Evaluation of antibacterial activity of sesquiterpene Ar-Turmerone from Curcuma soloensis Val. rhizomes

S D Marliyana¹, F R Wibowo¹, M W Wartono¹ and G Munasah¹
¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Sebelas Maret University, Jl. Ir. Sutami 36 A Surakarta, Indonesia
E-mail: msoerya@staff.uns.ac.id

Abstract. Curcuma soloensis Val. is a family plant Zingiberaceae and is known as "temu glenyeh". This plant has potential as a medicinal plant but has not been widely reported about its bioactivities. One of them was antibacterial activity. Therefore, the aim of this study was to evaluate the antibacterial activity of ar-turmerone, a sesquiterpenoid which was successfully isolated from Curcuma soloensis Val. rhizomes. Antibacterial test was carried out by using disc diffusion method and evaluated against four bacteria namely Staphylococcus aureus ATCC 25923, Escherichia coli ATCC 25922, Klebsiella pneumonia ATCC 13883, and Pseudomonas aeruginosa ATCC 27853. The result showed that ar-turmerone was not active against all of these bacteria.

1. Introduction
Infection caused by various drug-resistant bacteria is an increasing problem due to the appearance of and microbial drug resistance propagation and lack of new antimicrobial development [1]. With the increase in resistance of bacteria to antibiotic treatment, it is necessary to find new compounds that have antibacterial activity as alternatives to the antimicrobial therapies [2]. One source of the search for new compounds is from natural products, especially from plants because they have a variety of secondary metabolites with antimicrobial activities [3, 4]. From time to time many medicinal plants exhibit a source of new antimicrobial agents [5].

Curcuma is member of the family Zingiberaceae. It has been reported to have various bioactivities, including as an antioxidant [6], anti-inflammatory [7] antimicrobial [8], antiarthritic, antidepressant, antiatherosclerotic, antiaging [9], antidiabetic [10] and anticancer [11]. One of them is Curcuma soloensis Val. which has the potential to be widely used as phytopharmaeca. It is a medicinal plant that grows in Java, Indonesia, and the rhizome of C. soloensis Val. is called "temu glenyeh". Phytochemically, the bisacuraon, and curcuminoid were compounds that successfully isolated from C. soloensis Val. have been reported previously [12]. In addition, we have successfully isolated ar-turmerone, a sesquiterpene derivative.

Given the increasing resistance to the antibiotics, the search for new antimicrobial compounds with variety of chemical structures and novel mechanism of action are urgently needed [13]. Therefore, this study aimed to evaluate the antibacterial activity of ar-turmerone that has possibility antibacterial activity from C. soloensis Val. rhizome against four common pathogenic bacteria by disc diffusion method.
2. Experimental

2.1 General procedures

We have previously obtained ar-turmerone from the C. soloensis Val. Rhizome. All chemicals were purchased from E. Merck and used directly without any purification. They were Mueller-Hinton Agar (MHA), DMSO, NaCl, ethanol, and paper disc.

2.1.1 Antibacterial testing

The antibacterial test of the compound was determined by the disc diffusion method against four bacteria, i.e Klebsiella pneumonia ATCC 13883, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 25923, and Escherichia coli ATCC 25922. The sample was prepared to be 1000, 500, 250 dan 125 µg/mL in DMSO. The media used was Mueller-Hinton agar. The bacteria were cultured on agar media and incubated for 24 hours at 37 °C. The bacterial cultures were mixed with 9% NaCl solution and turbidity compared to McFarland 0.5 turbidity standard (108 CFU / mL). Afterward, the agar to be poured over the Petri dishes and the bacterial suspension was swab upon the agar. As much as 10 μL of the compound was added to sterile paper discs of 6 mm in diameter, which were put in agar that has been inoculated with bacterial strain. Then the plates were incubated at 37 °C for 24 hours. The test was done in triplicate. The inhibition zone represents as the antibacterial action. Chloramphenicol was used as positive control antibiotic.

3. Results and Discussion

Previously, we have been isolated ar-turmerone, a sesquiterpenoid from C. soloensis Val. The structure of ar-turmerone shows in Figure 1.

![Figure 1. Structure of ar-turmerone](image)

The results of the antibacterial testing of ar-turmerone from C. soloensis Val. rhizome against four bacterial species mainly Klebsiella pneumonia ATCC 13883, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 25923, and Escherichia coli ATCC 25922 are presented in Table 1 (inhibition zones).

| Bacteria               | Inhibition zones (mm) | Chloramphenicol |
|------------------------|-----------------------|-----------------|
| K. pneumonia ATCC 13883| -                     | -               |
| S. aureus ATCC 25923   | -                     | -               |
| E. coli ATCC 25922     | -                     | -               |
| P. aeruginosa ATCC 27853| -                    | -               |

The concentration of compound was in µg/mL.
The data in Table 1 shows that ar-turmerone did not have antibacterial activity against all test bacteria. It is because the activity of the bacteria is influenced by the structure of the compound and the characteristics of the bacteria. Each bacterium has a different level of sensitivity to certain compounds. Besides that, it can also be caused by the nature of synergism, which will be antibacterial if it was in the extract instead of pure compounds [14]. The mechanism of action of sesquiterpenoids cannot be known with certainty. Various mechanisms of action of antibacterial activity of sesquiterpenoids have been proposed. Sesquiterpenoids will first penetrate the cell wall and damage the membrane, disrupting cell activity such as membrane transport, increased permeability, and other metabolic regulatory functions [15].

Chloramphenicol as a positive control inactive to P. aeruginosa, maybe the chloramphenicol is resistant to P. aeruginosa. Skocibusic’ et al. stated that gram-negative P. aeruginosa is known to have a high level of intrinsic resistance to almost all recognized antimicrobials and antibiotics for the extremely tight combination of outer membrane barriers, even very resistant to synthetic drugs [16].

4. Conclusion
The evaluation of antibacterial activity showed that ar-turmerone, a sesquiterpenoid from Curcuma soloensis Val. rhizome was not active against four bacteria, namely Klebsiella pneumonia ATCC 13883, Pseudomonas aeruginosa ATCC 27853, Staphylococcus aureus ATCC 25923, and Escherichia coli ATCC 25922.

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