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THE POTENTIAL OF KASTURI AS HAND ANTISEPTIC

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ABSTRACT

Diarrhea is still one of the most important public health problems because it is the third cause of morbidity and mortality rate of children in various countries including Indonesia and South Kalimantan, especially for those who live around the banks of the Kuin river, Banjarmasin. Handwashing with hand antiseptics can reduce the amount of hand bacteria and the risk of having diarrhea as well. One of the typical plants of South Kalimantan that is known to contain active compounds such as flavonoids, terpenoids, and steroids is Kasturi, which can inhibit the growth of Gram-positive bacteria and Gram-negative bacteria. This research examines the activity of Kasturi juice against the bacteria on hands, so that kasturi can be developed as one of hand antiseptics. This research used pure laboratory experimental method (true experimental) with post test-only group design.The samples of this research are 30 swabs of students' hands of Kuin Cerucuk Banjarmasin Elementary School, taken randomly, and this sampling was done before and after the hand washing activity using kasturi juice..The result of identification on hand bacteria shows that 2 types of bacteria isolates were found, which are 22 isolates Staphylococcus aureus (73,3%) and 8 isolates Escherichia coli (26,7%). The result of the dilution test shows the minimal inhibitory concentration (MIC) of kasturi juice on hand bacteria is at concentration 12,5% and there is a significant difference between the mean number of bacteria colonies of hands before and after washing hands using the kasturi juice (sig. 0.000 <0,05). The result of diffusion test shows that there is a difference in inhibitory activity from each treatment of kasturi juice at concentration 25%, 37,5%, 50%, 62,5%, 75%, 87,5%, 100%, compared to antiseptic control, which is chlorexidine gluconate (Anova p <0.05). The treatment at concentration 75% showed the same inhibitory activity as the antiseptic activity of chlorexidine gluconate (LSD α = 0.05). The conclusion of this research is that there is a decrease in the number of hand bacteria colonies after washing hands with kasturi juice. The concentration of kasturi juice at 75% is the optimum concentration in inhibiting the growth of hand bacteria. Kasturi can be developed as a hand antiseptic.

Keywords : *elementary school, hand bacteria, number of bacteria colony, Kasturi, Kuin Cerucuk Banjarmasin*

INTRODUCTION

Diarrhea is still one of the most important public health issues as it is the third cause of morbidity and

mortality in many countries including Indonesia and South Kalimantan. According to South Kalimantan Riskesdas report in 2007, the

prevalence of diarrhea is most found in the age group of children under five and elementary school age. The cause of the high incidence of diarrheal infections in Banjarmasin in 2014 is related to the high number of people who still utilize river water that is contaminated by diarrhea-causing agents, especially for those who live around the banks of the Barito and Kuin river. The incidence of diarrheal infections in primary school-aged children may increase due to the habitual pattern of "snacking" in a stall with an open space system or a "career" agent that spreads diarrheal infections through unclean hands; hands that contain 39,000-460,000 CFU/cm^2 of bacteria, is potential to transmit seeds of disease including diarrhea. The results of research in 2014 and 2015 found the type of bacteria *Staphylococcus aureus* and *Escherichia coli* on the hands of elementary school students in the Pekapuran river and South Kuin as a "career" that potentially transmits gastrointestinal diseases.

Hand washing activity using antiseptics such as hand-washing soap decreases the amount of hand bacteria and can reduce the risk of diarrhea by up to 45%. In addition to the use of hand antiseptics or hand-washing soap, there is an Indonesian society's behavior of washing hands by adding natural ingredients, like fruit juice such as lime or wuluh star fruit. In addition to wuluh star fruit, typical fruit of South Kalimantan, which has been widely known is Kasturi. The skin and fruit of the kasturi are known to contain active compounds such as terpenoids, steroids and safonin, which can inhibit the growth of Gram-positive and Gram-negative bacteria. One effort that can be done to prevent transmission of diarrheal infections through the hands is by using natural ingredients that are efficacious as hand antiseptic.

Previous research found that washing hands with hand-washing soap can lower the number of bacteria colonies in the hands of elementary school students in the Kuin River of Banjarmasin. Budiarti (2014). This research was done to determine the potential of kasturi as hand antiseptic, it is expected that the activity of kasturi can reduce the number of bacteria colonies on hands. This research used the students of Kuin Cerucuk Banjarmasin Elementary School as many as 30 students as the subject; this is based on the fact that the general students who live around the banks of the Kuin river of Banjarmasin, are vulnerable to experience and transmit diarrheal infections or other infections that are transmitted through the hands.

