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Protective Effects of Tea Polysaccharides and Polyphenols on Skin

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The protective effects of tea polysaccharides (TPS) and polyphenols (TPP) on skin were investigated. TPS1 (92% TPS), crude TPS2 (20% TPS), and TPP (98%) were tested. The abilities of TPS and TPP to protect the skin were assessed in four aspects: moisture absorption and retention, sunscreen, promoting the proliferation of fibroblast cells, and tyrosinase inhibitory effect. TPS and TPP absorbed and reserved moisture perfectly. TPS with higher purity had better moisture absorption and retention abilities. TPS1 hardly protected the skin from the sun's ultraviolet (UV) rays and had little promoting effect on fibroblasts proliferation. TPP, however, protected skin against UV rays and enhanced proliferation of fibroblast cells significantly. TPP had good tyrosinase inhibitory effects; TPS showed weaker tyrosinase inhibitory effects with the purity increased. The results indicated that TPP and TPS had complementary advantages and they should be appropriately combined to achieve higher performance when applied as active components of cosmetics.

KEYWORDS: Tea polysaccharides and polyphenols; moisture absorption and retention; sunscreen; fibroblast cell proliferation; whitening

INTRODUCTION

Green tea, made from the leaf of *Camellia sinensis*, is one of the most popular beverages in the world. The consumption of green tea is especially popular in Asian countries, and its association with human health benefits has resulted in the application of green tea extracts as common botanical ingredients in dietary supplements, nutraceuticals, and functional foods (*1*). Tea polyphenols (TPP) and tea polysaccharides (TPS) are both important bioactive components extracted from green tea. TPP play a key role in preventing the risk and pathogenesis of several chronic diseases, especially cardiovascular disease and cancer, and related pathologies (*2*−*5*). Most of these bioactivities are likely related to the antioxidant abilities of flavonoids. The antioxidant activity of TPP has been investigated extensively (*6*, *7*), which is significantly suggested to be applied as an antiaging material for cosmetics. TPP could decrease the lipid peroxidation of the skin cells by improving the level of GSH-Px and prevent the free radical from damaging skin cells (*8*). TPS are other main bioactive components of green tea other than TPP, especially in low-grade green tea. The content decreases with the quality grade improvement of the tea. The contents of TPS were from 0.8 to 1.5% in low-grade tea and from 0.4 to 0.9% in high-grade tea (*9*). Great advances have been made in chemical and bioactive studies of TPS in recent decades. TPS is reported to possess a hypoglycemic effect (*10*), immunostimulating activity (*11*), and antibacterial activity (*12*). TPS have become secondary but important bioactive substances after TPP found in tea.

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Moisture retention capability is the basic function of skin-care cosmetics and skin health products. The moisture retention tissue of skin is damaged with age and exposure to external environments. When the water in the cuticle is reduced to $< 10\%$, the skin will be dry, nonelastic, wrinkled, which will quicken skin aging. Thus, water is important for skin, and moisture retention is an important part of delaying skin aging. Moreover, many natural plant extracts not only possess good moisture retention capability but also have a definite nutritional effect on human skin. Thus, natural active plant extracts have been replacing chemosynthetic moisturizers in accordance with the customers' demand for naturalness. Among the numerous external factors promoting skin aging, photoaging induced by ultraviolet (UV) light has been proved to be the main factor. Skin exposed to sunlight appears to be 30 years older than unexposed skin. Thus, sun protection is an important method to delay skin aging. UV light is artificially divided into three ranges: (1) UV-A is radiation in the 320−400 nm range; (2) UV-B is radiation in the 290−320 nm range; and (3) UV-C is radiation in the 100−290 nm range. Generally, UV-B has been blamed for sunburn, and some studies indicate that UV-A may cause skin damage; these findings indicated that the development of sunscreens which block UV-A and UV-B rays has great market potential. Skin aging normally reflected cell aging at the cellular level. Fibroblasts are principal cell components in the dermis. According to Bayreuther's theory of phenotype system, it could be concluded that the suppression of fibroblast growth was an important cause of skin aging. In the skin aging procedure, fibroblasts converted from the cleavage type with active dividing ability to the postcleavage type with inactive dividing ability. Due to a series of changes appearing in fibroblasts during skin aging, many methods have been adopted

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to improve the biological characteristics of fibroblasts and to delay the aging of skin and wrinkle formation. Therefore, fibroblasts have been used as experimental materials to study antiaging activity for bioactive components. Effects of TPS and TPP on skin aging were assessed by in vitro fibroblast cell proliferation.

In recent years, many studies have been done to discover safe and natural ways of getting rid of skin discolorations and achieving a beautiful and balanced skin tone. Therefore, in the present market of cosmetics, the exploration of skin-lightening substances has become a hot-spot in the cosmetic field. Visibly pigmented skin results from the synthesis and distribution of melanins. Melanin is synthesized in mammals in the melanosomes of melanocytes. Melanin synthesis is regulated by melanogenic enzymes such as tyrosinase, tyrosinase-related protein 1 (TRP-1), and tyrosinase-related protein 2 (TRP-2). Tyrosinase (EC 1.14.18.1) is the rate-limiting enzyme for melanogenesis and catalyzes the hydroxylation of L-tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and oxidizes DOPA to dioaquinone, which is the first stage for melanogenesis (*13*). Most melanin synthesis inhibitors inhibit melanogenesis by inhibiting tyrosinase activity (*14*). Inhibition of tyrosinase activity was applied in evaluating the skin-lightening activity of components in cosmaceuticals. Therefore, now more attention has been paid to the use of natural plant extracts such as tyrosinase inhibitors in the cosmetic industry.

