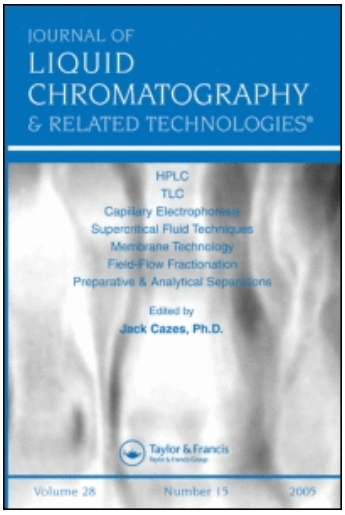


Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

SOLVENT COMPOSITION EFFECTS ON EFFICIENCY OF PRESSURIZED LIQUID EXTRACTION OF BIOACTIVE ISOFLAVONOIDS FROM *BELAMCANDA CHINENSIS* RHIZOMES

Hee Ju Lee[a]; Eun Ha Lee[a]; Sang Hoon Jung[a]; Dong-Un Lee[b]; Chul Young Kim[a]
[a] Natural Products Research Center, KIST Gangneung Institute, Gangneung, Gangwon-do, Korea [b] Department of Food Science and Technology, Chung-Ang University, Anseong, Korea

Online publication date: 07 January 2011

To cite this Article Lee, Hee Ju , Lee, Eun Ha , Jung, Sang Hoon , Lee, Dong-Un and Kim, Chul Young(2011) 'SOLVENT COMPOSITION EFFECTS ON EFFICIENCY OF PRESSURIZED LIQUID EXTRACTION OF BIOACTIVE ISOFLAVONOIDS FROM *BELAMCANDA CHINENSIS* RHIZOMES', Journal of Liquid Chromatography & Related Technologies, 34: 2, 143 — 154
To link to this Article: DOI: 10.1080/10826076.2011.540921
URL: http://dx.doi.org/10.1080/10826076.2011.540921

Journal of Liquid Chromatography & Related Technologies, 34:143–154, 2011

ISSN: 1082-6076 print/1520-572X online
DOI: 10.1080/10826076.2011.540921

SOLVENT COMPOSITION EFFECTS ON EFFICIENCY OF PRESSURIZED LIQUID EXTRACTION OF BIOACTIVE ISOFLAVONOIDS FROM *BELAMCANDA CHINENSIS* RHIZOMES

Hee Ju Lee,[1] Eun Ha Lee,[1] Sang Hoon Jung,[1] Dong-Un Lee,[2] and Chul Young Kim[1]

[1]*Natural Products Research Center, KIST Gangneung Institute, Gangneung, Gangwon-do, Korea*
[2]*Department of Food Science and Technology, Chung-Ang University, Anseong, Korea*

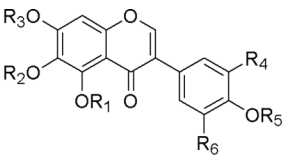
□ *A pressurized liquid extraction (PLE) technique was developed for the extraction of bioactive isoflavonoids including tectoridin (***1***), iridin (***2***), tectorigenin (***3***), iristectorigenin A (***4***), irisflorentine (***5***), and irigenin (***6***) from* Belamcanda chinensis *using aqueous ethanol as the extraction solvent. The effects of various key factors of PLE, including solvent composition (0–100% ethanol in water), temperature (80–180° C) and extraction time (5–20 min), were evaluated with respect to the extraction efficiency. Only solvent compositions altered the extraction yields of isoflavonoids with 60% ethanol providing the best extraction efficiency. When compared with conventional extraction methods, such as reflux or sonication extraction, PLE resulted in higher extraction efficiency with shorter extraction time and lower solvent consumption. We also tested the thermal stability of tectoridin, isoflavonoid glycoside, at various temperatures (80–180° C) in 60% ethanol for a 20 min extraction time to mimic conditions that could be encountered during PLE. Tectoridin was degraded to tectorigenin (aglycone) at temperatures over 150° C, therefore, care should be taken in the choice of temperature used for PLE.*

Keywords *Belamcanda chinensis*, isoflavonoids, pressurized liquid extraction, tectoridin, tectorigenin, thermal stability

INTRODUCTION

Rhizomes of *Belamcanda chinensis* (L.) DC. (Iridaceae) have been used as an Oriental traditional medicine to treat inflammation, asthma, and throat troubles such as tonsillitis.[1] Isoflavonoids, especially tectoridin (**1**) and tectorigenin (**3**) (Figure 1), are the main biological constituents of the

Address correspondence to Chul Young Kim, Natural Products Research Center, KIST Gangneung Institute, Daejeon-dong, Gangneung, Gangwon-do 210-340, Korea. E-mail: kcy98@kist.re.kr or Dong-Un Lee, Department of Food Science and Technology, Chung-Ang University, Anseong 456-756, Korea. E-mail: dong-un.lee@cau.ac.kr



