

## Research Article

### Studies on the Extracting Technology of Flavones in *Ginkgo* Leaves Assisted by Microwave

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**Abstract:** Flavones from *Ginkgo* leaves have special pharmacological effects and bioactivities. This study was focused on extraction process through single factor tests and orthogonal experiments method to investigate the influence of experimental factors on the yield of flavones. The results indicated that under microwave condition the optimal extraction conditions were concentration of ethanol 70% (v/v), weight ratio of liquid/solid 7: 1 (w/w), extraction time 7 min, the extraction percentage of flavones reaches maximum 82%.

**Keywords:** Extraction process, flavone, *Ginkgo biloba* leaves, microwave-assisted

#### INTRODUCTION

*Ginkgo* trees are widely distributed in China, whose leaves contained a mixture of effective agents such as flavones and lactones, which can aggrandize the blood vessel, increase the outflow of blood, improve the kinetic of blood-circulating, decrease the resisting force of cerebrovascular, suppress thrombosis and clean free radical of human body (Mahadevan and Park, 2008; Altiok *et al.*, 2006). Considering its special medicine effects, therefore its product has wide prospect of market.

Although there have been a lot of researches about different extraction methods and separation processing of the total flavones from natural *Ginkgo* leaves (Hasler *et al.*, 1992), there are still meaningful of studying extraction of flavones from *Ginkgo* leaves assisted by microwave method. It has been demonstrated that microwave-assisted method has the advantages of high efficiency, saving energy, less time and environment friendly compared with other traditional method (Ganzler *et al.*, 1990). Thus, it is utilized in this study to extract flavones from *Ginkgo* leaves to investigate the optimal extraction conditions based on single factor experiments and orthogonal experiments method.

#### MATERIALS AND METHODS

**Materials:** *Ginkgo* leaves were bought from *Ginkgo biloba* science and technology development center of Henan province. Ethanol, sodium hydroxide and aluminium nitrate were purchased from Chemical Reagent Co. Ltd. (Tianjin, China) and troxerutin was obtained from Halin Shengwu Co. Ltd. (Shanghai, China).

**Instruments:** The extraction was done in a microwave oven (MKX-J1A2, MKW Co. Ltd. Qingdao, China).

UV-visible absorption spectrum was obtained on UV-Visible spectrophotometer (Perkin Elmer Inc., USA).

**Preparation of sample powder of *Ginkgo* leaves:** After cleaned and dried to constant weight at 50°C for 24 h in an air-circulating oven, the leaves were ground to the particle size of 60 meshes.

**Determination of the drawing of standard curve:** 50.0 mg troxerutin was dissolved in ethanol (70%), then transferred into a 250 mL volumetric flask to calibrate, the standard solution was shaken up and placed still. Afterwards taken 0.0, 5.0, 10.0, 15.0 and 20.0 mL, respectively of the standard solution by measuring pipets to different 50 mL volumetric flasks, added 1.4 mL of sodium nitrite (5%) and 1.4 mL of aluminium nitrate (5%). 10 min later, added 8.0 mL sodium hydroxide (5%), shaken up and calibrated with ethanol (30%). Used reagent ethanol as a reference to measure its absorbance at 510 nm, thus obtained the standard curve under different concentrations (Gebner *et al.*, 1985). The equation between Absorbance (A) and concentration of troxerutin (X) was:  $A = 8.98432 X - 0.0706$ ,  $R^2 = 0.9985$ , in the scope of measurement.

**Measurement of total flavones in the sample solution:** Two mL sample solution treated under different extraction conditions was transferred into 50 mL volumetric flasks, then it was dealt with the same procedure mentioned above to measure its absorbance, furthermore, to calculate its mass.

