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Photoprotection effect of Pu'er tea and *Curcuma longa* L. extracts against UV and blue lights

Doyeong Son · Ji-Su Jun · KwangWon Hong

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Abstract Plant extracts have been studied due to their potential as photoprotective agents against UV and blue light exposure. Previous studies have revealed that several plant extracts have photoprotection capacities and synergistic effects with synthetic products. However, such results for pu'er tea and *Curcuma longa* L. have not been reported yet for a cosmetic formulation. Thus, the objective of this study was to evaluate photoprotection capacities of pu'er tea and *C. longa* L. extracts for a sunscreen compound. The pu'er tea extract improved sun protection factor value of 2-ethyl-hexyl methoxycinnamate (a synthetic sunscreen compound) by 46% and showed a high antioxidant capability that could help skin recover from photo-induced damage. *C. longa* L. extract also showed a potential to protect skin from blue light-induced damage because it not only had a maximum absorption peak at the blue light range, but also protected human fibroblasts from blue light-induced damage. The addition of both extracts shifted the critical wavelength of 2-ethyl-hexyl methoxycinnamate from 350 nm to 386 nm, giving it a broad-spectrum feature. Thus, pu'er tea and *C. longa* L. extracts may enhance the photoprotection ability of synthetic sunscreen products.

Keywords Blue light · *Curcuma longa* L. · 2-ethyl-hexyl methoxycinnamate · Photoprotection · Pu'er tea, Sun protection factor

KwangWon Hong (✉)
E-mail: hkwon@dongguk.edu

Department of Food Science and Biotechnology, College of Life Science and Biotechnology, Dongguk University, Goyang-si 10326, Republic of Korea

Introduction

Exposure to ultraviolet (UV) lights has been researched for decades since it not only can lead to intracellular DNA damage, but other can lead to issues such as photoaging, reduced human skin barrier function, and generation of reactive oxygen species leading to oxidative stress [1,2]. Such physiological changes may cause collagen damage and leave skin dehydrated [3].

There are three types of UV lights: UV-A (400-315 nm), UV-B (315-280 nm), and UV-C (280-100 nm). It is known that the Earth's ozone layer absorbs most UV-C lights but doesn't absorb UV-B and UV-A lights [2]. UV-B radiation is related to sunburn and skin darkening while UV-A radiation has a longer wavelength that can penetrate deeper into the skin with negative outcomes such as skin aging and DNA damage.

Bhattacharjee et al. [4] have reported that synthetic sunscreen agents have side effects such as genotoxicity, cytotoxicity, and endometriosis, although they are efficient in protecting UV rays. They have also suggested that natural sunscreen products in certain formulations are equally efficient as synthetic ones without side effects that synthetic filters have [4]. Other previous study has stated that some natural products can be used to reduce the quantity of synthetic sunscreen ingredients or as substitutes [5]. Along with their UV absorption property, most natural compounds from plant extracts also can act as antioxidants and anti-inflammatory agents to further protect skins from UV light-induced damage while synthetic filters could not offer such benefits [5]. Natural products also seem to have merits over synthetic products as they are generally more compatible with all skin types and that they can protect skin from early-maturing [4]. Blue light is a visible light that has wavelengths of 400 to 500 nm. The term of 'blue light' searched in Google has increased since 2004 [6], indicating that there are growing concerns about potential harm from blue light exposure. Although the exact mechanism of how blue light damages human skin has not been clarified, researchers have found that its major mode of action is

by giving skin cells oxidative stress by generating reactive oxygen species [7]. The main source of blue light that causes oxidative stress and other skin dysfunctions is sunlight [8]. Daily protection from blue light is a rising issue as not all sunblock products in the market can provide protection from the blue light.

