PENELITIAN ASLI

JIMKI : JURNAL ILMIAH
MAHASISWA KEDOKTERAN
INDONESIA

IN VITRO EVALUATION OF PHYTOCHEMICAL COMPOUND FOR ANTIBACTERIAL ACTIVITY AT *MURRAYA KOENIGII* LEAF EXTRACT AGAINST THE GROWTH OF *ESCHERICHIA COLI*

Elbert[1], Cut Adeya Adella[2], Dwi Faradina[2], Lokot Donna Lubis[3], Felix Khosasi[1]

[1] Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia
[2] Division of Gynecologic Oncology, Department of Obstetrics and Gynecology , Faculty of Medicine, Universitas Sumatera Utara, Haji Adam Malik General Hospital, Medan, Indonesia
[3] Department of Histology, Faculty of Medicine, Universitas Sumatera Utara, Medan, Indonesia

Korespondensi:

Elbert

Email Korespondensi:

elbert.sumarto14@gmail.com

Riwayat Artikel

Diterima: 16 – 07 – 2024
Selesai revisi: 31 – 12 – 2024

DOI :

10.53366/jimki.v11i1.767

Introduction: In developing nations, like Indonesia, urinary tract infection, or UTI, is a frequent infection in women that results from the growth of microorganisms in the urinary tract. *Escherichia coli* is the most common cause. Antibiotics are the major form of treatment; however, misuse of these drugs has led to resistance. Innovation in potential medical plants is crucial. The curry leaf plant (*Murraya koenigii*) is one of them.
Methods: The agar diffusion method was used to assess antibacterial activity. *Murraya koenigii* leaves were gathered from a garden in Tanjong Morawa. *Escherichia coli* ATCC 25922 is the type of bacteria that is used. Using 96% ethanol as the solvent, *Murraya koenigii* leaves were extracted using the maceration method. There were six treatments with concentrations of 6.25%, 12.5%, 25%, and 50%, positive control levofloxacin, and negative control DMSO. **Results**: The results of the phytochemical screening of the extracts showed that metabolites like flavonoids, tannins, and triterpenes had antibacterial effects. Data on the diameter of the inhibition zone were examined using the Kruskal-Wallis statistical test. The findings demonstrated that all treatments had statistically significant differences ($p<0.05$). **Discussions**: The presence of compounds such as flavonoids, tannins, and triterpenes in curry leaf extract has an antibacterial effect on *Escherichia coli*. This compound can prevent the creation of nucleic acids, inhibit cell membrane function, damage the permeability of bacterial cells, and deactivate bacterial enzymes. **Conclusion**: The ethanol extract of curry leaves has an inhibitory effect on the growth of *Escherichia coli* bacteria.

Keywords: Antibacterial; *Escherichia coli*; Inhibitory effect; Phytochemical screening; *Murraya koenigii* leaf extracts

e-ISSN : 2721-1924
ISSN : 2302-6391

1. INTRODUCTION

Escherichia coli is one of the bacteria that can cause infections in humans. *Escherichia coli* is a gram-negative, rod-shaped, facultatively anaerobic bacteria. These bacteria are capable of living in highly acidic conditions in the human body and also outside the body, where feces transmit them. Normal intestinal flora, *Escherichia coli*, can turn pathogenic if it enters tissues outside of the digestive tract, including the lining of the brain, bile ducts, and urinary tract[1]. *Escherichia coli* is responsible for over 90% of urinary tract infections[2]. Symptoms and signs of urinary tract infection consist of frequency of urination, dysuria, hematuria, pyuria, pain in the waist, and flank pain, which mark the occurrence of an ascending infection to the upper urinary tract[3].

Due to the prevalence of bacterial infections, antibiotics are the most commonly used drugs in the world. When the growth of bacteria is not inhibited by the use of antibiotics, antibiotic resistance can occur. This resistance is influenced by a number of factors, including stakeholder factors like a lack of government attention to monitoring the distribution and use of antibiotics, hospital factors like the widespread use of antibiotics in inpatient wards, and patient factors like a lack of patient knowledge about the disease and its management[4]. In 2014, the WHO stated that the problem of antibiotic resistance is a serious threat to public health, including in Indonesia. According to Basic Health Research (Riskesdas) from 2014, ten percent of people stored antibiotics at home, and eighty-six percent of them bought antibiotics without a prescription[5].

