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Tyrosinase Inhibition and Mutagenicity of Phenolic Compounds from Mulberry Leaves

·Research Note·

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Abstract

The tyrosinase inhibition activity and mutagenicity as assessed by the Ames test on phenolic antioxidants (5-Caffeoyl quinic acid, 3,4-Dihydroxy cinnamic acid, Quercetin 3-O-β-D-glucopyranose, Kaempferol 3-O-β-D-glucopyranose) and the ethyl acetate fraction isolated from mulberry leaves were examined. The ethyl acetate fraction and chlorogenic acid exhibited weaker tyrosinase inhibitory activities than kojic acid. In addition, the ethyl acetate fraction from mulberry leaves, containing phenolic antioxidants, showed no mutagenicity by the Ames test.

Key words: mulberry leaves, chlorogenic acid, tyrosinase inhibition, mutagenicity

INTRODUCTION

Plant leaves usually have many polyphenolic compounds, such as simple phenolic acids, phenyl propanoids and flavonoids which provide plant tissue protection tissue from UV radiation. These polyphenolic compounds act as phytoalexins, preventing plant diseases that can be caused by fungi and bacteria. As the sericultural industry has been gradually decreasing in Korea as a source of functional bioactive compounds, mulberry (*Morus alba* L.) leaves are increasingly used as health-promoting nutritional ingredients in such foods as noodles, cakes and tea products. Mulberry leaves contain many nutritional and functional compounds, and have exhibited antihyperglycemic activity in diabetic mice (1), and contain bioactive compounds, including flavonoids (2-5) and 1-deoxynojirimycin (6), with demonstrated pharmacological properties. Tyrosinase is a copper containing mixed-function polyphenol oxidase widely distributed in microorganisms, animals, and plant tissues, which catalyzes the hydroxylation of monophenol which facilitates melanin synthesis and formation of pigments (7,8). The tyrosinase inhibition activity of some flavonoids such as kampferol, quercetin, and morin have been examined for their copper chelating ability in the binuclear active center of the enzyme (9,10). Furthermore, quercetin showed inhibitory effects on the melanogenesis using an assay using melan-a melanocyte cell and mushroom tyrosinase (11). We isolated and identified some phenolic antioxidants from the ethyl acetate fraction of the mulberry leaves (12) by assaying the antioxidant activity of commercially grown leaves. The tyrosinase inhibition activity and the mutagenicity of these phenolic compounds and the ethyl acetate fraction were also examined.

MATERIALS AND METHODS

Phenolic compounds and ethyl acetate fractionation of mulberry leaves

Dried and powdered leaves of *M. alba* L. (Chongil) were extracted with methanol and then fractionated with chloroform, ethyl acetate, and *n*-butanol. The active compound of the ethyl acetate fraction was isolated using silica gel column chromatography with chloroform/water/methanol (upper layer) as an eluting solvent. Four phenolic compounds (Compound 1: 5-Caffeoyl quinic acid, Compound 2: 3,4-Dihydroxy cinnamic acid, Compound 3: Quercetin 3-O-β-D-glucopyranose; Compound 4: Kaempferol 3-O-β-D-glucopyranose) were isolated and purified with ODS-preparatory HPLC, and their chemical structures were identified with instrumental analyses including FAB-MS and NMR (12).

Tyrosinase inhibition activity

Tyrosinase inhibition by the purified compounds was examined by the method of Kubo et al. (13) using L-3,4-dihydroxyphenylalanine (L-DOPA) as substrate.

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Mutagenicity by Ames test

The mutagenic effects of the compounds and extract were evaluated using the Ames test with *S.* Typhimurium strains TA98, frame shift mutant and TA100, a base pair exchange mutant (14). The assay was performed with and without the S9 mixture. The S9 mixture was prepared from Sprague-Dawley male rats weighing approximately 200 g which were treated with Aroclor 1254 (500 mg/kg). The criteria for a positive response were a doubling frequency for TA98 and TA100, of the revertant solvent control value. Data were expressed as the mean and standard deviation of three plates.

