African Journal of Biotechnology Vol. 10(25), pp. 5020-5026, 8 June, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2544
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Ultrasonic extraction of flavonoids and phenolics from loquat (*Eriobotrya japonica* Lindl.) flowers

Chun-hua Zhou[1,2]*, Xian Li[2], Chong-de Sun[2] and Kun-song Chen[2]

[1]College of Horticulture and Plant Protection, Jiangsu Key Laboratory of Crop Genetics and Physiology, Yangzhou University, Yangzhou 225009, People's Republic of China.
[2]Laboratory of Fruit Quality Biology, College of Agriculture and Biotechnology, Zhejiang University/The Ministry of Agriculture's Laboratory of Horticultural Plant Growth and Quality Regulation, Hangzhou 310029, People's Republic of China.

Accepted 18 March, 2011

Ethanol was used to extract flavonoids and phenolics from loquat *(Eriobotrya japonica* Lindl. cv. Ruantiaobaisha) flowers with ultrasonic pharmaceutical managing machine. Single-factor and orthogonal experiment were used to investigate the optimum extraction condition. The results showed that, the combination of 30°C, 80 min, 60% ethanol and 1:40 material ratio was optimum extraction condition with the highest yields of flavonoids and phenolics at 47 kHz/500 W. Under the optimum extraction condition, two consecutive extractions was enough, the extraction rates of flavonoids and phenolics were all more than 90%, with the contents of 10.59 and 102.02 mg/g dry weight, respectively.

Key words: *Eriobotrya japonica*, flower, flavonoids, phenolics, ultrasonic extraction.

INTRODUCTION

Loquat (*Eriobotrya japonica* Lindl.), a small evergreen arbor, is one of the species of genus *Eriobotrya* (Rosaceae) (Pottier-Alapetite, 1979). The fruit, leave, flower and root of loquat plant are all proved with medicinal values. According to literatures, loquat flowers have been used usually to treat cold, cough, sputum and so on (Xiong et al., 1993). At present, the researches about functional components extraction and identification and their pharmacological action of loquat plant were mainly focused on the leaves (Bae et al., 2010; Chen et al., 2008; Huang et al., 2009; Ito et al., 2001; Kobayashi and Takamatsu, 2000; Jung et al., 1999; Li et al., 2009; Louati et al., 2003) and seeds (Kawahito et al., 2008; Sun et al., 2010; Yokota et al., 2006; Yoshioka et al., 2010), while few studies related to the flowers were reported.

Concerning the functional components of loquat flowers, Cheng et al. (2001) isolated four compounds from the ethanol extracts and identified as oleanolic acid, ursolic acid, 2α, 3α, 19α-trihydroxyurs-5,12-dien-28-acid and 2β, 3β, 23α-trihydroxyolean-12-en-28-acid by chemical methods and spectroscopic (IR, MS, ^{1}HNMR, ^{13}CNMR) analysis. Zhou et al. (2007) determined oleanolic acid and ursolic acid contents in flowers of five loquat cultivars. Until now, no other bioactive component was separated and no reports about functional component extraction were released. We found that there were much flavonoids and phenolics in the loquat flowers besides triterpenoic acid by analyzing different solvent extracts of loquat flower in preliminary experiments.

Different extraction techniques, such as hydrodistillation, Soxhlet, ultrasonic, etc., are widely used for obtaining extractable substances from different parts of a number of plants (Ammar et al., 2010; Li et al., 2010; Peres et al., 2006). As a novel technique, ultrasonic extraction has recently been shown to be very promising and effective for obtaining bioactive components, ensuring higher yields at much shorter times (Yue et al., 2010). The beneficial effects of ultrasonic extraction are attributed to the formation and asymmetrical collapse of microcavities in the vicinity of cell walls leading to the generation of microjets rupturing the cells in plant. The aim of this paper was to explore the optimum ultrasonic extraction condition of flavonoids and phenolics and provide some theoretical basis for integrative usage of loquat flower resources.

*Corresponding author. E-mail: chzhou@yzu.edu.cn. Tel: (+) 86-514-87991566. Fax: (+) 86-514-87347537.

