



## Potential Ekstract of Red Betel Leaf (*Piper crocatum* Ruiz & Pav.) as a Natural Hand Sanitizer

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Article Info	Abstract
<p><b>Article history:</b> Received 24 February 2022 Revised 03 August 2022 Accepted 17 August 2022 Available 01 August 2023</p> <p><b>Keywords:</b> Red Betel Extract, <i>Staphylococcus aureus</i>, <i>Streptococcus pyogenes</i>, <i>Salmonella typhi</i>, natural hand sanitizer</p> <p><b>Correspondence:</b> innm209@ums.ac.id</p> <p><b>How to cite this article:</b> MM Manurung, TH Subekti, NDA Sawitri, IN Nurhidayati Mahmuda. Potential Ekstract of Red Betel Leaf (<i>Piper crocatum</i> Ruiz &amp; Pav.) as a Natural Hand Sanitizer. MAGNA MEDIKA Berk Ilm Kedokt dan Kesehat. 2023;10(2): 141-149</p>	<p><b>Background:</b> Hands are the most common medium for spreading disease. Red betel leaf has antibacterial bioactive compounds such as flavonoids, alkaloids, polyphenols, tannins, and essential oils. The most common way to clean hands is to use hand sanitizer.</p> <p><b>Objective:</b> To determine the antibacterial potential of red betel leaf extract (<i>Piper crocatum</i> Ruiz &amp; Pav.) against the growth of <i>Staphylococcus aureus</i>, <i>Streptococcus pyogenes</i>, and <i>Salmonella typhi</i> as an active ingredient in hand sanitizers</p> <p><b>Methods:</b> Each bacterium, namely <i>Staphylococcus aureus</i>, <i>Streptococcus pyogenes</i>, and <i>Salmonella typhi</i>, were divided into six groups, PEG 5% as a negative control, clindamycin for <i>Staphylococcus aureus</i> and <i>Streptococcus pyogenes</i> and chloramphenicol for <i>Salmonella typhi</i> as a positive control, 70% alcohol as a comparison for hand sanitizer, extract 20%, 40%, and 80% as treatment groups. The obstacle zone is measured using a ruler.</p> <p><b>Results:</b> Zones of inhibition against <i>Staphylococcus aureus</i>, <i>Streptococcus pyogenes</i>, and <i>Salmonella typhi</i> were formed at concentrations of 20%, 40%, and 80%. Kruskal Wallis's statistical analysis showed significant results with <math>p=0.000</math>.</p> <p><b>Conclusion:</b> Red betel leaf extract has statistically significant antibacterial activity against <i>Staphylococcus aureus</i>, <i>Streptococcus pyogenes</i>, and <i>Salmonella typhi</i>. The extract concentration of 40% is the optimal raw material for hand sanitizer.</p>
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## INTRODUCTION

Hands are the most common medium for spreading disease. Because the human hand periodically comes into contact with the environment, then touches the area around the eyes, nose, or mouth as a route for disease to enter<sup>1</sup>. There are various kinds of pathogens on human palms. For example, pathogenic bacteria in children's palms are found with *Acinetobacter*, *Pseudomonas*, *Enterococcus*, *Klebsiella*, *Flavobacterium*, *Escherichia coli*, and *Enterobacter*<sup>2</sup>. While at the bottom of the human fingernails were found bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Acinetobacter sp.*, *Streptococcus sp.*, *Pseudomonas aeruginosa*, and *Klebsiella sp.*<sup>3</sup>. In addition, bacteria such as *Salmonella* and *Shigella* can contaminate food, causing infection<sup>4</sup>.

Therefore, maintaining hand hygiene is essential in preventing the transmission of disease-causing microorganisms<sup>5</sup>. The most widely used method is to use of hand sanitizer<sup>6</sup>. Hand sanitizer can be used if there is no visible dirt on the surface of the hand. The most effective hand sanitizers on the market, contain 60% to 90% alcohol and 0.05% triclosan as the active ingredients. 70% alcohol concentration and 0.05% triclosan are the best concentrations of hand sanitizer that can inhibit the growth of 72.45% *S. aureus* bacteria for 30 seconds<sup>7</sup>.

