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Tyrosinase inhibitory activity of some edible plants

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Abstract

Tyrosinase catalyzes melanin biosynthesis that protects the skin from harmful effects of UV radiation. If melanin generates abnormal production, it leads to hyperpigmentation, freckle and blemish. Therefore, tyrosinase inhibitors are used to stop production of melanin. The aim of this work is to evaluate the tyrosinase inhibitory activity of 20 edible plants. Plant material was extracted with methanol and water. Then, the methanol and aqueous crude extracts were tested for their *in vitro* tyrosinase inhibitory activity using *L*-DOPA as a substrate. Methanol crude extracts of pod peels of *Parkia speciosa* Hassk. (Petai) showed the strongest tyrosinase inhibitory activity among the tested plants with percentage of inhibition of 66.22 ± 1.29% at concentration of 1.0 mg/mL compared to positive control of kojic acid (83.46 ± 0.35% inhibition) and arbutin (23.35 ± 0.95% inhibition) at same concentration of 1.0 mg/mL. Further studies are needed to isolate, characterize and elucidate the structure of tyrosinase inhibitors and other medicinal properties of this plant.

Introduction

Tyrosinase (EC 1.14.18.1) is a multifunctional oxidase that is widely distributed in nature. It is an important enzyme in melanin biosynthesis and is involved in determining the color of mammalian skin.[1] Melanin is produced in melanocytes which are found in stratum basale of the epidermis.[2] Reaction of tyrosinase is initiated when skin is exposed to UV radiation by the hydroxylation of *L*-tyrosine to *L*-dihydroxyphenylalanine (*L*-DOPA), after that *L*-DOPA is oxidized to DOPAquinone. Finally, the melanin biosynthesis diverges to produce either eumelanin or pheomelanin.[3] Eumelanin is the most abundant type of melanin in humans. It can be found in two variations, black and brown eumelanin. Pheomelanin is a red-yellow pigment found both in lighter skinned humans and darker skinned humans.[4] The active site of tyrosinase is composed of two copper atoms. The two copper atoms are carried out to tyrosine hydroxylation from *L*-tyrosine to *L*-DOPA which is rapidly oxidized to DOPAquinone.[5] Melanin pigmentation is essential for protecting human skin against the radiation. However, this undesired accumulation in the basale layer leads to melanogenesis or skin pigmentation which causes serious aesthetic problems in humans like blemish, melisma, freckles and age spots.[6] So, tyrosinase inhibitors can eliminate these problems. Tyrosinase inhibitors are found in all parts of plants. Many species of plant which have polyphenols and flavonoids were found to contain tyrosinase inhibitory activity.[7] Therefore, it would be useful to investigate new tyrosinase inhibitors from natural sources for treatment of skin diseases.[8]

In this study, tyrosinase inhibitory activity of methanol and aqueous crude extracts of 20 edible plants were investigated. Kojic acid and arbutin were used as positive controls. These agents are potent and well-known tyrosinase inhibitors and are often used as the positive control for comparing the inhibitory strength.[9] Twenty edible plants were categorized into

Fabaceae and Solanaceae families. The family Fabaceae included *Erythrina suberosa* Roxb., *Crotalaria juncea* Linn., *Leucaena leucocephala* (Lam.) de Wit, *Neptunia oleracea* Lour., *Parkia speciosa* Hassk., *Psophocarpus tetragonolobus*, *Senegalia pennata*, *Sesbania grandiflora*, *Sesbania javanica* Miq., *Tamarindus indica* Linn. and *Vigna unguiculata* subsp. sesquipedalis. The family Solanaceae consisted of *Capsicum annuum* Linn., *Capsicum frutescens* Linn., *Physalis peruviana*, *Linociera parkinsonii* Hutch., *Solanum aculeatissimum* Jacq., *Solanum melongena* Linn., *Solanum virginianum* Linn., *Solanum trilobatum* Linn. and *Solanum wendlandii* Hook.

Methodology

Plant materials

Plant materials were collected and purchased from market in Bangkok. They were authenticated by a botanist of Chulalongkorn University.

Extract preparation

Fresh edible plant material was dried in hot air oven at 60 °C. Then, the dried plant material was ground to powder. The dried powder was soaked with methanol at room temperature for 24 h for methanol crude extract. In case aqueous crude extract, the dried powder was sonicated with deionized water at 30 °C for 30 min. The extract was filtered with Whatman no.1 filter paper and then was evaporated *in vacuo* to give crude extract.

