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RP-HPLC METHOD DEVELOPMENT FOR SIMULTANEOUS DETERMINATION OF PHENOLIC COMPOUNDS IN FRUIT EXTRACTS OF *MOMORDICA CHARANTIA* FROM DIFFERENT LOCATIONS IN MALAYSIA

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ABSTRACT

A sensitive, reproducible, and reliable reversed-phase high performance liquid chromatography (RP-HPLC) method with diode array detection (DAD) was developed and validated. Simultaneous determinations of five compounds; gallic acid, catechin, chlorogenic acid, epigallocatechin gallate and caffeic acid in four types of Momordica charantia extracts; water, ethanol, water:ethanol (1:1) and acetone were conducted. The compounds were successfully separated by C18 column (250 mm × 4.6 mm, 5 µm) with a gradient solvent system of 3% acetic acid in water:methanol:acetonitrile at flow rate of 1.0 mL/min. UV detection was carried out at 280 nm. The standard curves of the five compounds were linear in the range of 0.0396 µg/mL–100 µg/mL. The intra-assay relative standard deviation (RSD) was less than 4.97%, while the inter-assay RSD was less than 4.92%, whereas the accuracy was between 90.96% and 108.92%, respectively. Our optimised RP-HPLC-DAD method was capable to detect flavonoids and phenolic acid contents in four types of M. charantia fruits extracts simultaneously from five locations in Malaysia. The present method is recommended to be used for chemical analyses of phenolic compounds in other Momordica species.

Keywords: *Momordica charantia*, Phenolic compounds, Reversed-phase high performance liquid chromatography, Simultaneous determination

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INTRODUCTION

Momordica charantia (*M. charantia*) L., known as bitter gourd and bitter melon, belongs to the *Cucurbitaceae* family (Habicht *et al*. 2011). It is a vegetable indigenous to the East and Southeast Asian countries, particularly Sri Lanka, Bhutan, Nepal, Philippines, Indonesia, Malaysia, Pakistan, Bangladesh, India, Taiwan, Thailand, China and Japan. Over the past decades, there is a large volume of published studies investigating the health and pharmacological activities of *M. charantia*. More than 100 studies (Basch, Gabardi and Bricht 2003; Grover and Yadav 2004; Lucas *et al*. 2010) have demonstrated that *M. charantia* has a strong association with broad health benefits: anticancer (Nagasawa, Watanabe and Inatomi 2002; Deep *et al*. 2004; Yasui *et al*. 2005, Dia and Krishnan 2016), anti-inflammatory, analgaesic (Lin and Tang 2008, Ullah *et al*. 2012), hypolipidemic and hypocholesterolaemic effects (Nerurkar, Lee and Nerurkar 2010). Moreover, extensive research has been explored on the effectiveness of fresh, juiced or dried *M. charantia* in diabetic animals and in type 2 diabetic human subjects (Welihinda and Karunanayake 1986; Grover and Yadav 2004; Dans *et al*. 2007, Mahmoud *et al*. 2017).

Phenolics are secondary metabolites of plants, providing some organoleptic attributes and nutritional properties (Landete 2012). These compounds comprise of a heterogeneous group containing flavonoids, phenolic acids, stilbenes tannins, lignans and coumarins. Phenolic compounds in *M. charantia* have been recognised as the bioactive compounds accountable for its health effects, and among those have been reported are gallic acid, tannic acid, caffeic acid, ferulic acid, benzoic acid, +(-) catechin and *p*-coumaric acid (Kubola and Siriamornpun 2008, Svobodova *et al*. 2017). The chemical compounds of *M. charantia* have been extensively explored; nevertheless, to the best of our knowledge, information about phenolic compounds in Malaysian *M. charantia* are still scarce. The data obtained from this study will provide methodology reference and basis for the quality control of *M. charantia* extracts before the industrial application.

Appropriate identification of herbal plants, in fresh or dried form, is vital in quality control of the raw materials and extracts. Therefore, particular attention needs to be given to the development of identity standards and assurance profile, utilising combination of taxonomy, conventional microscopy, thin layer chromatography (TLC) and high performance liquid chromatography-diode array detector (HPLC-DAD) methods. HPLC analysis methods for flavonoids and phenolic acids in *M. charantia* had been reported (Kubola and Siriamornpun 2008; Choi *et al*. 2012). These works displayed good separations but consumed much time and reagents.

In this work, a sensitive, reproducible and reliable validated reversed-phase (RP)-HPLC-DAD optimised method was developed for simultaneous determination of five phenolic compounds in four different types of *M. charantia* extracts from five locations in Malaysia. Five chromatographic peaks were detected attributing to gallic acid, catechin, caffeic acid, epigallocatechin gallate and chlorogenic acid.

MATERIALS AND METHODS

Chemicals and Reagents

Gallic acid (purify > 98%), catechin (purify > 98%), epigallocatechin gallate (purify > 98%) were purchased from Chengdu Biopurify Phytochemicals, China. Chlorogenic acid (purify > 98%), caffeic acid (purify > 98%) were purchased from Indofine Chemical Co. Hillsborough, New Jersey, USA. *M. charantia* dried fruits were purchased from Herbagus

Sdn. Bhd., Pulau Pinang, Malaysia. All solvents were HPLC grade and acquired from Merck Sdn. Bhd., Selangor, Malaysia. Distilled water was prepared using Mili-Q purification system.

