European Journal of Medicinal Plants
5(2): 156-164, 2015, Article no.EJMP.2015.015
ISSN: 2231-0894

SCIENCEDOMAIN *international*
www.sciencedomain.org

HPLC Determination of the Eight Constitutes in *Portulaca oleracea* L. from Different Locations

Junjun Ai[1,2], Aijing Leng[2*], Xiaomei Gao[3], Wenjie Zhang[1], Di Li[1], Liang Xu[1], and Xixiang Ying[1]**

[1]School of Pharmacy, Liaoning University of Traditional Chinese Medicine, 116600 Dalian, China.
[2]First Affiliated Hospital of Dalian Medical University, 116011, China.
[3]School of Pharmacy, Beijing University of Traditional Chinese Medicine, 100102 Beijing, China.

Authors' contributions

This work was carried out in collaboration between all authors. Authors XY and AL designed the study, authors WZ and LX performed the statistical analysis, author JA wrote the protocol and wrote the first draft of the manuscript. Author XG managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2015/13253
Editor(s):
(1) Marcello Iriti, Plant Biology and Pathology, Department of Agricultural and Environmental Sciences, Milan State University, Italy.
Reviewers:
(1) Anonymous, Fujita Health University, Japan.
(2) Anonymous, University of Concepción, Chile.
(3) Anonymous, Università degli Studi Gabriele D'Annunzio, Italy.
(4) Anonymous, Federal University, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=685&id=13&aid=6461

Original Research Article

Received 9th August 2014
Accepted 13th September 2014
Published 10th October 2014

ABSTRACT

Aims: The objective of the present research was to simultaneously determine the eight constituents in *Portulaca oleracea* L. from different locations by a sensitive and specific high-performance liquid chromatographic (HPLC) method, including caffeic acid (CA), *p*-coumaric acid (*p*-CA), scopoletin (SCOP), ferulic acid (FA), quercetin-3-*O*-rhamnoside (QR), quercetin (QUER), apigenin (APIG) and bergapten (BERG) using rhein as the internal standard.
Methodology: With gradient elution procedure and mobile phase A (0.1% formic acid) and B (acetonitrile) ratio linear changing, the wavelength was set to 320 nm to determinate the eight constituents.
Result: The method validation presented good accuracy with recoveries in the range of 93.67-113.3% and good precision with RSD values less than 12.1%.The results indicated that

**Corresponding author: Email: ** yingxixiang@163.com, * aijingleng@hotmail.com;*

there existed the significant differences of the eight constituents in *P. oleracea* L. from 20 different locations.
Conclusion: The results can be used to reasonably assess the drug quality and exploit *P. oleracea* L.

Keywords: High performance liquid chromatography; Portulaca oleracea L.; determination; different locations.

1. INTRODUCTION

Portulaca oleracea L. is a common herbaceous, succulent annual plant, which is extensively distributed in temperate and tropical regions in worldwide and has many active ingredients such as flavonoids [1], alkaloids [2], terpenes [3], phenolic acids [4] and coumarins [5]. *P. oleracea* L. has been used as a kind of food or a medicinal plant for thousands of years in many countries, especially, being a kind of traditional Chinese medicine in China, it was commonly used for the treatment of dysentery with bloody stools and externally for boils and sores, eczema, erysipelas, and insect and snake bites [1]. Many researches show that it exhibits a wide range of biological effects, i.e. skeletal muscle relaxant effect [6], analgesic and anti-inflammatory effects [7], antifungal activity [8], antifertility effect [9] and anti-aging effect [10]. In addition, many compounds in *P. oleracea* L. have been studied, i.e. the fatty acid and *β*-carotene of a number of Australian varieties of *P. oleracea* L. by gas chromatography (GC) and high performance liquid chromatography (HPLC) [11]; the flavonoids, noradrenaline and dopamine in *P. oleracea* L. by capillary electrophoretic method [12]; the four compounds in rat plasma after intravenous administration of *P. oleracea* L. by HPLC [13]. More recently, the chromatographic fingerprint and quantification of the four compounds of *P. oleracea* L from 11 different sources with external standard method was determined by ultra performance liquid chromatography (UPLC) [14]; A fingerprint approach was developed by gas chromatography mass spectrum (GC-MS) and infrared spectroscopy (IR) for the quality control of *P. oleracea* L. from 11 different sources [15]. However, little information can be obtained about simultaneous determination the eight components using internal standard method in *P. oleracea* L. from 20 different locations.

