Biological screening of 100 plant extracts for cosmetic use (II)
Inhibitory activities of tyrosinase and DOPA autooxidation

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Abstract

In eastern Asian region, cosmetic preparations containing the molecules possessing the whitening activity are promising. In this study, 100 plant extracts were screened in order to elucidate the whitening effects using in vitro tyrosinase assay and DOPA autooxidation. Several plant extracts such as Morus alba, Glycyrrhiza glabra showed the inhibitory activity (>50%) against mushroom tyrosinase at the concentration 333μg/ml. And plant extracts including Morus alba and Bupleurum falcatum showed the inhibitory activity (>50%) at the concentration of 500(μg/ml against DOPA autooxidation. The IC_{50} values of the several extracts were also found.

Key words : Whitening effect; Tyrosinase; DOPA autooxidation; Inhibition; Plant extracts.

Introduction

In cosmetic preparations, many plant extracts such as Morus alba and Glycyrrhiza galbra have been used as whitening purposes. Melanin formation is believed to be nduced mainly by UV-light and other stimulus such as toxic chemicals [1]. Melanin ormed by tyrosine pathway including autooxidation serves as light absortion compound to protect harmful UV-light. However, whitening of facial skin and/or protection skin darkening (melanin formation) is favorable for cosmetic purpose, especially in Asia. And

plant extracts having an inhibitory activity of melanin formation may be the better choice for this purpose because of their relatively less side effects. Therefore, in this investigation, 100 plant extracts were evaluated for their inhibitory activity of tyrosinase and DOPA autooxidation.

Materials and Methods

Plant extract

One hundred plants were obtained from the oriental medicinal market located in Chuncheon, South Korea. Each powered plant (100g) was soaked in 300ml of 80% methanolic aquous solution at room temperature for 7 days. After filteration, the methanolic filtrate was evaporated to dryness under vacuum. These extracts were used for the further biological study including tyrosinase assay and DOPA autooxidation.

Tyrosinase assay

In an assay tube, tyrosine (0.1mg/ml) and mushroom tyrosinase (70 units/ml) were added to 0.05μM sod. phosphate buffer, pH 6.8. Total reaction volume per tube was 1.5ml. Each plant extract dissolved in the same buffer was added and incubated at 37o C for 10min. The absorbance was measured at 475nm according to the procedure of Vanni et al.[2]

DOPA autooxidation

In an assay tube, DOPA (500(M) in 0.05M sod. phosphate buffer, pH 6.8 was incubated at 37°C for 2 days. Total reaction volume per tube was 2ml. Each plant extract disolved in the same buffer was added before starting incubation. The absorbance was measured at 475nm according to the procedure of Joshi et al. [3].

Results and Discussion

We have screened 100 plant extracts for their inhibitory activities of tyrosinase and DOPA autooxidation. Table 1 represented the results of initial screening. Twelve extracts including *Agastache rugosa, Bupleurum falcatum, Chaenomeles speciosa, Dryopteris crassirrhizoma, Glycyrrhiza glabra and Morus alba* showed the higher inhibitory activity (>50%) of mushroom tyrosinase at the concentration of 333μg/ml. And it was also found that nine plant extracts such as *Morus alba, Caragana sinica and Bupleurum falcatum* showed more than 50% inhibition against DOPA autooxidation. For comparing the potencies of the several plant extracts selected, IC_{50} values were found.

Arbutin and kojic acid currently used as whitening agents were also compared.

Fig. 1 showed the concentration-dependent inhibition of tyrosinase by several selected plant extracts. It was revealed that IC_{50} values of *Morus alba, Gycyrrhiza glabra, Rheum palmatum, Sophora japonica and Chaenomeles speciosa* were 16.6, 71.0, 86.3, 93.4 and 328.8(g/ml, respectively, compared to the reference compounds, arbutin (65.2μg/ml) and kojic acid (5.8μg/ml). Fig.2 showed the concentration-dependent inhibition of DOPA autooxidation. IC_{50} values of Trichosanthes kirilowii (root), Lycium chinensis, Morus alba, Buplum falcatum and Liriope platypylla were found to be 155.6, 297.6, 329.3, 335.7, and 362.4μg/ml, respectively, compared to the reference compound, kojic acid (197.2μg/ml). Among the extracts tested, *Morus alba* showed the highest inhibitory activity against tyrosinase and DOPA autooxidation.