Preparation of Raw Material

M. charantia fruits were collected from five locations: Pulau Pinang, Perlis, Selangor, Pahang and Johor. The plant was authenticated by Rahmad Zakaria (PhD) from School of Biological Sciences, Universiti Sains Malaysia (USM). The voucher specimen was deposited at USM Herbarium with voucher number of 11727. The fruit was cut into small pieces and the seeds were removed. The fruits were washed, dried and ground into powder form utilising an electric grinder SM-100 (Retsch, Germany).

Preparation of *M. charantia* Extracts and Standard Solution

The extraction process was performed by using four types of solvents, namely water, ethanol, water:ethanol (1:1) and acetone. An amount of 20 g powder of dried fruit of *M. charantia* was extracted with 200 mL of different types of solvents (water, ethanol, water:ethanol (1:1) and acetone) separately utilising soxhlet apparatus for 6 h at 100°C, 78°C, 92°C, and 56°C, respectively. The extract solutions were evaporated to dryness using rotary evaporator R100 (Buchi, Switzerland) at 40°C, and were then put in the oven at 45°C for 12 h to ensure complete dryness.

Standard stock solutions of gallic acid, catechin, chlorogenic acid, epigallocatechin gallate and caffeic acid were prepared at 1 mg/mL in methanol as mobile phase. Serial dilutions of standard stock solutions were prepared in the range of 0.039 µg/mL–20 µg/mL to obtain calibration standard solutions. *M. charantia* extracts were also dissolved in mobile phase at a concentration of 10 mg/mL. The samples were filtered through 0.45 µm polytetrafluoroethylene (PTFE) syringe filter (UniFlo® RC, 13 mm, Whatman®).

Chromatographic Condition

The analysis was carried out using Agilent Technologies 1260 Infinity (USA) HPLC system comprised of photo diode array detector, quaternary solvent delivery system, column incubator and online degasser. Separation was achieved using ZORBAX Eclipse Plus C-18 (250 mm × 4.6 mm, 5µm) (Agilent Technologies, USA) with the column temperature was kept at 40°C.

The ultraviolet (UV) detection was set at 280 nm. The injection volume was 10 µL. The flow rate was 1 mL/min. The mobile phase consisted of 3% acetic acid in water (A), acetonitrile (B) and methanol (C). The gradient elution was employed as follows: 0 min–2 min, 98% (A) 0% (B); 2 min–6 min, 75% (A) 10% (B); 6 min–10 min, 75% (A) 10% (B); 10 min–17 min, 60% (A) 15% (B); 17 min–26 min, 60% (A) 15% (B) and 26 min–30 min, 98% (A) 0% (B).

Method Validation

The analytical method was validated for linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ) according to the International Conference on Harmonization (ICH) guideline (ICH 1994/2005). The linearity was evaluated with standard solutions. The calibration standard solutions were prepared as mentioned in the

sample preparation section. The calibration curves were then evaluated with five known concentrations. The determinations of intra- and inter-day precisions were performed by replicate analysis (n = 5) of standard solutions at five concentrations in the range of 0.039 µg/mL–20 µg/mL. Recovery was employed to determine the accuracy of the method and conducted by the standard addition method. Three different concentrations of standard mixtures were added to each sample types. Determinations of the limit of detection (LOD) and limit of quantification (LOQ) were performed based on the lowest detectable peak in the chromatogram. Signal-to-noise ratio was computed under the chromatographic condition. The LOD was defined as a signal/noise ratio of 3:1 and the LOQ was defined as signal/noise ratio of 10:1.

RESULTS

Selection of Detection Wavelengths

Wavelengths for the flavonoids and phenolic acids were detected by ultraviolet spectrophotometer in the range of 200 nm–400 nm (Figure 1). These results indicated that the common maximum absorbance of each compound was at the wavelength of 210 nm–330 nm. According to the previous studies (Somers and Ziemelis 1985; Cuvelier, Richard and Berset 1996; Chen, Zuo and Deng 2001; Mohammadzadeh Kakhki and Bazi 2015), phenolic compounds showed significant absorption bands in the range 220 nm–340 nm as a result of the conjugated property of benzene ring. In this study, based on the UV data, eight wavelengths were selected to improve the detection through the HPLC-DAD system. The results indicated that 280 nm was the best detection for the investigated compounds.

Optimisation of High Performance Chromatographic Condition

Figure 2 shows that HPLC profile of the standard markers was typical to *M. charantia* extract with regard to retention times and peak width of the peaks. The mobile phase system composition, detection wavelength and gradient elution procedure were optimised in order to acquire the most beneficial chemical information and the best separation in the fingerprint chromatograms of *M. charantia* extracts. Initially, various ratios of methanol-water system had been selected as mobile phase, however, the peaks separation was not satisfactory. Then, different ratios of methanol, acetonitrile and water combinations were investigated. Eventually, it was discovered that methanol-acetonitrile-water system showed an improved separation only for gallic acid, caffeic acid and catechin, whereas chlorogenic acid and epigallocatechin gallate did not show any good baseline separation. Acetic acid was added to the water to improve the separation and to restrain the ionisation of marker compounds. Different concentrations of glacial acetic acid (1%, 2% and 3%) in the aqueous phase were investigated to obtain higher efficiency and better selectivity. It was observed that addition of 3% acetic acid produced the best separation of *M. charantia* extract. Consequently, a mobile phase system containing 3% of acetic acid in aqueous phase, methanol and acetonitrile was selected.

