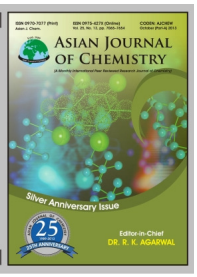
Asian Journal of Chemistry; Vol. 25, No. 18 (2013), 10191-10194

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.15223



Ultrasonic-Assisted Semi-Bionic Extraction of Tannins from Wild Persimmon Leaves

XIANCHUN YU[1,2,*], DELIN SUN[2], XIJUN FU[1] and SIROU ZHOU[1]

[1]Comprehensive Utilization Key Laboratory of Chinese Herbal Medicine of Yueyang Dongting Lake Area, Yueyang Vocational & Technical College, Yueyang 414000, Hunan Province, P.R. China
[2]College of Material and Engineering, Central South University of Forestry and Technology, Changsha 410004, Hunan Province, P.R. China

*Corresponding author: Fax: +86 730 8677405; Tel: +86 13037302032; E-mail: sdlyxc@163.com

(*Received*: 28 January 2013; *Accepted*: 11 November 2013) AJC-14364

Tannin was extracted from wild persimmon leaves (*Diospyros kaki L.f*) using the ultrasonic-assisted semi-bionic approach. Infrared spectrum analysis showed that tannins from wild persimmon leaves contain many proanthocyanidin-like substances with 3-O-gallate acyl structure. Free radical scavenging capacity assay showed that the ultrasonic-assisted semi-bionic approach can improve the scavenging activity of tannins on radicals, including $OH^{\bullet}$, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$. The half maximal inhibitory concentration values were reduced by 0.62, 0.116 and 1.19 mg mL^{-1}, respectively, compared with the water reflux extraction method. Experimental results of orthogonal optimization showed that the tannin extraction quantity can reach 226.8 mg g^{-1}, which was much higher than the sample obtained without ultrasonic assistance under the following experimental conditions: pH values of 1.8, 7.5 and 8.4, respectively; 50 % solvent mass fraction; 35 min extraction time; 50 ºC extraction temperature; 300 W ultrasonic power and 30 KHz ultrasonic frequency. These results suggested that this method could increase the activity and extraction yield of tannins.

Key Words: Ultrasonic-assisted extraction, Semi-bionic approach, Wild persimmon leaves tannin, Process optimization.

INTRODUCTION

Tannins are water-soluble compounds with a polyphenol hydroxyl structure found in plants. They exhibit strong antioxidant capacity, excellent antibacterial property and antiviral activity[1]. They are widely used in the medicine, food, leather, print and dye industries[2-4]. Currently, tannins are mainly extracted through water boiling or water bath heating using water-ethanol or acetone as solvent and black wattle bark or pine bark as raw material[5]. Although this method is simple, a long time is needed to complete the process. Tannins are easily oxidized and different methods affect their purity and activity. In addition, using bark as a raw material easily damages trees.

With the development of chemical technology, semi-bionic extraction and ultrasonic-assisted extraction have demonstrated unique advantages in many fields. Semi-bionic extraction is a new plant extraction process designed for the biopharmaceutical industry. It simulates the transit process of oral drugs in the gastrointestinal tract and ensures the biological activity of extracts using activity-guided separation methods[6]. The multi-level physical effects of ultrasonic assistance, including cavitation, mechanical vibration, micro-jet and micro-acoustic streaming, can deform and break plant cells under low temperatures[7]. Therefore, using ultrasonic-assisted extraction in semi-bionic extraction process can greatly enhance extraction volume, shorten extraction time and maintain the biological activity of products.

The present study used wild persimmon leaves (*Diospyros kaki L.f*) from western Hunan Province in China as raw materials in the extraction of tannins *via* the ultrasonic-assisted semi-bionic approach. This study also discussed the effects of extraction solution pH, solvent mass fraction, extraction temperature and time, ultrasonic power and frequency on the quantity of extracted tannins. The extraction process was optimized using orthogonal experiments.

