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Research Article

Optimizing Water Extraction Conditions of the Antidiabetic Compounds from Cinnamon

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Abstract: An orthogonal experiment L_9 (3^4) in triplicate was used to optimize the conditions for water extraction of the antidiabetic compounds in cinnamon. The antidiabetic compounds were polyphenols, cinnamaldehyde and cinnamic acid. The effect of temperature, solvent: material ratio, extraction time and frequency were determined on the total yields of the active compounds (polyphenols, cinnamaldehyde and cinnamic acid). The results showed that extraction temperature and frequency were the main variables that influenced the yields of extracts. Based on canonical analysis, the optimal conditions were at 60°C for one hour and solvent: material ratio of 10:1 (v/w) for three times and the maximizing extract yield was 17.52 mg/g.

Keywords: Antidiabetic compounds, cinnamon, extract yield, orthogonal experiment

INTRODUCTION

Cinnamon is the bark of the *Cinnamomum cassia* Presl. (Pharmacopoeia, People's Republic of China, 2000). It is cultivated widely in the southern part of China. And it contains cinnamaldehyde, cinnamic acid, tannin and polyphenols, etc. As a functional food, cinnamon was also widely used as a flavouring agent and has many applications in perfumery and pharmaceutical industries. In Asian countries, cinnamon has long been used as one of the traditional folk herbs for diabetes mellitus (Bailey and Day, 1989), such as China, Indian (Babu *et al*., 2007) and Korea.

In recent years, researchers had shown that cinnamon extract had a moderate effect in reducing fasting plasma glucose concentrations in diabetic patients with poor glycaemic control (Mang *et al*., 2006; Khan *et al*., 2003). *In vivo* studies, the oral treatment with aqueous cinnamon extract would enhances glucose utilization in rats in a dose-dependent fashion by potentiating insulin-stimulated tyrosine phosphorylation of IR-β, IR Substrate (IRS)-1 and IRS-1 association with phosphatidylinositol 3-kinase (Qin *et al*., 2003). Then Kim *et al*. (2006) also found that the water extract of cinnamon would lower the blood glucose in db/db mice by improving insulin sensitivity or slowing absorption of carbobydrates in the small intestine. *In vitro* studies, Broadhurst *et al*. (2000) and Jarvill-Taylor *et al*. (2001) revealed that the cinnamon extract mimics the effect of insulin, which can potentiate insulin action in isolated adipocytes.

Researchers not only reported the antidiabetic mechanism of cinnamon, but also studied the active compounds in it. To date, the antidiabetic compounds in cinnamon as reported were polyphenols, cinnamaldehyde and cinnamic acid. Jarvill-Taylor *et al*. (2001) and Anderson *et al*. (2004) and had found that water-soluble polyphenol type-A polymers in cinnamon can function as a mimetic for insulin in 3T3-L1 adipocytes *in vitro* and these polyphenols polymers may be beneficial in the control of glucose intolerance and diabetes. Then Cao *et al*. (2007) found that cinnamon extract and polyphenols could increase the amount of insulin receptor, glucose transporter 4 and tristetraprolin in mouse 3T3-L1 adipocytes. Babu *et al*. (2007) reported that cinnamaldehyde produced a significantly reduction in blood glucose concentration in a dose-dependent manner in streptozotocin (STZ)-induced diabetic rats. Cinnamic acid could reduce the blood glucose in both STZ-induced and alloxan-induced diabetic mice and it can be used to produce the antidiabetic medicine (Xiang, 1999). These results suggested that these compounds present in cinnamon may have beneficial effects on glucose and insulin levels. And the hypoglycemic effect and the treatment for the diabetes were associated with contents of the three active compounds in cinnamon extract.

As to the contents of the antidiabetic compounds, extraction is the first important step and the extraction conditions may affect the clinical efficacy of active compounds of cinnamon. Many extract techniques have been developed to extract the effective components

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Table 1: Orthogonal experimental design L_9 (3^4) to assess effects of temperature, solvent: material ratio, extraction time and frequency

Levels	Solvent: material (A, v/w [a])	Temperature (B,°C)	Time (C, h)	Frequency (D, times)
1	8:1	60	1	1
2	10:1	80	1.5	2
3	12:1	100	2	3

[a]: v/w: the ratio of water volume to material weight

from cinnamon (Hui *et al*., 2006; Zhu and Li, 2006). But the usage of cinnamon for diabetes treatment was always the aqueous extract. However, there has been no report on the optimization of water extraction conditions for cinnamon so far. So we studied the water extraction technique of cinnamon, in order to obtain highly contents of active compounds.