Specific objectives of this research are: (1) to identify the bacteria types from hand swab samples of students of SDN Kuin Cerucuk Banjarmasin based on bacteriological laboratory examination results; (2)to compare and analyze minimal inhibitory concentration of skin and

fruit of kasturi in reducing the number of bacteria colonies on hands; and (3) to compare and analyze the inhibitory zones of some treatment of various kasturi concentrations and hand-wash activity with kasturi in inhibiting the growth of hand bacteria.

RESEARCH METHODS

This research uses experimental method. The design of this research uses pretest-posttest with control group design. The population of this research are children of 5th and 6th grade students of Elementary School of Kuin Cerucuk Banjarmasin. The sample used are the palm swab, fingers and back of the hand before and after treatment using the juice of kasturi. The sampling technique used in this research is simple random sampling method, consists of the population of 5th and 6th grade students of Elementary School of Kuin Cerucuk Banjarmasin as many as 30 students.

Inclusion criteria in this research are students of 5th and 6th grade of Elementary School of Kuin Cerucuk Banjarmasin, who will and are able to cooperate to wash hands using kasturi juice. Exclusion criteria in this research are students of 5th and 6th grade of Elementary School of Kuin Cerucuk Banjarmasin who have skin diseases, fungal diseases or wounds on the fingers and hands, and students who do outdoor activity that is considered dirty.

The tools and materials used for data collection in this research consist of handscoons, masks, sterile cotton swabs, stationery, aluminium foil, incubator, tissue, label sticker, dry ice, thermos, petri dish, pipette, Bunsen burner, laminary air flow, oven, and colony counter. The materials used in this research are hand swab samples of 5th and 6th graders of North Alalak Utara 1 Banjarmasin elementary school, kasturi (bean and fruit), broth, nutrient agar, aquades, spiritus, and alcohol. The variables observed are minimal inhibitory concentration, number of bacteria colonies on hands before and after washing hands with kasturi juice and inhibitory zone of each treatment of the kasturi juice on isolates of hand bacteria.

Research procedure.

a. **Application to Be a Research Subject.** Students of Kuin Cerucuk Banjarmasin elementary school who attended the class at the time of sampling woud be asked to be the subject of research after previously explained about the objectives and research procedures. Students who are the subject of this research are students who agreed and signed the informed consent.
b. **Sampling.** Sampling was done by rubbing sterile swab cotton stick (Swab) onto the area of both palms (palm fingers, back of hand) of each research subject. The sampling results are taken into the Builon medium, then the test tube was covered using cotton and aluminium foil. After that, the sample was inserted in an ice thermos and taken to the laboratory. Sampling on each subject was conducted twice.
c. **Planting and Breeding Hand Bacteria Isolates.** After the sample arrived at the laboratory, the sample was put into the nutrient agar medium, then incubated at 37^{o}C for 18-24 hours. Colonies of growing

bacteria were counted and then followed by bacterial identification. Identification is done in 3 stages:

- The macroscopic identification, includes the structure, shape, nature, morphology of bacteria colonies.
- The microscopic identification of colonies that grow on blood agar media and Mac Conkey using Gram coloring. Next, identification is done by looking at the structure of bacteria by using a microscope with 100 X objective magnification.

d. Identification of Bacteria culture. The result of culture/incubation was colored by gram coloring, Gram-positive bacteria will look purple while Gram-negative bacteria will look red. The catalase test, the mannitol test, and the novobiocin test were tested for Gram-positive bacteria, then biochemical test was tested for the Gram-negative bacteria by scratching the culture material with a sterile spherical inoculating loop and placing it in each test tube. The biochemical tests performed consist of: (a) Citrat, a semi-solid media, to determine whether bacteria use citrate as the only carbon source or not. Positive results would turn blue. (b) SIM (Sulfit Indol Motility), a semi-solid media, to see the movement of bacteria. The result is positive if there is a movement of colonies from base to the top of media which means the bacteria are motile. (c) KIA (Kliger Iron Agar), which is a solid medium/slightly skewed medium, to know whether the bacterias ferment glucose and lactose and to see the formation of positive H_2S if it turns black.

