The Thai Journal of Pharmaceutical Sciences

Volume 38
Issue 0 *supplement issue*

Article 24

1-1-2013

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

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KEYWORDS: Alpha-mangostin, *Garcinia mangostana*, Antibacterial activity, Resistant bacteria

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 givers[1]. *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 cefoxitin due to the production of amp C β-lactamases[2]. Similarly, the emergence of fecal *Escherichia coli* isolates exhibiting resistance to extended-spectrum cephalosporins has been reported among pigs in Spain[3]. Furthermore, 19% antibiotics resistance of *E. coli* in hospital sewage water was resistant to ampicillin, ceftazidime, ceftriaxone, cefotaxime, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid and trimethoprim-sulphamethoxazole among the resistant isolates from the wells[4]. *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 reported[5]. Thus, development of novel antibacterial agents that can reverse the resistance to β-lactam antibiotics are research objectives of far reaching importance and urgently needed.
Mangosteen (*Garcinia mangostana* Linn.) (GML), belonging to the family Guttifera, has been used as a traditional medicine in Southeast Asia for the treatment of diarrhea, inflammation, and chronic ulcers[6] as well as abdominal pain, dysentery, infected wound and suppuration[7]. The xanthone derivertives, α-mangostin, is a major bioactive compound found in the pericarp of the mangosteen, has been discovered to possess antimicrobial activities against *Helicobacter pylori* and Methicillin-resistant *Staphylococucus aureus* (MRSA), anti-inflammatory activities, inhibition of oxidative damage[8, 9]. However, no works have been investigated the effect of GML extract on some drug resistant bacteria such as *S. saprophyticus, E. cloacae* and *E. coli*. The purpose of this investigation was to examine antibacterial activity of bioactive compounds from the pericarp of GML extract against those drug resistant bacteria, when 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 methods[10, 11] 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 reported[12]. 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 25922 from the American Type Culture Collection were used as control. Oxacillin and ceftazidime were obtained from Sigma.
Bacterial suspension standard curve In order to select bacterial suspensions with a known viable count, the method of Liu *et al*[13] 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 stain was added to 0.9 ml MHB, plus serial dilutions of the antibacterial agents, to give approximately 1x10^7 CFU/ml. Antibacterial-free tubes were use as control. Aliquots (2μl) of each inoculum were spotted on agar surface (the final concentration was approximately 1 x 10^4 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 spots[13, 14].

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 similar 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 $\leq$ 0.5 denoting synergistic; FICI > 0.5–4.0 denoting no interaction; FICI > 4.0 denoting antagonism[15].

Killing curve determinations (Viable counts) Viable counts for the determination of killing curve was performed as previously described[16] 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[12].

RESULTS AND DICUSSION

MIC and checkerboard determinations The MICs of CH_2Cl_2 crude extract, Fr_3, and α-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 ceftazidime-resistant *E. coli* and ceftazidime-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[17]. The results of checkerboard determinations demonstrated that MICs of CH_2Cl_2 crude extract, Fr_3, and α-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 (CH_2Cl_2 crude extract, Fr_3 extract, and α-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 Ceftazidime at 5 μg/ml in combination with 5 μg/ml of tested flavonoids reduced the CFU/mL of MRSA strain by 5x10^3 over 6 h. The reduced counts did not recover in 24 h[18].

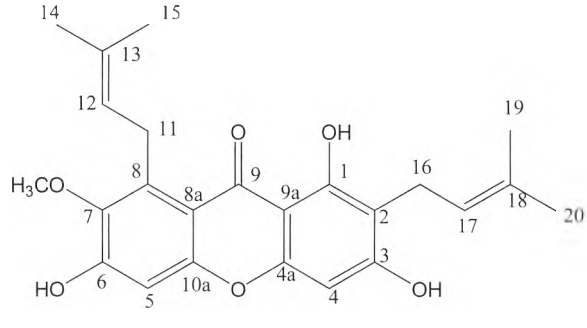

Fig. 1 The structure of α-mangostin

TEM study Electronmicroscope investigations clearly exhibited that the combination of oxacillin with α-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 α-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[18]. Similarly, TEM study clearly demonstrated that galangal extract caused both outer, inner membrane damage and cytoplasm coagulation of *S. aureus* strain[19].

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

Strains	agents	MIC alone (μg/ml)	MIC Combination (μg/ml)	FIC index	Outcome
S. saprophyticus **DMST 27055**	Oxacillin Crude extract	128 50	16 6.25	0.25	synergism
	Oxacillin Fr_3	128 31	16 3.875	0.138	synergism
	Oxacillin α-mangostin	128 8	16 2	0.375	synergism
E. coli **DMST 19629**	Ceftazidime Crude extract	>1,024 >10,000	>1,024 >10,000	>2	no interaction
	Ceftazidime Fr_3	>1,024 >10,000	>1,024 >10,000	>2	no interaction
	Ceftazidime α-mangostin	>1,024 >1,024	>1,024 >1,024	>2	no interaction
E. cloacae **DMST 21394**	Ceftazidime Crude extract	>1,024 >10,000	>1,024 >10,000	>2	no interaction
	Ceftazidime Fr_3	>1,024 >10,000	>1,024 >10,000	>2	no interaction
	Ceftazidime α-mangostin	>1,024 >1,024	>1,024 >1,024	>2	no interaction
S. aureus **ATCC 29213**	Oxacillin	2	NT	NT	NT
E. coli **ATCC 25922**	Ceftazidime	8	NT	NT	NT

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.

