HPLC Determination of Mangostin and Its Application to Storage Stability Study

Pathom Jujun[1], Krisana Pootakham[1], Yanee Pongpaibul[1], Prasit Tharavichitkul[2] and Chadarat Ampasavate[1*]

[1]Department of Pharmaceutical Sciences, Faculty of Pharmacy, Chiang Mai University, Chiang Mai 50200, Thailand

[2]Department of Microbiology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand

**Corresponding author. E-mail: chadarat@pharmacy.cmu.ac.th*

ABSTRACT

A reverse phase high performance liquid chromatography (RP-HPLC) was developed and validated for the determination of mangostin in mangosteen rind crude extract and throat spray preparation. The column was a C-18 analytical column, the mobile phase consisted of methanol-water (95:5% v/v), flow rate 1.5 ml/min and UV detector was set at 319 nm. The resulting chromatograms showed good resolution with a short retention time without interfering peak. Standard curves were constructed in the concentration range of 25-125 µg/ml (R^2>0.998). The percentage recoveries at 3 levels of mangostin addition (30, 50 and 70 µg/ml) were 94.73 to 98.39% for the crude extract and 94.14 to 99.87% for the throat spray with RSDs below 2% (n=5) in all analyzed concentrations. Stability of mangostin in the throat spray containing mangosteen rind extract was also investigated by keeping the products at 4°C, 30°C, 40°C and room temperature for 180 days. The throat spray samples were found to be quite stable up to 180 days at all tested conditions. The validated HPLC method for determination of mangostin in crude mangosteen rind extract and throat spray was simple, rapid, selective and should be suitable for the quality control of mangosteen rind hydroalcoholic crude extract and antibacterial throat sprays.

Key words: Mangostin, HPLC, Mangosteen rind, Throat spray, Validate, Stability

INTRODUCTION

Garcinia mangostana L. of the family Clusiaceae (Guttiferae) is a tree found in Thailand and other Southeast Asian countries. Its delicious and unique flavor has made this fruit highly popular. Utilization of this plant as herbal medicine has been dated back many years. In Thai traditional medicine, pericarp has been used to treat diarrhea, dysentery, skin infections and as an anti-inflammatory agent (Farnsworth and Bunyapraphatsara, 1992). Xanthones, terpenoids and

sugars have been reported from the fruit hulls of *G. mangostana*, and some of them have shown a variety of biological activities (Mahabusakam and Wiriyachitra, 1987, Praveen et al., 1991, Suksamran et al., 2002). The major components of fruit hulls are xanthones, such as α-mangostin, β-mangostin, and γ-mangostin (Bennett and Lee, 1989). Xanthone derivatives have been reported to possess several pharmacological activities, such as anti-inflammatory and anti-bacterial activities (Iinuma et al., 1996,Keigo et al., 2002). Among them, α-mangostin exhibited the most potent activity against methicillin resistant *Staphylococcus aureus* (MRSA), *Staphylococcus aureus* and *Streptococcus pyogenes* (Iinuma et al., 1996). Several pharmaceutical preparations containing mangosteen crude extract had been developed for treatment of periodontal disease (Pawinee, 2003) or sore throat (Kongchanmitkul, 2002). However, those products were evaluated by physical and biological method, but not by chemical analysis. In the recent years, rigorous quality control of the pharmaceutical formulations in terms of their active constituents in the products has been required. Therefore, the aim of this study is to develop and validate a simple HPLC method for the quantitative determination of α-mangostin in a crude extract and throat spray solution, since there is still no official one. The validation procedure used in this study followed the ICH guidelines (ICH Q2A,1995 ; ICH Q2B,1996). The validated RP-HPLC method for the determination of mangostin has been applied to the quality control of mangostin in mangosteen rind extract and stability study of mangosteen rind extract throat spray.

