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Effect of extracts of traditional Chinese medicines on anti-tyrosinase and antioxidant activities

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Fifty important cosmetic skin-whiting traditional Chinese medicines (TCMs) were investigated for their anti-tyrosinase and antioxidant (or DPPH-free-radical-scavenging) activities. The water and 70% ethanol extracts (WEs and 0.7EtEs, respectively) of TCMs were tested for tyrosinase inhibitory activities and DPPH-free-radical-scavenging activities. The 10 mg/ml WEs of 6 TCMs, *Angelica dahurica*, *Anredera cordifilia* Moq., *Cinnamomum aromaticum*, *Glycyrrhiza glabra*, *Melia toosendan*, and *Prunus davidiana*, presented over 50% inhibitory effect (referred to as the positive control of 0.5 mg/ml vitamin C) in tyrosinase activity, while *Prunus davidiana* showed the best anti-tyrosinase activity (94.0%). Only 3 TCMs of 0.7EtEs, *Cinnamomum aromaticum*, *Quisqualis indica*, *Areca catechu*, exhibited over 50% anti-tyrosinase activity. Among the TCMs screened, the 10mg/ml 0.7EtEs of *Evodis rutaecarpa*, *Leonurus heterophyllus*, *Nardostachys chinensis*, and *Quisqualis indica*, had strong DPPH-free-radical-scavenging effects or antioxidant activities (96.0, 90.5, 92.6 and 80.6%, respectively), while all the WEs of TCMs, except *Uncaria sessilifructus* Roxb, showed low antioxidant activities (65.6%). The anti-tyrosinase and antioxidant activities of these two TCM extracts may be due to direct linkage to the contents of their active compounds.

Key words: Traditional Chinese medicines, inhibition, anti-tyrosinase, antioxidant, free radical scavenging.

INTRODUCTION

The search for natural active compounds from natural herbal medicines or traditional Chinese medicines (TCMs) provides an interesting, largely unexplored area for development of new skin-care cosmetics (Kiken and Cohen, 2002; Kadekaro et al., 2003; Wang et al., 2006), such as natural whitening agents like melanin biosynthesis or tyrosinase inhibitors, which are able to modulate the metabolism of pigmentation for color of human skin and play a crucial protective role in skin whiteness, whereas antioxidants active in the oxidative stress of skin aging cells may support skin health. Melanin, which is biosynthesized by melanocyte cells in the basal layer of the epidermis (Hearing, 2005; Chen et al., 2015), may be overproduced with chronic sun exposure, melasma, or other hyperpigmentation diseases (Briganti et al., 2003). Therefore, whitening agents

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reduce melanin overproduction like hyperpigmentation of darkened age spots, whereas pigmenting agents such as melanins are designed to increase pigmentation for sun protection. However, the inhibition of melanin biosynthesis has already been described by avoiding ultraviolet (UV) exposure, inhibiting melanocyte metabolism and proliferation (Seiberg et al., 2000; Chang, 2012), inhibiting tyrosinase activity, or removing melanin with corneal ablation (Wang et al., 2006).