e. Preparation of Bacteria Isolate Suspension. In each culture of pure bacterial isolate, in the identification result, one scratch of isolate was taken using inoculating loop and each one of them was inserted into a test tube containing 10 ml of physiological salt solution (NaCl) at concentration 0.85% then incubated for 24 hours. The results of the bacteria colony isolate growth in the form of turbidity were later standardized (compared) to the solution turbidity series, Mac Farland Standard 0,5 (or equivalent to 0.5 × 108 CFU/ml of bacteria), which appears on the background of white paper. If the bacterial suspension is less turbid, more colony is added, whereas if it is more turbid, 0.85% Physiological NaCl is added up to the standard of turbidity.

f. Effectiveness Test on experimental plant as an antiseptic

- Planting on Muller Hinton Agar (MHA) medium. First, Muller Hinton Agar (MHA) medium and suspension of bacteria isolate whose turbidity had been standardized were prepared. Sterile cotton swabs were dipped into the suspension of bacteria, wait a moment so that the liquid can seep into the sterile cotton swabs. Then lift the cotton swab and squeeze it by pressing the wall of the inner tube while rotating it. Spread the cotton swab on the surface of Muller Hinton Agar (MHA) medium until the entire surface is covered with the suspension of the bacteria suspension. The spreading was done by performing 90% rotation on the1st spread to the 2nd

spread and 45% rotation on the 2nd spread to the 3rd spread, then the petri dish is rotated up to 45%. Put the Muller Hinton Agar (MHA) medium which has been covered with the bacteria suspension on the table for 15 minutes to allow the bacterial suspension to diffuse into the agar.

-Soak the Paper discs in experimental plant as natural antiseptic fluids for 15 minutes (until saturated or until paper discs are unable to absorb natural antiseptic liquids). The soaked paper discs were then placed on an aseptic Muller Hinton Agar (MHA) medium and incubated for 24 hours at 37^0C.

- The reading is done by measuring the inhibitory zone diameter using callifer, the diameter of the resistance zone is measured from one edge to another through the center of the paper disc and the value of the inhibitory zone is measured in millimeters.

Data collecting technique. The data is collected in the form of primary data based on the observation of the number of bacteria colonies and measurement of inhibitory zone around the paper disc that already contains kasturi juice at various concentrations placed on the growth of hand bacteria isolates. The treatment result is the number of bacteria colonies and the inhibitory zone diameter. The data is then added in the data table.

Data analysis. Data analysis is processed using SPSS 21. Saphiro Wilk test is used for normality test to verify the normal distribution of this research data. Data of the number of bacteria colonies and the size of the inhibitory zone (millimeters) are evaluated by performing a Saphiro-Wilk normality test, homogeneity test is done with Levene's test. If the data is normally distributed and homogeneous, parametric analysis of One Way Annova and Post-hoc LSD test with 95% confidence interval (α = 0,05) are performed.

RESULT AND DISCUSSION

The result of identification of hand bacteria is as seen in the table 5.1

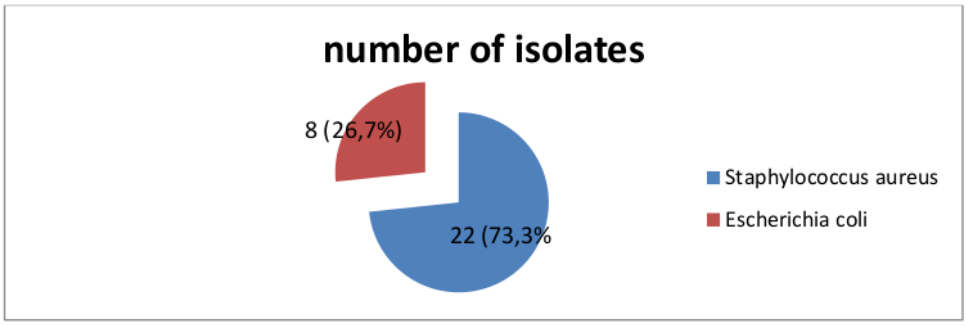

Figure 1. The types of bacteria on the hands of Kuin Cerucuk Banjarmasin elementary school students (N=30)

The results of identification in this research is there are 2 types of bacteria isolates found namely *S. aureus* (73.3%) and *E. coli* (26.7%). The results of this research does not differ much from the previous research on the type of bacteria on the hands of the students of Pekapuran Raya and North Alalak Banjarmasin elementary school, which obtained *S. aureus* as the most common bacteria isolates (Budiarti, 2016).

