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Antibacterial Activity of *Imperata cylindrica* Root Fractions against Pathogenic Bacteria

Aktivitas Antibakteri Fraksi Akar Alang-Alang terhadap Bakteri Patogen

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ABSTRACT

Antibiotic resistance has increased the need for new antibacterial agents from natural sources. This study evaluated the antibacterial activity of ethanolic root extract fractions of *Imperata cylindrica* against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Root powder was extracted with 70% ethanol and sequentially fractionated using n-hexane, ethyl acetate, n-butanol, and distilled water. Antibacterial activity was determined by the disk diffusion method at concentrations of 62.5–500 mg/mL. The most active fraction was further analyzed using thin-layer chromatography and silica gel column chromatography. The n-butanol fraction exhibited the strongest antibacterial activity, producing inhibition zones of 11.50, 9.00, and 8.17 mm against *S. aureus*, *E. coli*, and *P. aeruginosa*, respectively, at 500 mg/mL. Column chromatography generated 92 vials that were combined into nine subfractions. These findings indicate that the n-butanol fraction is a promising source of antibacterial compounds for further isolation and characterization.

ABSTRAK

Resistensi antibiotik mendorong pencarian sumber antibakteri baru dari bahan alam. Penelitian ini bertujuan mengevaluasi aktivitas antibakteri fraksi ekstrak etanol akar alang-alang (*Imperata cylindrica*) terhadap *Staphylococcus aureus*, *Escherichia coli*, dan *Pseudomonas aeruginosa*. Ekstrak diperoleh melalui maserasi dengan etanol 70%, kemudian difraksinasi menggunakan n-heksana, etil asetat, n-butanol, dan akuades. Aktivitas antibakteri diuji menggunakan metode difusi cakram pada konsentrasi 62,5–500 mg/mL. Fraksi paling aktif selanjutnya dianalisis dengan kromatografi lapis tipis dan dipisahkan menggunakan kromatografi kolom silika gel. Fraksi n-butanol menunjukkan aktivitas antibakteri tertinggi dengan zona hambat 11,50 mm, 9,00 mm, dan 8,17 mm terhadap *S. aureus*, *E. coli*, dan *P. aeruginosa* pada konsentrasi 500 mg/mL. Pemisahan kromatografi menghasilkan 92 vial yang dikelompokkan menjadi sembilan subfraksi. Fraksi n-butanol berpotensi sebagai sumber senyawa antibakteri untuk penelitian lanjutan.

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1. INTRODUCTION

Antimicrobial resistance has become a major global health concern because it reduces the effectiveness of commonly used antibiotics and increases the risk of treatment failure. Antimicrobial resistance remains a major health challenge in Indonesia, highlighting the need for effective surveillance and alternative antibacterial strategies (Kementerian Kesehatan Republik Indonesia, 2024). Natural products remain an important source of bioactive compounds and may provide structurally diverse candidates for the development of new antibacterial agents.

Imperata cylindrica is a perennial grass widely distributed across tropical Asia and is commonly regarded as an invasive weed because of its extensive spread and persistence in disturbed areas (Garrity et al., 1996). Despite its ecological status, the underground parts of *I. cylindrica* have long been used in traditional medicine (Hidayat & Rachmadiyanto, 2017). Phytochemical studies have identified glycosides, flavonoids, saponins, phenolic compounds, and coumarins as major constituents of this species (Jung & Shin, 2021). Previous studies have demonstrated the antibacterial activity of *I. cylindrica* preparations against Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Mulyadi et al., 2017). A methanolic extract of the underground parts, comprising rhizomes and roots, has also shown activity against *P. aeruginosa*, *Klebsiella pneumoniae*, and *Bacillus subtilis* (Lalthanpuii et al., 2019). Methanolic root extract of *I. cylindrica* has also shown antibacterial activity against several multidrug-resistant Gram-negative bacterial strains, including *E. coli* and *P. aeruginosa* (Voukeng et al., 2012).

Most previous studies, however, have focused on crude methanolic or ethanolic extracts, and the active constituents responsible for the observed antibacterial effects remain insufficiently localized. Previous studies have shown that semipolar fractions of *I. cylindrica* may modify the antibacterial activity of amoxicillin and chloramphenicol against *Staphylococcus aureus* and *Escherichia coli* (Desita et al., 2019). Similar variation among fractions was also reported when *I. cylindrica* fractions were combined with these antibiotics against *S. aureus* (Maulana et al., 2021). However, these studies focused primarily on interactions between plant fractions and conventional antibiotics rather than on the direct antibacterial activity of sequential solvent fractions. Therefore, systematic fractionation followed by direct antibacterial screening is needed to identify the fraction in which the active constituents are concentrated.

This study aimed to evaluate the antibacterial activity of the ethanolic extract and solvent fractions of *I. cylindrica* roots against *S. aureus*, *E. coli*, and *P. aeruginosa* and to identify the fraction with the highest activity. To achieve this objective, the ethanolic root extract was sequentially partitioned using n-hexane, ethyl acetate, n-butanol, and distilled water. Each fraction was evaluated using the disk diffusion method, and the most active fraction was subsequently profiled by thin-layer chromatography and separated using silica gel column chromatography to obtain subfractions for further investigation.

2. METHODS

2.1. Materials

A total of 1 kg of dried *Imperata cylindrica* roots was used in this study. The plant material was obtained from Wisata Kesehatan Jamu, Kabupaten Tegal, Jawa Tengah, Indonesia. The roots were authenticated by Universitas Bhamada Slawi, and a voucher specimen was deposited under the number 001/F-BMD/Bahan Alam/VI.2025.

