The Thai Journal of Pharmaceutical Sciences

Volume 43
Issue 2 *2019*

Article 2

1-1-2019

Physicochemical Characters and Fatty Acid Composition of Payena lucida Seed Oils

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Original Article

Thai Journal of Pharmaceutical Sciences

Physicochemical characters and fatty acid composition of *Payena lucida* seed oils

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Received: May 30, 2018
Accepted: Jan 09, 2019
Published: Jun 02, 2019

ABSTRACT

Introduction: *Payena lucida* A.DC. seed oil has long been used as edible oil and treating various kinds of diseases as oral and topical applications in some local areas of Myanmar. **Objectives:** This research aimed to identify and qualify the physicochemical characters and fatty acid composition of *P. lucida* seed oil. **Materials and Methods:** Two authentic seed oils of *P. lucida* were prepared and evaluated in comparison with two commercial seed oils. The physicochemical characters of the seed oils were determined by methods described in the USP 39, and the fatty acid composition was analyzed by gas chromatography-mass spectroscopy. **Results:** The physicochemical characters of authentic and commercial seed oil samples were not much different. Their specific gravities, refractive indices, acid values, saponification values, ester values, and iodine values were 0.9117–0.9138, 1.4645–1.4652, 3.88–12.03 mg KOH/g, 210–213 mg KOH/g, 199–208 mg KOH/g, and 68.85–84.85 g I_2/100 g, respectively. The amount of saturated, monounsaturated, and polyunsaturated fatty acids were 38–41%, 53–55%, and 7–8%, respectively, with oleic acid (53–55%) as the major fatty acid. **Conclusions:** Quality of *P. lucida* seed oil was concluded in terms of physicochemical characters and fatty acid composition. Commercially available seed oils showed comparable quality with those of authentic seed oils.

Keywords: *Payena lucida*, seed oil, fatty acid composition, physicochemical character, GC-MS analysis

INTRODUCTION

Plant seed oils have a great value for their usefulness and are produced by cold or hot expression, solvent extraction, and centrifugation method.[1] The cold pressing method provides seed oils with purity, safety, and nutritiousness but the yield of this oil is normally lesser than oil produced from hot pressing and solvent extraction methods. Refining processes are required in some cases to improve the quality of the oils.[1,2] The oils are principally composed of different types of fatty acids esterified with glycerol.[2] Since time immemorial, Myanmar people have conventionally prepared and used many plant seed oils for cooking purpose. Some of plant seed oils have been employed in traditional medicine preparations to prevent, alleviate, and cure various diseases of human beings and animals. Moreover, Myanmar people are usually using various kinds of herbs and medicinal plants for self-medication since these kind of treatments are low-cost. Nowadays, the incident rate of cancer is increasing in Myanmar[3] and many patients are utilizing traditional medicines for the treatment. *Payena lucida* A.DC. (family Sapotaceae)[4,5] is one of the locally reputed traditional remedies for Myanmar cancer patients. It is a kind of hard woody tree usually reaches 100 feet tall and widely distributed in Myanmar, Thailand, Andaman, West Malaysia, Sumatra, Banka, and Borneo. In Myanmar, it is widely distributed in Tanintharyi Division, especially in Myeik district and is well known as “Kan Zaw” and its other vernacular names are “Taung-min-gut” and “Thit-kya-poe.” In Thailand, it is called “Phikun Thuean.”[4-6] Its heartwood is usually used for building houses and timber production.[4,5] The commercial oil is produced by mechanical expression of the seeds and has been employed as edible oil in some local areas of Tanintharyi Division. The oil is used for treating various kinds of diseases such as respiratory diseases, gastrointestinal diseases and gynecological problems, and other ailments such as abrasion,

boils, abscess, neuralgia, knee pain, and inflammation as oral and topical applications.[7] The seed oil is popular remedy among Myanmar cancer patients with high reputation.[7] To the best of our knowledge, very little scientific study of the seed oil of this plant has been reported. This study concerned mainly on its physicochemical characters and chemical constituents in terms of fatty acid composition for its quality and identity of the authentic and available commercial seed oils.

