Antibacterial activity of essential oils from twenty *Curcuma aeruginosa* genotypes

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**Abstract.** *Curcuma aeruginosa* is a rhizome-like medicinal plant with many pharmacological properties. This research aimed to evaluate the antibacterial activity in essential oils from *C. aeruginosa* genotypes. Using hydrodistillation process, essential oils were acquired from twenty genotypes of *C. aeruginosa* rhizomes. Antibacterial activity of the samples has been evaluated using agar disk diffusion against *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus mutants*. Using the broth dilution process, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were measured for the selected bacteria. Twenty *C. aeruginosa* genotypes (excluding the G4, G8, G14 and G20 genotypes) demonstrated antibacterial activity against *S. aureus* with varying levels of inhibition. The genotype G15 showed inhibition against *E. coli*, while the genotype G1, G2, G4, G12 and G16 presented inhibition against *S. mutants*. These findings revealed that, compared with *E. coli* and *S. mutants*, the essential oils of *C. aeruginosa* exhibited good antibacterial activity in *S. aureus*. The MIC and MBC of twenty essential oils of *C. aeruginosa* genotypes against *S. aureus* ranged from 7.81 to 2000 μg/ml and 250 to 2000 μg/ml, respectively. These findings suggest that the essential oils of *C. aeruginosa* may be used as a natural antibiotic to treat many infectious diseases caused by gram-positive bacteria.

1. Introduction

Medicinal plants essential oils are considered to have pharmacological properties, especially antimicrobe and antioxidants [1]. The pharmacological properties of essential oils depend on their genotype-determined chemical composition, which is affected by agronomic and environmental conditions [2,3]. *Curcuma aeruginosa* is a rhizome-like medicinal plant native to the Southeast Asia region, belonging to the Zingiberacea family [4,5]. Its rhizome part is used to treat fungal infection and diarrhea in traditional medicine [6]. *C. aeruginosa* rhizome has been identified as a source of essential oils for its diversity [6–8]. The *C. aeruginosa* essential oils for pharmacological properties have been extensively studied and showed antimicrobial activity [7,8], antibacterial and biofilm degradation activity [9], anti-androgenic activity [6], and anticancer activity [10]. These works have demonstrated pharmacological differences in the volatile constituents of *C. aeruginosa*. Studying the pharmacological activity of *C. aeruginosa* genotype naturally occurring populations may help find a new elite genotype that can be used in plant breeding programs and the pharmaceutical industry. To this day, little research has been carried out of the natural populations on the antibacterial efficacy of collecting *C. aeruginosa* genotypes. Thus, the aim of the present research was to evaluate the antibacterial activity in essential oils from *C. aeruginosa* genotypes.
2. Materials and Methods

2.1 Plant material and obtaining the essential oils

The rhizome of twenty C. aeruginosa genotypes (G1 – G20) were collected from Tropical Biopharmaca Research Center, IPB University, Indonesia, 6°32’25.47” N and 106°42’53.22” E with 142.60 m above the sea level.

Every fresh genotype rhizome (3 kg) was washed with tap water, reduced in size, and then subjected to hydro-distillation with added water in a ratio of 1:5 using a Cleavenger-type system for five h [8]. The essential oils have been drained with anhydrous sodium sulphate and processed until antibacterial analysis in air-tight containers at 4 °C.

2.2 Bacterial strains

Cultures of gram-negative (Escherichia coli) and gram-positive (Staphylococcus aureus and Streptococcus mutants) bacteria were obtained from collection at the Tropical Biopharmaca Research Center Laboratory and maintained at 4 °C on nutrient agar (NA) and tryptic soy agar (TSA), respectively.

2.3 Agar-disc diffusion method

The antibacterial property was performed using the agar-disc diffusion assay. A bacterial inoculum was inoculated using a loop needle in TSA for S. aureus and S. mutants and NA for E. coli. Then, the inoculated agar's surface placed sterile paper discs (diameter, 6 mm) impregnated with samples of the essential oils (10 μL, with concentration of 1000 μL/mL in DMSO 20%). After inoculation for 24 h at 37 °C, the diameter zone of inhibition (discs diameter not included) were measured. Chloramphenicol and DMSO 20% were used as positive and negative controls, respectively.

2.4 Determination of minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations

As defined by Batubara et al [11] with modification, the MIC and MBC have been calculated using the micro-dilution method. Briefly, the essential oil sample (100 μL) with concentration 7.81 - 2 000 μL/mL were placed in 96 well plates. Then, the sample in each well was added with tryptic soy broth (100 μL) and S. aureus inoculants (10 μL) in a concentration of 10^2 CFU/ mL. The mixture was incubated for 24 h at 37 °C. The MIC was defined as sample concentrations at which no visually detectable bacterial growth occurred. In addition, 100 μL aliquot without visually observable bacterial growth was inoculated in a new medium TSB, incubated for 24 h at 37 °C. The sample concentration at which bacterial growth was not visually detectable was defined as MBC.

