Photochemistry and Photobiology, 2014, 90: 214–224

Protection from UVB Toxicity in Human Keratinocytes by Thailand Native Herbs Extracts

Visa Thongrakard[1,2], Nijsiri Ruangrungsi[3], Maneerat Ekkapongpisit[2], Ciro Isidoro*[2] and Tewin Tencomnao*[4]

[1]Ph.D. Program in Clinical Biochemistry and Molecular Medicine, Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand
[2]Laboratorio di Patologia Molecolare, Dipartimento di Scienze della Salute, Università del Piemonte Orientale, Novara, Italy
[3]College of Public Health Sciences, Chulalongkorn University, Bangkok, Thailand
[4]Center for Excellence in Omics-Nano Medical Technology Development Project, Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Bangkok, Thailand

Received 25 June 2013, accepted 23 July 2013, DOI: 10.1111/php.12153

ABSTRACT

Thai traditional medicine employs a wide range of indigenous herbs in the forms of tincture or tea for the cure of skin and systemic inflammatory diseases. The protection by Thai plants extracts against UVB DNA damage and cytotoxicity was investigated in human keratinocytes. Petroleum ether, dichloromethane and ethanol extracts were prepared from 15 Thai herb species, and the total phenolic and flavonoid contents, the antioxidant and UV-absorbing properties were assessed by standard procedures. Cytoprotective effects were evaluated on the basis of cell survival, caspase-3 activity and pyrimidine dimers determination. High total phenolic and flavonoid contents were found in the ethanol and dichloromethane fractions. Dichloromethane extract of turmeric was shown to possess the highest antioxidant activity. The maximum UV absorptions were found in the ethanol extract of turmeric and in the dichloromethane extract of ginger. These extracts stimulated the synthesis of Thioredoxin 1, an antioxidant protein, and could protect human HaCaT keratinocytes from UV-induced DNA damage and cytotoxicity. The present data support the utilization of turmeric and ginger extracts in anti-UV cosmetic pharmaceuticals.

INTRODUCTION

Exposure of the human skin to UV radiation induces many adverse effects such as DNA damage, gene mutation, oxidative stress, inflammation and immunosuppression. All of these events are mediated by free radicals, mainly reactive oxygen species (ROS), and cause many skin diseases including the development of skin cancer (1,2). Several studies suggest that plant polyphenols have the great potential to reduce adverse effects of UV-induced cellular damages (3–5). The followings are some examples. Luteolin, one of the most potent antioxidant plant polyphenols, was shown to possess UV absorption properties and to protect human keratinocytes from the deleterious effects of UV radiation (6). Glycyrrhizic acid, a triterpenoid saponin glycoside from the roots and rhizomes of licorice, protected human dermal fibroblasts from UVB irradiation-induced photoaging (5). Ginsenoids from traditional Chinese medicinal herbs were shown to attenuate the cell growth arrest of human fibroblasts induced by repeated subcytotoxic UVB exposure (4). Finally, the extract of *Calendula officinalis* was shown able to preserve the antioxidant system in UV-irradiated skin by preventing the UV-induced depletion of reduced glutathione (7). The potential benefits of plant components in preventing or attenuating UV-associated skin damages extend to their anti-inflammatory and antiaging activities. For instance, curcumin, a polyphenol enriched in *Curcuma longa* (commonly known as turmeric), effectively inhibited the production of NF-κB and of metalloproteases in human dermal fibroblasts exposed to UVB irradiation (8). Furthermore, the water extract of ginger (*Zingiber officinale*) rhizomes was shown to inhibit the UV-induced production of cytokines by HaCaT cells and to also attenuate inflammation in the skin of UV-irradiated mice (9).

