



α -Mangostin-Rich Extract: A Potential Oral Antibacterial Agent Prepared by Green Extraction

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Abstract

The purpose of this study was to prepare α -mangostin-rich extract (AME) using a green microwave extraction together with a simple one-step green fractionation method on a hydrophobic resin column. Based on the established method, AME containing 95% w/w α -mangostin content was obtained with an extract yield of 0.46% w/w dry plant powder. In addition, investigation of its antibacterial activity against oral pathogenic bacteria, including *Streptococcus mitis*, *S. mutans*, *S. pyogenes* and *Bacillus subtilis*, compared with the standard marker, α -mangostin found that AME exhibited strong antibacterial activities, which were equivalent to those of α -mangostin (MICs and MBCs of 0.5-2 and 4-16 μ g/mL, respectively). Moreover, AME exhibited equivalent antibacterial activity against *Propionibacterium acnes* when compared to the pure α -mangostin (MIC and MBC of 2 and 8 μ g/mL, respectively). A chemical stability evaluation found that AME was stable through a period of 16 weeks, when kept in a well-closed container protected from light and stored either at 4 ± 2 or $30 \pm 2^\circ\text{C}$. On the other hand, AME was unstable when exposed to light after 3 weeks of storage. The stability evaluation under accelerated conditions (75% relative humidity, at 45°C) implied that AME has a shelf-life of at least two years.

Keywords: α -Mangostin; Green Extraction; Microwave Extraction; Stability; Antibacterial; *Garcinia mangostana*

Introduction

General methods for preparation of herbal extracts involve uses of organic solvents such as methanol, ethyl acetate, acetone, chloroform, dichloromethane, hexane, etc. Their volatile, flammable, and toxic properties are of concern for human beings and the environment [1]. The conventional methods used for preparation of herbal extracts, i.e. maceration and heat reflux extraction, are time-consuming and require large amounts of organic solvents [2]. Therefore, a green and efficient extraction that is free from toxic organic solvents is essential for the preparation of active constituent-rich extract for pharmaceutical and cosmetic applications [3]. A green extraction concept has recently been applied to the development of commercialized herbal products. It is based on the design of extraction procedures that can

reduce energy consumption, promote the use of alternative green solvents, and produce a safe and high-quality extract [4]. Recently, a microwave-assisted extraction has been proven as a green extraction method used for commercial preparation of herbal extracts [5-7]. This method not only produces a high-quality herbal extract, but also reduces production costs.

Generally, a pure phytochemical exhibits higher biological activity than its plant extract. However, the applications of pure phytochemicals for phytopharmaceutical industries are rare due to their cost. A purification of phytochemicals involves multi-stage, time-consuming and energy-intensive processes that require a large amount of toxic organic solvents, ultimately increasing their production costs. In addition, most pure bioactive phytochemicals are not

available commercially. Recently, an active constituent-rich extract has been introduced to overcome this problem. Some active constituent-rich extracts have been reported to produce the equivalent biological effects like their pure marker compounds [8]. This phenomenon may be due to their high contents of bioactive compounds and a synergistic effect with other derivatives.

Garcinia mangostana L. (mangosteen; Clusiaceae family) is an important economic plant grown in Eastern and Southern Thailand. The pericarps of *G. mangostana* are enriched in α -mangostin (Figure 1), a bioactive prenylated xanthone [9,10]. α -Mangostin possess various biological effects, such as antibacterial, antifungal, anti-inflammatory and anti-cancer activities [11]. The extract of pericarps has been applied in a mouthwash, which exhibited antibacterial activity against *S. mutans* with a MIC of 0.625 $\mu\text{g}/\text{mL}$ equivalent to chlorhexidine, an antiseptic used for dental plaque control [12,13]. The dichloromethane extract of mangosteen pericarp has been reported as effective against dental plaque forming bacteria such as *Staphylococcus*, *Streptococcus*, *Streptobacillus* and *Monococcus* spp. [14]. Moreover, α -mangostin has exhibited antibacterial activity against *S. pyogenes* and *P. acnes* with MICs of 6.25 and 0.78 $\mu\text{g}/\text{mL}$, respectively [15].

