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Antioxidant and Antibacterial Activity of *Plectranthus scutellarioides* (L.) R.Br. Ethanol Extract against *Staphylococcus aureus*

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A B S T R A C T

Plectranthus scutellarioides (L.) R.Br., commonly known as *Coleus scutellarioides*, has been traditionally recognized for its medicinal properties, yet the synergy between its antioxidant capacity and its antibacterial efficacy against clinical pathogens, using modern extraction techniques remains limited and underexplored. This study aimed to evaluate the antioxidant activity and antibacterial efficacy of the ethanol extract of *P. scutellarioides* leaves against *Staphylococcus aureus*. The extract was obtained by ultrasonic-assisted extraction (UAE) using 96% ethanol as the solvent. Antioxidant activity was assessed through DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays. The antibacterial potential was determined by minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using the dilution method. The ethanolic extract of P. *scutellarioides* exhibited antioxidant activity with an IC_{50} value of 154.54 ppm for DPPH and 146.47 ppm for FRAP assays, compared to vitamin C controls with IC_{50} values of 3.54 ppm and 3.57 ppm, respectively. Furthermore, the extract showed antibacterial activity against *S. aureus* with MIC and MBC values of 1.95 mg/mL and 3.90 mg/mL, respectively. These findings demonstrate that the ethanol extract of *P. scutellarioides* leaves possesses moderate antioxidant and significant antibacterial activities, highlighting its potential as a natural source for therapeutic agents.

Contribution to Sustainable Development Goals (SDGs):
SDG 3: Good Health and Well-Being
SDG 9: Industry, Innovation, and Infrastructure
SDG 12: Responsible Consumption and Production
SDG 15: Life on Land

1. INTRODUCTION

1.1. Research Background

Infectious diseases caused by antibiotic-resistant bacteria represent an escalating global health emergency. Among the most clinically significant pathogens, *Staphylococcus aureus* stands out as a Gram-positive coccus responsible for a wide spectrum of community- and hospital-acquired infections, including skin, mucous membranes, the nose, armpits, and the perineum [1]. In addition, methicillin-resistant *Staphylococcus aureus* (MRSA) cases increase the risk of death due to its antibiotic resistance [2].

Concurrently, oxidative stress arising from an imbalance between the production of reactive oxygen species (ROS) and the capacity of the endogenous antioxidant defense system plays a role in various pathogenic diseases, including bacterial infection [3]. During bacterial infection, the host immune response eliminates the pathogen, leading to tissue damage via oxidative pathways [4]. In the modern era of medicine, there is an urgent need to identify novel dual therapeutic strategies that simultaneously attenuate ROS-mediated cellular damage and exert direct antimicrobial activity, making them highly attractive

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candidates for integrated agent development from alternative natural products.

Plectranthus scutellarioides (L.) R.Br. (family Lamiaceae), synonymously classified as *Coleus scutellarioides* (L.) Benth., and vernacularly known as Miana or Iler, is a widely cultivated aromatic herb indigenous to Southeast Asia [5]. In traditional West Javanese medicine, the leaves of *P. scutellarioides* are commonly utilized as a remedy, often consumed as a herbal tea prepared by boiling the leaves in water [6]. Previous studies showed that *P. scutellarioides* contains diterpenoids, flavonoids, rosmarinic acid, caffeic acid, salvinorin A, and salvinorin B [5], [7], [8]. In addition, aqueous and methanol extracts of *P. scutellarioides* contain alkaloids, flavonoids, terpenoids, and phenolic compounds [9]. These compounds consistently reveal the presence of pharmacological potential.

Maceration, Soxhlet, and hydrodistillation, as conventional extraction technologies, have limitations such as increades time required, degradation of thermolabile compounds, and excessive solvent use. In contrast, ultrasound-assisted extraction (UAE) is recognized as a green extraction method that minimizes the use of organic solvent. The mechanism of UEA is the use of cavitation energy from ultrasound waves to disrupt plant cell walls and enhance solvent penetration, thereby yielding higher concentrations of bioctive metabolites, reduced extraction times, and lower temperatures [10]. Based on these advantages, the application of UEA for efficient extraction and the comprehensive evaluation of both antioxidant and antibacterial properties of *P. scutellarioides* ethanolic extract against *S. aureus*, contribute valuable insights into the natural product's pharmacological potential.