Orthogonal experimental design had been widely used in the extraction process for plants (Wei *et al*., 2007; Hu *et al*., 2007; Chen *et al*., 2007). Orthogonal arrays are fractioned factorial designs, which could test multiple independent processes variables within a single experiment. It would reduce the amount of experiments and costs. Orthogonal experimental design has been already used to optimize the extract process of volatile oil from cinnamon (Huo *et al*., 2004), but it has not yet been applied to optimize water extraction conditions for polyphenols, cinnamaldehyde and cinnamic acid. In this study, we selected orthogonal experimental design to optimize water extraction conditions for the antidiabetic compounds in cinnamon.

The objective of this study was to determine the best water extraction conditions for the antidiabetic compounds in cinnamon. And the effect of temperature, water solvent: Material ratio, extraction time and extraction frequency were determined on the total yields of the active compounds (polyphenols, cinnamaldehyde and cinnamic acid).

MATERIALS AND METHODS

Materials and reagents: Cinnamon was kindly purchased from Tongrentang Chinese medicine-Since 1669 (Beijing, China). They were dried at 40°C to constant weight in vacuum drying oven (Model ZKF030, Shanghai Laboratory Instrument Works, China), broke to small pieces and passed through a 4 mesh sieve. Folin-Ciocalteu's phenol reagent was purchased from Sigma-Aldrich (USA). Gallic acid, cinnamaldehyde and cinnamic acid were supplied by National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China); Acetonitrile was chromatographic grade and other reagents were analytical grade.

Extracts preparation: Cinnamon samples were immersed in cold water for 0.5 h and 10 g of the samples was used for each treatment. Various water solvent: material ratio, extraction time and frequency were according to the orthogonal experimental design (Table 1). Cinnnamon samples were extracted on water bath. The extraction solutions were filtered through a filter paper (Whatman No. 541, Whatman International Ltd. Maidstone, England) using Eyela vacuum aspirator (Model A-3s, Tokyo rikakikai Co., Ltd., Japan). And the filtrate obtained from each treatment was combined, concentrated at 40°C in vacuum drying oven to a certain volume and diluted with water to 100 mL volumetric flask, as the test solution. Then the test solutions were stored at 4°C until use.

Determination of polyphenols by Folin-Ciocalteu chromatometry method: Measure accurately 1 mL of the test solution to a 25 mL brown volumetric flask, add water to volume and mix well. It was used for the measurement of polyphenols. And the polyphenols were measured by Folin-Ciocalteu chromatometry method (Emmons and Peterson, 1999). Briefly, 1mL of the test solution or the gallic acid solution was mixed with 1mLof 1N Folin-Ciocalteu reagent and 3 mL Na_2CO_3 (1 mol/L) was added to the mixture, then stand still for 15 min. Finally, 5 mL diluted water was supplied to the mixture. Then the absorbance was measured at 725 nm with the ultraviolet spectrophotometer (BIO-RAD, USA) and the results were expressed as standard Gallic Acid Equivalent (GAE). The standard gallic acid was dissolved in distilled water to prepare eight various concentrate standard solutions (10.5, 21, 31.5, 42, 52.5, 63, 73.5, 84 μg/mL), which were used to build the calibration curve. And 1mL of the solution was substituted by distilled water as the blank solution.

Determination of cinnamaldehyde and cinnamic acid by HPLC method: Measure accurately 1 mL of the test solution to a 10 mL brown volumetric flask, add water to volume and mix well. And this solution was filtered through a 0.45 μm filter membrane and used for the measurement of cinnamaldehyde and cinnamon acid. High Performance Liquid Chromatography (HPLC) was applied to determine cinnamaldehyde and cinnamon acid, using Shimadzum LC-20AT system equipped with a Shimadzum LC-20AT autosampler (Tokyo, Japan). Thermo BDS column (4.6×250 mm, 5 μm, Thermo, USA) was used. The solution system was acetonitrile (solution A) and purified water with (phosphate buffer, adjust pH to 4.7) as solution B. Solution A: solution B was at 45:55 (Wang *et al*., 2003); the flow rate was set at 1 mL/min. And the wavelength of the detector was 285 nm.

