Original Research Article

Korean J. Plant Res. 24(3) : 314～318 (2011)

Antioxidant Activity of *Saururus chinensis* Pretreated by Mechanochemical Technology
- Nitrite Scavenging and Electron Donating Ability -

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Abstract - *Saururus chinensis* is used as a raw material of an anti-rheumatic, poultice, sedative and stomachic. The tea of dried *Saururus chinensis* leaves has a function of relaxing pains in back and breast and of treating stomach ailments. The plant has the functions of anti-inflammatory, depurative, diuretic, febrifuge and refrigerant. The extraction of functional material is carried out by methanol and ethanol solvents. Mechanochemical grinding is applied as a pretreatment process before extraction to enhance the extraction efficiency by increasing the surface area of the materials while changing the chemical properties of the materials. Extraction of functional materials from the *Saururus chinensis* after grinding as a pretreatment using the mechanochemical technology was accomplished in this study to investigate the effect of grinding on the antioxidant activities of the extract. *Saururus chinensis* was ground by the planetary ball mill and the morphology was analyzed by SEM. Mechanochemical pretreatment increased the yields of functional materials from 5.9 g to 6.4, 7.0, and 8.1 g after grinding of 30 minutes, 1 hour and 2 hours, respectively. Nitrite scavenging ability increased from 53.0-71.0% to 61.0-79.0% for the methanol extraction in the solution of pH 1.2. Also, Electron donating abilities were increased from 7.90-33.51% to 11.97-38.51% for the methanol extraction. The extract concentration for the half inhibition of DPPH radicals was reduced from 278 μg/mL in the original sample to 263 μg/mL in the sample after grinding for the methanol extraction.

Key words - Mechanochemical grinding, *Saururus chinensis*, Antioxidant activities, Nitrite scavenging ability, Electron donating ability (EDA)

Introduction

Saururus chinensis is used as a raw material of an anti-rheumatic, poultice, sedative and stomachic. An infusion of the roots has been used for treating rheumatism. The root is roasted then mashed and made into a poultice to cure abscesses and boils. A boiled extract of the roots is used as a poultice to heal flesh wound. The dried leaves are used to make a tea, which has a function of relaxing pains in back and breast and of treating stomach ailments. The plant has the functions of anti-inflammatory, depurative, diuretic, febrifuge and refrigerant. A decoction of *Saururus chinensis* is used in the treatment of nephritis-associated oedema, inflammatory conditions and calculus of the urinary system, rheumatoid arthritis, boils and abscesses, rashes and fungal infections of the skin.

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The extraction of functional material is carried out by the complicated process using organic solvents such as methanol and ethanol resulting in time consumption. Mechanochemical technology is applied to grind the material reducing its size while changing the chemical properties by the impact and stress during the grinding process. Because the plant is destroyed in the cell if applied mechanochemical activation, functional material is extracted easily in a short time and without chemical solvent. Crushing machines using mechanochemistry technology are classified as planetary mill (PM), vibrating centrifugal mill (VCM), vortex mill according to the structure and operating principle. In PM, three or four inner containers are revolving (clock wise) around the main axis while rotating (counter clock wise) around its own axis as shown in Fig. 1. The materials to be crushed and some balls are inserted into the containers to make the crushing operation while they are revolving and rotating. Mechanochemical operation not only

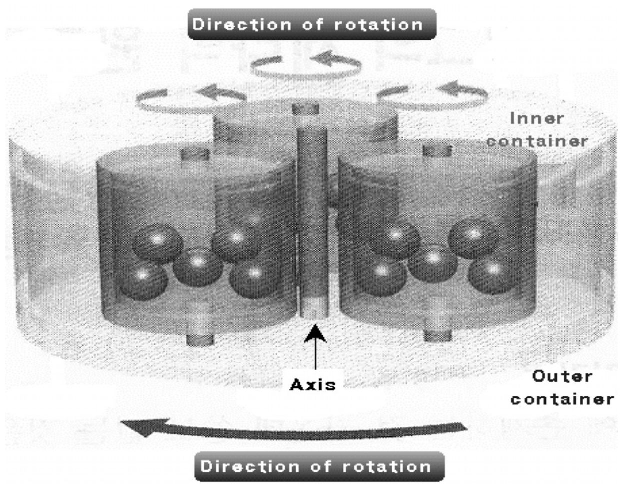

Fig. 1. Structure and operating principle of planetary mill.

increases surfaces of the materials but also destroys the cells for the next extraction. This technology is widely used as a pretreatment process before the extraction of functional materials.

