

The Effectiveness of Kirinyuh (*Chromolaena odorata* L.) Leaf Essential oil as an Antibacterial *Staphylococcus aureus* and *Escherichia coli*

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Abstract: One of the plants with antibacterial activity is Kirinyuh (*Chromolaena odorata* L.). The essential oil from these plants has the potential as a natural antibacterial against pathogenic bacteria, such as *Staphylococcus aureus* and *Escherichia coli*. This study aims to analyze the antibacterial ability of kirinyuh leaf essential oil (*Chromolaena odorata* L.) originating from Martapura, Indonesia, by the diffusion method, especially in its ability to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*. The study used five variations of the concentration of essential oils, namely 10 ul, 15 ul, 20 ul, 25 ul, and 30 ul, carried out five times. Essential oil distillation using water and steam distillation method. Antibacterial activity test using well diffusion method. The results showed that the diameter of the inhibition zone against *Staphylococcus aureus* had a diameter of 9.4mm at a concentration of 10uL, 10.8mm at a concentration of 15uL, 11.6mm at a concentration of 20uL, 13mm at a concentration of 25uL, and 14.4mm at a concentration of 30uL. The diameter of the inhibition zone against *Escherichia coli* had a diameter of 11.6mm at a concentration of 10uL, 12.8mm at a concentration of 15uL, and 14mm at a concentration of 20uL, 14.8mm at a concentration of 25uL, 15.8mm at a concentration of 30uL. Conclusion The most significant inhibition zone of kirinyuh leaf essential oil (*Chromolaena odorata* L.) against *Staphylococcus aureus* was 14.4mm at a concentration of 30uL and against *Escherichia coli* was 15.8mm at a concentration of 30uL. Further research is needed on the antibacterial effectiveness of kirinyuh leaf essential oil (*Chromolaena odorata* L.) against other types of bacteria and also against antibiotic-resistant bacteria.

Keywords: Essential oil; kirinyuh (*Chromolaena odorata* L.) Leaf; *Staphylococcus aureus*; *Escherichia coli*

INTRODUCTION

Indonesia has a variety of biodiversity that can be used as a source of traditional medicinal ingredients. One of the plants that have antibacterial activity is Kirinyuh (*Chromolaena odorata* L.)¹. Traditionally, the leaves of *Chromolaena odorata* L. have been used as medicine in wound healing to treat sore throat, malaria medicine, headache, antidiarrheal, and antiplasmodial astringent, antihypertensive and anti-inflammatory². Several studies on *Chromolaena odorata* L. showed various pharmacological properties

such as wound healing activity, antioxidant, stomach, hemostatic, anti-malarial, and antimicrobial activity^{3,4,5}.

Kirinyuh (*Chromolaena odorata* L.) is a wild plant considered a nuisance plant and is easily found around us. Its height reaches 2-3 m and can reach 5-10 m. There is variability in plant morphology regarding flower color, leaf shape, and odor⁶. Many diseases caused by pathogenic bacterial infections can be cured by some antibacterial drugs⁷. One of the processing of kirinyuh leaves can be made into essential oil. Essential oils are organic compounds derived from plants and are volatile. The compounds in these essential oils have the potential as natural antibacterials against pathogenic bacteria, for example, *Staphylococcus aureus* and *Escherichia coli*⁸.

Staphylococcus aureus is a Gram-positive bacterium that attacks humans and other mammals⁹. In contrast, *Escherichia coli* is a Gram-negative bacteria that grow as normal flora but can become pathogenic if the number of these bacteria in the digestive tract increases or is outside the intestine¹⁰. Research conducted by Toure et al. (2014) on the effectiveness test with the disc diffusion method showed that the best concentration of kirinyuh leaf essential oil (*Chromolaena odorata* L.) was 20 ul effective in inhibiting the growth of *Staphylococcus aureus* resulting in a zone diameter of the average standard deviation, namely 13.66 ± 0.58 and *Escherichia coli* resulted in an average standard deviation zone diameter of 12.67 ± 1.15 ¹¹.