MATERIALS AND METHODS

Plant material and crude extract from *G. mangostana* rind

Ripe mangosteens were purchased from a local market in Chiang Mai province, Thailand. The ripe mangosteens in size of 6-8 centimeters in diameters,12-15 fruits per kilograms and the dark brown purple color exocarp were selected. The rind were reduce to small pieces, dried in a circulating air oven (40°C) (Hot air oven, Binder®, Germany) and ground into powder (Hammer mill, Polymex®, Thailand) The dried powder of mangosteen rind was extracted by maceration with 95% ethanol (100 g dried powder / 120 ml of 95% ethanol) for 24 h (X5) at room temperature by continuous shaking (Water bath with shaker, Taitec®, Thailand). The filtrates of each time were pooled and the solvent was removed under vacuum at 45°C using rotary evaporator ((Eyela®, Japan) The obtained crude extracts were stored in a refrigerator at 4°C.(Toshiba®, Thailand)

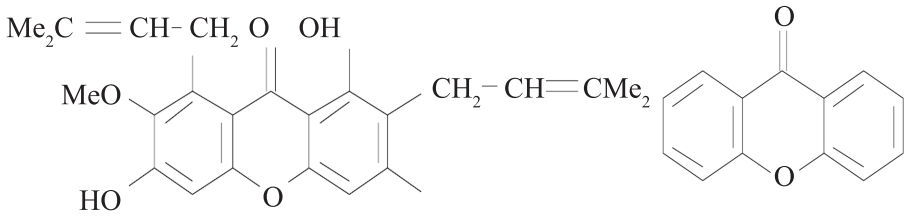
Throat spray

Two throat spray formulations contained 1% of crude extract according to an effective concentration previously observed from antibacterial assays were formulated. The formulation A(SA) and formulation B(SB) composed of 95 % ethanol (33.4, 29.15) propylene glycol (34.55, 31.11) sorbitol (14.35, 19.83) glycerine (9.98, 10.28) peppermint oil (0.49, 0.50) menthol (0.41, 1.06) cinnamond (0.21, 0.51) citric acid (0.21, 0.40 ascorbic acid (0.10, 0.20) saccharide sodium

(0.39, 0.41) and water adjust to 100 % w/w respectively.

Chemicals and Reagents

Mangostin and xanthone (an internal standard), (Figure I) were purchased from Indofine Chemical (New Jersey, USA). Acetonitrile and methanol were of a HPLC grade (Lab scan®, Stillorgan, Ireland).



mangostin xanthone

Figure 1. Structures of mangostin and xanthone (Geeta et, al).

High-Performance Liquid Chromatographic system (HPLC)

The HPLC (UV 1000/P2000, Thermo Separation Products, California, USA), equipped with an UV-detector was set at 319 nm. The chromatographic separation was performed at room temperature on an Alltima® C-18 analytical column (250 mm x 4.6 mm, i.d., Ilinoid, USA). injection volume 20 µl. at flow rate 1.5 ml/min.

Optimization of experimental parameters for HPLC

Maximum absorption of Mangostin and Xanthone

Standard solutions of mangostin and xanthone (10 µg/ml) in methanol were investigated by means of spectrophotometry. The solutions were scanned for their absorption maxima between 200-400 nm.

Preparation of standard solutions

The standard stock solutions of mangostin and xanthone, the internal standard (I.S.), were prepared by dissolving accurately-weighed compounds in methanol to make 1 mg/ml. The mangostin stock solution was sequentially diluted with methanol to prepare a series of working standard solutions in the concentration range of 25-125 µg/ml. Working solutions of the I.S. were prepared by diluting the xanthone stock solution with methanol and adding to the working mangostin standard solutions to yield the final concentration of 25 µg/ml. All solutions were stored at 4°C and brought to room temperature before use.

Method validation

Linearity

From the recorded peak areas, the ratios of mangostin to I.S. were calculated and plotted against mangostin concentrations. The calibration curves were prepared in triplicate. The linearity was evaluated by the least square regression method.

Precision

Standard addition method was employed for the evaluation of precision and accuracy of the analytical method. The amounts of standard mangostin solutions containing 30, 50 or 70 µg/ml and each solution containing 25 µg/ml of xanthone were added to known aliquots of crude extract or throat spray. The intra-day and inter-day precisions of analytical procedure were evaluated through the repeatability of the method by assaying five samples of crude extract and five samples of throat spray, at same concentration, during the same day, under the same experimental conditions or repeatedly over three assay days.

Accuracy

The recoveries were determined at three concentration levels, by adding known amounts of mangostin in the beginning of the process.

For crude extract samples, aliquots (100 µl) of the crude extract were transferred into 10.0 ml volumetric flasks, followed by making up to volume with methanol to give a stock solution. Portions of 0.25 ml of this stock solution were transferred to a 1.8 ml vial where 0, 30, 50 or 70 µg/ml of mangostin reference solutions and xanthone solution were added. Mobile phase was added to make up the volume.

