Available online at www.sciencedirect.com
SCIENCE DIRECT®
ELSEVIER
Journal of Ethnopharmacology 106 (2006) 353–359
Journal of ETHNO-PHARMACOLOGY
www.elsevier.com/locate/jethpharm

Cosmetic applications of selected traditional Chinese herbal medicines

Kuo-Hsien Wang [a,b], Rong-Dih Lin [c], Feng-Lin Hsu [d], Yen-Hua Huang [e], Hsien-Chang Chang [f], Ching-Yi Huang [d], Mei-Hsien Lee [d,*]

[a] *Department of Dermatology, Taipei Medical University Hospital, Taipei 110, Taiwan*
[b] *Department of Dermatology, Taipei Municipal Wan-Fang Hospital, Taipei 116, Taiwan*
[c] *Department of Internal Medicine, Ho-Ping Branch, Taipei City Hospital, Taipei 100, Taiwan*
[d] *Graduate Institute of Pharmacognosy Science, Taipei Medical University, Taipei 110, Taiwan*
[e] *Department of Biochemistry, Taipei Medical University, Taipei 110, Taiwan*
[f] *Bureau of Food and Drug Analysis, Department of Health, Taipei 110, Taiwan*

Received 30 September 2005; received in revised form 4 January 2006; accepted 13 January 2006
Available online 23 February 2006

Abstract

Because tyrosinase catalyzes melanin synthesis, tyrosinase inhibitors are important in cosmetic skin-whitening. Oxidative stress contributes to skin aging and can adversely affect skin health, which means antioxidants active in skin cells may support skin health. We examined 25 traditional Chinese herbal medicines that might be useful for skin-whitening and skin health. Extracts (100 μg/mL) were tested for cytotoxicity on human epidermal melanocytes (HEMn); 12 exhibited low cytotoxicity. Their effects on tyrosinase and melanin inhibitory activities and free radical scavenging activities were further assessed. Phenolic contents were evaluated using Folin–Ciocalteu reagent. Four herbs, *Pharbitis nil*, *Sophora japonica*, *Spatholobus suberectus*, and *Morus alba*, exhibited potent inhibitory effects on tyrosinase (IC_{50} values 24.9, 95.6, 83.9, and 78.3 μg/mL, respectively). Melanin inhibition was not dose-dependent. *Sophora japonica* (IC_{50}: 14.46 μg/mL, 1,1-diphenyl-2-picrylhydrazyl (DPPH); 1.95 μg/mL, hydroxyl radical) and *Spatholobus suberectus* (IC_{50}: 10.51 μg/mL, DPPH; 4.36 μg/mL, hydroxyl radical) showed good antioxidative activities and high phenolic contents (255 and 189 mg of gallic acid/g extract, respectively). Among active anti-tyrosinase extracts, *Sophora japonica* and *Spatholobus suberectus* were especially potent in HEMn cells in terms of free radical scavenging effects and high phenolic contents, making them the strongest candidates for cosmetic application found in the current study.
© 2006 Elsevier Ireland Ltd. All rights reserved.

Keywords: *Pharbitis nil*; *Sophora japonica*; *Spatholobus suberectus*; Tyrosinase; Antioxidative; Human epidermal melanocytes

1. Introduction

Melanin is the major pigment for color of human skin. It is secreted by melanocyte cells in the basal layer of the epidermis (Hearing, 2005). Melanin may be overproduced with chronic sun exposure, melasma, or other hyperpigmentation diseases (Briganti et al., 2003). Therefore, a number of depigmenting agents have been developed for cases of undesirable skin discoloration. Tyrosinase, a copper-containing monooxygenase, is a key enzyme that catalyzes melanin synthesis in melanocytes (Sturm et al., 2001). It catalyzes two major reactions, including hydroxylation of tyrosine and oxidation of the *o*-diphenol product, *l*-dopa. Dopa oxidation produces a highly reactive intermediate that is further oxidized to form melanin by a free radical-coupling pathway. If free radicals are inappropriately processed in melanin synthesis, hydrogen peroxide (H_2O_2) is generated, leading to production of hydroxyl radicals ($HO^{\bullet -}$) and other reactive oxygen species (ROS) (Perluigi et al., 2003). Melanin biosynthesis can be inhibited by avoiding ultraviolet (UV) exposure, by inhibition of melanocyte metabolism and proliferation (Seiberg et al., 2000), by inhibition of tyrosinase, or by removal of melanin by corneal ablation. Apart from avoiding UV exposure, application of tyrosinase inhibitors may be the least invasive procedure for maintaining skin whiteness; such

* Corresponding author at: Graduate Institute of Pharmacognosy Science, Taipei Medical University, 250 Wu-Hsing Street, Taipei 110, Taiwan.
Tel.: +886 2 2736 1661x6151.
E-mail address: lmh@tmu.edu.tw (M.-H. Lee).

0378-8741/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.jep.2006.01.010

agents are increasingly used in cosmetic products (Kadekaro et al., 2003).

Oxidative stress may be induced by increasing generation of ROS and other free radicals. UV radiation can induce formation of ROS in skin such as singlet oxygen and superoxide anion, promoting biological damage in exposed tissues via iron-catalyzed oxidative reactions (Yasui and Sakurai, 2003). These ROS enhance melanin biosynthesis, damage DNA, and may induce proliferation of melanocytes. A previous study (Yamakoshi et al., 2003) also found evidence for a role of oxidative stress in pathogenesis of skin disorders. It is known that ROS scavengers or inhibitors such as antioxidants may reduce hyperpigmentation (Ma et al., 2001).