MATERIALS AND METHODS

Materials and chemicals

The flowers of *E. japonica* cv. Ruantiaobaisha were collected at winter from Yuhang District, Hangzhou, Zhejiang Province, People's Republic of China. Rutin and chlorogenic acid were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All the other reagents used in the present study were of analytical grade.

Pretreatment of loquat flowers

The whole inflorescences were cut into pieces and dried with microwave oven (Panosonic) until the weight reached unchangeable. The flowers were then powdered in a grinder to 40-mesh size and stored at -20°C until analysis.

Extraction of flavonoids and phenolics

Flower powder of 0.2 g was extracted in TBT/C-YCL 500Tt/3P (D) ultrasonic pharmaceutical managing machine (Sinobest electronic Co. Ltd., Jining, Shangdong Province, China) with a certain volume solvent under the selected frequency and power combination of 47 kHz/500 W and then the extracts were filtrated and diluted to 10 ml. The diluted solution was used to determine the contents of flavonoids and phenolics. Effects of ethanol concentration (0, 20, 40, 60, 80 and 100%), material ratio (1:10, 1:20, 1:30, 1:40, and 1:50), extraction time (20, 40, 60, 80, 100 and 120 min) and extraction temperature (20, 30, 40, 50, 60 and 70°C) to extraction efficiency were investigated. Finally, orthogonal experimental design was used to select optimal extraction condition after single factor experiments. During the ultrasonic treatment, the temperature was maintained by a temperature controlling system.

Determination of total flavonoids content

Total flavonoids were determined by the method of Zhou et al. (2007b) with some modification. Aliquots (1 ml) of loquat flower extracts were placed in two test tubes, respectively. 7 ml methanol was added to one tube. In the other tube, 1 ml 2% $ZrOCl_2 \cdot 8H_2O$ and 6 ml methanol was added. The solution was mixed well again and placed into water bath at 30°C for 1 h. The absorbance was measured at 420 nm with DU-8000 UV-Vis spectrophotometer (Beckman Coulter, USA) and ΔOD was calculated. The amount of total flavonoids was calculated as a rutin equivalent from the standard curve and expressed as mg rutin/g dry plant material. All investigations were repeated three times.

Determination of total phenolics contents

Total phenolics were determined by Folin–Ciocalteu procedure (Maksimovic et al., 2005). Aliquots (0.5 ml) of loquat flower extracts were transferred into the test tubes and their volumes made up to 4.5 ml with distilled water. After addition of 0.5 ml Folin–Ciocalteu reagent and 1 ml 7% aqueous sodium carbonate solution, tubes were vortexed and placed into water bath at 30°C for 1 h. Then absorbance of mixtures was recorded at 760 nm against a blank containing 0.5 ml of extraction solvent. The amount of total phenolics was calculated as a chlorogenic acid equivalent from the calibration curve and expressed as mg chlorogenic acid/g dry plant material. All measurements were done in triplicate.

Statistical analysis

Results were represented as mean ± standard error. Statistical comparisons between groups were performed with a standard analysis of variance (ANOVA). The Duncan multiple range test was used to estimate significant differences among mean values of the treatments. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Ultrasonic frequency and power selection

The ultrasonic pharmaceutical managing machine used in this study has three frequency levels (26, 47 and 70 kHz) and two power levels (250 and 500 W). The contents of flavonoids and phenolics at different combination of frequency and power levels were shown in Table 1. Combination of 47 kHz/500 W had the highest extraction efficiency, but there was no significant difference between this combination with 47 kHz/250 W and 70 kHz/250 W. Combination of 70 kHz/500 W had the lowest extraction efficiency and was significantly different with other combinations. Therefore, flavonoids and phenolics extractions were performed at 47 kHz/500 W in the following experiments.

Effects of ethanol concentration on extraction efficiencies of flavonoids and phenolics

Ethanol concentration had obvious impact on extraction efficiencies of functional components from loquat flower. The extraction efficiencies of these two components with absolute ethanol were all lower than those with ethanol adding water. Among the four ethanol concentration (20, 40, 60 and 80%), the highest extraction efficiencies of flavonoids and phenolics were all with 60% ethanol, but were not significantly different from those of 40% ethanol (Figure 1). The results was similar with the reports of Wang et al. (2004) on *Sophora alopecuraides* flavonoids, Li et al. (2003) on apple peel flavonoids, Zhang et al. (2004) on hawkthorn flavonoids and Yang et al. (2002) on tea polyphenols. Whether 60% ethanol is the optimum extraction solvent, yet there is still need for concentration screening researches.