Polyphenols are natural compounds found in plants with skin photoprotection and other beneficial effects such as antioxidative ability [9,10]. This indicates that plants rich in polyphenols might have photoprotective effects. Studies on some natural plants such as guava and green coffee beans known to be abundant in polyphenols have revealed that they not only have photoprotective effects, but also have synergistic effects with 2-ethyl-hexyl methoxycinnamate (EHM), a synthetic sunscreen compound [11,12]. Previous studies have found that pu'er tea and *C. longa* L. extracts have antioxidative abilities because of their polyphenols [13,14]. Thus, they might possess photoprotective abilities against UV light and might also possess synergistic effects when they are used with a synthetic sunscreen agent together. Additionally, *C. longa* L. might have skin-protecting effect against blue light given that a recent study has found that it is protective against blue light-induced damage of retinal pigment epithelial cells [15]. Thus, the objective of the present study was to assess photoprotective effects of pu'er tea and *C. longa* L. extracts against UV and blue light when used with 2-ethyl-hexyl methoxycinnamate, a synthetic sunscreen compound.

Materials and Methods

Plant Materials

Powdered samples of Pu'er tea and *C. longa* L. used in this study were purchased from a market of medicinal herbs, Dongdaemun-gu, Seoul.

Extract Sample Preparation

Powdered pu'er tea and *C. longa* L. samples were placed in distilled water (10% w/v) and 70% ethanol (10% w/v), respectively. After 24 h of incubation at 55 ºC, liquid extracts were centrifuged at 10,000× g for 30 min. Supernatants were collected and filtered with a Whatman 597 1/2 filter paper (Whatman, Buckinghamshire, UK). All extract samples were stored at 4 ºC until used. A further step of lyophilization was used for cosmetic formulation described in Table 1. The weight of lyophilized extract compared to the liquid extract state of pu'er tea or *C. longa* L. was 7.4% or 1.2%, respectively. The lyophilized powder of *C. longa* L was diluted to a volume corresponding to the same yield rate of pu'er tea (7.4%) in 70% ethanol before it was used in cosmetic preparation while pu'er tea was used as a lyophilized powder.

Total Phenolic Contents and Total Flavonoid Contents

Total phenolic contents were determined using the Folin-Ciocalteu assay [16]. Briefly, an aliquot (1 mL) of the extract or standard solution of gallic acid (100, 200, 300, 400, and 500 µg/mL) was added to a 25 mL volumetric flask containing 9 mL of distilled water. A blank sample was prepared using distilled water. One milliliter of Folin-Ciocalteu phenol reagent was added to the mixture followed by vortexing at room temperature. After 5 min, 10 mL of 7% (w/v) Na_2CO_3 solution was added to the mixture before the volume was made up to 25 mL with distilled water and incubated for 90 min at room temperature. The absorbance was determined against a blank sample at 550 nm with a 6705 UV/vis spectrophotometer (Jenway, Staffordshire, UK). Results are expressed as mg of gallic acid equivalents per gram (mg GAE/g) of extract powder.

Total flavonoid contents were determined according to the Dowd method [17,18]. Briefly, an aliquot (1 mL) of extract or standard solution of quercetin (10-250 µg/mL) was mixed with

Table 1 Compositions of cosmetic formulations

Components (INCI) [a]	Concentration % (w/w)									
	C1	C2	S1	S2	S3	S4	S5	S6	S7	S8
Phase 1										
Disodium EDTA	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Purified water	84.9	82.9	74.9	81.9	77.9	75.4	72.9	74.9	72.9	62.9
Phase 2										
Self-emulsifying wax (Cetearyl alcohol, Polyoxyethlene sorbitan monostearate)	15	15	15	15	15	15	15	15	15	15
Phase 3										
2-Ethyl-hexyl methoxycinnamate	-	2	-	2	2	2	2	-	2	2
Pu'er tea extract	-	-	10	1	5	7.5	10	-	-	10
Curcuma longa L extract	-	-	-	-	-	-	-	10	10	10

[a]INCI, International Nomenclature of Cosmetic Ingredients.