In this study, we determined the total phenolic and total flavonoid contents and the antioxidant activity of the organic extracts of 15 species of Thai plants that are part of the traditional medicine pharmacopea. In addition, we examined the UV absorption properties and the potential of these extracts to protect from UV-induced DNA damage and cytotoxicity in cultured human keratinocytes. We found that the extracts of turmeric and of ginger (dichloromethane fraction) could absorb UVB and could protect HaCaT keratinocytes from DNA damage and apoptosis induced by a toxic dose of UVB irradiation. Of note, these extracts greatly increased the expression of Thioredoxin 1, a protein involved in the protection of mitochondria from oxidative stress. We propose that the preexposure to turmeric and ginger extracts activates several antioxidant defenses, including the thioredoxin system, which render the skin cells able to face the oxidative stress induced by UVB.

MATERIALS AND METHODS

Chemicals and reagents. Analytical grade reagents were used in this study. Folin–Ciocalteu's phenol reagent, 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS), potassium persulfate, quercetin and fetal bovine serum (FBS) were purchased from Sigma-Aldrich Co. (St Louis, MO, USA). Aluminum chloride ($AlCl_3$), 2,2-diphenyl-1-picrylhydrazyl (DPPH),

*Corresponding author emails: isidoro@med.unipmn.it (Ciro Isidoro); tewin.t@chula.ac.th (Tewin Tencomnao)

gallic acid, sodium carbonate (Na_2CO_3), sodium acetate ($NaC_2H_3O_2$), ascorbic acid (vitamin C), methanol, ethanol, petroleum ether, dichloromethane, dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) were purchased from Merck (Darmstadt, Germany). Dulbecco's modified Eagle medium/high glucose (DMEM/HG) and penicillin-streptomycin solution were purchased from Hyclone (Logan, UT). ApoTarget™ caspase-3 protease assay and trypan blue solution were purchased from Invitrogen (Carlsbad, USA). Cell Death Detection ELISA plus kit was purchased from Roche (Roche Diagnostic GmbH, Mannheim, Germany). OxiSelect™ Cellular UV-Induced DNA Damage ELISA Kit (cyclobutane pyrimidine dimers [CPD]) was purchased from Cell Biolabs (San Diego, USA).

Plant materials and extract preparation. Fifteen species of Thai plants in this study were collected from Bang pra subdistrict, Chonburi province, Thailand. They were authenticated by Professor Dr. Thaweesakdi Boonkerd and deposited at the Professor Dr. Kasin Suvatabhandhu Herbarium, Department of Botany, Faculty of Science, Chulalongkorn University, Thailand. The scientific names and parts used for this study are presented in Table 1 (note that, for simplicity, in the text the common name is used). All Thai plants were successively extracted using a Soxhlet extractor. Briefly, 100 g of fresh sample was extracted in organic solvents (1:4, w/v) using series of organic solvents with increasing polarities (petroleum ether, dichloromethane and ethanol) until exhaustion. The plant extracts were concentrated in a vacuum rotary evaporator under reduced pressure using the MiVac Quattro concentrator. Finally, the plant extracts were dissolved in DMSO in the concentration of 100 mg mL^{-1} as stocks, and stored with protection from light at −20°C.

Determination of phenolic and flavonoid contents. The total phenolic content of plant extracts was determined by the Folin–Ciocalteu method as described by Singelton *et al.* (10). Briefly, 50 μL of the extracts (1 mg mL^{-1}) or gallic acid (100–6.25 μg mL^{-1}) was added to 50 μL of 10% Folin–Ciocalteu's phenol reagent followed by 50 μL of Na_2CO_3 (10% w/v). After incubation at room temperature for 1 h in the dark, the absorbance of the reaction mixture was measured at a wavelength of 760 nm. Gallic acid was served as a standard. The results are expressed as mg gallic acid equivalent (GAE) per g fresh weight of sample. All samples were analyzed in triplicate.

Aluminum chloride colorimetric method was used to determine the total flavonoid content of plant extracts as described previously (11). Briefly, 50 μL of the extracts (1 mg mL^{-1}) or quercetin (200–1.56 μg mL^{-1}) was mixed with 150 μL of absolute ethanol and 10 μL of 1 M $NaC_2H_3O_2$. Thereafter, 10 μL of $AlCl_3$ solution (10% w/v) was added to mixture. The absorbance of the reaction mixture was measured immediately at a wavelength of 415 nm after incubation for 40 min in the dark at room temperature; quercetin was served as a standard. The results are expressed as mg quercetin equivalent (QE) per g fresh weight of sample.

