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The Antibacterial Activity Of Conventional Serum And Nano Face Serum From Pineapple Stem Extract (Ananas Comosus (L.) Merr) Against Staphylococcus Epidermidis

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ABSTRACT
Pineapple hump has been identified to contain secondary metabolites in saponin, flavonoid, and steroid/triterpenoid which show potential as antibacterial agents against *Staphylococcus epidermidis*. These bacteria are part of the normal composition of gram-positive bacteria on human skin and do not display any pathogenic characteristics. Even so, these bacteria can trigger facial skin infections, especially in the form of pimples which are often difficult to treat. This study aimed to determine the differences in bacterial inhibition of cosmetic preparations in the form of conventional serum and nano serum from pineapple hump extract (*Ananas comosus* (L.) Merr) against bacteria *Staphylococcus epidermidis.* This research uses the method of *True Experimental.* In this study, pineapple hump was extracted and formulated into conventional serum preparations and nano serum with various concentrations, then tested for its antibacterial activity against the bacteria *Staphylococcus epidermidis.* Diameter of antibacterial inhibition of conventional Serum Pineapple hump Extract against bacteria *Staphylococcus epidermidis* i.e., Blank has no inhibition, 2% concentration (10.10 mm), 10% concentration (11.28 mm) and 20% concentration (13.26 mm). The diameter of inhibition of Nano serum Pineapple Hump Extract, namely Blank, had no inhibition, 2% concentration (10.44 mm), 10% concentration (11.56 mm) and 20% concentration (15.14 mm). The positive control for Clindamycin had an inhibitory effect (32.8 mm), the positive control for the circulating Pineapple Hump Extract (15.52 mm) and the negative control had no inhibition.

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INTRODUCTION

Staphylococcus epidermidis is a gram-positive bacterium that is part of the human skin's commensal flora. Although it is not intrinsically pathogenic, it can adopt invasive behavior in the context of altered skin conditions. In such situations, Staphylococcus epidermidis can become an agent causing several pathological disorders, including acne (Acne vulgaris)

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(Jewetz et al., 2015). Acne is a dermatological disorder that appears on the skin and can be triggered by the presence of Staphylococcus epidermidis. This bacterium naturally resides in the skin follicles and plays a role in the formation of acne. Acne involves various types, including comedones, papules, pustules, nodules, and cysts, all marked by varying degrees of severity. Effective approaches to addressing acne issues can be implemented through the application of topical products (Fitriani et al., 2022).

Facial skin care using cosmetic products is a significant step in maintaining the condition and balance of facial skin. This practice aims to protect the facial skin from the accumulation of dead cells, dust particles, contaminants, and residual cosmetics, and also to prevent the possible emergence of various dermatological disorders (Rachmi, 2001). To enhance the efficiency of acne treatment, the choice of dosage form needs to be considered to ensure optimal delivery of therapeutic agents, while the supporting materials used do not induce the emergence of additional acne (Yuniarti et al., 2021).

One of the innovations in cosmetic products that has been developing recently is serum. The main advantage of serum lies in its high concentration of active ingredients, which can provide quick results in the absorption process by the skin. This property also positively impacts the comfort of use and ease of spreading on the skin surface due to its low viscosity (Kurniawati et al., 2018). The use of plants with positive health effects has been a common practice in society since ancient times. This approach is based on empirical experience, as plants tend to have minimal side effects and are more economical due to their abundant availability (Sari, 2006). One example of a promising plant in terms of health benefits is pineapple (Ananas comosus (L.) Merr). Pineapple contains the enzyme bromelain, which has been proven to have potential as an antibacterial and anti-inflammatory agent (Wiharningtias et al., 2016). So far, the part of the pineapple most commonly utilized is its flesh, while other components such as the skin, leaves, and stem are often considered waste and discarded. If not managed properly, this can potentially pollute the environment. However, it is important to note that the pineapple stem actually contains a higher concentration of the enzyme bromelain, even though it is often overlooked (Supartono, 2004). According to research by Lubis et al. (2021), the pineapple stem contains various types of secondary metabolites such as saponins, flavonoids, and steroids/triterpenoids.

