**IN VITRO ANTIBACTERIAL ACTIVITY OF KAEMPFERIA PANDURATA ROXB. AND CURCUMA XANTHORRIZA ROXB. EXTRACTS IN COMBINATION WITH CERTAIN ANTIBIOTICS AGAINST MSSA AND MRSA**

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**ABSTRACT**

**Objective:** The aim of this study was to find an alternative treatment of MRSA infection. We evaluated the interaction of *in vitro* antibacterial activity of *Kaempferia pandurata* Roxb. (rhizome) or *Curcuma xanthorrhiza* Roxb. (rhizome) ethanolic extracts combined with commonly used antibiotics, namely penicillin V, ampicillin, ciprofloxacin HCl, and tetracycline HCl.

**Methods:** Interaction between the plant extracts and antibiotics against *Methicillin-sensitive Staphylococcus aureus* (MSSA) and *Methicillin-resistant Staphylococcus aureus* (MRSA) were tested by checkerboard method and interpreted as Fractional Inhibitory Concentration (FIC) index.

**Results:** *Kaempferia pandurata* showed a synergistic effect on all antibiotics tested against MSSA. Against MRSA, however, only combinations of *Kaempferia pandurata* and penicillin V or ampicillin which showed synergism, while others were antagonisms. Meanwhile, with *Curcuma xanthorrhiza*, only its combination with ampicillin which resulted in synergistic effect against both MSSA and MRSA, others resulted in various interaction responses against MSSA and MRSA.

**Conclusion:** Interaction between *Kaempferia pandurata* and penicillin V or ampicillin and that between *Curcuma xanthorrhiza* and ampicillin resulted in synergistic effect against MRSA. These combinations are promising to be developed as an alternative strategy to treat MRSA.

**Keywords:** Antibacterial activity, Plants extract–drug interaction, MSSA, MRSA.

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**INTRODUCTION**

*Methicillin-sensitive Staphylococcus aureus* (MSSA) has developed to become resistant bacteria, known as *Methicillin-resistant Staphylococcus aureus* (MRSA) [1]. The current treatments for MSSA are limited. MRSA has been reported to be resistant to beta-lactams, aminoglycosides, fluoroquinolones and macrolides [2]. Therefore, the need in finding a new drug to treat bacterial infection is increased due to the occurrence of resistant bacteria. Finding a new compound derived from the plant as an antibacterial drug might be one alternative. An earlier study has reported that by combining the plant-derived drug with certain antibiotics reduced the minimum inhibitory concentrations of the antibiotics [3]. Such combination strategy could be a new approach to treating bacterial infection and reduce the occurrence of bacterial resistance.

Among medicinal plants well-known to have potent antibacterial activities are *Kaempferia pandurata* Roxb. and *Curcuma xanthorrhiza* Roxb. These plants are, therefore, expected to have beneficial effects when combined with antibiotics. *Kaempferia pandurata* Roxb or fingerroot, belonging to the family Zingiberaceae, is a ginger species that is widely found in Southeast Asia, Sri Lanka, India, and Southern China. The fresh rhizomes are used to treat illnesses such as swelling, wounds, dental caries, dermatitis, dry cough and cold, tooth and gum disease, diarrhoea, dysentery, and as diuretic. Fingerroot rhizomes contain many compounds, ranging from the chalcone derivatives, flavonoid derivatives, kawains, terpenes, esters, and terpenoids. These compounds have been shown to exhibit high medicinal potential. Previous studies have reported that *K. pandurata* showed antimicrobial, antiparasitic, antioxidant, anti-inflammatory, anti-angiogenic, antitumor, antifungal, anti-inflammatory, antiviral, and wound healing activities [4]. Furthermore, an early work reported that the ethanol extract of fingerroot rhizome had antibacterial activity against MSSA, MRSA, Methicillin-resistant coagulase-negative *Staphylococci* (MRGNS), *Bacillus subtilis*, and *Salmonella typhi*, with MIC of 2-16 μg/ml [5]. Panduratin A, a natural chalcone compound isolated from the fingerroot rhizomes was also found to possess strong *in vitro* antimicrobial activity against clinical *Staphylococcus* and *Enterococcus* strains [6, 7].

