ANTIBACTERIAL ACTIVITY OF ARECA NUT SOAP FORMULATION AGAINST STAPHYLOCOCCUS AUREUS

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ABSTRACT

***Background**: Areca nuts are widely grown in Jambi and areca seeds have antibacterial activity, antioxidant, skin aging and cosmetics. It has the potential to be developed as antibacterial soap.*

***Objective:** This study was to compare the antibacterial effect of betel nut concentration powder and extract in our soap formulation against Staphylococcus aureus using the in vitro test.*

***Method:** Soap formulation was made with 3 concentration of areca nut powder in soap, namely 1,5 grams/soap (soap 1); 2,3 grams/soap (soap 2) and 3 grams/soap (soap 3). also with extract of areca nut 1,2 grams/soap (soap 4). The final weight of soap was obtained 50 grams. Antiseptic soap brand X and Y as a control. Antibacterial activity test using Staphylococcus aureus ATCC 25923 with the well method.*

***Results:** The diameter of the clear zone produced by each areca nut soap was not much different (p>0,05). The clear zone diameter of soap 1, 2, 3 and 4 were 24.28 ± 7,95 mm, 23,96 ± 6,41 mm, 23,87 ± 6,14 mm, and 23,08 ± 1,52 mm respectively. While, diameter of clear zone in brand X and Y were zero. **Conclusion:** The betel nut soap formulation in this study has better antibacterial activity against Staphylococcus aureus ATCC 25923 than control.*

***Keywords:** soap, areca nut, antibacterial, Staphylococcus aureus*

ABSTRAK

Latar Belakang: . Pohon pinang banyak ditanam di Jambi dan biji pinang memiliki aktivitas antibakteri, antioksidan, anti penuaan dan kosmetik. Biji pinang berpotensi untuk dikembangkan sebagai sabun antibakteri.

Tujuan: Penelitian ini membandingkan efek antibakteri sabun pinang dengan bahan aktif dalam bentuk serbuk dan eksktrak terhadap Staphylococcus aureus menggunakan uji in vitro.

Metode: Formulasi sabun dibuat dengan 3 kadar serbuk pinang dalam sabun, yaitu 1,5 gram / sabun (sabun 1); 2,3 gram / sabun (sabun 2) dan 3 gram / sabun (sabun 3), serta ekstrak biji pinang 1,2 gram/sabun. Berat akhir sabun diperoleh 50 gram. Sabun antiseptik merek X dan Y sebagai kontrol. Uji aktivitas antibakteri menggunakan Staphylococcus aureus ATCC 25923 dengan metode sumur.

Hasil: Diameter zona bening yang dihasilkan masing-masing sabun pinang tidak jauh berbeda (p>0,05). Diameter zona bening sabun 1, 2 , 3 dan 4 masing-masing adalah 24.28 ± 7,95 mm, 23,96 ± 6,41 mm, 23,87 ± 6,14 mm, dan 23,08 ± 1,52 mm. Sedangkan diameter zona bening pada merek X dan Y adalah nol. **Kesimpulan:** Formulasi sabun pinang pada penelitian ini memiliki aktivitas antibakteri yang lebih baik terhadap Staphylococcus aureus ATCC 25923 daripada sabun control.

Kata kunci: sabun, pinang, antibakteri, Staphylococcus aureus

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INTRODUCTION

Areca catechu L is a plantation plant that is widely grown in Indonesia. Areca nut have medicinal actions as antioxidant, antibacterial, anti-inflammatory, anti aging and cosmetics[1]. Ethanolic extract from areca nut showed anti-oxidative higher than ascorbic acid[2]. Areca nut may reduce inflammatory on

skin problem, skin whitening effect[3], increase in collagen synthesis, improvement in skin hydration, the skin elasticity and skin wrinkles[4].

Areca nuts have long been used to treat fungal infections such as tinea versicolor, candidiasis, and scabies[1]. Areca catechu also can be used as a disinfectant[5]. Several types of bacteria that are known to be inhibited by areca catechu include gram-negative with levels of 3.3-7 µg/ml and gram-positive with an inhibitory level of 16 µg/ml[6].

Areca nut has the potential to be developed as antibacterial soap for skin diseases. Soap is the one of topical dosage form that can reach a wider area of the body[7]. Skin disease is one of the most common diseases in tropical countries, including Indonesia. Its prevalence in developing countries can range from 30% - 70%[8]. Most skin diseases are not deadly but can affect the quality of life of the sufferer[9]. Staphylococcus aureus is a gram-positive bacteria that often colonizes on the surface of the skin. About 20% of the population has persistent colonization of Staphylococcus aureus that often causes skin infections. Staphylococcus aureus is a common bacterium that causes furuncles, carbuncles, and other skin infections[10].

