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The Antibacterial Effectiveness Of N-Hexane Garlic Peel Extract Against *Staphylococcus aureus*

Salsabila Fauza Rahima[1], Trisnawaty K[2], Valentino Haksajiwo[3],
Bambang Nuryadi[4*]

[1]Student of Dentistry Study Program, Faculty of Medicine, Universitas Sriwijaya
[2,3,4]Department Of Oral and Maxillofacial Surgery, Faculty of Medicine, Universitas Sriwijaya

*Correspondence author email: nuryadi.bambang@yahoo.com

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Abstract : Background: Odontogenic infections are a common disease in dental practice that is caused by bacteria *S. aureus*. One of herbal ingredients that has antibacterial properties is garlic peel which can be extracted with n-hexane. This has not been widely studied and it is still considered waste. **Purpose**: This study aimed to determine the effectiveness of antibacterial extract of garlic peel against *S. aureus*. **Materials and Methods**: This study is an experimental in vitro laboratory study. The test group used an extract of garlic peel with concentrations of 25%, 50%, 75%, and 100% obtained by maceration method with n-hexane, povidone iodine 1% was used as a positive control and aquades as a negative control. The antibacterial potency was tested using disc diffusion method to determine the value of inhibition zone, the dilution method to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). The inhibition zone values were then analyzed statistically using Kruskal Wallis and Mann-Whitney tests. **Results**: This study showed that average inhibition zone diameter for the 25% and 50% concentrations was 0 mm, indicating no antibacterial activity. Meanwhile, the 75% and 100% concentrations exhibited inhibition zones of 0.06 mm and 0.07 mm, respectively, which were classified as weak inhibition and lower than that of povidone iodine 1%. The MIC and MBC values could not be determined in this study. **Conclusion**: N-hexane garlic peel extract has antibacterial activity against *S. aureus* at high concentrations (75% and 100%) and the activity is classified as weak.

Keywords: antibacterial, garlic peel, n-hexane, *Staphylococcus aureus*

INTRODUCTION

Odontogenic infections are common in dental practice and occur in the oral cavity. It is caused by bacteria, one of which is *S. aureus*.[1] *S. aureus* is a Gram-positive coccus with significant virulence factors, facilitating the dissemination of infection into adjacent or deeper tissues.[2] Infection management can involve the use of antiseptics, such as povidone-iodine.[3] However, its application may lead to side effects, including skin irritation.[4]

Garlic has strong antibacterial activity and the garlic peel is often considered waste despite containing bioactive compounds such as allicin, alkaloids, steroids, and terpenoids.[5] The study by Marliza et al., (2023) on methanolic extract of garlic peel showed that a 75% concentration produced an average inhibition zone of 7.6 mm, while a 100% concentration produced an average inhibition zone of 8.5 mm, both categorized as moderate against *Escherichia coli*.[6] In other studies, Aprilia et al., (2024) reported that the ethanolic extract of garlic peel at a 75% concentration exhibited an average inhibition zone of 2.96 mm against *S. aureus*, which was classified as weak.[7]

Previous studies have extensively reported the antibacterial efficacy of garlic peel extracts obtained using polar solvents against various bacterial pathogens. Nonetheless, data on the extraction and antibacterial properties of garlic peel using non-polar solvents, such as n-hexane, remain limited. N-hexane is a non-polar solvent with a lower boiling point compared to ethanol, which facilitates the separation of the final extract from the solvent.[8] Preliminary studies demonstrate that n-hexane extraction can isolate bioactive compounds with significant antibacterial potential.

Based on the above background, this study aimed to evaluate the antibacterial effectiveness of garlic peel extract using n-hexane as a solvent against *S. aureus* by measuring the inhibition zone diameter, minimum inhibitory concentration (MIC), and minimum bactericidal concentration (MBC).

RESEARCH METHODS

This type of research is a laboratory experimental study using a post-test only control group design and ethical feasibility test has been approved by the Medical and Health Research Ethics Committee (KEPKK) Faculty of Medicine, Sriwijaya University with ethical certificate number 477-2024. The research was conducted in January 2025 at the Chemistry Laboratory of Politeknik Negeri Sriwijaya for the manufacture of garlic peel extract and Faculty of Medicine, Muhammadiyah University for the calculation of antibacterial activity. Garlic peel was dried in the sun then ground using an electric blender to obtain 300g of dry garlic peel powder. The powder (50g) was soaked in n-hexane solvent (80 mL) through a maceration process for 3x24 hours and stirred occasionally then filtered. The filtrate from the maceration was concentrated using a rotary evaporator at a temperature of 40°C until a concentrated extract was obtained.

