BIOMEDIKA
ISSN: 2085-8345, E-ISSN: 2541-2582
Vol 17, No. 1 Februari 2025
doi: 10.23917/biomedika.v17i1.7033

Antibacterial Activity of Aqueous Extract Combination of Garlic (*Allium sativum*) and Red Ginger (*Zingiber officinale* var. *Rubrum*) Against *Staphylococcus aureus*

Rizka Aulia Rahma, Ayu Lidya Paramita, Syafarinah Nur Hidayah Akil

AFFILIATIONS
Faculty of Medicine, Muhammadiyah University of Surabaya

ABSTRACT

Background: *Irrational use of antibiotics can increase the resistance, so it is necessary to find alternative solutions by using herbals that have antibacterial activity, such as garlic which contains allicin, alliin, and sulfide derivatives, and red ginger which contains gingerol, flavonoids, and phenols. For traditional use, garlic and red ginger need to be extracted with aqueous solvents.* ***Objective:*** *Proving the antibacterial activity of the combination of garlic extract (Allium sativum) and red ginger extract (Zingiber officinale var. Rubrum) with aqueous solvents against Staphylococcus aureus.* ***Methods:*** *Each garlic extract and red ginger extract were made by the kinetic maceration with aqueous solvents, then mixed in a ratio of 1:1, 1:2, and 2:1.* ***Results:*** *The combination of garlic and red ginger extracts has strong antibacterial activity to inhibit the growth of Staphylococcus aureus. The average diameter of the inhibition zone at a ratio of 1:1, 1:2, and 2:1 was 30.40 mm, 24.51 mm, and 37.67 mm, respectively. The ANOVA test showed a P value of 0.000.* ***Conclusion:*** *The combination of garlic extract (Allium sativum) and red ginger extract (Zingiber officinale var. Rubrum) with aqueous solvent has strong antibacterial activity against Staphylococcus aureus, with the strongest combination at a ratio of 2:1.*

KEYWORDS:
Garlic, Red Ginger, Aqueous extract, Antibacterial, *Staphylococcus aureus*

CORRESPONDING AUTHOR:
Rizka Aulia Rahma
rizka.aulia.rahma-2021@fk.um-surabaya.ac.id

INTRODUCTION

Health issues that often occur in tropical countries like Indonesia are health issues due to infections [1]. The irrational use of antibiotics increases the incidence of resistance, so alternative solutions using herbs with antibacterial activity that are widely used in traditional medicine, such as garlic (*Allium sativum*) and red ginger (*Zingiber officinale* var. *Rubrum*), need to be sought [2–4].

Garlic extract and red ginger extract with nonpolar organic solvents such as ethanol and acetone have antibacterial activity, but for simpler use, extraction with water as a solvent is necessary [4–6]. The difference in the type of solvent used can affect the antibacterial effect produced. It is influenced by the polarity match between the solvent and the compounds contained in garlic and red ginger [7].

Garlic (*Allium sativum*) is a plant in the Amaryllidaceae family, which is one of the aromatic herbs widely used around the world as a traditional medicine or spice in food [8]. The main phytoconstituent components of garlic consist of phenolic compounds, polysaccharides, and organosulfur compounds such as allicin, alliin, ajoenes, and vinyldithiins. These phytoconstituents influence various biological activities of garlic [8,9]. Garlic has various biological activities such as anticarcinogenic, antibacterial, antifungal, anti-inflammatory, and antioxidant. The antimicrobial activity of garlic is influenced by the compound Allicin. Allicin is a dose-related biocide that can affect the essential metabolism of cysteine protease. In addition to its antibacterial activity, garlic has been reported to prevent toxins produced by bacterial infections [10].

Ginger (*Zingiber officinale*) is an important herbal medicinal plant from the Zingiberaceae family. Red ginger contains various complex chemical constituents.These chemical constituents include terpenoid compounds, flavonoids, and polyphenol components such as phenolic acids, gingerol, paradol, and shogaol. The biological activity of red ginger can also be attributed to the synergistic or additive effects of the phytochemical compounds it contains. The phytochemical components contained in red ginger are responsible for its antioxidant, antimicrobial, anti-inflammatory, and analgesic properties [9,11]. Essential oil compounds, gingerol flavonoids, terpenoids, and phenolic compounds are identified as having antibacterial activity [12,13]. Essential oil compounds and flavonoid compounds in red ginger have antibacterial activity with mechanisms that can disrupt the metabolism of bacterial cell membranes [14].