At high alcohol concentrations, the ability to inhibit bacteria will be better. However, the high concentration of alcohol in hand sanitizer can harm the skin. Alcohol can dry the skin and make it easier for microorganisms to stick. At the same time, triclosan in hand sanitizer can trigger dermatitis<sup>8</sup>. Solutions can be

sought by using natural ingredients as active ingredients in hand sanitizer products<sup>9</sup>.

The red betel leaf plant (*Piper crocatum* Ruiz & Pav.) is one of the native Indonesian plants that grows vines or attaches to other tree trunks<sup>10</sup>. Chromatography showed that red betel leaf contains flavonoids, polyphenolics, tannins, alkaloids, and essential oils. The ethanol extract in red betel leaf has been found to have antibacterial activity. The activity of these compounds can disrupt and damage bacterial cell membranes<sup>11</sup>.

The negative impact of alcohol and triclosan increases people's desire to switch to safer active ingredients. The alternative solution is to make a hand sanitizer from natural ingredients<sup>12</sup>. Therefore, red betel leaf (*Piper crocatum* Ruiz & Pav.) can be developed as a natural hand sanitizer.

## METHODS

This research is an experimental laboratory study with a post-test only with a controlled group design method to test the concentration of red betel leaf extract (*Piper crocatum* Ruiz & Pav.) on the growth of *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Salmonella typhi* bacteria which will be used as hand sanitizers. Antibacterial activity was determined by the disk diffusion method (*Kirby Bauer*) with Mueller Hinton Agar media based on the diameter of the inhibition zone generated around the disc.

Red betel leaf extract group with concentrations of 20%, 40%, and 80% as the experimental group, 5% PEG as the negative control group, and the positive control group di-

vided into two; 70% alcohol was used for comparison control of hand sanitizer and antibiotic disc group. PEG 5% was used as a negative control because it could dissolve the extract well and did not have antibacterial activity at that percentage. Antibiotic disc clindamycin 30 µg/disk is used for *Staphylococcus aureus* and *Streptococcus pyogenes*, and antibiotic disc chloramphenicol 30 µg/disk for *Salmonella typhi*.

Data analysis includes inferential statistical analysis and hypothesis testing using SPSS 25 software. If the sample group is more than two and the data is less than 50, then the Shapiro-Wilk test's normality distribution is

used. Because the data distribution was not expected, the test was carried out using *Kruskal Wallis*. This research is under the Ethical Clearance issued by the Komisi Etik Penelitian Kesehatan (KEPK) RSUD Dr. Moewardi with letter number 706/VI/HREC/2021.

## RESULTS

After testing the antibacterial activity of red betel leaf extract (*Piper crocatum* Ruiz & Pav.) against the growth of *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Salmonella typhi* bacteria in vitro, the following results were obtained:

**Table 1.** Results of Measurement of Inhibitory Zone Diameter of *Staphylococcus aureus*.

<i>Staphylococcus aureus</i> bacteria replication	PEG 5% (mm)	Alcohol 70% (mm)	Clindamycin (mm)	Red betel leaf extract		
				20% (mm)	40% (mm)	80% (mm)
1	6	9	24	9	9	12
2	6	15	24	9	12	14
3	6	10	26	9	10	12
4	6	9	25	9	9	15
5	6	10	25	10	10	15
Mean±SD	6±0	10,6±2,50	24,8±0,83	9,2±0,44	10±0,12	13,6±1,51



**Figure 1.** Diameter of Inhibitory Zone of *Staphylococcus aureus*.



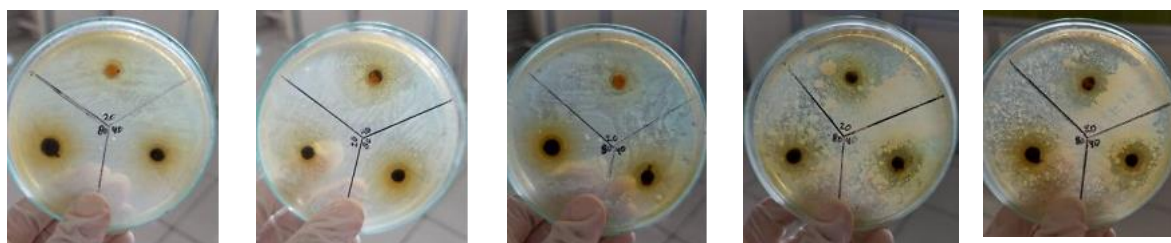
**Figure 2.** Diameter of Inhibitory Zone of *Streptococcus pyogenes*.

