کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

اصول تنظیم قراردادها

آموزش مهارت‌های کاربردی در تدوین و چاپ مقاله
Caffeic acid derivatives from *Bupleurum chinense*

G. Haghi\(^1\)*, A. Hatami\(^1\), M. Mehran\(^1\) and H. Hosseini\(^2\)

\(^1\)Phytochemistry Group, Barij Essence Medicinal Plants Research Center, Kashan, I.R. Iran.
\(^2\)Agriculture Group, Barij Essence Medicinal Plants Research Center, Kashan, I.R. Iran.

**Abstract**

In this study, caffeic acid (CA) and its three derivatives including 3-caffeoylquinic acid (3-CQA, neochlorogenic acid), 4-caffeoylquinic acid (4-CQA, cryptochlorogenic acid), and 5-caffeoylquinic acid (5-CQA, chlorogenic acid) were identified in *Bupleurum chinense* aerial parts using reverse-phase high-performance liquid chromatography (RP-HPLC) with photodiode array (PDA) detector, reference compounds and chemical reactions. Separation was performed on a C18 column using gradient elution with 4% (v/v) aqueous acetic acid and acetonitrile as mobile phase at ambient temperature. In addition, the flavonoid aglycones were characterized and quantified after acid hydrolysis of the plant material. The flavonols profile showed quercetin (0.36 g per 100 g), kaempferol (1.11 g per 100 g) and isorhamnetin (0.16 g per 100 g). Total phenolic and total flavonoid contents ranged from 7.3 to 18.7% and 0.58 to 2.72% in dry plant material, respectively.

**Keywords:** *Bupleurum chinense*; Apiaceae; Caffeic acid derivatives; Flavonoids; HPLC

**INTRODUCTION**

The genus *Bupleurum* belongs to the plant family Umbeliferae or Apiaceae. This genus includes 14 species in Iran 3 of which are endemic namely, *B. flexile*, *B. wolffianum* and *B. ghahremani* (1). Some species in the family are used as medicines, foods, and ornamental herbs. Most species of this genus have been previously analyzed in numerous studies concerning pharmacological properties and their chemical composition. There are some reports regarding pharmacological activities of this genus including antioxidant and hepatoprotective (2,3), anti-fibrotic (4,5), anti-inflammatory (6), antimicrobial (7,8), analgesic (9), immunomodulatory (10), cytotoxicity and anti-tumour (11), inhibition of growth of liver cancer cells and promotion of liver regeneration (12). Phytochemical investigations of approximately 50 *Bupleurum* species led to the isolation and identification of almost 250 natural compounds from all major phytochemical classes. The saikosaponin triterpenes (13), sterols (14,15), essential oil (16), fatty acids (17), polysaccharides (18), lignans (19), polyacetylenes (20,21), coumarins (22) and flavonoids (23,24) have demonstrated in some *Bupleurum* species. *B. Chinense* represents one of the most popular and widely used herbal drugs in Asia for the treatment of many diseases over the past 2000 years (25). In recent years, increasing researches have been carried out on *B. chinense*, and the results revealed that the extracts and its isolated compounds possess a wide variety of biological effects. This species does not grow in Iran. Due to its medicinal importance, the seeds of *B. chinense* was purchased from China and cultivated for its propagation. In order to screen some bioactive constituents, we investigated the flavonoids and CA derivatives of *B. chinense* aerial parts. Flavonoids are widely used as chemotaxonomical markers to distinguish between different *Bupleurum* species and different geographical sources (23). *B. chinense* aerial parts is phytochemically characterized by the occurrence of flavonol glycosides including quercetin, kaempferol, isorhamnetin and their free aglycones with several biological properties (24).