Traditional herbal medicines provide an interesting, largely unexplored source for development of potential new drugs. The potential use of traditional herbal medicines for development of new skin-care cosmetics has been emphasized recently (Kiken and Cohen, 2002). It is of great interest to know whether preparations used cosmetically in folk medicine have activities that might be useful in modern formulations. In the present study, the anti-tyrosinase effects of 95% ethanol extracts of some traditional Chinese herbal medicines used for skin-care in ancient books were evaluated in cultures of human melanocytes. Their antioxidant abilities and phenolic contents were also tested.

2. Materials and methods

2.1. Reagents

Triton X-100, *l*-3,4-dihydroxyphenylalanine (*l*-DOPA), sodium hydroxide (NaOH), luminol, melanin, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), ethylenediaminetetraacetic acid (EDTA), and Folin–Ciocalteu reagent were purchased from Sigma (St. Louis, MO). Other chemicals were of the highest grade commercially available.

2.2. Materials

Chinese herbal medicines were purchased from Taipei local medicinal markets in 2004, and identity was confirmed by Prof. Chang, H.C. at Bureau of Food and Drug Analysis, Department of Health, Taiwan. The specimens were deposited in the Graduate Institute of Pharmacognosy, Taipei Medical University, Taipei, Taiwan.

2.3. Preparation of extracts

Dried traditional Chinese herbal medicines were pulverized in a grinder and extracted with 10-fold 95% ethanol solution at room temperature for 3 days and then filtered. The procedure was repeated two times. The filtrates were combined and were concentrated under reduced pressure, freeze-dried, and stored in a closed container until use.

2.4. Cell culture

Primary cultures of normal human epidermal melanocytes (HEMn, Cat. C-102-5C, Cascade Biologics, Inc., Portland, OR) derived from neonatal foreskin were purchased from Cascade Biologics Company. They were grown in Medium 254 (Cat. M-254-500, Cascade Biologics) supplemented with Human Melanocyte Growth Supplement (HMGS, Cat. S-002-5, Cascade Biologics).

2.5. Assay of cell viability

Cell viability was determined using the MTT method. For experiments, cells were plated in 24-well plates at 1×10^5 cells/well. After 24 h, the test sample was added to each well and incubated for 24 h. Cell survival was determined in a colorimetric assay using mitochondrial dehydrogenase activity in active mitochondria to form purple formazan. Cell viability was calculated as follows: cell viability (%) = (absorbance of the sample tested/absorbance of the medium only) × 100.

2.6. Assay of cellular tyrosinase activity

Tyrosinase activity was measured as described previously, with slight modification (Jones et al., 2002). Briefly, normal human melanocyte cells were cultured in 24-well plates. After being treated with an individual herbal preparation for 24 h, cells were washed with potassium phosphate buffered saline (PBS) and lysed with PBS (pH 6.8) containing 1% Triton X-100. Then, cells were disrupted by freezing and thawing, and lysates were clarified by centrifugation at 10,000 × *g* for 10 min. Protein content was determined using a Bio-Rad protein assay kit (Bio-Rad, Hercules, CA, U.S.A.) after quantifying protein levels and adjusting concentrations with lysis buffer until each lysate contained the same amount of protein (40 μg). Each well of a 96-well plate contained 40 μg protein, 2.5 mM *l*-DOPA, and 0.1 M PBS (pH 6.8). After incubation at 37 °C for 1 h, absorbance (as optical density, OD) was measured at 450 nm using an ELISA (enzyme-linked immunosorbent assay) reader. Tyrosinase inhibitory activity was calculated with the following formula:

$$\text{Tyrosinase inhibition (\%)} = \left[1 - \frac{\text{OD}_{450}\ \text{of sample}}{\text{OD}_{450}\ \text{of control}}\right] \times 100$$

2.7. Measurement of melanin content in melanocytes

Melanin contents were measured as described previously, with slight modification (Jones et al., 2002). Cells were treated with tested individual preparations for 24 h. Cell pellets were dissolved in 1N NaOH at 37 °C overnight and centrifuged for 10 min at 10,000 × *g*. The optical density (OD) of each supernatant was measured at 450 nm using an ELISA reader.

2.8. Assay for 1,1-Diphenyl-2-picrylhydrazyl (DPPH) scavenging activity in vitro

Each individual test preparation underwent reaction with 160 μM DPPH in methanol solution. After incubating for 20 min at room temperature in the dark, absorbance was read at 517 nm as previously described (Hou et al., 2003).

2.9. Assay for hydroxyl radical scavenging activity in vitro

The hydroxyl radical was generated by the Fenton reaction using the luminescence method as modified by Cheng et al. (2003). The reaction mixture contained 40 μM luminol, 4.17 mM phosphate buffer (pH 7.5), 4.6 μM iron(III)–2.3 μM EDTA, test preparation, and 96 mM H_2O_2. The chemiluminescent reaction proceeded in KH_2PO_4–NaOH-buffered solution (pH 7.5) at room temperature. Initiation of reaction was achieved by adding Fe(III)–EDTA and then H_2O_2 into the mixture. Luminescence intensity was monitored in the wavelength range of 200–900 nm.