C1 is a base cream sample. C2 is a base cream sample supplemented with 2% 2-ethyl-hexyl methoxycinnamate (EHM). S1 is a base cream sample containing 10% pu'er tea extract (PE). S2 to S5 contain various concentrations (1, 5, 7.5, and 10%) of PE and 2% EHM. S6 is a base cream sample containing 10% *Curcuma longa* L. extract (CE). S7 contains 10% CE and 2% EHM. S8 sample contains 10% CE, PE, and 2% EHM. Results are shown as mean ± standard deviation (n =3)

0.2 mL of 10% (w/v) $AlCl_3$ solution in methanol, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water. After incubating at room temperature for 30 min, the absorbance at 415 nm was measured against the blank. Results are expressed as mg of quercetin equivalents per gram (mg QE/g) of extract powder.

Scavenging ability for 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH)

The antioxidant activity of each liquid extract sample was measured based on the scavenging ability for DPPH radicals as described by Shimada et al. with some slight modifications [19]. Briefly, two milliliters of pu'er tea extract (3-7 μg/mL), *C. longa* L. extract (10-50 μg/mL), or standard solution of ascorbic acid (0.4-1 μg/mL) was mixed with 0.5 mL of 0.05 M DPPH in methanol and incubated at room temperature for 30 min in the dark. After the incubation, the absorbance at 517 nm was measured against the blank with a spectrophotometer. The scavenging activity (%) was calculated with the following equation: [(absorbance of control-absorbance of sample)/absorbance of control]×100. Results are expressed as RC_{50} value (μg extract/mL), the effective concentration of the sample when scavenging activity was 50%. RC_{50} value was calculated from a linear regression analysis.

Reducing Power

The reducing power was determined using the method of Oyaizu [20]. Briefly, various concentrations of 2.5 mL of each extract sample and standard solution of ascorbic acid were mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After the addition of 2.5 mL 10% (w/v) trichloroacetic acid, the mixture was centrifuged at 1660×g for 10 min. The supernatant (2.5 mL) was mixed with the same volume of deionized water and 0.5 mL of 0.1% (w/v) ferric chloride. The absorbance at 700 nm was then measured against a blank with a spectrophotometer. Results are shown as EC_{50} value (μg extract/mL), the effective concentration of the sample when the absorbance was 0.5 for reducing power. EC_{50} value was calculated from a linear regression analysis.

Absorbance Spectrum and Sun Protection Factor (SPF) Determination

Absorbance spectra of lyophilized extract samples and EHM were measured using a spectrophotometer at a wavelength of 240 to 500 nm with 1 nm intervals. The lyophilized powder of pu'er tea extract or *C. longa* L. extract was diluted in ethanol to final concentrations of 0.003% and 0.0005% (w/w) for measurement. The final concentration of EHM was 0.003% (w/w).

The SPF value is an indicator of the photoprotective effect of sunscreen cosmetic formulations against UV-B rays. It was measured using the following equation (1) proposed by Mansour et al. [21].

$$SPF = CF \times \Sigma_{290}^{320} EE(\lambda) \times I(\lambda) \times ABS(\lambda) \quad (1)$$

where SPF is sun protection factor, $EE_{(\lambda)}$ is erythematogenic effect of wavelength radiation (λ), $I_{(\lambda)}$ is the intensity of sunlight at a wavelength (λ), $ABS_{(\lambda)}$ is the absorbance of the sample at the wavelength (λ); CF is the correction factor (=5) estimated from the sample containing 8% homomenthyl salicylate corresponding to SPF value of 4 by Mota et al. [11]. EE × I values are constant numbers. They were measured previously by Agin et al. [22].

The formulation was based on the Brazilian Pharmacopoeia National Form [23]. However, additional emollients and adjuvants were taken out to avoid interaction with the results. Control samples named C1 and C2 corresponded to the base cream formulation only and base cream supplemented with an EHM, respectively. Experimental samples named S1 to S5 corresponded to the base cream supplemented only with pu'er tea extract or EHM along with four different concentrations of pu'er tea. Experimental samples named S6 to S7 corresponded to the base cream supplemented only with *C. longa* L. extract or with EHM and *C. longa* L. extract. Sample S8 was the base cream supplemented with EHM, pu'er tea, and *C. longa* L. extract.