EXPERIMENTAL

Wild persimmon leaves were collected from western Hunan Province. The leaves were allowed to dry for the experiments. The main instruments included an ultrasonic cell disruptor (JY92-IID, China), an ultraviolet spectrophotometer (UV-2550, Shimazu, Japan), an infrared Fourier transform spectroscope (Varian 660, America), a digital water bath temperature oscillator (SHZ-82, China) and herbal medicine grinding machines (FW117, China).

Methods: According to the design principle of semi-bionic experiment, the pH value in the gastrointestinal tract was simulated and ethanol-water was used as the extract solution. The pH values were adjusted to produce acidic, weakly alkaline and alkaline environments (Table-1) for preliminary experiments.

TABLE-1
pH VALUE DESIGN USING THE SIMULATED HUMAN GASTROINTESTINAL TRACT ENVIRONMENT

Acidic (simulated stomach) pH value	Weakly alkaline (simulated small intestine) pH value	Alkaline (simulated large intestine) pH value
1.4	7.5	8.3
1.4	7.5	8.4
1.4	7.5	8.5
1.8	7.5	8.3
1.8	7.5	8.4
1.8	7.5	8.5
2.4	7.5	8.3
2.4	7.5	8.4
2.4	7.5	8.5

Dried wild persimmon leaves were crushed to 0.5 mm and extracted three times under the designed temperature (*i.e.*, 48, 50, 53 ºC) in acidic, weakly alkaline and alkaline environments. Different ultrasonic powers and frequencies were used in the ultrasonic-assisted approach. The three extracts were then combined, centrifuged to remove residues and dried at 50-60 ºC to obtain the tannins from wild persimmon leaves.

Contrast experiments were performed under the same conditions without ultrasonic assistance.

Determination of tannin: Tannin content was measured using the zinc acetate complexometric titration method[8]. The zinc acetate solution was mixed with the sample solution until excess Zn^{2+} precipitated all the tannins through complexation. The remaining Zn^{2+} in the reaction system was determined using standard solution of disodium edetate titration to calculate tannin content.

Free radical scavenging capability: The $OH^{\bullet}$, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging capacities were determined according to Smimoff Cumbe[9], Brand-Williams *et al.*[10], Cervato *et al.*[11], Miller *et al.*[12] and Re *et al.*[13], respectively (Table-2).

TABLE-2
FREE RADICAL SCAVENGING CAPABILITY OF TANNINS EXTRACTED USING DIFFERENT METHODS

Extraction method	IC_{50} value of $OH^{\bullet}$ (mg/mL)	IC_{50} value of $DPPH^{\bullet}$ (mg/mL)	IC_{50} value of $ABTS^{\bullet+}$ (mg/mL)
Water reflux method	0.85	0.13	1.56
Ultrasonic-assisted semi-bionic method	0.23	0.014	0.37

Effect of extract pH : The effect of extract pH was determined under the following conditions: 50 % extract mass fraction, 40 min extraction time, 50 ºC extraction temperature, 250 W ultrasonic power and 30 KHz ultrasonic frequency. The results are shown in Fig. 1. Sample 5 exhibited the highest quantity of extracted tannin (*i.e.*, 209.3 mg g^{-1}), with pH values of 1.8, 7.5 and 8.5 for the acidic, weak alkaline and alkaline environments, respectively. Thus, the pH values of the three extracts were set at 1.8, 7.5 and 8.5 in this experiment.

Effect of solvent mass fraction: Ethanol with mass fractions of 30, 40, 50, 60 and 70 % was used as the extract solution. The pH values of the acidic, weak alkaline and alkaline environments were set at 1.8, 7.5 and 8.4, respectively, with 40 min extraction time, 50 ºC temperature, 250 W ultrasonic