The materials included technical-grade 70% and 96% ethanol and dimethyl sulfoxide (CV Nurul Jaya Medicallabsains, Banyumas, Indonesia), technical-grade n-hexane, ethyl acetate, n-butanol, sulfuric acid, and distilled water (CV Kimia Jaya Labora, Cilacap, Indonesia), analytical-grade methanol (99.980% purity) and glacial acetic acid (99.989% purity) (PT Smart-Lab Indonesia, South Tangerang, Indonesia), chloroform (100% purity, Thermo Fisher Scientific, Massachusetts, USA), silica gel 60 with a particle size of 0.063–0.200 mm (Merck, West Point, USA), silica gel plates and sterile paper disks (Macherey-Nagel GmbH & Co. KG, Düren, Germany), Nutrient Agar and Nutrient Broth (HiMedia Laboratories, Thane, India), and streptomycin sulfate (Meiji, Pasuruan, Indonesia). The bacterial strains used were *Staphylococcus aureus* (FNCC 0047), *Escherichia coli* (FNCC 0091), and *Pseudomonas aeruginosa* (ATCC 9027), obtained from Universitas Bhamada Slawi, Kabupaten Tegal, Indonesia.

2.2. Instruments

The instruments included a UV–visible spectrophotometer UV-1280 (Shimadzu, Kyoto, Japan), water bath XMTD-702 (Biobase, Jinan, China), rotary evaporator B-ONE 2000 Series RE 2000 VN (PT Prioritas Bangun Nusantara, Tangerang, Indonesia), chamber furnace CY-M1200-X (Zhengzhou CY Scientific Instrument Co., Ltd., Zhengzhou, China), laminar airflow cabinet SW-CJ-1D (Sugold, Suzhou, China), drying oven YNC-OV-53L (Xi'an Zealchon Electronic Technology Co., Ltd., Xi'an, China), vortex mixer MX-S (DLAB Scientific, Beijing, China), colony counter J-2 (Jiangsu Kangjian Medical Apparatus Co., Ltd., Taizhou, China), analytical balance FA 5003 (Joanlab, Huzhou, China), moisture analyzer DSH-50-1 (Hangzhou Tuoheng Weighing Instrument Co., Ltd., Zhejiang, China), and blender HR 2115 (Philips, Amsterdam, the Netherlands). Standard laboratory glassware, including graduated pipettes, graduated cylinders, test tubes, beakers, Petri dishes, chromatography chambers, and porcelain crucibles, was also used.

2.3. Preparation of the Ethanolic Root Extract

The *I. cylindrica* roots were dried in an oven at 50–55 °C for 24 h and subsequently ground into a fine powder, following general procedures for the preparation of herbal raw materials described in the Indonesian Herbal Pharmacopoeia (Kementerian Kesehatan

Republik Indonesia, 2017). A 500 g portion of the powdered roots was macerated with 5 L of 70% ethanol at a material-to-solvent ratio of 1:10 (w/v) for 72 h with occasional stirring. The maceration procedure was adapted from Dillasamola et al. (2015), with modifications. The macerate was filtered, and the residue was not remacerated. The filtrate was concentrated using a rotary evaporator at 60 ˚C and further evaporated on a water bath at 70 ˚C until a viscous extract was obtained. The extraction yield was calculated using the following equation:

$$\text{Extraction yield (\%)} = \frac{\text{weight of viscous extract}}{\text{weight of dried root powder}} \times 100$$

The moisture content of the viscous extract was determined using a moisture analyzer. A 1 g sample was placed in the sample pan and analyzed at 105ºC until a constant value was obtained.

2.4. Liquid–Liquid Fractionation

Liquid–liquid fractionation was performed sequentially according to solvent polarity using n-hexane, ethyl acetate, and n-butanol. The procedure was adapted from Sudarwati and Fernanda (2019), with modifications to the sample amount, solvent types, solvent volumes, and number of partitioning cycles. A 120 g portion of the viscous ethanolic extract was dissolved in 1200 mL of distilled water and transferred into a separatory funnel. An equal volume of n-hexane was added, and the mixture was shaken thoroughly with periodic venting. After complete phase separation, the n-hexane phase was collected. The residual aqueous phase was then successively partitioned with equal volumes of ethyl acetate and n-butanol.

Each he n-hexane, ethyl acetate, n-butanol, and residual aqueous fractions were separately concentrated on a water bath at 50°C until the solvents had evaporated. The yield of each fraction was calculated using the following equation:

$$\text{Fraction yield (\%)} = \frac{\text{weight of dried fraction}}{\text{weight of extract subjected to fractionation}} \times 100$$

2.5. Preparation and Standardization of Bacterial Suspensions

All glassware and culture media were sterilized in an autoclave at 121 ˚C for 20 min. Each bacterial strain was inoculated into Nutrient Broth and incubated at 36˚C for 24 h. The turbidity of each bacterial suspension was measured at 600 nm using a UV–visible spectrophotometer and adjusted to an absorbance 0,08 wich represent approximately 1.5×10^{8} colony-forming units (CFU) per milliliter (cells/mL).

2.6. Antibacterial Activity Assay

The antibacterial activity of the ethanolic extract and solvent fractions was evaluated using the disk diffusion method. A 100 µL aliquot of each standardized bacterial suspension was inoculated into or distributed over 15 mL of sterile Nutrient Agar. The inoculated medium was (bacterial suspension was mixed with molten nutrient agar before pouring) poured into sterile Petri dishes and allowed to solidify under aseptic conditions.