The aim of this study is to develop an HPLC method with internal standard to fully evaluate the contents of the eight constituents in the methanol extract of *P. oleracea* L. from 20 different locations. It was found that the eight components determined in our study have many pharmacological effects, such as anti-inflammatory activity for ferulic acid (FA) [16], antibacterial effects for caffeic acid (CA) and *p*-coumaric (*p*-CA) [17], and so on, and the eight components could be related to the traditional effect of *P. oleracea* L. used to clear heat and relieve toxicity. Therefore, the *P. oleracea* L. can be exploited and utilized reasonably *via* HPLC analyzing the eight components related to antibacterial and anti-inflammatory activities.

2. MATERIALS AND METHODS

2.1 Plant Materials and Reagents

Standard substances of quercetin-3-*O*-rhamnoside (QR), quercetin (QUER), apigenin (APIG) and the internal standard (rhein) were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), and CA, *p*-CA, FA, scopoletin (SCOP) and BERG were obtained from Sichuan Weikeqi Biological Technology Co., Ltd (Chengdu, China). All standards have purities above 98%. The chemical structures were shown in Fig. 1.

The samples of dried *P. oleracea* L. were collected from 20 different locations listed in Table 1. All of the plant materials were collected from June 2013 to September 2013. The botanical origins of all the collected samples were identified by Professor Yanjun Zhai and the voucher specimens of all these materials (No.20111101-20) were deposited at School of Pharmacy, Liaoning University of Traditional Chinese Medicine. Water was purified with Milli-Q® Biocel Ultrapure Water System (Millipore, Bedford, MA, USA). Methanol and acetonitrile were all of HPLC grade provided by Damao Chemical Reagent Plant (Tianjin, China). All other reagents were of analytical grade purchased from Jinfeng Chemical Factory (Tianjin, China).

2.2 Chromatographic System and Conditions

The analysis was performed on an Agilent 1100 series HPLC (Palo Alto, CA) incorporating a UV detector at a flow rate of 1 mL/min with the detection wavelength set at 320 nm. The separation was obtained using a Kromasil C18 column (5 μm, 150×4.6 mm, Dalian Jiangshen Separation Science and Technology Co., Ltd, Dalian, China) and the chromatographic system suitability test showed that the column had a high theoretical plates with 9000 and the resolution was more than 1.5, which was fully suited for the requirement of the chromatographic. The column temperature was maintained at 30℃. The separation was carried out with gradient elution procedure and mobile phase A (0.1% formic acid) and B (acetonitrile) ratio linear changed as follows: 0-15 min, 95%A; 15-35 min, 88% A; 35-55 min, 75% A; 55-65 min, 60% A. The mobile phase was passed under vacuum through a 0.45 μm membrane filter before use.

2.3 Standard Preparation

The eight standard compounds and IS were accurately weighed and dissolved in methanol by ultrasound and then diluted to appropriate concentration ranges for method validation. All stock and working standard solutions were stored in brown bottles at 4℃ until used for analysis.

2.4 Sample Preparation

The dried and powdered *P. oleracea L*. (10 g) samples from different locations were respectively refluxed for twice with 100 ml anhydrous methanol, each for 1 h, and the two extracted solutions were merged then evaporated to dryness under reduce pressure. The residue was washed in petroleum ether and removed the petroleum ether. The washed residue was evaporated to dryness before it was dissolved in methanol and transferred into a 10 mL volumetric flask. The solutions were filtered under vacuum through a 0.45 μm membrane filter before injection into the HPLC system for analysis.

