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Original Article

Synergistic effect of α-mangostin on antibacterial activity of tetracycline, erythromycin, and clindamycin against acne involved bacteria

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ABSTRACT

Objective: To evaluate the synergistic effect of α-mangostin with tetracycline, erythromycin, and clindamycin against bacteria involved in acne production.
Methods: A broth microdilution method was used to determine the minimum inhibitory concentration (MIC) of α-mangostin and a range of antibiotics. Synergistic effects on antibacterial activity were determined based on their own MIC, and then using a checkerboard method and a time-kill assay at 37 °C for 24 h.
Results: α-Mangostin exhibited antibacterial activity against *Propionibacterium acnes, Staphylococcus aureus, S. epidermidis* and *S. pyogenes* with MIC values of 0.78, 3.13, 0.78, and 6.25 μg/mL, respectively. The results of the checkerboard assay showed that α-mangostin produced synergistic effects with tetracycline, erythromycin, and clindamycin against all tested bacteria, with a fractional inhibitory concentration index (FICI) between 0.09 and 0.32. Moreover, time-kill curve data indicated that α-mangostin increased the antibacterial activity of tetracycline, erythromycin, and clindamycin.
Conclusion: These findings suggested that α-mangostin may be used to enhance the antibacterial activity of some antibiotics against bacteria involved in acne production.

1. Introduction

Acne vulgaris is one of the most common dermatological disorders affecting adolescents and adults, and affects more than 90% of individuals at some point in their lives (Joo et al., 2008). It is a chronic inflammatory condition with increased sebum production that results in altered keratinization, inflammation, and bacterial colonization under hair follicles by *Propionibacterium acnes* (Williams, Dellavalle, & Garner, 2012). Acne persists for a long time, and although it does not present a life-threatening condition, its consequences are very significant and long lasting. As such there is no ideal treatment for acne, but a suitable regimen for reducing acne lesions is being prescribed to patients. Benzoyl peroxide, retinoid, and antibiotics are being used in combination for topical application to improve mild to moderate acne (Leccia et al., 2015).

During the last decade, bacterial resistance to antibiotics has become a public health crisis throughout the world. Bacterial resistance can be due to abuse, non-adherence with a dosage regimen, or due to the bacteria's ability to overcome the effect of drugs because of its short life cycle (Rao, De Waelheyns, Economou, & Anné, 2014). To respond to this resistance, development of new antibiotics with new targets and modes of action is urgently needed. However, it is very challenging to find new antibiotics due to the high cost and time needed to search them, which may then also lead to further resistance when they are frequently used in the subsequent clinical treatments. Therefore, there is a need for extensive research into combination therapies of antibiotics with natural products for effective and safe administration of acne treatment. Natural products are a major source of chemical diversity and have provided important therapeutic agents for many bacterial-based infectious diseases. It has been well documented that many plant extracts possess considerable antibacterial, anti-inflammatory, and antioxidant effects. The interaction of different compounds in combination synergizes their biological action, due to targeting at different sites and influencing each site to achieve an enhanced response in cells (Yang et al., 2014). Although the synergistic antibacterial activities of α-mangostin

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have been demonstrated for oxacillin against oxacillin-resistant *Staphylococcus saprophyticus* (Phitaktim et al., 2016), and for gentamicin and vancomycin against vancomycin-resistant *Enterococci* (Sakagami, Iinuma, Piyasena, & Dharmaratne, 2005), there is no report on antibiotics used against bacteria involved in acne production.