Tyrosinase is known to be the key enzyme in the anabolism of melanin biosynthesis in melanocytes (Sturm et al., 2001; Parvez et al., 2007; Chang, 2012), catalyzing the initial two steps of this pathway, including hydroxylation of tyrosine (one of monophenolic compounds) to L-dopa (L-3,4-dihydroxyphenylalane; one of *o*-diphenols) and oxidation of L-dopa to *o*-dopaquinone (one of *o*-quinones). These *o*-quinones are then transformed into melanin in a series of non-enzymatic reactions (Prota, 1988). Therefore, tyrosinase inhibitors are important constituents of cosmetics and skin-lightening agents (An et al., 2005), and tyrosinase becomes the key target enzyme for screening and discovery of new inhibitory compounds. This is why a constant search for tyrosinase inhibitors obtained by extraction from natural plants or TCMs is underway in the hope of preventing the occurrence of these melanin overproductions or hyperpigmentation disorders (No et al., 1999). The highly reactive intermediate produced by dopa oxidation, and the reactive oxygen species (ROS) and other free radicals induced by oxidative stress in skin cells or by UV radiation exposure have been presented to be inappropriately processed in enhancing melanin biosynthesis, damaging DNA, probably inducing proliferation of melanocytes (Yamakoshi et al., 2003; Yasui and Sakurai, 2003). The free radicals or ROS scavengers such as antioxidants may be known to reduce hyperpigmentation (Ma et al., 2001). Although the plant-derived antioxidants scavenge free-radicals, it is assumed that their nature and concentration vary among different kinds of plants. However, 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a stable radical and the DPPH free radical-scavenging assay is a simple and widely popular method for screening free radical-scavenging ability of compounds or antioxidant activity of plant extracts. In this study, the biological evaluations of water and 70% ethanol extracts (WEs and 0.7EtEs, respectively) of 50 TCMs were investigated for the inhibition of tyrosinase activity and the antioxidation of DPPH-free-radical-scavenging activity. The comparison of the anti-tyrosinase and antioxidant activities of these two TCM extracts was also studied.

MATERIALS AND METHODS

Reagents and TCM materials

Tyrosine, L-dopa, mushroom tyropsinase (Prod. No. T7755), DPPH were purchased from Sigma (St. Louis, MO). Other chemicals were of the highest grade commercially available. TCMs were purchased from local medicinal markets in Taichung County, Taiwan.

Preparation of TCM extracts

Dried TCMs were pulverized in a grinder and 10 g of the pulverized TCM powders were extracted with 100 ml of distilled water (WEs) or 70% ethanol solution (0.7EtEs) at 28°C for 24 h. Extraction was carried out under shaking (100 rpm). The WEs was filtered with 0.22 µm membrane and the filtrate was stored at 4°C until use. The 0.7EtEs filtrate was completely dried at 50°C and then re-dissolved with distilled water. The samples of 0.7EtEs were obtained after filtration with 0.22 µm membrane.

Assay for tyrosinase inhibitory activity

Tyrosinase activity was determined by spectrophotometry (Masamoto et al., 1980; Bernard and Berthon, 2000) with slight modification. Briefly, 1.0 ml of the test sample (10 mg/ml extract) with or without WEs or 0.7EtEs, 1.0 ml of 20 mM phosphate buffer (pH 6.2) and 0.25 ml of 10 mM tyrosine were mixed at 37°C for 10 min. Subsequently, 25 ml of mushroom tyrosinase (200 U/ml) was added into the mixture. After incubation at 37°C for 25 min, the absorbance was measured at 475 nm (OD_{475}) using a SHIMADZU UV-Visible spectrophotometer (Model UV-1201). The same procedure was applied with 0.5 mg/ml of vitamin C (as the positive controls for comparison with those of the WEs). To test the concentration required for 50% inhibition (IC_{50}) values of TCMs on anti-tyrosinase activity, 1.0 ml of the test samples with different concentrations was performed as described above. For 0.7EtEs, the inhibition percentage of tyrosinase activity was calculated as follows:

Inhibition (%) = [1 - (OD_{475} with test sample) / (OD_{475} without test sample)] × 100

For WEs, the tyrosinase inhibitory activity was calculated with the following formula:

Inhibition (%) = [(% inhibition of test sample) / (% inhibition of vitamin C)] × 100

Assay for DPPH-free-radical-scavenging activity

The sample (either 50 µl of 10 mg/ml test sample or 50 µl of serial dilution concentrations of test samples mixed with 950 µl of 25 mM DPPH in methanol) test preparation underwent reaction in the dark at room temperature for 30 min. Fifty microliters of methanol was used as a positive control. The same procedure was applied with 0.2 mg/ml of vitamin C for comparison with those of the WEs. The decrease in absorbance at 517 nm (A_{517}) induced by the samples was compared to that of the positive controls (Yamaguchi et al., 1998). The effect percentage of DPPH-free-radical-scavenging activity was calculated as follows:

Scavenging effect (%) = [1 - (A_{517} of test sample) / (A_{517} of control)] × 100%