Tea has been used as a beverage in China and Japan for thousands of years. TPS and TPP are the main components in tea beverage. There were no data showing that TPS and TPP were harmful to human body. These two components have been proven to be safe and nontoxic (*15*−*17*). However, TPS and TPP have not been studied in detail with regard to skin-protecting effect. To further develop the application of green tea, the protective effects of TPS and TPP on skin were investigated from the following aspects: moisture absorption and retention, sunscreen, promotion of the proliferation of fibroblast cells, and tyrosinase inhibitory effect.

MATERIALS AND METHODS

Materials. TPS (TPS1, 92% purity; and TPS2, 20% purity) and TPP (98% purity) were prepared from green tea leaves in our laboratory. Rutin (>98%), glycerol, DMEM high-glucose culture medium, fetal bovine serum (FBS), and MTT was provided from Amresco Inc. (Solon, OH). Trypsinase (0.25%), SDS, mushroom tyrosinase, EGCG, EGC, ECG, EC, caffeine, vitamin C, and L-DOPA were purchased from Sigma Chemical Co. (St. Louis, MO).

Sample Preparation. (*1*) *Preparation of TPS1*. Lower grade green tea (500 g) was mixed with 2500 mL of 80% ethanol (v/v) and refluxly heated at 30 °C for 24 h. This treatment was repeated twice. After the mixture was filtered, the residue material was extracted with 5 L of 0.5% (m/m) plant hydrolase (Novozymes Co., Beijing, China) solution at 40 °C, pH 5.5, for 3 h. After filtration, the tea leaf residues were extracted again with 5 L of distilled water at 60 °C for another 2 h. Then the combined filtered solutions were centrifuged (10 min, 8000 rpm) to remove the contaminants, and the supernatant was filtered through a 0.45 μm microfiltration equipment. The permeated solution was concentrated to about 2 L under reduced pressure, and 3 volumes of 95% ethanol (v/v) was added slowly by stirring to precipitate the polysaccharides and kept at 4 °C overnight. The polysaccharide pellets were obtained by centrifugation at 4800 rpm for 15 min and repeatedly washed sequentially with possibly less amounts of ethanol, acetone, and ether, respectively. The refined polysaccharide pellets were completely dissolved in an appropriate volume of distilled water and intensively dialyzed for 2 days against distilled water (cutoff M_w = 3500 Da). The retentate portion was concentrated and deproteinated with Sevag reagent ($CHCl_3$/BuOH = 4:1, v/v) for 30 min under the magnetic force stirring, and the procedure was repeated twice. Finally, the extracts were centrifuged to remove insoluble material, and the supernatant was precipitated by 3 volumes of 95% ethanol (v/v) again. The polysaccharide pellets were obtained by centrifugation at 4800 rpm for 15 min and repeatedly washed sequentially with ethanol, acetone, and ether, respectively. The polysaccharide pellets were redissolved in distilled water and lyophilized to give TPS1 (about 4.5 g).

(*2*) *Preparation of TPS2*. Lower grade green tea (500 g) was extracted with 5 L of 0.5% (m/v, pH5.5) plant hydrolase (Novozymes Co.) solution at 40 °C for 3.0 h. After filtration, the tea leaf residues were extracted again with 5 L of distilled water at 60 °C for another 2 h. Then the combined filtered solution was centrifuged to remove the contaminants. The supernatant was concentrated under reduced pressure to about 2 L and precipitated by adding 8 L of 95% (v/v) ethanol at 4 °C for 24 h and then centrifuged at 5000 rpm for 10 min. The sediment was vacuum-dried, and 15.0 g of crude TPS2 was obtained.

(*3*) *Preparation of TPP*. Lower grade green tea (500 g) was extracted with 0.5% (m/v) plant hydrolase (Novozymes Co.). After filtration, the tea leaf residues were extracted again with distilled water at 90 °C for another 1 h. Then the combined solution was centrifuged (10 min, 8000 rpm), and the supernatant was filtered through a 0.45 μm microfiltration equipment. The permeate was concentrated under reduced pressure. The concentrate were first decaffeinated by chloroform and then extracted by ethyl acetate, citric acid buffer solution, and butyl acetate successively. The extraction phase was vacuum concentrated for solvent recovery and redissolved in water and then lyophilized to give TPP (about 19.5 g).

General Analysis. The content of tea polyphenols were determined according to the ferrous tartrate method (*18*). The compositions of tea polyphenols (catechins) and caffeine in TPP were determined by reverse-phase HPLC analysis (*19*, *20*).

