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Inhibitory Effects of Herbal Extracts on Dopa Oxidase Activity of Tyrosinase

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Abstract – Tyrosinase catalyzes the rate-limiting steps in melanin biosynthesis which is involved in skin-coloring and local hyperpigmentation of human beings, and unfavorable darkening of food products. Inhibitory effects on 3,4-dihydroxyphenylalanine (dopa) oxidase activity of tyrosinase by 594 kinds of herbal extracts prepared from herbal medicines and wild plants in Korea were estimated. Two herbal extracts prepared from radicis cortex of *Morus alba* and rhizoma of *Curcuma longa* were selected as those exhibiting potent inhibitory effects on the enzyme activity. These herbal extracts were subjected to sequential fractionations with methylene chloride, ethyl acetate, *n*-butanol, and polar residue. The inhibitory effects on dopa oxidase activity of tyrosinase were shown in ethyl acetate fraction of *Morus alba*, and in methylene chloride fraction of *Curcuma longa*. The ethyl acetate fraction of *Morus alba* exhibited 50% of inhibition on dopa oxidase activity of tyrosinase at the concentration of 12 μg/ml, and methylene chloride fraction of *Curcuma longa* at 51 μg/ml.

Key words – Tyrosinase inhibition, herbal extracts, *Morus alba, Curcuma longa.*

Introduction

Tyrosinase (EC 1.14.18.1) called as polyphenol oxidase is widely distributed in the animal and plant kingdoms. The enzyme catalyzes two different reactions: hydroxylation of monophenolic compounds to *o*-diphenols and oxidation of the *o*-diphenols to *o*-quinones. The hydroxylation activity of the enzyme in the presence of its substrate is shown to have a lag period, while the oxidation activity of *o*-diphenols shows no slow transition phenomena (Garcia-Carmona *et al.*, 1992; Jimenez-Cervantes *et al.*, 1993).

Mammalian tyrosinase catalyzes the hydroxylation of tyrosine to form 3,4-dihydroxyphenylalanine (dopa) and oxidation of dopa to form dopaquinone, which plays an important role in the process of melanin biosynthesis (Hearing and Tsukamoto, 1991). This process is a determinant of human skin color and involved in local hyperpigmentations such as melasma, ephelide, and lentigo (Fitzpatrick *et al.*, 1979; Iwata *et al.*, 1990). Therefore, the enzyme inhibitors have been of great concern as cosmetic products to have skin-whitening effects and as medical products to have preventive and therapeutic effects on the local hyperpigmentation diseases.

Unfavorable darkening of food products resulting from enzymatic oxidation of phenolic compounds to *o*-quinones by the polyphenol oxidase causes a decrease in their market values because it connotes spoilage although innocuous to consumers (Flurkey and Jen, 1978). Anti-melanosis agents to food products by inhibi-

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ting the activity of polyphenol oxidase or reduction of *o*-quinones to *o*-diphenols have been identified (Chen *et al.*, 1991; Golan-Goldhirsh and Whitaker, 1984; Walker, 1976). However, a number of the anti-melanosis agents are not allowed to use in food systems due to off-flavors, off-odors, and toxicity.

These previous observations have led us to search for naturally occurring tyrosinase inhibitors. In this study, inhibitory effects on dopa oxidase activity of tyrosinase by 594 kinds of herbal extracts prepared from herbal medicines and wild plants in Korea were analyzed, where mushroom tyrosinase was used as the enzyme source because of its easy availability although the enzyme differs somewhat from human tyrosinase (Jimenez-Cervantes *et al*, 1993). Two herbal extracts prepared from radicis cortex of *Morus alba* and rhizoma of *Curcuma longa* were identified as those exhibiting potent inhibitory effects on dopa oxidase activity.

Experimental

Herbal extracts – Herbal medicines were purchased from a drug strore (Dongyang Yakup), and wild plants were collected from the hills and mountains in Korea. The plants were taxonomically identified, and voucher specimens were deposited at College of Pharmacy, Chungbuk National University. Each of the dried plants was sliced, extracted twice with 80% MeOH at room temperature, evaporated under reduced pressure at 50℃, and completely dried by lyophilization. Each of the MeOH extracts was used as test samples. Some of the extracts exhibiting inhibitory effects on tyrosinase activity were subjected to sequential fractionations with methylene chloride, ethyl acetate, and *n*-butanol as follows. About 0.5 g of the MeOH extract was resuspended in 200 ml of water, and then extracted several times with the same volume of methylene chloride until no colored constituents were transferred to the methylene chloride layer. The resulting aqueous layer was sequentially extracted with ethyl acetate, and then *n*-butanol as the same procedure with methylene chloride extraction. The methylene chloride, ethyl acetate, *n*-butanol layers and aqueous layer after the *n*-butanol extraction were evaporated under reduced pressure, and then completely dried by lyophilization. The dried solvent-fractionated extracts and aqueous extract were used as test samples.

Assay of tyrosinase activity – As the enzyme activity, dopa oxidase activity was colorimetrically determined as described previously with minor modifications (Masamoto *et al.*, 1980). Fourty μl of 0.05% dopa (Sigma), 80 μl of 67 mM phosphate buffer (pH 6.8) and 40 μl of the same buffer with or without of test sample were added to a 96-well microplate (Nunc), and then 40 μl of mushroom tyrosinase (125 units/ml, Sigma) was mixed. After incubation at 37℃ for 20 min, the amount of dopachrome formed in the reaction mixture was determined as the optical density at 492 nm (OD_{492}) by using a microplate reader. The OD_{492} before adding the enzyme was also measured in order to eliminate the colorimetric interference by sample itself.

