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Comparison of techniques for extraction of isoflavones from the root of Radix Puerariae: Ultrasonic and pressurized solvent extractions

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Abstract

Radix Puerariae (RP), a traditional Chinese medicinal herb, has been used for a variety of disease prevention and treatment purposes. Three isoflavones, puerarin, daidzin and daidzein, isolated from RP are responsible for its broad therapeutic effects. In the present study, we demonstrate the application of three extraction methods, traditional, pressurized solvent extraction (PSE) and ultrasonic techniques, for preparing ethanolic RP extract. A comparison of the three extraction methods showed preferential higher yields of the three major isoflavones when the ultrasonic technique was applied. The extraction yield became higher as the mean size of RP particles decreased while the total accumulated power varied from 20 to 80 MJ at the solid-to-solvent ratios of 1:5 and 1:10 (g/ml). The relationship among accumulated energies, extraction yields and mean particle sizes under different extraction ratios using ultrasound was also discussed. This study proves that using the ultrasonic method should be the most economic way for enhancing the extraction yield of isoflavones-containing herbal extract in a short time with a reduced amount of solvent at a lower temperature.

Keywords: Radix Pueraria; Isoflavones; Ultrasound; Pressurized solvent extraction; Mean particle size; Herbal extracts

1. Introduction

Radix Pueraria (RP, *Pueraria lobata* or Ge-Gen), a traditional Chinese medicinal herb, has been used as an antimicrobial, pain-releasing and appetite-inducing agent, as recorded in ancient Chinese literatures (Fang, 1980; Keung & Vallee, 1998; Yasuda et al., 2005). In modern times, RP has proven useful in the treatment of alcohol abuse, hypertension and cardiovascular diseases from pharmacological and clinical studies (Fan, O'Keefe, & Powell, 1985; Keung, Klyosov, & Vallee, 1997; Lukas et al., 2005). Several isoflavones, mainly puerarin, daidzin and daidzein, isolated from RP, are responsible for the broad therapeutic effects identified in RP (Fig. 1). Puerarin possesses antioxidant and anticancer activities and may protect ischemic myocardium as well as improving memory impairment; while daidzin exerts an antidipsotropic effect by inhibiting mitochondrial aldehyde dehydrogenase and decreasing ethanol consumption in rats (Fan et al., 1985; Keung et al., 1997). A study of metabolism of puerarin and daidzin to their aglycone, daidzein, has been reported.

The concern of the effect of estrogen on human health has raised the issue of using phytoestrogenic isoflavones as a natural alternative to estrogen replacement therapy (ERT) in the pharmaceutical industry. Numerous reports have shown that phytoestrogens, especially soy products, may exert physiological effects in the prevention of breast cancer, osteoporosis and cardiovascular disease (Choo, Park, Yoon, & Kim, 2002). However, their potential action as endocrine disruptors still remains as a major adverse effect on human health. Isoflavones have been shown to prevent bone loss in ovariectomized animals and are considered to act as selective estrogen receptor modulators (SERM) by exerting both estrogen agonist and antagonist

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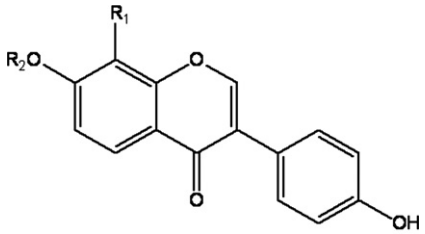

	R1	R2
1. Puerarin	glc	H
2. Daidzin	H	glc
3. Daidzein	H	H

Fig. 1. Chemical structures of the three major isoflavones in Radix Puerariae.

actions (Anthony, Clarkson, Hughes, Morgan, & Burke, 1996; Arjmandi et al., 1996; Cornwell, Cohick, & Raskin, 2004; Herman et al., 1995). Ipriflavone, a synthetic analogue of daidzein shows great promise in the prevention and treatment of osteoporosis as well as other metabolic bone diseases (Head, 1999).