The total sugar content of TPS was determined as follows: (1) The contents of uronic acids (CUA) in TPS were determined by *m*-hydroxybiphenol colorimetry using galacturonic acids as standard (*21*). (2) The absorbance of tea polysaccharides (AT) in TPS were determined according to the phenol−sulfuric acid method (*22*) using galactose as standard. The absorbance of uronic acids (AUA) in TPS was determined according to the phenol−sulfuric acid method using galacturonic acids as standard. Then the actual absorbance of neutral sugars was the difference value of AT and AUA. The actual contents of neutral sugars (CNS) were obtained by means of the standard curve. (3) The contents of total sugars of TPS were the sum of neutral sugars and acid polysaccharides contents (CUA + CNS).

Monosaccaride Composition Analysis. Monosaccharide was determined by GC, and sample preparation was carried out according to the reference by Blakeney et al. (*23*). TPS sample (20 mg) was hydrolyzed at 118 °C for 2 h with 2 mL of 2 mol L^{-1} TFA. The supernatant was evaporated to dry under reduced pressure. The residue sample was stirred with inositol (2 mg), hydroxylamine hydrochloride (10 mg), and pyridine (0.5 mL) at 90 °C for 30 min and then cooled to room temperature. After acetic anhydride was added at 90 °C for 30 min, the mixture was subjected to GC analysis. GC was performed on a Shimadzu GC-14A gas chromatograph (Tokyo, Japan) equipped with an OV1701 flexible silica capillary column (30 m × 0.53 mm × 1.0 μm) and a flame ionization detector. The detailed experimental conditions were as follows: N_2 (2 mL/min), H_2 (40 mL/min); air (550 mL/min); the elution was realized by a temperature gradient from 180 °C (5 min) to 240 °C (25 min) with a slope of 3 °C/min. The vaporizer temperature was 280 °C, and the detector temperature was 260 °C. A 10 μL aliquot was injected for each run.

Moisture Absorption and Retention Tests. The effects of TPS1, TPS2, TPP, and glycerol (positive control) on moisture absorption and retention were evaluated by a weighting method through controlling relative humidity of the desiccators (*24*). Prior to the moisture absorption test, the samples were dried in a vacuum for 24 h. The water absorption ability was assessed by the percentage of mass increase of dry sample (R_{ab})

$$R_{ab} = [(m_1 - m_0)/m_0] \times 100\% \quad (1)$$

where m_0 and m_1 are the masses of the dry samples before and after being put into a saturated $(NH_4)_2SO_4$ desiccator (81% relative humidity) or in a saturated K_2CO_3 desiccator (43% relative humidity) at 20 °C for about 40 h, respectively.

In the moisture retention test, wet samples were prepared by adding water (10%) to each sample. The moisture retention ability was assessed by the percentage of residual water of the wet sample (R_{re})

$$R_{re} = (m_2/m_0) \times 100\% \quad (2)$$

where m_2 and m_0 are the masses of water in the sample before and after placement into a desiccator with silica gel at 20 °C for 48 h, respectively.

Sunscreen Function Assessment. The efficacy of sunscreen of purified TPS and TPP was measured according to the ultraviolet absorption values, which were determined by the Cary 100 UV−vis spectrophotometer (*25*). TPS and TPP were dissolved in double-distilled water at the concentration of 100 mg/L. Rutin was used as the positive control, which had a strong ability to absorb ultraviolet light. It was dissolved in a 1:4 methanol−water solution to the concentration of 100 mg/L.

Effect on Proliferation of Skin Fibroblasts. Effects of TPP and TPS on proliferation of fibroblast cells were examined in phase-contrast microscopy and were determined morphologically by cell proliferation and MTT assays (*26*).

Cell Culture. Fibroblasts were obtained from mice skin and were cultured by the medium of DMEM with 10% FBS supplemented with penicillin−streptomycin (100 U/mL/100 μg/mL) and 2 mM L-glutamine. The cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. The media were replaced every 2 or 3 days until confluence was reached. Fibroblasts used for this study were between their third and fifth passage in culture.

MTT Assay. Cell survival was analyzed by using a non-radioactive cell proliferation assay system (MTT assay) consisting of 3-(4,5-dimethylthiaziazol-2-yl) 2,5-diphenyltetrazolium bromide. The cells were plated at 1×10^5 cells/mL per well into 96-well plates, and various concentrations (500, 200, 100, 50, and 20 μg/mL) of sample were added with serum-free DMEM in a total volume of 100 μL/well. After further culture for 48 h at 37 °C in a humidified atmosphere of 5% CO_2 in air, fibroblast growth induction activity was determined by MTT solution (5 mg/mL), which was added to each well of the plate, and plates were incubated for 4 h at 37 °C in a humidified atmosphere of 5% CO_2 in air. The medium in each well was changed by 100 μL of DMSO and mixed thoroughly to dissolve the dark blue crystals for 10 min at room temperature to ensure all crystals could be dissolved. The plates were read with an ELISA reader, using a test wavelength of 570 nm and a reference wavelength of 630 nm. All assays were repeated two times. Its cell proliferation rate was set at 100%. If the proliferation rate is lower than 100%, the tested sample inhibited cell proliferation; otherwise, it promoted cell proliferation.

