Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Extraction and HPLC Characterization of Chlorogenic Acid from Tobacco Residuals

Yuru Chen[a]; Qiming Jimmy Yu[b]; Xuemei Li[a]; Yaojun Luo[a]; Hui Liu[c]
[a] Key Laboratory of Microbial Engineering, College of Life Science, Nanjing Normal University, Nanjing, P. R. China [b] Environmental Engineering, Griffith School of Engineering, Griffith University, Brisbane, Queensland, Australia [c] Department of Environment Engineering, College of Dynamic Engineering, Nanjing Normal University, Nanjing, P. R.China

To cite this Article Chen, Yuru , Yu, Qiming Jimmy , Li, Xuemei , Luo, Yaojun and Liu, Hui(2007) 'Extraction and HPLC Characterization of Chlorogenic Acid from Tobacco Residuals', Separation Science and Technology, 42: 15, 3481 — 3492
To link to this Article: DOI: 10.1080/01496390701626677
URL: http://dx.doi.org/10.1080/01496390701626677

Separation Science and Technology, 42: 3481–3492, 2007

ISSN 0149-6395 print/1520-5754 online
DOI: 10.1080/01496390701626677

Extraction and HPLC Characterization of Chlorogenic Acid from Tobacco Residuals

Yuru Chen
Key Laboratory of Microbial Engineering, College of Life Science, Nanjing Normal University, Nanjing, P. R. China

Qiming Jimmy Yu
Environmental Engineering, Griffith School of Engineering, Griffith University, Brisbane, Queensland, Australia

Xuemei Li and Yaojun Luo
Key Laboratory of Microbial Engineering, College of Life Science, Nanjing Normal University, Nanjing, P. R. China

Hui Liu
Department of Environment Engineering, College of Dynamic Engineering, Nanjing Normal University, Nanjing, P. R.China

Abstract: Chlorogenic acid is a highly valuable natural polyphenol compound used in medicine and industries. Its current commercial sources are from plant extracts of *Lonicera japonica Thunb* and *Eucommia ulmoides Oliver*. These sources are limited and expensive. On the other hand, tobacco residuals contain chlorogenic acid and other natural polyphenol compounds. Large quantities of tobacco residuals are produced each year as waste materials from tobacco manufacturing, potentially providing an alternative commercial source of chlorogenic acid and other valuable compounds. In this paper, microwave and ultrasound extractions of chlorogenic acid with mixed solvent were studied. Total polyphenol concentrations in extract solutions obtained with different extraction methods were analyzed with the method

Received 27 March 2007, Accepted 5 June 2007
Address correspondence to Qiming Jimmy Yu, Environmental Engineering, Griffith School of Engineering, Griffith University, Nathan Campus, Brisbane, Queensland 4111, Australia. Tel.: +61 737355289; Fax: +61 737357459; E-mail: jimmy.yu@griffith.edu.au

of ferrous tartrate and UV-Vis spectrophotometry and compared. The extraction solutions were also characterized for polyphenol compositions with the method of HPLC. Experimental results indicated that high extract concentrations of chlorogenic acid were obtained with a mixed solvent of acetone and water (1:2 v/v). A total polyphenol concentration of up to 4.87 mg/ml and a chlorogenic acid concentration of up to 2.12 mg/ml were achieved. The application of microwave and ultrasound significantly increased the extract concentrations. The extraction time needed was also much reduced. HPLC analysis indicated that acetone water mixed solvent extraction achieved much higher relative concentrations of chlorogenic acid to other compounds in the extract solutions. These results indicted that fast and effective extraction of chlorogenic acid from tobacco residuals were achieved.

Keywords: Tobacco residues, polyphenol, cholorgenic acid, ferrous tartrate method, solvent extraction, HPLC

INTRODUCTION

Chlorogenic acid and many other polyphenol compounds are extensively used in medicine and industries such as in consumer chemicals and food industries (1). Chlorogenic acid is used as various additives in beverage, cosmetics, tea products, and foods as well as medical substances (2, 3). Chlorogenic acid has antibacterial and antiviral properties, and it is a natural antioxidant and anticancer agent (4). It is also a promising precursor compound for the development of medicine that can resist AIDS virus HIV (5). The current commercial sources of chlorgenic acids are from plant extracts of plants such as *Lonicera japonica Thunb* and *Eucommia ulmoides Oliver* etc. (6–9). These sources are generally limited and therefore expensive.

