Evaluation of Antimicrobial Activity of Alocasia Cordfolia Rhizomes Extract

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Abstract

Objective: Discovering new antibacterial drugs with high efficacy and low toxicity from medicinal plants has received increasing attention worldwide. The aim of this study was to investigate the antimicrobial activity of ethanolic extract of Alocasia cordfolia rhizomes (ACRE). Methods: S.aureus, E.coli and C.albicans were selected as the tested microorganisms. The diameter of the inhibition zone was determined by the disc diffusion method, and MIC and MBC were measured using the broth microdilution method. Results: The diameter of the inhibition zone, MIC and MBC values of ACRE against S. aureus were 7.67±0.58 mm, 160 μg/μL and 320 μg/μL, and that of ACRE against C.albicans were 8.33±1.15 mm, 80 μg/μL and 160 μg/μL, respectively. However, ACRE did not show antimicrobial activity against E. coli. Conclusion: ACRE may be a potentially promising natural antimicrobial product, and further studies are needed to identify the specific active compounds present in this extract and to elucidate their antimicrobial mechanisms.

Keywords: Alocasia cordfolia, rhizomes, antimicrobial activity, disc diffusion method, MIC, MBC

1. Introduction

The discovery and use of antibiotics were one of the greatest achievements of the 20th century. Fleming’s accidental discovery of penicillin in the 1920s, followed by the development of synthetic antibiotics, which have made great contributions to the diagnosis and treatment of infectious diseases [1]. In recent years, the misuse and overuse of antibiotics has become a rising public health concern globally. The indiscriminate use of antimicrobial drugs not only results in drug resistance and the emergence of “superbugs”, but also brings serious side effects to the human body [2, 3]. It is estimated that without interventions against bacterial resistance, 10 million people will die annually from drug-resistant infections by 2050, and the resulting economic losses will be more than 1.5 times the world’s gross domestic product (GDP) today [4]. Furthermore, some studies have shown that the current rate of bacterial resistance is much faster than the rate of new drug development, and the discovery rate of antibacterial drugs is declining [5]. Therefore, it is of great significance to screen new antibacterial drugs.

Medicinal plants are invaluable resources for maintaining health for the majority of world’s population [6], and are also very significant resources in the research of alternative new drug active ingredients [7]. Medicinal plants have a long usage history in the prevention and treatment of infectious diseases, due to their effectiveness, low toxicity and easy availability. Thus, the search for natural antibacterial drugs from medicinal plants has gradually become a hot topic in recent years.

Alocasia cordfolia, locally known in Malaysia as “Birah negeri”, is an herbaceous plant belonging to the Araceae family. Different parts of this plant have been used traditionally to treat cough, toothache rheumatic, typhoid, tuberculosis, diuretic, laxative, astringent, malaria, typhoid and tuberculosis for a very long time in many countries [8]. Despite being the most abundant natural material in Malaysia, Alocasia cordfolia has not been utilized sufficiently due to a lack of basic research. The leaves of Alocasia cordfolia have been reported to possess antimicrobial, antifungal, antioxidant, anti-inflammatory, antidiabetic, hypolipidemic and hepatoprotective activities [9]. However, few researches have been performed on pharmacological activity of Alocasia cordfolia rhizomes, especially the
antimicrobial activity has not yet been reported. Hence, the present study was conducted to investigate the antimicrobial activity of ethanolic extract of *Alocasia cordfolia* rhizomes, which provides the theoretical basis for the further exploration, development, and utilization of *Alocasia cordfolia*.

2. Materials And Methods

Plant materials

The fresh whole plant of *Alocasia cordfolia* was purchased from a local market (Katsura Garden Center, Petaling Jaya, Selangor, Malaysia). Authentication was performed by Dr. Sreenoy Kanti Das at Faculty of Pharmacy, Lincoln University College, where a voucher specimen (No. SBID: 001/21) was deposited. The rhizomes of *Alocasia cordfolia* were washed with distilled water, chopped into small pieces, and then transferred to a hot air oven at 50°C until completely dried after 3 days under sunlight. Next, the dried rhizomes were smashed into powder by using a Philips HR2221/01 blender.