Magnolol from *Magnolia officinalis* was extracted using the mechanochemical technique, using a high intensive activator, AGO-2. Extraction time and temperature were reduced by applying mechanochemical extraction method, and a higher magnolol yield and content in the crude extracts were attained compared with superfine grinding extraction and heat-reflux extraction (Xie and Li *et al*., 2011). The extraction of flavonoids and terpene trilactones from Ginkgo leaves (Zhu *et al*., 2011) was accomplished using the mechanochemical technololgy. Also, the extraction of rutin from *Hibiscus mutabilis* (Xie and Shi *et al*., 2011) was conducted by the mechanochemical-assisted extraction technology. Mechanochemical grinding was also conducted to make the quartz particle to be a surface-active material for the efficient extraction of desired materials (IImura *et al*., 2007; Palaniandy and Azizli, 2009; Zubrik *et al*., 2007).

Nitrite is used as additive to protect acidification. When nitrite reacts with amine in the stomach, it produces cancer-causing substance. However, nitrite scavenging ability by natural plant extracts is high in the solution of pH 1.2 which is similar condition of gastric juice of human body (Boo *et al*., 2011).

This study was aimed to investigate the effect of mechano-chemical pretreatment before solvent extraction on the yields of functional materials and antioxidant activities by comparing the extraction process.

Materials and Methods

Plant material collection

Saururus chinensis is a perennial plant with intrinsic smell and grows in the wet soil near the stream in Korea, Japan, China and etc. The stalk is white and 50-100 cm in length. Flowers open from June and August. *Saururus chinensis* was purchased from the medicinal stuff market of Geumsan, Chungbuk, in Korea and kept under room temperature.

Mechanochemical pretreatment

Before grinding by planetary mill, preliminary crushing was carried out using domestic crusher to make an original dried plant to small pieces of 1-2 mm. Mechanochemical grinding of *Saururus chinensis* was carried out in a planetary ball mill (Pulverisette-5, Fritsch, Germany) with grinding speed of 350 rpm and the grinding periods of 30 min, 1 hour and 2 hours. About 15 g of materials and seven ZrO_2 balls (15 mm in diameter) were inputted in each ZrO_2 container (100 cm^3 inner volume each) for the optimal grinding. The ground products were characterized by scanning electron microscopy (S-4800, Hitachi, Japan) and extraction of functional materials analysis.

Extraction of functional materials

The ground materials were dissolved in the solvent of methanol or ethanol for 5 weeks and three samples were taken for each treatment and filtered using Whatman No.2 filter. After evaporation of the filtrate under reduced pressure using evaporation apparatus (Eyela, Rotary evaporator NE-series, Japan), the powders of functional materials were attained and subsequently analyzed for the extraction efficiency and antioxidant activities.

Nitrite scavenging ability

According to the standard method (Boo *et al*., 2011), after 1 mL of sample was inserted into the reagent 1 mM of $NaNO_2$, and using the 0.1N HCl and 0.2M buffering citric acid, pH was controlled to have 1.2, 3.0 and 6.0 each making the total volume of 10mL. After incubating above solution in a water bath at 37℃ for 1 hr, took 1 mL of solution and added 5 mL of 2% acetic acid and 0.4 mL of Griess reagent (a 1:1 ratio of 1% sulfanilic acid in 30% acetic acid and 1% naphthylamine in 30% acetic acid). Afterward, the mixture was incubated for 15 minutes in room temperature. The residual nitrite capacity was determined by spectrophotometric analysis at 520 nm. Control vial was made using the 1 mL of distilled water instead of sample. Nitrite scavenging ability was acquired by comparing the vial with extraction and the one without as follows:

Nitrite scavenging ability (%) = (1 − absorbance of the solution with extraction/absorbance of the solution without extraction) x 100

Electron donating ability (EDA)

Antioxidant ability for the functional materials are determined by the measurement of free radical scavenging using DPPH (2,2-diphenyl−1−pycrylhydrazyl) radical, inhibition effect of MDA-BSA conjugation reaction, inhibition effect of tyrosinase, hydroxyl radical scavenging and etc. Among them, DPPH radical is commonly used for evaluating antioxidant effect in a relatively short time. The inhibition of DPPH radicals is determined by the decrease in their absorbance due to the antioxidant. Electron donating ability was determined by the method of DPPH (Blois, 1958; Yang *et al*., 2011). After dissolving 12 mg of DPPH reagent into ethanol, added 50 % of ethanol to have absorbance 1 of DPPH solution at 517 nm. Afterwords, 0.5 mL of extract was mixed with 5 mL of DPPH solution and measured absorbance. Electron donating ability (EDA) was calculated by the following equation using the absorbance data with three replications.

