MAGNA MEDIKA
Berkala Ilmiah Kedokteran dan Kesehatan
e-ISSN 2774-2318
p-ISSN 2407-0505

Research Article
MAGNA MEDIKA
Berkala Ilmiah Kedokteran dan Kesehatan
Journal Page: https://jurnal.unimus.ac.id/index.php/APKKM

Effectiveness of 96% Ethanol Extract of Clove Leaves (*Syzygium aromaticum* (L.) Merr & Perry) as an Antibacterial Agent Against *Staphylococcus epidermidis* And *Providencia stuartii*

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Article Info

Article history:

Received 03 August 2021
Revised 03 February 2022
Accepted 18 February 2022
Available online 01 August 2022

Keywords:

Clove Leaves; antibacterial; *Staphylococcus epidermidis; Providencia stuartii*

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How to cite this article:

1. Dewi L M, Nafi'ah M Q. Effectiveness of 96% Ethanol Extract of Clove Leaves (*Syzigium aromaticum* (L.) Merr & Perry) as an Antibacterial Agent Against *Staphylococcus epidermidis* and *Providencia stuartii*. MAGNA MEDIKA Berk Ilm Kedokt dan Kesehat. 2022; 9(2):139-146

Abstract

Background: The incidence of antibiotic resistance as a consequence of antibiotic abuse becomes a barrier to accessing effective care. Bacteria's ability to form biofilms is an internal factor that can prevent contact between bacteria and antibiotic agents. The use of plant extracts as an antibiotic is thought to be a promising solution, and one of them is the clove leaves (Syzygium aromaticum (L.) Merr & Perry).

Objective: This study aims to determine the effectiveness of 96% ethanol extract of clove leaves as an antibacterial agent against Staphylococcus epidermidis and Providencia struartii

Methods: This was an experimental analytic study with a post-test-only control group design method on four treatment groups with a concentration of 2.5%, 5%, 10%, and 20%, as well as a positive and negative control group. Data were analyzed by the Kruskal Wallis test and Post Hoc Mann Whitney test.

Results: The Kruskal Wallis test's result on both germs obtained a p-value <0.05. Post-Hoc Mann Whitney test results, for both germs, the comparison of the data on each concentration to the negative control obtained a p-value <0.05.

Conclusion: 96% ethanol extract of clove leaves has activity as an antibacterial agent against Staphylococcus epidermidis and Providencia stuartii.

e-ISSN 2774-2318
p-ISSN 2407-0505

INTRODUCTION

Several antibiotic resistance has occurred worldwide. Infections caused by bacterial resistance have also been reported in developed countries such as the United States, with a prevalence of 2.8 million people per year, while 35,000 of them died [1,2]. Easy over-the-counter access to antibiotics also leads to an increase in antibiotic consumption. This aggravates the problem of antimicrobial resistance in low- and middle-income countries, including Indonesia[3]. Besides, bacterial ability to produce biofilms also plays a role in the occurrence of antibiotic resistance[4].

The ability of bacteria to form biofilms is an internal factor that may prevent contact between bacteria and antibiotic agents, facilitating the development of antibiotic resistance[4]. *Staphylococcus epidermidis* is a bacteria that often causes opportunistic infections in patients who use medical implants and is the most common cause of nosocomial sepsis[5]. Nosocomial infections can also occur in urinary tract infections due to prolonged use of catheters. The primary etiology of this type of infection is *Providencia stuartii* [6].

Alternative medicine to overcome several challenges related to antibiotic resistance is increasingly being investigated. WHO also recommends traditional medicine, including medicinal plants, if it has been proven safe and effective, it can be incorporated into the national health system[4,7]. One of Indonesia's native plants that can be used as an antibiotic is the clove plant (*Syzygium aromaticum* (L.) Merr & Perry). However, clove leaves are more often used as a component in the manufacture of cigarettes and eventually become waste[8].