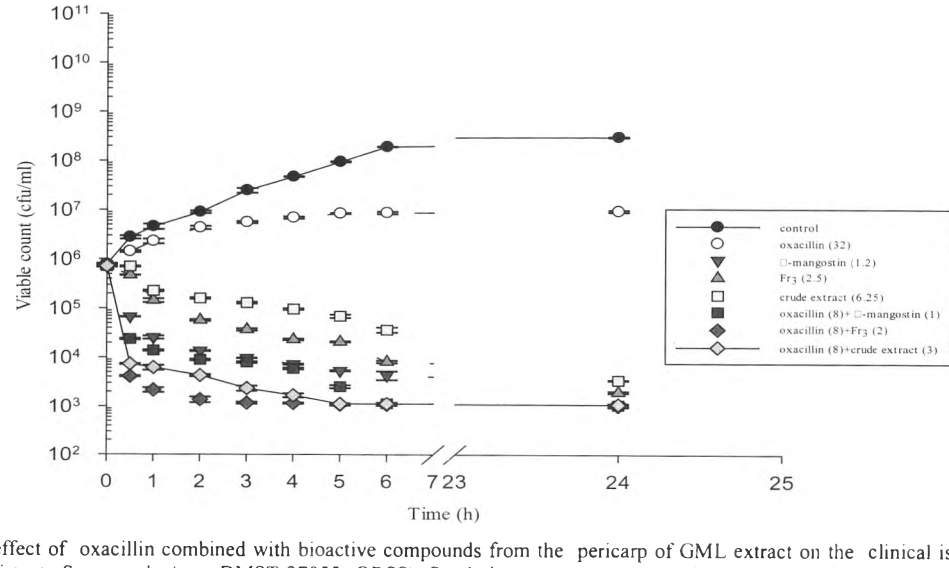

Fig. 2 The effect of oxacillin combined with bioactive compounds from the pericarp of GML extract on the clinical isolates of oxacillin-resistant *S. saprophyticus* DMST 27055 (ORSS). Symbol represents: (●) control (antibacterial free); (○) oxacillin (32 μg/ml); (▼)α-mangostin (1.2 μg/ml) ;(▲) Fr3 (2.5 μg/ml); (◦) crude extract (6.25 μg/ml);(■) oxaxillin (8 μg/ml)+ mangostin (1μg/ml); (♦)oxaxillin (8 μg/ml) + Fr3 (2 μg/ml); (♦)oxaxillin (8 μg) + Fr3 (3 μg/ml). The values plotted are the means of 3 observations, and the vertical bars indicate the standard errors of the means

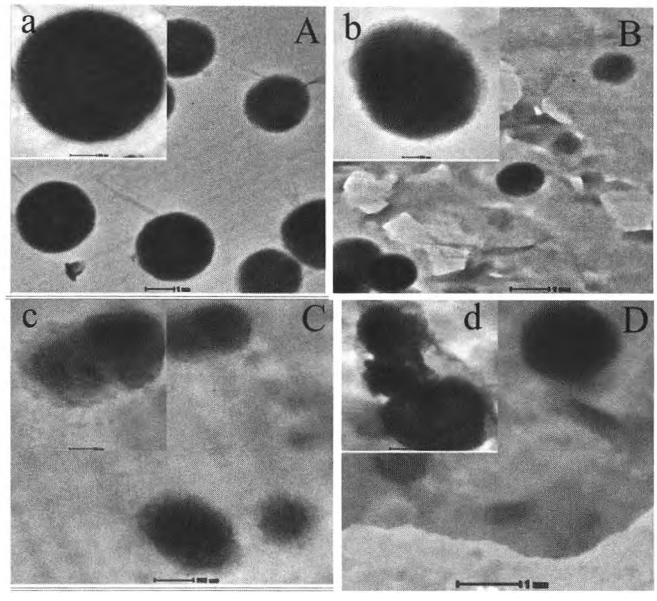

Fig. 3 Ultrathin sections of log phase clinical isolates of oxacillin-resistant *S. saprophyticus* DMST 27055 (ORSS) grown in MHB: Control (A: bar = 1μm, x4,000; a: bar=500 nm,x15,000); 32 μg/ml oxaxillin (B:bar=1μm, x4,000; b: bar=200 nm, x19,500); 1.2 μg/ml α-mangostin (C: bar= 500 nm, x9,900; c: bar 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.

ACKNOWLEDGEMENTS

The authors are indebted and highly appreciative to the National Research Council of Thailand for invaluable assistant in research funds support.

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