$$EDA (\%) = (A_0 - A)/A_0 * 100$$

Where A_0 : Absorbance of DPPH solution without sample
A : Absorbance of DPPH solution mixed with extract

Results and Discussion

SEM photos were acquired for the ground materials of *Saururus chinensis* with magnification of 500 and 2,000 times. The materials were divided into small pieces from 1-3 mm before mechanochemical grinding to 1-100 μm after two hours of grinding. The particle size has not changed so much after 30 minutes of grinding as shown in Fig. 2.

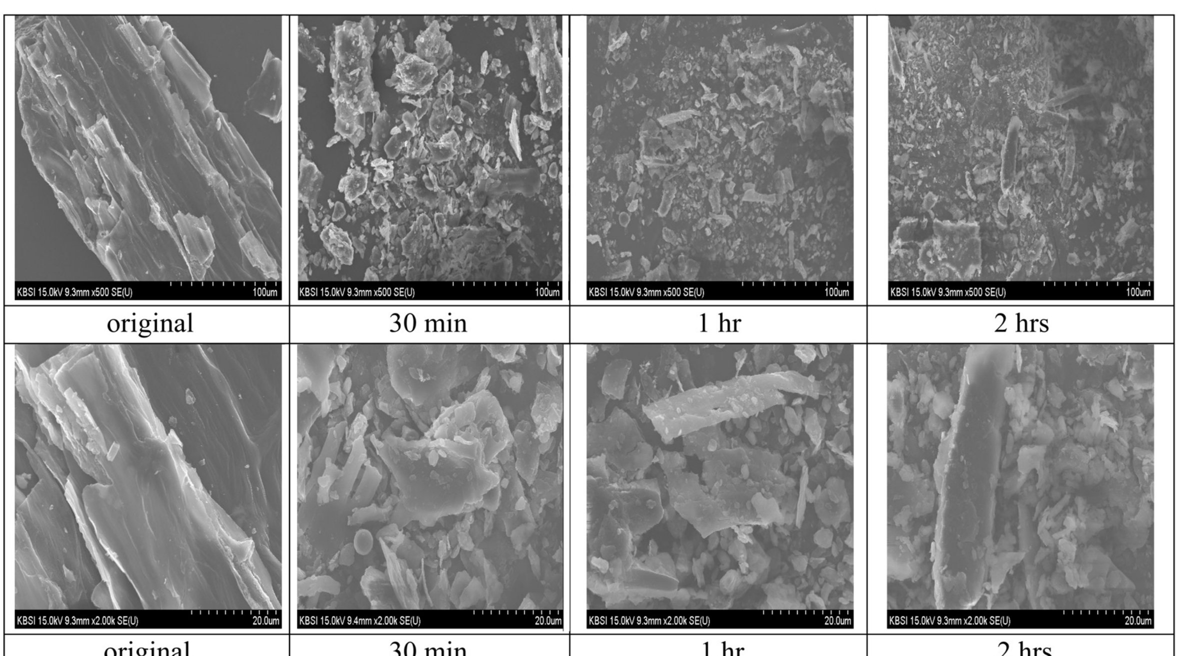

Fig. 2. SEM photos of *Saururus chinensis* (Upper: x500, Lower: x 2,000 times).

Table 1. Nitrite scavenging abilities from leaf extracts of *Saururus chinensis*.

Concentration (μg/mL)		Nitrite-scavenging ability (%)			
		Methanol extract		Ethanol extract	
		Control	2 hr	Control	2 hr
pH 1.2	50	57 ± 0.21[a]	69 ± 0.84[a]	58 ± 1.47[a]	64 ± 1.47[a]
	100	63 ± 1.36[ab]	71 ± 1.48[ab]	61 ± 1.69[ab]	67 ± 1.69[ab]
	300	68 ± 1.42[ab]	75 ± 1.65[ab]	64 ± 1.51[ab]	69 ± 1.51[ab]
	600	77 ± 1.88[b]	86 ± 1.96[b]	71 ± 1.63[b]	77 ± 1.63[b]
pH 3.0	50	11 ± 1.48[a]	10 ± 1.46[a]	6.0 ± 1.11[a]	7.1 ± 1.11[a]
	100	25 ± 1.42[ab]	27 ± 1.52[ab]	8.8 ± 1.49[ab]	13 ± 1.49[ab]
	300	30 ± 1.39[ab]	31 ± 1.78[ab]	15 ± 1.58[ab]	20 ± 1.58[ab]
	600	32 ± 1.70[b]	39 ± 1.72[b]	18 ± 1.77[ab]	28 ± 1.77[ab]
pH 6.0	50	0.00 ± 0.0[a]	0.00 ± 0.0[a]	0.00 ± 0.0[a]	0.00 ± 0.0[a]
	100	0.00 ± 0.0[a]	0.00 ± 0.0[a]	0.00 ± 0.0[a]	0.00 ± 0.0[a]
	300	0.00 ± 0.0[a]	0.00 ± 0.0[a]	0.00 ± 0.0[a]	0.00 ± 0.0[a]
	600	1.7 ± 1.18[b]	3.1 ± 1.29[b]	1.06 ± 1.39[b]	2.07 ± 1.39[b]