Determination of antioxidant activities. The DPPH assay was performed according to the method of Brand-Williams *et al.* (12). To generate the stable free radical DPPH (DPPH•), DPPH was dissolved in absolute methanol. Fresh DPPH solution was prepared daily, and absorbance of the DPPH solution was adjusted to 0.650 ± 0.020 at 517 nm using absolute methanol. Thereafter, 20 μL of the extracts (1 mg mL^{-1}) or Ascorbic acid (serving as a standard) (25–1.56 μg mL^{-1}) was mixed with 180 μL of DPPH solution and incubated for 30 min in the dark at room temperature. The absorbance of the reaction mixture was measured at a wavelength of 517 nm. The results are expressed as mg ascorbic acid equivalent per g fresh weight of sample. The IC_{50} was determined in comparison with ascorbic acid. The % scavenging activity (% SC) was calculated using the following formula:

% SC = {[Abs. control − (Abs. sample − Abs. blank sample)]/Abs. control} * 100

Control contained 180 μL of DPPH solution and 20 μL of absolute methanol; whereas, blank sample contained 180 μL of absolute methanol and 20 μL of extracts.

The ABTS assay was performed according to the previously described method (13). The working solution or ABTS•+ solution was prepared by mixing 8 mL of 7.4 mM ABTS•+ and 12 mL of 2.45 mM potassium persulfate solution, and was subsequently incubated for 16–18 h at room temperature in the dark. After that, ABTS•+ solution was diluted by mixing with absolute ethanol to obtain an absorbance of 0.700 ± 0.020 at 734 nm. A quantity of 20 μL of the extracts (1 mg mL^{-1}) or ascorbic acid (serving as a standard) (50–1.56 μg mL^{-1}) was reacted with 180 μL of ABTS•+ solution for 45 min in the dark. The absorbance of the reaction mixture was measured spectrophotometrically at 734 nm. The results are expressed as mg ascorbic acid (vitamin C) equivalent per g fresh weight of sample. The IC_{50} was determined in comparison with ascorbic acid. The % SC was calculated using the following formula:

% SC = {[Abs. control − (Abs. sample − Abs. blank sample)]/Abs. control} * 100

Control contained 180 μL of ABTS•+ solution and 20 μL of absolute ethanol; whereas, blank sample contained 180 μL of absolute ethanol and 20 μL of extracts.

Determination of UV absorption. The UV absorption properties of extracts were investigated spectrophotometrically at wavelengths 315–400 nm for UVA and 280–315 nm for UVB. The extracts were measured using a quartz cuvette at a distance of 1 mm. DMSO (0.1% in PBS) was used as a blank solution for this assay. Plant extract samples were measured at the concentration of 100 μg mL^{-1} by dissolving the stocks in PBS.

UVB irradiation. A narrowband TL 20W/01 lamp (Philip, Holland), which emits at 305 and 315 nm with a peak emission at 311 nm, was used to irradiate the cells as described previously (14). A spectro-radiometer (Solarmeter) was used to monitor UVB dosage (mJ cm^{-2}) at a fixed distance.

Cells and treatments. Immortalized human epidermal keratinocyte HaCaT cell line was purchased from Cell line service (Heidelberg, Germany). The cells were cultured in DMEM/HG, supplemented with 10% FBS and antibiotics (100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin) at 37°C in a humidified atmosphere at 5% CO_2. The cells were seeded and let adhere on cell culture plate for 24 h prior to start the treatments. HaCaT cells were irradiated with UVB by two different experimental protocols. In the first protocol, the cells were pretreated with plant extracts for 30 min, 2, 8, 16 and 24 h, followed by exposition to UVB and further incubated for 24 and 48 h. In the second protocol, the cells were exposed to UVB followed by treatment of plant extracts for 24 h. At the end, cytotoxicity and DNA damage were determined.

Table 1. Details of common species of Thai plants.

