EFFECTIVENESS TEST COMBINATION OF ROSELLE FLOWER (*HIBISCUS SABDARIFFA L*) AND GREEN TEA (*CAMELLIA SINENSIS*) EXTRACT WITH VANCOMYCIN ANTIBIOTIC AGAINST METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) BACTERIA IN VITRO

Mahliga Harum Hanindita[1], Irma Suswati[2]
[1] Medical Faculty Students, University of Muhammadiyah Malang
[2] Lecturer in Microbiology Departement of Medical Faculty, University of Muhammadiyah Malang

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ABSTRACT

Background: MRSA is a strain of *Staphylococcus aureus* bacteria that is resistant to methicillin antibiotics. These bacteria can cause various infections of the skin, soft tissue, Etc. The incidence of resistance to MRSA is currently increasing significantly, including in Indonesia. Inappropriate use of vancomycin as MRSA therapy makes resistance to *Staphylococcus aureus* bacteria and causes a high risk of complications. **Purpose:** This research aims to compare the inhibitory effect between the combination of roselle flower extract (*Hibiscus sabdariffa* L) and green tea leaf (*Camellia sinensis*) with vancomycin antibiotics against MRSA bacteria. **Methods:** True experimental: post-test only control design, with the Kirby Bauer disc diffusion method with extract concentrations of 100%, 50%, 25%, and 12.5%, which will then be determined the Minimum Inhibitory Content (MIC). Statistical data analysis used One Way ANOVA and Post Hoc Bonferroni tests, and descriptive analysis used the Greenwood classification (1995). **Results:** MIC combination of roselle and green tea extract against MRSA bacteria's growth was 12.5%. One Way ANOVA test showed significant differences (p=0.000) in the inhibition zone of the combination of roselle and green tea extract against MRSA bacteria growth. Post-Hoc Bonferroni test showed no significant difference (p=1,000) between roselle and green tea extract at a concentration of 12,5% and vancomycin 30µg, and there were significant differences (p=0,000) at a concentration of 100%, 50%, and 25% against MRSA bacteria growth and vancomycin 30µg. **Conclusion:** The combination of roselle and green tea extract has a stronger sensitivity than vancomycin antibiotics to MRSA bacteria's in vitro

Corresponding Author:

Irma Suswati
Medical Faculty University of Muhammadiyah Malang
Jl. Bendungan Sutami No 188A, Malang, East Java Indonesia

ORCID : https://orcid.org/0000-0002-9934-3297

Email: irma@umm.ac.id

1. INTRODUCTION

Methicillin-Resistant Staphylococcus aureus or MRSA is a strain of *Staphylococcus aureus* bacteria that is resistant to methicillin antibiotics. This bacterial strain also has cross-resistance to all beta-lactam antibiotics

such as penicillin, ampicillin, and amoxicillin [1]. These bacteria can cause various infectious diseases, such as skin and soft tissue infections, sepsis, endocarditis, Etc [2].

The incidence of antimicrobial resistance also causes delays ineffective treatment for infectious diseases. Patients often experience failure in the right treatment and generate a high risk of complications and death [3]. The prevalence of MRSA infections has increased in sub-Saharan Africa, Australia, Latin America (90%), and India (47%) [4]. MRSA prevalence in clinical isolates Dr. Saiful Anwar, Malang City, there was a reasonably high increase. There was a change in the pattern of sensitivity to several antibiotics, namely 37% in 2014 using a pus specimen trial [5].

One of the drugs of choice to treat MRSA infections is the antibiotic vancomycin. However, the vancomycin antibiotic's excessive and inappropriate use makes this antibiotic-resistant to Staphylococcus aureus bacteria [6]. These antibiotics can cause side effects such as nephrotoxicity and ototoxicity, so it is necessary to adjust the dosage based on inflammation level [2].