According to Owolabi et al. (2010), Kirinyuh essential oil has chemical compounds, namely -pinene, -pinene, germacrene, -copaen-4 α -ol, (E)-caryophyllene, and geijerene/pregeijerene¹². There is a diversity of essential oils from the leaves of *Chromolaena odorata* L. As in India¹³, the main ingredients are geijerene, -copaene, 3-carene, and -caryophyllene. The main content of -pinene and p-cymene in Cameroon and Congo¹⁴, while in Pantai Gading¹⁵ identified pregeijerene and germacrene-D as the main compounds. The difference in essential oil content is significantly different in different regions, so there may be variations in the antibacterial effects of essential oils from *Chromolaena odorata* L. plants originating from different regions. Kirinyuh leaf essential oil (*Chromolaena odorata* L.) from Martapura, Indonesia, has not been widely known for its effectiveness against bacteria. Thus, this study aimed to analyze the antibacterial ability of kirinyuh leaf essential oil (*Chromolaena odorata* L.) from Martapura, Indonesia, by the diffusion method, especially in its ability to inhibit the growth of *Staphylococcus aureus* and *Escherichia coli*.

MATERIALS AND METHOD

The research material used was kirinyuh leaf (*Chromolaena odorata* L.) from the Martapura area, South Kalimantan province, Indonesia. The leaves used are whole, fresh leaves with no rotten parts and clean from pests. The bacterial suspension used was *Staphylococcus aureus* and *Escherichia coli* obtained from pure cultures of the Bacteriology Laboratory, Technology Study Program, Medical Laboratory, Health Polytechnic, Banjarmasin, Indonesia. The media used for culturing were Tryptic Soy Broth Media (Merck), Mannitol Salt Agar Media (Merck), Nutrient Agar Media (Merck), Eosin Methylene Blue (Merck), Mac Farland 0.5, sterile Aquades, 70% alcohol, chloramphenicol, sterile yellow tip, cotton. The study used five variations of the concentration of essential oils, namely 10 ul, 15 ul, 20 ul, 25 ul, and 30 ul, carried out five times. This treatment was used for each type of bacteria.

Essential oil distillation using water and steam distillation method. The production of kirinyuh leaf essential oil (*Chromolaena odorata* L.) is by using kirinyuh leaves selected based on the criteria by weighing, washing thoroughly with running water, and drying in the open air. Kirinyuh leaves are heated in the oven for 45 minutes at 70°C. The distillation is added to water under the stimulus to the limit mark, then 6 kg of dried kirinyuh leaves are added above the stimulus so that they do not come into direct contact with the water—distillation time for 4 hours at a temperature of 98°C. A separating funnel separates the liquid that comes out. The resultant essential oil is then stored in a dark bottle.

The diffusion test was carried out by making a bacterial suspension, dissolving the bacterial colonies in physiological salt (0.9% NaCl) so that the cell density of the suspension reached 0.5 McFarland. The bacterial suspension was spread evenly on the surface of the Muller Hinton agar medium and allowed to dry for 4-5 minutes. Wells were made, and various concentrations of essential oil were added. Petri dishes were incubated at 37°C for 24 hours. The inhibition zone formed was measured.

RESULTS AND DISCUSSION

Tabel 1. Inhibitory Zone Diameter Average of Kirinyuh Leaf Essential Oil (*Chromolaena odorata* L.) against *Staphylococcus aureus*

Repetition	Inhibitory Zone Concentration (mm)					Control	
	10 ul	15 ul	20 ul	25 ul	30 ul	Positive	Negative
1	10	11	12	12	14		
2	9	10	11	12	13		
3	9	12	13	15	16	23	0
4	10	11	11	13	14		
5	9	10	11	13	15		
Average	9,4	10,8	11,6	13	14,4		

In table 1, it can be seen that there was an increase in the diameter of the inhibition zone at each concentration. The diameter of the inhibition zone against *Staphylococcus aureus* had a diameter of 9.4mm at a concentration of 10uL, 10.8mm at a concentration of 15uL, and 11.6mm at a concentration of 20uL, 13mm at a concentration of 25uL, and 14.4mm at a concentration of 30uL.

Tabel 2. Inhibitory Zone Diameter Average of Kirinyuh Leaf Essential Oil (*Chromolaena odorata* L.) against *Escherichia coli*

Repetition	Inhibitory Zone Concentration (mm)					Control	
	10 ul	15 ul	20 ul	25 ul	30 ul	Positive	Negative
1	10	11	12	12	14		
2	9	10	11	12	13		
3	9	12	13	15	16	23	0
4	10	11	11	13	14		
5	9	10	11	13	15		
Average	9,4	10,8	11,6	13	14,4		

In table 2, it can be seen that there was an increase in the diameter of the inhibition zone at each concentration. The higher the concentration added, the larger the diameter

of the resulting inhibition zone. The diameter of the inhibition zone against *Escherichia coli* had a diameter of 11.6mm at a concentration of 10uL, 12.8mm at a concentration of 15uL, and 14mm at a concentration of 20uL, 14.8mm at a concentration of 25uL, 15.8mm at a concentration of 30uL.

