Methods Article

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A Rapid Method for the Retrieval of Bioactive Xanthone from *Garcinia Mangostana*: A Case Study of α-Mangostin

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Abstract: Natural products have been used as the functional food and alternative treatment to combat various diseases for centuries. A handful of bioactive compounds that are available clinically such as antibiotic, anti-neurodegeneration, and anticancer drugs were derived from natural products. Recent study showed that α-mangostin, a natural occurring prenylated xanthone, possesses a wide variety of pharmacological activities including anti-cancer, anti-inflammation, anti-bacterial, anti-viral, and anti-neurodegenerative. In view of the pharmacological importance of α-mangostin, a rapid extraction method is paramount importance to recover the α-mangostin from natural resources to cope the therapeutic needs and potential for future clinical translation. The yield of α-mangostin was 9.2 g/kg DW. This study presents a simple, cost effective, and rapid extraction with identification of α-mangostin from the pericarp of the mangosteen fruit.

Keywords: Xanthones; α-mangostin; extraction; *Garcinia mangostana*; mangosteen

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Introduction

Phytochemicals can be categorised into carbohydrate, lipids, terpenenoids, phenolic, and alkaloids (nitrogen containing metabolites)[1–2] . The xanthones are phenolic compounds that can be derived from fungi, lichen and higher plants[3]. These metabolites are commonly found in the higher plant families such as Gentianaceae, Guttiferae, Moraceae, Clusiaceae, and Polygalaceae. There are more than 285 xanthones identified from plant species of the family Guttiferae. The main groups of xanthones from natural sources include basic xanthone, xanthone glycosides, prenylated xanthones, xanthonolignoids, and bisxanthones[4]. Among all, prenylated xanthones showed a wide range of pharmacological properties including anti-cancer, anti-inflammatory, anti-cholinesterase, anti-diabetic, anti-microbial and anti-neurodegenerative[5–8].

The α-mangostin, β-mangostin, γ-mangostin, gartanin, 8-deoxygartanin, garcinone C and garcinone D are the prenylated xanthone commonly found in the pericarps of *Garcinia mangostana*. The α-mangostin (Figure 1) is the major prenylated xanthone found in the fruit hull of mangosteen. It was first isolated in 1855 with its structure first revealed after 113 years later[9]. The first total synthesis of α-mangostin was reported by Likubo *et al*. (2002)[10]. Since then, several research groups have reported the modification of α-mangostin, aiming to enhance its bioactivity and pharmacokinetics[11–13].

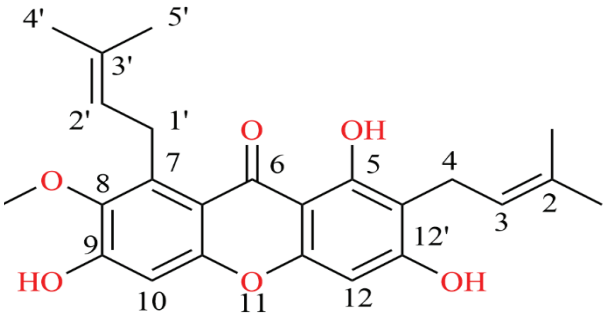

Figure 1. Chemical structure of α-mangostin.

To extract α-mangostin, several conventional methods such as column chromatography, preparative thin layer chromatography, semi-preparative HPLC, and

some recent environmental-friendly techniques such as supercritical fluid extraction have been reported. The use of supercritical carbon dioxide in the Lee WJ, Ng CC, Ng JS, et al. Supercritical carbon dioxide extraction of alpha-mangostin from mangosteen pericarp with virgin coconut oil as co-extractant and in-vitro bio-accessibility measurement. The use of supercritical carbon dioxide in the extraction has been advocate as the "green solvent"

as it is considered as harmless to the environment as compared to organic solvents that may contribute to environmental pollutions[14]. However, the drawback of this technology including high setup cost and relative low yield. Therefore, this study proposed a rapid workflow that might overcome these limitations to purify the α-mangostin by the means of pilot scale ultrasonic homogenizer. The extract was subsequently dried-up by rotary evaporator and fractionated with chloroform to obtain xanthone-rich fraction. Lastly, α-mangostin was obtained in high yield by column chromatography. Figure 2 shows the workflow of extraction and purification of α-mangostin from the pericarp of the mangosteen fruit.