MATERIALS AND METHODS

Sample Materials

Two authentic seed samples of *P. lucida* were obtained in 2016 from a traditional medicine practitioner in Myeik (Authentic-1) and a local supplier in Mandalay, Myanmar (Authentic-2). Plant material was identified by Professor Soe Myint Aye (Department of Botany, Mandalay University, Department of Higher Education, Myanmar). The voucher specimens (Pcogsu-KZSe/2018) are deposited at the Herbarium of the Faculty of Pharmacy, Silpakorn University, Thailand. Two different commercial seed oil products were purchased from a local supplier of the seed oil in Myeik (Commercial-1) and another local supplier in Mandalay, Myanmar (Commercial-2).

Extraction of the Seed Oils

Each authentic seed sample was dried, deshelled and ground into fine powder with an electric grinder. The ground seed kernels (45 g) were extracted with 600 ml of hexane by sonication at room temperature for 2 h for 3 times. For sedimentation, the extracted solution was allowed for 2 h and then the supernatant was filtered through a cotton filter. The filtrate was centrifuged at 4000 rpm at 25°C for 10 min. The upper clear supernatant was further evaporated under vacuum. 2 mL of the seed oil was shaken with anhydrous sodium sulfate and filtered through a 0.45 μm nylon membrane filter to get the anhydrous clear oil for preventing hydrolysis during transesterification. The yields of two authentic samples were 51.44% w/w and 48.22% w/w.

Determination of Physicochemical Characters

The physicochemical characters of the seed oils were determined according to the methods stated in the USP 39.[8] All oil samples were centrifuged at 4000 rpm at 25°C for 30 min, and the clear supernatants were used for the determination.

Specific gravity

Both the weight of empty and distilled water filled pycnometer (10.265 cm^3) was first determined at 25°C. Then, the weight of the pycnometer filled with the oil was determined at the same temperature. Specific gravity was calculated as follow:

$$\text{Specific gravity} = \frac{Wc - Wa}{Wb - Wa}$$

where, *Wa* is weight of the empty pycnometer, *Wb* is weight of the pycnometer filled with distilled water, and *Wc* is weight of the pycnometer filled with seed oil.

Refractive index

Refractive indices were determined by refractometer (ATAGO NAR 1T Liquid, Japan) at 25°C. A drop of each oil sample was applied to the center part of the main prism of the refractometer, and the measurements of the oil samples were done according to the guidelines stated by the supplier.

Acid value

Each oil sample (10.0 g) was accurately weighed and dissolved in 50 ml of a mixture of equal volumes of absolute ethanol and ether in a 250 ml Erlenmeyer flask. Then, 1.0 ml of phenolphthalein test solution (TS) was added into the oil solution and titrated with potassium hydroxide (0.1 N) until the solution remains faintly pink after shaking for 30 s. The result of the acid value of the oils was expressed in terms of the volume of the titrant used and calculated as follow:

$$\text{Acid value} = \frac{M \times V \times N}{W}$$

where *M* is molecular weight of potassium hydroxide (56.11), *V* is volume of potassium hydroxide (ml), *N* is normality of potassium hydroxide, and *W* is weight of the oil sample taken for the test (g).

Saponification value

Each oil sample (1.5 g) was accurately weighed in 250 ml flask and added with 25.0 ml of 0.5 N alcoholic potassium hydroxide. The flask was heated on a steam bath, making sure to reflux for 30 min, and frequently shaken. Then, 1.0 ml of phenolphthalein TS was added and titrated the excess potassium hydroxide with 0.5 N hydrochloric acid volumetric solution (VS). A blank determination under the same conditions was performed previously. The saponification value was calculated as follow:

$$\text{Saponification value} = \frac{(Vb - Va) \times 56.11 \times N}{W}$$

where, *Vb* is volume of hydrochloric acid VS of the blank (ml), *Va* is volume of hydrochloric acid VS of the sample (ml), *N* is exact normality of hydrochloric acid, and *W* is weight of the oil sample taken for the test (g).

Ester value

The ester value of the oil samples was determined by subtracting the acid values from the respective saponification values.