2.5 Method Validation

Stock solutions of the eight reference standards and IS were prepared with methanol. The concentrations of CA, *p*-CA, SCOP, FA, QR, QUER, APIG, BERG and IS were 0.231, 0.182, 0.199, 0.156, 0.286, 0.251, 0.192, 0.249 and 0.190 mg/mL respectively. The highest concentration of CA, *p*-CA, SCOP, FA, QR, QUER, APIG, BERG in the calibration curve diluted from the stock solution were 10.0, 10.0, 20.0, 10.0, 30.0, 40.0, 15.0, 10.0 μg/mL respectively, and then diluted to aliquot seven calibrators to curve plotting. The concentration of CA, *p*-CA, SCOP, FA, QR, QUER, APIG, BERG were successively 2.50, 2.50, 5.00, 2.50, 7.50, 10.0, 3.75, 2.50; 1.00, 1.00, 2.00, 1.00, 3.00, 4.00, 1.50, 1.0; 0.40, 0.40, 0.80, 0.40, 1.20, 1.60, 0.60, 0.40; 0.20, 0.20, 0.40, 0.20, 0.60, 0.80, 0.30, 0.20; 0.10, 0.10, 0.20, 0.10, 0.30, 0.40, 0.15, 0.10; 0.05, 0.05, 0.10, 0.05, 0.15, 0.20, 0.075, 0.05 μg/mL respectively. The amount of IS was added 0.2 mL from the concentration of 0.190 mg/mL when seven calibrators of the eight reference standards were prepared by dilution of stock solutions. The calibration curves for each analyte were generated by plotting their peak area ratio of the eight constituents to IS vs the nominal concentrations. The regression equation was obtained by weighted ($1/C^2$) least-square linear regression. LOD and LOQ were determined by stepwise dilution of CA, *p*-CA, SCOP, FA, QR, QUER, APIG, BERG at low concentration level with 0.10, 0.1, 0.2, 0.1, 0.3, 0.4, 0.15, 0.1 μg/mL, respectively. The limit of detection (LOD) was determined in signal to noise ratio (S/N) of 3:1, and the lower limit of quantitation (LOQ) was determined in signal to noise ratio (S/N) of 10. The precision of the method were evaluated with the mixture standard compounds at one concentration and using six replicates in one day for intra-day variation and on three consecutive days for inter-day variation. The precision was expressed as the RSD. The recovery was determined by adding known amounts of the standard compounds, approximately 1 times the levels detected in unspiked samples prior to extraction, and these spiked samples were prepared as described in the “Sample preparation” section. The found amount of the reference standards subtracted that of unspiked sample was divided by the added known amount of the reference standards, and the extraction recovery was calculated as a percentage. The stabilities of the eight constituents in the methanol were investigated by comparing their peak area ratios of the eight constituents to IS at 0, 12, 24, 36 and 48 h.

3. RESULTS AND DISCUSSION

3.1 Optimization of Chromatographic Conditions

In previous documents, there are many reports about the quantification of the eight components such as FA using an ODS column (5μm particle size) eluted with a methanol, acetonitrile and water gradient by HPLC in the cell wall of higher plants [18], CA with a gradient elution system of methanol/water containing orthophosphoric acid by reverse phase column in the aromatic herbs [19], bergapten (BERG) also using the same column and a tetrahydrofuran-MeOH elution gradient from plant tissue cultures of *Ammi majus* hairy roots and *Ruta graveolens* cell suspensions [20] and so on.

To obtain a good chromatographic peak for each analyte and simple mobile phase, during the method development, the different combinations of several simple mobile phases including methanol-water and acetonitrile-water were chosen, a good separation and elution among the eight analytes, IS and interferences was finally obtained after the mobile phase consisting of acetonitrile-0.1% formic acid was applied, in which the formic acid was added to improve the peak shape and restrain the peak tailing. The UV absorption spectra of them were recorded from 210 to 400 nm and it was found that only the wavelength 320 nm which was set for detection can simultaneously obtain high sensitivities of the eight analytes. The typical chromatograms of the reference standards and a sample (Pingdingshan) were shown in Fig. 2.

