Chiang Mai J. Sci. 2017; 44(4) : 1453-1462
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

Standardization of *Gardenia jasminoides* Fruits and Crocin Content Analysis Using UV/visible Spectrophotometry

Onuma Zongram [a], Nijsiri Ruangrungsi [a,b], Chanida Palanuvej [a] and Kanchana Rungsihirunrat* [a]

[a] College of Public Health Sciences, Chulalongkorn University, Bangkok 10330, Thailand.

[b] Faculty of Pharmacy, Rangsit University, Pathumthani 12000, Thailand.

*Author for correspondence; e-mail: kanchana.r@chula.ac.th

Received: 24 September 2015
Accepted: 24 December 2015

ABSTRACT

Crocin is an important source of food colorant that is found in the fruit of *Gardenia jasminoides* which exhibited various biological activities for the treatment of inflammation, jaundice, headache, edema, fever, hepatitis and hypertension. Standardization of herbal medicines is the process carries out for an assessment of quality, efficacy, safety and reproducibility using various methods. The aim of the current study was to evaluate the microscopic characteristics, physicochemical properties, TLC fingerprint and UV/visible spectrophotometry method for the quantitative estimation of crocin content in the fruit of *G. jasminoides*. Microscopic inspection of powdered herbal drug showed the presence of plant structural tissue including multicellular trichomes. Physicochemical parameters and TLC fingerprinting were also established. Percentage of total ash value, acid insoluble ash value, loss on drying and moisture content should not more than 4.93, 0.71, 8.85 and 5.02% w/w, respectively while water soluble extractive value and ethanol soluble extractive value should not less than 26.91 and 22.53 % w/w, respectively. UV/visible spectrophotometry method was validated for determination of crocin content. The method validation was performed according to the ICH guideline. The linearity was in the range between 5 and 100 µg/ml (r^2=0.999) and exhibited suitable accuracy, precision and robustness. The result of crocin content analysis of *G. jasminoides* fruits was 7.59 ± 2.64 mg/g of dried crude drug. The established methods in this study can be applied for assessing the standardization and the crocin content in *G. jasminoides* fruits.

Keywords: *Gardenia jasminoides* fruit, microscopic examination, physicochemical parameters, crocin, UV/visible spectrophotometry

1. INTRODUCTION

Medicinal plants have been an important source of medicinal substances for primary health care [1]. World Health Organization (WHO) recommends, encourages, promotes and supports the use of traditional medicines because of their availability and affordability

[2-3]. Therefore, a system of standardization is necessary for quality control and quality assurance [4]. Standardization parameters such as macroscopic and microscopic examinations, physicochemical analysis, fluorescence analysis and phytochemical screening for plant drugs are mentioned in pharmacopoeia [5].

The fruit of *Gardenia jasminoides* Ellis (Family Rubiaceae) (Figure 1) is an important traditional medicine exhibited various biological activities for the treatment of inflammation, jaundice, headache, edema, fever, hepatitis and hypertension[6]. The *Gardenia* fruit contain crocin (crocetin di-gentiobiose ester) which is a water soluble carotenoid, yellow pigment and used as food colorants in oriental countries in products such as noodles and confectioneries [6-7]. Moreover, the major constitutions containing such as gardenoside, genipin, geniposide, chlorgenic acid, gentiobioside, crocetin, gardenin, mannitol and beta-sitosterol were also found in *G. jasminoides* fruit [8].

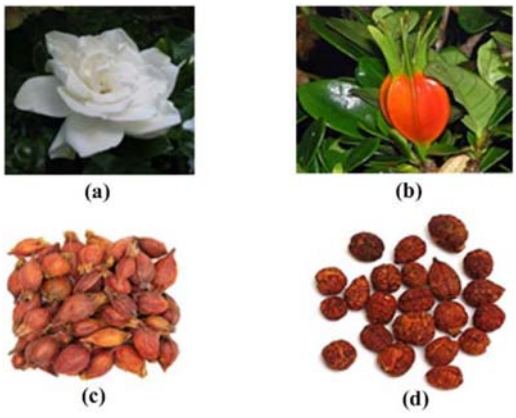

Figure 1. *Gardenia jasminoides*: flower (a); ripened fruit (b); dried fruit (c) and seed (d).