	R_1	R_2	R_3	R_4	R_5	R_6
Tectoridin (**1**)	H	CH_3	Glc	H	H	H
Iridin (**2**)	H	CH_3	Glc	OCH_3	CH_3	OH
Tectorigenin (**3**)	H	CH_3	H	H	H	H
Iristectorigenin A (**4**)	H	CH_3	H	OCH_3	H	H
Irigenin (**5**)	H	CH_3	H	OCH_3	CH_3	OH
Irisflorentine (**6**)	CH_3	-CH_2-		OCH_3	CH_3	OCH_3

FIGURE 1 The structures of isoflavonoids (**1-6**) from *B. chinensis*.

rhizomes and show anti-inflammatory,[2] anti-angiogenic, anti-tumor,[3] anti-fungal,[4] and anti-diabetic properties.[5]

Organic solvent extraction methods conventionally used for the isolation of isoflavonoids from plant tissues are time consuming, require high volumes of frequently toxic organic solvents, and can leave harmful solvent residues in the final product. In this study, we have tested an alternative method, pressurized liquid extraction (PLE), and the use of a more environmentally acceptable extraction solvent, aqueous ethanol, to replace more toxic forms such as methanol, chloroform, or dichloromethane. PLE is an extraction technique that uses liquid solvents under elevated temperature and pressure to achieve fast and efficient extraction of analytes from solid and semi-solid samples.[6–8] Many experiments have demonstrated the successful extraction of active ingredients from medicinal plants using PLE.[9–14]

In the present study, the effects of the main factors affecting extraction efficiency, namely solvent composition (mixtures of ethanol and water), temperature, and extraction time, were evaluated with respect to PLE of six isoflavonoids markers in *B. chinensis* rhizomes. As a further test, we also subjected tectoridin, an isoflavonoid glycoside, to 20 min extractions in 60% ethanol at various temperatures (80–180°C) to determine its thermal stability under conditions that could be encountered during a PLE procedure.

EXPERIMENTAL

Plant Materials and Chemicals

Rhizomes of *B. chinensis* were purchased from a Kyungdong oriental medicine market, Seoul, Korea, in September 2006. The voucher specimen

(BC-001) was stored at the Natural Products Research Center, KIST Gangneung Institute, Gangneung, Korea. The sample was ground to powder and passed through a 25–mesh (710 μm) sieve. The ground powder was stored at room temperature until use. Standard compounds (**1-6**) were isolated from the power according to the method described previously and the structures were identified by comparing their ^{1}H- and ^{13}C-NMR data in the literature.[5] HPLC grade ethanol, methanol, water, and acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA, USA). Sea sand (15–20 mesh) for pressurized liquid extraction was purchased from Junsei (Tokyo, Japan). Analytical grade trifluoroacetic acid (TFA) was purchased from the Sigma-Aldrich Company (St. Louis, MO, USA).

HPLC Analysis

All of the extracts were analyzed using an Agilent Series 1200 liquid chromatography system, equipped with a G1379B vacuum degasser, a G1312A binary pump, a G1329A autosampler, a G1316A column oven, and a G1315B DAD detector, connected to Agilent ChemStation software (Agilent, Waldbronn, Germany). A SunFire C18 column (4.6 × 150 mm, 3.5 μm, Waters, USA) and a J'sphere ODS H-80 C18 guard column (4.0 × 20 mm, 4 μm, YMC, Japan) were used for the separation. The gradient program was as follows: 0–5 min, initial mobile phase acetonitrile/0.1% TFA in water (18:82, %, v/v); 5–15 min, linear gradient 40:60 (%, v/v); 15–30 min, isocratic 40:60 (%, v/v); and reconditioning steps to initial condition for 15 min. Standard solutions of tectoridin (**1**) (127.19–2002.67 μg/mL) iridin (**2**) (53.91–994.71 μg/mL); tectorigenin (**3**) (14.42–498.57 μg/mL); iristectorigenin A (**4**) (30.37–499.72 μg/mL); irigenin (**5**) (9.79–251.60 μg/mL); and irisflorentine (**6**) (29.84–497.97 μg/mL) were prepared in methanol and 10 μl of each was injected into the HPLC column via the auto-injector three separate times (n = 3).

The standard curves of samples were calibrated using the linear least squares regression equation derived from the peak area. Flow rate was 1.0 mL/min and the oven temperature was set at 40°C. The analysis was performed at a wavelength of 280 nm and the injection volume of standards and extracts was 10 μl. The limits of detection (LOD) and quantification (LOQ) for each analyte were determined as the standard deviation of the response and the slope (σ/S) of approximately 3.3 and 10.

