Nusantara Scientific Medical Research Journal (NSMRJ)
Volume 1, No. 01 (2022), Page 01 – 08
https://nusantarascientificjournal.com/index.php/nsmrj/article/view/15

Antibacterial Activity Ethanolic Extract of *Ocimum basilicum* L. Leaves in Inhibiting the Growth of *Escherichia coli* and *Pseudomonas aeruginosa*

Muhammad Fauzan Lubis[1*], Hafid Syahputra[2], Ririn Astyka[3]

[1]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Sumatera Utara, 20155, Indonesia.
[2]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Sumatera Utara, 20155, Indonesia.
[3]Bachelor Program, Faculty of Pharmacy, Universitas Sumatera Utara, Medan, Sumatera Utara, 20155, Indonesia.

Coressponding authors: fauzan.lubis@usu.ac.id

Abstract

Infectious disease is the biggest problem in the world. The problem with this infection is related to antibiotic resistance if its use is not consistent. Utilization of kemangi leaves, which are always used as fresh vegetables, has potential as an antibacterial in inhibiting the growth of *Escherichia coli* and *Pseudomonas aeruginosa* bacteria. This study aims to analyze the inhibition of an ethanol extract of kemangi leaves against *Escherichia coli* and *Pseudomonas aeruginosa*. The Kirby-Bauer method with the disc diffusion method was used to determine the minimum inhibitory diameter and to calculate the activity index value. The test results at a concentration of 500 mg/mL showed activity in the strong category, namely 13.70 ± 0.10 mm (*Escherichia coli*) and 12.93 ± 0.06 mm (*Pseudomonas aeruginosa*), and the minimum inhibitory concentration was at a concentration of 3.125 mg/mL. Conclusion the ethanolic extract of kemangi leaves shows antibacterial activity.

Keywords: *Kemangi leaves, antibacterial, Pseudomonas aeruginosa, Escherichia coli*

Abstrak

Penyakit infeksi merupakan masalah tersebasar didunia. Masalah infeksi ini berkaitan dengan resistensi antibiotik dimana bila penggunaannya tidak patuh dalam pengonsumsiannya. Pemanfaatan daun kemangi yang selalu digunakan sebagai lalapan sebagai sayuran memiliki potensi sebagai antibakteri dalam menghambat pertumbuhan bakteri Escherichia coli dan Pseudomonas aeruginosa. Penelitian ini bertujuan untuk menganalisa daya hambat ekstrak etanol daun kemangi terhadap Escherichia coli dan Pseudomonas aeruginosa. Metode Kirby-Bauer dengan metode difusi cakram digunakan untuk penentuan diameter hambat minimum dan dilakukan perhitungan nilai aktivitas indeks. Hasil pengujian pada konsentrasi 500 mg/mL menunjukkan aktivitas dengan kategori kuat yaitu 13,70 ± 0,10 mm (*Escherichia coli*) dan 12,93 ± 0,06 mm (*Pseudomonas aeruginosa*) dan konsentrasi hambat minimum pada konsentrasi 3.125 mg/mL. Kesimpulan ekstrak etanol daun kemangi menunjukkan aktivitas antibakteri.

Kata kunci: *Daun kemangi, antibakteri, Pseudomonas aeruginosa, Escherichia coli*

Received: *December 2022* ***Accepted:*** *December 2022*

Nusantara Scientific Medical Research Journal (NSMRJ)
Volume 1, No. 01 (2022), Page 01 – 08
https://nusantarascientificjournal.com/index.php/nsmrj/article/view/15

INTRODUCTION

Infectious diseases caused by pathogens are a big problem in the world. Some bacteria are normal flora in the host's body, such as *Escherichia coli*; however, in large numbers, they will cause infectious diseases such as diarrhea and urinary tract infections (UTI)[1].

Other dangerous pathogens, such as *Pseudomonas aeruginosa*, show a high degree of pathogenicity. This bacterium will cause pneumoniae, otitis media, UTI, and other infections[1]. Treatment with antibiotics is always used but has the effect of developing resistance to pathogenic bacteria if non-compliance with the use of antibiotics continuously occurs[2]. Utilization of plants as a natural resource for inhibiting the growth of pathogenic bacteria has the potential to be developed[3,4].

The discovery of antibacterial compounds from plants continues; one example is the use of kemangi leaves, which are typically used as fresh vegetables but have antibacterial potential[5]. Previous studies have proven that the ethanol extract of kemangi leaves has the potential to inhibit the growth of *Staphylococcus aureus*[6]. Based on this description, the researchers were interested in analyzing the antibacterial activity of the ethanol extract of kemangi leaves against Gram-negative pathogenic bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*.