Various analytical methods such as, UV/visible spectrophotometry, thin layer chromatography (TLC), GC-MS and HPLC have been developed for quantitative analysis of crocin content from saffron stigmas [9-10] but crocin content analysis from *Gardenia jasminoides* fruits using UV/visible spectrophotometry which is a rapid, simple, economic, accurate and reproducible method has not been established [9]. Despite the medicinal importance of the *G. jasminoides* fruit, scientific information on the standardization parameters of this plant is unavailable. This study aimed to investigate the microscopic evaluation, physicochemical analysis, TLC fingerprint and to develop the validation of UV/visible spectrophotometric method for determination of crocin content in *G. jasminoides* fruits. This study can assist in standardization and also can be used to develop a monograph for the proper identification of the plant material.

2. MATERIALS AND METHODS

Plant Materials

Dried fruits of *G. jasminoides* were collected from 12 different traditional Thai drug stores located in different provinces throughout four parts of Thailand. Plant samples were authenticated by one of the authors (N.R.) and voucher specimens were deposited at College of Public Health Sciences, Chulalongkorn University. The dried fruits were pulverized with mechanical pulverizer for size reduction. The dried fruits powders were used for microscopic study, physicochemical parameters determination, TLC fingerprint and crocin content analysis.

2.1 Powder Microscopy

A small quantity of the *G. jasminoides* fruit powders was heated with chloral hydrate for 10 min and mounted over the glass slide in 50% glycerin and then observed under a light microscope (Zeiss Axio Imager A2). The recorded images were illustrated in a size proportional to the original scale.

2.2 Physicochemical Constants

The physicochemical parameters including loss on drying, moisture, ashes and solvent extractive values were evaluated from 12 samples of *G. jasminoides* fruit powders in triplicate according to the WHO guideline on quality control method for medicinal plants materials with some modification.

2.3 TLC Fingerprint

One gram of each twelve different samples of *G. jasminoides* fruit powders was macerated in ethanol for 24 hrs and then 20 ml of ethanol extractive soluble was filtered and evaporated to dryness and re-dissolved in 1 ml of ethanol. Three microliter of the ethanolic extract was applied on the silica gel 60 GF_{254} TLC plate with 0.2 mm thickness (Merck, Germany). The plate was developed in a solvent system of ethyl acetate: iso-propyl alcohol: water (65:25:10). The TLC fingerprint profile was visualized under daylight, UV light at 254 nm and 366 nm and sprayed with p-anisaldehyde reagent.

2.4 Crocin Content Analysis Using Spectrophotometric Method: Isolation of Crocin from *G. jasinoides* Fruit Powders

Ten gram of *G. jasminoides* fruit powders from 12 samples were individually extracted in soxhlet apparatus with 90% ethanol until it was exhausted. The extracts were filtered, evaporated to dryness under reduced pressure in a rotary evaporator. The percentage yields of the extracts were calculated. The extracts were then stored in a refrigerator and protected from light for crocin content and component analysis.

Preparation of Standard Stock Solution and Calibration Curve

Standard crocin stock solution (Sigma, USA) was dissolved in methanol to make a final concentration of 1 mg/ml. A SHIMADZU UV/visible Spectrophotometer (UV1800) was used for scanning the absorption spectrum of standard crocin ranging from 300-600 nm and the λmax was recorded. A series of five different concentrations of standard crocin ranging from 5-100 mg/ml were prepared and the calibration curve was obtained by plotting the absorbance at λmax *versus* five different concentrations of crocin.

2.5 Method Validation

Spectrophotometric method for quantitative analysis of crocin in *G. jasminoides* fruit was validated with respect to linearity, range, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ) and robustness according to the ICH guideline [11].

Linearity, Range, LOD and LOQ

The series of five different concentrations of standard crocin (5-100 µg/ml) were prepared from the stock solution and were scanned for absorbance at λmax 434 nm. Least square regression analysis was performed from the obtained data. The LOD and LOQ were determined using calibration standards. LOD and LOQ were calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively, where σ is the standard deviation of y-intercept of regression equation and S is the slope of the calibration curve.