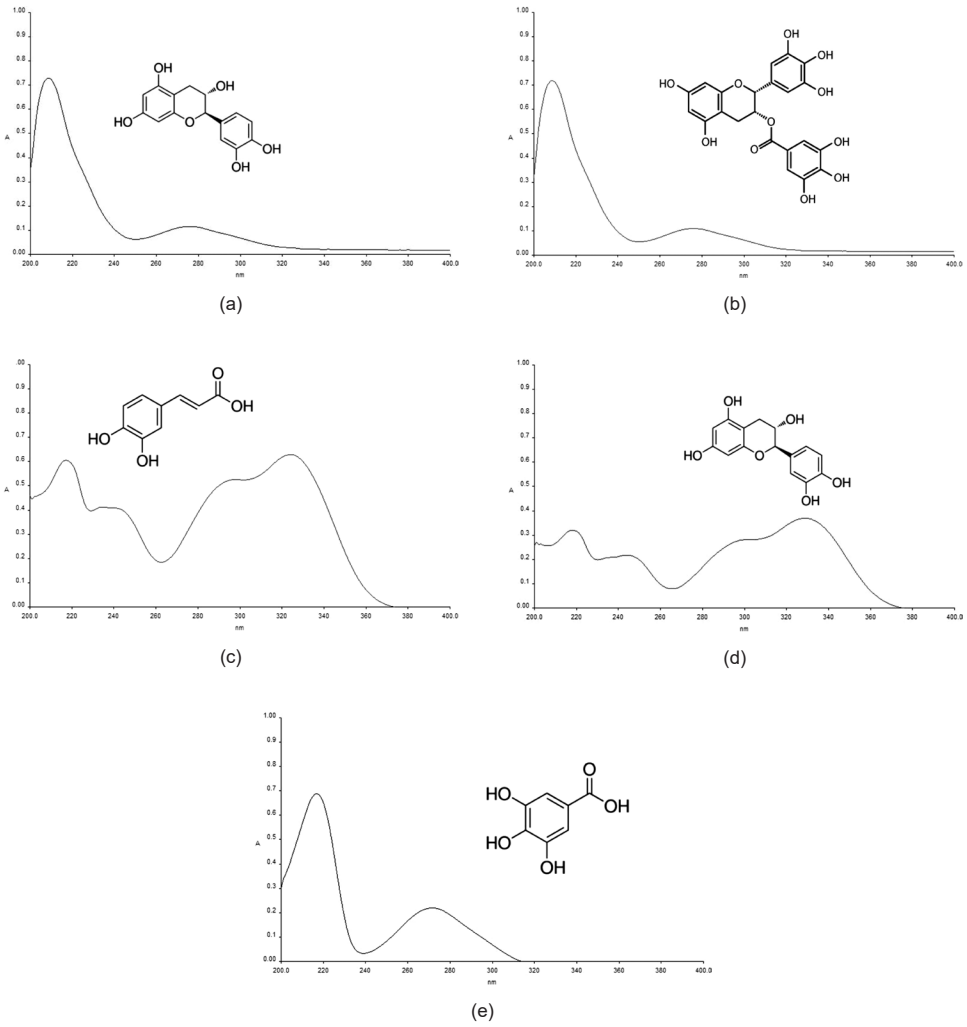

Figure 1: Chemical structures of the five compounds namely (a) catechin, (b) epigallocatechin gallate, (c) caffeic acid, (d) chlorogenic acid and (e) gallic acid as well as their UV absorption spectra.