Pressurized Liquid Extraction

PLE was carried out using a Dionex ASE 300 Accelerated solvent extractor (Sunnyvale, CA, USA). The dried powder of *B. chinensis* (3 g) was placed

in a 34 mL stainless steel extraction cell. A filter paper was placed at the bottom of the extraction cell. Sea sand (35 g) was used as supporting material in the extraction cell. The extraction procedures were as follows: (1) extraction cell was loaded into the oven; (2) cell was filled with solvent; (3) initial heat-up time was applied; (4) an extraction with all system valves closed was performed; (5) the cell was rinsed with extraction solvent equal to 40% of the cell volume; (6) solvent was purged from cell with N_2 gas for 120 s; and (7) the system was depressurized. Approximately 50 mL of solvent was used for each extraction. The extract was collected into glass collection vials and then transferred to 100 mL volumetric flasks. Extractions were performed using four different mixtures of ethanol and water (0%, 30%, 60%, and 100% ethanol in water, v/v) to determine the optimal amount of ethanol required for extraction at 100 °C, 1500 psi and 10 min. The experiments then were carried out to determine the optimal temperature (80, 100, 120, 150, and 180 °C) and extraction time (5, 10, 15, and 20 min) using 60% ethanol as the extraction solvent.

Thermal Stability of Tectoridin

Thermal stability of tectoridin was tested using the Dionex ASE 300 Accelerated solvent extractor. Tectoridin (3 mg) was placed in a 34 mL stainless steel extraction cell and extracted in 60% ethanol at various temperatures (80, 100, 120, 150, and 180°C) for 20 minutes. The extracts were analyzed by HPLC and TLC. HPLC conditions were as described in the previous section.

Reflux Extraction

Three grams of ground samples (n = 3) were refluxed with 50 mL of 60% ethanol three times for 3 hr and filtered through filter paper into a 100 mL volumetric flask. The volume of extract was adjusted with methanol to 100 mL and filtered prior to injection into the HPLC system as described previously. All experiments were carried out in triplicate.

Sonication Extraction

Sonication extractions were carried out in an ultrasonic cleaning bath (model RK 158s, Bandelin, Germany). Three grams of ground samples were extracted with 50 mL of 60% ethanol (n = 6) and methanol (n = 6) three times at room temperature for 3 hr and filtered through filter paper into 100 mL volumetric flasks. All experiments were carried out in triplicate.

RESULTS AND DISCUSSION

HPLC Analysis of Isoflavonoids from *B. chinensis* Rhizomes

Six major isoflavonoids including tectoridin (**1**), iridin (**2**), tectorigenin (**3**), iristectorigenin A (**4**), irisflorentine (**5**), and irigenin (**6**), were detected by comparison of their HPLC retention times and UV spectra with those of authentic standards (Figure 2). The sum of these major isoflavonoids was used as a marker of extraction efficiency. The standard curves of the six isoflavonoids (**1**-**6**) were calibrated using the linear least squares regression equation derived from the peak area. The concentration of these compounds in the samples was calculated according to the regression parameters derived from the standard curve. Three replicate measurements were carried out. The linear regression data, LODs, and LOQs of the six isoflavonoids are shown in Table 1.

