



Research Article



Red onion (*Allium ascalonicum* L.) skin as an antibacterial on the growth of *Staphylococcus aureus*



Reza Anindita ^{1,a,*}, Elva Dwi Pamungkas ^{1,b}, Maulin Inggraini ^{2,c}, Melania Perwitasari ^{1,d}, Maya Uzia Beandrade ^{1,e}, Intan Kurnia Putri ^{1,f}, Dede Dwi Nathalia ^{1,g}, Nofria Rizki Amalia Harahap ^{1,h}, Ariska Deffy Anggarany ^{1,i}

⁸ Department of Pharmacy, STIKES Mitra Keluarga, Bekasi, Indonesia

² Department of Medical Laboratory Technology, STIKES Mitra Keluarga, Bekasi, Indonesia

Email: rezaanindita@gmail.com ^{a,*}, elvadwip@gmail.com ^b, maulin.inggraini@stikesmitrakeluarga.ac.id ^c, melania.perwitasari@stikesmitrakeluarga.ac.id ^d, maya.uzia@stikesmitrakeluarga.ac.id ^e, intan.kurnia.p@stikesmitrakeluarga.ac.id ^f, dededwi.nathalia@stikesmitrakeluarga.ac.id ^g, nofria.rizki@stikesmitrakeluarga.ac.id ^h, ariska.deffy@stikesmitrakeluarga.ac.id ⁱ

* Corresponding author

Article Information	ABSTRACT
Submitted: 2022-12-26 Accepted: 2023-10-14 Published: 2023-10-25	<p>The main bacteria that cause infection in humans is <i>Staphylococcus aureus</i>. Efforts to prevent infectious diseases caused by bacteria can be made by using plant parts that have medicinal potential. One part of the plant that needs to be explored for its potential is the skin of shallot bulbs (<i>Allium ascalonicum</i> L.). This research aimed to determine the effect of red onion skin extract on the growth of <i>S. aureus</i> bacteria. The research design is experimental. The samples in this study were 1 kg of red onion skin and a bacterial sub-culture of <i>S. aureus</i> ATCC: 25923. Extraction was carried out using the maceration method using 96% ethanol solvent and evaporated using a rotary evaporator and water bath. The antibacterial test was carried out by administering 30 µg chloramphenicol (positive control), sterile distilled water (negative control), and red onion skin extract with concentrations of 60%, 65%, 70%, and 75% with 3 repetitions. All treatments were given to <i>S. aureus</i> at 30 µL each using the Kirby-Bauer method. The research instrument was an observation sheet of the diameter of the inhibition zone. Data analysis used One-Way ANOVA. The results of this study show that ethanol extract of red onion skin with concentrations of 60%, 65%, 70%, and 75% was able to produce <i>S. aureus</i> inhibition zone diameters of 3.5 mm, 4 mm, 4.2 mm, and 4.3 mm. The results of the One-Way ANOVA test showed a significance value of 0.15 (sig>0.05), not proven to be able to significantly inhibit the growth of <i>S. aureus</i>. The conclusion shows that red onion skin extract as an antibacterial is not able to significantly inhibit the growth of <i>S. aureus</i>.</p>
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INTRODUCTION

Pathogenic bacterial infection is the second highest cause of death in the world after ischemic heart disease (Negara, 2014). The data for 2019 reported that the number of deaths due to pathogenic bacteria was 7.7 (13.6%) of the total deaths of the world's population (Ikuta et al., 2022). The results of the study reported that as many as 5 out of 33 pathogenic bacteria that were the cause of 54.9% of the deaths of the world's population, including *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Murray et al. (2022) explain *S. aureus* is the dominant bacteria that causes the most deaths in 135 countries worldwide.

Results of the literature review by Rungelrath and Deleo (2021) show that *S. aureus* is the dominant cause of infection due to its ability to produce molecules that promote evasion of host defense, including the ability to avoid killing by neutrophils. Ali-Alghamdi et al. (2023) added that this dominance was exacerbated by the ability of *S. aureus* to produce beta-lactamase which destroys beta-lactam antibiotics thereby triggering resistance of *S. aureus* to antibiotics. The research results by Tyasningsih et al. (2022) proved that *S. aureus* isolated from raw milk in Indonesia showed 19.6% resistance to antibiotics. The results of a meta-analysis of 98 studies on *S. aureus* in Nigeria showed the prevalence of *S. aureus* resistance to antibiotics was 13%-98% (Ezeh et al., 2023).