Weighed accurately standard cinnamaldehyde and cinnamic acid, dissolved in methanol to produce a solution containing cinnamaldehyde 0.0244 mg, cinnamic acid 0.0254 mg each per mL and mixed well as the reference solution. Inject accurately 4, 6, 8, 10,

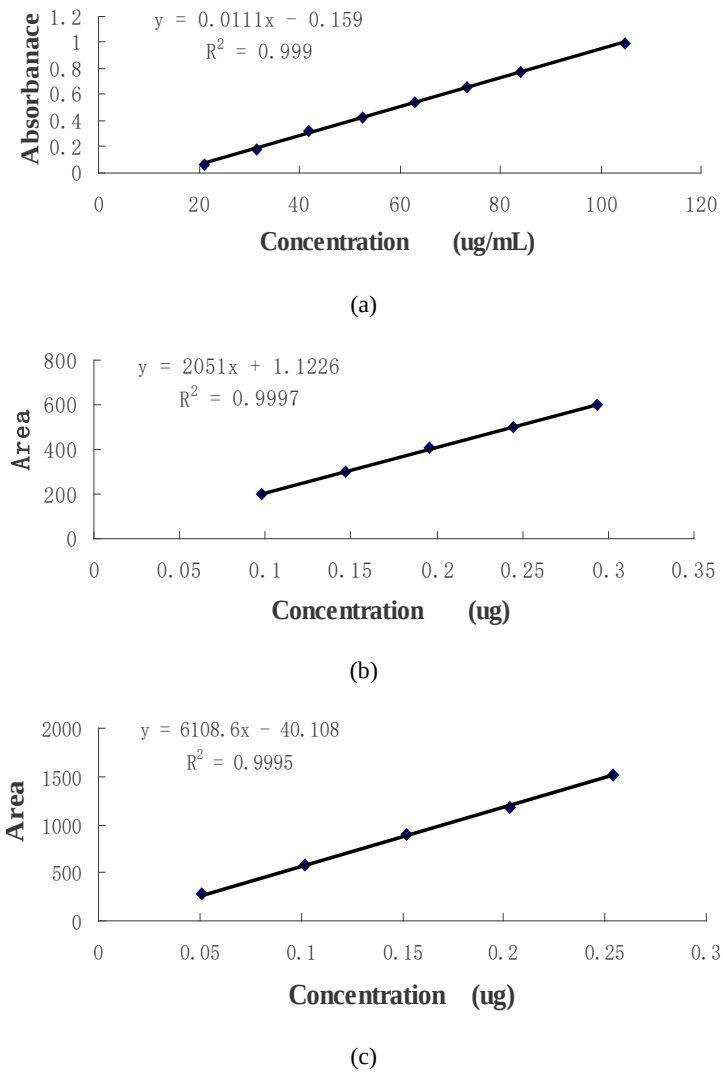


Fig. 1: (a): The calibration curve of gallic acid; (b): cinnamaldehyde and; (c): cinnamic acid

12 μL of the reference solution of cinnamaldehyde respectively into the column and the standard curve of it was obtained. And inject accurately 2, 4, 6, 8, 10 μL of the reference solution of cinnamic acid respectively into the column, to obtain the standard curve.

Orthogonal experimental design: An orthogonal design L_9 (3^4) was conducted to optimize the extract conditions (Table 1). Experiment runs numbered from 1 to 9 were done to 9 samples of 10 g cinnamon per bag. All the extraction trials were carried out in triplicate. The effect of temperature, solvent: material ratio, extraction time and frequency were determined on the total yield of cinnamaldehyde, polyphenols and cinnamic acid.