1.2. Literature Review

1.2.1. Plectranthus scutellarioides

Plectranthus scutellarioides (L.) R.Br. or Coleus scutellarioides (L.) Benth. belonging to the family Lamiaceae, it is a versatile plant widely distributed across tropical regions, particularly in Southeast Asia. In Indonesia, it is commonly known as "Iler" or "Miana" and is used in folk medicine to treat skin diseases and abdominal pain [5]. There is a wide array of P. scutellarioides varieties cultivated in Indonesia, including the Beale Street, Color Blaze Dark Star, Flamingo, Trailing Psycholeus, Trailing Queen, and Trailing Rose [11].

The existing literature indicates that the plant has been reported to display several pharmacological properties, including antioxidant, anti-inflammatory, anticancer, antihypertensive, antibacterial, anti-ageing, anti-peri-implantitis, and anti-pneumonia. Bioactive flavonoid and diterpenoid compounds in P. scutellarioides exhibit significant antioxidant and hyaluronidase inhibition activities [8]. The ethanolic, ethyl acetate, and aqueous extracts of P. scutellarioides exhibited anti-inflammatory activity through the inhibition of xanthine oxidase, cyclooxygenase-1 (COX-1), and cyclooxygenase-2 (COX-2) [6]. In addition, the ethanol extract of dehydrated P. Scutellarioides leaves contains phenolic and flavonoid compounds that play a key role as an angiotensin-converting enzyme-1 inhibitor [12]. The essential oil of P. scutellarioides obtained by microwave-assisted hydrodistillation showed potential antibacterial activity against multidrug-resistant strains of S. aureus and E. coli [13]. Diterpenoid compounds from the dichloromethane extract of P. scutellarioides inhibit the proliferation of RPMI 8226 tumor cells and human multiple myeloma cancer stem cells via the NF-κB pathway [14]. In addition, diterpenoids isolated from P. scutellarioides exhibited cytotoxicity against cervical, pancreatic, and breast cancer cell lines [5]. Fish gelatin films incorporated with ethanol-water P. scutellarioides extract demonstrate potential as active packaging materials, capable of enhancing food shelf life through their phenolic properties [15]. A previous study reported that the lyophilized extract contains flavonoid compounds, including quercetin 3-(6"-acetylglucoside), quercetin-3-glucoside, and quercetin 3-O-acetyl-rhamnoside, which show potential for treating peri-implantitis [16]. The leaf extract of P. scutellarioides increased mRNA HMGB1 expression in mice infected with K. pneumonia [17].

1.2.2. In vitro antioxidant assay

In general, in vitro antioxidant assays consist of hydrogen atom transfer (HAT) and single-electron transfer (SET). HAT measures the capacity of antioxidants to release a hydrogen atom by inhibiting free radicals, such as oxygen radical absorbance capacity (ORAC), total peroxyl radical trapping antioxidant parameter (TRAP), and lipid peroxidation. While, based on the SET mechanism, the release of electrons to a free radical to produce an anion, including cupric ion reducing antioxidant capacity (CUPRAC), ferric iron reducing antioxidant power (FRAP), and Folin-Ciocalteu test. The combination of HAT-SET, such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay, Trolox equivalent capacity (TEAC), and 2,2'-azinobis[3-ethylbenzothiazoline-6-sulfonic acid] (ABTS) [18], [19].

1.2.3. Staphylococcus aureus

Staphylococcus aureus is a Gram-positive anaerobic bacterium, primarily residing in the anterior nares, mucosal, and on the skin surface [20]. Recent studies have identified novel species within the S. aureus complex, including S. aureus sensu stricto, S. aureus subspecies aureus, S. aureus subspecies anaerobius, S. argenteus, S. schweitzeri, S. singaporensis, and S. roterodami that are capable of causing severe infections [21]. The immune response plays a key role in the pathogenesis of S. aureus by activating macrophages and neutrophils. Neutrophils produce ROS such as hydrogen peroxide and superoxide via NADPH oxidase. The production of ROS enhanced bactericidal activity against S. aureus. Macrophages play a role in the innate immune response by phagocytosis of cellular debris and foreign pathogens, including S. aureus. Macrophages as antigen-presenting cells (APCs) recognize bacterial antigens and activate adaptive immune responses [22].

1.3. Research Objective

This study aimed to evaluate the antioxidant activity and antibacterial potential of the ethanolic extract of *P. scutellarioides* leaves against *Staphylococcus aureus*.