Iodine value

Iodine value was determined by Method I (Hanus Method). The accurately weighed of each oil sample (0.2 g) was dissolved with 10 ml of chloroform in a 250 ml iodine flask. After that, 25.0 ml of iodobromide TS was added and the stopper was inserted in the vessel securely, and allowed to stand for 30 min with occasional shaking and protecting from light. Then, 30 ml of potassium iodide TS and 100 ml of water were added in the order, and titrated the liberated iodine with 0.1 N sodium thiosulfate VS, shaking thoroughly after each addition of sodium thiosulfate. Starch TS (3 ml) was added at the time of quite pale iodine color was observed and the

titration was continued until the blue color was disappeared. The iodine value was calculated as follow:

$$\text{Iodine value} = \frac{A \times (Vb - Va) \times N}{10 \times W}$$

where *A* is atomic weight of iodine (126.90), *Vb* is volume of sodium thiosulfate VS of the blank (ml), *Va* is volume of sodium thiosulfate VS of the sample (ml), *N* is exact normality of the sodium thiosulfate VS, and *W* is weight of the oil sample taken for the test (g).

Analysis of Fatty Acid Composition

Preparation of standard

Two alkane standard mixtures (40.0 μg/ml in hexane) of alkane C_8 to C_{20} and alkane C_{21} to C_{40} (Fluka®, Germany) were used. They were mixed and 1.0 ml of the mixture was used as the standard solution.

Transesterification of the seed oils

Transesterification of fatty acid composition was slightly modified from the method described by Nkwonta *et al*.[9] Each seed oil (100.0 mg) was separately mixed with hexane (2.0 ml) and methanolic potassium hydroxide (2 N, 200 μl). The mixture was vigorously shaken for 2 min. Then, it was allowed to stratify and 0.5 ml of the clear upper layer containing fatty acid methyl esters (FAMEs) was carefully taken out for gas chromatography-mass spectroscopy (GC-MS) analysis.

GC-MS[9]

The transesterified seed oils were analyzed using GC-MS (GC 6890N/MS 5973 [G2589A], Agilent Technologies, USA) with DB-5 (122-5032, J&W Scientific, Agilent Technologies, USA), 5%-phenyl and 95% dimethylpolysiloxane polymer column of 30 m length, 0.25 mm internal diameter, and film thickness 0.25 μm. The initial oven temperature was 50°C, held for 2 min, then increased to 90°C at 20°C/min and was held again for 1 min and heated to a final temperature of 280°C for 10 min. The injector temperature was kept at 250°C and electron ionization occurred at 70 eV at 250°C with a full scan range of 50–500 *m/z*. Helium was used as a carrier gas with a flow rate of 1.0 ml/min and 52.7 KPa. No solvent delay was employed for the detector. Mode of splitless injection of samples and the full scan mode were employed. Identification of each FAME was done on the basis of the similarity of its mass spectrum with those in the Wiley 7n.l MS library and Kovats Index (NIST SRD 69).[10] Calculation of Kovats Index (Kovats Retention Index, KI) was done as follow:

$$KI = 100 \times [n + (N - n)\frac{Rt\ (unknown) - Rt\ (n)}{Rt\ (N) - Rt\ (n)}\ ]$$

where the *Rt* (unknown) is the retention time of each peak. It was measured at the same conditions as those of the *n*-alkanes (C_8-C_{40}) and then compared with the two *n*-alkanes which eluted in front and behind *n* and *N* which are the number of carbon atoms of alkanes in the smaller and larger, respectively. The relative amount (%) of each identified FAME was determined by area normalization measurement.

Statistical Analysis

All results were expressed as the mean and standard deviation of three independent determinations. An analysis of variance was performed for statistical significance of the tested samples using Microsoft Excel 2013 and $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

Physicochemical characters and fatty acid composition of two authentic and two commercial seed oils of *P. lucida* were studied and compared. All samples were clear yellow oily liquid with a characteristic odor. Their physicochemical characters are shown in Table 1. In general, most of seed oils show the character of specific gravity at the value <1 ranging from 0.9100 to 0.9400.[11] The specific gravity of *P. lucida* seed oil samples was in the range of 0.9117–0.9138. The refractive indices of two authentic seed oils were the same with 1.4645, and those of two commercial seed oils also showed identical value at 1.4652. This very negligible difference indicated that molecular weight, fatty acid chain length, degree of unsaturation, and degree of conjugation of the chemical compositions of all oil samples were similar ($P < 0.01$).[12-14] The acid values of authentic seed oils were higher than those of the commercial seed oils. Higher acid values indicated that more free fatty acids were liberated from triglycerides due to the factors such as light, heat and the activity of lipase during harvesting, storage, and processing of the oil.[15-19] The acid value of the seed oils may vary depending on the methods of extraction, modes of treatments before extraction[13,18] and hydrolysis of the oils during storage and processing.[16] For the use as edible oils, the acid value should not be >4.0 mg KOH/g.[15,20,21] To

