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A Preliminary Screening Study with Dermal Tea Formulations Against 311 nm Ultraviolet B Radiation

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Synopsis

One potential health benefit of *Camellia sinensis* extracts on the skin is protection from the detrimental effects of UV radiation. Tea polyphenols both absorb the UV and also alleviate the UV-induced damage in human skin. In this study, we aimed to test the protection of black and green tea gels against UV. The gels were prepared using a carbomer resin and freeze-dried black or green tea extracts. In formulations, total phenolic content, antioxidant activity, and free radical scavenging activity were tested. The sites were irradiated with an artificial narrow band UVB source 30 min after topical application of the formulations on separate regions of the upper back of 21 subjects, Black and green tea gels, and the commercial sunscreen protected the skin of volunteers against the UV erythema. Caffeine gel and carbopol control gels did not provide any protection. Commercial sunscreens can only protect the skin by absorbing or scattering UV radiation. However, tea extracts both absorb the UV radiation and also have the potential to repair the UV damage inside the skin due to their strong antioxidant contents. Tea extracts are safe for humans as well as for aqueous environments without toxicological concerns.

INTRODUCTION

The tea plant, *Camellia sinensis*, contains polyphenolic compounds. The majority of health benefits related to this plant are due to the effects of these polyphenols (1). Tea polyphenols, also called catechins, have been reported to regulate inflammatory responses. Studies display tea polyphenols when applied orally or topically, improve destructive skin reactions following ultraviolet (UV) exposure, including erythema and lipid peroxidation (2). Polyphenols are effective radical oxygen species (ROS) scavengers (3) and they are more potent antioxidants than ascorbic acid and α-tocopherol (4). Besides polyphenols, the tea plant also contains caffeine, l-theanine, minerals, trace amounts of vitamins, amino acids, and carbohydrates. The description of polyphenols contained in tea is determined by the level of fermentation to which it has been subjected (5). White tea consists of minimally processed young leaves and green tea minimally processed mature leaves. While oolong tea is semifermented, black tea is completely fermented. Throughout the

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world, approximately 78% of the tea production is black tea and 20% is green tea, while oolong tea and white tea comprise about 2% of total output (6). Green tea polyphenols generally attribute approximately 35% of the dry solids in brewed form. Epicatechin, epicatechin-3-gallate, epigallocatechin (EGC), and epigallocatechin-3-gallate (EGCG) are the four major classes of green tea polyphenols. The most plentiful among these polyphenols is EGCG with a ratio of about 65% (7). It has been widely reported that the main active ingredient in green tea, EGCG, has a well-established performance as an anti-inflammatory and antioxidant (2). It was found to reduce UV-induced DNA damage dose-dependently in fibroblasts and keratinocytes (8). During the fermentation of black tea, from the smaller catechins, polyphenoloxidase elicits the formation of larger molecules such as orange-red colored dimeric theaflavins (TFs), and dark–brown polymeric thearubigins (TRs) (9,10). The major change was reported to be the oxidation and condensation of EGC and EGCG. Thus, the main difference between green tea and black tea solutions, in terms of polyphenols, is the higher concentration of condensed gallocatechin in black tea (9). The black tea extract inhibited UV radiation-induced tyrosine phosphorylation in the mouse skin and also prevented erythema in human skin after UV exposure (11). Although the great majority of studies on the antioxidative and photoprotective effects of tea and its polyphenols are performed with green tea, it has been shown that TFs and TRs in black tea, like green tea catechins, are also effective in the inhibition of lipid peroxidation. Antioxidative activities of black tea and green tea are reported to have similar potencies (12).

Previously, we reported that black or green tea extracts protected the skin from direct UV exposure preventing sunburn and erythema on a small group of subjects (13,14). In this trial, we studied with a larger volunteer population and also included an SPF 50 commercial sunscreen along with green and black tea treatment gels.

MATERIALS AND METHODS

PREPARATION OF TEA EXTRACT

The water-soluble fraction of tea was used in this study and 10 g of black and green dry tea leaves (Çaykur, Rize, Turkey) were weighed. In a separate glass vessel, 100 g of deionized water was boiled. Tea samples were added to water, mixed for 20 min with a mechanical stirrer, and cooled to room temperature. Extracts were filtered using filter paper under a vacuum. The infusions were frozen at −18°C before further processing. Using a laboratory freeze-dryer (Alpha 1-2 LD Plus, Martin Christ, Germany) frozen tea samples were lyophilized at −52°C/0.1 mBar. Freeze-dried samples were stored at −18°C for further quality control tests.