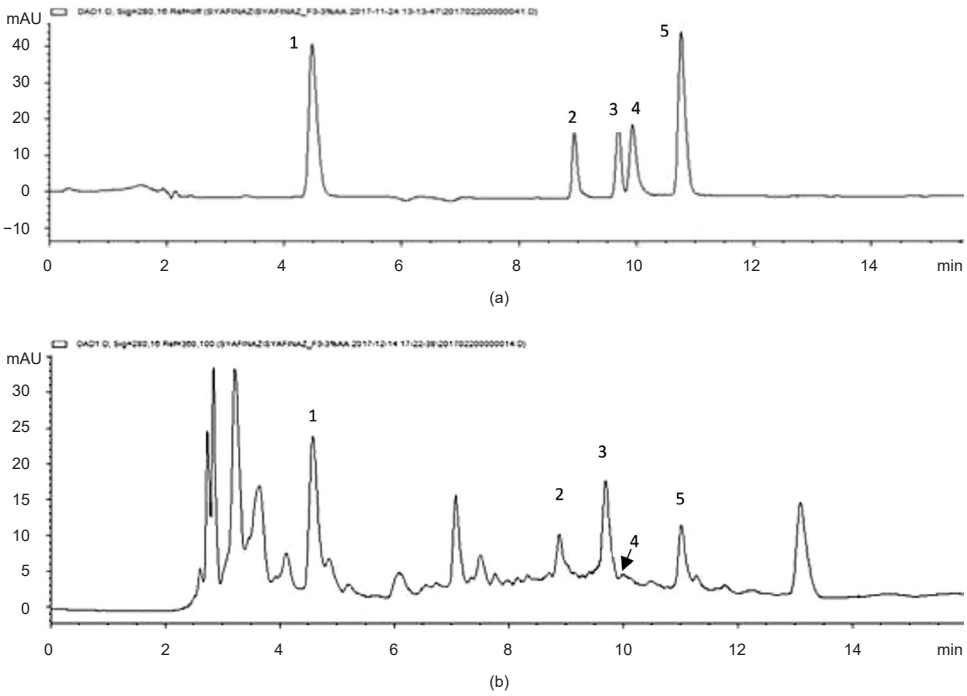

Figure 2: Representative HPLC chromatograms of (A) the standard mixtures and (B) *M. charantia* fruit extract. 1 = gallic acid; 2 = catechin; 3 = chlorogenic acid; 4 = epigallocatechin gallate and 5 = caffeic acid at concentration of 10 mg/mL.

Analytical Method Validation

Validation of the HPLC method was conducted for the analysis of gallic acid, catechin, chlorogenic acid, epigallocatechin gallate and caffeic acid in *M. charantia* extracts. Linearity, accuracy, precision, LOD and LOQ were investigated (Han, Tian and Row 2009; Alquadeib 2019). Linearity was analysed using five levels of concentration in the range of 0.0396 µg/mL–100 µg/mL with five replicates. All calibration curves showed high linear correlation coefficients (R^2) which were higher than 0.999, reflecting good linearity, as shown in Table 1. The calibration graphs were obtained based on linear regression analysis of the integrated peak areas (y) versus concentration of standards (x).

Table 1: Calibration data of the reported HPLC method.

Compounds	Regression equation	R^2	Linear range*	LOD*	LOQ*
Gallic acid	y = 26.352x−5.2485	1.0000	0.040–100	0.012	0.040
Catechin	y = 6.5082x−3.6682	0.9999	0.158–100	0.048	0.158
Chlorogenic acid	y = 15.085x−5.4135	1.0000	0.040–100	0.012	0.040
EGCG	y = 9.8302x−12.0700	0.9998	0.323–100	0.098	0.323
Caffeic acid	y = 29.765x−7.3317	0.9999	0.040–100	0.012	0.040

Note: *Value is in µg/mL.

Table 2: Intra- (n = 5) and inter-day (n = 5) precision and accuracy for determination of five compounds.

Standards	Nominal concentration*	Intra-day (n = 5) (%)			Inter-day (n = 5) (%)		
		Observed concentration* (n = 5)[a]	Accuracy (%)	RSD (%)	Observed concentration* (n = 5)[a]	Accuracy (%)	RSD (%)
Gallic acid	0.039	0.038 ± 0.002	98.30	4.94	0.041 ± 0.001	104.21	4.28
	0.156	0.150 ± 0.009	96.25	4.96	0.149 ± 0.002	95.54	1.68
	2.5	2.650 ± 0.052	105.99	1.96	2.596 ± 0.052	103.84	2.03
	10	10.050 ± 0.190	100.54	0.54	9.901 ± 0.129	99.01	1.31
	20	20.040 ± 0.139	100.20	0.69	19.956 ± 0.204	99.78	1.02
Catechin	0.156	0.170 ± 0.011	108.92	3.37	0.155 ± 0.008	99.67	4.91
	0.313	0.326 ± 0.015	104.02	4.49	0.316 ± 0.016	101.04	4.90
	2.5	2.497 ± 0.040	99.88	0.04	2.611 ± 0.098	104.43	3.75
	10	10.001 ± 0.048	99.01	0.05	10.133 ± 0.135	101.34	1.33
	20	19.758 ± 0.496	98.79	0.50	19.593 ± 0.240	97.96	1.22
Chlorogenic acid	0.039	0.038 ± 0.002	98.30	4.94	0.040 ± 0.002	102.71	4.62
	0.078	0.076 ± 0.003	98.30	4.33	0.074 ± 0.003	95.49	3.66
	0.625	0.589 ± 0.008	94.30	1.36	0.644 ± 0.028	103.09	4.42
	5	4.820 ± 0.040	96.40	0.83	4.870 ± 0.036	97.40	0.74
	10	10.026 ± 0.057	100.26	0.57	10.044 ± 0.042	100.44	0.42
EGCG	0.313	0.318 ± 0.008	101.62	2.60	0.326 ± 0.014	104.29	4.22
	0.625	0.597 ± 0.010	95.54	1.67	0.623 ± 0.018	99.76	2.89
	2.5	2.274 ± 0.036	90.96	1.59	2.359 ± 0.212	100.28	4.10
	10	9.480 ± 0.174	94.80	1.84	9.912 ± 0.418	99.12	4.21
	20	19.980 ± 0.170	99.90	0.85	20.277 ± 0.609	101.39	3.00
Caffeic acid	0.039	0.038 ± 0.002	96.99	4.76	0.039 ± 0.001	99.29	2.61
	0.078	0.073 ± 0.003	93.74	3.84	0.077 ± 0.003	98.85	4.52
	1.25	1.194 ± 0.019	95.53	1.60	1.205 ± 0.017	96.46	1.38
	5	4.980 ± 0.072	99.58	1.44	4.970 ± 0.026	99.41	0.51
	10	10.026 ± 0.019	100.26	0.19	10.135 ± 0.124	101.35	1.23

Notes: *Concentration is in µg/mL; [a]Expressed as mean ± SD.