Roselle flowers and green tea leaves contain high flavonoids and have potential ingredients as antibacterial, but research using a combination of these two natural ingredients is still very rare. Based on this background, researchers are interested in researching the antimicrobial effect of various roselle flower extract (*Hibiscus sabdariffa* L) and green tea (*Camellia sinensis*) with an antibiotic often used in cases of MRSA, namely vancomycin antibiotics using MRSA bacteria.

2. METHOD

This research is an experimental study using a combination of roselle flower extract (*Hibiscus Sabdariffa* L) and Green Tea (*Camellia Sinensis*) with vancomycin antibiotics against the growth of Methicillin-Resistant Staphylococcus aureus (MRSA) bacteria in vitro, with true experimental method: post-test-only control design using Kirby Bauer disc diffusion and used six treatments in this study with a concentration of 12.5%, 25%, 50%, and 100%, positive control (vancomycin 30µg), and negative control (DMSO 10%). This research was conducted in September 2020, starting with combining roselle flowers and green tea and research activities in the Laboraturium Biomedicine Faculty of Medicine, University of Muhammadiyah Malang.

The tools used were Petri dishes, disc paper, sterile ose, sterile cotton stick, incubator, autoclave, calipers, laminar airflow, tweezers, beaker glass, stirring rod, refrigerator, oven, micropipette, blue tip, test tube, stirrer, rack coloring, Erlenmeyer, bunsen, Eppendorf, analytical balance, rotary evaporator, standard 0.5 mc Farland BaCl2 + H2SO4 0.1m. The materials used were ethanol extract of a combination of roselle flowers and green tea, MRSA bacteria, Mueller Hinton Agar (MHA), 30g vancomycin antibiotic discs, blank disks (blank paper discs), sterile distilled water, 10% Dimethyl Sulfoxide (DMSO), and emersion oil.

The research began with the preparation of each extract, namely roselle flowers and green tea. 500gr of dried simplicia in roselle flowers soaked using 96% ethanol as much as 1L in a closed vessel for 24 hours. After that, the soaking results are filtered, and the filtrate obtained is concentrated using a rotary evaporator to 1/3 part, then weighed on an analytical balance. Green tea did the same process, where each extract was diluted with 96% ethanol to a concentration of 50%, 25%, and 12.5%.

The zone of inhibition was then observed and measured by the Kirby Bauer disc diffusion method with four repetitions according to the Resource Equation Approach formula to see the antibacterial activity produced from a combination of roselle flower extract and green tea on the growth of MRSA bacteria [7]. Then immersed the blank disk for 1 minute and then streaked the bacteria on the entire surface so that MHA. Then place the blank disk containing the agar's extract combination, which has been streaked and incubated for 24 hours. The inhibition zone formed around the blank disk is from the antibacterial activity of the combined extract of roselle flowers and green tea.

3. RESULTS AND DISCUSSION

This study used four kinds of extract concentrations, namely 12.5%, 50%, and 100%, with four repetitions. The antibacterial activity of the combination of roselle flower and green tea extract against MRSA is shown in Table 1. Table 1 shows that the diameter of the inhibition zone formed can be seen starting from a concentration of 12.5%.

Table 1. Diameter of Inhibition Zone Effectiveness of Combination of Roselle Flower Extract and Green Tea with Vancomycin Antibiotic Against MRSA Bacteria

Combined concentration roselle and green tea extract (%)	Repetition				Average (mm)
	I (mm)	II (mm)	III (mm)	IV (mm)	
100%	24	24,5	24,5	23	24
50%	19,5	24	21	20,5	21,25

25%	20	19	19,5	18,5	19,25
12,5%	16,1	16,5	16,5	17	16,53
Control + (Vankomisin 30µg)	16,8	16,1	16,5	16,4	16,45
Control – (DMSO 10%)	0	0	0	0	0

(Primary Source, 2020)

Based on Table 1, it was found that the higher the concentration of the combination of roselle flower extract (*Hibiscus sabdariffa* L) and green tea (*Camellia sinensis*), the greater the resulting inhibition of MRSA bacterial growth. Based on descriptive analysis of research data using the Greenwood classification (1995) [8], the author's study showed that the combination of roselle and green tea extracts at a concentration of 12.5% and 25% with an inhibition zone of 16.53mm and 19.25mm had an antibacterial activity that was comparable to vancomycin 30g, which was included in the moderate category (intermediate). At concentrations of 50% and 100%, the inhibition zones were 21.25mm and 24mm, included in the strong criteria (sensitive).