3.2 Optimization of Extraction Procedure

To obtain satisfactory extraction efficiency of each analyte (the sample from Pingdingshan), two kinds of extraction methods (reflux and ultrasound), different solvents (anhydrous methanol, 70% methanol, 50% methanol), and extraction times (once, twice, three times) were optimized. The optimized conditions were at last obtained: refluxing for twice with anhydrous methanol, each for 1 h. Petroleum ether was used to wash the extract in order to remove the nonpolar compounds polluting the column. Simultaneous determination of the eight analytes was carried out with the internal standard method to prevent possible errors brought on by the changes of injection volume. To obtain a suitable IS, several compounds including rhein were chosen. Rhein, as it is commercial available and has the similar structure and property to the analytes, was chosen as the IS, a good baseline separation was obtained between the analytes and the adjacent peaks.

3.3 Method Validation

The results of calibration curves, LOD and LOQ are listed in Table 2, indicating that the linear ranges of the eight analytes were wide with high sensitivities. To evaluate the precision, the mixture standard solution was analyzed six times in one day for intra-day precision and on three successive days for inter-day precision. Table 3 indicated that the RSDs of intra-, inter-day precisions were respectively less than 2.09% and 2.98%, and the extraction recoveries for each compound carried out in six replicates were acceptable with RSD less than 12.1% at Table 4, suggesting that there was negligible loss during the extraction process. The stabilities of the eight constituents in the methanol were investigated during the storing period of 0–48 h, of which the eight constituents were considered to be stable with RSD less than 3% after the samples were treated as in the “Sample preparation” section under the experimental conditions of the regular analytical procedure.

3.4 Simultaneous Determination of the Eight Constituents

HPLC incorporating UV detector using the IS method was employed to simultaneously determine the eight constituents in *P. oleracea* L. Considering to the output of the herbs, the plant materials were collected from June 2013 to September 2013. The dried and powdered herbs were treated as in the “Sample preparation” section. The results of the variation of the eight constituents in *P. oleracea* L. from 20 different locations were listed in Table 1, in which *p*-CA, FA and QR were abundant in most locations and the maximal contents of them were 572.9, 146.5, 935.1 μg/g respectively. In addition, the contents of CA and *p*-CA in Beijing were higher than that of the other locations, and the contents of FA, QUER in Ji’an and APIG, BERG in Ningbo were the highest in the all samples. The maximal contents of SCOP and QR were respectively in Liu’an and Zhangjiajie. All the results aforementioned can be as the basis of separating and utilizing the analytes from *P. oleracea* L. Compared to some previous analytical methods analyzed few constituents and locations without internal standard [14], in

our study, a simple, accurate HPLC method combined with the internal standard with the simple mobile phase composition was used to simultaneously determine the eight compounds from 20 different locations. and the results obtained *via* HPLC analyzing the eight components of the herb can be applied to the quality assessment of *P. oleracea* L. with a good antibacterial and anti-inflammatory activities in clinic [7].