Statistical analysis: All data were expressed as means of triplicate measurements. Statistical analyses were performed using *F*-test and one way analysis of variance. Multiple comparisons of means were done by the LSD (least significance difference) test. Differences were considered significant, when a probability value was less than 0.05 ($p<0.05$). All computations were made by employing the statistical software using ANOVA analysis (SAS, version 8.2).

RESULTS AND DISCUSSION

The calibration curve of polyphenols: Gallic acid was used as the standard to produce the standard curve (Fig. 1a) and determine the content of polyphenols in cinnamon. The *X*-axis was the concentration of the gallic acid and the *Y*-axis was the absorbance value. The amount of polyphenols was determined with the Folin-Ciocalteu chromatometry method. This method was employed to evaluate the phenolic content of the samples. A standard gallic acid solutions (ranging from 10.5 to 84 μg/mL) were prepared in distilled water to

Table 2: The yield obtained in each run for the polyphenols, cinnamaldehyde and cinnamic acid and the total yield of the three active compounds

Run	Polyphenols yield (mg/g)	Cinnamaldehyde yield (mg/g)	Cinnamic acid yield (mg/g)	Total yield[a] (mg/g)
1	12.60	1.03	2.83	16.46
2	5.31	0.63	0.20	6.14
3	10.18	0.75	0.13	9.56
4	9.51	1.05	0.36	10.92
5	7.35	0.78	0.13	7.27
6	8.90	0.69	0.21	14.20
7	12.40	1.40	0.51	9.80
8	7.25	0.81	0.10	11.15
9	5.70	0.86	0.23	6.79

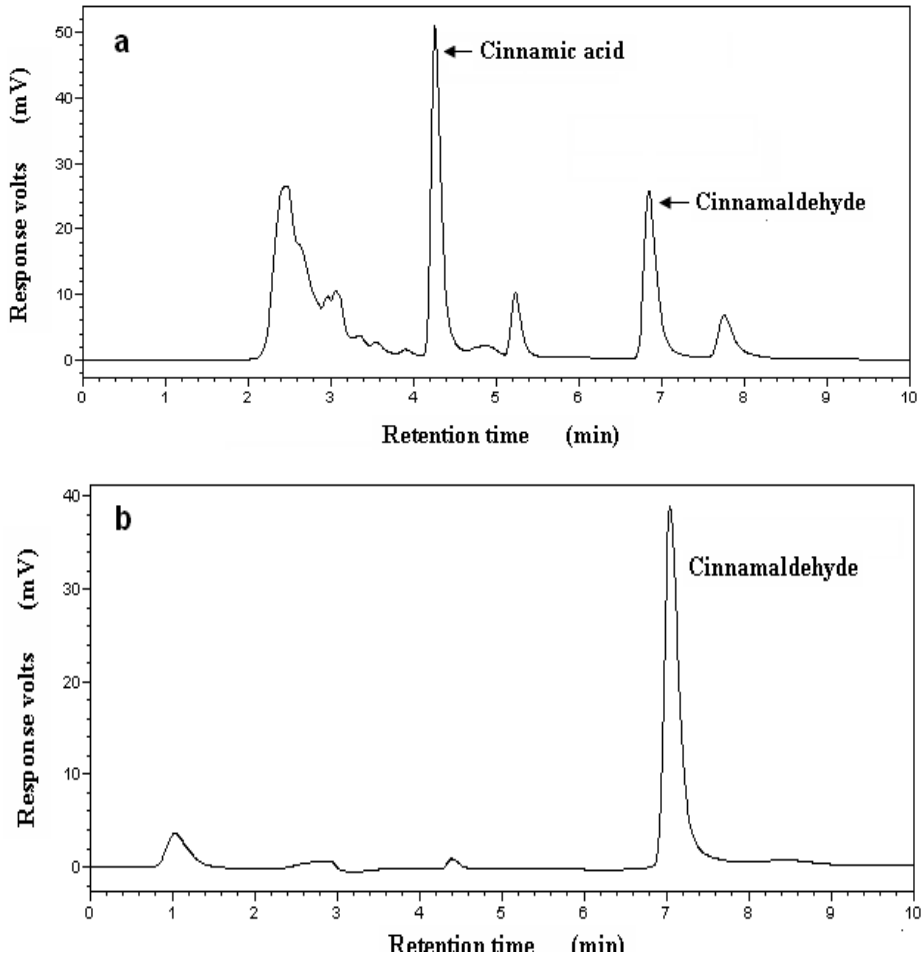
[a]: Total yield was the sum of the three active compounds (polyphenols, cinnamaldehyde and cinnamic acid)

produce the regression equation of the calibration curve (y = 0.0111x-0.159, R^2 = 0.999). The polyphenols content of the test solutions was expressed as mg gallic acid equivalents per gram dry weight of raw material (mg GAE/g dry weight) (Hayouni *et al*., 2007). The yield of polyphenols was shown in Table 2.

