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Tyrosinase Inhibitory Activity of 80 Plant Extracts (II)

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Abstract – The purpose of this study was to evaluate tyrosinase inhibitory activity of plant extracts for cosmetic use. When 80 plant extracts were tested, the methanol extracts of *Allium thunbergi, Asparagus oligoclonos, Ixeris dentate, Salvia plebeia, Sophora flavescens* and *Sophora japonica* showed more than 30% inhibition of mushroom tyrosinase activity at 100 μg/mL. Although less active than the reference compound, kojic acid (IC_{50}=7.0–16.3 μg/mL), these plant extracts may be used as tyrosinase inhibitors in cosmetics.

Keywords – tyrosinase, plant, skin whitening, *Salvia plebeia, Sophora flavescens.*

INTRODUCTION

The color of human skin is determined mainly by melanin content. Melanin is synthesized from tyrosine by enzymatic oxidative as well as non-enzymatic autooxidation processes. Among these sequential reactions, the most important and rate-limiting step is oxidation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) by a mixed function oxidase, tyrosinase (EC 1.14.18.1) (Sanchez-Ferrer *et al.*, 1995). Therefore, the inhibition of tyrosinase activity may lead to reduction of skin darkness. Especially, in East Asia, skin whitening is an important issue for cosmetic industry. Several tyrosinase inhibitors including arbutin, kojic acid and Glycyrrhizae radix extract have been widely used. But, there is always a need for new skin whitening agents. Thus, as our continual efforts to search useful skin whitening agents (Lee K.T. *et al.*, 1997), the methanol extracts from 80 plants were prepared and evaluated for their tyrosinase inhibitory activity in this investigation. It is found that the several plant materials such as *Salvia plebeia* and *Sophora flavescens* possess considerable inhibitory activity and may be used as skin whitening ingredients in cosmetics.

MATERIAL AND METHODS

Preparation of Plant Extracts

The plant materials used in this study were obtained from the Eui-Sung botanical garden of herbal drug, Kyungbuk Province, Korea and identified by Dr. Kyu Young Chung (Dept. Resources and Environment, Andong National University). 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.

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 unit, 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

Eighty 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 if any at the concentrations tested. Among the extracts tested, some plant extracts such as *Actinidia arguta, Allium thunbergi, Angelica gigas, Asparagus oligoclonos, Chrysanthemum zawadskii, Dianthus chinensis, Ixeris dentate, Salvia plebeia, Sophora flavescens* and *Sophora japonica* showed considerable inhibition at 10 and 100 μg/mL.

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Table 1. Tyrosinase inhibitory activity of the plant extracts

