The Journal of Applied Pharmacology, 11, 245-248(2003)

Tyrosinase Inhibitory Activity of Plant Extracts (III): Fifty Korean Indigenous Plants

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(Received , September 29 2003; Accepted November 10, 2003)

Abstract – The purpose of this study was to evaluate tyrosinase inhibitory activity of plant extracts, especially Korean indigenous plants, for the cosmetic use of skin whitening. When 50 plant extracts were tested, the methanol extracts of *Agrimonia pilosa, Aster scaber, Dianthus sinensis, Fatsia japonica, Hemistepta lyrata, Lespedeza cuneata, Osmunda japonicum, Pyrola japonica, Rodgersia phodophylla and Veratrum grandiforum* possessed the considerable tyrosinase inhibitory activity at 3-300 μg/mL. Especially, *L. cuneata*, aerial part of *O. japonicum* and *V. gandiforum* exhibited the strong inhibition (>50% inhibition at 300 μg/mL). In particular, the methanol extract of *V. grandiforum* and its ethylacetate fraction showed potent inhibition (IC_{50} = 30 and 13 μg/mL, respectively), while the reference compound, kojic acid, showed IC_{50} value of 26 μg/mL. These plant extracts may be used as tyrosinase inhibitors in cosmetics.

Keywords □ Tyrosinase, plant, skin whitening, *Lespedeza cuneata, Osmunda japonicum, Veratrum grandiforum*

INTRODUCTION

The color of human skin is determined mainly by melanin content. Enzymatic oxidative as well as non-enzymatic autooxidation processes lead to the formation of melanin from tyrosine. The rate-limiting enzymatic oxidation process is carried out by a mixed function oxidase, tyrosinase (EC 1.14.18.1) converting tyrosine to 3,4-dihydroxyphenylalanine (DOPA) (Sanchez-Ferrer *et al.*, 1995). Therefore, a regulation of skin tyrosinase activity may change skin darkness. Since skin whitening is one of the important issues for cosmetic industry, especially in North-East Asia, several tyrosinase inhibitors have been used. Examples are the synthetic chemical inhibitors such as arbutin and kojic acid. In addition, the plant extracts from *Glycyrrhiza glabra* and *Morus alba* have been also used. Recently, *Ramulus mori* extract has been demonstrated as a potent tyrosinase inhibitor and currently used as a skin whitening agent (Lee *et al.*, 2003). But, there is always a need for new skin whitening agents. Thus, as our continual efforts to search useful skin whitening agents (Lee *et al.*, 1997a and Kim *et al.*, 2003) and others (Lee *et al.*, 1997b; Chi *et al.*, 1998; Choi *et al.*, 2001), the methanol extracts from 50 Korean indigenous plants were prepared and evaluated for their tyrosinase inhibitory activity in this investigation.

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MATERIAL AND METHODS

Preparation of plant extracts

The plant materials used in this study were obtained from the various parts of the Korean peninsula, and identified by one of the authors, Dr. K. Bae. All specimens were deposited at the Herbarium of Chungnam National University under the serial numbers for each plant materials. They were dried and finely chopped. Each material (100 g) was soaked in 300 mL methanol at room temperature for 3 days. After filtration, the filtrates were evaporated to dryness under vacuum and used throughout this study. The methanol extract of *Veratrum grandiforum* was dried, and dissolved in distilled water. The solution was further partitioned serially with n-hexane, ethylacetate and n-butanol.

Tyrosinase assay

Tyrosinase activity was determined essentially based on the previously described procedure (Vanni *et al.*, 1990) with slight modification. In brief, the test reaction mixture comprised of each plant extract, mushroom tyrosinase (105 units, Sigma-

Aldrich) and 0.15 mg L-tyrosine in 0.05 mM sodium phosphate buffer (pH 6.8). The reaction mixture (1.5 mL) was incubated at 37°C for 10 min, and the absorption at 475 nm was measured. And the absorbance of the same mixture without tyrosinase was used as the control.

RESULTS AND DISCUSSION

Fifty plant extracts were tested for their inhibitory activity against tyrosinase. Table 1 summarized the experimental results. Majorities of the extracts did not possess tyrosinase inhibitory activity, or possessed weak activity (less than 30% inhibition) if any at the concentrations tested (3-300 μg/mL). Among the extracts tested, several plant extracts such as *A. pilosa, A. scaber, F. japonica, H. lyrata, P. japonica* and *V. grandiforum* showed the considerable inhibition (more than 20%) at lowest concentration (3 μg/mL). And plant extracts including *A. pilosa, A. scaber, D. sinensis, H. lyrata, L. cuneata, O. japonicum, P. japonica, R. phodophylla* and *V. grandiforum* possessed the strong inhibition at 30 and 300 μg/mL. These plant materials have advantages for further investigation, although most of them did not show the comparable

