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Combined microwave-assisted extraction and high-speed counter-current chromatography for separation and purification of xanthones from *Garcinia mangostana*

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ABSTRACT

A microwave-assisted extraction (MAE) method is presented for the extraction of xanthones, α-mangostin and γ-mangostin from *Garcinia mangostana*. The MAE conditions including extraction temperature, liquid/solid ratio, extraction time and concentration of ethanol were optimized with an orthogonal test, and 5 g sample was extracted with the optimized conditions. The crude extraction of MAE was successfully isolated and purified by high-speed counter-current chromatography (HSCCC) with a two-phase solvent system composed of petroleum ether–ethyl acetate–methanol–water (0.8:0.8:1:0.6, v/v) in one-step separation. The separation yielded 75 mg of α-mangostin at 98.5% purity, and 16 mg of γ-mangostin at 98.1% purity from 360 mg crude extract of *G. mangostana* in less than 7 h. The purity of the two xanthones was determined by HPLC. Their structures were further identified by ESI-MS, ^{1}H NMR and ^{13}C NMR.

1. Introduction

Garcinia mangostana L. (Clusiaceae), commonly known as mangosteen, is imported from Thailand and cultivated South China to produce a popular refreshing juicy fruit in the summer. The fruit hulls of mangosteen have been used in Thailand folk medicine for the treatment of diarrhea, trauma, and skin infections [1–3]. Modern phytochemical research has revealed that *G. mangostana* is rich in a variety of oxygenated and prenylated xanthones [4,5]. The xanthones, α- and γ-mangostins are the major bioactive compounds [6,7] found in the fruit hulls of mangosteen, and possess a wide range of biological activities, such as antimicrobial activity [8], antioxidant activity [9], anti-inflammatory activity [10], prevention oxidative damage of LDL [11], and inhibition on HIV infection [12]. In view of these beneficial biological properties and broad applications, the study on the separation and purification of α- and γ-mangostins from *G. mangostana* is necessary.

The xanthones, α- and γ-mangostins have been obtained by isolating from the fruit hulls of mangosteen with conventional methods, such as maceration, heat reflux, and soxhlet extraction, followed with separation and purification by column chromatography [13–16]. However, these conventional methods are consumed longer extraction time, large quantities of organic solvents, and often offered low recoveries of the target products. Microwave-assisted extraction (MAE), an improved method with good extraction efficiency and environmental-friendliness, has been successfully used for the effective separation of various natural products such as quercetin from *Anoectochilu roxburghii* [17], isofraxidin from *Sarcandra glabra* [18], bergenin from *Ardisia crenata* [19], ferulic acid from *Angelicae sinensis* [20], and dehydrocavidine from *Corydalis saxicola* [21]. High-speed counter-current chromatography (HSCCC), being as a kind of liquid–liquid partition chromatography, eliminates irreversible adsorption of samples on solid support in conventional column chromatography and offers excellent recovery of target compounds. It has been successfully applied to the separation and purification of different kinds of natural products [22,23], which also has been used to the pre-isolation of xanthones [24,25]. In this study, we report a simple and efficient method for the separation and purification of xanthones from *G. mangostana* by MAE combined with HSCCC directly. The critical parameters, including MAE conditions and solvent system of HSCCC, were optimized.

2. Experimental

2.1. Apparatus

The microwave-assisted extraction of *G. mangostana* was carried on Excel Microwave system (EU Microwave Chemistry Technology Co., Ltd, Shanhai, China). The power ranged from 0 to 1500 W,

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and the temperature ranged from 0 °C to 250 °C. The temperature precision was controlled at ±1 °C.

Preparative HSCCC was carried out using a Model GS10A-2, with a multilayer coil of 1.6 mm I.D. and 110 m in length with a total capacity of 230 ml. The β values of this preparative column range from 0.5 at internal to 0.8 at the external ($\beta = r/R$, where r is the rotation radius or the distance from the coil to the holder shaft, and R is the revolution radius or the distances between the holder axis and central axis of the centrifuge) (Beijing Emilion Science & Technology Co., Beijing, China). The solvent was pumped into the column with a Model NS-1007 constant-flow pump (Beijing Emilion Science & Technology Co., Beijing, China). Continuous monitoring of the effluent was achieved with a Model 8823A-UV Monitor (Beijing Emilion Science & Technology Co., Beijing, China) at 254 nm. A manual sample injection valve with a 10-ml loop (for the preparative HSCCC) (Tianjin High New Science Technology Company, Tianjin, China) was used to introduce the sample into the column. A portable recorder (Yokogawa Model 3057, Sichuan Instrument Factory, Chongqing, China) was used to draw the chromatogram.