Determining IC_{50} of TCM extracts

To calculate the concentration required for 50% inhibition (IC_{50}) value of TCM extracts, the data of anti-tyrosinase or antioxidant activities (yi) obtained from a series of concentration (xi) of TCM extracts were put in the software of ICEstimator (ICEstimator Version 2.1, http://www.antimalarial-icestimator.net/index.htm) and

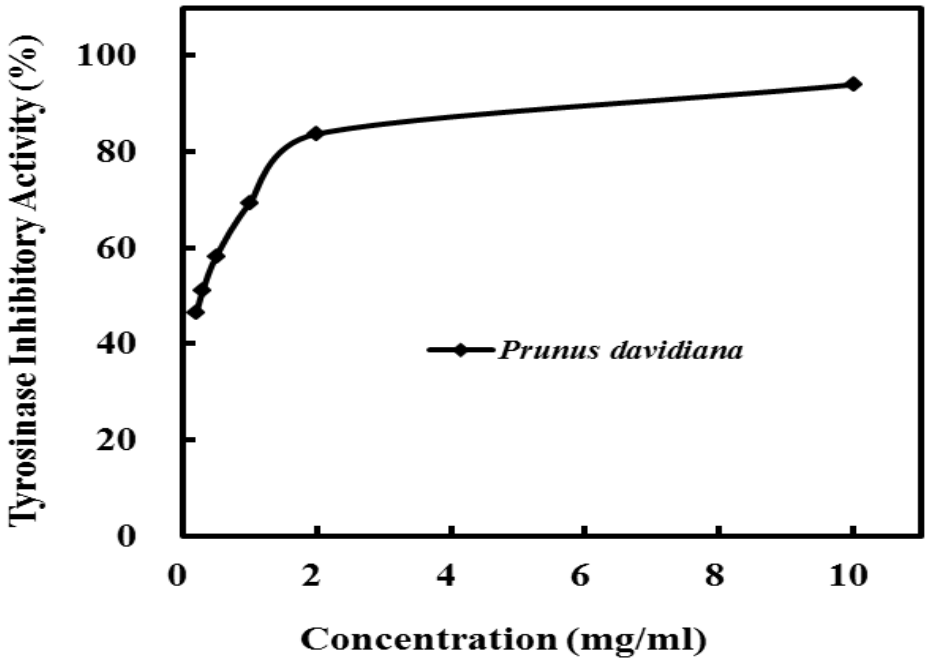

Figure 1. Effect of concentrations of *Prunus davidiana* water-extracts (WEs) on tyrosinase inhibitory activity.

a nonlinear regression ($yi=a+bxi+cxi^2$) was carried out to get the IC_{50} value. Herein, yi is the inhibition activity in relative concentration of TCMs (Le Nagard et al., 2010).

Statistical analysis

Data were subjected to the analysis of variance (ANOVA) using the software package (SAS 8.1, Cary, NC, USA). In the case of significant treatment effects, a comparison of means was performed by means of the least significant difference (LSD) test at a significance level of 5% (p = 0.05).

RESULTS

Tyrosinase inhibitory activity

The summarized results of mushroom tyrosinase inhibition of the 10 mg/ml extracts (WEs and 0.7EtEs) of 50 TCMs are shown in Table 1. Over 50% of anti-tyrosinase activity were observed in the WEs of 6 TCMs, *Angelica dahurica*, *Anredera cordifilia* Moq., *Cinnamomum aromaticum*, *Glycyrrhiza glabra*, *Melia toosendan* and *Prunus davidiana* (61.7, 68.7, 54.7, 70.4, 88.0 and 94.0%, respectively) referred to as that of vitamin C (0.5 mg/ml). Yet, only *P. davidiana* WEs exhibited the highest inhibition of tyrosinase with value over 90%. Figure 1 shows the dose-response curve for the inhibitory effect of tyrosinase activity of the *P. davidiana* WEs with different concentration (mg/ml). The tyrosinase inhibitory activity of *P. davidiana* WEs at a dilution concentration of 2 mg/ml was still about 84%. Linear dose-dependent behavior on tyrosinase activity inhibitions (ranged from 51 to 84%) affected by *P. davidiana* WEs was found to be between the dilution concentration 0.3 and 2 mg/ml (Figure 1). The tyrosinase inhibitory effect was increased with increasing dilution concentration of *P. davidiana* WEs. The IC_{50} of *P. davidiana* on tyrosinase activity inhibitions was 0.24 mg/ml. Among the 50 tested 0.7EtEs, only 3 TCM extracts, *Areca catechu*, *C. aromaticum* and *Q. indica*, presented good anti-tyrosinase activities with values at 50-70%, as shown in Table 1.