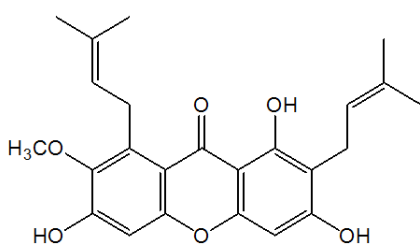


Figure 1: Chemical structure of α -mangostin.

The present study, therefore, used α -mangostin as the bioactive marker for preparation of α -mangostin-rich extract (AME) using a microwave-assisted extraction and alternative green solvent together with a simple one-step fractionation on a hydrophobic resin column. The extract was then evaluated for antibacterial activity compared with its standard marker. In addition, the stability of α -mangostin in AME during storage was determined. These results are essential for further drug development.

Materials and Methods

Plant Materials and Chemicals

Dried powders of *G. mangostana* were purchased from Charoensuk Pharma Supply (Nakhon Pathom, Thailand). Ampicillin and Diaion HP-20 resin were from Sigma-Aldrich (Steinheim, Germany). α -Mangostin was previously purified

by the method described by Ahmad, et al. [15]. Formic acid, brain heart infusion (BHI) and agar were from the Becton, Dickinson, and Company (New Jersey, USA). Polyethylene glycol 400 (PEG 400, pharmaceutical grade), acetonitrile and methanol (HPLC grades) were from RCI Labscan (Bangkok, Thailand). Ethanol (commercial grade) was from High Science Ltd. (Hat-Yai, Thailand). Water was purified using a Milli-Q system (Millipore, Bedford, MA, USA).

Microorganisms

Streptococcus mutans (DMST 26095), *S. pyogenes* (DMST 17020), *S. mitis* (ATCC 49456T), *B. subtilis*, *P. acnes* (DMST 14916) were obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand.

Preparation of α -Mangostin Enriched Extract

G. mangostana powders (1.5 kg) was extracted with PEG 400 (3 L) using a microwave-assisted extraction (frequency of 1,450 MHz and electric power of 900 Watt) at 90°C, for 24 min. The extract was then filtered through a filter paper and subjected to fractionation using a Diaion HP-20 column (8 cm in diameter and 100 cm in height). The column was eluted with 60 and 70% (v/v) ethanol to obtain AME from the 70% (v/v) ethanol elution after evaporated by a rotary evaporator at 45°C.

Quantitative HPLC Analysis of α -Mangostin

The content of α -mangostin in MAE was analyzed using a HPLC method previously described by Bundeasomchok, et al. [16], with some modification. Briefly, the quantitative analysis was performed on a binary HPLC pump (Shimadzu 33236, Tokyo, Japan) equipped with a photodiode array detector (Shimadzu 12623, Tokyo, Japan), an autosampler (Shimadzu 03224, Tokyo, Japan) and a 150 \times 4.6 mm ACE 5 C18-PFP column (Advanced Chromatography Technologies, Aberdeen, Scotland). The sample injection volume was 20 μL , and the column was eluted with a solution of acetonitrile and 0.2% formic acid (70:30, v/v), at a flow rate of 1 mL/min. The concentration of α -mangostin was quantified at a wavelength of 240 nm using a calibration curve of the authentic α -mangostin. The calibration curve was established at five concentrations of α -mangostin (12.5-200 $\mu\text{g}/\text{mL}$) by plotting peak areas against concentrations to obtain the linear equation of $Y = 95349X - 98846$ ($R^2 = 0.9995$). All experiments were performed in triplicate.