*Curcuma xanthorrhiza* Roxb, commonly known as temulawak or Javanese turmeric is one type of herbs derived from family Zingiberaceae. It is used in Southeast Asian countries for food and medicinal purposes. *C. xanthorrhiza* has been traditionally used to treat stomach disease, liver disorder, constipation, dysentery, diarrhoea, children’s fever, and hemorrhoids [8]. The rhizomes contain two classes of characteristic constituents. The main substances are curcuminoïds (1-2%) such as curcumin, desmethoxycurcumin, and bisdemethoxycurcumin, and volatile oil (3-12%) composed mainly of sesquiterpenes, xanthorrhizol, and a small amount of camphor. Curcumin and xanthorrhizol are the principal components of *C. xanthorrhiza* that have a wide range of pharmacological activities including hepatoprotective, antioxidant, antinflammatory, immunomodulatory, antimutagenic, antitumorigenic, hypolipidaemic, antimicrobial, antifungal, insecticidal, anti aging, and anticoagulation [9]. An ethanol extract of *C. xanthorrhiza* has been demonstrated to inhibit the growth of *Staphylococcus aureus*, *Streptococcus mutans*, and *Bacillus cereus* with a MIC of 0.1-2.0% (w/v) [10]. Xanthorrhizol, a natural sesquiterpenoid compound isolated from the turmeric rhizomes have been known to have powerful antibacterial activity against *S. mutans* and oral pathogens [8, 11].

In the present study, we evaluated the interaction of *in vitro* antibacterial activity of *Kaempferia pandurata* Roxb. (rhizome) or *Curcuma xanthorrhiza* Roxb. (rhizome) ethanolic extracts combined with commonly used antibiotics, namely penicillin V, ampicillin, ciprofloxacin HCl, and tetracycline HCl, in an attempt to find an alternative treatment of MRSA infection.

**MATERIALS AND METHODS**

**Chemicals**

Mueller Hinton Agar (MHA) and Mueller-Hinton Broth (MHB) (Oxoid Ltd., Hampshire, England) were used as growth media for bacteria.
96% ethanol and dimethylsulfoxide (DMSO, Merck, Germany) were obtained from School of Pharmacy, Bandung Institute of Technology. Penicillin V, ampicillin, ciprofloxacin HCl, and tetracycline HCl were obtained from Brataco Chemical, Indonesia.

**Plant collection and identification**

Fresh rhizomes of *Kaempferia pandurata* were collected from the garden in Tanjungkerta village, Sumedang, Indonesia. *Curcuma xanthorrhiza* rhizome was freshly harvested and collected in the garden of Cipendey village, Majalengka, Indonesia. Identification of plants was performed in Herbarium Bandungense, School of Life Sciences and Technology, Bandung Institute of Technology. Rhizomes and leaves were sorted, washed, chopped, and dried using the oven (WTB binder) at 40-45 °C for several days. The dried rhizomes then were ground to a fine powder.

**Microorganism tested and culture**

Methicillin-sensitive *Staphylococcus aureus* (MSSA) was obtained from the culture collection of School of Pharmacy, Bandung Institute of Technology. Methicillin-resistant *Staphylococcus aureus* (MRSA) was obtained from clinical patient isolate at Hasan Sadikin Hospital, Bandung. All of bacteria strains were cultured aerobically in MHA and MHB medium.

**Extraction**

The rhizome of *Kaempferia pandurata* and *Curcuma xanthorrhiza* were extracted by reflux using 96% ethanol as solvent. The ethanol extracts were filtered through filter paper (Whatman No. 1) and concentrated by a rotary evaporator (Buchi R-125) to obtain viscous extracts. The viscous extracts were stored at room temperature and protected from sunlight.

**Determination the susceptibility of bacteria**

*In vitro* susceptibility test was performed in a 96-well microtiter plate to determine minimum inhibitory concentrations (MICs) of plant extracts and antibiotics against tested bacterial strains using standard broth microdilution methods with an inoculum of 5 x 10^5 CFU/ml, according to the guidelines of CLSI standard M7-A8 [12]. Briefly, the extracts and antibiotics were first diluted in 20% DMSO, followed by two-fold dilutions in the test wells and tested over a range of concentrations from 0.5 to 4096 μg/ml against overnight broth cultures of MSSA and MRSA. There was positive control (only medium and inoculum) and negative control (only medium) for all samples in each plate. Microtiter plates were incubated aerobically at 37 °C for 24 h. The MIC was defined as the lowest concentration of an antibiotic that resulted in the complete inhibition of visible growth. Minimum bactericidal concentrations (MBCs) were established for each test sample. Briefly, medium (approximately 100 μl) from each well showing no visible growth was spread onto MHA plates. Plates were incubated at 37 °C for 24 h until growth was seen in the growth-positive control plates. The MIC was defined as the lowest concentration of an antimicrobial agent at which all bacteria in the culture are killed or the lowest concentration of drug that kills 99.9% of the total initially viable cells [13]. Experiments were conducted in triplicate.