2.10. Determination of total phenolic content

Total phenolic content of each extract was determined by a modified Folin–Ciocalteu method (Yeh and Yen, 2005). The sample solution (50 μL) was mixed with an equal volume of 1N Folin–Ciocalteu reagent–20% sodium carbonate (Na_2CO_3). After a 25-min incubation period at room temperature, the reaction mixture was centrifuged at 5000 rpm for 10 min. The supernatant was measured at 730 nm using a spectrophotometer. Total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram (mg/g) dry plant extract.

2.11. Statistical analysis

Data are presented as the mean ± standard deviation (S.D.) of each triplicate test. Differences between groups were tested for significance by means of the non-parametric Mann–Whitney *U*-test. A *P*-value < 0.5 was considered to be statistically significant.

Table 1
Selected traditional Chinese herbal medicines used on the skin, and their recommended uses

No.	Family	Scientific name	Used part	Traditional uses (Yen, 1992)	Voucher specimen	Yield (%)
1	Aristolochiaceae	*Asarum heterotropoides* Fr. Schumidt var. *mandshuricum* (Maxim.) Kitag.	Entire plant	Wind-cold-effusing agent	M128	5.3
2	Boraginaceae	*Lithospermum erythrorhizon* Sieb. Et Zucc.	Root	Heat-clearing and blood-cooling agent	M103	3.7
3	Convolvulaceae	*Pharbitis nil* (L.) Choisy	Seed	Potent precipitating and water-expelling agent	M94	6.4
4	Cucurbitaceae	*Trichosanthes kirillowii* Maxim.	Root	Heat-clearing and fire-draining agent	M154	2.1
5	Gentianaceae	*Gentiana macrophylla* Pall.	Root	Wind-damp-dispelling agent	M91	17.7
6	Labiatae	*Elsholtzia ciliate* (Thunb.) Hyland	Entire plant	Exterior-resolving agent	M101	2.5
7	Labiatae	*Leonurus heterophyllus* Sweet	Entire plant	Blood-rectifying agent	M126	4.5
8	Labiatae	*Agastache rugosa* (Fisch. et Mey) O. Ktze.	Entire plant	Abducting dispersion agent	M129	3.8
9	Labiatae	*Prunella vulgaris* L.	Spike of common self-heal	Heat-clearing and fire-draining agent	M97	2.3
10	Leguminosae	*Astragalus membranaceus* (Fisch.) Bunge	Root	Qi-boosting agent	M25	17.1
11	Leguminosae	*Glycyrrhiza uralensis* Fisch.	Root and rhizome	Qi-supplementing agent	M21	24.0
12	Leguminosae	*Sophora japonica* L.	Flower	Blood-staunching agent	M108	6.5
13	Leguminosae	*Spatholobus suberectus* Dunn.	Stem	Blood-rectifying agent	M99	6.2
14	Liliaceae	*Polygonatum odoratum* (Mill.) Druce	Rhizome	Yin-supplementing agent	M123	2.5
15	Moraceae	*Cannabis sativa* L.	Seed	Moist precipitating agent	M127	10.4
16	Moraceae	*Morus alba* L.	Leaves	Wind-heat-effusing agent	M100	5.9
17	Phytolaccaceae	*Phytolaacca acinosa* Roxb.	Root	Potent precipitating and water-expelling agent	M106	3.5
18	Ranunculaceae	*Paeonia suffruticosa* Andr.	Root-bark	Heat-clearing and blood-cooling agent	M109	4.7
19	Rosaceae	*Crataegus pinnatifida* Bge. var. *major* N. E. Br.	Fruit	Abducting dispersion agent	M93	33.5
20	Rosaceae	*Prunus persica* (L.) Batsch	Seed	Blood-quickening and stasis-dispelling agent	M124	10.1
21	Rutaceae	*Citrus reticulata* Blanco	Pericarp	Qi-rectifying agent	M89	20.1
22	Rutaceae	*Dictamnus dasycarpus* Turcz.	Root-bark	Heat-clearing and toxin-resolving agent	M125	2.4
23	Saururaceae	*Houttuynia cordata* Thunb.	Entire plant	Heat-clearing and toxin-resolving agent	M92	9.2
24	Vitaceae	*Ampelopsis japonica* (Thunb.) Makino	Root	Heat-clearing agent	M102	4.7
25	Zingiberaceae	*Amornurn villosurn* Lour.	Fruit	Aromatic dampness-transforming agent	M105	13.2

3. Results and discussion

In previous papers, inhibition of tyrosinase by a variety of compounds has been studied, with the result that several inhibitors are now used as cosmetic additives or as medicinal products for hyperpigmentation (Rescigno et al., 2002; An et al., 2005). Recently, natural substances such as green-plant products have been in increased demand in the global market for new agents for depigmenting, cosmeceutical, and skin-lightening purposes (Aburjai and Natsheh, 2003). Traditional Chinese herbal medicines have been used in clinical practice for centuries; they are often used to maintain good health or used to treat various diseases. In the present study, these materials were selected based on compiled ethnobotanical data that revealed the agents are usually used clinically as skin applications (Table 1). Therefore, we evaluated their effects on tyrosinase and melanin content in human skin melanocytes, HEMn, as well as their antioxidant activities. The 25 selected traditional Chinese herbal medicines were extracted with 95% ethanol, with extract yields ranging from 2.1 to 33.5% (Table 2).

