

Antibacterial Activity of *Polygonum pulchrum* Blume Ethanol Extract on *Staphylococcus aureus* and *Escherichia coli*

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Abstract

The emergence of resistant bacteria strain has become a global health concern. It encourages the exploration of potential antibacterial agents, particularly from natural sources. The aim of this study was to investigate the antibacterial activity of ethanol extract of root, stems, leaves, and flowers of *Polygonum pulchrum* Blume against *Staphylococcus aureus* and *Escherichia coli*, through disc diffusion method using cup-plate method. Inhibition zone against *S. aureus* from roots, stems, leaves, and flowers ethanol extract were 3.5 mm, 2.5 mm, 2.25 mm, and 2.62 mm, respectively, while the inhibition zone against *E. coli* were 2.25 mm, 2.12 mm, 1.62 mm, and 1.75 mm, respectively. In conclusion, ethanol extract of root, stem, leaves, and flower of *P. pulchrum* Bl possessed weak antibacterial activity against *S. aureus* and *E. coli*.

Keywords: *P. pulchrum* Bl, antibacterial, *E. coli*, *S. aureus*, cup-plate technique

Introduction

The emergence of resistant bacteria strain has become a global health concern. It encourages the exploration of potential antibacterial agents, particularly from natural sources. Antibiotic resistance is caused by overuse and misuse of antibiotics, inappropriate prescribing, limited availability of new generation of antibiotics, and extensive agricultural use. During the last several decades, the focus of development of new antibacterial agent has shifted towards ethnomedicinal plants.¹

Medicinal plants play an important role in the treatment of several diseases, particularly in developing countries. Many plant-derived medicines have been documented in pharmacopeias for herbal medicines because of their efficacy against microbial pathogens. The antibacterial activity of plants are obtained from the ability to synthesize secondary metabolites and its derivative compounds *i.e.*, phenolics group, terpenoid, alkaloids, lectins, polypeptides, and polyacetylenes.²

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Twenty-four species of Polygonum plants contains more than a hundred of biologically active compounds.³ For instance, extract of *P. perforliatum* has been used in China to treat fever, rheumatoid arthritis, joint pain, oedema, and importantly against bacterial infection.⁴ *P. pulchrum* BI has been predicted to have antibacterial activity due to the secondary metabolites contained in the plants. Five steroids (*i.e.*, stigmasterol-4,25-diene-3,6-diol; stigmasterol-4,22-diene-3-one; ergosterol peroxide; stigmasterol; 6 β -hydroxystigmasterol-4,22-diene-3-one) obtained from stems methanol extract of *P. pulchrum* BI have been identified and then isolated. Those isolated compounds have been proven to possess radical scavengers activity and anticancer activity against WiDr cells lines.³

Therefore, in this study the ethanol extract of roots, stems, leaves, and flowers of *P. pulchrum* BI have been evaluated for potential antibacterial activity against *E. coli* ATCC 35218 and *S. aureus* ATCC 25923 for further pharmacological activity study.

Methods

Instruments and materials

Materials used in this study included 96% ethanol (Merck®), nutrient agar (Merck®), nutrient broth (Merck®), chloramphenicol (Brataco®), *S. aureus* ATCC 25923, *E. coli* ATCC 35218, *P. pulchrum* BI plants. Plant samples were collected from the Faculty of Fisheries and Marine Sciences, Halu Oleo University, Kendari, South East Sulawesi.

Extraction

Each part of the plants was macerated in a closed container for 3x24 hours using ethanol solvent. The residue separation and filtrate were performed every 24 hours accompanied by the same solvent replacement to obtain the filtrate. The filtrate was collected

and concentrated using a rotary vacuum evaporator at 58 °C to obtain a viscous extract.

Antibacterial activity

Media preparation

Antibacterial activity was evaluated using two types of medium for bacterial growth, *i.e.*, nutrient agar (NA) and nutrient broth (NB). The NA and NB medium were prepared by weighing 2.8 g and 1.3 g, respectively. Each medium was then dissolved in 100 ml of distilled water into an Erlenmeyer flask. The medium was heated over a hot plate until completely dissolved and sterilized in an autoclave at 121 °C for 15 minutes.

