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Antibacterial Potential of Fermented and Non-Fermented *Allium sativum* var. *Solo* Against *Staphylococcus epidermidis*

I Gede Ngurah Putra Nata Sudana[1], Sabariah[2]✉, Musyarrafah[3], Wiwin Mulianingsih[4]

[1-4] Medical faculty of Universitas Islam Al-Azhar Mataram, Indonesia

✉hussabariah@yahoo.co.id, Phone: +6281338582710

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Abstract

Acne vulgaris is one of the most prevalent skin diseases worldwide, often treated with antibiotics such as clindamycin to suppress *Staphylococcus epidermidis* growth. However, irrational antibiotic use has increased antimicrobial resistance (AMR), necessitating alternative solutions. Herbal plants like single bulb garlic (*Allium sativum* var. Solo) possess antibacterial properties due to their allicin content, and fermentation has been reported to enhance these effects. This study aimed to compare the antibacterial potential of non-fermented and fermented single bulb garlic extracts against *S. epidermidis*.A true experimental design with posttest-only control group was employed, using the well diffusion method on 24 samples divided into six treatment groups (non-fermented and fermented extracts at 60%, 80%, and 100%) plus positive (clindamycin) and negative (sterile aquadest) controls, each in triplicate. The bacterial strain was obtained from the Balai Laboratorium Kesehatan Pengujian dan Kalibrasi (BLKPK-BLUB) in Mataram, Indonesia. Fermentation was conducted for 96 hours at room temperature (~27–30°C) using *Rhizopus sp.*, in covered glass containers. Inhibition zones were measured with a digital caliper,and data were analyzed using the Kruskal–Wallis and Mann–Whitney tests.All garlic extract groups showed sensitivity (>21 mm). Mean ± SD inhibition zones for non-fermented extracts were 29 ± X mm, 33 ± X mm, and 33 ± X mm; fermented extracts showed 38 ± X mm, 40 ± X mm, and 40 ± X mm, respectively. Fermented garlic exhibited significantly greater inhibition than non-fermented at all concentrations ($p < 0.05$). These findings suggest that fermentation enhances the antibacterial activity of single bulb garlic, supporting its potential as a complementary alternative for managing *S. epidermidis*-related skin infections.

Keywords: Antibacterial; Solo Garlic; Fermentation; Non-Fermented

INTRODUCTION

Acne vulgaris is a disease that affects the sebaceous glands and hair follicles on the surface with blockage by dead cells (Fajryana et al. 2022). Acne vulgaris is among the eight most common skin diseases, affecting about 9% of the population worldwide (Eichenfield, Sprague, dan Eichenfield 2021). There are more than 60 million people with acne vulgaris, dominated by adolescents and adults (Junita 2020). Acne vulgaris is the 3rd most common disease after scabies and dermatitis in Indonesia every year (Rizqi et al. 2022).

The incidence of acne vulgaris is due to an increase in the number and activity of the flora (Saleh et al. 2023). Some of the bacteria that cause acne vulgaris are *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Staphylococcus epidermidis* is often found in acne lesions, with a percentage of 63.3% (Dewi, Habibah, dan Mastra 2020). *Staphylococcus epidermidis* is a gram-positive, coagulase-negative cocci bacteria. (Lee dan Anjum 2023).

Anti-acne drugs in circulation are retinoids and some also contain topical antibiotics in them such as

clindamycin and erythromycin (Wardani 2020). The continuous and irrational use of topical and oral antibiotics leads to antimicrobial resistance (AMR). Antibiotic resistance is a condition where the action of antimicrobial agents is inhibited and antibiotics lose their efficiency to inhibit bacterial growth. (Putri et al. 2023). A recent systematic review and meta-analysis in Indonesia reported that acne-causing bacteria exhibit high levels of resistance to macrolide and Lincosamide antibiotics. The resistance to macrolides and clindamycin in Indonesia is among the highest globally, with clindamycin resistance reaching approximately 60.1% and clindamycin 53.3%. This rate is considerably higher than the global average of 30-40%. (Legiawati et al. 2023). This phenomenon forces researchers to find alternative antibiotics from herbal plants (Sharma dan Nagar 2021).

Herbal plants have lower or no side effects when compared to chemical drugs. (Kumontoy et al. 2023). Garlic (*Allium sativum*) has been tested to have antibacterial activity and is effective against gram-positive bacteria (Al-Kubeisi 2022). Garlic has more than 600 sub-varieties, one of which is single garlic (Lestari 2021). Single bulb garlic is thought to be 5 times higher and there are 15 additional ingredients compared to compound onions (Multi bulb garlic) (Lestari 2021).