In the present study, we used human skin melanocytes as an in vitro model because of the need to measure cytotoxic effects. An MTT assay for cytotoxicity was employed before further in vitro testing in skin melanocytes was done to test tyrosinase inhibition and melanin content. Among the 25 tested extracts, 12 extracts, *Lithospermum erythrorhizon* (M103), *Pharbitis nil* (M94), *Gentiana macrophylla* (M91), *Glycyrrhiza uralensis* (M21), *Sophora japonica* (M108), *Spatholobus suberectus* (M99), *Polygonatum odoratum* (M123), *Cannabis sativa* (M127), *Morus alba* (M100), *Phytolaacca acinosa* (M106), *Citrus reticulata* (M89), and *Amornurn villosurn* (M105), showed relatively lower cytotoxicity, with cell viability above 80% with a concentration of 100 μg/mL (Fig. 1). After low cytotoxicity was identified as a first test of possible clinical usefulness, the 12 appropriate extracts were further examined for inhibition of tyrosinase activity and melanin content.

Tyrosinase inhibitors are important constituents of cosmetics and skin-lightening agents (An et al., 2005). We used *l*-DOPA as the substrate to detect any tyrosinase inhibitory effect in HEMn cells (protein content per well, 40 μg). Among extracts, *Pharbitis nil* (M94), *Sophora japonica* (M108), *Spatholobus suberectus* (M99), and *Morus alba* (M100) showed potent tyrosinase inhibitory effects. Their IC_{50} values were 24.9, 95.6, 83.9, and 78.3 μg/mL, respectively. In comparison, arbutin, a naturally occurring cosmetic vehicle and whitening agent with tyrosinase inhibitory activity (Nihei and Kubo, 2003; Roh et al., 2004), has an IC_{50} value of 3.0 mM in HEMn cells (Table 3). Thus, our tested extracts exhibited greater inhibitory activity than arbutin.

Melanin formation is the most important determinant of mammalian skin color (Hearing, 2005). Melanin is synthesized in a multi-step biochemical pathway that operates within a specialized intracellular organelle, the melanosome. In melanogenesis, the proximal pathway consists of the enzymatic oxidation of tyrosine or *l*-DOPA to its corresponding *o*-dopaquinone catalyzed by tyrosinase. After multi-biosynthesis steps, further polymerization yields melanin (Kim and Uyama, 2005). In

Table 2
The extracted yields and IC_{50} values of selected Chinese herbal medicines against DPPH and OH free radicals

No.	Scientific name	Yield (%)	Total phenolic (mg GA/g)	IC_{50} (μg/ml)	
				DPPH radical scavenging activity	Hydroxyl radical scavenging activity
1	*Asarum heterotropoides* var. *mandshuricum*	5.3	21.4	>100	<1.00
2	*Lithospermum erythrorhizon*	3.7	156.9	24.1	2.4
3	*Pharbitis nil*	6.4	39.5	>100	45.9
4	*Trichosanthes kirillowii*	2.1	25.4	>100	16.6
5	*Gentiana macrophylla*	17.7	8.9	>100	61.1
6	*Elsholtzia ciliate*	2.5	83.4	48.7	1.5
7	*Leonurus heterophyllus*	4.5	29.9	>100	4.5
8	*Agastache rugosa*	3.8	29.3	>100	4.6
9	*Prunella vulgaris*	2.3	60.0	24.8	1.5
10	*Astragalus membranaceus*	17.1	8.1	>100	27.7
11	*Glycyrrhiza uralensis*	24.0	95.2	–	64.5
12	*Sophora japonica*	6.5	254.5	14.5	1.9
13	*Spatholobus suberectus*	6.2	188.7	10.5	4.4
14	*Polygonatum odoratum*	2.5	12.8	>100	–
15	*Cannabis sativa*	10.4	29.3	>100	2.8
16	*Morus alba*	5.9	50.9	>100	2.3
17	*Phytolaacca acinosa*	3.5	99.5	56.0	5.6
18	*Paeonia suffruticosa*	4.7	149.2	15.4	1.6
19	*Crataegus pinnatifida* var. *major*	33.5	71.1	81.6	5.8
20	*Prunus persica*	10.1	2.9	>100	>100
21	*Citrus reticulata*	20.1	37.9	>100	33.4
22	*Dictamnus dasycarpus*	2.4	11.0	>100	>100
23	*Houttuynia cordata*	9.2	126.0	72.5	4.2
24	*Ampelopsis japonica*	4.7	19.3	>100	22.4
25	*Amornurn villosurn*	13.2	88.0	78.8	1.1

–: enhances the generation of radicals.