Because of antibiotic resistance, other alternatives are crucial, such as using plants in Indonesia that have antibacterial properties. In addition to being an alternative, the advantage of using plants is that they are relatively cheap and easy to obtain. The curry plant (*Murraya koenigii*) is one of the plants that is frequently used as medicine. In Indonesia, the curry plant (*Murraya koenigii*) is extensively obtainable. It is frequently used as a spice to add flavor, and it has a variety of uses in traditional medicine. The part that contains antibacterials is the leaf. Antibacterial compounds contained in curry leaves are alkaloids, saponins, tannins, polyphenols, quinones, and triterpenoids[6]. Curry leaf extract showed antibacterial effects on a wide variety of microorganisms. Curry leaves can be used as a natural remedy in everyday meals for the prevention of many bacterial diseases since the methanol and ethanol extracts of curry leaves have been shown to be effective against strains of *Escherichia coli*, *Staphylococcus*, *Streptococcus*, and *Proteus bacteria*[7].

In response to the various descriptions provided above, I, as a researcher, am motivated to investigate the impact of curry leaf extract (*Murraya koenigii*) on *Escherichia coli* growth inhibitors in order to help other researchers better understand and investigate the potential therapeutic benefits of this plant against the bacterial infection disease *E. coli*. The study has three objectives: (1) in vitro testing to determine whether phytochemical components in curry leaf extract may inhibit the development of Escherichia coli bacteria; (2) identifying the curry leaf extract's phytochemical profile; and (3) determining the amount of curry leaf extract (*Murraya koenigii*) needed to inhibit the development of *Escherichia coli* bacteria in vitro.

2. MATERIALS AND METHODS

This research has an experimental design with a posttest-only control group design. The Biology Laboratory, Faculty of Pharmacy, Universitas Sumatera Utara, is where the Murraya koenigii leaf extract was created. The *Murraya koenigii* extract's phytochemical profile was examined in the Biology Laboratory of the Faculty of Pharmacy at Universitas Sumatera Utara. The research on the antibacterial activity of *Murraya koenigii* leaf extract against the growth of *Escherichia coli* bacteria was conducted in the Microbiology Laboratory, Faculty of Medicine, Universitas Sumatera Utara. This study was conducted from July to October 2021.

The sample used in this study is *Escherichia coli* bacteria that have been exposed to various concentrations of *Murraya koenigii* leaf extract. The *Murraya koenigii* leaf is taken from a garden in Tanjong Morawa, whereas *Escherichia coli* ATCC 25922 is obtained from the Laboratory of Microbiology, Faculty of Medicine, Universitas Sumatera Utara.

In this study, the sample received six treatments consisting of levofloxacin as the positive control, *Murraya koenigii* leaf extract at concentrations of 6.25%, 12.5%, 25%, and 50%, as well as DMSO as the negative control. These six treatments are repeated four times, so the total number of samples is 24.

Ethical approval

This study had been approved by the Research Ethics Committees of Universitas Sumatera Utara, Indonesia (approval number: No: 944/KEPK/USU/2021).

Collection of plant material

The fresh leaves of *Murraya koenigii* were collected in July 2021 from a garden in Tanjong Morawa, Medan, Indonesia. The plant specimen was identified and authenticated at the Herbarium Medanese Laboratory, Universitas Sumatera Utara. The plant material was prepared for the extraction technique after identification.

Preparation of the plant extract

Two kilograms of leaves were properly washed under running water, then dried in the shade for a full day. The leaves were then dried for five days in a cabinet with a 40-watt bulb. The leaves were then crushed into little bits and finally powdered using a grinder. The leaf powder was then put into plastic bags and kept for further use. 500 grams of dried, powdered leaves were macerated in 5 L of 96% ethanol, which was then tightly closed and left to soak for 24 hours while being occasionally stirred. The extract solution was filtered twice using Whatman paper No. 1 after being in solution for 24 hours. After that, all the filtrate was collected and evaporated using the rotary vacuum evaporator, and the leftover filtrate was concentrated in a glass container in the oven to create a viscous extract. For later usage, the extract was kept chilled at 4 °C in the refrigerator.