PREPARATION OF TEA GELS

Gels were obtained using a carbomer resin (Carbopol Ultrez 21, Noveon, USA). 100 mL of 0.75% (w/w) carbomer solution were prepared and 3 g freeze-dried black or green tea extract was added to the carbomer dispersion. By monitoring the pH, 18% sodium hydroxide solution was added drop by drop until a viscous gel was obtained at pH 5.5. Acting as a preservative, 1% benzyl alcohol was used. The gels were stored in glass jars at

Table I
The Contents of the Gels

g/100g	Black tea gel	Green tea gel	Caffeine gel	Vehicle gel
Black tea extract	3			
Green tea extract		3		
Caffeine			0.3	
Carbomer	1	1	1	1
Sodium hydroxide	qs	qs	qs	qs
Benzyl alcohol	1	1	1	1
Pure water	qs	qs	qs	qs

qs: quantum sufficit.

room temperature. A 0.3% caffeine gel (the amount of caffeine in tea extracts) and a gel-base were also prepared as described above. The contents of the gels were given in Table I.

PHYTOCHEMICAL ANALYSIS

Gels were weighed as 1 g and diluted to 10 mL in a beaker with distilled water. All solutions were filtered through a 45 μm filter before high-performance liquid chromatography (HPLC) analysis. The analytical procedure was adapted from the literature (15) with slight changes. The system was Perkin Elmer Series 200 and the column was C18 reversed-phase 5 μm (250 × 4.6 mm). System conditions were as follows: Mobile Phase A: 0.15% hydrochloric acid in water (v/v); Mobile Phase B: 0.15% hydrochloric acid in acetonitrile/water (v/v); Flow, 1 mL/min; Injection Volume, 10 μL; Column Temperature, 25°C. Detection was accomplished with a diode array detector and chromatograms were recorded at 280 nm.

TOTAL PHENOLIC CONTENT ANALYSIS

The total phenolic content of the extracts was determined using the Folin-Ciocalteu reagent (Sigma Aldrich, USA). The reagent was diluted at a volume ratio of 1:3 with 96% EtOH before use. Trolox (Sigma Aldrich, USA) was used as the standard. For this analysis, a calibration curve was made with 7 different concentrations of standard Trolox solution between 0.05–2.0 mM. The results were calculated using the regression equation of the obtained curve and defined as mg of Trolox equivalent, 1 g of tea gel was diluted to 10 mL with distilled water. 1 mL of this solution was mixed with 1 mL diluted Folin-Ciocalteu reagent, and 2 mL 35% sodium carbonate was added to the latter solution which was later diluted to 6 mL with distilled water. The final solution was incubated for 30 min at room temperature and the absorbance was measured at 700 nm.

ANTIOXIDANT ACTIVITY ANALYSIS

The cupric ion-reducing antioxidant capacity of samples was determined using an assay previously described (16). Briefly, 1 g of gel sample was diluted to 10 mL with distilled water. Subsequently 0.2 mL of 10 mM $CuCl_2$, 0.2 mL of 7.5 mM neocuproine, and 0.2 mL of 1 M ammonium acetate were added into a test tube. After vortex mixing, a 100 μL sample, and 120 μL ultrapure water was added, and the absorbance at 450 nm was read

30 min later. Trolox equivalent antioxidant capacity was calculated based on a calibration curve obtained by the serial dilution of 1 mM Trolox.

FREE RADICAL SCAVENGING ACTIVITY ANALYSIS

The free radical scavenging activity of samples was measured with 1,1-diphenyl-2 picrylhydrazil using an assay described formerly (17). Briefly, 1,1-diphenyl-2 picrylhydrazil was dissolved in ethanol (4 mg/100 mL) and 100 μL of this solution was added to an equal volume of a sample. The mixture was shaken vigorously and the decrease in absorbance was measured at 515 nm after 30 min. Water was used as a control. The percent inhibition activity was calculated using the following equation: Inhibition activity (%) = $[(A_0 - A_1)/A_0 \times 100]$, where A_0 and A_1 are the absorbances of the control and sample, respectively.