According to Sirajudin & Soraya (2016), the antibacterial effectiveness of nanoscale serum formulations is closely related to the characteristics of the nanoparticle dimensions that form them. These characteristics are influenced by the physical properties of the nanomaterials, including size, shape, and surface properties. Furthermore, the smaller the particle size, the greater the surface area-to-volume ratio, resulting in more efficient antibacterial capability on a nanoscale. Nano serum is a serum formulated with active ingredient particles having dimensions of less than 1000 nm (Octarika, 2017). One advantage of nano serum is its ability to interact with targets more efficiently and quickly due to its larger surface area (Agustin et al., 2010). Based on the above explanation, the researchers are interested in conducting a study to test the antibacterial activity of conventional serum and

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nano face serum derived from pineapple stem extract (Ananas comosus (L.) Merr) against Staphylococcus epidermidis.

METHODS

This research will be conducted from January to May 2023. The study will take place in various laboratories: the Botanical Laboratory for Extract Preparation, the Pharmacy Laboratory for the Formulation of Conventional Serum, the Chemistry Laboratory and NRELAB for using the Homogenizer and Ultrasonic Homogenizer to reduce the serum size to nanoscale, and the Antibacterial Activity Testing will be carried out at the Integrated Research Laboratory of Universitas Muslim Nusantara Al-Washliyah Medan. The particle size of the serum and nano serum will be measured using a Particle Size Analyzer (PSA) at the Nanomedicine Laboratory of Universitas Sumatera Utara.

The samples used in this study are pineapple stems (Ananas comosus (L.) Merr.), collected from fruit salad vendors on Mahkamah Kolam Sri Deli Street, Medan Kota district, North Sumatra. The sampling method is purposive, with samples taken from only one location and not compared with other regions. The pineapple stem samples are extracted and then formulated into conventional serum and nano serum preparations containing ingredients such as pineapple stem extract at concentrations of 2%, 10%, and 20%, tween 80, TEA, glycerin, methylparaben, propylparaben, 70% ethanol, Viscolom MAC-10, and distilled water as a solvent. Subsequently, antibacterial activity tests of the conventional serum and nano serum formulations containing pineapple stem extract will be performed against Staphylococcus epidermidis.

RESULTS AND DISCUSSION

Based on the results of the antibacterial activity test of Conventional Pineapple Stem Extract Serum against Staphylococcus epidermidis, the average inhibition zone sizes were as follows: In the control group (Blank), no bacterial growth inhibition was observed. At a 2% concentration, an inhibition zone of 10.10 mm was observed; at a 10% concentration, an inhibition zone of 11.28 mm was observed; and at a 20% concentration, an inhibition zone of 13.26 mm was observed. Meanwhile, the results of the antibacterial activity test of Nano Serum Pineapple Stem Extract showed that in the control group (Blank), there was no inhibitory effect. At a 2% concentration, an inhibition zone of 10.44 mm was observed; at a 10% concentration, an inhibition zone of 11.56 mm was observed; and at a 20% concentration, the largest inhibition zone of 15.14 mm was observed.

In petri dishes containing the positive control clindamycin, the marketed pineapple stem extract preparation control, and the negative control, the inhibition zones were 32.8 mm, 15.52 mm, and no inhibition, respectively. The inhibition categories obtained were based on CLSI (Clinical and Laboratory Standards Institute, 2018).

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Table 1. Inhibition Zone Categories According to CLSI (Clinical and Laboratory Standards Institute, 2018)

Interpretive Category	Minimum Inhibitor Concentration (MIC)	Zone Diameter (mm)
Susceptible	≤4	≥ 20 mm
Intermediate	8-16	15-19 mm
Resistant	≥32	≤ 14 mm

Based on the table above, the inhibition zone categories for the antibacterial activity test results are shown in Table 2.

