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Original Article

Mangiferin content in different parts of mango tree (*Mangifera indica* L.) in Thailand

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Abstract

Mangiferin is a xanthone glucoside that possesses a broad range of therapeutic uses and has no reports of adverse effects. It is abundant in the leaves and stems of the mango tree (*Mangifera indica* L.). In order to evaluate and standardize plant raw materials containing mangiferin and their extracts, the high-performance liquid chromatography analytical method was developed to analyze mangiferin content in *M. indica* extracts. Separation was carried out on a Hypersil BDS C-18 column using 0.5% acetic acid in water and methanol (75:25, v/v) as the mobile phase. Various extracting solvents were employed to optimize the mangiferin yield from the *M. indica* leaves using sonication. The results showed that 70% methanol in water was the optimal solvent for mangiferin extraction. Mangiferin quantification in various parts of the *M. indica* collected from different locations of Thailand was performed. Our results indicated the optimal solvent, part, and stage of *M. indica* raw materials for further development.

Keywords: *Mangifera indica*, mangiferin, xanthones, HPLC, standardization, quantitative analysis

1. Introduction

Mangiferin is a natural *C*-glucoside xanthone [2-*C*-*β*-D-glucopyranosyl-1,3,6,7-tetrahydroxyxanthone] (Figure 1). It is a major component found in many species. The mango tree (*Mangifera indica* L) is one of the primary sources. It is reported to possess a broad range of therapeutic uses (Jyotshna, Khare, & Shanker, 2016). The polyphenols have potent free radical scavenging activity and an antioxidant effect as well as other beneficial biological properties that include anti-inflammatory, anti-oxidative, immunomodulatory, anti-obesity, anti-bacterial, anti-viral, anti-depression, and anti-diabetic effects (Ganogpichayagrai, Palanuvej, &

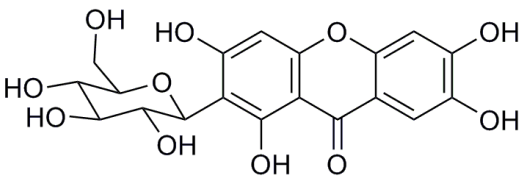

Figure 1. Chemical structure of mangiferin.

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Ruangungsi, 2017; Gong *et al.*, 2013; Hirwani, 2013; Imran *et al.*, 2017; Jyotshna, Khare, & Shanker, 2016; Marquez, Garcia-Bueno, Madrigal, & Leza, 2012; Pardo-Andreu *et al.*, 2008; Telang, Dhulap, Mandhare, & Ramirez *et al.*, 2017; Zhao *et al.*, 2017). Mangiferin supplementation could improve serum lipid profiles by reducing serum triglycerides and free fatty acids in overweight patients with hyperlipidemia (Na *et al.*, 2015). It also modulated key enzyme expression involved in inhibiting lipogenesis and promoting fatty acid oxidation in the liver (Guo *et al.*, 2011; Niu *et al.*, 2012). Moreover, mangiferin showed anti-diabetic action through activation of mammalian dehydrogenase complex, which ultimately increased insulin sensitivity (Apontes *et al.*, 2014; Jyotsha, Khare, & Shanker, 2016). It also decreased insulin resistance in KK-Ay mice, an animal model of type-2 diabetes, by lowering the glucose level after oral administration with no effect on the blood glucose level in normal mice (Miura *et al.*, 2001).

Mango is now commercially grown in more than 87 countries with an area of approximately 3.7 million hectares worldwide (Masud Parvez, 2016). Every part of the mango tree, including leaves, bark, fruit, juice, seed, and kernel, has been used for centuries in traditional medicines (Shah, Patel, Patel, & Parmar, 2010). Mangiferin is present mostly in the leaves and stems. It has been used in therapeutic and cosmetic applications with no adverse effects so far (Telang, Dhulap, Mandhare, & Hirwani, 2013; Yalena *et al.*, 2015; Zhang *et al.*, 2014a). To date, the chemical synthesis of mangiferin has been scarcely reported and the other viable methods provided extremely low yields (Bhatia & Seshadri, 1968; Ehianeta, Laval, & Yu, 2016; Nott & Roberts, 1967; Wu, Wei, Lian, & Yu, 2010). Therefore, the primary sources of mangiferin have been the mango tree and other natural sources.