Effects of material ratio on extraction efficiencies of flavonoids and phenolics

The extraction efficiencies of flavonoids and phenolics from loquat flower rose with material ratio increase under certain conditions, which were similar with the results of Li et al. (2009) and Zhang et al. (2009). When the ratio reached 1:40, the increase extents of bioactive compounds was not too much (Figure 2). Considering the cost and efficiency of extraction simultaneously, 1:40 was an appropriate ratio.

Effects of extraction time on extraction efficiencies of flavonoids and phenolics

Effects of extraction time on extraction efficiencies of

Table 1. The effects of frequency/power combination on flavonoids and phenolics extraction (mg/g DW).

Frequency/power	Flavonoid	Phenolic
26 kHz /500 W	2.832±0.514[a]	5.419±0.752[bc]
26 kHz /250 W	2.808±0.711[a]	5.184±0.907[c]
47 kHz /500 W	3.115±0.106[a]	6.972±0.357[a]
47 kHz /250 W	2.804±0.313[a]	6.160±0.659[ab]
70 kHz /500 W	1.978±0.069[b]	3.837±0.213[d]
70 kHz /250 W	3.092±0.255[a]	6.752±0.334[a]

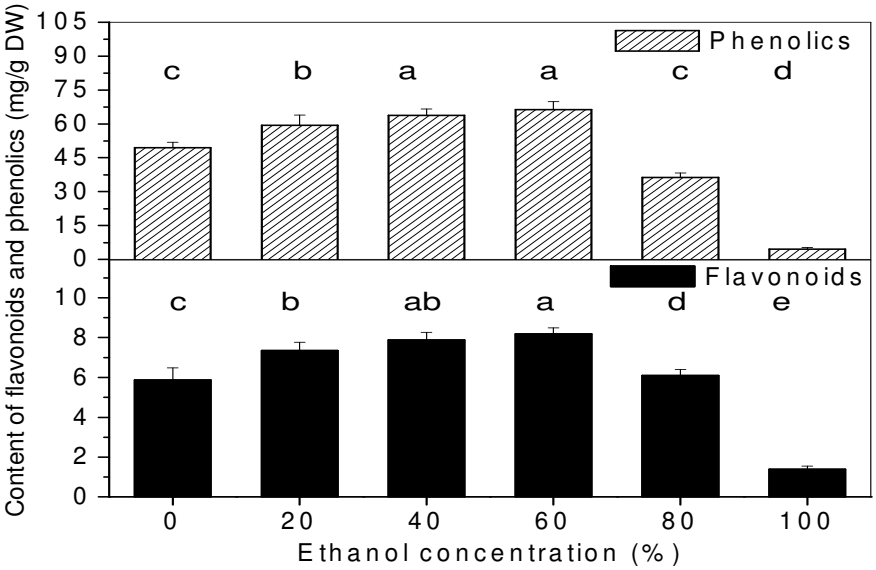

Figure 1. Effects of ethanol concentration on flavonoids and phenolics extract.

