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The HPTLC Validated Method Development for the Quantification of Naringin from the Partially Purified Labisia Pumila Dichloromethane Extract

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Abstract

Partially purified fraction E, dichloromethane extract of leaves from *Labisia Pumila* has been shown to possess anti-ulcer, anti-inflammatory, anti-asthmatic activities among others. Naringin has been identified by LC-MS and HPLC as the major compound present in fraction E. The present study reports the development and validated TLC densitometric method for the quantification of Naringin present in Fraction E. ICH guidelines were followed to develop this method. CAMAG-HPLTC system comprising of a TLC Visualizer and Linomat 5 sample applicator was used in this study. The separation was performed using TLC aluminum plates pre-coated with silica gel 60 F254. Optimized mobile phase consisted of Methanol: Ethyl Acetate (60:40 v/v). Win-CATS-V 1.2.3 software and Video Scan were used to identify and quantify Naringin at 366nm in fluorescence mode. The peak heights at Rf 0.648 ± 0.01 gave a linear range from 200-1000µg/ml correlation coefficient R2± SD = 0.973 ± 0.024. The LOD and LOQ were found to be Naringin 0.74 ± 0.29 µg and 7.73 ± 0.26 µg, respectively. Repeatability gave CV % < 2.0. We recovered 92.56% of Naringin and 642.4 mg/g (64.24%) was found to be present in Fraction E. The HPTLC method was found to be reproducible, accurate and convenient for rapid screening of bioactive constituents present in Fraction E. This developed method will be used for analysis and quality control of drug formulations containing *Labisia Pumila*.

Keywords: HPTLC; *Labisia Pumila;* ICH guidelines

Introduction

Labisia Pumila (LP) (var. Alata) a member of the Myrsinaceae family is a traditional herb found widely throughout the rainforest of Indochina [1]. Its other names include Kacip Fatimah, Sangkoh (Iban), Mata Pelanduk Rimba, Selusoh Fatimah and Tadah Matahari [1]. Indigenous women of the Malay Archipelago use this herb to increase libido, improve post-partum health and ease menstrual problems [1, 2]. This plant has been reported by many authors to possess; anti-bacterial, oestrogenicity, anti-inflammatory, anti-photoaging, antioxidant and gastro-protective with positive results [3-6]. The partially purified extracts of LP have been extensively studied [7-9] and Fraction E has been shown to possess antioxidant and anti-inflammatory activity. Different studies on the constituents of the different varieties of LP have reported them to contain variable patterns of flavonoids, phenolic and various bioactive volatile compounds [10, 11]. Phytochemical compounds like naringin, quercertin, rutin, kaempferol, vanillic acid and hesperetin among others have been reported to be constituted in LP extract [11]. Naringin (Cas: 10236-47-2) (4′,5,7-Trihydroxyflavanone 7-rhamnoglucoside) is a flavanone glycoside that has been reported to possess *in vitro* [12] and *in vivo* [13, 14] antioxidant properties. It was also reported to improve diabetic foot ulcers its down-regulation of anti-inflammatory, inhibition of oxidative stress and hyperglycemia and up regulation of growth factors (IFG-1, TGF-β and VEGF-c) expression. Preliminary phytochemical analysis on the partially purified extracts showed Fraction E tested positive for phenolic compounds while LCMS and HPLC (unpublished data) confirmed the presence of Naringin its major constituent.

HPTLC is quickly becoming the leading chromatographic technique used to quantify the amount of different compounds in complex samples [15]. The objective of this experiment was to report a new high performance-thin layer chromatography (HPTLC)-densitometric procedure for the separation and quantitative determination of Naringin in fraction E of partially purified DCM leaf extract of *Labisia Pumila*. The Validation of this chromatographic procedure was according to the International Conference on Harmonisation of Technical Requirements For Registration of Pharmaceuticals For Human Use guideline (ICH guideline) [16].

Materials and Methods

Plant samples and chemicals

The leaves of LP var. alata (Kacip Fatimah) were obtained and identified from a forest in Sungai Perak, by Dr. Shamsul Khamis, a research officer (plant taxonomy) from the Laboratory of Natural Products (NATPRO), Institute of Bioscience in University Putra Malaysia. Naringin (Cas: 10236-47-2) (>90%), Pre-coated aluminum silica gel TLC plates (F_{254} 20 x 20 cm) and 2-amino-ethyldiphenylborinate (Neu's reagent) were obtained from (Merck, Germany). All solvents used were of analytical grade.