Garlic fermented by *Rhizopus sp* fungus from processed soybeans and yeast has a greater antibacterial effect compared to those that are not fermented. In a previous study, using the same concentration and bacterial strain, fermented garlic for 96 hours created an inhibition zone of 24.5mm. It is mentioned that there are antibacterial substances during the fermentation of tempeh, namely glycogen that inhibits the growth of Gram-positive bacteria (Fajryana et al. 2022).

This study was conducted to determine the comparison of non-fermented and fermented single garlic (*Allium sativum var. Solo garlic*) antibacterial activity test against the growth of *Staphylococcus epidermidis* bacteria.

METHOD

This study employed a true experimental design with a posttest-only control group. The antibacterial activity of single bulb garlic (*Allium sativum* var. Solo) was evaluated using the well diffusion method across six treatment groups (non-fermented and fermented extracts at 60%, 80%, and 100%) and two control groups (positive control: clindamycin; negative control: sterile aquadest). Eight treatment groups were tested as follows:

Table 1. Treatment groups in the experiment

Group	Treatment description
NF60	Non-fermented garlic extract 60%
NF80	Non-fermented garlic extract 80%
NF100	Non-fermented garlic extract 100%
F60	Fermented garlic extract 60%
F80	Fermented garlic extract 80%
F100	Fermented garlic extract 100%
PC	Positive control (clindamycin)
NC	Negative control (sterile aquadest)

NF=non-fermented, F=fermented, PC=positive control, NC=negative control

All experiments were conducted in triplicate for each treatment group (n = 3). Data were analyzed for normality using the Shapiro–Wilk test. As the data were not normally distributed, non-parametric tests were applied. Differences among groups were first assessed using the Kruskal–Wallis test, followed by pairwise comparisons with the Mann–Whitney U test. A *p*-value < 0.05 was considered statistically significant.

Procedure

The single bulb garlic used was obtained from a garlic farm in Sembalun, Lombok. The single garlic was cleaned using water to remove any dirt.

Preparation of non-fermented garlic extracts

Single bulb garlic (*Allium sativum* var. Solo) weighing 250 g was obtained in stored form, peeled, and blended. The blended material was placed in a muslin cloth and squeezed, after which the filtrate was centrifuged twice at 10,000 rpm for 5 minutes using a Dlab D1524R centrifuge. The supernatant was collected as the crude extract at 100% concentration (50 ml) (Fajryana et al., 2022).

Preparation of fermented garlic extracts

Single garlic (*Allium sativum var. Solo garlic*) as much as 40 grams was peeled and then ground and mixed with 0.8 grams of soybeans that had been ground and mixed with 40 ml of distilled water. Soybeans are used as a medium for the growth of tempeh yeast containing *Rhizopus sp* fungus. After mixing, it is brought into a dark glass bottle and mixed with tempeh yeast containing *Rhizopus sp* fungus with a predetermined concentration of 15%, then fermented for a period of 96 hours. The supernatant was taken after centrifugation at 3500 rpm for 15 minutes. The results obtained in 100% concentration were 50ml (Fajryana et al. 2022).

After forming a 100% standard solution of 50ml, then proceed to dilute the standard solution so that the results of dilution of each concentration are 60%, 80% and 100%.

Negative control uses sterile distilled water. While the manufacture of positive control solutions is made from clindamycin 300 mg antibiotic preparations. A total of 10 clindamycin capsules were opened then weighed one by one and calculated the average, the positive control was diluted using 10 ml distilled water and 20 mg of clindamycin powder was needed. So it was calculated that the weighing of clindamycin used was 3 mg: (Hana, Gerung, dan Antasionasti 2021)

The method of testing antibacterial activity used is the well diffusion method.

1. Staphylococcus epidermidis was obtained as a clinical isolate from PME BBLK Surabaya (2021), and a suspension was prepared in accordance with the 0.5 McFarland standard, then taken with a sterile cotton swab.
2. The cotton swab was streaked evenly on the surface of Mueller–Hinton Agar (MHA; Oxoid, UK) plates.
3. Make 3 wells measuring 6 mm using a micropipette cup with a distance between the wells of about 20 mm except for the control group cup which has only two wells.
4. The wells were filled with extracts using a micropipette as much as 20µl with a concentration of 60%, 80%, and 100% respectively. For negative control, the wells were dripped with 20µl of distilled water and for positive control the wells were dripped with 20µl of clindamycin.
5. The inoculated plates were then incubated at 37 °C for 24 hours using an incubator (POL-EKO Aparatura, Poland).
6. Observed whether there is a clear zone formed around the wells.
7. If there is an inhibition zone, measure the diameter of the inhibition area using a ruler (CLSI 2023; Hasanah 2023).

