



Original Article

## Formulation and evaluation of antibacterial activity of hand sanitizers containing red betel (*Piper crocatum*) and white galangal (*Alpinia galanga*) extract

Faridah BD <sup>1✉</sup>, Lita Angelina Saputri <sup>1</sup>, Iin Prima Fitriah <sup>1</sup>, Lisa Rahmawati <sup>1</sup>, Nike Sari Oktavia <sup>1</sup>, Fithriani Amin <sup>2</sup>

<sup>1</sup> Diploma Program of Midwifery, Department of Midwifery, Padang Health Polytechnic, Padang, West Sumatera, Indonesia

<sup>2</sup> Bachelor of Pharmacy, Department of Pharmacy, Andalas University, Padang, West Sumatera, Indonesia

### ARTICLE INFORMATION

Received: February 00, 00

Revised: March 00, 00

Accepted: April 00, 00

### KEYWORDS

Hand Sanitizers; *Alpinia galanga*; *Piper crocatum*

### CORRESPONDENCE

Phone: +6281270620611

E-mail: faridahbd140@gmail.com

### A B S T R A C T

**Background:** The COVID-19 outbreak has changed how people live worldwide, making hand hygiene a must, whether it involves hand sanitizer (HS). The majority of HS are made of alcohol. Several side effects of alcohol are dryness and allergies. Alternative natural ingredients and HS are needed. Red betel (*Piper crocatum*) and white galangal (*Alpinia galanga*) are familiar and easy to be found. These two plants have the potential to be the composition of HS because of their compounds, such as antioxidants, antibiotics, antifungals, and antivirals. However, there is a need for further research on their formulation and effectiveness in germ-killing.

**Purpose:** To formulate and identify the antibacterial activity of HS containing red betel and white galangal extract.

**Method:** This research was an in vitro laboratory experiment with several steps, including extraction, specific and nonspecific tests, formulation, and antibacterial activity tests.

**Results:** Red betel and white galangal leaves had been extracted at a concentration of 10% and formulated into HS. The HS containing 10% red betel extract produced an inhibition zone of 0 mm against *E. coli* and 1.05 mm against *S. aureus*, and the HS containing 10% White galangal extract produced an inhibition zone of 1.47 mm against *E. coli* and 1.40 mm against *S. aureus*.

**Conclusion:** The formulation of HS containing 10% of white galangal extract is more effective than the HS containing 10% of Red betel extract.

### INTRODUCTION

The COVID-19 outbreak hit the world, including Indonesia. This condition has changed the way of living, resulting in a need for hand hygiene. Millions of people in Indonesia need access to public hand sanitary facilities. Hand sanitizers (HS) have emerged as an alternative to soap and water. <sup>1-5</sup> Most HS available in the market are alcohol-based, and frequent use could lead to dryness of the hands, allergies, and dermatitis. <sup>6</sup> There can also be skin toxicity if a concentration of alcohol is too high. The research found that participants experienced hand crack (36.47%), redness (24.71%), and skin disease (8.40%) while using alcohol-based HS. <sup>7</sup> Given these concerns, it is

crucial to make an alternative HS made from natural ingredients, which is considered safer, consists of no dangerous compounds, and is less toxic.

Various studies indicate that certain plants contain antioxidants, antibiotics, antifungals, and antivirals. Red betel (*Piper crocatum*) is familiar and widely used in Indonesia as ornament plant, vegetables, medicine or material in ceremonial events. This plant contains antiseptic properties twice as potent as those found in green betel leaves. The composition of red betel extract is identified to have antibacterial activity. <sup>8</sup> A study found that red betel serves as a disinfectant and antifungal agent, making it effective in

<https://doi.org/10.30595/medisains.v22i1.21357>

©(2024) by the Medisains Journal. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. More information is available at [Attribution-NonCommercial 4.0 International](https://creativecommons.org/licenses/by-nc/4.0/).

treating conditions such as bad breath and vaginal discharge.<sup>9</sup> Research has found that red betel contains chemical compounds such as flavonoid, alkaloid, polyphenols, tannin, and essential oil with high potential as an antibacterial agent.<sup>10</sup> Another research found that ethanol extract of red betel has an antibacterial effect toward gram-positive and gram-negative bacteria.<sup>11</sup>