Extraction of plant materials

The fresh leaves were air-dried for 7 days before being powdered by dry mill. About 500 g of plant material was kept in 6 L of

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dichloromethane for 2 days. This extraction process was repeated six times before filtering with No. 1 Whatman filter paper. The filtrate was rotary evaporated at 40°C and kept to dry in a fume cupboard. The extract was kept in a desiccator for further separation by column chromatography. The partial purification of the DCM extract by column chromatography used a 42 x 2.5 cm vertical column equipped with a stop cock and glass frit to support the silica gel, pore size 60 Å, 200-400 mesh. 100% hexane was used to pre-elute the column. A decreasing polarity solvent ratio (100% hexane (Hx) – Hx: Ethyl acetate (EA) – EA: Methanol (MeOH) – 100% MeOH) was used to obtain Fraction E (60:40 Hx:EA to 100% MeOH). The fractions were collected based on their chromatogram profiles analyzed on (TLC plates Silica gel 60 F_{254}) and mobile phase 60:40 Hx:EA.

Preparation of standard and extract solution

Naringin and the Fraction E sample (5 mg/ml) were prepared by transferring accurately weighed 5mg into a 1.5ml centrifuge tube; the extract was dissolved in 1 mL of HPLC analytical grade methanol and ultra-sonicated for 20 minutes and filtered with 0.45 µm millipore sterile syringe filter. Standards and sample were prepared daily immediately before use.

HPTLC Instrumentation and chromatography conditions

20 µl of the extracts were separately applied (Samples and standard) onto the TLC plate with 6 mm wide band or spotted with an automatic TLC applicator Linomat-V with N_2 flow (Camag, Switzerland), 8 mm from the bottom with instruction input defined from win-CATS-V 1.2.3 software. After sample application, the plates were developed in a 10 x 20 cm horizontal Camag twin glass chamber pre-saturated with the mobile phase (10 ml each side) for 20 minutes at room temperature (25 -27°C). The mobile phase consisted of MeOH: EA (60:40 v/v). Linear ascending development was carried out until the 8 cm mark. The plates were observed after 30 minutes air drying under the Camag UV visualizer (366 nm) as shown in Figure 1. The plates were sprayed with Neu's reagent spray. Video Scan software in fluorescence mode was used to quantify the plates post derivation.

Validation method

ICH guidelines were followed for the validation of the quantitative method developed for precision, repeatability and accuracy of naringin in Fraction E [16]. A concentration range of 200 to 5000 µg/ml was spotted on the TLC plate in triplicates. Linearity, LOD and LOQ was done according to methods described by [17]. Precision was evaluated by the analysis of replicate (n = 3) applications of freshly prepared standard solution at concentrations (200, 600 and 1000 µg/ml) by methods described by [18]. The repeatability of sample application and measurement of peak height was expressed in terms of coefficient of variation (CV%). Accuracy was measured by performing recovery experiments by spiking (200, 600, 1000 µg/ml) of Naringin with a single known concentration of Fraction E (600 µg/ml) and percentage recovery was calculated according to the formula below Figure 2:

% Recovery = (Peak height of Spiked sample – Peak height of Known Sample)

(Peak height of known standard concentration)

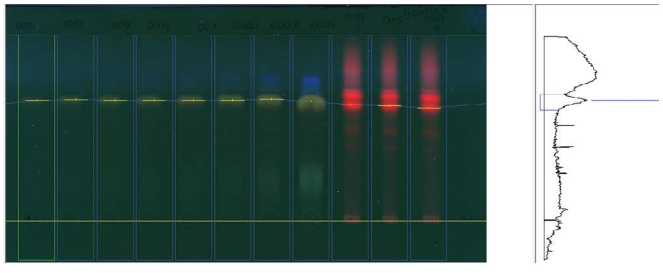


Figure 1: Video scan capture of TLC plate at 366 nm of Naringin and SNP E.

Results and Discussion

The amount of herbal drugs used worldwide has risen dramatically in the recent decade. Natural health products and Chinese herbal remedies are two examples from among the variety of plant drugs in the market [15]. High performance thin layer chromatography has been reported to be a universally accepted method for evaluating the chemical composition of natural products [19], specifically in phytochemical analysis [15]. This is due to advantages HPTLC offers like; image result presentation, it's simple, cost efficiency, rapid obtainable results and high sample capacity [15].