Table 1: Physicochemical characters of *P. lucida* seed oils

Physicochemical characters	Oil samples				*P* value
	Authentic-1	Authentic-2	Commercial-1	Commercial-2	
Specific gravity at 25°C	0.9117±0.00	0.9123±0.00	0.9138±0.00	0.9127±0.00	P=0.47
Refractive index at 25°C	1.4645±0.00	1.4645±0.00	1.4652±0.00	1.4652±0.00	P<0.01
Acid value (mg KOH/g oil)	12.03±0.19	8.34±0.12	6.28±0.00	3.88±0.02	P<0.01
Saponification value (mg KOH/g oil)	211.03±1.37	209.51±1.93	210.19±0.05	211.10±1.60	P=0.65
Ester value (mg KOH/g oil)	199.42±1.32	201.17±1.92	203.91±0.05	207.22±1.61	P=0.01
Iodine value (g I_2/100g oil)	75.52±3.91	68.85±0.53	84.85±0.57	81.96±1.43	P<0.01

P. lucida: *Payena lucida*

Table 2: Peak identification and relative amount of chemical composition of *P. lucida* seed oils

Peak no.	Average R_t(min)	Calculated KI	Literature KI	Constituents	Molecular weight	Characteristic ions *m/z*	Relative amount (%)			
							Authentic-1	Authentic-2	Commercial-1	Commercial-2
1	6.13	961	964[22]	Benzaldehyde	106	106, 105, 77, 51, 43	nd	nd	0.11±0.00	0.14±0.00
2	23.61	1726	1727[23]	Myristic acid methyl ester (C14:0)	242	242, 211, 199, 143, 129, 101, 87, 74	nd	0.01±0.00	0.01±0.00	0.01±0.00
3	27.32	1906	1879[24]	Palmitoleic acid methyl ester (C16:1)	268	268, 236, 194, 55	0.15±0.02	0.15±0.01	0.15±0.00	0.13±0.00
4	27.76	1928	1928[23] 1929[25,26]	Palmitic acid methyl ester (C16:0)	270	270, 239, 227, 143, 87, 74	22.34±0.16	24.91±0.13	23.45±0.17	23.97±0.17
5	29.66	2027	2028[23,27]	Margaric acid methyl ester (C17:0)	284	284, 253, 241, 143, 87, 74	0.07±0.01	0.07±0.01	0.07±0.00	0.07±0.00
6	30.94	2096	2097[28]	Linoleic acid methyl ester (C18:2)	294	294, 263, 220, 109, 95, 81, 67, 55, 41	6.85±0.06	6.61±0.07	7.53±0.08	7.62±0.05
7	31.11	2106	2106[29]	Oleic acid methyl ester (C18:1)	296	296, 264, 222, 55	55.07±0.32	52.64±0.18	53.72±0.40	53.16±0.34
8	31.53	2129	2130[23,25,30]	Stearic acid methyl ester (C18:0)	298	298, 267, 255, 143, 87, 74	14.83±0.15	14.90±0.06	14.30±0.19	14.23±0.10
9	34.57	2304	ur	Gondoic acid methyl ester (C20:1)	324	324, 292, 281, 250, 207, 55	0.09±0.01	0.09±0.01	0.08±0.00	0.07±0.00
10	35.00	2330	2329 2333[23,27]	Arachidic acid methyl ester (C20:0)	326	326, 295, 283, 143, 87, 74	0.61±0.02	0.62±0.01	0.59±0.00	0.60±0.01

R_t - retention time, KI - Kovats Index (Kovats Retention Index), nd - not detected, ur - unavailable reference with DB 5 column. *P. lucida: Payena lucida*

lower the higher acid value and to improve the oil quality, refining process can be exploited.[19,20] Higher acid values of authentic seed oils obtained from solvent extraction without refining process in this study might be the major contributing factor. The saponification value of all samples was in the range of 210–213 mg KOH/g indicating non-significant difference of average molecular mass of different fatty acids present in the oils.[13,18] Consequently, high ester values were observed in the commercial seed oil samples. The degree of unsaturation of the oils can be determined by iodine value. The iodine values in the range of 68.85–84.85 g I_2/100 g of all samples showed the contribution of unsaturated fatty acids to the fatty acid profiles. On the other hand, it also indicated that their sensitivity to oxidation.[12,14,19] However, authentic seed oils showed some lower iodine values than those of commercial oils.