METHODS

This is an experimental analytic study with a post-test-only control group design method, with ethical clearance number 2438/A.1/KEPK-FKUMS/X/2019. We use colonies of *Staphylococcus epidermidis* and *Providencia stuartii,* obtained from the Microbiology Laboratory of the Faculty of Medicine, Universitas Muhammadiyah Surakarta, which were taken by purposive sampling method. The research group consisted of 4 treatment groups with a concentration of 2.5%; 5%; 10%; and 20%, a positive control group and a negative control group, for each bacteria studied. The minimum number of samples in each group for each bacterium studied was 6, determined by the Federer formula (n-1)(t-1)>15. Statistical analysis was carried out by the Kruskal Wallis test and followed by the Post Hoc Mann Whitney test. The positive control used was chloramphenicol for *Staphylococcus epidermidis* and ciprofloxacin for *Providencia stuartii.* At the same time, the negative control was Na-CMC 0.1% for both groups.

RESULTS

The results of *Staphylococcus epidermidis* and *Providencia stuartii* inoculation on Muller Hinton media that have been given clove-leaf extract in various concentrations are summarized in the following Table 1:

e-ISSN 2774-2318
p-ISSN 2407-0505

Table 1. Diameter of Bacterial Inhibitory Zone on Various Concentrations of Clove Leaf Extract

Group	Diameter of Bacterial Inhibitory Zone (mm) on each Replication						Mean
	1	2	3	4	5	6	
Staphylococcus epidermidis							
1	20	15	15	20	20	15	17.5
2	20	15	25	25	25	20	21.7
3	25	25	25	20	25	20	23.3
4	30	25	25	20	20	25	24.17
5	15	10	15	15	20	10	14.17
6	0	0	0	0	0	0	0
Providencia stuartii							
1	0.80	0.75	0.70	0.80	0.70	0.75	0.75
2	1.20	1.00	1.00	0.90	0.80	0.90	0.96
3	1.40	1.40	1.40	1.30	1.30	1.20	1.3
4	2.00	1.70	1.60	1.50	1.50	1.60	1.65
5	37	36	35	35	33	32	34.7
6	0	0	0	0	0	0	0

Note:
Group 1: Clove leaf extract with a concentration of 2,5%
Group 2: Clove leaf extract with a concentration of 5%
Group 3: Clove leaf extract with a concentration of 10%
Group 4: Clove leaf extract with a concentration of 20%
Group 5: Positive control
Group 6: Negative control

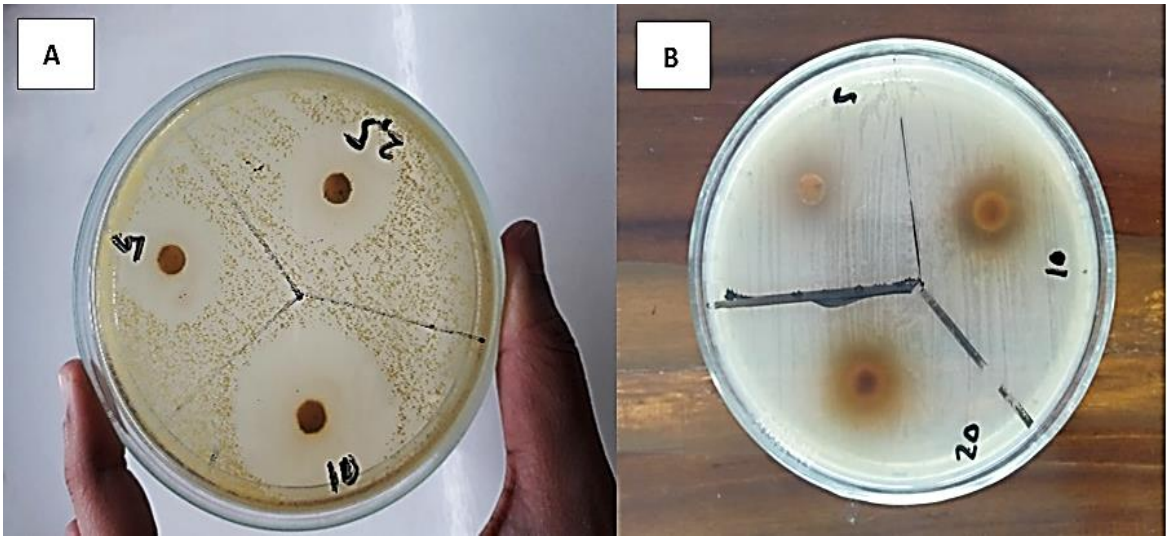

Figure 1. Inhibition Zone in *Staphylococcus epidermidis* (A) and *Providencia stuartii* (B)

doi: 10.26714/magnamed.9.2.2022.139-146
9 (2) August 2022

e-ISSN 2774-2318
p-ISSN 2407-0505

Table 2. The Result of Post Hoc Mann Whitney Test on *Staphylococcus epidermidis*