The cosmetic samples to determine absorbance measurements were made by following methods. Components of Phase 1, disodium EDTA and Purified water, were added together to a 100 mL beaker and heated to 75 °C. Components of Phase 2, self-emulsifying wax, were added to a 100 mL beaker and heated to 80 °C. When phase 1 and 2 were heated, they were mixed under mechanical stirring. Components of Phase 3 for each cosmetic formulation were added to the mixture of phase 1 and 2 after the temperature was 55 °C under mechanical stirring. Absorbances of each cosmetic formulation sample described in Table 1 were determined at 290 to 320 nm with 5 nm intervals to estimate SPF values. The cosmetic samples made were diluted in ethanol to a final concentration of 0.20 mg/mL before absorbance measurements.

Critical Wavelength Determination

Critical wavelength was defined as a wavelength where the area under the curve of absorbance spectrum was 90% of the integral of the spectrum from 290 to 400 nm. The U.S Food and Drug Ministration has announced the over-the-counter sunscreen labeled as broad-spectrum sunblock should have a critical wavelength of 370 nm or greater than that [24]. The critical wavelength of cosmetic formulation sample containing EHM with or without mixture of pu'er tea or *C. longa* L. extract was determined and calculated with a SPF-290s analyzer (Laser Components, Gothenburg, Sweden). Results from triplicate experiments were obtained and calculated with an intrinsic software, Win SPF 4 (Laser Components, Gothenburg, Sweden), of the SPF-290s analyzer.

Cell Culture

Human fibroblast skin cells (CCD-986sk) were purchased from

Korean Cell Line Bank, Seoul. These cells at passage numbers of 13 to 16 were used in this study. Dulbecco's modified eagle medium (DMEM) used for cell culture was supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (100 U/mL). These cells were incubated in a 5% CO_2 incubator at 37 ℃.

Cytotoxicity Test

Fibroblasts were seeded into a 96-well plate at a density of 5,000 cells/well and treated with different concentrations (0, 100, 150, and 200 μg/mL) of *C. longa* L extract. These cells were then incubated in a 5% CO_2 incubator at 37 ℃ for 24 h. The viability of cells was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) tetrazolium assay.

Blue Light Irradiation

Fibroblasts were cultured in a 96-well plate (5,000 cells/well) in DMEM medium supplemented with 10% FBS and 1% penicillin/streptomycin (100 U/mL) and incubated at 37 ℃ for 24 h. After incubation, cells were irradiated at a total dose of 150 J/cm^2 with an LED model TGC-2835B (Shenzhen avatar optoelectronic technology, Shanghai, China) that could emit 420 nm blue light with a spectral width of 405-435 nm and has a 692 mW/cm^2 irradiance. Following the irradiation, the medium was replaced with a fresh culture medium with or without *C. longa* L extract and incubated at 37 ℃ in a 5% CO_2 incubator for 24 h for further analysis.

MTT Assay

The MTT assay determines the cell viability of live cells by metabolic reduction of MTT to an insoluble, purple formazan product by the mitochondria of viable cells. This assay was performed to determine the viability of cells treated with or without *C. longa* L. extract according to the method described by Mosmann with slight modifications [25]. Following blue light irradiation and 24 h of incubation, the medium was removed and 100 μL of complete DMEM containing MTT was added to each well to a final concentration of 0.5 mg/mL. After incubation for 3 h in a cell culture incubator, complete DMEM was removed and 100 μL of dimethyl sulfoxide was added to each well. The microplate was then placed in a microplate shaker model 2D 300 (Finepcr, Seoul, Korea) at 100 rpm for 10 min. The absorbance of each well was measured at 570 nm using an AMR 100 microplate reader (Hangzhou Allsheng Instruments, Hangzhou, China). The assay was carried out in triplicates.