HPLC was carried out on a Waters Empower system (Milford, MA, USA) including a model 600 pump, a model 600 system controller, a model 600 multi-solvent delivery system, a model 996 diode-array detector (DAD), a sample injector with a 20 μL loop and an Empower workstation. The identification of α- and γ-mangostins was performed on a Q-Trap LC/MS/MS spectrometer (Aglient, USA) and a Varian 600 MHz NMR spectrometer (Varian, Palo Alto, USA).

2.2. Reagents and materials

Organic solvents including ethanol, ethyl acetate, petroleum ether (60–90 °C), and methanol were all of analytical grade (Juye Chemical Factory, Jinan, China). Acetonitrile used for HPLC was chromatographic grade (Merck, Germany), and water used was distilled water.

The fruit hulls of *G. mangostana* were collected in Hainan, China, and identified by Prof. F. Zhou (College of Pharmacy, Shandong University of Traditional Chinese Medicine, Shandong, China). The fruit hulls were air-dried and then powdered before use.

2.3. Optimization of MAE conditions

In order to determine a suitable extraction condition, an orthogonal test design $L_9(3)^4$ was employed where the concentration of ethanol, extraction temperature, extraction time, and liquid/solid ratio were considered to be four major factors for effective extraction [17,18]. Combinations of the three different levels of each factor were listed in Table 1. In each test, 5 g of the powdered sample was added to a 100 mL extraction vessel, and the extractions were carried out under different MAE conditions according to the pre-designed trial (see Table 1). After extraction, the vessels were cooled down to room temperature before opening. Sample extracts were collected, evaporated to dryness under reduced pressure, and dissolved in 100 mL methanol for HPLC analysis.

2.4. Selection of two-phase solvent system

The selection of two-phase solvent system is the crucial point for a successful separation by HSCCC. The solvent system was selected according to the partition coefficient (K) of target compound. The K values were determined by HPLC as follows: approximately 2 mg of crude extract was added to the test tube, to which 2 mL of each phase of the two-phase solvent system was added. The test tube was shaken violently for several minutes. Then an equal volume of each phase was analyzed by HPLC to obtain the partition coefficients. The K value was expressed as the peak area of the compound in the upper phase divided by that in the lower phase.

2.5. HSCCC separation procedure

In the present study, the HSCCC experiments were performed with a two-phase solvent system of petroleum ether–ethyl acetate–ethanol–water (0.8:0.8:1:0.6, v/v). Solvent mixture was thoroughly equilibrated in a separation funnel by repeatedly vigorously shaking at room temperature. The two phases were separated shortly prior to use. The upper phase was used as the stationary phase, while the lower phase was used as the mobile phase. The sample solution was prepared by dissolving the dried extract in the mixture solution of lower phase and upper phase (1:1, v/v) of the solvent system.

HSCCC separation was performed as follows: the multiplayer coiled column was first filled entirely with the upper organic phase as the stationary phase. The lower aqueous phase was then pumped into the head end of the column at a suitable flow-rate of 2 ml/min, while the apparatus was rotated at a speed of 800 rpm. After hydrodynamic equilibrium was reached, as indicated by a clear mobile phase eluting from the tail outlet, the sample solution was injected into the column through the inject valve. The effluent of the column was continuously monitored with a UV detector at 254 nm and the chromatogram was recorded. Each peak fraction was collected according to the elution profile and determined by HPLC. After the separation was completed, retention of the stationary phase was measured by collecting the column contents by forcing them out of the column with pressurized nitrogen gas.

2.6. HPLC analysis and identification of HSCCC fractions

The crude extract and the fractions from the preparative HSCCC separation were analyzed by HPLC. Chromatographic separations were accomplished with a Shim-pack VP-ODS column (250 mm × 4.6 mm, I.D.) at room temperature. The mobile phase was acetonitrile and water (90:10, v/v) and performed at a flow-rate of 1.0 ml/min. The effluent was monitored at 320 nm by a photodiode array detector. Routine sample calculations were made by comparison of the peak area with that of the standard. The identification of HSCCC peak fractions was carried out by electrospray ionization mass spectrometry (ESI-MS) on an Agilent 1100/MSG1946 (Agilent, CA, USA) and ^{1}H NMR and ^{13}C NMR spectra on a Varian-600NMR spectrometer (Varian, Palo Alto, USA).