Accuracy

Accuracy was determined by spiking methods of crocin at 3 levels (lower, intermediate and higher concentration) from independent standard stock solutions of 1 mg/ml (N = 9). Three different concentrations of standard crocin (20, 40, 60 mg/ml) were added to crocin sample and

the total crocin content was determined. The accuracy was determined as percentage recovery of the spiked standard crocin in the sample. Percentage recovery (% Recovery) = [(Cs-Cu)/Ca] × 100, where Cs is the total crocin concentration measured after standard addition; Cu is crocin concentration in the sample and Ca is standard crocin concentration added to the sample. Each test was done in triplicate.

Precision

The precision of the method was determined by repeatability and intermediate precision and reported as percent relative standard deviation (% RSD) of 3 concentrations of sample and 3 replicate each.

Robustness

Robustness was determined by analyzing absorbance of crocin in the extract sample added with 30 ug/ml of standard crocin at slightly different wavelength (433, 434, and 435 nm) and 6 replicates of each wavelength were determined.

2.6 Crocin Components Analysis Using TLC Densitometric Method

Crocin Content Analysis

The extracted of *G. jasminoides* fruit powders from twelve samples were prepared in methanol to obtain a concentration of 1 mg/ml and determined for their total crocin content using spectrophotometry. The experiment was performed in triplicate.

The extracted of *G. jasminoides* fruits and standard crocin were separated by TLC. Three microliter of 3 concentration levels (7.5, 10, 12.5 µg/spot) of standard crocin and extracted crocin sample solutions (100 mg/ml) were applied on an aluminium sheet of silica gel G60 F_{254} (20 × 10 cm with 0.2 mm thickness; E. Merck, Darmstadt, Germany) as 7 mm band with a Linomat V automatic sample spotter (Camag, Switzerland). The mobile phase ratio of n-butanol: acetic acid: water is 4: 1: 1 [12]. Densitometric analysis was carried out at 434 nm using a TLC Scanner 3 (Camag, Switzerland) with winCATS software.

3. RESULTS

3.1 Powder Microscopy

The powder microscopy revealed the presence of pieces of epidermal cell, fiber, parenchyma, epidermis of seed coat, fragment of spiral vessels, sclereids, fragment of pitted vessels and multicellular trichomes (Figure 2).

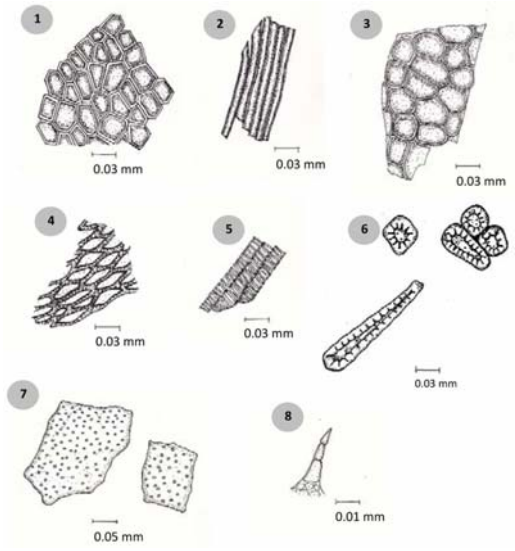

Figure 2. Microscopic characters of powdered *Gardenia jasminoides* fruit: epidermal cell (1), fiber (2), parenchyma (3), epidermis of seed coat (4), fragment of spiral vessels (5), sclereids (6), fragment of pitted vessels (7), and multicellular trichomes (8).

3.2 Physicochemical Constants

The percentage of loss on drying, total ash, acid-insoluble ash, water soluble extractive, ethanol soluble extractive and moisture contents are presented in Table 1.

Table 1. Physicochemical parameters analysis of *G. jasminoides* fruits.

Parameter	Contents* (%w/w)
Total ash	4.93±0.16
Acid insoluble ash	0.71±0.13
Loss on drying	8.85±1.13
Water soluble extractives	26.91±2.41
Ethanol soluble extractives	22.53±1.64
Moisture	5.02±0.57

*Results were expressed as grand mean ± pooled SD from 12 samples in triplicate.