Meanwhile, white galangal (*Alpinia galanga*) is familiar in Indonesia, widely known as a cooking spice in West Sumatera, and traditionally used to cure gastrointestinal and skin infections.<sup>12</sup> White galangal contains chemical compounds such as flavonoid, quinone, essential oil and phenol.<sup>13</sup> A study found that white galangal extract can inhibit microbial growth such as *E. coli*, *S. enteritidis*, and *S. aureus*.<sup>14</sup> Red betel and White galangal have the potential to be the natural active ingredients in HS because of their capability to kill bacteria as proven by many previous types of research. However, only a few researchers have identified their antibacterial activity as a HS formula.

A previous study formulated HS from red betel and aloe vera, which resulted in a HS with no allergic reaction. However, there was no result regarding its antibacterial activity.<sup>15</sup> Another research found that hand antiseptic gel containing white galangal effectively reduces the hand colony. However, no data were found about its antibacterial activity that proved with inhibition zone to specific germs.<sup>16</sup> Considering these facts, it is necessary to formulate HS containing red betel and white galangal and investigate their antibacterial activity to compare their effectiveness in killing specific germs. This study aims to formulate and identify the antibacterial activity of HS containing red betel and white galangal extract.

## METHOD

### Study Design

This research was an in vitro experiment with a post-test-only control group design.

### Study Site

This research was conducted from June until November 2022 at the pharmacy laboratory of Baiturrahmah University Padang West Sumatera.

### Materials

The materials used were red betel and white galangal leaves, ethanol 70%, aquadest, NaCl 0.9%, nutrient agar, DMSO 50 ml, glycerin, glycol propylene, carbophol, methylparaben, nipagin and triethanolamine (TEA).<sup>17</sup>

### Red Betel and White Galangal Extraction

The amount of 20 kg of red betel leaves is dried using a cabinet dryer at 50°C for three days, and 20 kg of white galangal is cut into smaller pieces before drying using a

50°C cabinet dryer. After drying, the two ingredients are processed into simplicia by grinding them using a chopper, resulting in approximately 1000 gr of each.<sup>15</sup> About 1 kg of red betel simplicia is macerated with Et-OH (1:10) for 24 hours (during the first 6 hours while stirring occasionally, then let stand for 18 hours). After that, filter it to obtain macerate 1. Then proceed with the second maceration with Et-OH (1:5) for 24 hours (during the first 6 hours, the chili is stirred occasionally, then let stand for 18 hours). After that, filter it to obtain macerate 2. Combine macerate 1 and 2 and evaporate the solvent in-vacuo to obtain a thick extract.

### Specific and Nonspecific Test of The Extracts

A specific test is carried out by quantifying total flavonoid levels. Simplicia powder is added with ethanol and extracted for 1 hour with magnetic stirring. Filter the mixture into a 25-mL volumetric flask, adjusting the volume with ethanol P through the filter.<sup>18</sup> Accurately weigh about 0.25 g of the extract, transfer it to an Erlenmeyer flask, add 50 mL of ethanol P, and stir for 30 minutes using a magnetic stirrer. Filter the solution into a 50-mL graduated flask, adjusting the volume with ethanol P. Preparation of the reference solution: Weigh approximately 10 mg of quercetin reference, transfer it to a 25-mL volumetric flask, dissolve it, and add ethanol P to reach the desired volume. If necessary, perform quantitative dilution in 20, 40, 60, 80, and 100 µg/mL increments. Measurement process: Pipette 0.5 mL of the Test solution and the standard Comparison solution separately, add 1.5 mL of ethanol P to each, along with 0.1 mL of aluminum chloride at 10%, 0.1 mL of 1 M sodium acetate, and 2.8 mL of distilled water. Shake the solutions and let them stand for 30 minutes at room temperature. Measure the absorbance at a wavelength of 438 nm. Similarly, blank measurements should be conducted, excluding adding aluminum chloride. Construct a calibration curve and calculate the concentration of the test solution.