The difference in the results obtained is known due to the use of the ethanol solvent used in the author's study, where ethanol is a universal polar solvent that can dissolve almost all secondary metabolite compounds that are polar and nonpolar and have better absorption ability with low toxicity values compared to organic solvents [9]. Besides, it is known that the higher the ethanol concentration used, the higher the ability to attract bioactive compounds in the extracted material so that more bioactive compounds are extracted [9,10]. Another difference shown from the results of the inhibition zone of 100% concentration in the author's study with previous research is due to differences in viscosity extract level. The author's study used high concentrations of two natural ingredients, such as a combination of roselle flower extract and green tea. This combination will produce a level of viscosity and high density, which affects the diffusion process of the extract into disc paper and agar media, where this will affect the formed inhibition zone to be less than optimal [11]. The good method is known to produce a wider inhibition zone because this method uses a tip or cork borer to perforate the agar medium so that the extract can enter the hole up to 100µg. This method makes the extract used more and can diffuse maximally throughout the agar layer that contains bacteria and makes the extract osmolarity occur as a whole, homogeneous, and more potent in inhibiting growth bacteria [12,13].

The use of a combination of these two natural ingredients results in better inhibition due to the presence of complementary elements, in which roselle flowers contain anthocyanins and, proanthocyanidins which green tea leaves do not have, and the presence of catechins such as Epicatechin Gallate (ECG), Epigallocatechin (EGC), and Epigallocatechin Gallate (EGCG) which are owned by green tea leaves but are not owned by roselle flowers [14,15,16]. The unique content of the two ingredients is thought to have a synergistic effect.

The Shapiro-Wilk test shows a significance value of $p > 0.05$ for all data to be normally distributed. In the homogeneity test using the Levene test, a significance value of $p > 0.05$ means that all data is homogeneous. Data analysis can be continued using the One Way ANOVA test. In the One Way ANOVA test, the significant result was $p = 0.000$ ($p < 0.05$), which means a significant difference between the inhibition zone results in the treatment group derived from the combination of roselle and green tea extract on the growth of MRSA bacteria. This result is due to the presence of compounds possessed by roselle flowers and green tea, which provide synergistic antibacterial effects such as the use of antibiotics, namely the content of phenols, alkaloids, saponins, and tannins [17,18]. The phenol content is known to interfere with the permeability of the bacterial cell membrane and the transportation process, and this results in the loss of macromolecules and cations from the cell so that it can interfere with the growth of bacterial cells and will cause cell death [19]. The alkaloid content is known to have antibacterial activity by inhibiting peptidoglycan constituent components in bacterial cells. The cell wall layer is not formed entirely and causes death in bacterial cells (Suhendar & Sogandi, 2019). The saponin content is known to have an antibacterial mechanism that causes protein and enzyme leakage from within bacterial cells, and this is because the surface of the saponin compound is similar to the detergent content so that it can reduce the surface tension of the cell wall, which can ultimately damage the permeability of the bacterial cell membrane. Furthermore, saponins will diffuse through the walls and membranes of bacterial cells to disturb the stability of the membrane and cause leakage in the cell cytoplasm, which eventually causes death in gram-positive and negative bacteria. The content of tannins has activities that can interfere with bacterial cell walls' permeability, resulting in inhibition of bacterial cell growth and causing bacterial cell death [20].