Data Analysis

Data were analyzed univariately and bivariately using the Statistical Packages for Social Science (SPSS) program. The non-parametric Kruskal Wallis test was used followed by the Mann Whitney test (Hasanah 2023).

RESULT AND DISCUSSION

Diameter measurement of the inhibition zone is determined by measuring the inhibition zone of bacterial growth due to the diffusion of antibacterial substances. This measurement is made using a ruler by observing the clear zone that will not be overgrown by *Staphylococcus epidermidis* bacteria. The measurement results of the diameter on the inhibition zone of non-fermented and fermented garlic extract can be found in Table 2.

The results that have been obtained then interpreted the diameter of the inhibition zone ≥21 mm is considered sensitive, 15-20 mm intermediate, and ≤14 mm resistent (CLSI 2023). Based on the observations in table 2, it is known that all single garlic groups, both non-fermented and fermented, have inhibition and are considered sensitive to *Staphylococcus epidermidis* bacteria. On the other hand, the negative control, namely aquadest, has no inhibitory power which is characterized by the absence of inhibition zones, while clindamycin as a positive control has the highest inhibitory power among all groups, namely 58 mm (Table 3).

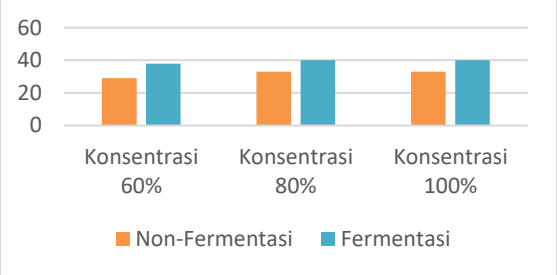
Table 2. Results of the number of measurements of the inhibition zone of *Staphylococcus epidermidis* bacteria

Treatment groups	n	Area of resistance zone (mm)			Mean (mm) ± SD	Interpretation
		1	2	3		
60%	3	33	33	22	29.33 ± 6.35	Sensitif
80%	3	30	35	33	32.67 ± 2.52	Sensitif
100%	3	30	35	33	32.67 ± 2.52	Sensitif
F60%	3	40	38	36	38.00 ± 2.00	Sensitif
F80%	3	38	40	38	38.67 ± 1.15	Sensitif
F100%	3	40	40	40	40.00 ± 0.00	Sensitif
NC	3	0	0	0	0.00 ± 0.00	Resistant
PC	3	58	58	58	58.00 ± 0.00	Sensitif

Table 3. Ranking of inhibition zones (largest to smallest)

Rank	Group	Mean ± SD (mm)
1	Positive control	58.00 ± 0.00
2	F 100%	40.00 ± 0.00
3	F 80%	38.67 ± 1.15
4	F 60%	38.00 ± 2.00
5	NF 80%	32.67 ± 2.52
6	NF 100%	32.67 ± 2.52
7	NF 60%	29.33 ± 6.35
8	Negative control	0.00 ± 0.00

In this study, there was an increase in the inhibition zone area in each concentration and the fermentation group was superior to the non-fermentation group. As shown in the graph below.



Picture 1: Comparison of inhibition of non-fermented and fermented single garlic (*Allium sativum var. Solo garlic*) against *Staphylococcus epidermidis* bacteria

Data Analysis

The Kruskal-Wallis test was used to determine whether there was a difference in the average between each treatment group. Meanwhile, the Mann-Whitney test was used to determine differences between non-fermented and fermented groups.

Table 4. Shapiro–Wilk test for normality of inhibition zone diameter data

Treatment Groups	Shapiro–Wilk *p*-value	Normality assumption
NF 60 %	0.000	Not normal
NF 80 %	0.780	Normal
NF 100 %	0.780	Normal
F 60 %	1.000	Normal
F 80 %	0.000	Not normal
F 100 %	-	Not available (zero variance)
NC	-	Not available (zero variance)
PC	-	Not available (zero variance)

The data distribution was tested with *Shapiro-wilk*: the data was partially undistributed normal $p < 0.05$

Table 5. Results of inhibition zones between treatment groups

Treatment Groups	Average (mm)	P value	Kruskal-Wallis H
NF 60%	29[a]	0,003	22.010
NF 80%	33[a]		
NF 100%	33[a]		
F 60%	38[b]		
F 80%	40[b]		
F 100%	40[b]		
NC	0[c]		
PC	58[d]		

The data is presented in averages. *Kruskal-Wallis* test: p 0.003 ($p \leq 0.05$). *Mann-Whitney* with significant results is characterized by superscript notation.[a-b-c-d] Values with different superscripts show significant differences ($p < 0.05$)