Preparation of extract of *Alocasia cordfolia* rhizomes

The powder of *Alocasia cordfolia* rhizomes was extracted by a continuous hot percolation process in a Soxhlet apparatus, with three solvents of increasing polarity, that is, chloroform, acetone and 70% ethanol. After completion of extraction, the ethanol extraction solution was evaporated to complete dryness using a water bath, thereby the extract of *Alocasia cordfolia* rhizomes (ACRE) was obtained. The extract was dissolved in 70% ethanol to a concentration of 2560 μg/μL as stock, and further diluted with 70% ethanol to the desired concentration for use in subsequent experiments.

Preparation of Mueller-Hinton (MH) agar plate and MH broth

MH agar powder (38 g) was dissolved in distilled water (1 L) and stirred until fully dissolved. The medium was sterilized in an autoclave for 30 min at 121°C. After autoclaving, the agar solution was cooled down to approximately 50°C in a super clean bench and then poured into each sterile plate (diameter, 90 mm; thickness, 4 mm). MH agar plates were thus prepared. 10.5 g of MH broth powder was added to 500 mL of distilled water. The mixture was stirred until completely dissolved, sterilized by autoclaving at 121°C for 30 min, and then divided into 20 mL sterile bottles. MH agar plates and MH broth were stored in a refrigerator at 4°C until use.

Disc diffusion method

Antimicrobial activities of ACRE were initially determined using the paper disc diffusion method. The test organisms used in this assay are as follows: *Staphylococcus aureus* (*S.aureus*, ATCC 29737), *Escherichia coli* (*E.coli*, ATCC 35218), and *Candida albicans* (*C.albicans*, ATCC 10231) were provided by the microbiology laboratory of Lincoln University College. *S.aureus* and *E.coli* were grown on MH agar plates, whereas *C.albicans* was grown in MH broth. Briefly, 100 μL of microorganism suspensions (10³ CFU/mL) was uniformly spread on the surface of MH agar plates with sterile cotton swabs in the super clean bench, and 6 mm sterile paper disks were added to the surface of agar medium. 10 μL of ACRE (100 μg/μL) was dropped on the filter paper. Besides, 70% ethanol was employed as the blank control, and standard antibiotic discs of gentamicin (10 μg) and ampicillin (10 μg) were served as the positive control, respectively. The diameter of the inhibition zone surrounding each dis was measured after 22-24 h incubation in a 37°C incubator. The above assay was repeated in triplicate, and the results were averaged.

Determination of minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ACRE were determined using the broth microdilution method described by the CLSI [10]. Sterile tubes were arranged in three rows with each row containing ten tubes, and 100 μL of sterile MH broth was transferred into each test tube. 100 μL of ACRE stock solution were added to the first column of each row and then used twofold dilution to obtain 8 different gradient concentrations (1280, 640, 320, 160, 80, 40, 20, 10 μg/μL). Next, 100 μL suspensions (10⁷ CFU/mL) of tested microorganism were added to each tube and the inoculated tubes were incubated for 18-24 h at 37°C. In the present assay, tubes (column 9) with only MH broth (without inoculum and drugs) were designed as the blank control and tubes (column 10) with MH broth and inoculum (without drugs) were used as the positive control. The MIC was considered as the lowest concentration where no visible growth (no visual turbidity) in the test tubes. Determination of MBC was carried out directly according to results of the MIC assay. A portion of liquid (10 μL) from each tube that exhibited no growth was taken and streaked on MH agar plates, and then incubating 37°C for 18-24 h. The MBC was defined as the lowest concentration that completely kills organisms, where no bacterial growth was observed.

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Statistical Analysis
Data are expressed as the mean ± SD and analyzed by SPSS 20.0 statistical analysis software. Comparison between groups was carried out using one-way analysis of variance (ANOVA) with Duncan’s multi-range test. $P<0.05$ was considered to be statistically significant.

3. Results and Discussion
Although antibiotics have been the most widely accepted treatment for bacterial infectious diseases, the abuse of antibiotics has brought great threats to human health. On the one hand, the extensive use of antibiotics has led to the emergence of multidrug resistant “superbugs”, which may decrease drug efficacy and even result in no available antibiotics forthcoming in the near future [11]. On the other hand, antibiotics misuse can produce severe side effects. For example, while killing pathogenic bacteria, antimicrobials also damage or inhibit normal bacteria, causing dysbiosis, or even leading to secondary infections with drug-resistant pathogens, thus reducing the resistance of the human body [12]. It has been reported that some antibiotics can alter the interaction between mitochondria and lysosomes, leading to apoptosis [13]. Some antibiotics even cause rare side effects such as hypoglycemia, myelosuppression and hyponatremia [14]. Therefore, there is an urgent need to search for new antibacterial drugs with high efficacy and low toxicity.