DPPH-free-radical-scavenging activity

The effect results of DPPH-free-radical-scavenging activity of the 50 TCM extracts were summarized in Table 2. The higher scavenging activity implies the higher DPPH-free-radical-scavenging ability or antioxidant activity. Of 50 TCM WEs, only *Uncaria sessilifructus* Roxb had a good antioxidant activity (65.6%). In contrast, 4 TCM 0.7EtEs, *Evodis rutaecarpa*, *Leonurus heterophyllus*, *Nardostachys chinensis* and *Quisqualis indica*, exhibited strong antioxidant activities (96.0, 90.5, 92.6 and 80.6%, respectively) (Table 2). Similar to the tyrosinase inhibitory activity of the *P. davidiana* WEs, these 0.7EtEs exhibited dose-response curves for scavenging effect or antioxidant activity as shown in Figure 2. The antioxidant activity was increased with

Table 1. Effect of water and 70% ethanol extracts of traditional Chinese medicines on tyrosinase inhibitory activity.

Sample	Tyrosinase inhibitory activity (%)*	
	Water extracts (WEs)	70% ethanol extracts (0.7EtEs)
Tribulus terrestris L.	9.9	20.5
Litharge (PbO)	7.8	18.9
Coix lacryma-iobi L.	9.7	18.7
Atractylodes macrocephala Koidz	7.7	22.6
Angelica dahurica Bentham et Hook.	61.7	18.1
Nelumbo nucifera Gaertn	20.5	16.4
Cyperus rotundus L.	20.4	24.1
Glycyrrhiza glabra L.	70.4	37.0
Ligusticum chuanxiong Hort.	14.0	29.0
Rheum officinale Baill	35.0	19.2
Lonicera japonica Thunb.	11.1	32.2
Nardostachys chinensis Bat.	16.1	22.5
Prunus armeniaca L.	32.9	16.8
Paeonia anomala L.	7.8	27.6
Polygonum aviculare L.	24.6	29.3
Typhonium giganteum Engl.	8.8	12.9
Bombyx mori L.	9.1	11.5
Kaempferia galangal L.	18.9	48.6
Akebia trifoliate Thunb.) Koidz.	-8.0	24.8
Forsythia suspense (Thunb.) Vahl	17.1	17.7
Trichosanthes kirilowii Maxim.	14.5	18.0
Vigna radiate (L.) R. Wilcz.	12.9	16.5
Angelica sinensis (Oliv.) Diels	20.7	30.6
Zingiber officinale Rosc.	26.0	37.2
Benincasa hispida (Thunb.) Cogn.	12.9	24.7
Elsholtzia splendens Nakai et F.Maek.	39.5	17.4
Rubus chingii Hu	0.9	34.6
Cinnamomum aromaticum Nees	54.7	64.4
Ipomoea biflora (L.) Persoon	19.4	32.1
Artemisia capillaries Thunb.	-18.8	-25.4
Plantago asiatica L.	-0.9	24.0
Salvia miltiorrhiza Bunge	15.8	23.5
Quisqualis indica L.	7.9	52.8
Ageratum conyzoides L.	37.8	37.7
Poria cocos (Schw.) Wolf.	17.6	17.6
Prunus davidiana Franch	94.0	16.7
Scutellaria baicalensis Georgi	11.4	20.6
Ampelopsis japonica (Thunb.) Makino	6.2	30.0
Areca catechu L.	29.3	67.1
Saposhnikovia divaricata (Turcz.) Schischk.	-4.7	18.0
Talinum triangulare Willd.	16.8	27.8
Trichosanthes kirilowii Maxim.	44.8	15.9
Bletilla striate (Thunb.) Rchb. f.	27.2	36.4
Leonurus heterophyllus Sweet	13.8	15.9
Evodia rutaecarpa (Juss.) Benth.	18.8	16.3
Melia toosendan Sieb. et Zucc.	88.0	24.9
Anredera cordifilia Moq.	68.7	25.7
Homo sapiens L. (placenta of..)	40.0	16.0
Uncaria sessilifructus Roxb.	-70.2	17.0
Lycium chinenses Mill.	28.0	15.3