α-Mangostin is a major bioactive xanthone found in the pericarps of *Garcinia mangostana* L. It has been used as an antibacterial agent, especially in anti-acne preparations made from the extract of *G. mangostana* (Obolskiy, Pischel, Siriwatanametanon, & Heinrich, 2010). The emergence of resistance of *P. acne* to erythromycin, clindamycin, and tetracycline has added an increased risk to the general population for dermal diseases like acne (Mendoza, Hernandez, Tyring, Haitz, & Motta, 2013). Thus, there is an urgent need to develop a combination therapy of antibiotics with natural products to address acne resistance. The aim of the present study was therefore to evaluate the synergistic effect of α-mangostin with tetracycline, erythromycin, and clindamycin against bacteria involved in acne production, namely *P. acnes, S. aureus, S. epidermidis,* and *S. pyogenes*.

2. Materials and methods

2.1. Chemicals and bacterial strains

The solvents used, dichloromethane, ethyl acetate, hexane and ethanol, were of commercial grade from RCI Labscan, Bangkok, Thailand. Brain heart infusion (BHI) and agar were from the Becton, Dickinson and Company (Franklin Lakes, New Jersey, USA). *Propionibacterium acnes* (DMST 14916), *Staphylococcus aureus* (ATCC 25923), *S. epidermidis* (ATCC 14990), and *S. pyogenes* (ATCC 17020) were obtained from the Department of Medical Science Center, Thailand.

2.2. Plants materials

Garcinia mangostana fruit were bought from a local market in Hat-Yai, Songkhla, Thailand. The edible and internal parts of fruits were removed. The pericarps were washed and cut into small pieces, and dried at 60 °C for 72 h in a hot air oven. The dried pericarps were then grated into a fine powder utilizing a grinder, and sifted through a No. 45 sieve.

2.3. Extraction of G. mangostana pericarps

An ultrasonic extraction method was used for preparation of *G. mangostana* pericarp extract. The dried pericarp powder (500 g) was extracted with 2.5 L dichloromethane using an ultrasonic bath (Crest Ultrasonic, Florida, USA) at 35 °C for 10 min at 45 kHz. After sonication, the extract was filtered and concentrated under reduced pressured using a rotary vacuum evaporator at 40 °C, and subsequently dried in a desiccator. The extract was stored in airtight container and protected from light until use.

2.4. Purification of α-mangostin

The crude pericarp extract (60 g) was fractionated using silica gel vacuum chromatography. The extract was triturated with silica then added to the top of a silica gel column (13 cm in diameter and 6 cm in height), and the column was subsequently eluted with a 500 mL-fraction of solvent using a step-gradient elution, starting from hexane and followed by the mixture of hexane and ethyl acetate (99%–90% hexane). Based on the TLC chromatograms of each fraction, the fractions that contained mainly α-mangostin were pooled and subjected to further purification of α-mangostin on a silica gel column (3 cm in diameter and 60 cm in height); Eluted with a mixture of hexane and ethyl acetate (90% hexane) to obtain a yellow crystal of α-mangostin. Identification of α-mangostin was performed using NMR spectral data compared with the previous report (Pothitirat, Chomnawang, & Gritsanapan, 2010).

2.5. Determination of minimum inhibitory concentrations

MICs were determined using a microdilution method (NCCLS, 2008) with slight modifications. The sample was dissolved in DMSO, diluted with BHI broth to reach the desired final concentration, and then two-fold dilutions were prepared in a 96-well plate. The bacterial suspensions were prepared in normal saline solution (0.85%), and the turbidity was adjusted to the 0.5 McFarland standards [equivalent to (1×10^8) CFU/mL]. The suspension was diluted with normal saline solution to contain (1×10^6) CFU/mL, and added into each well. The final cell concentration was (5×10^5) CFU/mL. The plate was kept in an incubator for 24 h at 37 °C. The MIC was considered to be the lowest concentration of the sample that inhibited the growth of microbes compared to the control.