METHODOLOGY

Planing Research

The research was conducted starting with collecting raw materials or samples of kemangi leaves, then extracting them and testing their antibacterial activity in inhibiting the growth of *Escherichia coli* and *Pseudomonas aeruginosa*.

Sampel Preparation

Sampling was carried out purposively without comparing samples from one region to another. Fresh kemangi leaves were purchased from the Jamin Ginting market, washed, dried, and powdered using a blender[1].

Preparation of Kemangi Leaves Ethanol Extract

The ethanol extract of kemangi leaves was prepared using the maceration method with 96% (pa) ethanol as the solvent. As much as 200 g of kemangi leaf powder was soaked in 1500 mL of ethanol for 5 days, then filtered and macerated again for 2 days with 500 mL of ethanol[7]. The extract obtained was concentrated using a rotary vacuum evaporator[7,8].

Preparation of Apparatus and Materials

The glassware used was sterilized using an oven at 170^{o}C for 1 hour[9]. The test medium used was sterilized using an autoclave at 121^{o}C for 15 minutes[10].

Nusantara Scientific Medical Research Journal (NSMRJ)
Volume 1, No. 01 (2022), Page 01 – 08
https://nusantarascientificjournal.com/index.php/nsmrj/article/view/15

Preparation of Bacterial Stock Cultures

Prepare 1 tube of sterile slanting agar and then streak 1 ose of pure culture of *Escherichia coli* and *Pseudomonas aeruginosa* on the surface of the agar slants for 24 hours at 37°C ± 2°C [11,12].

Preparation of the Various Concentration

A condensed extract of kemangi leaves was weighed at 1 g and then dissolved in 2 mL of DMSO for a concentration of 500 mg/mL. Then it was diluted with various concentrations of 250, 100, 50, 25, 12.5, 6.25, and 3.125 mg/mL[8,11].

Preparation of Bacterial Suspension

One sample of revitalized bacteria was suspended in a test tube containing 10 mL of MHB medium, vortexed, and turbidity was adjusted using 0.5 McFarland standards [7,13].

Antibacterial Activity Test

The method used in this test is the Agar Diffusion Method (Kirby-Bauer)[12]. A total of 0.1 mL of the bacterial suspension was pipetted and placed in a sterile petri dish, and then 15 mL of MHA medium was added[14,15]. Pour into a Petri dish and homogenize; place on the surface of the disc of paper media that contains each concentration, and use chloramphenicol antibiotic disc paper containing 30 mcg. The treatment was carried out over three repetitions[1,9].

Calculation of Index Activity Value

Index activity value is calculated using the following formula: [8]

$$Index\ Activity = \frac{extract\ inhibition\ zone\ diameter}{positive\ control\ inhibition\ zone\ diameter}$$ [16]

Data Analysis

Data is presented in 3 treatments with the average and standard deviation values, and the difference is seen from the control using SPSS v.22 software[9].

RESULT AND DISCUSSION

The results of testing the antibacterial activity of the ethanol extract of kemangi leaves in inhibiting the growth of *Escherichia coli* and *Pseudomonas aeruginosa* showed positive inhibition results. This is indicated by the presence of a clear zone around the disc, which is dripped with a concentrated ethanol extract of kemangi leaves[17,18]. The results of measuring the diameter of the inhibition zone on the two bacteria can be seen in Table 1 and Figure 1.

At Table 1. it can be seen that at a concentration of 500 mg/mL, it showed an inhibition zone of 12.93 ± 0.06 mm (*Pseudomonas aeruginosa*) and 13.70 ± 0.10 mm (*Escherichia coli*).

At a concentration of 500 mg/mL, the inhibition zone was the largest among all concentrations. At the smallest concentration of 3.125 mg/mL, the diameter of the inhibition zone was 6.57 ± 0.15 mm (*Pseudomonas aeruginosa*) and 6.90 ± 0.10 mm (*Escherichia coli*).

Nusantara Scientific Medical Research Journal (NSMRJ)
Volume 1, No. 01 (2022), Page 01 – 08
https://nusantarascientificjournal.com/index.php/nsmrj/article/view/15

Table 1. Inhibition Result of Ethanolic Extract of *Ocimum basilicum* in inhibiting the growth of *Escherichia coli* and *Pseudomonas aeruginosa*.