Assessment of antibacterial activity

Escherichia coli ATCC 25922, which were collected from the Microbiology Laboratory, Faculty of Medicine, Universitas Sumatera Utara, were used in this study. The antibacterial activity of the leaf extracts was evaluated by the agar-well diffusion method. *Escherichia coli* cultures that had been growing for 24 hours were placed in each petri dish with Muller-Hinton agar. A sterile cork's pit with a diameter of about

6 mm was used to bore wells, and various extract concentrations were applied. Dimethyl sulfoxide (DMSO) was used to dissolve the extract at concentrations of 6.25%, 12.5%, 25%, and 50%. Levofloxacin was used as a positive control and DMSO as a negative control. The plates were incubated in an upright position for 24 hours at 37 °C. The antibacterial activity was evaluated by measuring the diameter of the inhibition zone that formed around the well using a caliper.

Statistical analysis

SPSS Statistics version 25 was used to perform the statistical analysis. The Kruskal-Wallis one-way ANOVA statistical test was used for analyzing the data.

3. RESULT

Once the curry leaf extract is complete, it should be checked and analyzed to see if it contains any active ingredients, such as flavonoids, tannins, and other substances that have the potential to prevent bacteria from growing in the extract that has been described in the Das and Madhavan study, 2021. Therefore, the phytochemical test is carried out in the Biological Laboratory of Universitas Sumatera Utara School of Pharmacy using a six-parameter method and a qualitative test method using multiple reagents. According to the results of the phytochemical test, the parameters of the substances are shown in Table 1 below.

Table 1. Phytochemical test of curry leaf extract *(Murraya koenigii)*

Compound	Reagent	Interpretation of Results
Flavonoid	Mg powder + Alcohol + HCl Amil	+
Alkaloid	Dragendroff	-
	Bouchardat	-
	Meyer	-
Tannin	$FeCl_3$	+
Saponin	Hot water/ whipped	-
Glycosides	Molish + H_2SO_4	+
Triterpene/ Steroid	Lieberman-Bourchat	+

The diameters of the inhibition zones of *Escherichia coli* that have been given Murraya koenigii leaf extract with concentrations of 6.25%, 12.5%, 25%, and 50%, the positive control and negative control, are shown in table 2.

Table 2. The diameter of the inhibition zone of the ethanolic leaf extract of *Murraya koenigii*

	Diameter of inhibition zone (mm)					
Repetition	C - DMSO	Extract 50%	Extract 25%	Extract 12.5%	Extract 6.25%	C + Levofloxacin
I	0	13.40	12.45	11.80	10.90	53.55
II	0	12.65	11.80	11.60	10.45	50.55
III	0	12.50	12.35	11.45	11.85	49.5
IV	0	13.60	12.85	12.65	10.10	50.02
Mean	**0**	**13,037**	**12.362**	**11.875**	**10.825**	**50.905**
Mean ± SD	**0**	**13.037 ±0.54371**	**12.362 ±0.18729**	**11.875 ±0.2875**	**10.825± 0.57416**	**50.905± 1,81469**