Table 1. Cont'd

Vitamin C (L-ascorbic acid) (0.5mg/mL)	100.0	-----
$LSD_{0.05}$	6.2	5.7

* Measurements were performed in triplicate.

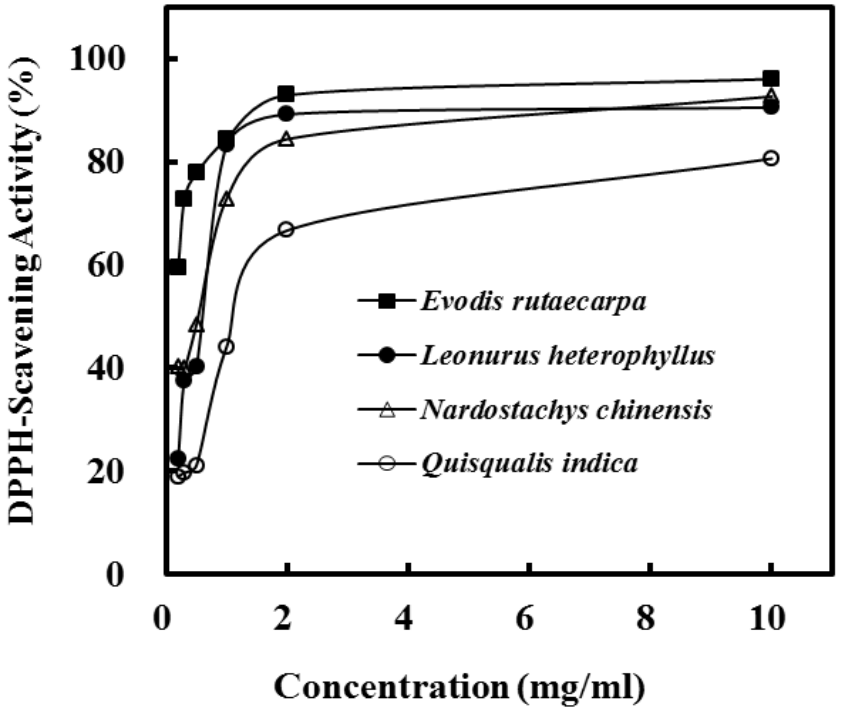

Figure 2. Effect of concentrations of 70% ethanol extracts (0.7EtEs) of 4 TCMs, *Evodis rutaecarpa*, *Leonurus heterophyllus*, *Nardostachys chinensis*, and *Quisqualis indica,* on DPPH-free-radical-scavenging activity (antioxidant activity).

increasing dilution concentration of these 4 0.7EtEs. The IC_{50} of 4 0.7TtEs, *E. rutaecarpa*, *L, heterophyllus*, *N. chinensis* and *Q. indica*, on antioxidant activity were 0.12, 0.41, 0.34, 0.79 mg/ml, respectively. The 7 other 0.7EtEs, *Angelica sinensis*, *Atractylodes macrocephpala* Koidz, *C. aromaticum*, *Cyperus rotundus*, *G. glabra*, *Zingiber officinale*, *Plantago asiatica*, also present good antioxidant activities with values between 50 and 80% (Table 2).