For more detail on chemical composition, all seed oil samples were extensively analyzed by GC-MS. All samples revealed identical nine peaks. One additional peak (peak 1) was found in two commercial seed oils. Identification of each peak was done on the basis of the similarity of its mass spectrum with those in the Wiley 7n.l MS library and calculating of KI and compared with references.[22-30] The total ion chromatograms of the analyzed seed oils and the results were shown in Figure 1 and Table 2, respectively.

Identification of each GC-MS peak was also confirmed by interpretation of its mass fragmentation. All identified saturated FAMEs revealed the base peak at *m/z* 74 due to the McLafferty rearrangement, and two obvious peaks due to α-cleavage (loss of a methoxy group, $[M-31]^+$) and a hydrogen atom and propyl radical loss $[M-43]^+$. In addition, according to the *β*-cleavage of the carbomethoxy ion series, a difference of 14 amu with each characteristic peaks suggested the structure of saturated hydrocarbon chain.[31-34] These data and molecular ion of each FAME supported the identification of five saturated FAMEs as myristic acid methyl ester (peak 2), palmitic acid methyl ester (peak 4), margaric acid methyl ester (peak 5), stearic acid methyl ester (peak 8), and arachidic acid methyl ester (peak 10).

Three monounsaturated FAMEs were identified in all seed oils as palmitoleic acid methyl ester (peak 3), oleic acid methyl ester (peak 7), and gondoic acid methyl ester (peak 9). Their characteristic peak was the base peak at *m/z* 55 according to the McLafferty rearrangement. Prominent peaks at *m/z* 194, 236 of palmitoleic acid methyl ester, at *m/z* 222 and 264 of oleic acid methyl ester, and at *m/z* 250 and 292 of gondoic acid methyl ester were loss of the McLafferty ion $[M-74]^+$ and loss of a methoxy group plus a hydrogen atom $[M-32]^+$.[31-33] One polyunsaturated FAME identified in all seed oil samples was linoleic acid methyl ester (peak 6). The characteristic fundamental peak was observed at *m/z* 67 due to the α-cleavage. Moreover, other prominent peaks at *m/z* 263 $[M-31]^+$ as the result of the *α*-cleavage of the carbonyl and *m/z* 220 $[M-74]^+$ due to loss of the McLafferty ion were observed.[31-34]

One volatile hydrocarbon aldehyde, benzaldehyde (peak 1) was identified only in two commercial seed oil samples with the characteristic peaks at *m/z* 105 (base peak) due to loss of hydrogen atom and *m/z* 77 due to the inductive, *i*-cleavage $[M-29]^+$.[31]

Overall, fatty acid composition of authentic and commercial oils was the same in quality. The minor difference between them was the trace amount of benzaldehyde (0.11–0.14%) which was observed only in commercial samples. It was assumed as the naturally hydrolyzed product of free unsaturated fatty acids present, and therefore, it could not be