Standardization of raw materials containing mangiferin and their extracts is needed for pharmaceutical and phytochemical quality controls. The assurance test using a validated analytical method is necessary. Several analytical techniques for mangiferin quality assurance have been developed such as high-performance liquid chromatography (HPLC) (Barreto *et al.*, 2008; Gowda, Kumar, Paghal, & Rajshree, 2010; Naveen, Lingaraju, & Prasad, 2017; Zhang *et al.*, 2014b), liquid chromatography technique coupled with tandem mass spectrometry (Cai *et al.*, 2014; Liu *et al.*, 2010), high-performance thin-layer chromatography densitometric assay (Jyotshna, Srivastava, Killadi, & Shanker, 2015; Khurana, Rao, Beg, Katare, & Singh, 2016), capillary zone electrophoresis (Nong, He, Flemning, Pan, & Huang, 2005), and enzyme-linked immunosorbent assay (Yusakul, Kitirattrakarn, Tanwanichkul, Tanaka, & Putalun, 2012). However, a simple and rapid method is required. Therefore, this current research studied and optimized the extracting solvents which are the crucial factors affecting the efficiency of mangiferin extraction. The HPLC method in this study was validated and applied for the quantification of mangiferin in various parts of the *M. indica* that were collected from different locations in Thailand. Our study provided a basis for rich sources of mangiferin, quality assessment, and standardization for further development of phytopharmaceutical products.

2. Materials and Methods

2.1 Plant materials

Young and old leaves, twigs, and ripe and unripe fruits of *M. indica* fresh samples were collected from different locations in Thailand that included Chiang Mai (1-3), Chiang Rai (1-3), Lamphun (1-2) and Uttaradit provinces in northern Thailand and Samut Sakhon Province in central Thailand. Identification was done by the authors by comparison with the authentic samples. Voucher specimens (SKMG001-010) were deposited at Drug Discovery and Development Center, Thammasat University (Rangsit Campus), Thailand. The samples were washed by tap water, dried at 50 °C using a hot air oven, cut into small pieces, and ground into powder using an electronic mill. The samples were kept in air-tight plastic bags at room temperature until use.

2.2 Chemical and reagents

HPLC grade methanol and acetic acid were purchased from RCI Labscan Limited (Bangkok, Thailand). Mangiferin standard was purchased from Sigma-Aldrich Co. LLC. (MO, USA). Deionized water was purified by Ultra Clear, Siemen Water Technologies Corp (Erlangen, Germany). All reagents were of analytical grade unless stated otherwise.

2.3 HPLC apparatus and conditions

HPLC was performed on an Agilent 1260 Series (Agilent Technologies) equipped with a 1260 Quat pump VL quaternary pump, 1260 ALS autosampler, 1260 TCC column thermostat and 1260 DAD VL diode array detector. The separation was done on a Hypersil BDS C_{18} column (4.6 × 100 mm i.d., 3.5 µm) with a C_{18} guard column. The elution was performed on isocratic solvent system using a mixture of 0.5% acetic acid in water and methanol (75:25, v/v). The flow rate was set at 1.0 mL/min with controlled temperature at 25 °C. The diode array detector was set at the wavelength of 258 nm and the injection volume was 10 µL.

2.4 Stock and working solutions of standard compounds

A standard stock solution of mangiferin was prepared by dissolving an accurately weighed amount (5.0 mg) of mangiferin standard in 5.0 mL of methanol-water (50:50, v/v). Working standard solutions were obtained by appropriate dilution of the stock solution with methanol-water (50:50, v/v).