Nonspecific tests include moisture content tests and ash content tests. A moisture content test is carried out by measuring 1-2 grams of the sample and placing it in a bottle with a known weight. Subject the sample to drying in an oven at 105°C for 3 hours. Allow the sample to cool in a desiccator. Weigh the sample and repeat the process until a consistent weight is achieved. Ash content test is carried out by weighing 2 grams of the test material, which has been refined, and putting it in a silicate crucible that has been heated and tarred, heated slowly until the charcoal runs out, cooled and weighed, heated to a constant weight. The total ash content is calculated by weight of the test material, expressed in % b/b.<sup>19</sup>

### Hand Sanitizer Formulation

HS formulation is conducted by adding carbomer, glycolpropylene, glycerin, nipagin, and TEA.<sup>17</sup> At this stage,

making the right formula for HS uses 10 % of red betel extract and 10 % of white galangal extract.

### ***In Vitro Procedure***

#### ***Sterilization of Tools and Materials***

The tools used are first washed and dried. Petri dishes, test tubes, Erlenmeyer, measuring cups, vials, disc paper, and pipettes were closed with cotton wrapped in gauze; after being encased in parchment, the items were subjected to sterilization in an autoclave at 121°C and 15 lbs pressure for 5 minutes. Tweezers and needle loops undergo sterilization through flaming using a spirit lamp. The laminar airflow cabinet is cleared of dust and sterilized by activating the UV lamp for 5 minutes. Similarly, the aseptic cabinet is cleaned and then treated with 70% ethanol, allowing it to sit for 15 minutes.<sup>20</sup> The media were placed in an Erlenmeyer flask, covered with gauze-wrapped cotton, further enclosed in parchment paper, and subsequently subjected to sterilization in an autoclave at 121°C and 15 lbs pressure for 15 minutes.

#### ***Production of Seed Media Nutrient Agar***

Twenty grams of nutrient agar powder is dissolved in 1 liter of distilled water in an Erlenmeyer, then heated while stirring until a clear solution is formed. Then Erlenmeyer's mouth was gagged with cotton wrapped in gauze. Then sterilized in an autoclave at 121°C and 15 lbs pressure for 15 minutes.<sup>21</sup>

#### ***Microbial Rejuvenation Test***

The microbes from pure culture stock were planted on NA slanted agar media by scraping one of the microbial culture on the surface of the slanted agar, then incubated for 18-24 hours at 37°C. Rejuvenation is carried out every two weeks. The bacteria used in this study were *Escherichia coli* and *Staphylococcus aureus*.<sup>22</sup>

#### ***Preparation of Suspension Culture Stock and Preparation of Test Bacterial Suspensions***

Bacterial colonies were taken from pure culture and then suspended in 10 ml of 0.9% NaCl. The transmittance of this suspension was measured with a UV-Vis Spectrophotometer at a wavelength of 580 nm at 25%. Transmittance was adjusted by adding bacteria or physiological NaCl.

#### ***Experimental Procedure***

15 mL of sterile NA was poured into a petri dish and allowed to solidify. Next, the bacterial suspension was inoculated using the sterile cotton swap method and then left for 5 minutes. A sterile disc was placed on the surface of the test microbial inoculum, which was dripped with 10  $\mu$ L of the test solution and then incubated for 24 hours. After that, measure the diameter of the inhibition formed (indicated by the clear area around the disc). DMSO was used as a negative control, and 3 mg/mL chloramphenicol was used as a positive control.<sup>23</sup> The antimicrobial activity test

in this research uses a well-diffusion method. This method allows the samples to be absorbed by the bacteria and left for 18-24 hours in a 37°C incubator. The measurement of the transparent/inhibition zone reflects the activity of the bacteria.<sup>24</sup>

#### ***Statistical Analysis***

All experiments were performed in triplicate, and results were expressed as mean $\pm$ SD. Mean and standard deviation were calculated in Microsoft Excel.

#### ***Ethical Consideration***

This research has been approved by the research ethics committee of Perintis University, certificate number 185/KEPK.F2/EIK/2022.

## **RESULTS**

### ***Results of Extraction***

Figure 1a and Figure 1b show the result of the extraction of red betel and white galangal. Both of these extracts resulted in a thick and dark-colored appearance. The red betel extract is blackish red-colored, and the white galangal extract is blackish green-colored.