The ethanolic extract and each fraction were dissolved in 10% dimethyl sulfoxide to obtain concentrations of 125, 250, and 500 mg/mL. Sterile paper disks with a diameter of 6 mm were immersed in solution with 100 µL of each test solution. The disks were allowed to absorb the solutions for 30 min before being placed on the inoculated agar.

Streptomycin at 5 mg/ml was used as the positive control, whereas 10% dimethyl sulfoxide was used as the negative control. The plates were incubated at 36˚C for 24 h. The inhibition-zone diameter, including [or excluding] the disk diameter, was measured in millimeters using ruler. Each treatment was performed in three replicates. the paper disk diameter was not included in the reported inhibition zone values.

2.7. Thin-Layer Chromatographic Profiling

Thin-layer chromatographic profiling was performed according to standard phytochemical analysis principles (Hanani, 2015). Silica gel 60 GF254 plates (Macherey-Nagel GmbH&Co.KG, Duren, Germany) were marked to provide an 8 cm development distance and activated at 110 ˚C for 30 min. The n-butanol fraction was dissolved in chloroform and methanol (1:1), then applied to the plate.

The mobile phase consisted of chloroform, 96% ethanol, and glacial acetic acid at a ratio of 9.4:0.5:0.1 (v/v/v). The chromatographic chamber was saturated with the mobile phase for 60 min before development. After elution, the plate was dried and observed under ultraviolet light at 254 and 366 nm. The plate was subsequently sprayed with 10% sulfuric acid and heated at 100˚C for 2 min. The retention factor was calculated using the following equation:

$$Rf = \frac{\text{distance traveled by the compound}}{\text{distance traveled by the solvent front}}$$

2.8. Silica Gel Column Chromatography

The n-butanol fraction was separated by silica gel column chromatography. Silica gel 60 was dried at 40 ˚C for 30 min before use. A total of 80 g of silica gel (Merck, West Point, USA) was suspended in chloroform–methanol (95:5, v/v) and packed into a glass column with an internal diameter of 3 cm and length 27 cm. A polyethylene sintered frit was placed at the bottom of the column. The column wall was gently tapped during packing to remove air bubbles and prevent channel formation. A solvent layer of approximately 3 cm was maintained above the silica bed to prevent drying.

The packed column was left submerged in the initial mobile phase for 24 h before sample loading. A 300 mg portion of the n-butanol fraction was dissolved in a sufficient volume of an n-butanol. The solution was mixed with 3 g of silica gel and evaporated until a dry, free-flowing sample was obtained. The dry-loaded sample was then placed on top of the packed silica bed.

Elution was performed using a stepwise chloroform–methanol gradient comprising 95:5, 95:5 (intentional, each 190 ml), 90:10, 85:15, 80:20, 75:25, 70:30, 65:35, 60:40, 55:45, and 50:50 (v/v).

A total volume of 190 mL was used for each gradient step. The eluate was collected in 20 mL portions, yielding 92 vials.

2.9. Monitoring and Pooling of Column Fractions

From 92 vials, 8 vials colored fraction (vial number 6,7,8,10,11,16,19 and 39) was selected. Each colored collected vial was monitored by thin-layer chromatography using silica gel 60 GF254 plates and chloroform–methanol (9.6:0.4, v/v) as the mobile phase. The chromatograms were visualized under ultraviolet light at 254 and 366 nm. Vials showing similar spot patterns, colors, and Rf values were combined into subfractions.

2.10. Statistical Analysis

Inhibition-zone data were expressed as mean ± standard deviation from three replicates. Differences among samples at each concentration were analyzed using one-way analysis of variance followed by Tukey's test. Statistical significance was set at $p < 0.05$. The streptomycin control was presented descriptively and was not included in the statistical analysis.

3. RESULTS AND DISCUSSION

3.1. Extraction Yield and Moisture Characteristics

The preparation and extraction procedures for *I. cylindrica* roots are summarized in **Figure 1**. Freshly prepared root simplicia were dried at 55 °C, ground into powder, and extracted by maceration using 70% ethanol in two consecutive batches. The first extraction employed 500.00 g of powdered roots with 5.00 L of 70% ethanol, whereas the second extraction used 427.91 g of powder with 4.23 L of the same solvent. After filtration, the combined filtrates were concentrated using a rotary evaporator followed by evaporation on a water bath to obtain a viscous ethanolic extract.

The first extraction yielded 75.34 g of concentrated extract, while the second yielded 65.46 g, corresponding to extraction yields of 15.07% and 15.30%, respectively (**Table 1**). The comparable extraction yields indicate that the maceration procedure provided consistent recovery despite the difference in the amount of starting material. The moisture content of the first extract was 3.97% and the second extract 3.89%, indicating that most of the residual water had been removed during concentration. The overall experimental workflow, including sample preparation, maceration, filtration, concentration, and extract recovery, is presented in **Figure 1**.

Table 1. Extraction yield of the ethanolic root extract of *Imperata cylindrica*

Batch	Root powder (g)	70% ethanol (L)	Extract weight (g)	Extraction yield (%)	Extract moisture content (%)
1	500.00	5.00	75.34	15.07	3.97
2	427.91	4.23	65.46	15.30	3.89

Note: Extraction yield was calculated relative to the initial weight of root powder.