Tyrosinase Inhibitory Assays. The tyrosinase inhibitory activity was measured as previously described, using L-DOPA as the substrate (*27*) with some modifications. Samples TPS1, TPS2, and TPP were respectively dissolved into deionized water and prepared at different concentrations (5, 20, 100, and 500 μg/mL). A 2.0 mL sample solution was mixed with 2.0 mL of substrate solution containing 0.02% L-DOPA in 25 mM phosphate buffer (pH 6.8), and then 1.0 mL of enzyme solution (100 U/mL) and 5 mL of phosphate buffer (pH 6.8) were added to this mixture solution. The absorbance was recorded after 6 min at 25 °C at 475 nm using a Purkinje spectrophotometer (Beijing, China). The phosphate buffer (pH 6.8) was used as a blank, whereas 1.0 mL of distilled water was used as the control. Results were compared with the control and the blank containing distilled water in place of the sample solution. Vitamin C was used as the positive control. Percent tyrosinase inhibitory activity was calculated using the following formula: $[(OD_{control} - OD_{sample})/OD_{control}] \times 100$. The IC_{50} value was obtained through extrapolation from linear regression analysis and denoted the concentration of sample required to inhibit 50% of tyrosinase activity. The data presented are means ± SD of three determinations.

Statistical Analysis. SPSS 11.0 programs were used in the statistical analysis. The significance of differences among values was analyzed by one-way analysis of variance (ANOVA). Significant differences ($P < 0.05$) between means were determined using Dunnett's t test. The criterion for significance was $p < 0.05$ or $p < 0.01$, as specified.

Table 1. Purity and Composition of TPS

sample	yield/%	purity/%	polyphenol/%	neutral sugar/%	uronic acid/%
TPS1	0.9	92.0		64.27	27.95
TPS2	3.3	20.0	35.60	17.05	3.95

Table 2. Effects of TPS1, TPS2, and TPP on Moisture Absorption and Retention

	RH = 43%		RH = 81%	
sample	R_{ap} (RSD[a])/%	R_{re} (RSD[a])/%	R_{ap} (RSD[a])/%	R_{re} (RSD[a])/%
TPS1	14.31 (2.4)	111.03 (0.3)	12.66 (1.8)	103.63 (0.1)
TPS2	9.36 (5.5)	104.25 (0.1)	5.33 (2.5)	102.73 (0.1)
TPP	10.07 (0.2)	107.77 (0.1)	9.23 (3.7)	103.26 (0.3)
glycerol	27.73 (2.2)	111.40 (0.9)	20.16 (1.0)	112.37 (0.2)

[a] RSD was the relative standard deviations of the three parallel experimental groups.

RESULTS AND DISCUSSION

Purity and Composition of TPS and TPP. The purity and composition of TPS obtained are shown in **Table 1**. TPS1 (yield = 0.9%) was obtained by a series of purification procedures such as ethanol infusion, water extraction, ethanol sedimentation, deproteination, and dialysis. The actual neutral sugars content of TPS1 was 64.27%, the uronic acids content of TPS1 was 27.95%, and the purity of TPS1 was calculated as 92.0%. No polyphenols were detected in TPS1. TPS1 consisted of rhamnose, ribose, arabinose, mannose, glucose, galactose, and galacturonic acid with the molar ratio of 4.8:1.6:15.4:7.3:6.6:44.9:19.4.

TPS2 (yield = 3.3%) was obtained by simple extraction procedures such as water extraction and ethanol sedimentation. The actual neutral sugars content of TPS2 was 17.05%, the uronic acids content of TPS2 was 3.95%, and the purity of TPS2 was calculated as 20.00%. The polyphenols content of TPS2 was 35.60%. Besides the polysaccharides and polyphemols, TPS2 also contained proteins, ashes, pigments, amino acids, and other components.

The content of tea polyphenols in TPP was 98.25% when measured by methods of ferrous tartrate. The level of total catechins was 81.83%. TPP consisted of EGCG (60.97%), EGC (3.35%), EC (7.62%), ECG (3.05%), C (1.40%), and caffeine (0.67%) determined by RP-HPLC.

Moisture Absorption and Retention Properties. The moisture absorption ability and moisture retention property of TPS and TPP were examined and compared with those of glycerol. As shown in **Table 2**, the RSD values of three parallel groups are all very low, which means that the method has high accuracy and good repeatability. In all cases, the glycerol has the best moisture absorption and retention activities among the samples tested. Comparison of the results between TPS1 and TPS2 showed that both moisture absorption ability and moisture retention capacity increased with the increasing content of polysaccharides in samples. Moisture absorption ability and moisture retention capacity of TPP were lower than those of TPS1, but higher than those of TPS2. TPS and TPP both had better moisture absorption ability and moisture retention capacity under low relative humidity condition (RH = 43%) than under high relative humidity (RH = 81%). Thus, they are suitable for moisture retention in relatively dry environments when used as components in cosmetics.

The good moisturizing function of TPS was primarily due to the molecular structures (*28*). The hydroxyl groups, carboxyl groups, and some other polar groups in polysaccharide molecules can form hydrogen bonds with water molecules. In this way,

polysaccharides can bind a great deal of water. Besides, polysaccharide molecular chains wind together to form a web and combine hydrogen bonds of water. Thus, polysaccharides have good water retention. Furthermore, tea polysaccharides can provide water by being constituted of an extracellular colloidal matrix containing a great deal of water in an extracellular matrix with other polysaccharide components and fibrous protein in skin (*28*).