Determination of Antibacterial Activity

A broth microdilution assay was used to determine the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) against the tested bacteria [17]. Briefly, the samples were dissolved in

dimethyl sulfoxide and diluted with BHI broth to reach the concentration of 1,000 $\mu\text{g/mL}$. Two-fold dilutions were then prepared in a 96-well plate. The bacterial suspension was prepared in 0.85% normal saline and the turbidity of the suspension 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 then added into each well. The final cell concentration was 5×10^5 CFU/mL and the plates were incubated at 37°C , for 24 h. The MICs were defined as the lowest concentration of compound that produced suppression of visible growth and the MBCs were defined as the lowest concentration of the compound to kill the microorganisms.

Stability Evaluation of AME

Stability of α -mangostin in AME was determined using the method previously described by Puttarak, et al. [18].

Effect of Light

Two samples of AME were kept in a well-closed container and stored separately, one under protection from light and the other under exposure to fluorescent light, at room temperature ($30 \pm 2^\circ\text{C}$), for 16 weeks. An aliquot of the sample was taken at 0, 1, 2, 3, 4, 6, 8, 12 and 16 weeks and subjected to the quantitative HPLC analysis of α -mangostin. The experiments were performed in triplicate.

Effect of Temperature

AME was kept in a well-closed container, protected from

light, and stored separately at $4 \pm 2^\circ\text{C}$ and room temperature ($30 \pm 2^\circ\text{C}$) for 16 weeks. An aliquot of the sample was taken at 0, 1, 2, 3, 4, 6, 8, 12 and 16 weeks and subjected to the quantitative HPLC analysis of α -mangostin. The experiments were performed in triplicate.

Effect of Accelerated Conditions

AME was kept in a well-closed container, protected from light, and stored in a stability chamber at 45°C , with 75% relative humidity for 16 weeks. An aliquot of sample was taken at 0, 1, 2, 3, 4, 6, 8, 12 and 16 weeks and subjected to the quantitative HPLC analysis of α -mangostin. The experiments were performed in triplicate.

Statistical Analysis

The results are expressed as mean \pm S.D. Significant differences were analyzed using an ANOVA followed by Tukey's test. Values of $p < 0.05$ were considered statistically significant.

Results and Discussion

Development of a Green Method for AME

A green method for preparation of AME was achieved by a microwave-assisted extraction using PEG 400, an alternative green solvent, for extraction of α -mangostin. Although some alternative solvents, i.e. ethyl lactate, dimethyl carbonate, and 2-methyltetrahydrofuran, have been reported for extraction of α -mangostin [16], they are too expensive for commercialization of α -mangostin extract.