Statistics – Inhibitory effect on dopa oxidase activity of the tyrosinase was represented as % of inhibition, [1-(sample OD_{492}/control OD_{492})] $\times 100$. Data were collected as mean or mean $\pm$ standard error (n=3), and their significances were analyzed by the Student's t-test.

Results and Discussion

Dopa oxidase activity of tyrosinase was colorimetrically determined by using a microplate reader. Below 3 units/ml of mushroom tyrosinase, dopa oxidase activity detected was statistically insignificant. Dopa oxidase activity was in a dose-dependent manner when the tyrosinase with 6 units/ml to 50 units/ml was added to the standard reaction mixture. Thus, 25 units/ml of tyrosinase exhibiting submaximal dopa oxidase activity was used to estimate the

inhibitory effects by test samples in this study.

Inhibitory effects of 594 kinds of herbal extracts on mushroom tyrosinase were estimated (Table 1). At a concentration of 100 μg/ml, 18 extracts of them exhibited more than 20% of inhibition on the enzyme activity. The

Table 1. Inhibitory effects of herbal extracts on tyrosinase. Each of the herbal extracts was treated at the final concentration of 100 μg/ml. Inhibitory effects on the enzyme activity are represented as % of inhibition, mean of three independent tests

Plant (part of use)	Inhibition (%)
Abies holophylla (aerial part)	5
Abutilon avicennae (aerial part)	9
Acalypha australis (whole plant)	21
Acanthopanax gracilistylus (aerial part)	9
Acanthopanax gracilistylus (cortex)	<0
Acanthopanax koreanum (aerial part)	4
Acanthopanax sessiliflorus (aerial part)	4
Acer ginnala (aerial part)	29
Acer mono (aerial part)	<0
Acer pseudo-sieboldianum (aerial part)	12
Achillea sibirica (aerial part)	<0
Achyranthes japonica (aerial part)	<0
Aconitum koreanum (whole plant)	6
Acorus calamus var. *angustatus* (whole plant)	6
Acorus gramineus (rhizoma)	4
Actaea asiatica (aerial part)	5
Actinidia arguta (aerial part)	7
Actinidia arguta var. *rufinervis* (aerial plant)	3
Actinidia polygama (whole plant)	2
Adenophora liliifolia (whole plant)	11
Adenophora trachelioides (radix)	5
Adenophora triphylla var. *japonica* (whole plant)	13
Aeschynomame indica (whole plant)	2
Aesculus turbinata (aerial part)	1
Agastache rugosa (aerial part)	13
Agrimonia pilosa (whole plant)	9
Agrostis clavata var. *nukabo* (whole plant)	21
Ailanthus altissima (aerial part)	9
Ainsliaea acerifolia (whole plant)	6
Akebia quinata (caulis)	8
Alangium platanifolium var. *macrophyllum* (aerial part)	18
Albizzia julibrissin (aerial part)	9
Albizzia julibrissin (cortex)	6
Alisma orientale (rhizoma)	12
Allium cepa (bulbus)	13

Table 1. Continued

Plant (part of use)	Inhibition (%)
Alnus mayri (aerial part)	<0
Alopecurus aequalis var. *amurensis* (herba)	12
Alpinia oxyphylla (fructus)	5
Amaranthus mangostanus (aerial part)	8
Ambrosia artemisiifolia var. *elatior* (whole plant)	11
Amomum cardamomum (fructus)	<0
Amomum tsao-ko (fruit)	3
Amomum villosum (semen)	<0
Ampelopsis brevipedunculata var. *heterophylla* (aerial part)	10
Amphicarpaea edgeworthii var. *trisperma* (aerial part)	3
Anemarrhena asphodeloides (rhizoma)	<0
Anemarrhena asphodeloides (whole plant)	6
Angelica dahurica (aerial part)	2
Angelica dahurica (radix)	1
Angelica decursiva (whole plant)	7
Angelica gigas (aerial part)	3
Angelica gigas (radix)	3
Aquilaria agallocha (lignum)	<0
Arabis pendula (whole plant)	4
Aralia continentalis (aerial part)	6
Aralia elata (aerial part)	<0
Arctium lappa (aerial part)	12
Arctium lappa (semen)	2
Areca catechu (pericarpium)	4
Areca catechu (semen)	7
Arisaema amurense var. *serratum* (aerial part)	2
Aristolochia contorta (aerial part)	10
Armoracia lapathifolia (whole plant)	13
Artemisia argyi (folium)	14
Artemisia capillaris (aerial part)	11
Artemisia iwayomogi (aerial part)	12
Artemisia japonica (aerial part)	14
Artemisia keiskeana (whole plant)	<0
Artemisia montana (aerial part)	11
Artemisia princeps var. *orientalis* (aerial part)	20
Artemisia selengenesis (aerial part)	10
Artemisia sieversiana (aerial part)	12
Arthraxon hispidus (whole plant)	22
Arundinella hirta (aerial part)	1
Asiasarum mandshuricum var. *heterotropoides* (radix)	15
Asiasarum sieboldii (whole plant)	<0
Asparagus cochinchinensis (tuber)	10
Asparagus schoberioides (whole plant)	11
Aster ageratoides (aerial part)	10
Aster pinnatifidus (whole plant)	12