2.6. Checkerboard assay

The checkerboard method described by Chang, Chen, Luh, and Hsieh (1995) was used, with slight modification, to evaluate the synergistic effect of different combinations of α-mangostin and antibiotics against the tested bacteria. Two-fold dilutions of α-mangostin were prepared in BHI along the x-axis, while two-fold dilutions of the antibiotics were diluted along the y-axis in a 96-well plate. Each well was subsequently inoculated with a bacterial suspension of (1×10^6) CFU/mL. The plates were then incubated at 37 °C for 24 h. The fractional inhibitory concentration index (FICI) was quantified as the fractional inhibitory concentration (FIC) of α-mangostin and the FIC of the antibiotic, where the FIC of α-mangostin was the MIC of α-mangostin in combination divided by the MIC of α-mangostin alone, while the FIC of the antibiotic was the MIC of the antibiotic in-combination divided by the MIC of the antibiotic alone (Milne & Gould, 2012).

$$FICI = FIC \text{ of } \alpha\text{-mangostin} + \text{FIC of the antibiotics}$$

$$\text{FIC} = \frac{\text{MIC of } \alpha\text{-mangostin or antibiotics in combination}}{\text{MIC of a } \alpha\text{-mangostin or antibiotics alone}}$$

The results were interpreted as synergistic (FICI $\leq$ 0.5), additive (0.5 $\leq$ FICI $\leq$ 1), indifferent (1 $\leq$ FICI $\leq$ 4), or antagonistic (FICI $>$ 4).

2.7. Time-kill assay

A time kill assay was performed to confirm the antibacterial and synergistic effects of α-mangostin when used singly and in combination with each antibiotic for inhibition of bacterial growth (Mun et al., 2013). Compounds were used at the half minimal inhibitory concentration (1/2-MICs) when each compound was assessed alone. However, to study the effect of the compounds in combination, each compound was used at the MIC that produced synergism. The samples were collected at eight different periods (0, 1, 2, 4, 6, 8, 12, and 24 h) during the time-kill assay. A bacterial suspension at a concentration of (1×10^6) CFU/mL was added to BHI broth containing the mixture of samples, to reach a final cell concentration of (5×10^5) CFU/mL. These were then incubated at 37 °C for the course of the experiment. Aliquots (50 µL) of the cultures were removed at eight time intervals, diluted (1:10) with 450 µL of normal saline, and ten-fold serial dilutions were prepared in normal saline solution. Subsequently, 20 µL of each dilution was cultured on BHI agar and the number of viable colonies were calculated after incubating for 24 h. A compound was considered to be a bactericidal agent when it was able to reduce colony

forming units per mL (CFU/mL) to less than $3 \times \log_{10}$ (0.1%). A combination involving antibacterial agents was considered to be synergistic when the CFU/mL was reduced $> 2 \log_{10}$ (1%) (Hamoud, Zimmermann, Reichling, & Wink, 2013).

3. Results and discussion

α-Mangostin showed effective antibacterial activity against *P. acne, S. aureus, S. epidermidis*, and *S. pyogenes* with MICs of 0.78, 3.13, 0.78, and 6.25 μg/mL, respectively. All tested bacteria were still susceptible to the antibiotics, although some of them exhibited less or equal antibacterial activity than α-mangostin, i.e. tetracycline and clindamycin against *P. acne*, and tetracycline against *S. pyogenes* (Table 1). Although antibiotics have been used for a long time to treat acne, resistance to antibiotics has become noticeably prevalent since 1970 (Leyden, Marples, Mills, & Kligman, 1973). Resistance to erythromycin, clindamycin and tetracycline were the most common forms observed, and cross-resistance has also been

Table 1
Effects of α-mangostin on antibacterial activity of antibiotics against bacteria involved in acne production.