From all of these results, it is clear that some plant extracts possessed the inhibitory activity of melanin formation, at least in vitro. This study suggests that several plant extracts may have a potential for whitening agents by single use or some combination of above active extracts.

References

1) Kubo M. and Matsuda H. : Development studies of cuticle and medicinal drugs from natural sources on melanin biosynthesis. Fragrance J., 8, 48 (1995).

2) Vanni,A.,Gastaldi,D. and Giunata,G.: Annali di Chimica, 80,35(1990).

3) Joshi, P.C., Carraro, C. and Pathak, M.A. : Biochem. Biophys. Res. Comm., 142(1),265(1987).

Table 1. Inhibitory activities of tyrosinase and DOPA autooxidation by 100 plant extracts.

Name of plant	Part of plant	Inhibition (%) used Tyrosinase		Inhibition (%) DOPA autooxidation	
		3.3μg/ml	333μg/ml	100μg/ml	500μg/ml
Acorus gramineus	Rhizoma	-	-	-	-
Agastache rugosa	Leaves	5	51	-	-
Akebia quinata	Stem	-	-	-	-
Alisma orientale	Rhizoma	-	-	-	40
Alpinia officinarum	Rhizoma	-	-	-	-
Amomum cardamomum	Fruit	9	40	-	9
Amomum xanthioides	Seed	7	16	-	-
Anemarrhena asphodeloides	Rhizoma	-	-	-	-
Angelica dahurica	Roots	-	36	-	26
Angelica koreana	Root	-	16	-	19
Angelica tenuissima	Root	-	-	-	30
Anthriscus sylvestris	Root	-	47	-	31
Aralia cordata	Root	15	33	-	13
Areca catechu	Seed	2	34	-	-
Areca catechu	Peel	-	-	-	-
Arisaema heterophyllum	Tuber	-	-	-	-
Asiasarum sieboldii	Root	-	5	-	30
Astragalus membranaceus	Root	-	-	23	55
Atractylodes japonica	Rhizoma	-	10	-	11
Belamcanda chinensis	Rhizoma	-	-	-	-
Brassica alba	Seed	-	-	-	-
Bupleurum falcatum	Root	25	50	22	71
Cannabis sativa	Seed	-	3	-	5
Caragana sinica	Root	3	37	37	83
Chaenomeles speciosa	Fruit	-	62	-	-
Chrysanthemum indicum	Flower	7	10	-	-
Cinnamomum cassia	Bark	-	5	-	-
Citrus aurantium	Fruit	-	13	-	-
Citrus unshiu	Peel	-	-	-	-
Codonopsis pilosula	Root	-	16	-	-
Commiphora molmol	Resin	-	-	-	-
Cornus officinalis	Fruit	-	-	-	-
Coptis japonica	Rhizoma	-	-	70	46
Corydalis ternata	Tuber	-	20	-	24
Curcuma longa	Rhizoma	-	-	-	-