2.5 Investigation of suitable solvent for extraction

Water, methanol, different ratios of water and methanol, ethanol, and tetrahydrofuran were evaluated as the extracting solvents to obtain the highest mangiferin content from *M. indica* extraction. Dried *M. indica* leaves (7.5 mg) were accurately weighed and separately extracted with 5 mL

of each solvent by sonication at the ambient temperature. Each extract was prepared and analyzed five times by HPLC. The solvent yielding the highest content of mangiferin in the extract was chosen as the appropriate solvent for mangiferin extraction.

2.6 Preparation of the plant sample solutions

Dried powders from each of the young and old leaves, twigs, and ripe and unripe fruits of *M. indica* were extracted with methanol-water (70:30, v/v), which was the most efficient solvent for mangiferin extraction. Sonication was used to assist the extraction for 30 min and each sample was done in five replicates. Prior to the injection and analysis by HPLC, all extracts were filtered through a 0.22 µm nylon membrane filter.

2.7 Method validation

Validation of the method was done according to the International Conference on Harmonization guideline (International Conference on Harmonization [ICH], 1996/2005). The method was validated for linearity, precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ).

2.7.1 Linearity

Linearity of the method was determined by injecting seven known concentrations of the mangiferin standard in the range of 7-500 µg/mL in triplicate. The calibration curves were obtained by plotting the peak areas versus the amounts of the standard.

2.7.2 Precision

Measurements of intra- and inter-day precisions were done by analysis of 100 µg/mL of the mangiferin standard solution. The intra-day precision was determined by analysis of seven injections within 1 day while the inter-day precision was determined using the same method as intra-day for three consecutive days. The precision was expressed as a percentage of relative standard deviation (% RSD).

2.7.3 Accuracy

Recovery was used to evaluate the accuracy of the method. Standard addition was performed with a pre-analyzed standard solution. Three different levels of standard mixtures were added to the sample extracts. Spiked samples were prepared in triplicate. Recovery was calculated as follows: recovery (%) = 100 x (detected amount – original amount) /spiked amount.

2.7.4 Limit of detection (LOD) and limit of quantitation (LOQ)

Determination of signal-to-noise ratio was performed by comparing signals from samples with known low concentrations of the analytes with those of blank samples and establishing the minimum concentration at which the analyte could be reliably detected under the proposed chromatographic condition. A concentration of the analyte which established a signal-to-noise ratio of 3:1 was considered as the LOD and 10:1 as the LOQ.

2.8 Statistics analysis

SPSS statistics software version 24 was used to calculate the statistical significance ($P<0.05$) of defined groups using paired t-test. Values are expressed as mean±SD of five replicates. Bars labeled with different letters are significantly different.

3. Results and Discussion

3.1 HPLC method development

The HPLC method was developed to analyze the mangiferin content in the young and old leaves, twigs, and ripe and unripe fruits of *M. indica* extracts. From various mobile-phase trials, an isocratic solvent system using a mixture of 0.5% acetic acid in water and methanol (75:25, v/v) was the optimal condition. It provided symmetrical peaks and the most efficient separation and speed. The maximum absorbance of mangiferin at 258 nm was used for wavelength detection. The chromatogram of different parts of *M. indica* extracts and mangiferin standard compound is shown in Figure 2.

3.2 Appropriate solvent for mangiferin extraction from *M. indica* leaves

Optimized extraction is the crucial step in quantitative analysis. Selection of a suitable solvent is an essential part for recovery of desired components from a complex matrix. In this study, water, methanol, different ratios of water and methanol, ethanol, and tetrahydrofuran were tested to optimize mangiferin extraction from *M. indica* leaf samples. Sonication was chosen as an extraction method because it is simple, rapid, and compatible with various solvents. After quantification by HPLC, the highest mangiferin content was obtained from the 70% methanol extract (Table 1). Thus, the mixture of methanol-water (70:30, v/v) was chosen as a suitable solvent for extraction.