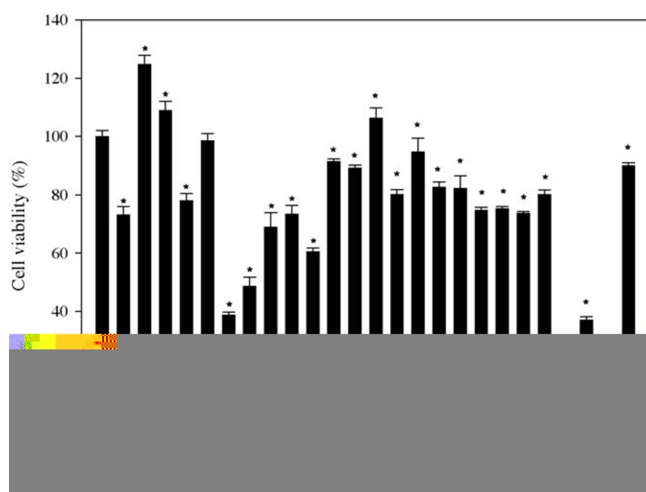

Fig. 1. Cell viabilities of human epidermal melanocytes (HEMn) treated with the Chinese herbal medicines. Differences in data were evaluated for statistical significance (P-value <0.5) with the non-parametric Mann–Whitney U-test. C: Control; 1: *Asarum heterotropoides* var. *mandshuricum*; 2: *Lithospermum erythrorhizon*; 3: *Pharbitis nil*; 4: *Trichosanthes kirillowii*; 5: *Gentiana macrophylla*; 6: *Elsholtzia ciliate*; 7: *Leonurus heterophyllus*; 8: *Agastache rugosa*; 9: *Prunella vulgaris*; 10: *Astragalus membranaceus*; 11: *Glycyrrhiza uralensis*; 12: *Sophora japonica*; 13: *Spatholobus suberectus*; 14: *Polygonatum odoratum*; 15: *Cannabis sativa*; 16: *Morus alba*; 17: *Phytolaacca acinosa*; 18: *Paeonia suffruticosa*; 19: *Crataegus pinnatifida* var. *major*; 20: *Prunus persica*; 21: *Citrus reticulata*; 22: *Dictamnus dasycarpus*; 23: *Houttuynia cordata*; 24: *Ampelopsis japonica*; 25: *Amornurn villosurn*.

the present study, normal human melanocytes were used for determination of extract effects and cellular melanin content (Table 3). The most active anti-tyrosinase extracts, *Pharbitis nil* (M94), *Sophora japonica* (M108), *Spatholobus suberectus* (M99), and *Morus alba* (M100), did not show dose-dependent inhibition of melanin production (Table 3). The extracts of *Phytolaacca acinosa* (M106) and *Citrus reticulata* (M89) showed little tyrosinase inhibitory activity, and cellular melanin content was actually enhanced. Thus, in the present study, inhibition of melanin synthesis was not related to level of tyrosinase inhibition. This finding may due to the fact that melanin is synthesized by a multi-step pathway. Apart from tyrosinase, synthesis is also controlled by other enzymes such as 5,6-dihydroxyindole-2-carboxylic acid oxidase (DHICA oxidase) and dopachrome tautomerase (DCT) (Kobayashi et al., 1995; Kim and Uyama, 2005). The increase in melanin content after treatment with two extracts may be due to effects on other steps of melanin biosynthesis.

DPPH is a stable radical that is used in a popular method for screening free radical-scavenging ability of compounds or antioxidant activity of plant extracts. The five extracts *Lithospermum erythrorhizon* (M103), *Prunella vulgaris* (M97), *Sophora japonica* (M108), *Spatholobus suberectus* (M99), and *Paeonia suffruticosa* (M109) showed dose–response curves for DPPH radical-scavenging activity. The IC_{50} values were calculated and are presented in Table 2. The results imply that these active extracts may contain constituents with strong proton-donating abilities (Sawai and Moon, 2000).

The hydroxyl radical is one of the most reactive radicals generated from biologic molecules and can damage living cells

Table 3
Inhibition of tyrosinase activity and melanin production in HEMn cells by the selected Chinese herbal medicines

Voucher specimen	Sample	Concentration (μg/ml)	Tyrosinase inhibition (%)	Melanin inhibition (%)
	Arbutin	5 mM	67.1 ± 1.8	5.7 ± 0.1
		2.5 mM	43.5 ± 3.2	7.1 ± 2.0
		1 mM	36.0 ± 3.2	6.5 ± 1.2
		0.5 mM	29.8 ± 1.2	−1.7 ± 1.6
M103	*Lithospermum erythrorhizon*	100	26.4 ± 0.3	6.5 ± 0.6
M94	*Pharbitis nil*	100	56.6 ± 0.2	25.7 ± 1.9
		80	55.6 ± 0.6	16.8 ± 4.2
		40	53.1 ± 0.1	24.2 ± 2.8
		10	47.4 ± 4.8	23.8 ± 0.7
M91	*Gentiana macrophylla*	100	–	–
M21	*Glycyrrhiza uralensis*	100	19.6 ± 4.2	9.5 ± 6.8
M108	*Sophora japonica*	100	54.4 ± 1.3	12.1 ± 0.1
		80	38.2 ± 1.5	9.0 ± 5.2
		60	24.9 ± 0.7	8.1 ± 5.1
		40	14.8 ± 1.9	7.6 ± 7.5
M99	*Spatholobus suberectus*	100	57.2 ± 0.2	3.5 ± 3.7
		80	49.6 ± 0.5	11.1 ± 5.3
		60	38.5 ± 2.6	4.5 ± 3.6
		40	22.9 ± 0.7	6.6 ± 2.8
M100	*Morus alba*	100	70.3 ± 0.2	16.3 ± 4.8
		80	49.6 ± 8.7	14.19 ± 5.9
		60	35.4 ± 1.1	12.1 ± 5.1
		40	14.3 ± 6.1	12.5 ± 3.0
M106	*Phytolaacca acinosa*	100	2.2 ± 1.10	–
M89	*Citrus reticulata*	100	7.4 ± 3.23	–

–: enhances of the activity.