*Corresponding author: G. Haghi
Tel. 0098 8643621121, Fax. 0098 8866436 2187
Email: g.haghikashani@gmail.com
A literature survey revealed that there is no report about the presence of CA and its derivatives in genus *Bupleurum* and other genera of Umbeliferae family except the occurrence of 1-O-caffeoylglycerol and ethyl caffeate in *B. chinense* aerial parts (26,27). This study elucidates the occurrence of CA and its derivatives for the first time and three known flavonols in the aerial parts of *B. chinense* using several techniques. The chemical structures of CA and its derivatives has been shown in Fig. 1.

**MATERIALS AND METHODS**

**Chemicals and reagents**

High-performance liquid chromatography (HPLC) grade acetonitrile, HPLC grade water, ethyl acetate and caffeic acid (CA) were from Merck (Darmstadt, Germany). Quercetin, kaempferol and isorhamnetin were provided from Sigma-Adrich. 5-caffeoylquinic acid (5-CQA) was purchased from Carl Roth (Karlsruhe, Germany). Among the isomers, only 5-CQA is available commercially. 5-CQA was isomerized in a saturated aqueous sodium hydrogen carbonate (NaHCO₃) solution at 60 °C (28) or in 0.2% aqueous sodium carbonate (29). The 5-CQA yields a mixture of 3-caffeoylquinic acid (3-CQA), 4-caffeoylquinic acid (4-CQA) and 5-CQA by isomerization. To obtain 3-CQA and 4-CQA from 5-CQA, isomerization was performed according to the procedure reported in the literature (34).

Twenty-five milligrams of 5-CQA was dissolved in 25 ml of distilled water and 25 ml of 0.2% aqueous sodium carbonate was added. After 30 min, 1 ml of 10% (v/v) acetic acid and 5 ml of methanol were added to 4 ml of the reaction solution.

**Plant materials**

*Bupleurum chinense* seeds was obtained from Chongoing Alcon Bio-Engineer Co. Ltd., China and cultivated in greenhouse of Barij Essence Pharmaceutical Company at 20 °C and moisture content of 55%. *B. chinense* seedlings raised in the nursery. The aerial parts of *B. chinense* were picked in August and October (seedling step) 2012 and identified by Dr. Mozaffarian, an expert taxonomist from the Research Institute of Forest and Rangelands, Tehran, Iran. Voucher specimen was deposited in the Herbarium of Agriculture Department of Pharmaceutical Barij Essence Company under number 196/1.

**Extraction of caffeic acid derivatives**

Powdered plant aerial parts (0.25 g) were extracted with 70% (v/v) aqueous methanol (20 ml) using a 50 ml round-bottom flask at 75 °C under reflux for 1 h. The solution was filtered into a 25 ml volumetric flask and the residue rinsed with 10 ml of the same solvent and added to the previous solution. The filtrate volume was adjusted with the solvent to the mark. The solution (3 ml) was filtered through a syringe filter of 0.45 µm membrane for HPLC analysis and for total phenolic content measurement (30).
Caffeic acid derivatives hydrolysis

3-caffeoylquinic acid, 4-CQA and 5-CQA were hydrolyzed in acid or alkaline media and converted to CA and quinic acid (QA). The above extract was subjected to alkaline hydrolysis, using the procedure reported by others (31). Briefly, 1 ml of 5-CQA (0.1 mg/ml) and 1 ml of crude extract were dried under vacuum and were separately exposed to alkaline hydrolysis. The aglycones were extracted with ethyl acetate. The solvent was evaporated under vacuum to dry. The residue was dissolved in 1 ml of 70% methanol, centrifuged, and filtered through a syringe filter (Chromafil, 0.45 µm pore size). A 20 µl volume of this solution was injected into the HPLC column.

Determination of flavonoid aglycones

Quantitative determination of individual flavonoid glycosides in food and plants is difficult because of diversity and the lack of most reference compounds. Thus, flavonol glycosides are converted to related aglycones by acid hydrolysis to overcome this problem. The 50% aqueous methanol (7 ml) containing 1.2 M HCL and 0.04% (w/v) ascorbic acid as the antioxidant was added to 0.1 g of dried material. The hydrolysis was performed at 80 °C under reflux for 2 h. The extract was cooled and diluted in a 10 ml volumetric flask with methanol. The extract (3 ml) was filtered through a 0.45 µm syringe filter and injected three times into the HPLC system (32).

