OPEN ACCESS
Bioactivities Vol. 1 No. 1 (2023) https://doi.org/10.47352/bioactivities.2963-654X.182
Check for updates
Research Article

Antibacterial Activity of Several Types of Weed Extracts on The Growth of *Escherichia coli*

Dwi Nur Rikhma Sari, Septarini Dian Anitasari*, and Intan Choirul Uliyah

Received : February 26, 2023	Revised : June 26, 2023	Accepted : June 27, 2023	Online : June 30, 2023

Abstract
Escherichia coli is a type of Gram-negative bacteria that is commonly found in the human digestive tract. It has been reported as one of the bacteria that has been resistant to several antibiotics. This study aims to determine four (4) types of weed extracts that are considered but have not been used as antibacterial compounds against *E. coli*. This experimental study used a completely randomized design with several types of plant extracts, namely *Acalypha indica* L., *Ageratum conyzoides*, *Phyllanthus niruri* L., and *Amaranthus spinosios* at various concentrations (0, 50, and 100%). The results showed that the plant extract of *A. indica* L. had the ability as an antibacterial against the growth of *E. coli* at concentrations of 50% (1.41 ± 0.12) and 100% (1.53 ± 0.01) compared to other extracts. Meanwhile, the lowest average diameter of the inhibition zone for *E. coli* bacteria was found in the treatment of *A. spinosios* grass leaf extract 50% (1.17 ± 0.05).

Keywords: *Escherichia coli*, antibacterial, weed extract, *Acalypha indica*

1. INTRODUCTION

Escherichia coli is commonly found in the human and poultry digestive tracts [1] with a colony count of 10^4-10^5 CFU/mL. In addition, *E. coli* bacteria cause many gastrointestinal infections which are strongly influenced by environmental hygiene [2]. *E. coli* bacteria are pathogenic if they are outside the intestinal digestive tract, one of which can infect the urinary tract, bile duct, and other cavities in the stomach [3]. This bacterium belongs to the Gram-Negative bacteria which have a 3 layers of cell walls, namely lipopolysaccharide, protein, and phospholipids, which are highly resistant to antibacterial compounds compared to Gram-Positive bacteria [3].

E. coli bacteria have been reported as one of the bacteria that has been resistant to several antibiotics [4]. There was an increase from 7.2% (in 1950 in America) to 63.6% (2000) which also increased in several other countries [5]. Resistance to *E. coli* pathogenic bacteria are very dangerous because they can cause sporadic and endemic infections [6]. Thus, various studies are needed on new antibiotics that are potential to inhibit or kill resistant bacteria at affordable prices.

Several studies have been carried out to overcome the resistance of pathogenic bacteria by utilizing several antimicrobial compounds. One of which is by utilizing several plant extracts. Plants are one type of natural resource that has a lot of antimicrobial agents and still needs a lot of exploration in finding new drugs [7]. One of which is weeds which are considered weeds and they are rarely used.

Several types of extracts of weeds or weeds that have been researched to inhibit the growth of pathogenic bacteria that are resistant to antibiotics include *Lophatherum gracile* Brongn [8], *Mimosa pudica* [9], *Cyperus rotundus* [10], *Imperata cylindrica* [11], *Acalypha indica L.* L. [12][13], *Ageratum conyzoides* [14][15], and *Phyllanthus niruri* L. [16]. This study aims to determine four types of weed extracts that are considered but have not been used as antibacterial compounds against *E. coli*.

2. MATERIALS AND METHODS

This experimental study aims to determine the potential of some grass extracts as antibacterial compounds against *E. coli* at concentrations of 0, 50, and 100%; and using the agar diffusion method. The materials used were Nutrient Agar (NA) media, and wild grass extracts, namely: (1) *Acalypha*