3. Results and discussion

3.1. Optimization of MAE conditions

The first step in the MAE is to optimize the operating conditions to obtain an efficient extraction of the target compounds. The concentration of ethanol, extraction temperature, extraction time, and liquid/solid ratio are generally considered to be the most important factors and optimized by using an orthogonal $L_9(3)^4$ test design. The results shown in Table 2 indicated that there were great yield differences among each set of MAE conditions. The influence to the mean extraction yields of α- and γ-mangostins decreases in the order: A > B > C > D according to the R values. The best MAE conditions was $A_2B_3C_1D_2$ according to the extraction of yields of α- and γ-mangostins, and the maxium yield of α- and γ-mangostins was 27.03 and 5.56 mg/g of dry fruit hulls, respectively.

The concentration of ethanol was found to be the most important determinant of yields of α- and γ-mangostins. The results in Table 3 showed that the yields of xanthones increased with the concentration of ethanol significantly when the concentration was lower than 95%. However, the extraction yields decreased sharply

Table 1
$L_9(3)^4$ orthogonal test design.

Test no.	Factors					
	(A) Percent ethanol (%)		(B) Temperature (°C)		Extraction time (min)	Ratio of solid/liquid
1	A1	100	B1	50	C1 10	D1 1:6
2	A1	100	B2	60	C2 15	D2 1:8
3	A1	100	B3	70	C3 20	D3 1:10
4	A2	95	B1	50	C2 15	D3 1:10
5	A2	95	B2	60	C3 20	D1 1:6
6	A2	95	B3	70	C1 10	D2 1:8
7	A3	90	B1	50	C3 20	D2 1:8
8	A3	90	B2	60	C1 10	D3 1:10
9	A3	90	B3	70	C2 15	D1 1:6

Table 2
Analysis of $L_9(3)^4$ orthogonal test results.

Test no.	Factors				Yield (%)[a]	Yield (mg/g)[b]	
	A	B	C	D		α-Mangostin	γ-Mangostin
1	A1	B1	C1	D1	8.8	23.74	4.77
2	A1	B2	C2	D2	9.1	24.27	5.02
3	A1	B3	C3	D3	9.3	25.09	4.92
4	A2	B1	C2	D3	8.9	23.80	4.99
5	A2	B2	C3	D1	8.7	23.56	4.74
6	A2	B3	C1	D2	9.9	27.03	5.56
7	A3	B1	C3	D2	6.8	18.36	3.54
8	A3	B2	C1	D3	7.2	19.80	3.92
9	A3	B3	C2	D1	7.5	20.19	3.98

[a] Extraction yield (%) = (the amount of extract/sample mass) × 100.
[b] Extraction yield (mg/g) = the amount of α-mangostin or γ-mangostin/sample mass.

Table 3
Analysis of $L_9(3)^4$ orthogonal test results.

	α-Mangostin yield (mg/g)				γ-Mangostin yield (mg/g)			
	A	B	C	D	A	B	C	D
K_1	73.083	65.901	70.569	67.491	14.709	13.299	14.250	13.491
K_2	74.391	67.629	68.259	69.660	15.291	13.680	13.989	14.121
K_3	58.350	72.309	67.011	68.691	11.439	14.460	13.200	13.830
k_1	24.367	21.967	23.523	22.497	4.903	4.433	4.750	4.497
k_2	24.797	22.543	22.753	23.220	5.097	4.560	4.663	4.707
k_3	19.450	24.103	22.337	22.897	3.813	4.820	4.400	4.610
R	5.347	2.317	1.186	0.723	1.284	0.387	0.350	0.210
Optimal Level	A2	B3	C1	D2	A2	B3	C1	D2

$K_i^A = \sum$ extraction yield at A_i. $k_i^A = \frac{K_i^A}{3}$. $R_i^A = \max\{k_i^A\} - \min\{k_i^A\}$.

Table 4
Partition coefficients (K) and separation factors (α) of α- and γ-mangostins.