Standard curve, LOD and LOQ

The methods linearity was determined with a specified range to obtain test results in direct proportion to the concentrations of the analyte. Naringin had linear range from 200-1000µg/ml with correlation coefficient R^2± SD = 0.973 ± 0.024 as reported in Table 1. The limit of quantification (LOQ) is the lowest amount of the analyte that can be quantitatively determined in sample with defined precision and accuracy under standard conditions. LOQ is the amount of loaded sample producing a peak area that is equal to the sum mean blank area and ten times its standard deviation [20]. Sample concentration at 900 µg/ml was interpolated with the linear regression curve using graphpad prism 6 Figure 3. The amount of naringin found in Fraction E was found to be 642.39 ± 22.9 mg/g (64.2%) The LOD and LOQ have been reported in Table 1.

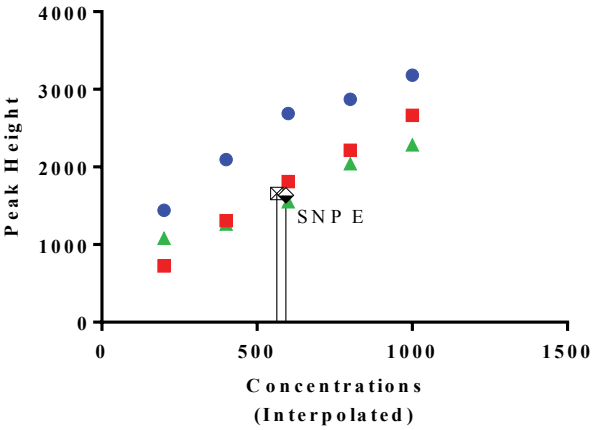
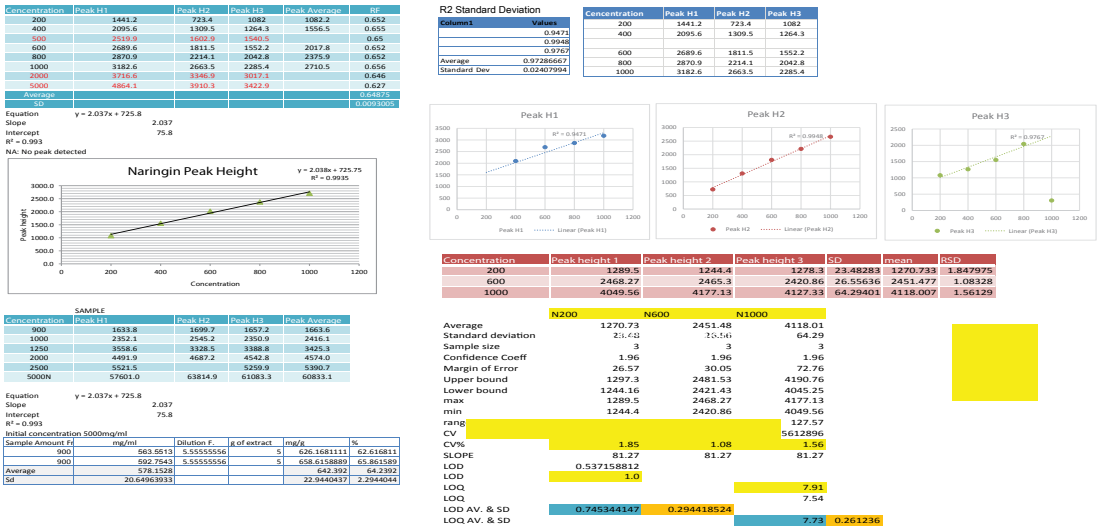


Figure 2: Interpolation of amount of naringin in SNP E from the standard curve.

Parameters	Results (mean ± SD) (n=3)
Regression equation	y = 2.037x + 725.8
Correlation coefficient	0.973 ± 0.024
Linearity range	200 to 1000 µg/ml
Limit of detection	0.75 ± 0.29 µg
Limit of quantification	7.73 ± 0.26 µg
Amount naringin (mg/g)	642.39 ± 22.9 mg/g (64%)

Table 1: Amount of Calibration Parameters.