Table 1. Continued

Plant (part of use)	Inhibition (%)
Aster scaber (whole plant)	29
Aster tataricus (aerial part)	2
Aster yomena (aerial part)	10
Astragalus membranaceus (radix)	6
Athyrium yokoscense (aerial part)	16
Atractylodes japonica (aerial part)	2
Atractylodes ovata (rhizoma)	3
Belamcanda chinensis (aerial part)	6
Benincasa hispida (semen)	6
Berberis amurensis var. *quelpaertensis* (aerial part)	<0
Berberis koreana (aerial part)	14
Berberis poiretii (aerial part)	6
Betula davurica (aerial part)	<0
Betula schmidtii (aerial part)	<0
Bidens bipinnata (aerial part)	<0
Bidens cernua (whole plant)	1
Bidens tripartita (whole plant)	10
Bilderdykia dentato-alata (aerial part)	10
Biota orientalis (semen)	2
Boehmeria nivea (aerial part)	18
Boehmeria sieboldiana (aerial part)	2
Boehmeria spicata (aerial part)	5
Boehmeria tricuspis (whole plant)	3
Boswellia carterii (resin)	<0
Brassica alba (semen)	8
Brassica campestris subsp. *napus* var. *nippo-oleifera* (herba)	10
Bulbostylis barbata (whole plant)	16
Bupleurum falcatum (radix)	<0
Buxus microphylla var. *koreana* (aerial part)	1
Caesalpinia sappan (lignum)	<0
Callicarpa japonica (aerial part)	<0
Calystegia soldanella (whole plant)	5
Campanula glomerata var. *dahurica* (aerial part)	2
Campanula takesimana (whole plant)	15
Cannabis sativa (aerial part)	12
Capsella bursa-pastoris (whole plant)	7
Caragana sinica (aerial part)	3
Cardamine lyrata (aerial part)	5
Carduus crispus (aerial part)	3
Carex humilis (underground part)	24
Carex kobomugi (whole plant)	19
Carex maackii (whole plant)	8
Carex siderosticta (whole plant)	16
Carpesium divaricatum (whole plant)	13
Carthamus tinctorius (flos)	2
Caryopteris divaricata (aerial part)	10
Cassia tora (aerial part)	14
Catalpa ovata (aerial part)	8
Cayratia japonica (aerial part)	<0

Table 1. Continued

Plant (part of use)	Inhibition (%)
Cedrus deodara (aerial part)	6
Celastrus flagellaris (aerial part)	2
Celastrus orbiculatus (aerial part)	2
Celastrus stephanotifolius (aerial part)	5
Centipeda minima (whole plant)	3
Cephalotaxus koreana (aerial part)	4
Cersis chinensis (aerial part)	22
Chaenomeles sinensis (aerial part)	10
Chelidonium majus var. *asiaticum* (aerial part)	7
Chemnopodium virgatum (whole plant)	<0
Chenopodium album var. *centrorubrum* (aerial part)	2
Chenopodium glaucum (aerial part)	4
Chrysanthemum indicum (aerial part)	<0
Chrysanthemum indicum (flos)	<0
Chrysanthemum zawadskii var. *latilobum* (whole plant)	7
Cimicifuga heracleifolia (aerial part)	<0
Cimicifuga heracleifolia (rhizoma)	4
Cinnamomum cassia (cortex)	3
Cinnamomum japonicum (aerial part)	10
Cirsium pendulum (aerial part)	6
Cirsium setidens (aerial part)	3
Cistanche deserticola (herba)	6
Citrus aurantus var. *tachibana* (fructus)	15
Clematis apiifolia (aerial part)	4
Clematis chinensis (semen)	4
Clematis fusca var. *violacea* (aerial part)	3
Clematis heracleifolia var. *davidiana* (whole part)	1
Clematis mandshurica (aerial part)	11
Clematis trichotoma (aerial part)	3
Clerodendron trichotomum (aerial part)	<0
Clinopodium chinense var. *parviflorum* (aerial part)	8
Cnidium monnieri (fruit)	6
Cnidium officinale (rhizoma)	2
Cocculus trilobus (aerial part)	5
Coix lachryma-jobi var. *mayuen* (aerial part)	3
Coix lachryma-jobi var. mayuen (semen)	<0
Colocasia antiquorum var. *esculenta* (aerial part)	9
Commelina communis (whole plant)	<0
Commiphora molmol (resin)	<0
Convallaria keiskei (aerial part)	8
Coptis chinensis (rhizoma)	<0
Corchorus capsularis (aerial part)	5
Cornus controversa (aerial part)	8
Cornus officinalis (fructus)	2
Corydalis speciosa (aerial part)	<0