The antibacterial activity of garlic extract has been reported to have a stronger inhibitory effect compared to the inhibitory effect of red ginger extract [6], but there has been no research that combines these two substances to determine the synergistic effects of their combined use. Additionally, the combination of extracts aims to expand the spectrum of antibiotic activity and reduce the risk of resistance to certain compounds [15,16]. The use of water as a solvent is also a factor that can influence the resulting antibacterial activity.

METHODS

This research is a true experimental laboratory in vitro study with a Post Test Only Control Group Design. Primary data were obtained during the experimental process in the Microbiology Laboratory of the Faculty of Medicine, Muhammadiyah University of Surabaya. Data analysis used ANOVA and Tukey's HSD post hoc test. The number of samples used is 5 samples for each treatment group, so the total number of research samples is 25 petri dishes.

Extraction of garlic and red ginger was done separately using distilled water following standard procedures [4]; with slight modifications. The sample preparation was carried out by peeling the garlic skin and then washing it with running water until clean, while the ginger rhizome was soaked first for 30 minutes and then washed with running water until clean, after that each ingredient was ground. A total of 25 grams of the material was placed into different Erlenmeyer flasks and 100 ml of distilled water was added. After that, stirring was carried out at a speed of 300 rpm for 24 hours. After that, filtration and evaporation were carried out using a rotary evaporator with a vacuum system at a temperature of 40°C. Before examination, the extract can be stored at a temperature of 4 ± 1°C in an airtight container. The extract results were combined in a 1:1 concentration ratio by mixing 5 ml of garlic extract with 5 ml of red ginger extract. Mixing with similar calculations was done to create extracts with a 1:2 and 2:1 ratio. The ratio of this extract combination adapts previous research[17] by using only two herbal plants (garlic and red ginger) and employing a different solvent from the previous study.

The object bacteria are *Staphylococcus aureus* cultures from the Microbiology Laboratory of the Faculty of Medicine, Muhammadiyah University of Surabaya. A cotton swab is dipped into a 0.5 MFU *Staphylococcus aureus* bacterial suspension and then spread over the entire surface of the MHA medium in the petri dish. Then, in 20 petri dishes, a single well was made in the center.

The antibacterial activity test was conducted on petri dishes inoculated with *Staphylococcus aureus*, with 150 μL of a combination of garlic extract (*Allium sativum*) and red ginger extract (*Zingiber officinale* var. *Rubrum*) (25 mg/mL) added at each concentration ratio, and 150 μL of distilled water as a negative control added to the wells, along with a 25 μg amoxicillin antibiotic disc as a positive control. The petri dishes were then incubated at 37 °C for 24 hours. This study had been approved by the Health Research Ethics Committee at the Muhammadiyah University of Surabaya, with reference number 025/KET/II.3/AU/F/2024.

RESULT AND DISCUSSION

The research was conducted at the Pharmacy Laboratory of the Faculty of Health Sciences and the Microbiology Laboratory of the Faculty of Medicine, Muhammadiyah University of Surabaya, from August 7-16, 2024.

The extraction results show that the thick garlic extract is in the form of a viscous yellow transparent liquid, while the thick red ginger extract is in the form of a brownish turbid liquid (Figure 1). The results of mixing the two extracts in various proportions did not show significant differences in the resulting characteristics, where all three mixtures produced a liquid extract with a murky brown color (Figure 2).

Figure 1 Garlic Extract (GE) and Red Ginger Extract (RGE)

Figure 2 Extracts from the Combination of Garlic and Red Ginger

Water is considered the safest solvent, but generally, water is not the most optimal solvent for extracting compounds contained in plants due to the incompatibility of polarity properties. To address this incompatibility, several methods can be employed to enhance the solubility of compounds in water, such as applying physical treatments [18]. In the extraction process using water as a solvent, it is generally hindered in the process of making a thick extract, because the evaporation of water solvent at high temperatures risks damaging the active compounds [19]. Thus, in this study, a kinetic maceration process was carried out to improve extraction efficiency, and during the evaporation process, a rotary evaporator with a vacuum system was used to avoid damage to active compounds.

Observation of the petri dishes showed that all three test groups and the positive control amoxicillin produced zones of inhibition against the growth of *Staphylococcus aureus* (Figure 3).

