

แมงจิเฟอรินในใบมะม่วงไทยสามสายพันธุ์

Mangiferin in Leaves of Three Thai Mango (*Mangifera indica* L.) Varieties

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บทคัดย่อ

มะม่วงเป็นแหล่งหนึ่งของสารแมงจิเฟอรินซึ่งเป็นสารที่มีฤทธิ์ทางชีวภาพ โครงสร้างเคมีเป็นแซนโทนไกลโคไซด์ ในการศึกษาเป็นการแยกสารแมงจิเฟอรินจากส่วนใบของมะม่วง 3 สายพันธุ์ที่ปลูกในประเทศไทย ได้แก่ พันธุ์น้ำดอกไม้ (N) พันธุ์เขียวเสวย (K) และพันธุ์แก้ว (G) โดยใช้ เมทานอล เอทานอลและสารละลาย 70% อะซีโตน เป็นตัวทำละลายในการสกัด การสกัดโดยใช้เมทานอลเป็นตัวทำละลายให้สารแมงจิเฟอรินจากใบมะม่วงทั้ง 3 สายพันธุ์ ออกมาในปริมาณสูงที่สุด (2.80 %w/w, N; 2.40 %w/w, K; 1.30 %w/w, G) ตามด้วยการสกัดโดยใช้เอทานอลและสารละลาย 70% อะซีโตน เป็นตัวทำละลาย ตามลำดับ และมะม่วงน้ำดอกไม้เป็นสายพันธุ์ที่ให้สารแมงจิเฟอรินออกมาในปริมาณมากที่สุด วิธีการแยกสารแมงจิเฟอรินที่ใช้ในการศึกษานี้ เป็นวิธีที่สามารถกำจัดเมดสีและสารปนเปื้อนอื่นๆ ได้โดยอาศัยขั้นตอนที่ไม่สลับซับซ้อนและราคาถูก ซึ่งประกอบไปด้วยการหมัก การแยกสลายด้วยน้ำและกรด การใช้ตัวทำละลายอินทรีย์เพื่อแยกส่วน และการตกผลึกซ้ำ

คำสำคัญ: แมงจิเฟอริน แซนโทนไกลโคไซด์ มะม่วง แมงจิเฟอริน อินดิคา ลินน์ วงศ์มะม่วง.

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Abstract

Mango is a rich source of biological active compound, mangiferin that is a C-glycosyl xanthone structure. In the present study, mangiferin was isolated from leaves of three mango varieties cultivated in Thailand including: Nam Doc Mai (N), Keow Savoey (K), and Gaew (G). Methanol, ethanol and 70% acetone were used as extraction solvents. Extraction with methanol yielded the highest amount of mangiferin from all

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of 3 varieties (2.80 %w/w, N; 2.40 %w/w, K; 1.30 %w/w, G), followed by extraction with ethanol and 70% acetone, respectively. And Nam Doc Mai was the variety that provided largest amount of mangiferin. The isolation method for preparing mangiferin in this study could eradicate pigments and other impurities with unsophisticated and inexpensive processes comprising maceration, acid hydrolyzation, partition with organic solvent, and recrystallization.

Keywords : Mangiferin, C-glycosyl xanthone, mango, *Mangifera indica* L., Anacardiaceae

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Introduction

Mango (*Mangifera indica* L), the plant classified in family Anacardiaceae, is cultivated in many tropical and subtropical regions for commercial fruit production and as a garden tree. There are over a thousand mango varieties around the world (Bally, 2006). The ripe and unripe fruits are recognized and eaten throughout the world. In addition, mango has also been used as traditional medicine i.e. a bark infusion used in Samoa for mouth infections in children, mango leaves used as a remedy for treatment of relapse sickness in Tonga (Odyek et al., 2007), in India (Scartezzini et al., 2000), unripe fruit taken as a remedy for exhaustion and heat stroke, half-ripe fruit used for a treatment of gastrointestinal disorders, bilious disorders, blood disorders and scurvy, fresh leaves for treatment of diabetes, dried seed ground into flour for treatment of diarrhea, and bark extract for treatment of diarrhea and throat disorders (Bally, 2006).