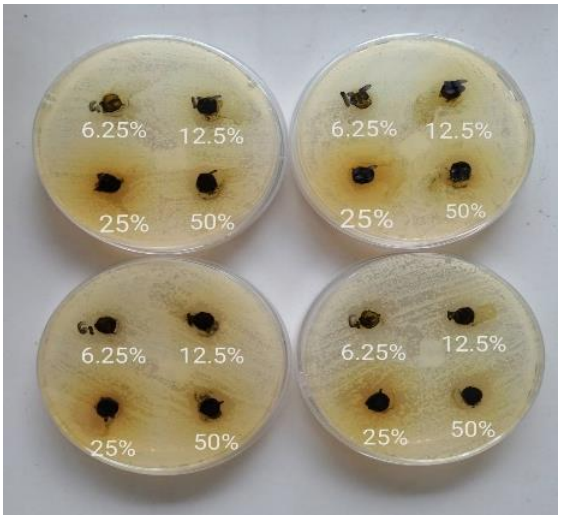

Figure 1. Inhibition zone of *Murraya koenigii* extract

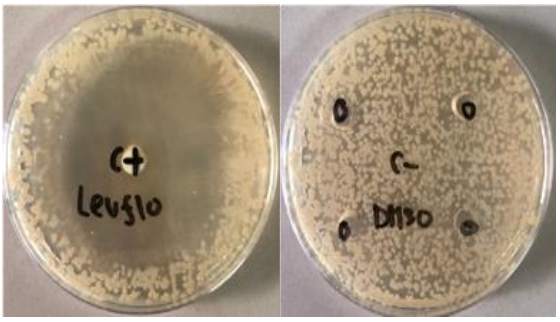

Figure 2. Inhibition zone of control positive and negative

Table 2, figure 1 and 2, show the inhibition zone, and numbers from the measurement of curry leaf extract inhibition zones against the growth of E. coli bacteria from extracts with concentrations of 6.25%, 12.5%, 25%, and 50% allow for the conclusion that the higher the concentration, the bigger the diameter of the inhibition zone. Even though, from the data, there is only a difference of about one millimeter between each concentration, it can be concluded that there is indeed an effect on concentration in the zone. However, the study was described under different concentrations of curry leaf extract as having a smaller, milder area compared to some of the positive controls performed. This reveals that curry leaf extract contains compounds that can inhibit the growth of *E. coli* bacteria but are less effective than the positive controls.

Table 3. Kruskal-Wallis Test Results

Kruskal-Wallis Test

Ranks

	Kelompok	N	Mean Rank
ZonaHambat	kontrol negatif	4	2,50
	Ekstrak 12.5%	4	6,50
	Ekstrak 25%	4	11,00
	Esktrak 50%	4	14,38
	Ekstrak 75%	4	18,13
	kontrol positif	4	22,50
	Total	24	

Test Statistics[a,b]

	ZonaHambat
Kruskal-Wallis H	21,978
df	5
Asymp. Sig.	,001

a. Kruskal Wallis Test

b. Grouping Variable: Kelompok

Based on the results of the Kruskal-Wallis one-way ANOVA variant test, we obtained a significance value of 0.000 (P < 0.05). This indicates that there is a significant difference in the various concentrations of *Murraya koenigii* leaf extract in inhibiting the growth of the *Escherichia coli* bacteria.

4. DISCUSSION

Due to the presence of secondary metabolites in the extract, curry leaf extract can produce inhibition zones. This claim has been supported by phytochemical results, in which components like flavonoids, glycosides, tannins, and triterpenes performed well in

a qualitative test of their phytochemical content. Flavonoids work as antibacterial substances by preventing the creation of nucleic acids, preventing the function of cell membranes, and preventing the metabolism of energy. The antibacterial mechanism of flavonoid compounds prevents the synthesis of nucleic acid rings A and B, which play a significant part in the hydrogen bonding process, which prevents the development of DNA and RNA by accumulating nucleic acid bases. The interaction between flavonoids and bacterial DNA will damage the permeability of bacterial cell walls, microsomes, and lysosomes. Flavonoids disrupt cell membranes by forming complex compounds from extracellular and dissolved proteins, preventing the function of cell membranes, and allowing intracellular compounds to get out. Flavonoids also prevent the production of energy in the cytoplasmic membrane and inhibit the motility of bacteria that are involved in antimicrobial activity and extracellular proteins, which contribute to the inhibition of energy metabolism and bacterial oxygen[8].

Tannin substances have an antibacterial mechanism that works by blocking the reverse transcriptase and DNA topoisomerase enzymes, preventing the formation of bacterial cells [9]. Tannins have the ability to disrupt the proper construction of bacterial walls, deactivate bacterial enzymes, and obstruct the movement of proteins in the inner layer of cells[10].

Terpenoids work as antibacterial agents by decreasing the permeability of the wall by creating a powerful polymer connection with the porin, which harms the porin. Bacterial cells that lack nutrients will either stop growing or die as a result of damage to the porin, which serves as the entrance and exit for compounds[11]. Terpenoids harm cell membranes, which causes the cytoplasm to congeal and membrane permeability to rise. These effects lead to intracellular component leakage and decreased ATP generation[12].