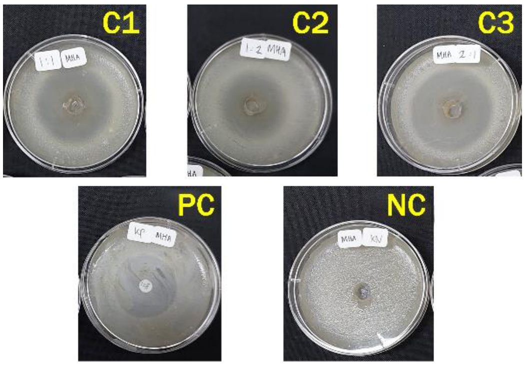

Figure 3 Inhibition Zone Results of Antibacterial Activity Test

The average inhibition zone was calculated and classified according to Table 1[1].

Table 1. Classification of Bacterial Growth Inhibition Strength

Diameter of the Inhibition Zone	Inhibition Strenght
> 20 mm	Strong
16 – 20 mm	Medium
10 – 15 mm	Weak
< 10 mm	None

Based on the average results of the inhibition zone diameter, a classification of the antibacterial activity against *Staphylococcus aureus* shown in Table 2.

Table 2. Summary of Inhibition Zone in the Antibacterial Activity Test of Combined Garlic and Red Ginger Extracts

Treatment Group	Average ± SD (mm)	Inhibition Strenght
C1	30,40 ± 2,0670	Strong
C2	24,51 ± 2,1695	Strong
C3	37,67 ± 1,8291	Strong
PC (+)	28,79 ± 1,9581	Strong
NC (-)	0,00 ± 0,0000	Strong

Note:
C1: Extracts Combine Ratio 1:1
C2: Extracts Combine Ratio 1:2
C3: Extracts Combine Ratio 2:1
PC (+): Positive Control (Amoxicillin)
NC (-): Negative Control (Aquadest)

The antibacterial activity possessed by various types of plants is considered an alternative to antibiotics to avoid the effects of antibiotic resistance. Various compounds have been indicated to possess antibacterial activity, such as phenolic compounds, flavonoids, and sulfide derivatives [8,9].

The homogeneity test and normality test were conducted as prerequisites for the ANOVA test. The normality test resulted in a P value >0.05, indicating that the data is normally distributed. The homogeneity test resulted in a P value of 0.074>0.05, indicating that the data is homogeneous.

An ANOVA test is conducted to determine whether there is a difference in results between the test groups. The P value shows 0.000<0.05, indicating that there is a significant difference between the test groups. Then a post hoc Tukey's HSD test was conducted (Table 3). Based on the results of the post hoc test, it was found that almost all test groups had significant differences with each other, except for the C1 test group, which did not show a significant difference from the positive control group, indicated by a p-value of 0.625 > 0.05.

Table 3. Post Hoc Analysis of Antibacterial Activity Test

	C1	C2	C3	PC	NC
C1		0,000*	0,000*	0,625*	0,000*
C2	0,000*		0,000*	0,010*	0,000*
C3	0,000*	0,000*		0,000*	0,000*
PC	0,625	0,010*	0,000*		0,000*
NC	0,000*	0,000*	0,000*	0,000*	

*The mean difference is significant
C1: Extracts Combine Ratio 1:1
C2: Extracts Combine Ratio 1:2
C3: Extracts Combine Ratio 2:1
PC (+): Positive Control (Amoxicillin)
NC (-): Negative Control (Aquadest)

Based on the results of the ANOVA test, the test group had significantly different results compared to the negative control group. In the post hoc test results, the C1 test group (extracts combine ratio 1:1) did not show a statistically significant difference from the positive control group, indicating that the combination extract group with a 1:1 ratio has an inhibitory power comparable to 25 μg of amoxicillin against the growth of *Staphylococcus aureus*. Based on these results, the use of a combination of garlic and red ginger extracts in a 1:1 ratio can be considered as an antibacterial substitute comparable to 25 μg of amoxicillin against the growth of *Staphylococcus aureus*.

