

## Research Article

**Antibacterial Activity of Bandotan (*Ageratum conyzoides* L) Leaves Extracts Against Methicillin-Resistant *Staphylococcus aureus***

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Garut, Garut, West Java, Indonesia\*email: [doni@uniga.ac.id](mailto:doni@uniga.ac.id)**Keywords:***Ageratum conyzoides*  
Antibacterial  
MRSA**Abstract**

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infections throughout the world and can be life-threatening as well. This study aimed to determine the antibacterial activity of Bandotan (*Ageratum conyzoides* L) leaves ethanolic extract against MRSA's growth. *Ageratum conyzoides* leaves were extracted by ethanol and screened for their phytochemical constituent. Ethanolic extracts of *A. conyzoides* leaves were evaluated for their potential antibacterial activity using disc diffusion assay. The minimum inhibitory concentration (MIC) value was determined using the agar dilution method. Phytochemical screening shows that the extracts contain alkaloids, flavonoids, saponins, tannins, and steroids or triterpenoids. *Ageratum conyzoides* leaves extract shows a 25.1 mm inhibitory zone at 12.5% extract concentration with MIC value equivalents to  $4.46 \times 10^{-6}$  g of gentamicin. This study concludes that *A. conyzoides* leaves ethanolic extracts have potential antibacterial activity against MRSA.

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**INTRODUCTION**

Antibiotic resistance is one of the biggest threats to global health. It is rising to dangerously high levels in all parts of the world (Aslam *et al.*, 2018). Methicillin-resistance *Staphylococcus aureus* (MRSA) is a common cause of severe nosocomial infections (Choo & Hambers, 2016). It has developed resistance to numerous antibiotics caused by the misuse and overuse of antibiotics. The MRSA can hydrolyze almost any type of lactams, and its strains spread quickly, leading to a high mortality rate (Hu *et al.*, 2019). World Health Organization (WHO)'s first global report on antibiotic resistance reveals that more than one-quarter of *S. aureus* infections in the south-east Asia region are reported to be MRSA, which is home to a quarter of the world's population (Prestinaci *et al.*, 2015).

Therefore, alternatives antibacterial against MRSA infections is still sought-after investigation.

Many antibacterial drugs were firstly isolated from natural sources (Rossiter *et al.*, 2017). Many studies revealed that medicinal plants provide antibacterial compounds from its secondary metabolites (Gorlenko *et al.*, 2020; Othman *et al.*, 2019; Voravuthikunchai & Kitpipit, 2005). Indonesia is a rich archipelago with an abundance of natural plants to explore. One of them is bandotan (*Ageratum conyzoides* L.) plants, which grow in many Indonesian regions and are classified as tropical weeds (Kotta *et al.*, 2020; Atisha & Mita, 2018). It is easy to find the weeds because it thrives in any garden and agricultural soils. It is also ubiquitous in disturbed sites and degraded areas (Marks & Nwachuku, 1986). The weeds are also noxiously regarded as harmful for crops,

but on the contrary, *A. conyzoides* have been known since ancient times for their therapeutic benefits (Garg *et al.*, 2015). The weed is traditionally used to treat new wounds, bleeding wounds, ulcers, eczema, bacterial infection diseases, arthrosis, headaches, pneumonia, analgesic, antispasmodic, anti-inflammatory, leprosy, and other skin diseases (Kamboj & Saluja, 2008; Achmad *et al.*, 2020). Recent studies have shown that bandotan leaves have antibacterial activity against *Staphylococcus aureus*, *Porphyromonas gingivalis*, *Streptococcus mutans*, and *Escherichia coli* (Mentari *et al.*, 2020; Achmad *et al.*, 2020; Sugara *et al.*, 2016).

Many research shows that *A. conyzoides* have various health benefits. However, there is still limited research on *A. conyzoides* leaves antibacterial activity specifically towards MRSA. Therefore, this study focused on assessing the antibacterial activity of *A. conyzoides* leaves extract towards MRSA.

## MATERIALS AND METHODS

### *Plant material*

*Ageratum conyzoides* plants were collected from the rice field at Limbangan, Garut, West Java, Indonesia. The plants were authenticated and determined at the Herbarium Unit, Department of Biology, Universitas Padjadjaran, Indonesia.