(Bergamini et al., 2004). Some plant extracts have the ability to scavenge hydroxyl radicals and may protect cellular lipids against free radical reactions (Reiter et al., 2001). The data in Table 2 show that *Asarum heterotropoides* var. *mandshuricum* (M128), *Lithospermum erythrorhizon* (M103), *Elsholtzia ciliate* (M101), *Leonurus heterophyllus* (M126), *Agastache rugosa* (M129), *Prunella vulgaris* (M97), *Sophora japonica* (M108), *Spatholobus suberectus* (M99), *Cannabis sativa* (M127), *Morus alba* (M100), *Paeonia suffruticosa* (M109), *Houttuynia cordata* (M92), and *Amornurn villosurn* (M105) can effectively inhibit formation of •OH in a concentration-dependent manner. The IC_{50} values were lower than 5 μg/mL (Table 2).

In current epidemiological studies, phenolics have been found to be one of the most plentiful classes of constituents in the plant kingdom, and they have been reported to have multiple biological effects (Rao, 2003). Previous papers have also noted that many phenolic compounds are in plants showing tyrosinase inhibitory activity (Sugumaran, 2002; Boissy and Manga, 2004; Victor et al., 2004). Therefore, we used the Folin–Ciocalteu method to determine the total phenolic content of the 25 extracts (Table 2). Total phenolic content was expressed as gallic acid equivalents (GAEs) per gram of dried plant extract. The results showed that, in general, the stronger the antioxidant and tyrosinase inhibitory activities of these extracts, the higher the phenolic content. Thus, phenolics present in the extracts may play a major role in producing the results we obtained with the present studies. There are also reports that phenolic compounds may be used as depigmenting agents because they have a similar chemical structure to tyrosine, the substrate of tyrosinase (Boissy and Manga, 2004).

With all test findings taken together, we saw that *Pharbitis nil* (M94), *Sophora japonica* (M108), *Spatholobus suberectus* (M99), and *Morus alba* (M100) exhibited low cytotoxicity, potent tyrosinase inhibitory activity, and the ability to reduce cellular melanin content. They also possessed higher phenolic content and good hydroxyl radical scavenging activities, except for *Pharbitis nil* (M94). In previous papers, phenolic compounds have been reported to have tyrosinase inhibitory activity and have been studied as depigmenting agents (Boissy and Manga, 2004). Additionally, antioxidants may prevent or delay pigmentation by different mechanisms, such as by scavenging ROS and reactive nitrogen species (RNS) (Seo et al., 2003), or by reducing *o*-quinones or other intermediates in melanin biosynthesis, thus delaying oxidative polymerization (Karg et al., 1993). The antioxidant α-tocopherol is usually considered to inhibit oxidative polymerization of phenylalanines such as DOPA during melanin formation (Nilsson et al., 2003). Therefore, the potency of substances used as whitening agents in skin-care products may due at least in part to phenolic components.

Although some papers have studied the effects and constituents of traditional Chinese herbal medicines, *Pharbitis nil* (M94) (Szmidt-Jaworska et al., 2003, 2004), *Sophora japonica* (M108) (Wu et al., 1997; Kim et al., 2003; Lao et al., 2005), *Spatholobus suberectus* (M99) (Lam et al., 2000; Li et al., 2003; Yoon et al., 2004), and *Morus alba* (M100) (Fujimoto et al., 2000), few papers have reported that these Chinese herbal medicines exhibit tyrosinase inhibitory activity. Only *Morus alba* (M100) has been reported to show in vitro mushroom tyrosinase inhibition (Shin et al., 1998; Baurin et al., 2002) and inhibitory effects on tyrosinase activity and melanin formation in B-16 melanoma cells (Lee et al., 2003) and melan-a cells (Lee et al., 2002). This herbal medicine has been used as a cosmetic additive for skin-whitening.

4. Conclusion

In the present study, 25 selected Chinese herbal medicines were investigated for potential effectiveness as skin-whitening agents and in maintaining skin health. Extracts of four herbal preparations were shown to be potent tyrosinase and melanin synthesis inhibitors in human skin melanocyte cells. In addition to extracts of *Morus alba* (M100), which are currently in use as cosmetic additives, results of this study indicate that extracts of *Pharbitis nil* (M94), *Sophora japonica* (M108), and *Spatholobus suberectus* (M99) are likely to be useful for cosmetic applications and products. Their bio-guided isolated components may prove to have considerable value as cosmetics additives in the future.

Acknowledgments

This study was sponsored by the Taipei Medical University-Wan Fang Hospital (grant no. TMU92-AE1-B51) and CCMP93-RD-034. The authors also express thanks for partially financial support from the Juridical Person of Yen's Foundation, Taiwan.

References

Aburjai, T., Natsheh, F.M., 2003. Plants used in cosmetics. Phytotherapy Research 17, 987–1000.