**Figure 1a.** Red betel extract

**Figure 1b.** White galangal extract

### ***Specific and Nonspecific Test***

Table 1 shows that the white galangal extract's level of flavonoid is higher than the flavonoid level of the red betel extract. Red betel extract's moisture content level is higher than white galangal extract's. Besides that, the ash content level of red betel extract is higher than that of white galangal extract. The higher the ash content, the higher the mineral content (Table 2).

### ***Hand Sanitizer Formulation***

Figure 2a shows the appearance of HS containing 10% red betel extract, and Figure 2b shows the appearance of HS containing 10% white galangal extract. As seen in the pictures, both HS have a cloudy appearance. This is caused by the color of the extract used from the red betel

and white galangal leaves that have a dark color, as seen in Figures 1a and 1b. The HS color remains dark after mixing the extracts and materials to make the formulas.



**Figure 2a.** HS Containing Red Betel 10% **Figure 2b.** HS Containing White Galangal 10%

**Antibacterial Activity Test**

The antimicrobial activities are identified by measuring the inhibition diameter against *E. coli* and *S. aureus*. Figure 3a shows no inhibition zone formed by adding 10% red betel HS against *E. coli*, which means no antibacterial activity is observed. Meanwhile, positive control forms an inhibition zone, and no inhibition zone is formed by negative control. Figure 3b, it can be seen that an inhibition zone formed in 10% red betel HS, which implies that there is antibacterial activity. Chloramphenicol also forms an inhibition zone, while DMSO shows no antibacterial activity, proving that no inhibition zone has been formed.



**Figure 3a.** Antimicrobial activity test of HS containing 10% Red betel against *E. coli* **Figure 3b.** Antimicrobial activity test of HS containing 10% Red betel against *S. aureus*

Figures 4a and 4b show that HS containing 10% white galangal forms an inhibition zone against *E. coli* and *S. aureus*. This implies that 10% of white galangal extract contained in HS has antibacterial activity. Positive control also forms an inhibition zone, while negative control does not show antibacterial activity.



**Figure 4a.** Antimicrobial activity test of HS containing 10% white galangal against *E. coli* **Figure 4b.** Antimicrobial activity test of HS containing 10% White galangal against *S. aureus*

Table 3 shows that HS with 10% red betel extract effectively inhibits *S. aureus* growth. The observed inhibition zone is about 1.08 mm, but no antibacterial activity is observed against *E. coli*, and no inhibition zone is observed. Unlike the Red betel, the HS with 10% White galangal extract is effective against both *S. aureus* and *E. coli*. The inhibition zones resulted in 1.40 mm and 1.47 cm, while the positive control used in this research resulting an inhibition zone of 1.98 mm for *E. coli* and 2.00 mm for *S. aureus*.

**DISCUSSION**

This study found that HS containing 10% white galangal extract effectively inhibits the growth of *E. coli* and *S. aureus* bacteria with inhibition diameters of 1.43 mm and 1.40 mm, respectively. The results of the study were in line with the previous research, which stated that white galangal extract was effective for inhibiting bacterial growth and that the administration of 15% *Alpinia galanga* extract to *E. coli* bacteria could produce an inhibition of 0.46 mm.<sup>25</sup> Several studies also have shown the activity of white galangal extract as an antibacterial. White galangal causes damage to the inner and outer membranes and causes coagulation of the cytoplasmic. A study found that the essential oil contained in white galangal can inhibit the growth of microbes in food caused by *E. coli*, *S. aureus*, and *S. typhi*.<sup>26</sup> Another research found that hand antiseptic gel containing white galangal extract effectively kills hand germs.<sup>16</sup>

This study also found that HS containing 10% Red betel is effective against *S. aureus* with a 1.08 mm inhibition zone but resulted in no antibacterial activity against *E. coli*. A Previous study found that using red betel extract and vancomycin therapy could have a four times greater inhibitory effect on the growth of *S. aureus*.<sup>25</sup> Another study found that the use of irrigation fluid containing 20% red betel extract can effectively reduce the number of bacteria in white