Solvent system: petroleum ether –ethyl acetate–methanol–water	Partition coefficients (K)		Separation factors (α)
	α-Mangostin K_1	γ-Mangostin K_2	
1:1:1:1	25.13	8.61	2.92
1:0.8:0.8:1	11.95	4.12	2.90
1:0.5:1:0.5	1.49	0.42	3.55
0.8:0.8:1:0.6	2.07	1.41	1.46
0.7:0.9:1:0.6	1.73	1.72	1.00

when extracted with anhydrous alcohol. From these results, it is clear that the proper amount of water in alcohol improved the extraction efficiency, probably due to the proper effects on the absorption, transferability of microwave energy and good solubility of xanthones. Therefore, 95% ethanol showed the best extraction yield and was chosen as the isolation solvent in the following experiments.

Extraction temperature also had significant influence on the yields of xanthones. It could be observed that the increase of extraction temperature in range of 50–70 °C resulted in the increase of the yields of xanthones. This is probably because that higher temperature could cause opening cell matrix, increase the diffusivity of the solvent, decrease the solvent viscosity, and improve the solubility of xanthones.

The extraction time and liquid/solid ratio only showed slightly influence on the extraction yields of xanthones in comparison with the other two factors. It is seen in Table 3 that the yield of xanthones decreased with the increase of extraction time, because xanthones could be destroyed with the increasing time. Meanwhile, the yields of experiments at different levels of liquid/solid ratio showed no significant difference. A liquid/solid ratio of 10/1 (mL/g) and extraction time of 10 min were adopted in the present study.

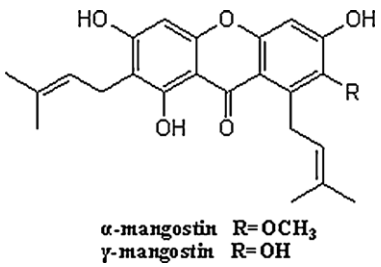

Fig. 1. Chemical structures of α- and γ-mangostins from *G. mangostana*.

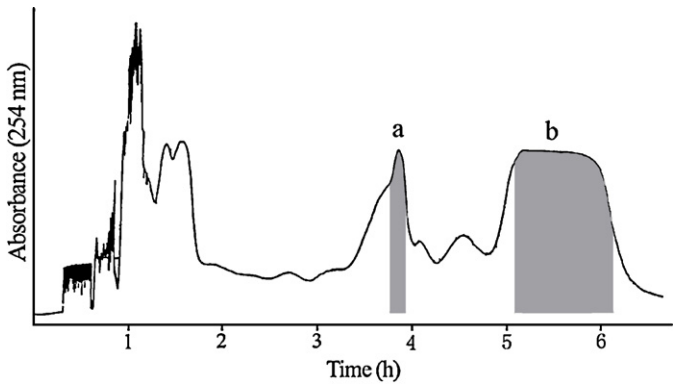

Fig. 2. HSCCC chromatogram of the crude sample of *G. mangostana*. Solvent system: petroleum ether–ethyl acetate–methanol–water (0.8:0.8:1:0.6, v/v); revolution speed: 800 r/min; flow rate: 2.0 mL/min; sample size: 360 mg; UV detection wavelength: 254 nm; retention of stationary phase: 75%; a: γ-mangostin; b: α-mangostin.

These results indicated that the optimal conditions for extraction of the two xanthones by MAE were 95% ethanol, 70 °C of temperature, 10 min of extraction and 10/1 (mL/g) of liquid/solid ratio. Under the optimum MAE conditions, the extraction yields of α- and γ-mangostins were 27.03 and 5.56 mg/g, respectively. Moreover, to evaluate the extraction efficiency of MAE, the two xanthones were also extracted with the typical traditional heat reflux