Total phenolic content

Total phenolic content (TPC) in the crude extract was estimated by a spectrophotometer (Perkin–Elmer Lambda EZ-210 UV/VIS), with dual-beam, using the Folin-Ciocalteu’s reagent based on a standard curve generated with 5-CQA at 740 nm. The crude extract (0.2 ml) was transferred into a 5 ml volumetric flask and swirled with 3 ml of deionised water. Folin-Ciocalteu’s reagent (0.25 ml) was added and swirled. After 3 min, 0.75 ml of 20% (w/v) sodium carbonate solution was added and mixed. This was recorded as time zero. Deionised water was added to make up the volume to 5 ml exactly. The solution was mixed thoroughly and allowed to stand at ambient temperature for 2 h until the characteristic blue colour developed; the UV absorption was then recorded at the range of 550-850 nm. The maximum absorption wavelength was 740 nm. The TPC in the crude extract expressed as gram (g) of 5-CQA equivalent per 100 g of dry plant material (33).

Total flavonoid content

Stock solution of quercetin (0.5 mg/ml) prepared in methanol was diluted with 80% (v/v) ethanol (ranging from 25 to 100 µg/ml) to make calibration curve. The standard solutions and the crude extract (each 0.5 ml) were separately transferred into a 5 ml volumetric flask containing 2 ml of 80% ethanol, 0.1 ml of 10% (w/v) aluminum chloride and 0.1 ml of 1 M potassium acetate and diluted with distilled water to the mark and mixed. After 30 min, the absorbance was measured at 415 nm with a Perkin-Elmer Lambda EZ-210 spectrophotometer. The total flavonoid content (TFC) in the crude extract expressed as gram of quercetin equivalent per 100 g of dry plant material (34).

Standard solutions

The chromatographic purity of standard substances was scanned by PDA detector at 200-700 nm. Standard stock solutions of chlorogenic acid, quercetin, kaempferol and isorhamnetin were freshly prepared in methanol and diluted for the plotting of calibration curves. The concentration range of standards was according to the levels of analytes expected in the plant material.

Chromatographic conditions

The HPLC analysis was performed using a Knauer UHPLC/HPLC PLATINblue system (Knauer, Germany), equipped with a binary pump, column compartment, and PDA detector. The HPLC separation was achieved on a Eurospher II 100-3 C18 column (250 × 4.6 mm) at ambient temperature. Optimal separation of CA derivatives was achieved using linear gradient elution with 4% (v/v) aqueous acetic acid (eluent A) and acetonitrile (eluent B). The gradient elution was started with A from 8 to 26% in 40 min and then 26 to 44% in 10 min at a flow rate of 1.0 ml/min.
The UV spectra obtained by PDA detector showed the maximum absorption wavelength at 330 and 370 nm for the CA derivatives and flavonols, respectively. Therefore, detections were set at 330 and 370 nm to obtain the highest sensitivity. Flavonols separation was performed according to our earlier work (32) under isocratic condition 35:65 (A:B). The volume of all injections was 20 µl.

**Method validation**

The method was validated for linearity, sensitivity, repeatability and recovery. To establish calibration curves, linearity was tested at five different concentrations. Detection limits (LODs) and quantification limits (LOQs) were measured on the basis of visual evaluation of the amounts for which signal-to noise ratios were 3 and 10, respectively.

The precision of the method was determined by repeatability (intra-day) and intermediate precision (inter-day) and was expressed as relative standard deviation (RSD). The repeatability was assessed by analyzing samples within one day (four parallel samples) and intermediate precision on three consecutive days (two parallel samples each day). Recovery was evaluated by the standard addition method at three concentration levels to the sample.

