ANTIBACTERIAL ACTIVITY OF α-MANGOSTIN FROM THE PERICARP EXTRACT OF GARCINIA MANGOSTANA L. AGAINST DRUG RESISTANT BACTERIA

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INTRODUCTION
In the recent years, incidence of multidrug resistance in pathogenic and opportunistic bacteria has been increasingly documented. These bacteria pose life-threatening risks to the hospitalized patients and their care givers1. *Enterobacter cloacae* are significant cause of nosocomial infections. mainly causes of pneumonia, wound infection and urinary tract infection in the most hospital. Antimicrobial resistance in this strain has increasingly emerged, including resistant to aminopenicillins, cefazolin, and cefotaxin due to the production of amp C β-lactamases2. Similarly, the emergence of fecal *Escherichia coli* isolates exhibiting resistance to extended-spectrum cephalosporins has been reported among pigs in Spain3. Furthermore, 19% antibiotics resistance of *E. coli* in hospital sewage water was resistant to ampicillin, cefazidime, ceftriaxone, cefotaxime, chloramphenicol, ciprofloxacin, gentamicin, na idic acid and trimethoprim-sulphamethoxazole among the resistant isolates from the wells4. *Staphylococcus saprophyticus* is implicated in 10-20% of urinary tract infections (UTI), usually susceptible to antibiotics commonly prescribed for patients with UTI. However, resistance of *S. saprophyticus* to penicillins such as oxacillin has also been reported5. Thus, development of novel antibacterial agents that can reverse the resistance to β-lactam antibiotics is research objectives of far reaching importance and urgently needed. *Garcinia mangostana* (GML), belonging to the family Guttifera, has been used as a traditional medicine in Southeast Asia for the treatment of diarrhea, inflammation, and chronic ulcers6 as well as abdominal pain, dysentery, infected wound and suppuration7. The xanthone derivatives, α-mangostin, is a major bioactive compound found in the pericarp of the mangosteen, has been discovered and used alone and in combination with β-lactam antibiotics. 

**MATERIALS AND METHODS**

**Isolation and purification of α-mangostin** α-mangostin from pericarp of GML was isolated and purified according to previous methods8,9 with some modifications. The dichloromethane crude extract was used to separate in silica gel column chromatography to yield 11 fractions. Fraction 3 was subjected to HPLC and purified using preparative thin layer chromatography to obtain the major compound. This compound was elucidated the chemical structure by NMR and structure spectrum data was compared with previously reported10. The structure of α-mangostin is shown in figure 1. 

**Bacterial strains and antibiotics** Clinical isolates of *S. saprophyticus* DMST 27055, *E. cloacae* DMST 21394 and *E. coli* DMST 19629 were obtained from Department of Medical Science, National Institute of Health, Ministry of Public Health, Thailand. The reference strains *S. aureus* ATCC 29213 and *E. coli* ATCC 29222 from the American Type Culture Collection were used as control. Oxacillin and cefotaxime were obtained from Sigma. 

**Bacterial suspension standard curve** In order to select bacterial suspensions with a known viable count, the method of Liu et al11 was followed. Mueller-Hinton agar and Cation-adjusted Mueller-Hinton broth (CAMHB) were used as medium.

**Minimum inhibitory concentration determination (MIC)** To determine MICs of α-mangostin and antibiotics against these strains, the standard agar dilution 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 approximately 1x10^3 CFU/ml. Antimicrobial-free tubes were used as control. Aliquots (2µl) of each inoculum were spotted on agar surface (the final concentration was approximately 1 x 10^6 CFU/ml). Then, agar plates were incubated at 37 °C for 24 h. The MICs was defined as the lowest concentration of antibiotic at which there is no visible growth in the triplicate spots12,13.
Checkerboard determination

Combinations of antibacterial agents were performed using checkerboard assay in order to examine synergistic antibacterial activity of the extract and antibiotics. Cultured bacteria and antibacterial agents were prepared and performed similarly to previously described in MIC determination. Differently, in this method, combinations of antibacterial agents plus GML extract in each spot were employed. The interactions between antibacterial agents and these extracts were determined by the fractional inhibitory concentration (FIC). The FIC index (FICI) was calculated and interpreted in accordance with Odds’s described: FICI ≤ 0.5 denoting synergistic; FICI > 0.5-4.0 denoting no interaction; FICI > 4.0 denoting antagonism.