Table 6. Pairwise Mann–Whitney U comparisons of inhibition zones

Sample 1-Sample 2	Mann-Whitney U	Value Sig.	Interpretation
NF 60%-F 60%	0,000	0,046[a-b]	There are differences
NF 80%-F 80%	0,000	0,046 [a-b]	There are differences
NF 100%-F 100%	0,000	0,037 [a-b]	There are differences
NC-PC	0,000	0,025 [c-d]	There are differences

The Kruskal–Wallis test indicated significant overall differences among groups ($H = 22.010$, df = 7, $p = 0.003$). Post-hoc pairwise comparisons using the Mann–Whitney U test are summarized in Table 6, with exact *p*-values reported. Groups not sharing the same superscript letter in Table 5 differ significantly ($p < 0.05$). No correction for multiple comparisons (e.g., Bonferroni) was applied, which is acknowledged as a limitation.

Based on the description above, it can be said that superscript groups a and b have significant differences in meaning, indicating that non-fermented and fermented groups have statistically different antibacterial activities against bacterial tests.

The ability of single garlic extract (*Allium sativum var. Solo garlic*) to inhibit the growth of *Staphylococcus epidermidis* bacteria is due to the content of bioactive substances (Bhatwalkar et al. 2021). Lestari (2021) identified more than 70 compounds in single garlic extract through LC-MS analysis, but only a subset (such as allicin, alkaloid, tanin, 1 hexanol, Alpha Phellandrene, Geraniol, and diallyl sulfides) are strongly associated with antibacterial effects.

The higher the concentration, the wider the inhibition zone formed. The difference in the diameter of the inhibition zone obtained by each concentration

occurs because it is influenced by the different concentrations of single garlic used, the higher the concentration of single garlic, the more the content of active compounds that denature proteins in bacteria, and break down cells and inhibit bacterial growth (Sofyanita 2023).

The fermentation group was more optimal in inhibiting the growth of *Staphylococcus epidermidis* bacteria compared to the non-fermentation group. This difference is because the fermentation process with the help of rhizopus sp will break down allicin compounds that cause the aroma of garlic to disappear and break down into antioxidant compounds such as S-Alycysteine (SAC), tetrahydro-β-carbolin, alkaloids and flavonoids so that they can more effectively inhibit the growth of *Staphylococcus epidermidis* bacteria (Fajryana et al. 2022).

The concentration-dependent increase in inhibition zones further supports the hypothesis that higher levels of active compounds exert stronger antibacterial effects. For example, fermented extracts at 100% concentration showed inhibition zones comparable to mid-range antibiotic performance, while lower concentrations (60%) were significantly less effective. This indicates that both fermentation and concentration contribute synergistically to antibacterial potency.

The mechanism of single garlic when compared to the control group, relies on active compounds as antibacterials. Flavonoids have activity as antimicrobial compounds because they are able to form complex compounds with extracellular proteins, change the physical and chemical properties of the cytoplasm and denature the bacterial cell wall through hydrogen bonds. This activity will interfere with the function of selective permeability, active transport function, and control of protein composition, causing death to bacteria (Dewi et al. 2020).

Allicin compounds can increase the permeability of the bacterial wall which causes the SH group (sulfihydryl and disulfide) on the amino acids cystine and cysteine to be destroyed, the destroyed SH group can inhibit the synthesis of protease enzymes that damage the cytoplasmic membrane on the bacterial wall and interfere with protein and nucleic acid metabolism so that no proliferation occurs in bacteria (Purwantiningsih, Rusae, dan Freitas 2019).

Tannin activity is thought to work by forming hydrophobic complexes with proteins, inactivating enzymes and cell wall transport proteins, thereby disrupting bacterial growth. In addition, tannin can also wrinkle the cell wall so that it disrupts the permeability of the cell wall as a result it inhibits bacterial growth or even dies (Rahmawatiani, Mayasari, dan Narsa 2020).

The mechanism of action of alkaloids as antibacterials is thought to be by interfering with the constituent components of peptidoglycan in bacterial cells, so that the cell wall layer is not formed intact and causes the death of these cells (Saptowo, Supriningrum, dan Supomo 2022).