**RESULTS**

**Total phenolic and flavonoid contents**

The TPC and TFC contents were calculated based on of 5-CQA and quercetin and expressed as equivalents of gram of 5-CQA and quercetin per 100 g of dry plant material. A considerable difference was observed for chlorogenic acid, quercetin, kaempferol, isorhamnetin TFC and TPC contents during grow of plant (Table 1). As shown in Table 1, the highest TPC and TFC and analyte values were found at seedling step. The 5-COA at seedling stage found to be 3.48% in dry sample. The quercetin, kaempferol and isorhamnetin amounts were 0.36, 1.11 and 0.16 g per 100 g of dry plant material, respectively (Table 1).

**Table 1.** Total phenolic, total flavonoid contents and amount of analytes in dry plant material.

| Sample       | Chlorogenic acid % (w/w) | Quercetin % (w/w) | Kaempferol % (w/w) | Isorhamnetin % (w/w) | Total flavonoid % (w/w) | Total phenolic % (w/w) |
|--------------|--------------------------|-------------------|--------------------|----------------------|-------------------------|------------------------|
| B. chinense  | 0.43                     | 0.11              | 0.05               | 0.18                 | 0.58                    | 7.3                    |
| B. chinenseb | 3.48                     | 0.36              | 1.11               | 0.16                 | 2.72                    | 18.7                   |

a: at seedling step.

**Table 2.** Linearity and sensitivity data.

| Compound                  | Linearity range (µg/ml) | Regression equation | LOD (ng/ml) | LOQ (ng/ml) | Correlation coefficient (r) |
|---------------------------|-------------------------|----------------------|-------------|-------------|-----------------------------|
| Chlorogenic acid (5-CQA)  | 5-30                    | y = 145, 864x - 101, 208 | 84          | 250         | 0.9997                      |
| Quercetin                 | 5-30                    | y = 360, 546x - 97, 875  | 70          | 200         | 0.9998                      |
| Kaempferol                | 5-30                    | y = 432, 954x - 47, 357  | 52          | 160         | 0.9999                      |
| Isorhamnetin              | 5-30                    | y = 413, 858x - 90, 844  | 60          | 180         | 0.9998                      |

**Table 3.** Intra- and inter-day precision data.

| Compound         | Intraday% (w/w) | RSD (%) | Interday% (w/w) | RSD (%) |
|------------------|----------------|---------|----------------|---------|
| Chlorogenic acid | 3.48           | 1.82    | 3.46           | 1.89    |
| Quercetin        | 0.36           | 1.68    | 0.34           | 1.75    |
| Kaempferol       | 1.12           | 1.63    | 1.14           | 1.68    |
| Isorhamnetin     | 0.17           | 1.73    | 0.15           | 1.78    |

a: relative standard deviation.
Validation results

Table 2 shows the results of the linearity and sensitivity. The calibration curves were linear in the ranges of 5-30 µg/ml for standards. Regression equations and correlation coefficients revealed linear response for the used method. The LODs and LOQs for the reference compounds were in the range of 52-84 ng/ml and 160-250 ng/ml, respectively. Repetitability (expressed in terms of RSD) was obtained in the range of 1.63-1.89%. The recovery tests were carried out on the crude extract and acidic hydrolysis of the plant sample using standard addition method. Known amounts of standard solutions of 5-CQA and the mixture of quercetin, kaempferol and isorhamnetin at three levels were added to the samples. The intra- and interday precisions (expressed in terms of %RSD) were obtained in the range of 0.17-1.48 and 0.15-1.46%, respectively. The recovery of flavonoids and phenolic acids was determined by the method of standard addition as above-reported. Table 3 shows the results of precision in this study. The recovery percent and RSD (%) were in the range of 97.6-101.3% and 1.86-3.23%, respectively. This study revealed the presence of compounds 3-CQA, 4-CQA, 5-CQA and CA in genus Bupleurum for the first time. Major phenolic compounds found were 5-CQA, quercetin, kaempferol and isorhamnetin. There was a considerable difference for phenolic compounds, TFC and TPC contents and it can be related to the pick time, cultivation and climatic conditions, environment, geographical origin and other factors.