Table 1. Continued

Plant (part of use)	Inhibition (%)
Corydalis yanhusuo (tuber)	<0
Corylus heterophylla var. *thunbergii* (aerial part)	10
Crataegus pinnatifida (aerial part)	9
Crataegus pinnatifida (fructus)	1
Croton tiglium (semen)	3
Cryptotaenia japonica (whole plant)	<0
Cucurbita moschata (aerial part)	1
Curcuma longa (rhizoma)	44
Curcuma zedoaria (rhizoma)	<0
Cuscuta australis (aerial part)	5
Cuscuta japonica (aerial part)	1
Cynomorium songaricum (herba)	7
Cyperus amuricus (whole plant)	3
Cyperus nipponicus (whole plant)	1
Cyperus rotundus (rhizoma)	4
Cyrtomium fortunei (aerial part)	6
Datura stramonium (whole plant)	5
Desmodium oldhami (whole plant)	23
Desmodium oxyphyllum (aerial part)	8
Deutzia parviflora (aerial part)	5
Deutzia prunifolia (aerial part)	3
Dianthus sinensis (aerial part)	9
Dicentra spectabilis (aerial part)	4
Dictamnus dasycarpus (aerial part)	10
Digitaria sanguinalis (aerial part)	7
Dioscorea batatas (rhizoma)	1
Dioscorea batatas (semen)	16
Dioscorea quinqueloba (whole plant)	12
Dioscorea tokoro (aerial part)	1
Disporum viridescens (aerial part)	16
Draba nemorosa var. *hebecarpa* (herba)	14
Dryopteris crassirhizoma (whole plant)	23
Duchesnea chrysantha (whole plant)	5
Echinochloa crus-galli var. *frumentacea* (aerial part)	2
Elaeagnus umbellata (aerial part)	7
Eleusine indica (whole plant)	18
Elsholtzia ciliata (whole plant)	13
Elsholtzia splendens (whole plant)	6
Ephedra sinica (herba)	9
Epimedium grandiflorum (herba)	2
Equisetum arvense (whole plant)	2
Equisetum hyemale (aerial part)	9
Erigeron annuus (whole plant)	7
Erigeron canadensis (whole plant)	7
Eriobotrya japonica (folium)	8
Eucommia ulmoides (aerial part)	1
Eucommia ulmoides (cortex)	<0
Eugenia caryophyllata (flos)	<0
Euonymus alatus for. *ciliato-dentatus* (aerial part)	<0
Euonymus alatus (aerial part)	10

Table 1. Continued

Plant (part of use)	Inhibition (%)
Euonymus japonica (aerial part)	12
Euonymus pauciflorus (aerial part)	6
Euonymus sachalinensis (aerial part)	14
Eupatorium chinense for. *tripartitum* (aerial part)	2
Eupatorium chinense var. *simplicifolium* (whole plant)	17
Eupatorium fortunnei (aerial part)	8
Eupatorium lindleyanum (aerial part)	11
Euphorbia jolkini (aerial part)	2
Euphorbia kansui (radix)	7
Euphorbia pekinensis (aerial part)	9
Euphorbia pekinensis (radix)	6
Euphorbia supina (whole plant)	13
Euphoria longana (fructus)	4
Evodia officinalis (fructus)	7
Festuca myuros (aerial part)	<0
Festuca ovina (whole plant)	5
Filipendula glaberrima (whole plant)	2
Foeniculum vulgare (fructus)	1
Forsythia koreana (aerial part)	<0
Forsythia viridissima (fructus)	<0
Fraxinus rhynchophylla (aerial part)	6
Fraxinus sieboldiana (aerial part)	1
Fritillaria verticillata (tuber)	<0
Galium spurium (whole plant)	2
Galium verum var. *asiaticum* (aerial part)	<0
Gardenia jasminoides (fruit)	<0
Gastrodia elata (rhizoma)	10
Gentiana scabra (radix)	<0
Gentiana uchiyamai (whole plant)	8
Geranium nepalense subsp. *thunbergii* (whole plant)	11
Geranium sibiricum (aerial part)	8
Geum aleppicum (aerial part)	6
Geum japonicum (whole plant)	6
Ginko biloba (aerial part)	4
Glechoma hederacea var. *longituba* (aerial part)	<0
Gleditsia japonica var. *koraiensis* (aerial part)	7
Gleditsia sinensis (spina)	1
Glycyrrhiza grabra (aerial part)	2
Glycyrrhiza uralensis (radix)	9
Gossipium nanking (semen)	1
Helianthus annuus (aerial part and semen)	4
Hemerocallis fulva (whole plant)	7
Hemerocallis fulva var. *kwanso* (whole plant)	9
Hepatica asiatica (whole plant)	1
Heracleum moellendorffii (whole plant)	<0