**Table 1.** Flavanoid Level

Sample	Absorbancy			Mean±SD	Flavanoid level (mgQE/g)
	I	II	III		
Red betel extract	0.558	0.569	0.576	0.567±0.11	7.86
White galangal extract	0.030	0.034	0.033	0.032±0.004	11.21

**Table 2.** Moisture and Ash Content

Sample	Moisture Content Test (Level %)			Mean±SD	Ash Content Test (Level %)			Mean±SD
	I	II	III		I	II	III	
	Red betel simplicia	9.443	9.269		9.305	9.339±0.00091	3.357	
Red betel extract	19.203	24.213	12.150	20.852±0.0291	4.190	3.347	2.721	3.420±0.0073
White galangal simplicia	15.899	12.150	12.332	12.226±0.00094	2.680	2.330	2.330	2.447±0.0020
White galangal extract	15.169	16.380	16.147	15.899±0.00642	2.004	3.821	4.085	3.304±0.0113

**Table 3.** Antimicrobial Activity

Sample	Average Inhibition Zone ± SD (mm)			
	HS Red Betel 10%	HS White Galangal 10%	Control + (Chloramphenicol 0.3%)	Control – (DMSO)
	E. coli.	0	1.47±0.06	1.98 ±0.02
S. aureus	1.08±0.04	1.40±0.10	2.00±0.0	0

rat diabetic ulcer isolates.<sup>27</sup> A study found that red betel has an antibacterial effect on gram-positive and gram-negative bacteria such as *S. aureus*, *S. pyogenes*, and *S. typhi*.<sup>8,28</sup>

Red betel can inhibit bacterial growth by inhibiting nucleic acid synthesis, changing cytoplasmic membrane function, inhibiting energy metabolism, reducing cell attachment and biofilm formation, inhibiting porin in cell membranes, and disrupting permeability of cell walls and membranes to cause bacterial cell lysis. However, another study found the opposite, as red betel extract is ineffective against *S. aureus* and *E. coli*. Similar to this study, another study found that red betel extract is ineffective for *E. coli*. It might be caused by different specific defense mechanisms possessed by bacteria to survive in any pressured condition.<sup>24</sup> Previous studies found that there are differences in the characteristics of the cell wall between gram-positive and gram-negative bacteria. The structure of the cell wall of gram-positive bacteria is simpler, with only a peptidoglycan layer. In contrast, the cell wall of gram-negative bacteria is more complex as it consists of 3 layers: lipopolysaccharide, protein, and peptidoglycan, which can inhibit the infiltration of antibacterial agents into the cell.<sup>22,24</sup>

According to this research, 10% white galangal extract has more potential as a HS natural ingredient than 10% red betel extract. This result can be caused by the higher level of white galangal flavonoid than red betel's flavonoid level. This study finds that the flavonoid level in white galangal is 11.21 mmQE/g, while 7.86 mmQE/g for the red betel. Similar results were found from previous studies, as the flavonoid level of red betel ethanol extract is 4,07mmQE/, lower than the level of flavonoid in white galangal (14.12 mmQE/g).<sup>29,30</sup> Flavanoid is a phytochemical compound

that can suppress nuclei acid synthesis, cytoplasmic membrane function, and energy metabolism. Flavonoids have also been found to reduce adhesion and biofilm formation, porin on the cell membrane, membrane permeability, and pathogenicity, so it is a natural compound with high potency as an antibacterial agent.<sup>31</sup>

## CONCLUSIONS AND RECOMMENDATION

HS containing 10% white galangal is more effective than HS containing 10% red betel against *S. aureus* and *E. coli*. Researching further to produce HS with 10% white galangal with a more attractive appearance (color and smell) is recommended.

