Antibacterial activity of *Stephania suberosa* extract against methicillin-resistant *Staphylococcus aureus*

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**ABSTRACT**

Bacterial resistance to \(\beta\)-lactam antibiotics is a global problem. *Staphylococcus aureus* (*S. aureus*) strains are resistant to penicillin, methicillin around the world and in most of the Asian countries nowadays. Strain of methicillin-resistant *S. aureus* (MRSA) is now pose serious problem to hospitalized patients, and their care providers. Antibiotics available for the treatment of MRSA infection are fairly toxic and their use is frequently associated with unwanted side-effects. Novel antibiotics and/or new generation of phytopharmaceuticals approaches that can reverse the resistance to well tried agents which have lost their original effectiveness are research objectives of far reaching importance. The aim of this investigation was to examine antibacterial and synergistic activities of *Stephania suberosa* extract (SSE) against MRSA when used singly and in combination with ampicillin. The minimum inhibitory concentrations (MICs) determination of ampicillin and SSE against MRSA were 455 \(\mu\)g/ml and 4,000 \(\mu\)g/ml, respectively. Synergistic activity was observed in the combination of ampicillin and SSE with fractional inhibitory concentration index (FICI) 0.5. The viability of MRSA was determined using time-killing assay showed dramatically reduced from \(5 \times 10^5\) CFU/ml to \(10^3\) CFU/ml within 6 h and throughout 24 h. Electronmicroscopic study revealed that 0.125 \(\mu\)g/ml ampicillin in combination with 2,000 \(\mu\)g/ml SSE caused severe damage to MRSA envelopes. This study establishes evidence that SSE may be used combination with ampicillin, as a new generation of phytopharmaceuticals, to treat MRSA that currently almost untreatable microorganism. These *in vitro* results have to be confirmed in an animal test or in humans.

**Keywords:** \(\beta\)-lactam antibiotics, MRSA, *Stephania suberosa* Forman, Synergistic activity, Ampicillin
1. INTRODUCTION

Plant-derived antibacterials are an interesting source of novel therapeutics. *Stephania suberosa* Forman belongs to the family Menispermaceae, has been traditionally used in folk Medicine as a tonic, carminative, and expectorant [1]. Drug-resistant bacteria has emerged to be one of the greatest test to human health worldwide. MRSA is a major cause of community and health care associated infections. Recently, this strain has acquired resistant to practically antibiotics, its primary antibiotics cannot be effectively used against this strain. Thus, the development of a novel antibacterial agent MRSA strain is urgently needed. There is no a wealth of evidence regarding on antibacterial activity of *S. suberosa* against MRSA. Therefore, the purpose of this study was to determine antibacterial and synergistic activities of *S. suberosa* against MRSA when used alone and in combination with ampicillin.

2. MATERIALS AND METHODS

Preparation of plant extracts

Root of *S. suberosa* was dried and powdered. 50 g *S. suberosa* powdered was extracted with ethanol at 75°C for 8 hours and each extract was concentrated using a rotary evaporator. Then, freeze dryer was performed to yield a brown powder and a dark brown sticky oil of ethanol extract [1, 2].

Bacterial strains and antibiotics

Clinical isolates of MRSA DMST 20651 were obtained from Department of Medical Science, National Institute of Health, Ministry of Public Health, Thailand. *S. aureus* ATCC 29213 obtained from the American Type Culture Collection (ATCC) was used as reference strain. Ampicillin was obtained from Sigma.

Bacterial suspension standard curve

In order to select bacterial suspensions with a known viable count [3] the method was followed.

Minimum inhibitory concentration determination (MIC)

To determine MIC of crude extract and antibiotic against these strains, the broth macrodilution method was performed. Briefly, bacterial suspension was adjusted to approximately 1 x 10^8 CFU/ml. Inoculum (0.1 ml) of each strain was added to 0.9 ml MHB, plus serial dilutions of the antibacterial agents, to give final concentration approximately 1x10^5 CFU/ml. The lowest concentration that showed no visible growth was recorded as the MIC [3, 4].

Checkerboard determination

Checkerboard determinations of antimicrobial combinations were performed in accordance with Odds’s method [5]. The interactions between antibacterial agents and crude extracts were determined by the FICI was calculated and interpreted by previously described: FICI ≤ 0.5 denoting synergistic; FICI > 0.5–4.0 denoting no interaction; FICI > 4.0 denoting antagonism [5].

Killing curve determinations (viable counts)

Viable count of MRSA was examined using determination of killing curve in according to previously described [6]. Inocula (5 x 10^5 CFU/ml) were exposed to the antibacterials either singly or in combination with crude extract after contact time of 0, 0.5, 1, 2, 4, 6 and 24 h incubation at 37°C for 18 h were allowed counting of growing colonies. The lowest detectable limit for counting is 10^3 CFU/ml.

Transmission electron microscopy (TEM)

To assess the effect of *S. suberosa* induced MRSA envelope damage when either used alone or in combination with ampicillin, the TEM study was performed in accordance to previously described [6, 7].