Treatment using antibiotics is still the main choice in treating infectious diseases caused by *S. aureus*. However, the use of antibiotics often causes *S. aureus* to become resistant to various types of antibiotics or Multi-Drug Resistance Antibiotics (MDR) so that strains of *S. aureus* appear that are resistant to methicillin antibiotics or Methicillin Resistance *Staphylococcus aureus* (MRSA) which are difficult to treat (Setiawati, 2015). Considering the use of antibiotics in preventing and treating *S. aureus* bacterial infections needs to be evaluated, so as an alternative effort to prevent the emergence of *S. aureus* bacterial infections, it is necessary to search for plant simplicia that has antibacterial properties.

One of the plants that have been widely used by the community to treat diseases is red onion (*Allium ascalonicum* L.). However, there are still many who have not used red onion skin considering that its efficacy has not been proven empirically or scientifically. Even though the compilation of scientific evidence regarding the results of the phytochemical screening conducted by Elsyana and Tutik (2018), Elsyana et al. (2019), Suryandari and Kusumo (2022), Prabowo and Noer (2020), Rosyada (2022), and Nandasari (2020) found that the ethanol extract of red onion skin positively contained alkaloids, flavonoids, saponins, tannins, phenols, glycosides and steroids or triterpenoids. The results of maceration extraction of shallot skins yielded flavonoid and phenol values of 5.48 and 2.46 ppm. The combination of these metabolites can damage the bacterial cell wall (Adiwibowo et al., 2023). This was proven in research by Anh et al. (2023) who reported that administration of 100% concentration of red onion skin ethanol extract was able to inhibit *S. aureus* with an inhibition zone of 14.75 mm and an MIC value of 224 µg/ml. The antibacterial effect of red onion skin was reported by Octaviani et al. (2019) that the ethanol extract of red onion skin was able to inhibit the growth of the bacteria *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Salmonella thypi*, *Escherichia coli*, and the fungus *Trichophyton mentagrophytes*. Sa'adah et al. (2020) added that the aqueous extract of red onion skin can inhibit the growth of the *Propionibacterium acnes* bacteria in the strong category.

The research gap between this research and previous research is the concentration level of red onion skin extract. In previous research, it was still carried out at low concentrations with intervals of 1.5625% to 40%. Therefore, researchers tried to continue previous research by using concentrations of 60%, 65%, 70%, and 75%. The aim of this research was to determine the effect of shallot skin extract on the growth

of *Staphylococcus aureus*. It is hoped that the results of this research will provide information regarding the efficacy of red onion skin extract on the growth of *S. aureus*.

RESEARCH METHODS

This research is a type of quantitative research. The research design was laboratory experimental with treatment with red onion skin ethanol extract concentrations of 60%, 65%, 70%, and 75%. The positive control in the study was chloramphenicol, and the negative control in the study was sterile distilled water. The samples in this study were 1 kg of red onion skin and a bacterial sub-culture of *S. aureus* ATCC: 25923 (Figure 1). The tools used are autoclaves, Petri dishes, test tubes, micropipettes, incubators, Laminar Air Flow (LAF), rotary evaporators, digital analytical scales, and vortexes. The materials used were red onion skin, pure isolate *Staphylococcus aureus* ATCC: 25923 purchased from the microbiology laboratory at the University of Indonesia, Media Nutrient Agar (NA), Mueller Hinton Agar (MHA), Ethanol 96%, and Aquades.

The research sample was 1 kg of red onion skin purchased from the Bekasi market. Determination of red onion plants was carried out at the Indonesian Institute of Sciences (LIPI), Bogor, West Java. The sample preparation stage includes wet sorting, drying in the sun for 5 days, dry sorting, and pollination using a blender. The extraction stage was carried out by the maceration method by weighing 100 grams of simplicia powder and then soaking it in a closed Erlenmeyer containing 500 ml of 96% ethanol for 5 days while stirring occasionally. After 5 days, the sample was filtered with filter paper to produce filtrate 1 and residue 1. Residue 1 was added with 250 ml of 96% ethanol, then covered with aluminum foil and waited for 2 days while stirring occasionally. After 2 days, the samples were filtered using filter paper to produce filtrate 2 and residue 2. Filtrate 1 and filtrate 2 were mixed and then evaporated using a rotary evaporator (temperature 40 °C, pressure 200 bar, and speed 60 rpm) for 3 days and a water bath until a thick extract was obtained. free from ethanol solvent. The concentrated extract was then weighed and stored in a closed glass container before being used for antibacterial testing (Fauziah & Isnawati, 2023).

