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Inhibitory Power of Liquid Soap Made from Hibiscus Flower (*Hibiscus rosasinensis* L.) on The Growth of Bacterial Colonies on Hands

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Abstract:

Background*: One of the easiest ways to prevent and control direct transmission is to maintain hand hygiene by hand washingusing water and antibacterial soap. It is recommended to use antibacterial agents from natural ingredients, one of which has this potential, namely hibiscus flower (Hibiscus rosasinensis* L.*).*

Objectives*: Analyzing the differences in the inhibitory power of non-antiseptic soap, antiseptic soap, and soap made from hibiscus flower on the growth of bacterial colonies on hands.*

Methods*: An experimental pre and post-test control group design study was conducted on 57 participants who were grouped into a hand washing group with: non-antiseptic soap, antiseptic soap, and hibiscus soap (containing 40% hibiscus flower extract). Samples were pre and post-hand swabs using sterile cotton swabs. Colony growth was assessed from the culture results on Nutrient Agar for 24 hours. Differences in inhibition between groups were analyzed using the Kruskal-Wallis test.*

Results*: There was a significant difference between the number of pre and post-hand washing bacterial colonies in the hibiscus soap group (p = 0.03) and in the antiseptic soap group (p = 0.042). The use of non-antiseptic soap did not show a significant difference in the number of pre-post handwashing colonies (p = 0.717).Nonetheless, it can be proved that the effectiveness of hibiscus flower soap, antiseptic soap, and non-antiseptic soap in reducing the number of hand bacteria by 84.2%; 68.4%; and 68.4%.*

Conclusion*: Hibiscus soap which contained 40% of hibiscus flower extract effective in reducing the number of colonies of hand bacteria (84.2%).*

Keywords *-Hibiscus rosasinensis* L.*, hibiscus soap, hand washing, hand bacterial colonies*

I. INTRODUCTION

Direct contact transmission is a method that plays an important role in the transmission of respiratory infectious diseases such as influenza, tuberculosis, and covid-19. In addition, direct contact transmission also plays a role in several digestive diseases such as diarrhea, skin and mucosa diseases, including sexually transmitted diseases, viral conjunctivitis, and Ebola.[1] Direct contact transmission can be prevented and controlled by keeping hand hygiene through handwashing using running water and antibacterial soap.[2] The existence of hand hygiene promotion can reduce the incidence of 31% of digestive tract infections and 21% of

respiratory disease.[3] Research in 2017 regarding the effectiveness of hand hygiene education in women in the community, found that hand washing 6 times a day have better hand hygiene, a little bacterial growth, and no *Escherichia coli* and *Klebsiella* spp. found in the hands.

Hand washing can be done based on the WHO standard, namely with soap and running water.[4] 2 kinds of soap can be used, namely antiseptic soap containing antibacterial compounds and soap without antimicrobial soap (non-antiseptic soap).[5] Research conducted in 2019 regarding the comparison of the effectiveness of handwashing using a hand sanitizer with antiseptic soap in health workers, finding the effectiveness of antiseptic soap (73%) is higher than the hand sanitizer (63%).[6] Triclosan is one of the antibacterial materials that are often used in soap, hand sanitizer, pasta teeth, and mouthwash,[7] but their use as a handwashing soap material has been banned by Food and Drug Administration (FDA) because triclosan cannot show security in long-term use.[8] A study declares antibacterial soap has no significant difference compared to non-antiseptic soap in reducing bacterial growth.[9]

Hibiscus flower have antibacterial components, namely alkaloids, glycosides, saponins, tannins, phenols, and flavonoids.[10] The natural antibacterial content in hibiscus flower can be an additional ingredient of potential substitutes for triclosan on a soap. Research in 2016 regarding the antibacterial activity of hibiscus leaf extract (*H. rosasinensis* L.) in an ointment, finding hibiscus leaf extract has antibacterial activity against injured injuries infected with *Staphylococcus aureus*.[11] The antibacterial activity of hibiscus flower extract has also been proven in vitro against other bacteria that also contaminate hands, such as *S. epidermidis, Streptococcus mutans, E. coli, Klebsiella pneumoniae, Bacillus subtilis,* and *Pseudomonas aeruginosa.*[10,12–14]

This research was conducted to analyse the inhibitory power of non-antiseptic soap, antiseptic soap, and hibiscus flower (*H. rosasinensis* L.) on the growth of hand bacterial colonies.