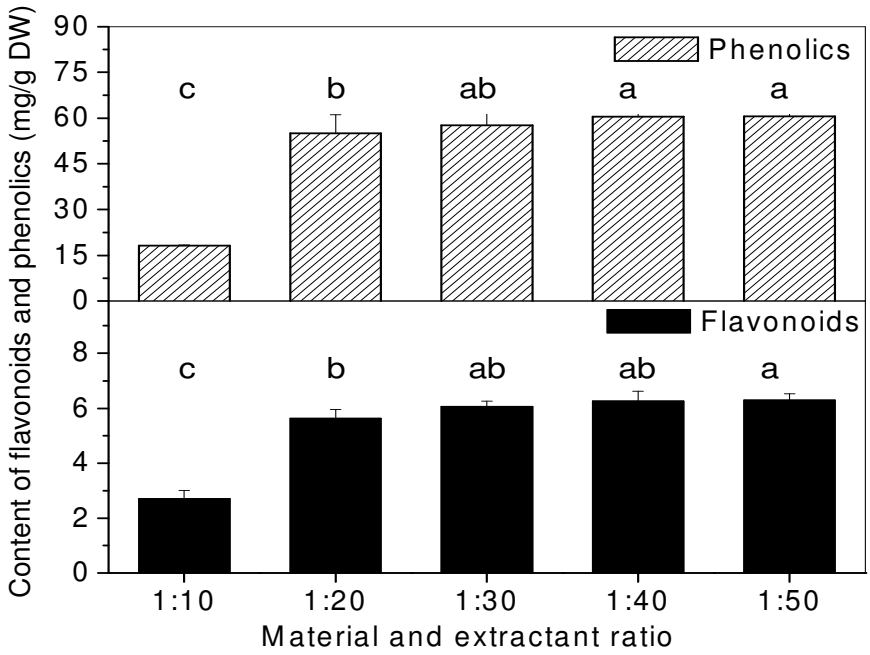

Figure 2. Effects of material and extractant ratio on flavonoids and phenolics extract.

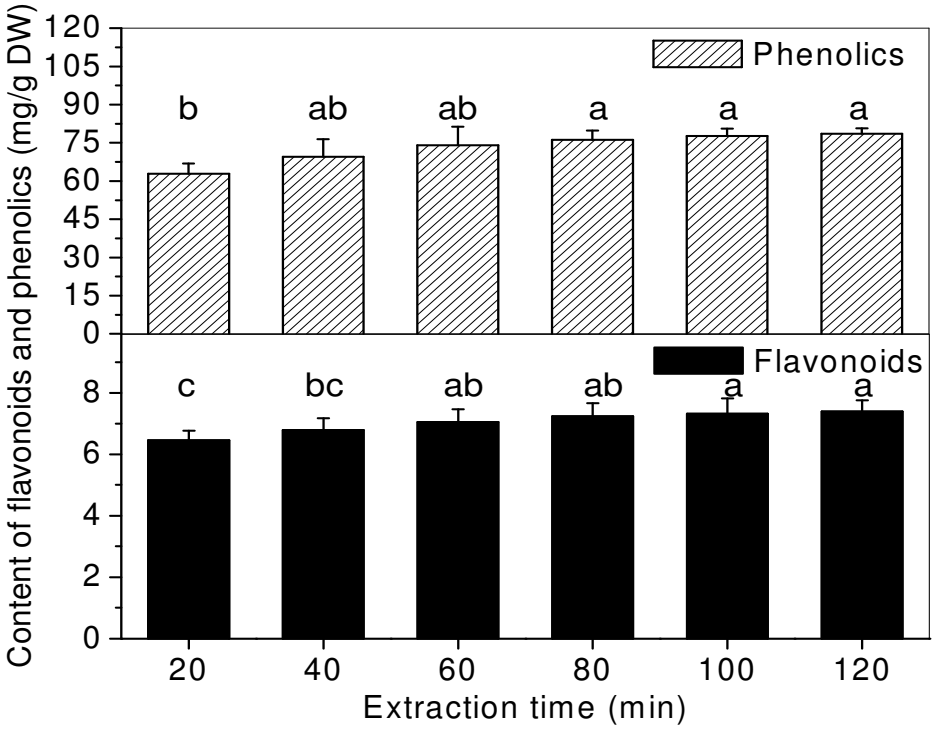

Figure 3. Effects of extraction time on flavonoids and phenolics extract.

flavonoids and phenolics were similar with those of material ratio. The extraction efficiencies of flavonoids and pheno-lics were also increased with extraction time prolonged (Figure 3). From the point of view of increase degrees, the increase ratio became slow after 80 min. Therefore, 80 min is an appropriate extraction time for the sake of saving time and energy.

Effects of temperature on extraction efficiencies of flavonoids and phenolics

Bioactive component extraction efficiency of plant was greatly affected by temperature. In a certain temperature range, the extraction efficiencies of flavonoids and phenolics in loquat flowers were enhanced with temperature increase and extraction at 50℃ had highest efficiencies (Figure 4). The extraction efficiency declined sharply when the temperature was higher than 50℃, which may be due to the damages of high temperature to flavonoids and phenolics.