The test group C3 (extracts combine ratio 2:1) has the greatest inhibitory effect on the growth of *Staphylococcus aureus*. This means that the antibacterial activity of garlic is greater than that of red ginger. The results of this study are in line with the findings of previous research[6] which show that garlic has a greater growth inhibition capacity compared to red ginger. This may be due to the fact that garlic contains organosulfur compounds that are water-soluble, whereas the active compounds in red ginger, which have antibacterial activity, are oil-soluble and less water-soluble [4].

The difference in compounds that are believed to affect antibacterial activity between garlic and red ginger are organosulfur compounds such as allicin and alliin, as well as sulfide derivative compounds like diallyl disulfide and diallyl trisulfide[20]. The compound allicin is the most effective antibiotic compound found in garlic[20]. The compound allicin has a broad-spectrum antimicrobial effect influenced by its thiol structure, which allows easy penetration of cell membranes and subsequently inactivates various intracellular and extracellular targets[21]. Generally, zingiberene, which is a terpenoid compound in red ginger, is the component with the most active antibacterial activity. However, zingiberene is an oily compound that is poorly extracted into aqueous solvents [4].

In this study, it can also be observed that the inhibition zone results were greater compared to the studies by Rahmatika and Oktaria[6] and Akullo *et al.*[4], indicating a synergistic effect between garlic and red ginger extracts compared to when each extract was used separately.

The limitation of this study is the use of a high extract concentration, namely 25 mg/mL, and the amount of extract used, which is 150 µl. Although this study shows strong inhibitory activity, the concentration is considered too high compared to the standard concentrations used in most previous studies. Nevertheless, the difference in solvents used can be a consideration because the suitability of the solvent also affects the antibacterial effectiveness produced [4,7].

CONCLUSION

The combination of garlic extract (*Allium sativum*) and red ginger extract (*Zingiber officinale* var. *Rubrum*) with an aqueous solvent has antibacterial activity against the growth of *Staphylococcus aureus*. The combination of extracts in a 2:1 ratio has the highest antibacterial effectiveness, while the combination of extracts in a 1:1 ratio has antibacterial effectiveness comparable to 25 µg of amoxicillin against the growth of *Staphylococcus aureus*.

It is hoped that in future research, a smaller concentration of extract, namely <1000 µg/ml, can be used, and each extract can be tested individually as well as using several levels of extract concentration to ensure the effectiveness of using garlic and red ginger extracts with water as the solvent.

FUNDING

This research did not receive any external funding.

REFERENCES

1. Amalia N, Pratiwi Hasanuddin A, Studi Analis Kesehatan P, Panrita Husada Bulukumba S. Uji Antibakteri Ekstrak Jahe emprit (Zingiber officinale) Terhadap Pertumbuhan Bakteri Staphylococcus aureus Dengan Metode Difusi Cakram. *Nuhela J Inj*. 2022;1(1):31–39. https://journal.pdpt-nusantara.org/injury
2. Azkiyah SZ. Pengaruh Uji Antibakteri Ekstrak Rimpang Jahe Terhadap Pertumbuhan Staphylococcus Aureus Dan Escherichia Coli Secara In Vitro. *J Farm Tinctura*. 2020;1(2):71–80. doi:10.35316/tinctura.v1i2.1003
3. Tandanu E, Rambe PW. Efektivitas Antibakteri Ekstrak Rimpang Jahe Merah (Zingiber officinale var rubrum) Terhadap Pertumbuhan Bakteri Staphylococcus aureus Secara In Vitro. *Prima Med J*. 2020;5(1).
4. Akullo JO, Kiage B, Nakimbugwe D, Kinyuru J. Effect of aqueous and organic solvent extraction on in-vitro antimicrobial activity of two varieties of fresh ginger (Zingiber officinale) and garlic (Allium sativum). *Heliyon*. 2022;8(9):e10457. doi:10.1016/j.heliyon.2022.e10457

