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ORIGINAL ARTICLE

Antibacterial activity of palm leaf extract against *Propionibacterium acnes* and *Staphylococcus aureus*

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Abstract

Acne vulgaris is the most common skin disorder in adults and young adults. The growth of acne is caused by many factors, including excess sebum secretion and changes in microbial flora, mainly the colonization of *Propionibacterium acnes* and *Staphylococcus aureus*. This study aimed to determine the anti-bacterial activity of palm leaf extract against *Propionibacterium acnes* and *Staphylococcus aureus*. The research procedure includes palm leaf extraction and anti-bacterial tests. Palm leaf extraction was carried out using ultrasonication, while the anti-bacterial test was carried out using disc diffusion. The palm leaf extract obtained was diluted to 10, 20, 30, 50, and 50%, and each variation of this concentration was tested for anti-bacterial activity. The results of the anti-bacterial test showed that all palm leaf extract samples from 10% to 50% concentration could inhibit the growth of *P. acnes* and *S. aureus* bacteria. The 50% extract of ultrasonicated palm leaves had the largest inhibition zone diameter of 13.8 mm for *P. acnes* bacteria and 10.1 for *S. aureus* bacteria. From the results of this study, palm leaf extract has the potential to be used as a product for the prevention or treatment of acne vulgaris.

Keywords: anti-bacterial activity; palm oil leaves extract; *Propionibacterium acnes*; *Staphylococcus aureus*; ultrasonication

Introduction

Acne vulgaris (AV) is the most prevalent skin ailment, affecting up to 80% of teenagers and young adults in their lifetime. Several studies imply that the emotional burden of acne is equivalent to debilitating conditions, such as diabetes or epilepsy.[1] According to the Global Burden of Disease (GBD) study, AV affects around 85% of young individuals aged 12 to 25 years. In the United States, more than 85% of patients suffering with AV are adolescents, regardless of gender or race. AV is more frequent in male teenagers, while in the post-adolescent time it is more common in girls. A study of Indonesian youths found that anxiety and quality of life were negatively impacted by AV, regardless of its intensity. A study found a link between an AV patient's quality of life and the duration of AV.[2]

Typically, AV results physical discomfort, mental anguish, deformity, and possibly irreversible scarring. Patients may also experience emotions of humiliation and worry, which both exacerbate mental depression. The pathogenesis of acne is multifactorial due to the previously mentioned exposome factors. The four main causes of acne are excessive sebum production, hyperproliferation of the bacteria known as *Cutibacterium acnes* (formerly known as *Propionibacterium acnes*), abnormal hyperkeratinization of the pilosebaceous follicles, and inflammatory mechanisms.[3] *P. acnes*, a type of cutaneous propionibacteria, is the most common cause of opportunistic infection. It is associated with a variety of seemingly unrelated conditions such as acne vulgaris, progressive macular hypomelanosis (PMH), infections related to medical devices and dental procedures, sarcoidosis, cervical disc disease, prostate cancer, and different types of soft tissue infections.[4] Besides *P.acnes, Staphylococcus aureus* causes pyogenic infections. These infections

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usually present with typical signs of inflammation, necrosis, and abscess formation and can cause a wide variety of infections, such as acne, boils, or pus.[5]

Antibiotics have a crucial role in treating acne by inhibiting bacterial activity and providing an anti-inflammatory impact. Antibiotics can be utilized in both topical and systemic formulations. Oral antibiotics are exclusively prescribed for cases of moderate to severe acne and are often taken for a duration of 6 to 8 weeks, with a maximum treatment period of 12 to 18 weeks. In cases of mild to severe acne, oral antibiotics are sometimes used in combination with topical antibiotics.[6] Over the course of time, there was a progressive rise in the resistance of *P.acne* bacteria to antibiotics. This resistance was initially observed in 1979 at a rate of 20%, and it subsequently escalated to 67% by 1996. There is a growing trend of moving away from antibiotic therapy and it is necessary to conduct research on utilizing natural compounds as a substitute.[7]

Palm oil leaves account for at least 53% of the dry weight of waste produced during harvesting, trimming, replanting, and mill processing in the business. Furthermore, it has been discovered that palm oil leaves contain bioactive compounds such as antioxidants, antihyperglycemic, and hypertensive. Previous studies have reported that palm leaf extract has antibacterial activity against *S. aureus* with inhibition zones from 0 to 20 mm, with MIC and MBC values of 3.906 mg/mL and 7.813 mg/mL, respectively.[8] In this study, researchers will test the antibacterial activity of palm leaf aqueous extract against *P.acnes* and *S.aureus* bacteria where the extraction process is carried out using the ultrasonication method.