Table 1. Continued

Plant (part of use)	Inhibition (%)
Hibiscus mutabilis (aerial part)	6
Hibiscus trionum (whole plant)	3
Hordeum vulgare (new born plant)	<0
Hosta japonica var. *lancifolia* (whole plant)	<0
Houttuynia cordata (whole plant)	7
Humulus japonicus (whole plant)	4
Humulus lupulus (aerial part)	<0
Hydrangea serrata for. *acuminata* (whole plant)	7
Hypericum ascyron (aerial part)	11
Hypericum erectum (aerial part)	<0
Hypocaeris ciliata (aerial part)	<0
Ilex macropoda (aerial part)	<0
Impatiens balsamina (aerial part)	7
Impatiens noli-tangere (aerial part)	<0
Impatiens textori (aerial part)	6
Imperata cylindrica var. *koenigii* (whole plant)	5
Inula britannica var. *chinenasis* (aerial part)	4
Iris ensata var. *spontanea* (whole plant)	2
Iris koreana (whole plant)	3
Iris nertschinskia (aerial part)	5
Isodon excisus (whole plant)	5
Isodon japonicus (aerial part)	29
Isodon japonicus (whole plant)	15
Ixeris dentata (aerial part)	7
Ixeris polycephala (whole plant)	8
Juglans sinensis (aerial part)	<0
Juniperus chinensis (aerial part)	<0
Kalopanax pictus (aerial part)	<0
Kalopanax septemlobus (cortex)	6
Kerria japonica (aerial part)	5
Koelreuteria paniculata (aerial part)	11
Kummerowia stipulacea (whole plant)	15
Kummerowia striata (whole plant)	19
Kyllinga brevifolia var. *leiolepis* (whole plant)	13
Lactuca indica for. indivisa (whole plant)	4
Lactuca triangulata (whole plant)	4
Lathyrus davidii (aerial part)	7
Lathyrus japonica (whole plant)	1
Leonurus sibiricus (aerial part)	2
Lespedeza cuneata (aerial part)	12
Lespedeza cyrtobotrya (aerial part)	1
Lespedeza maximowiczii (aerial part)	20
Lespedeza pilosa (aerial part)	4
Lespedeza tetraloba (aerial part)	1
Ligularia jaluensis (aerial part)	14
Ligusticum tenuissimum (radix)	2
Ligustrum obtusifolium (aerial part)	7
Lilium tigrinum (underground part)	23

Table 1. Continued

Plant (part of use)	Inhibition (%)
Lindera erythrocarpa (aerial part)	7
Lindera obtusiloba (aerial part)	6
Lindera strychnifolia (radix)	10
Liriodendron tulipifera (aerial part)	6
Liriope spicata (whole plant)	3
Lobelia chinensis (aerial part)	9
Loncicera japonica (aerial part)	9
Lonicera japonica (flos)	3
Lonicera maackii (aerial part)	15
Loranthus parasiticus (aerial part)	2
Lycium chinense (aerial part)	<0
Lycium chinense (fructus)	<0
Lycium chinense (radicis cortex)	8
Lycoris koreana (aerial part)	12
Lycoris squamigera (aerial part)	2
Lyndera erythrocarpa (aerial part)	4
Lysimachia davurica (whole plant)	8
Lythrum salicaria (whole plant)	4
Maackia amurensis (aerial part)	8
Machilus thunbergii (cortex)	18
Magnolia kobus (aerial part)	<0
Magnolia liliflora (flos)	3
Magnolia salicifolia (aerial part)	12
Magnolia siebolbii (aerial part)	7
Malus asiatica (aerial part)	5
Malus baccata (aerial part)	<0
Malus pumila var. *dulcissima* (aerial part)	<0
Melampyrum roseum (aerial part)	12
Melandryum firmum (whole plant)	15
Melilotus suaveolens (aerial part)	4
Mentha arvensis (herba)	<0
Mentha arvensis var. *piperascens* (whole plant)	<0
Metaplexis japonica (aerial part)	1
Microstegium vimineum (whole plant)	<0
Mirabilis jalapa (aerial part)	8
Miscanthus sacchariflorus (aerial part)	<0
Miscanthus sinensis (aerial part)	<0
Morus alba (radicis cortex)	81
Mosla dianthera (whole plant)	12
Mosla punctulata (whole plant)	7
Nelumbo nucifera (semen)	16
Nicotiana tabacum (aerial part)	16
Oenanthe javanica (aerial part)	<0
Oenothera lamarckiana (whole plant)	8
Oenothera odorata (whole plant)	5
Ophiopogon japonicus (tuber)	3
Oplismenus undulatifolius (whole plant)	22
Orixa japonica (aerial part)	<0
Orostachys japonicus (aerial part)	2
Osmunda japonica (aerial part)	4
Ostericum koreanum (aerial part)	<0

Table 1. Continued

Plant (part of use)	Inhibition (%)
Ostericum koreanum (rhizoma)	<0
Ostericum sieboldii (aerial part)	13
Ostericum sieboldii (whole plant)	2
Pachyma hoelen (sclerotia)	5
Paederia scandens (aerial part)	1
Paeonia albiflora (radix)	2
Paeonia suffruticosa (cortex)	8
Panax ginseng (radix)	<0
Parthenocissus tricuspidata (whole plant)	15
Patrinia scabiosaefolia (whole plant)	8
Paulownia coreana (aerial part)	6
Pedicularis resupinata (whole plant)	1
Perilla frutescens var. *acuta* (herba)	6
Persicaria conspicua (whole plant)	9
Persicaria filiforme (whole plant)	1
Persicaria hydropiper (whole plant)	2
Persicaria lapathifolia (aerial part)	5
Persicaria perfoliata (aerial part)	<0
Persicaria posumbu var. *laxiflora* (aerial part)	9
Persicaria senticosa (aerial part)	2
Persicaria sieboldii (aerial part)	5
Persicaria thunbergii (whole plant)	3
Persicaria viscosa (whole plant)	5
Petasites japonicus (whole plant)	4
Peucedanum japonicum (aerial part)	9
Peucedanum japonicum (radix)	3
Peucedanum praeruporum (radix)	2
Peucedanum terebinthaceum (whole plant)	7
Pharbitis nil (aerial part)	3
Pharbitis nil (semen)	6
Phaseolus nipponensis (aerial part)	6
Phaseolus radiatus (whole plant)	12
Phaseolus vulgaris (aerial part)	10
Phellodendron amurense (aerial part)	<0
Phellodendron amurense (cortex)	<0
Phlox paniculata (aerial part)	1
Phlox subulata (whole plant)	2
Phragmites communis (aerial part)	1
Phragmites japonica (aerial part)	14
Phryma leptostachya var. *asiatica* (aerial part)	2
Phtheirospermum japonicum (whole plant)	2
Phyllostachys nigra var. *henonis* (stem)	<0
Phyllostachys pubescens (aerial part)	1
Phyteuma japonicum (aerial part)	6
Phytolacca americana (aerial part)	15
Phytolacca americana (underground part)	16
Phytolacca esculenta (radix)	1
Picea abies (aerial part)	9