5. Aernan PT, Odo JI, Ado BV, Epor JO. Antibacterial Activities of Three Commonly Used Spices; Garlic (Allium moly), Ginger (Zingiber officinale), and Turmeric (Curcuma longa) Extracts on Bacterial Isolates From Spoilt Tomatoes (Lycopersicumesculatum). *IPHOPEN*. 2023;02(09):1–9.
6. Rahmatika D, Oktaria S. Perbedaan Uji Daya Antibakteri Jahe Merah (Zingiber Officinale Var. Rubrum) Dan Bawang Putih (Allium Sativum) Terhadap Pertumbuhan Bakteri Staphylococcus Aureus. *J Kedokt Ibnu Nafis*. 2021;10(1):1–8. doi:10.30743/jkin.v10i1.94
7. Awan UA, Ali S, Shahnawaz AM, et al. Biological activities of Allium sativum and Zingiber officinale extracts on clinically important bacterial pathogens, their phytochemical and FT-IR spectroscopic analysis. *Pak J Pharm Sci*. 2017;30(3):729–745.
8. Batiha GES, Beshbishy AM, Wasef LG, et al. Chemical Constituents and Pharmacological Activities of Garlic (Allium sativum L.): A Review. *Nutr 2020, Vol 12, Page 872*. 2020;12(3):872. doi:10.3390/NU12030872
9. Parham S, Kharazi AZ, Bakhsheshi-Rad HR, et al. Antioxidant, Antimicrobial and Antiviral Properties of Herbal Materials. *Antioxidants 2020, Vol 9, Page 1309*. 2020;9(12):1309. doi:10.3390/ANTIOX9121309
10. Majewski M. Allium sativum: facts and myths regarding human health. *Rocz Państwowego Zakładu Hig*. 2014;65(1):1–8.
11. Zhang S, Kou X, Zhao H, Mak KK, Balijepalli MK, Pichika MR. Zingiber officinale var. rubrum: Red Ginger's Medicinal Uses. *Mol 2022, Vol 27, Page 775*. 2022;27(3):775. doi:10.3390/MOLECULES27030775
12. Sari D, Nasuha A. Kandungan Zat Gizi, Fitokimia, dan Aktivitas Farmakologis pada Jahe (Zingiber officinale Rosc.): Review. *Trop Biosci J Biol Sci*. 2021;1(2):11–18. doi:10.32678/TROPICALBIOSCI.V1I2.5246
13. Sharma S, Shukla MK, Sharma KC, et al. Revisiting the therapeutic potential of gingerols against different pharmacological activities. *Naunyn Schmiedebergs Arch Pharmacol*. 2023;396(4):633. doi:10.1007/S00210-022-02372-7
14. Guo Y, Song G, Sun M, Wang J, Wang Y. Prevalence and Therapies of Antibiotic-Resistance in Staphylococcus aureus. *Front Cell Infect Microbiol*. 2020;10. doi:10.3389/FCIMB.2020.00107
15. Nabi SG, Iqbal M, Aslam A, Khadim SF, Ghafoor S, Majeed F. Antimicrobial Activity of Zingiber officinale (Ginger) and Allium sativum (Garlic) Against Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. *J Sharif Med Dent Coll Lahore, Pakistan*. 2022;8(1):26–30.
16. Sikrodia R, Chhabra D, Audarya SD, Sharda R. Efficacy of Antimicrobial Activity of Aqueous Extract of Garlic (Allium sativum) and Ginger (Zingiber officinale) against Different Bacterial Species. *Int J Curr Microbiol Appl Sci*. 2018;7(09):3021–3025. doi:10.20546/ijcmas.2018.709.376
17. Munira M, Sarfica D, Zakiah N, Sri mulyani N. Aktivitas Antibakteri Kombinasi Ekstrak Bawang Putih (Allium sativum Linn), Jahe (Zingiber officinale Roscoe) Dan Kunyit (Curcuma domestica Val) Terhadap Pertumbuhan Bakteri Staphylococcus aureus Dan Escherichia coli. *J Ilm Farm SIMPLISIA*. 2021;1(2):68–73. doi:10.30867/jifs.v1i2.76
18. Lajoie L, Fabiano-Tixier AS, Chemat F. Water as Green Solvent: Methods of Solubilisation and Extraction of Natural Products—Past, Present and Future Solutions. *Pharmaceuticals*. 2022;15(12):1507. doi:10.3390/ph15121507
19. Ibrahim N, Kebede A. In vitro antibacterial activities of methanol and aqueous leave extracts of selected medicinal plants against human pathogenic bacteria. *Saudi J Biol Sci*. 2020;27(9):2261–2268. doi:10.1016/j.sjbs.2020.06.047
20. Block E. *Garlic and Other Alliums The Lore and The Science*. Royal Society of Chemistry; 2015.
21. Murad F, Rahman A ur, Bian K. *Herbal Medicine: Back to the Future: Volume 4, Infectious Diseases*. 4 Ed. Bentham Science Publishers; 2021.