Table 2. Antibacterial Activity Test Results

Concentration	Average Inhibition Zone (mm)	Category
Conventional Serum		
Blank	-	-
2%	10.10	Resistant
10%	11.28	Resistant
20%	13.26	Resistant
Nano Serum		
Blank	-	-
2%	10.44	Resistant
10%	11.56	Resistant
20%	15.14	Intermediate
Positive Control		
Clindamycin	32.8	Susceptible
Marketed Product		
Marketed Product	15.52	Intermediate
Negative Control		
Aquadest	-	-

This data indicates that while both the conventional serum and nano serum showed antibacterial activity against Staphylococcus epidermidis, the nano serum at a 20% concentration exhibited a higher inhibition zone, falling into the intermediate category, suggesting a potentially more effective formulation compared to the conventional serum.

In the antibacterial activity testing of Conventional Serum and Nanoserum Pineapple Stem Extract, the initial stage involves sterilization steps. This stage includes the sterilization of glassware and discs using an oven at 180℃ for one hour. Mueller Hinton Agar (MHA) medium is sterilized using an autoclave at 121℃ for 15 minutes. Meanwhile, equipment made of metal, such as inoculating needles and tweezers, is heated using a spirit lamp. The main purpose of this sterilization step is to ensure that all equipment and media used are free from unwanted microbial contamination during the testing process.

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Staphylococcus epidermidis is a gram-positive microorganism that is a natural component of the skin flora and is inherently non-pathogenic. However, if there are changes in skin conditions, this bacterium can become more likely to cause infections. Consequently, conditions like acne (Acne vulgaris) can be triggered by infections from this bacterium (Jewetz et al., 2015).

The basic principle of this research is the administration of conventional preparations and nanoserum pineapple stem extract with four concentration variations: Blank, 2% concentration, 10% concentration, and 20% concentration, tested against Staphylococcus epidermidis bacteria on agar medium to observe bacterial growth inhibition. According to Arlian (2021), pineapple stem extract, which is the active substance in these formulations, positively contains secondary metabolites such as saponins, flavonoids, and steroids/triterpenoids. These secondary metabolites are suspected to have antibacterial activity. Flavonoids exhibit antibacterial properties through three main mechanisms: inhibition of nucleic acid synthesis, disruption of cell membrane function, and disruption of cell metabolism. The action of flavonoids in inhibiting nucleic acid synthesis involves interactions from the B-ring on the flavonoid structure, which plays a crucial role in forming hydrogen bonds. This interaction occurs when nucleic acid bases accumulate, resulting in inhibition effects on the formation of hydrogen bonds essential for DNA and RNA synthesis (Cushnie, T.P & Lamb, A.J., 2005). On the other hand, the antibacterial effect of steroids involves damage to the bacterial cell membrane layer, describing a different mechanism of action from flavonoids (Monalisa et al., 2011). The antibacterial properties of secondary metabolite saponins arise from their ability to disrupt cell membrane permeability, causing disturbances in cell transport and leading to cell lysis (Herwandi et al., 2019).

Bacterial growth inhibition can be observed through the inhibition zone, which appears as a transparent area around the discs on the agar medium. The appearance of a transparent area around the paper discs indicates the absence of bacterial growth, which also suggests that the extract sample has the ability to inhibit bacterial growth. Additionally, there are variables affecting this antibacterial activity test, such as the diffusion rate of various substances and the bacterial response to the test substance (Dwidjoseputro, 2005).