Furthermore, the concentrated extract was diluted with distilled water to obtain garlic peel extract with concentrations of 25%, 50%, and 75% using the formula M1.V1 = M2.V2.

Identification of secondary metabolite compounds was done qualitatively using test tubes assays. The following is the procedure for the identification of secondary metabolite compounds:

1. Alkaloid Test

Two milliliters of garlic peel extract were placed into a test tube, followed by the addition of 1 mL of 1% HCl and mixed thoroughly. The upper phase was collected, and 4–5 drops of Mayer's reagent were added. The formation of a yellow-red precipitate indicated the presence of alkaloids in the extract.

2. Steroid and Terpenoid Test

Two milliliters of garlic peel extract were transferred into a test tube, then 10 drops of anhydrous acetic acid and 2 drops of concentrated H_2SO_4 were added. A positive terpenoid test was indicated by the formation of a reddish-brown ring, whereas a positive steroid test was indicated by the formation of a blue-green ring.

3. Tannin Test

Two milliliters of garlic peel extract were placed in a test tube, followed by the addition of 3 drops of $FeCl_3$. A color change to dark blue-black indicated the presence of tannins.

Povidone iodine is a mouthwash that will be used as a positive control in this study with the concentration of 1%. *S. aureus* bacterial colonies were placed in a test tube containing Nutrient Agar (NA) mediaum, which had been sterilized by autoclave at 121°C for 15 minutes. Inoculate *S. aureus* bacteria into Nutrient Agar (NA) media and incubate in an incubator for 24 hours at 37°C. A suspension of *S. aureus* was prepared by taking 1 mL of bacterial culture and adding it to a test tube containing 5 mL of 0.9% NaCl then vortexed. Before testing the antibacterial test, turbidity of the suspension was measured using the McFarland standard.

The inhibitory activity of n-hexane garlic peel extract was tested using the *Kirby-Bauer* method. The bacterial suspension was evenly spread on Mueller Hinton Agar

(MHA) using a sterile cotton swab. Each petri dish was marked with a permanent marker to indicate the placement of paper discs for garlic peel extract concentrations of 25%, 50%, 75%, 100%, and control groups. The discs were pre-soaked in their respective extract concentrations and control solutions, then placed on the marked areas. The test was performed in four replicates and incubated at 37°C for 24 hours. A clear zone around the disc indicated bacterial growth inhibition. The inhibition zone diameter was measured using a caliper in millimeters (mm).

The solid dilution method was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using Mueller Hinton Agar (MHA). Garlic peel extract (25%, 50%, 75%, and 100%) was mixed with molten MHA (2 mL extract + 18 mL agar) and allowed to solidify. *Saureus* was spread onto the surface and incubated at 37°C for 24 hours. MIC was indicated by visible bacterial growth, while MBC was defined by the absence of growth. Colonies were counted using a colony counter. Data processing and analysis were performed using SPSS software. Normality test using Shapiro-Wilk test, homogeneity test using Levene test, parametric statistical test with Kruskal Wallis and Mann-Whitney.

RESULTS

Table 1. Phytochemical Identification for N-hexane Garlic Peel Extracts

Compound	Results	Description
Alkaloids	Positive (+)	Red precipitate
Steroids	Positive (+)	Green ring
Terpenoids	Positive (+)	Red ring
Tannin	Negative (-)	No change

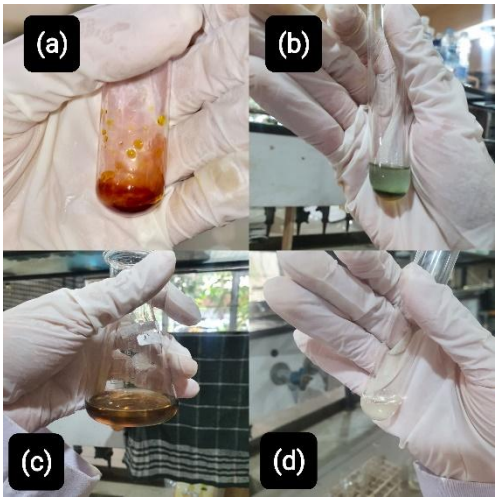

Figure 1. Phytochemical Assays (a)Alkaloids (b)Steroids (c)Terpenoids (d)Tannin

Table 2. Results of Inhibition Zone

Concentrations	Average (mm)	Inhibition response
100%	0,07	Weak
75%	0,06	Weak
50%	0	Absent
25%	0	Absent
Negative control (aquades)	0	Absent
Positive control (povidone iodine 1%)	0,155	Weak