Statistical Analysis

All assays were carried out in triplicates. Results are shown as mean ± standard deviation. One-way analysis of variance followed by Tukey's post hoc test was performed for multiple comparisons. All analyses were performed with GraphPad Prism 5 software (GraphPad Software, San Diego, CA, USA).

Results and Discussion

Total Phenolic and Flavonoid Contents

Pu'er tea is known to contain polyphenols and flavonoids such as gallic acid and quercetin [25]. Such compounds possess antioxidative and photoprotective functions [26,27]. *C. longa* L. also contain polyphenols such as curcumins. These polyphenols are known to have photoprotective effects [28,29]. The pu'er tea extract used in this study showed total phenolic content and total flavonoid content of 100.93±0.974 mg GAE/g and 8.48±0.037 mg QE/g, respectively (Table 2). The *C. longa* L. extract used in this study showed total phenolic content and total flavonoid content of 23.71±0.307 mg GAE/g and 4.89±0.070 mg QE/g, respectively.

Antioxidant Ability

It is known that oxidative stress-induced from UV rays contribute to photoaging besides direct effects of UV rays on DNA molecules with subsequent structural changes [30]. Thus, extracts used in this study were tested for their antioxidant abilities. Although sunscreen products can provide significant protection from UV light, some UV light can still reach the skin and cause oxidative damage. This can also occur for blue lights as they can damage the skin by generating superoxides [7,31]. This indicates that sunscreen products containing antioxidant agents can protect skin from damages due to photo-induced oxidative stresses by UV and blue lights. RC_{50} values of pu'er tea, *C. longa* L., and ascorbic acid were found to be 4.70±0.30, 40.12±3.07, and 0.66±0.01 μg/mL, respectively (Table 3). EC_{50} values of pu'er tea, *C. longa* L., and ascorbic acid were 323.48±2.04, 3087.67±44.78, and 51.19±0.19 μg/mL, respectively. Ascorbic acid, a powerful antioxidant,

Table 2 Total phenolic and flavonoid contents of pu'er tea and *Curcuma longa* L. extracts

Metabolites	Estimated concentration	
	Pu'er tea	*Curcuma longa* L.
Phenolic Contents (mg GAE/g)[a]	100.93±0.974	23.71±0.307
Flavonoids (mg QE/g)[b]	8.48±0.037	4.89±0.070

[a]mg GAE/g, Gallic acid equivalents in milligrams per gram (mg GAE/g) of extract powder.
[b]mg QE/g, Quercetin equivalents in milligrams per gram (mg QE/g) of extract powder.
Results are shown as mean ± standard deviation (n =3)

Table 3 Antioxidant capacities of pu'er tea and *Curcuma longa* L extracts

	DPPH (RC_{50}[a] μg/mL)	Reducing Power (EC_{50}[b] μg/mL)
Pu'er tea	4.70±0.30	323.48±2.04
Curcuma longa L.	40.12±3.07	3087.67±44.78
Ascorbic acid	0.66±0.01	51.19±0.19

[a]RC_{50}, Concentration required for 50% reduction of 1,1-diphenyl-2-picrylhydrazyl (DPPH).
[b]EC_{50}, Concentration when the absorbance of sample at 700 nm was 0.5.
Results are shown as mean ± standard deviation (n =3)

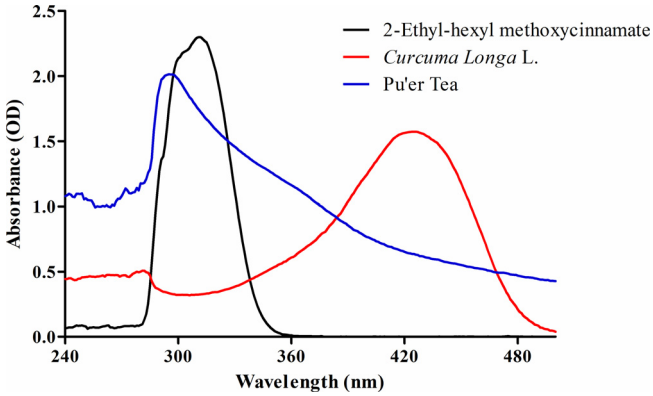

Fig. 1 UV absorption spectrum of 2-ethyl-hexyl methoxycinnamate, lyophilized extract of *Curcuma longa* L., and pu'er tea. Samples were diluted in ethanol before absorbance determination. Results were read at a concentration (w/w) of 0.003% except for *C. longa* L. (at 0.0005%)