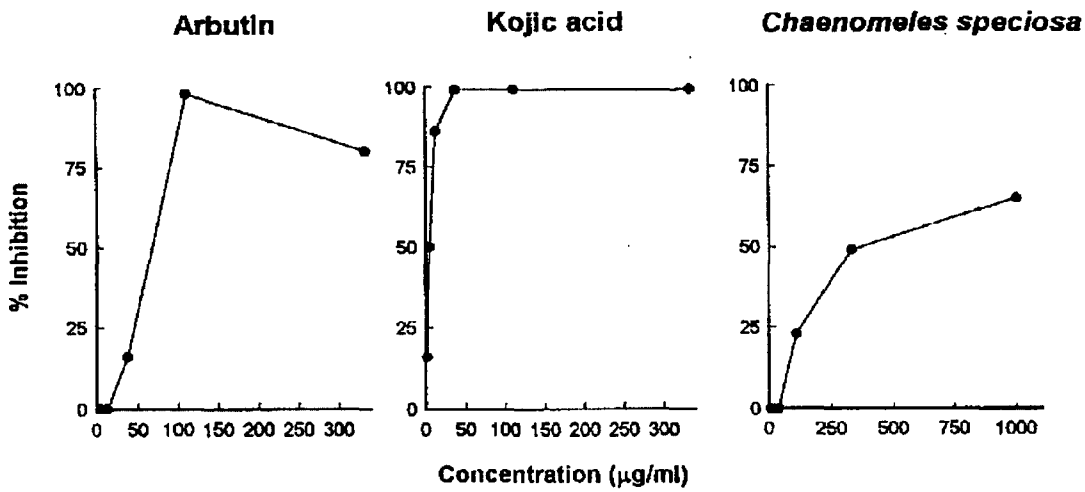
Curcuma longa	Rhizoma	-	-	-	-
Curcuma zedoaria	Rhizoma	-	-	-	-
Cuscuta chinensis	Seed	-	-	-	-
Cyperus rotundus	Rhizoma	-	-	-	48
Dendrobium moniliforme	Leaves	-	25	-	-
Dioscorea batatas	Leaves	-	-	18	26
Dryopteris crassirrhizoma	Rhizoma	16	72	-	-
Equisetum hyemale	Leaves	-	-	-	-
Eucommia ulmoides	Bark	-	49	-	-
Eugenia caryophyllata	Flower	12	10	-	-
Evodia officinalis	Fruit	-	-	-	-
Foeniculum vulgare	Fruit	-	-	12	53
Gardenia jasminoides	Fruit	12	40	-	23
Gastrodia ellata	Rhizoma	-	85	-	-
Gleditsia sinensis	Spina	-	8	-	-
Glycyrrhiza glabra	Root	25	68	-	-
Hordeum vulgare	Fruit	-	34	-	14
Kochia scoparia	Fruit	-	15	-	-
Leonurus sibricus	Leaves	9	48	-	22
Liriope platyphylla	Tuber	-	15	14	52
Lindera strychnifolia	Root	-	-	-	-
Lycium chinensis	Fruit	-	41	-	-
Morinda officinalis	Root	-	24	-	-
Morus alba	Stem	75	85	8	100
Myristica fragrans	Seed	-	69	-	31
Nepeta japonica	Leaves	-	-	-	31
Paeonia suffruticosa	Bark	-	-	-	-
Perilla frutescens	Leaves	-	-	-	-
Perilla frutescens	Seed	10	-	-	5
Phellodendron amurense	Bark	-	52	-	5
Phragmites communis	Root	3	12	50	49
Phylostachys nigra	Stem	-	-	-	-
Pinellia ternata	Tuber	20	62	8	49
Plantago asiatica	Seed	-	-	-	-
Platicodon grandiflorum	Roots	-	-	10	47
Polygala tenuifolia	Roots	-	-	-	20
Poncirus trifoliata	Fruit	-	-	-	-
Poria cocos (red)	Hoelen	-	-	-	-
Poria cocos (alba)	Hoelen	-	-	-	-
Prunus armeniaca	Seed	-	26	-	-
Prunus mume	Fruit	-	-	-	3
Prunus persica	Seed	-	28	-	39

