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Effectiveness of Pineapple and Lemon Peel Extracts as Antimicrobial Paper Soap

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ABSTRACT

Health is still an issue of concern around the world. This is also supported by a response in the form of efforts to realize large-scale development in the health sector with a target of achieving it by 2030, namely the Sustainable Development Goals, ensuring a healthy life. Indonesia is a country that is still experiencing crucial environmental problems, especially waste. One of them is the organic waste of pineapple (*Ananas comosus* L.) peels. The addition of lemon extract (*Citrus limon* L.) which is formulated will provide natural aromatherapy and have an effect on feelings of calm. On the other hand, the development of paper soap is an innovation in efficiency and ability to create a habit of washing hands in Indonesian society. The purpose of this study was to determine the manufacture of paper soap with natural ingredients, namely lemon and pineapple waste and to determine the bioactivity of the combination as an antiseptic paper soap. This research method is an experimental method by testing the antimicrobial activity carried out by the paper disc technique diffusion method. From this study it can be concluded that paper soap made from natural ingredients lemon and pineapple peels can be realized and has an antiseptic effect with an average total inhibition zone against *S. aureus* bacteria of 5.7 mm and against *E. coli* bacteria of 20 mm so that soap paper is classified as effective in inhibiting the growth of pathogenic bacteria.

Introduction

Health is an issue that is still in the spotlight of the world. Ensuring healthy lives and promoting well-being at all ages is essential to realizing sustainable development. In response, support was also held for efforts to realize large-scale development in the health sector through the third point of the SDGs (Sustainable Development Goals) target, namely ensuring

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a healthylife with a target achievement in 2030.

Entering mid-2022, more than 500 million people worldwide have been infected with COVID-19. Even the latest estimates show that at the end of 2021, the global "excess deaths" directly and indirectly caused by COVID-19 had reached 15 million people (United Nations, no date). The pandemic has severely disrupted essential health services, fueled an increase in the prevalence of anxiety and depression, lowered global life expectancy, derailed global health progress, and hampered two decades of recovery efforts to achieve universal health coverage. As a result, the coverage of health protocols and healthy living habits must be increased to prevent the risk of health decline. Urgent and integrated action is needed to get the world back on track toward achieving SDGs goal 3, one of which is to support and innovate handwashing habits.

Efforts to ensure public health supported by clean living habits are certainly based on maintaining hand hygiene. This is because the hand is the organ that touches the most and has the greatest potential for transmission. So it is important for each individual to have awareness of the habit of washing hands properly and correctly. Based on data from the World Health Organization, it was found that a 50% reduced risk of upper respiratory tract infections and bird flu can be prevented by washing hands using soap (Ridwanuloh and Nurwulandari, 2022) compared to using hand sanitizers (Putri, 2020). The application of handwashing with soap culture is proven to reduce the risk of typhoid fever (Sumiati et al., 2022), intestinal worms, irritation due to germs, and various other diseases (Makarim, 2020).

Related to the explanation above, Indonesia's health problems are also closely related to environmental problems. The Indonesian Ministry of Environment and Forestry reported that the amount of landfill has increased in 2022 with the percentage of organic waste reaching 57% (Mulachela, 2021). Pineapple (*Ananas comosus* L.) and lemon (*Citrus limon* L.) waste, including the peel, is one of the organic waste that is the source of the problem. Though pineapple waste has the potential to be the main ingredient in making effective soap because it contains bromelain enzymes, carotenoids, and vitamin C which can be used as antioxidants, antiallergic, anti-inflammatory and antibacterial in protecting the skin and preventing the emergence of free radicals. In addition, the addition of lemon extract (*Citrus limon* L.) which is formulated in addition to its good essential oil content, lemon is also able to act as an antiseptic that will provide natural aromatherapy and have an effect on feelings of calm (Azizah et al., 2022).

On the other hand, the development of the paper soap trend has become an efficiency and capability innovation that supports the creation of handwashing habits of the Indonesian people (Awaluddin et al., 2022). The use of paper soap can be an alternative to handwashing facilities, especially soap, which is often not provided in some places. Its size which tends to be small and light to carry can be an effective choice for each individual to provide their own paper soap while traveling. In addition, the shape is uniquely designed in such a way that it will add interest and economic value that is higher than the paper soap that existed before. From the explanation of the problem above, this study was conducted to determine the manufacture of Paper Soap with natural ingredients, namely a combination of lemon peel and pineapple

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Materials and Methods

This research will be conducted for 1 (one) month to achieve the planned target. This type of research is experimental, namely by conducting experiments to determine the existing influences, as a result of certain treatments or experiments (Soesilo, 2015). The research was carried out in January 2022 at the Microbiology Laboratory, Department of Biology, Faculty of Mathematic and Science, Medan State University.