### *Extract preparation*

*Ageratum conyzoides* leaves were washed and dried for ten days at 40°C. Dried leaves were grinded into powder and evaluated by the distillation method to analyze the moisture content. Extraction was performed by the maceration method. As much as 250 g of *A. conyzoides* leaves were extracted by ethanol 96% at room temperature. The solvent was replaced three times with fresh solvent every 24 hours. After filtration of total extracts, *A. conyzoides* leaves extract were evaporated by

rotary evaporator until it dry and were weighed to determine the yield.

### *Phytochemical screening*

The extract was subjected to various phytochemical screening to identify its chemical constituents, including alkaloids, flavonoids, saponins, tannins, quinones, steroids, or triterpenoids. The procedures for detecting those secondary metabolites are referred to *Materia Medika Indonesia* volume IV (1980).

### *Bacterial culture*

Methicillin-resistance *Staphylococcus aureus* ATCC 43300 isolates were obtained from the Department of Pharmacy, Universitas Padjadjaran, Indonesia. The bacteria were maintained on Nutrient Agar (NA) slope and then subcultured on NA at 37°C for 18-24 hours.

### *Antibacterial activity*

Antibacterial activity of *A. conyzoides* leaves extract was evaluated by disc diffusion method. Extract was diluted on dimethyl sulfoxide (DMSO) solution to yield 5%, 7.5%, 10% and 12.5% concentration. Bacteria inoculum was introduced onto the sterile NA plates' surface using a sterile loop and spread over the media for even distribution. The plates were divided into five sections: four sections for extract and one section for gentamicin. Blank sterile paper discs were placed on the NA surface and impregnated with 15 mL of the extracts. The plates were incubated at 37°C for 18-24 hours. The antibacterial activity was expressed as clear inhibition zones produced by the extracts. The test was repeated three times.

The minimum inhibitory concentration (MIC) was investigated using the agar dilution method. As much as 1 mL of *A. conyzoides* leaves extracts with various concentration (1%, 2%, 3%, 4%, and 5%) were added into 10 mL NA. Bacterial suspensions were inoculated onto each plate with a sterile loop, and the presence or absence of bacteria growth is recorded after suitable incubation. Incubation lasted for 18-24 hours at 37°C. The MIC was

determined as the lowest concentration of *A. conyzoides* leaves extracts, which completely inhibited bacterial growth. Furthermore, the MIC was converted by regression linear equation into its antibiotic dose.

## RESULTS AND DISCUSSION

This study is experimental laboratory research. The moisture content of *A. conyzoides* L. leaves was 5%, which less than 10%. The yields extract obtained with maceration was 17.16 g from 250 g simplicia. The phytochemical screening showed that *A. conyzoides* leaves contained various secondary metabolites, such as alkaloids, flavonoids, saponins, tannins, steroids, or triterpenoids as shown in **Table I**. These results agree with the previous study of phytochemical screening of *A. conyzoides* leaves. It shows alkaloids, saponins, flavonoids, polyphenols, tannins, glycosides, resins, phenols, and essential oils (Achmad *et al.*, 2020; Chew *et al.*, 2018; Amadi *et al.*, 2012). Plants synthesized the secondary metabolites to protect them from predators such as herbivores, insects, and microorganisms. It could kill or inhibit microorganism growth via different mechanisms (Chew *et al.*, 2018).

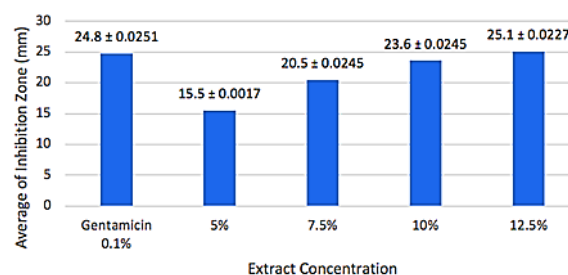
**Table I.** Phytochemical screening of *A. conyzoides* leaves

Phytochemical	Simplicia	Extract
Alkaloids	+	+
Flavonoids	+	+
Saponins	+	+
Tannins	+	+
Quinones	-	-
Steroids/Triterpenoids	+	+

(+): presence; (-): absence of phytochemicals

The results of the antibacterial activity were revealed using the disc diffusion method. This is a standard qualitative assay to evaluate the antimicrobial activity of extracts or phytochemicals. *Ageratum conyzoides* leaves ethanolic extracts have demonstrated antibacterial activity against MRSA isolate. From **Figure 1**, it can be seen that the higher extract concentration produced a more expansive inhibition zone. The inhibition zone at

5% concentration of *A. conyzoides* leaves extract had the lowest average inhibition zone of 15.47 mm, while 12.5% had the largest average inhibition zone of 25.1 mm. *Ageratum conyzoides* leaves extract with 12.5% concentration, giving almost similar results compared to gentamicin, which was used as a comparative antibiotic. This activity may be attributed to the rich tannins and flavonoid contents of *A. conyzoides* leaves. Flavonoids and tannins have been reported to possess antimicrobial activity due to their ability to complex with the bacteria cell wall and inactivate enzymes, microbial adhesion, and cell envelopes proteins (Cowan, 1999). These results confirmed the evidence in previous studies that reported that the extract of *A. conyzoides* has potential antibacterial activity against *S. aureus* (Garg *et al.*, 2015; Sugara *et al.*, 2016).