Due to the above reasons, the isoflavones-enriched Radix Puerarin might be a valuable source for nutraceutical herbal products. Therefore, developing a fast and efficient way for production of high quality botanical products has become an issue of concern in pharmaceutical and food industries. However, application of novel techniques in the extraction of natural products has not been studied extensively. In addition to the traditional extraction methods, i.e. maceration, percolation, Soxhlet extraction that have been used for decades, pressurized solvent extraction (PSE) was developed for its application in natural product extraction in recent years (Kaufmann & Christen, 2002). This technique was reported to be a reliable analytical method in the analysis of isoflavones in a variety of soybean foods (Klejdus et al., 2004; Rostagno, Palma, & Barroso, 2004). PSE offers advantages with regard to extraction time, yields and reproducibility by using elevated pressure and temperature, which facilitate better penetration to the matrix pores. In spite of that, large particle sizes of plant cells and the difficulty of applying traditional or PSE methods in continuous mode for manufacturing production has stimulated the usage of ultrasonic technique for natural product extraction.

The use of ultrasound for the enhancement of extraction yield was advanced in the1950s (Vinatoru, 2001). The technique is also widely used in the food industry for emulsification, crystallization and freezing, among others (Mason, Paniwnyk, & Lorimer, 1996). Ultrasound, the term used to describe sounds above 20 kHz and power ultrasound, usually generated by a transducer which converts mechanical or electrical energy into high frequency vibrations (Romdhane & Gourdon, 2002). The majority of transducers are designed to deliver ultrasonic frequencies of between 20 kHz and 35 kHz. The enhancement of extraction efficiency of organic compounds using ultrasound is attributed to a phenomenon called cavitation produced in the solvent by the passage of an ultrasonic wave. Cavitation bubbles are produced and compressed during the application of ultrasound, allowing higher penetration of the solvent into the raw plant materials and the intracellular products released by disrupting the cell walls (Romdhane & Gourdon, 2002). The ultrasound has been shown to aid extraction in a number of plant materials by significantly reducing extraction time and increasing maximum extraction yield (Albu, Joyce, Paniwnyk, Lorimer, & Mason, 2004; Rostagno, Palma, & Barroso, 2003; Valachovic, Pechova, & Mason, 2001; Wu, Lin, & Chau, 2001).

Most of the previous studies regarding the extraction of isoflavones from natural products primarily aimed at optimization of the extraction conditions as well as improvement of the analytical methods (He et al., 2005; Klejdus et al., 2004; Rostagno et al., 2003, 2004). In the present study, the application of three extraction methods, traditional, PSE and ultrasonic techniques, for preparing ethanolic RP extract is demonstrated. The extracted amounts of three isoflavones, particle size distributions under various extraction ratios and conditions were compared. The relationship among the accumulated energies, extraction yields and mean particle sizes under different extraction ratios using ultrasound are also discussed.

2. Materials and methods

2.1. Plant material

The roots of Radix Puerariae were collected from the Yan-Min mountain in the Taipei suburban region. The dried pale-yellow fibrous roots were chopped, and ground into powders. These rough fibers and coarse powders were strained through a 250 μm sieve (mesh no. 60 base on ASTM classifications) to afford a mean size of 143.8 μm of RP fine powders using a vibrating screen.

2.2. Traditional extraction method

RP fine powders (1 g) were soaked in 10 ml of 95% EtOH for 2 h with ultrasonic agitation (Branson 5210). The sample was filtered through a 0.45 μm filter for high performance liquid chromatography (HPLC) analysis and the resultant fine powders were subjected to particle analysis. The ethanolic extract was stored in refrigerator and used within 2 weeks.