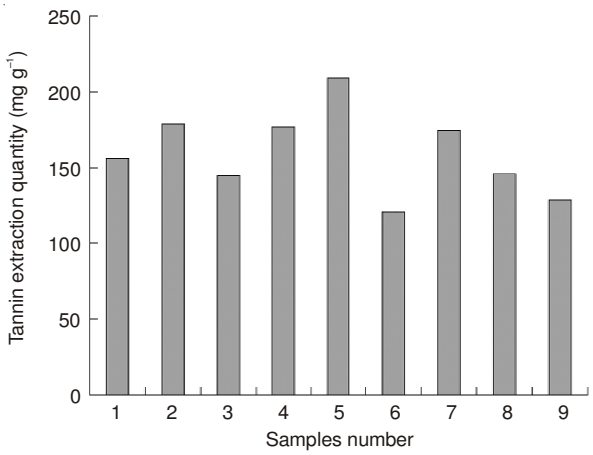


Fig. 1. Effect of pH value on extraction quantity in different samples (the number of samples corresponds with Table-1)

power and 30 KHz frequency. The experimental results are shown in Fig. 2. The highest extraction quantity was obtained from the ethanol solution with 50 % solvent mass fraction, exhibiting a downward trend when the solvent mass fraction increased by more than 50 %. This result may be attributed to the composition and properties of tannins. The maximum solubility of tannins in ethanol was reached at 50 % solvent mass fraction. The ethanol-water solution showed selectivity on tannin solubility[14] when the ethanol volume fraction was not equal to 50 %. That is, the extracted tannin may either be water or alcohol soluble when the ethanol solvent mass fraction was below 50 % or above 50 %, respectively. Thus, 50 % ethanol was selected as the extract solvent mass fraction.

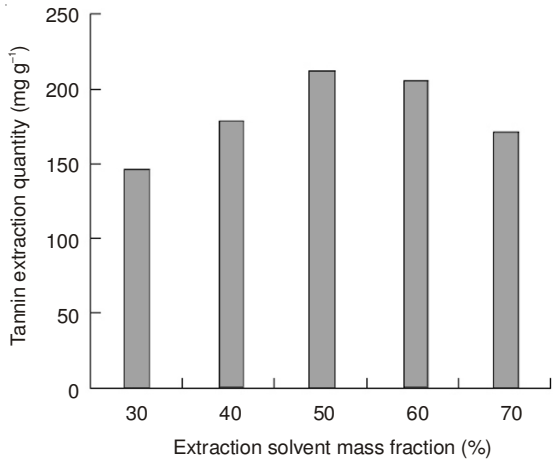


Fig. 2. Effect of solvent mass fraction on extraction quantity

Effect of extraction time: The effect of different extraction times and ultrasonic frequencies on the extraction quantity of tannin is shown in Fig. 3. The extraction quantity increased quickly with time and then decreased after 40 min. This result may be attributed to the unstable molecular structure of tannins. The amount of tannin that underwent oxidation and decomposition processes increased with time. Fig. 3 also shows that the extraction quantity without ultrasonic assistance was significantly lower than that with ultrasonic assistance during the same time period. In addition, a relatively higher tannin extraction quantity was obtained at 30 KHz.