The LOD for gallic acid, catechin, chlorogenic acid, epigallocatechin gallate, and caffeic acid were 0.012, 0.048, 0.012, 0.098 and 0.012 µg/mL, respectively, indicating that the present method was acceptable with sufficient sensitivity. The LOQ for gallic acid, catechin, chlorogenic acid, epigallocatechin gallate and caffeic acid were 0.040 µg/mL, 0.158 µg/mL, 0.040 µg/mL, 0.323 µg/mL and 0.040 µg/mL, respectively. This result demonstrated that the developed analytical method can be applied to identify and quantify trace amounts of the phenolic compounds in crude extract of *M. charantia.*

Assessment of intra-assay precision was performed by analysing three sets of samples, separately prepared at three different concentrations: low, middle and high. The determination of inter-assay precision, using the same method as intra-assay precision, was investigated on five successive days and the precision results are shown in Table 2. The relative standard deviation (RSD) values for intra-assay and inter-assay precisions were less than 4.97% and 4.92% whereas the accuracy was between 90.96% and 108.92%, respectively. The obtained precision and accuracy data were acceptable for quantitative analysis of the phenolic compounds in *M. charantia* fruit extracts.

The recoveries were performed using standard addition method. The results are depicted in Table 3. The average recoveries were in the range of 96.89%–126.12% with RSD values less than 2.69 %. The results showed that the method and conditions applied in the quantitative analysis were appropriate.

Table 3: Recovery studies of five investigated compounds (*n* = 5).

Standards	Concentration*	Fortified concentration*	Observed concentration*	Recovery (%)	RSD (%)
Gallic acid	0.039	0.053	0.055	107.91	1.80
	2.5	1.611	1.690	104.92	1.11
	20	14.203	13.918	97.99	0.38
Catechin	0.156	0.271	0.285	104.21	2.33
	2.5	1.968	2.024	102.83	0.69
	20	15.753	15.532	98.60	1.34
Chlorogenic acid	0.039	0.075	0.074	99.53	0.0002
	0.625	0.645	0.625	96.89	0.67
	10	9.230	9.008	97.59	2.21
EGCG	0.313	0.635	0.688	108.64	2.24
	2.5	3.975	4.092	102.99	2.32
	5	1.672	2.109	126.12	0.03
Caffeic acid	0.039	0.045	0.044	97.45	2.68
	1.25	1.021	1.028	100.75	1.36
	10	8.154	8.226	100.88	0.63

Note: *Concentration is in µg/mL.

Table 4: Quantifications of five compounds in different extracts of *M. charantia* (n = 5).

Sample	State	Gallic acid*	Catechin*	Chlorogenic acid*	EGCG*	Caffeic acid*
Acetone	Perlis	0.423 ± 0.001	0.039 ± 0.005	0.395 ± 0.003	0.064 ± 0.001	ND
	Pulau Pinang	0.778 ± 0.018	ND	0.456 ± 0.019	0.062 ± 0.001	ND
	Selangor	0.352 ± 0.001	ND	0.204 ± 0.002	0.056 ± 0.011	ND
	Pahang	0.208 ± 0.001	ND	0.448 ± 0.003	0.055 ± 0.001	ND
	Johor	0.805 ± 0.008	ND	0.627 ± 0.014	0.115 ± 0.001	ND
Ethanol	Perlis	0.349 ± 0.001	0.035 ± 0.002	0.219 ± 0.002	0.067 ± 0.001	ND
	Pulau Pinang	0.319 ± 0.001	0.080 ± 0.001	0.196 ± 0.001	0.062 ± 0.001	0.121 ± 0.005
	Selangor	0.336 ± 0.001	ND	0.257 ± 0.002	0.073 ± 0.005	ND
	Pahang	0.238 ± 0.002	0.321 ± 0.005	0.256 ± 0.001	0.073 ± 0.014	0.184 ± 0.002
	Johor	0.275 ± 0.003	0.055 ± 0.005	0.073 ± 0.009	0.113 ± 0.012	0.078 ± 0.001
Water:ethanol (1:1)	Perlis	0.060 ± 0.001	ND	0.036 ± 0.002	0.049 ± 0.001	0.214 ± 0.005
	Pulau Pinang	0.055 ± 0.001	ND	0.138 ± 0.010	0.056 ± 0.001	0.257 ± 0.002
	Selangor	0.092 ± 0.002	0.127 ± 0.003	0.138 ± 0.001	0.050 ± 0.001	0.454 ± 0.011
	Pahang	0.111 ± 0.001	0.125 ± 0.003	0.152 ± 0.003	0.053 ± 0.005	0.325 ± 0.006
	Johor	0.064 ± 0.001	0.066 ± 0.015	0.161 ± 0.003	ND	0.480 ± 0.001
Water	Perlis	0.070 ± 0.001	0.085 ± 0.006	0.117 ± 0.003	ND	0.050 ± 0.001
	Pulau Pinang	0.061 ± 0.001	0.061 ± 0.007	0.086 ± 0.002	0.058 ± 0.001	0.368 ± 0.001
	Selangor	0.089 ± 0.016	0.125 ± 0.032	0.090 ± 0.001	0.056 ± 0.001	0.330 ± 0.005
	Pahang	0.077 ± 0.001	0.344 ± 0.007	0.150 ± 0.001	0.057 ± 0.001	0.114 ± 0.001
	Johor	0.095 ± 0.001	0.119 ± 0.003	0.063 ± 0.001	ND	0.113 ± 0.002

Notes: *The content of compound is in mg/ML; ND = Not detected.