The phytochemical test results obtained flavonoid compounds, tannins, glycosides, and triterpenes, which were also found in the Madhavan study of 2021, where these compounds have the potential to inhibit bacterial growth. However, there are differences in the phytochemical results from the Madhavan study (2021), where the results of the Madhavan phytochemical test found alkaloid active compounds, while in this study they were not found. The difference in the content of active compounds in this study with other studies may be due to differences in curry leaf extract due to the location, condition, and weather of the growth of this curry plant, where the content of metabolites in a plant is influenced by several factors, both internal and external. Internal factors are the influence of genes, and external factors such as the influence of light, temperature, humidity, pH, nutrient content in the soil, and altitude at different locations will certainly have different conditions[13].

In this study, curry leaf extract was diluted into four concentrations, namely 6.25%, 12.5%, 25%, and 50%. With each increase in concentration, the inhibition zone expands. The findings of this study are consistent with those of Jelita's research regarding the antibacterial properties of curry leaf extract, which found that the antibacterial activity increased with curry leaf content. This study has found that the amount of antibacterial chemicals in an extract increases with its concentration, allowing

it to more effectively suppress bacterial development[14].

In a study conducted by Jelita et al. (2019) using concentrations starting from 10%, 20%, and 40% with the Kirby Bauer diffusion method, the researchers conducted another trial with the diffusion method of the well and used a lower concentration of 6.25%, and the results are sufficient to provide data that the concentration of 6.25% can also be effective and has bacterial growth inhibitory activity[14].

According to Salim et al. (2016) research, it was found that soil nutrient content is inversely proportional to the amount of secondary metabolite production but directly proportional to the number of secondary metabolites produced, and flavonoid compounds are formed in areas with high calcium content [15]. Calcium is an enzyme activator, especially for protein-related enzymes, which helps facilitate the formation of secondary metabolites. Because low-pH soils and waters are typically found in nutrient-deficient conditions followed by poor productivity levels, variations in soil pH also affect the concentration of secondary metabolites in the extracts[16].

According to the results of the normality test, the data has a normal distribution because it has a significance value ($p>0.05$), followed by the data homogeneity test, which found a significance value ($p<0.05$), which indicates the data is not homogeneous or the data group comes from a population with a different variance, so it does not meet the requirements for the one-way ANOVA test, so it continues with another alternative test, the Kruskal-Wallis test. It was discovered that there was a significant difference in the concentration of curry leaf extract in preventing the development of *Escherichia coli* bacteria, with a significance value of 0.001 ($p<0.05$).

5. CONCLUSIONS

As a conclusion, the ethanolic extract of *Murraya koenigii* leaves has antibacterial activity against the growth of *Escherichia coli* bacteria. Results of six-parameter phytochemical testing revealed the presence of substances such as flavonoids, tannins, glycosides, and triterpenes that have the potential to inhibit the growth of bacteria. The inhibitory zone grew larger as the extract concentration increased.

REFERENCES

1. Haribi, R., & Yusron, K. (2010). PEMERIKSAAN Escherichia coli PADA AIR BAK WUDHLU 10 MASJID DI KECAMATAN TLOGOSARI SEMARANG. J*urnal Kesehatan*, 3(1), *JURNAL*, 21-26.
2. Sari, R. P., & Muhartono. (2018). Angka Kejadian Infeksi Saluran Kemih (ISK) dan Faktor Resiko Yang Mempengaruhi Pada Karyawan Wanita di Universitas Lampung. *Majority | Volume 7*, 1150120.
3. Jawetz, Melnick, Adelberg's Riedel, S., Morse, S., Mietzner, T., & Miller, S. (2019). Jawetz Microbiology [28th Edition]. New York: McGraw-Hill.
4. Putra, D. P., & Kusmiati, T. (2015). Manajemen Pemberian Antibiotik dengan Hasil Uji Kepekaan Resisten. *JURNAL RESPIRASI*, 7-14.
5. KEMENKES. (2018, November 28). *Pengendalian Resistensi Antimikroba Jadi Perhatian Dunia*. Diakses pada 4 Mei 2021, dari www.kemkes.go.id:https://www.ke

mkes.go.id/article/view/181129000002/pengendalian-resistensi-antimikroba-jadi-perhatian-dunia.html