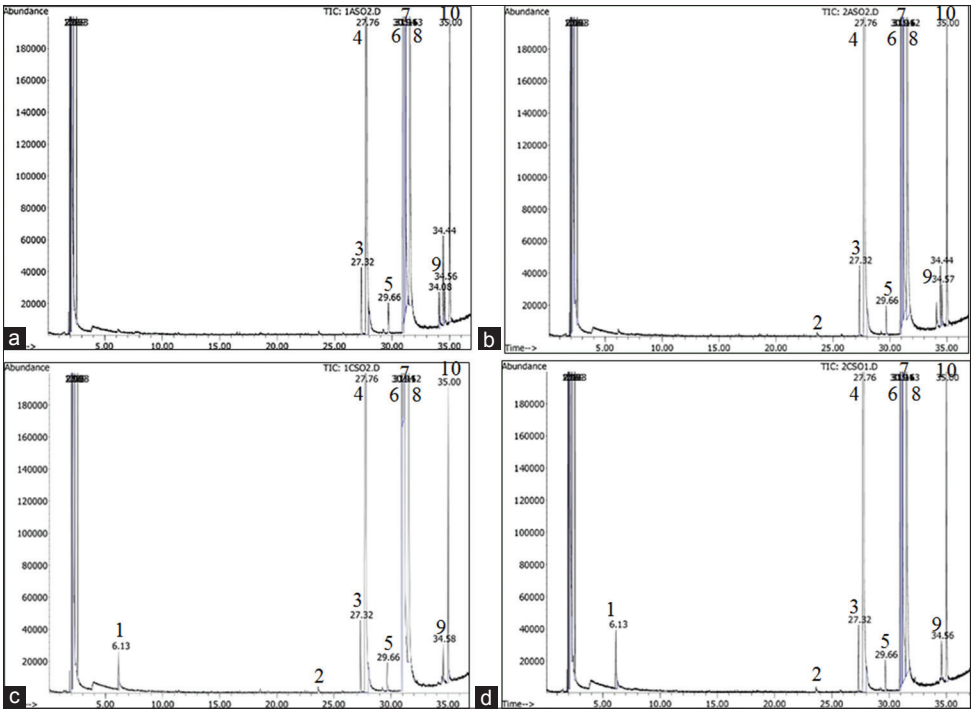

Figure 1: Total ion chromatogram of (a) Authenic-1, (b) Authentic-2, (c) Commercial-1, (d) Commercial-2 of *P. lucida* seed oil

Table 3: Relative amount (%) of fatty acids of the seed oils analyzed by GC-MS

Relative amount (%) of fatty acids	Oil samples			
	Authentic-1	Authentic-2	Commercial-1	Commercial-2
SFA	37.84	40.52	38.43	38.88
MUFA	55.31	52.87	53.94	53.36
PUFA	6.85	6.61	7.53	7.62
UFA: SFA	1.64	1.47	1.60	1.57

SFA: Saturated fatty acid, MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid, UFA: Unsaturated fatty acid

detected in two authentic seed oil samples due to loss on the removal of the solvent during the extraction process.[35] Among the fatty acid composition, oleic acid was the principal fatty acid. The ratio of fatty acids of each degree of unsaturation is shown in Table 3. High content of monounsaturated and polyunsaturated fatty acids reflects their iodine value, and the identification of them in all tested seed oil samples was observed as the same. As a result, the fatty acid composition of all seed oils analyzed proved the agreement with their physicochemical characters.

As *P. lucida* seed oil is the locally reputed traditional remedies for cancer patients in Myanmar and is becoming a high-priced entity, preliminary *in vitro* cytotoxicity testing on a commercial sample against two cancer cell lines (NCI-H187-Small Cell Lung Cancer and MCF-7 Breast Cancer Cell) was performed by our research group. No cytotoxicity against the tested cancer cell lines was determined, and therefore, it could be supposed to be unbeneficial for cancer cure. However, more types of cancer cell lines should be used to confirm its cytotoxic activity for the benefits of cancer cure as a traditional mention. Indeed, its physicochemical characters and fatty acid composition were quite similar to those of the well-known olive oil.[1,36] Olive oil provided a lot of health benefits and contributed low incidence of chronic diseases including breast cancer, colorectal cancer, prostate cancer, chronic heart diseases, and diabetes and enhanced gastric ulcer healing.[37-40] Therefore, *P. lucida* seed oil might be exploitable as a nutrient and profitable as healthy vegetable oil like olive oil.

CONCLUSIONS

Physicochemical characters and fatty acid composition of two authentic and two commercial seed oils of *P. lucida* were analyzed for the quality and identity. Physicochemical characters of the seed oils determined could serve as criteria for the quality assessment. Overall, fatty acid compositions of authentic and commercial seed oils were the same in quality. Commercial seed oil samples revealed comparable characters with authentic seed oils. Consequently, they could be accepted as reliable and quality products to use and also as a good nutrient and healthy vegetable oil.

ACKNOWLEDGMENTS

The authors are grateful to the Department of Pharmacognosy, the Graduate School, and the Research and Creative Fund (Grant no. RG 002/2561), Faculty of Pharmacy, Silpakorn University, Thailand, for research facilities and financial support.

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