Identification of caffeic acid derivatives

A methanol/water (70/30 V/V) extract from the dried aerial parts of B. chinense was analyzed by RP-HPLC to characterize the chemical structure of CA acid and its derivatives. Four compounds were identified as CA, 3-CQA, 4-CQA and 5-CQA (Figs. 2a and 2b). The recorded UV spectra by PDA detector, elution sequence of peaks, and retention times in chromatogram of the crude extract were the same as chromatogram of their standards and comparison with recorded data (30,40). UV spectra of peaks 1-4 recorded by PDA detector were identical to the UV spectra of CA and its derivatives (λ_max at approx. 240, 300sh, 328 nm) standards. Some authors have named CA derivatives pre-IUPAC numbering (35). Increasing the standard solutions to the crude extract increased the peak area of analytes (peaks 1-4). The chromatograms of standard materials and the crude methanol/water (70/30 V/V) extract have been exhibited in Figs. 2a and 2b. The crude extract was subjected hydrolyse and isomerization. The chromatograms of 5-CQA and the extract isomerized and hydrolyzed have been showed in Fig. 3. The hydrolysis process showed the presence of quercetin, kaempferol and isorhamnetin in dry plant material. Figs. 4a and 4b exhibit the chromatograms of standard materials and acid hydrolysis of plant sample.

**Fig. 2.** High-performance liquid chromatography chromatograms of standard materials (a); methanol/water (70/30 v/v) extract (b); 1:3-caffeoylquinic acid, 2:4-caffeoylquinic acid, 3: 5-caffeoylquinic acid, 4:caffeic acid.
DISCUSSION

Extraction of phenolics by 70% methanol solvent followed by PLC-PDA revealed the presence of CA and its derivatives in the herb. The chemical structures were confirmed by chromatographic data, isomerization and hydrolysis of 5-CQA, as reported in our previous study (30). Conversion of 5-CQA, 3-CQA and 4-CQA to CA by hydrolytic procedure and isomerization of 5-CQA is a strategy employed to confirm the occurrence of the analytes. Four compounds were identified as CA, 3-CQA, 4-CQA and 5-CQA. The below methods are important parameters for a definite assessment of analytes in the crude extract. To clarify the structural features of the CA compounds the isomerization and hydrolysis of 5-CQA and the methanol/water (70/30 v/v) extract were subsequently performed to complete the identification of CA, 5-CQA and its isomers and in B. chinense. The isomerization of 5-CQA yielded a mixture of 3-CQA, 4-CQA and 5-CQA. Isomerization
led to decrease of 5-CQA peak area and increase of peaks heights of 3-CQA and 4-CQA. For further confirmation, a mixture of 3-CQA, 4-CQA, 5-CQA and also the methanol/water (70:30 v/v) extract were separately subjected to alkaline hydrolysis treatment. Saponification of esteric bounds realized their aglycones. The 3-COA, 4-CQA and 5-CQA were disappeared and yielded peak 4 corresponding to CA.

The three flavonol aglycones were identified by comparison with retention times, spiking experiments with the respective reference standards, UV spectra of standard materials recorded by PDA detector and the literature (23,24,32). The flavonoid aglycones identified were quercetin, kaempferol and isorhamnetin. Zhang and colleagues (23) and Tiang and coworkers (24) reported the occurrence of flavonol glycosides including quercetin, kaempferol, isorhamnetin and their free aglycones with several biological properties. These results were consistent with our findings.