Making a test bacterial suspension involves inserting several rounds of pure bacterial subculture into a 0.9% NaCl solution and vortexing it until it is homogeneous. The results will be compared with McFarland 0.5 solution (equivalent to a bacterial suspension of 1.5×10^8 CFU/ml). If the comparison results show that the bacterial suspension is still too clear, several rounds of bacterial testing are needed, and if it is too cloudy, then you need to add 0.9% NaCl to obtain a 0.5 McFarland standard suspension solution. The antibacterial test was carried out using the Kirby-Bauer method with 4 quadrants of MHA media, sterile cotton and left for ± 5 minutes (Nassar et al., 2019). Next, prepare a blank disk with extract concentrations of 60%, 65%, 70%, and 75% in the amount of 30 μ l and leave for ± 15 minutes (Figure 2).

All discs were placed in a petri dish using sterile tweezers. Each petri dish required 3 repetitions of this treatment. Next, all Petri dishes were incubated for 24 hours at 37°C. After 1x24 hours, the diameter of the inhibition zone in the form of a clear zone was measured using a ruler. The measurement results will be compared with the guidelines of Clinical Laboratory and Standard Institute (2020) to see the sensitivity category of the tested bacteria in response to each treatment disc. The instrument used in this research was an observation sheet for the diameter of the *S. aureus* growth inhibition zone. The research data is the average value of the diameter of the *S. aureus* growth inhibition zone. Research data analysis was carried out using a one-way ANOVA test.



Figure 1. Pure Isolate of *S. aureus* ATCC: 25923 (left), and Turbidity Level of *S. aureus* Subculture in 0.9% NaCl Compared to Mcfarland Standard 0.5 (right)

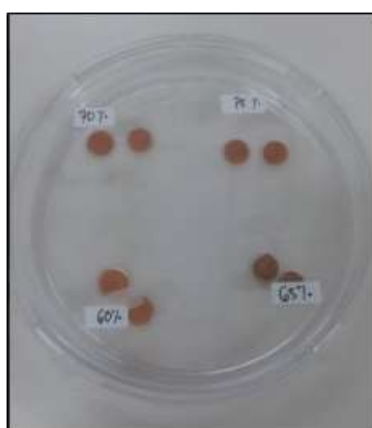


Figure 2. Blank Discs Dripped with Concentration Solutions of 60%, 65%, 70%, and 75%

FINDING AND DISCUSSION

The results of the determination test at the Indonesian Institute of Sciences, Bogor, showed that the red onion bulbs to be peeled belonged to the *Amaryllidaceae* family with the latin name *Allium ascalonicum*. The extraction of 100 grams of leek skin powder with 96% ethanol solvent using the maceration method yielded a viscous extract weight of 9.13 grams. The percentage of viscous extract yield in this study was 9.13%. The results of the liquid extract from the maceration method and the viscous extract in which the solvent has been evaporated using a rotary evaporator and a water bath can be shown in Figure 3. The Figure 3 shows that the liquid and viscous red onion skin extract has a blackish red color with a characteristic red onion odor. The results of the liquid and viscous extracts obtained in the study are in accordance with the organoleptic observations of red onion skin extract by Badriyah and Fahriah (2022) which showed that the thick extract of shallot skin has the form of a viscous liquid, red-black in color, and has a characteristic shallot odor.

The yield value of red onion peel ethanol extract complies with the Indonesian Herbal Pharmacopoeia, which is not less than 7.2%. The results of this study complement the results of the study of Badriyah and Farihah (2022) who reported that samples of red onion skin extracted by maceration using 1000 ml of 96% ethanol and water were able to produce a yield percentage of viscous extract of 10.66% and 10.29%. The use of an ethanol-water mixture will produce a yield percentage of 13.27%. Other results were shown in the research of Pranata et al. (2021) which reported that the extraction of 300 grams of red onion skin using the percolation method with ethyl acetate and N-heksane solvents

respectively resulted in a percent yield of 7.84% and 6.50%. Suryandari and Kusumo (2022) added that maceration extraction of 50 grams of red onion skin powder in 50 ml of solvent n-hexane, chloroform, ethyl acetate, acetone, 96% ethanol resulted in a yield percentage sequentially of 0.96%, 1.62%, 2.66%, 3.90%, and 4.80%.



Figure 3. Liquid Extract (left), and Viscous Extract (right)

The suitability of the percentage of yield that complies with the herbal pharmacopoeia reference is due to the polar nature of 96% ethanol which is capable of attracting the polar properties of the secondary metabolites contained in red onion skins such as alkaloids, flavonoids, tannins and terpenoids. The polar nature of ethanol can dissolve phytochemical compounds with high, medium and low polarity. The polarity similarity causes ethanol to easily enter the simplicia cell membrane and attract the secondary metabolites in it, thereby affecting the yield value obtained during the maceration extraction process. The viscous extract of red onion skin obtained by maceration method was then used to test the antibacterial ability of *S. aureus* bacteria. The results of the viscous extract test of red onion skin on the growth of *S. aureus* are presented in Table 1.