No	Concentration	Diameter Inhibition Zones (mm)	
		P. aeruginosa	*E. coli*
1	Negative Control	6,00 ± 0,00	6,00 ± 0,00
2	3,125 mg/mL	6,57 ± 0,15*	6,90 ± 0,10*
3	6,25 mg/mL	7,73 ± 0,06*	7,67 ± 0,15*
4	12,5 mg/mL	8,63 ± 0,15*	8,20 ± 0,20*
5	25 mg/mL	9,00 ± 0,10*	8,93 ± 0,25*
6	50 mg/mL	9,63 ± 0,15*	10,13 ± 0,21*
7	100 mg/mL	10,30 ± 0,10*	11,40 ± 0,26*
8	250 mg/mL	11,73 ± 0,15*	12,63 ± 0,25*
9	500 mg/mL	12,93 ± 0,06*	13,70 ± 0,10*
10	Positive Control	23,17 ± 0,21	25,13 ± 0,15

*Significantly difference with negative and positive control.

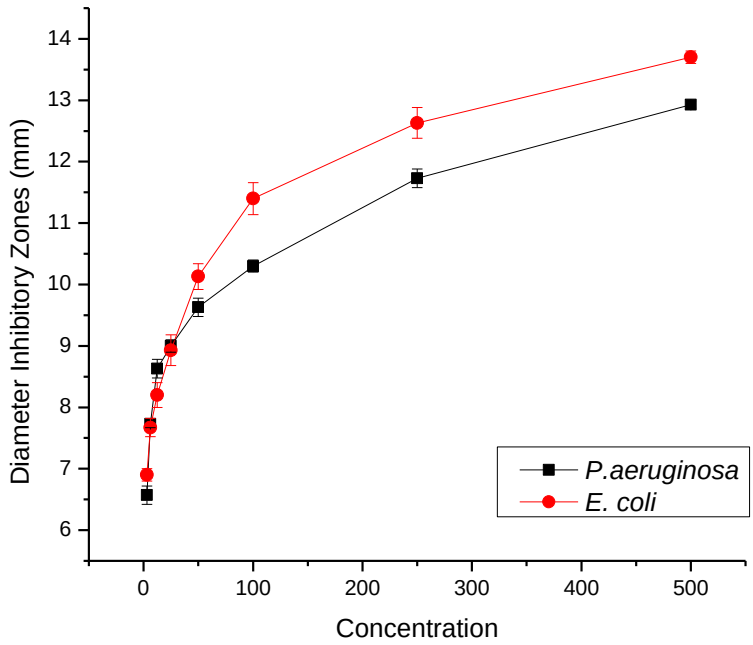

Figure 1. Graph of diameter inhibitory zones versus concentration against *Pseudomonas aeruginosa* and *Echerichia coli*

Nusantara Scientific Medical Research Journal (NSMRJ)
Volume 1, No. 01 (2022), Page 01 – 08
https://nusantarascientificjournal.com/index.php/nsmrj/article/view/15

Table 2. Activity Index Value

No	Concentration	Activity Index	
		P. aeruginosa	*E. coli*
1	Negative Control	0,26 ± 0,00	0,24 ± 0,00
2	3,125 mg/mL	0,28 ± 0,07	0,27 ± 0,06
3	6,25 mg/mL	0,33 ± 0,02	0,31 ± 0,10
4	12,5 mg/mL	0,37 ± 0,07	0,33 ± 0,13
5	25 mg/mL	0,39 ± 0,04	0,36 ± 0,16
6	50 mg/mL	0,42 ± 0,07	0,40 ± 0,13
7	100 mg/mL	0,44 ± 0,06	0,45 ± 0,17
8	250 mg/mL	0,51 ± 0,05	0,50 ± 0,16
9	500 mg/mL	0,56 ± 0,02	0,55 ± 0,06

The formation of this inhibition zone is due to the diffusion power from high concentrations to lower concentrations[19]. In this case, the movement of the active substance or secondary metabolite compounds from the disc paper moves and diffuses into the medium so as to provide a zone of inhibition around the disc paper[20,21].

Table 2 shows the value of the index activity calculation results. Index activity is a comparison between the diameter of the inhibition zone of each concentration and that of the positive control[22]. If the index activity value is close to 1, then the test concentration activity is close to the activity of the positive control[23]. In Table 2, it can be seen that at the largest concentration of 500 mg/mL, it shows an activity index value of 0.56 ± 0.02 (*Pseudomonas aeruginosa*) and 0.55 ± 0.06 (*Escherichia coli*). This value means that *Pseudomonas aeruginosa* has an activity of 56% of the test concentration against the positive control, while *Escherichia coli* shows an activity of 55% of the test concentration against the positive control of chloramphenicol.