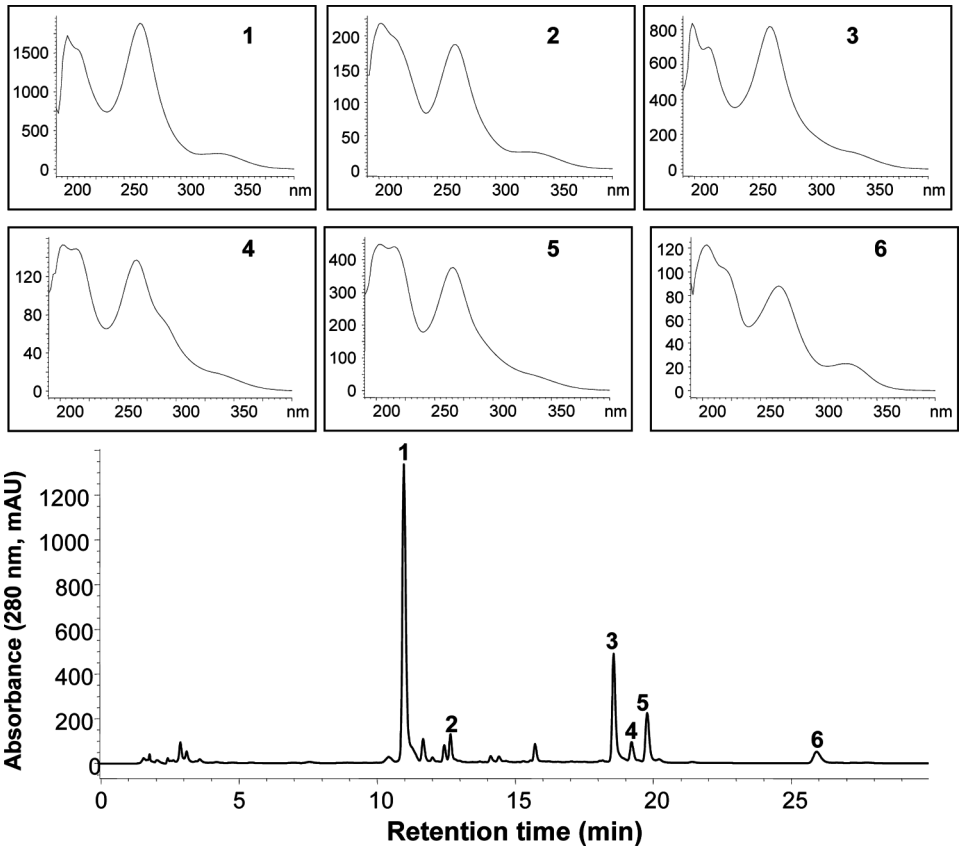


FIGURE 2 HPLC chromatogram of a PLE extract from *B. chinensis*. Peak identification: (**1**) tectoridin; (**2**) iridin; (**3**) tectorigenin; (**4**) iristectorigenin A; (**5**) irigenin; and (**6**) irisflorentine. Chromatographic conditions are described in the HPLC Analysis section.

TABLE 1 Calibration Curves, LODs, and LOQs for the Six Isoflavonoids in *B. chinensis*

Compounds	Calibration curve	r^2	Linear range (μg/mL)	LOD (μg/mL)	LOQ (μg /mL)
Tectoridin (**1**)	Y = 13.6629 x − 10.9792	1.0000	127.19–2002.67	4.18	12.67
Iridin (**2**)	Y = 10.6355 x + 126.8181	0.9995	53.91–994.71	9.53	28.89
Tectorigenin (**3**)	Y = 25.1899 x + 23.7979	0.9999	14.42–498.57	0.29	0.89
Iristectorigenin A (**4**)	Y = 21.1052 x + 35.6844	1.0000	30.37–499.72	0.86	2.61
Irigenin (**5**)	Y = 24.6700 x − 71.9837	0.9996	9.79–251.60	9.95	30.14
Irisflorentine (**6**)	Y = 20.6991 x + 4.3349	0.9998	29.84–497.97	2.55	7.73

Optimization of PLE Conditions

The parameters that can affect the extraction efficiency of PLE include solvent type, temperature, and extraction time. Above all, extraction solvent is the main factor that determines extraction efficiency.[14,15] Water