Tobacco leaves contain chlorogenic acid and many other natural ploypheol compounds (10). Analytical data also indicated that the concentration of chlorogenic acid and rutin is the highest (75%–95%) among the polyphenol compounds in tobacco leaves (11, 12). Therefore, this potentially provides an alternative commercial source for chlorogenic acid and other valuable polyphenol compounds (13). In addition, the tobacco industry produces large quantities of tobacco residues as waste materials, such as low grade tobacco leaves and tobacco powders. The extraction of solansol from tobacco leaves also produces large quantities of residues, which still contain the polyphenol compounds. The utilization of these waste materials for the extraction of valuable compounds such as polyphenols and nicotine has the potential of significant environmental and economic benefits (14, 15).

The objectives of this paper were two-fold. The first was to study the extraction of chlorgenic acid and other polyphenol compounds from tobacco residues with a number of methods. The effectiveness of mixed solvent extraction with acetone and water and the use of ultrasound and microwave as extraction aids were investigated. The total concentrations of polyphenols in the extract solutions were analysed with the method of

ferrous tartrate and UV-Vis spectrophometry. The second objective was to characterize the polyphenol compositions of the extract solutions with the method of HPLC (16) and to study the effects of the extraction methods on the polyphenol compositions.

MATERIALS AND METHODS

Tobacco Residues and Reagents

The dry tobacco residues used in the experiments were obtained from Bangbu Cigarette Plant, Anhui, P. R. China. The tobacco leaves had been oven-dried, sliced, re-moisturized, and flavorized, and re-dried during the tobacco manufacturing processes. The residues were crushed into powders (2–3 mm diameter) for use in the extraction experiments. Acetone, ethanol, and $FeSO_4 \cdot 7H_2O$, $Na_2HPO_4 \cdot 2H_2O$, $KH_2PO_4 \cdot 2H_2O$ (analytical grade) were purchased from Nanjing Chemical Company. Rochelle salt (sodium potassium tartrate, $KNaC_4H_4O_6 \cdot 4H_2O$; analytical grade) was purchased from Shenyang Chemical Reagent No. 2 Plant. Chlorogenic acid standard was obtained from China Biological Products Assay Institute. Phosphoric acid and acetonitrile (chromatography grade) were purchased from Sigma Co.

A standard chlorogenic solution of 2 mg/ml was prepared by dissolving weighed amounts in ethanol. A ferrous tartrate solution was prepared by dissolving 0.1 g $FeSO_4 \cdot 7H_2O$ and 0.5 g $KNaC_4H_4O_6 \cdot 4H_2O$ in 100 ml distilled water. A phosphate buffer solution of pH 7.5 was prepared by adding 85 ml of a solution A (2.969 g $Na_2HPO_4 \cdot 2H_2O$ dissolved in 250 ml distilled water) to 15 ml of a solution B (2.2695 g $KH_2PO_4 \cdot 2H_2O$ dissolved in 250 ml of distilled water).

Analytical Instruments and Apparatus

The following analytical instruments and apparatus were used in the experiments: HPLC system with UV detector and C18 reversed phase column (Model HP1100, Hewlett-Packard), UV-Vis spectrophotometer (Model 722, Shanghai No. 3 Analytical Instrument Plant), Incubator (Model PYX-DHS-50X65-S, Shanghai Yuejing Medical Appliance Plant), constant temperature water bath (Model HH-4, Changzhou Guohua Appliance Company), Microwave oven (Model WD900SL23-2,900 Watt), and Ultrasound Cleaner (Model KQ-100DB, 100 Watt, Kunshan Ultrasound Instrument Company).

Analysis of Phenol Concentrations in Extract Solutions

The total polyphenol concentrations in the extract solutions were determined by the method of ferrous tartrate and UV-Vis spectrophotometry (16–18).

Chlorogenic acid standard solutions were used as the analysis standard. As the extract solutions contained chlorogenic acid and other polyphenol compounds, the measured concentrations can be regarded as the chlorogenic acid equivalent concentrations for the total concentrations of all the polyphenols in the extraction solutions. This equivalent concentration was referred as the total polyphenol concentration in the paper.