**Table 2.** Results of Measurement of Inhibitory Zone Diameter of *Streptococcus pyogenes*.

Streptococcus pyogenes bacteria replication	PEG 5% (mm)	Alcohol 70% (mm)	Clindamycin (mm)	Red betel leaf extract		
				20% (mm)	40% (mm)	80% (mm)
1	6	10	30	9	11	16
2	6	11	28	10	13	15
3	6	11	28	10	14	16
4	6	10	29	10	13	15
5	6	11	29	10	11	15
Mean±0	6±0	10,6±0,54	28,5±0,83	9,8±0,44	12,4±1,34	15,4±0,54

**Table 3.** Results of Measurement of Inhibitory Zone Diameter of *Salmonella typhi*.

<i>Salmonella typhi</i> bacteria replication	PEG 5% (mm)	Alcohol 70% (mm)	Chloram- phenicol (mm)	Red betel leaf extract		
				20% (mm)	40% (mm)	80% (mm)
1	6	10	21	8	9	12
2	6	10	20	8	9	10
3	6	10	20	9	10	12
4	6	11	21	9	10	12
5	6	11	20	9	10	12
Mean±SD	6±0	10,4±0,54	20,4±0,54	8,6±0,54	9,6±0,54	11,6±0,89



**Figure 3.** Diameter of Inhibitory Zone of *Salmonella typhi*.

## DISCUSSION

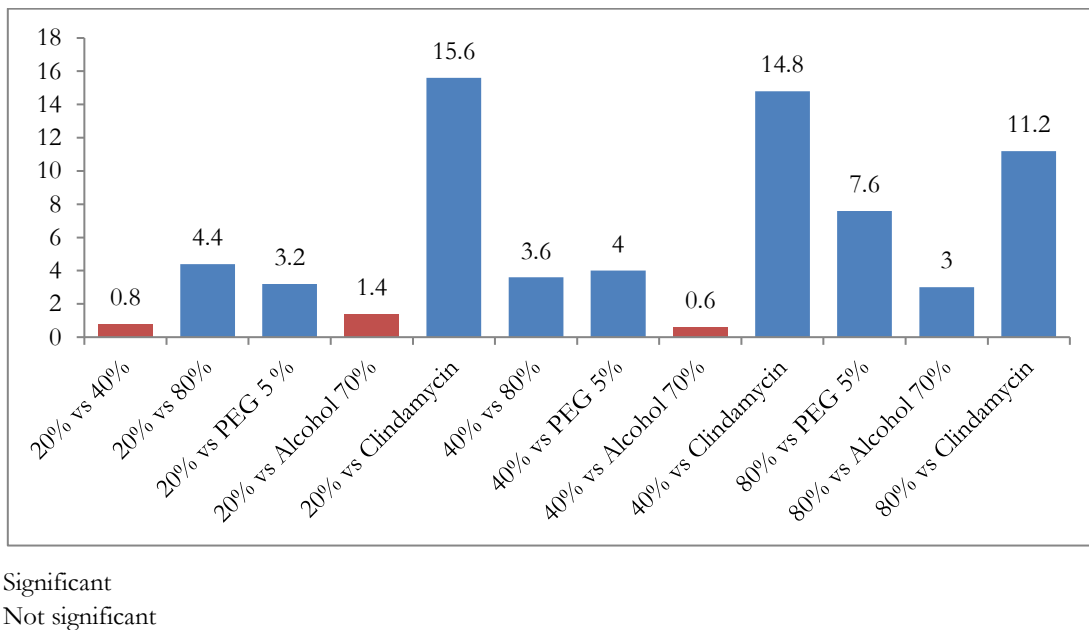
From the third table above, it can be seen that the negative control group did not form the diameter of the inhibition zone, which means it did not produce an antibacterial effect. The antibacterial effect of red betel leaf extract (*Piper crocatum* Ruiz & Pav.) increased with increasing concentration. The effectiveness of the three antibacterial concentrations was not maximal. The positive control was clindamycin for *Staphylococcus aureus*, *Streptococcus pyogenes*, and chloramphenicol for *Salmonella typhi* bacteria. 70% alcohol used as a comparison for the active ingredients of hand sanitizers formed an inhibition zone diameter that was not much different from red betel leaf extract (*Piper crocatum* Ruiz & Pav.) with a concentration of 40% for each bacterium.