CONCLUSION

This study revealed the presence of four CA derivatives including 3-CQA, 4-CQA, 5-CQA and CA in B. chinense for the first time. Major phenolic compounds found were 5-CQA, quercetin, kaempferol and isorhamnetin. There was a considerable difference for TFC and TPC during grow of plant and it can be according to the pick time, cultivation and climatic conditions and geographical origin.

ACKNOWLEDGMENTS

This work financially was support by the Barij Essence Pharmaceutical Company. Authors are thankful to Agriculture Group of Pharmaceutical Barij Essence Center Research for providing of plant samples.

REFERENCES

1. Mozaffarian V. A Dictionary of Iranian Plant Names. Farhang Moaser, Tehran, 2007. p. 86-87.
2. Liu CT, Chuang PT, Wu CY, Weng YM, Chen W, Tseng CY. Antioxidative and in vitro hepatoprotective activity of Bupleurum kaori leaf infusion. Phytother Res. 2006;20:1003-1008.
3. Zhao W, Li JJ, Yue SQ, Zhang LY, Dou KF. Antioxidant activity and hepatoprotective effect of a polysaccharide from Bei Chaihu (Bupleurum chinense DC) Carbohydr Polym. 2012; 89:448-452.
4. Chiang LC, Ng LT, Liu LT, Shieh DE, Lin CC. Cytotoxicity and anti-hepatitis B virus activities of saikosaponins from Bupleurum species. Planta Med. 2003;69:705-709.
5. Chang JS, Wang KC, Liu HW, Chen MC, Chiang LC, Lin CC. Sho-Saiko-to (Xiao-Chai-Hu-Tang) and crude saikosaponins inhibit hepatitis B virus in a stable HBV- producing cell line. Am J Chinese Med. 2007;35:341-351.
6. Just MJ, Recio MC, Giner RM, Cuéllar MJ, Máñez S, Bilá AR, et al. Anti-inflammatory activity of unusual lupine saponins from Bupleurum fruticosens. Planta Med. 1998;64:404-407.
7. Li Y, Xu C, Zhang Q, Liu JY, Tan RX. In vitro anti-Helicobacter pylori action of 30 Chinese herbal medicines used to treat ulcer diseases. J Ethnopharmacol. 2005;98:329-333.
8. Shafaghat A, Antioxidant, antimicrobial activities and fatty acid components of leaf and seed of Bupleurum lancifolium. Hormen. J Med Plants Res. 2011;5:3758-3762.
9. Xie D, Jia X, Cai B, Zhang L. Experimental study on anti-inflammatory and analgesic effects of volatile oil of Bupleurum chinense and B. scorzonerifolium. Pharm Cli Res. 2007;15:108-110.
10. Wang Z, Li H, Xu H, Yue XL, Cheng XQ, Hou WJ, et al. Beneficial effect of Bupleurum polysaccharides on autoimmune disease induced by Campylobacter jejuni in BALB/c mice. J Ethnopharmacol. 2009;124:481-487.
11. Cheng YL, Chang WL, Lee SC, Liu YG, Lin HC, Chen CJ et al. Acetone extract of Bupleurum scorzonerifolium inhibits proliferation of A549 human lung cancer cells via inducing apoptosis and suppressing telomerase activity. Life Sci. 2003;73:2383-2394.
12. Yin F, Pan RX, Chen RM, Hua LH. Saikosaponins from Bupleurum chinense and inhibition of HBV DNA replication activity. Nat Prod Commun. 2008;3:155-157.
13. Huang HQ, Zhang X, Lin M, Shen YH, Yan SK, Zhang WD. Characterization and identification of saikosaponins in crude extracts from three Bupleurum species using LC-ESI-MS. J Sep Sci. 2008;31:3190-3201.
14. Ebata N, Nakajima K, Hayashi K, Okada M, Maruno M. Saponins from the root of Bupleurum falcatum. Phytochemistry. 1993;41:895-901.