Quantification of Five Compounds in *M. charantia* Extracts

The established analytical method was successfully utilised for simultaneous determination of five compounds in *M. charantia* extracts. The contents of the five compounds (gallic acid, catechin, chlorogenic acid, epigallocatechin gallate and caffeic acid) from five different locations, extracted with four different types of solvent, are demonstrated in Table 4. The acetone extract from Pulau Pinang showed the highest value of gallic acid (0.778 mg/mL), the water extract from Pahang showed the highest value of catechin (0.344 mg/mL), the acetone extract from Johor showed the highest chlorogenic acid content (0.627 mg/mL) as well as epigallocatechin gallate (0.115 mg/mL), while the water:ethanol (1:1) extract from Johor showed the highest caffeic acid content (0.480 mg/mL).

DISCUSSION

HPLC is one of the selected chromatographic methods in pharmaceutical analysis for qualitative and quantitative analysis of herbal chemical compounds. In this work, a RP-HPLC method was developed for simultaneous analysis of phenolic content of four different *M. charantia* fruit extracts from five locations. The findings indicated that the analytical method was linear within the investigated concentration range as well as accurate and precise.

HPLC with a reversed-phase C18 column is probably the most extensively used chromatographic method for the analysis of phenolic content. Although C18 stationary phase is the most frequently used in determination of phenolic components in various types of matrices with excellent separation capability for diverse families of compounds (Schieber, Keller and Carle 2001; Abu-Reidah *et al*. 2013), optimisation of the mobile phase system is still a vital step in the HPLC method development. In this study, the system was successfully optimised by conducting various ratios of mobile phase trials. It was discovered that addition of 3% acetic acid produced good resolution and separation for both standard mixtures and *M. charantia* extracts.

In this study, both elution programs, isocratic and gradient had been tested. However, the gradient elution was chosen to be performed since isocratic elution had not shown any good spectral resolution. Tertiary system of methanol/acetonitrile/water was used due to the simple binary systems (water/methanol) do not fulfil the requirement of the analysis in the most of cases as reported by (Zheng and Row 2007). However, the peaks resolution obtained was not satisfactory in certain extracts. This could be due to the fact that most of the separations are almost impossible without application of mobile phase additives, for example salts, acids and organic compounds (Han, Tian and Row 2007).

The basicity or acidity of the mobile phase system contributes to significant effect in separation of the peaks (Ma *et al*. 2012). In this study, acetic acid, one of the traditional mobile phase additives in RP-HPLC, was used to provide the acidic condition during the optimisation. The concentrations of acetic acid in the mobile phase were evaluated from 1%–3%, and we established that 3% acetic acid produced the best resolution and separation for both standard mixtures and *M. charantia* extracts. The finding was in agreement with (Li, Tian and Ho Row 2010) who reported that acetic acid was efficient in increasing the resolution of those compounds since gallic acid, caffeic acid and chlorogenic acid are organic acids that consist of carboxyl and hydroxyl groups. Furthermore, the interaction between stationary phase and target compounds was also reduced when the concentration

of the acetic acid had been increased in the mobile phase system. High concentration of acetic acid lead to competitive adsorption of the acetic acid molecules with the target compounds on the binding sites. Generally, the detection of binding sites to the target compounds based on the interaction of hydrogen bond is reduced by the addition of proton donor solvent (Li *et al*. 2005). Thus, 3% of acetic acid in mobile phase was selected for this optimisation. Similar usage of acetic acid concentration had been reported by Zuo, Chen and Deng (2002) and Ma *et al*. (2012).

M. charantia fruits collected from five different locations in Malaysia had been extracted with water, ethanol, water:ethanol (1:1) and acetone, and were then subjected to simultaneous quantitative analysis utilising RP-HPLC based on our optimised mobile phase system. The findings indicated wide ranges of gallic acid, catechin, chlorogenic acid, epigallocatechin gallate and caffeic acid contents that possibly due to differences in humidity, soil, environmental condition and harvesting time of the raw plant material and others in their habitat (Ghasemzadeh and Jaafar 2013). The inconsistency in extraction recovery could have also contributed to the variances. Hence, our optimised reverse-phased HPLC method successfully performed simultaneous determination of phenolic contents in four types of *M. charantia* fruits extracts from five locations.

CONCLUSION

In this study, an optimised RP-HPLC method with DAD for simultaneous determination of flavonoids and phenolic acid contents in four types of *M. charantia* fruits extracts from five locations had been developed and validated. This method was found to be sensitive, reproducible and reliable, thus benefiting the standardisation method of *M. charantia* samples for future applications in pharmaceutical industry, commercial sectors and herbal product development.