Optimization of ultrasonic extraction condition of loquat flower

The influence of single parameter on extraction efficiencies of flavonoids and phenolics from loquat flowers was discussed in early chapters. However, there was an interaction between every single parameter in the practical extraction operations. In order to optimizing the ultrasonic extraction condition, orthogonal experimental design was introduced with four parameters and three levels on the basis of single factor results (Table 2). Table 3 showed the results and mean standard deviation analysis of the orthogonal experiment.

As seen from Table 3, we found that the influence to the mean extraction efficiency of loquat flower decreases in the order: D > A > B > C according to the R values. Material ratio was found to be the most important determinant of extraction yield of loquat flower. The best combination shown was A1B3C1D3 and A1B3C2D3 for flavonoids and phenolics, respectively. Comparing with these two combinations, only ethanol concentration was found different. Because flavonoids is a group of phenolics, therefore, A1B3C2D3 (30℃, 80 min, 60% ethanol and 1:40 material ratio) combination was finally selected as the optimum ultrasonic extraction condition of flavonoids and phenolics in loquat flowers on the basis of previous single-factor test with ethanol concentrations.

Flavonoids and phenolics contents with optimum extraction condition

Loquat flowers were extracted three successive times under the optimum condition, with extraction yield gradually decreased. The ratio of each extraction was calculated according to the total amount of three consecutive extraction yields. The results demonstrated that, extract

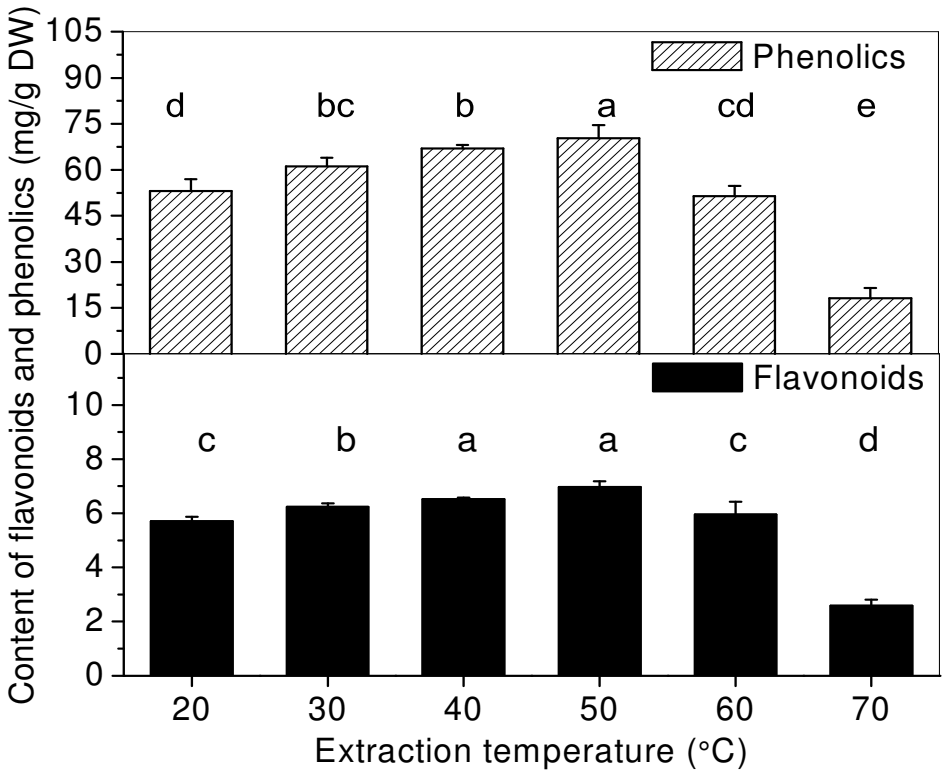

Figure 4. Effects of extraction temperature on flavonoids and phenolics extract.

Table 2. $L_9(3^4)$ orthogonal experimental design.

Level	Temperature	Extraction time	Ethanol concentration	Material ratio
	A (ºC)	B (min)	C (%)	D
1	30	40	50	1:20
2	40	60	60	1:30
3	50	80	70	1:40

Table 3. Results of orthogonal experiment.

