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오동나무 추출물의 항산화 효능※

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Antioxidative activities of *Paulownia coreana* extractives※

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요 약

오동나무 잎, 열매, 내수피, 외수피 그리고 목질부를 채취하여 건조시킨 후 분말로 제조하여 각 시료 약 5.0 kg을 아세톤-물(7:3, v/v) 혼합용액으로 추출하고 유기용매를 제거한 후 헥산, 메틸렌클로라이드, 에틸아세테이트 및 물 등 네 개의 분획으로 분리하였다. 오동나무 각 부위별 추출물 수율은 내수피(11.35%)가 가장 높았고 잎(7.87%), 열매(6.94%), 외수피(4.24%)의 순 이었으며 목질부가 4.10%로 가장 낮게 나타났다.

오동나무 각 부위별 조추출물, 고형분과 분획물을 가지고 DPPH radical 소거법을 이용하여 기초적인 항산화 실험을 실시하였다. 기준물질로는 천연 항산화제인 α-tocopherol과 합성항산화제인 BHT를 사용하여 항산화효능을 비교하였다. 그 중에서는 모든 EtOAc용성 분획이 기준물질 보다 더 우수한 항산화 활성을 나타내었다.

ABSTRACT

Approximately each 5 kg dried *Paulownia coreana* leaves, seeds, outer barks, inner barks and wood were ground, extracted with acetone-H_2O (7:3, v/v), concentrated under reduced pressure and successively fractionated using *n*-hexane, CH_2Cl_2, EtOAc and H_2O on a separation funnel. Each fraction was then freeze dried and weighed. The extraction yield of each sample was investigated and the yield of inner barks was the highest, while the wood was the lowest.

Antioxidative activities of crude extracts, precipitates and partitioned fractions were evaluated by DPPH radical scavenging method. The results indicated that all EtOAc soluble fractions, including some of H_2O soluble fractions, showed significantly high antioxidative potential compared with α-tocopherol and BHT used as controls.

Key words : Paulownia coreana, antioxidative activities, extract, DPPH

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Ⅰ. INTRODUCTION

Paulownia coreana (Scrophulariaceae) is a deciduous tree indigenous to the Korean peninsula, especially to Ullungdo of South Korea and has been cultivated together with *Paulownia tomentosa* throughout the sightseeing places and villages as a honey and ornament species. Its wood is widely used for construction, furniture making, musical instrument and handicrafts (Kim, 1994; Ayan *et al.*, 2003). The tree has also been used as traditional medicines for a long time to treat symptoms such as bronchitis, cough, phlegm, carbuncle, traumatic bleeding, hemorrhoid, gonorrhea, erysipelas, asthma, high blood pressure, upper respiratory tract infection, bronchopneumonia, tonsillitis, bacteriologic diarrhea, enteritis, conjunctivitis, parotitis and swelling (Jiangsu New Medical College, 1977).

Some studies on the chemical compositions of *P. coreana* flowers (Oh *et al.*, 2000) and young plant (Damtoft and Jensen, 1993) have been reported. We have been previously investigated the chemical composition of *P. coreana* outer barks (Si *et al.*, 2005a), leaves (Si *et al.*, 2005b; Si *et al.*, 2006a; Si *et al.*, 2006b) and fruits (Si *et al.*, 2006c). However, the biological activities are still one of research topics to be investigated and as a part of our researches on *P. coreana*, we report the antioxidative activities of extracts of this species in this paper.

Ⅱ. MATERIALS and METHODS

1. Plant materials

The leaves, seeds, inner barks, outer barks and wood of *P. coreana* (10 years old, DBH, 35 cm) were collected from the experimental forest of Kangwon National University in September and October of 2002. A voucher specimen of each sample was deposited in the Department of Wood Science and Engineering, College of Forest Science, Kangwon National University. Sample of each part was air-dried at room temperature for three weeks and then ground to fine powder by Wiley mill to be extracted.

2. Extraction and Fractionation

Each ground sample (5 kg) was soaked in acetone-H_2O (7:3, v/v) in a glass jar (20 ℓ) at room temperature for more than 3 days and then the acetone-H_2O solution was decanted and filtered to give a crude extract. Each batch was extracted four times to obtain enough extracts. The combined extractants were then concentrated on a rotary evaporator under reduced pressure at 35~40 ℃. Then mixture was sequentially fractionated with *n*-hexane, CH_2Cl_2, EtOAc and H_2O in a separation funnel. Each fraction was concentrated and freeze dried to get precipitates, *n*-hexane soluble fraction, CH_2Cl_2 soluble fraction, EtOAc soluble fraction and H_2O soluble fraction.

3. DPPH free radical scavenging assay

The antioxidative activities were determined on the basis of the scavenging activity of the stable DPPH free radical method firstly introduced by M. S. Blois (Blois,

1958) with slight modification. Samples of different concentrations (20~160 ug/ml) were added to a solution of DPPH ($1.5x10^{-4}$ M, 1 ml) in 4 ml MeOH. After mixing gently and standing at room temperature for 30 min, the optical density was measured at 517 nm with a UV-visible spectrophotometer (Libra S32, Biochrom LTD). The results were calculated by taking the mean of all triplicated values. IC_{50} values were obtained through extrapolation from concentration of sample necessary to scavenge 50% of the DPPH free radicals. BHT and α-tochopherol were used as controls.