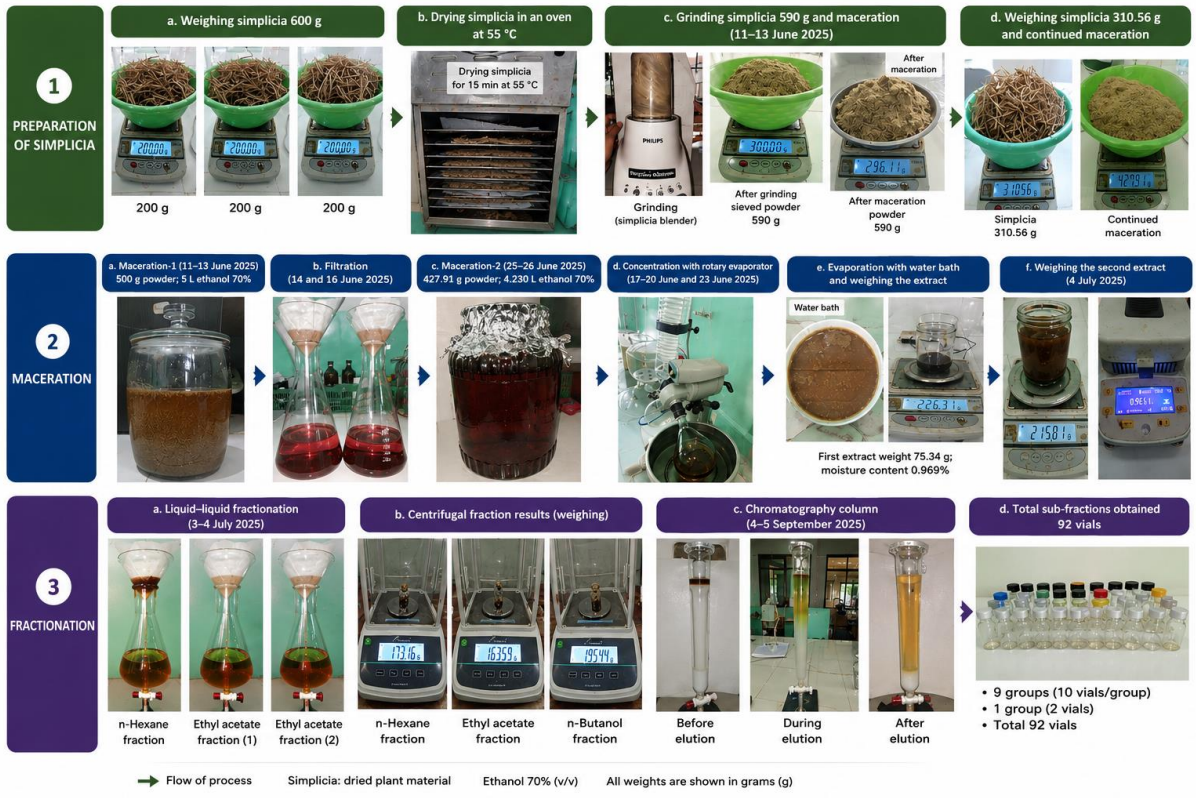


Figure 1. Experimental workflow for the preparation, ethanol extraction, liquid–liquid fractionation, and chromatographic separation of *Imperata cylindrica* roots. The workflow includes drying and grinding of the root material, two-stage maceration with 70% ethanol, filtration, concentration using a rotary evaporator and water bath, liquid–liquid fractionation with n-hexane, ethyl acetate, n-butanol, and water, followed by silica gel column chromatography of the selected n-butanol fraction.

The extraction yields obtained in the present study are consistent with the ability of aqueous ethanol to recover a broad range of polar and moderately polar phytochemicals from *I. cylindrica* roots. Previous phytochemical investigations have identified flavonoids, phenolic compounds, glycosides, coumarins, and saponins among the major constituents reported in *I. cylindrica* (Jung & Shin, 2021; Nayim et al., 2023). In the present study, 70% ethanol was selected to extract compounds across a relatively broad polarity range. The low moisture content of the concentrated extract also indicates adequate solvent removal prior to fractionation.

3.2. Fractionation Yield

The ethanolic extract was partitioned successively with n-hexane, ethyl acetate, n-butanol, and water. The aqueous fraction gave the highest yield, accounting for 78.68% of the extract subjected to fractionation. Much smaller yields were obtained for the n-butanol, n-hexane, and ethyl acetate fractions, at 2.88%, 1.09%, and 0.54%, respectively (**Table 2**). The large aqueous fraction suggests that most of the extractable constituents were highly polar or remained in the aqueous phase after partitioning. This pattern is consistent with the reported presence of glycosides, saponins, and other polar metabolites in *I. cylindrica* (Jung & Shin, 2021). By contrast, the low yields of the n-hexane and ethyl acetate fractions indicate that only small amounts of nonpolar and semipolar constituents were recovered.

Table 2. Yield of solvent fractions obtained from the ethanolic root extract of *Imperata cylindrica*

Fraction	Extract subjected to fractionation (g)	Fraction weight (g)	Yield (%)
n-Hexane	120.00	1.31	1.09
Ethyl acetate	120.00	0.66	0.54
n-Butanol	120.00	3.47	2.88
Aqueous	120.00	94.42	78.68

Note: Fraction yield was calculated relative to the initial weight of the ethanolic extract subjected to fractionation.