The analysis of data variants of the three bacteria, namely *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Salmonella typhi*, shows that the

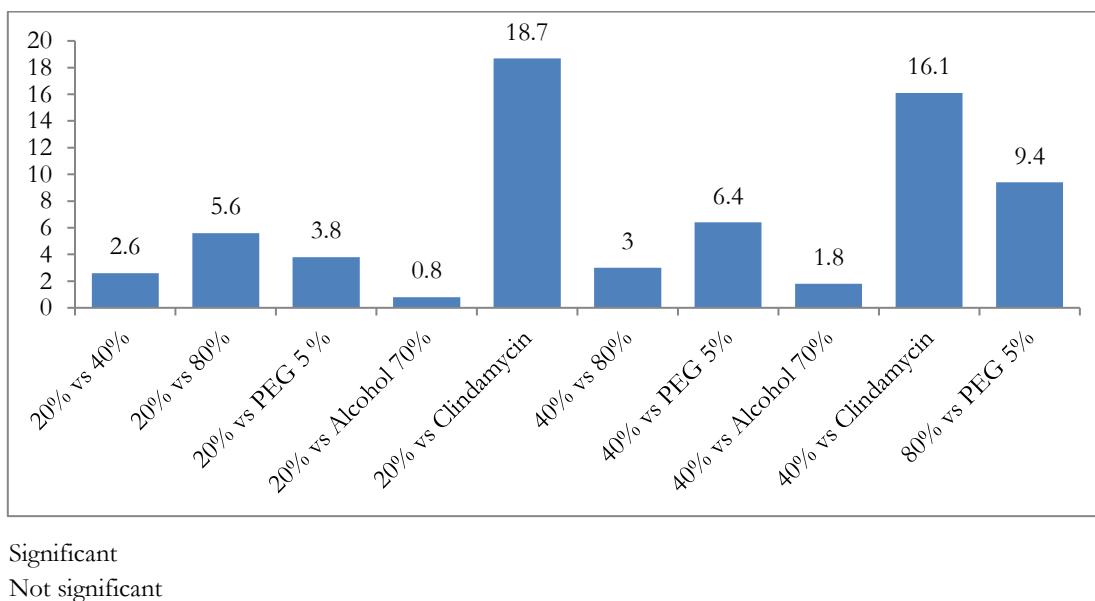
data are not normally distributed and are not homogeneous. Therefore, the *Kruskal Wallis* non-parametric test was chosen. This test was chosen as an alternative to the *ANOVA test*. The *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Salmonella typhi* produced the same value in this test,  $p=0.000$ . The result means that the  $p$ -value  $<0.05$ , and it can be

concluded that there are significant differences in the antibacterial effect of each treatment group on each bacterium.