REFERENCES

ABU-REIDAH, I. M., ARRÁEZ-ROMÁN, D., SEGURA-CARRETERO, A. & FERNÁNDEZ-GUTIÉRREZ, A. (2013) Profiling of phenolic and other polar constituents from hydro-methanolic extract of watermelon (*Citrullus lanatus*) by means of accurate-mass spectrometry (HPLC–ESI–QTOF–MS), *Food Research International*, 51(1): 354–362. https://doi.org/10.1016/j.foodres.2012.12.033

ALQUADEIB, B. T. (2019) Development and validation of a new HPLC analytical method for the determination of diclofenac in tablets, *Saudi Pharmaceutical Journal*, 27(1): 66–70. https://doi.org/10.1016/j.jsps.2018.07.020

BASCH, E., GABARDI, S. & BRICHT, C. (2003) Bitter melon (*Momordica charantia*): A review of efficacy and safety, *American Journal of Health-System Pharmacy*, 60: 356–359. https://doi.org/10.1093/ajhp/60.4.356

CHEN, H., ZUO, Y. & DENG, Y. (2001) Separation and determination of flavonoids and other phenolic compounds in cranberry juice by high performance liquid chromatography*, Journal of Chromatographic A*, 913: 387–395. https://doi.org/10.1016/S0021-9673(00)01030-X

CHOI, J. S., KIM, H. Y., SEO, W. T., LEE, J. H. & CHO, K. M. (2012) Roasting enhances antioxidant effect of bitter melon (*Momordica charantia L.*) increasing in flavan-3-ol and phenolic acid contents, *Food Science and Biotechnology*, 21(1): 19–26. https://doi.org/10.1007/s10068-012-0003-7

CUVELIER, M., RICHARD, H. & BERSET, C. (1996) Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary, *Journal of the American Oil Chemists' Society*, 73: 645–652. https://doi.org/10.1007/BF02518121

DANS, A. M. L., VILLARRUZ, M. V. C., JIMENO, C. A., JAVELOSA, M. A. U., CHUA, J., BAUTISTA, R. *et al*. (2007) The effect of *Momordica charantia* capsule preparation on glycemic control in type 2 diabetes mellitus needs further studies, *Journal of Clinical Epidemiology*, 60: 554–559. https://doi.org/10.1016/j.jclinepi.2006.07.009

DEEP, G., DASGUPTA, T., RAO, A. R. & KALE, R. K. (2004) Cancer preventive potential of *Momordica charantia* L. against benzo(a)pyrene induced fore-stomach tumourigenesis in murine model system, *Indian Journal of Experimental Biology*, 42: 319–322.

DIA, V. P. & KRISHNAN, H. B. (2016). BG-4, a novel anticancer peptide from bitter gourd (*Momordica charantia*), promotes apoptosis in human colon cancer cells, *Scientific Reports*, 6(1): 1–12. https://doi.org/10.1038/srep33532

GHASEMZADEH, A. & JAAFAR, H. Z. (2013) Profiling of phenolic compounds and their antioxidant and anticancer activities in pandan (*Pandanus amaryllifolius* Roxb.) extracts from different locations of Malaysia, *BMC Complementary and Alternative Medicine*, 13(1): 1–9. https://doi.org/10.1186/1472-6882-13-341

GROVER, J.K. & YADAV, S.P. (2004) Pharmacological actions and potential uses of *Momordica charantia*: A review, *Journal of Ethnopharmacology*, 93: 123–132. https://doi.org/10.1016/j.jep.2004.03.035

HABICHT, S. D., KIND, V., RUDLOFF, S., BORSCH, C., MUELLER, A. S., PALLAUF, J. *et al*. (2011) Quantification of antidiabetic extracts and compounds in bitter gourd varieties, *Food Chemistry*, 126: 172–176. https://doi.org/10.1016/j.foodchem.2010.10.094

HAN, D., TIAN, M. & ROW, K. H. (2009) Isolation of four compounds from *Herba Artemisiae Scopariae* by preparative column HPLC, *Journal of Liquid Chromatography and Related Technologies*, 32: 2407–2416. https://doi.org/10.1080/10826070903188120

INTERNATIONAL CONFERENCE ON HARMONIZATION (ICH). (2005) Q2 (R1), Validation of analytical procedures: Text and methodology, ICH Harmonised Tripartite Guideline. International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Chicago, USA.

KUBOLA, J. & SIRIAMORNPUN, S. (2008) Phenolic contents and antioxidant activities of bitter gourd (*Momordica charantia L.*) leaf, stem, and fruit fraction extracts *in vitro*, *Food Chemistry*, 110: 881–890. https://doi.org/10.1016/j.foodchem.2008.02.076

LANDETE, J. M. (2012) Updated knowledge about polyphenols: Functions, bioavailability, metabolism, and health, *Critical Reviews in Food Science and Nutrition*, 52(10): 936–948. https://doi.org/10.1080/10408398.2010.513779

LI, H., LIU, Y., ZHANG, Z., LIAO, H., NIE, L. & YAO, S. (2005) Separation and purification of chlorogenic acid by molecularly imprinted polymer monolithic stationary phase, *Journal of Chromatography A*, 1098(1–2): 66–74. https://doi.org/10.1016/j.chroma.2005.08.046