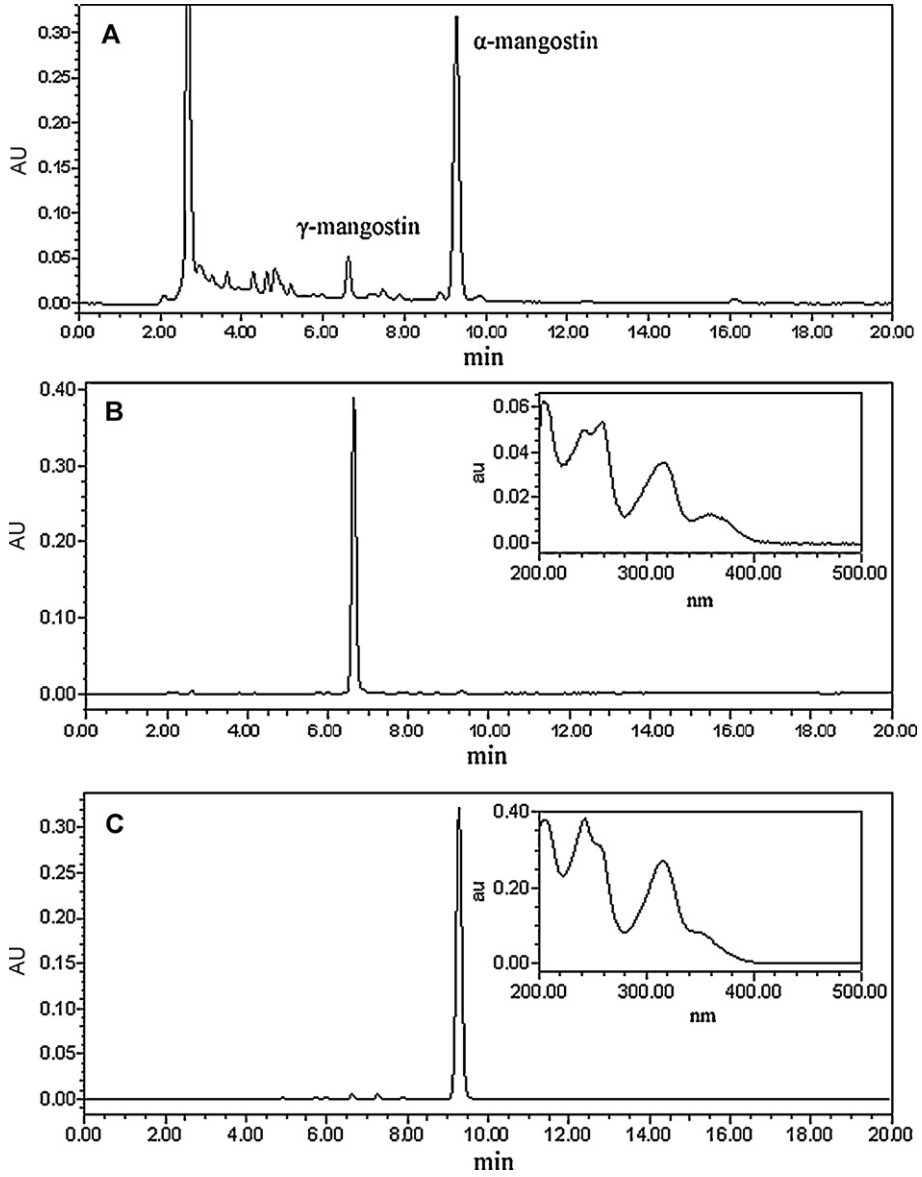

Fig. 3. (A) HPLC chromatogram of the extract from preparative MAE; (B) HPLC analyses and UV spectrum of γ-mangostin purified with HSCCC; (C) HPLC analyses and UV spectrum of α-mangostin purified with HSCCC. Conditions-a Shim-pack VP-ODS column (250 mm × 4.6 mm i.d.); column temperature: 25 °C; mobile phase: acetonitrile–water (90:10, v/v); flow-rate: 1.0 ml/min; detection wavelength: 320 nm.

method, and the yields of α- and γ-mangostins were 24.49 and 4.94 mg/g, respectively. Compared with heat reflux method, similar extraction yields of xanthones by MAE were obtained with the greatly reducing time (10 min instead of 5 h). Therefore, MAE can be used as a rapid, efficient and reliable method for extraction of xanthones from *G. mangostana*.

3.2. Selection of HSCCC separation conditions

The choice of a suitable two-phase solvent system is the first and critical step in a HSCCC experiment. To achieve a successful separation using HSCCC, the suitable solvent system should provide an ideal range of partition coefficient (*K*, 0.5–2) for xanthones and a proper separation factor ($\alpha > 1.5$) between the two components. In our research, several kinds of solvent systems were tested, and the values of *K* and α for the two xanthones in different solvent systems were summarized in Table 4. The results indicated that good separation results could be obtained when petroleum ether–ethyl acetate–ethanol–water with the volume ratio of 0.8:0.8:1:0.6 was used as the two-phase solvent system Fig. 1.