The extraction process can affect the content of phytochemical compounds in the extracted results. The extraction method and the solvent are several factors that affect the phytochemical content of the extracted results[11]. When the soap is to be commercialized, the extraction process can increase production costs. So, it is necessary to compare the antibacterial effect of soap with active ingredients areca nut between powder or extract. This study aims to compare the antibacterial effect of betel nut concentration powder and extract in our soap formulation against Staphylococcus aureus using the in vitro test.

METHODS

2.1 Betel Nut Processing

We used areca nuts which are yellowish-green in color that obtained from one of the areca plantations in Jambi City. Areca nuts were chopped and then dried in the oven at 50°C for 24 hours. It was blended into powder. Ethanol 70% were used as a solvent for extraction. The dry powder of Areca seeds was mixed with solvent in a ratio of 1:3 for the Areca seeds and the solvent. The mixture was kept in a dark bottle at room temperature for seven days and shaken occasionally. The filtrate was filtered with Whatman no.1 filter paper. Then, the filtrate was processed at a rotary evaporator at 40°C and dried on a water-bath at 70°C to obtain concentrated and dried form.

2.2. Areca Soap Formulation

The areca soap ingredients was composed of 60 mL coconut oil, 2,5 mL olive oil, 25 mL NaOH 5% solution, 2 mg stearic acid, areca nut powder, 2,5 mL liquid milk, 2,5 mL honey and 2,5 mL perfume. Soap was made in 3 doses of areca powder, namely soap 1 was 1.5 grams/soap, soap 2 was 2.3 grams/soap and soap 3 was 3 grams/soap. Also, soap 4 was made with extract of areca nut 1,2 grams/soap.The final weight of the soap was 50 grams. First, amount of coconut oil, olive oil and preheated stearic acid were mixtured. Then, Areca powder was mixed into the mixture solution according to the predetermined doses. This mixture was added with mixture liquid consist of milk and honey then stirred until homogeneous. Finally, parfume was added,

stirred for about 20 seconds. Final mixture was poured into a soap mold.

2.3. Phytochemical Testing

The phytochemical test was carried out quantitatively using a spectrophotometer to see the alkaloid and polyphenol content in the extract.

2.4. Antibacterial Activity Test

This study used a Staphylococcus aureus ATCC 25923. The turbidity suspension of Staphylococcus aureus was compared with an Mc Farlan reagent of 0.5%. Using a cotton line, the suspension was taken and spread evenly over the previously prepared sterile Mueller Hinton Agar medium. A well was made in the media about 4 mm ini diameter to place a soap piece. The media was incubated at 37°C for 24 hours. Assessment of the inhibition of areca nut soap against Staphylococcus aureus was carried out by measuring the clear zone that arose after 24 hours of incubation. Measurement of the clear zone uses a caliper in millimeters.

RESULTS AND DISCUSSION

The three areca soap preparations have a clear zone diameter that is not much different. The areca soap formulation in this study has an antibacterial effect against Staphylococcus aureus. Antiseptic soap brand X and Y used in this study did not result a clear zone (figure 3). This indicated that this brand soap has no antibacterial effect against Staphylococcus aureus.

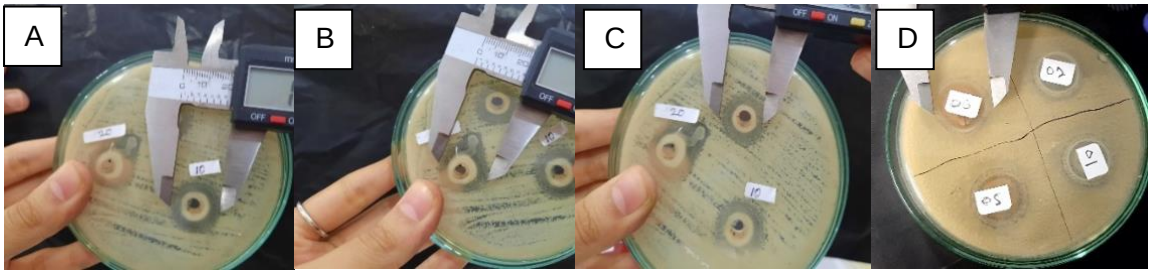

Figure 1. *Clear zone of areca nut soap. (A) is the clear zone of areca nut powder soap with a content of 1.5 grams/soap, which was 24,28 ± 7,95 mm. (B) is the clear zone of 2.3 grams of areca nut powder soap, which is 23,96 ± 6,41 mm. (C) is the clear zone of 3 grams of areca seed powder soap, which was 23,19 ± 6,78 mm. (D)) is the clear zone of areca nut extract soap with a content of 1.5 grams/soap, which was 23,08 ± 1,52 mm. There were no statistically significant different of diameter mean among the soaps.*