A calibration curve was prepared for the spectrophotometer analysis. The standard solutions for the calibration curve were prepared by diluting the 2 mg/ml chlorogenic acid standard solution. To each 25 ml test tube, 0, 0.25, 0.5, 0.75, 1.0, 1.25, and 1.5 ml chlorogenic acid standard solutions, respectively, were added. Distilled water was added to make up the liquid volume in each test tube to 1.5 ml. Then, 5 ml of ferrous tartrate solution and 10 ml of phosphate buffer solution were added to each test tube, and absorbance values at 540 nm were obtained to plot the calibration curve. Extract solution samples of 1.5 ml each were analyzed with the same procedure.

Analysis of Polyphenol Composition in Extract Solutions

The polyphenol compositions of the extract solutions were analyzed with the method of HPLC. The HPLC conditions and procedures were as follows: MetaChem Polaris 5 μ C18-A silica gel column (5.0 μm, 4.6 mm id × 250 mm), column temperature: 30°C, mobile phase: 4:1 mixture solution of phosphate buffer solution (0.4%) ethylcyanide, flow rate: 0.7 ml/min, and detection wavelength: 327 nm. All samples solutions were filtered with Cellulose acetate filter paper (pore size 0.8 μm) before HPLC analysis.

Extraction Experiments

A number of methods were used for the extraction of chlorogenic acid and polyphenols from the tobacco residues. The extraction experiments were carried out as follows. The ratios of tobacco residue to solvent volume were fixed at 10 g to 90 ml in all extraction experiments. At the end of each extraction, the solutions were separated by filtration using filter paper and used for analyses. The extraction conditions used in the experiments were determined from a preliminary optimization process and the extraction times for each of the methods were selected based on the estimation that most of the extractable polyphenols were extracted into the liquid phase and equilibrium conditions were achieved.

Ethanol Extraction

A sample of 10 g of tobacco residue powder was added into 90 ml of 95% ethanol in a flask with a reflux tube. The flask was then put in a hot water bath at 78°C for 2 hr.

Acetone Water Mixed Solvent Extraction

A sample of 10 g of tobacco residue powder was added into 90 ml of acetone and water mixed solvent (1:2 v/v) in a beaker. The extraction was carried out for 2 days under static conditions.

Acetone Water Mixed Solvent Extraction with Ultrasound

A sample of 10 g of tobacco residue powder was added into 90 ml of acetone and water mixed solvent (1:2 v/v) in a beaker. The extraction in the contents of the beaker was then carried out with ultrasound treatment (100 W) for a period of 15 min treatment.

Acetone Water Mixed Solvent Extraction with Microwave

A sample of 10 g of tobacco residue powder was added into 90 ml of acetone and water mixed solvent (1:2 v/v) in a beaker. The extraction in the contents of the beaker was then carried out with microwave treatment (900 W) for a period of 90 sec. The temperature of the extract solution was increased because of the application of the microwave. However, due to the short duration of the extraction processes, there was no noticeable loss of the solvents.

RESULTS AND DISCUSSION

Polyphenol Concentrations in Extract Solutions

The effectiveness of a number of methods for the extraction of chlorogenic acid and other polyphenol compounds from tobacco residues was studied first. The total effective concentrations of polyphenol in the extraction solutions relative to standard chlorogenic acid as determined with the method of ferrous tartrate were obtained. These results were compared as in Table 1. The data given in Table 1 were average values from triplets and the standard deviation was in the order of 0.09 mg/ml.

It can be seen from Table 1 that all the extraction methods could effectively extract the polyphenol compounds from tobacco residues. However, there were large differences in extraction times that were needed. There were also some differences in the concentrations of total polyphenol compounds in the extraction solutions. Ethanol is the most commonly used solvent in commercial extraction processes of chlorogenic acid products (5). The results in Table 1 showed that a concentration of 3.36 mg/ml was obtained with ethanol extraction for 2 hr at 78°C. This was slightly less than

Table 1. Total concentration of polyphenol compounds in extract solutions obtained with different extraction methods (tobacco residue: 10 g; solvent: 90 ml)

Extraction method	Extraction temperature (°C)	Extraction time	Polyphenol concentration (mg/ml)
Ethanol extraction	78	2 hr	3.36
Acetone water (1:2 v/v) extraction	25	2 day	3.55
Acetone water (1:2 v/v) ultrasound	25	15 min	4.42
Acetone water (1:2 v/v) microwave	25[a]	90 sec	4.87

[a]Microwave extraction temperature increased to 95°C.