Bacteria	Compounds	MIC*a*/(μg·mL^{-1})	MIC*c*/(μg·mL^{-1})	FIC	FICI	Interactions
P. acne	α-Mangostin Tetracycline	0.78 1.56	0.20 0.10	0.26 0.06	0.32	Synergistic
	α-Mangostin Clindamycin	0.78 0.78	0.10 0.05	0.13 0.06	0.19	Synergistic
	α-Mangostin Erythromycin	0.78 0.39	0.20 0.02	0.26 0.05	0.31	Synergistic
S. aureus	α-Mangostin Tetracycline	3.13 0.10	0.20 0.01	0.06 0.10	0.16	Synergistic
	α-Mangostin Clindamycin	3.13 0.10	0.20 0.01	0.06 0.10	0.16	Synergistic
	α-Mangostin Erythromycin	3.13 0.78	0.20 0.05	0.06 0.06	0.12	Synergistic
S. epidermidis	α-Mangostin Tetracycline	0.78 0.39	0.10 0.02	0.13 0.05	0.18	Synergistic
	α-Mangostin Clindamycin	0.78 0.01	0.10 0.01	0.13 0.10	0.23	Synergistic
	α-Mangostin Erythromycin	0.78 0.39	0.20 0.02	0.26 0.05	0.31	Synergistic
S. pyogenes	α-Mangostin Tetracycline	6.25 6.25	0.39 0.39	0.06 0.06	0.12	Synergistic
	α-Mangostin Clindamycin	6.25 0.02	0.10 0.002	0.03 0.10	0.13	Synergistic
	α-Mangostin Erythromycin	6.25 3.13	0.20 0.20	0.03 0.06	0.09	Synergistic

Note: MIC*a* (MIC of one sample alone); MIC*c* (MIC of samples in combination); MIC of samples in combination is lowest concentration of compounds in combination that inhibit visible bacterial growth; FIC (Fractional inhibitory concentration); FICI (Fractional inhibitory concentration index).

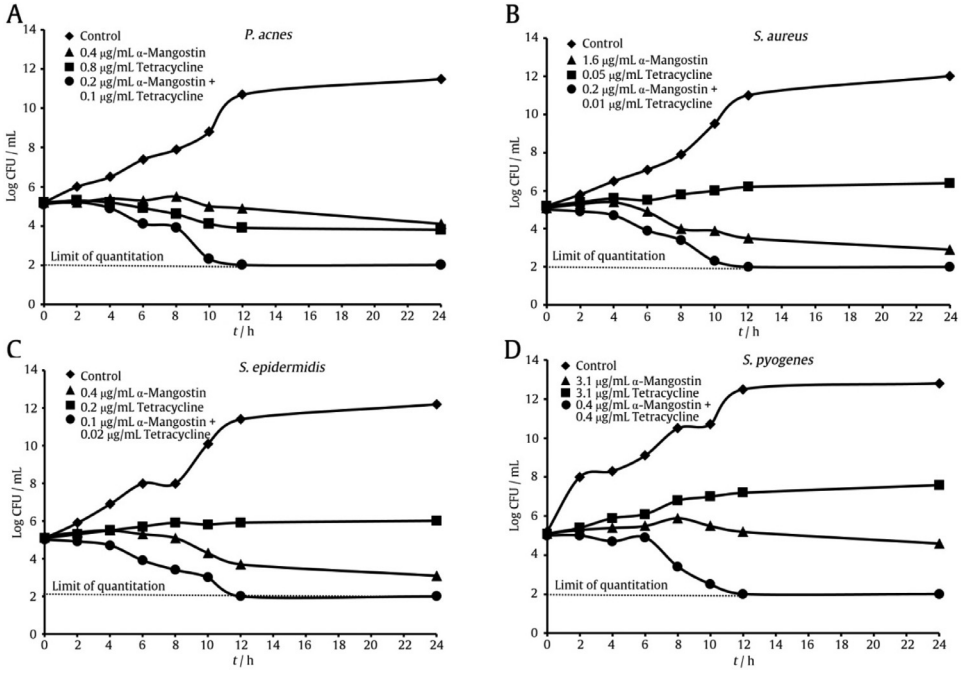

Fig. 1. Time kill curves of α-mangostin, tetracycline, and their combination against *P. acne* (A), *S. aureus* (B), *S. epidermidis* (C), and *S. pyogenes* (D).

found (Pannu et al., 2011). Synergistic effects from any combination of natural compounds and these common antibiotics may overcome the bacterial resistance to antibiotics (Septama & Panichayupakaranant, 2015).