Phytochemical studies on various parts of *M. indica* L. revealed that it contains phenolic acids, phenolic esters, flavonols and the C-glycosyl xanthone, mangiferin (Andreu et al., 2005; Hernandez et al., 2007; Ribeiro et al., 2008; Ling et al, 2009). Mangiferin (2-beta-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthen-9-one; Fig. 1) has significant pharmacological properties including antidiabetic (Garcia et al, 2003; Miura et al., 2001), antioxidant (Garrido et al., 2004; Sanchez et al, 2000; Martinez et al, 2001), antitumor (Leiro et al.,

2003; Sarkar et al., 2004; Yoshimi et al, 2001), vascular modulatory (Beltran et al, 2004), immunomodulatory, and antiviral activities (Dar et al., 2005; Nong et al., 2005; Ribeiro et al., 2008). There are quite a number of mango varieties cultivated in Thailand. Among them, the variety Nam Doc Mai, Keow Savoey, and Gaew have been generally grown. Therefore, they are the interesting sources of a pharmacologically active compound, mangiferin.

The present study is to isolate mangiferin from the leaves of these three mango varieties using different extraction solvents and the isolated mangiferin identified by thin layer chromatography and UV spectroscopy. Furthermore, comparison of the quantification of the C-glycosyl xanthone, mangiferin, obtained from each mango variety was performed in the study.

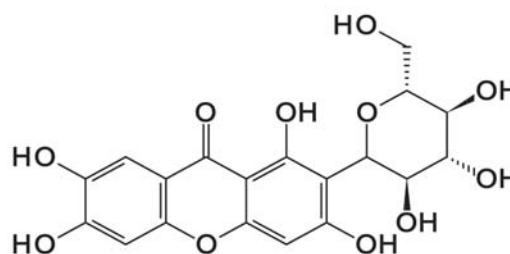


Figure 1 Mangiferin

Methods

1. Reagents

Analytical grade methanol, ethanol, acetone, dichloromethane and ethyl acetate were used as solvents for extraction and isolation. Mangiferin from *Mangifera indica* L. stem bark was purchased from SIGMA-ALDRICH (Cat. No. M3547, USA).

2. Plant materials

The leaves of mango variety Nam Doc Mai, Keow Savoey, and Gaew were collected in May, 2008 from Kram sub-district, Klaeng district, Rayong province, Thailand. After cleaning by rinsing with tap water, the leaves were dried at 40–50°C in an oven. Then, they were ground to powder with a cutting mill and the powdered leaves were stored at room temperature in dark and dry place.

3. Instrumentation

The extract solutions were concentrated with a vacuum rotary evaporator (Buchi Rotavapor® model R-210, Switzerland). The magnetic stirrer with heating (Heidolph® model MR 3001, Germany) was used in hydrolysis process. A UV/VIS spectrophotometer (JASCO® model V-630, Japan) was used in characterization of isolated compound compared to a reference standard with the wavelength range of 200–400 nm. In addition, identification of isolated compound using Thin Layer Chromatography (TLC) analysis was performed on silica gel 60 F254 TLC aluminium sheets (Merck® Cat. No. 105554, Germany) and cellulose TLC plates (Merck® Cat. No. 105716, Germany)

4. Isolation of mangiferin

Each 100 g of the powdered leaves of mango variety, Nam Doc Mai, Keow Savoey, and Gaew, was subjected to extract by maceration with 500 mL of three different extraction solvents including methanol, ethanol and 70% acetone. The maceration was made up at room temperature and the extraction solvents were changed every week for 3 weeks. Each extract was filtered and evaporated at 40°C in vacuum until