Table 1. Continued

Plant (part of use)	Inhibition (%)
Picrasoma quassioides (aerial part)	11
Picris hieracioides var. *glabrescens* (whole plant)	<0
Pilea mongolica (aerial part)	4
Pinellia ternata (tuber)	<0
Pinus densiflora (aerial part)	3
Pinus koraiensis (aerial part)	15
Pinus parviflora var. *pentaphylla* (aerial part)	4
Pinus strobus (aerial part)	17
Plantago asiatica (semen)	6
Platanus occidentalis (aerial part)	9
Platycarya strobilacea (aerial part)	13
Platycodon grandiflorum (radix)	<0
Pleuropterus cilinervis (aerial part)	<0
Pleuropterus multiflorum (radix)	7
Poa sphondylodes (whole plant)	3
Polygala tenuifolia (radix)	4
Polygonatum odoratum var. *pluriflorum* (rhizoma)	5
Polygonatum odoratum var. *pluriflorum* (whole plant)	18
Polygonatum sibiricum (aerial part)	14
Polygonum aviculare (whole plant)	13
Polystichum tripteron (whole plant)	2
Poncirus trifoliata (aerial part)	9
Poncirus trifoliata (fructus)	<0
Populus davidiana (aerial part)	16
Populus maximowiczii (aerial part)	10
Populus tomentiglandulosa (aerial part)	8
Portulaca oleracea (aerial part)	<0
Potentilla chinensis (whole plant)	9
Potentilla freyniana (aerial part)	4
Potentilla paradoxa (aerial part)	25
Prunella vulgaris (herba)	<0
Prunus armeniaca var. *ansu* (aerial part)	<0
Prunus armeniaca var. *ansu* (semen)	3
Prunus ishidoyana (aerial part)	9
Prunus leveilleana var. *pendula* (aerial part)	12
Prunus mume (aerial part)	<0
Prunus persica (aerial part)	<0
Prunus persica (semen)	<0
Prunus salicina (aerial part)	9
Prunus sargentii (aerial part)	<0
Psoralea corylifolia (semen)	<0
Pteridium aquilinum var. *latiusculum* (aerial part)	3
Pterocarya stenoptera (aerial part)	6
Pueraria thubergiana (aerial part)	5
Pueraria thubergiana (radix)	<0
Pulsatilla koreana (aerial part)	<0
Quamoclit angulata (aerial part)	8

Table 1. Continued

Plant (part of use)	Inhibition (%)
Quamoclit pennata (aerial part)	3
Quercus acutissima (aerial part)	9
Quercus aliena (aerial part)	16
Quercus dentata (aerial part)	3
Ranunculus sceleratus (aerial part)	8
Ranunculus tachiroei (whole plant)	12
Raphanus sativus (semen)	5
Rehmannia glutinosa (rhizoma)	2
Reynoutria elliptica (aerial part)	2
Rhamnus davurica (aerial part)	2
Rhapontica uniflora (aerial part)	4
Rheum undulatum (rhizoma)	11
Rhododendron mucronulatum (aerial part)	7
Rhododendron schlippenbachii (aerial part)	17
Rhododendron yedoense var. *poukhanense* (aerial part)	18
Rhus chinensis (aerial part)	7
Rhus verniciflua (semen)	19
Ricinus communis (whole plant)	20
Robinia pseudo-acacia (aerial part)	7
Rorippa islandica (whole plant)	9
Rosa laevigata (fructus)	<0
Rosa multiflora (aerial part)	5
Rubia akane (aerial part)	7
Rubus coreanus (fructus)	3
Rubus crataegifolius (whole plant)	7
Rubus parvifolius (aerial part)	<0
Rubus phoenicolasius (aerial part)	<0
Rumex longifolius (whole plant)	3
Salix floderusii (aerial part)	3
Salix gilgiana (aerial part)	14
Salix glandulosa (aerial part)	5
Salix hallaisanensis (aerial part)	4
Salvia chanroenica (whole plant)	6
Salvia plebeia (aerial part)	1
Sambucus williamsii var. *coreana* (aerial part)	7
Sanguisorba hakusanensis (whole plant)	1
Sanguisorba longifolia (aerial part)	5
Sanguisorba officinalis (radix)	11
Sasa borealis (aerial part)	4
Saxifraga manshuriensis (aerial part)	4
Schizandra chinensis (semen)	11
Schizonepeta tenuifolia (herba)	2
Scirpus wichurae (aerial part)	11
Scrophularia buergeriana (whole plant)	7
Scrophularia ningpoensis (radix)	6
Scutellaria baicalensis (aerial part)	1
Scutellaria baicalensis (radix)	<0
Secale cereale (whole plant)	12
Securinega suffruticosa (aerial part)	8