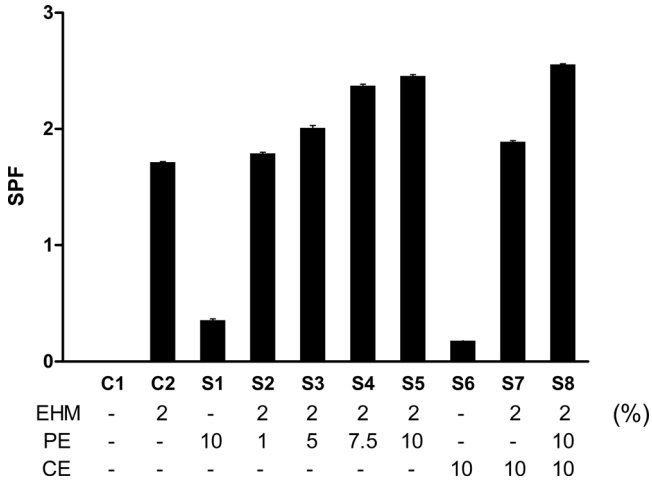

Fig. 2 Sun protection factor (SPF) values of control and test cosmetic samples determined with a spectrophotometer. C1 and C2 are base cream samples with (C1) and without (C2) 2% 2-ethyl-hexyl methoxycinnamate (EHM), respectively. S1 contains 10% pu'er tea extract (PE). S2 to S5 contain various concentrations (1, 5, 7.5, and 10%) of PE and 2% EHM. S6 is a sample with 10% *Curcuma longa* L. extract (CE). S7 contains 10% CE and 2% EHM. S8 sample contains 10% CE, PE, and 2% EHM

was used as a positive control [32]. Pu'er tea showed higher antioxidant capacity than *C. longa* L. in both assays. A previous study has reported that antioxidant capacities of natural plants can contribute to their photoprotective effects besides their direct absorption of UV rays [26]. Green tea extract is well known for its antioxidant capability. According to Oh et al., green tea powder has shown 15.5% of antioxidant capacity of ascorbic acid in DPPH assay [33,34]. Pu'er tea extract from this study showed 14% and 16% of DPPH radical scavenging activity and reducing power of antioxidant capacity of ascorbic acid. This indicates that pu'er tea has an antioxidant ability corresponding to that of green tea. Such a high antioxidant capacity of pu'er tea extract can contribute to its photoprotective effect in a cosmetic formulation.

UV Absorption Spectrum and SPF Determination

The absorption spectra of EHM, pu'er tea, and *C. longa* L. extract is shown in Fig. 1. The EHM had the highest absorption at the UV-B region (310 nm). However, it did not show any absorption property in the UV-A range. Likewise, pu'er tea extract also showed a peak at the UV-B region (305 nm). The absorption spectrum of *C. longa* L. extract showed an upslope graph uprising from the wavelength of 320 nm with the highest absorption at 420 nm. A previous study has revealed that blue light (420 nm) irradiation can cause intracellular oxidative stress and toxic effects on human dermal fibroblasts [35]. Despite the low absorption at the UV-B region of *C. longa* L. extract, it is noteworthy that it might protect skin from blue light-induced damage.

