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Evaluation Antibacterial Activity of Rosary Pea (*Abrus precatorius L.*) Leaf Extract against the Growth of *Staphylococcus aureus and Shigella sonnei*

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Abstract: Bacteria are major contributors to infectious diseases, including *Staphylococcus aureus* and *Shigella sonnei*. Although antibiotics remain the primary treatment, prolonged use can lead to antimicrobial resistance, highlighting the need for alternative agents capable of inhibiting pathogenic bacteria. Scientific validation of medicinal plants is crucial for complementing traditional knowledge in bioprospecting efforts. The Rosary pea (*Abrus precatorius L.)* is one such plant with potential antibacterial properties. This study evaluates the inhibitory activity of *Abrus precatorius L.* leaf ethanol extract against *S. aureus* and *S. sonnei* at concentrations of 3.125%, 6.25%, 12.5%, 25%, 50%, and 100%. The extract demonstrated optimal activity at 100%, exhibiting moderate inhibition against *S. aureus* and weak inhibition against *S. sonnei*. These findings underscore the potential of *Abrus precatorius L* as a source of antibacterial compounds and provide a foundation for developing improved formulations with enhanced antibacterial efficacy.

Keywords: antibacterial, *Abrus precatorius L.,* rosary pea, *Staphylococcus aureus, Shigella sonnei*

1. INTRODUCTION

Bacterial pathogens such as Staphylococcus aureus and Shigella sonnei continue to pose significant public health challenges [1]. Staphylococcus aureus is frequently linked to skin and respiratory infections and Shigella sonnei is a prominent cause of shigellosis [2], [3]. Staphylococcus aureus and Shigella sonnei have shown a rapid rise in antimicrobial resistance, particularly in low- and middle-income countries [4]. This growing resistance profile makes Staphylococcus aureus and Shigella sonnei a relevant and increasingly important target in the search for new antibacterial agents.

Although antibiotics remain the primary therapeutic option, widespread and inappropriate use has contributed to escalating antimicrobial resistance [5], [6]. Consequently, natural products—especially medicinal plants have gained attention due to their rich content of bioactive metabolites such as flavonoids, alkaloids, saponins, and tannins, which exhibit antibacterial mechanisms through membrane disruption, interference with nucleic acid synthesis, and inhibition of metabolic pathways [7].

Abrus precatorius L. (rosary pea) has long been used in traditional medicine, particularly to treat coughs, sore throats, wounds, and diarrhea, suggesting its potential antibacterial activity [8], [9]. Previous studies have demonstrated inhibition of Staphylococcus aureus and Shigella dysenteriae by its ethanolic leaf extract [5]. However, studies specifically evaluating its activity against Shigella sonnei are still limited, despite the increasing clinical importance and distinct resistance characteristics of this species. This gap highlights the need to assess whether A. precatorius exhibits comparable or differential efficacy against Gram-negative pathogens with emerging resistance concerns. Furthermore, previous studies have not directly compared its antibacterial effects between Gram-positive and Gram-negative bacteria; thus, its broader antibacterial spectrum has not been sufficiently characterized. Therefore, this study aimed to evaluate the antibacterial activity of the ethanolic leaf extract of A. precatorius against S. aureus, as a gram-positive bacterium, and S. sonnei,

as a gram-negative bacterium using the disc diffusion method. This study is expected to provide new insights into the antibacterial potential of A. precatorius and explain how its activity differs across clinically significant bacterial groups (gram-positive and gram-negative).

2. MATERIALS AND METHOD

2.1. Tools and Materials

In this study, the tools and materials used *A. precatorius* L. leaves, distilled water, 96% ethanol, Mueller–Hinton Agar (MHA), 10% DMSO, filter paper, aluminum foil, blank paper discs, Ciprofloxacin 5 μg antibiotic discs, and bacterial cultures of *S. aureus* and *S. sonnei*. Brown bottles, sieve (mesh no. 40), Petri dishes, test tubes, analytical balance, beaker glasses, Erlenmeyer flasks, hot plate, oven, incubator, autoclave, evaporating dish, micropipettes and tips, test tube rack, Bunsen burner, inoculating loop, measuring cylinder, blender, and ruler.

2.2. Plant determination, preparation and sampel extraction of Rosary Pea

Fresh *A. precatorius* L. leaves were collected, washed under running water, air-dried, and taxonomically identified at the Herbarium Depokensis (UIDEP), Universitas Indonesia. One hundred grams of dried leaves were macerated in 500 mL of 96% ethanol for five days in brown bottles. Ethanol 96% was selected as the extraction solvent because it efficiently dissolves a wide range of secondary metabolites, including flavonoids, alkaloids, tannins, and saponins, while also preventing microbial growth during the extraction process. The mixture was filtered, and the filtrate was concentrated in an oven at 60 °C for one week. The extract was dried at 60°C because this temperature remains within the stability range of most phytochemicals and allows gradual evaporation of ethanol without causing significant degradation of heat-sensitive compounds [10]. Drying at this temperature for one week ensured complete solvent removal while minimizing contamination risk [10]. Extracts were prepared at concentrations of 100%, 50%, 25%, 12.5%, 6.25%, and 3.125%.