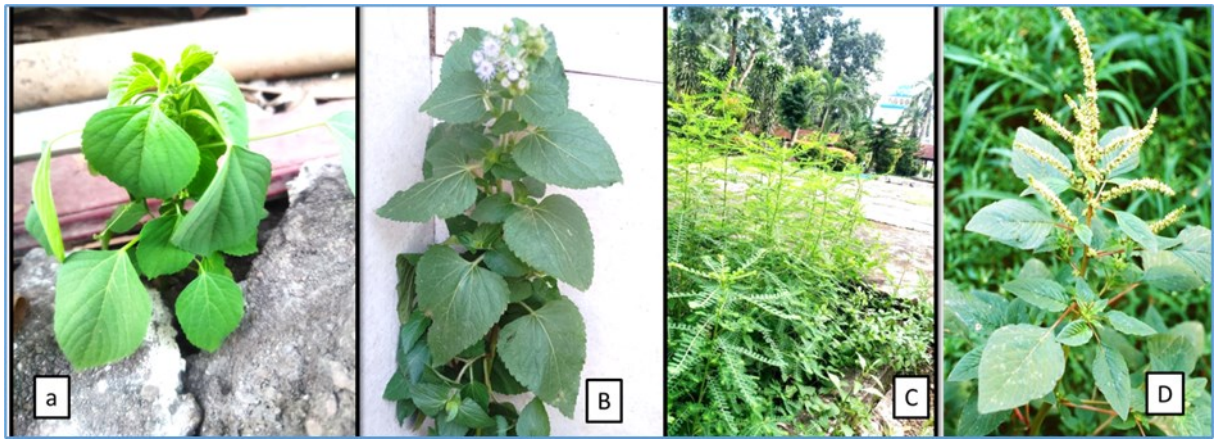

Figure 1. Leaves of *Acalypha indica* L (a), *Ageratum conyzoides (b)*, *Phyllanthus niruri* L. (c), and *Amaranthus spinosios* (d).

indica L., (2) *Ageratum conyzoides*, (3) *Phyllanthus niruri* L., and (4) *Amaranthus spinosios*. Apart from that, the materials needed are cotton, spirit, aluminum foil, distilled water, 70% and 95% alcohol [17], and *E. coli*. The tools needed are incubator, Bunsen, volume pipette, Erlenmeyer, beaker glass, 1000 mL micropipette, Petri disk, paper disk, ose, hot plate, analytical balance, mortal, crusher, test tube, stirrer, and tweezers.

The first stage is washing the grass leaves prior to use. Clean grass leaves are then air-dried at room temperature. The material is then crushed with a blender into smaller pieces, sifted to obtain powder, stored in a glass beaker, and covered with aluminum foil [18].

The NA media is prepared with the appropriate procedure listed on the label where the media is. For NA media, weighed as much as 2.8 g NA and then dissolved in 100 mL of distilled water each. After that, put into a 500 mL Erlenmeyer flask and sterille with other equipment using an autoclave of 121 ºC, 1 atm for ± 15 min [18].

Rejuvenation of bacterial isolates in this study used *E. coli* which had previously been obtained as the parent of the bacterial culture, by taking ± 1 ose and growing it, and streaking it on NA media so that it was slanted in a test tube. After that, the rejuvenation results were incubated at room temperature for 24 h and the growth of bacteria was observed by measuring the diameter of the inhibition zone [17].

The antibacterial activity test stage (Diffusion Method) was carried out by taking as much as 1 mL of bacterial starter from the dilution and then pouring it into 9 mL of NA media in a sterile agar plate. While waiting for it to solidify, soak the paper disc for ± 10 min in each of the grass extracts that have been prepared with various treatment concentrations of 0, 50, and 100%. Next, place the paper disc on the surface of the NA media in a petri dish which has been mixed with bacterial isolates and incubated at room temperature for 24 h. The antibacterial observation was carried out by looking at the clear zone around the disc paper. Afterward, the observed data were further analysed [18]. The data obtained in the form of diameters of the inhibition zones of several extracts at various concentrations were analyzed using the 5% Kruskall Wallis Test which had previously been analyzed for Normality and Homogeneity.

3. RESULTS AND DISCUSSIONS

Leaves of *A. indica* L., *A. conyzoides*, *P. niruri*

Table 1. Mean Diameter of Inhibition Zone of Leaf Extract against *Escherichia coli*.