3.3 TLC Fingerprint

The TLC fingerprint profile of *G. jasminoides* showed three yellow spots under daylight, four major spots under 254 nm, one major spot under 366 nm and four major spots under daylight after spraying with p-anisaldehyde (Figure 3).

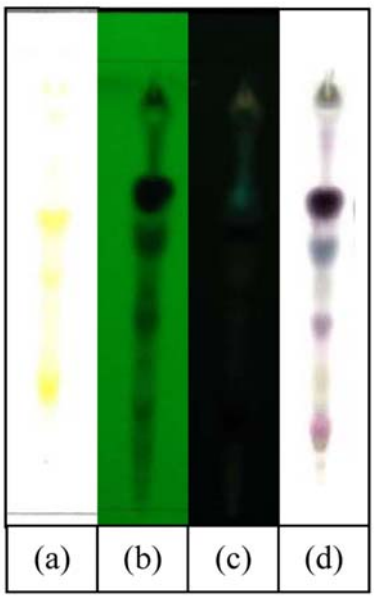

Figure 3. TLC fingerprint profile of *G. jasminoides* fruits under daylight (a), under 254 nm (b), under 366 nm (c), and under daylight after spray with p-anisaldehyde reagent (d).

3.4 Crocin Content Analysis Using Spectrometric Method

The yields of ethanolic extracted by soxhlet extraction and crocin contents determined by spectrophotometric method of 12 samples of *G. jasminoides* fruits collected from different locations in Thailand were presented in Table 2.

3.5 Method Validation

Method validation with respect to linearity, range, accuracy, precision, LOD, LOQ and robustness was performed according to the ICH guideline. The λmax of crocin was found to be 434 nm. Linear correlation parameters obtained from plotting absorbance *versus* concentration of standard crocins in the range of 5-100 mg/ml was shown in Table 3.

3.6 Crocin Components Analysis Using TLC Densitometric Method

The TLC analysis of 12 *G. jasminoides* fruits extracted developing in n-butanol: acetic acid: water (4 : 1 : 1) was shown in Figure 4. Standard crocin (Lane 1-3) showed six distinguishable bands indicated that standard crocin was composed of six crocin components with different Retention factor (Rf) value while 12 *G. jasminoides* fruits extracted (Lane 4-15) showed less than six separated bands in yellow observed under daylight. Crocin component 6 (Rf 0.23) was not found in all 12 *G. jasminoides* fruit extracted while crocin component 1 (Rf 0.63), 3 (Rf 0.43), 4 (Rf 0.32) and 5 (Rf 0.28) were found in all 12 samples with different contents. Crocin component 2 (Rf 0.51) was found in 7 samples. The major crocin component content was crocin component 1 which was observed in all 12 samples ranging from 58.66-83.91% (Table 4).

Table 2. Yield of ethanolic extracted by soxhlet extraction and crocin content by UV/visible spectrophotometry of *G. jasminoides* fruits.

Sample	Extracted yield (mg/g of dried crude drug)	crocin content (mg/g of dried crude drug)
S1	383.1	4.74±0.06
S2	386.4	7.52±0.20
S3	351.5	5.54±0.53
S4	376.6	7.66±0.19
S5	368.0	7.19±0.34
S6	353.7	7.22±0.79
S7	360.9	6.46±0.16
S8	373.6	13.34±0.32
S9	375.6	10.11±0.32
S10	374.1	4.39±0.25
S11	434.7	8.95±0.52
S12	455.7	7.55±0.27
Mean±SD	382.8±31.3	7.55±2.39

Table 3. The validities of quantitative analysis of crocin in *G. jasminoides* fruits using spectrophotometric method.

Method validities	
λmax (nm)	434
Range (mg/ml)	5-100
Regression equation (y= mx+c)	y= 0.0084x-0.0045
R^2	0.999
LOD (mg/ml)	1.36
LOQ (mg/ml)	4.12
Accuracy (%Recovery)	88.96
Repeatability (%RSD)	0.971.35
Intermediate precision (%RSD)	0.45
Robustness (%RSD)	

Table 4. Crocin component of 12 *G. jasminoides* fruits extracted samples using TLC densitometry.