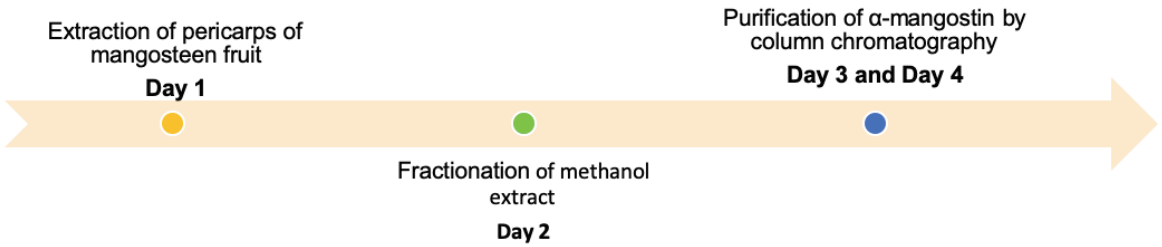

Figure 2. Workflow of recovery of α-mangostin from the pericarps of mangosteen fruit.

Materials and Methods

Extraction of pericarps of mangosteen fruit

Materials:

Dried pericarp powder

Analytical grade methanol

5L beaker

Hielscher ultrasonic homonizer

Rotary Evaporator

Whatmann filter paper (No. 4)

Procedures:

1. Pericarp powders were soaked with analytical grade methanol in the ratio of 1:10 (w/v) for one hour.
2. The extracts were decanted and filtered with Whatman filter paper.
3. The process was repeated three times and the solvent was removed by rotary evaporator to obtained yellowish powder.

Fractionation of methanol extract

Materials:

Analytical grade chloroform

Rotary evaporator

Liquid extraction funnel (1L)

Procedures

1. 30 g of methanol extract was reconstituted in water and further liquid-liquid extraction with equal volume of chloroform (250 ml).
2. The solvent was removed to obtain yellowish chloroform fraction.

PURIFICATION OF α-MANGOSTIN BY COLUMN CHROMATOGRAPHY

Materials:

N-hexane

Ethyl acetate

Column chromatography

15 ml test tube

α-mangostin standard

Silica gel 60-F254 plates (Merck No. 5544; Darmstadt, Germany)

Silica gel 60 (0.040-0.060 mm) (Merck No.9385; Darmstadt, Germany)

Bruker NMR AscendTM 400 instrument

High-resolution mass spectroscopy

High performance liquid chromatography

Procedures:

1. 100 g of silica gel was added with hexane to make into slurry and loaded into column chromatography and packed overnight.
2. 3 g of chloroform fraction was dissolved in (5 ml) of ethyl acetate and was loaded onto 100 g of column and eluted with 200 ml of hexane and 500 ml of

hexane-ethyl acetate mixtures in a sequent of 9:1 to 2:3.

3. The eluents were collected by test tube and the progress was monitored by TLC with the mobile phase on hexane-ethyl acetate (3:2) and compared with α-mangostin.

4. The eluents with similar Rf (single spot) value were combined and dried by rotary evaporator.

5. The sample was structural confirmed by comparison of the mass, ^{1}H, ^{13}C NMR with standard.

Results and Discussion

The yield of α-mangostin is 9.2 g/kg of dry weight (DW) or 0.92 % of DW. The total cost of producing 1 g of α-mangostin is about RM 1541 taking into consideration of solvent used and salary for research personnel in year 2020. Table 1 shows ^{1}H and ^{13}C chemical signals of α-mangostin. The signal at δ 3.79 representing methoxy group, signal at δ 5.29 corresponding to olefinic methane, signal in the range of δ 6.30–6.84 corresponding to aromatic group, signal in the range δ 3.44–4.13 corresponding to methylene group, signal in the range of δ 1.70–1.88 corresponding to methyl group, and δ 13.79 representing hydroxyl group (Figure 3). The ^{13}C NMR spectrum (Figure 4) of α-mangostin has twenty-four carbon signals, inclusive of one methoxy, two methylene, four methine, four methyl and thirteen quaternary carbons. In addition, the molecular formula of α-mangostin was $C_{24}H_{26}O_6$ and the mass was mass for α-mangostin was 411.1821 in positive mode.

Table 1. ^{1}H-NMR and 13C Spectra data for α-mangostin (400 MHz, deuterated chloroform $CDCl_3$).