Based on the results of phytochemical screening by Angga Nugraha et al. (2022)[6], positive results were observed for the presence of secondary metabolites in the form of alkaloids, flavonoids, steroids, saponins, tannins, and glycosides in kemangi leaf extract. This is consistent with the findings of Angga Nugraha et al. (2022), who tested the antibacterial activity of the ethanol extract of kemangi leaves against Staphylococcus aureus at a concentration of 500 mg/mL and found an inhibition zone diameter of 11.93 ± 0.25 mm.[6].

The mechanism of secondary metabolites as antibacterials, namely tannins, works by inhibiting the reverse transcriptase and DNA topoisomerase enzymes so that bacterial cells cannot form[24,25]. Likewise, flavonoids function by forming complex compounds with extracellular and dissolving proteins, which then disrupt the bacterial cell membrane and lead to the release of intracellular chemicals[26,27]. It is supported by saponin chemicals and has a method of action involving the reduction of surface tension, which results in enhanced permeability or cell leakage and the release of intracellular substances[28]. Likewise, other secondary metabolites synergize in providing antibacterial effects.

CONCLUSION

Strong antibacterial activity was demonstrated by the ethanolic extract of kemangi leaves, which had an activity index value of 55–56% against the positive control.

REFERENCES

1. Nasri, N., Kaban, V. E., Syahputra, H. D. & Satria, D. Aktivitas Antibakteri Ekstrak Etanol Daun Alpukat (Persea americana Mill) Terhadap Escherichia coli, Salmonella typhi, dan Pseudomonas aeruginosa. *Herb. Med. J.* **5**, 13–19 (2022).

2. Dugassa, J. & Shukuri, N. Review on antibiotic resistance and its mechanism of development. *J. Health Med. Nurs.* **1**, 1–17 (2017).

3. Bhalodia, N. R. & Shukla, V. J. Antibacterial and antifungal activities from leaf extracts of Cassia fistula l.: An ethnomedicinal plant. *J. Adv. Pharm. Technol. Res.* **2**, 104 (2011).

4. Nasri, N., Harahap, U., Silalahi, J. & Satria, D. Antibacterial activity of lactic acid bacteria isolated from Dengke Naniura of Carp (Cyprinus carpio) against diarrhea-causing pathogenic bacteria. *Biodiversitas J. Biol. Divers.* **22**, (2021).

5. Nasri, N., Harahap, U., Silalahi, J. & Satria, D. Antidiarrheal Effect of Lactobacillus fermentum Isolated from Dengke Naniura on Escherichia coli-induced Rats. *Indones. J. Pharm. Clin. Res.* **5**, 47–54 (2022).

6. Sanjaya, A. N., Harahap, U. & Dalimunthe, A. Phytochemical Screening and Antibacterial Potential Ethanol Extract of Kemangi Leaves (Ocimum basilicum L.) Towards Staphylococcus aureus. *Int. J. Sci. Technol. Manag.* **3**, 1520–1526 (2022).

7. Kaban, V. E., Nasri, N., Dharmawan, H. & Satria, D. Formulasi dan Uji Efektivitas Sabun Pencuci Tangan dari Ekstrak Daun Pandan (Pandanus amaryllifolius Roxb.) Terhadap Bakteri Salmonella sp. *Herb. Med. J.* **5**, 8–12 (2022).

8. Nasri, N., Kaban, V. E., Gurning, K., Syahputra, H. D. & Satria, D. Aktivitas Antibakteri Ekstrak Etanol Daun Pepaya (Carica papaya Linn.) Terhadap Bakteri Pseudomonas aeruginosa. *INSOLOGI J. Sains Dan Teknol.* **1**, 252–259 (2022).

9. Haro, G., Iksen, I. & Nasri, N. Identification, characterization and antibacterial potential of probiotic lactic acid bacteria isolated from naniura (A traditional batak fermented food from carp) against Salmonella typhi. *Rasayan J. Chem.* **13**, 464–468 (2020).

10. Lay, W. B. Analisis mikroba di laboratorium (Jakarta: PT. Raja Grafindo Persada). (1994).

11. Muhammad, M., Nasri, N., Kaban, V. E., Satria, D. & Cintya, H. Antibacterial Potential Ethanol Extract of Papaya Leaves (Carica papaya Linn.) Towards Salmonella typhi. *BEST J. Biol. Educ. Sains Technol.* **5**, 265–270 (2022).

12. Harahap, U. *et al.* Antioxidant and antibacterial activities of ethanol extract of Vernonia amygdalina Delile. Leaves. in *AIP Conference Proceedings* vol. 2342 080011 (AIP Publishing LLC, 2021).