Mushroom tyrosinase inhibitory assay

The tyrosinase inhibitory activity was performed using the method of Piao *et al*.[10] The determination of tyrosinase inhibitory activity was performed using *L*-DOPA as a substrate. First, phosphate buffer (150 µL of 0.05 M, pH 6.8), sample (50 µL, 1.0 mg/mL in 20% dimethyl sulfoxide (DMSO)) and tyrosinase (50 µL, 1.0 mg/mL in phosphate buffer) were mixed and incubated at room temperature for 10 min, then *L*-DOPA (50 µL, 1.0 mg/mL in phosphate buffer) was added and the absorbance was measured at 490 nm. The reaction solution was incubated for 20 min and then absorbance was measured at 490 nm. The percentage of tyrosinase inhibition was calculated using the following equation:

$$\%\text{Tyrosinase inhibition} = \left[\frac{\Delta A_{control} - \Delta A_{sample}}{\Delta A_{control}}\right] \times 100$$

Where $\Delta A_{control}$ is the change of absorbance at 490 nm without a test sample ($A_{control} - A_{blank\ of\ control}$) and ΔA_{sample} is the change of absorbance at 490 nm with a test sample ($A_{sample} - A_{blank\ of\ sample}$).

Statistical analysis

The values of percentage of tyrosinase inhibition were expressed for statistical analysis as the mean ± standard deviation for n = 3.

Results and Discussion

The percentage of tyrosinase inhibition of methanol and aqueous crude extracts of twenty edible plants is shown in **Table 1**. This assay used kojic acid and arbutin as positive controls which are often used as standard for comparing the strength of skin-lightening agent.[9] Methanol crude extracts of *P. speciosa* (pod peels) showed strong tyrosinase inhibitory activity with percentage of inhibition of 66.22 ± 1.29% at concentration of 1.0 mg/mL. Methanol crude extracts of *S. trilobatum* (fruits), *L. leucocephala* (pods), *V. unguiculata* (pods) and *S. wendlandii* (fruits) showed moderate tyrosinase inhibitory activity with percentage of

inhibition of 53.26 ± 0.72%, 51.87 ± 0.60%, 38.86 ± 1.03% and 33.49 ± 1.39%, respectively at concentration of 1.0 mg/mL. Methanol crude extracts of *S. javanica* (shoots), *P. tetragonolobus* (pods), *E. suberosa* (leaves), *C. annuum* (fruits), *C. juncea* (shoots), *P. speciosa* (seeds), *P. peruviana* (fruits), *S. aculeatissimum* (fruits), *C. frutescens* (fruits), *S. virginianum* (fruits), *L. parkinsonii* (fruits), *T. indica* (baby fruits) and *S. melongena* (fruits) showed weak tyrosinase inhibitory activity with percent inhibition in range of 26.54 ± 0.51% to 0.54 ± 0.27% at concentration of 1.0 mg/mL. Aqueous crude extract of *S. trilobatum* (fruits), *V. unguiculata* (pods), *S. wendlandii* (fruits) and *S. javanica* (shoots) exhibited moderate tyrosinase inhibitory activity with percentage of inhibition of 38.99 ± 0.31%, 30.66 ±1.39%, 30.41 ± 0.32% and 29.41 ± 0.11%, respectively at concentration of 1.0 mg/mL. Aqueous crude extracts of *C. juncea* (shoots), *L. leucocephala* (pods), *P. speciosa* (seeds), *P. speciosa* (pod peels), *P. peruviana* (fruits), *C. annuum* (fruits), *S. grandiflora* (flowers), *S. virginianum* (fruits), *S. aculeatissimum* (fruits), *L. parkinsonii* (fruits), *C. frutescens* (fruits) and *P. tetragonolobus* (pods) showed weak tyrosinase inhibitory activity with percent inhibition in range of 22.45 ± 0.27% to 2.15 ± 1.13% at concentration of 1.0 mg/mL. Both methanol and aqueous crude extracts of *N. oleracea* and *S. pennata* exhibited no activity on tyrosinase inhibition.

Table 1. Tyrosinase inhibitory activity of methanol and aqueous crude extracts of some edible plants at concentration of 1.0 mg/mL using *L*-DOPA as a substrate.