Table 1. The Diameter of the *S. aureus* Inhibition Zone

Treatment	Inhibition Zone Diameter (mm)			Mean	Category
	Repetition 1	Repetition 2	Repetition 3		
Chloramphenicol	18.5	24	22	21.5	Sensitive
Sterile Distilled Water	-	-	-	-	Resistant
60%	3	3.5	4	3.5	Resistant
65%	3.5	4	4.5	4	Resistant
70%	4	4.5	4	4.2	Resistant
75%	4	4.5	4.5	4.3	Resistant

Table 1 shows that the average diameter of the inhibition zone for the growth of *S. aureus* is 3.5 mm, 4 mm, 4.2 mm, and 4.3 mm with concentrations the ethanol extract on red onion skin of 60%, 65%, 70%, and 75%. The diameter of the inhibition zone around the treatment disc was visible due to the influence of the ethanol extract on red onion skin. The zone of inhibition is the main benchmark for the effect of ethanol extract of shallot peel on the growth of *S. aureus*. Based on these results, red onion skin extract can inhibit the growth of *S. aureus* as indicated by a sensitive response (resistant category). A one-way ANOVA test was carried out to test the effect of the concentration of red onion skin ethanol extract on the diameter of the growth inhibition zone of *S. aureus* (Table 2).

Table 2. The Results of The One-Way ANOVA Test

The One-Way ANOVA		Result
N		12
Mean		4
Standard deviation		0.476
Significance		0.150

Table 2 shows a significance value (sig) of 0.15 (sig>0.05). Based on these values, it can be seen that there is a clear zone in the growth of *S. aureus*. However, the effect of ethanol extract on red onion skin was not able to significantly inhibit the growth of *S. aureus* (Figure 4). The clear zone in the diameter of the inhibition zone is evidence of inhibition of *S. aureus* growth from the ethanol extract of red onion skin. The higher the diameter of the inhibition zone, the more effective the extract is in inhibiting the growth of *S. aureus*. The ability of ethanol extract from red onion skin (Figure 4) is also shown in the results of Misna and Diana (2016) showing that administration of red onion skin ethanol extract with concentrations of 5%, 10%, 20%, 40%, 60%, 80% can produce diameter inhibition zones. Other results were shown by Octaviani et al. (2019) proved that ethanol extract of red onion skin with concentrations of 1.562%, 3.125%, 6.25%, 12.5%, 25%, and 50% produced an inhibitory zone diameter for the growth of *S. aureus*.

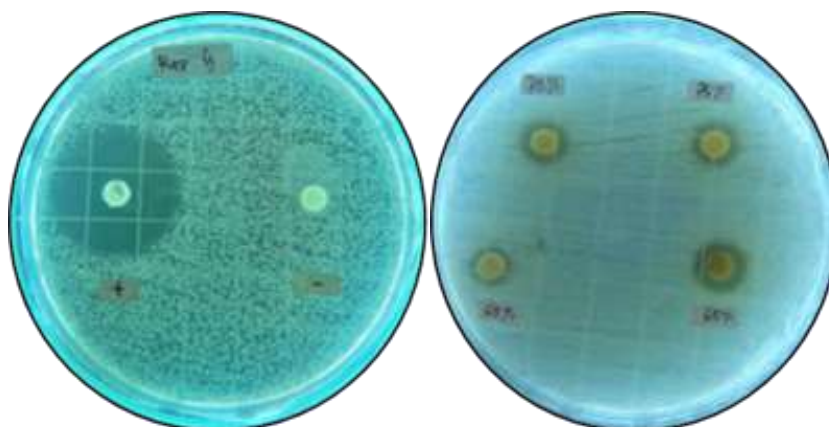


Figure 4. The diameter of the growth inhibition zone of *S. aureus* with positive control, negative control, and shallot peel ethanol extract concentrations of 60%, 65%, 70%, and 75%

Comparison of the effect of shallot skin extract on other bacteria, it was shown in the study of Wulaisfan et al. (2018) which gave treatment of 96% ethanol extract of red onion peel 10%, 20%, 30% to *Streptococcus mutans* bacteria capable of causing an inhibition zone diameter of 1.33 mm, 2.12 mm, and 2.51 mm. Another study by Sa'adah et al. (2020) reported that red onion water extract of 5%, 10%, 20%, and 40% was proven to provide a diameter of inhibition zone for *Propionibacterium acnes* bacteria of 12.8 mm, 13 mm, 14.33 mm, and 15.50 mm.