## REFERENCES

1. Kementerian Kesehatan RI. Infeksi Emerging. Penyakit Infeksi Emerging. Published 2022.
2. Haleem A, Javaid M, Vaishya R. Effects of COVID-19 pandemic in daily life. *Curr Med Res Pract.* 2020;10(2):78-79. doi:10.1016/j.cmrp.2020.03.011.
3. Suryani D, Nuraeni T, Buston E, et al. Hand Hygiene Effectiveness in Controlling the Spread of COVID-19: A Scoping Review. *J Penelit Pendidik IPA.* 2023;9(10):800-809. doi:10.29303/jppipa.v9i10.4686
4. Karana KP. Untuk kalahkan COVID-19, kebersihan tangan harus menjadi bagian kehidupan setiap penduduk Indonesia. UNICEF Indonesia. 2020.
5. Prajapati P, Desai H, Chandarana H. Hand sanitizers as a preventive measure in COVID-19 pandemic, its characteristics, and harmful effects: a review. *J Egypt Public Heal Assoc.* 2022;97(6). 206-209. doi: 10.1186/s42506-021-00094-x.



6. Dey D. Effects of Alcohol Based Hand Sanitizers on Human Life and Environment. In: *International Webinar on "Geography, Environment and Sustainable Development"*. 2021. doi: 10.13140/RG.2.2.13676.90248
7. Alhalwani A, Husain A, Saemaldahar A, et al. The impact of alcohol hand sanitizer use on skin health between healthcare worker: Cross-sectional study. *Ski Res Technol.* 2024;30(1):1-7. doi:10.1111/srt.13527
8. Hartini YS, Nugroho LH. Short communication: Antibacterial effect of red betel (*Piper crocatum*) extract in combination with vancomycin against *Staphylococcus aureus*. *Biodiversitas.* 2020;21(7):3271-3274. doi: 10.13057/biodiv/d210750
9. Damarini S, Eliana, Mariati. The Effectiveness of Red Betel in Healing Perineal Wound in Independent. *J Kesehat Masy Nas.* 2013;8(3):39-44. doi:10.21109-kesmas.v8i1.340.
10. Fadlilah M. Benefit of Red Betel (*Piper Crocatum* Ruiz & Pav.) As Antibiotics. *J Major.* 2015;4(3):71-74.
11. Rachmawaty JF, Akhmad MM, Pranacipta SH, et al. Optimasi Ekstrak Etanol Daun Sirih Merah (*Piper Crocatum*) sebagai Antibakteri terhadap Bakteri *Staphylococcus Aureus*. *Mutiara Med J Kedokt dan Kesehat.* 2018;18(1):13-19. doi:10.18196/mm.180109
12. Mursyida E, Alfiola T. Pengaruh Pemberian Ekstrak Lengkuas Putih (*Alpinia galanga*) Terhadap Pertumbuhan *Escherichia coli*. *Klin Sains J Anal Kesehat.* 2020;8(1):8-16. doi:10.36341/klinikal\_sains.v8i1.1237
13. Wu Y, Wang Y, Li ZH, et al. Composition of the essential oil from *alpinia galanga* rhizomes and its bioactivity on *lasioderma serricorne*. *Bull Insectology.* 2014;67(2):247-254.
14. Hiala MA, Aspatria U, Riwu RR. Uji Efektivitas Ekstrak Lengkuas (*Alpinia Galanga*) Sebagai Antibakteri *Escherichia Coli*. *Lontar J Community Heal.* 2019;1(2):48-52. doi:10.35508/ljch.v1i2.2159
15. Yaturramadhan H, Putri CL. Formulation of Natural Handsanitizer From Red Belt (*Piper Crocatum*) Leaves and Aloe Vera (*Aloe Vera*). *J Public Heal Pharm.* 2021;1(2):43-45. doi:10.56338/jphp.v1i2.3718
16. Wijayanto BA, Kurniawan DW, Sobri I. Formulasi dan Efektivitas Gel Antiseptik Tangan Minyak Atsiri Lengkuas (*Alpinia galanga* (L.) Willd.). *J Ilmu Kefarmasian Indones.* 2013;11(2):102-107.