3.3 Method validation

Method validation was performed according to the ICH guideline (ICH, 1996/2005) to ensure that the method was suitable for its intended use. Linearity, precision, accuracy, LOD, and LOQ were examined. The calibration curve was constructed from the peak area versus the concentration of the standards and showed that the developed method was linear across the range of 7.8-504 µg/mL with a correlation coefficient (r^2) of 0.9999 (Table 2). Precision of the method was studied using the 100 µg/mL standard solution. Percent relative standard deviation (% RSD) values lower than 2% showed the acceptable precision of the method (Table 2). Specificity of the method was assessed by peak purity using the UV spectrum obtained from the diode array detector. The accuracy of the method was determined by the recovery values, which were in the range of 100.0-100.8%

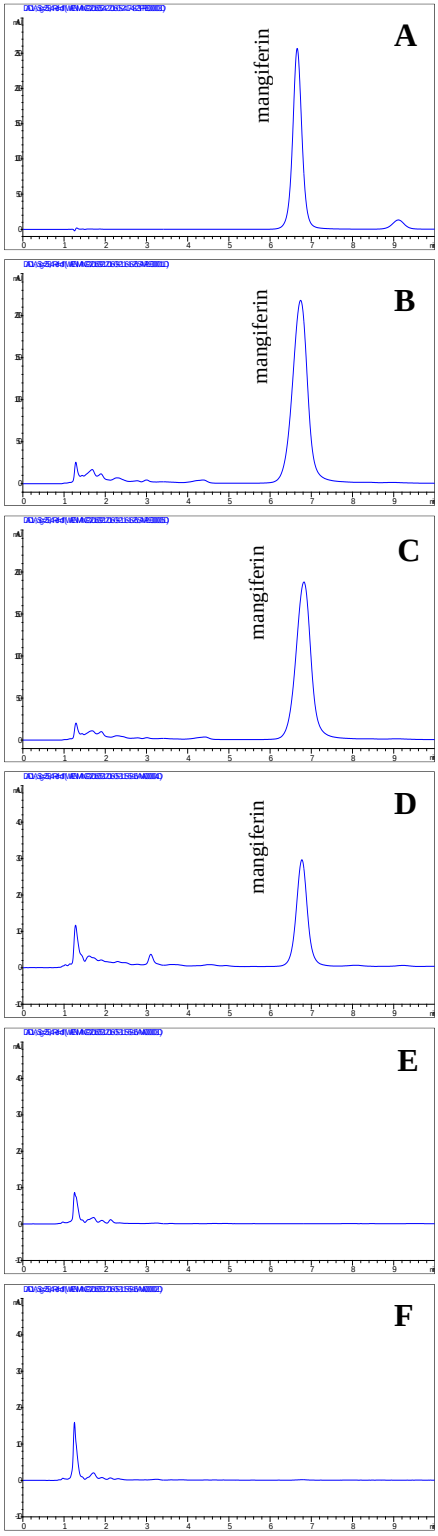

Figure 2. HPLC chromatograms of (A) standard, (B) young leaves of *M. indica* (C) old leaves of *M. indica*, (D) twigs of *M. indica*, (E) ripe fruit of *M. indica*, and (F) unripe fruit of *M. indica* extracts. Peak identification: t_R 6.8 min = mangiferin.

(average 100.5%) (Table 3). The LOD and LOQ, determined by a signal-to-noise ratio of 3:1 for LOD and 10:1 for LOQ, were 0.15 and 0.50 µg/mL, respectively.

Table 1. Mangiferin contents in dried leaves of *M. indica* obtained from various extracting solvents.