Many natural plant extracts not only possess good moisture retention but also have a definite nutritional effect on human skin. The dual effective components extracted from natural plants have replaced some synthetic moisturizers. This alternation is the main trend for the future for developing moisturizers.

Sunscreen Function. Sunscreens are cosmetic formulations that block UV rays. According to **Figure 1c**, rutin was found to strongly absorb ultraviolet light of UV-A, UV-B, and UV-C, which is in accordance with the literature. As shown in **Figure 1a**, TPS can absorb UV-A weakly and hardly absorbs UV-B. TPP, however, can intensively absorb UV-A and UV-B (**Figure 1b**). From these results, it can be concluded that TPP can be used as an additive in sunscreens, which absorb the solar UV energy on the skin's surface and prevent deeper penetration and damage. The significant antioxidant ability of TPP is also considered to stop penetration of free radicals and to heal any damage as it may occur within the skin. According to **Figure 1b**, TPP strongly absorbs UV rays within the range of 280−320 nm, which resulted from the benzene rings in the TPP. TPP mainly consists of catechins such as EGCG, EGC, EC, and ECG, which contain two or three benzene rings.

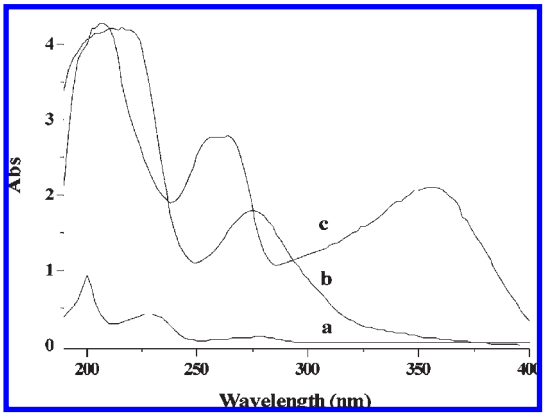

Figure 1. UV adsorption spectrograms of TPS1 (**a**), TPP (**b**), and rutin (**c**).

Effects of TPS and TPP on Fibroblast Cell Proliferation. In microscopic examination, a low concentration of TPP (500 μg/mL) stimulated fibroblast cell growth after 36 h of incubation (**Figure 2c**,**d**). The number of cells increased after incubation for 36 h with TPP (**Figure 2a**,**d**).

In MTT assay, it was shown that the effect of TPS1 on skin fibroblast proliferation was not siginificant ($p < 0.05$) (**Figure 3a**). Cell viability was obviously reduced in a dose-dependent manner after exposure of fibroblasts to TPS2. The low concentrations (20−200 μg/mL) of TPS2 showed a stimulation of cell proliferation, but with concentration increasing, the effect of TPS2 on skin fibroblasts proliferation decreased rapidly. The highest cell viability of TPS2 was 163.75% at the concentration of 20 mg/L. The high concentration (500 μg/mL) of TPS2 was cytotoxic and inhibited cell proliferation (**Figure 3b**).

In the TPP group, the effect was extremely significant on cell proliferation ($p < 0.01$). Cell viability was obviously increased in a dose-dependent manner after exposure of fibroblasts to TPP.