Staphylococcus aureus is one of many normal flora of the skin, nasal mucosa, and respiratory tract. Transmission can occur either directly or indirectly. Clinical manifestations of this bacteria in humans include pneumonia, endocarditis, scalded skin syndrome, impetigo, meningitis, and sepsis.

Escherichia coli is a bacteria that is found in the human's colon as a normal flora. *Escherichia coli* is associated with intestinal disease in humans (diarrhea), this is associated with enterotoxin released by this bacteria, namely LT toxin (termolable) and ST toxin (thermostable). *Escherichia coli* is found in the hands due to direct contact of the hands with areas that are contaminated by this bacteria such as genitals and anus.

The skin of the palm is contaminated by this type of bacteria, because there is much food that supports the growth of bacteria such as fat, nitrogen, and minerals, on the skin surface . The presence of bacteria on human skin is affected by age, diet, health hormones, and hygiene. The important factors in reducing / removing microorganisms that do not include skin normal flora are low pH, fatty acids in sebaceous gland secretions, and the presence of lysozyme. Actions to prevent the presence of bacteria on the hands can be done by maintaining hand hygiene, for instance by washing hands with soap / antiseptic and flowing water in the proper hand washing order. The use of antibacterial or antiseptic substances can lower the bacteria colonies on the hand. Differences in the nature and characteristics of Gram-positive and Gram-negative bacteria may affect the colonization and effectiveness of the action of antibacterial agents.

The activity of kasturi in reducing the number of bacteria colonies on the hand is known by observing and comparing the number of bacteria colonies on the hands before and after washing hands with kasturi juice. Data on the mean number of bacteria colonies before and after washing hands using kasturi juice are shown in Figure 2 and the result of Saphiro-Wilk as normality test on this research data is shown in table 1.

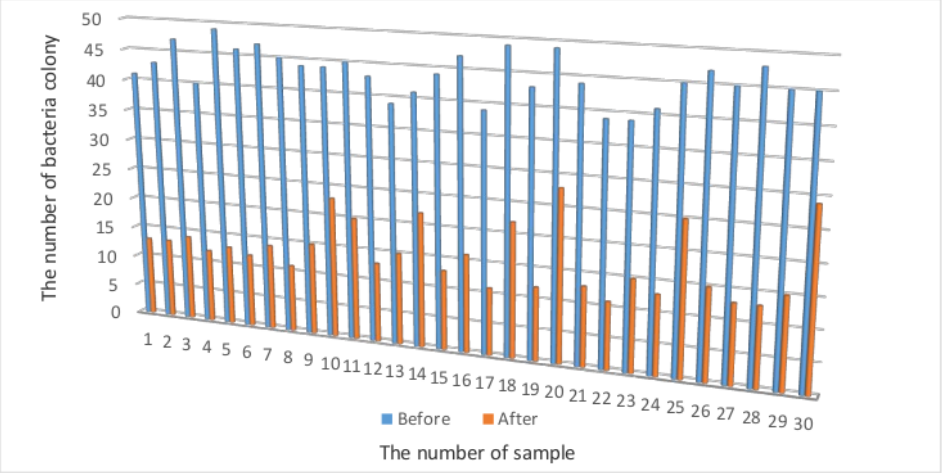

Figure 2. Mean Number of Colonies of Hand Bacteria Before and After Washing Hands Using Kasturi Juice

Table 5.1. The Result of Normality Test of the Activity of Kasturi Juice in Lowering Number of Bacteria Colonies on Hand based on Saphiro-Wilk (α.0,05)
Normality test

Normality Test

The mean number of treatments	Shapiro-Wilk		
	Number of samples	Mean number of bacteria colonies	P .0,05
Mean number before treatment	30	44	,076
Mean number after treatment	30	16	,000

Figure 2 shows a decrease in the number of bacteria colonies on the hands after washing hands with kasturi juice. The Saphiro Wilk test results (table 5.1) obtains a significance value of p = 0.076 for the mean number of bacteria colonies before treatment and p = 0,000 for the average number of bacteria colonies after treatment. These tests indicate that the data of this research is not normally distributed, so to know whether there is a difference of influence before and after washing hands with kasturi juice, Wilcoxon test is done. Wilcoxon test results are listed in table 5.2.