Table 1. Continued

Plant (part of use)	Inhibition (%)
Sedum Zokuriense (aerial part)	14
Sedum aizoon (whole plant)	5
Sedum erythrostichum (aerial part)	<0
Sedum sarmentosum (whole plant)	5
Sedum verticillatum (aerial part)	2
Senecio integrifolius var. *spathulatus* (whole plant)	11
Sesamum indicum (whole plant)	11
Setaria chondrachne (whole plant)	8
Setaria glauca (whole plant)	<0
Setaria viridis (whole plant)	2
Siegesbeckia glabrescens (aerial part)	3
Siegesbeckia pubescens (aerial part)	<0
Silene armeria (aerial part)	5
Siphonostegia chinensis (aerial part)	7
Sium suave (aerial part)	9
Smilax china (underground part)	5
Smilax sieboldii (aerial part)	14
Solanum nigrum (herba)	<0
Sophora flavescens (aerial part	1
Sophora flavescens (fructus)	6
Sophora japonica (fruit)	12
Spiraea blumei (aerial part)	<0
Spiraea japonica (aerial part)	<0
Spiraea prunifolia (aerial part)	13
Spiraea salicifolia (aerial part)	3
Staphylea bumalda (aerial part)	3
Stellaria alsine var. *undulata* (whole plant)	10
Stellaria aquatica (aerial part)	9
Stephania tetrandra (radix)	1
Stephnandra incisa (aerial part)	3
Stewartia koreana (aerial part)	5
Streptolirion cordifolium (aerial part)	5
Styrax japonica (aerial part)	5
Styrax obassia (aerial part)	5
Symphytum officinale (aerial part)	<0
Symplocos chinensis for. *pilosa* (aerial part)	2
Syneilesis palmata (whole plant)	16
Synurus exelsus (aerial part)	11
Taraxacum mongolicum (whole plant)	7
Taraxacum platycarpum (whole plant)	5
Taxodium distichum (aerial part)	9
Taxus cuspidata (aerial part)	7
Teucrium japonicum (aerial part)	<0
Thalictrum aquilegifolium (aerial part)	3
Thalictrum filamentosum (aerial part)	4
Thalictrum minus var. *hypoleucum* (aerial part)	11
Thuja orientalis (aerial part)	3
Torilis japonica (aerial part)	2
Tradescantia reflexa (whole plant)	7

Table 1. Continued

Plant (part of use)	Inhibition (%)
Trichosanthes kirilowii (aerial part)	9
Trichosanthes kirilowii (radix)	<0
Trifolium repens (aerial part)	11
Trigonotis peduncularis (whole plant)	5
Tussilago fartara (whole plant)	1
Ulmus davidiana (aerial part)	8
Ulmus parvifolia var. *coreana* (aerial part)	8
Uncaria rhynchophylla (ramulus)	11
Urtica angustifolia (aerial part)	3
Vaccinium koreanum (aerial part)	3
Veronica persica (aerial part)	15
Viburunum sargentii (aerial part)	1
Vicia bungei (aerial part)	2
Vinca major (whole plant)	13
Viola dissecta var. *chaerophylloides* (aerial part)	14
Viola patrinii (whole plant)	9
Viola verecunda (whole plant)	7
Viola yedoensis (whole plant)	11
Vitex rotundifolia (fructus)	<0
Vitis amurensis (aerial part)	6
Vitis coignetiae (aerial part)	13
Weigela subsessilis (aerial part)	9
Wistaria floribunda (aerial part)	4
Xanthium strumarium (folium)	3
Youngia chelidonifolia (whole plant)	1
Youngia denticulata (whole plant)	5
Youngia japonica (aerial part)	7
Yucca smalliana (aerial part)	8
Zanthoxylum bungeanum (pericarpium)	15
Zanthoxylum schinifolium (aerial part)	15
Zingiber officinale (rhizoma)	10
Zizyphus jujuba var. *inermis (fructus)*	3

active extracts were prepared from whole plant of *Acalypha australis*, aerial part of *Acer ginnala*, whole plant of *Agrostis clavata* var. *nukabo*, aerial part of *Artemisia princess*, whole plant of *Arthraxon hispidus*, whole plant of *Aster scaber*, underground part of *Carex humilis*, aerial part of *Cersis chinensis*, rhizoma of *Curcuma longa*, whole plant of *Desmodium oldhami*, whole plant of *Dryopteris crassirhizoma*, aerial part of *Isodon japonicus*, aerial part of *Lespedeza maximowiczii*, underground part of *Lilium tigrinum*, radicis cortex of *Morus alba*, whole plant of *Oplismanus undulatifolius*, aerial part of *Potentilla paradoxa*, and whole plant of *Ricinus communes*. The highest inhibitory effect on the dopa oxidase activity of tyrosinase was shown in the extract prepared from radicis cortex of *Morus alba*, and next in that prepared from rhizoma of *Curcuma longa*.