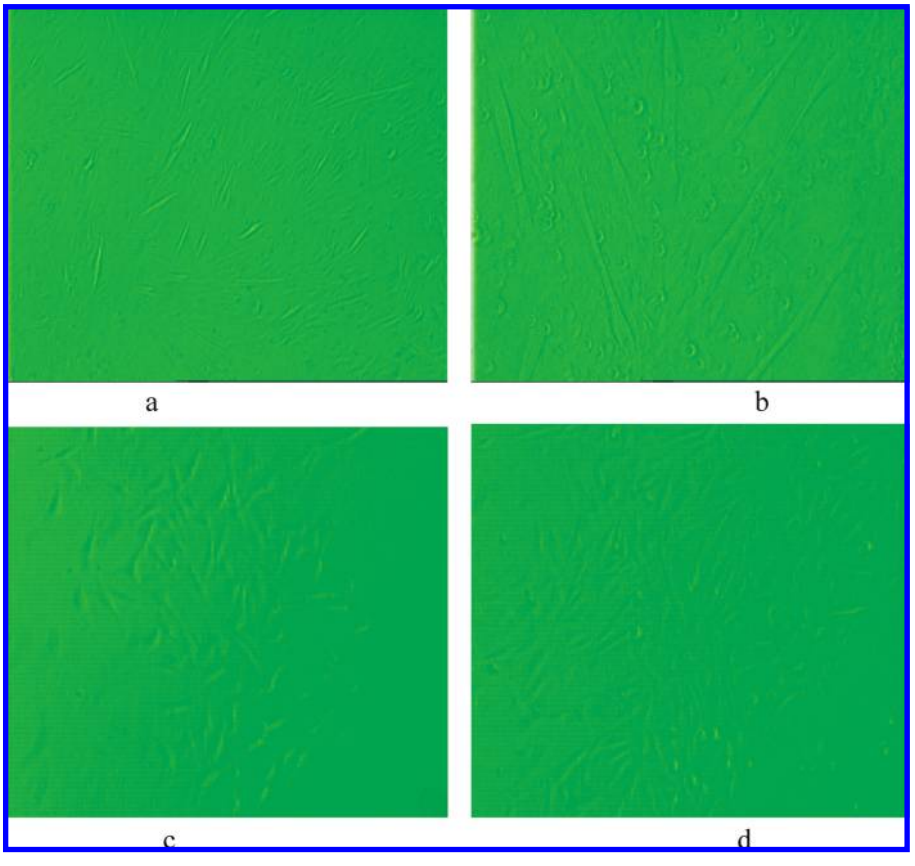

Figure 2. Fluorescent microscope picture of skin fibroblasts cultures [**a**, 0 h (400×); **b**, 0 h (1000×)]and fibroblast cultures after 36 h of incubation with 0 μg/mL TPP (**c**, 400×) and 500 μg/mL TPP (**d**, 400×). The number of cells increased after incubation for 36 h with TPP (**a**, **d**).

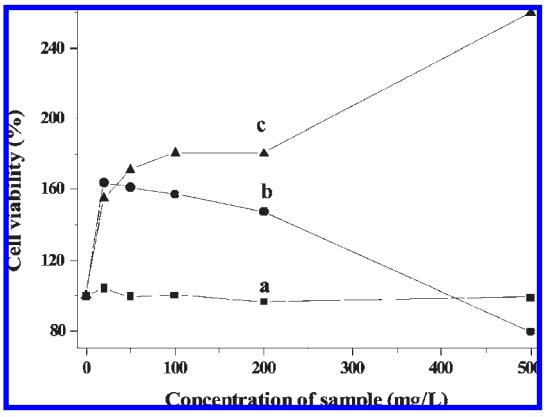

Figure 3. Concentration−cell viability curves of TPS1 (**a**), TPS2 (**b**), and TPP (**c**) exposure on mice skin fibroblasts using the MTT assay.

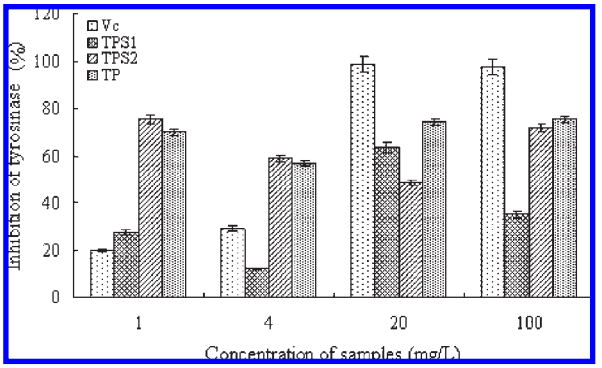

Figure 4. Inhibitory effects of Vc, TPP, TPS1, and TPS2 on tyrosinase. Tyrosinase activity was estimated in the presence of L-DOPA as the substrate, represented as inhibition percent at various concentrations. Values are means of three separate experiments.

The cell viability of TPP was up to 259.85% at the concentration of 500 mg/L, which indicated that TPP can greatly promote fibroblast cell proliferation. With the increase of age and UV radiation, fibroblast cells reduced and their activities declined; thus, collagen synthesis reduced correspondingly. Collagen proteins, which are synthesized by fibroblast cells, are the main materials in the maintenance of skin water and elasticity. The reduction of collagen proteins will lead to skin wrinkling and roughness. Therefore, the studies of natural active components promoting fibroblast cell proliferation are worthwhile. It is suggested that TPP has good application in antiaging cosmetics.

Tyrosinase Inhibitory Effect. Tyrosinase inhibitory activities of TPS, TPP, and Vc are shown in **Figure 4**. In the positive group (Vc group), tyrosinase inhibition effects dose-dependently increased with the addition of Vc. The inhibitory effect of Vc on tyrosinase increased rapidly up to 98.52% at the concentration of 20 mg/L. It can be concluded from **Figure 4a** that the IC_{50} for Vc was between 4 and 20 mg/L. The inhibitory effects of TPP, TPS1, and TPS2 on tyrosinase were unrelated to the sample dose. However, inhibitory efficiency on tyrosinase was even more remarkable than that of Vc at a relatively low dose (1−4 mg/L). Within the range of 1−4 mg/L, the tyrosinase inhibitory rates of Vc were between 27.59 and 11.82%, the inhibitory rates of TPS2 were between 75.37 and 59.11%, and the inhibitory rates of TPP were between 69.65 and 57.14%, which indicated the tyrosinase inhibitory effects of TPP and TPS2 had good performance at the lower concentration. Within the range of 1−100 mg/L, the tyrosinase inhibitory rates of TPP were between 75.37 and 57.14%, the inhibitory rates of TPS2 were between 75.37 and 59.11%, and the inhibitory rates of TPS1 were between 11.82 and 48.27%, which indicated TPS2 and TPP both had good tyrosinase inhibitory effects, but TPS1 had very weak inhibitory effects. When raw extracts was used as test samples, some components in the crude extracts could react with tyrosinase, which interfered with the determination. This may be the reason the inhibitory curves of TPP, TPS1, and TPS2 on tyrosinase were irregular. However, Vc appeared to have a more regular tyrosinase inhibition property. As shown in **Figure 4**, the tyrosinase inhibitory effect of TPP is almost the same as that of TPS2. However, the inhibitory effect of TPS1 is much lower than that of TPP and TPS2.