Bacterial cultures preparation

E. coli ATCC 35218 and *S. aureus* ATCC 25923 were each rejuvenated on NA medium in a tube and were incubated for 24 hours at 37 °C.

Bacterial suspension preparation

Both of the bacteria were suspended in NaCl 0.9% solution. Turbidity of bacterial suspension was compared to standard McFarland 0.5 solution (0.05 ml BaCl₂ 1% and 9.95 ml H₂SO₄ 1% compositions, equivalent to 150x10⁶ CFU/ml).

Antibacterial activity assay

Antibacterial activity was assayed using disc diffusion method with cup-plate technique. This method was done in two stages; 1) preparation of NA medium by pouring 10 ml of sterile NA medium into a sterile petri dish; 2) preparation of indicator medium by mixing 1 ml of bacterial suspension into 5 ml of NB medium in a reaction tube and adding 10 ml of semi-solid liquid media. It was then homogenized and placed in petri dish. Several wells were formed using cylinder cup. Each well was filled with 50 μ l ethanol extract of roots, stems, leaves, and flower samples with concentrations of 12.5 μ g/ml,

25 µg/ml, 50 µg/ml, 100 µg/ml, 125 µg/ml, 250 µg/ml, 500 µg/ml, 1000 µg/ml, 2000 µg/ml, and 4000 µg/ml; 50 µl of chloramphenicol with a concentration of 30 µg/ml; and 50 µl of ethanol solvent. The petri dish was placed into the refrigerator at 10 °C for 30 minutes, and was incubated at 37 °C for 24 hours. The inhibitory activity against *E. coli* and *S. aureus* were observed by measuring inhibition zone.

Results and Discussion

The antibacterial activity of ethanol extract of roots, stems, leaves, and flowers ethanol extract of *P. pulchrum* Bl. were evaluated on *S. aureus* ATCC 25923 and *E. coli* ATCC 35218 using disc diffusion method with cup-plate technique. The result can be seen in Table 1.

The highest antibacterial activity against *S. aureus* and *E. coli* was found in the roots ethanol extract 4000 µg/ml with 3.50 mm and 2.25 mm inhibition zone, respectively. It is known that all of the plant parts contained alkaloids, but in a different quantity. Previous study showed that leaves contained highest concentration of alkaloids, followed by the stems and then the roots.^{5,6} However, the root extracts exhibited more significant antibacterial activity with larger zone of inhibitions if compared to the leaves and stem extracts.

The effectiveness of an antibacterial agent is classified into 4 categories based on the inhibitory zone. Inhibitory zone ≥ 20 mm indicates very strong antibacterial activity, 11-19 mm indicates strong activity, 5-10 mm indicates moderate activity, <5 mm indicates weak activity. However, based on the result, the ability of all extracts to inhibit *S. aureus* and *E. coli* were classified as weak if compared to chloramphenicol. The inhibitory activity against Gram-positive bacteria was stronger than the Gram-negative bacteria.

Our study is compared with that of Agbafor

et al, who reported that the secondary metabolites was more active against Gram-positive bacterium compared to Gram-negative bacteria.⁷

Extract of *P. pulchrum* Bl. roots and leaves was reported contained alkaloids.⁸ Most studies indicated that alkaloids showed bactericidal activity.⁹⁻¹¹ Mechanisms of action (MOA) of alkaloids (indolizidine, isoquinoline, quinolone, agelasine and polyamine classes) have been reported investigated.¹² The indolizidines act by inhibiting nucleic acid synthesis, as they inhibit the dihydrofolate reductase.¹² The isoquinolines perturbate of the Z-ring and inhibition cell division.^{13,14} The quinolones, which is lack of the 3-carboxyl group, unable to inhibit the type II topoisomerase enzymes (Heeb).¹⁵ Agelasines inhibit BCG 3185c enzyme, a suspected dioxygenase which thereby allows disruption of bacterial homeostasis.¹⁶ Lastly, polyamines compromise outer membrane and cytoplasmic membrane integrity.¹⁷