Interestingly, both radicis cortex of *Morus alba* and rhizoma of *Curcuma longa* are herbal medicines. Tyrosinase inhibitors isolated and identified from oriental traditional medicines are glabridin from *Glycyrrhiza glabra*, cinnamaldehyde from *Cinnamomum cassia*, eugenol from *Syzygium aromaticum*, and yakuchinone A and B from *Alpinia oxyphylla*, and those from bolivian medicinal plants are agrimoniin, and buddlenoid A and B from *Buddleia coriacea*, gnaphalin and leuteolin 4'-β-D-glucoside from *Gnaphalium cheiranthifolium*, and *p*-hydroxybenzoic acid from *Scheelea princeps* (Kubo *et al.*, 1995; Shirota *et al.*, 1994). The extracts prepared from *Glycyrrhiza glabra*, and *Cinnamomum cassia* were also used as samples in this study, but their inhibitory effects on dopa oxidase activity of mushroom tyrosinase were not significant at 100 μg/ml of concentration.

The total MeOH extracts prepared from radicis cortex of *Morus alba* and rhizoma of *Curcuma longa* were subjected to sequential fractionations with methylene chloride, ethyl acetate, *n*-butanol, and polar residue. Inhibitory effects of each of the solvent-fractionated extracts on dopa oxidase activity of tyrosinase were estimated (Table 2). At a concentration of 100 μg/ml, significant inhibitory effects on the enzyme activity were shown in all fractions except polar residue of the radicis cortex of *Morus alba*, and methylene chloride fraction of the rhizoma of *Curcuma longa*. The ethyl acetate fraction of radicis cortex of *Morus alba* and methylene chloride fraction of rhizoma of *Curcuma longa* exhibited more than 90 % of inhibition on the enzyme activity at a concentration of 100 μg/ml, and ethyl acetate and *n*-butanol fractions of radicis cortex of *Morus alba* did 42% to 47% of inhibition at the same concentration. As shown in Fig. 1,

Table 2. Inhibitory effects on tyrosinase by solvent-fractionated extracts of *Morus alba* and *Curcuma longa*

Plant	Fraction	% of inhibition at 100 μg/ml	IC_{50} (μg/ml)
Morus alba	MC	47±1*	>100
	EtOAc	92±1*	12
	BuOH	42±1**	>100
	Polar residue	7±2	>100
Curcuma longa	MC	93±3*	51
	EtOAc	7±2	>100
	BuOH	11±1	>100
	Polar residue	10±3	>100

Fractions are methylene chl- oride (MC), ethyl acetate (EtOAc), *n*-butanol (BuOH) layers and polar residue. Inhibitory effects on dopa oxidase activity of tyrosinase are represented as % of inhibition, mean±standard error (n=3) and their significances compared with the control are $p<0.001$ (*) and $p<0.01$ (**).

both ethyl acetate fraction of radicis cortex of *Morus alba* and methylene chloride fraction of rhizoma of *Curcuma longa* exhibited dose-dependent inhibitions on dopa oxidase activity of mushroom tyrosinase. The ethyl acetate fraction of radicis cortex of *Morus alba* exhibited 50% of inhibition (IC_{50}) on the enzyme activity at the concentration of 12 μg/ml, and methylene chloride fraction of rhizoma of *Curcuma longa* at 51 μg/ml.

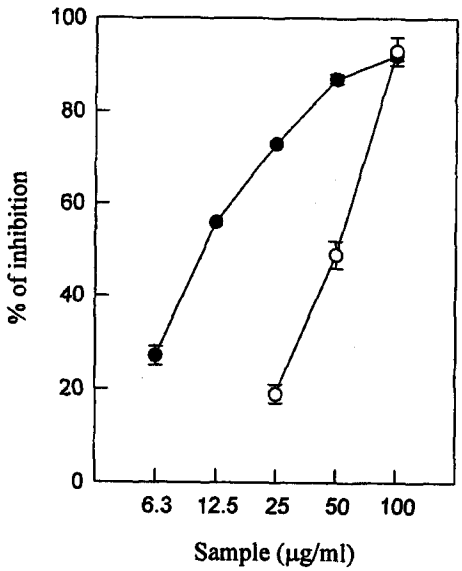

Fig. 1. Dose-dependent inhibitions on tyrosinase by ethyl acetate fraction of *Morus alba* (solid circle) and methylene chloride fraction of *Curcuma longa* (open circle).

As the major biological activities, *Morus* species are known to have hypoglycemic activity, and *Curcuma* species to have anti-inflammatory and anti-mutagenic activities (Anto *et al.*, 1996; Chen *et al.*, 1995; Srivastava *et al.*, 1995). Bioactive secondary metabolites of *Morus* species identified are nitrogen-containing sugars including deoxynojirimycin and fagomine, and glycoproteins including moran A (Asano *et al.*, 1994; Hikino *et al.*, 1985). Major bioactive constituents of *Curcuma* species are identified as curcuminoids (Anto *et al.*, 1994). Curcumin, one of the curcuminoids from *Curcuma* species, is known to have potent inhibitory effect on tyrosinase (Shirota *et al.*, 1994). However, the principle of inhibitory effect on tyrosinase by *Morus* species is still unknown. Active constituent(s) exhibiting inhibitory effect on dopa oxidase activity of tyrosinase will be elucidated from the ethyl acetate fraction of *Morus alba* in a future study.

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