3.55 mg/ml obtained from acetone water (1:2 v/v) mixed solvent extraction at room temperature for 24 hr. In contrast, the applications of ultrasound and microwave not only dramatically reduced the extraction time needed, but also produced significantly higher concentrations of polyphenol compounds of 4.42 and 4.87 mg/ml, respectively, in the extraction solutions, indicating improvements in the recovery rates. It appears that ultrasound and microwave effectively disrupted the cellular organizational structures of tobacco leaves such that the polyphenol compounds were rapidly released into the solvent solutions. In addition, the improved extraction with ultrasound and microwave is also due to the improved mass transfer processes by such factors as cavitational effect, mechanic agitation effect and heat effect (19). Therefore, both ultrasound and microwave were demonstrated to be excellent aids for solvent extraction of compounds from tobacco residues, simultaneously with much reduced extraction time and significantly higher recovery rates.

Polyphenols Compositions in Extract Solutions

Although the level of chlorogenic acid is relatively higher among the polyphenol compounds in tobacco leaves, the extraction solutions contain mixtures of polyphenol compounds. The actual compositions of the polyphenol compounds, as well as the effects of extraction on the resultant compositions are important and need to be characterised. In this paper, the characterizations of extraction solutions were achieved through the use of HPLC chromatograms, which were compared to the chromatogram of the standard solution of chlorogenic acid.

The chromatogram of the standard solution (2 mg/ml) is shown in Fig. 1, in which the retention time of chlorogenic acid was at 5.6 min. There were no other peaks observed in the chromatogram, as standard chlorogenic acid solution was used.

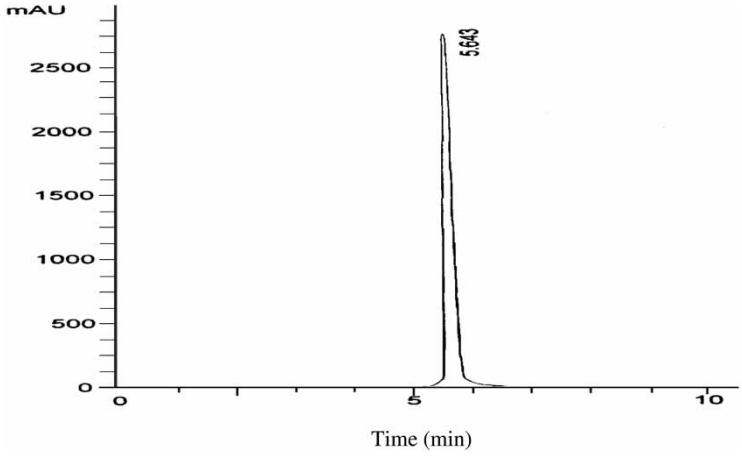

Figure 1. HPLC chromatogram of chlorogenic acid standard solution (2 mg/ml).

The chromatogram of the extraction solution from ethanol extraction is shown in Fig. 2. In Fig. 2, it can be observed that in addition to the chlorogenic acid peak at 5.6 min, there are at least another 3 significant peaks before, and 2 after, the chlorogenic acid peak at retentions of 3.8, 4.2, 4.9, 8.9, and 9.9 min, indicating the presence of other substances such as other polyphenol compounds. The chlorogenic acid concentration in the extraction solution can be calculated from its peak area to be 0.54 mg/ml, which represents 26.6% of the total concentrations as calculated from the total peak areas in

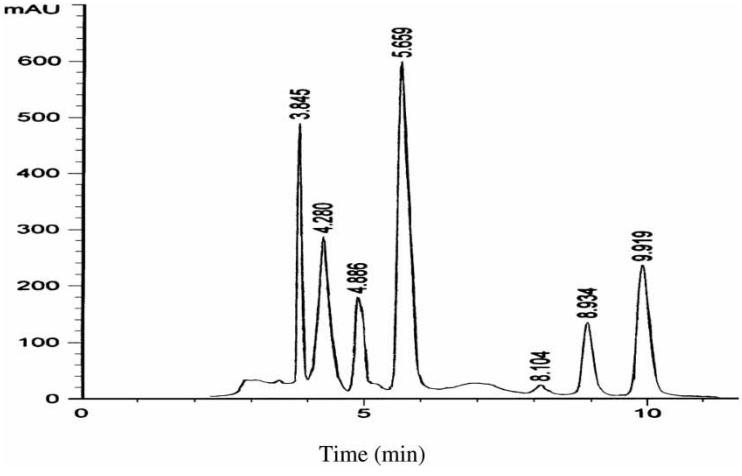

Figure 2. HPLC chromatogram of extraction solution from ethanol extraction at 78°C (2 hr).

