

Biological and Chemical Stability of Mangosteen Fruit Rind Extract

Werayut Pothitirat¹ • Pimolpan Pithayanukul² • Mullika Traidej Chomnawang³ •
Roongtawan Supabphol⁴ • Wandee Gritsanapan^{1*}

¹ Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand

² Department of Pharmacy, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand

³ Department of Microbiology, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand

⁴ Department of Physiology, Faculty of Medicine, Srinakharintarawit University, Bangkok, Thailand

Corresponding author: * pywgs@mahidol.ac.th

ABSTRACT

The stability of α -mangostin, antiradical activity against DPPH radical and antibacterial activity against bacteria-inducing acne, i.e. *Propionibacterium acnes* and *Staphylococcus epidermidis* of *Garcinia mangostana* fruit rind extract were studied. The extract was kept in an amber glass vial and in an aluminium foil bag at different temperatures (4-8, 25-28 and 45°C) for 120 days. The content of α -mangostin in the extract stored at each condition was analyzed by the validated RP-HPLC method, while free radical scavenging and antibacterial activity were performed using the DPPH scavenging assay and broth microdilution method, respectively. The results revealed that packaging material and storage temperature had no effect on the content of α -mangostin, antiradical and anti-acne activities of the *G. mangostana* fruit rind extract. However, a small change in color and characteristic of the extract was observed when the extract was kept at 25-28 and 45°C. These data indicate good chemical and biological stabilities of *G. mangostana* fruit rind extract for appropriate antioxidant and anti-acne raw materials in pharmaceutical preparations.

Keywords: acne vulgaris, antioxidant, *Propionibacterium acnes*, stability, *Staphylococcus epidermidis*

INTRODUCTION

A stability test of herbal extracts is necessary to ensure the quality of herbal products throughout its storage period. Determination of chemical and biological stabilities of raw materials and finished products are important in the process of quality assessment. The purpose of the stability test is to provide evidence on how the quality of herbal products varies with time under the influence of environmental factors, especially temperature and humidity (Thakur *et al.* 2008).

Mangosteen (*Garcinia mangostana* Linn.) of the Guttiferae family, is a tropical fruiting tree. The fruit rind has a deep reddish purple color when ripening, while the edible flesh is white and sweet, tangy and citrusy in taste. In Asian countries, the fruit rind of this plant has been used as a medicine for treatment of skin infection, wounds, and diarrhea (Gritsanapan and Chulasiri 1983; Nakatani *et al.* 2002). The fruit rinds contain various phenolic compounds such as tannins, flavonoids, and xanthenes, supporting its medicinal properties (Council of Scientific and Industrial Research 1956; Perry and Metzger 1980; Nonaka *et al.* 1983; Tapiero *et al.* 2002; Yu *et al.* 2007). Moreover, α -mangostin, a major xanthone in mangosteen fruit rind, was reported to have antioxidant activity (Williams *et al.* 1995) and a strong inhibitory effect against *Propionibacterium acnes* and *Staphylococcus epidermidis*, which are the common pus-forming bacteria that trigger inflammation in acne (Chomnawang *et al.* 2005; Pothitirat *et al.* 2009a). These biological activities are interesting for the potential therapeutic application of *G. mangostana* fruit rind extract. Recently, the extract of mangosteen fruits is popularly used in dietary antioxidants and pharmaceutical products for the treatment of acne. Although many publications concerning this plant were reported, there is no information on the stability of *G. mangostana* fruit rind extracts from chemical and biological

viewpoints. Therefore, the purpose of this study was to evaluate the stability of α -mangostin in the extract of *G. mangostana* fruit rind kept in an amber glass vial and in an aluminium foil bag at different temperatures for 120 days. In addition, the antiradical activity against DPPH radical, and antibacterial activity against *P. acnes* and *S. epidermidis*, which are bacteria involved in acne development, of the extract were investigated.

MATERIALS AND METHODS

Preparation of *G. mangostana* fruit rind extract

Mature fruits of *G. mangostana* were collected from Lang Suan District, Chumphon Province in the South of Thailand in June 2006. The samples were identified by Dr. Wandee Gritsanapan at the Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. A voucher specimen (WGM0806) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University. The fruits were cleaned and the edible aril parts were removed. The fruit rinds were cut into small pieces and dried in a hot air oven at 50°C for 72 hrs. The dried samples were ground into powder, passed through a sieve (20 mesh). The powdered sample was kept in air-tight container protected from light until used.

The dried powder of *G. mangostana* fruit rind (10 g) was placed into a thimble and extracted with 400 mL of 95% ethanol in a Soxhlet apparatus. Extraction was carried out for 15 hrs at 5 cycles/h. The extract was filtered through a Whatman no. 1 filter paper and then concentrated under reduced pressure at 50°C using a rotary vacuum evaporator.

Stability study of *G. mangostana* fruit rind extract

1. Stability study condition

The extract of *G. mangostana* fruit rind was separately weighed (1 g) and kept in an amber glass vial with a rubber stopper and aluminium cap, and in an aluminium foil bag. The samples were kept for 120 days at room temperature (25-28°C), in a refrigerator (4-8°C), and in an oven at 45°C. 75% relative humidity was maintained throughout the study.