2. MATERIALS AND METHODS

2.1. Materials and reagents

The simplicia of P. scutellarioides were obtained and identified at the Research and Development Center for Medical and Traditional Medicinal Plants, Karanganyar, Central Java. 2,2-

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diphenyl-1-picrylhydrazyl (DPPH) and 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) were bought from Sigma-Aldrich. The ascorbic acid standard was obtained from EMSURE® (Merck, 1.00468.0100). The microorganism (S. aureus ATCC 25923) and Nutrient Broth (NB), and Mueller Hinton Agar (MHA) media used in this study were obtained from the collection of the Integrated Research and Testing Laboratory, Universitas Gadjah Mada.

2.2. Extraction of P. scutellarioides

Dried simplicia was extracted using 96% ethanol at a 1:10 ratio via ultrasound-assisted extraction (UAE). Two hundred and fifty grams of the powdered plant material were then soaked in 2.5 L of 96% ethanol. The sonication condition for extraction: 40 °C for 30 minutes. The resulting liquid was vacuum-filtered through a Buchner funnel. Finally, the filtrate was concentrated using a rotary evaporator, then dried in a water bath to yield a thick extract. The yield was calculated as follows:

$$\text{Extract yield (\%)} = \frac{Total\ weigh\ \ of\ extract}{Total\ weight\ of\ simplicia} \text{ x } 100$$

2.3. Antioxidant DPPH assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity was determined according to the method of Gurning et al. (2021), with minor changes. One millilitre of freshly prepared DPPH in methanol (0.4 mM) was mixed with 1.0 mL of the sample solution at different concentrations (50-250 ppm), then diluted to 5 mL. The mixture was incubated in the dark at room temperature for 30 minutes (operating time) to ensure the reaction reaches equilibrium. Absorbance was measured at maximum wavelength (515 nm) using a Shimadzu UV-1780 spectrophotometer. The results were expressed as ascorbic acid equivalents in the range 2-10 ppm. Ethanol is used as a blank, and a DPPH solution without the extract serves as the control [23]. The data was measured in triplicate.

$$\text{Inhibition (\%)} = \frac{Acontrol - Asam}{Acontrol}\ x\ 100$$

where Acontol is the absorbance of the DPPH blank solution, and A sample is the absorbance of the extract/standard solution.

2.4. Antioxidant FRAP assay

The ferric reducing power assay was conducted as described by Fernandes et al. (2016), with a slight modification. Two millilitres of the FRAP reagent prepared by mixing 300 mM acetate buffer (pH 3.6), 20 mM FeCl3.6H2O solution, and 10 mM TPTZ solution in proportions of 10:1:1 (v/v/v) was added to the 0,1 mL samples with various concentrations (50-250 ppm) or ascorbic acid (2-10 ppm). The absorbance of the solution was measured at 596 nm using a Shimadzu UV-1780 spectrophotometer. Ethanol was used as a blank, and a reagent solution without the extract served as the control [24]. The data was measured in triplicate.

2.5. Antibacterial activity assay

2.5.1. Preparation and subculture of S. Aureus

The S. aureus bacteria were subcultured in Nutrient Broth (NB) and incubated for 18-24 hours at 36 ± 1 °C. Following the incubation period, the bacterial cells were harvested by centrifugation, and the resulting pellet was resuspended in phosphate buffer. The bacterial suspension was standardized to a cell density of 108 CFU/mL, equivalent to a 0.5 McFarland standard (optical density of 0.1 at 530 nm), and subsequently diluted to a final concentration of 106 CFU/mL.

2.5.2. Microdilution assay for MIC and MBC determination

The assay was performed in a 96-well microtiter plate with a total final volume of 200 µL per well. To prepare the stock solution, exactly 1.0 g of the crude ethanol extract was weighed and dissolved in 1 mL of NB medium to achieve an initial concentration of 1,000 mg/mL. A two-fold serial dilution was performed to obtain test concentrations ranging from 250 mg/mL down to 1.95 mg/mL. Each well was inoculated with 20 µL of the standardized bacterial suspension. The plates were then incubated at 37 °C for 18-24 hours. Following incubation, the minimum inhibitory concentration (MIC) was determined as the lowest extract concentration that completely inhibited visible bacterial growth. The minimum bactericidal concentration (MBC) was defined as the lowest extract concentration that prevented visible bacterial colony growth on the agar medium.