Plant Species	Concentration Extract		
	0% (Control)	50%	100%
Ageratum conyzoides	1.02 ± 0.02^{a}	1.31 ± 0.09^{c}	1.47 ± 0.07^{de}
Acalypha indica L. L.	1.00 ± 0.00^{a}	1.41 ± 0.12^{cde}	1.53 ± 0.01^{e}
Phyllanthus niruri L.	1.00 ± 0.00^{a}	1.18 ± 0.07^{b}	1.38 ± 0.22^{cd}
Amaranthus spinosios	1.00 ± 0.00^{a}	1.17 ± 0.05^{b}	1.53 ± 0.09^{e}

L., and *A. spinosios* (Figure 1) were dried naturally for ± 3-4 d. Then each leaf was extracted using sterilled distilled water according to the concentration treatment (0, 50, and 100%). The antibacterial activity test of E. coli was evaluated using the diffusion method. The diameter of the inhibition zone of each treatment was observed for 24 h of the incubation period (Figure 2). The results obtained on the diameter of the inhibition zone for *E. coli* growth at various can be seen in Table 1. Based on Table 1, it can be seen that the average diameter of the inhibition zone is the highest for *A. spinosios* (1.53 ± 0.09) and *A. indica* L. (1.53 ± 0.01) with 100% concentration (Table 1). Meanwhile, the lowest average diameter of the inhibition zone for *E. coli* was found in the treatment of *A. spinosios* grass leaf extract (1.17 ± 0.05) with a concentration of 50%. Table 1 also shows that the average diameter of the inhibition zone of all types of leaf extracts was greater than the control treatment. This shows that the higher the concentration of leaf extract gave the larger the diameter of the inhibition zone for bacterial growth. The increase in inhibition zone activity is possible because the higher the concentration of the extract used, the more antibacterial substances it contains.

Homogeneity and normality analysis showed that the data was not homogeneous, so the analysis in this study used the Kruskal Wallis test (Sig 0.00). Based on the results of the SPSS analysis with a confidence level of 95%, it showed that the concentrations of some grass leaf extracts showed a significant difference (Table 2).

Based on the mean, Table 1 shows that the growth inhibition ability of grass extracts against *E. coli* at a concentration of 50% was highest in *A. indica* L. leaves and the lowest average was found in *A. spinosios* leaf extract. At 100% concentration, the inhibition ability of grass extracts against *E. coli* was highest in *A. indica* L. plants and the lowest mean diameter was found in *P. niruri* L. plants (Table 1). *Acalypha indica* L., leaves have greater

Table 2. Mean Diameter of Inhibition Zone of Leaf Extract against *Escherichia coli*

	Diameter of inhibition zone (mm)
Chi-square	52.596
df	11
Asymp. Sig.	.000

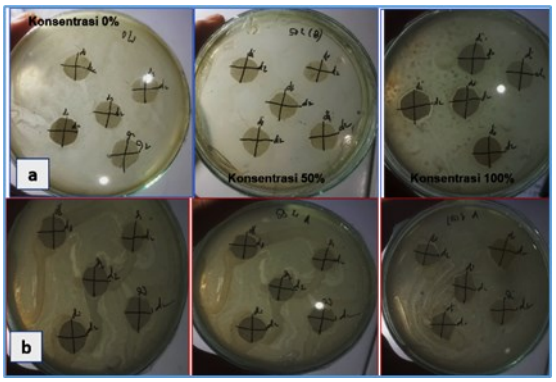

Figure 2. Zone of Inhibition of leaf extracts of (a) *Ageratum conyzoides* and (b) *Amaranthus spinosios* at various concentrations (0, 50, and 100%).

antibacterial ability than the other three types of grasses. This is because *Acalypha indica* L. contains secondary metabolites, namely alkaloid compounds, flavonoids, catechol, phenolic compounds, steroids, and saponins [13] which have been shown to be effective as an antioxidant, anticancer, antiviral, antidiabetic, and antifungal compounds [19]. In addition, this grass also contains acalyphines, polyphenols, tannins, and essential oils which are beneficial to health and have been shown to have abilities as antioxidant compounds [20]. This is supported by other research, which states that the *A. indica* L. plant has a flavonoid content of 18.84 mg QE/g extract [21] and 21.50 ± 3 mg QE/g extract and 19 .0 ± 3 mg QE/g extract [12]. The flavonoid content of *A. indica* L. has the ability as an antibacterial compound that works by damaging the bacterial cell walls [12] and has antifungal activity [22].