13. Fitri, R., Reveny, J., Harahap, U. & Dharmawan, H. Anti-Acne Activity From Biocellulose Mask Formula Containing (Aloe Vera (L.) Burm. F) Essence Combined With Vitamin E. *Indones. J. Pharm. Clin. Res.* **4**, 1–7 (2021).

14. Situmorang, R. F. R., Gurning, K., Kaban, V. E., Butar-Butar, M. J. & Perangin-Angin, S. A. B. Determination of Total Phenolic Content, Analysis of Bioactive Compound Components, and Antioxidant Activity of Ethyl Acetate Seri (Muntingia calabura L.) Leaves from North Sumatera Province, Indonesia. *Open Access Maced. J. Med. Sci.* **10**, 240–244 (2022).

15. Syahputra, H. D., Nasri, N. & Kaban, V. E. Pengujian Potensi Aktivitas Antibakteri dari Daun Cep-cepan (Saurauia cauliflora DC.) dalam Formulasi Sediaan Gel Terhadap Propionibacterium acnes. *Herb. Med. J.* **5**, 28–32 (2022).

16. Kuspradini, H., Putri, A. S., Egra, S. & YANTI, Y. In vitro antibacterial activity of essential oils from twelve aromatic plants from East Kalimantan, Indonesia. *Biodiversitas J. Biol. Divers.* **20**, (2019).

17. Mere, J. K., Bintang, M. & Safithri, M. Antibacterial Effectiveness of Syzygium cumini (L.) Skeels Leaves to Escherichia coli pBR322. *Indones. J. Chem. Res.* **9**, 8–14 (2021).

18. Lubis, M. F. *et al.* ACUTE TOXICITY AND ANTIFUNGAL ACTIVITY OF THE OINTMENT Murraya koenigii ETHANOL EXTRACT. **15**, 256–261 (2022).

19. Chantadee, T., Santimaleeworagun, W., Phorom, Y., Chuenbarn, T. & Phaechamud, T. Vancomycin HCl-loaded lauric acid in situ-forming gel with phase inversion for periodontitis treatment. *J. Drug Deliv. Sci. Technol.* **57**, 101615 (2020).

20. Rodrigues, J. P., Prova, S. S., Moraes, L. A. B. & Ifa, D. R. Characterization and mapping of secondary metabolites of Streptomyces sp. from caatinga by desorption electrospray ionization mass spectrometry (DESI–MS). *Anal. Bioanal. Chem.* **410**, 7135–7144 (2018).

21. Rani, Z., Nasution, H. M., Kaban, V. E., Nasri, N. & Karo, N. B. Antibacterial activity of freshwater lobster (Cherax quadricarinatus) shell chitosan gel preparation against Escherichia coli and Staphylococcus aureus. *J. Appl. Pharm. Sci.* (2022).

22. Barabadi, H. *et al.* Green synthesis, characterization, antibacterial and biofilm inhibitory activity of silver nanoparticles compared to commercial silver nanoparticles. *Inorg. Chem. Commun.* **129**, 108647 (2021).

23. Sharififar, F., Moshafi, M. H., Mansouri, S. H., Khodashenas, M. & Khoshnoodi, M. In vitro evaluation of antibacterial and antioxidant activities of the essential oil and methanol extract of endemic Zataria multiflora Boiss. *Food Control* **18**, 800–805 (2007).

24. Rofida, S. Antibacterial and Characterization of Secondary Metabolite Compound from Ethyl Acetate and Ethanol Fraction of Leaves Moringa oliefera L. in *In Proceedings of the 2nd Health Science International Conference (HSIC 2019)* 233–239 (Science and Technology Publications, 2019).

25. Simaremare, E. S., Gunawan, E., Yarangga, I., Satya, M. D. & Yabansabra, Y. R. Antibacterial and toxicity activities itchy leaves (Laportea decumana, Roxb. Wedd) extract. in *Journal of Physics: Conference Series* vol. 1503 012041 (IOP Publishing, 2020).

26. Anisa, A., Ambarwati, M., Triani, A. A. & Tarigan, I. L. Modification of Nanocellulose as Conjugate of infection-causing Antibacterial Hydrogel. *Fuller. J. Chem.* **6**, 58–70 (2021).

27. Suryani, R. & Owbel, O. PENTINGNYA EKSPLORASI DAN KARAKTERISASI TANAMAN PISANG SEHINGGA SUMBER DAYA GENETIK TETAP TERJAGA. *Agro Bali Agric. J.* **2**, 64–76 (2019).

28. Salton, M. R. J. Lytic agents, cell permeability, and monolayer penetrability. *J. Gen. Physiol.* **52**, 227–252 (1968).