Scientific name	Family	Common name	Part used	Herbarium number
Curcuma longa L.	Zingiberaceae	Turmeric	Rhizome	013396 (BCU)
Centella asiatica (L.) Urb.	Umbelliferae	Asiatic pennywort	Leaf	013400 (BCU)
Zingiber officinale Roscoe	Zingiberaceae	Ginger	Rhizome	013407 (BCU)
Coccinia grandis L.	Cucurbitaceae	Ivy gourd	Rhizome	013439 (BCU)
Allium ascalonicum L.	Alliaceae	Shallot	Whole body	013440 (BCU)
Ocimum basilicum L.	Labiatae (Lamiaceae)	Common basil	Leaf	013441 (BCU)
Moringa oleifera Lam.	Moringaceae	Horseradish tree	Fruit	013442 (BCU)
Morinda citrifolia L.	Rubiaceae	Indian mulberry	Leaf	013443 (BCU)
Mentha cordifolia Opiz ex Fresen	Labiatae (Lamiaceae)	Kitchen mint	Leaf	013445 (BCU)
Solanum torvum Sw.	Solanaceae	Common Asiatic weed	Fruit	013446 (BCU)
Citrus hystrix DC.	Rutaceae	Leech lime	Leaf	013447 (BCU)
Ocimum tenuiflorum L.	Labiatae (Lamiaceae)	Holy basil	Leaf	013448 (BCU)
Cucumis sativus L.	Cucurbitaceae	Common cucumber	Fruit	013449 (BCU)
Solanum aculeatissimum Jacq.	Solanaceae	Yellow-berried nightshade	Fruit	013450 (BCU)
Aloe vera (L.) Burm. f.	Asphodelaceae	Aloe	Gel	013451 (BCU)

MTT assay, cell counting and trypan blue dye exclusion assay. The cytotoxic effect of UVB and of plant extracts treatments was preliminary assessed by MTT assay. This assay is based on measuring the activity of the mitochondrial enzyme NADH-dependent succinate dehydrogenase that reduces MTT to the purple-colored formazan insoluble product in metabolically active cells. Briefly, at indicated time, MTT was added in each well (final concentration 0.5 mg mL^{-1}) and the cells were incubated at 37°C in 5% CO_2 incubator for 4 h, afterward the medium was removed and 200 μL of DMSO was added to each well to dissolve formazan. The absorbance was measured at a wavelength of 550 nm. The results were expressed as% cell survival, assuming as 100% the absorbance in control untreated cells. To discriminate viable cells and necrotic cells, the trypan blue dye exclusion test based on permeability of cell membrane was used. The cells were counted with a hemocytometer.

Detection of apoptotic cells. Apoptosis was estimated by photometric enzyme-immunoassay of mono- and oligonucleosomes with the Cell Death Detection ELISA plus kit, following manufacturer's instructions. In brief, the cells were plated in microplate and treated as described in the Results section. Necrotic cells in suspension were discharged. Apoptosis in the cell lysate was determined as histone-associated DNA fragments detected by specific monoclonal antibodies labeled with peroxidase and revealed with an appropriate chromogenic substrate. Absorbance was determined with a spectrophotometer. Negative and positive controls were included in the test. Cells unexposed to UVB are referred to as "sham."

Caspase-3 activity. The activity of caspase-3 was determined by the ApoTarget™ caspase-3 protease assay kit. The assay is based on the caspases-3 cleavage of the amino acid sequence DEVD (Asp-Glu-Val-Asp) which is labeled at its C-terminus with para-nitroaniline (pNA). The absorption of free pNA was quantified using a spectrophotometer at 405 nm.

Detection of CPDs. Assay of UVB-induced CPDs was performed by OxiSelect™ Cellular UV-Induced DNA Damage ELISA Kit according to the manufacturer's instructions. Briefly, after UVB irradiation, the cells were fixed and denatured, and the cell containing CPDs was probed with anti-CPD antibody. The absorbance was measured at a wavelength of 450 nm. Cells unexposed to UVB are referred to as "sham."