Test number		Factor				Result (mg/g DW)	
		A	B	C	D	Flavonoid	Phenolic
1		1 (30 ºC)	1 (40 min)	1 (50%)	1 (1:20)	5.638	58.985
2		1 (30 ºC)	2 (60 min)	2 (60%)	2 (1:30)	6.381	65.123
3		1 (30 ºC)	3 (80 min)	3 (70%)	3 (1:40)	7.895	77.081
4		2 (40 ºC)	1 (40 min)	2 (60%)	3 (1:40)	6.510	69.895
5		2 (40 ºC)	2 (60 min)	3 (70%)	1 (1:20)	4.608	36.389
6		2 (40 ºC)	3 (80 min)	1 (50%)	2 (1:30)	6.382	59.013
7		3 (50 ºC)	1 (40 min)	3 (70%)	2 (1:30)	5.575	51.532
8		3 (50 ºC)	2 (60 min)	1 (50%)	3 (1:40)	6.932	66.515
9		3 (50 ºC)	3 (80 min)	2(60%)	1 (1:20)	5.465	50.559
K1	Flavonoids	19.914	17.723	18.952	15.710		
	Phenolics	201.191	180.414	184.515	145.934		

Table 3. Continues

K2	Flavonoids	17.499	17.921	18.356	18.337
	Phenolics	165.297	168.027	185.577	175.669
K3	Flavonoids	17.972	19.741	18.077	21.337
	Phenolics	168.606	186.653	165.002	213.491
k1	Flavonoids	6.638	5.908	6.317	5.237
	Phenolics	67.064	60.138	61.505	48.645
k2	Flavonoids	5.833	5.974	6.119	6.112
	Phenolics	55.099	56.009	61.859	58.556
k3	Flavonoids	5.991	6.580	6.026	7.113
	Phenolics	56.202	62.218	55.001	71.164
R	Flavonoids	0.805	0.673	0.292	1.876
	Phenolics	11.965	6.209	6.858	22.519

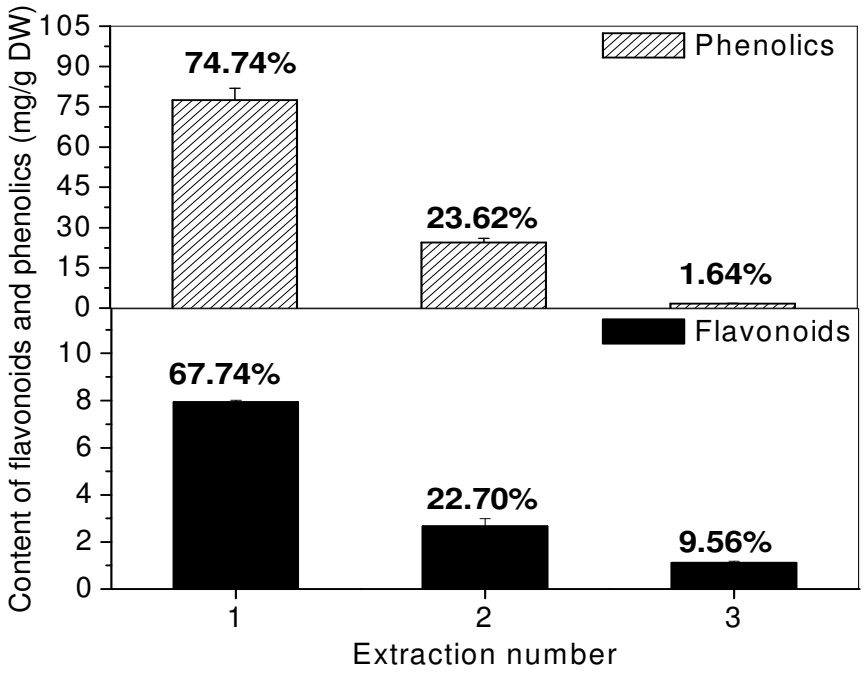

Figure 5. Flavonoids and phenolics content of different extract time under the optimal extraction condition.

twice may be enough under the best condition, the extraction rates of flavonoids (90.44%) and phenolics (98.33%) were all more than 90%, with the contents of 10.59 and 102.02 mg/g dry weight, respectively (Figure 5).

