicrobial that is sensitive to MSSA bacteria. The alkaloid extract was dried and then applied to paper disks in various phases in duplicate to test its inhibitory power against the growth of MSSA bacteria. The average diameter of the inhibition zone of areca nut alkaloid extract in various phases is presented in Table 3.

Table 3. Diameter of the Zone of Inhibition of Areca Seed Extract against MSSA Bacteria

Substances on disk discs	**Inhibition zone diameter (mm)**
Areca nut extract phase 1	20,33
Areca nut extract phase 2	19,40
Areca nut extract phase 3	16,73
Amoxcicillin clavulanate 30 mg (positive control)	22,80
Ethanol (negative control)	0

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

It can be seen in Table 3 that all phases of the alkaloid extract showed strong activity against MSSA bacteria as evidenced by an inhibition zone diameter of more than 16 mm. In the table it can also be concluded that phase 1 has stronger MSSA antibacterial activity than phases 2 and 3. Phase 2 also shows stronger MSSA antibacterial activity than phase 3. This means that the higher the level of alkaloids contained in the extract, the better the activity. in inhibiting the growth of MSSA bacteria.

DISCUSSION

In previous studies, it has been proven that ethanol extracts of young and old areca seeds provide inhibition zones against MSSA bacteria with moderate activity and strong activity respectively (20). Old areca seeds contain secondary metabolite compounds alkaloids, flavonoids, phenols. Alkaloids are one of the natural compounds that can be chosen as antibacterials, because 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 formed completely and the cells will experience lysis.(18) One journal even states that alkaloids can fight MRSA bacteria by inhibiting pyruvate kinase enzymes, Quorum quenching effect, alteration in efflux pump in MRSA and intercalating of bacterial DNA.(21,22) However, the extract produced by the fractionation method in this study is not a pure alkaloid extract, this is a limitation of the study, so that in addition to alkaloids this extract also still contains flavonoids and tannins. Flavonoids are phenolic compounds that have a tendency to cause changes in the arrangement of membrane phospholipids followed by swelling and lysis of cell walls.(9) A study states that flavonoids and tannins can also inhibit biofilm formation and hemolytic activity of Staphylococcus aureus.(23) Tannin alone also shows bacteriostatic and bactericidal effects against MRSA bacteria by disrupting protein synthesis in MRSA and affecting the integrity of the bacterial cell membrane.(24,25) Antibacterial activity is influenced by several factors, namely extract concentration, antibacterial compound content, extract diffusion power and the type of bacteria inhibited. Simultaneously, the three active compounds contained in areca nut extract are also found in several other plants that also have the potential as anti MSSA, namely in *Aloe secundiflora, Bulbine frutescens, Tagetes minuta, Vernonia lasiopus, Anredera cordifolia* and *Terminalia avicennioides*. (22,26,27)

Similarly, the areca nut extract in this study, which contains both alkaloids, flavonoids and tannins, the higher the concentration of the extract, the greater the diameter of the inhibition zone of bacterial growth, this is because the content of active compounds of areca nut at high concentrations is greater than at low concentrations. In this study, it can be proven that areca nut alkaloid extract has an inhibitory effect on the growth of MSSA bacteria with strong activity.

CONCLUSIONS

Areca nut alkaloid extract has strong activity against the growth of MSSA bacteria. This means that the areca nut alkaloid extract has great potential as an antimicrobial agent for MSSA bacteria.

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