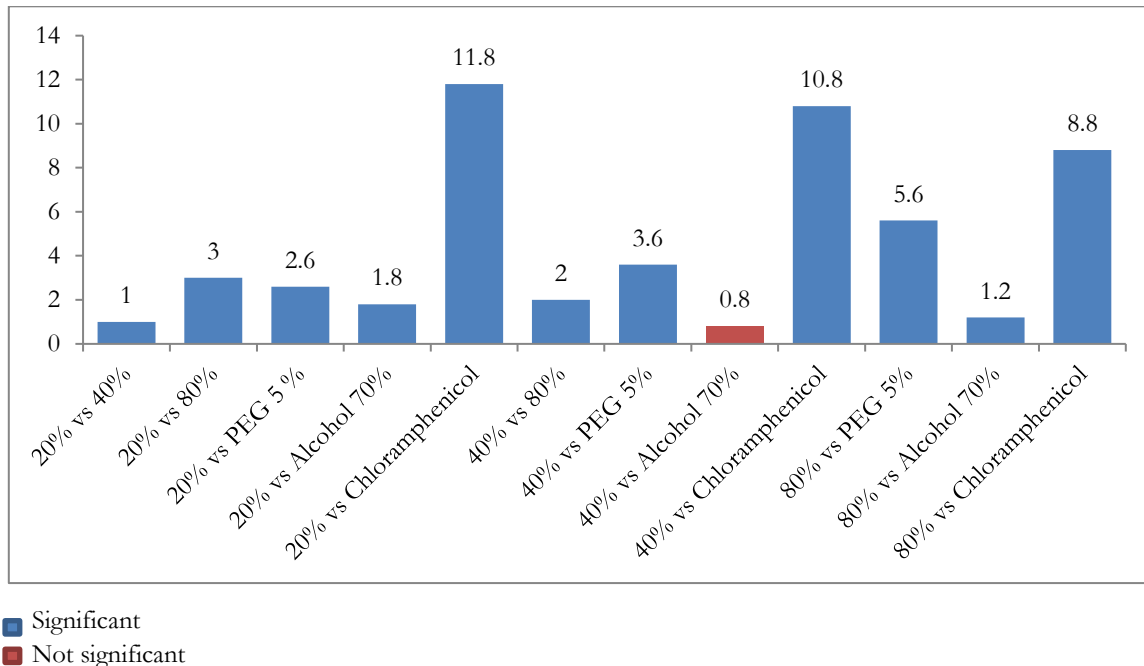
Furthermore, the *Mann-Whitney* non-parametric test was carried out by comparing each treatment group which is shown in Chart 1, Chart 2, and Chart 3 as follows:



**Chart 1.** Results of Post-Hoc Test of *Staphylococcus aureus*.



**Chart 2.** Results of Post-Hoc Test of *Streptococcus pyogenes*.



**Chart 3.** Results of *Post-Hoc* Test of *Salmonella typhi*.

This study obtained data that red betel leaf extract (*Piper crocatum* Ruiz & Pav.) with concentrations of 20%, 40%, and 80% had higher effectiveness in suppressing the growth of *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Salmonella typhi* bacteria when compared to controls. Negative Polyethylene glycol (PEG) 5%. However, the three concentrations were not as good as the positive control, namely clindamycin for *Staphylococcus aureus* and *Streptococcus pyogenes* and chloramphenicol for *Salmonella typhi* bacteria. 70% alcohol, compared with the active ingredient of hand sanitizer, has an inhibition zone diameter that is almost the same as red betel leaf extract with a concentration of 40%. So, this study chose concentration as the active ingredient of hand sanitizer.

If the concentration of 20% is chosen as the concentration of the active ingredient of the hand sanitizer, the antibacterial activity produced is weak and will not be comparable to 70% alcohol. Meanwhile, if the concentration

of 80% is chosen as the concentration of the active ingredient of the hand sanitizer, the antibacterial activity produced will be more potent. However, the extract required at a concentration of 80% will be more. In addition, too much extract in the formulation will make the results of the hand sanitizer too concentrated and affect the resulting preparation.

Alcohol with a concentration of 70% and triclosan with a concentration of 0.05% is the best formulas for making hand sanitizers. This formula can suppress the growth of 72.45% *Staphylococcus aureus* bacteria for 30 seconds<sup>7</sup>. Alcohol, commonly used as an antiseptic and disinfectant, is alcohol with 70% because it is effective at breaking down proteins in microorganisms<sup>13</sup>. That is why 70% alcohol was chosen as a hand sanitizer comparison.

Making hand sanitizer begins with developing carbopol in warm water, then stirring. The red betel leaf extract was mixed with 1% glycerin, and korigen odoris 0.2% sodium metabisulfite

until well mixed, then put into carbopol. Then, a little alcohol is added to the mixture so that the extract does not leave color on the skin when used—followed by adding water until the desired volume, then adding TEA dropwise while stirring slowly until a clear gel is formed.