Fig. 2. When compared to the total polyphenol concentration as determined by the ferrous tartrate method (3.36 mg/ml), the percentage chlorogenic acid concentration is 16.1% (wt). It is noted that the two percentage concentrations obtained from the two methods are different. This is mainly because of the fact that concentrations of all compounds are measured against a single chlorogenic acid standard. Different spectroscopic properties of different compounds in different analytical conditions were not taken into account. Therefore, the equivalent concentrations here only provide a relative measure for the purpose of comparisons between results obtained from different extraction methods.

The chromatogram in Fig. 2 showed that the extraction solution obtained from ethanol extraction contained a mixture of a number of polyphenol substances at relatively high concentrations. If the extraction solution is to be used as an antibacterial agent, it may be advantageous to have a mixture of polyphenol compounds. However, chlorogenic acid is one of the components in the mixture, if pure chlorogenic acid is the final product required, the presence of other polyphenol compounds in high concentrations became undesirable. Although ethanol is the most commonly used solvent for chlorogenic acid extraction, it may not be the best solvent selection (16). The effectiveness of other extraction solvents can be studied to increase the relative concentration of the chlorogenic acid in the extraction solution.

Figure 3 shows the HPLC chromatogram of extraction solution from acetone water (1:2 v/v) extraction at 25°C. As can be seen in Fig. 3, the peak characteristics are quite different from those shown in Fig. 2. In particular, the peak for chlorogenic acid is much larger than peaks for other compounds. Therefore, both relative and the absolute concentration of the

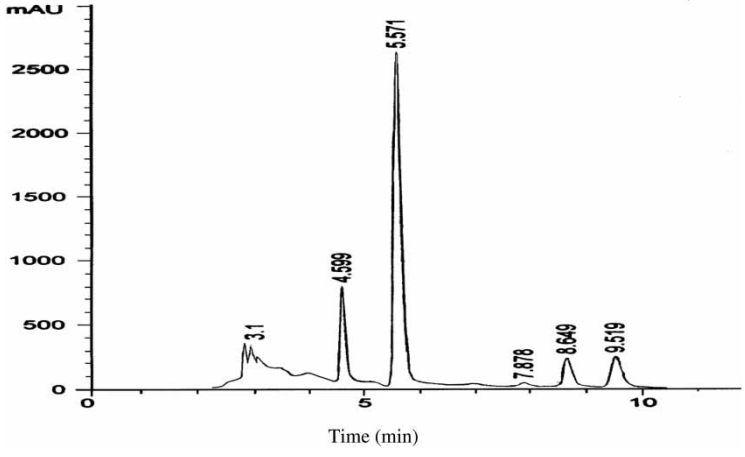

Figure 3. HPLC chromatogram of extraction solution from acetone water (1:2 v/v) extraction at 25°C (2 days).

chlorogenic acid were higher. Or, the purity of the chlorogenic acid in the extraction solution from the mixed solvent extraction was higher, indicating the mixed solvent has a higher selectivity for chlorogenic acid over other extracted compounds.

The chlorogenic acid concentration in Fig. 3 was calculated to be 1.64 mg/ml, which represents 39.7% (peak area) of total concentrations from the HPLC method, or 46.1% (wt) of the total concentrations determined from the ferrous tartrate method. As the mixed solvent extraction was carried at 25°C, the extraction time needed was long at 24 hr. As it was shown earlier, the extraction time can be much reduced by the use of higher extraction temperatures, or extraction aids such as ultrasound and microwave treatment.

Figure 4 is the HPLC chromatograms from extraction solutions from acetone water (1:2 v/v) extraction with ultrasound treatment. It can be seen that peak characteristics of the chromatograms in Fig. 4 are similar to those observed in Fig. 3. Therefore, while the extraction time was much reduced with the use of ultrasound, the composition of the extract solution, or the selectivity of the mixed solvent extraction for chlorogenic acid was not significantly changed by the ultrasound used. The chlorogenic acid concentration in Fig. 4 was 2.12 mg/ml, which is 43.5% (peak area) of the total concentration from the HPLC method, or 48.0% (wt) of the total concentration from the ferrous tartrate method. It is noted here that the percentage concentrations are also similar to the case of mixed solvent extraction without the use of ultrasound.

