Growth Inhibiting Activity Of Staphylococcus Aureus ATCC 25923 Kirinyuh Leaves (Chromolaena Odorata L.) Using The Soxhletation Method

Anna Fitriawati[1], Alenda Rahmad Adji[2], Bagas Ardiyantoro[3] Azimah Abdul Wahab Sabrina[4] Karim[5]
Universitas Duta Bangsa Surakarta, Faculty of Health Sciences, Bachelor of Pharmacy Education Program[123] Institute of Medical Science Technology, Universiti Kuala Lumpur[45]
Email Correspondence : anna_fitriawati@udb.ac.id

ABSTRACT

Kirinyuh leaves (Chromolaena odorata L.) have the potential as a medicinal plant because of the active compounds contained in kirinyuh leaves (Chromolaena odorata L.). The purpose of this study was to determine the antibacterial activity of ethanol extract of kirinyuh leaves (Chromolaena odorata L.) against the growth of Staphylococcus aureus ATCC 25923 bacteria. Kirinyuh leaf extract (Chromolaena odorata L.) was carried out using the soxhletation method and the solvent used was 96% ethanol. The concentrations used for each treatment were 30%, 40% and 50%. Each concentration was tested for antibacterial activity against Staphylococcus aureus bacteria using the disc diffusion method. The results showed that the extract of kirinyuh leaves (Chromolaena odorata L.) with a concentration of 30% had an average diameter of the inhibition zone of 9.2 mm, a concentration of 40% had an average diameter of the inhibition zone of 12.36 mm, and a concentration of 50% had an average diameter of the inhibition zone of 13.71 mm. The research conducted can be concluded that the extract of kirinyuh leaves can provide the best effect of inhibiting bacterial growth at a concentration of 50% with the results of the anova test p <0.05 which means there is a significant difference compared to other concentrations.

KEYWORDS Kirinyuh Leaf Extract, Antibacterial, Staphylococcus aureus

INTRODUCTION

Infectious diseases are diseases where bacteria enter and multiply in the body, one of which is bacteria, causing organ damage. Microorganisms that cause infectious diseases are also called pathogens. Staphylococcus aureus is one of the bacteria that causes infectious diseases.(Selvia & Wahyuni, 2022).

Staphylococcus aureus is commonly found on the skin, almost everyone experiences Staphylococcus aureus infection, with symptoms ranging from food poisoning or mild skin infections to serious, life-threatening infections. Common treatment is by giving antibiotics. Improper use of antibiotics can cause various problems, namely it can cause bacterial resistance to the antibiotic (Putri, 2021). The use of antibiotics often causes side effects such as allergic reactions, toxic reactions, and biological and metabolic changes in the host (Selvia & Wahyuni, 2022). This makes people choose medicines from natural ingredients such as plants found around them to be used as traditional medicine (Fadia *et al*., 2020).

One of the plants that can be used as an antibacterial medicine is the kirinyuh leaf. Kirinyuh leaves (Chromolaena odorata L.) are efficacious as a wound medicine, mouthwash to treat sore throats, cough medicine, malaria medicine, headaches, antidiarrhea, astringent, antispasmodic, antihypertensive, anti-inflammatory and diuretic (Fadia *et al*., 2020). Kirinyuh leaves are known to contain several active compounds such as flavonoids, phenols, tannins and saponins, and produce essential oils or volatile oils containing cadinene, α-pinene, β-cariophyllena, camphora and candino isomers (Andika et al., 2020). The mechanism of antibacterial compounds is usually carried out by damaging cell walls, changing membrane permeability, disrupting protein synthesis, and inhibiting enzyme activity (Septiani *et al*., 2017).

Flavonoids have the ability to denature bacterial cell proteins and damage cell membranes, causing cells to lyse. Flavonoids work as antibacterials by forming complex compounds against extracellular proteins that disrupt the integrity of bacterial cell membranes. The mechanism of action is to denature bacterial cell proteins and damage cell membranes beyond repair (Rahmawatiani *et al*., 2020).

The activity of an antibacterial can be determined by determining its inhibitory and killing power against bacterial growth using the diffusion and dilution methods. Based on research (Selvia & Wahyuni, 2022) It is known that the extract of kirinyuh leaves using the maceration method has antibacterial activity against the growth of Staphylococcus aureus bacteria at a concentration of 100% using the disk diffusion method has the potential to moderately inhibit the growth of Staphylococcus aureus bacteria with an average inhibition zone diameter of 6.6 mm. Other research conducted by (Fadia et al., 2020) kirinyuh leaf extract on the growth of Staphylococcus aureus bacteria Minimal Inhibitory Concentration (MIC) of ethanol extract of kirinyuh leaves using the maceration method against Staphylococcus aureus: 20%. Minimal Bacterial Concentration (MBC) of ethanol extract of kirinyuh leaves against Staphylococcus aureus: 40%.