Table 1. Tyrosinase inhibitory activity of the plant extracts

plant name	part used	inhibition of tyrosinase[a]		
		3 μg/mL	30 μg/mL	300 μg/ml
Adenocaulon himalaicum	whole	-[b]	-	7
Agrimonia pilosa	whole	28	29	38
Aster scaber	whole	20	20	25
	aerial	25	36	40
	root	-	-	-
Breea segetum	whole	1	-	-
Callicarpa japonica	stem	-	-	-
Catalpa ovata	stem	-	-	34
Cercis chinensis	aerial	-	-	11
Chelidonium majus	aerial	-	7	5
Chrysanthemum boreale	flos	-	8	23
Cimicifuga simplex	root	-	8	25
Clematis apiifolia	aerial	-	-	-
Cocculus trilobus	stem	3	15	17
Convallaria keiskei	whole	9	6	6
Daphniphyllum macropodum	fruits	-	-	-
Dianthus sinensis	whole	13	18	32
Eupatorium chinensis	whole	6	-	10
Euphorbia siebaldiana	whole	5	-	-
Fatsia japonica	aerial	21	-	22
Geum japonicum	whole	-	-	-
Hemistepta lyrata	whole	26	30	36
Hydrocharis dubia	whole	10	8	12
Hypericum erectum	aerial	-	-	-
Kerria japonica	aerial	1	3	10
Lespedeza cuneata	whole	-	19	60
Ligularia fischerii	whole	-	-	39
Lindera obtusiloba	stem	6	8	13
Lychnis cognata	whole	-	-	28
Magnolia denudata	fruits	-	-	-
Melandryum firmum	whole	-	2	17
Metaplexis japonica	whole	-	-	18
Osmunda japonicum	aerial	18	24	54
	root	-	3	1

Table 1. Continued

plant name	part used	inhibition of tyrosinase[a]		
		3 μg/mL	30 μg/mL	300 μg/ml
Parnassia palustris	whole	-	1	2
Peucedanum terebinthaceum	aerial	-	-	35
Phryma leptostachya	root	7	-	7
Pleuropterus cilinervis	rhizoma	-	-	-
Potentilla chinensis	whole	-	-	-
Pyrola japonica	whole	27	20	38
Rhamnus davurica	branch	16	-	47
Rodgersia podophylla	root	-	31	32
Rubia akane	aerial	-	-	1
	root	-	-	5
Salsola collina	whole	17	-	34
Salvinia natans	whole	-	-	2
Siegesbeckia glabrescens	stem	-	-	-
Solidago virga-aurea	whole	9	7	12
Sorbus commizta	stem cortex	-	-	1
Syneilesis palmate	whole	3	5	8
Tripterygium regelii	stem	-	9	-
Veratrum grandiflorum	root	36	54	85
Xanthium strumarium	whole	-	-	19
Youngia denticulate	whole	-	-	-
Kojic acid		36	58	96

[a]Tyrosinase (105 U) showed 0.22-0.43 abs. at 475 nm by 10 min incubation in our experiments. Percent inhibition shown here represents an arithmetic mean of triplicate experiments. [b]No inhibition was observed.

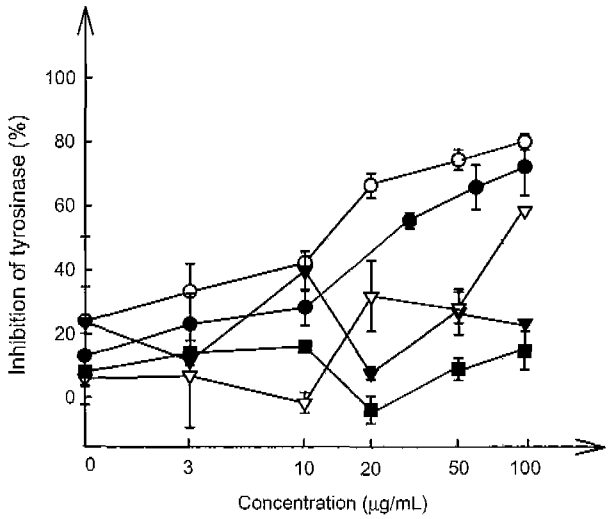

Fig. 1. Tyrosinase inhibition of fractions from the methanol extract of *V. grandiforum*. Kojic acid (●), hexane (▼), ethylacetate (○), butanol (▽) and water (■) fractions. Points and bars represent the arithmetic mean ± standard deviation (SD) from triplicate experiments.

activity to kojic acid. To the best of our knowledge, this is the first report demonstrating tyrosinase inhibitory activity of the above mentioned plant materials. Especially, *L. cuneata*, arial part of *O. japonicum* and *V. grandiforum* exhibited more than 50% tyrosinase inhibitory activity at 300 μg/mL. In particular, the methanol extract of *V. grandiforum* showed the potent inhibition (IC_{50} = 30 μg/mL), comparable to kojic acid. Fig. 1 showed concentration-dependent tyrosinase inhibition of four different fractions from *V. grandiforum* extract. Of these fractions tested, the ethylacetate fraction gave the strongest inhibition of tyrosinase (IC_{50} = 13 μg/mL), being twice more potent than kojic acid (IC_{50} = 26 μg/mL). The butanol fraction also showed potent inhibition of tyrosinase (IC_{50} = 86 μg/mL). Considering inhibitory potency of the methanol extract and its ethylacetate fraction, *V. grandiforum* has a potential to develop as a skin whitening agent.

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