3.3. Antibacterial Activity of the Ethanolic Extract and Solvent Fractions

The ethanolic extract and its aqueous, n-hexane, ethyl acetate, and n-butanol fractions were tested against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* at concentrations of 62.5–500 mg/mL. The inhibition-zone data are presented as mean ± standard deviation from three replicates, and differences among samples at the same concentration are indicated by different superscript letters (Table 3). Representative inhibition zones at 500 mg/mL are shown in **Figure 2**. The laboratory record also reported a 24 mm inhibition zone for streptomycin concentration 5 mg/ml as the positive control. streptomycin was obtained in the same assay series as the extract and fraction tests.

Table 3. Antibacterial activity of the ethanolic extract and solvent fractions of Imperata cylindric roots

Bacterium	Sample	500 mg/mL	250 mg/mL	125 mg/mL
Staphylococcus aureus	Ethanolic extract	2.58 ± 0.38^{c}	0.67 ± 1.15^{b}	0.00 ± 0.00^{b}
	Aqueous fraction	2.00 ± 1.73^{c}	0.67 ± 1.15^{b}	0.00 ± 0.00^{b}
	n-Hexane fraction	2.83 ± 0.29^{c}	2.33 ± 0.58^{b}	2.00 ± 0.50^{a}
	Ethyl acetate fraction	6.00 ± 1.00^{b}	3.33 ± 1.15^{b}	2.58 ± 0.38^{a}
	n-Butanol fraction	11.50 ± 1.32^{a}	6.90 ± 1.65^{a}	3.17 ± 0.76^{a}
	Streptomycin (5 mg/ml), positive control	24.00	—	—
Escherichia coli	Ethanolic extract	3.33 ± 1.53^{bc}	1.33 ± 1.15^{ab}	0.00 ± 0.00^{a}
	Aqueous fraction	1.00 ± 1.73^{c}	0.00 ± 0.00^{b}	0.00 ± 0.00^{a}
	n-Hexane fraction	2.50 ± 0.50^{bc}	2.17 ± 0.29^{ab}	0.67 ± 1.15^{a}
	Ethyl acetate fraction	6.00 ± 1.00^{ab}	2.50 ± 0.87^{ab}	2.17 ± 0.29^{a}
	n-Butanol fraction	9.00 ± 2.00^{a}	4.33 ± 2.31^{a}	2.50 ± 1.80^{a}
	Streptomycin (5 mg/ml), positive control	24.00	—	—
Pseudomonas aeruginosa	Ethanolic extract	3.75 ± 0.66^{bc}	3.00 ± 0.00^{ab}	1.67 ± 1.53^{a}
	Aqueous fraction	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{a}
	n-Hexane fraction	1.33 ± 1.15^{c}	1.33 ± 1.15^{bc}	0.83 ± 0.76^{a}
	Ethyl acetate fraction	7.00 ± 1.32^{ab}	4.00 ± 1.00^{a}	1.67 ± 0.58^{a}
	n-Butanol fraction	8.17 ± 2.93^{a}	2.67 ± 1.61^{ab}	1.67 ± 1.26^{a}
	Streptomycin (5 mg/ml), positive control	24.00	—	—

Note: Values are mean ± standard deviation, $n = 3$. Different lowercase superscript letters within the same bacterium and concentration indicate significant differences at $p < 0.05$. The positive control was not included in the statistical comparison.

Against *S. aureus*, the n-butanol fraction produced the largest inhibition zone at 500 mg/mL, reaching 11.50 ± 1.32 mm, and differed significantly from all other samples. The ethyl acetate fraction ranked second at 6.00 ± 1.00 mm, whereas the ethanolic extract, aqueous fraction, and n-hexane fraction produced smaller inhibition zones. The activity of the n-butanol fraction declined with concentration, indicating a clearer concentration-related response than that observed for the other fractions. Its stronger activity suggests that moderately polar antibacterial constituents were concentrated in this fraction. Flavonoid glycosides, saponins, and polar phenolic compounds may contribute to the observed effect through disruption of bacterial membranes or interaction with cellular proteins. The greater susceptibility of *S. aureus* may also be related to its Gram-positive cell envelope, which lacks the outer membrane found in Gram-negative bacteria. Similar antibacterial activity of *I. cylindrica* roots against *S. aureus* has been reported previously (Mulyadi et al., 2017).

Against *E. coli*, the n-butanol fraction also produced the largest mean inhibition zone at 500 mg/mL, reaching 9.00 ± 2.00 mm, although it did not differ significantly from the ethyl acetate fraction, which produced 6.00 ± 1.00 mm. The ethanolic extract, aqueous fraction, and n-hexane fraction showed weaker activity. At lower concentrations, the differences among samples became less distinct because the inhibition zones were smaller and more variable. The lower activity against *E. coli* than against *S. aureus* may reflect the permeability barrier formed by the lipopolysaccharide-containing outer membrane of Gram-negative bacteria. Nevertheless, the measurable activity of the n-butanol and ethyl acetate fractions indicates that moderately polar and semipolar constituents were able to penetrate or disturb this barrier. Previous studies have likewise reported activity of *I. cylindrica* root extracts against *E. coli*, including multidrug-resistant strains (Mulyadi et al., 2017; Voukeng et al., 2012).