Concentration	Peak height 1			SD	mean	
200	1609	1629	1634	13.22876	1624	0.814579
600	3870	3869.5	3796	42.58032	3845.167	1.107372
1000	6004.4	5993	5893	61.29154	5963.467	1.027784

	N200	N600	N1000
Average	1624.00	3845.17	5963.47
Standard deviation	13.23	42.58	61.29
Sample size	3	3	3
Confidence Coeff	1.96	1.96	1.96
Margin of Error	14.97	48.18	69.36
Upper bound	1639.0	3893.35	6032.82
Lower bound	1624.00	3796.98	5894.11
max	1634	3870	6004.4
min	1609	3796	5893
range	25	74	111.4
CV	0.0081	0.011	0.01027784
CV%	0.81	1.11	1.03
SLOPE	81.27	81.27	81.27
LOD	0.5		
LOQ			7.54

Naringin
SAMPLE Height

CONCENTRATIC	PA1	PA2	PA3	MEAN	SD	CV%
200	2881.9	2864.3	2820.4	2855.5	31.7	1.11
1000	3122.7	3162.7	3202.7	3162.7	40.0	1.26
1250	4995.3	4987.3	4955.3	4979.3	21.2	0.43

NARINGIN ONLY

CONCENTRATIC	PA1	PA2	PA3	MEAN	SD	CV%
200	1749	1709	1734	1730.7	20.2	1.17
600	3970	3969.5	3896	3945.2	42.6	1.08
1000	6354.4	6693	6793	86726.7	229.9	0.27

SPIKED AREA (sample + standard)

CONCENTRATIC	PA1	PA2	PA3	MEAN	SD	CV%
200	4470.2	4498.0	4368.0	4445.4	68.5	1.54
600	6498.9	6688.9	6667.9	6618.6	104.2	1.57
1000	8638.4	8878.4	8982.4	8833.1	176.4	2.00

RECOVERY

CONCENTRATIC	PA1	PA2	PA3	MEAN	SD	CV%
200	92.32	96.11	87.22	91.88	4.46	4.85
600	91.77	96.57	97.85	95.40	3.21	3.36
1000	91.01	89.99	90.19	90.40	0.54	0.60

Table Recovery studies of Naringin by the proposed TLC densitometric method (n=3)

Concentration (µg/ml)		Height		Total height (sample + standard)	% Recovery
Sample	Spiked amount	Sample height	Spiked height		
600	200	2855.533333	1730.7	4445.4	91.88
600	600	3162.7	3945.2	6618.6	95.40
600	1000	4979.3	86726.7	8833.1	90.40
Average Recovery					92.56

Figure 3: Hptlc Validation of Partially Purified Labisia Pumila Extract

Amount (µg/ml)	Intraday Precision		Interday Precision	
	SD in height	%CV	SD in height	%CV
200	13.23	0.81	23.48	1.85
600	42.58	1.11	26.56	1.08
1000	61.29	1.03	64.29	1.56

Table 2: Intra and Inter day precision of naringin (n=3).

Concentration (µg/ml)		Sample height		Total height (sample + standard)	% Recovery
Sample	Spiked amount	Sample height	Spiked height		
600	200	2855.5	1730.7	4445.4	91.88
600	600	3162.7	3945.2	6618.6	95.40
600	1000	4979.3	86726.7	8833.1	90.40
Average					92.56

Table 3: Recovery studies of naringin by the proposed TLC densitometric method (n=3).

Precision and accuracy

Precision of comparison (intra-day and inter-day) were determined under different conditions, different day, different reagents, on the same sample. Intra and Intra-day was carried out using the standard on three different days where results were expressed in CV %. In terms of repeatability of the measurement peak area this method obtained a coefficient of variation not more than 2% (Table 2). The percent recovery was found to be 92.56%. The results are shown in Table 3.

Conclusion

A Quantitative HPTLC method for estimating the amount of Naringin in Fraction E from the partially purified DCM leaf extract from *Labisia Pumila* has been described in this paper. This method was able to obtain precise and accurate results. The data could be used as a quality control technique for the evaluation of Naringin in Fraction E. The method gave good peak resolution in the analysis of bioactive constituents present in the sample. Linearity gave an R_2 value of more than 0.9. Precision and recovery was able to give CV% less than 2%. The proposed HPTLC method for the analysis of fraction E from the partially purified leaf DCM extract of *Labisia Pumila* reported here is simple, sensitive, economic and suitable for rapid routine quality control analysis and quantification of Naringin in herbal drug preparation and may be useful for standardization purposes.