Based on this study, the effectiveness of the essential oil of kirinyuh leaf (*Chromolaena odorata* L.) at a concentration of 20 L against *Staphylococcus aureus* (Table 1) had an average diameter of the inhibition zone of 11.6 mm, while the study of Toure et al. (2014) at a concentration of 20 L averaged zone diameter. The mean, standard deviation is 13.66 ± 0.58 mm (11). The results of this inhibition zone diameter are not too much different; as well as for *Escherichia coli*, an inhibition zone of 14mm was obtained at a concentration of 20 ul (Table 2), while Toure et al.'s (2014) study of *Escherichia coli* produced an average standard deviation zone diameter of 12.67 ± 1.15 at the same concentration¹¹. The difference in the diameter of the inhibition zone can be caused by the concentration and composition of the oil, which varies depending on the area of origin, collection period, stage of plant growth, and the distillation process.

Another study on kirinyuh leaf extract and its effectiveness against *Staphylococcus aureus* showed varying inhibition zone results, namely 10.41 mm at a concentration of 50%¹⁶, and 11.5 mm at a concentration of 90%¹⁷, 8mm at a concentration of 100%¹⁸. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of kirinyuh leaf extract against *Staphylococcus aureus* were found at a concentration of 20% for MIC and 40% for MBC¹⁹.

The limitation of this study is that the main content of kirinyuh leaf essential oil (*Chromolaena odorata* L.) is not known from Martapura Indonesia, so further research is needed on the primary active substance contained in kirinyuh leaf essential oil (*Chromolaena odorata* L.) so that it can be used for treatment antibacterial.

CONCLUSION

The most significant inhibition zone of kirinyuh leaf essential oil (*Chromolaena odorata* L.) against *Staphylococcus aureus* was 14.4mm at 30uL. The most significant inhibition zone of kirinyuh leaf essential oil (*Chromolaena odorata* L.) against *Escherichia coli* was 15.8mm at 30uL. Further research is needed on the antibacterial effectiveness of kirinyuh leaf essential oil (*Chromolaena odorata* L.) against other types of bacteria and also against antibiotic-resistant bacteria.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper.

REFERENCES

1. Frastika, D., Pitopang, R. and Suwastika, I.N. Uji Efektivitas Ekstrak Daun Kirinyuh (*Chromolaena odorata* (L.) RM King Dan H. Rob) Sebagai Herbisida Alami Terhadap Perkecambahan Biji Kacang Hijau (*Vigna Radiata* (L.) R. Wilczek) Dan Biji Karuilei