In addition, the leaves of *A. spinosios*, *A. conyzoides*, and *P. niruri* L., have the ability as antibacterial compounds in *E. coli*. This is because these grasses contain secondary metabolites that can inhibit the growth of bacteria. Based on the results of research using a gas chromatography-mass spectrometry on the *n*-hexane extract, the *A. conyzoides* plant contains coumarin and ageratochromen compounds which have antibacterial abilities, namely inhibiting bacterial growth [14]. In addition, *A. conyzoides* also contains saponins, flavonoids, polyphenols, and essential oils which are known as disinfectants to kill pathogenic microorganisms [23]. Other studies also support that the polyphenol compounds in the *A. conyzoides* plant have been shown to have antibacterial activity and coumarin compounds [24].

P. niruri L. also has benefits as a medicine for fever, canker sores, hepatitis, gastrointestinal disorders, skin diseases, and diarrhea [25] because secondary metabolites have antibacterial activity. This is in accordance with the results of phytochemical tests kaur et al. [26] which showed that this plant contains bioactive compounds that have antibacterial activity, namely terpenoids, alkaloids, flavonoids, saponins, and tannins. Meanwhile, based on table 1, *A. spinosios* grass also showed antibacterial activity. This is consistent with the results of the study that the *A. spinosios* plant contains secondary metabolites, namely alkaloids, saponins, flavonoids, tannins, phenols, anthraquinones, and steroids which function as antibacterial compounds [27].

Based on the results of the research above, it was shown that extracts of grass plants *A. indica* L., *A. conyzoides*, *P. niruri* L., and *A. spinosios* could inhibit the growth of *E. coli* which are Gram-Negative bacteria. This is because the cell wall of Gram-Negative bacteria has a more complex chemical composition, due to the presence of a peptidoglycan layer and three polymer layers, namely lipoprotein, outer membrane, and lipopolysaccharide, making it more difficult for active drug compounds to penetrate the cell walls of Gram-negative bacteria than Gram-positive bacteria [15].

4. CONCLUSIONS

The antibacterial activity of several types of weed extracts on the growth of *E. coli* study concluded that the plant extract of *Acalypha indica* L. had the ability to be an antibacterial against the growth of *E. coli* at a concentration of 50% (1.41 ± 0.12) and 100% (1.53 ± 0.01) compared to other grass extracts. Meanwhile, the lowest average diameter of the inhibition zone for *E. coli* was found in the treatment of *Amaranthus spinosios* at a concentration of 50% (1.17 ± 0.05).

AUTHOR INFORMATION

Corresponding Author

Septarini Dian Anitasari — Department of Biology, University of PGRI Argopuro Jember, Jember–68121 (Indonesia);
Email: septarinidian87@gmail.com
orcid.org/0000-0002-2790-7986

Authors

Dwi Nur Rikhma Sari — Department of Biology, University of PGRI Argopuro Jember, Jember–68121 (Indonesia);
orcid.org/0009-0009-1176-3103

Intan Choirul Uliyah — Department of Biology, University of PGRI Argopuro Jember, Jember–68121 (Indonesia);
orcid.org/0009-0006-4304-6895

Author Contributions

D. N. R. S. Validation, Formal Analysis, Investigation, Writing – Original Draft Preparation; S. D. A. Writing – Original Draft Preparation, Review & Editing, and Project Administration; I. C. U. Investigation.

Conflicts of Interest

The authors declare no conflict of interest.

ACKNOWLEDGEMENT

This research was conducted by Lecturers and Students, Department of Biology, Faculty of Science and Technology, which is fully supported by the Integrated Laboratory, especially the Biology Laboratory, University of PGRI Argopuro Jember.

REFERENCES

[1] A. Bichon, C. Aubry, G. Dubourg, H. Drouet, J. C. Lagier, D. Raoult, and P. Parola. (2018). "Escherichia coli spontaneous community-acquired meningitis in adults: A case report and literature review". *International Journal of Infectious Diseases.* **67** : 70-74. 10.1016/j.ijid.2017.12.003.

[2] D. Stanley, R. J. Hughes, and R. J. Moore. (2014). "Microbiota of the chicken gastrointestinal tract: influence on health, productivity and disease". *Applied Microbiology and Biotechnology.* **98** (10): 4301-10. 10.1007/s00253-014-5646-2.