Table 5.2. Number of Colonies of Bacteria Before and After Washing Hands Using Kasturi Juice based on Wilcoxon Test (α.0,05)

Mean number of treatment	Significance Value (α.0,05)
Z Significance	-4,787b ,000

Table 5.2 shows Wilcoxon test results, in which p = 0,000 and z = -4.787 with significance of 0.000 (p <0.005). These results indicate a significant difference between the mean number of bacteria colonies before and after washing hands using kasturi juice. The occurrence of the decreased number of bacteria colonies after washing hands with kasturi juice, is due to antibacterial content in the kasturi such as flavonoid and alkaloids. Flavonoids can inhibit the activity of DNA gyrase enzyme and ATPase enzyme causing nucleic acid synthesis disturbance and plasma membrane damage that lead to the leakage of potassium ions and bacterial cell lysis. Flavonoid has various activities beside being an antibacterial agent, such as antiviral, anticancer, anti-inflammatory, antioxidants, and antidiabetes. Xanthone is a yellow phenolic pigment whose color reaction and chromatography are similar to flavonoids. The derivatives of xanthones have a remarkable effect on antiulserogenic activity as antibacterial, antifungal, inhibitor of protein kinase, anticancer, antioxidant, and antiinflammatory. Alkaloids kill bacteria by inhibiting nucleic acid synthesis and prevent cell division. Saponins can increase membrane permeability by forming saponin-cholesterol complexes and causing protein denaturation so that cell membranes are damaged and eventually bacterial cell lysis occurs. (Setiawan, Darusman, 2008; Zarena, Udaya, 2009; Yoswathana Eshtiaghi, 2015)

Kasturi juice activity in inhibiting the growth of hand bacteria. Based on the result of the dilution test of the kasturi to the hand bacteria, the minimum inhibitory concentration (MIC) of kasturi is at a concentration of 12.5%. Test of inhibitory activity of kasturi juice to hand bacteria uses paper disc diffusion method. The concentrations of kasturi juiced used are 25%, 37,5%, 50%, 62,5%, 75%, 87,5%, 100% with liquid hand antiseptic as positive control. In each treatment, repetition is done three times. The observed parameters are the inhibitory zones. The results can be seen in Table 3.

Table 3. Mean of Inhibitory Zone Diameter of Hand Bacteria Growth in various Kasturi juice Concentration

No	Treatments	Inhibitory zones (mm)			Mean (mm)
		1	2	3	
1	Positive control	15	15	15	15
2	Aquadest	0	0	0	0
3	25% kasturi juice	6	8	7	7
4	37,5% kasturi juice	8	9	10	9
5	50% kasturi juice	10	10	10	10
6	62,5% kasturi juice	11	12	13	12
7	75% kasturi juice	17	15	16	16
8	87,5% kasturi juice	18	19	20	19
9	100% kasturi juice	22	22	22	22

Table 3 shows the effect of mean number of inhibitory zone from the treatment of kasturi juice to hand bacteria, there is an increase in the diameter of the inhibitory zone along with the increase of concentration of Kasturi juice. the lowest inhibitory zone is at 25% (7 mm) of concentration and the largest inhibitory zone is at 100% (22 mm)of concentration. The hand antiseptic as positive control treatment gave a mean inhibitory effect of 15 mm. Terpenoid compounds, flavonoids, essential oils contained in kasturi can inhibit the growth of bacteria on the hands by reducing the surface tension of bacterial cell walls and damaging the permebiality of cell membranes and interfere with the transport of proteins on the inner layer of bacterial cells.

Flavonoids have various activities beside antibacterial activity, such as antiviral, anticancer, anti-inflammatory, antioxidant, and antidiabetic effects. Xanthones are yellow phenolic pigments whose color reaction and chromatography are similar to flavonoids. The derivatives of xanthones have a remarkable effect on antiulserogenic activity as antibacterial, antifungals, inhibitors of protein kinase, anticancer, antioxidants, and antiinflammatory. Terpenoids are an active component in medicinal plants that have physiological activity and are used in the treatment of diabetes, menstrual disorders, snake venom, skin disorders, liver damage and malaria. Some triterpenoid compounds show antibacterial and antiviral activity.