An, B.J., Kwak, J.H., Park, J.M., Lee, J.Y., Park, T.S., Lee, J.T., Son, J.H., Jo, C., Byun, M.W., 2005. Inhibition of enzyme activities and the antiwrinkle effect of polyphenol isolated from the persimmon leaf (*Diospyros kaki folium*) on human skin. Dermatologic Surgery 31, 848–854.

Baurin, N., Arnoult, E., Scior, T., Do, Q.T., Bernard, P., 2002. Preliminary screening of some tropical plants for anti-tyrosinase activity. Journal of Ethnopharmacology 82, 155–158.

Bergamini, C.M., Gambetti, S., Dondi, A., Cervellati, C., 2004. Oxygen, reactive oxygen species and tissue damage. Current Pharmaceutical Design 10, 1611–1626.

Boissy, R.E., Manga, P., 2004. On the etiology of contact/occupational vitiligo. Pigment Cell Research 17, 208–214.

Briganti, S., Camera, E., Picardo, M., 2003. Chemical and instrumental approaches to treat hyperpigmentation. Pigment Cell Research 16, 101–110.

Cheng, Z., Yan, G., Li, Y., Chang, W., 2003. Determination of antioxidant activity of phenolic antioxidants in a Fenton-type reaction system by chemiluminescence assay. Analytical and Bioanalytical Chemistry 375, 376–380.

Fujimoto, Y., Morisaki, M., Ikekawa, N., 2000. Studies on the biosynthesis of sterol side chain in higher plants. Journal of the Pharmaceutical Society of Japan 120, 863–873.

Hearing, V.J., 2005. Biogenesis of pigment granules: a sensitive way to regulate melanocyte function. Journal of Dermatological Science 37, 3–14.

Hou, W.C., Lin, R.D., Cheng, K.T., Hung, Y.T., Cho, C.H., Chen, C.H., Hwang, S.Y., Lee, M.H., 2003. Free radical-scavenging activity of Taiwanese native plants. Phytomedicine 10, 170–175.

Jones, K., Hughes, J., Hong, M., Jia, Q., Orndorff, S., 2002. Modulation of melanogenesis by aloesin: a competitive inhibitor of tyrosinase. Pigment Cell Research 15, 335–340.

Kadekaro, A.L., Kanto, H., Kavanagh, R., Abdel-Malek, Z.A., 2003. Significance of the melanocortin 1 receptor in regulating human melanocyte pigmentation, proliferation, and survival. Annals of the New York Academy of Sciences 994, 359–365.

Karg, E., Odh, G., Wittbjer, A., Rosengren, E., Rorsman, H., 1993. Hydrogen peroxide as an inducer of elevated tyrosinase level in melanoma cells. Journal of Investigative Dermatology 100, 209–213.

Kiken, D.A., Cohen, D.E., 2002. Contact dermatitis to botanical extracts. American Journal of Contact Dermatitis 13, 148–152.

Kim, B.H., Chung, E.Y., Ryu, J.C., Jung, S.H., Min, K.R., Kim, Y., 2003. Anti-inflammatory mode of isoflavone glycoside sophoricoside by inhibition of interleukin-6 and cyclooxygenase-2 in inflammatory response. Archives of Pharmacal Research 26, 306–311.

Kim, Y.J., Uyama, H., 2005. Tyrosinase inhibitors from natural and synthetic sources: structure, inhibition mechanism and perspective for the future. Cellular and Molecular Life Sciences 62, 1707–1723.

Kobayashi, T., Vieira, W.D., Potterf, B., Sakai, C., Imokawa, G., Hearing, V.J., 1995. Modulation of melanogenic protein expression during the switch from eu- to pheomelanogenesis. Journal of Cell Science 108, 2301–2309.

Lam, T.L., Lam, M.L., Au, T.K., Ip, D.T., Ng, T.B., Fong, W.P., Wan, D.C., 2000. A comparison of human immunodeficiency virus type-1 protease inhibition activities by the aqueous and methanol extracts of Chinese medicinal herbs. Life Sciences 67, 2889–2896.

Lao, C.J., Lin, J.G., Kuo, J.S., Chao, P.D., Cheng, C.Y., Tang, N.Y., Hsieh, C.L., 2005. Microglia, apoptosis and interleukin-1beta expression in the effect of *Sophora japonica* L. on cerebral infarct induced by ischemia-reperfusion in rats. American Journal of Chinese Medicine 33, 425–438.

Lee, S.H., Choi, S.Y., Kim, H., Hwang, J.S., Lee, B.G., Gao, J.J., Kim, S.Y., 2002. Mulberroside F isolated from the leaves of *Morus alba* inhibits melanin biosynthesis. Biological and Pharmaceutical Bulletin 25, 1045–1048.

Lee, K.T., Lee, K.S., Jeong, J.H., Jo, B.K., Heo, M.Y., Kim, H.P., 2003. Inhibitory effects of *Ramulus mori* extracts on melanogenesis. Journal of Cosmetic Science 54, 133–142.

Li, R.W., David Lin, G., Myers, S.P., Leach, D.N., 2003. Anti-inflammatory activity of Chinese medicinal vine plants. Journal of Ethnopharmacology 85, 61–67.