3.3. Purification ofα- and γ-mangostins by HSCCC

The crude extract (360 mg) was separated and purified in one step by the preparative HSCCC with petroleum ether–ethyl acetate–ethanol–water (0.8:0.8:1:0.6, v/v) as a solvent system (Fig. 2). The retention of the stationary phase was 75.0%, and the separation time was within 7 h in each separation run. The HSCCC fractions were analyzed by HPLC, and the HPLC chromatograms of the collected fractions are shown Fig. 3, respectively. The separation produced 75 mg of α-mangostin at 98.5% purity and 16 mg of γ-mangostin at 98.1% purity according to HPLC analysis. These results demonstrate the high resolving power of HSCCC.

3.4. Identification ofα- and γ-mangostins

The structural identification of α-mangostin (Peak b) was carried by ESI-MS, ^{1}H NMR and ^{13}C NMR spectra. The ESI-MS data is *m*/*z* 409 [M−H]$^-$. ^{1}H NMR (acetone-d_6, 600 MHz) δ: 1.62, 1.63 (3H each, s, H-5′ and H-5″), 1.78 (3H, s, H-4″), 1.79 (3H, s, H-4′), 3.48 (2H, d, *J* = 7.2 Hz, H-1″), 3.92 (3H, s, OCH_3), 4.25 (2H, d, *J* = 6.0 Hz, H-1′), 5.40 (2H, m, H-2′ and H-2″), 6.53 (1H, s, H-8), 6.95 (1H, s, H-1), 13.92 (1H, s, C-5-OH). ^{13}C NMR (acetone-d_6, 150 MHz) δ: 17.2 (C-4″), 17.6 (C-4′), 21.3 (C-1″), 25.2 (C-5′), 25.4 (C-5″), 26.2 (C-1′), 60.5 (OCH_3), 92.4 (C-8), 101.9 (C-1), 102.9 (C-5a), 110.3 (C-6), 111.3 (C-4a), 122.8 (C-2″), 124.19 (C-2′), 130.7 (C-3′ and C-3″), 137.4 (C-4), 143.8 (C-3), 155.0 (C-7), 155.5(C-2), 161.0 (C-5), 162.3 (C-8a), 182.4 (C-10). The ^{1}H NMR and ^{13}C NMR data were accorded with the literature [26], indicating the structural identification of α-mangostin.

The structural identification of γ-mangostin (Peak a) was carried by ESI-MS, ^{1}H NMR and ^{13}C NMR spectra. The ESI-MS data is *m*/*z* 395 [M−H]$^-$. ^{1}H NMR (acetone-d_6, 600 MHz) δ: 1.62, 1.63 (3H each, s, H-5′ and H-5″), 1.76 (3H, s, H-4″), 1.82 (3H, s, H-4′), 3.32 (2H, d, *J* = 7.2 Hz, H-1″), 4.16 (2H, d, *J* = 6.0 Hz, H-1′), 5.27 (2H, m, H-2′ and H-2″), 6.38 (1H, s, H-8), 6.80 (1H, s, H-1), 13.92 (1H, s, C-5-OH). ^{13}C NMR (acetone-d_6, 150 MHz) δ: 17.2 (C-4″), 17.6 (C-4′), 21.3 (C-1″), 25.2 (C-5′), 25.4 (C-5″), 26.2 (C-1′), 92.2 (C-8), 100.3 (C-1), 103.0 (C-5a), 110.0 (C-6), 111.3 (C-4a), 122.9 (C-2″), 123.9 (C-2′), 130.6 (C-3′ and C-3″), 140.9 (C-3), 151.6 (C-1a), 152.8 (C-2), 155.1 (C-7), 156.7 (C-1a), 161.0 (C-5), 162.0 (C-8a), 182.4 (C-10). The above NMR data was accorded with the literature [26], suggesting the structural identification of γ-mangostin.