SPF values of cosmetic formulations are shown in Fig. 2. The *C. longa* L. extract did not show a significant synergistic effect with EHM. The C2 sample (2% EHM) showed a SPF value of 1.71. The SPF value of S1 (10% pu'er tea extract) was 0.36. When the sample containing 2% EHM was supplemented with 10% pu'er tea extract, the SPF value was increased by about 46% to 2.49. FDA recommends all sunscreen products should have a SPF value of 2 or higher [36]. The S1 sample by itself does not meet the standard of FDA, although it has the potential to increase the SPF value of EHM by about 46% which is remarkable. Although the synergistic effect of an EHM based sunscreen can be obtained up to 59% with other synthetic sunscreen agents such as octocrylene and 2-ethyl-hexyl salicylate [11], there are benefits of using natural sunscreen agents over synthetic ones such as soothing effect, relief of oxidative stress, and anti-inflammation effects [4,5]. Thus, pu'er tea extract could be a potential natural sunscreen agent for boosting SPF value of the EHM while providing other properties to the cosmetic product that natural plant extracts have.

Critical Wavelength

Critical wavelengths of the control and mixture samples were 349.73±2.67 nm and 386.03±0.05 nm, respectively (Table 4). The co-treatment of pu'er tea and *C. longa* L. extract at the same time to the sample supplemented with an EHM made the critical wavelength shift to greater than 370 nm. According to the FDA document, when the critical wavelength of a sunscreen product is greater than or equal to 370 nm, the product can be labeled as a 'broad-spectrum', meaning that it gives protection from a broad range of UV rays including UV-B and UV-A [24]. The EHM only provides protection from UV-B rays, but not from UV-A rays that account for 95% of solar UV rays reaching the earth [37]. However, with the addition of pu'er tea and *C. longa* L. extracts, the mixture could protect from both UV rays. This suggests that pu'er tea and *C. longa* L. extracts can give an ability to protect against UV-A rays to synthetic or natural sunscreen agents that

Table 4 Critical wavelengths[a] of cosmetic samples with or without extracts of pu'er tea and *Curcuma longa* L. and their compositions

Components % (w/w)	Control sample	Mixture sample
Purified Water	82.9	62.9
Disodium EDTA	0.1	0.1
Self-emulsifying wax	15	15
2-Ethyl-hexyl-methoxycinnamate	2	2
Pu'er tea extract	-	10
Curcuma longa L. extract	-	10
Critical Wavelength (nm)	349.73±2.67	386.03±0.05

[a]Critical wavelength, a wavelength where the area under the curve of absorbance spectrum is 90% of the integral of the spectrum from 290 to 400 nm. Results are shown as mean ± standard deviation (n =3). FDA has announced that a sun cream product with a critical wavelength of 370 or greater can protect skin from both UV-B and UV-A

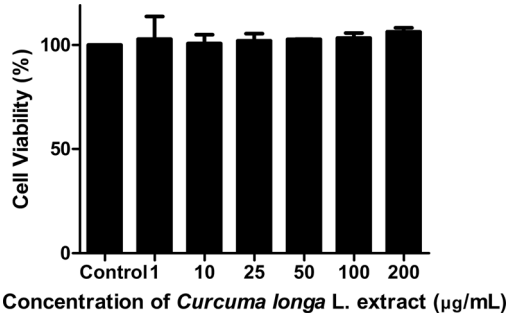

Fig. 3 Cytotoxicity of *Curcuma longa* L. extract. Human fibroblasts were treated with or without different concentrations of C. *longa* L extract. Results are shown as mean ± standard deviation (n =3)

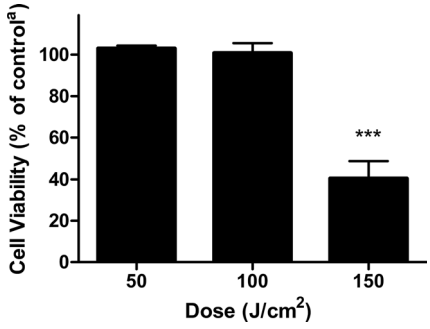

Fig. 4 Cytotoxicity of different doses of blue light to CCD986-sk cells. Human fibroblasts were irradiated with three different doses of blue light to determine their cytotoxicities. Asterisk (*) represents a statistically significant difference compared with the sample only treated with blue light (no extract) (***p<0.001). [a]Human fibroblasts without a treatment of blue light were used as a control

only protect skin from UV-B rays.