Conclusions

The combination of 30℃, 80 min, 60% ethanol and 1:40 material ratio was proved to be the optimum ultrasonic extraction condition of flavonoids and phenolics in loquat flowers under 47 kHz/500 W according to the results of single-factor and orthogonal experiments. Loquat flowers were extracted two consecutive times under the optimum extraction condition, which was enough and the contents of flavonoids and phenolics were 10.59 and 102.02 mg/g DW, respectively.

ACKNOWLEDGEMENTS

We greatly thank Tangxi Xitaiyang Loquat Production Co. Ltd., Zhejiang Province, People's Republic of China for kindly providing the loquat flower materials. This research was financially supported by the Special Scientific Research Fund of Agricultural Public Welfare Profession of China (200903044), the Priority Academic Program Development from Jiangsu Government, International Co-operation Project of the Science and Technology Department of Zhejiang Province (2006C24005), the 111 project (B06014) and Science and Technology Infrastructure Construction Project of Jiangsu Province (BM2010590).

REFERENCES

Ammar AH, Zagrouba F, Romdhane M (2010). Optimization of operating conditions of Tunisian myrtle (*Myrtus communis* L.) essential oil extraction by a hydrodistillation process using a 2(4) complete factorial design. Flavour Frag. J. 25(6): 503-507.

Bae D, You Y, Yoon HG, Kim K, Lee YH, Kim Y, Baek H, Kim S, Lee J, Jun W (2010) Protective effects of loquat (*Eriobotrya japonica*) leaves against ethanol-induced toxicity in HepG2 cells transfected with CYP2E1. Food Sci. Biotechnol. 19(4): 1093-1096.

Chen J, Li WL, Wu JL, Ren BR, Zhang HQ (2008). Hypoglycemic effects of a sesquiterpene glycoside isolated from leaves of loquat (*Eriobotrya japonica* (Thunb.) Lindl.). Phytomedicine 15(1-2): 98-102.

Cheng L, Liu Y, Chen LY, Luo J (2001). Studies on the triterpenoidal saponins from flowers of *Eriobotrya japonica* (In Chinese with English Abstract). J. Western Med. Univ. China 32(2): 283-285.

Huang Y, Li J, Meng XM, Jiang GL, Li H, Cao Q, Yu SC, Lv XW, Cheng WM (2009). Effect of triterpene acids of *Eriobotrya japonica* (Thunb.) Lindl. leaf and MAPK signal transduction pathway on inducible nitric oxide synthase expression in alveolar macrophage of chronic bronchitis rats. Am. J. Chinese Med. 37(6): 1099-1111.

Ito H, Kobayashi E, Li SH, Hatano T, Sugita D, Kubo N, Shimura S, Itoh Y, Yoshida T (2001). Megastigmane glycosides and an acylated triterpenoid from *Eriobotrya japonica*. J. Nat. Prod. 64(6): 737-740.

Jung HA, Park JC, Chung HY, Kim J, Choi, JS (1999). Antioxidant flavonoids and chlorogenic acid from the leaves of *Eriobotrya japonica*. Arch. Pharm. Res. 22(2): 213-218.

Kawahito Y, Kondo M, Machmudah S, Sibano K, Sasaki M, Goto M (2008). Supercritical CO_2 extraction of biological active compounds from loquat seed. Sep. Purif. Technol. 61(2): 130-135.

Kobayashi E, Takamatsu Y (2000). Polyphenols from *Eriobotrya japonica* and their cytotoxicity against human oral tumor cell lines. Chem. Pharm. Bull. 48(5): 687-693.

Li EN, Luo JG, Kong LY (2009). Qualitative and quantitative determination of seven triterpene acids in *Eriobotrya japonica* Lindl. by high-performance liquid chromatography with photodiode array detection and mass spectrometry. Phytochem. Anal. 20(4): 338-343.

Li H, Zhang YH (2003). Optimization of extraction method of flavonoids from apple peel and its determination (In Chinese with English Abstract). J. Shandong Agri. Univ. (Natural Science), 34(4): 471-474.

Li W, Li T, Tang KJ (2009). Flavonoids from mulberry leaves by microwave-assisted extract and anti-fatigue activity. Afr. J. Agric. Res. 4(9): 898-902.