Hydrogen	ppm	Carbon	Present study
3	5.29	2	132.13
4	3.44	3	123.18
10	6.84	4	26.54
12	6.30	4a	110.50
1’	4.10	5	160.56
2’	5.28	5a	101.64
4’	1.88	6	182.00
5’	1.84	6a	108.60
2-CH_3	1. 77	7	137.03
	1.70	8	142.60
8-OCH_3	3.79	9	157.00
5-OH	13.79	10	103.74
		10a	155.90
		11a	155.00
		12	93.30
		12’	161.67
		1’	21.44
		2’	123.18
		3’	132.13
		4’	17.92
		5’	25.84
		2-CH_3	18.23
			25.90
		8-OCH_3	61.97

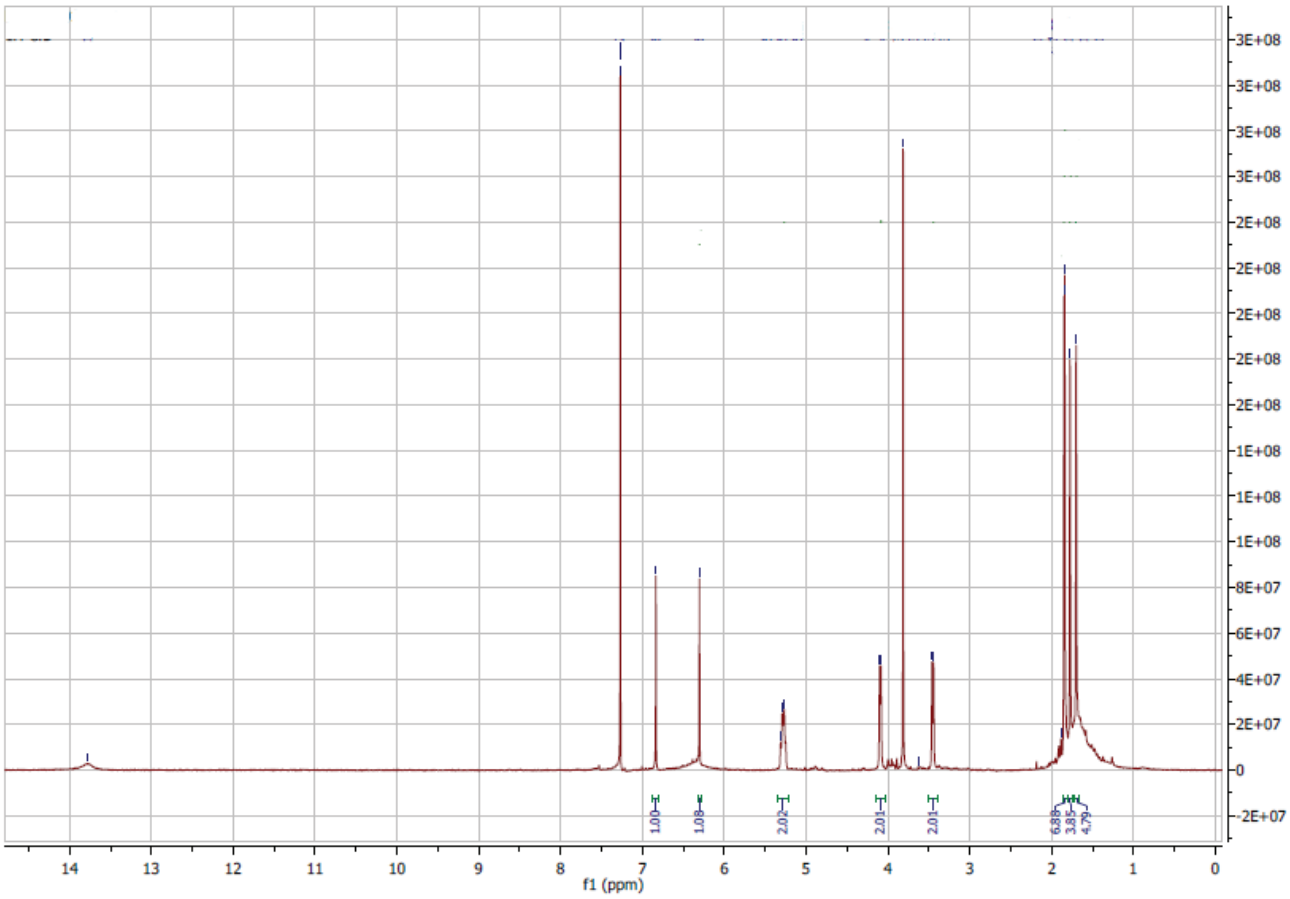