Table 2. Electron donating abilities from leaf extracts of *Saururus chinensis*.

Sample	Concentration (μg/mL)	Electron donating ability (%)		IC_{50}[1)] (μg/mL)	
		Control	2 hr	Control	2 hr
Methanol extract	10	10.01 ± 2.72[a2)]	11.01 ± 2.90[a2)]	241	223
	20	11.47 ± 2.79[b]	13.47 ± 2.92[b]		
	40	15.39 ± 1.87[c]	28.39 ± 1.99[c]		
	60	40.29 ± 1.49[d]	49.29 ± 1.87[d]		
Ethanol extract	10	10.10 ± 1.86[a]	9.84 ± 1.79[a]	278	267
	20	11.35 ± 1.63[b]	12.35 ± 1.87[b]		
	40	24.49 ± 1.78[c]	26.49 ± 1.81[c]		
	60	37.69 ± 2.00[d]	43.69 ± 2.07[d]		

[1)] IC_{50} values represent the concentration required for 50% inhibition DPPH.
[2)] All values are mean ± SD of triplicate determinations. Different letters (a-d) within the same groups differ significantly ($P<0.05$).

Extraction of functional materials

The extraction amount of functional materials from *Saururus chinensis* was increased from 5.9 g in the sample before grinding to 6.4, 7.0, and 8.1 g after grinding of 30 minutes, 1 hour and 2 hours, respectively.

Nitrite scavenging ability

Nitrite acid reacts with amine resulting in the generation of nitrosamines some of which are transferred into diazoalkane in the human body and cause the cancer. To investigate the effect of mechanochemical grinding, the sample pulverized for 2 hrs by planetary ball mill was compared with the sample without grinding (control). The nitrite scavenging ability was determined by spectrophotometric analysis at 520 nm as table 1.

Nitrite scavenging ability was a little higher for the methanol extraction than that for the ethanol extraction. As pH increased from 1.2 to 6.0, nitrite scavenging ability decreased so much showing almost zero at pH 6.0. This result is consistent with the previous results for the extraction of onion peel, redbeet, yellow gardenia, mature foxglove and etc. (Al-Dabbas *et al*., 2007; Boo *et al*., 2011).

For pH value of 1.2, as the concentration varied from 50 μg/mL to 600 μg/mL, nitrite scavenging ability increased from 53.0-71.0% to 61.0-79.0% for the methanol extraction while from 43.0-68.0% to 59.0-73.0% for the ethanol extraction. For pH value of 3.0, as the concentration varied from 50 μg/mL to 600 μg/mL, nitrite scavenging ability increased from 8.0-23.0% to 10.0-33.0% for the methanol extraction while from 6.6-19.0% to 7.3-29.0% for the ethanol extraction. For pH value of 6.0, nitrite scavenging abilities were 0% for all the extract concentration except 800 μg/mL.

Electron donating ability (EDA)

Antioxidant ability of *Saururus chinensis* was evaluated by the method using DPPH (2,2-diphenyl−1− pycrylhydrazyl) radical. This method measures the spectrophotometric absorbance by DPPH radicals in the solution. DPPH scavenging abilities were increased from 7.90-33.51% to 11.97-38.51% for the methanol extraction, and from 8.77-35.97% to 9.56-39.97% for the ethanol extraction as a result of mechanochemical grinding. The extract concentration for the half inhibition of DPPH radicals was reduced from 278 μg/mL in the original sample to 263 μg/mL in the sample after grinding for the methanol extraction, and from 311 μg/mL to 299 μg/mL for the ethanol extraction.

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(Received 15 May 2011 ; Accepted 18 June 2011)