Li SA, Zhu RH, Zhong M, Zhang YP, Huang KL, Zhi X, Fu ST (2010). Effects of ultrasonic-assistant extraction parameters on total flavones yield of *Selaginella doederleinii* and its antioxidant activity. J. Med. Plants Res. 4(17): 1743-1750.

Louati S, Simmonds MSJ, Grayer RJ, Kite GC, Damak M (2003). Flavonoids from *Eriobotrya japonica* (Rosaceae) growing in Tunisia. Biochem. Syst. Ecol. 31(1): 99-101.

Maksimovic Z, Malencic D, Kovacevic N (2005). Polyphenol contents and antioxidant activity of *Maydis stigma* extracts. Bioresource Technol. 96: 873–877.

Peres VF, Saffi J, Melecchi MIS, Abad FC, Jacques RD, Martinez MM, Oliveira EC, Caramao EB (2006). Comparison of soxhlet, ultrasound-assisted and pressurized liquid extraction of terpenes, fatty acids and Vitamin E from *Piper gaudichaudianum* Kunth. J. Chromatogr. A 1105(1-2): 115-118.

Pottier-Alapetite G (1979). Flore de la Tunisie (premiere partie). Imprimerie officielle de la republique Tunisienne, Tunis, p. 269.

Sun GC, Liu YQ, Zhu JL, Iguchi M, Yoshioka S, Miyamura M, Kyotani S (2010). Immunomodulatory effect of *Eriobotrya japonica* seed extract on allergic dermatitis Rats. J. Nutr. Sci. Vitaminol. 56(2): 145-149.

Wang SH, Wang SJ, Gou JB (2004). Study on technology of extracting flavonids from *Sophora alopecuraides* (In Chinese with English Abstract). Ningxia Engineering Technol. 3(4): 358-359.

Xiong WY, Wang JZ, Shi TD, Li WF (1993). (Ed.) Officinal wood plant of China. Shanghai, Educational Publishing house of Shanghai Science and Technology, pp. 239-246.

Yang AP, Wang QJ, Suo SL, Yuan YL, Wang YS, Zhu LH (2002). Research on the extraction and isolation technology of tea-polyphenols (In Chinese with English Abstract). J. Laiyang Agri. College, 19(2): 106-107.

Yokota J, Takuma D, Hamada A, Onogawa M, Yoshioka S, Kusunose M, Miyamura M, Kyotani S, Nishioka Y (2006). Scavenging of reactive oxygen species by *Eriobotrya japonica* seed extract. Biol. Pharmaceu. Bull. 29(3): 467-471.

Yoshioka S, Hamada A, Jobu K, Yokota J, Onogawa M, Kyotani S, Miyamura M, Saibara T, Onishi S, Nishioka Y (2010). Effects of *Eriobotrya japonica* seed extract on oxidative stress in rats with non-alcoholic steatohepatitis. J. Pharm. Pharmacol. 62(2): 241-246.

Yue L, Zhang F, Wang ZX (2010). Study on ultrasonic extraction of gastrodin from *Gastrodia elata* Bl. Sep. Sci. Technol. 45(6): 832-838.

Zhou CH, Chen KS, Sun CD, Chen QJ, Zhang WS, Li X (2007a). Determination of oleanolic acid, ursolic acid, and amygdalin in the flower of *Eriobotrya japonica* Lindl. by HPLC. Biomed. Chromatogr. 21(7): 755-761.

Zhou CH, Sun CD, Li X (2007b). Study on method for flavonoids determining of plant rich in chlorogenic acid (In Chinese with English Abstract). Plant Physiol. Commun. 42: 902- 904.

Zhang J, Cheng L, Kang TG (2004). Optimum extraction technology Selection of flavonoids from hawkthorn (In Chinese with English Abstract). J. Liaoning College of TCM, 6(3): 218-219.

Zhang L, Shan Y, Tang KJ, Putheti R (2009). Ultrasound-assisted extraction flavonoids from Lotus (*Nelumbo nuficera* Gaertn) leaf and evaluation of its anti-fatigue activity. Int. J. Phys. Sci. 4(8): 418-422.