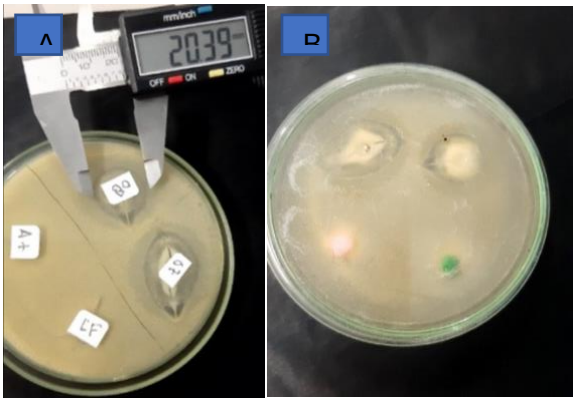

Figure 2. *Comparation clear zone of areca soap (08, 09) and control, antiseptic soap brand X (A+) and Y (LF). There were no*

clear zone in antiseptic soap brand X and Y.

Areca nuts contain many compund such as polyphenols (catechins, epicatechin, leucocyanidin, and procyanidins), alkaloids (arecoline, arecaidine, guvacoline, and guvacine), fatty acids (lauric acid, myristic acid, palmitic acid, stearic acid, decanoic acid, oleic acid, dodecenoic acid, tetradecenoic acid and hexadecenoic acid) and several minerals and vitamins (calcium, phosphorus, iron, vitamin B6 and vitamin C) (1). The content of fatty acids from areca nuts, namely myristic and oleic acid, and procyanidins are the main antibacterial compounds against Streptococcus mutans through the inhibition mechanism of the glucosyltransferase enzyme. Other study has also stated that the tannic acid in areca nuts can suppress the growth of bacteria in the mouth[1].

Polyphenolic compounds are also known to have antibacterial effects[12]. The antibacterial mechanism of polyphenol is not well known yet. Several studies mention the antibacterial activity of polyphenols through changes in cell membrane permeability, hydrogen bonding with intracellular enzymes or changes in cell walls. Flavonoid compounds, is a polyphenols, can also bind to soluble proteins on the membrane surface and create a complex that take role in inhibiting energy metabolism and DNA synthesis[12,13,14]. In Gram-positive bacteria, polyphenols changes bacterial intracellular pH that can lead to bacterial death[14].

Results of Phytochemical testing showed that the areca powder had tanin 30,791 ± 0,240 %w/w, phenols 39,513 ± 0,645 %w/w and alcaloids 1,757 ± 0,049 %w/w. Meanwhile, the areca extract had tanin 49,094 ± 0,241 %w/w, phenols 58,784 ± 0,423 %w/w and alcaloids 1,177 ± 0,049 %w/w. Although, the phytochemical substances were quite different between powder and extract, there were no significant different in clear zone. The clear zone arising on the agar with diffusion method is influenced by several factors, such as the growth rate of the testing bacteria, the active compounds contain in the soap and the diffusion of active compound in the soap into the agar and the susceptibility of the testing bacteria to the active compound[15,16]. The lipophilicity characteristics of herbal active compounds will affect the interaction of compounds with bacterial cell membranes, thereby affecting the strength of their antibacterial activity[13,17].

In this study, it was found several germ colonies in the clear zone. The presence of bacterial colonies in the clear zone probably due to bacterial persistence, namely the dormant phase of the bacteria. The antibacterial compounds could not bind to the surface of the dormant bacterial[18]. Another possibility is the lack of homogeneity of the extract so that the diffusion process of antibacterial compounds could not be uniform in the media. The speed of distribution of antibacterial compounds from the soap into the agar media could also affect the clear zone resulted in the media.

CONCLUSIONS

The formulation of areca nut soap in this study had an antibacterial effect against Staphylococcus aureus better than two branded antiseptic soap. Further research needs to be done to see the quality and the effectiveness of areca nut soap for human skin disease caused by Staphylococcus aureus.

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