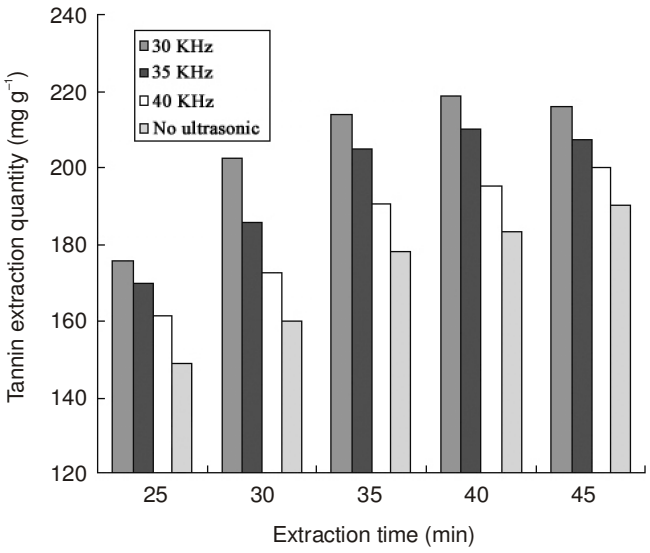


Fig. 3. Effect of extraction time on extraction quantity

Effect of extraction temperature: The effects of extraction temperature and ultrasonic frequency on extraction quantity are shown in Fig. 4. The tannin extraction quantity substantially increased and reached a relatively higher level at 30 KHz. The tannin extraction quantity also increased with increasing extraction temperature, reaching its maximum at approximately 50 ºC and subsequently declined at higher temperatures. This result may be attributed to the unstable tannin structure produced by oxidation or hydrolysis at high-temperature heating conditions, which decreased the extraction quantity. Therefore, 50 ºC was selected as the extraction temperature under the auxiliary ultrasonic frequency of 30 KHz.

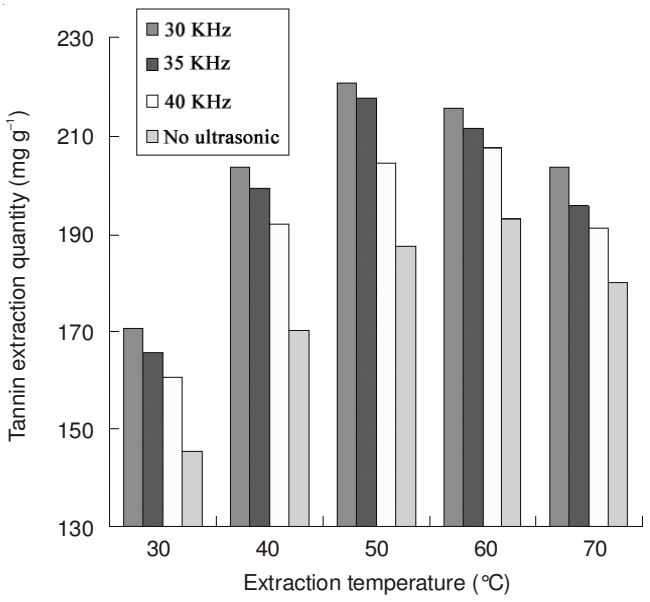


Fig. 4. Effect of extraction temperature on extraction quantity

Effect of ultrasonic power: The effects of different ultrasonic powers and frequencies on tannin extraction quantity are shown in Fig. 5. The tannin extraction quantity increased with increasing ultrasonic power, reaching a maximum of 220.2 mg g^{-1} at approximately 250 W and then subsequently decreased at higher ultrasonic power. This result may be attributed to plant tissue breakage or deformation caused by multi-level physical effects, including cavitation, mechanical vibration, micro-jet and micro-acoustic streaming, thereby enhancing the tannin extraction quantity[15]. However, the formation of cavitation bubbles may reach an unprecedented level when the ultrasonic power increased to a certain extent. As a result, the energy dissipation between the cavitation bubbles is decreased and the cavitation bubble is unable to collapse fully. Consequently, energy transfer efficiency and extraction quantity are reduced. Fig. 5 also shows that the relatively higher extraction quantity appeared at 30 KHz ultrasonic frequency.