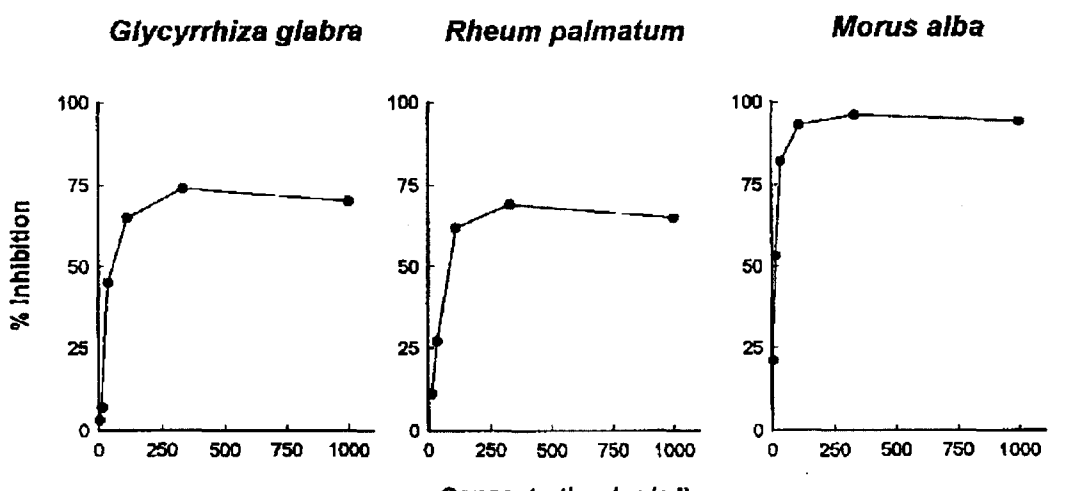
Pueraria thunbergiana	Root	22	-	-	12
Pueraria thunbergiana	Flower	17	-	-	8
Rehmannia glutinosa	Root	22	48	-	-
Rhaphanus sativus	Seed	-	-	-	-
Rheum palmatum	Rhizoma	45	68	-	-
Rhus verniciflua	Resin	-	-	-	-
Rubus coreanus	Fruit	-	-	-	-
Sanguisorba officinalis	Root	-	19	-	-
Saussurea lappa	Root	-	28	-	11
Schizandra chinensis	Fruit	-	30	-	-
Sophora flavescens	Root	-	26	-	-
Sophora japonica	Flower	-	80	-	-
Sparganium stoloniferum	Rhizoma	-	20	-	15
Taraxacum platycarpum	Root	-	-	-	33
Trapa bispinosa	Fruit	-	-	-	-
Terminalia chebula	Fruit	25	38	-	-
Torilis japonica	Fruit	-	-	-	-
Trichosanthes kirilowii	Root	8	18	35	56
Trichosanthes kirilowii	Seed	-	-	20	32
Tussilago farfara	Flower	-	-	-	61
Valeriana fauriei	Root	-	-	-	-
Zanthoxylum piperitum	Peel	-	-	-	-
Zingiber officinale	Rhizoma	-	41	-	-

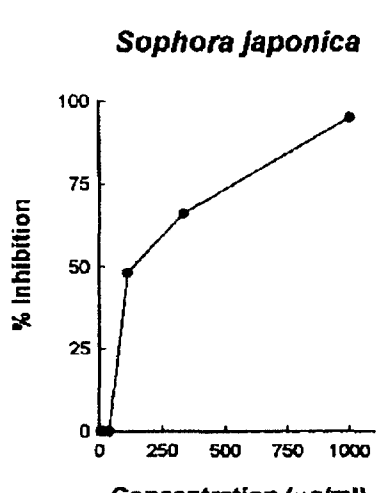
Figure legends

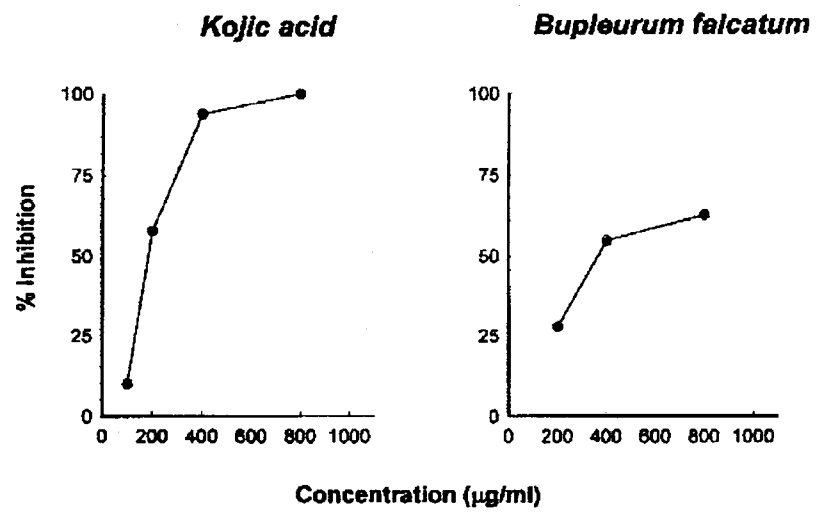
Fig. 1. Concentration-dependant inhibition of tyrosinase.