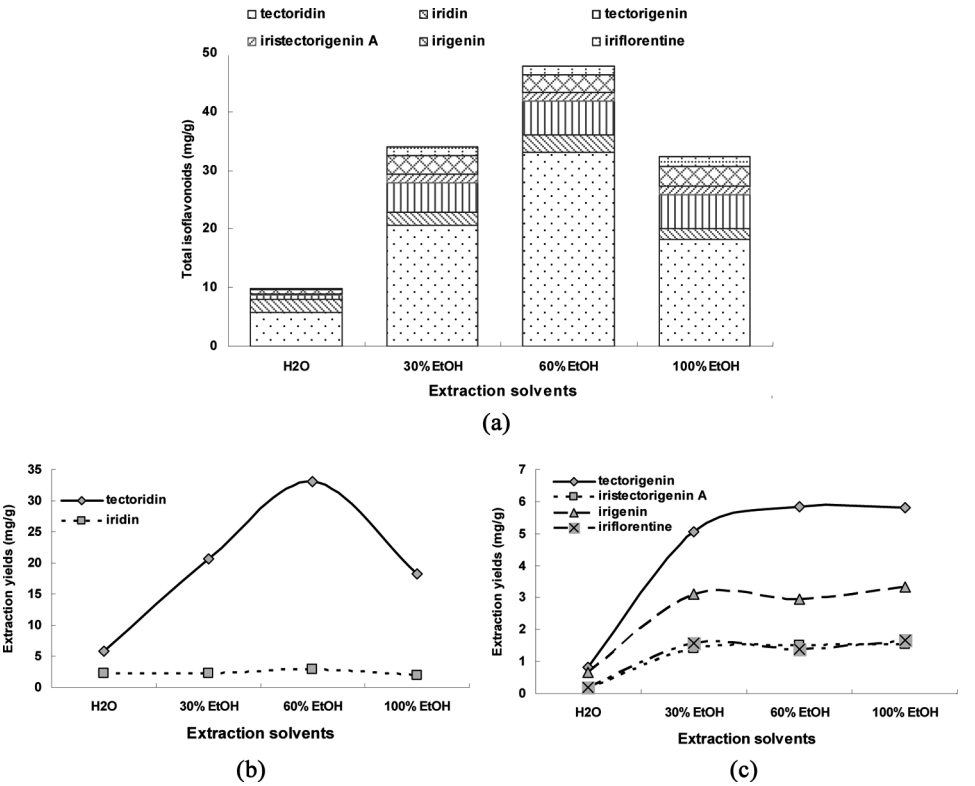


FIGURE 3 Effect of different water and ethanol mixtures on extraction efficiency of isoflavonoids from *B. chinensis*: (A) total isoflavonoids, and (B) isoflavone glycosides (tectoridin and iridin), (C) isoflavone aglycones (tectorigenin, iristectorigenin A, irigenin and irisflorentine). PLE conditions: temperature, 100°C; extraction time, 10 min; pressure, 1500 psi; flush volume, 40%; purge, N_2 gas for 120 s; extraction cycle, 1; replicates, n = 3.

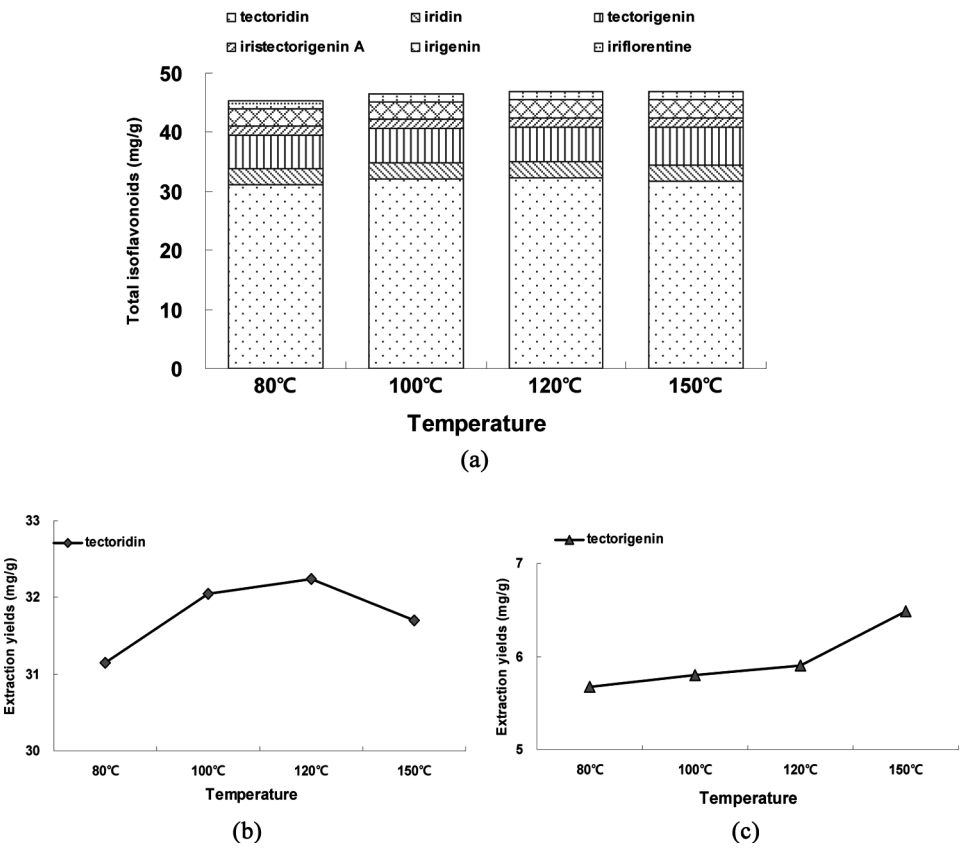


FIGURE 4 Effect of extraction temperature on the extraction efficiency of (A) total isoflavonoids, (B) tectoridin, and (C) tectorigenin from *B. chinensis*. PLE conditions: solvent, 60% aqueous ethanol; extraction time, 10 min; pressure, 1500 psi; flush volume, 40%; purge, N_2 gas for 120 s; extraction cycle, 1; replicates, n = 5.

miscible solvents such as methanol, ethanol, and acetonitrile were considered for use in the present study, based on the polarity of the target isoflavonoids and literature reports.[2–5] Among these solvents, ethanol was selected as it was considered safer and more environmentally friendly than the other organic solvent choices.