6. Purwanitiningsih, E., Nurbaiti, & D.L, A. L. (2021). UJI DAYA HAMBAT DAUN SALAM KOJA (Murraya koenigii (L.) Spreng) TERHADAP PERTUMBUHAN BAKTERI Escherichia coli DAN Staphylococcus aureus DENGAN METODE KIRBY BAUER. *Jurnal Pro-Life Volume 8*, 1-11.
7. Balakrishnan, R., Vijayraja, D., Jo, S., Ganesan, P., Su-kim, I., & Choi, D. (2020). Pharmacological Activities of Murraya koenigii and Its Primary Bioactive Compounds. *Antioxidants*, *9*, 101.
8. Nomer, N., Rakasari, G., Duniaji, A. S., Nocianitri, K. A., Pertanian, F. T., Pertanian, F. T., & Jimbaran, K. B. (2019). *KANDUNGAN SENYAWA FLAVONOID DAN ANTOSIANIN EKSTRAK KAYU SECANG (Caesalpinia sappan L .) SERTA AKTIVITAS ANTIBAKTERI TERHADAP Vibrio cholerae*. *8*(2), 216–225.
9. Egra, S., Mardhiana, ., Rofin, M., Adiwena, M., Jannah, N., Kuspradini, H., & Mitsunaga, T. (2019). Aktivitas Antimikroba Ekstrak Bakau (Rhizophora mucronata) dalam Menghambat Pertumbuhan Ralstonia Solanacearum Penyebab Penyakit Layu. *Agrovigor: Jurnal Agroekoteknologi*, *12*(1), 26. https://doi.org/10.21107/agrovigor.v12i1.5143
10. Sapara, T. U., & Waworuntu, O. (2016). Efektivitas Antibakteri Ekstrak Daun Pacar Air (Impatiens Balsamina L.) Terhadap Pertumbuhan Porphyromonas Gingivalis. *Pharmacon*, *5*(4), 10–17. https://doi.org/10.35799/pha.5.2016.13968
11. Rachmawati, F., Nuria, M. C., & Sumantri. (2011). UJI AKTIVITAS ANTIBAKTERI FRAKSI KLOROFORM EKSTRAK ETANOL PEGAGAN (Centella asiatica (L) Urb) SERTA IDENTIFIKASI SENYAWA AKTIFNYA. *Jurnal Ilmu Farmasi & Farmasi Klinik*
12. Anon,2019.[online]Available at:https://www.researchgate.net/publication/333702920_Inhibition_Activity_of_Water_Hyacinth_Leaf_Extract_Eichhornia_Crassipes_Against_Aggegatibacter_Actinomycetemcomitans [Accessed 7 Nov. 2019]
13. Katuuk, R. H. H. *et al.* 2019. ‘Mahasiswa Program Studi Agroteknologi Fakultas Pertanian Universitas Sam Ratulangi Manado 2) Dosen Program Studi Agroteknologi Fakultas Pertanian Universitas Sam Ratulangi Manado Jl. Kampus Unsrat Manado
14. Jelita, J., Wirjosentono, B., Tamrin, T., & Marpaung, L. (2019). Aktivitas Antibakteri dan antioksidan dari Ekstrak Daun Kari (Murraya koeginii) Ditinjau dari Waktu Penyimpanan. *Talenta Conference Series: Science and Technology (ST)*, *2*(1), 29–36. https://doi.org/10.32734/st.v2i1.308
15. Julianto, T. S. (2019). *FITOKIMIA Tinjauan Metabolit Sekunder dan Skrining Fitokimia*. Yogjakarta: Universitas Islam Indonesia.

16. Salim, M., Yahya, Y., Sitorus, H., Ni'mah, T., & Marini, M. (2017). Hubungan Kandungan Hara Tanah dengan Produksi Senyawa Metabolit Sekunder pada Tanaman Duku (Lansium domesticum Corr var Duku) dan Potensinya sebagai Larvasida. *Jurnal Vektor Penyakit*, *10*(1), 11–18. https://doi.org/10.22435/vektorp.v10i1.6252.11-18