The effect of red onion skin as an antibacterial in various studies was explained by Badriyah and Farihab (2022) who reported that extraction of red onion skin using 96% ethanol solvent was able to extract secondary metabolites such as alkaloids, flavonoids, phenols, saponins, and tannins. The existence of these secondary metabolites causes red onion skin to have efficacy in inhibiting the growth of pathogenic microorganisms. Roza et al. (2017) explained that the working principle of phenol and tannins as antibacterial agents is to prevent the formation of cell walls, damaging cell walls, preventing protein synthesis, interfering with the permeability function of cell membranes and active transport so that *S. aureus* bacterial cells become lysed (ruptured).

Flavonoids, alkaloids, and terpenoids were able to inhibit the growth of *S. aureus* by preventing nucleic acid synthesis, energy formation, inhibiting the formation of FabZ and fimbriae enzymes (Khusnia, 2020); Pudiarifanti & Farizal (2022). The presence of flavonoids in the ethanol extract of red onion skin was proven in the study of Setiani et al. (2017) who reported that the levels of flavonoids extracted by the maceration method were 14.92%, while the MAE (Microwave Assisted Extraction) method was 17.18%. The saponins work by destroying the stability of the *S. aureus* cell membrane (Kabrah et al., 2016). According to Khashan (2014), it is necessary to screen allicin and allin components contained in red onion skins, bearing in mind the presence of allicin and allin contained in red onion bulbs has proven to be effective in inhibiting the growth of *S. aureus*. The results of different inhibition zones between studies were caused by various factors, namely the amount of composition of phytochemical compounds, extraction methods, environmental factors, genetic differences in the betel nut used, and the type of solvent.

The average diameter of the growth inhibition zone of *S. aureus* after administration of 60%, 65%, 70%, and 75% viscous red onion skin extract ranged from 3.5 mm - 4.3 mm with the resistant category. In this study the category of sensitivity response of *S. aureus* to ethanol extract of red onion skin refers to the standard of Clinical Laboratory and Standard Institute (2020) regarding the effect of the antibiotic chloramphenicol as a positive control on the sensitivity of *S. aureus* bacteria, that is, if the diameter of the inhibition zone ≥ 18 mm is classified as sensitive, 13 - 17 mm is in the intermediate category, and ≤ 12 mm is in the resistant category. The sensitive response category indicated that the red onion skin extract inhibited the growth of *S. aureus*, the intermediate category had moderate effectiveness while the resistant category indicated that the red onion skin viscous extract was not effective in inhibiting the growth of *S. aureus*. According to Mulyani et al. (2020), the antibacterial ability of the concentration of plant secondary metabolites, it is influenced by several factors, including growing location, type solvent, extraction method, and type of bacteria used. However, in this study, it was suspected that the low concentration of secondary metabolite compounds contained in shallot skin was unable to significantly inhibit the growth of *S. aureus*.

In this study the positive control used the antibiotic chloramphenicol 30 μ g which was able to inhibit the growth of *S. aureus* with an inhibition zone diameter of 21.5 mm with the sensitive category. Chloramphenicol is the antibiotic chosen as the gold standard for the treatment of typhoid fever. The mechanism of action of chloramphenicol is to inhibit the peptidyl transferase enzyme which plays a role in the formation of peptide bonds in the process of protein synthesis in *S. aureus* bacteria. Peptide bond formation will continue to be inhibited as long as chloramphenicol remains bound to the ribosome of *S. aureus* bacteria (Jamilah, 2015). The advantages of this study are the use of concentrations of 60%, 65%, 70%, and 75% which complement the concentration data from previous studies, and research outputs that can provide information about the efficacy of red onion skin as an antibacterial so that it can be used as a candidate for natural ingredients in the manufacture of pharmaceutical products. However, the limitations include the Kirby Baur method for antibacterial testing for clinicians, allicin and allin have not been identified both qualitatively and quantitatively and phytochemical screening for secondary metabolites qualitatively and quantitatively, as well as the examination of damage to the structure of the *S. aureus* bacteria has not been carried out microscopically due to extract treatment.

CONCLUSION

Treatment with ethanol extract of red onion skin at concentrations of 60%, 65%, 70%, and 75% can inhibit the growth of *S. aureus* with inhibitory zone diameters of 3.5 mm, 4 mm, 4.2 mm, and 4.3 mm (resistant category), but does not inhibit significantly. Based on the research results, the treatment of

ethanol extract from red onion skin in the Bekasi market could not significantly inhibit the growth of *S. aureus*. Therefore, the ethanol extract with the source, concentration, and administration volume in the study cannot be recommended as an antibacterial against *S. aureus*.

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