For throat spray samples, aliquots (100 µl) of the throat spray were transferred volumetrically into 10.0 ml volumetric flasks, followed by making up to volume with methanol to give a stock solution. Amounts of 0.25 ml of throat spray were accurately pipetted and placed in five 1.8 ml vials. The samples was added 0.0, 30.0, 50.0 or 70.0 µg/ml of mangostin reference solution, xanthone solution and methanol was added to make up the volume

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Standard mangostin (1 µg/ml) was sequentially diluted with methanol and analyzed by HPLC. The LOD concentration was obtained when signal of peak height was at least three times the noise or measurement of the signal to noise peak height ratio of 3 : 1 (S/N=3, injection volume 20 µl). The LOQ was achieved by measurement of the signal-to-noise peak height ratio equalled 10, injection volume 20 µl. In addition, precision and accuracy of the mangostin determination at the LOQ concentration must be in the acceptable range following the ICH guidelines.

Determination of mangostin in *G mangostana* crude extract and throat sprays by HPLC

Crude extract (100 µl) or throat spray (100 µl) were accurately pipetted into a 10.0 ml volumetric flask and adjusted to the mark with methanol. The solutions were filtered through a 0.45 µm membrane (Millipore®).The chromatographic analysis was performed at room temperature with a flow rate of 1.5 ml/min and the eluate was monitored at 319 nm. The mobile phase consisted of methanol-water (95 : 5% v/v). After the throat spray or crude extract were injected onto the HPLC column, amounts of mangostin present in crude extract or in throat spray were calculated by reference to the calibration curve.

Study of the stability of a throat spray preparation

Mangosteen rind throat spray containing 1% of the crude extract was formulated. The chemical stability test were observed after storage at 4°C, 30°C, 40°C and room temperature. The amounts of mangostin content were analysed at 0, 30, 60, 90, 120, 150 and 180 days of storage

RESULTS AND DISCUSSION

Selection of wave length, mobile phase composition and mobile phase flow rate

The absorption spectra of mangostin and xanthone solutions were investigated by UV-visible spectrophotometry. Results indicated that mangostin and xanthone exhibited maximum absorption at 319 and 337.5 nm respectively. The optimized HPLC condition was achieved after determination of mangostin with different combinations of acetronitrile, methanol and water. The mobile phase containing methanol : water at the ratio of 95 : 5% v/v with the flow rate 1.5 ml/min was found to be the most appropriate condition. Good resolution, short analysis time, high peak area with sharp peaks at the retention of time less than 6 minutes were observed. The overall separation was complete within 13 minutes per 1 sample and was considerably more rapid than the previous describe method (Sirikatitham et al., 2007) (Xiuhong et al., 2007) with out compromising the sensitivity. In addition, methanol was selected for an economical reason as its price is almost half of acetonitrile. The representative chromatograms of mangostin and xanthone standard, mangostin crude extract and mangosteen throat spray are shown in Figure 2.

