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Response surface optimization of mechanochemical-assisted extraction of flavonoids and terpene trilactones from Ginkgo leaves

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ABSTRACT

Mechanochemical-assisted extraction (MCAE) method was developed for extraction of flavonoids and terpene trilactones from Ginkgo leaves. The MCAE parameters and the antioxidant activities of Ginkgo biloba extract (GBE) were investigated. Through response surface methodology design experiments, the processing conditions were optimized as follows: amount of solid reagent ($NaHCO_3$) 21%, milling time 7.5 min, and ratio of solvent to solid 33 mL/g. Under these conditions, the yields of flavonoids and terpene trilactones in GBE were 6.83 mg/g and 1.72 mg/g, respectively. Compared with the heat reflux extraction method, the yields of flavonoids and terpene trilactones are higher, and the extraction time was significantly shortened. What is more, the MCAE method only used water as solvent. GBE of MCAE possessed notable antioxidant activity with IC_{50} values of 11.7 and 13.8 μg/mL, respectively.

1. Introduction

The living fossil tree *Ginkgo biloba* L. is well-known for its unique properties including its long lifetime and resistance to diseases (Miao et al., 2010). Ginkgo leaves contain various species of active ingredients (van Beek, 2002). The standardized Ginkgo biloba extract (GBE) contains 22–24% flavonoids along with 5–7% terpenoids (Fig. 1) (Singh et al., 2008; Jaracz et al., 2004), and it is helpful in inhibiting the onset of dementia, slowing down cognitive decline and functional disability, and reducing the incidence of cardiovascular disease due to its ability to prevent free radical damage, improve brain function, and support microcirculation (Maurer et al., 1997; Oyama et al., 1996; Nooshinfar et al., 2008; Rodriguez et al., 2007).

The quality of GBE is highly dependent on the manufacturing process. Conventional methods of producing GBE usually use mixtures of water–ethanol (Thompson et al., 1996; van Beek et al., 1991) or water–acetone (Chauret et al., 1991) as solvent by refluxing. These methods can obtain mostly active composition, but low selectivity and organic solvent remnant make the latter purification difficult. At the same time, volatile components may be destroyed and lost in the heating process. New kinds of methods including supercritical fluid extraction (Yang et al., 2002), pressurized water extraction (Lang and Wai, 2003), microwave-assisted extraction (Liu et al., 2008) and ultrasound-assisted extraction (Wu et al., 2001) have been reported with various yields.

Mechanochemical processing is the term applied to powder processing in which chemical reaction and phase transformations take place during milling due to the application of mechanical energy (Delogu et al., 2004; Suryanarayana, 2001). Mechanochemical-assisted extraction (MCAE) is an alternative extraction method which implements mechanochemical processing to the material with solid reagent to obtain mechanochemical composites before extraction in solvent. MCAE has been developed as an effective method in extracting triterpene acids from *Siberian Fir Needles* (Korolev et al., 2003), extracting phytoecdy steroids from *Serratula coronata* L., isolating lappaconitine from *Aconitum Septentrionale Roots* (Goncharov et al., 2006), extracting chondroitin sulfate from shark cartilage (Wang and Tang, 2009), extracting isofraxidin from *Eleutherococcus Senticosus* (Liu et al., 2007), etc. However, application of MCAE in extraction of GBE has not been reported.

In this paper, MCAE has been developed on extraction of GBE to improve the conventional methods. The effects of main operating parameters, namely solid reagent concentration, milling time, ratio of solution to solid, on the extraction yields of GBE were investigated. The MCAE method was compared with the traditional heat reflux extraction (HRE). Furthermore, antioxidant activity of GBE under optimized conditions was determined by means of hydroxyl radical-scavenging assay and superoxide anion radical-scavenging assay.

2. Materials and methods

2.1. Materials and reagents

Ginkgo leaves were purchased from Zhejiang CONBA Pharmaceutical Co., Ltd. which owns a standard ginkgo planting