The structural significance of the phenol ring configuration in inhibitory actions on tyrosinase has been reported (*29*). Although green tea has been reported to inhibit mushroom tyrosinase activity, especially the major active constituents in the green tea (−)-epicatechin-3-gallate (ECG), (−)-gallocatechin-3-gallate (GCG), and (−)-epigallocatechin-3-gallate (EGCG), which showed strong inhibitory effects (*30*), the comparison of inhibitory effects of crude TPS and TPP on tyrosinase in vitro has not yet been reported.

On the basis of the results above, it can be obviously concluded that TPS and TPP both had positive protective effects on skin. TPS and TPP both had good moisture retention capability in relatively dry environment for at least 48 h. TPP can intensively absorb UV-A and UV-B, whereas TPS showed weak absorbance of UV-A and UV-B. In skin cell cultures, TPP and crude TPS had strong proliferation activity, whereas TPS with high purity had hardly any effect on it. As for the tyrosinase inhibition assay, TPP had good tyrosinase inhibitory effects; TPS showed weaker tyrosinase inhibitory effects as the purity increased. The data provided a useful support for developing skin-protecting cosmetic additives and information for being added in the proper proportion and amounts to cosmetics. This indicated that combination of TPS and TPP would be a favorable possibility for protecting skin, especially for the good moisture retention ability of TPS and fibroblast proliferation effect and strong UV absorbance ability of TPP. The results suggested that TPS and TPP extracted from green tea may potentially be applied in cosmetics. The experiments in the paper were all done in vitro, and the human clinical effects need further in vivo trials.

ABBREVIATIONS USED

TPS, tea polysaccharide; TPP, tea polyphenol; DMEM, Dulbecco's modified Eagle's Medium; FBS, fetal bovine serum; MTT, methylthiazolyl tetrazolium; SDS, sodium dodecyl sulfonate; Vc, vitamin C; L-DOPA, L-3,4-dihydroxyphenylalanine; UV, ultraviolet; R_{ab}, water absorption rate; R_{re}, moisture retention rate; DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assy; EGCG, (−)-epigallocatechin 3-gallate; EGC, (−)-epigallocatechin; EC, (−)-epicatechin; C, catechin; ECG, (−)-epicatechin 3-gallate; DL-C, DL-catechin.

LITERATURE CITED

(1) Jayabalan, R.; Marimuthu, S.; Thangaraj, P.; Sathishkumar, M.; Binupriya, A. R.; Swaminathan, K.; Yun, S. E. Preservation of Kombucha tea. Effect of temperature on tea components and free radical scavenging properties. *J. Agric. Food Chem.* **2008**, *56*, 9064–9071.

(2) Anesini, C.; Ferraro, G. E.; Filip, R. Total polyphenol content and antioxidant capacity of commercially available tea (*Camellia sinensis*) in Argentina. *J. Agric. Food Chem.* **2008**, *56*, 9225–9229.

(3) Kang, N. J.; Lee, K. M.; Kim, J. H.; Lee, B. K.; Kwon, J. Y.; Lee, K. W.; Lee, H. J. Inhibition of gap junctional intercellular communication by the green tea polyphenol (−)-epigallocatechin gallate in