DISCUSSION

In this study, WEs and 0.7EtEs of 50 TCMs were measured for the performance of anti-tyrosinase and antioxidant activities. The result indicated that 10 mg/ml aqueous extract of *P. davidiana* showed the best anti-tyrosinase activity (94.0%) similar to 0.5 mg/ml of vitamin C. Concentration required for 50% inhibition (IC_{50}) values of *P. davidiana* on anti-tyrosinase activity was 0.24 mg/ml. In terms of antioxidant activity, 0.7EtEs of *E. rutaecarpa* and *L. heterophyllus* were the best two TCMs, even though at 1.0 mg/mL concentration, they could still maintain more than 80% of the free radical scavenging ability. IC_{50} of two TCMs were 0.12 and 0.41 mg/ml, respectively.

In terms of Chinese herbal whitening, effect of *Bletilla striate*, *Ampelopsis japonica*, *A. dahurica, Atractylodes macrocephala* and *Typhonium giganteum* widely used in cosmetic products were considered. However, the present study found that the tyrosinase inhibition activities of these TCMs were lower than 50% whether in WEs or 0.7EtEs. This result is inconsistent with other studies (Chun et al., 2003; Ye et al., 2010). In addition, the tyrosinase inhibitory effect of *Ligusticum chuanxiong* has been reported in different polarization results. For example, Deng et al. (2007) reported the ethanol extract of *L. chuanxiong* has stronger inhibitory effect on

Table 2. Effect of water and 70% ethanol extracts of traditional Chinese medicines on DPPH-free-radical-scavenging activity (antioxidant activity).

Samples	Antioxidant activity (%)*	
	Water extracts (WEs)	70% ethanol extracts (0.7EtEs)
Tribulus terrestris L.	15.1	7.6
Litharge (PbO)	14.3	19.6
Coix lacryma-iobi L.	2.94	32.2
Atractylodes macrocephala Koidz	23.2	63.5
Angelica dahurica Bentham et Hook.	0.5	35.9
Nelumbo nucifera Gaertn	23.8	13.6
Cyperus rotundus L.	20.6	53.7
Glycyrrhiza glabra L.	25.5	56.8
Ligusticum chuanxiong Hort.	29.2	30.7
Rheum officinale Baill	26.4	42.6
Lonicera japonica Thunb.	32.9	20.2
Nardostachys chinensis Bat.	28.8	92.6
Prunus armeniaca L.	-2.4	7.6
Paeonia anomala L.	29.7	16.0
Polygonum aviculare L.	36.3	37.0
Typhonium giganteum Engl.	22.1	17.8
Bombyx mori L.	27.5	23.8
Kaempferia galangal L.	15.9	22.6
Akebia trifoliate Thunb.) Koidz.	32.2	22.6
Forsythia suspense (Thunb.) Vahl	32.8	46.6
Trichosanthes kirilowii Maxim.	24.7	2.82
Vigna radiate (L.) R. Wilcz.	31.3	4.6
Angelica sinensis (Oliv.) Diels	28.7	60.1
Zingiber officinale Rosc.	27.2	68.8
Benincasa hispida (Thunb.) Cogn.	15.0	10.6
Elsholtzia splendens Nakai et F.Maek.	29.8	29.8
Rubus chingii Hu	24.8	25.6
Cinnamomum aromaticum Nees	29.3	74.1
Ipomoea biflora (L.) Persoon	19.4	49.0
Artemisia capillaries Thunb.	18.1	46.0
Plantago asiatica L.	23.0	54.6
Salvia miltiorrhiza Bunge	24.1	33.8
Quisqualis indica L.	19.0	80.5
Ageratum conyzoides L.	25.1	32.2
Poria cocos (Schw.) Wolf.	15.5	19.0
Prunus davidiana Franch	18.0	20.8
Scutellaria baicalensis Georgi	35.7	35.2
Ampelopsis japonica (Thunb.) Makino	29.2	25.6
Areca catechu L.	23.2	33.4
Saposhnikovia divaricata (Turcz.) Schischk.	22.8	28.0
Talinum triangulare Willd.	41.8	12.4
Trichosanthes kirilowii Maxim.	21.7	8.8
Bletilla striate (Thunb.) Rchb. f.	39.8	20.2
Leonurus heterophyllus Sweet	34.6	90.5
Evodia rutaecarpa (Juss.) Benth.	31.2	96.0
Melia toosendan Sieb. et Zucc.	28.3	37.0
Anredera cordifilia Moq.	31.6	31.6
Homo sapiens L. (placenta of..)	46.8	20.4
Uncaria sessilifructus Roxb.	65.6	32.3

Table 2. Cont'd.