2.3. Pressurized solvent extraction

Accelerated solvent extraction (ASE®) is a registered technique of PSE that combines elevated temperature and pressures with solvents to achieve efficient extraction. The instrument, Accelerated Solvent Extractor (ASE100®, Dionex Corporation, Sunnyvale, CA) was used for pressurized solvent extraction. 1 g of RP powders were placed in a 10 ml extraction cell. The extraction was conducted under

the following condition: solvent: 95% EtOH; extraction volume: 10 ml; temperature: 60, 80, 100 °C; static time: 10 min; purge time: 60 s; static cycle: 1. The extraction was maintained at 1400 psi. The extract was diluted 10-fold and then subjected to HPLC analysis.

2.4. Ultrasonic extraction

RP fine powders (3 g or 6 g) were added to 30 ml of 95% EtOH and then mixed with a magnetic stirrer in a 250 ml beaker for 5 min. The previous slurry was subjected to ultrasound probe (Sonics, Ultrasonic Processor VCX 750, CT, USA) with a 19 mm high gain probe at 20 kHz and 750 W. The extraction was carried out at less than 37 °C by means of an integrated temperature controller, which precludes harmful overheating of the sample and guarantees process integrity by terminating the ultrasound when the sample temperature reaches a predetermined limit during the processing cycle.

The extraction was conducted by applying various accumulated powers, 20, 40, 60 and 80 MJ, which correspond to 27, 53, 80 and 102 min, respectively, of extraction time over the whole process, respectively. The extract was then decanted from a beaker and filtered through a 0.45 μm filter for HPLC analysis. The residual slurry added 200 ml of deionized water (the resistively = 18.0 MΩ cm) to prevent agglomeration. Approximately 1.5 ml of sample was loaded directly into the small module for the determination of size distribution by a dynamic light scattering analyzer (Coulter LS230, Fullerton, CA).

2.5. HPLC apparatus and conditions

The HPLC system (Shimadzu, Kyoto, Japan) consisted of two LC-10AD pumps, a SCL-10A system controller, and a SIC-10 A auto injector connected to a SPD-10A UV–Vis detector.

The separation was performed on a Cosmosil 5C 18 MS column (5 μm, 25 cm × 4.6 mm ID, Nacalai Tesque, Kyoto, Japan). The mobile phase was composed of 0.1% H_3PO_4 (solvent A) and acetonitrile (solvent B). The sample (10 μl) was eluted with a gradient profile as follows: 0–15 min, from 85% A, 15% B to 80% A, 20% B; 15–30 min, from 80% A, 20% B to 50% A, 50% B; 30–35 min, from 50% A, 50% B to 10% A, 90% B. The flow rate and detection wavelength were set at 1.0 ml/min and 254 nm, respectively. The calibration curves (correlation coefficient) for puerarin, daidzin and daidzein were $y = 2874x + 536\,981$ ($R^2 = 0.9943$), $y = 3082x + 14\,021$ ($R^2 = 0.9998$), $y = 6066x + 4064$ ($R^2 = 0.9991$), respectively.

3. Results and discussion

3.1. Analysis of the extracted amounts of puerarin, daidzin and daidzein using the traditional, ASE and ultrasonic methods

Preparation of ethanolic RP extract with the three extraction methods, traditional, PSE and ultrasonic techniques, was conducted. Analysis of the extracted amounts of three major isoflavones is presented in Table 1. High performance liquid chromatography was used for quantitative analysis with a good separation of puerarin, daidzin and daidzein achieved within 30 min. It has been reported that using deactivated C-18 column and a mobile phase containing acetonitrile and a weak organic acid would result in the optimal separation of flavonoids. The contents of puerarin, daidzin and daidzein in RP extract with the traditional method were 1.19 ± 0.17, 0.658 ± 0.004 and 0.073 ± 0.001 mg/g of powder, respectively. In PSE group, various temperatures, 60, 80 and 100 °C, were applied to the extraction with increased yields of 1.8–2.4 fold for puerarin, 1.4–1.8 fold for daidzin, and 2.6–3.7 fold for daidzein, correspondently. A remarkable increase in extraction yield was observed as the temperature was raised in accelerated solvent extraction. In the case of ultrasonic extraction, the yield increased as the applied energy was increased at