The extractions were performed using four different mixtures of ethanol and water (0%, 30%, 60%, and 100% ethanol in water, v/v) to determine the optimum extraction solvent at 100°C, 1500 psi, 10 min, 40% of flush volume and one extraction cycle. AS seen in Figure 3A, 60% ethanol showed the highest efficiency, and the extraction yield was significantly higher than with the other mixtures. The extraction yields of isoflavone glycosides (tectoridin and iridin) increased with the increasing percentage of ethanol in the solvent mixture until 60%, but then declined when 100% ethanol was used as the extraction solvent (Figure 3B). However, the

extraction efficiency for isoflavone aglycones (tectorigenin, iristectorigenin A, irigenin, and irisflorentine) continuously increased with increasing ethanol content (Figure 3C). Since the total extraction yields of isoflavonoids were highest using 60% ethanol, 60% ethanol was chosen as the extraction solvent for subsequent investigations.

The effect of temperature on extraction efficiency was examined over the range of 80–180°C, using 60% ethanol as the extraction solvent. As shown in Figure 4A, extraction temperatures did not affect the extraction yield of total isoflavonoids. However, extraction temperatures influenced the extraction efficiency for tectoridin (isoflavonoid glycoside) or tectorigenin (aglycone). The extraction yield of tectoridin increased with increasing temperature from 80 to 120°C, however, over 150°C, the extraction efficiency slightly decreased (Figure 4B). Increasing the temperature (from 80 to 180°C) increased the amount of tectorigenin, the aglycone of tectoridin, slightly (Figure 4C). The extraction efficiency for other isoflavonoids showed similar increases in extraction yields with increasing temperature. The extraction yields were reduced at temperatures above 150°C.

As shown in Figure 5, extraction times (5, 10, 15, and 20 min) at a temperature 120°C and 60% aqueous ethanol (v/v) had no significant effect on extraction efficiency.

Thermal Stability of Tectoridin

The amounts of tectoridin and tectorigenin appearing in the extracts were influenced by temperatures (Figure 4B and 4C). For this reason, we

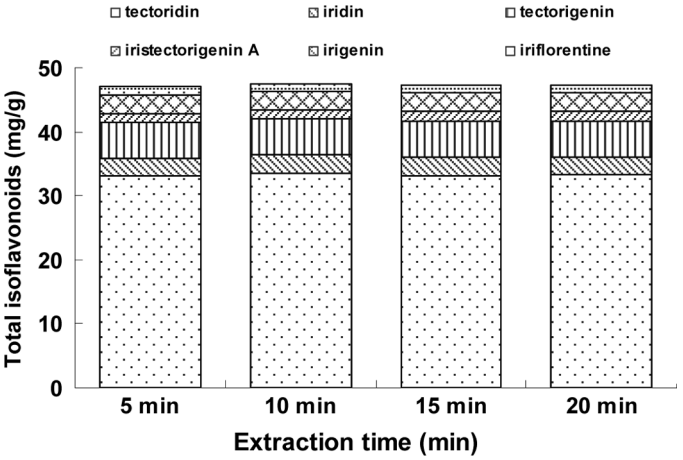


FIGURE 5 Effect of extraction time on the extraction efficiency of total isoflavonoids from *B. chinensis*. PLE conditions: solvent, 60% aqueous ethanol; temperature, 120°C; pressure, 1500 psi; flush volume, 40%; purge, N_2 gas for 120 s; extraction cycle, 1; replicates, n = 3.

examined the thermal stability of tectoridin during extraction at various temperatures (80, 100, 120, 150, and 180°C). Tectoridin was stable at temperatures between 80°C and 120°C. At 120°C, 3.8% degradation to tectoridin (aglycone) and glucose was observed, but this increased to 11.0% of at 150°C and 41% at 180°C (Figure 6). However, as seen in Figures 4B and 4C, actual rhizome samples showed a decline in tectoridin content of only 6.7% at 180°C compared with 120°C and tectorigenin increased only 21.9%. These results may reflect a protective effect of the *B. chinensis* rhizome matrix. Alternatively, a change in the dielectric constant of aqueous ethanol at elevated temperature may have affected solvent polarity and extraction yields of the aglycone or glycoside of the isoflavonoids. At high temperature, aglycones were extracted more easily compared with