Medicinal plants represent a rich source of antimicrobial agents, and it’s a novel strategy to discover new antibacterial drugs from medicinal plants. It has been discovered that secondary metabolites of medicinal plants [15]. The leaves of Alocasia cordifolia were previously demonstrated to exhibit relatively good antimicrobial activity. In addition, our previous studies found that the rhizomes of Alocasia cordifolia contain various bioactive ingredients, such as phenols, flavonoids, and alkaloids. Based on the findings presented above, we hypothesized that the rhizomes of Alocasia cordifolia also have antimicrobial potential.

In this study, the in vitro antimicrobial activity of ACRE was evaluated by two most commonly used methods: the disc diffusion method and the broth microdilution method. As shown in Table 1, the diameter of the inhibition zone of ACRE against S. aureus and C.albicans were 7.67±0.58 and 8.33±1.15 mm, respectively, whereas that of ACRE against E.coli was not observed. In addition, gentamicin and ampicillin formed a larger bacteriostatic circle compared with ACRE, the diameter of the inhibition zone of gentamicin against S. aureus, E. coli and C.albicans were 13.33±0.58, 17.33±1.15, and 25.33±1.15 mm. The diameter of the inhibition zone of ampicillin against S. aureus and C.albicans were 18.67±1.53 and 21.33±0.58 mm. Interestingly, however, we found that standard antibiotic ampicillin also did not exhibit antimicrobial effect towards E.coli. By consulting the literature, it was found that E. coli ATCC 35218 showed resistance to ampicillin [16]. The results demonstrated that ACRE exhibited antimicrobial activity against delete the space between, and a C.albicans while no antimicrobial effect against E.coli. Next, MIC and MBC of ACRE against three microorganisms were determined. According to Table 2, the MIC and MBC values of ACRE against S. aureus were 160 and 320 μg/μL, and that of ACRE against C.albicans were 80 and 160 μg/μL. Nevertheless, the MIC and MBC values of ACRE against E. coli were not measured, which indicated no antimicrobial effect was observed at 10-1280 μg/μL concentration range. These results were also consistent with the results of the disc diffusion assay. Collectively, the above findings indicated that ACRE had antimicrobial activity against S.aureus and C.albicans, no antimicrobial effect against E.coli. Additionally, the antimicrobial activity of ACRE may be attributed primarily to the presence of phenols, flavonoids, and alkaloids.

Table 1 Antimicrobial activity of ACRE against three microorganisms (Disc diffusion method)

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Diameter of the inhibition zone (mm)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ACRE</td>
</tr>
<tr>
<td>S.aureus (ATCC 29737)</td>
<td>7.67±0.58c</td>
</tr>
<tr>
<td>E. coli (ATCC 35218)</td>
<td>-</td>
</tr>
<tr>
<td>C.albicans (ATCC 10231)</td>
<td>8.33±1.15c</td>
</tr>
</tbody>
</table>

-,- indicates no inhibition zone was observed. Values are presented as mean ± SD (n = 3). Different letters within the same row indicate significant differences (P < 0.05).

Table 2 Antimicrobial activity of ACRE against three microorganisms (Broth microdilution method)

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>MIC (μg/μL)</th>
<th>MBC (μg/μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.aureus (ATCC 29737)</td>
<td>160</td>
<td>320</td>
</tr>
<tr>
<td>E.coli (ATCC 35218)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Moreover, the specific active ingredients and mechanisms of action are still needed to be further explored. In recent years, there has been an increased interest worldwide in natural antimicrobial agents, particularly those obtained from medicinal plants. In the present study, ACRE showed antimicrobial activity against S.aureus and C.albicans, and can be used as a potent source of antimicrobials. Moreover, the specific active ingredients and mechanisms of action are still needed to be further explored.

References:

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**C.albicans** (ATCC 10231)  80  160

* -, indicates that the value was not measured.