Table 1
Comparison of the extracted amounts of three isoflavones and the mean sizes of Radix Puerariae using ultrasonic and ASE methods

Extraction method[a]	Puerarin (mg/g of powder)	Daidzrin[b] (mg/g of powder)	Daidzein (mg/g of powder)	Mean size (μm)
Tradition	1.19 ± 0.17	0.658 ± 0.004	0.073 ± 0.001	263.00
ASE-60[c]	2.18 ± 0.09	0.929 ± 0.012	0.189 ± 0.007	157.60
ASE-80	2.44 ± 0.09	0.979 ± 1.017	0.224 ± 0.007	155.15
ASE-100	2.74 ± 0.09	1.21 ± 0.05	0.272 ± 0.008	135.25
Ultra-10-20M[d]	3.00 ± 002	1.18 ± 0.08	0.248 ± 0.012	28.00
Ultra-10-40M	3.57 ± 0.55	1.42 ± 0.13	0.301 ± 0.025	12.57
Ultra-10-60M	3.70 ± 0.25	1.37 ± 0.10	0.287 ± 0.014	11.09
Ultra-5-20M[e]	2.87 ± 0.16	1.01 ± 0.05	0.205 ± 0.007	32.88
Ultra-5-40M	2.98 ± 0.42	1.15 ± 0.04	0.243 ± 0.011	16.22
Ultra-5-60M	3.32 ± 0.01	1.36 ± 0.01	0.323 ± 0.001	13.77
Ultra-5-80M	3.06 ± 0.13	1.25 ± 0.03	0 291 ± 0.006	12.36

[a] The extraction methods were described in Section 2.
[b] The contents were presented as mean ±S.D. ($n = 3$).
[c] ASE-60: accelerated solvent extraction at 60 °C.
[d] Ultrasonic method with 1 g powder/10 ml EtOH ratio, total energy = 20 MJ.
[e] Ultrasonic method with 1 g powder/5 ml EtOH ratio, total energy = 20 MJ.

both extraction ratios. Interestingly, in comparison with the amounts extracted by traditional method, the increased amount (over fourfold) of the aglycon, daidzein, was much more than those of daidzein glycoside, puerarin and daidzin, when the ultrasonic technique was applied.

3.2. Comparison of particle size distributions by different extraction methods and conditions

The size distributions of RP powders following traditional and ASE treatments at 60 and 100 °C are shown in Fig. 2. It can be seen that the curves of size distribution among these three conditions differed within the size range from 1 to 1000 μm. In the PSE group, there was a sharp decrease in the percentage of powder within 400–1000 μm, compared to the traditional one. In addition, as the extraction temperature increased from 60 to 100 °C, the maximum peak decreased from 0.013 to 0.01, while the peak value of the traditional one reached 0.03 within the higher size range. This phenomenon could be explained by the high pressure (1400 psi) in PSE causing the powder to be readily crushed and the particle size distribution moved from 400–1000 to 10–100 μm of range.

As shown in Table 1, the mean sizes following treatment with both traditional and PSE methods were correlated with the extracted amounts of the three isoflavones in RP. In the case of PSE, the mean sizes at 60, 80 and 100 °C were 157.60 ± 12.0, 155.15 ± 15.1 and 135.25 ± 12.1 μm, respectively, compared to 263 μm from the traditional method. The extracted amounts of puerarin, daidzin and daidzein increased as the average particle sizes decreased. It is reasonable to conclude that the aggregated RP powders disintegrated under high pressure, resulting in reduced particle sizes as well as the bioactive ingredients being released much more readily. Also, as expected, extraction temperature in PSE facilitated the mass-transfer process of bioactive components in a temperature-dependent manner, as the extracted amounts of daizein, puerarin and daidzin increased from 2.6 to 3.7, 1.8 to 2.3 and 1.4 to 1.8 when the extraction temperature was raised from 60 to 100 °C.