Crocin component	Rf	Percentage of each component (%)											
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12
1	0.63	73.10	70.15	68.67	72.55	58.66	67.08	60.42	75.82	83.91	74.04	78.27	78.93
2	0.51	0	0	6.11	4.70	8.97	6.17	7.49	4.86	3.97	0	0	0
3	0.43	5.33	7.66	7.78	5.74	9.90	5.45	10.40	6.37	4.03	9.09	7.70	10.78
4	0.32	11.22	12.04	10.38	10.18	10.26	12.09	14.08	8.14	3.32	8.30	9.25	6.55
5	0.28	10.35	10.15	7.06	6.83	12.21	9.21	7.61	4.81	4.77	8.57	4.78	3.74
6	0.23	0	0	0	0	0	0	0	0	0	0	0	0

* Rf=Retention factor

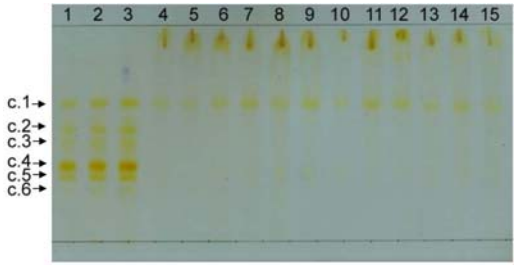

Figure 4. TLC chromatogram of crocin components from *G. jasminoides* fruit extracts: standard crocin (Lane 1-3), 12 samples of *G. jasminoides* fruit extracts (Lane 4-15), c.1-c.6 (crocin component 1 - crocin component 6).

4. DISCUSSION

Various analytical methods were elaborated for medicinal plant identification. Most of these methods were based on plant macroscopic, microscopic and chemical composition. According to WHO guideline, the quality evaluation of the starting material is a fundamental requirement of manufacturing herbal medicinal products. The development of authentic analytical methods including quantitative analyses of marker/bioactive compounds and other major constituents, is a major challenge to scientists. To prove the consistent composition of herbal preparations, adequate analytical methods have been applied including TLC and UV/visible pectrophotometry. This present study proposed a standardization of *G. jasminoides* fruits and crocin content analysis using UV/visible spectrophotometry.

Microscopic can be considered both for powders and ungrounded drugs. The types of epidermal parenchyma, stomata, trichomes, fibers, vessels and calcium oxalate crystals have been used for the identification [13-15]. According to the results, microscopic analysis of *G. jasminoides* fruit powders revealed of various important histological characters which can serve as useful parameters for the identification of this plant. Physicochemical parameters can also serve as valuable information in evaluation of purity and quality of plant materials [16]. This study is the first report of physicochemical parameters determination of *G. jasminoides* fruits. The water extractive matter (26.91 ± 2.41%) was higher than ethanol extractives (22.53 ± 1.64%). It indicates that *G. jasminoides* fruit has large amount of polar compounds or water soluble constituents like phenols, alkaloids, steroids, glycosides, flavonoids [1]. Percentage of weight loss on drying and moisture contents were found to be 8.85 ± 1.13 and 5.02 ± 0.57, respectively. The less value of moisture contents could prevent bacterial, fungal or yeast growth [17]. The total ash value and acid insoluble ash value were found to be 4.93 ± 0.16% and 0.71 ± 0.13%, respectively. Ash values are useful in determining authenticity and purity of plant material and also these values are important quantitative standards [17]. Evaluation of physicochemical parameters is an important part in the preparation of modern monograph [18] . TLC fingerprint is a simple, rapid and inexpensive method for qualitative determination of small amounts of impurities. However, the visualization of developed TLC fingerprint remains a subjective interpretation. The subsequent use of UV/visible spectrophotometry has shown to be adequate for conclusive interpretation. UV/visible spectrophotometry is the absorption spectroscopy using the UV and visible spectral region for the quantitative of the chemical compounds. Due to its relatively fast, cheap, simple and routinely used, the TLC and UV/visible spectrophotometry methods were employed in this study for the quantification of crocin content in *G. jasminoides* fruits.