While the fermentation group has a slightly different mechanism of action, Rhizopus sp. plays a crucial role by breaking down carbohydrate and protein components in garlic, thereby providing carbon, nitrogen, and phosphorus sources that support the growth of lactic acid bacteria. These lactic acid bacteria subsequently produce lactic acid, which contributes to the inhibition of *Staphylococcus epidermidis*. The antibacterial effect arises from both the lactic acid generated during fermentation and the active components of fermented single-bulb garlic. Weak acids such as lactic acid are only partially dissociated in water; the undissociated molecules can diffuse into the cytoplasm of *S. epidermidis*. Once inside the cell, these molecules dissociate and release H^+ ions, lowering the intracellular pH and leading to cellular damage under acidic conditions (N Parhusip 2023)

Fermented garlic also exhibits enhanced antibacterial activity through additional mechanisms. Lactic acid bacteria (LAB) produce lactic, acetic, and propionic acids during fermentation, lowering the pH and creating unfavorable conditions for pathogenic bacterial growth (Zapaśnik, Sokołowska, dan Bryła 2022). This antimicrobial effect is largely dependent on organic acids, while hydrogen peroxide produced by certain LAB strains provides an additional bacteriostatic effect (Ren et al. 2018). Moreover, fermentation increases the flavonoid content of solo garlic, which has been linked to wider inhibition zones against *Staphylococcus aureus* and *Escherichia coli* (Setiyoningrum et al. 2021). Taken together, the combined effects of nutrient breakdown by Rhizopus sp., acid production, hydrogen peroxide activity, and enhanced bioactive compounds provide a comprehensive rationale for the stronger inhibitory potential of fermented garlic against Gram-positive bacteria.

The negative control group, namely aquadest, did not produce an inhibition zone at all. This explains that the negative control group has no antibacterial activity. Sterile aquadest has no inhibition zone at all, meaning that the extract solvent has no effect on the antibacterial activity of non-fermented and fermented single garlic. This is in accordance with the results in this study, where the wells given aquadest had no inhibition zone. Aquadest is used as a negative control because it does not provide inhibition against test bacteria, is stable, non-toxic, non-volatile, and easily available (Saleh et al. 2023).

In this study, the diameter of the inhibition zone produced by clindamycin as a positive control against *Staphylococcus epidermidis* bacteria was 58 mm so that it was categorized as sensitive, while the negative control produced an inhibition zone of 0 mm. The selection of clindamycin as a positive control is because Clindamycin works against anaerobic bacteria, most gram-positive aerobic cocci bacteria, gram-positive and gram-negative bacilli, and some protozoa. *Staphylococcus epidermidis* bacteria are gram-positive bacteria (Saleh et al. 2023).

When compared with the positive control, clindamycin, the fermented extract showed notable improvement over the non-fermented form, yet clindamycin remained superior, producing inhibition zones approximately 45% larger than the best-performing fermented group. This comparison underscores the practical significance: while fermentation enhances garlic's antibacterial activity, it is not yet equivalent to standard antibiotic therapy.

Positive control clindamycin is a semisynthetic antibiotic derived from lincomycin which is used for the treatment of various serious infections due to susceptible microorganisms and topically for acne vulgaris. The mechanism of action of clindamycin is to inhibit the translocation of tRNA of the 50S ribosomal sub unit (Achyar 2020).

These findings suggest that fermented single bulb garlic extract possesses enhanced antibacterial activity against *Staphylococcus epidermidis* compared to the non-fermented extract. This highlights its potential as a natural alternative or adjunct to conventional antibiotics such as clindamycin, particularly in the context of rising antimicrobial resistance. However, further studies involving standardized fermentation conditions, larger sample sizes, and in vivo models are required to validate these results. Future research should also explore the identification and quantification of active compounds responsible for the antibacterial effect, as well as their possible synergistic interactions with existing antibiotics.

CONCLUSION

This study demonstrates that both non-fermented and fermented extracts of single garlic (*Allium sativum var. Solo garlic*) exhibit antibacterial activity against *Staphylococcus epidermidis*. However, the fermented garlic extract, particularly after 96 hours of fermentation, showed significantly higher effectiveness in inhibiting bacterial growth compared to its non-fermented counterpart. The results highlight a clear difference in inhibition zones, with the fermentation group forming larger zones of inhibition at various concentrations: 60% (38 mm vs. 29 mm), 80% (40 mm vs. 33 mm), and 100% (40 mm vs. 33 mm). These findings suggest that the fermentation process enhances the antibacterial potential of single garlic extract, making it a promising candidate for natural antibacterial agents. The study supports the potential application of fermented garlic as a more effective alternative in combating *Staphylococcus epidermidis*.

These findings highlight the potential of fermented garlic extract as a supplementary approach in the search for alternative antibacterial agents. Future research should focus on standardizing fermentation conditions, identifying and quantifying the specific bioactive compounds responsible for the enhanced activity, and testing efficacy in in vivo models or clinical settings.

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