4. Conclusions

The present study demonstrated a simple method for the extraction, separation and purification of xanthones from *G. mangostana* by MAE coupled with HSCCC. The xanthones was efficiently extracted by MAE, which showed the most prominent advantages of MAE over conventional heat reflux extraction were less time used. The crude extract of MAE was separated and purified directly by HSCCC using petroleum ether–ethyl acetate–ethanol–water (0.8:0.8:1:0.6, v/v) solvent system. Under optimal conditions, 75 mg of α-mangostin and 16 mg of γ-mangostin were obtained from 360 mg dried extract of *G. mangostana* within 7 h with purity over 98% in one-step separation. The results indicated that the present method of MAE coupled with HSCCC is suitable for the extraction, separation and purification ofα- and γ-mangostins from *G. mangostana*.

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References

[1] F.W. Martin, Durian and Mangosteen, Tropical and Subtropical Fruits, Westport, 1980.
[2] P. Moongkarndi, N. Kosem, S. Kaslungka, O. Luanratana, N. Pongpan, N. Neungton, J. Ethnopharmacol. 90 (2004) 161.
[3] K. Nakatani, N. Nakahata, T. Arakawa, H. Yasuda, Y. Ohizumi, Biochem. Pharmacol. 63 (2002) 73.
[4] V. Peres, J.T. Nagem, F F.F. de Oliveira, Phytochemistry 55 (2000) 683.
[5] V. Peres, T.J. Nagem, Phytochemistry 44 (1997) 191.
[6] N. Chairungsrilerd, K.I. Furukawa, T. Ohta, S. Nozoe, Y. Ohizumi, Eur. J. Pharmacol. 314 (1996) 351.
[7] W. Jinsart, B. Ternai, D. Buddhasukh, G.M. Polya, Phytochemistry 31 (1992) 3711.
[8] M. Iinuma, H. Tosa, T. Tanaka, F. Asai, Y. Kobayashi, R. Shimano, K.I. Miyauchi, J. Pharm. Pharmacol. 48 (1996) 861.
[9] N. Chairungsrilerd, K.I. Furukawa, T. Ohta, S. Nozoe, Y. Ohizumi, Planta Med. 62 (1996) 471.
[10] Z.X. Lu, M. Hasmeda, W. Mahabusarakam, B. Ternai, P.C. Ternai, G.M. Polya, Chem. Biol. Interact. 114 (1998) 121.
[11] K. Iikubo, Y. Ishikawa, N. Ando, K. Umezawa, S. Nishiyama, Tetrahedron Lett. 43 (2002) 291.
[12] S.X. Chen, M. Wan, B.N. Loh, Planta Med. 62 (1996) 381.
[13] G. Gopalakrishnan, B. Banumathi, G. Suresh, J. Nat. Prod. 60 (1997) 519.
[14] Y.L. Huang, C.C. Chen, Y.J. Chen, R.L. Huang, B.J. Shieh, J. Nat. Prod. 64 (2011) 903.
[15] H.R.W. Dharmaratne, K.G.N.P. Piyasena, S.B. Tennakoon, Nat. Prod. Res. 19 (2005) 239.
[16] N. Chairungsrilerd, K. Takeuchi, Y. Ohizumi, S. Nozoe, T. Ohta, Phytochemistry 43 (1996) 1099.
[17] L. Huang, Y. Cao, G. Chen, Sep. Purif. Technol. 64 (2008) 101.
[18] X.H. Xiao, Z.N. Guo, J.C. Deng, G.K. Li, Sep. Purif. Technol. 68 (2009) 250.
[19] J.C. Deng, X.H. Xiao, X. Tong, G.K. Li, Sep. Purif. Technol. 74 (2010) 155.
[20] Z.L. Liu, J. Wang, P.N. Shen, C.Y. Wang, Y.J. Shen, Sep. Purif. Technol. 52 (2006) 18.
[21] J.C. Deng, X.H. Xiao, G.K. Li, G.H. Ruan, Phytochem. Anal. 20 (2009) 498.
[22] I.A. Sutherland, D. Fisher, J. Chromatogr. A 1216 (2009) 740.
[23] Y. Ito, J. Chromatogr. A 1065 (2005) 145.
[24] A. Urbain, A. Marston, D. Batsuren, O. Purev, K. Hostettmann, Phytochem. Anal. 19 (2008) 514.
[25] A.E. Hay, J.J. Hélesbeux, O. Duval, M. Labaïed, P. Grellier, P. Richomme, Life Sci. 75 (2004) 3077.
[26] L.G. Chen, L.L. Yang, C.C. Wang, Food Chem. Toxicol. 46 (2008) 688.