Group	1	2	3	4	5	6
1		0.073	0.011	0.011	0.116	0.002
2	0.073		0.465	0.337	0.016	0.002
3	0.011	0.465		0.715	0.005	0.002
4	0.011	0.337	0.715		0.005	0.002
5	0.116	0.016	0.005	0.005		0.002
6	0.002	0.002	0.002	0.002	0.002	

Table 3. The Result of Post Hoc Mann Whitney Test on *Providencia stuartii*

Group	1	2	3	4	5	6
1		0.006	0.003	0.004	0.004	0.002
2	0.006		0.004	0.004	0.004	0.002
3	0.003	0.004		0.004	0.004	0.002
4	0.004	0.004	0.004		0.004	0.002
5	0.004	0.004	0.004	0.004		0.002
6	0.002	0.002	0.002	0.002	0.002	

Note:
Group 1: Clove leaf extract with a concentration of 2,5%
Group 2: Clove leaf extract with a concentration of 5%
Group 3: Clove leaf extract with a concentration of 10%
Group 4: Clove leaf extract with a concentration of 20%
Group 5: Positive control
Group 6: Negative control

DISCUSSION

The data in Table 1 shows an increase in the diameter of the inhibition zone of each bacterium along with the increase in the concentration of the clove-leaf extract. Thus, it can be interpreted that the greater concentration of the extract used, the more compelling content of active ingredients in the extract so as to increase the effect of each active ingredient.

Kruskal Wallis test on the two bacteria tested obtained a p-value <0.05, which means that there are at least two groups that have significant differences in the diameter of the inhibition zone formed. Based on the Post-Hoc Mann Whitney test on *Staphylococcus epidermidis* and *Providencia stuartii,* the results of the comparison of data on each concentration to the negative control obtained a p-value <0.05. These results indicate that there is a significant difference in the size of the inhibition zone, and in other words, 96% ethanol extract of clove leaves is effective as an antibacterial agent against the two bacteria. This is also in line with several other previous studies which state that clove leaves have antibacterial activity against certain bacte-ria[9,10,11].

Clove leaves contain several active substances with antibacterial properties, such as eugenol,

e-ISSN 2774-2318
p-ISSN 2407-0505

flavonoids, tannins, saponins, and triterpenoids[9,10,11,12,13]. Eugenol appears as a yellow oil with a characteristic clove odor and a spicy taste[9,10]. Eugenol is classified as a phenolic compound that can inhibit bacterial growth. Eugenol lyses bacterial cell membranes, resulting in cell leakage. Thus, the essential metabolites needed by bacteria will be wasted. Eugenol also causes denaturation and inhibition of protein and nucleic acid synthesis. This may lead to disruption of the metabolic function of bacteria, then cause bacterial death[14]. Flavonoids and tannins work as antibacterial by forming complex compounds against extracellular proteins that disrupt the integrity of the bacterial cell membrane without being repaired[12,13,15]. Triterpenoids are secondary metabolites that act on porins on the outer membrane of the bacterial cell wall. Triterpenoids from strong polymeric bonds cause damage to porins so that bacterial cells will lack nutrients, and their growth will be stunted or die[12,13,17]. Tannins form covalent and non-covalent bonds with proteins or bacterial polysaccharides, thereby destroying the integrity of the bacterial cell wall, inhibiting their growth, and inhibiting protease activity[16,17].

However, the data on the *Staphylococcus epidermidis* group showed that the average size of the inhibition zone of the clove-leaf extract concentration was more significant than the size of the inhibition zone on the positive control. In contrast, the data on the *Providencia stuartii* group showed the opposite. Therefore, it can be interpreted that clove-leaf extract has a more substantial antibacterial effect than chloramphenicol but is weaker than ciprofloxacin.