Lycium chinenses Mill.	33.3	40.3
Vitamin C (L-ascorbic acid) (0.2 mg/mL)	98.6	------
$LSD_{0.05}$	6.8	5.6

* Measurements were performed in triplicate.

tyrosinase activity (Deng et al., 2007), but Chen et al. (2008) reported that ethanol extract of *L. chuanxiong* enhances the activity of tyrosinase. In this study, tyrosinase inhibition rate of *L. chuanxiong* was much less than 50%. Wang et al. (2002) reported that the 50% ethanol extract of *Prunus persica* (L.) Batsch did not show a good tyrosinase inhibitory effect. In contrast, the WEs of *P. davidiana* showed the best anti-tyrosinase activity with the inhibition rate of 94% in the present study. Thus, the differences in tyrosinase inhibitory activity for TCM WEs and 0.7EtEs may be due to the various active components of different TCM sources and their polarities and other properties related to the extract methods and the solvent constituents (Ko et al., 2002; Wang et al., 2002).

To determine the antioxidant activity, 50 TCM extracts were screened for their DPPH-free-radical-scavenging activity. Of 11 TCM, 0.7EtEs showed good antioxidant activities with values more than 50%. Among them, *E. rutaecarpa, L. heterophyllus*, *N. chinensis* and *Q. indica*, exhibited strong antioxidant activities with values more than 80%. *E. rutaecarpa* is commonly used as an analgesic, antiemetic, anti-inflammatory and astringent drug in TCMs (Tang and Eisenbrand, 1992). The total alkaloids obtained from the fruits of *E. rutaecarpa* was found and the total antioxidant capacity and inhibitory lipid peroxidation are superior to synthetic antioxidant 2, 6-di-ter-butyl-4-methylphenol (BHT), but scavenging activity on DPPH radical is lower than that of BHT at the same condition (Tan et al., 2011). Wang et al. (2006) examined 25 TCMs that might be useful for antioxidant agents and pointed out that *L. heterophyllus* can effectively inhibit formation of •OH in a concentration-dependent manner. The IC_{50} value of *N. chinensis* essential oil to scavenge DPPH radical was 637.47 µg/ml (Wang et al., 2010). Anu Kiruthika and Sornaraj (2014) reported the methanolic crude extracts of *Q. indica* flower possess significant antioxidant activity which might be due to the presence of alkaloids, tannins, flavonoids and saponin. However, all the WEs of TCMs, except *U. sessilifructus* showed a poor antioxidant activity as compared to all the 0.7EtEs. This finding maybe due to the fact that 70% ethanol solution is an active solvent constitute for extraction of these good or strong TCM antioxidants. The result suggested that these 4 TCM 0.7EtEs may contain constituents with strong proton-donating abilities (Sawai and Moon, 2000). In addition, to the authors' knowledge, there is no report regarding the antioxidant activity of *U. sessilifructus* extracts.

Conclusion

In the present study, there is no doubt that the extract methods and the solvent constituents play important roles in extraction of natural active compounds of 50 selected TCMs, described for anti-tyrosinase activity and antioxidant activity. The *P*. *davidiana* WEs and the *E. rutaecarpa* 0.7EtEs were investigated for potential effectiveness as skin-whitening agents and in maintaining healthy skin, respectively. In the future, the isolation and structural elucidation of the active bio-guided isolated compounds of these 2 TCMs will likely have considerable value as cosmetics additives and be useful for cosmetic applications and products.

Conflict of Interests

The authors have not declared any conflict of interests.

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