In the Bonferroni Post Hoc test, it was found that the concentrations of 100%, 50%, and 25% showed significant values, namely 0.000 and 0.011 ($p < 0.05$) compared to the vancomycin 30g group, this indicates that there is a significant difference between the zone averages. Inhibition produced by concentrations of 100%,

50%, and 25% against the mean vancomycin inhibition zone 30g. Meanwhile, at a concentration of 12.5%, it shows a significance value of 1,000 (p> 0.05), which indicates that there is no significant difference to the mean vancomycin 30g inhibition zone so that it has an inhibition zone value that is comparable to that of the antibiotic. The growth of MRSA bacteria is influenced by various factors, such as the acidity of the culture media (pH), incubation temperature, light radiation, and atmospheric hydrostatic pressure [21].

4. CONCLUSION

The combination of roselle flower extract (*Hibiscus sabdariffa* L) and green tea (*Camellia sinensis*) at a concentration of 12.5% had a stronger sensitivity than the antibiotic vancomycin 30g to the growth of MRSA bacteria. The MIC combination of roselle flower extract (*Hibiscus sabdariffa* L) and green tea (*Camellia sinensis*) against MRSA bacteria's growth is at a concentration of 12.5%. The vancomycin antibiotic with a level of 30g can produce an inhibition zone against the growth of MRSA bacteria of 16.45mm. The 12.5% concentration in the combination of roselle flower extract (*Hibiscus sabdariffa* L) and green tea (*Camellia sinensis*) has an inhibitory level comparable to vancomycin 30g.