Staphylococcus epidermidis colonizes on the epidermis layer of the skin, which has the main constituent in the form of keratinocytes. In the presence of these colonies, pattern recognition receptors (PRRs) such as TLR2 on keratinocytes bind to soluble proteins secreted by *Staphylococcus epidermidis* to stimulate the production of antimicrobial peptides. Colonies of *Staphylococcus epidermidis* induce a CD4+ FOXP+ Treg response critical in immunetolerance to *Staphylococcus epidermidis* as a commensal organism. Unintact skin conditions allow *Staphylococcus epidermidis* to enter the bloodstream and cause bacteremia and acute sepsis. The body's immune response to sepsis highly depends on neutrophils, the comple-ment system, and the immune response to biofilm-associated infections is often ineffec-tive because biofilms inhibit phagocytic mechanisms by PMNs and macrophages. The production of anti-inflammatory cytokines such as IL-13 also tends to increase, while the secretion of proinflammatory cytokines such as IL1-beta, IL-12, and IFN-gamma is deficient[5,17,18].

Providencia stuartii may produce biofilm cells resistant to calcium, magnesium, and high concentrations of urea and show the ability to grow over a wide range of pHs. Omp-Pst2 porin sheds light on its role in the early stages of growth and the adaptation to high concentrations of urea and varying pH[6,19].

In addition to looking at the level of bacterial pathogenicity, primary health care services need to be more active in tackling antimicrobial resistance. Revitalizing primary health care in low- and middle- income countries are needed to incorporate more vital systems to prevent and manage infections in the community and health facilities. Community involvement and empowerment are critical to effective behavior change that can prevent and

e-ISSN 2774-2318
p-ISSN 2407-0505

manage common diseases without unnecessarily using antimicrobials[4, 20].

CONCLUSION

The 96% ethanol extract of clove leaves contained active compounds that could act as antibacterial agents against *Staphylococcus epidermidis* and *Providencia stuartii* bacteria and were more potent than chloramphenicol but weaker than ciprofloxacin.

REFERENCES

1. Ventola, Lee, C. 2015. The Antibiotics Resistance Crisis. *Pharmacy and Therapeutics*. Volume 40. No. 04. April 2015. Pp 277-83. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4378521/pdf/ptj4004277.pdf
2. CDC. 2019. *Antibiotic/Antimicrobial Resistance. Biggest Threats and Data*. 2019 Antibiotic Resistance Threats Report. https://www.cdc.gov/drugresistance/biggest-threats.html
3. Hall, C., W., dan Mah, T-F. 2017. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. *FEMS Microbiology Reviews*. Vol.41. No. 3. pp. 276-301
4. World Health Organization. 2018. *Antimicrobial Resistance and Primary Health Care: Technical Series on Primary Health Care*. World Health Organization. https://www.who.int/docs/default-source/primary-health-care-conference/amr.pdf?sfvrsn=8817d5ba_2
5. Nguyen, Thuan, H. Park, Matthew, D. Otto, Michael. 2017. Host Response to Staphylococcus epidermidis Colonization and Infections. *Front. Cell. Infect. Microbiol.* https://www.frontiersin.org/articles/10.3389/fcimb. 2017.00090 /full
6. Khatib, M. E., Tran, Q-T., Nasrallah, C., Lopes, J., Bolla, J-M., Vivaudou, M., Pages, J-M., Colletier, J-P. 2017. Providencia stuartii form biofilms and floating communities of cells that display high resistance to environmental insults. *PLoSONE*. https://journals.plos.org/plosone/article/file?id =10.13 71/journal.pone.0174213&type=printable
7. Marasini, Bishnu, P., Baral, P., Aryal, P., Ghimire, Kashi, R., Neupane, S., Dahal, N., Singh, A., Ghimire, L., Shrestha, K. 2015. Evaluation of Antibacterial Activity of Some Traditionally Used Medicinal Plants against Human Pathogenic Bacteria. *BioMed Research International*. Hindawi Publishing Corporation. http://downloads.hindawi.com/journals/bmri/2015/265425.pdf
8. Wahyulianingsih. Handayani, Selpida. Malik, Abd. 2016. Penetapan Kadar Flavonoid Total Ekstrak Daun Cengkeh (Syzygium aromaticum (L.) Merr & Perry). *Jurnal Fitofarmaka Indonesia*. Volume 3. No. 2. http://www.jurnal.farmasi.umi.ac.id/index.php/fitofarmakaindo/article/view/221
9. Jimoh, S., O., Arowolo, L., A., Alabi, K., A., 2017. Phytochemical Screening and Antimicrobial Evaluation of Syzygium aromaticum Extract and Essential oil. *Int.J.Curr.Microbiol.App.Sci*. Volume 6. No.7. Pp: 4557-67. https://www.researchgate.net/profile/Kazeem_Al-

e-ISSN 2774-2318
p-ISSN 2407-0505

abi/publication/3191 04781 Phytochemical Screening and Antimicrobial Evaluation of Syzygium aromaticum Extract and Essential oil/links/5a43ac02458515f6b052bfc1/Phytochemical-Screening-and-Antimicrobial-Evaluation-of-Syzygium-aromaticum-Extract-and-Essential-oil.pdf