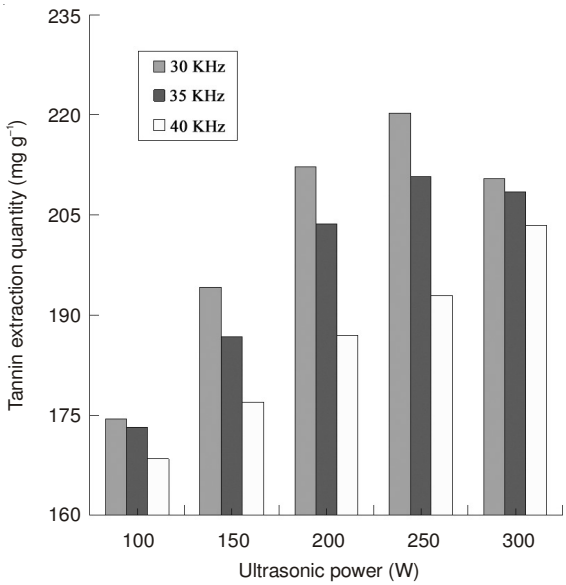


Fig. 5. Effect of ultrasonic power on extraction quantity

Effect of ultrasonic frequency: Figs. 3-5 show that the ultrasonic frequency affected the tannin extraction quantity. The extraction quantity reached the highest at 30 KHz, followed by 35 KHz and reached the lowest at 40 KHz. This result may be attributed to the relationship between the amplitudes of extract solution and ultrasonic frequency. The amplitude was larger at lower frequencies, the solid material and fluid were mixed more evenly and a higher extraction quantity was obtained. However, the lowest frequency did not exhibit good results because low frequencies require high energy consumption and equipment. Thus, 30 KHz was selected as the appropriate ultrasonic frequency under the given experimental conditions.

RESULTS AND DISCUSSION

Orthogonal experiment: Single-factor analysis was performed under the following experimental conditions to make the extraction process more scientific and reasonable: pH values of 1.8, 7.5 and 8.5 for the three extracts. An L_{16} (4^5) orthogonal test was employed to design the five factors exhibiting major effects *i.e.*, extraction temperature (A), ultrasonic power (B), solvent mass fraction (C), ultrasonic frequency (D) and extraction time (E). The orthogonal experimental design and range analysis are shown in Table-3.

The orthogonal experimental results showed the sequence of factors affecting the tannin extraction quantity in the following trend: A > B > D > C > E. The most appropriate technology combination was $A_2B_4D_2C_2E_2$ (*i.e.*, 50 ºC extraction

TABLE-3
ORTHOGONAL EXPERIMENTAL RESULTS OF THE EXTRACTION QUANTITY OF TANNINS

No.	A (ºC)	B (W)	C (%)	D (KHz)	E (min)	Extraction quantity (mg/g)
1	1(40)	1(150)	1(40)	1(0)	1(30)	179.4
2	1	2(200)	2(50)	2(30)	2(35)	224.6
3	1	3(250)	3(60)	3(35)	3(40)	191.1
4	1	4(300)	4(70)	4(40)	4(45)	186.5
5	2(50)	1	2	3	4	195.4
6	2	2	1	4	3	199. 4
7	2	3	4	1	2	205.4
8	2	4	3	2	1	209.5
9	3(60)	1	3	4	2	187.4
10	3	2	4	3	1	197.8
11	3	3	1	2	4	201. 2
12	3	4	2	1	3	196. 5
13	4(70)	1	4	2	3	208. 7
14	4	2	3	1	4	210. 1
15	4	3	2	4	1	214. 9
16	4	4	1	3	2	209.6
k_1	195.4	192.7	197.4	197.9	200.4	–
k_2	202.4	207.9	207.9	211.0	206.8	–
k_3	195.7	203.2	199.5	198.5	198.9	–
k_4	210.8	200.5	199.6	197.1	198.3	–
Rj	15.4	15.2	10.5	13.9	8.5	–

temperature, 300 W ultrasonic power, 30 KHz ultrasonic frequency, 50 % solvent mass fraction and 35 min extraction time). Three additional experiments were performed using the above optimal conditions, with an average tannin extraction quantity of 226.81 mg g^{-1}. However, only 197.23 mg g^{-1} was obtained under the same conditions without ultrasonic assistance.

Infrared spectroscopy analysis: The most distinct infrared bands of tannin included the aryl ring vibration at 1540 and 1600 cm^{-1}, the C-H deformation and aryl ring vibration at 1450 and 1470 cm^{-1} and the C-H stretching vibration at 1030 and 1170 cm^{-1}. In addition, the peak near 3360 cm^{-1} became wider and stronger, indicating that it belongs within the range of the stretching vibration of phenolic hydroxyl (O-H) group. A stretching vibration near 1680 cm^{-1} belonging to phenyl ester (C=O) was also observed, indicating that the products belong to proanthocyanidin substances containing 3-O-gallate acyl structure.