The checkerboard method was performed to evaluate the antibacterial interaction when α-mangostin combined with antibiotics against bacteria involved in acne formation. On the basis of FICI determination, α-mangostin synergized with the antibacterial activity of tetracycline, erythromycin and clindamycin by markedly lowering their individual MIC values to the FICI values of 0.09–0.32 (Table 1). A time-kill assay was performed to confirm their synergistic effects. These results agreed with those from the checkerboard method (Figs. 1–3). The combination of α-mangostin and the antibiotics at their synergistic concentrations completely inhibited bacterial growth after 10–12 h of incubation, while neither the individual α-mangostin nor antibiotics at half their MICs completely inhibited the bacterial growth until 24 h of incubation. This finding indicates that α-mangostin may help overcome the problems associated with some multidrug-resistant pathogens, e.g. bacteria involved in acne formation, when used in a combination with commonly prescribed antibiotics i.e. tetracycline, clindamycin, and erythromycin.

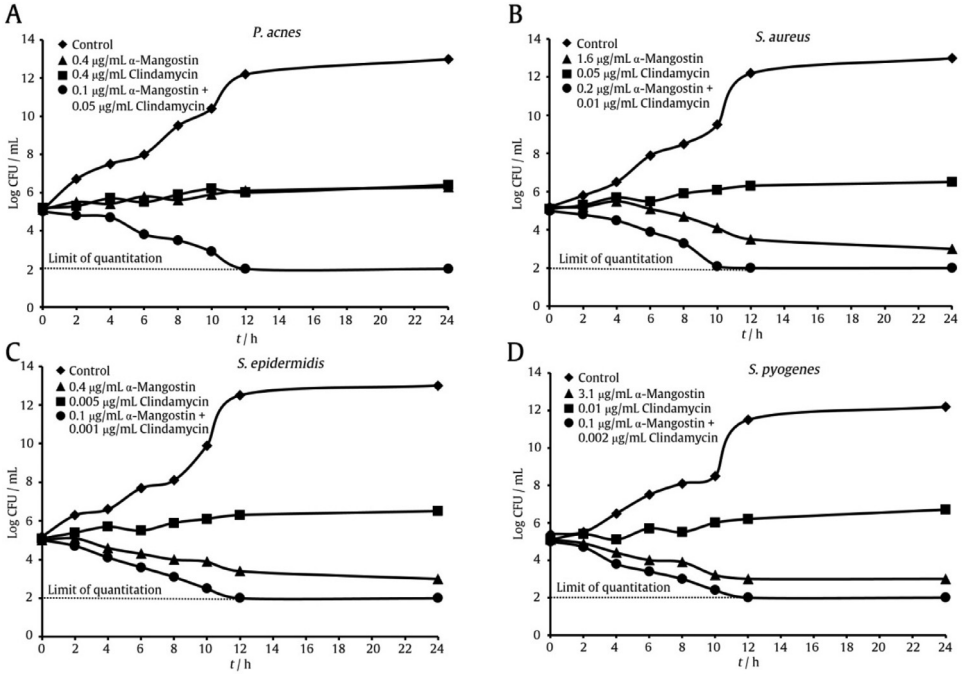

Fig. 2. Time kill curves of α-mangostin, clindamycin, and their combination against *P. acne* (A), *S. aureus* (B), *S. epidermidis* (C), and *S. pyogenes* (D).