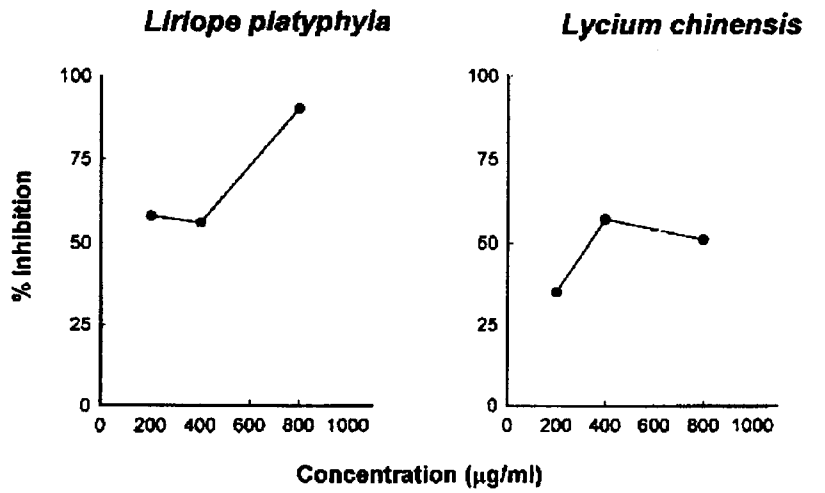
Fig. 2. Concentration-dependant inhibition of DOPA autooxidation.

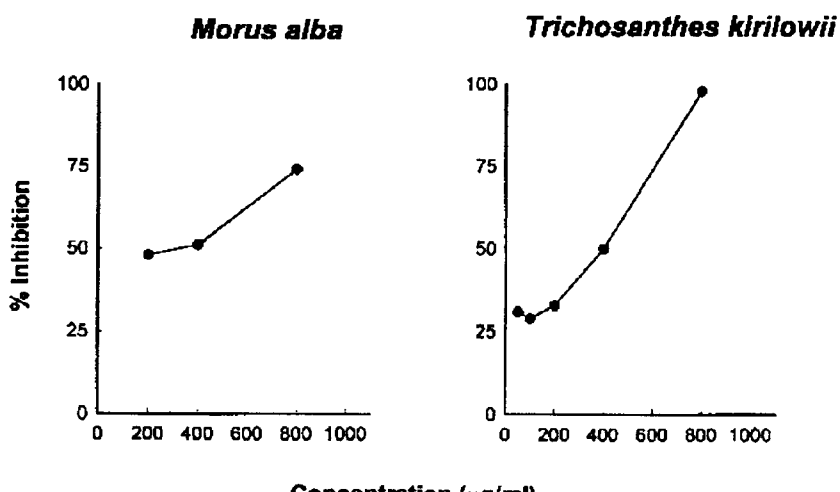

Arbutin
Kojic acid
Chaenomeles speciosa
% Inhibition
100
75
50
25
0
0 50 100 150 200 250 300
0 50 100 150 200 250 300
0 250 500 750 1000
Concentration (μg/ml)


Glycyrrhiza glabra
Rheum palmatum
Morus alba
% Inhibition
100
75
50
25
0
0 250 500 750 1000
Concentration (μg/ml)


Sophora japonica
% Inhibition
100
75
50
25
0
0 250 500 750 1000
Concentration (μg/ml)


Kojic acid
Bupleurum falcatum
% Inhibition
100
75
50
25
0
0 200 400 600 800 1000
Concentration (μg/ml)


Liriope platyphyla
Lycium chinensis
% Inhibition
100
75
50
25
0
0 200 400 600 800 1000
Concentration (μg/ml)


Morus alba
Trichosanthes kirilowii
% Inhibition
100
75
50
25
0
0 200 400 600 800 1000
Concentration (μg/ml)