CLINICAL STUDY

SUBJECTS

The group consisted of 21 caucasian subjects, 15 females and 6 males, 20–48 years old, with no known disease enrolled in the trial with written informed consent. The subjects were chosen under the control of a dermatologist based on the inclusion/exclusion criteria: The subjects were not using any topical or systemic medications, they had all healthy dermatologic appearance, they had no photosensitivity history, and none of them were pregnant or lactating. All of the trial procedures were performed in line with the ethical principles laid down for medical research (Helsinki Declaration of World Medical Association, 1964, and amendments). The study protocol was approved by the local ethics committee. After grading the skin types, the minimal erythema dose (MED) of each subject was determined on the upper back skin with the artificial narrow band UVB source (Gigatest UVB-311, Germany) and its emission spectrum is shown in Figure 1 (18). This handheld MED tester has five test fields, each with a diameter of 15 mM which emits different doses of UVB. The MED was determined as the lowest UVB dose that caused just perceptible erythema at 24 h.

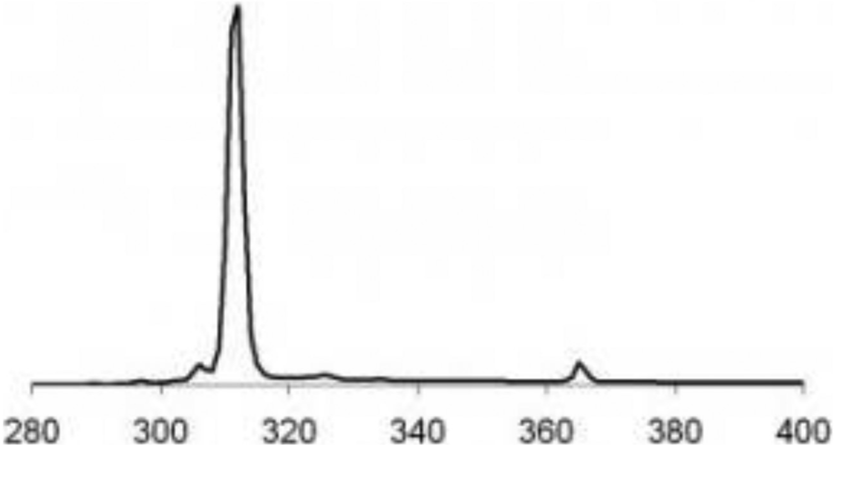

Figure 1. The emission spectrum of the lamp (UVB) (18).

APPLICATION OF FORMULAS

Six separate regions 2 × 2 cm in size at least 1 cm apart were marked on the dorsal trunk. Green tea gel, black tea gel, caffeine gel, vehicle gel, and an SPF 50 commercial sunscreen were applied to 5 areas at an amount of 2 mg/cm^2 whereas one area was kept blank as control. The area of each topical application was randomly chosen on each subject. After 30 min of gel application, each of the six areas was irradiated with narrow-band UVB at a 2MED dose. Irradiation was performed by a dermatologist using the MED tester and another dermatologist evaluated the degree of reaction at 24, 48, and 72 h. In addition, volunteers were asked to apply tea gels to areas with erythema twice daily: the black tea gel to the control area and the green tea gel to the vehicle applied area.

ASSESSMENT CRITERIA

Skin erythema was assessed via clinical evaluation based on visual scoring of the reactions according to a standardized procedure in which severity of the reaction increases from 0 (no reaction) to 9 (severe reaction). The positive reactions were graded beginning from 1 as the mildest (very light, patchy pink hue) to 9 as the most severe (dark red color with vesicles or edema).

TRANSPORE® TEST

The efficacy of sunscreen filtering agent formulations *in vitro* was evaluated in a variety of ways. Transpore® (Pennsylvania, United States) test commonly used is one of the methods (19). Transpore® testing was performed after *in vivo* testing to support human studies and to give an idea about the absorption spectrum of different gels and the potential use of gels as sunscreens. In our previous studies, the gel formulation of Kenyan black and Indonesian green teas (Lipton, Unilever, Turkey), which had high UV absorbance and antioxidant capacity, was prepared at a concentration of 3%. New formulations were prepared by increasing the concentration of black and green tea used in the study to 5%. The concentration of the caffeine gel was also increased to 2%. The Transpore® tape was adhered to the quartz cuvette, 4 mg of gel was applied, and then dried for 15 min. The analysis was performed by taking the spectrum with a UV spectrophometer (Shimadzu UV Mini) aganist the reference with Transpore tape attached. The procedure was repeated 3 times for each sample.