For the validation assay, aliquots of 50 µL were taken from the wells showing no visible growth after the initial 18-24 h incubation period. These aliquots were transferred into a 24-well plate, and 500 µL of sterile Mueller-Hinton Agar (MHA) was added to each well. The plates were incubated again at 37 °C for 18–24 hours. The negative control contained only sterile NB medium to ensure reagent sterility; the growth control contained the bacterial suspension in NB medium without extract to verify the viability of the bacterial inoculum.

2.5.3. Analysis Data

Antioxidant activity calculated based on percentage inhibition. A standard calibration curve was constructed by plotting absorbance (y) against concentration (x). Linearity was expressed by the equation y = bx + a, where b and a represent the slope and intercept, respectively. IC50 value (the concentration required to inhibit 50% of the radicals) was calculated. The sample was evaluated in triplicate, and the average ± SD was calculated. At the same time, the antibacterial activity was determined by the MBC/MIC ratio. A bactericidal agent is considered if the MBC/MIC ratio is less than 4; otherwise, it is bacteriostatic.

3. RESULT AND DISCUSSION

3.1. Extraction results of P. scutellarioides

The extraction of P. scutellarioides leaves using the ultrasound-assisted extraction method resulted in a yield of 2.97% (w/w) with a dark green, thick color. According to Chemat et al. (2017), the UAE is highly regarded for its ability to reduce extraction time and solvent consumption while increasing the recovery of thermolabile compounds due to its high efficiency at lower temperatures [25]. In addition, the extraction yield and phytochemical profile of plant extracts are significantly influenced by a combination of genetic factors and environmental variables, including geographical location, lighting, soil composition, and temperature [26], [27].

3.2. Antioxidant activity

Oxidative stress occurs when there is abnormal body homeostasis between reactive oxygen species (ROS) production and antioxidants [28]. Antioxidants are bioactive substances that protect the body against oxidative stress, a condition intimately associated with cell damage and various diseases, including chronic inflammation, cancer, diabetes, atherosclerosis, hypertension, kidney failure, neurodegenerative diseases, Parkinson's disease, and Alzheimer's disease [29], [30]. Endogenous antioxidant defenses are often insufficient to neutralize overwhelming levels of free radicals or reactive oxygen species. Therefore, dietary consumption of exogenous antioxidants enhances the body's innate defense mechanism by promoting the synthesis of antioxidant enzymes, which prevent the overaccumulation of free radicals [31], [32].

3.2.1. Radical scavenging activity

The DPPH radical exhibits a peak light absorption at maximum wavelenght of 515 nm (Figure 1), characterized by a distinct purple coloration. The antioxidant activity acts as a free-radical scavenger by reducing DPPH to DPPH-H. The chemical transition results in a marked decrease in absorbance and a visible color shift to yellow, reflecting the neutralization of the radical through hydrogen-atom or electron transfer [33], [34].

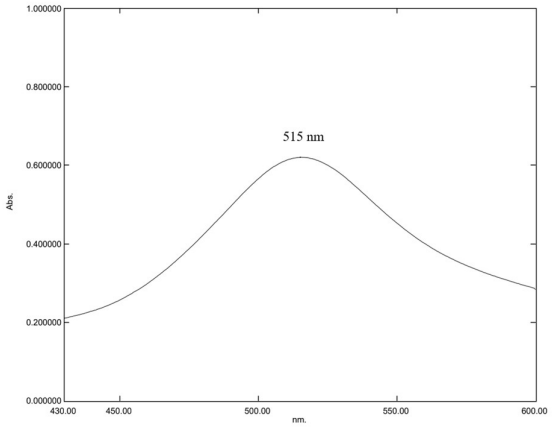

Figure 1. Maximum wavelength of ascorbic acid in the DPPH assay

The ethanol extract of *P. scutellarioides* demonstrated concentration-dependent DPPH radical scavenging activity across the tested range (50-250 ppm). The IC_{50} value for ascorbic acid showed an inhibitory effect of 3.64 ppm. Meanwhile, the ethanolic extract of *P. scutellarioides* leaves showed an IC_{50} value of 154.54 ± 0.43 ppm, indicating moderate antioxidant radical scavenging (100-150 ppm). The notably lower IC_{50} of vitamin C reflects the structural efficiency of its single ascorbate radical intermediate, which is substantially more stable than the mixed radical-stabilizing mechanism operative in a complex polyphenolic extract.