II. METHOD

The study was conducted after obtaining ethical clearance from the Health Research Ethics Commission (KEPK) of the Faculty of Medicine Diponegoro University with No.258/EC/KEPK/FK-UNDIP/VII/2021.

The experimental research of the pre and post-test with control group design was conducted from August to September 2021. A total of 57 participants in Gedawang, Banyumanik, Semarang, Indonesia, who met the inclusion and exclusion criteria were used as research/sample subjects. The inclusion criteria for the selection of the subjects of this study are can wash their hands with the method of 6 WHO steps, can follow instructions, and agree to participate in the research, while the exclusive criteria are there are injuries in hand and having skin diseases.

Participants are divided, stated, and asked for handwashing using different soap, namely non-antiseptic soap (not containing antibacterial material), antiseptic soap (containing chloroxylenol as an antibacterial active ingredient), and soap flower shoes (containing 40% hibiscus flower extract). Hibiscus flower extract is made using the maceration method until it is obtained by a concentration of 40%. Hibiscus flower soap are made with components of flower extract 40%, SLS, Na_2SO_4, NaCl, CAPB, glycerin, fixative, perfume, and food coloring. Before use, let the soap sit for 12 hours first and put it in a soap bottle (Fig. 1).

Figure 1. Hibiscus flower soap (*H.* rosa*sinensis*L.)

The subjects' hands were swabbed using sterile cotton before and after hand washing, and the subjects were educated on how to wash hands based on the WHO standard. The swab was done using a sterile cotton swab at 5x5cm^2 of the palms, 5x5cm^2 of the backhand, 4x5x3x1cm^2 of the fingers and between fingers, also 5x1x1cm^2 of the tip of the fingers.[15] The palms are swabbed vertically, horizontally, and diagonally in one direction and parallelly.[16] The swab results are subsequently inoculated on Nutrient Agar (NA) and incubated for 24 hours at 37^{o}C, then calculated the number of granted bacterial colonies. Sampling was carried out twice and inoculated on different NA media. The data obtained was then tested for normality using the Kolmogorov-Smirnov test and continued with the Wilcoxon hypothesis test and the Kruskal Wallis test because the data was not normally distributed.

III. Result

Data analysis of differences in the number of colonies before and after handwashing in the three treatment groups is presented in Table 1. In this study, there was a significant difference between pre-test and post-test in the hibiscus flower soap group (p = 0.03) and antiseptic soap (p = 0.042). In the handwashing group with non-antiseptic soap, there was no significant difference between pre-test and post-test (p = 0.717). The analysis of the difference in the number of bacterial colonies before and after handwashing (delta) on the three types of soap used in this study was obtained there was no significant difference (p = 0.991).

Table 1. Growth of the Hand Bacteria Colony

Variable	*Mean* (SD)	Median(Min – *Max*)	*p*[a]	*p*[b]
Hibiscus Soap				
- Pre test	228.28 (250.6)	114 (23 – 886)	0.030*	
- Post test	141.84 (167.5)	78 (10 – 621)		
Antiseptic Soap				
- Pre test	396.86 (468.12)	188 (8,5 – 1659)	0.042*	
- Post test	236.68 (354.55)	82 (5 – 1506)		
Non-Antiseptic Soap				
- Pre test	528.52 (548.67)	322 (70 – 2227)	0.717	
- Post test	607.84 (651.17)	297.5 (15 – 2104.5)		
Changes in the Number of Bacterial Colonies				0.991
- Hibiscus Soap	86.44 (263.32)	48 (-493 – 838)		
- Antiseptic Soap	160.18 (351.55)	105.5 (-243.5 – 1364)		
- Non-Antiseptic Soap	-79.31 (653.53)	120 (-1774 – 696)		

[a] Wilcoxon test
[b] Kruskal Wallis test
*significant p (< 0.05)