**Antimicrobial combination testing of plant extracts and antibiotics**

The effects of interactions between plant extracts and antibiotics against bacterial strains were evaluated using the modification and simplified checkerboard technique. The test for the antimicrobial combination was performed using a 96-well microtiter plate, round bottom microdilution plate with MHB. Antimicrobial activity of each plant extract in combination with antibiotic was tested (in quadruplicate) against each microorganism. The highest concentration of extract A and antibiotic B used in combination was 2 times of each MIC. Furthermore, dilutions of plant extract and antibiotic were made with a series of twofold dilution. The extract A and antibiotic B were diluted twofold along the x-axis and y-axis of the plate, respectively. Overnight broth cultures of each strain (MSSA and MRSA) were adjusted to 10^6 CFU/ml and subsequently were added to the wells. Negative control (only MHB) and growth control (MHB and inoculum) were also included in each plate. The plates were then incubated at 37 °C for 24 h and monitored for growth. At the end of incubation period, the MICs of each extract and antibiotic alone and in the combination were recorded. The MIC was defined as the lowest concentration of the plant extract or antibiotic at which visible growth is inhibited. To evaluate the effect of combinations, the fractional inhibitory concentration index (FIC index) was calculated for each of the combinations according to the formula below.

\[
\text{FIC index} = \frac{\text{MIC in combination}}{\text{MIC alone}} + \frac{\text{MIC in combination}}{\text{MIC alone}}
\]

The results were interpreted as synergism (FIC index ≤ 0.5), additive (FIC index 1), and antagonism (FIC index>2) [14].

**RESULTS AND DISCUSSION**

**Antimicrobial activity of plant extracts and antibiotics alone against MSSA and MRSA**

*In vitro* antibacterial activity of plant extract and antibiotics was quantitatively investigated in terms of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) and results are represented in table 1. The MIC of plant extracts and antibiotics was varied, depending on the type of plant extract and antibiotic and the strain of bacteria tested. In general, all the plant extracts and antibiotics exerted antibacterial effects.

The strongest antibacterial activity against MSSA for plant extract was shown by ethanolic extract of *Kaempferia pandurata* (MIC = 128 μg/ml), while, for antibiotics, ciprofloxacin HCl showed the strongest activity [MIC = 0.5 μg/ml]. Furthermore, the MIC for all tested plant extracts and antibiotics against MRSA had higher MIC level compared to MSSA, suggesting that the antimicrobial activity of the plant extracts and antibiotics were lower against MRSA than MSSA.

<table>
<thead>
<tr>
<th>Plant extract and antibiotic</th>
<th>MSSA</th>
<th>MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Kaempferia pandurata</em> extract</td>
<td>128</td>
<td>256</td>
</tr>
<tr>
<td><em>Curcuma xanthorrhiza</em> extract</td>
<td>256</td>
<td>512</td>
</tr>
<tr>
<td>Penicillin V</td>
<td>8</td>
<td>16</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Ciprofloxacin HCl</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Tetracycline HCl</td>
<td>8</td>
<td>16</td>
</tr>
</tbody>
</table>

**Table 1: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of plant extracts and antibiotics against MSSA and MRSA**

Note: Experiments were conducted in triplicate.

Minimum bactericidal concentrations (MBCs) were also determined for each plant extract and antibiotic. The results showed that in general, the MBC values were equal to (MBC = MIC) or two times greater than the MIC (MBC = 2 x MIC). The MIC and MBC values of the ethanol plant extracts were higher than the antibiotics.
Effects of combination between plant extracts and antibiotics in antimicrobial activity

The antibacterial activity of the combination of each plant extract and antibiotics against MSSA and MRSA are shown in Table 2 and Table 3.