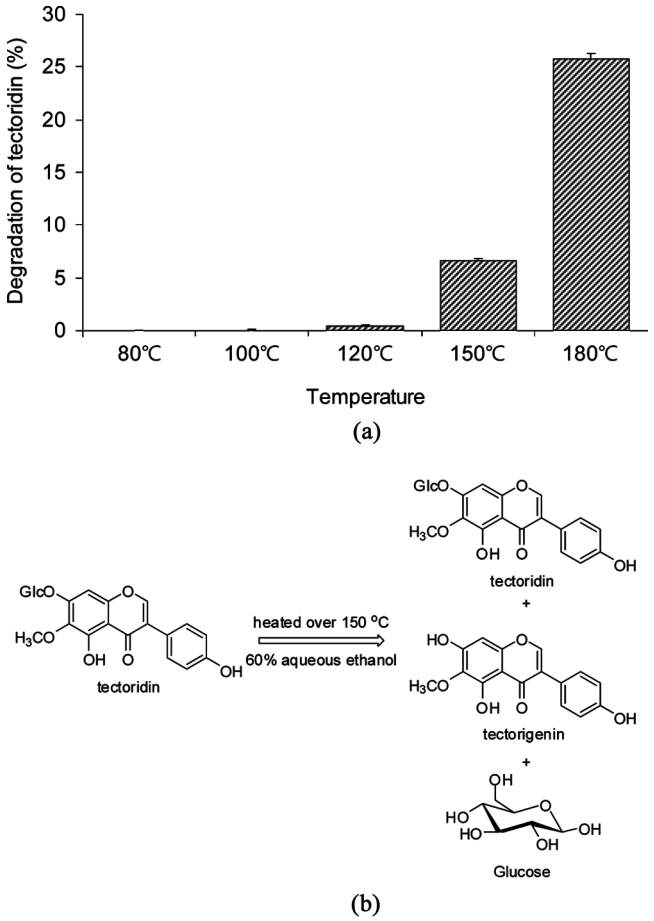


FIGURE 6 (A) Degradation of tectoridin at various temperatures, solvent, 60% aqueous ethanol; temperature, 80–180°C. (B) Structural depiction of the degradation of tectoridin to tectorigenin and glucose at higher temperatures.

TABLE 2 Comparison of PLE, Reflux and Sonication Method for the Extraction of Six Isoflavonoids in *B. chinensis*

Method	Time	Extraction solvent (mL)	Extraction yields (mg/g)					
			1[a]	**2**[a]	**3**[a]	**4**[a]	**5**[a]	**6**[a]
PLE	10 min	60% ethanol (50 mL)	33.13 ± 0.65	2.87 ± 0.02	5.84 ± 0.11	1.42 ± 0.22	2.96 ± 0.12	1.39 ± 0.08
Reflux	3 h	60% ethanol (50 mL)	31.25 ± 0.81	2.66 ± 0.05	5.18 ± 0.18	1.42 ± 0.08	2.69 ± 0.09	1.18 ± 0.02
Sonication	3 h	60% ethanol (50 mL)	28.47 ± 1.19	2.41 ± 0.07	4.62 ± 0.13	1.22 ± 0.05	2.49 ± 0.07	1.08 ± 0.05
Sonication	3 h	Methanol (50 mL)	21.50 ± 0.31	2.46 ± 0.12	4.53 ± 0.07	1.20 ± 0.02	2.65 ± 0.03	1.07 ± 0.03

[a]Compounds were (**1**) tectoridin; (**2**) iridin; (**3**) tectorigenin; (**4**) iristectorigenin A; (**5**) irigenin; (**6**) irisflorentine.

polar glycosides, as the dielectric constant of aqueous ethanol is decreased due to the weakened polarity and hydrogen bonding at elevated temperature.[16–18] As shown in Figures 3B and 3C, extraction yields of the glycosides (tectoridin and iridin) were decreased in 100% ethanol, which those of the aglycones (tectorigenin, iristectorigenin A, irigenin, and irisflorentine) were increased.

Comparison of PLE, Reflux, and Sonication Extraction Methods

The extraction efficiency of PLE for isoflavonoids from *B. chinensis* was compared with that achieved by reflux and sonication extraction. Reflux and sonication extraction were carried out at a boiling point above 70°C and at room temperature, respectively. The PLE experimental conditions were extraction temperature of 120°C, 1500 psi and 10 min. As seen in Table 2, higher amounts of isoflavonoids were extracted with PLE and the PLE procedure required less extraction time and a smaller volume of solvents compared with reflux and sonication extraction methods.

CONCLUSIONS

PLE appears to be a very effective alternative extraction method that is simple, rapid, and uses smaller quantities of low toxicity organic solvents to give a high efficiency extraction of bioactive isoflavonoids from *B. chinensis* rhizomes. Solvent composition appears to be the key variable that determines the final extraction yields and 60% ethanol gave the best extraction efficiency for isoflavonoids under the conditions described here. Care must also be taken in selecting proper extraction conditions, as tectoridin (isoflavonoid glycoside) was found to be degraded to tectorigenin (aglycone) at temperatures above 150°C.

ACKNOWLEDGMENTS

This work was supported by grant no. RTI05-01-02 from the Regional Technology Innovation Program of the Ministry of Knowledge Economy, Republic of Korea.

REFERENCES

1. Lee, S. O.; Woo, W. S.; Woo, E. H.; Kim, K. S. Isoflavonoids of *Belamcanda chinensis*. *Kor. J. Pharmacog.* **1989**, *20*, 219–222.
2. Shin, K. H.; Kim, Y. P.; Lim, S. S.; Lee, S. H.; Ryu, N.; Yamada, M.; Ohuchi, K. Inhibition of Prostaglandin E2 Production by the Isoflavones Tectorigenin and Tectoridin Isolated from the Rhizomes of *Belamcanda chinensis*. *Planta Med.* **1999**, *65*, 776–777.