IV. Discussion

The results of this study prove that hand washing using hibiscus flower soap can reduce the number of hand bacterial colonies significantly (p = 0.03). This is following the journal about the components of chemical compounds, pharmacological effects, and therapy from *H. rosasinensis* L. which suggests that hibiscus flower extract has antibacterial activity,[12] because it contains antibacterial compounds such as flavonoids, saponins, polyphenols, and tannins.[11,17]

Flavonoids in the hibiscus flower (*H. rosasinensis* L.) will inhibit the growth of bacteria by denying proteins, inhibiting nucleic acid synthesis, and damaging cell membranes.[10,17] Saponins will interfere with the stability and reduce the surface of the cell membrane so that the cytoplasmic fluid will exit the bacteria.[17] Polyphenols will increase the permeability of bacterial cell walls by denaturing the proteins and reducing surface tension,[11] while tannins work on cell membrane polypeptides and shrink the cell membrane so that the permeability will be disrupted.[17]

Meaningful difference (p = 0.042) was found in the number of bacterial colonies between before and after washing hands with antiseptic soap. This is following the study in 2019 which compares the effectiveness of hand sanitizer with antiseptic soap, which antiseptic soap is effective in reducing the number of bacterial colonies with a decrease in the number of bacterial colonies by 73%.[6] The antiseptic soap used in this study contains chloroxylenol as an antibacterial agent. Chloroxylenol works by activating enzymes and changing bacterial cell walls.[4]

In the non-antiseptic soap group, there was no significant difference (p = 0.717) between the number of hand bacterial colonies before and after washing hands. The decrease in the number of bacterial colonies after washing hands with non-antiseptic soap was caused by a mechanical or friction process during handwashing. The mechanical process during handwashing will help in removing hand bacteria, especially when hand washingusing warm water because the pores will open and more and more microorganisms are removed.[4,18]

The non-antiseptic soap used in this study contained 15% biodegradable surfactant. Biosurfactants play a role in removing dirt, fat, and sweat on the skin. In addition, biosurfactants also have antibacterial properties.[19] The average number of hand bacterial colonies after hand washing was higher than before hand washing on the non-antiseptic soap group. These results are in line with the results of 2020 research comparing two handwashing soaps on the growth of carbapenemase-producing and non-carbapenemase-producing Enterobacterales, which finds an increase in the number of bacterial colonies after hand washing. It is because some bacteria can use soap as a food source.[20]

The increase in the number of colony bacteria in the three groups can be caused by water contamination when hand washing because the water source used in this study comes from a spring and has not been studied the quality of the water. Handwashing using contaminated water sources can add to the number of hand bacteria.[21] The increase in the number of bacterial colonies in the research subjects can also be caused by the ring used during handwashing so there could be bacterial contamination after hand washing properly. Other factors that can affect the band's colonization of hands is age, gender, race, health status, chronic disease, diabetes, use of antibiotics, smoking, and immunological status.[22]

Some studies concluded that handwashing using antiseptic soap was no more effective than non-antiseptic soap.[9] This study did not find a significant difference (p = 0.991) between the difference before and after hand washing in the three treatment groups. This proves the ability of hibiscus flower soap in reducing the number of hand bacterial colonies does not differ significantly from the two other types of soap. This can be caused by the swab method used in this study being less sensitive than sampling using the *glove juice method* in assessing the three-hand hygiene group.[23]

This study has been able to prove that hibiscus flower extract (*H. rosasinensis* L.) with a concentration of 40% is an effective hand washing soap material in reducing the number of hand bacterial colonies in the general public in vivo. It can reduce the number of colonies of hand bacteria by 84.2%, higher than antiseptic soap and non-antiseptic soap (68.4% and 68.4%).

This study has limitations not to research the characteristics of subject demographics (age, gender, occupation, socio-economic, and education) and some subjects use rings that can be a source of contamination. It is also not shown the quality of water used for washing hands that can cause contamination.

V. Conclusion

This study obtained a significant difference in the number of bacterial colonies between before and after washing hands on the hibiscus flower soap group, which contained 40% hibiscus flower extract, and antiseptic soap. There was no significant difference between before and after washing hands in the non-antiseptic soap group. This research also shows that hibiscus soap which contained 40% of hibiscus flower extract effectively reduce the number of colonies of hand bacteria by 84.2%.

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