2.3. Antibacterial Activity Test by Disc Diffusion Method

The disc diffusion method was used to determine the antibacterial activity of *A. precatorius* leaf extract against *S. aureus* and *S. sonnei* by measuring the diameter of inhibition zones. Bacterial suspensions prepared in 0.9% NaCl were inoculated onto MHA plates. Blank paper discs soaked in leaf extract at the various concentrations were placed on the medium surface. Ciprofloxacin 5 μg discs served as the positive control, while blank discs with 10% DMSO served as the negative control. Plates were incubated at 37°C for 24 hours. Each extract concentration, along with the positive and negative controls, was tested in triplicate (n = 3). The inhibition zone diameter from each replicate was measured, and the mean and standard deviation (SD) were calculated.

3. RESULTS AND DISCUSSION

Plant identification conducted at the Herbarium Depokensis (UIDEP), Universitas Indonesia, confirmed that the specimen used was *Abrus precatorius L.* The extraction was performed using the maceration method with 96% ethanol as the solvent at a simplicia-to-solvent ratio of 1:1. Maceration was employed as the extraction method in this study to obtain flavonoids, saponins, alkaloids, and tannins from *Abrus precatorius L.* (Rosary Pea). This technique involves soaking powdered plant material (simplicia) in a suitable solvent, which in this case was 96% ethanol. The selection of 96% ethanol is supported by its high organic character, which enables the efficient dissolution of semi-polar and non-polar constituents, such as terpenoids, essential oils, steroids, and aglycone flavonoids [5]. Its low water content enhances selectivity, reduces the co-extraction of highly polar impurities, and helps maintain the stability of water-sensitive compounds, making it a reliable solvent for extracting lipophilic phytochemicals [11]. The extraction process yielded more than 25%, meeting the Indonesian Herbal Pharmacopoeia requirement of a minimum 10% yield. This percentage reflects the proportion of extractable compounds present in the *A. precatorius* simplicia when processed using 96% ethanol.

Phytochemical screening conducted using reagent-based test tube assays confirmed that the ethanol extract contained flavonoids, saponins, alkaloids, and tannins.

Table 1. Phytochemical Screening Results

Active Compounds	Screening Result
Flavonoid	Positive
Saponin	Positive
Terpenoid	Negative
Alkaloid	Positive
Tanin	Positive

The antibacterial activity of the extract was evaluated by measuring inhibition zones formed at various concentrations of *Abrus precatorius* leaf extract. Ciprofloxacin was used as the reference antibiotic due to its broad-spectrum activity against both Gram-positive and Gram-negative bacteria [12]. A 10% DMSO solution served as the extract solvent, as this concentration is known to lack antibacterial effects, ensuring that the observed inhibition zones reflected the activity of the ethanol extract rather than solvent interference. The assay was performed using the disc diffusion method, with inhibition zones measured after 24 hours of incubation at 37 °C and reported as mean diameters in millimeters. The results showed that the ethanol extract of *A. precatorius* inhibited the growth of *Staphylococcus aureus* at all concentrations tested, but the best was at a concentration of 100%, which was included in the medium category. For *Shigella sonnei*, the ethanol extract of *A. precatorius* inhibited only at concentrations of 50% and 100% as presented in Tables 2 and 3.

Table 2. Mean inhibition zone diameters (mm) of *A. precatorius* leaf extract against *Staphylococcus aureus* at various concentrations

Treatments	Concentration	Replication (mm)			Mean±SD	Interpretation
	(%)	I	II	III		
	100	8.5	7.5	6	7.33±1.26	Medium
	50	3.5	3.5	3	3.33±0.29	Weak
Abrus precatorius L	25	2	1.5	1.5	1.67±0.29	Weak
Leaf extract	12.5	1.5	2	1	1.50±0.50	Weak
	6.25	1	0.5	1	0.83±0.29	Weak
	3.125	0.5	0.5	0.5	0.50±0.00	Weak
Ciprofloxacin	1	22.5	23.25	22.5	22.7±0.43	Powerful
DMSO	10	0	0	0	0±0	None

Values represent the mean of replicate measurements

Table 3. Mean inhibition zone diameters (mm) of *A. precatorius* leaf extract against *Shigella sonnei* at various concentrations