RESEARCH METHOD

This type of research is descriptive. This research was conducted in March 2024 – August 2024. This research was conducted at Duta Bangsa University, Surakarta.

The extraction process of kirinyuh leaves (Chromolaena odorata L.) is carried out using the soxhletation method. The soxhletation extraction method is a method of separating substances from their mixtures by heating, the solvent used will experience circulation, compared to the maceration method, soxhletation extraction provides higher extract results (Wijaya et al., 2019).

Kirinyuh leaf extract is a thick extract obtained from the extraction of kirinyuh leaves (Chromolaena odorata L.) using the soxhletation method using 96% ethanol solvent with concentrations of 30%, 40% and 50% which is used to inhibit the growth of Staphylococcus aureus bacteria.

Antibacterial activity test was conducted using the disc diffusion method. Observation of antibacterial activity was conducted after 1 x 24 hours of incubation period by measuring the diameter of the clear inhibition zone formed around the disc (Anggraini *et al*., 2021). The samples used in the research were kirinyuh leaves (Chromolaena odorata L.) obtained from Sundoluhur Village, Kayen District, Pati Regency.

Tools used: Blender, 40 mesh sieve, Measuring cup, Erlenmeyer flask, stirring rod, Digital scales, filter paper, thread, soxhlet, Rotary evaporator, Autoclave, Loop needle, Petri dish, Test tube rack, Test tube, Tripod, Bunsen burner, Matches, Measuring pipette, Incubator, Tweezers, LAF (Laminar air flow), Beaker glass, Sterile disc paper, wrapping paper, aluminum foil, caliper, microscope, object glass.

Materials used: Simplisia leaves of kirinyuh (Chromolaena odorata L.) bacteria Staphylococcus aureus ATCC 25923, 96% ethanol, Dragendorff reagent, Mayer reagent, Wagner, chloroform, 2N sulfuric acid, anhydrous acetic acid, Mg powder, concentrated HCl, 1% FeCl3, NA media (nutrient agar), McFarland 0.5%, Spiritus, lugol, safranin, NaCl, crystal violet, DMSO.

RESULTS AND DISCUSSION

Kirinyuh leaf powder (Chromolaena odorata L.) as much as 100 grams that has been sieved using a 40 mesh sieve wrapped in filter paper adjusted to the size of the soxhlet apparatus then inserted into the soxhlet apparatus. 96% ethanol solvent as much as 1½ cycles is inserted into the soxhlet flask and soxhletation is carried out at a temperature of 700C until the cycle drops are almost colorless (perfectly extracted)(Maryam *et al*., 2023). Soxhlet extraction was carried out 4 times, because the extract obtained from the first soxhlet extraction was insufficient, so soxhlet extraction was carried out 4 times.

Research on the inhibitory power of ethanol extract of kirinyuh leaves (Chromolaena odorata L.) at concentrations of 30%, 40% and 50% on the growth of Staphylococcus aureus bacteria produced data that can be seen in Table 1.

Table 1. Diameter of Inhibition Zone of Antibacterial Activity Test

Test Solution	Inhibition Zone Diameter (mm)			
	1	2	3	Average
30%	7,55	8,45	11,4	9,2
40%	9,25	13,65	14,2	12,36
50%	10,8	15,65	14,6	13,71
K +	23,65	25,2	26,45	25,1
K -	0	0	0	0

The results of the observation showed that kirinyuh leaves (Chromolaena odorata L.) with the soxhletation method were able to inhibit the growth of Staphylococcus aureus bacteria. This activity is due to the presence of flavonoid compounds in the extract of kirinyuh leaves (Chromolaena odorata L.). The mechanism of action of flavonoids is to damage the bacterial cell membrane, inhibit the work of bacterial enzymes that damage the bacterial cell wall and change the molecular structure of bacterial cell proteins. (Rahman *et al*., 2017). Flavonoid compounds are thought to be able to damage cell membranes in Staphylococcus aureus bacteria. Giving ethanol extract with concentration variations of 30%, 40%, 40% showed the presence of an inhibition zone with an average inhibition zone of 9.2 mm, 12.36 mm, 13.71 mm with all moderate inhibition responses. The smallest inhibition zone was found at a concentration of 30% and the largest zone at a concentration of 50%. The inhibition zone formed can be seen in Figure 1.