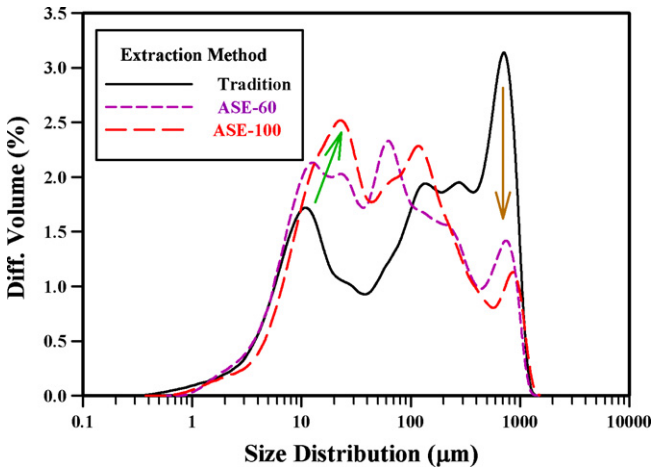

Fig. 2. Comparison of the size distributions of RP powders after treatment at different temperature using ASE method.

3.3. The relationship among accumulated energies, extraction yields and mean particle sizes under different extraction ratios using ultrasound

The relations of total accumulated energies vs. particle size distributions at two extraction ratios of RP exact using ultrasonic technique are demonstrated in Fig. 3. As indicated in both extraction ratios of 3 g/30 ml EtOH (1:10) and 6 g/30 ml EtOH (1:5), the size distributions shifted from the range of 50–200 μm to accumulation at 8–20 μm. The averaged sizes under different ultrasonic treatments were also calculated in Table 1 and plotted against the total accumulated energies shown in Fig. 4. The mean size of RP particles ranging from 11 to 28 μM following ultrasonic agitation was mainly related to the total accumulated power of ultrasonic agitation. The extraction yield became higher as the mean size of RP particles decreased,

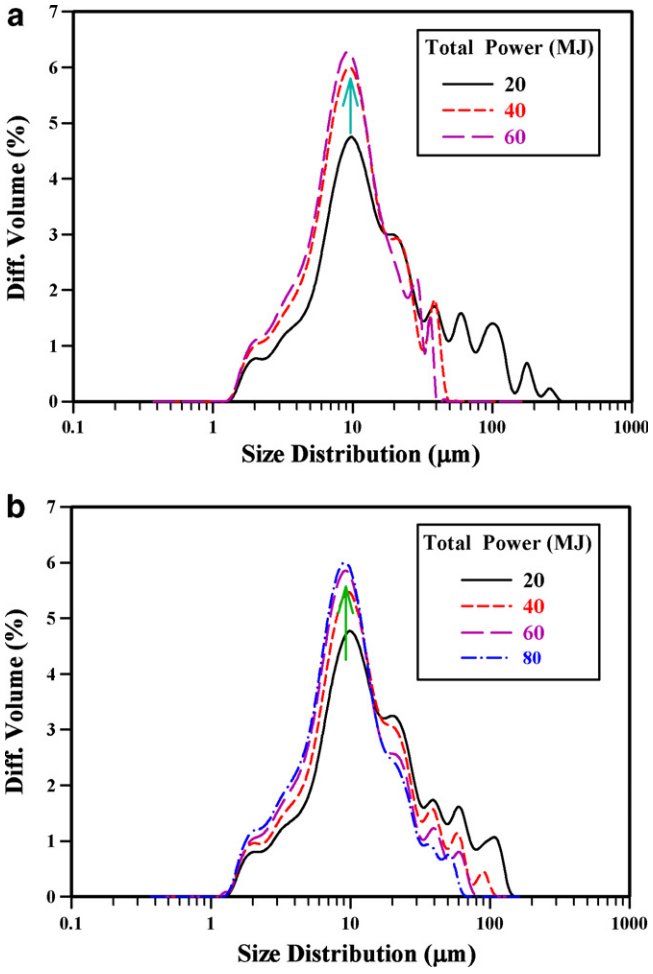

Fig. 3. Comparison of the size distributions of RP powders after treatment with different ultrasonic accumulated energies. (a) Extraction ratio = 1:10, (b) extraction ratio = 1:5.