Extracting solvent	Mangiferin Content (% w/w)
Water	5.86±0.26 [c]
Methanol : Water (30:70, v/v)	6.62±0.58 [d]
Methanol : Water (50:50, v/v)	6.93±0.35 [d]
Methanol : Water (70:30, v/v)	7.45±0.12 [e]
Methanol	3.17±0.25 [b]
Ethanol	2.97±0.11 [b]
Tetrahydrofuran	1.87±0.16 [a]

Values are expressed as mean ± SD of five replicates. Statistical significance was calculated using paired t-test comparing all pairs of means ($P<0.05$). Different letters indicate significant differences.

Table 2. Validation of parameters for the quantification of mangiferin in *M. indica*.

Parameters	Results
Regression equation[a]	Y = 41.38X + 7.5967
Correlation coefficient (r^2)	0.9999
Linear range, µg/mL	7.8 – 504
Precision (% RSD)	
Intra-day	0.29
Inter-day	0.12
LOD, µg/mL	0.15
LOQ, µg/mL	0.50

[a] X is the concentration of mangiferin in µg/mL Y is the peak area at 258 nm.

Table 3. Recovery study of mangiferin.

Serial No.	Original amount (µg/mL)	Amount spiked (µg/mL)	Found[b] (µg/mL)	Recovery[a] (%)
1	61.02	32.22	93.26±0.38	100.02±0.39
2	61.02	62.03	123.90±0.78	100.70±0.63
3	61.02	93.39	155.60±0.78	100.78±0.50
Average				100.50

[a] Expressed as mean ± SD (n=3).

3.4 Analysis of mangiferin contents in various parts of *M. indica* from different locations

Quantification of mangiferin was performed in samples from various parts of the *M. indica* collected from 10 different locations of Thailand. The mangiferin contents are shown in Table 4. Young and old leaves were characterized by the color of the leaves which vary from light-green to slightly brownish or purplish when the plants are young and acquire a dark green as it develops and becomes mature. The contents of mangiferin in dried powders of young leaves, old leaves, and twigs of *M. indica* ranged from 4.82% to 8.85% (average 6.78%), 3.32% to 7.78% (average 5.89%), and

Table 4. Contents of mangiferin in various parts of *M. indica* collected from different locations in Thailand.

Samples	Mangiferin content (% w/w)				
	Young leaves	Old leaves	Twig	Ripe fruit	Unripe fruit
Samuth Sakhon	5.69± 0.45***	3.32± 0.46**	0.69±0.08*	ND	ND
Uttaradit	6.79± 0.21**	-	1.65±0.04 *	ND	ND
Chiang Mai 1	8.85± 0.69***	7.78± 0.16**	3.48±0.57*	-	-
Chiang Mai 2	7.50± 0.25***	4.32± 0.14**	3.29±0.23*	-	-
Chiang Mai 3	4.82± 0.08***	4.34± 0.49**	2.22±0.17*	-	-
Chiang Rai 1	8.02± 0.21***	6.88± 0.26**	2.00±0.17*	-	-
Chiang Rai 2	8.50± 0.20***	6.29± 0.33**	2.16±0.16*	-	-
Chiang Rai 3	6.51± 0.31***	4.61± 0.66**	3.02±0.19*	-	-
Lamphun 1	5.38± 0.08***	4.38± 0.20**	3.09±0.07*	-	-
Lamphun 2	7.80± 0.12***	5.35± 0.16**	2.74±0.21*	-	-
Average	6.78± 1.76***	5.89± 1.41**	2.75±0.56*	-	-

ND = not detected. - = data not available. Values are expressed as mean±SD of five replicates.
Statistical significance was calculated using paired t-test comparing means of mangiferin from various parts between each location ($P<0.05$). Different symbols indicate significant differences. ND=not detected.

0.69% to 1.65% (average 2.75%) w/w, respectively. Mangiferin could not be detected in the ripe fruit or unripe fruit in the two samples that were analyzed.