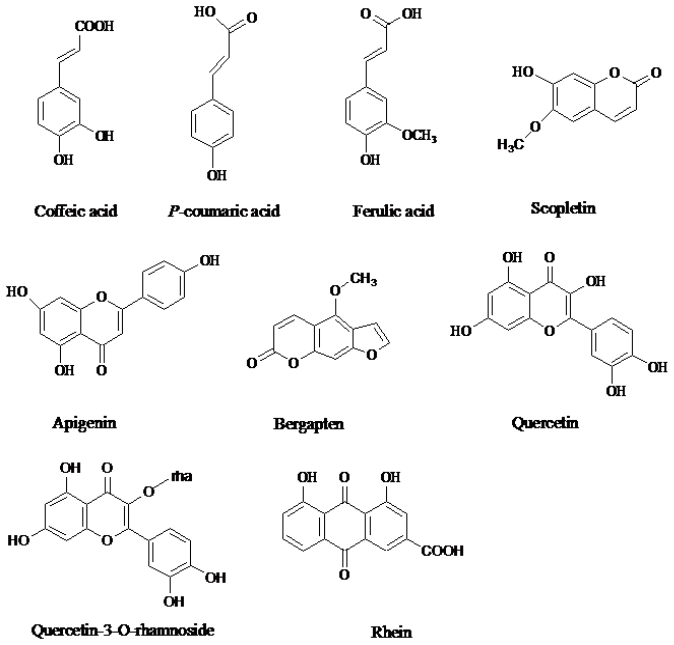

Fig. 1. The structures of the eight analytes and the internal standard

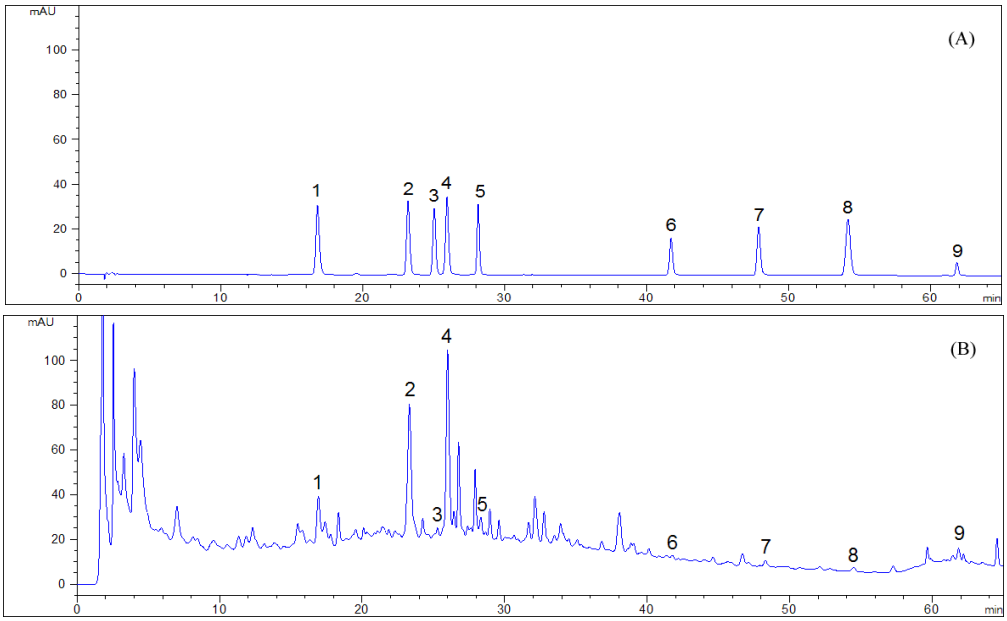

Fig. 2. The chromatograms of the standard analytes (A) and the sample (B) (1: caffeic acid; 2: *p*-coumaric acid; 3: scopoletin; 4: ferric acid; 5: quercetin-3-*O*-rhamnoside; 6: quercetin; 7: apigenin; 8: bergapten; 9: the internal standard)

Table 1. The contents of the eight analytes in *P. oleracea* L from different locations (μg/g)