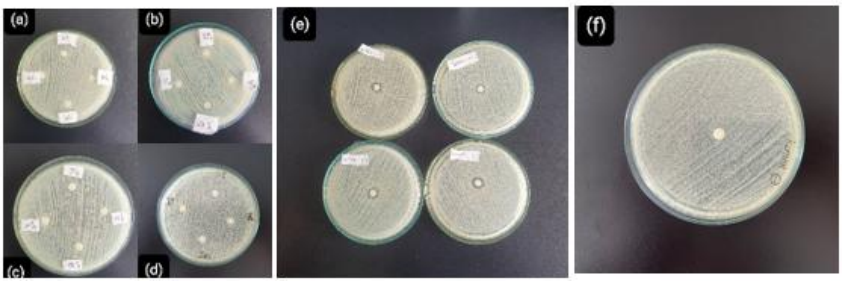

Figure 2. Results of Inhibition Zone (a,b,c,d) Extracts (e) Positive Control (f) Negative Control

Table 3. Results of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Concentrations	Bacterial Colony Count (CFU/ml)
100%	
75%	

50%	TNTC
25%	
Negative control (aquades)	
Positive control (povidone iodine 1%)	

TNTC (too numerous to count, >300 colony)

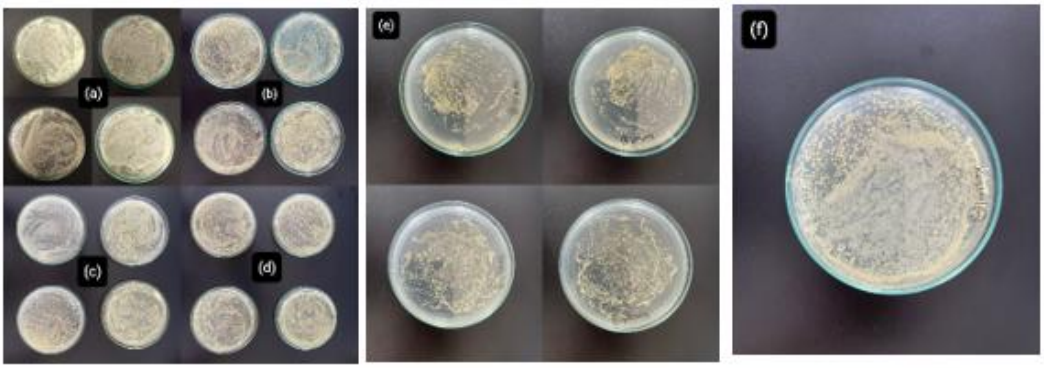

Figure 3. Results of MIC and MBC (a,b,c,d) Extracts (e) Positive Control (f) Negative Control

Table 4. Results of Normality Test and Homogeneity Test

Groups	Shapiro-Wilk test		Levene test	
	P	**Distribution**	***p***	**Information**
100%	0,007	Abnormal		
75%	0,373	Normal	0,078	Homogeneous
Control (+)	0,861	Normal		

p> 0,05 (Normally distributed and Homogeneous Variance)

Table 4 shows the results of the Kruskal Wallis test has a p value (<0.001) < 0.05, it can be concluded that there is a significant difference in the number of *S. aureus* bacterial growth at each concentration. Therefore, the next stage is to conduct a Post Hoc test Mann-Whitney test to determine the difference in the growth of the number of colonies in each treatment group.