- (*Mimosa Invisa* Mart. ex Colla). *Natural Science: Journal of Science and Technology*, 2017; 6(3).
2. Vaisakh M, Pandey. The Invasive Weed With Healing Properties: A review On *Chromolaena odorata*. Department Of Pharmaceutical Science, 2012; 3 (1): 80–83
 3. Akinmoladun A.C., Ibukun E.O., Dan-Ologe I.A. Phytochemical constituents and antioxidant properties of extracts from the leaves of *Chromolaena odorata*. *Sci. Res. Essays*, 2007; 2:191–194.
 4. Idu M., Onyibe H.I. Medicinal Plants of Edo State, Nigeria. *Res. J. Med. Plant*. 2007; 1:32–41.
 5. Nanadini N., Nagababu P., Rao U., Venugopal N. Phytochemical, antimicrobial and antioxidant properties of an invasive weed-*Chromolaena odorata* (L.) King & Robinson. *Int. J. Phytomed*. 2014; 6:286–292.
 6. Zahara M. Description of *Chromolaena odorata* L. R.M King and H. Robinson as a medicinal plant: A Review. IOP Conference Series: Materials Science and Engineering. Volume 506 IOP Publishing; Aceh, Indonesia: 2019.
 7. Awoyinka, O.A., Balogun, I. O. Ongunnowo, A.A., 2007, Phytochemical Screening and Vitro Bioactivity *Cnidocolus acountifolius* (*Euphorbiaceae*), *J. of Medicinal Plants Res*, 2007; 1(3): 063- 065.
 8. Munte, N. and Lubis, R. Skrining Fitokimia dan Antimikroba Ekstrak Daun Kirinyuh terhadap Bakteri *Staphylococcus aureus* Dan *Escherichia coli*. *BIOLINK (Jurnal Biologi Lingkungan Industri Kesehatan)*, 2016; 2(2): 132-140.
 9. Gunawan, H.C., Yusliana, Y., Daeli, P.J., Sarwendah, S. and Chiuman, L. Uji Antibakteri Air Perasan Daging Buah Nanas (*Ananas Comosus* (L) Merr) terhadap Bakteri *Staphylococcus aureus*. *Jurnal Kedokteran dan Kesehatan*, 2019; 15(2): 170-177.
 10. Kusuma SAF. *Escherichia coli*. Bandung: Fakultas Farmasi Universitas Padjajaran; Bandung, 2010.
 11. Toure, D., Kouamé, B.K.F.P., Bedi, G., Joseph, A., Guessennd, N., Oussou, R., Chalchat, J.C., Dosso, M. and Tonzibo, F. Effect of geographical location and antibacterial activities of essential oils from Ivoirian *Chromolaena odorata* (L) RM King & Robinson (*Asteraceae*). *Journal of Pharmacognosy and Phytotherapy*, 2014; 6(6): 70-78.
 12. Owolabi, M.S., Ogundajo, A., Yusuf, K.O., Lajide, L., Villanueva, H.E., TUTEN, J.A. And Setzer, W.N. Chemical composition and bioactivity of the essential oil of *Chromolaena odorata* from Nigeria. *Record of natural product*, 2010; 4(1): 72
 13. Gogoi R., Sarma N., Begum T., Pandey S.K., Lal M. North-East Indian *Chromolaena odorata* (L. King Robinson) Aerial Part Essential Oil Chemical Composition, Pharmacological Activities—Neurodegenerative Inhibitory and Toxicity Study. *J. Essent. Oil Bear. Plants*, 2020; 23: 1173–1191. doi: 10.1080/0972060X.2020.1867009.
 14. Lamaty G., Menut C., Zollo P.H.A., Kuate J.R., Bessiere J.M., Ouamba J.M., Silou T. Aromatic plants of tropical central Africa IV. Essential oils of *Eupatorium odoratum* L. from Cameroon and Congo. *J. Essent. Oil Res*, 1992; 4: 101–105. doi: 10.1080/10412905.1992.9698028.

15. Tonzibo Z.F., Wognin E., Chalchat J.C., N'Guessan Y.T. Chemical investigation of *Chromolaena odorata* L. King Robinson from ivory coast. J. Essent. Oil Bear. Plants. 2007; 10: 94–100. doi: 10.1080/0972060X.2007.10643525.
16. Priono A, Yanti N, Lili D. Perbandingan Efektivitas Antibakteri Ekstrak Etanol Daun Kelor (*Moringaoleifera lamck.*) dan Ekstrak Daun *Chromolaena odorata* L.. Jurnal Ampibi, 2016; 1(2): 1-6 .
17. Rahayu RS. Aktivitas Ekstrak Etanol Daun kirinyuh (*Chromolaena odorata*) sebagai Antibakteri terhadap *Escherichia coli*, *Staphylococcus aureus* dan *Pseudomonas aeruginosa*, Skripsi. Universitas Negeri Medan, 2017.
18. Rasyid, S. A., Sugireng, Surya, R. A., Sanatang, Rosdarni, & Natalia, W. O. R. The antibacterial activity of Tembelekan leaf (*Lantana Camara* L.) and Kopasanda leaf (*Chromolaena odorata* L.) extracts against *Staphylococcus aureus*. Infectious Disease Reports, 2020; 12(1s). <https://doi.org/10.4081/idr.2020.8734>
19. Fadia, Nurlailah, Helmiah TE, Lutpiatina L. Efektivitas Ekstrak Etanol Daun Kirinyuh (*Chromolaena odorata* L) sebagai Antibakteri Salmonella Typhi dan *Staphylococcus aureus*. Jurnal Riset Kefarmasian Indonesia 2020; 2(3):158-168. DOI: 10.33759/jrki.v2i3.104.