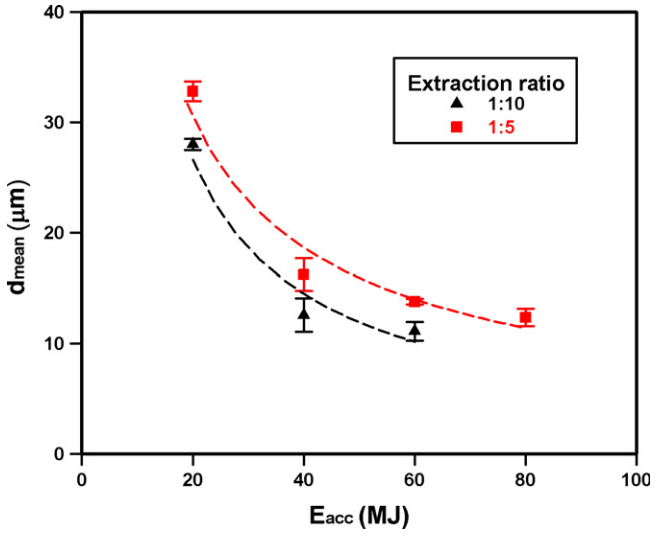

Fig. 4. Relations of accumulated energies vs. mean particle sizes under different extraction ratios using ultrasonic technique.

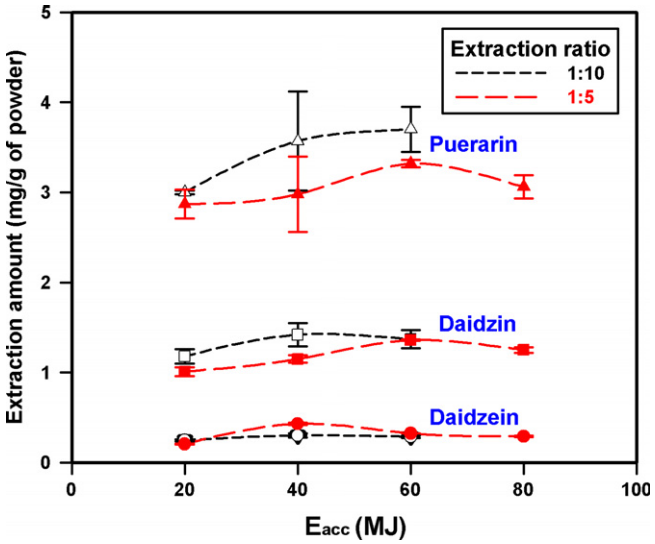

Fig. 5. Relations of accumulated energies vs. extracted amounts of isoflavones from RP extracts under different extraction ratios using ultrasound.

while the total accumulated power varied from 20 to 80 MW at the extraction ratio of 0.1 and 0.2 g of RP powder/ml of ethanol. We derived a mathematical relationship between these two arrays of data as the regression equations of $dmean = 367.347^{*}Eacc^{**}(-0.876669)$ (R-squared = 0.945555) and $dmean = 256.115^{*}Eacc^{**}(-0.710303)$ (R-squared = 0.939473) with respect to the extraction ratios of 1:10 and 1:5. The results indicate that the mean size decreased as the ultrasonic accumulated power increased, while the decreasing rate declined the most between 20 and 40 MJ and then turned to slow after 40 MJ. It is also observable that under the same accumulated energy, the lower the extraction ratio is, the lower the average particle size is.