Table 5. Kruskal Wallis Test Results

Concentration	***P***	Information
100% 75% 50%	<0,001	Significantly Different

Concentration	*P*	Information
100%		
25%		
Control (-)		
Control (+)		

Information: SD = Standard Deviation

Table 6. Post-Hoc Test Results (Mann-Whitney)

Groups	100%	75%	50%	25%	Control (-)	Control (+)
100%						
75%	0,772*					
50%	0,013*	0,014*				
25%	0,013*	0,014*	1,000*			
Control (-)	0,013*	0,014*	1,000*	1.000*		
Control (+)	0,020*	0,043*	0,014*	0,014*		

Description: Sign (*) = Significantly different at a significance level of 5% ($p < 0.05$)

The Mann-Whitney test results demonstrated that increasing the extract concentration was associated with a greater antibacterial effect. No significant difference was observed between the 25% and 50% concentrations. However, the 25% concentration showed a statistically significant difference compared to the 75%, 100%, and positive control groups. Similarly, antibacterial activity at 75% and 100% concentrations did not differ significantly, indicating comparable efficacy at these levels. Nevertheless, both 75% and 100% concentrations exhibited significantly lower antibacterial activity compared to the positive control, suggesting that the positive control remains more effective than the extract.

DISCUSSION

The results of this study demonstrated that garlic peel extract using n-hexane as a solvent exhibited antibacterial activity against Staphylococcus aureus at higher concentrations (75% and 100%), although the inhibitory effect was categorized as weak. No inhibition zones were observed at 25% and 50% concentrations, while the 75% and 100% concentrations produced mean inhibition zone diameters of 0.06 mm and 0.07 mm,

both markedly lower than the positive control, 1% povidone iodine, which yielded a mean diameter of 0.155 mm.

The results of the MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bactericidal Concentration) tests also showed no significant findings. No minimum concentration was identified that could definitively inhibit or kill the bacteria, as indicated by the presence of too numerous to count (TNTC) bacterial colonies in all groups, including the control. This further supports the conclusion that, although the extract exhibited weak antibacterial activity at high concentrations, it was not effective in completely inhibiting bacterial growth.

Previous research conducted by Aprilia et al., (2024) demonstrated that the ethanol extract of garlic peel exhibited weak antibacterial activity against *S. aureus*, with inhibition zones of 0.90 mm at a 50% concentration and 2.96 mm at a 75% concentration.[7] Meanwhile, a study by Syamsi et al., (2020) reported that the aqueous extract of garlic peel at a 15% concentration produced an average inhibition zone of 15.67 mm, indicating moderate activity against *Streptococcus mutans*.[9] A comparison of the present study with previous findings reveals variable results depending on the type of solvent and extraction method used.

The weak antibacterial activity of this extract may be influenced by several factors, such as the low solubility of the active compounds in the n-hexane solvent and the concentration of the extracted active antibacterial compounds. This is consistent with the phytochemical test results, which showed that the extract contained only alkaloids, steroids, and terpenoids, while tannins, which also have antibacterial properties, were not detected.

The mechanism of action of alkaloids involves disrupting the integrity of bacterial cell components, interfering with homeostasis, and inhibiting nucleic acid synthesis.[6,10] Steroids exert their antibacterial effect by causing leakage in bacterial cell liposomes.[11] Terpenoids act as antibacterial agents by reducing the permeability of the bacterial cell wall and causing degradation of the bacterial membrane.[11,12]

The quality of the extract influences its ability to inhibit bacterial growth, which can be affected by several factors including the type of solvent, extraction method, particle size, drying technique, and storage conditions. Maceration is a simple extraction technique involving the soaking of the sample in a solvent for a specific period to allow

the diffusion of bioactive compounds.[13] The selection of n-hexane as the solvent was based on its ease of evaporation and its effectiveness in extracting non-polar compounds such as steroids and terpenoids.[14]

In this study, garlic peel powder was sieved to obtain a simplicia particle size of 100 mesh. Smaller particle sizes provide a greater surface area for contact between the sample and the solvent, thereby facilitating the extraction of compounds from the sample.[15,16] Sun drying is a simple and cost-effective method. However, it tends to retain higher moisture content compared to oven drying, which may affect the quality and shelf life of the sample.[17,18] The extract was stored in amber glass bottles at low temperatures to preserve the stability of bioactive compounds by minimizing exposure to oxygen and light, thereby reducing the risk of degradation.[19]

The n-hexane garlic peel extract demonstrated antibacterial activity against *S. aureus* at higher concentrations, specifically at 75% and 100%. Garlic peel has potential as a source of antibacterial agents. However, further research and formulation development are necessary to optimize its efficacy and support its practical application in antimicrobial therapy.

CONCLUSION

Based on the research results, it can be concluded that the n-hexane garlic peel extract has antibacterial activity against *S. aureus* at high concentrations (75% and 100%) and the activity is classified as weak.

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