STATISTICAL ANALYSES

A one-way analysis of variance was used to test the differences among groups. For multiple comparisons, Tukey's test was performed.

RESULTS

The results of the HPLC analysis were given in Table II. In general, catechins quantities were comparable in both tea gels. But, unlike green tea, black tea gel contained TFs as the result of condensation of smaller catechins due to the fermentation process. Table III shows the results of total phenolic content, antioxidant activity, and free radical scavenging activity.

Table II
The Quantities of Catechins in Tea Gels

Catechins	GA (mM)	EGCG (mM)	CAF (mM)	EC (mM)	EGC (mM)	ECG (mM)	TF (mg/mL)
Black tea gel	0.0060	0.8900	0.0005	0.1900	0.3000	0.0600	0.1500
Green tea gel	0.0020	0.8700	0.0005	0.1700	0.3000	0.0500	0.0000

GA: *Gallic acid*, **EGCG**: *Epigallocatechingallat*, **CAF**: *Caffeine*, **EC**: *Epicatechin*, **EGC**: *Epigallocatechin*, **ECG**: *Epicatechingallat,* **TF**: *Theaflavin.*

Mean ± standard deviation (SD) age of subjects was 34.4 ± (8.1). Out of 21, Subjects 12, 5, and 4 had Fitzpatrick skin types II, III, and IV, respectively. Reactions revealed by a male subject at 24 h were given in Figure 2. In the photo, from 1 to 6, the applied formulations were: Caffeine gel, vehicle gel, control (no formulation), black tea gel, green tea gel, and sunscreen respectively. In Table IV, the mean and SD of erythema values for each formula on each day were given. The graphic presentation of decreasing erythema severity for each formula on each day was shown in Figure 3. The F ratio was significant (<0.0001) for the formulas in a one-way analysis of variance test.

Table III
Total Phenolic Contents, Antioxidant Activities, and Free Radical Scavenging Activities of Tea Gels

	Total phenolic content (mM TR/mg)	Antioxidant activity (mM TR/mg)	Free radical scavenging activity (inhibition %)
Black tea gel*	4.9800 ± 0.8760	5.0480 ± 0.5450	49.96 ± 3.87
Green tea gel*	4.2700 ± 0.7650	8.5680 ± 1.7650	51.02 ± 0.54

*mean ± SD.

Figure 2. Reactions revealed by a male subject at 24 h.

Table IV
The Erythema Grades Obtained Based on Formulas

	Day	Black tea gel	Green tea gel	Caffeine gel	Vehicle gel	Control	SPF 50 sunscreen
Mean ± SD	1	**1.81 ± 1.5**	**1.76 ± 1.6**	**5.62 ± 2.0**	**6.57 ± 1.6**	**6.62 ± 1.8**	**0.10 ± 0.30**
	2	1.29 ± 1.3	1.43 ± 1.6	5.48 ± 2.0	6.48 ± 1.7	6.52 ± 1.6	0.00 ± 0.00
	3	0.67 ± 1.1	1.05 ± 1.6	4.10 ± 2.5	4.86 ± 2.3	5.05 ± 2.0	0.00 ± 0.00