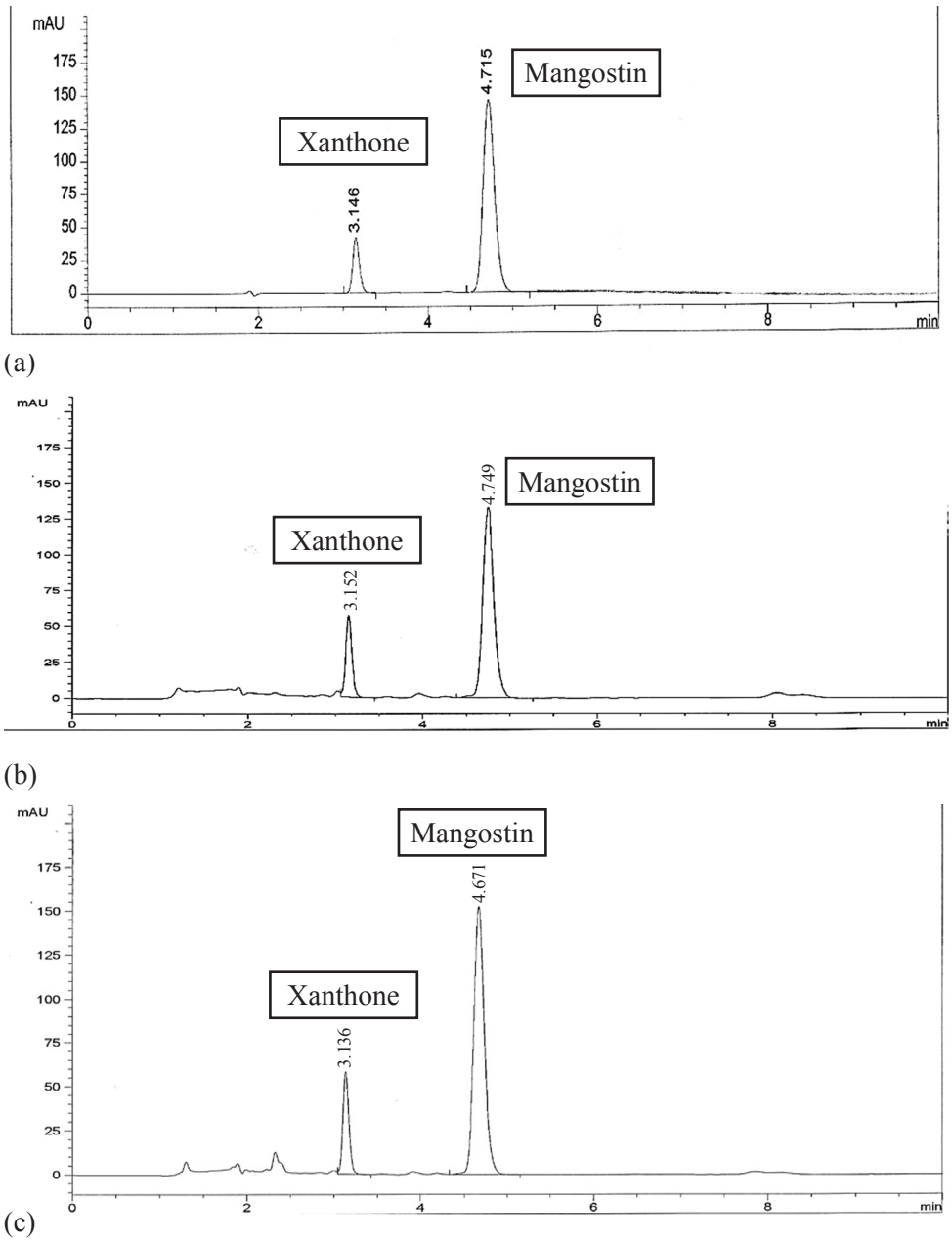


Figure 2. Representative HPLC chromatograms : (a) mangostin and xanthone standard (b) crude extract from *G. mangostana* (c) mangosteen throat spray.

Validation method

Linearity and Range

Calibration curve for the mangostin assay constructed with the peak area ratios of mangostin to internal standard versus mangostin concentration were found to be linear over the concentration range of 25 - 125 µg/ml. The linear regression equation of the calibration curve was Y = 0.1032x-0.0015, and $R^2 \geq 0.998$. The representative linear calibration curve is shown in Figure 3.

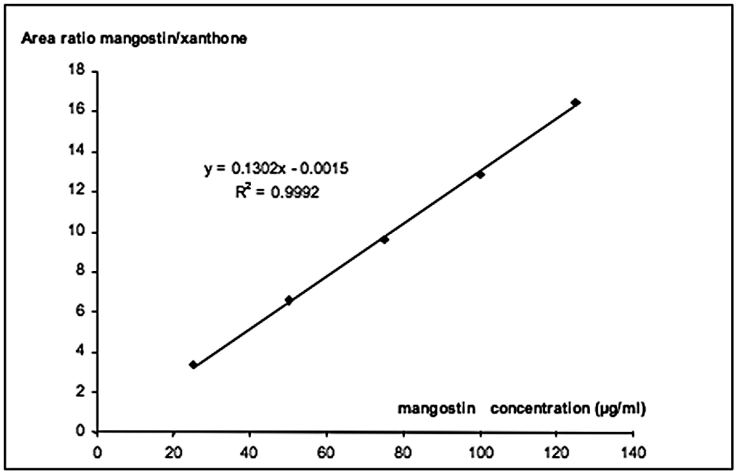


Figure 3. Calibration curve of mangostin standard concentration ranging from 25 to 125 µg/ml.

Accuracy and Precision

The accuracy evaluation was performed by preparing samples of mangostin crude extract and throat sprays spiked with 30, 50 or 70 µg/ ml of mangostin. Each sample was prepared in triplicate. The recovery was determined by comparing the peak area of the control matrix preparation with those samples spiked with mangostin, the expected and observed quantities are shown in Table 1. The results of the accuracy test demonstrated low interference of the matrix onto the recovery of mangostin. The method showed mangostin recovery of 94.73 to 98.39% for crude extract and 94.14 to 99.87% for throat spray with RSD below 2% in all analyzed concentrations.