Against *P. aeruginosa*, the n-butanol fraction produced the largest mean inhibition zone at 500 mg/mL, at 8.17 ± 2.93 mm, followed closely by the ethyl acetate fraction at 7.00 ± 1.32 mm; the difference between these fractions was not significant. The ethanolic extract and n-hexane fraction were less active, while the aqueous fraction showed no measurable inhibition. At 250 mg/mL, the ethyl acetate fraction produced the largest mean inhibition zone, whereas no significant differences were observed among samples at 125 and 62.5 mg/mL. The activity of the n-butanol and ethyl acetate fractions is notable because *P. aeruginosa* possesses low outer-membrane permeability, efficient efflux systems, and several adaptive resistance mechanisms. However, the relatively large standard deviation of the n-butanol fraction at 500 mg/mL indicates considerable variation among replicates. Activity of *I. cylindrica* roots against *P. aeruginosa* has also been documented using methanolic and ethanolic preparations (Lalthanpuii et al. 2019; Nurhalimmah, 2021; Voukeng et al., 2012).

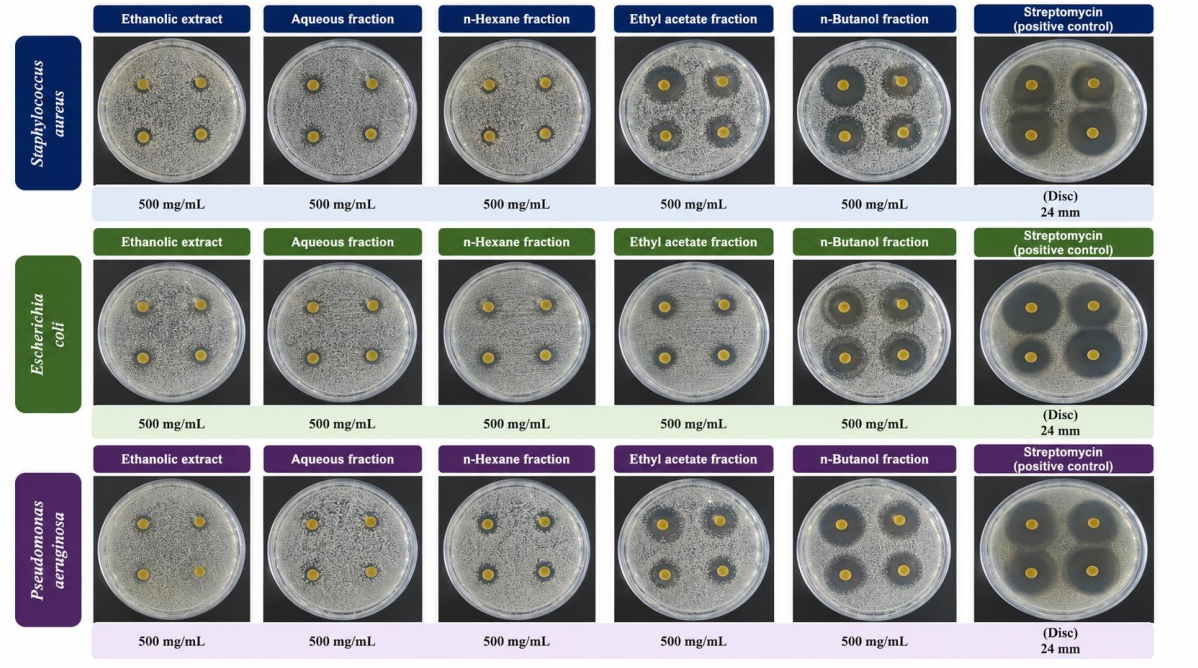


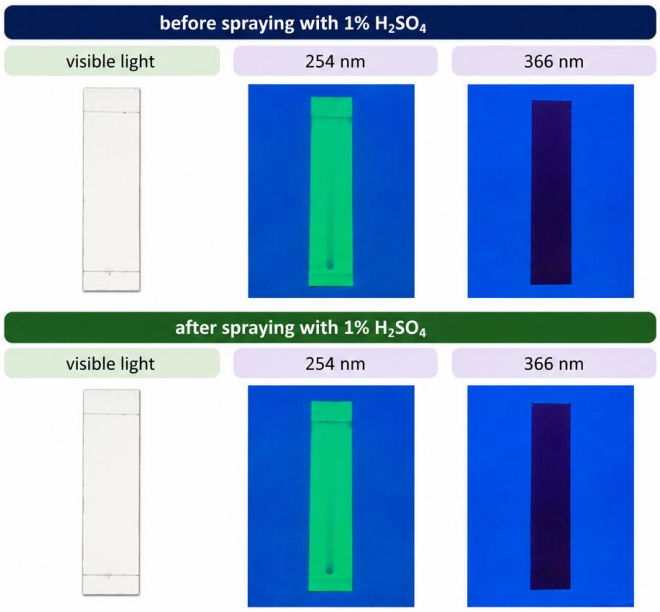
Figure 2. Antibacterial activity of the ethanolic extract and solvent fractions of *Imperata cylindrica* roots against *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa* at a concentration of 500 mg/mL using the disc diffusion method. Streptomycin served as the positive control.

Across the three bacteria, the n-butanol fraction consistently produced the largest mean inhibition zone at 500 mg/mL. Its superiority was statistically clear against *S. aureus*, while its activity was comparable to that of the ethyl acetate fraction against *E. coli* and *P. aeruginosa*. These findings are consistent with previous reports demonstrating the antibacterial potential of *I. cylindrica* extracts prepared using different extraction procedures and tested against various bacterial species (Mulyadi et al., 2017; Rejeki et al., 2024). The 24 mm inhibition zone produced by streptomycin was substantially larger than those produced by all

plant-derived samples, showing that the reference antibiotic remained more active under the applied test conditions. Even so, the consistent activity of the n-butanol fraction supported its selection for further chromatographic separation.