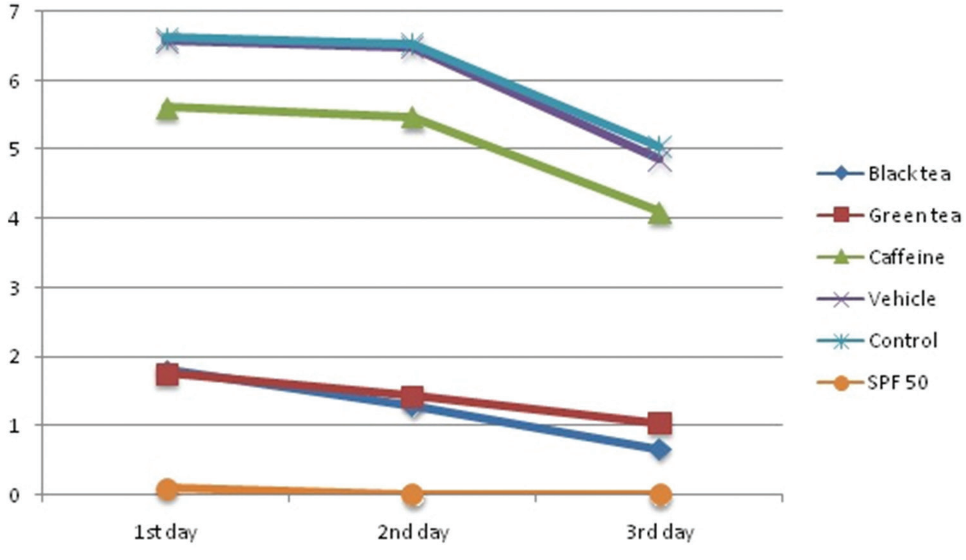

Figure 3. The change in erythema severity for each formula by time.

The clinical study results observed 3 distinct groups: (1) The commercial SPF 50 sunscreen showed the highest protection; (2) black tea and green tea gels were effective in protecting against UV erythema; and (3) the vehicle gel, control gel, and caffeine gel (which had no UV protection). The p values obtained after multiple comparison post hoc tests were presented in Table V. In terms of UV protection, there was no statistical difference between black tea and green tea gels. No erythema reducing activity was found in control and vehicle gel areas after the exposure to UV and no erythema reducing activity was found after the exposure to UV. The UV absorbance of all gels was examined with a Transpore® test. The results are given in Figure 4.

DISCUSSION

In our previous studies, we reported the erythema prevention effect of tea extracts due to UV exposure of the skin (13,14). In this study, with a larger group ($n = 21$) of volunteers, we demonstrated the UV protective effect of black or green tea gel formulations. We also recently reported the anabolic effect of green tea extract in the cell cultures of keratinocytes (20). The literature is rich with the health benefits of tea or tea extracts, especially on

Table V
The Multiple Comparisons for Formulas

	Day	Black tea	Green tea	Caffeine	Vehicle	Control	SPF 50
Black tea	1	NA					
	2						
	3						
Green tea	1	NS	NA				
	2	NS					
	3	NS					
Caffeine	1	<0.0001	<0.0001	NA			
	2	<0.0001	<0.0001				
	3	<0.0001	<0.0001				
Vehicle	1	<0.0001	<0.0001	NS	NA		
	2	<0.0001	<0.0001	NS			
	3	<0.0001	<0.0001	NS			
Control	1	<0.0001	<0.0001	NS	NS	NA	
	2	<0.0001	<0.0001	NS	NS		
	3	<0.0001	<0.0001	NS	NS		
SPF 50	1	=0.0083	=0.0114	<0.0001	<0.0001	<0.0001	NA
	2	NS	=0.0332	<0.0001	<0.0001	<0.0001	
	3	NS	NS	<0.0001	<0.0001	<0.0001	

NA: Nonapplicable; NS: Nonsignificant.

the skin. UV enhances skin damage mainly by increasing the production of ROS and by raising the levels of matrix metalloproteases that degrade the collagen and elastin fibers of the skin matrix. These reactions lead to wrinkle formation and reduced integrity of the skin, which are overall defined as photoaging (5). It is confirmed with numerous *in vitro* studies and animal models that topical treatment with green tea polyphenols reduce UV-induced oxidative stress and inflammatory response (5). It has been reported that the suggested positive effects of tea extracts were also determined with systemic treatment. The signs of UV-induced photoaging (e.g., wrinkle formation were decreased and collagen synthesis) were promoted in mice fed with green tea seed extracts (21). It can be suggested that the tea plant (*C sinensis*) has a wholistic influence on the skin consisting of anti-inflammatory, antioxidative, immunoregulatory, antiproliferative, DNA repairing, and anticancerogenic effects. Green tea extract was found to demonstrate anabolic effects on hyaluronic acid, collagen, and elastin by causing upregulation of hyaluronan synthase-2, and downregulations of matrix metalloproteinase-9 and elastase gene expressions (20). On histologic examination, skin treated with green tea extracts decreased the number of sunburn cells and protected epidermal Langerhans cells from the detrimental effects of UV (22). Tea components significantly delay the signs of skin aging through the inhibition of lipoxygenase, metalloproteinase, hyaluronidase, and collagenase (23). It is reasonable to claim that these results are mainly because of the plant's strong antioxidant activity associated with its phenolic and flavonoid ingredients (Tables II and III). In the light of the literature data and the results of this study, with routine use of the tea extracts in the form of cosmetic formulations daily, the concentration of tea actives will increase in the skin, and it can be speculated that flavonoids and polyphenols will protect the skin, both from the effects of acute UV damage by absorbing the incoming UV radiation. Additionally, this might contribute to repairing the chronic UV damage that causes collagen destruction and photoaging. This study indicates that both forms of tea, black or green, have almost similar