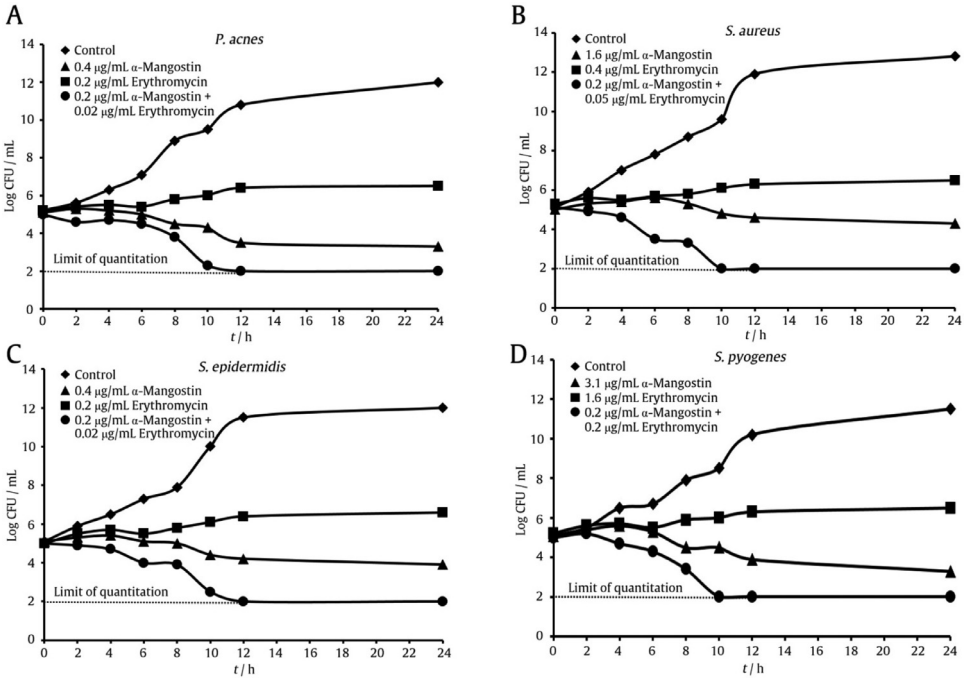

Fig. 3. Time kill curves of α-mangostin, erythromycin, and their combination against *P. acne* (A), *S. aureus* (B), *S. epidermidis* (C), and *S. pyogenes* (D).

α-Mangostin possesses a broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria (Sakagami et al., 2005), especially those associated with skin infection. It has been reported that the 1,4-benzopyrone skeletal (chromone) and isoprenyl side chains of α-mangostin, as well as their substituted positions, may play an important role in its antibacterial activity (Phitaktim et al., 2016). In addition, the combination of C-6 and C-3 hydroxyl groups, along with the isoprenyl substitution at C-2, are essential to increase its antibacterial activity (Dharmaratne, Sakagami, Piyasena, & Thevanesam, 2013). It has been reported that α-mangostin exhibits an inhibitory activity against β-lactamase, thus it may be synergizing the antibiotic activity of β-lactam antibiotics. Moreover, α-mangostin exhibited disruption of both peptidoglycan and the cytoplasmic membrane, resulting in leakage of intracellular materials. The later mechanism of action may play an important role in the synergistic activity of the tested antibiotics, i.e. tetracycline and erythromycin, which have bacteriostatic activity by inhibiting protein synthesis, and clindamycin, which exhibits bacteriostatic activity by binding to 50 s RNA.

4. Conclusion

The α-mangostin isolated from the pericarp of *G. mangostana* does not only possess antibacterial effects of its own against bacteria involved in acne production, such as *P. acnes, S. aureus, S. epidermidis* and *S. pyogenes*, but also exhibits enhanced activity when in combination with the commonly used antibiotics for treating acne, i.e. tetracycline, clindamycin, and erythromycin. This trait may also help to abate the antibiotic resistance in bacterial strains such as these, through synergy with the above antibiotics. However, toxicity tests in animals and humans should be the topic of further studies during the course of developing this research into a viable treatment option.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest regarding the publication of this article.

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