The relations of total energies vs. the extracted amounts of the three isoflavones under different extraction ratios are demonstrated in Fig. 5. Under the same total accumulated energy, the extraction efficiencies at 1:10 were higher than those at 1:5 of the extraction ratio in the three bioactive components. At 1:10 of the extraction ratio, the extracted amounts of daidzin and daidzein reached a maximum under 40 MJ of accumulated energy, however, it required higher energy to achieve the saturation in the case of puerarin. At 1:5 of the extraction ratio, all three components reached the maximum extracted amounts under 60 MJ of accumulated energy.

Moreover, the results in Figs. 3 and 4 and Table 1 indicate that at the same extraction ratio, the mean particle size decreased as the total accumulated energy increased. The extracted amounts of puerarin, daidzin and daidzein reached a maximum with the averaged particle sizes of 12.58 and 13.77 μm at the extraction ratio of 1:10 and 1:5, respectively. However, a decline in extraction efficiency was observed at 80 MJ of accumulative energy. We speculate that the ultrasonic agitation produced high accumulated energy that led to the degradation of bioactive components when the ultrasonic energy reached 80 MJ in this case.

Finally, we make a comparison of the extraction yields of the three isoflavones under three extraction treatments, traditional, ASE-100 and Ultra-10-40M. The result shows that the increased ratios of the puerarin, daidzin and daidzein in ASE-100 were 2.4, 1.8 and 3.7, while those in Ultra-10 -40M were 3, 2.1 and 4.1, accordingly, when the value in traditional treatment was set at 1. It can be concluded that for the extraction of RP isoflavones at high efficiency, the ultrasonic technique works better than the ASE and the traditional one is not recommended. In Table 2, three extraction methods for crude natural products are compared with respect to time, solvent consumption, extraction condition and cost. It is easy to see that PSE and ultrasonic methods are superior to the traditional one in all respects. PSE would be more suitable for small-scale routine quantitative analysis of heat stable components, while the ultra-

Table 2
Comparison of the traditional, ASE and ultrasonic extraction techniques

Tradition	ASE	Ultrasonic
Time consuming (several hours to days)	Time saving (within 1 h)	Moderate (1–2 h)
Low extraction yields	Medium to high extraction yields	High extraction yields
Solvent wasting	Large quantity of exhaustive solvent for maintenance of high pressures	Moderate solvent consuming
High or room temperature	High temperature, high pressure	Room temperature
Low cost	High cost	Moderate cost

sonic method is applicable to the preparation of a large quantity of heat-labile herbal extracts.

References

Albu, S., Joyce, E., Paniwnyk, L., Lorimer, J. P., & Mason, T. J. (2004). Potential for the use of ultrasound in the extraction of antioxidants from *Rosmarinus officinalis* for the food and pharmaceutical industry. *Ultrasonics Sonochemistry, 11*, 261–265.

Anthony, M. S., Clarkson, T. B., Hughes, C. L., Morgan, T. M., & Burke, G. L. (1996). Soybean isofalvones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. *Journal of Nutrition, 126*, 43–50.

Arjmandi, B. H., Alekel, L., Hollis, B. W., Amin, D., Stacewicz-Sapuntzakis, M., Guo, P., et al. (1996). Dietary soybean prevents bone loss in ovariectomised rat model of osteoporosis. *Journal of Nutrition, 126*, 161–167.

Choo, M. K., Park, E. K., Yoon, H. K., & Kim, D. H. (2002). Antithrombotic and antiallergic activities of daidzein, a metabolite of puerarin and daidzin produced by human intestinal microflora. *Biological & Pharmaceutical Bulletin, 25*, 1328–1332.

Cornwell, T., Cohick, W., & Raskin, I. (2004). Dietary phytoestrogens and health. *Phytochemistry, 65*, 995–1016.

Fan, L. L., O'Keefe, D. D., & Powell, W. W. Jr., (1985). Pharmacologic studies on radix puerariae: effect of puerarin on regional myocardial blood flow and cardiac hemodynamics in dogs with acute myocardial ischemia. *Clinical Medicine Journal, 98*, 821–832.