Crocin is an importance bioactive compound which can be isolated from saffron (*Crocus sativus*) and gardenia fruits (*G.*

jasminoides) [9, 19-20]. In the present study, soxhlet extraction was used to isolated crocin from gardenia fruits due to its continuous character, simple method, not expensive and possibility to extract more sample mass than the other methods [21]. The percentage of extraction yield of 12 *G. jasminoides* fruits samples were ranging from 35.15-45.57 g. Various analytical methods have been developed for determination of crocin content in saffron such as UV/visible spectrophotometry [22], HPLC [19],ultra-performance liquid chromatography [23], HPLC-ELSD [24], TLC, UV-Spectrophotometer, infrared spectrophotometer and NMR [25], HPLC-DAD-ESI-MS [26], LC-MS [27]. However, such equipments and instruments are expensive and they are not available in most laboratories. As a result, a method for determination of crocincontent was developed using UV/visible spectrophotometry.

Method validation plays a major role in achieving consistent, reliable and accurate data. Thus the analytical procedure obtain from method validation can be used to judge the quality standard result. According to the ICH guideline for quantitative of major compound, the parameters such as linearity, range, LOD, LOQ, accuracy, precision, and robustness should be validated. The λmax of crocin was found to be 434 nm and the standard linear calibration curve showed a good linear relationship with accepted value (0.999). The quantitative amount of crocin was in the range from 44.36 to 140.59 mg. Six components of crocins were detected in standard crocin (Sigma) by TLC densitometry method as well as other standard crocin (Fluka) has been detected six components of crocin by HPLC [28]. However, four to five crocin component were determined in 12 *G. jasminoides* fruits extracted using TLC densitometry which similar to the previous reported less than six crocin components (crocin 1, crocin 2, crocin 3) in gardenia fruits by HPLC analysis [29], five crocin components in gardenia fruits by MS, UV/visible and 1D NMR [30].

CONCLUSION

The finding on evaluation of powder microscopy, physicochemical parameters and TLC fingerprint of *G. jasminoides* fruits can serve as an important tool for the identification and authentication parameters of this plant. Moreover, the simple, rapid, sensitive and specific UV/visible spectrophotometric method were developed for determination of crocin content in *G. jasminoides* fruits. This precisely and accurately analytical method was suitable for the quality control of crocin in herbal medicine.

ACKNOWLEDGEMENTS

This research was scholarly supported by the scholarship from College of Public Health Sciences, Chulalongkorn University, and The 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund). The authors would like to thanks College of Public Health Sciences, Chulalongkorn University for their facilities.

REFERENCES

[1] Kadam P.V., Deoda R.S., Shivatare R.S., Yadav K.N. and Patil M.J., *Der. Pharmacia Lett.*, 2012; **4(2)**: 607-613.

[2] Rose E.N., Namdeo K.P. and Samal P.K., *Res. J. Pharm. Technol.*, 2008; **1(4)**: 310-312.

[3] Bigoniya P., Singh C.S. and Srivastava B., *Asian Pacific J. Trop. Biomed.*, 2012; **2(1)**: S290-S295.

[4] Vijay K.P., Laxman B.C., Balasaheb S.R., Yuvraj N.R. and Janardhan M.P., *J. Biol. Sci. Opin.*, 2013; **1(1)**: 21-24. DOI 10.7897/2321-6328.01124.

[5] World Health Organization, *Quality Control Methods for Herbal Materials*, Geneva, Switzerland, 1998.

[6] Han J., Zhang W., Cao H., Chen S. and Wang Y., *Biochem. Syst. Ecol.*, 2007; **35(3)**: 138-145. DOI 10.1016/j.bse. 2006.05. 021.

[7] Ding Y., Xiang L.Y., Zhang T., Ji S. and Xu L.Y., *J. Food Drug Anal.*, 2010; **18(3)**: 180-190. DOI 10.1016/j.foodchem. 2015.05.039.

[8] Singla R.K. and Giliyaru V.B., *Indo. Global J. Pharm. Sci.,* 2011; **1(4)**: 281-286.

[9] Zhang H., Zeng Y., Yan F., Chen F., Ahang X., Liu M. and Liu W., *Chromatographia,* 2004; **59**: 691-696. DOI 10.1365/s10337-004-0311-z.