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References

1. Abdullah N, Chermahini SH, Suan CL, Sarmidi MR (2013) *Labisia pumila*: A review on its traditional, phytochemical and biological uses. World Appl Sci J 27:1297- 1306.
2. Al-Wahaibi A, Wan Nazaimoon WM, Norsyam WN, Farihah HS, Azian AL (2008) Effect of water extract of *Labisia pumila* Var Alata on aorta of ovariectomized Sprague Dawley rats. Pakistan J Nutr 7:208- 213.
3. Nadia ME, Nazrun AS, Norazlina M, Isa NM, Norliza M, et al. (2012) The Anti-Inflammatory, Phytoestrogenic, and Antioxidative Role of *Labisia pumila* in Prevention of Postmenopausal Osteoporosis. Adv Pharmacol Sci 2012: 706905.
4. Okechukwu NP, Marunga J (2011) Gastro-protective effect of dichloromethane crude extract of *labisia pumila*. Int J Pharmacol Toxicol Sci 2:1- 7.
5. Choi HK, Kim DH, Kim JW, Ngadiran S, Sarmidi MR, et al. (2010) *Labisia pumila* extract protects skin cells from photoaging caused by UVB irradiation. See comment in PubMed Commons below J Biosci Bioeng 109: 291-296.
6. Sanusi RAM, Shukor NAA, Sulaiman MR (2013) Anti-inflammatory Effects of *Labisia pumila* (Blume) F . Vill-Naves . Aqueous Extract. Sains Malaysiana 42:1511- 1516.
7. Samuagam L, Akowuah GA, Okechukwu PN (2011) Partial purification and antinociceptive investigation of leaves of *Labisia Pumila*. Asian J Pharm Clin Res 4: 44- 46.
8. Okechukwu PN, Ekeuku SO, Loshnie S, Akowuah GA (2014) Anti-inflammatory, Analgesic, Antinociceptive and Antipyretic Investigation of Bioactive Constituents from Partial Purified Dichloromethane Crude Extracts from Leaves of *Labisia Pumila*. Int J Pharm Res Sch 3: 743- 751.
9. Okechukwu NP, Lee JA, Akowuah AG (2011) *In-vitro* antihistaminergic, anticholinergic, antioxidant and mast cell stabilizing effects of partially purified *Labisia pumila* leaf extract. FASEB J 25: 1020- 1027.
10. Karimi E, Jaafar HZ (2011) HPLC and GC-MS determination of bioactive compounds in microwave obtained extracts of three varieties of *Labisia pumila* Benth. Molecules 16: 6791-6805.
11. Chua LS, Latiff NA, Lee SY, Lee CT, Sarmidi MR, et al. (2011) Flavonoids and phenolic acids from *Labisia pumila* (Kacip Fatimah). Food Chem 127: 1186-1192.
12. Das R, Dutta A, Bhattacharjee C (2013) Assessment on on the Antibacterial Potential of Phytochemical Naringin-An *in vitro* Evaluation. Int J Emerg Technol Adv Eng 3: 353- 357.
13. Thangavel P, Muthu R, Vaiyapuri M (2012) Antioxidant potential of naringin - a dietary flavonoid-in N-Nitrosodiethylamine induced rat liver carcinogenesis. Biomed Prev Nutr 2:193- 202.
14. Jeon SM, Bok SH, Jang MK, Kim YH, Nam KT, et al. (2002) Comparison of antioxidant effects of naringin and probucol in cholesterol-fed rabbits. Clin Chim Acta 317: 181- 190.
15. Ciesla L, Zaluski D, Smolarz HD, Hajnos M, Waksmundzka-hajnos M (2011) HPTLC-Densitometric Method for Determination of Eleutherosides B , E , and E 1 in Different Eleutherococcus Species. J Chromatogr Sci 49:182- 188.
16. Q2A I (1994) Text on validation of analytical procedures. In: Proceedings of the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use. Japan.
17. Kumar P, Dwivedi SC, Kushnoor A (2011) Development and Validation of HPTLC Method for the Determination of Nelfinavir as Bulk Drug and in Tablet Dosage Form. 2:1783- 1788.
18. Maheshwari S, Khandhar AP, Jain A (2010) Quantitative Determination and Validation of Ivabradine HCL by Stability Indicating RP-HPLC Method and Spectrophotometric Method in Solid Dosage Form. EJAC 5:53- 62.
19. Schoenbart B (1999) Comparative Chromatographic Analysis of Dan Shen (Salvia miltiorrhiza) Roots Grown in Different Regions of the World. USA 1-14.
20. Dhandhukia PC, Thakker JN (2011) Quantitative Analysis and Validation of Method Using HPTLC. High-Performance Thin-Layer Chromatogr pp 203- 221.

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