2. Chemical stability evaluation

The extract of *G. mangostana* fruit rind at each storage condition was analyzed for α -mangostin content by the validated HPLC method (Pothitirat *et al.* 2009b). α -Mangostin was purchased from Chroma Dex Inc. (Santa Ana, CA). All other chemicals and solvents were of analytical grade, purchased from Labscan Asia (Thailand) and M&B Chemical Laboratory (England).

For preparation of the standard solution, the reference standard of α -mangostin was accurately weighed and dissolved in methanol to give serial concentrations from 10 to 200 μ g/mL, and three injections into the HPLC were performed for each dilution. For preparation of the sample solution, each extract was accurately weighed and dissolved in methanol to make a concentration of 150 μ g/mL. Prior to analysis, the solutions were filtered through 0.45 μ m membrane filters. α -Mangostin content in the extract was calculated using its calibration curve with regard to the dilution factor. The contents of α -mangostin in the extract were expressed as g/100 g. Each determination was carried out in triplicate.

A Shimadzu SCL-10A HPLC system, equipped with a model LC-10AD pump, UV-vis detector SPD-10A, Rheodyne injector fitted with a 20 μ L loop and auto injector SIL-10A were used for all analysis. Chromatographic separations were carried out on a Hypersil[®] BDS C-18 column (4.6 \times 250 mm, 5 μ m size) with 0.1 % (v/v) ortho phosphoric acid (solvent A) and acetonitrile (solvent B) as the mobile phase at a flow rate of 1.0 mL/min at room temperature. The gradient programme was as follows: 70% B for 0-15 min, 70% B to 75% B for 3 min, 75% B to 80% B for 1 min, constant at 80% B for 6 min, 80% B to 70% B for 1 min. There was 11 min of post-run for reconditioning. The sample injection volume was 10 μ L while the wavelength of the UV-vis detector was set at 320 nm. The compound was quantified using CLASS VP software.

3. Determination of minimum inhibitory and bactericidal concentrations

P. acnes (ATCC 6919) and *S. epidermidis* (ATCC 14990) were obtained from the American Type Culture Collection, USA. For preparation of inoculum, *P. acnes* was incubated in brain heart infusion medium for 72 hrs at 37°C under anaerobic conditions,

while *S. epidermidis* was incubated in tryptic soy broth for 24 hrs at 37°C, and then adjusted to yield approximately 10⁸ CFU/mL. Minimal inhibitory concentration (MIC) values was determined by the broth microdilution assay. Briefly, the extracts were incorporated into the media to obtain concentrations of 500 to 0.24 μ g/mL. A standardized suspension (10 μ L) of each tested organism was transferred to each well. The broth cultures of *S. epidermidis* and *P. acnes* were incubated for 24 and 72 hrs, respectively. The MIC, defined as the lowest concentration of compound that inhibits the microorganism, was determined. The minimal bactericidal concentration (MBC), defined as the lowest concentration of compound that kills the microorganism, was recorded. This gave the lowest concentration of compound that showed no visible growth after subculture of each clear well onto a new plate containing suitable media.

4. DPPH radical scavenging activity of *G. mangostana* fruit rind extract

Briefly, a stock solution of the extract (5 mg/mL) was diluted to make a two-fold dilution series and then the solution of DPPH (152 μ M) was added to the sample solution in the same volume (750 μ L). The mixture was measured for absorbance at 517 nm after 30 min of the reaction by a UV-visible spectrophotometer (PerkinElmer, USA). The corresponding blank readings were also taken, and percent inhibition was then calculated as follows:

$$\% \text{ Inhibition} = [A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}] \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test sample) and A_{sample} is the absorbance of the test sample.

The EC₅₀ value, the concentration of sample required for 50% scavenging of the DPPH free radical, was determined from the plot between percent inhibition and concentration (Mensor *et al.* 2001). Each sample was done in triplicate. EC₅₀ values were converted to antiradical activity defined as 1/EC₅₀. The higher scavenging activity shows the higher value of antiradical activity.

RESULTS AND DISCUSSION

The sample used in this study were collected from Chumphon Province in the South of Thailand where the area demonstrates good quality of *G. mangostana* fruit rinds (Pothitirat and Gritsanapan 2008; Pothitirat *et al.* 2009c). In our previous study, Soxhlet extraction was found to be the appropriate extraction method for *G. mangostana* fruit rinds, yielding high content of bioactive compounds, and promoting good anti-acne and DPPH scavenging activities. Therefore, Soxhlet extraction was used to prepare the extract sample in this study (Pothitirat *et al.* 2009d).

Table 1 shows the content of α -mangostin, anti-acne inducing bacteria and DPPH scavenging activities of the extract from *G. mangostana* fruit rinds after being stored at

Table 1 The content of α -mangostin, anti-acne inducing bacteria effect, and DPPH scavenging activity of the extract from *G. mangostana* fruit rinds at various storage conditions for 120 days.