The treatment is given in this study against bacteria Staphylococcus aureus and *E.coli*. Positive control (+) used in the form of the commercial liquid soap brand Dettol and negative control (-) was used Aquadest. The total treatment in this study was 4 treatments with 2 repeats. The stages of research are as follows:

1. Simplisia Creation

   Samples are collected and then cleaned of dirt attached to running water. After cleaning the leaves are drained, then dried in the sun and in the oven for 3 hours, the sample is mashed with a blender until it becomes a fine powder, then sifted. Both samples are simplisia separately. The simplisia powder is put in a separately closed glass container.
2. Making Ethanol Extract from Pineapple Peel and Lemon Peel

   Extraction by maceration method using 96% ethanol solvent. Pineapple peel simplisia powder (100 grams) and lemon peel (50 grams) macerated with 750 ml (500+250 ml) of 96% ethanol solvent. The maceration container is covered with aluminum foil and left for 3 days while occasionally stirring. Filtrate filtration is carried out and evaporated with the help of a water bath until a thick ethanol extract of pineapple and lemon peel is obtained.
3. Paper Soap Making

   Making paper soap begins by putting HPMC into a beaker and dissolved using hot water while stirring until it becomes a gel. In a separate beaker heat olive oil, and put propilenglikol stirring until homogeneous. Next, put KOH 30% and aquadest and added with SLS that has been dissolved with aquadest and then stirred until perfectly mixed. Add 2 drops of essential oil and finally add the active substance. After homogeneous then the mixture is poured into a beaker containing HPMC gel, then stirred until homogeneous. On the next day, the liquid material of paper soap was applied to oil paper (Awaluddin et al., 2022). The process of making paper soap is done by pouring liquid soap on oil paper that has been prepared on the table.
4. Sterilization of Tools and Materials

   Tools and materials to be used are first cleaned, packaged, and then sterilized. Petri dishes, test tubes, tweezers, and other glassware are put into an autoclave (wet heating) and sterilized at 121°C for 15 hours (Widyawati, 2017).
5. NA Media Creation

   The media used is NA (Nutrient agar). Making NA media is done by weighing 2.8 g, then adding 100 mL aquadest, then sterilizing using an autoclave for 15 minutes at a temperature of 121 °C. Furthermore, the media is poured into a petri dish aseptically as much as 15-20 ml, then left at room temperature until the media solidifies (Juariah, 2021).
6. Antibacterial Test

   Antibacterial activity tests were performed using the Kirby-Bauer disc method. *Staphylococcus aureus* bacteria are inoculated by dipping a cotton swab into a tube of bacterial isolate and then into solid NA media, scraping evenly. Then the disc paper is dipped in each treatment (sample), namely 2.5 gr paper soap, 5 gr paper soap, and 7.5 gr paper soap each dissolved in 9 mL aqua dest. The preparation used is paper soap from commercial liquid soap (positive control), and aqua dest (negative control). The preparation of negative control is done by dripping the aqua dest onto a paper disc.

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Then the petri dishes are incubated at 370C for 24 hours. Measurement of the inhibitory zone after a 24-hour incubation period around the paper disc for each treatment. Likewise, the treatment of *Escherichia coli* bacteria (Puspa Dewi, 2020). Antibacterial activity is observed based on the diameter of the inhibitory zone, indicated by the formation of a clear area around the paper disc. The diameter of the inhibitory zone is measured using a caliper

Results and Discussion

The purpose of antibacterial testing is to determine the effectiveness of paper soap added with extracts from pineapple and lemon peels in inhibiting the growth of bacteria in the form of *Staphylococcus aureus* (*S. aureus*) and *Eschericia coli (E.coli).* The calculation of the diameter of the inhibitory zone uses millimeters (mm) using a caliper. The antibacterial test is calculated by measuring the diameter of the bacterial inhibitory zone. The formula calculates the diameter of the inhibitory zone as follows:

$$\frac{(Dv - Dc) + (Dh - Dc)}{2}$$

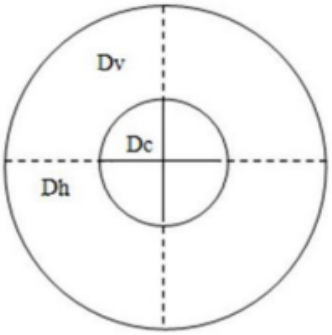

Figure 1. Calculation of Inhibition Zone Diameter (Kandoli et al., 2016)

The concentration contained in paper soap preparations is pineapple peel extract and lemon added to other active ingredients such as KOH 30%, SLS, HPMC gel, and others in accordance with the design of research methods in making paper soap that has been described. Based on the calculation of the diameter of the inhibitory zone that has been done, the average results are obtained in the table below:

Table 1. Average inhibitory power Pineapple and lemon peel extract Paper soap

Bacterial preparations	Inhibitory Zone Diameter (mm)		
	***Paper soap* preparation**	**Positive Control**	**Negative control**
Escherichia Coli	25,30	19,20	0
	16,10	21,80	0
Staphylococcus aureus	5,23	4,20	0
	6,80	7,80	0

Based on the test results, the data obtained showed that there was an effect of adding pineapple and lemon peel extracts on the ability of paper soap to inhibit the growth of test bacteria. *Escherichia coli* bacteria have a greater average inhibitory power than *Staphylococcus aureus* bacteria. Antibacterial tests are closely related to the diameter of the inhibitory zone formed as an indicator that the test material has the ability to inhibit bacterial growth. The diameter of the inhibitory zone does not always increase with high

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concentrations of antimicrobial agents, this possibility can be attributed to differences in the diffusion rates of antimicrobial compounds in agar media as well as differences in types and concentrations of antimicrobial compounds. The clear zone produced in the antibacterial activity of paper soap pineapple peel extract and lemon against *S.aureus* and *E.coli* can be seen in the figure below.

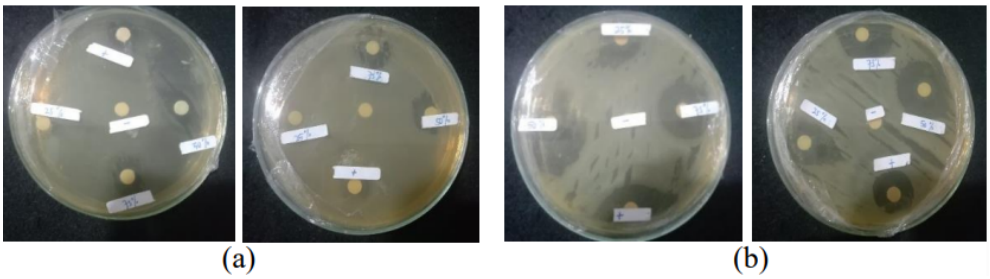

Figure 2. Paper soap inhibition zone of pineapple peel extract and lemon against (a) *E.coli* bacteria and (b) *S. aureus* bacteria

According to David and Stout (1971) in Kandoli et al., (2016), a very strong response occurs when the results of the inhibitory zone diameter measurement reach more than 20 mm, a strong response between 10 and 20 mm, a moderate response when the diameter of the inhibitory zone reaches 5 to 10 mm, and a weak response when the diameter of the inhibitory zone is less than 5 mm. Through the value of the diameter of the inhibitory zone that has been determined, the antibacterial effectiveness value of the preparation used can be measured.

Based on the category of inhibitory zones that have been studied, it is known that paper soap preparations, pineapple peel extract, and lemon against the growth of E. coli bacteria have a very strong inhibitory power (25.3 mm). The positive control showed strong inhibitory power (19.2 mm) while the negative control did not show inhibition against the test bacteria.

The existence of this bacterial growth inhibition shows that there are active compounds in pineapple skin, namely flavonoids and bromelain enzymes, that can inhibit bacterial growth by breaking down proteins on bacterial cell membranes. In addition, tannin compounds and saponins can also inhibit bacterial growth. The use of high concentrations can affect the size of the diameter of the resistance in bacteria (Halima et al., 2020).

From the data and research results that have been described, it can be proven that pineapple and lemon peel extracts have antibacterial activity against gram-positive bacteria, so they have the potential to be the main ingredient in making antibacterial soap. This is in accordance with the results of research by Arsyada et al. (2018) which found that antibacterial activity is indicated by the large diameter of strong bacterial growth inhibition. Paper soap formulations with the addition of pineapple and lemon peel extracts can qualify to handwash paper soap with differences in inhibition of the growth of *Staphylococcus aureus* and *Escherichia coli.*

Conclusions

Bioactive compounds in pineapple skin such as bromelain enzymes, flavonoids, saponins, and tannins where flavonoids are phenol compounds that are antibacterial and antifungal so that pineapple and lemon

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peels can be used as basic ingredients for making paper soap.

Paper soap preparations of pineapple peel extract and lemon are able to inhibit *Staphylococcus aureus* and *Eschericia coli* bacteria seen from the resulting inhibitory zone. Paper soap with a total average inhibitory zone of against *S. aureus* bacteria of 5.7 mm and against *E. coli* bacteria of 20 mm so that paper soap is effective in inhibiting the growth of *S. aureus and E. coli* bacteria based on the inhibitory zone produce.

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