Protection Effect of *Curcuma longa* L. Extracts against Blue Light

The SPF value and critical wavelength can indicate the level of protective effect of a sunscreen agent from UV-A and UV-B rays, but not from blue light. Therefore, cell viability test was conducted using human dermal fibroblasts to see the protective effect of *C. longa* L. extract against blue light. The concentration of *C. longa* L. extract used in this experiment was below 200 µg/mL as such concentration did not show cytotoxicity (Fig. 3). The blue light dose of 150 J/cm^2 was selected as it significantly decreased cell viability (Fig. 4). Results showed that *C. longa* L. extract at a concentration of 200 µg/mL enhanced the viability of human dermal fibroblasts by 38% compared to blue light-irradiated cells without treatment of extract (Fig. 5). Curcuminoids are polyphenol compounds in *C. longa* L. They have been found to have protective effects against blue light-induced damage in human retinal pigment epithelial cells [15]. Moreover, a previous study has revealed that *C. longa* L. has polyphenols that can reduce oxidative stresses in adipose cells [14]. Avola et al. have also found that blue light-induced damage can be reduced by hydroxytyrosol, a strong antioxidant compound [39]. Taken together, these findings suggest that polyphenols and curcuminoids

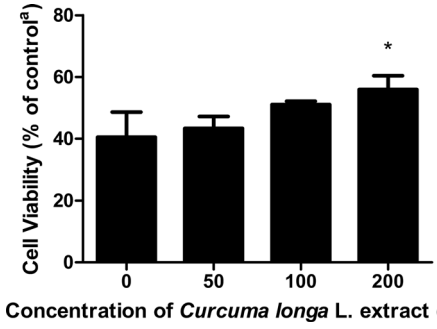

Fig. 5 Protection effect of *Curcuma longa* L. extract on human fibroblast cells against blue light (420 nm, 150 J/cm^2). All cells were seeded at a density of 5×10^3 cells per well in a 96-well plate. Results are shown as mean ± standard deviation (n =3). Asterisk (*) represents a statistically significant difference compared with the sample only treated with blue light (no extract) (*p<0.05). [a]Human fibroblasts without a treatment of blue light or *C. longa* L. extract were used as a control

of *C. longa* L. might play a significant role in protecting human dermal fibroblasts from blue light-induced damage. They also suggest that *C. longa* L. extract can potentially give a protective effect to a sunscreen product from blue light.

Our results showed that pu'er tea and *C. longa* L extracts contributed to the photoprotection of sunscreen products against UV and blue lights. This gives new options to those who seek alternatives or synergistic agents to reduce the use of synthetic photo-protectors. Especially, the ability of *C. longa* L. extract to reduce blue light-induced damage is noteworthy now that blue light is gaining much attention due to its potential threat to human skin.

In conclusion, Pu'er tea extract showed an UV absorbance spectrum in UV-B rays while *C. longa* L. showed an absorbance spectrum in UV-A rays and blue light range. The addition of both extracts to an EHM in a cosmetic formulation resulted in an increase of SPF value by 46%. It also gave the mixture a broad-spectrum absorption property as the critical wavelength shifted from 349.7 to 386.0 nm. Pu'er tea showed a high antioxidant capability in DPPH and reducing power assay. Such property of pu'er tea indicates that it can protect skin from UV and blue light-induced oxidative stress when it is used in a cosmetic formulation. On the other hand, *C. longa* L. extract may protect human skins from blue light as it has an absorption peak at 420 nm and that it can increase the viability of human fibroblasts after blue light irradiation. Further research is needed to identify chemical compounds of both extracts and to understand if the same improvement or photoprotective effect can be observed *in vivo*. Also, synergistic effects of pu'er tea and *C. longa* L. extracts with other synthetic or natural sunscreen compounds need to be tested.

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