[3] N. Suryati, E. Bahar, and I. Ilmiawati. (2018). "Uji Efektivitas Antibakteri Ekstrak Aloe vera Terhadap Pertumbuhan Escherichia coli

Secara In Vitro". *Jurnal Kesehatan Andalas.* **6** (3). 10.25077/jka.v6i3.732.

[4] U. Sharaha, E. Rodriguez-Diaz, K. Riesenberg, I. J. Bigio, M. Huleihel, and A. Salman. (2017). "Using Infrared Spectroscopy and Multivariate Analysis to Detect Antibiotics' Resistant Escherichia coli Bacteria". *Analytical Chemistry.* **89** (17): 8782-8790. 10.1021/acs.analchem.7b01025.

[5] D. A. Tadesse, S. Zhao, E. Tong, S. Ayers, A. Singh, M. J. Bartholomew, and P. F. McDermott. (2012). "Antimicrobial drug resistance in Escherichia coli from humans and food animals, United States, 1950-2002". *Emerging Infectious Diseases.* **18** (5): 741-749. 10.3201/eid1805.111153.

[6] L. W. Riley. (2019). "Differentiating Epidemic from Endemic or Sporadic Infectious Disease Occurrence". *Microbiology Spectrum.* **7** (4). 10.1128/microbiolspec.AME-0007-2019.

[7] M. A. Hayashi, F. C. Bizerra, and P. I. Da Silva Jr. (2014). "Antimicrobial Compounds from Natural Sources". *Frontiers in Microbiology.* **2**. 10.3389/fmicb.2013.00195.

[8] K. H. Lai, P. J. Chen, C. C. Chen, S. H. Yang, M. El-Shazly, Y. C. Chang, Y. H. Wu, Y. H. Wu, Y. H. Wang, H. L. Hsieh, and T. L. Hwang. (2021). "Lophatherum gracile Brongn. attenuates neutrophilic inflammation through inhibition of JNK and calcium". *Journal of Ethnopharmacology.* **264** : 113224. 10.1016/j.jep.2020.113224.

[9] L. Mehingko, H. Awaloei, and M. P. Wowor. (2013). "Uji Efek Antimikroba Ekstrak Daun Putri Malu (Mimosa Pudica Duchaas & Walp) Secara in Vitro". *Jurnal Biomedik.* **2** (1). 10.35790/jbm.2.1.2010.842.

[10] Q. P. Hu, X. M. Cao, D. L. Hao, and L. L. Zhang. (2017). "Chemical Composition, Antioxidant, DNA Damage Protective, Cytotoxic and Antibacterial Activities of Cyperus rotundus Rhizomes Essential Oil against Foodborne Pathogens". *Scientific Reports.* **7** : 45231. 10.1038/srep45231.

[11] R. D. T. Nago, P. Nayim, A. T. Mbaveng, J. D. S. Mpetga, G. T. M. Bitchagno, B. Garandi, P. Tane, B. N. Lenta, N. Sewald, M. Tene, V. Kuete, and A. S. Ngouela. (2021). "Prenylated Flavonoids and C-15 Isoprenoid Analogues with Antibacterial Properties from the Whole Plant of Imperata cylindrica (L.) Raeusch (Gramineae)". *Molecules.* **26** (16). 10.3390/molecules26164717.

[12] S. H. B. Aulia Nuanza Alam, Ibnul Mubarok. (2017). "Penentuan Konsentrasi Minimum Ekstrak Daun Anting-Anting (Acalypha indica L.) sebagai Antibakteri pada Staphylococcus aureus". *LIife Science.* **6** (1): 34-39.

[13] A. Majid and N. Majid. (2020). "Identifikasi Senyawa Antibakteri Pada Ekstrak Akar Herba Acalypha Indica L. Asal Kota Kupang". *CHMK Applied Scientific Journal.* **3** (3): 87-92.

[14] T. Aminingsih, H. Nashrianto, and A. Syaiful Rohman. (2012). "Potensi antibakteri terhadap Escherichia coli dan Staphylococcus aureus dan identifikasi senyawa ekstrak heksana bandotan (Ageratum conyzoides L.)". *FITOFARMAKA: Jurnal Ilmiah Farmasi.* **2** (1): 18-26. 10.33751/jf.v2i1.163.