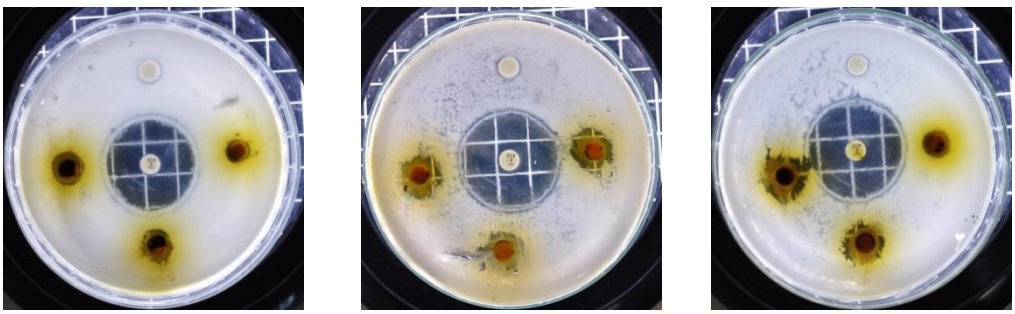

Figure 1. Results of Antibacterial Activity Inhibition Zone

CONCLUSION

Ethanol extract of kirinyuh leaves (Chromolaena odorata L.) has antibacterial activity against Staphylococcus aureus ATCC 25923 bacteria using the soxhletation method. The best concentration of kirinyuh leaf extract (Chromolaena odorata L.) that can inhibit the growth of Staphylococcus aureus ATCC 25923 bacteria using the soxhletation method is a concentration of 50% with an average inhibition zone diameter of 13.71 mm.

REFERENCES

Andika, B., Halimatussakdiah, H., & Amna, U. (2020). Analisis Kualitatif Senyawa Metabolit Sekunder Ekstrak Daun Gulma Siam (*Chromolaena odorata* L.) di Kota Langsa, Aceh. *QUIMICA: Jurnal Kimia Sains Dan Terapan*, *2*(2), 1–6. https://doi.org/10.33059/jq.v2i2.2647

Fadia, Nurlailah, Herlina, T. E., & Lutpiatina, L. (2020). Efektivitas Ekstrak Etanol Daun Kirinyuh (*Chromolaena Odorata* L) Sebagai Antibakteri Effectiveness of Kirinyuh Leaf (*Chromolaena Odorata* L) Ethanol Extract As an Antibacterial of *Salmonella Typhi* and *Staphylococcus Aureus*. *Jurnal Riset Kefarmasian Indonesia*, *2*(3), 158–168.

Ilmiah, J., & Pendidikan, W. (2023). *3 1,2,3*. *9*(25), 624–639.

Maryam, F., Taebe, B., & Toding, D. P. (2020). Pengukuran Parameter Spesifik Dan Non Spesifik Ekstrak Etanol Daun Matoa (*Pometia pinnata* J.R & G.Forst). *Jurnal Mandala Pharmacon Indonesia*, *6*(01), 1–12. https://doi.org/10.35311/jmpi.v6i01.39

Putri, anisa yustikka. (2021). Uji Aktivitas Dan Efektivitas Antibakteri Ekstrak Dan Fraksinasi Herba Sirih Cina (*Peperomia pellucida* L. Kunth) Terhadap *Staphylococcus aureus* Skripsi Diajukan kepada Program Studi S1 Farmasi Sekolah Tinggi Ilmu Kesehatan Borneo Cendekia Medika untuk mem. *Skripsi*.

Rahman, F. A., Haniastuti, T., & Utami, T. W. (2017). Skrining fitokimia dan aktivitas antibakteri ekstrak etanol daun sirsak (*Annona muricata* L.) pada *Streptococcus mutans* ATCC 35668. *Majalah Kedokteran Gigi Indonesia*, *3*(1), 1. https://doi.org/10.22146/majkedgiind.11325

Rahmawatiani, A., Mayasari, D., & Narsa, A. C. (2020). Kajian Literatur: Aktivitas Antibakteri Ekstrak Herba Suruhan (*Peperomia pellucida* L.). *Proceeding of Mulawarman Pharmaceuticals Conferences*, *12*, 117–124. https://doi.org/10.25026/mpc.v12i1.401

Selvia, D., & Wahyuni, A. (2022). Jurnal Kesehatan Saintika Meditory Jurnal Kesehatan Saintika Meditory. *Jurnal Kesehatan Saintika Meditory*, *4*(4657), 78–84.

Septiani, S., Dewi, E. N., & Wijayanti, I. (2017). Aktivitas Antibakteri Ekstrak Lamun (*Cymodocea rotundata*) Terhadap Bakteri *Staphylococcus aureus* Dan *Escherichia coli* (Antibacterial Activities of Seagrass Extracts (*Cymodocea rotundata*) Against *Staphylococcus aureus* and *Escherichia coli*). *SAINTEK PERIKANAN : Indonesian Journal of Fisheries Science and Technology*, *13*(1), 1. https://doi.org/10.14710/ijfst.13.1.1-6

Wijaya, D. R., Paramitha, M., & Putri, N. P. (2019). C. Kata kunci: Oleoresin, jahe, ekstraksi, soklet. *Jurnal Konversi*, *8*(1), 9–16.