Compound location	Caffeic acid	*p*-coumaric	Scopoletin	Ferulic acid	Quercetin-3-*O*-rhamnoside	Quercetin	Apigenin	Bergapter
Liu'an	17.86 ± 1.158	169.8 ± 16.24	81.89 ± 10.51	26.89 ± 4.013	20.40 ± 2.569	29.49 ± 4.235	0.476 ± 0.037	2.710 ± 0.258
Beijing	27.20 ± 4.451	572.9 ± 84.59	16.5 9 ± 0.931	46.13 ± 5.057	320.0 ± 32.46	11.82 ± 1.061	0.895 ± 0.075	_
Changde	10.88 ± 1.114	42.84 ± 7.332	28.04 ± 4.711	68.48 ± 8.049	385.3 ± 35.18	31.11 ± 3.054	46.17 ± 4.586	3.402 ± 0.305
Chengdu	1.011 ± 0.087	4.450 ± 0.099	0.121 ± 0.001	5.380 ± 0.446	7.710 ± 0.581	13.45 ± 1.265	23.91 ± 2.168	1.831 ± 0.157
Yantai	1.883 ± 0.124	292.4 ± 25.22	33.95 ± 5.937	40.72 ± 4.859	227.2 ± 26.49	6.450 ± 0.512	0.496 ± 0.058	_
Shijiazhuang	1.982 ± 0.144	25.61 ± 2.956	14.08 ± 0.884	6.032 ± 0.535	75.97 ± 8.486	_	2.963 ± 0.249	_
Chifeng	7.772 ± 0.677	66.94 ± 9.552	7.080 ± 0.588	21.58 ± 3.542	170.5 ± 17.59	16.51 ± 1.529	5.790 ± 4.275	2.881 ± 0.245
Huan'an,	2.645 ± 0.052	24.53 ± 4.110	4.852 ± 0.072	15.35 ± 1.154	11.12 ± 1.001	26.24 ± 2.549	52.87 ± 4.915	1.842 ± 0.148
Huanggang	11.96 ± 1.211	76.99 ± 9.957	4.596 ± 0.071	47.12 ± 5.106	68.35 ± 7.652	15.84 ± 1.326	8.292 ± 0.748	2.003 ± 0.215
Ji'an	7.130 ± 0.954	73.36 ± 8.963	7.435 ± 0.610	146.5 ± 15.75	895.5 ± 89.49	32.71 ± 3.164	108.2 ± 12.52	4.692 ± 0.413
Shenzhen	0.062 ± 0.001	12.32 ± 1.414	2.023 ± 0.161	9.980 ± 1.057	0.572 ± 0.043	23.01 ± 2.016	9.230 ± 0.849	3.131 ± 0.304
Suzhou	0.911 ± 0.061	15.19 ±1.086	7.720 ± 0.410	3.921 ± 0.088	20.25 ± 4.623	6.840 ± 0.618	0.702 ± 0.060	_
Xiangyang	1.568 ± 0.214	57.57 ± 7.446	17.62 ± 1.079	5.411 ± 1.059	32.48 ± 6.486	10.07 ± 0.975	43.97 ± 3.915	3.211 ± 0.324
Ha'erbin	14.70 ± 1.022	23.54 ± 3.882	14.63 ± 0.952	19.54 ± 3.109	195.9 ± 19.79	28.42 ± 2.713	34.38 ± 3.012	_
Ningbo	1.988 ± 0.141	10.61 ± 0.918	2.246 ± 0.061	15.98 ± 1.235	3.092 ± 0.349	7.971 ± 0.618	164.7 ± 15.48	6.133 ± 0.548
Yuncheng	1.994 ± 0.155	17.90 ± 1.380	25.71 ± 3.915	7.320 ± 0.559	25.29 ± 4.823	6.050 ± 0.549	1.450 ± 0.102	_
Zhangjiajie	24.22 ± 3.799	56.24 ± 7.014	16.67 ± 1.135	39.02 ± 4.649	935.1 ± 90.16	_	1.342 ± 0.091	_
Pingdingshan	0.896 ± 0.055	183.9 ± 38.85	49.98 ± 5.232	17.25 ± 1.549	127.2 ± 13.04	31.35 ± 2.185	9.801 ± 0.815	1.581 ± 0.123
Dalian	0.178 ± 0.001	15.21 ± 1.195	8.982 ± 0.706	9.555 ± 1.106	11.92 ± 1.095	6.623 ± 0.548	4.933 ± 0.348	1.534 ± 0.112
Liaozhong	0.163 ± 0.003	20.57 ± 1.521	1.541 ± 0.201	7.115 ± 1.026	14.01 ± 1.504	5.428 ± 0.459	10.44 ± 0.916	1.591 ± 0.142