Free radical scavenging capacity: Table-3 shows the free radical scavenging capability of tannins extracted *via* water reflux and ultrasonic-assisted semi-bionic methods. The $OH^{\bullet}$, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ clearance rates of tannins extracted *via* the ultrasonic-assisted semi-bionic method were all higher than that extracted *via* the water reflux method. The half maximal inhibitory concentration (IC_{50}) was 0.23, 0.014 and 0.37 mg mL^{-1}, respectively, under the experimental conditions. The free radical scavenging capacity was much higher than the sample from water reflux extraction, which may be attributed to the rapid oxidation, isomerization and other changes in the tannins obtained from persimmon leaf under the effect of external conditions such as heating and lighting. Ultrasonic-assisted semi-bionic technology can reduce the extraction temperature and shorten the extraction time. As a result, the oxidation of tannin under high temperatures is prevented. Thus, excessive effects on the molecular structure and physicochemical properties of tannins are prevented and the physiological activity of tannins is maintained.

Conclusion

Ultrasonic-assisted semi-bionic method was used in extracting tannins from wild persimmon leaves to increase tannin extraction quantity and maintain its biological activity. The pH value of the extract, solvent mass fraction, temperature, time, ultrasonic power, ultrasonic frequency and other factors exhibit great effects on the extraction quantity of tannin. The optimal experimental conditions were the following: pH values of 1.8, 7.5 and 8.4 for the three extracts; 50 % solvent mass fraction, 50 ºC extraction temperature, 35 min time, 300 W ultrasonic power and 30 KHz ultrasonic frequency. The tannin extraction quantity can reach 226.8 mg g^{-1}, which was 29.6 mg g^{-1} higher than the extraction quantity obtained without ultrasonic assistance. The scavenging capability of the extracted tannins on $OH^{\bullet}$, $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ was higher than that of the samples obtained using the water reflux method.

ACKNOWLEDGEMENTS

This work received financial aid from the Science and Technology Project of Hunan Province (No. 2013GK3184).

REFERENCES

1. T. Katsube, H. Tabata, Y. Ohta, Y. Yamasaki, E. Anuurad, K. Shiwaku and Y. Yamane, *J. Agric. Food Chem.*, **52**, 2391 (2004).
2. E.P. Lansky and R.A. Newman, *J. Ethnopharmacol*., **109**, 177 (2007).
3. M.H. Chen, C.M. Li and Y.S. Yang, *Food Sci. Technol*., **34**, 120 (2009).
4. H.F. Gu, C.M. Li, Y.J. Xu, J. Zhang and H.L. Dou, *Trans. Chinese Soc. Agric. Eng*., **23**, 241 (2007).
5. Z.H. Huang, B. Zhang and G.Z. Fang, *J. Northeast Forestry Univ.*, **37**, 67 (2009).
6. X.J. Chen and C.S. Zhou, *Fine Chem*., **23**, 35 (2006).
7. X.C. Yu, D.L and Sun, X.S. Li, *Asian J. Chem*., **24**, 409 (2012).
8. W.Y. Yang, Z.G. Li, H. Wu, Y.H. Zhao and Z.H. Zhang, *J. Southwest Forestry College*, **19**, 249 (1999).
9. N. Smimoff and Q.J. Cumbes, *Phytochemistry*, **28**, 1057 (1989).
10. W. Brand-Williams, M.E. Cuvelier and C. Berset, *LWT-Food Sci. Technol*., **28**, 25 (1995).
11. G. Cervato, M. Carabelli, S. Gervasio, A. Cittera, R. Cazzola and B. Cestaro, *J. Food Biochem*., **24**, 453 (2000).
12. N.J. Miller, J. Sampson, L.P. Candeias, P.M. Bramley and C.A. Rice-Evans, *FEBS Lett*., **384**, 240 (1996).
13. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang and C. Rice-Evans, *Free Radic. Biol. Med*., **26**, 1231 (1999).
14. B. Tong and D.C. Liu, *China Oils Fats*, **33**, 47 (2008).
15. M. Parvinzadeh, N. Memari, M. Shaver, B. Katozian, S. Ahmadi and I. Ziadi, *J. Surfact. Detergents*, **13**, 135 (2010).