3. Jung, S. H.; Lee, Y. S.; Lee, S. H.; Lim, S. S.; Kim, Y. S.; Ohuchi, K.; Shin, K. H. Anti-Angiogenic and Anti-Tumor Activities of Isoflavonoids from the Rhizomes of *Belamcanda chinensis*. *Planta Med.* **2003**, *69*, 617–622.
4. Oh, K. B.; Kang, H. J.; Matsuoka, H. Detection of Antifungal Activity in *Belamcanda chinensis* by a Single-Cell Bioassay Method and Isolation of Its Active Compound, Tectorigenin. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 939–942.
5. Jung, S. H.; Lee, Y. S.; Lee, S. H.; Lim, S. S.; Kim, Y. S.; Shin, K. H. Isoflavonoids from the Rhizomes of *Belamcanda chinensis* and Their Effects on Aldose Reductase and Sorbitol Accumulation in Streptozotocin Induced Diabetic Rat Tissues. *Arch. Pharm. Res.* **2002**, *25*, 306–312.
6. Ahmed, F. E. Analyses of Pesticides and Their Metabolites in Foods and Drinks. *Trends Anal. Chem.* **2001**, *20*, 649–661.
7. Huie, C. W. A Review of Modern Sample-Preparation Techniques for the Extraction and Analysis of Medicinal Plants. *Anal. Bioanal. Chem.* **2002**, *373*, 23–30.
8. Ong, E. S. Extraction Methods and Chemical Standardization of Botanicals and Herbal Preparations. *J. Chromatogr. B* **2004**, *812*, 23–33.
9. Ong, E. S.; Len, S. M. Pressurized Hot Water Extraction of Berberine, Baicalein and Glycyrrhizin in Medicinal Plants. *Anal. Chim. Acta* **2003**, *482*, 81–89.
10. Ong, E. S.; Len, S. M. Evaluatin of Surfactant-Assisted Pressurized Hot Water Extraction for Marker Compounds in *Radix Codonopsis pilosula* using Liquid Chromatography and Liquid Chromatography/Electrospray Ionization Mass Spectrometry. *J. Sep. Sci.* **2003**, *26*, 1533–1540.
11. Attawadee, S. Y.; Chitchamai, O.; Arunporn, I.; Ruedeekorn, W. Extraction of Rotenone from *Derris elliptica* and *Derris malaccensis* by Pressurized Liquid Extraction Compared with Maceration. *J. Chromatogr. A* **2006**, *1125*, 172–176.
12. Li, P.; Li, S. P.; Lao, S. C.; Fu, C. M.; Kan, K. K. W.; Wang, Y. T. Optimization of Pressurized Liquid Extraction for Z-Ligustilide, Z-Butylidenephthalide and Ferulic acid in *Angelica sinensis*. *J. Pharm. Biomed. Anal.* **2006**, *40*, 1073–1079.
13. Wan, J. B.; Yang, F. Q.; Li, S. P.; Wang, Y. T.; Cui, X. M. Chemical Characteristics for Different Parts of *Panax notoginseng* using Pressurized Liquid Extraction and HPLC-ELSD. *J. Pharm. Biomed. Anal.* **2006**, *41*, 1596–1601.
14. Carbabias-Martinez, R.; Rodriguez-Gonzalo, E.; Revilla-Ruiz, P.; Hernandez-Mendez, J. Pressurized Liquid Extraction in the Analysis of Food and Biological Samples. *J. Chromatogr. A* **2005**, *1089*, 1–17.
15. Jiang, Y.; Li, S. P.; Chang, H. T.; Wang, Y. T.; Tu, P. F. Pressurized Liquid Extraction Followed by High-Performance Liquid Chromatography for Determination of Seven Active Compounds in Cortex Dictamni. *J. Chromatogr. A* **2006**, *1118*, 268–272.
16. Mannila, M.; Wai, C. M. Pressurized Water Extraction of Naphtodianthrones in St. John's Wort (*Hypericum perforatum* L.). *Green Chem.* **2003**, *5*, 387–391.
17. Bezman, R. D.; Cassassa, E. F.; Kay, R. L. The Temperature Dependence of the Dielectric Constants of Alkanols. *J. Mol. Liq.* **1997**, *73–74*, 397–402.
18. Harvey, A. H.; Prausnitz, J. M. Dielectric Constants of Fluid Mixtures Over a Wide Range of Temperature and Density. *J. Solut. Chem.* **1987**, *16*, 857–869.