Killing curve determinations (Viable counts)

Viable counts for the determination of killing curve was performed as previously described with slightly modifications. Inocula 5 x 10^6 CFU/ml were exposed to antibacterial agents at 0.5, 1, 2, 3, 4, 5, 6, 24 h. The cultures at each exposed time were plated on MHA in quadruplicate. Then, incubation at 37 °C for 24 h. Finally, these plates were allowed to count bacterial growth.

Transmission electronmicroscopy (TEM)

The combination of oxacillin plus GML extract that exhibited synergistic effect against oxacillin-resistant S. saprophyticus was chosen to investigate primarily mechanism of action by TEM. Oxacillin either alone or in combination with these extracts were prepared for TEM following previously described.

RESULTS AND DISCUSSION

MIC and checkerboard determinations
The MICs of CH2C12 crude extract, Fr3, and a-mangostin against clinical isolates of oxacillin-resistant S. saprophyticus (ORSS) were 50, 31 and 8 µg/ml, respectively. Whereas, the MICs of those GML extracts against both clinical isolates of ceftriaxone-resistant E. coli and ceftriaxone-resistant E. cloacae were the same value at >10,000, >10,000 and >1,024 µg/ml, respectively (Table 1). These results indicated that the bioactive compounds exhibit higher potency against ORSS than oxacillin alone but no effect on those of E. coli and E. cloacae, which are gram negative bacteria and have a multi-layered and complex structure. The outer membrane can act as a barrier to many environmental substances including antibiotics. The results of checkerboard determinations demonstrated that MICs of CH2C12 crude extract, Fr3, and a-mangostin in combinations with oxacillin against ORSS were dramatically decreased, exhibiting the synergistic effect at FICI 0.25, 0.138, and 0.375, respectively (Table 1). These results indicate that the bioactive compounds can reverse the resistant strain of ORSS to its primary susceptible antibiotic.

Killing curve assay

The control showed no reduction in the counts of CFU from control inoculum. The results showed that the combination of the bioactive compounds (CH2C12 crude extract, Fr3 extract, and a-mangostin) and oxacillin caused a reduction of 5 X 10^5 CFU/ml of ORSS to 10^3 CFU/ml within 6 h and throughout the remainder of a 24 h (Figure 2). These results provide evidence that bioactive compounds in combination with oxacillin have synergistic activity. The results of this study seem consistent with earlier findings that Ceftriaxone at 5 µg/ml in combination with 5 µg/ml of tested flavonoids reduced the CFU/ml of MRSA strain by 5x10^5 over 6 h. The reduced counts did not recover in 24 h.

Fig. 1 The structure of n-mangostin

TEM study

Electronmicroscope investigations clearly exhibited that the combination of oxacillin with a-mangostin from the pericarp of GML markedly showed great synergism activity against ORSS and caused damage to ultrastructures of this strain, the majority of these cells undoubtedly exhibited cell shape and cell envelope damage (Figure 3D). Similarly, most of these cells exposed to a-mangostin alone were considerably smaller than those of control cells and exhibited cell envelope damage and morphological change (Figure 3C). Whereas, cells treated with oxacillin alone seemed slightly smaller.
than control cells (Figure 3B). The results of TEM in this investigation suggest that oxacillin had weaker activity against ORSS. Whereas, biochemical compounds of GML extracts showed rather higher potency than oxacillin against this strain. These results are consistent with previously reported that TEM clearly showed damage to the ultrastructures of MRSA strain after exposure to the combination of galangin and ceftazidime. Similarly, TEM study clearly demonstrated that galangal extract caused both outer, inner membrane damage and cytoplasm coagulation of S. aureus strain.