Scientific name	Common name	Plant part	Family	Tyrosinase inhibition (%)	
				Methanol crude extract	Aqueous crude extract
Crotalaria juncea Linn.	Sunn hemp	Shoots	Fabaceae	16.12 ± 1.96	22.45 ± 0.27
Erythrina suberosa Roxb.	Indian coral tree	Leaves	Fabaceae	18.68 ± 1.35	N.D.
Leucaena leucocephala	White leadtree	Pods	Fabaceae	51.87 ± 0.60	21.95 ± 0.13
Neptunia oleracea Lour.	Water mimosa	Shoots	Fabaceae	N.A.	N.A.
Parkia speciosa Hassk.	Petai	Pod peels	Fabaceae	66.22 ± 1.29	14.69 ± 0.60
		Seeds	Fabaceae	14.08 ± 0.71	18.10 ± 1.56
Psophocarpus tetragonolobus	Winged bean	Pods	Fabaceae	24.68 ± 0.20	2.15 ± 1.13
Senegalia pennata	Climbing wattle	Leaves	Fabaceae	N.A.	N.A.
Sesbania grandiflora	Humming bird tree	Flowers	Fabaceae	N.A.	9.99 ± 0.58
Sesbania javanica Miq.	Dhaincha	Shoots	Fabaceae	26.54 ± 0.51	29.41 ± 0.11
Tamarindus indica Linn.	Tamarind	Baby fruits	Fabaceae	5.06 ± 0.61	N.A.
Vigna unguiculata subsp. sesquipedalis	Yardlong bean	Pods	Fabaceae	38.86 ± 1.03	30.66 ± 1.39
Capsicum annuum Linn.	Chili spur pepper	Fruits	Solanaceae	17.63 ± 1.21	13.37 ± 1.33
Capsicum frutescens Linn.	Bird pepper	Fruits	Solanaceae	9.38 ± 0.51	3.72 ± 0.34
Linociera parkinsonii Hutch.	Thai eggplant	Fruits	Solanaceae	8.25 ± 1.34	3.97 ± 0.51
Physalis peruviana	Yellow berried nightshade	Fruits	Solanaceae	12.96 ± 2.53	14.57 ± 1.40

Solanum aculeatissimum Jacq.	Thorny nightshade	Fruits	Solanaceae	12.32 ± 1.14	8.62 ± 1.54
Solanum melongena Linn.	Eggplant	Fruits	Solanaceae	0.54 ± 0.27	N.D.
Solanum trilobatum Linn.	Thoodhuvalai	Fruits	Solanaceae	53.26 ± 0.72	38.99 ± 0.31
Solanum virginianum Linn.	Dutch eggplant	Fruits	Solanaceae	9.33 ± 1.66	9.79 ± 0.54
Solanum wendlandii Hook.	Eggplant	Fruits	Solanaceae	33.49 ± 1.39	30.41 ± 0.32
Kojic acid				83.46 ± 0.35	
Arbutin				23.35 ± 0.95	

Results are displayed with means ± standard deviations for n = 3
N.A.: No activity
N.D.: No detection

Methanol crude extract of pod peels of *P. speciosa* (Petai) exhibited the strongest tyrosinase inhibitory activity among the test plants. *P. speciosa* is a plant of the genus Parkia in family Fabaceae. Methanol crude extract of pod peels of *P. speciosa* was reported as a source of natural antioxidant. It contained phenolic compounds, flavonoids, alkaloids, tannins and saponins.[11] In addition, methanol crude extract of pod peels of *P. speciosa* had a potential to prevent hypertension cardioprotective and hypoglycemic effects because of it consisted of β-sitosterol and stigmasterol.[12,13] Ethanol crude extract of pod peels of *P. speciosa* contained high amount of polyphenols, particularly quercetin.[14] Quercetin was reported to exhibit antioxidant and anti-inflammatory properties by reduction of intracellular reactive nitric oxide.[14] Therefore, methanol crude extract of this plant might be contained tyrosinase inhibitors. Further studies are needed to isolate, characterize and elucidate the structure of tyrosinase inhibitors and other medicinal properties of this plant.

Conclusion

Tyrosinase inhibitory activity of methanol and aqueous crude extracts of 20 edible plants were investigated using *L*-DOPA as a substrate. The methanol crude extract of pod peels of *P. speciosa* (Petai) showed the strongest tyrosinase inhibitory activity. It might be contained chemical constituents which are tyrosinase inhibitors.

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