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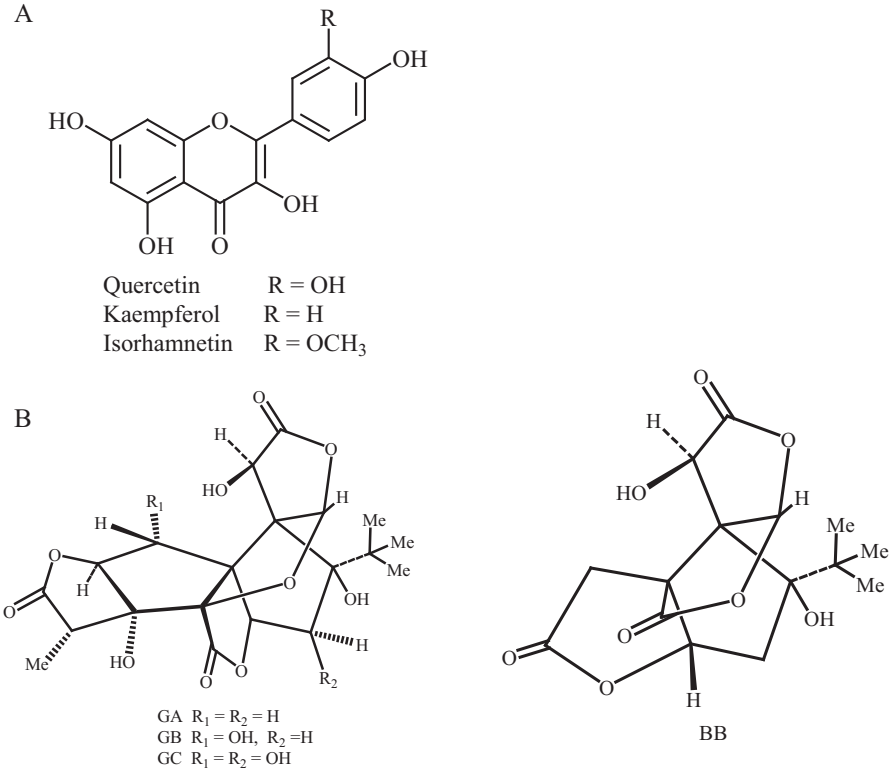

Fig. 1. Molecular structures of the ginkgo flavonoids (A) and ginkgo terpene trilactone (B).

Table 1
Code and level of factors chosen for the trials.

Factor	Levels		
	−1	0	1
Amount of solid reagent (%)	10	20	30
Ratio of solution to solid (mL/g)	20:1	30:1	40:1
Milling time (min)	3	6	9

base. The leaves were dried at 50 °C, pulverized into an average size of 2 mm by a disintegrator, and kept in a dry place until use. Standard flavonoids (99% purity), terpene trilactones (98% purity) were purchased from Chinese Medical and Biological Products Institute. Analytical-grade reagents were purchased from Tianjin Yongda Chemical Reagent Development Centre and HPLC grade solvents were purchased from Tedia Company Inc.

2.2. Mechanochemical-assisted extraction

Ginkgo leaves (20.0 g) and different amounts of solid reagent were added into AGO-2 high-intensity planetary activator. After co-grinding for several minutes, the powder was dissolved in water for a short time. Then the mixtures were centrifuged (4700 rpm, 10 min). The solution pH was adjusted to 5 with dilute acetic acid, then the solution was absorbed into a macroporous resin (ADS-17). The flavonoids and terpene trilactones were eluted with 70% (v/v) ethanol. The GBE product was obtained after the eluant was concentrated and dried, then weighted and analyzed by HPLC. The extraction yield is expressed as the percent ratio of the mass of

Table 2
Experimental conditions for the BBD and the corresponding responses measured.

Exp. no.	X_1	X_2	X_3	A (%)	R (mL/g)	T (min)	Yield of flavonoids (mg/g)	Yield of terpene trilactones (mg/g)
1	−1	−1	0	10	20	6	5.09	1.31
2	−1	1	0	10	40	6	6.13	1.57
3	1	−1	0	30	20	6	5.87	1.43
4	1	1	0	30	40	6	5.97	1.59
5	0	−1	−1	20	20	3	5.34	1.38
6	0	−1	1	20	20	9	6.1	1.56
7	0	1	−1	20	40	3	5.72	1.46
8	0	1	1	20	40	9	5.89	1.58
9	−1	0	−1	10	30	3	6.16	1.62
10	1	0	−1	30	20	3	6.23	1.52
11	−1	0	1	10	30	9	6.45	1.65
12	1	0	1	30	30	9	6.92	1.77
13	0	0	0	20	40	6	6.69	1.84
14	0	0	0	20	40	6	6.83	1.79
15	0	0	0	20	40	6	6.85	1.79
16	0	0	0	20	40	6	6.81	1.78
17	0	0	0	20	40	6	6.97	1.78