[10] Carmona M., Zalacain A., Sanchez A.M., Novella J.L. and Alonso G.L., *J. Agric. Food Chem.*, 2006; **54**: 973-979. DOI 10. 1021/jf052297w.

[11] ICH harmonized tripartite guideline, validation of analytical procedures: text and methodology Q2(R1), in International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use, 2005.

[12] Bolhasani A., Bathaie S.Z., Yavarit A.A., Movahedi M. and Ghaffari M., *Asian J. Chem.,* 2005; **17(2)**: 725-729.

[13] Khare P., Chaudhary S., Kumar A., Yadav G. and Thakur N., *Middle-East J. Scientific Res.,* 2013; **15(10)**: 1472-1477. DOI 10.5829/idosi.mejsr. 2013.15.10. 2111.

[14] Sanmugarajah V., Thabrew I. and Sivapalan S.R., *J. Pharm.,* 2013; **3(2)**: 52-58.

[15] Parmar S. and Patel P.M., *Am. J. Pharm. Technol. Res.*, 2013; **3(1)**: 526-537.

[16] Kumar D., Kumar A. and Prakash O., *Beni-Suef Univ. J. Basic Appl. Sci.,* 2014; **3(1)**: 32-36. DOI 10.1016/j.bjbas.2014. 02.004.

[17] Mulla S.K. and Swamy P., *Int. J. Pharm. Technol. Res.,* 2010; **2(3)**: 1699-1702.

[18] Deb N.K. and Dash G.K., *Der. Pharmacia Lett.,* 2014; **6(4)**: 267-271

[19] Papandreou M.A., Kanakis C.D., Polissiou M.G., Efthimiopoulos S., Cordopatis P., Marqurity M. and Lamari F.N., *J. Agric. Food Chem.,* 2006; **54(23)**: 8762-8768. DOI 10.1021/jf061932a.

[20] Hassani F.V., Naseri V., Razavi B.M., Mehri S., Abnous K. and Hosseinzadeh H., *J. Pharmaceut. Sci.,* 2014; **22**: 1-9. DOI 10.1186/2008-2231-22-16.

[21] Woz'niak K.S., Widelski J. and Gtowniak K., *Thin Layer Chromatography in Phytochemistry,* 2008; Vol. 99., The United State of America: CRC Press. DOI 10.3390/molecules17044133.

[22] Dar R.A., Brahaman P.K., Tiwari S. and Pitre K.S., *E-J. Chem.*, 2012; **9(2)**: 918-925. DOI 10.1155/2012/967079.

[23] Yang W., Wang J., Li X. and Du Z., *J. Chromatography A,* 2011; **1218(11)**: 1423-1428. DOI 10.1016/j.chroma. 2010.12.121.

[24] Chen Y., Yang Z.L., Zhang L.H., Liu S.J. and Zhang X.T., *Zhong Yao Cai*, 2011; **34(5)**: 687-690.

[25] Siwawej S. and Jarayapun A., *Kasetsart J. (Nat. Sci.),* 1998; **32**: 520-528.

[26] Wu X., Zhou Y., Mao C., Li L., Cai B. and Lu T., *J. Phytomed.*, 2014; **21(4)**: 551-559. DOI 10.1016/j.phymed.2013.10.002.

[27] Xiaoling Z., *Adv. Mater. Res.,* 2014; 1073-1076.

[28] Hadizadeh F., Mohajeri S.A. and Seifi M., *Pakistan J. Biol. Sci.,* 2010; **13(14)**: 691-698. DOI 10.3923/pjbs.2010.691.698.

[29] Chen Y., Zhang H., Li Y.X., Cai L., Huang J., Zhao C., Jia L., Buchanan R., Yang T. and Jiang L.J., *Fitoterapia,* 2010; **81(4)**: 269-273. DOI 10.1016/j.fitote.2009.09.011.

[30] Chen Y., Cai L., Zhao C., Xu H.C., Cao C.Y., Liu Y., Jia L., Yin H.X., Chen C. and Zhang H., *Food Chem.*, 2008; **109**: 269-277. DOI 10.1016/j.foodchem.2007.10.023.