Ma, W., Wlaschek, M., Tantcheva-Poor, I., Schneider, L.A., Naderi, L., Razi-Wolf, Z., Schuller, J., Scharffetter-Kochanek, K., 2001. Chronological ageing and photoageing of the fibroblasts and the dermal connective tissue. Clinical and Experimental Dermatology 26, 592–599.

Nihei, K., Kubo, I., 2003. Identification of oxidation product of arbutin in mushroom tyrosinase assay system. Bioorganic and Medicinal Chemistry Letters 13, 2409–2412.

Nilsson, S.E., Sundelin, S.P., Wihlmark, U., Brunk, U.T., 2003. Aging of cultured retinal pigment epithelial cells: oxidative reactions, lipofuscin formation and blue light damage. Documenta Ophthalmologica 106, 13–16.

Perluigi, M., De Marco, F., Foppoli, C., Coccia, R., Blarzino, C., Marcante, M.L., Cini, C., 2003. Tyrosinase protects human melanocytes from ROS-generating compounds. Biochemical and Biophysical Research Communications 305, 250–256.

Rao, B., 2003. Bioactive phytochemicals in Indian foods and their potential in health promotion and disease prevention. Asia Pacific Journal of Clinical Nutrition 12, 9–22.

Reiter, R.J., Acuna-Castroviejo, D., Tan, D.X., Burkhardt, S., 2001. Free radical-mediated molecular damage. Mechanisms for the protective actions of melatonin in the central nervous system. Annals of the New York Academy of Sciences 939, 200–215.

Rescigno, A., Sollai, F., Pisu, B., Rinaldi, A., Sanjust, E., 2002. Tyrosinase inhibition: general and applied aspects. Journal of Enzyme Inhibition and Medicinal Chemistry 17, 207–218.

Roh, J.S., Han, J.Y., Kim, J.H., Hwang, J.K., 2004. Inhibitory effects of active compounds isolated from safflower (*Carthamus tinctorius* L.) seeds for melanogenesis. Biological and Pharmaceutical Bulletin 27, 1976–1978.

Sawai, Y., Moon, J.H., 2000. NMR analytical approach to clarify the molecular mechanisms of the antioxidative and radical-scavenging activities of antioxidants in tea using 1,1-diphenyl-2-picrylhydrazyl. Journal of Agricultural and Food Chemistry 48, 6247–6253.

Seiberg, M., Paine, C., Sharlow, E., Andrade-Gordon, P., Costanzo, M., Eisinger, M., Shapiro, S.S., 2000. Inhibition of melanosome transfer results in skin lightening. Journal of Investigative Dermatology 115, 162–167.

Seo, S.Y., Sharma, V.K., Sharma, N., 2003. Mushroom tyrosinase: recent prospects. Journal of Agricultural and Food Chemistry 51, 2837–2853.

Shin, N.H., Ryu, S.Y., Choi, E.J., Kang, S.H., Chang, I.M., Min, K.R., Kim, Y., 1998. Oxyresveratrol as the potent inhibitor on dopa oxidase activity of mushroom tyrosinase. Biochemical and Biophysical Research Communications 243, 801–803.

Sturm, R.A., Teasdale, R.D., Box, N.F., 2001. Human pigmentation genes: identification, structure and consequences of polymorphic variation. Gene 277, 49–62.

Sugumaran, M., 2002. Comparative biochemistry of eumelanogenesis and the protective roles of phenoloxidase and melanin in insects. Pigment Cell Research 15, 2–9.

Szmidt-Jaworska, A., Jaworski, K., Tretyn, A., Kopcewicz, J., 2003. Biochemical evidence for a cGMP-regulated protein kinase in *Pharbitis nil*. Phytochemistry 63, 635–642.

Szmidt-Jaworska, A., Jaworski, K., Tretyn, A., Kopcewicz, J., 2004. The involvement of cyclic GMP in the photoperiodic flower induction of *Pharbitis nil*. Journal of Plant Physiology 161, 277–284.

Victor, F.C., Gelber, J., Rao, B., 2004. Melasma: a review. Journal of Cutaneous Medicine and Surgery 8, 97–102.

Wu, A.M., Song, S.C., Sugii, S., Herp, A., 1997. Differential binding properties of Gal/GalNAc specific lectins available for characterization of glycoreceptors. Indian Journal of Biochemistry and Biophysics 34, 61–71.

Yamakoshi, J., Otsuka, F., Sano, A., Tokutake, S., Saito, M., Kikuchi, M., Kubota, Y., 2003. Lightening effect on ultraviolet-induced pigmentation of guinea pig skin by oral administration of a proanthocyanidin-rich extract from grape seeds. Pigment Cell Research 16, 629–638.

Yasui, H., Sakurai, H., 2003. Age-dependent generation of reactive oxygen species in the skin of live hairless rats exposed to UVA light. Experimental Dermatology 12, 655–661.

Yeh, C.T., Yen, G.C., 2005. Effect of vegetables on human phenolsulfotransferases in relation to their antioxidant activity and total phenolics. Free Radical Research 39, 893–904.

Yen, K.Y., 1992. The Illustrated Chinese Materia Medica, SMC Publishing Inc., Taipei.

Yoon, J.S., Sung, S.H., Park, J.H., Kim, Y.C., 2004. Flavonoids from *Spatholobus suberectus*. Archives of Pharmacal Research 27, 589–592.