Figure 3. [1]H NMR spectrum of α-mangostin

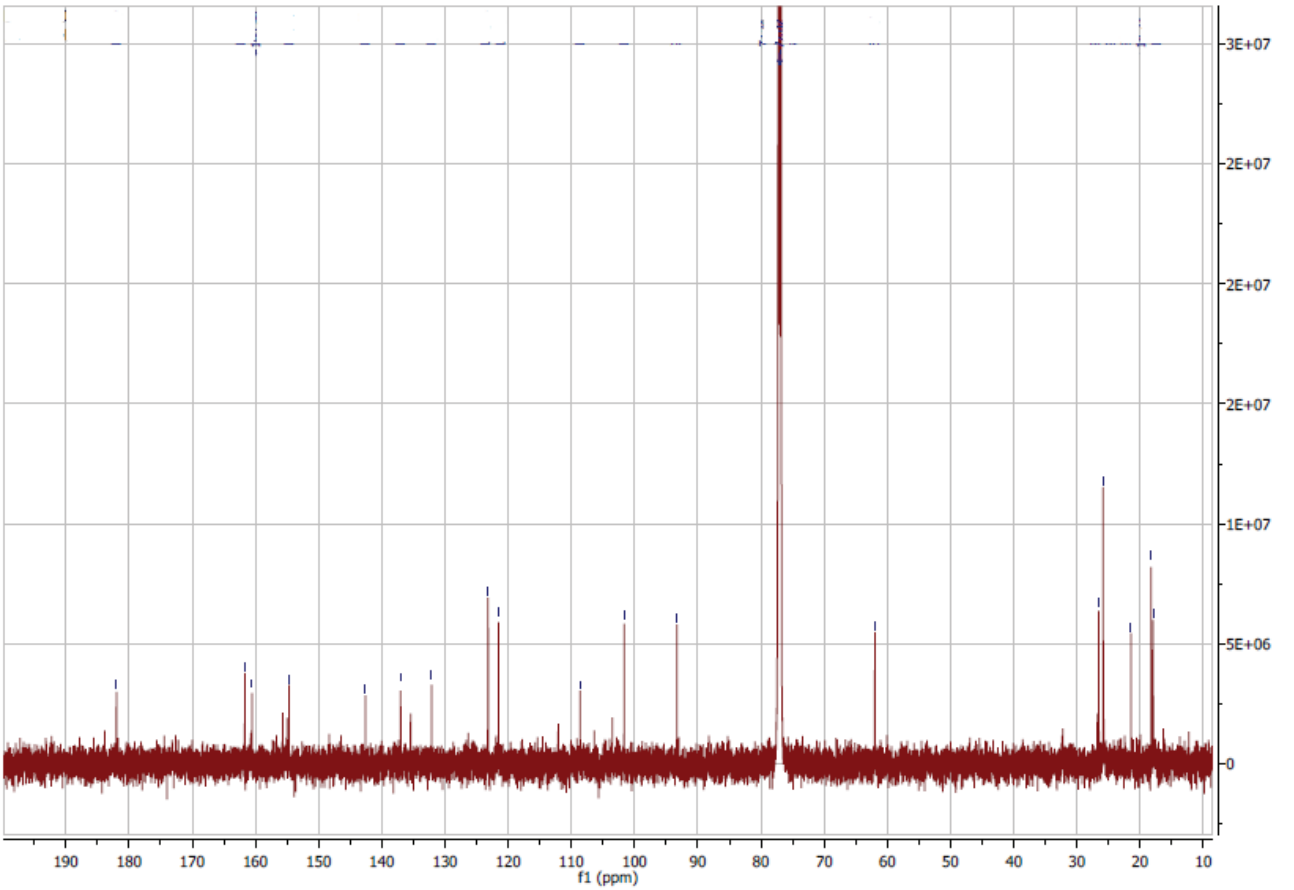

Figure 4. [13]C NMR spectrum of α-mangostin

Summary

In conclusion, a simple and cost-effective method has been developed to extract and purify α-mangostin which can be used for future biodiscovery and drug development.

Ethics statement

Not applicable.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

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