17. Fathoni DS, Fadhillah I, Kaavessina M. Efektivitas Ekstrak Daun Sirih Sebagai Bahan Aktif Antibakteri Dalam Gel Hand Sanitizer Non-Alkohol. *Equilib J Chem Eng.* 2019;3(1):9. doi:10.20961/equilibrium.v3i1.43215
18. Utami YP, Umar AH, Syahrani R, Kadullah I. Standardisasi Simplisia dan Ekstrak Etanol Daun Leilem (*Clerodendrum minahassae* Teijsm. & Binn.). *J Pharm Med Sci.* 2017;2(1):32-39.
19. Depkes RI. *Farmakope Herbal Indonesia Edisi I*. Departemen Kesehatan Republik Indonesia; 2008.
20. Luntungan BM, Wewengkang DS, Rumondor E. Uji aktivitas antibakteri ekstrak dan fraksi spon mycale vansoesti dari perairan pulau mantehage minahasa utara terhadap pertumbuhan bakteri *Escherichia coli* dan *Staphylococcus aureus*. *Pharmac.* 2021;10(2):889. doi:10.35799/pha.10.2021.34040
21. Nofita AD. Uji efektivitas antibakteri ekstrak etanolik bawang merah (*Allium cepa* L.) terhadap bakteri *Staphylococcus aureus* dalam media mueller hinton agar (MHA). *Media Inf.* 2021;16(1):1-7. doi:10.37160/bmi.v16i1.355
22. Puspita, Tri I, Muflihah CH. Uji Aktivitas Antibakteri Ekstrak Dan Fraksi Rimpang Lengkuas Putih (*Alpinia Galanga*) Terhadap Bakteri *Pseudomonas Aeruginosa* Dan *Bacillus Subtilis* Serta Bioautografinya. *J Pharm.* 2023;2(2):144-162.
23. Noor AS, Triatmoko B, Nuri. Uji Aktivitas Antibakteri Ekstrak Metanol dan Fraksi Daun Kenikir (*Cosmos caudatus* Kunth) terhadap *Salmonella typhi*. *Pustaka Kesmas.* 2020;8(3):177-182. <https://doi.org/10.19184/pk.v8i3.13008>
24. Puspita PJ, Safithri M, Sugiharti NP. Antibacterial Activities of Sirih Merah (*Piper crocatum*) Leaf Extracts. *Curr Biochem.* 2019;5(3):1-10. doi:10.29244/cb.5.3.1-10
25. Rini, Rohmah J, Widyaningrum LY. The antibacterial activity test galanga (*Alpinia galangal*) on the growth of bacteria *Bacillus subtilis* and *Escherichia coli*. In: *2nd Nommensen International Conference on Technology and Engineering.* 2018:1-5 doi:10.1088/1757-899X/420/1/012142
26. Silalahi M. Essensial oil pada *Alpinia galanga* (L.) Willd. dan pemanfaatannya. *J Pro-Life.* 2017;4(1):287-296. <https://doi.org/10.33541/jpvol6lss2pp102>
27. Purwaningsih I, Suryani L, Permata Sari YI. The effectiveness of red betel leaf (*Piper crocatum*) cleansing infusa in reducing the number of total bacteria of ulcer diabeticum isolates in alloxan induced white rats. *Proc Int Nurs Conf Chronic Dis Manag Pekalongan.* 2019;(2012):255-262.
28. Manurung MM, Subekti TH, Sawitri NDA, Mahmuda INN. Potential Ekstrak Of Red Betel Leaf (*Piper crocatum* Ruiz & Pav.) as a Natural Hand Sanitizer. *MAGNA MEDICA Berk Ilm Kedokt dan Kesehat.* 2023;10(2):141. doi:10.26714/mag-named.10.2.2023.141-149
29. Najmudin GA, Lukmayani Y, Yuliawati KM. Penetapan Kadar Flavonoid Ekstrak Etanol Daun Sirih Merah (*Piper Ornatum* N.E.Br.). *Bandung Conf Ser*

- Pharm.* 2023:250-257. doi:10.29313/bcsp.v3i2.8614
30. Aljobair MO. Chemical composition, antimicrobial properties, and antioxidant activity of galangal rhizome. *Food Sci Technol.* 2022;42:1-8. doi:10.1590/fst.45622
31. Shamsudin NF, Ahmed QU, Mahmood S, et al. Antibacterial Effects of Flavonoids and Their Structure-Activity Relationship Study: A Comparative Interpretation. *Molecules.* 2022;27(4):1-43. doi:10.3390/molecules27041149