Ethanol extract of red betel leaf (*Piper crocatum* Ruiz & Pav.) started to form the diameter of the inhibition zone or started to have antibacterial activity on *Staphylococcus aureus* bacteria at a concentration of 10%<sup>14</sup>. So in this study, the initial concentration series was increased, namely the concentration of 20%, to get more optimal results.

At high concentrations, the diameter of the inhibition zone formed will be even greater. This condition is because the amount of concentration affects the growth of bacteria. The higher the concentration, the greater the bacterial inhibition process. The higher the concentration, the more concentrated the solution and the more antibacterial substances it contains<sup>15</sup>.

Red betel leaf extract (*Piper crocatum* Ruiz & Pav.) can suppress the growth of *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Salmonella typhi* bacteria because it contains flavonoids, alkaloids, polyphenols, tannins, and essential oils. The mechanism of flavonoids as antibacterial agents includes increasing cell membrane permeability, suppressing DNA and RNA synthesis, suppressing ATPase enzyme activity, and disrupting the function of bacterial cell walls<sup>11</sup>. Alkaloids can reduce the integrity of the peptidoglycan constituent particles of bacterial cells<sup>16</sup>. Polyphenols, as antibacterial, can coagulate and denature bacterial cell proteins<sup>17</sup>. Polyphenols in low concentrations,

phenolic protein complexes that have weak bonds are formed and degraded rapidly, followed by the entry of phenols into cells triggering denaturation and precipitation of proteins. Protein coagulation and membrane lysis occur at high phenol concentrations<sup>18</sup>. Tannins can make complex compounds with enzymes or substrates to interfere with and destroy bacterial cell membranes<sup>19</sup>. The antibacterial mechanism of essential oils breaks down the process of compiling cell membranes that cannot be formed completely<sup>18</sup>. Essential oils act as antibacterial agents, which have been proven to inhibit the growth of gram-positive and gram-negative bacteria<sup>20</sup>.

## CONCLUSION

From the results of this study, it can be seen that red betel leaf extract (*Piper crocatum* Ruiz & Pav.) at concentrations of 20%, 40%, and 80% had antibacterial effects against *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Salmonella typhi* bacteria. The most suitable concentration for the active ingredient of hand sanitizer is red betel leaf extract 40%. It is necessary to develop further research on the effect of red betel leaf extract on more varied bacteria and in test animals. Further development of hand sanitizer formulations is also needed to obtain an even better formula.

## REFERENCES

1. Arisanty A, Abdullah T, Sukmawaty M. Formulasi dan Efek Anti Bakteri Sediaan Gel Pencuci Tangan dari Minyak Atsiri Daun Kemangi (*Occimum basilicum* L.) Terhadap *Escherichia coli*. *Media Farm*. 2019;15(2):163–70.