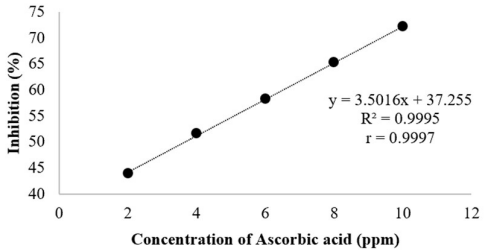

Figure 2. Regression of ascorbic acid standard in the DPPH assay

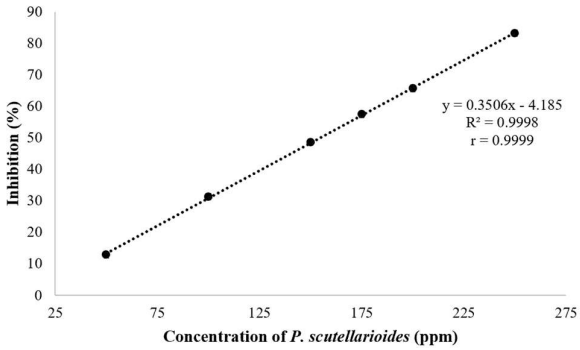

Figure 3. Regression of *P. scutellarioides* ethanolic extract in the DPPH assay

3.2.2. Ferric reducing antioxidant power

The FRAP assay measures the ability of antioxidants to reduce the Fe^{3+}-TPTZ complex to its blue-colored Fe^{2+}-TPTZ form. The conversion of Fe^{3+} to Fe^{2+} is facilitated by reducing agents of pharmaceuticals and plant extracts, such as phenolic compounds [35]. In this study, the FRAP assay was monitored at the maximum wavelength of 596 nm after a 5-minute incubation period to ensure optimal color development and stable absorbance readings (Figure 4).

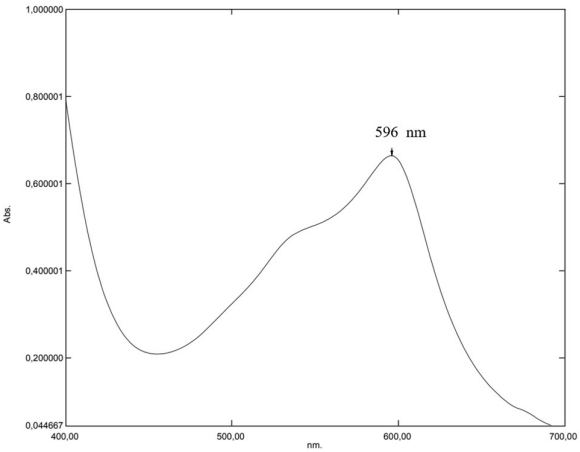

Figure 4. Maximum wavelength of ascorbic acid in the FRAP assay

The ethanolic extract of *P. scutellarioides* leaves exhibits measurable antioxidant activity in the FRAP assay with an IC_{50} value of 146.47 ± 5.90 ppm. While the 50% inhibition of ascorbic acid at 3.57 ppm.

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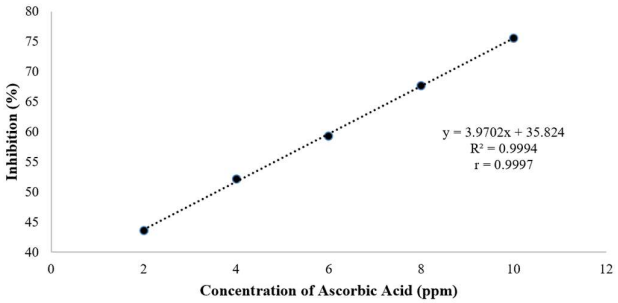