dryness to obtain the thickening mass of methanol extracts (Nam Doc Mai, NM; Keow Savoey, KM; Gaew, GM), ethanol extracts (Nam Doc Mai, NE; Keow Savoey, KE; Gaew, GE), and acetone extracts (Nam Doc Mai, NA; Keow Savoey, KA; Gaew, GA). The dried methanol extracts of each mango variety (NM, KM, and GM) were resuspended in 50 mL of 50% methanol then partitioned with 100 mL dichloromethane for 4 times. The aqueous methanolic phases each were hydrolyzed by reflux with 2N sulfuric acid at pH 3 for an hour with continuous stirring. After cooled to room temperature, it was partitioned with 100 mL ethyl acetate for 3 times. Subsequently, the combined ethyl acetate layer was dried at 40°C using a vacuum rotary evaporator. The dried ethyl acetate fraction was dissolved in methanol and left in a refrigerator (4–8°C) over night. After that the precipitate came out and was isolated by filtration. For crystallization, the precipitate was dissolved in 70% aqueous methanolic solution and left in a refrigerator (4–8°C) over night. Lastly, the pale yellow needle-shaped crystals of mangiferin were isolated and dried. The dried ethanol extracts (NE, KE, and GE) each were resuspended in 50 mL of 50% ethanol then partitioned with 100 mL dichloromethane for 4 times. The aqueous ethanolic phases each were subjected to hydrolyze and partition with ethyl acetate followed by the same processes as present above until the pale yellow mangiferin was obtained. Likewise, the dried acetone extracts (NA, KA, and GA) each were resuspended in 50 mL of 50% acetone then partitioned with 100 mL dichloromethane for 4 times and the aqueous acetone phases each were subjected to hydrolyze followed by the same processes as present above until the pale yellow mangiferin was obtained. The isolated mangiferins from each extract (NM, KM, GM, NE, KE, GE, NA, KA, and GA) were further characterized using TLC and UV/VIS spectrophotometry scanning compared to reference standard mangiferin.

5. Identification of the isolated mangiferins by TLC and UV spectroscopy

Identification of the isolated mangiferins obtained from each mango variety and isolation method were performed on TLC analysis compared to reference standard mangiferin. Silica gel 60 F254 was used as an adsorbent for normal phase and two solutions of ethyl acetate: acetone: formic acid: water (8:2:1:1) and ethyl acetate: methanol: formic acid: water (8:2:1:1) were used as developing solvents. Furthermore, cellulose was used as an adsorbent for reverse phase and the solution of ethyl acetate: formic acid: water (67:13:20) as a developing solvent. The plates were detected by spraying with sulfuric acid (10% in methanol) and heat at 110°C for 10 minutes. In addition, spectral scanning of the isolated mangiferins was performed at the wavelength range of 200–400 nm. The isolated mangiferins were prepared at concentration of 40 µg/mL by dissolving in methanol. Comparison of the spectrums between isolated mangiferins and its reference standard were also reported.

Results

The results from the present study showed that the extraction of 100 g of the powdered leaves of three mango varieties, Nam Doc Mai (N), Keow Savoey (K), and Gaew (G) using three different extraction solvents including methanol (M), ethanol (E), and 70% acetone (A) yielded 12.30, 11.85, and 8.72 g of the methanol extracts, 8.04, 6.36, and 7.21 g of the ethanol extracts and 33.18, 27.01, and 24.90 g of 70% acetone extracts, respectively. And the compounds isolated from each crude extract were pale yellow powder. The amount of these compounds obtained from each variety and each isolation method were reported in table 1. Three extraction solvents used in this study were methanol, ethanol, and 70% acetone. When using methanol as an extraction solvent, Nam Doc Mai gave the largest

amount of isolated compound (2.80 g) followed by Keow Savoey (2.40 g) and Gaew (1.30 g), respectively. When using 70% acetone as an extraction solvent, the sequence of the amount of isolated compounds from highest to lowest amounts were Nam Doc Mai (0.66 g), Keow Savoey (0.15 g), and Gaew (0.13 g), respectively. While using ethanol as an extraction solvent, the sequence from highest to lowest amounts were Nam Doc Mai (1.00 g), Gaew (0.90 g), and Keow Savoey (0.30 g), respectively.