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REFERENCES

1. Liana, P. (2014). Gambaran Kuman Methicillin-Resistant Staphylococcus aureus (MRSA) di Laboratorium Mikrobiologi Departemen Patologi Klinik Rumah Sakit. Majalah Kedokteran Sriwijaya. Vol.3:171–175.
2. Khan, A., Wilson, B., & Gould, I. M. (2018). Current and Future Treatment Options for Community-Associated MRSA Infection. *Expert Opinion on Pharmacotherapy*. Vol.19(5):457–470. https://doi.org/10.1080/14656566.2018.1442826
3. World Health Organization. (2014). Antimicrobial Resistance: Global Report on Surveillance. WHO Publications. pp.1–9. Retrieved February 10, 2021, from https://www.who.int/drugresistance/documents/surveillancereport/en/https://www.cdc.gov/epiinfo/ index.html
4. Chaudhary, A. S. (2016). A Review of Global Initiatives to Fight Antibiotic Resistance And Recent Antibiotics Discovery. *Acta Pharmaceutica Sinica B*. Vol.6(6):552–556.https://doi.org/10.1016/j.apsb.2016.06.004
5. Erikawati, D., Santosaningsih, D., & Santoso, S. (2017). Tingginya Prevalensi MRSA pada Isolat Klinik Periode 2010- 2014 di RSUD Dr. Saiful Anwar Malang, Indonesia. *Jurnal Kedokteran Brawijaya*. Vol.29(2):149–156. http://dx.doi.org/10.21776/ub.jkb.2016.029.02.9
6. Afifurrahman, Samadin, K. H., & Aziz, S. (2014). Pola Kepekaan Bakteri Staphylococcus aureus terhadap Antibiotik Vancomycin di RSUP Dr. Mohammad Hoesin Palembang. *Majalah Kedokteran Sriwijaya*. Vol.46(4):266–270.https://doi.org/10.36706/mks.v46i4.2716
7. Arifin, W. N., & Zahiruddin, W. M. (2017). Sample size calculation in animal studies using resource equation approach. Malaysian Journal of Medical Sciences, 24(5), 101–105. https://doi.org/10.21315/mjms2017.24.5.11
8. Greenwood, D., Finch, R., Whitley, R., & Norrby, S. R. .(1995). *Antibiotic susceptibility (sensitivity) test, anti- microbial and chemotherapy*. USA: Mc Graw Hill Company.
9. Utami, L. A., & Putri, D. H. (2020). The Effect of Ethanol Solvent Concentration on Antimicrobial Activities The Extract of Andalas Endophytic Bacteria (Morus Macroura Miq.) Fermentation Product. *EKSAKTA*. Vol.21(01):1–6.
10. Marpaung, M. P., & Handayani, D. W. (2018). The Effect of Solvent Concentration on Antioxidant Activity of Akar Kuning (Fibraurea Chloroleuca Miers) Extract. *AIP Conference Proceedings*. Vol.2049:1-5. http://dx.doi.org/10.31602/dl.v3i1.3109
11. Hadi, D. K., Erina, Rinidar, Fakrurrazi, Rosmaidar, & Sayuthi, A. (2019). Daya Hambat Ekstrak Etanol Daun Mengkudu (Morinda citrifolia L.) terhadap Pertumbuhan Salmonella sp . dan Escherichia coli. *Jimvet*. Vol.3(2):87–97. https://doi.org/10.21157/jim%20vet..v3i2.10835
12. Arirahmayanti, I. G. A. E., Artini, I. G. A., & Ernawati, D. K. (2019). Perbandingan Aktivitas Antibakteri Ekstrak Etanol Kunyit (Curcuma longa) dan Bawang Putih (Allium sativum) Terhadap Escherichia coli ATCC 8739. *Jurnal Medika Udayana*. Vol.8(11):1-5. ISSN 2303-1395. Available at: <https://ojs.unud.ac.id/index.php/eum/article/view/55068>. Date accessed: 10 feb. 2021.
13. Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of Pharmaceutical Analysis*. Vol.6(2):71–79.https://doi.org/10.1016/j.jpha.2015.11.005
14. Abdel-shafi, S., Sitohy, M., & Mosa, B. (2019). Antimicrobial Activity and Chemical Constitution of the Crude, Phenolic-Rich Extracts of Hibiscus sabdariffa, Brassica oleracea and Beta vulgaris. *Journal Molecules.* Switzerland. Vol.24(23):4280.https://doi.org/10.3390/molecules24234280
15. Murukan, G., Lawarence, B., & K., M. (2018). Microbicidal Potentiality of Purified Anthocyanin From in Vitro Culture of Clerodendron Infortunatum L. Against Selected Pathogens. *International Journal of Pharmacy and Pharmaceutical Sciences*. Vol.10(6):68. https://doi.org/10.22159/ijpps.2018v10i6.18649
16. Reygaert, W., & Jusufi, I. (2013). Green Tea As an Effective Antimicrobial For Urinary Tract Infections Caused by Escherichia Coli. *Frontiers in Microbiology*. Vol.4:1–4. DOI: 10.3126/ijasbt.v8i1.27313
17. Ananthi, J; Giri, R. (2018). Antibacterial Potential of Green Tea (Camellia sinensis) Against Urinary Trect Infection. *World Journal of Pharmaceutical and Medical Research*. 4(9):181–185.

18. Fadahunsi, I. F., & Adeyina, O. E. (2019). Antimicrobial Susceptibility of Spoilage Bacteria of Water Melon Juice to Ethanol Extract of Roselle. Vol.11(2019):1140–1153.
19. Larasati, D. A., & Apriliana, E. (2016). Efek Potensial Daun Kemangi (Ocimum basilicum L .) sebagai Pemanfaatan Hand Sanitizer. *Majority*. Vol.5(5):124–129.
20. Suhendar, U., & Sogandi, S. (2019). Identifikasi Senyawa Aktif Ekstrak Daun Cengkeh (Syzygium aromaticum) Sebagai Inhibitor Streptococcus mutans. *Al-Kauniyah: Jurnal Biologi*. Vol.12(2): 229–239. https://doi.org/10.15408/kauniyah.v12i2.12251
21. Cowan, M. K., & Smith, H. 2018. *Microbiology A System Approach* (Fifth Edition). New York: McGraw-Hill Education.