Table 1. Accuracy and intra- and inter-day precision for the assay of mangostin.

Sample	Added mangostin conc. (µg/ml)	1st day (*n*=5)		2nd day (*n*=5)		3rd day (*n*=5)		Pooled (*n*=3)
		CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)	Accuracy (%)	CV (%)
Crude extract	30	1.58	95.35	1.58	95.35	2.02	92.32	1.72
	50	1.91	99.36	1.91	99.36	1.38	97.52	1.73
	70	2.18	94.73	1.81	94.94	0.41	98.39	1.46
Throat spray	30	1.52	94.14	1.65	94.14	1.52	94.14	1.56
	50	0.59	97.9	0.64	97.9	0.59	97.67	0.60
	70	2.00	99.87	1.24	99.87	1.24	99.87	1.49

Limit of Detection and Limit of Quantitation

The limits of detection and quantitation were of 1.56 ng/ml (S/N = 3) and 6.25 ng/ml (S/N = 10), respectively.

Determination of mangostin in G. mangostana crude extract and throat spray by HPLC

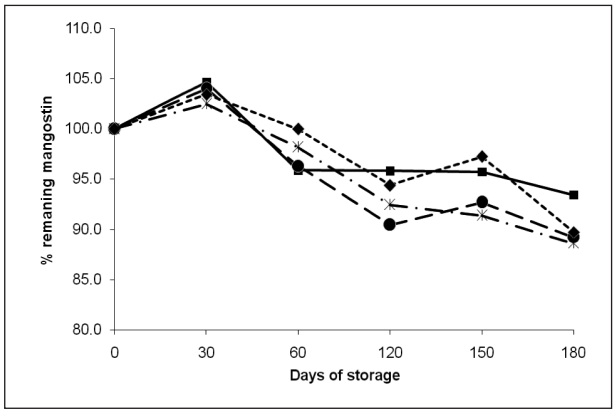
The validated method was tested for the quantitative analysis of mangostin in two different sources of crude extract and two throat spray preparations. Identification of mangostin in all samples was confirmed with the retention time obtained from the standard solution. Percentage of mangostin in crude extracts which were formulated in formulation A (SA) and formulation B (SB) was 11.45 and 11.39 % w/w, respectively. Subsequently, amounts of mangostin were quantitated in the throat spray formulations, A and B, resulting in the concentrations of 837.4 and 894.6 µg/ml, respectively (Table 2).

The stability study of throat spray FA and FB was also investigated by keeping the formulations at room temperature, refrigerator (4°- 8°C), 30°C with 65±5 % RH and 40°C with 75±5% RH for 6 months (Figure 4). The contents and stability of mangostin were analyzed and evaluated. The mangostin throat spray samples were found to be quite stable at all tested conditions. For stability study, the mangostin did not change more than ± 10 % and no significant change occurred at any time during 6 months, Thus, the shelf life of this formulation could be estimated to be around 2 years (ICH O1A(R2),2003).

Table 2. Mangostin in crude extracts and 1% mangosteen throat spray formulations.

Sample	Conc. mangostin
Crude extract sample A (% w/w)	11.45
Crude extract sample B (% w/w)	11.39
1% Mangosteen throat spray formulation A (µg/ml)	837.4
1% Mangosteen throat spray formulation B (µg/ml)	894.6

Formulation A



Formulation B

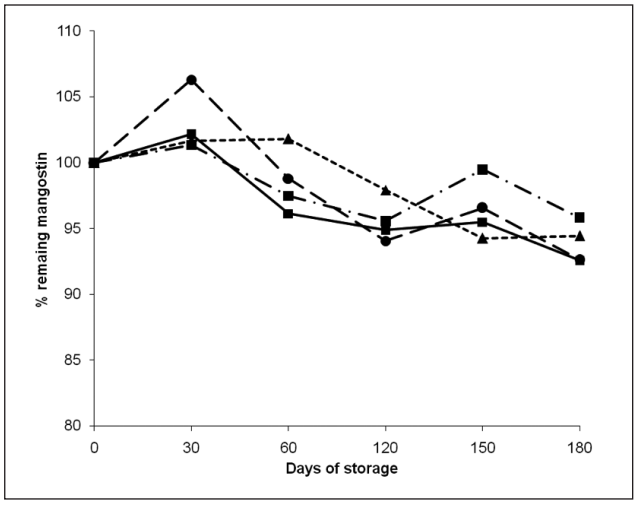


Figure 4. Percentage of mangostin remaining in antibacterial throat spray formulation A and formulation B in accelerated stability study for 180 days ((■) storage at 4°C; (●) room temperature; (♦) 30°C; (*) 40°C).