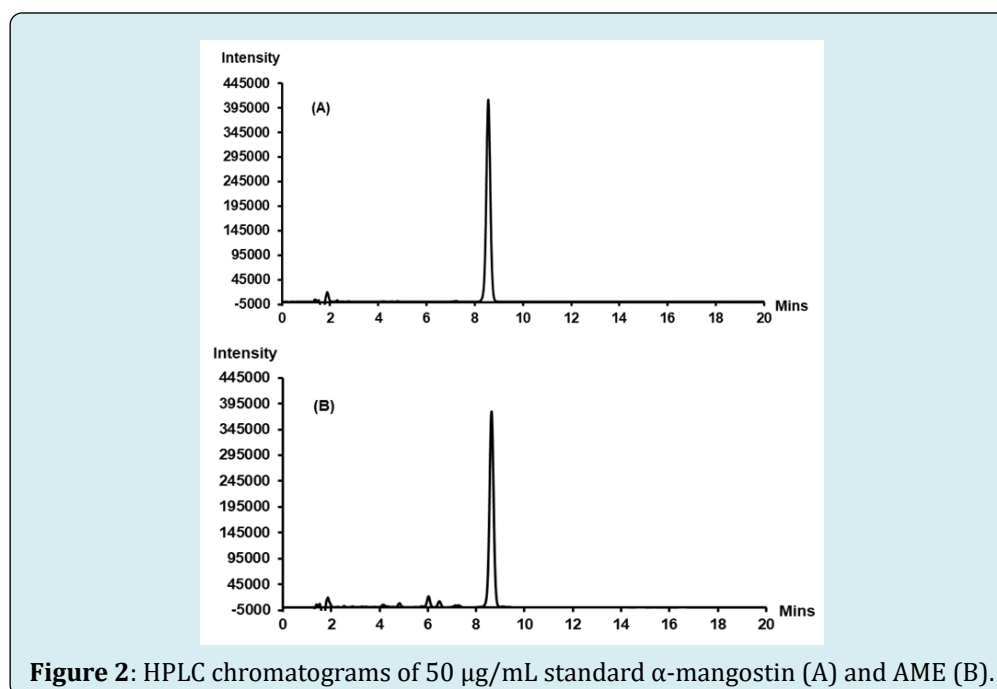


Figure 2: HPLC chromatograms of 50 $\mu\text{g/mL}$ standard α -mangostin (A) and AME (B).

The present study indicated that PEG 400, usually used as a co-solvent in pharmaceutical products and cosmetics, exhibited selectivity for extraction of α -mangostin. Although PEG 400 is miscible with water, α -mangostin can be dissolved freely in PEG 400. This is an advantage of PEG 400 for extraction of α -mangostin. Therefore, the obtained extract was further purified without the step of solvent evaporation. According to a non-polar molecule of α -mangostin, the extract was subjected to a reusable hydrophobic copolymer styrene-divinylbenzene (Diaion HP-20) column, which is an adsorbent used in reverse phase chromatography. By this column, other polar compounds were excluded by 60% (v/v) ethanol elution, while α -mangostin was eluted and highly accumulated in the 70% (v/v) ethanol fractions. The pooled 70% (v/v) ethanol fractions were evaporated to obtain a yellow powder of AME (4.6 g per 1 kg plant powders). Based on HPLC analysis (Figure 2), AME contained a very high content of α -mangostin ($95.6 \pm 0.59\%$ w/w). No toxic organic solvents were used in the process. The established method is very simple, cost effective, and environmentally friendly, which is practically used for development of commercialized products from α -mangostin extract.

Antibacterial Activity of AME

The ethanol extract of mangosteen pericarp has been

reported to possess anticariogenic activity against *S. mutans* and was suggested as a good candidate for further development of an anti-plaque agent for prevention of dental caries [13]. In addition, the active constituent enriched extracts usually produce biological effects equivalent to their pure marker compounds [8]. Therefore, the present study determined antibacterial activity of AME against the oral pathogen, including *S. mitis*, *S. mutans*, *S. pyogenes*, and *B. subtilis* compared with its pure marker compound, α -mangostin. It was found that both of them revealed potent antibacterial activity against all tested bacteria with MICs and MBCs of 0.5-2 $\mu\text{g/mL}$ and 4-16 $\mu\text{g/mL}$, respectively (Table 1). The results indicated that AME may be used for the development of oral care products, such as mouthwash and toothpaste. It has been reported that α -mangostin at a concentration of 4 mg/mL does not show any toxicity on human gingival fibroblast over a period of 8 h [19].

Moreover, α -mangostin has exhibited strong anti-*P. acnes* activity [15]. The present study therefore determined anti-*P. acnes* activity of AME compared with α -mangostin and found that AME and α -mangostin exhibited equivalent anti-*P. acnes* activity with MIC and MBC of 2 and 8 $\mu\text{g/mL}$, respectively (Table 1). A chromone (1,4-benzopyrone) skeletal as well as isoprenyl groups and their substituted positions may play a significant role on antibacterial activity of α -mangostin [20].