#### RESULTS AND DISCUSSION

**Effect of weight ratio of solid/liquid on the extraction yield of flavones:** (10.0 g) powders and 70% concentration of different mass of ethanol were

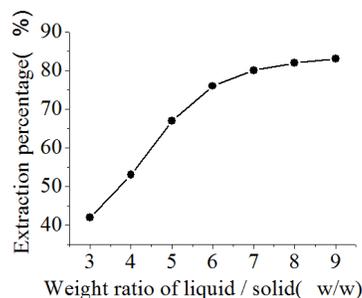


Fig. 1: Effect of weight ratio of liquid/solid on the extraction yield of flavones

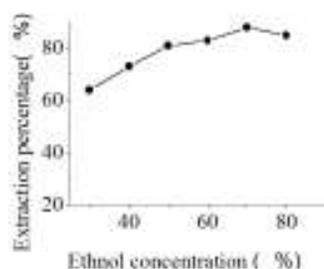


Fig. 2: Effect of ethanol concentration on the extraction yield of flavones

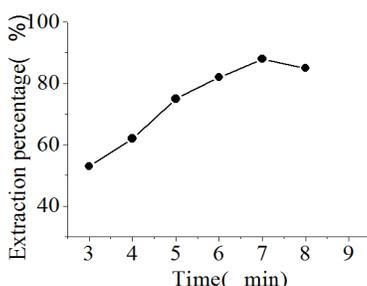


Fig. 3: Effect of time on the extraction yield of flavones

Table 1: Experimental results in orthogonal test of extraction

No.	Weight ratio of liquid/solid (w/w)	Concentration (v/v)	Time (min)	Extraction yield (%)
1	1	1	1	73
2	1	2	2	78
3	1	3	3	80
4	2	1	2	82
5	2	2	3	79
6	2	3	1	75
7	3	1	3	72
8	3	2	1	74
9	3	3	2	80
$x_1$	0.770	0.757	0.740	
$x_2$	0.787	0.770	0.800	
$x_3$	0.753	0.783	0.770	
R	0.034	0.026	0.060	

Table 2: Experimental factors and levels in orthogonal test

Level	Weight ratio of liquid/solid (w/w)	Concentration (v/v)	Time (min)
1	6	60	6
2	7	70	7
3	8	80	8

added in a flask under radiation for 8 min. As is shown in Fig. 1, the extraction rate is increasing in accordance with the augment of ethanol weight. Considering the cost and difficulty of separation, the ratio 7:1 is preferred.

**Effect of ethanol concentration on the extraction yield of flavones:** (10.0 g) powders and 30 g ethanol of different concentration were added in a flask under microwave radiation for 8 min. The extraction rate is improving along with the increment of ethanol concentration from 30-70% (Fig. 2). According to the theory of "similarity and intermiscibility", the polarity of the 70% ethanol is the most similar to flavones solution.

**Effect of microwave radiation time on the extraction yield of flavones:** (10.0 g) powders and 700.0 g ethanol of 70% concentration were added into a flask followed by radiation for some time. The results (Fig. 3) indicate that the extraction yield is increasing with the time length varying from 3 to 7 min, the reason is that the enough radiation would raise temperature of solution to enhance the solubility of flavones. But the extra radiation would cause the decomposition of flavones.

**The results of orthogonal test:** Based on the results of single factor tests, we choose weight ratio of liquid/solid, ethanol concentration and time as three factors to study the extraction yield of flavones (Table 1). Each factor has 3 levels shown in Table 2.

It can be concluded from above results of Table 1 that the weight ratio of solid/liquid affected the yield the most significantly in the 3 factors, while time the least. Therefore, the optimum experimental conditions were 70% ethanol, the ratio of liquid/solid 7:1 and time 7 min to obtain the maximum yield 82%. In order to confirm the results, the mean extraction percentage was 81.5% under the selected conditions for 3 times repeated.

## CONCLUSION

Based on single-factor and orthogonal tests, it was investigated that the optimization of extraction of flavone from *Ginkgo* leaves using microwave radiation method in this study. Under the optimum experimental conditions microwave power 500 W, 70% ethanol as extraction solvent, the ratio of liquid/solid 7:1 and extraction time 7 min, the maximum extraction rate of flavones reaches 89.2%, which demonstrates the method is efficient, energy-saving and environmentally friendly.

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