Method

Extraction of palm leaf samples

In this study, researchers used the ultrasonic method to extract palm leaves. Five thousand grams of palm leaves were cleaned using running water and dried in a drying cabinet for three days. The dried palm leaves were then blended into powder. The ultrasonication method was done by dissolving the palm leaf powder in distilled water with a composition of 1:10. The mixture was then shaken for 12 hours at 180 rpm. The palm leaf powder and water mixture were ultrasonicated using an ultrasonicator (Omnisonic Ruptor) for 40 minutes. The extract was then centrifuged at 4000 rpm for 10 min to separate the remaining solid components of the plant. The resulting extract is then concentrated using a rotary evaporator.[9]

Antibacterial activity

Antibacterial activity was performed using the agar diffusion method. P. acnes and S. aureus isolates were cultured into nutrient agar media and incubated for 24 hours at 37°C. Bacterial isolates were added with physiological NaCl and measured with a spectrophotometer at λ 600 nm until reaching an absorbance of 0.1. The *P.acnes* and *S.aureus* cultures were swabbed onto the surface of nutrient agar media evenly, then dipped sterile discs into palm leaf extract cultures with various concentration variations (10%, 20%, 30%, 40%, and 50%) and placed on the surface of nutrient agar. The petri dish was incubated for 24 hours at 37°C. The zone of inhibition, the clear area around the discs where the bacteria did not grow, was measured with a caliper to determine the antibacterial activity of the palm leaf extract.[10]

Results

Palm oil leaves extraction

In this study, fresh palm leaves were taken weighing approximately 5 kg, cleaned using running water, cut into pieces, and dried in a drying cabinet for three days. The palm leaf powder obtained was extracted with distilled water using two methods: the ultrasonication method using an ultrasonicator (Omnisonic Ruptor). The extracts from ultrasonication can be seen in Figure 1.

Figure 1. The result of extraction of palm oil leaves

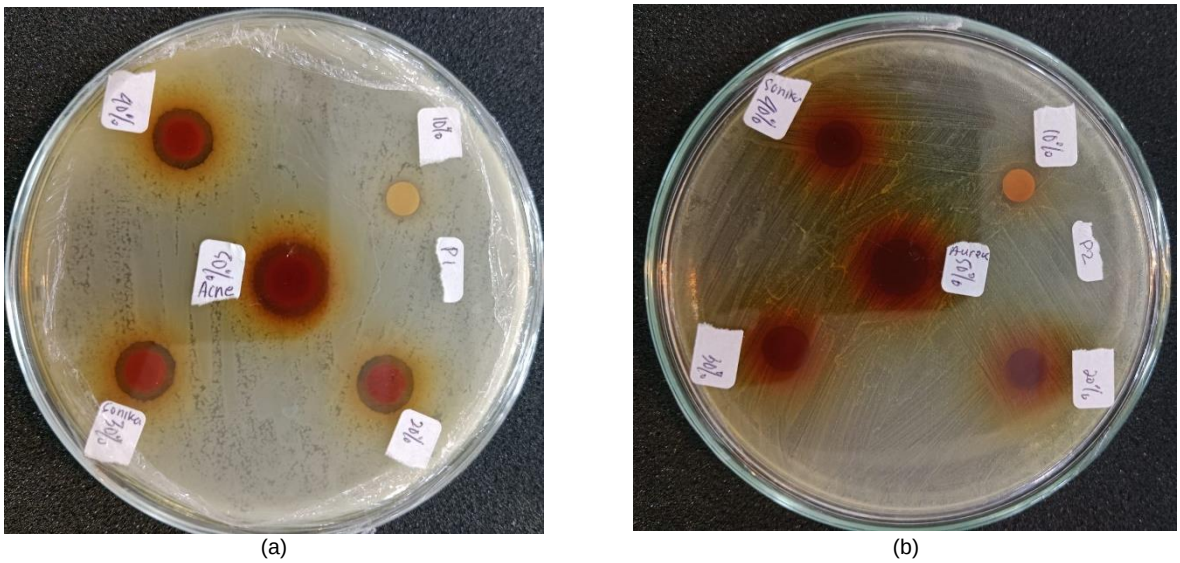
The ultrasonicated extract obtained was brown in color and was further diluted to 10%, 20%, 30%, 40%, and 50% for antibacterial activity testing.

Table 1. Antibacterial activity of ultrasonicated palm leaf extract against *P.acnes* and *S.aureus* bacteria

No	Concentration	Inhibition zones against *P. acnes* (mm)	Inhibition zones against *S.aureus* (mm)
1	10 %	7,2	7,8
2	20 %	10,2	8,4
3	30 %	10,5	8,5
4	40 %	11,3	8,9
5	50 %	13,8	10,1
6	Amoxycillin (positive control)	12,91	31,1

Palm leaf extracts produced from ultrasonication and maceration methods were then tested for antibacterial activity against *P. acnes* and *S. aureus* bacteria using the disc diffusion method. The results of antibacterial activity testing are shown in Table 1 below.