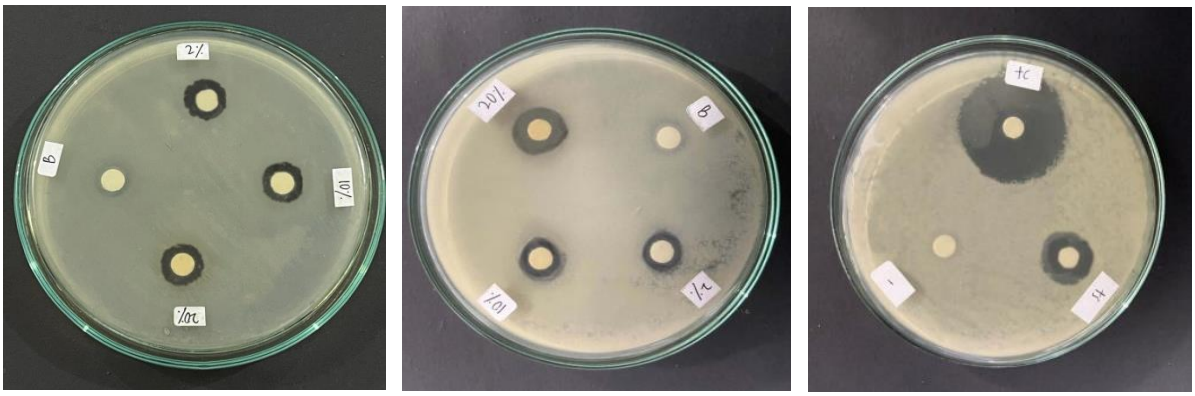

Figure 1. Antibacterial Activity of Conventional Serum, Naoserum, and Control

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The results of the antibacterial activity test of Conventional Serum Pineapple Stem Extract against Staphylococcus epidermidis bacteria showed that the average inhibition zone sizes were as follows: In the control group (Blank), no inhibitory activity was observed. At a concentration of 2%, the inhibition zone size reached 10.10 mm; at a concentration of 10%, the inhibition zone size was 11.28 mm; and at a concentration of 20%, the inhibition zone size increased to 13.26 mm. Meanwhile, the antibacterial activity test of Nanoserum Pineapple Stem Extract showed different results: In the control group (Blank), no inhibitory effect occurred. At a concentration of 2%, the inhibition zone size was 10.44 mm; at a concentration of 10%, the inhibition zone size reached 11.56 mm; and at a concentration of 20%, the largest inhibition zone size was 15.14 mm.

In petri dishes containing positive control clindamycin, positive control of the marketed pineapple stem extract preparation, and negative control, the inhibition results were 32.8 mm for clindamycin, 15.52 mm for the marketed pineapple stem extract preparation, and no inhibition for the negative control. While Conventional Serum and Nanoserum Pineapple Stem Extract do show differences in inhibitory effects, the difference in inhibitory power is not significant. This can be seen from their inhibition category, which remains the same from Resistant to Intermediate. The inhibition category of the clindamycin positive control falls under the Susceptible category, whereas the positive control product of the marketed pineapple stem extract serum shows the Intermediate category. The negative control, which is distilled water, does not show any inhibition zone.

CONCLUSION

Based on the analysis of the antibacterial activity test of Conventional Serum Pineapple Stem Extract against Staphylococcus epidermidis bacteria, the average inhibition zone results are as follows: the Blank has no inhibitory effect, while the 2%, 10%, and 20% concentrations have inhibition zones of 10.10 mm, 11.28 mm, and 13.26 mm, respectively. Meanwhile, the antibacterial activity test results of Nanoserum Pineapple Stem Extract are as follows: the Blank has no inhibitory effect, while the 2%, 10%, and 20% concentrations have inhibition zones of 10.44 mm, 11.56 mm, and 15.14 mm, respectively. The positive control clindamycin has an inhibition zone of 32.8 mm, and the positive control of the marketed pineapple stem extract serum preparation has an inhibition zone of 15.52 mm. Conventional Serum and Nanoserum Pineapple Stem Extract show differences in inhibitory effects, but the difference in inhibitory power is not significant, as indicated by their inhibition categories, which range from intermediate to resistant.

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