Finally, the HPLC chromatogram of extraction solution from acetone water (1:2 v/v) extraction with microwave is shown in Fig. 5. The peak characteristics as shown Fig. 5 are also somewhat similar to those shown in

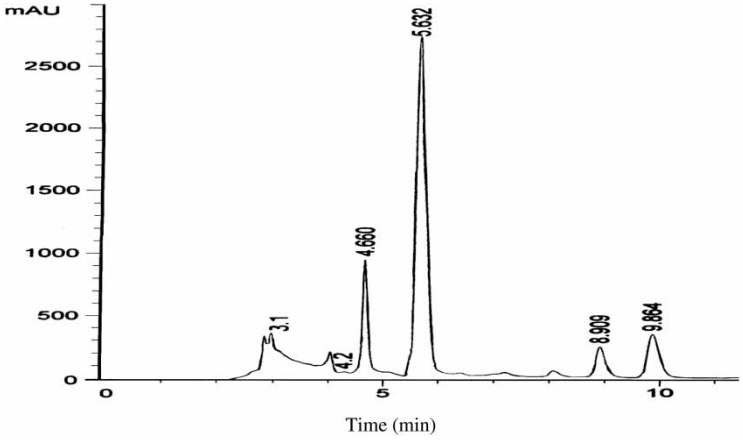

Figure 4. HPLC chromatogram of extraction solution from acetone water (1:2 v/v) extraction with ultrasound (15 min).

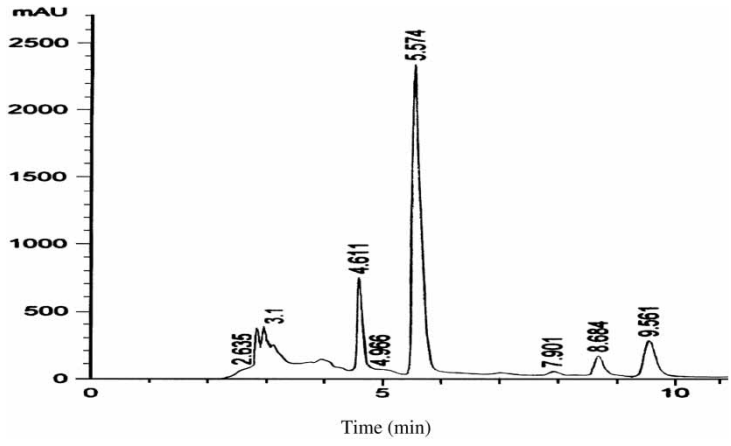

Figure 5. HPLC chromatogram of extraction solution from acetone water (1:2 v/v) extraction with microwave (90 sec).

Fig. 3 and Fig. 4. Or the use of microwave treatment did not greatly change the composition of the extraction solution. Similarly, the chlorogenic acid concentration in Fig. 5 was 1.47 mg/ml, and the corresponding percentage concentrations were 34.5% (peak area) and 30.2% (wt), respectively. The percentage concentrations for the case of microwave treatment seemed to be lower than the case of the ultrasound treatment.

CONCLUSIONS

The extraction of chlorogenic acid and other polyphenol compounds from tobacco residues with the method of ethanol extraction, acetone water (1:2 v/v) mixed solvent extraction, with and without the use of ultrasound and microwave as the extraction aids were effectively achieved. The compositions of the various extraction solutions were also characterized by the use of HPLC method. The use of the extraction aids dramatically reduced the extraction time. The total amount of polyphenol compounds obtained from extraction experiments with the use of ultrasound and microwave were also significantly higher than those obtained from simple solvent extractions.

Composition analysis results showed that the relative concentration, or the purity of the chlorogenic acid achieved from the acetone water (1:2 v/v) mixed solvent extraction was much higher than that from ethanol extraction, indicating better selectivity of the mixed solvent solution for chlorgenic acid. Overall, mixed solvent extraction with the use of ultrasound treatment achieved the best results in terms of both the relative and absolute concentrations chlorogenic acid of 48.0% (wt) and 2.12 mg/ml as determined from the HPLC method.

ACKNOWLEDGMENTS

The authors would like thank the Research Centre for Food Quality Safety and Detection, Academy of Agriculture Sciences of Jiangsu Province for their assistance in carrying out the HPLC analyses. This project was supported in part by a China National Tobacco Bureau and a Jiangsu High Tech Industry Development Fund.