4. Discussion

HPLC is the analytical method of choice for pharmaceutical analysis. It is considered efficient for plant chemical compound qualitative and quantitative analysis. In this study, the HPLC method was developed for mangiferin determination in *M. indica* extracts. The method was modified from previously reported data (Naveen, Lingaraju, & Prasad, 2017; Zhang *et al.*, 2014b). Compared to the existing HPLC-based methods (Gowda, Kumar, Paghal, & Rajshree, 2010; Naveen, Lingaraju, & Prasad, 2017; Zhang *et al.*, 2014b), the method we developed was an acetonitrile-free isocratic solvent system which was economical, simple, rapid, and suitable for routine use. Sensitivity of the method in terms of LOD (0.15 µg/mL) and LOQ (0.50 µg/mL) was comparable to the previously reported methods (Naveen, Lingaraju, & Prasad, 2017; Zhang *et al.*, 2014b) which had LOD values in the range of 0.21-0.48 µg/mL and LOQ values in the range of 0.63-1.95 µg/mL. The optimized rapid isocratic system could minimize running time and costs. The method was validated and confirmed to be suitable for the intended use. The results showed that the analytical method was linear within the tested concentration range, precise, and accurate.

Extraction is a process that separates and obtains chemical constituents of plant materials. Many techniques, which may vary in cost, extraction time, and level of complexity, can be used to extract plant samples. Ruiz-Montanez *et al.* (2014) evaluated the efficiency of different extraction methods and suggested that ultrasonic-assisted extraction was an efficient method for extracting mangiferin and could be considered a low-cost technique with low instrument requirements. Therefore, ultrasonication was chosen as the extracting method in this study. From previous studies (Kulkarni & Rathod, 2014a, 2014b; Zou *et al.*, 2014), various parameters such as solvent polarity, frequency, input power, and extraction temperature could affect the extraction yield of mangiferin. Solvent polarity played the most important role in the efficiency of extraction. Thus, water, methanol, different ratios of water and methanol, ethanol, and tetrahydrofuran were tested to optimize mangiferin extraction from the *M. indica* leaf samples. Despite the previously reported condition using 44% ethanol (Zou *et al.*, 2014), our study showed that 70% methanol in water was the optimal solvent mixture for mangiferin extraction.

The applicability of the method was done in quantification of mangiferin in various parts of *M. indica* samples collected from 10 different locations of Thailand. The amounts of mangiferin from different parts of the *M. indica* in Thailand was comparable to the samples from Brazil (Barreto *et al.*, 2008), which has the same tropical climate as Thailand, but the amounts of mangiferin were much greater than the samples from China (Zhang *et al.*, 2014b), which has a colder climate than Thailand. Our results demonstrated that the mango leaves were the primary source of abundant mangiferin. Variations in the amounts of mangiferin in the samples from the various locations were possibly affected by the different environmental factors including soil, humidity, age, and climate. Our results appeared to be well in line with a previous study (Baretto *et al.*, 2008) that reported young leaves of the mango tree had a greater amount of mangiferin than the old ones. The mangiferin contents in young and old leaves appeared to be well in line with a previous report (Yusakul *et al.*, 2012). The HPLC method in the current study can be applied in routine analysis for standardization of mangiferin containing products and its raw materials. We also suggest that the preferred climate for *M. indica* cultivation is a tropical climate. This study presented the optimal conditions, solvent, part, and stage of mango raw material for the development of functional foods and cosmetic products.

5. Conclusions

In order to evaluate and standardize plant raw materials containing mangiferin and their extracts, the HPLC method was developed to analyze mangiferin content in *M. indica* extracts. The extracting solvent for maximum recovery of mangiferin extraction was optimized. The method showed high sensitivity, precision, and accuracy. Quantification of

mangiferin in various parts of the *M. indica* samples collected from 10 different locations of Thailand was performed. This method could be of benefit in the routine analysis for standardization of mangiferin containing products and its raw materials. This study indicated the optimal requirements, solvent, part, and stage of the mango raw material for the development of functional foods and cosmetic products.

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