normal rat liver epithelial cells. *J. Agric. Food Chem.* **2008**, *56*, 10422–10427.
(4) Kato, A.; Minoshima, Y.; Yamamoto, J.; Adachi, I.; Watson, A. A.; Nash, R. J. Protective effects of dietary chamomile tea on diabetic complications. *J. Agric. Food Chem.* **2008**, *56*, 8206–8211.
(5) Xiao, J.; Chen, X.; Zhang, L.; Talbot, S. G.; Li, G. C.; Xu, M. Investigation the mechanism of enhanced effect of EGCG on huperzine A inhibiting acetylcholinesterase activity in rats by multi-spectroscopic method. *J. Agric. Food Chem.* **2008**, *56*, 910–915.
(6) Anesini, C.; Ferraro, G. E.; Filip, R. Total polyphenol content and antioxidant capacity of commercially available tea (*Camellia sinensis*) in Argentina. *J. Agric. Food Chem.* **2008**, *56*, 9225–9229.
(7) Kang, N. J.; Lee, K. M.; Kim, J. H.; Lee, B. K.; Kwon, J. Y.; Lee, K. W.; Lee, H. J. Inhibition of gap junctional intercellular communication by the green tea polyphenol (−)-epigallocatechin gallate in normal rat liver epithelial cells. *J. Agric. Food Chem.* **2008**, *56*, 10422–10427.
(8) Fu, Y.; Jin, X.; Wei, S.; Lin, H.; Zhang, Y. Effects of tea polyphenols on cell growth in primary cultured rat skin keratinoctytes and fibroblasts. *J. Shanghai Med. Univ.* **2000**, *27*, 24–27.
(9) Wang, D. F.; Xie, X. F.; Cai, C. Y.; Yang, M. Analysis of pharmacological components of coarse tea curing diabetes. *Chin. Tradit. Herb. Drugs* **1995**, *26*, 255–257.
(10) Zhou, X.; Wang, D.; Sun, P.; Bucheli, P.; Li, L.; Hou, Y.; Wang, J. Effects of soluble tea polysaccharides on hyperglycemia in alloxan-diabetic mice. *J. Agric. Food Chem.* **2007**, *55*, 5523–5528.
(11) Monobe, M.; Ema, K.; Kato, F.; Maeda-Yamamoto, M. Immunostimulating activity of a crude polysaccharide derived from green tea (*Camellia sinensis*) extract. *J. Agric. Food Chem.* **2008**, *56*, 1423–1427.
(12) Lee, J. H.; Shim, J. S.; Lee, J. S.; Kim, J. K.; Yang, I. S.; Chung, M. S.; Kim, K. H. Inhibition of pathogenic bacterial adhesion by acidic polysaccharide from green tea (*Camellia sinensis*). *J. Agric. Food Chem.* **2006**, *54*, 8717–8723.
(13) Hearing, V. J.; Jimenez, M. Analysis of mammalian pigmentation of at the molecular level. *Pigment Cell Res.* **1989**, *2*, 75–85.
(14) Aoki, Y.; Tanigawa, T.; Abe, H.; Fujiwara, Y. Melanogenesis inhibition by an oolong tea extract in B-16 mouse melanoma cells and UV-induced skin pigmentation in brownish guinea pigs. *Biosci., Biotechnol., Biochem.* **2007**, *71*, 1879–1885.
(15) Chow, H. H.; Cai, Y.; Hakim, I. A.; Crowell, J. A.; Shahi, F.; Brooks, C. A.; Dorr, R. T.; Hara, Y.; Alberts, D. S. Pharmacokinetics and safety of green tea polyphenols after multipledose administration of epigallocatechin gallate and polyphenon E in healthy individuals. *Clin. Cancer Res.* **2003**, *9*, 3312–3319.
(16) Chen, H. X.; Zhang, M.; Qu, Z. S.; Xie, B. J. Compositional analysis and preliminary toxicological evaluation of a tea polysaccharide conjugate. *J. Agric. Food Chem.* **2007**, *55*, 2256–2260.
(17) Kapetanovic, I. M.; Crowell, J. A.; Krishnaraj, R.; Zakharov, A.; Lindeblad, M.; Lyubimov, A. Exposure and toxicity of green tea polyphenols in fasted and non-fasted dogs. *Toxicology* **2009**, in press.
(18) Iwasa, K.; Torii, H. A colorimetric determination of tea tannin with ferrous tartrate. *Stud Tea* **1962**, *26*, 87–91.
(19) Lin, Y. S.; Wu, S. S.; Lin, J. K. Determination of tea polyphenols and caffeine in tea flowers (*Camellia sinensis*) and their hydroxyl radical scavenging and nitric oxide suppressing effects. *J. Agric. Food Chem.* **2003**, *51*, 975–980.
(20) Cabrera, C.; Gimnez, R.; Lpez, M. C. Determination of tea components with antioxidant activity. *J. Agric. Food Chem.* **2003**, *51*, 4427–4435.
(21) Blumenkrantz, N.; Asboe-Hansen, G. New method for quantitative determination of uronic acids. *Anal. Biochem.* **1973**, *54*, 484–489.
(22) Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* **1956**, *28*, 350–356.
(23) Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* **1983**, *113*, 291–299.
(24) Liu, Y. L.; Wang, J.; Zhang, J. P. The skin moisturizer and evaluation of its properties. *Chin. Surf. Deter. Cosm.* **1999**, *10*, 52–54.
(25) Li, M. X. Simultaneous determination of rutin and quercertin in flos sophorae buds by isoabsorptive point ultraviolet spectrophotometry. *Chin. J. Spectrosc. Lab.* **2005**, *22*, 42–45.
(26) Dechsakulthorn, F.; Hayes, A.; Bakand, S.; Joeng, L.; Winder, C. *In vitro* cytotoxicity assessment of selected nanoparticles using human skin fibroblasts. *AATEX* **2007**, *14*, 397–400.
(27) Kobayashi, Y.; Kayahara, H.; Tadasa, K.; Nakamura, T.; Tanaka, H. Synthesis of amino acid derivatives of kojic acid and their tyrosniase inhibitory activity. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 1745–1746.
(28) Sebti, I.; Coma, V. Active edible polysaccharide coating and interactions between solution coating compounds. *Carbohydr. Polym.* **2002**, *49*, 139–144.
(29) Yokozawa, T.; Kim, Y. J. Piceatannol inhibits melanogenesis by its antioxidative actions. *Biol. Pharm. Bull.* **2007**, *30*, 2007–2010.
(30) No, J. K.; Soung, D. Y.; Kim, Y. J.; Shim, K. H.; Jun, Y. S.; Rhee, S. H.; Yokozawa, T.; Chung, H. Y. Inhibition of tyrosinase by green tea components. *Life Sci.* **1999**, *65*, 241–246.

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