Fang, Q. (1980). Some current study and research approaches relating to the use of plants in the traditional chinese medicine. *Journal of Ethnopharmacology, 2*, 57–63.

He, J., Zhao, Z., Shi, Z., Zhao, M., Li, Y., & Chang, W. (2005). Analysis of isoflavone daidzein in Puerariae radix with micelle-mediated extraction and preconcentration. *Journal of Agricultural and Food Chemistry, 53*, 518–523.

Head, K. A. (1999). Ipriflavone: an important bone-building isoflavone. *Alternative Medicine Review, 4*, 10–22.

Herman, C., Adlercreutz, T., Goldin, B. R., Gorbach, S. L., Hockerstedt, K. A. V., Watanabe, S., et al. (1995). Soybean phytoestrogen intake and cancer risk. *Journal of Nutrition, 125*, 757S–770S.

Keung, W. M., Klyosov, A. A., & Vallee, B. L. (1997). Daidzin inhibits mitochondrial aldehyde dehydrogenase and suppresses ethanol intake of Syrian golden hamsters. *Proceedings of the National Academy of Sciences of the United States of America, 94*, 1675–1679.

Kaufmann, B., & Christen, P. (2002). Recent extraction techniques for natural products: microwave-assisted extraction and pressurised solvent extraction. *Phytochemical Analysis, 13*, 105–113.

Keung, W. M., & Vallee, B. L. (1998). Kudzu root: an ancient Chinese source of modern antidipsotropic agents. *Phytochemistry, 47*, 499–506.

Klejdus, B., Mikelova, R., Adam, V., Zehnalek, J., Vacek, J., Kizek, R., et al. (2004). Liquid chromatographic-mass spectrometric determination of genistin and daidzin in soybean food samples after accelerated solvent extraction with modified content of extraction cell. *Analytica Chimica Acta, 517*, 1–11.

Lukas, S. E., Penetar, D., Berko, J., Vicens, L., Palmer, C., Mallya, G., et al. (2005). An extract of the Chinese herbal root kudzu reduces alcohol drinking by heavy drinkers in a naturalistic setting. *Alcoholism Clinical & Experimental Research, 29*, 756–762.

Mason, T. J., Paniwnyk, L., & Lorimer, J. P. (1996). The uses of ultrasound in food technology. *Ultrasonics Sonochemistry, 3*, 2253–2260.

Romdhane, M., & Gourdon, C. (2002). Investigation in solid–liquid extraction: influence of untrasound. *Chemical Engineering Journal, 87*, 11–19.

Rostagno, M. A., Palma, M., & Barroso, C. G. (2003). Ultrasound-assisted extraction of soy isoflavones. *Journal of Chromatography A, 1012*, 119–128.

Rostagno, M. A., Palma, M., & Barroso, C. G. (2004). Pressurized liquid extraction of isoflavones from soybeans. *Analytica Chimica Acta, 522*, 169–177.

Valachovic, P., Pechova, A., & Mason, T. J. (2001). Towards the industrial production of medicinal tincture by ultrasound assisted extraction. *Ultrasonics Sonochemistry, 8*, 111–117.

Vinatoru, M. (2001). An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Ultrasonic Sonochemistry, 8*, 303–313.

Wu, J., Lin, L., & Chau, F. T. (2001). Ultrasound-assisted extraction of ginseng saponins from ginseng roots and cultured ginseng cells. *Ultrasonics Sonochemistry, 8*, 347–352.

Yasuda, T., Endo, M., Kon-no, T., Kato, T., Mitsuzuka, M., & Ohsawa, K. (2005). Antipyretic, analgesic and muscle relaxant activities of pueraria isoflavonoids and their metabolites from Pueraria lobata Ohwi – a traditional Chinese drug. *Biological & Pharmaceutical Bulletin, 28*, 1224–1228.