3.4. Thin-Layer Chromatographic Profile of the n-Butanol Fraction

The n-butanol fraction was examined by thin-layer chromatography using chloroform–ethanol–glacial acetic acid (9.4:0.5:0.1, v/v/v) as the mobile phase. The chromatogram was observed under visible light and ultraviolet light at 254 and 366 nm before and after spraying with 10% sulfuric acid (**Figure 3**). Six spots were recorded, with retention factor values of 0.2625, 0.3250, 0.4750, 0.5625, 0.7875, and 0.8250. The broad distribution of retention factors shows that the n-butanol fraction contained constituents with different affinities for the silica stationary phase and the selected mobile phase. Components with lower retention factors were retained more strongly on silica, whereas those with higher values migrated more readily with the solvent system. The chromatographic profile therefore confirmed that the n-butanol fraction remained chemically heterogeneous and required further separation before the antibacterial activity could be attributed to a specific constituent. Observation after sulfuric acid treatment provided additional visualization of compounds that were less apparent under visible or ultraviolet light alone, although the chromatogram did not permit identification of individual compounds.



spot retention factor (R_f)

spot	R_f
1	0.2625
2	0.3250
3	0.4750
4	0.5625
5	0.7875
6	0.8250

Figure 3. Thin-layer chromatographic profile of the n-butanol fraction developed with chloroform–ethanol–glacial acetic acid (9.4:0.5:0.1, v/v/v). The chromatogram was observed under visible light and ultraviolet light at 254 and 366 nm before and after spraying with 10% sulfuric acid. The recorded retention factor values were 0.2625, 0.3250, 0.4750, 0.5625, 0.7875, and 0.8250.

3.5. Column Chromatographic Separation and TLC Profiling of Selected Subfractions

The n-butanol fraction was further separated by silica gel column chromatography using a chloroform–methanol gradient with progressively increasing polarity. Elution started at a chloroform–methanol ratio of 95:5 and continued stepwise to 50:50 (v/v), with 190 mL used at each gradient step. During the separation, two visually distinct bands moved through the silica bed, indicating that constituents with different affinities for the stationary and mobile phases were being resolved (**Figure 4a**).

A total of 92 fractions was collected in 20 mL vials throughout the chromatographic run (**Figure 4b**). The relatively small collection volume allowed closer monitoring of the elution profile and reduced the likelihood of combining chemically different constituents in the same vial. The gradual increase in methanol content promoted the sequential elution of compounds with increasing polarity.

The collected fractions were monitored by thin-layer chromatography using silica gel 60 GF_{254} plates and chloroform–methanol (9.6:0.4, v/v) as the mobile phase. The chromatograms were examined under ultraviolet light at 254 and 366 nm (**Figure 4c**). Vials 6, 7, 8, 10, and 11 showed a major spot at an Rf value of 0.9125, indicating closely related chromatographic compositions. Vial 11 also showed an additional spot at Rf 0.4750, suggesting that it still contained more than one detectable constituent. Vial 16 produced a major spot at Rf 0.8300, vial 19 at Rf 0.8625, and vial 39 at Rf 0.9625.

The repeated occurrence of the same Rf value in several consecutive fractions suggests that one dominant constituent eluted over a relatively broad solvent volume. Such overlap is common in open-column chromatography, particularly when compounds have similar polarity or when the elution band is broad. By contrast, the distinct Rf values of vials 16, 19, and 39 indicate differences in chromatographic behavior and, therefore, likely differences in chemical composition.