Figure 5. Regression of ascorbic acid in the FRAP assay

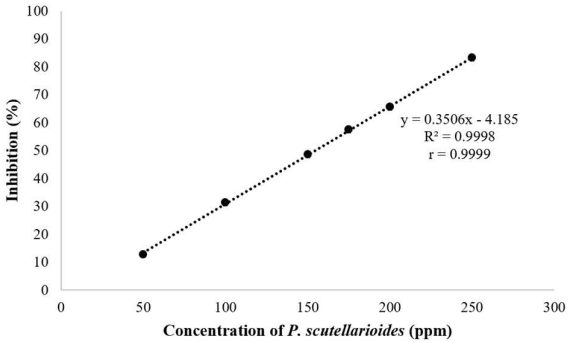

Figure 6. Regression of *P. scutellarioides* ethanolic extract in FRAP assay

The close agreement of the activity of the *P. scutellarioides* ethanolic extract between DPPH IC_{50} (154.54 ppm) and FRAP IC_{50} (146.47 ppm) values indicates that the antioxidant activity of this extract operates through both hydrogen atom transfer (HAT) and single-electron transfer (SET) mechanisms at comparable efficiencies. Based on a previous study, methoxyl and hydroxyl groups can enhance antioxidant activity more than the carboxyl group in phenolic compounds [36]. The hydroxyl group position of the flavonoid structure influences antioxidant activity [37]. The flavonoid, phenolic, alkaloid, and terpenoid compounds detected in *P. scutellarioides* leaves [9]. Therefore, flavonoid and phenolic compounds in the *P. scutellarioides* ethanolic extract may play a role in the HAT or SET mechanism. Results for both assays are consolidated in Table 1.

Table 1. The summary of the antioxidant activity of *P. scutellarioides* ethanolic extract

Samples	IC_{50} (ppm)		Category
	DPPH assay	FRAP assay	
Ascorbic acid (positive control)	3.64	3.57	Very strong
P. scutellarioides ethanolic extract	154.54	146.47	Moderate

3.3. Antibacterial activity against S. Aureus

Antibacterial activity can be classified as bacteriostatic and bacteriocidal based on the MBC/MIC ratio. MIC is the lowest concentration of an agent that inhibits visible growth, and the MBC is the lowest concentration required to kill 99.9% of bacteria [38]. The data mapping and visualization of the MIC and MBC results are shown in Figure 7.

(a)

Treatments				Negative control	
250 mg/mL	15.63 mg/mL			250 mg/mL	15.63 mg/mL
125 mg/mL	7.8 mg/mL	*S. aureus* control	Media control	125 mg/mL	7.8 mg/mL
62.5 mg/mL	3.9 mg/mL	*S. aureus* control	Media control	62.5 mg/mL	3.9 mg/mL
31.25 mg/mL	1.95 mg/mL			31.25 mg/mL	1.95 mg/mL

(b)

Figure 7. (a)The visualization and (b) mapping data of MIC and MBC results of *P. scutellarioides* against *S. aureus*

The ethanolic extract demonstrated potent antibacterial activity against *S. aureus* (ATCC 25923). The MIC and MBC values were 1.95 mg/mL and 3.90 mg/mL, respectively. The calculated MBC: MIC ratio of 2.05 (≤ 4) classifies the extract as exerting bactericidal rather than bacteriostatic activity against the *S. aureus* pathogen [39]. The bacterial MBC:MIC ratio of 2.05 confirms that the extract not only inhibits bacterial growth but also actively kills S. aureus at concentrations marginally above the MIC, a clinically desirable characteristic, particularly relevant for immunocompromised or systemic infections, where bacteriostatic agents may be insufficient. These values are summarized in Table 2.

Table 2. MIC, MBC, and MBC: MIC ratio of *P. scutellarioides* ethanolic extract against *S. aureus*

Treatment	MIC (mg/mL)	MBC (mg/mL)	MBC/ MIC	Activity Type
P. scutellarioides (96% EtOH)	1.95	3.90	2.05	Bactericidal

3.4. Correlation between antioxidant capacity and antibacterial activity

A noteworthy finding of the present study is the apparent dissociation between the moderate antioxidant and the remarkable antibacterial potency of the *P. scutellarioides* ethanolic extract. In the clinical management of staphylococcal skin and wound infections, oxidative stress generated during the inflammatory response exacerbates tissue damage and impairs wound healing. An ethanolic extract that simultaneously neutralizes ROS and eliminates *S. aureus* at mg/mL concentrations could therefore provide complementary therapeutic benefits not achievable with conventional antibiotic monotherapy.

4. CONCLUSION

This study demonstrated that *Plectranthus scutellarioides* leaves, when treated with 96% ethanol under UAE conditions, exhibit moderate antioxidant activity and potent bactericidal activity against *Staphylococcus aureus*. These findings collectively establish *P. scutellarioides* as a promising natural source for developing novel antimicrobial and antioxidant therapeutic agents. Rigorous *in vivo* assays, toxicological profiling, and bioassay-guided isolation of active compounds are recommended as future directions.

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