REFERENCES

1. Kweon, M.H., Hwang, H.J., and Sung, H.C. (2001) Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (*Phyllo stachys edulis*). *Agr. Food Chem.*, 49: 4646–4655.
2. Jiang, Y., Satoh, K., and Kusama, K. (2000) Interaction between chlorogenic acid and antioxidants. *Anticancer Res.*, 20: 2473–2476.
3. Jin, U.-H., Lee, J.-Y., Kang, S.-K., Kim, J.-K., Park, W.-H., Kim, J.-G., Moon, S.-K., and Kim, C.-H. (2005) A phenolic compound, 5-caffeoylquinic acid (chlorogenic acid), is a new type and strong matrix metalloproteinase-9 inhibitor: Isolation and identification from methanol extract of Euonymus alatus. *Life Sci.*, 77: 2760–2769.
4. Jiang, Y., Satoh, K., and Watanabe, S. (2001) Inhibition of chlorogenic acid induced cytotoxicity by $CoC1_2$. *Anticancer Res.*, 21: 3349–3353.
5. Ma, B. and Liang, S. (2003) Progress report on extraction and separation of chlorogenic acid from eucomiaulmoides. *Shanxi Forest Sci. and Tech.*, 4: 74–79.
6. Li, H., Chen, B., and Yao, S. (2005) Application of ultrasonic technique for extracting chlorogenic acid from *Eucommia ulmodies Oliv. (E. ulmodies)*. *Ultrasonics Sonochem.*, 12: 295–300.
7. Rønsted, N., Strandgaard, H., Jensen, S.R., and Mølgaard, P. (2002) Chlorogenic acid from three species of hydrostachys. *Biochem. Systematics Ecol.*, 30: 1105–1108.
8. Chun, O.K. and Kim, D.-O. (2004) Consideration on equivalent chemicals in total phenolic assay of chlorogenic acid-rich plums. *Food Res. Int.*, 37: 337–342.
9. Clifford, M.N., Wu, W., and Kuhnert, N. (2006) The chlorogenic acids of Hemerocallis. *Food Chem.*, 95: 574–578.
10. Yang, H., Zhou, J., Wang, Y., Yang, C., Duan, F., and Luo, Z. (2005) Study on the contents of chlorogenic acid and rutin in the different genotype of flue-cured tobacco leaves. *Tobacco Agri. Sci.*, 1: 187–191.
11. Chen, Y., Luo, Y., and Li, X. (2005) Study on combined treatment of pulp sludge and tobacco residue in compostion. *Jiangsu Agr. Sci.*, 1: 92–94.
12. Kao Corporation. (2005) Reduction of phenolic compound precursors in tobacco. WO 099493.
13. Chen, Y., Luo, Y., and Li, X. (2004) Method of manufacturing chlorogenic acid using tobacco as raw material. CN200410065240.6.
14. Juan, J., Cristobala, C., Lunarb, L., Lafontc, F., Baumertd, A., and Fontes, A.G. (2004) Boron deficiency causes accumulation of chlorogenic acid and caffeoyl polyamine conjugates in tobacco leaves. *J. Plant Phys.*, 161: 879–881.
15. Zhang, Y., Yang, T., and Qin, J. (2000) Utilization of waste tobacco in solid fermentation system as carrier. *Tobacco Tech.*, 23: 5–6.

16. Turkmen, N., Sari, F., and Velioglu, Y.S. (2006) Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin–Ciocalteu methods. *Food Chem.*, 99: 835–841.
17. Yuan, X., Koh, H.-L., and Chui, W.-K. (2005) A high performance liquid chromatography method for the simultaneous determination of arctiin, chlorogenic acid and glycyrrhizin in a Chinese proprietary medicine. *J. Pharma. Biomed. Anal.*, 39: 697–704.
18. Grubešić, R.J., Vuković, J., Kremer, D., and Vladimir-Knežević, S. (2005) Spectrophotometric method for polyphenols analysis: Prevalidation and application on Plantago L. species. *J. Pharm. Biomed. Anal.*, 39: 837–842.
19. Zeng, L. and Xia, Z. (2002) The improvement and influence of ultrasonic and microwave irradiation on the extraction of traditional Chinese medicine. *Chem. Res. Appl.*, 14: 245–249.