Plant Name	Part Used	% Inhibition of Tyrosinase[a]	
		10 μg/mL	100 μg/mL
Achillea sibirica	whole	–	11.1
Aconitum carmichaehi	whole	3.0	–
Acorus calamus	leaf	8.5	18.6
Actinidia arguta	leaf	9.0	25.0
Ademophora triphylla	radix	–	–
Allium thunbergi	whole	8.7	31.3
Allium tuberosum	whole	–	1.8
Althaea rosea	whole	16.6	17.9
Aneilema keisak	whole	7.7	7.2
Angelica gigas	whole	16.5	22.3
Arctium lappa	fruit	–	–
Artemisia capillaris	whole	–	13.4
Asparagus cochinchinesis	whole	–	–
Asparagus oligoclonos	whole	21.6	16.8
	stem	32.3	32.8
Aster koraiensis	whole	10.6	20.8
Aster tataricus	whole	–	7.8
Campsis grandiflora	leaf	–	5.6
Cassia tora	whole	15.3	17.8
Cassia occidentalis	aerial	–	20.0
Cedreal sinensis	leaf	11.9	0.6
Chrysanthemum zawadskii	whole	–	25.7
Clamatis manshurica	radix	–	–
Crataegus pinnatifida	fruit	–	–
Dianthus chinensis	whole	–	29.2
Dictamnus dasycarpus	whole	16.9	18.6
Disporum sessile	whole	–	14.0
Ecinopsis setifer	whole	–	12.5
Ficus carica	aerial	–	–
	leaf	–	5.6
Forsythia viridissima	whole	–	–
	fruit	–	–
Galium verum	whole	–	–
Glycyrrhizae uralensis	whole	–	–
	seed	–	7.1
Gossypium indicum	leaf	18.4	9.2
Hemerocallis aurantiaca	whole	–	8.4
Hemerocallis fulva	whole	20.7	21.2
Hemerocallis lilioasphodelus	whole	–	10.8
Hibiscus manihot	whole	4.2	9.8
Houttuynia cordata	aerial	9.8	–
Hosta plantaginea	whole	–	–
Inula helenium	aerial	–	–
Iris koreana	whole	–	–
Iris pallassii	leaf	9.3	9.8
Iris sanguinea	whole	–	–
Ixeris dentate	whole	14.0	35.1
Leonurus sibiricus	whole	28.9	13.8
Lotus corniculatus	whole	18.1	16.8
Lysimachia davurica	whole	17.6	12.4
Mentha arvensis	whole	–	–
Ostericum koreanum	whole	–	7.7
Patarinia scabiosaefolia	whole	1.4	25.6
Patrinia villosa	whole	–	–
Peucedanum japonicum	whole	7.5	19.7
Physalis alkekengi	leaf	6.1	22.3
Pleuroptems multiflorus	whole	–	–
Polygonatum stenophyllum	whole	7.5	21.2
Potentilla discolor	flower	4.7	–
Prunus persica	flower	–	9.2
Pteridium aquilinum	whole	–	9.3
Pulsatilla koreana	whole	–	12.1
Reynoutria elliptica	leaf	–	5.9
Rhodea japonica	leaf	–	–
Rhododendron mucronulatum	whole	–	12.5
Rumex acetocella	whole	1.7	13.3
Rumex crispus	whole	2.6	0.6
Ruta graveolens	aerial	2.0	1.2
Salvia mitiorrhiza	radix	–	–
Salvia plebeia	whole	8.1	36.1
Saururus chinensis	whole	5.6	11.1
Scilla chinensis	aerial	–	–
Sedum kamtschaticum	whole	–	7.9
Selaginella tamariscina	whole	13.3	18.2
Sophora flavescens	root	24.1	56.3
Sophora japonica	flower	–	38.7
Sophora subprostrata	radix	–	–
Symphytum officinale	whole	13.1	20.6
Trachelospermum asiaticum	whole	–	10.0
Veronica linariaefolia	whole	8.5	16.7
Viola mandshurica	whole	4.8	1.7
Zanthoxylum schinifolium	whole	–	11.0
	Leaf	2.6	14.0
Zingiber mioga	whole	–	–
Zizyphus jujuba	seed	–	–
Kojic acid[b]		63.5	98.4

[a]Tyrosinase (105 U) showed 0.38-0.53 abs. at 475 nm by 10 min incubation in our experiments. [b]IC_{50} values of kojic acid ranged at 7.0-16.3 μg/mL from five separate experiments. % Inhibition shown here represented one typical result of five experiments.

Among the extracts tested in this study, 11 plant extracts from *Aconitum carmichaehi*, *Actinidia arguta*, *Angelica gigas*, *Artemisia capillaries*, *Asparagus cochinchinesis*, *Crataegus pinnatifida*, *Forsythia viridissima*, *Leonurus sibiricus*, *Ostericum koreanum*, *Sophora flavescens* and *Zizyphus jujuba* were previously evaluated for their tyrosinase inhibitory activity and showed similar results as ours (Lee S.-H. *et al.*, 1997; Choi *et al.*, 1998; Choi *et al.*, 2001). Especially, the plant materials including *Allium thunbergi*, *Asparagus oligoclonos*, *Ixeris dentate*, *Salvia plebeia*, *Sophora flavescens* and *Sophora japonica* showed more than 30% inhibition at 100 μg/mL. These plant materials merit for further investigation, although no one showed the comparable activity with kojic acid (IC_{50} = 7.0 – 16.3 μg/mL). In particular, our results of *Sophora flavescens* were well correlated with the previous investigation describing that the methanol extract from *Sophora flavescens* possessed potent tyrosinase inhibitory activity (Lee S.-H. *et al.*, 1997). And some parts of active principles from the same plant material have been recently identified as kurarinone and kushnol F (Ha *et al.*, 2001). For further isolating active principles, *Salvia plebeia* and *Sophora flavescens* are now being under investigation.

From the results, it is clear that some plant extracts were able to inhibit melanin formation, at least *in vitro*. This study suggests that several plant extracts have a potential as skin whitening agents in cosmetics.

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