The results of this research are compatible with the results of the previous research which stated that mangoes at concentrations of 50%, 37.5%, 25%, 18.75% and 12.5% have antibacterial and antifungal activity. Kasturi plant is analogous to mango because it is a plant with the same genus or the same family and also has the same active substance. Increased antibacterial / antifungal activity is caused by the content of the dissolved active substances in kasturi that increases at higher concentrations. This is because natural medicines' pharmacological activity (herbal medicines) as in synthetic drugs, is determined by the presence of such drug bonds with receptors. The magnitude of the pharmacological intensity that arises depends on the

concentration / number of drugs that reach the receptor and the type of drug and receptor bonds, which can be both specific and non-specific. The result of statistical test using Shapiro -Wilk normality test (amount of data is less than 50), shows the normal distributed data, whose value is p = 1.00 (p> 0,05). Data homogeneity test using Levene's test, shows p = 0,106 (p> 0,05). To find out whether there is a difference of effect between treatments, One way ANOVA test is done and the result is p = 0,000 (p <0,05, meaning there is a significant difference in each treatment tested. Furthermore, to know which treatment has different meaningful effect, post hoc LSD test is done with results seen in Table 4.

Table 4. Differences in Inhibitory Zones Effects of Various Kasturi Treatment on Hand Bacteria Based on Post-Hoc LSD Test (α = 0.005)

Treatments	Controls		Kasturi juice concentrations treatments						
	Aq	K+	25%	37,5%	50%	62,5%	75 %	87,5%	100%
25% kasturi juice	M	M		NM	M	M	M	M	M
37,5% kasturi juice	M	M	NM		NM	M	M	M	M
50% kasturi juice	M	M	M	M		NM	M	M	M
62,5% kasturi juice	M	M	M	M	NM		M	M	M
75 % kasturi juice	NM	M	M	M	M	M		M	M
87,5% kasturi juice	M	M	M	M	M	M	M		M
100% kasturi juice	M	M	M	M	M	M	M	M	

Note: M = Meaningful NM = Not meaningful

Table 4 shows that there are significant and non-significant differences between the treatments being tested. There are no significant differences between the concentrations of 37.5% and 25%, concentrations of 50% and 37.5%, concentrations of 50% and 62.5%. or this result shows that the effect of the concentrations of 37.5%, 25%, and 37.5% are the same effects. At 75% of concentration, there is no significant difference with the positive control (hand antiseptic) and also significantly different with the concentration of 87,5% and 100%. Based on the results of this test it can be seen that the juice of kasturi at 75% concentration can give optimum effect in inhibiting hand bacteria growth, or Kasturi juice at 75% concentration can be used as hand antiseptic and the biggest inhibitory effect is obtained at 100% concentration treatment.

The hand antiseptic used in this research is the chlorexidine gluconate fluid, which works by inhibiting the synthesis of microbial cell walls and cause the lysis bacterial cell membranes. (Sari YP. 2012). Active substances contained in kasturi are relatively equal to the content of mangoes. The mango (*mangifera indica)* plant itself is has the same species with Kasturi (mangifera casturi), according to Rosyidah et al (2010), the antibacterial effect of Kasturi is greater than mangoes'. Furthermore, it is mentioned that the growth of *S.aureus* and *E. Coli* can be inhibited by stem bark extract of Kasturi with inhibitory diameter of 10.3 ± 0.5 mm against *E. coli* and 10.8 ± 0.3 mm against *S.aureus*. In order to remove microorganisms, the research material must penetrate into the bacterial cell wall. The results of this research shows that Kasturi juice has antibacterial activity against hand bacteria and can be used as a hand antiseptic.

CONCLUSIONS AND SUGGESTION

Conclusion. Based on the results of research on the activity of kasturi juice on the hand bacteria of students of Kuin Cerucuk Banjarmasin elementary school, it can be concluded that the juice of kasturi at concentration of 70% has the effect as hand antiseptic.

Suggestion. The results of this research can be used to utilize the waste of kasturi plants, especially the seeds of Kasturi as antibacterial agent. Further research on the potential of kasturi as hand antiseptic as well as antibacterial or antifungal agent that is safe for humans to consume, can be done in the future.

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