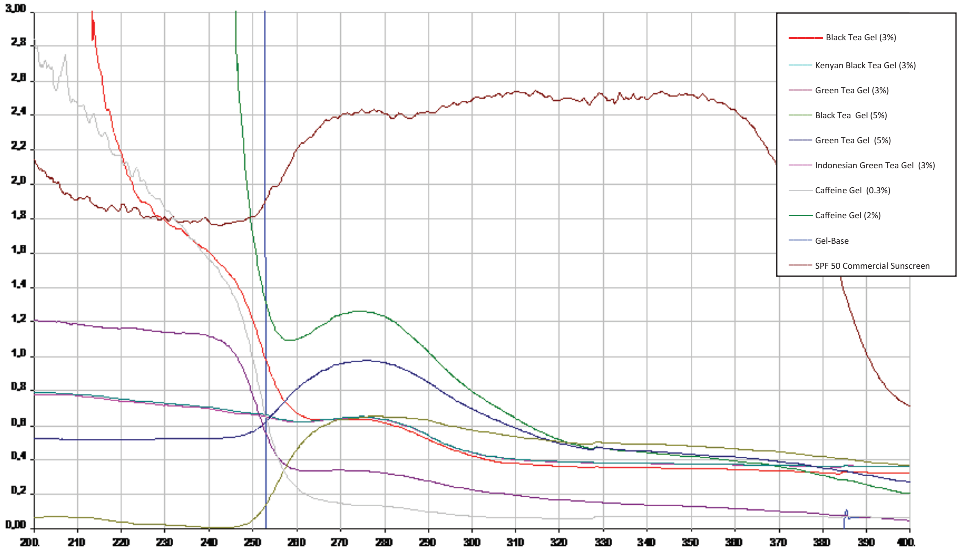

Figure 4. Transpore® test results. The axis represents the wavelength and the ordinate (each interval 0.2) represents the absorbance.

effects *in vitro* and *in vivo*. In addition to their comparable total phenolic content, antioxidant activity, and free radical scavenging activities (Table III), their resulting "erythema severity scores" in subjects after UV exposure were also similar (Figures 2 and 3, Tables IV and V). This could indicate that, without being selective, both forms of tea can be used in dermal formulations against UV.

The tea polyphenols may reduce the erythema intensity because they have anti-inflammatory properties (5); however, no erythema reducing activity was found after the exposure to UV. This may be due to insufficient time (only 2 d) or concentration (3%) to observe this activity.

Kenyan black, Indonesian green, black and green tea gels with the same concentration have a similar UV spectrum determined by the Transpore® test (Figure 4). The UV spectrum of SPF 50 commercial sunscreen was found to be very high compared to other gels. The UV spectrum of 2% caffeine gel and 5% tea gel was shown to be similar and higher. These results show us that caffeine also has UV protection potential. However, the amount of caffeine in tea is not enough for protection UV alone and the main UV protectors in tea are catechins. When the results of the Transpore® test are evaluated together with the results of the *in vivo* study, it has shown that formulations containing fewer physical filters and which can be prepared together with polyphenols for an effective formulation can be designed, since they show protection close to the commercial sunscreen.

CONCLUSION

Currently, commercial sunscreens can only protect the skin by absorbing or scattering UV radiation. We do not expect any damage repair inside the skin. On the contrary, skin toxicity is a major concern for commercial sunscreens. Whereas, tea extracts can absorb the UV radiation with their rich polyphenol content which is also strong chromophore molecules. Tea polyphenols are strong antioxidants, when inside the skin they will fight against oxidative stress. The safety of tea extracts for humans and the environment is also a big advantage.

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