Time (Day)	Packaging	Storage temperature (°C)	α -mangostin content (%w/w in crude extract)	Susceptibility of bacteria to extracts				Antiradical activity (1/EC ₅₀)	Color / Characteristics
				<i>P. acnes</i>		<i>S. epidermidis</i>			
				MIC (μ g/mL)	MBC (μ g/mL)	MIC (μ g/mL)	MBC (μ g/mL)		
0	-	-	16.19 \pm 1.05 (100)*	7.81	15.63	15.63	31.25	0.0790 \pm 0.0015 (100)*	Yellow / Fine powder
120	Amber glass vial	4-8	14.29 \pm 0.63 (91.41 \pm 2.05)	7.81	15.63	15.63	31.25	0.0800 \pm 0.0013 (99.94 \pm 1.57)	Yellow / Fine powder
		25-28	15.33 \pm 0.77 (98.98 \pm 0.47)	7.81	15.63	15.63	31.25	0.0808 \pm 0.0001 (100.96 \pm 0.13)	Brown / Semi-solid
		45	14.95 \pm 0.42 (94.54 \pm 3.13)	7.81	15.63	15.63	31.25	0.0776 \pm 0.0050 (96.98 \pm 6.29)	Brown / Semi-solid
	Aluminium foil bag	4-8	16.26 \pm 0.76 (101.23 \pm 4.85)	7.81	15.63	15.63	31.25	0.0791 \pm 0.0007 (98.92 \pm 0.90)	Yellow / Fine powder
		25-28	15.71 \pm 0.06 (97.00 \pm 0.37)	7.81	15.63	15.63	31.25	0.0798 \pm 0.0013 (99.75 \pm 1.57)	Brown / Semi-solid
		45	14.60 \pm 0.16 (90.20 \pm 1.21)	7.81	15.63	15.63	31.25	0.0827 \pm 0.0013 (103.40 \pm 1.69)	Brown / Semi-solid

(.....) α -mangostin content/antiradical activity of the extract when compared to 100 % of the initial ones

various conditions. After 120 days of storing, the characteristics and color of the extract were observed. The results showed stable characteristics of the extracts kept in both an amber glass vial and in aluminium foil bag at 4°C, while temperature at 25 to 28°C, and at 45°C affected the yellow-brown color powder of the extract and changed it to dark brown color (**Table 1**).

The initial values (day 0) of α -mangostin content, anti-radical scavenging activity (1/EC₅₀), and MIC and MBC for *P. acnes* and *S. epidermidis* were 16.19 ± 1.05% (w/w); 0.0790 ± 0.0015; 7.81 µg/mL (MIC), and 15.63 µg/mL (MBC) for *P. acnes*, while MIC at 15.63 µg/mL and MBC at 31.25 µg/mL were found for *S. epidermidis*.

After the extracts were kept at various conditions for 120 days, they were analyzed for α -mangostin content remained after storing period using the validated HPLC method. When the content of α -mangostin in the extract at day 0 was assumed to be 100%, the percentages of α -mangostin that remained after being stored at all conditions were found in the range of 90.20 to 101.23% of the initial content.

Antibacterial activity against *P. acne* and *S. epidermidis*, and antiradical activity against DPPH radical of the extract of *G. mangostana* fruit rinds from each storage condition were investigated at 120 days of storage period using broth microdilution method, and DPPH scavenging assay, respectively. Base on MIC and MBC values, the antibacterial activity against *P. acne* and *S. epidermidis* of the extracts kept in an amber glass vial and in aluminium foil bag at 4-8, 25-28 and 45°C for 4 months was not different from the initial activity (**Table 1**). The percentages of antiradical activity of the extracts stored in an amber glass vial and in aluminium foil bag at 4-8, 25-28 and 45°C were in the range of 96.98 to 103.40% of the initial activity. The increase of the percentages of remained α -mangostin and the scavenging activity of the extract over 100% might come from the interday variation of the assay, and the interference from other compounds occurred during the storage period in the extract.

These results indicate that the different on storage temperatures and types of container had small effect on the content of α -mangostin but no effect on antibacterial activity against *P. acne* and *S. epidermidis*, and antiradical activity against DPPH of the *G. mangostana* fruit rind extract during 120 days of storing. Because of the good chemical and biological stabilities, *G. mangostana* fruit rind extract was appeared to be appropriate for using as a raw material for antioxidant and anti-acne preparations.

CONCLUSIONS

The extract from *G. mangostana* fruit rinds had good chemical and biological stabilities for at least 120 days when stored in an amber glass vial with rubber stopper and aluminium cap, and in an aluminium foil bag at 4 to 45°C. The content of α -mangostin in the extract remained more than 90% of the initial content for all storage conditions. No significant change in DPPH scavenging activity and antibacterial inducing acne effect of all kept extracts were found. However, color and characteristic of the extract were changed from yellow fine powder to dark-brown semisolid,

for both packaging when stored at room temperature (25-28°C), and at higher temperature (45°C). Thus, the extract of *G. mangostana* fruit rinds should be stored in a refrigerator (4-8°C). Our data will be useful for applying *G. mangostana* fruit rind extracts in pharmaceutical production, especially for antioxidant and anti-acne preparations.

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