CONCLUSION

A simple, specific, precise, rapid and reproducible HPLC method was first time reported for identification and quantitation of mangostin, a relevant marker compound in crude extract from *G. mangostana* rind and throat spray solution. The validation procedure confirms that this is an appropriate method for the quality control of crude extract and preparation from *G. mangostana* that can be used in pharmaceutical industry. The mangosteen rind throat spray was quite stable up to six months. All data obtained from this investigation were important and beneficial for chemical control of mangosteen crude extract and throat spray.

ACKNOWLEDGEMENTS

The authors gratefully acknowledged the financial support from Faculty of Pharmacy, Graduate School of Chiang Mai University and National Research Council of Thailand (NRCT).

REFFERENCES

Bennett, G.J., and H.H. Lee. 1989. Xanthones from Guttiferae. Phytochemistry 28:967-998.

Farnsworth, N., and N. Bunyapraphatsara.. 1992. Thai medicinal plants : Recommended for primary health care system. Prachachon Bangkok.

Geetha, G., B. Banumathi, and G. Suresh.1997. Evaluation of the Antifungal Activity of Natural Xanthones from *Garcinia mangostana* and Their Synthetic Derivatives J. Nat. Prod. 60: 519-524

Iinuma, M., H. Tosa, T. Tanaka, F. Asai, Y. Kobayashi, R. Shimano, and K. Miyauchi. 1996. Antibacterial activity of xanthones from Guttiferaeous plants against methicillin-resistant *Staphylococcus aureus.* J. Pharm. Pharmacol. 48 : 861-865

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. 1995.Text on validation of analytical procedures (ICH Q2A), Geneva.

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. 1996. Validation of analytical procedures : Methodology (ICH Q2B). Geneva.

International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. 2003. Stability testing of new drug substances and products (ICH Q1A (R2)), Geneva.

Keigo, N., N. Norimichi, A.Tsutomu, and Y. Hideyuki. 2002. Inhibition of cyclooxygenase and prostaglandin E2 synthesis by γ-mangostin, a xanthone derivative in mangosteen, in C6 rat glioma cells.Biochem. Pharmaco. 63: 173-179.

Kongchanmitkul, W. 2002. Preparation and evaluation antibacterial throat sprays from mangosteen crude extract. Master's Thesis. Chiang Mai University, Chiang Mai, Thailand.

Mahabusarakam, W., and P. Wiriyachitra. 1987. Chemical constituents of *Garcinia mangostana*. J. Nat. Prod. 50 : 474-478.

Mahabusarakum, W., S. Phongpaichit, C. Jansakul, and P. Wiriyachitra,1983. Screening ofantibacterial activity of chemicals from *Garcinia mangostana*. Songklanakarin J. Sci. Technol. 5 : 337-339.

Pawinee, M. 2003. Clinical and microbiological effects of Garcinia mangostana pericarp gel as an adjunct in non-surgical periodontal treatment. Master's Thesis. Mahidol University. Bangkok, Thailand.

Praveen, M., N.U. Khan, B. Achari, and P.K.Dutta. 1991. A triterpene from Garcinia Mangostana. Phytochemistry 30: 361-362.

Sirikatitham, A., S. Yodhnu, and C. Wattanapiromsakulb. 2007. Validation method for quantification of alpha-mangostin, in mangosteen peel extract by high performance liquid chromatography. Grad research 7th, Prince of Songkla University.

Suksamran, S., N. Suwannapoch., P.Ratananukul, N. Aroonlerk, and A. Suksamrarn 2002. Xanthones from green fruit hulls of *Garcinia mangostana*. J. Nat. Prod. 65 (5) : 761-767.

Xiuhong, J., A. Bharathi, and A. K. Ikhlas. 2007. Quantitative and qualitative determination of six xanthones in *Garcinia mangostana* L. by LC-PDA and LC-ESI-MS. J. Pharm. Biomed. Anal. 43 : 1270-1276.

none