Table 1 MICs of bioactive compounds from the pericarp of GML extract when used singly and in combination with antibiotics against drug resistant bacteria

<table>
<thead>
<tr>
<th>Strains</th>
<th>agents</th>
<th>MIC alone (µg/ml)</th>
<th>MIC Combination (µg/ml)</th>
<th>FIC index</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. saprophyticus</td>
<td>Oxacillin</td>
<td>128</td>
<td>16</td>
<td>0.25</td>
<td>synergism</td>
</tr>
<tr>
<td></td>
<td>Crude extract</td>
<td>50</td>
<td>6.25</td>
<td>0.375</td>
<td>synergism</td>
</tr>
<tr>
<td></td>
<td>Oxacillin</td>
<td>128</td>
<td>31</td>
<td>0.138</td>
<td>synergism</td>
</tr>
<tr>
<td></td>
<td>a-mangostin</td>
<td>8</td>
<td>2</td>
<td>0.375</td>
<td>synergism</td>
</tr>
<tr>
<td>E. coli DMST 19629</td>
<td>Ceftazidime</td>
<td>&gt;1,024</td>
<td>&gt;1,024</td>
<td>&gt;4.0</td>
<td>no interaction</td>
</tr>
<tr>
<td></td>
<td>Crude extract</td>
<td>&gt;1,024</td>
<td>&gt;1,024</td>
<td>&gt;4.0</td>
<td>no interaction</td>
</tr>
<tr>
<td></td>
<td>Oxacillin</td>
<td>&gt;1,024</td>
<td>&gt;1,024</td>
<td>&gt;4.0</td>
<td>no interaction</td>
</tr>
<tr>
<td>E. cloaca DMST 21394</td>
<td>Ceftazidime</td>
<td>&gt;1,024</td>
<td>&gt;1,024</td>
<td>&gt;4.0</td>
<td>no interaction</td>
</tr>
<tr>
<td></td>
<td>Crude extract</td>
<td>&gt;1,024</td>
<td>&gt;1,024</td>
<td>&gt;4.0</td>
<td>no interaction</td>
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<tr>
<td></td>
<td>Oxacillin</td>
<td>&gt;1,024</td>
<td>&gt;1,024</td>
<td>&gt;4.0</td>
<td>no interaction</td>
</tr>
<tr>
<td>S. aureus ATCC 29213</td>
<td>Oxacillin</td>
<td>2</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>E. coli ATCC 25922</td>
<td>Ceftazidime</td>
<td>8</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

FICI ≤ 0.5 denoting synergistic; FICI > 0.5-4.0 denoting no interaction; FICI > 4.0 denoting antagonism; NT: not test; the references S. aureus ATCC 29213 and E. coli ATCC 25922 were used as control strains.

Fig. 2 The effect of oxacillin combined with bioactive compounds from the pericarp of GML extract on the clinical isolates of oxacillin-resistant S. saprophyticus DMST 27055 (ORSS). Symbol represents: (*) control (antibacterial free); (+) oxacillin (32 µg/ml); (x) a-mangostin (2 µg/ml); (A) Fr3 (2.5 µg/ml); (b) crude extract (0.25 µg/ml); (w) exacinil (8 µg/ml); (u) exacinil (8 µg/ml) + a-mangostin (1 µg/ml); (t) exacinil (8 µg/ml) + Fr3 (2 µg/ml); (+) oxacillin (8 µg/ml) + Fr3 (3 µg/ml). The values plotted are the means of 3 observations, and the vertical bars indicate the standard errors of the means.
Fig. 3 Ultrathin sections of log phase clinical isolates of oxacillin-resistant R. saprophyticus DMST 27055 (ORSS) grown in MHB: Control (A: bar = 1µm, x4,000; a: bar=500 nm, x15,000); 32 µg/ml oxacillin (B: bar=1µm, x4,000; b: bar=200 nm, x19,500); 12 µg/ml α-mangostin (C: bar= 500 nm, x9,900; c: bar 200 nm, x29,000); 8 µg/ml oxacillin plus 1 µg/ml α-mangostin (D: bar=1µm, x5,000 : d bar = 200 nm, x29,000).

CONCLUSION
The bioactive compounds from the pericarp of GML, especially α-mangostin, possess a potential antibacterial agent against clinical isolates of ORSS. Furthermore, the great synergistic activity between this compound and oxacillin against this strain was occurred. Our findings provide evidence that these GML extract compounds can reverse bacterial resistance to sensitive status for oxacillin. Interestingly, the antibacterial combination approach is an interesting avenue to combat drug resistant bacteria. This study provides evidence that the bioactive compounds from the pericarp of GML extract offer for the development of a valuable adjunct to oxacillin against ORSS, which currently almost penicillins resistance.

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REFERENCES