15. Pistelli L, Bilia AR, Marsili A, De Tommasi N, Manunta A. Triterpenoid saponins from Bupleurum fruticosum. J Nat Prod. 1996;59:2383-2394.
16. Li XQ, He ZG, Bi KS, Song ZH, Xu L. Essential oil analyses of the root oils of 10 Bupleurum species from China. J Essent Oil Res. 2007;19:234-238.
17. Li XQ, Song AH, Li W, Chen XH, Bi KS. Analysis of the fatty acid from Bupleurum chinense DC in China by GC-MS and GC-FID. Chem Pharm Bull. 2005;53:1613-1617.
18. Sun L, Feng K, Jiang R, Chen J, Zhao Y, Ma R, et al. Water-soluble polysaccharide from Bupleurum chinense DC: Isolation, structural features and antioxidant activity. Carbohydr Polym. 2010;79:180-183.
19. Si-Qi L, Long-Ze L, Cordell GA. Lignan glucosides from Bupleurum wenchuanense. Phytochemistry, 1993;33:193-206.
20. Huang HQ, Su J, Zhang X, Shana L, Zhang WD. Qualitative and quantitative determination of polyacetylenes in different Bupleurum species by high performance liquid chromatography with diode array detector and mass spectrometry. J Chromatogr. 2011;1218:1131-1138.
21. Barrero AF, Haidour A, Munoz-Dorado M, Aksiria M, Sedqui A, Mansour I. Polyacetylenes, terpenoids and flavonoids from Bupleurum spinosum. Phytochemistry, 1998;48:1237-1240.
22. Estévez-Braun A, González AG. Coumarins. Nat Prod Rep. 1997;14:465-475.
23. Zhang TT, Zhou JS, Wang Q. Flavonoids from aerial part of Bupleurum chinense DC. Biochem Syst Ecol. 2007;35:80-84.
24. Ting-Ting Z, Jin-Song Z, Qiang W. HPLC analysis of flavonoids from the aerial parts of Bupleurum species. Chin J Nat Med. 2010;8:0107-0113.
25. Ashoura ML, Winka M. Genus Bupleurum: a review of its phytochemistry, pharmacology and modes of action. J Pharm Pharmacol. 2011;63:305-321.
26. Wang M, Liu P, Feng X, Dong YF, Zhao YY, Chen Y, et al. Studies on the chemical constituents of the aerial part of Bupleurum chinense (II). J Chin Med Mater (Zhong Yao Cai). 2009;32:367-369.
27. Liu P, Feng X, Dong Y, Wang, M, Zhao Y, Zhao X. Chemical constituents of aerial part of Bupleurum chinense DC. Shizhen Guoyi Guoyao, 2008;19:2103-2104.
28. Fritsche JF, Beindorff CM. Isolation, characterization and determination of minor artichoke (Cinara scolymus L.) leaf extract compounds. Eur Food Res Technol. 2002;215:149-157.
29. Mader J, Rawel H, Kroh LW. Composition of phenolic compounds and glycoalkaloids a-solanine and a-chaconine during commercial potato processing. J Agric Food Chem. 2009;57:6292-6297.
30. Haghi G, Hatami A; Arshi R. Distribution of caffeic acid derivatives in Gundelia tournefortii L. Food Chem. 2011;124:1029-1053.
31. Nardini M, Cirillo E, Natella F, Mencarelli D, Comisso A, Scaccini C. Detection of bound phenolic acids: Prevention by ascorbic acid and ethylenediaminetetraacetic acid of degradation of phenolic acids during alkaline hydrolysis. Food Chem. 2002;79:119-124.
32. Haghi G, Hatami A. Simultaneous quantification of flavonoids and phenolic acids in plant materials by a newly developed isocratic high-performance liquid chromatography approach. J Agric Food Chem. 2010;58:10812-10816.
کارگاه‌های آموزشی مرکز اطلاعات علمی

مقاله نویسی علوم انسانی

آموزش مهارت های کاربردی در تدوین و چاپ مقاله

اصول تنظیم قراردادها