10. da Silva, F., F., M., Monte, F., J., Q., de Lemos, T., L., G., do Nascimento, P., G., G., Costa, A., K., M., de Paiva, L., M., M. 2018. Eugenol derivatives: synthesis, characterization, and evaluation of antibacterial and antioxidant activities. *Chemistry Central Journal*. 2018 Apr 3;12(1):34. doi: 10.1186/s13065-018-0407-4.
11. Yadav, M.K., Chae, S.W., Im, G.J., Chung, J.W., Song, J.J. 2015. Eugenol: A Phyto-Compound Effectiveagainst Methicillin-Resistant and Methicillin-Sensitive Staphylococcus aureus Clinical Strain Biofilms. PLoS ONE 10(3): e0119564.
12. Wankhede, T., B. 2015. Evaluation of antioxidant and antimicrobial activity of the Indian clove Syzygium aromaticum L. Merr. & Perr. *Int. Res. J. of Science & Engineering*. Vol. 3 (4): 166-172. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.735.7897&rep=rep1&type=pdf
13. Hosseini, M.; Jamshidi, A.; Raeisi, M.; Azizzadeh, M. 2019. The Antibacterial and Antioxidant Effects of Clove (Syzygium aromaticum) and Lemon Verbena (Aloysia citriodora) Essential Oils. *Journal of Human, Environment, and Health Promotion*. Volume 5. No. 2. Pp 86-93. http://zums.ac.ir/jhehp/browse.php?a_id=195&sid=1&slc_lang=en&ftxt=1
14. Raja, M. R. C., Srinivasan, V., Selvaraj, S., Mahapatra, S. K. 2015. Versatile and Synergistic Potential of Eugenol: A Review. *Pharmaceutica Analytica Acta*. Volume 6. No. 5. https://pdfs.semanticscholar.org/b56e/d9cc32095eca979a6f79473f35c708ed9a95.pdf
15. Farhadi, F., Khameneh, B., Iranshahi, M., Iranshahy, M. 2018. Antibacterial activity of Flavonoid and Their Structure-Activity Relationship: An Update Review. *Phytotheraphy Research*. 2019; 33:13–40 https://onlinelibrary.wiley.com/doi/epdf/10.1002/ptr.6208
16. Othman L, Sleiman A, Abdel-Massih R.M., 2019. Antimicrobial Activity of Polyphenols and Alkaloids in Middle Eastern Plants. *Front. Microbiol.* 10:911. https://www.frontiersin.org/articles/10.3389/fmicb.2019.00911/full
17. Manner, S., Skogman, M., Goeres, D., Vuorela, P., Fallarero, A. 2013. Systematic Exploration of Natural and Synthetic Flavonoids for the Inhibition of Staphylococcus aureus Biofilms. *Int. J. Mol. Sci.*, *14*(10), 19434-19451 **Kesalahan! Referensi hyperlink tidak valid.**
18. Büttner, H., Mack, D., Rohde, H. 2015. Structural basis of Staphylococcus epidermidis biofilm formation: mechanisms and molecular interactions. *Front. Cell. Infect.* Published online 2015 Feb 17. doi: 10.3389/fcimb.2015.00014
19. Wie, Seong-Hoen. 2015. Clinical Significance of Providencia Bacteremia or Bacteriuria. *Korean J Intern Med.* https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4351322/pdf/kjim-30-167.pdf

e-ISSN 2774-2318
p-ISSN 2407-0505

20. Kemenkes RI. 2005. Antimicrobial Resistance, Antibiotic Usage and Infection Control: A Self-Assessment Program for Indonesian hospitals. http://apps.who.int/medicinedocs/documents/s18010en/s18010en.pdf