"-" below the LOD

Table 2. Calibration curves of the eight reference standards

Compound	Linear equation	r	Linear range (μg/mL)	LOD (μg/mL)	LOQ (μg/mL)
Caffeic acid	y=1.0015X+0.0329	0.9999	0.05-10	0.013	0.044
p-coumaric acid	y=0.9715X+0.0522	0.9991	0.05-10	0.012	0.040
Scopoletin	y=0.4197X+0.0527	0.9995	0.10-20	0.030	0.098
Ferutic acid	y=1.3201X-0.0216	0.9991	0.05-10	0.012	0.041
Quercetin-3-*O*-rhamnoside	y=0.2277X+0.0251	0.9991	0.15-30	0.040	0.131
Quercetin	y=0.1974X-0.0188	0.9998	0.20-40	0.053	0.176
Apigenin	y=0.4565X+0.0413	0.9990	0.075-15	0.020	0.068
Bergapten	y=1.5305X-0.0449	0.9993	0.05-10	0.011	0.035

y, peak area ratio; x, concentration of the reference standard (μg/mL); r, correlation coefficient; LOD, limit of detection; LOQ, limit of quantification.

Table 3. Precision of the eight analytes (n=6)

Compounds		Intra-day			Inter-day		
	Added conc (μg/mL)	Mean (μg/ml)	RSD (%)	RE (%)	Mean (μg/ml)	RSD (%)	RE (%)
Caffeic acid	7.00	6.58	1.58	6.00	6.52	1.07	6.86
p-coumaric	7.00	6.51	0.470	7.00	6.48	1.06	7.43
Scopoletin	14.0	12.8	0.740	8.70	12.8	2.14	8.71
Ferutic acid	7.00	6.08	2.09	13.1	6.20	1.24	11.4
Quercetin-3-*O*-rhamnoside	21.0	19.9	0.300	5.33	19.8	1.88	5.57
Quercitrin	24.0	21.0	1.79	12.3	20.9	1.93	12.9
Apigenin	10.5	10.0	0.540	4.67	10.1	2.98	4.00
Bergapten	7.00	6.81	1.08	2.71	6.81	2.37	2.71

Table 4. The recovery of the eight analytes

Compounds	Initial (μg)	Added (μg)	Final (μg)	Recovery mean (μg)	Recovery mean (%)	RSD (%)
Caffeic acid	3.14	3.41	6.61	3.47	101.6	9.74
p-coumaric	15.1	15.6	29.7	14.6	93.67	9.93
Scopoletin	0.925	0.940	1.93	1.01	107.2	10.7
Ferutic acid	16.3	12.2	17.8	11.5	93.88	9.88
Quercetin-3-*O*-rhamnoside	13.9	13.3	28.9	15.0	113.3	12.0
Quercitrin	5.23	5.30	10.4	5.20	98.26	10.8
Apigenin	3.10	3.12	6.17	3.07	98.50	12.1
Bergapten	0.320	0.350	0.690	0.370	106.0	6.65