We also found that stems and leaves extract of *P. pulchrum* Bl contain flavonoids. Flavonoids have also been recognized for their antimicrobial activity. Many researchers have isolated and identified the structures of flavonoids which have potential properties of antifungal, antiviral and antibacterial activity. MOA of flavonoids is the formation of complex body with proteins through non-specific forces such as hydrogen bonding, hydrophobic effects, and covalent bond formation, which allows them to inactivate microbial adhesions, enzymes, cell envelope transport proteins, and enhance microbial membranes disruption.¹⁸ In addition, the leaves extract of *P. pulchrum* Bl also contains polyphenols.⁸ Polyphenols inhibit protease enzyme activity in bacterial cell envelope transport proteins and destruct or inactivate genetic material functions. Other metabolites, tannin, has the ability to shrink

Table 1. Antibacterial activity of *P. pulchrum* Bl. extract against *S. aureus*

Extract	Concentration (µg/mL)	Inhibition Zone (mm)
Root	12.5	0.25
	25.0	0.50
	50.0	0.75
	100.0	0.87
	125.0	1.12
	250.0	2.00
	500.0	2.25
	1000.0	2.37
	2000.0	3.12
	4000.0	3.50
	Control (+) Chloramphenicol	
Control (-) Ethanol 96%		-
Stem	12.5	0.25
	25.0	0.37
	50.0	0.50
	100.0	0.62
	125.0	0.75
	250.0	0.87
	500.0	1.00
	1000.0	1.12
	2000.0	1.87
	4000.0	2.50
	Control (+) Chloramphenicol	
Control (-) Ethanol 96%		-
Leaves	12.5	0.12
	25.0	0.25
	50.0	0.37
	100.0	0.62
	125.0	0.75
	250.0	0.87
	500.0	1.12
	1000.0	1.75
	2000.0	2.00
	4000.0	2.25
	Control (+) Chloramphenicol	
Control (-) Ethanol 96%		-
Flower	12.5	0.00
	25.0	0.12
	50.0	0.25
	100.0	0.50
	125.0	1.12
	250.0	1.50
	500.0	1.87
	1000.0	2.25
	2000.0	2.37
	4000.0	2.62
	Control (+) Chloramphenicol	
Control (-) Ethanol 96%		-

Diameter of well = 7 mm

Table 2. Antibacterial activity of *P. pulchrum* Bl. extract against *E. coli*

Extract	Concentration ($\mu\text{g/mL}$)	Inhibition Zone (mm)
Root	12.5	0.25
	25.0	0.37
	50.0	0.50
	100.0	0.62
	125.0	0.75
	250.0	0.87
	500.0	1.12
	1000.0	1.62
	2000.0	1.75
	4000.0	2.25
	Control (+) Chloramphenicol	
Control (-) Ethanol 96%		-
Stem	12.5	0
	25.0	0.12
	50.0	0.25
	100.0	0.50
	125.0	0.75
	250.0	0.87
	500.0	1.25
	1000.0	1.50
	2000.0	1.62
	4000.0	2.12
	Control (+) Chloramphenicol	
Control (-) Ethanol 96%		-
Leaves	12.5	0.12
	25.0	0.25
	50.0	0.37
	100.0	0.50
	125.0	0.62
	250.0	0.75
	500.0	0.87
	1000.0	1.12
	2000.0	1.37
	4000.0	1.62
	Control (+) Chloramphenicol	
Control (-) Ethanol 96%		-
Flower	12.5	0
	25.0	0
	50.0	0.25
	100.0	0.37
	125.0	0.50
	250.0	0.62
	500.0	0.87
	1000.0	1.50
	2000.0	1.62
	4000.0	1.75
	Control (+) Chloramphenicol	
Control (-) Ethanol 96%		-

Diameter of well = 7 mm

the bacteria cell wall, which disrupts cell permeability.¹⁹

Conclusion

Our study showed that root, stem, leaves, and flower ethanol extracts of *P. pulchrum* Bl possessed weak antibacterial activity against *S. aureus* and *E. coli*.

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Conflict of interest

Not declared.

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