TLC analysis of the isolated compounds from leaves of all mango varieties used in the study showed the relative factor (R_f) values range from 0.42 to 0.44 which were closely to R_f value of reference standard mangiferin (0.43) when using silica gel 60 F254 as an adsorbent and solution of ethyl acetate: acetone: formic acid: water (8:2:1:1) as developing solvent. While the R_f values of the isolated compounds were range from 0.61 to 0.63 and the R_f value of reference standard mangiferin was 0.61 when developing with solution of ethyl acetate: methanol: formic acid: water (8:2:1:1). Furthermore, the R_f values of the isolated compounds ranged from 0.51 to 0.53 when using cellulose as an adsorbent and solution of ethyl acetate: formic acid: water (67:13:20) as developing solvent and the R_f value of reference standard mangiferin was 0.51 (Table 2). The UV spectrums of isolated compounds and reference standard, mangiferin, in methanol showed three major peaks which were as follows: 263, 315, 364 (NM); 262, 314, 365 (KM); 261, 315, 364 (GM); 266, 315, 364 (NE); 264, 315, 364 (KE); 264, 315, 364 (GE); 266, 315, 365 (NA); 266, 315, 364 (KA); 265, 315, 364 (GA); 263, 315, 364 (reference standard mangiferin) displayed in Figure 2.

Tables 1 The amount of mangiferin and crude extract obtained from 100 g of dry powdered leaves of three mango varieties using different extraction solvents

Mango variety	Extraction solvent					
	MeOH		EtOH		70% acetone	
	mangiferin (g)	crude extract (g)	mangiferin (g)	crude extract (g)	mangiferin (g)	crude extract (g)
Nam Doc Mai	2.80	22.76	1.00	12.44	0.66	1.99
Keow Savoey	2.40	20.25	0.30	4.72	0.15	0.55
Gaew	1.30	14.91	0.90	12.48	0.13	0.52

Tables 2 The relative factor (Rf) values of the isolated compounds and reference standard mangiferin

TLC condition	Rf values of isolated compounds from									
	NM ¹	KM ²	GM ³	NE ⁴	KE ⁵	GE ⁶	NA ⁷	KA ⁸	GA ⁹	MF ¹⁰
Adsorbent:										
Silica gel 60 F254	0.43	0.43	0.42	0.44	0.44	0.44	0.43	0.43	0.43	0.43
Developing solvent:										
EtOAc: (CH ₃) ₂ CO: HCOOH: H ₂ O (8:2:1:1)										
Adsorbent:										
Silica gel 60 F254	0.61	0.61	0.61	0.61	0.61	0.61	0.61	0.63	0.62	0.61
Developing solvent:										
EtOAc: MeOH: HCOOH: H ₂ O (8:2:1:1)										
Adsorbent:										
Cellulose	0.53	0.53	0.53	0.51	0.51	0.51	0.51	0.51	0.51	0.51
Developing solvent:										
EtOAc: HCOOH: H ₂ O (67:13:20)										

¹ Nam Doc Mai methanol extract, ² Keow Savoey methanol extract, ³ Gaew methanol extract,⁴ Nam Doc Mai ethanol extract, ⁵ Keow Savoey ethanol extract, ⁶ Gaew ethanol extract,⁷ Nam Doc Mai 70% acetone extract, ⁸ Keow Savoey 70% acetone extract, ⁹ Gaew 70% acetone extract,¹⁰ reference standard mangiferin