[15] H. Astuti. (2015). "Uji aktivitas antibakteri ekstrak etanol dan ekstrak air daun bandotan (Ageratum conyzoides, L.) terhadap Staphylococcus aureus dan Escherichia coli". *Majalah Farmaseutik.* **11** (1): 290-293.

[16] P. N. Munfaati, E. Ratnasari, and G. Trimulyono. (2015). "Aktivitas Senyawa Antibakteri Ekstrak Herba Meniran (Phyllanthus niruri) terhadap Pertumbuhan Bakteri Shigella dysenteriae Secara in Vitro". *Lentera Bio.* **4** (1): 64-71.

[17] S. D. Anitasari and D. N. R. Sari. (2021). "The Activities Of Combination Citrus hystrix Peel Extract and Carica papaya Leaves Extract Against Candida albicans and Escherichia coli". *Medicra (Journal of Medical Laboratory Science/Technology).* **4** (1): 17-21. 10.21070/medicra.v4i1.1359.

[18] E. D. B. Karundeng, E. Hanizar, and D. N. R. Sari. (2022). "Potensi Ekstrak Daun Rhizophora mucronata Sebagai Antibakteri Pada Staphylococcus aureus". *BIOSAPPHIRE: Jurnal Biologi dan Diversitas.* **1** (1).

[19] H. S. Kirom and Z. M. Ramadhania. (2017). "Aktivitas Biologis Tanaman Kucing

Kucingan (Acalypha indica L.)". *Farmaka.* **15** (3): 162-169.

[20] P. Venkatachalam, N. Jayalakshmi, N. Geetha, S. V. Sahi, N. C. Sharma, E. R. Rene, S. K. Sarkar, and P. J. C. Favas. (2017). "Accumulation efficiency, genotoxicity and antioxidant defense mechanisms in medicinal plant Acalypha indica L. under lead stress". *Chemosphere.* **171** : 544-553. 10.1016/j.chemosphere.2016.12.092.

[21] A. Mullick, S. Mandal, R. Bhattacharjee, and A. Banerjee. (2013). "In-vitro assay of antioxidant and antibacterial activity of leaf extract and leaf derived callus extract of acalypha indica l". *International Journal of Pharmacy and Biological Sciences.* **3** (1): 504-510.

[22] N. S. Zahidin, S. Saidin, R. M. Zulkifli, Muhamad, II, H. Ya'akob, and H. Nur. (2017). "A review of Acalypha indica L. (Euphorbiaceae) as traditional medicinal plant and its therapeutic potential". *Journal of Ethnopharmacology.* **207** : 146-173. 10.1016/j.jep.2017.06.019.

[23] K. C. Chinsembu. (2016). "Plants and other natural products used in the management of oral infections and improvement of oral health". *Acta Tropica.* **154** : 6-18. 10.1016/j.actatropica.2015.10.019.

[24] A. Krishnan K and S. Thomas. (2019). "Recent advances on herb-derived constituents-incorporated wound-dressing materials: A review". *Polymers for Advanced Technologies.* **30** (4): 823-838. 10.1002/pat.4540.

[25] N. Y. Lee, W. K. Khoo, M. A. Adnan, T. P. Mahalingam, A. R. Fernandez, and K. Jeevaratnam. (2016). "The pharmacological potential of Phyllanthus niruri". *Journal of Pharmacy and Pharmacology.* **68** (8): 953-69. 10.1111/jphp.12565.

[26] N. Kaur, B. Kaur, and G. Sirhindi. (2017). "Phytochemistry and Pharmacology of Phyllanthus niruri L.: A Review". *Phytotherapy Research.* **31** (7): 980-1004. 10.1002/ptr.5825.

[27] K. Kusmiati, T. Rachmatiah, and A. A. Pertiwi. (2014). "Pengujian Ekstrak Aseton Daun Bayam (Amaranthus Sp) Sebagai Senyawa Antiradikal Dpph, Antibakteri Dan Identifikasi Senyawa Aktif Dengan KG SM". *Proceeding Biology Education Conference.* **11** (1).