4. CONCLUSION

An HPLC method using the internal standard was developed to evaluate the quality standard of *P. oleracea* L. *via* simultaneous determination of the eight constituents in the herb from 20 different locations. The result indicated that the contents of the eight components in *P. oleracea* L. from different locations varied significantly which can be as the means to assess the quality of *P. oleracea* L. from different sources when it was exploited and utilized.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Xu X, Yu L, Chen G. Determination of flavonoids in *Portulaca oleracea L.* by capillary electrophoresis with electrochemical detection. Journal of Pharmaceutical and Biomedical Analysis. 2006;41:493-9.
2. Zhong Y, Wang l. Xiang, Li, s. Chun, Xuan Determination of Alkaloids in *Portulaca oleracea L*. Journal Taiyuan University of Technology. 2001;32:197-198.
3. Xin HL, Xu YF, Hou YH, Zhang YN, Yue XQ, Lu JC, et al. Two Novel Triterpenoids from *Portulaca oleracea L*. Helvetica Chimica Acta. 2008;91:2075-2080.
4. Park J, Kim K. Identification of physiologically active compounds from Purslane (*Portulaca oleracea L*.). Korean Journal of Weed Science. 1988;8:169-175.
5. Awad N. Lipid content and antimicrobial activity of phenolic constituents of cultivated *Portulaca oleracea L.* Bull Fac Pharm Cairo Univ. 1994;32:137-142.
6. Parry O, Okwuasaba FK, Ejike C. Skeletal muscle relaxant action of an aqueous extract of *Portulaca oleracea L.* in the rat. Journal of Ethnopharmacology. 1987;19:247-53.
7. Chan K, Islam M, Kamil M, Radhakrishnan R, Zakaria M, Habibullah M, et al. The analgesic and anti-inflammatory effects of *Portulaca oleracea* L. subsp.sativa(Haw.) Celak. Journal of Ethnopharmacology. 2000;73:445-451.
8. Oh KB, Chang IM, Hwang KJ, Mar W. Detection of antifungal activity in *Portulaca oleracea L.* by a single cell bioassay system. Phytotherapy Research. 2000;14:329-332.
9. Verma OP, Kumar S, Chatterjee SN. Antifertility effects of common edible *Portulaca oleracea* L. on the reproductive organs of male albino mice. Indian Journal of Medical Research. 1982;75:301-10.
10. Chen CJ, Wang WY, Wang XL, Dong LW, Yue YT, Xin HL, et al. Anti-hypoxic activity of the ethanol extract from *Portulaca oleracea L.* in mice. Journal of Ethnopharmacology. 2009;124:246-250.
11. Liu L, Howe P. Zhou YF, Xu ZQ, Hocart C, Zhan R. Fatty acids and beta-carotene in australian purslane (*Portulaca oleracea L*.) varieties. Journal of Chromatography A. 2000;893:207-13.
12. Yue ME, Jiang TF, Shi YP. Simultaneous determination of noradrenaline and dopamine in *Portulaca oleracea L*. by capillary zone electrophoresis. Journal of Separation Science. 2005;28:360-4.
13. Cheng ZZ, Wang D, Zhang W, Du Y, Wang Y, Zhai Y, et al. LC determination and pharmacokinetic study of the main phenolic components of *Portulaca oleracea L.* extract in rat plasma after oral administration. Natural Product Research. 2012;26:2247-50.
14. Lin Y, Li Z, Wu B, Yang Q, Jiang J, Huang C. Quanlity assessment for *portulaca oleracea L.* by multi-component quantification, chromatographic fingerprint and related chemometric analysis. Journal of Liquid Chromatography. 2012;35:2655-2668.
15. Zhu H, Wang Y, Liang H, Chen Q, Zhao P, Tao J. Identification of *Portulaca oleracea L.* from different sources using GC-MS and FT-IR spectroscopy. Talanta. 2010;81:129-35.
16. Chawla AS, Singh M, Murthy MS, Gupta M, Singh H. Anti-inflammatory action of ferulic acid and its esters in carrageenan induced rat paw oedema model. Indian Journal of Experimental Biology. 1987;25:187-9.

17. Aziz NH, el-Fouly MZ, Abu-Shady MR, Moussa LA. Effect of gamma radiation on the survival of fungal and actinomycetal florae contaminating medicinal plants. Appl Radiat Isot. 1997;48:71-6.
18. Waldron KW, Parr AJ, NG A, Ralph J. Cell wall esterified phenolic dimers: identification and quantification by reverse phase high performance liquid chromatography and diode array detection. Phytochemical Analysis. 1996;7:305-312.
19. Wang H, Provan GJ, Helliwell K. Determination of rosmarinic acid and caffeic acid in aromatic herbs by HPLC. Food Chemistry. 2004;87:307-311.
20. Kaminski M, Kartanowicz R, Kaminski MM, KR Licka A, Sidwa□Gorycka M, Łojkowska E, Gorzen W. HPLC□DAD in identification and quantification of selected coumarins in crude extracts from plant cultures of Am mi majus and Ruta graveolens. Journal of Separation Science. 2003;26:1287-1291.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=685&id=13&aid=6461