Microorganisms	α -Mangostin		AME		Ampicillin	
	MIC ($\mu\text{g/mL}$)	MBC ($\mu\text{g/mL}$)	MIC ($\mu\text{g/mL}$)	MBC ($\mu\text{g/mL}$)	MIC ($\mu\text{g/mL}$)	MBC ($\mu\text{g/mL}$)
<i>S. mitis</i> (ATCC 49456T)	0.5	4	0.5	4	0.12	0.12
<i>S. mutans</i> (DMST 26095)	1	8	1	16	0.5	1
<i>S. pyogenes</i> (DMST 17020)	1	8	2	8	0.06	0.12
<i>B. subtilis</i>	1	4	2	4	0.5	0.5
<i>P. acnes</i> (DMST 14916)	2	8	2	8	0.12	0.12

Table 1: Antibacterial activity of α -mangostin, AME and ampicillin against bacterial pathogens.

Stability of MAE

The stability of MAE was evaluated by the content of α -mangostin after storing for 16 weeks in different conditions, including light, temperature, and accelerated conditions. The results revealed that the physical appearances of AME kept in both protected and unprotected from light at $30 \pm 2^\circ\text{C}$ were not changed throughout the period of 16 weeks. In contrast, the α -mangostin content of AME which was exposed to light markedly decreased from 95.6 to 83.6% w/w, while the extract kept in a light protected container was not changed throughout the storage period (Table 2). This indicated that α -mangostin is unstable by light accelerated oxidation, common for most phenolic compounds [21].

The effect of temperature on the stability of AME was investigated under protected from light conditions at 4 ± 2 and $30 \pm 2^\circ\text{C}$. Both physical appearance and α -mangostin content of AME stored at both temperatures remained unchanged throughout the period (Table 2). The result confirmed the previous report on the stability of α -mangostin in crude ethanolic extracts of mangosteen kept at 4°C and 25°C and protected from light for 120 days [22]. The results indicated that AME is stable under protected from light conditions even at room temperature ($30 \pm 2^\circ\text{C}$). These findings suggested that AME should be kept in a well-closed container, protected from light, and can be stored at room temperature ($30 \pm 2^\circ\text{C}$).

Weeks	α -Mangostin content (% w/w \pm SD)			
	Light	Protected from light		Accelerated conditions
	30 \pm 2°C	30 \pm 2°C	4 \pm 2°C	
0	95.6 \pm 0.59	95.6 \pm 0.59	95.6 \pm 0.59	95.6 \pm 0.59
1	95.6 \pm 0.62	95.6 \pm 0.61	95.6 \pm 0.61	95.6 \pm 0.58
2	95.5 \pm 0.60	95.6 \pm 0.63	95.5 \pm 0.62	95.5 \pm 0.61
3	94.1 \pm 0.69*	95.5 \pm 0.67	95.5 \pm 0.58	95.5 \pm 0.65
4	91.3 \pm 0.72*	95.5 \pm 0.75	95.5 \pm 0.57	95.5 \pm 0.74*
8	89.1 \pm 0.75*	95.5 \pm 0.64	95.5 \pm 0.49	94.2 \pm 0.66*
12	87.4 \pm 0.61*	95.5 \pm 0.58	95.5 \pm 0.53	93.9 \pm 0.53*
16	83.6 \pm 0.53*	95.4 \pm 0.57*	95.4 \pm 0.51*	92.6 \pm 0.56*

* Significant difference ($p < 0.05$) when compared with the content of α -mangostin at initial time.

Table 2: Stability evaluation of AME stored in different conditions.

Testing the stability of AME under the accelerated conditions was conducted to estimate the shelf-life of AME. If an extract is stable under the accelerated conditions or the remained content is higher than 90%, it is estimated to have a 2 year-shelf-life. It was found that the physical appearances of AME were not changed throughout the period, while the content of α -mangostin was a bit reduced from 95.6 to 92.6% w/w (Table 2). However, the remaining content of α -mangostin was 96.9%. Therefore, it can be implied that when kept in a well-closed container, protected from light and stored at room temperature, AME has a shelf-life of at least two years.

Conflict of Interest

The authors have declared that there is no conflict of interest.

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