Western blotting analyses. HaCaT cells were plated in petri dishes and incubated with Thai herbs extracts as indicated, and thereafter cell homogenates were prepared by freeze thawing and ultrasonication in a buffer containing detergents and protease inhibitors as previously reported (15). A quantity of 30 μg of cell proteins was denatured with Laemmli sample buffer, separated by electrophoresis on a 12% SDS-containing polyacrylamide gel and then electroblotted onto PVDF membrane (Biorad, Hercules, CA, USA). The filter was first probed with the rabbit polyclonal antithioredoxin 1 (ab26320, Abcam, UK) antibody, and after stripping it was reprobed with the mouse monoclonal anti-β-tubulin (T 5293, Sigma, Missouri, USA) antibody. β-Tubulin was used as homogenate protein loading control in the lanes. Immunocomplexes were revealed using an appropriate peroxidase-conjugated secondary antibody (cod. 170-6515 and 170-6516, respectively; Biorad), and subsequent peroxidase-induced chemiluminescence reaction (cod. NEL105001EA; PerkinElmer, Waltham, MA, USA). Western blotting data were reproduced three times independently. Intensity of the bands was estimated by densitometry (Quantity one software, Biorad; ImageJ software).

Statistical analysis. All experiments were performed independently at least three times and in triplicates or quadruplicates as indicated. Data are expressed as the mean ± SE of the mean (SEM). The correlation coefficient (R^2) between antioxidant contents and antioxidant activities was determined using MS Excel 2007. The statistical probability for correlation coefficients was calculated using the software "Statistic calculators," version 3.0 BETA. When two means were compared, significance was determined using Student's *t*-test (statistical level of significance was set at $P < 0.05$).

RESULTS

Extraction yields and total phenolic and total flavonoid contents

We took advantage of the successive extraction method to use small quantity of plant materials and solvents and to also reduce organic wastes. The percentage yield of all Thai plants that were successively extracted with petroleum ether, dichloromethane and ethanol ranged from 0.04% to 3.28% (Table 2). In general, the highest percentage yields were obtained from ethanol fractions. Total phenolic and total flavonoid contents (shown in Table 3) varied depending on the plant type and solvents used. Total phenolic content in extracts ranged from 85.66 ± 2.75 to 3.23 ± 0.09 mg GAE per g fresh weight of sample using the standard curve of GAE (R^2 = 0.9993). In each solvent used for extraction, turmeric was shown to contain the highest level of total phenol, followed by ginger. The lowest content of phenol was found in aloe extract from the petroleum ether fraction. Total flavonoid content ranged from 1092.71 ± 117.49 to 0.75 ± 0.38 mg QE per g fresh weight of sample using the standard curve of quercetin (R^2 = 0.9992). No total flavonoid content was detected in extracts from horseradish tree (petroleum ether and dichloromethane fractions), common cucumber (dichloromethane and ethanol fractions), yellow-berried nightshade (petroleum ether fraction) and aloe (all fractions). The lowest, but detectable, total flavonoid content was found in common Asiatic weed extract from the petroleum ether fraction. Although the richest total phenolic and total flavonoid contents were found in extracts derived from dichloromethane fractions, extracts from ethanol fractions showed higher total phenolic and total flavonoid contents than dichloromethane fraction extracts in most plants analyzed in this current study. The highest level of total flavonoid content was detected in turmeric extract from dichloromethane fraction, followed by turmeric extract from petroleum ether fraction.

Antioxidant activities

Two different experimental approaches were employed for determination of antioxidant activities. DPPH assay is based on hydrogen donor property of antioxidants and is widely used in natural antioxidant studies because of its simplicity and sensitivity. ABTS assay has also been widely used to evaluate antioxidant activities due to its applicability in both aqueous and lipid phases (16,17). Results of the DPPH and ABTS assays are listed in Tables 4 and 5, respectively. In all antioxidant activity assays,

Table 2. Extraction yield (%) of common species of Thai plants extracted successively in petroleum ether, dichloromethane and ethanol.