**Figure 1.** Antibacterial activity of *A. conyzoides* leaves extract against MRSA

The quantitative analysis using the agar dilution method showed that at a concentration of 1%, 2%, and 3% of *A. conyzoides* leaves extract still observed MRSA growth; meanwhile, the absence of MRSA growth can be seen at a concentration of 4% and 5% as shown in **Table II**. It was indicated that extract of *A. conyzoides* leaves active exhibiting the highest potency with MIC of 4%. Previously, the plant has been reported to have good antibacterial activity towards *S. aureus* with the MIC value of 2% (Budiman & Aulifa, 2020). According to research conducted by Astuti (2015), ethanolic extract of *A. conyzoides* leaves had the MIC value of 12.5 mg/mL against *S. aureus*. The MRSA was more challenging to

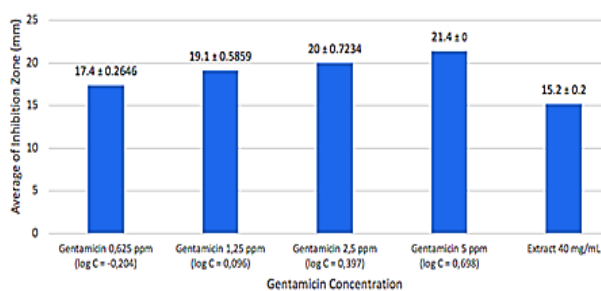
treat than most *S. aureus* because it is resistant to some commonly used antibiotics. Therefore, the MIC value for MRSA was higher than *S. aureus*.

**Table II.** Minimum inhibitory concentration of *A. conyzoides* leaves extract

Extract concentration (%)	Bacterial growth
1	+
2	+
3	+
4	-
5	-

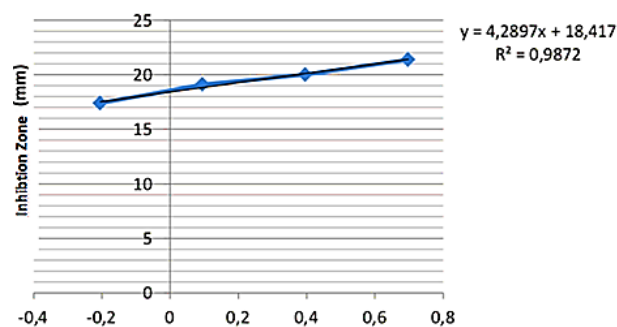
(+): growth of bacteria; (-): no growth of bacteria

The MIC value of *A. conyzoides* leaves extracts then converted into equivalency of antibiotic dose. The concentration of 4% *A. conyzoides* leaves extract concentration was analyzed to determine its antibiotic dosage equivalency using the agar diffusion method as shown in **Figure 2**. Gentamicin was used as a reference standard antibiotic. Gentamicin is an aminoglycoside that inhibits bacterial protein synthesis by binding to its ribosomes (Krause *et al.*, 2016). The standard dose of gentamicin is 3-6 mg/kg/day divided every eight hours to treat MRSA prosthetic valve endocarditis. Gentamicin is regularly added with rifampin for the first two weeks of treatment (Galar *et al.*, 2019).



**Figure 2.** Inhibition zone of *A. conyzoides* leaves extract compared with gentamicin

Furthermore, the data was calculated by linear regression, as seen in **Figure 3**. The obtained equation concluded that 4% of *A. conyzoides* leaves extract was equivalent to  $4.46 \times 10^{-6}$  g of gentamicin. This concentration could be considered when designing the next potential drug to treat nosocomial infection caused by MRSA.



**Figure 3.** Linear regression graphic and equation of gentamicin inhibition zone

## CONCLUSION

Ethanollic extracts of *A. conyzoides* leaves have potential antibacterial activity against MRSA. Further identification of the active constituents is needed to evaluate its efficacy and safety against MRSA.

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