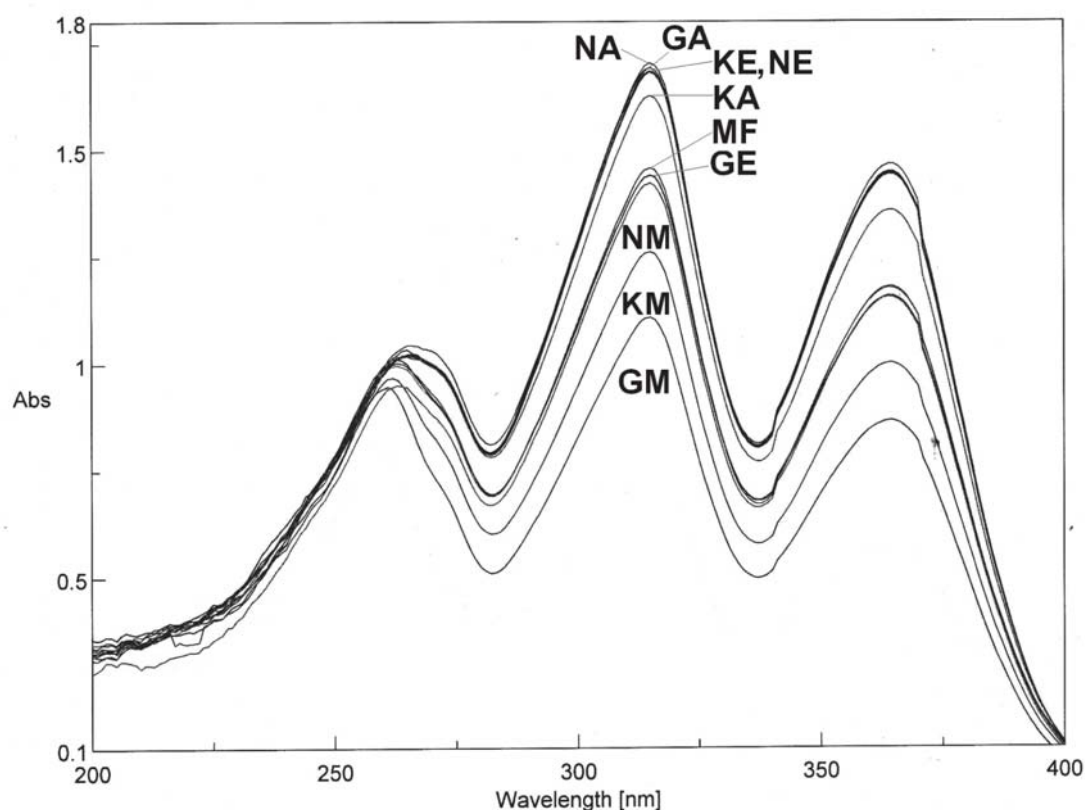


Figure 2 The UV spectrum (in MeOH) of isolated compounds and reference standard mangiferin.

NM = Nam Doc Mai methanol extract

KM = Keow Savoey methanol extract

GM = Gaew methanol extract

NE = Nam Doc Mai ethanol extract

KE = Keow Savoey ethanol extract

GE = Gaew ethanol extract

NA = Nam Doc Mai 70% acetone extract

KA = Keow Savoey 70% acetone extract

GA = Gaew 70% acetone extract

MF = reference standard mangiferin

Discussion and Conclusion

The present study demonstrated that the leaves of three mango varieties cultivated in Thailand including: Nam Doc Mai, Keow Savoey, and Gaew were interesting sources of the pharmacologically active C-glycosyl xanthone, mangiferin. The compound could be isolated using an uncomplicated method modified from Svetlana V. Rusakova's method, which used for preparing mangiferin from plants of the genus of *Hedysarum* (*H. alpinum* L. and *H. flavescens* Rgl. et Schmalh). Methanol, ethanol and 70% acetone were used as extraction solvents in macera-

tion process to obtain crude extracts. Partitioning with dichloromethane to eradicate some pigment matters and non-polar compounds contained in the plant then purified them. Consequently, hydrolyzation was achieved at pH 3 to remove some impurities such as O-glycosides before partitioning with ethyl acetate. After dryness the dried ethyl acetate fraction was dissolved in methanol and left at cool temperature (4–8°C) over night. Accordingly mangiferin precipitated. Finally, the precipitated mangiferin was recrystallized in 70% aqueous methanolic solution and left in a refrigerator (4°C) over night and the pale

yellow needle-shaped crystals of mangiferin was isolated and dried. It showed identical TLC chromatogram and UV spectrum to reference standard mangiferin. From the percentage amount of isolated mangiferin, methanol was the best solvent for maceration and variety Nam Doc Mai was a good source of mangiferin.

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