3. Results and Discussions

The antibacterial activity of different genotypes of C. aeruginosa essential oils was assessed against two gram-positive bacteria (Staphylococcus aureus and Streptococcus mutants) and one gram-negative bacteria (Escherichia coli) using a disc diffusion method. Compared with chloramphenicol as a positive control and DMSO as a negative control, the antibacterial activity of the different genotypes of C. aeruginosa essential oils was determined (Table 1). Chloramphenicol exhibited the strongest antibacterial activity in all strains examined compared to twenty genotypes of C. aeruginosa essential oils. The DMSO used to dissolve C. aeruginosa essential oils has no antibacterial effects on the strains tested. The effects of essential oil of C. aeruginosa genotypes on bacteria strain have helped us to identify them as sensitive: S. aureus > S. mutants > E. coli. S. aureus, consistent with findings obtained by Akarchariya et al [8], is the most susceptible strain. G12, G15, and G4 genotypes showed highest antibacterial activity against S. aureus (4.79 mm), E. coli (3.32 mm), and S. mutants (4.45 mm), respectively. The antibacterial classification according to their diameter of inhibitory value (resistant: D < 6 mm; intermediate: 13 mm > D > 6 mm; sensitive: D > 13 mm) was proposed by De Billerbeck [12]. This classification showed that twenty essential oils of the C. aeruginosa genotypes were in the category of resistance to the three tested bacteria. Thus, the results of twenty essential oils of the C. aeruginosa genotypes showed weak antibacterial activity. The high antibacterial activity of
C. aeruginosa essential oils was reported by Akarchariya et al [8]. They were found to be 21.94 ± 0.24 for S. aureus. Our result in line with Kamazeri et al [7] reported an inhibition zone of 7.0 ± 0.0 mm for S. aureus and no inhibition for E. coli.

### Table 1. Antibacterial activity of essential oils of twenty C. aeruginosa genotypes against S. aureus, E. coli, and S. mutants

| Sample (Genotypes) | Zone of inhibition (mm) average ± SD | S. aureus | E. coli | S. mutants |
|--------------------|--------------------------------------|-----------|---------|------------|
| G1                 | 0.90 ± 1.27                          | -         | 2.73 ± 3.85 |
| G2                 | 1.67 ± 2.36                          | -         | 3.39 ± 4.79 |
| G3                 | 0.18 ± 0.25                          | -         | -        |
| G4                 | -                                    | -         | 4.45 ± 6.29 |
| G5                 | 2.11 ± 2.98                          | -         | -        |
| G6                 | 1.10 ± 1.56                          | -         | -        |
| G7                 | 1.76 ± 2.48                          | -         | -        |
| G8                 | -                                    | -         | -        |
| G9                 | 1.27 ± 1.79                          | -         | -        |
| G10                | 2.46 ± 3.48                          | -         | -        |
| G11                | 1.64 ± 2.31                          | -         | -        |
| G12                | 4.79 ± 3.01                          | -         | 1.44 ± 2.04 |
| G13                | 0.71 ± 0.99                          | -         | -        |
| G14                | -                                    | -         | -        |
| G15                | 2.78 ± 3.92                          | 3.32 ± 4.69 | -        |
| G16                | 0.52 ± 0.73                          | -         | 2.74 ± 3.87 |
| G17                | 1.61 ± 2.28                          | -         | -        |
| G18                | 2.72 ± 1.15                          | -         | -        |
| G19                | 1.58 ± 2.23                          | -         | -        |
| G20                | -                                    | -         | -        |
| Chloramphenicol (positive control) | 6.48 ± 0.21 | 6.76 ± 1.97 | 8.66 ± 1.83 |
| DMSO 20% (negative control) | - | - | - |

Note: "-" means that there is no inhibitory activity of essential oil on the examined strain.

Most genotypes of C. aeruginosa have antibacterial activity against S. aureus compared to E. coli and S. mutants (Table 1). Therefore, S. aureus was selected bacterial to determine minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations of essential oils from twenty C. aeruginosa genotypes. Table 2 showed the MIC and MBC of essential oils of twenty C. aeruginosa genotypes against S. aureus. The MIC for essential oils of genotypes G1, G2, G3, and G4 were the highest, with the same value of 7.81 μg/mL measured for S. aureus. However, in genotypes G1 and G2, with a value of 250 μg/mL for S. aureus studied, the highest MBCs of essential oils were found. These findings are better than 125 to 1 000 μg/mL for S. aureus found as MIC for C. aeruginosa essential oil by Akarchariya et al [8]. The antibacterial activity differentials in each genotype essential oils are linked to its metabolite and functional composition (monoterpenes, sesquiterpenes, cycloisolongifolene, 8,9-dehydro formyl, and dihydrocostunolide) for major or minor components [7,8].
Table 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of essential oils of twenty C. aeruginosa genotypes against S. aureus

| Sample (Genotypes) | Minimum inhibitory concentration (µg/mL) | Minimum bactericidal concentration (µg/mL) |
|--------------------|------------------------------------------|-------------------------------------------|
| G1                 | 7.81                                     | 250.00                                    |
| G2                 | 7.81                                     | 250.00                                    |
| G3                 | 7.81                                     | 1,000.00                                  |
| G4                 | 7.81                                     | 2,000.00                                  |
| G5                 | 250.00                                   | -                                         |
| G6                 | 62.50                                    | -                                         |
| G7                 | -                                        | -                                         |
| G8                 | 2,000.00                                 | 2,000.00                                  |
| G9                 | 62.50                                    | 2,000.00                                  |
| G10                | 31.25                                    | 2,000.00                                  |
| G11                | 62.50                                    | -                                         |
| G12                | 125.00                                   | -                                         |
| G13                | 1,000.00                                 | -                                         |
| G14                | 125.00                                   | -                                         |
| G15                | 62.50                                    | -                                         |
| G16                | 2,000.00                                 | -                                         |
| G17                | 62.50                                    | 2,000.00                                  |
| G18                | 15.62                                    | -                                         |
| G19                | 250.00                                   | -                                         |
| G20                | 250.00                                   | -                                         |

4. Conclusion

Essential oils from twenty genotypes of C. aeruginosa showed antibacterial activity against S. aureus than E. coli and S. mutants. MIC and MBC against S. aureus showed highest in genotype G1 and G2 with value of 7.81 µg/mL and 250 µg/mL, respectively. These findings indicated C. aeruginosa essential oils potential as an antibacterial agent, especially for gram-positive bacteria.

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