The calibration curves of cinnamaldehyde and cinnamic acid: For the HPLC systems employed, the peaks of cinnamaldehyde and cinnamic acid in the samples were separated commendably as shown in Fig. 2a. And identification and quantification of cinnamaldehyde and cinnamic acid present in the samples were achieved by comparing each peak's retention time and area with their standards (Fig. 2b and c).

The calibration curves of cinnamaldehyde and cinnamic acid were used as the quantification of them in the samples respectively. Figure 1b showed the calibration curve of cinnamaldehyde and the regression equation was y = 2051x+1.1226 (R^2 = 0.9997). The calibration curves of cinnamic acid was shown in Fig. 1c with its regression equation (y = 6108.6x-40.108, R^2 = 0.9995).

All these results suggested that this HPLC method was suitable and precise for the determination of cinnamaldehyde and cinnamic acid in cinnamon. The yield of them was shown in Table 2.



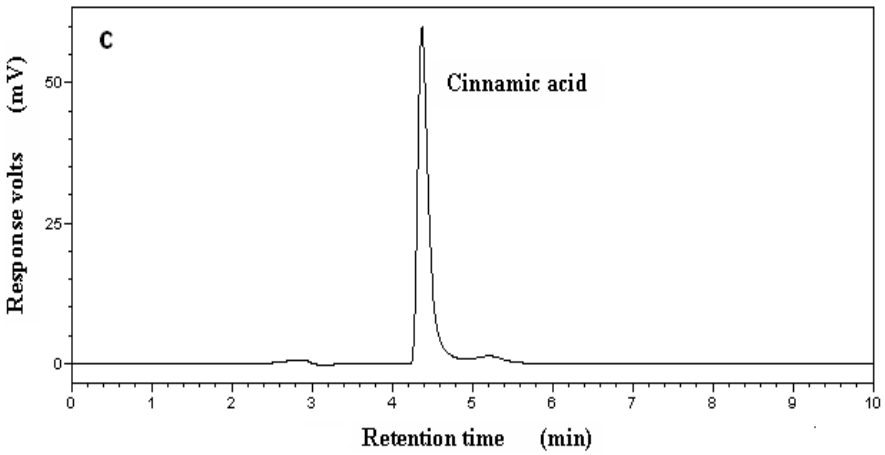


Fig. 2: Separation and detection of cinnamaldehyde and cinnamic acid by HPLC; (a): Chromatography present in cinnamon extracts; (b): Chromatography of cinnamaldehyde standard; (c): Chromatography of cinnamic acid standard

Table 3: Results obtained at the experimental condition using L_9 (3^4) orthogonal design

	Factors				
Run	A	B	C	D	Total yield[c] (mg/g)
1	1	1	1	1	16.46
2	1	2	2	2	6.14
3	1	3	3	3	9.56
4	2	1	2	3	10.92
5	2	2	3	1	7.27
6	2	3	1	2	14.20
7	3	1	3	2	9.80
8	3	2	1	3	11.15
9	3	3	2	1	6.79
I[a]	10.72	12.39	13.94	10.17	
II[a]	10.80	8.19	7.95	10.05	
III[a]	9.25	10.18	8.88	10.54	
R [b]	1.55	4.21	5.99	0.50	

[a]: Average response of each level about total yield; [b]: *R* value means range between four average responses of each level about total yield; [c]: Total yield was the sum of the three active compounds (polyphenols, cinnamaldehyde and cinnamic acid)