Ⅲ. RESULTS and DISCUSSION

1. Extraction yields of samples

The air-dried and ground samples were extracted with acetone-H_2O (7:3, v/v) for several days at room temperature.

The results, as shown in Table 1, indicated that the extraction yield of inner barks was the highest (11.35%), while that of the wood was the lowest (4.10%). In the *n*-hexane soluble fractions, the extraction yield were in order of leaves (0.59%), inner barks (0.52%), outer barks (0.32%), wood (0.10%) and seeds (0.10%). In the CH_2Cl_2 soluble fractions, the extraction yields were in order of inner barks (0.46%), outer barks (0.18%), wood (0.15%), leaves (0.13%) and seeds (0.10%). In the EtOAc soluble fractions, the extraction yields of inner barks, seeds, wood, outer barks and leaves were 0.48%, 0.42%, 0.39%, 0.30% and 0.23%, respectively. In the H_2O soluble fractions, the extraction yields were in order of inner barks (9.71%), leaves (4.86%), seeds (4.65%), wood (3.14%) and outer barks (1.80%). Precipitates were obtained only in the extracts of leaves, seeds and outer barks fractions and the yields were 1.99%, 1.61% and 1.52%, respectively.

Table 1. Extraction yields of *P. coreana* samples

Part	Sample weight (g)	Stored crude extract (g)	Precipitate (g)	Hexane soluble fraction (g)	CH_2Cl_2 soluble fraction (g)	EtOAc soluble fraction (g)	H_2O soluble fraction (g)	Total yield (g)
Leaves	5060	3.4 (0.07%)	100.7 (1.99%)	30 (0.59%)	6.7 (0.13%)	11.7 (0.23%)	245.9 (4.86%)	398.4 (7.87%)
Seeds	5000	4.6 (0.10%)	80.5 (1.61%)	4.4 (0.10%)	4.2 (0.10%)	20.8 (0.42%)	232.3 (4.65%)	346.8 (6.84%)
Outer barks	5020	6.5 (0.13%)	76.3 (1.52%)	15.8 (0.32%)	9.1 (0.18%)	15.0 (0.30%)	90.3 (1.80%)	213 (4.24%)
Inner barks	5000	8.6 (0.17%)	-	25.9 (0.52%)	23.2 (0.46%)	24.0 (0.48%)	485.6 (9.71%)	567.3 (11.35%)
Wood	5000	12.1 (0.24%)	-	4.9 (0.10%)	7.7 (0.15%)	19.7 (0.39%)	156.8 (3.14%)	201.2 (4.10%)

Table 2. Antioxidative activities (IC_{50} values) of crude extracts, precipitates and partitioned fractions of *P. coreana*

Samples			IC_{50} (μg)
Controls		α-Tocopherol	26
		BHT	30
Fractions	Leaves	Hexane soluble fraction	91
		CH_2Cl_2 soluble fraction	190
		EtOAc soluble fraction	14
		H_2O soluble fraction	90
		Precipitate	87
		Crude extract	121
	Seeds	Hexane soluble fraction	29
		CH_2Cl_2 soluble fraction	66
		EtOAc soluble fraction	20
		H_2O soluble fraction	25
		Precipitate	45
		Crude extract	31
	Outer barks	Hexane soluble fraction	42
		CH_2Cl_2 soluble fraction	43
		EtOAc soluble frcation	20
		H_2O soluble frcation	40
		Precipitate	35
		Crude extract	33
	Inner barks	Hexane soluble fraction	45
		CH_2Cl_2 soluble fraction	47
		EtOAc soluble fraction	18
		H_2O soluble fraction	17
		Crude extract	32
	Wood	Hexane soluble fraction	47
		CH_2Cl_2 soluble fraction	48
		EtOAc soluble fraction	16
		H_2O soluble fraction	21
		Crude extract	27

2. Antioxidative activities(IC_{50} values)

The DPPH radical scavenging capacity tests (IC_{50} values) on the crude extracts, precipitates and solvent partitioned fractions of leaves, seeds, outer barks, inner barks and wood were shown in Table 2. Results indicated that the antioxidative potentials of the crude extracts of leaves (121 μg), outer barks (33 μg), inner barks (32 μg), seeds (31 μg) and wood (27 μg) were more effective. Results also indicated that the EtOAc soluble fractions of all samples and the H_2O soluble fractions of seeds (25 μg), wood (21 μg) and inner barks (17 μg) exhibited significantly high activities, while the rest fractions, crude extracts and precipitates showed weak or none activities compared with BHT (30 μg) and α-tocopherol (26 μg). Therefore, most

of the EtOAc soluble fractions of *P. coreana* could be a useful source of natural antioxidants.

Ⅳ. CONCLUSION

The extraction yield of each sample was investigated and the yield of the inner bark was the highest (11.35%), while the wood was the lowest (4.10%). The DPPH radical scavenging effect of the samples indicated that all the EtOAc soluble fractions, including some of the H_2O soluble fractions, showed significantly high antioxidative potential compared with BHT and α-tocopherol as controls. This fact suggested that the extracts of *P. coreana* could be a new source of natural antioxidants.

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