RESULTS AND DISCUSSION

The tyrosinase inhibition activity

Tyrosinase is known to be a key enzyme for melanin biosynthesis through the hydroxylation of tyrosine and the oxidation of L-DOPA. The inhibition of tyrosinase by natural products has been widely studied with regard to the prevention of skin melanogenesis and in the development whitening cosmetic agents. Among the compounds isolated, only chlorogenic acid showed tyrosinase inhibition activity, although it was weaker than that of kojic acid (Table 1). Tyrosinase inhibition by some phenolic compounds may be due to interactions with metal ions in the catalytic site of the enzyme (15). Tada et al. (16) had also reported strong tyrosinase inhibitory activity of quinic acid derivatives such as chlorogenic acid. However, it was reported that flavonol glycosides did not show any effect on tyrosinase inhibition (17). Lee et al. (18) also reported that mulberroside F, isolated from the butanol fraction of mulberry leaves, could inhibit melanin biosynthesis. Hence, several lines of evidence suggest that the ethyl acetate fraction from mulberry leaves, with its strong tyrosinase inhibition activity, could be used as a whitening cosmetic agent (19).

Table 1. The tyrosinase inhibition activity of phenolic compounds from mulberry leaves

Compounds[1)]	Activity (%)
Compound 1	20.76±3.25
Compound 2	–[2)]
Compound 3	–
Compound 4	–
EtOAc fraction	23.43±5.52
Kojic acid	78.42±8.36
Ascorbic acid	18.12±6.23

[1)]Compound 1: chlorogenic acid, Compound 2: caffeic acid, Compound 3: quercetin 3-*O*-D-glucopyranoside, Compound 4: kaempferol 3-*O*-D-glucopyranoside.
[2)]No effect.
The final concentrations of all compounds tested were 0.16 mg/mL.

Table 2. The mutagenicity of the phenolic compounds from mulberry leaves by the Ames test

Compounds[1)]	Dose (mg/plate)	Revertants / plate			
		Without S-9 mix		With S-9-mix	
		TA98	TA100	TA98	TA100
Spontaneous		17±2	157±13	22±3	145±12
Compound 1	0.5	20±3	153±6	21±2	164±6
	1.0	21±4	151±7	21±3	159±14
	2.0	24±2	164±10	26±3	157±9
	4.0	21±2	160±5	25±1	170±7
Compound 2	0.5	19±2	157±10	24±4	162±12
	1.0	21±3	150±8	25±5	147±14
	2.0	23±0	168±12	24±3	168±10
	4.0	21±1	170±7	20±2	153±7
Compound 3	0.5	17±4	154±14	21±3	156±12
	1.0	23±2	150±6	22±5	157±9
	2.0	19±3	171±11	26±3	164±10
	4.0	20±0	150±10	27±2	161±8
Compound 4	0.5	21±2	153±9	21±3	151±12
	1.0	23±1	161±9	25±1	154±11
	2.0	22±3	170±10	23±2	162±10
	4.0	19±2	149±7	26±3	152±8
EtOAc fraction	0.5	20±2	152±8	22±3	154±11
	1.0	21±1	159±9	25±1	157±10
	2.0	24±4	165±11	27±3	164±9
	4.0	21±4	153±8	26±3	153±8

[1)]The same as in Table 1.

Mutagenicity by the Ames test

The mutagenecity of the four isolated compounds and ethyl acetate fraction from mulberry leaves were examined by an Ames test using *S.* Typhimurium TA98, a frame shift mutant (Table 2), and TA100, a base pair exchange mutant. They all showed a mutant frequency value below 2.0, and there was no dose-dependent increase within the range of the test solution (0.5~4.0 mg/plate). In previous studies, quercetin was reported to be a genotoxic compound (20,21); however, not all compounds found to be mutagenic by the Ames test are always carcinogenic *in vivo*. Moreover, quercetin might be the major flavonoid in most edible plants and in several studies it has exhibited no *in vivo* carcinogenicity (22). In conclusion, the ethyl acetate fraction from mulberry leaves, containing phenolic antioxidants had no mutagenicity and exhibited potential functionality as an antioxidant and whitening agent.

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