Against MSSA, the interaction between *Kaempferia pandurata* with each antibiotic tested showed a synergistic effect. The MIC value of *Kaempferia pandurata* and antibiotic in combination was reduced up to 1/4-1/8 MIC. Moreover, for *Curcuma xanthorrhiza*, only interaction with ciprofloxacin HCl and tetracycline HCl that showed a synergistic effect. While the interaction between *Curcuma xanthorrhiza* and penicillin V or tetracycline HCl showed additive and antagonistic effect, respectively. Against MRSA, a combination of *Kaempferia pandurata* or *Curcuma xanthorrhiza* with penicillin V or ampicillin gave a synergistic effect. An additive effect was found when *Curcuma xanthorrhiza* was combined with tetracycline HCl.

Moreover, the antagonistic effect was observed when ciprofloxacin HCl was combined with ethanolic extracts of *Kaempferia pandurata* or *Curcuma xanthorrhiza*, and when tetracycline HCl was combined with extracts of *Kaempferia pandurata*. According to FIC index, MRSA exerted the greatest resistance to most of the combinations between plant extract and antibiotic.

<table>
<thead>
<tr>
<th>Combinations</th>
<th>Antibiotics</th>
<th>MIC</th>
<th>FIC index</th>
<th>Interactions</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Kaempferia pandurata</em></td>
<td>Penicillin V</td>
<td>1/8+1/8</td>
<td>0.25</td>
<td>Synergism</td>
</tr>
<tr>
<td></td>
<td>Ampicillin</td>
<td>1/8+1/8</td>
<td>0.25</td>
<td>Synergism</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin HCl</td>
<td>1/8+1/8</td>
<td>0.25</td>
<td>Synergism</td>
</tr>
<tr>
<td></td>
<td>Tetracycline HCl</td>
<td>1/4+1/4</td>
<td>0.50</td>
<td>Synergism</td>
</tr>
<tr>
<td><em>Curcuma xanthorrhiza</em></td>
<td>Penicillin V</td>
<td>1/2+1/2</td>
<td>1.00</td>
<td>Additive</td>
</tr>
<tr>
<td></td>
<td>Ampicillin</td>
<td>1/8+1/8</td>
<td>0.25</td>
<td>Synergism</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin HCl</td>
<td>1/8+1/8</td>
<td>0.25</td>
<td>Synergism</td>
</tr>
<tr>
<td></td>
<td>Tetracycline HCl</td>
<td>1+1</td>
<td>2.00</td>
<td>Antagonism</td>
</tr>
</tbody>
</table>

Note: Experiments were conducted in quadruplicate.

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<tr>
<td></td>
<td>Ciprofloxacin HCl</td>
<td>1+1</td>
<td>2.00</td>
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</tr>
<tr>
<td></td>
<td>Tetracycline HCl</td>
<td>2+2</td>
<td>4.00</td>
<td>Antagonism</td>
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<tr>
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<td>1.00</td>
<td>Additive</td>
</tr>
</tbody>
</table>

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In this study, the synergistic effects resulted from the combination of antibiotics with plant extracts varied against different bacteria. These results were in agreement with a previous report [15]. In that report, it demonstrated the synergistic property of combination of doxycycline with ethanolic leaf extract of *Vangueria spinosa* against *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumonia* [15]. Such combination, however, showed additive interaction against *Pseudomonas aeruginosa* [15].

This synergistic action can occur due to the favorable interactions between the active component of plant extracts and antibiotics. The synergistic effect that was detected in this study suggests that plant extract is a mixture of compounds [16] that can enhance the activity of different antibiotics. One of the mechanisms of action of the synergistic interaction that has been reported was increasing the penetrability of the cell membrane which leads to the increased permeability of antibiotics [16]. These results revealed that the combined use of plant extracts and antibiotics could be useful in the treatment of infectious bacterial disease and useful in overcoming drug resistance problem although clinical controlled studies are needed to confirm the real efficacy and possible toxic effects in vivo. Therefore, understanding the mechanism of action of combination of antibiotics with plant extracts against bacteria is fundamental to the development of pharmacological agents to treat bacterial infections using the medicinal plant.

CONCLUSION

In conclusion, the results presented in this study showed that there were synergistic antibacterial effects between ethanolic plant extracts and antibiotics against MSSA and MRSA bacteria. The interaction between *Kaempferia pandurata* and penicillin V or ampicillin and interaction between *Curcuma xanthorrhiza* and ampicillin was shown to be synergistic against MRSA. These combinations can be used as an alternative strategy to treat MRSA.

ACKNOWLEDGEMENT

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CONFLICT OF INTERESTS

Declared None

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