The table shows that palm leaf extract with a 50% concentration shows the highest antibacterial activity against *P. acnes* (exceeding the activity of the positive control), which is 13.8 mm. The highest antibacterial activity against *S. aureus* was also shown by the 50% extract concentration. The antibacterial activity of ultrasonicated palm leaf extract against *P. acnes* and *S. aureus* bacteria in Petri dishes can be seen in Figure 2 below.



(a) (b)

Figure 2. Antibacterial activity of ultrasonicated palm leaf extract against P. acnes (a) and S. aureus (b)

Discussion

Based on Table 1, it can be seen that the palm leaf extract obtained via the ultrasonic method shows antimicrobial solid activity. Previous studies reported that ultrasonically assisted extraction has been shown to enhance the antibacterial activity of plant extracts. Studies on *Senna siamea* leaf extract[11] and *Salvia stepposa*[12] both reported higher antibacterial activity in ultrasonically assisted extracts compared to solvent extracts. This method was also shown to be effective in extracting antibacterial components from Eucalyptus globulus.[13] Ultrasonically-assisted extraction produces more effective antibacterial activity in plant extracts than other extraction methods. This is due to its ability to efficiently pick up and solubilize extractable compounds, resulting in higher yields and more significant antibacterial activity.[11]

In a previous study it was reported that the ultrasonic extracts of two chemotypes of T. serpyllum L. have a comparable composition of phenolic components, but they differ in the quantity of phenolic acids and flavonoids, with the exception of rosmarinic acid. The ultrasonic extracts possess a broad range of antimicrobial properties, demonstrating either bactericidal or bacteriostatic effects against all examined bacteria and fungus at concentrations ranging from 0.0625 to 20 mg/ml. However, the extracts vary in their potency against different strains of microorganisms.[14] The data also showed that the higher the concentration of plant extracts, the stronger the antibacterial activity. Increasing the concentration of plant extracts can indeed enhance their antimicrobial activity, as demonstrated by several studies. In a previous study, it was mentioned that the antimicrobial activity of *Spinacia oleracealeaf, Zingiberofficinalerhizome, Coriandrum sativum leaf, Allium sativum clove, Aloe vera* gel and leaf, was stronger with increasing concentration of plant extracts.[15] This is further supported by Vaou[16] who emphasized the importance of

optimized extraction methods and concentration levels in enhancing the antimicrobial activity of plant extracts.

Table 1 also show that the extract's antibacterial activity against *P. acnes* is more potent than that against *S. aureus*. This is likely due to the difference in the composition and structure of the two bacteria. The cell membranes or walls of *Propionibacterium acnes* and *Staphylococcus aureus* differ in their composition and structure, leading to variations in their susceptibility to inhibition. *P. acnes*, found in hair follicles, is more easily inhibited due to its anaerobic nature and prefers areas with high sebum production.[17] On the other hand, *S. aureus*, a nosocomial bacterium, forms complex biofilms that provide protection against unfavorable conditions and antibacterial drugs.[18] The presence of prophages on *S. aureus*, which interactively modulate bacterial growth and membrane composition, further complicates their inhibition.[19]

Recent studies have highlighted the significant antimicrobial activity of various phytochemical compounds, with terpenes, flavones, flavonols, and several alkaloids and phenylpropanoids showing promising results.[20] These compounds have been found to disrupt bacterial membranes, inhibit efflux pumps, and suppress virulence factors.[21] Among these, flavonoids are particularly effective, with some studies reporting strong antibacterial and antifungal effects.[22] Flavonoids, a class of bioactive compounds derived from plants, have been shown to have antibacterial properties. Their mechanism of action involves various activities, including inhibiting DNA gyrase.[23] Flavonoids can also suppress bacteria by affecting transcriptional and metabolic pathways and their lipophilicity is a key factor in their activity against gram-positive bacteria.[24] The degree of hydroxylation of flavonoids is thought to affect their antibacterial effect.[25] In addition, flavonoids can directly damage the bacterial envelope and interact with specific molecular targets that are essential for bacterial survival.[26]

Conclusion

The results show that palm leaf extract from ultrasonication showed antibacterial activity against *P.acnes* and *S.aureus* bacteria, with the highest activity being 13.8 mm against *P.acnes* and 10.1 mm against *S.aureus*. The results also showed that the higher the extract concentration, the stronger the antibacterial activity. Further research is needed on the topical anti-aging potential of palm oil leaf extract.

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