LI, S., TIAN, M. & HO ROW, K. (2010) Effect of mobile phase additives on the resolution of four bioactive compounds by RP-HPLC, *International Journal of Molecular Science*,11: 2229–2240. https://doi.org/10.3390/ijms11052229

LIN, J. & TANG, C. (2008) Strawberry, loquat, mulberry, and bitter melon juices exhibit prophylactic effects on LPS-induced inflammation using murine peritoneal macrophages, *Food Chemistry*, 107: 1587–1596. https://doi.org/10.1016/j.foodchem.2007.10.025

LUCAS, E. A., DUMANCAS, G. G., SMITH, B. J., CLARKE, S. L. & ARJMANDI, B.H. (2010) Health benefits of bitter melon (*Momordica charantia*). In: R. R. Watson & V. R. Preedy (Eds.). *Bioactive foods in promoting health*, pp. 525–549 (San Diego: Academic Press). https://doi.org/10.1016/B978-0-12-374628-3.00035-9

MA, T. T., XIE, J., ZHANG, Q. L., XU, H., LI, J., A. & CHEN, F. H. (2012) Analysis of fingerprint and bioactive components of *Bidens biternata* by HPLC, *Journal of Chinese Medicinal Materials*, 35(6): 892–896.

MAHMOUD, M. F., EL ASHRY, F. E. Z. Z., EL MARAGHY, N. N. & FAHMY, A. (2017) Studies on the antidiabetic activities of *Momordica charantia* fruit juice in streptozotocin-induced diabetic rats, *Pharmaceutical Biology*, 55(1): 758–765. https://doi.org/10.1080/13880209.2016.1275026

MOHAMMADZADEH KAKHKI, R. & BAZI, R. (2015) Application of mean centering spectra spectrophotometric method for simultaneous determination of salisylic acid and benzoic acid in fruit juice samples, *Russian Agricultural Sciences*, 41: 66–70. https://doi.org/10.3103/S1068367415010164

NAGASAWA, H., WATANABE, K. & INATOMI, H. (2002) Effects of bitter melon (*Momordica charantia L.*) or ginger rhizome (*Zingiber offifinale rosc*) on spontaneous mammary tumorigenesis in SHN mice, *American Journal of Chinese Medicine*, 30: 195–205. https://doi.org/10.1142/S0192415X02000302

NERURKAR, P. V., LEE, Y. K. & NERURKAR, V. R. (2010) *Momordica charantia* (bitter melon) inhibits primary human adipocyte differentiation by modulating adipogenic genes, *BMC Complementary and Alternative Medicine*, 10: 34. https://doi.org/10.1186/1472-6882-10-34

SCHIEBER, A., KELLER, P. & CARLE, R. (2001) Determination of phenolic acids and flavonoids of apple and pear by high-performance liquid chromatography, *Journal of Chromatography A,* 910(2): 187–398. https://doi.org/10.1016/S0021-9673(00)01217-6

SOMERS, T. C. & ZIEMELIS, G. (1985) Spectral evaluation of total phenolic components in *Vitis vinifera*: Grapes and wines, *Journal of the Science of Food and Agriculture*, 36: 1275–1284. https://doi.org/10.1002/jsfa.2740361212

SVOBODOVA, B., BARROS, L., CALHELHA, R.C., HELENO, S., ALVES, M.J., WALCOTT, S. *et al*. (2017) Bioactive properties and phenolic profile of *Momordica charantia* L. medicinal plant growing wild in Trinidad and Tobago, *Industrial Crops and Products*, 95: 365–373. https://doi.org/10.1016/j.indcrop.2016.10.046

ULLAH, M., SHOWKAT, M., AHMED, N. U., ISLAM, S., & ABSAR, N. (2012) Evaluation of *Momordica charantia* L. fruit extract for analgesic and anti-inflammatory activities using in vivo assay, *Research Journal of Medicinal Plant*, 6(3): 236–244. https://doi.org/10.3923/rjmp.2012.236.244

WELIHINDA, J. & KARUNANAYAKE, E. H. (1986) Extra-pancreatic effects of *Momordica charantia* in rats, *Journal of Ethnopharmacology*, 17: 247–255. https://doi.org/10.1016/0378-8741(86)90112-1

YASUI, Y., HOSOKAWA, M., SAHARA, T., SUZUKI, R., OHGIYA, S., KOHNO, H. *et al.* (2005) Bitter gourd seed fatty acid rich in 9c,11t,13t-conjugated linolenic acid induces apoptosis and up-regulates the GADD45, p53 and PPARγ in human colon cancer Caco-2 cells, *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 73(2): 113–119. https://doi.org/10.1016/j.plefa.2005.04.013

ZHENG, J. & ROW, K. H. (2007) Optimum of mobile phase condition for resolving isoflavones in RP-HPLC, *Chinese Journal of Chemical Engineering,* 15: 291–295. https://doi.org/10.1016/S1004-9541(07)60073-1

ZUO, Y., CHEN, H. & DENG, Y. (2002) Simultaneous determination of catechins, caffeine and gallic acids in green, Oolong, black and pu-erh teas using HPLC with a photodiode array detector, *Talanta*, 57(2): 307–316. https://doi.org/10.1016/S0039-9140(02)00030-9