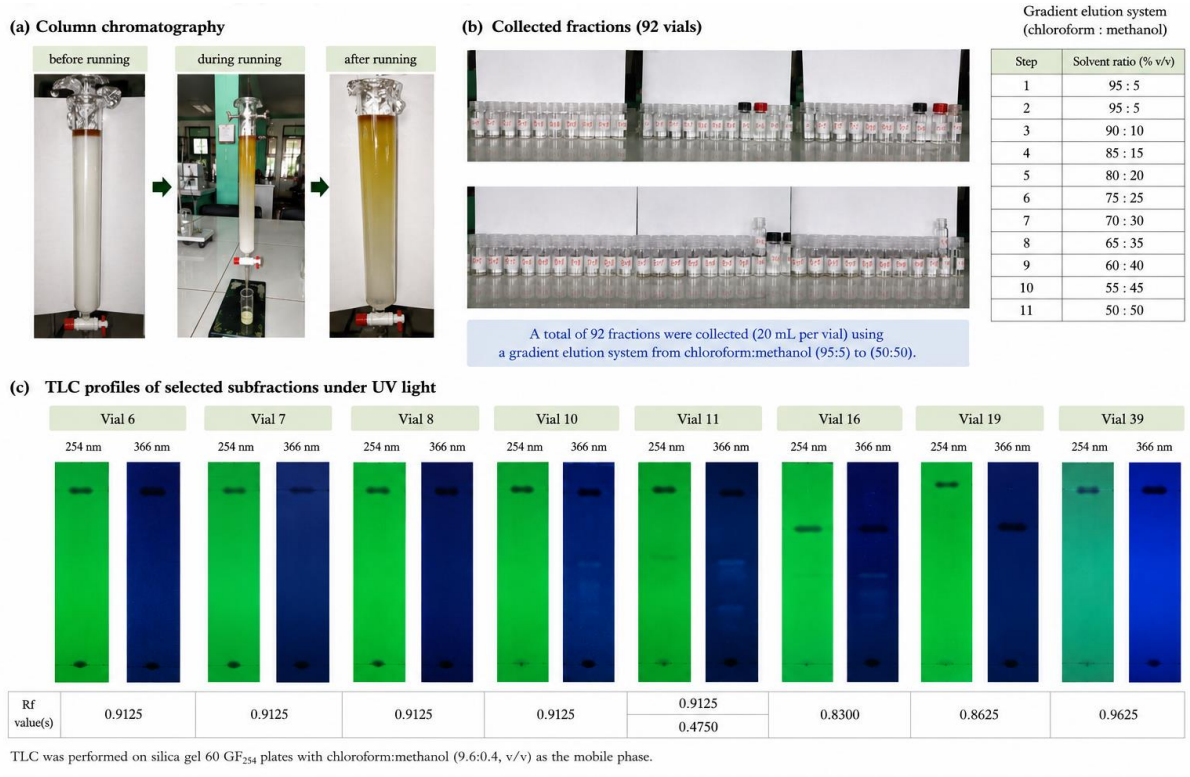


Figure 4. Column chromatographic separation and TLC profiling of selected n-butanol subfractions. (a) Column condition before, during, and after elution; (b) 92 fractions collected in 20 mL vials during gradient elution; and (c) TLC profiles of selected fractions under ultraviolet light at 254 and 366 nm, with the corresponding Rf values shown beneath each vial.

The TLC profiles confirm that column chromatography reduced the complexity of the parent n-butanol fraction and separated it into fractions with distinguishable migration patterns. However, several fractions were not chemically homogeneous, as shown by the additional spot detected in vial 11. The chromatographic data provide a reasonable basis for pooling fractions with similar Rf values before further purification.

The available antibacterial data establish the n-butanol fraction as the most active solvent fraction, but they do not identify which column fraction retained that activity. Therefore, the antibacterial effect can only be attributed to the parent n-butanol fraction at this stage. Further testing of the pooled subfractions is needed before the active component can be localized and structurally characterized.

3.6. Limitations and Implications

Several limitations should be considered when interpreting these findings. Although the n-butanol fraction showed the strongest antibacterial activity among the tested samples, the chromatographic subfractions obtained from this fraction were not evaluated individually. The active constituent therefore could not be assigned to a specific vial, pooled subfraction, or chromatographic band. In addition, the antibacterial assessment relied on inhibition-zone measurements, which are influenced not only by intrinsic antibacterial activity but also by solubility, molecular size, and diffusion through the agar medium. The absence of minimum inhibitory concentration and minimum bactericidal concentration data further limits comparison of antibacterial potency.

Despite these limitations, the results provide a clear basis for further bioactivity-guided fractionation. The n-butanol fraction consistently produced the largest inhibition zones at the highest tested concentration, while column chromatography and TLC monitoring separated this fraction into chemically distinguishable groups. Fractions sharing similar Rf values, particularly vials 6, 7, 8, 10, and 11, may be pooled for subsequent antibacterial screening. Fractions 16, 19, and 39 should also be evaluated separately because their distinct migration patterns suggest different chemical compositions.

Future work should therefore focus on testing the pooled subfractions against the same bacterial strains, followed by determination of minimum inhibitory and bactericidal concentrations. The most active subfraction can then be subjected to further purification and structural characterization using appropriate spectroscopic and chromatographic techniques. This stepwise approach would establish whether the observed antibacterial activity arises from a single compound, several active constituents, or synergistic interactions within the n-butanol fraction.

CONCLUSION

The ethanolic root extract of *Imperata cylindrica* was successfully partitioned into solvent fractions with distinct yields and antibacterial activities. Among the tested samples, the n-butanol fraction showed the strongest inhibition against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, particularly at 500 mg/mL, although its activity remained lower than that of

streptomycin. Thin-layer and column chromatographic analyses further demonstrated that this fraction contained several chemically distinguishable constituents. These findings support the n-butanol fraction as the most promising source for continued bioactivity-guided purification. Further antibacterial testing of the pooled subfractions, followed by minimum inhibitory concentration, minimum bactericidal concentration, and structural characterization, is required to identify the constituents responsible for the observed activity. Overall, this study provides a practical basis for the further development of *I. cylindrica* roots as a potential source of antibacterial compounds.

AUTHOR CONTRIBUTIONS

Conceptualization, D.S.R. and E.P.; methodology, D.S.R. and S.K.N.; validation, D.S.R., S.K.N., and E.P.; formal analysis, D.S.R. and S.K.N.; investigation, D.S.R. and S.K.N.; resources, D.S.R. and E.P.; data curation, D.S.R. and S.K.N.; writing—original draft preparation, D.S.R.; writing—review and editing, S.K.N. and E.P.; visualization, D.S.R. and S.K.N.; supervision, E.P.; project administration, D.S.R.; funding acquisition, D.S.R. All authors have read and agreed to the published version of the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable. This study did not involve human participants or experimental animals.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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The funder had no role in the design of the study; the collection, analysis, or interpretation of the data; the preparation of the manuscript; or the decision to publish the results.

DECLARATION OF GENERATIVE ARTIFICIAL INTELLIGENCE (AI) USE

During the preparation of this manuscript, the authors used ChatGPT (OpenAI) to assist in improving the clarity, structure, and readability of the text. After using this tool, the authors thoroughly reviewed, edited, and verified the manuscript to ensure that it accurately represented their data, ideas, and scientific interpretations. The authors take full responsibility for the integrity, accuracy, and originality of the published work.

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