Treatments	Concentration	Replication (mm)			Mean±SD	Interpretation
	(%)	I	II	III		
Abrus precatorius L	100	3.5	3	4	3.50±0.50	Weak
Leaf extract	50	2	2.5	1.5	2.00±0.50	Weak
	25	0	0	0	0±0	None
	12.5	0	0	0	0±0	None
	6.25	0	0	0	0±0	None
	3.125	0	0	0	0±0	None
Ciprofloxacin	1	24	23.75	24	23.9±0.14	Powerful
DMSO	10	0	0	0	0±0	None

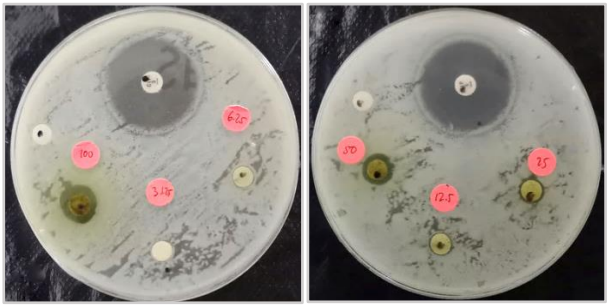

Figure 1. Inhibition zones against *Staphylococcus aureus* with Ciprofloxacin as the positive control and DMSO as the negative control

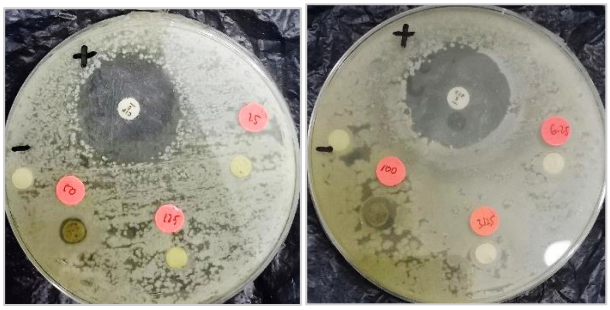

Figure 2. Inhibition zones against *Shigella sonnei* with Ciprofloxacin as the positive control and DMSO as the negative control

According to Table 2 and Figure 1, 100% extract concentration produced a moderate inhibitory effect against *Staphylococcus aureus*, while concentrations of 25%, 50%, and 75% showed weak inhibition. As shown in Table 3 and Figure 2, *A. precatorius* extract also exhibited antibacterial activity against *Shigella sonnei* at 50% and 100% concentrations, although the inhibition was weaker than that of S. aureus. This pattern indicates a dose-dependent response, with higher extract concentrations producing larger zones of inhibition. This trend aligns with findings from previous studies on medicinal plant extracts, which consistently report increased antibacterial activity at higher extract concentrations [13]**.** Beyond the dose-dependent response, the concentration of secondary metabolites can also be significantly modulated by geo-climatic conditions and the harvesting time of the plant [14].

Secondary metabolites present in *A. precatorius* are known to contribute to its antibacterial activity. Flavonoids exert their antibacterial effect through interactions with extracellular proteins, destabilizing cell membranes, while tannins inhibit bacterial growth by inactivating enzymes and disrupting intracellular protein function [15], [16]. Saponins are associated with increased membrane permeability, and alkaloids generally interfere with nucleic acid synthesis [16], [17]. The combined action of these metabolites likely underlies the observed inhibitory effects of the zone of inhibition against *Staphylococcus aureus and Shigella sonnei*.

This difference in susceptibility is also influenced by the structural characteristics of Gram-negative bacteria. S. sonnei has an outer membrane rich in lipopolysaccharide (LPS), which limits the penetration of various plant-derived polyphenols [18]. In addition, this bacterium expresses several efflux pump systems, such as MdtEF and AcrAB-TolC, which actively expel antibacterial compounds before they reach intracellular targets [19]. The presence of detoxification enzymes, including oxidoreductases and β-lactamases, further contributes to the reduced sensitivity to phytochemicals [20]. These mechanisms collectively explain the lower inhibitory response observed in *S. sonnei*.

Overall, the antibacterial activity observed in this study can be attributed to the secondary metabolites contained in *A. precatorius* extract, including flavonoids, saponins, alkaloids, and tannins. These findings strengthen the evidence that these compounds actively contribute to bacterial growth inhibition through mechanisms such as membrane disruption, interference with enzymatic

processes, inhibition of nucleic acid synthesis, and interaction with cell wall proteins. The inhibitory effects across all tested concentrations indicate that the ethanol extract of *A. precatorius* has antibacterial potential against both Gram-positive and Gram-negative bacteria, although with varying degrees of effectiveness depending on the bacterial cell structure and defense mechanisms.

4. CONCLUSION

Based on the results of this study, the ethanol extract of Abrus precatorius L. (Rosary Pea) leaves showed stronger antibacterial activity against Staphylococcus aureus than against Shigella sonnei. These findings highlight A. precatorius's potential as a source of antibacterial compounds and provide a scientific basis for the development of better formulations with higher antibacterial efficacy.

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