2. Vishwanath R, Selvabai AP, Shanmugam P. Detection of bacterial pathogens in the hands of rural school children across different age groups and emphasizing the importance of hand wash. *J Prev Med Hyg.* 2019;60(2):E103.
3. Risan MH. Isolation and Identification of Bacteria from under Fingernails. *Intl J Curr Microbiol Appl Sci.* 2017;6(8):3584–90.
4. Apriani L, Rahmawati R, Kurniatuhadi R. Deteksi Bakteri Salmonella Dan Shigella Pada Makanan Burger Di Sungai Raya Dalam Pontianak. *Protobiont.* 2019;8(3).
5. Karolina ME, Kusdiyah E, Aurora WID. Perbandingan Efektifitas Antibakteri Mencuci Tangan dengan Electrolyzed Oxidized Water (EOW) dan Dengan Sabun Antiseptik. *Electron J Sci Environ Heal Dis.* 2020;1(1).
6. Wasiaturrahmah Y, Jannah R. Formulasi dan Uji Sifat Fisik Gel Hand Sanitizer dari Ekstrak Daun Salam (*Syzygium polyanthum*). *Borneo J Pharmascientech.* 2018;2(2).
7. Asngad A, Subiakto DW. Potensi Ekstrak Biji Alpukat sebagai Hand Sanitizer Alami: Literatur Review. *Bioeksperimen J Penelit Biol.* 2020;6(2):106–15.
8. Olaniyan LWB, Mkwetshana N, Okoh AI. Triclosan in Water, Implications for Human and Environmental Health. *Springerplus.* 2016;5(1):1–17.
9. Alawiyah AL, Karmila A, Hajar DS, Pebriani F, Putri NLFH. Pelatihan Pembuatan Hand Sanitizer Alami dari Daun Sirih dan Jeruk Nipis di Desa Salamnunggal. *Educivilia J Pengabd pada Masy.* 2021;2(2):117–26.
10. Sitanggang IM. Analisis Dan Perbandingan Metode Sobel Dan Canny Pada Deteksi Tepi Citra Daun Sirih Merah. *J Ilmu Komput dan Sist Inf.* 2020;3(1.1):140–9.
11. Rachmawaty FJ, Akhmad MM, Pranacipta SH, Nabila Z, Muhammad A. Optimasi ekstrak etanol daun sirih merah (*Piper crocatum*) sebagai antibakteri terhadap bakteri *Staphylococcus aureus*. *J Kedokt dan Kesehat.* 2018;18(1):13–6.
12. Hurria H. Formulasi, Uji Stabilitas Fisik, dan Uji Aktifitas Sediaan Gel Hand Sanitizer dari Air Perasan Jeruk Nipis (*Citrus aurantifolia SWINGLE*) Berbasis Karbomer. *J Farm UIN Alauddin Makassar.* 2014;2(1):28–34.
13. Rahayu EDS. Efektivitas Daun Mimba (*Azadirachta indica A. Juss*) sebagai Desinfektan Alami terhadap Daya Hambat Bakteri Total di Ruang Penampungan Susu. *Gontor AGROTECH Sci J.* 2018;4(1):45–55.
14. Candrasari A, Romas MA, Astuti OR. Uji Daya Antimikroba Ekstrak Etanol Daun Sirih Merah (*Piper Crocatum Ruiz & Pav.*) Terhadap Pertumbuhan *Staphylococcus aureus* ATCC 6538, *Eschericia Coli* ATCC 11229 Dan *Candida Albicans* ATCC 10231 Secara In Vitro. *Biomedika.* 2012;4(1).
15. Afifi R, Erlin E, Rachmawati J. Uji Anti Bakteri Ekstrak Daun Belimbing Wuluh (*Averrhoa bilimbi L*) terhadap Zona Hambat Bakteri Jerawat *Propionibacterium acnes* Secara In Vitro. *Quagga J Pendidik dan Biol.* 2018;10(01):10–7.
16. Rahman FA, Haniastuti T, Utami TW. Skrining Fitokimia dan Aktivitas Antibakteri Ekstrak Etanol Daun Sirsak (*Annona muricata L.*) pada *Streptococcus*



- mutans ATCC 35668. Maj Kedokt Gigi Indones. 2017;3(1):1–7.
17. Afiff FE, Amilah S. Efektivitas Ekstrak Daun Mengkudu (*Morinda citrifolia* L.) dan Daun Sirih Merah (*Piper crocatum* Ruiz & Pav) Terhadap Zona Hambat Pertumbuhan *Staphylococcus aureus*. STIGMA J Mat dan Ilmu Pengetah Alam Unipa. 2017;10(01).
  18. Fadlilah M. Benefit of Red Betel (*Piper crocatum* Ruiz & Pav.) as Antibiotics. J Major. 2015;4(3).
  19. Januarti IB, Wijayanti R, Wahyuningsih S, Nisa Z. Potensi Ekstrak Terpurifikasi Daun Sirih Merah (*Piper crocatum* Ruiz & Pav) sebagai Antioksidan dan Antibakteri. J Pharm Sci. 2019;2:61.
  20. Wati YS, Zukhra RM, Permanasari I. Konsumsi Rebusan Daun Sirih Merah Efektif Terhadap Perubahan Kadar Gula Darah Penderita Diabetes Mellitus. Al-Insyirah Midwifery J Ilmu Kebidanan (Journal Midwifery Sci. 2020;9(2):91–9.