3. RESULTS

The present study reported the antibacterial activity of *S. suberosa* extract when used alone and in combination with conventional antibiotic. The MICs of the extract and ampicillin against MRSA were 4 mg/ml and 455 µg/ml, respectively, while against susceptible *S. aureus* strain were 16 mg/ml and 2 µg/ml, respectively (Table 1). These results indicated MRSA used in this study revealed high resistant level to ampicillin. Interestingly, synergistic effect was observed in crude extract and ampicillin combination (FICI = 0.5) (Table 1). These finding indicated that the ability of *S. suberosa* can reverse the resistance strains to be susceptible to primary antibiotics. The
alkaloids have been found to be the major bioactive compound of *S. suberosa* [8], thus alkaloids could probably be the bioactive compound of this extract against drug resistant bacteria.

Table 1. MICs of crude extract from *S. suberosa* when used singly and in combination with ampicillin against drug resistant bacteria

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Agents</th>
<th>MIC Combination</th>
<th>FICI</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em> DMST 20651 (MRSA)</td>
<td>Ampicillin</td>
<td>455 µg/ml</td>
<td>0.125 µg/ml</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>SSE</td>
<td>4 mg/ml</td>
<td>2 mg/ml</td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em> ATCC 29213</td>
<td>Ampicillin</td>
<td>2 µg/ml</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>SSE</td>
<td>16 mg/ml</td>
<td>NT</td>
<td></td>
</tr>
</tbody>
</table>

FICI ≤ 0.5 denoting synergism; FICI > 0.5–4.0 denoting no interaction; FICI > 4.0 denoting antagonism; *S. aureus* ATCC 29213 were used as control strains. NT = not test.

The killing curve assay is shown in Figure 1. The cell grown in absence of antibacterial agents (control) revealed no reduction in viable counts. No significant change was observed in cells treated with the SSE and ampicillin alone. Interestingly, the combination of the SSE and ampicillin exhibited steady reduction of $5 \times 10^5$ CFU/ml to $10^3$ CFU/ml within 6 h and did not recover within 24 h. These results establish evidence that the combination of the SSE and ampicillin have synergistic activity against MRSA. These findings seem consistent with previously reported that ceftazidime in combination with flavonoids caused markedly reduction in viable counts of MRSA [7].

Electron microscopic investigation clearly exhibited that the cytoplasmic membrane and cell wall of MRSA treated without antibacterial agents (control) can be undoubtedly distinguished (2A). MRSA treated with ampicillin alone showed minimal peptidoglycan damage to a minority of these cells (2B). Cells treated with the SSE alone caused quite rather cell wall damage to several of these cells (2C). These findings suggest that the SSE alone cause higher peptidoglycan damage than ampicillin alone. Interestingly, the combination of ampicillin plus SSE inhibited definitely damage to peptidoglycan and cytoplasmic membrane, cell shape distortion, cell wall and cytoplasmic membrane of these cells cannot be distinguished in a majority of these cells (Figure 2D). These results provide evidence that SSE exerts synergy effect with ampicillin by reversing ampicillin resistance to be susceptible to its primary antibiotic. These results are in substantial agreement with previously reported that TEM clearly showed damage to the ultrastructures of MRSA strain after exposure to the combination [6, 7].

![Figure 1](image_url)

Figure 1. The effect of ampicillin either alone or in combination with *S. suberosa* extract (SSE) on clinical isolates of Methicillin-resistant *Staphylococcus aureus* DMST 20651 (MRSA). Symbol represents: (●) control (antibacterial free); (○) SSE (2 mg/ml); (▲) ampicillin (227 µg/ml); (△) SSE (2 mg/ml) + ampicillin (0.125 µg/ml). The values plotted are the means of 3 observations, and the vertical bars indicate the standard errors of the means.
Figure 2. Ultrathin sections of log phase clinical isolates of Methicillin-resistant *Staphylococcus aureus* DMST 20651 (MRSA) grow in MHB: Control (A: bar=500 nm, x19500; a: bar=100 nm, x43000); 227 µg/ml ampicillin (B: bar=500 nm, x15000; b: bar=200 nm, x38000); 2 mg/ml SSE (C: bar=500 nm, x9900; c: bar=200nm, x38000); 2 mg/ml SSE plus 0.125 µg/ml ampicillin (D: bar=500 nm, x9900; d: bar=200 nm, x29000).

4. CONCLUSIONS

The results of present study support the traditional use of *S. suberosa* and also suggest that this plant possess antibacterial properties. Our results also demonstrate the ability of *S. suberosa* extract also have the ability to reverse the resistance of such bacterial strain to the activity of the primary antibiotic. Moreover, the combination of SSE plus ampicillin could be offered for the development of a valuable adjunct to ampicillin, as a new generation of phytopharmaceuticals, treatments against, MRSA, otherwise resistant strain of currently almost untreatable microorganism.

ACKNOWLEDGEMENTS

The authors are indebted and gratefully to Suranaree University of Technology invaluable assistant in research funds support.

REFERENCES