Table 4: Analyses of variance of total yield of three active compounds

Factor	SS[a]	*df* [b]	*F*	Significance[c]
A	4.56	2	11.46	
B	26.57	2	66.48	*
C	62.30	2	155.90	**
D	0.40	2	1.00	

[a]: Sum of square; [b]: Degree of freedom; [c]: $F_{0.05}(3, 2) = 19$, $F_{0.01}(3, 2) = 99$; *: Significant at $p<0.05$($F>19$); **: Significant at $p<0.01$($F>99$)

Effect of extract conditions on the total yield of antidiabetic compounds (polyphenols, cinnamaldehyde and cinnamic acid): An orthogonal design L_9 (3^4) was conducted to optimize the extract conditions and the factors and level were listed in Table 1. The effect of temperature, water solvent: Material ratio, extraction time and frequency were determined on the total yield of polyphenols, cinnamaldehyde and cinnamic acid. Table 3 showed the experimental design matrix and the total yield of the active compounds (polyphenols, cinnamaldehyde and cinnamic acid) obtained in each run. The last four rows gave the average yield of each level for the four parameters. For example, for II, the value of 10.8 at column "A" was the average of the yield at trials 4, 5 and 6.

In order to identify the significant effect of each process parameter on the performance characteristics, the *F*-test was calculated. High values for the calculated *F* mean a greater influence of factor on the experimental results. The significant varieties were evaluated by the value of *P*. If $p<0.05$, the factor was seemed to be significant; and if $p>0.05$, there was no significant effect. As shown in Table 4, it can be indicated that the effect of extraction variables on extraction yield decreased in the following order: temperature>time>solvent: material ratio>frequency. Temperature was strongly influent on the extract yields, next was the extraction time; and solvent: material ratio and extraction frequency was considered statistically as insignificant.

For each level of a factor, the average yield was calculated in the cases where the level occurred (see the last four rows in Table 4). The level corresponding to the maximum average yield among the three levels

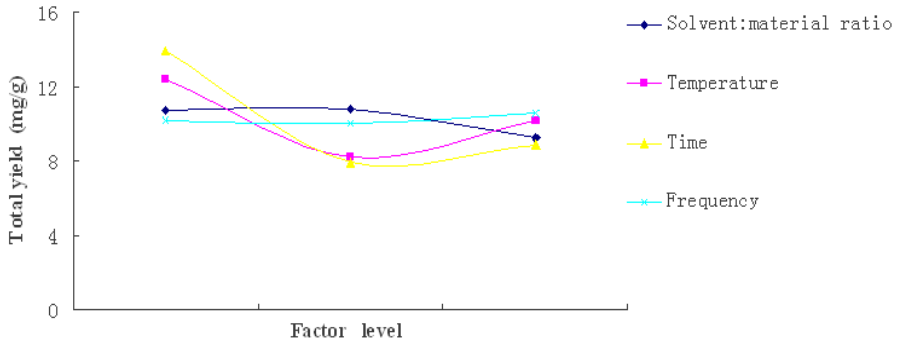


Fig. 3: Effect of extraction conditions: solvent: material ratio, temperature, extraction time and frequency on total yield of cinnamon

would be chosen for the optimal set of parameters as shown in Fig. 3.

In a word, from Table 4 and Fig. 3, we can see that the optimal parameters identified are $A_2B_1C_1D_3$. So the optimum design of variable combinations was considered with the factors: solvent: material ratio (10:1), time (1 h), temperature (60°C) and frequency (3 times). The extraction under the optimal conditions was carried out to validate the process optimal parameters. The total yield of three active compounds was 17.52 mg/g. Extraction yield was higher than the values obtained during the design of experiment, proving the reliability of the statistical analysis.

Finally, the best extraction conditions for the antidiabetic compounds in cinnamon were determined. Under this condition, the total yield of the antidiabetic compounds reached to the maximum and cinnamon extract in this process may improve the efficiency of diabetes treatment.

CONCLUSION

In this study, orthogonal experimental design was used to optimize the water extraction conditions for the antidiabetic compounds in cinnamon. This method would reduce the amount of experiments and costs. For all the factors, temperature was the most important factor and it can be strongly influent on the extraction yield of three antidiabetic compounds (polyphenols, cinnamaldehyde and cinnamic acid).

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