	% Yield of plant extractions (w/w)		
Types of plant	Petroleum ether	Dichloromethane	Ethanol
Turmeric	0.37	0.50	1.24
Asiatic pennywort	0.34	0.45	1.65
Ginger	0.80	0.33	0.79
Ivy gourd	0.06	0.16	0.79
Shallot	0.62	0.15	1.22
Common basil	0.35	0.56	1.87
Horseradish tree	0.69	0.84	1.81
Indian mulberry	0.14	0.61	3.28
Kitchen mint	0.12	0.15	0.42
Common Asiatic weed	0.19	0.18	2.04
Leech lime	0.43	0.19	2.75
Holy basil	0.42	0.51	1.07
Common cucumber	0.05	0.08	1.05
Yellow-berried nightshade	0.04	0.12	0.70
Aloe	0.34	0.63	0.39

Table 3. Total phenolic and flavonoid contents of common species of Thai plant extracts derived from different solvents.

	Total phenolic content*			Total flavonoid content†		
Types of plant	Petroleum ether	Dichloromethane	Ethanol	Petroleum ether	Dichloromethane	Ethanol
Turmeric	37.19 ± 2.76	85.66 ± 2.75	54.94 ± 4.57	281.50 ± 27.75‡	1092.71 ± 117.49‡	91.06 ± 12.37‡
Asiatic pennywort	4.59 ± 1.11	5.57 ± 1.13	8.28 ± 2.45	6.97 ± 3.28	4.99 ± 1.50	16.38 ± 3.24
Ginger	18.56 ± 2.76	78.49 ± 3.90	27.63 ± 1.59	4.96 ± 0.30	2.67 ± 0.32	2.14 ± 0.84
Ivy gourd	7.86 ± 1.46	10.64 ± 1.68	13.63 ± 0.25	4.27 ± 0.84	17.74 ± 3.14	19.17 ± 2.38
Shallot	6.97 ± 1.21	17.02 ± 1.76	12.10 ± 2.38	3.55 ± 0.72	20.18 ± 1.61	7.81 ± 1.13
Common basil	3.61 ± 0.24	9.05 ± 1.22	21.21 ± 2.93	3.35 ± 0.85	8.03 ± 1.97	18.84 ± 2.10
Horseradish tree	4.86 ± 0.82	5.34 ± 1.23	10.55 ± 1.22	NA	NA	2.07 ± 0.95
Indian mulberry	8.10 ± 0.69	16.02 ± 0.90	9.80 ± 1.03	13.20 ± 3.09	31.29 ± 2.02	27.32 ± 3.11
Kitchen mint	5.64 ± 0.91	17.20 ± 1.76	25.03 ± 2.52	3.88 ± 1.52	14.01 ± 2.76	41.73 ± 2.60
Common Asiatic weed	5.60 ± 1.70	10.37 ± 0.07	9.60 ± 1.16	0.75 ± 0.38	8.40 ± 1.69	3.83 ± 1.59
Leech lime	5.87 ± 1.61	20.00 ± 1.50	22.73 ± 1.58	8.55 ± 2.51	80.34 ± 3.90	14.61 ± 3.25
Holy basil	6.83 ± 0.18	10.51 ± 1.37	19.67 ± 1.82	3.31 ± 2.20	7.64 ± 1.35	22.94 ± 4.46
Common cucumber	8.51 ± 1.07	6.26 ± 1.48	7.50 ± 0.80	1.32 ± 0.48	NA	NA
Yellow-berried nightshade	8.29 ± 1.95	5.05 ± 0.12	8.37 ± 0.55	NA	1.28 ± 0.68	5.90 ± 1.56
Aloe	3.23 ± 0.09	4.55 ± 1.21	4.59 ± 1.07	NA	NA	NA

NA = not available; Values are means ± SD of triplicate independent analyses. *Total phenolic content expressed as mg gallic acid equivalent per g fresh weight of sample; †total flavonoid content expressed as mg quercetin per g fresh weight of sample; ‡Curcuminoids might interfere with this method.

Table 4. Antioxidant activities of 15 species of Thai plant extracts derived from different solvents by DPPH assay.

	% scavenging activity (% SC)			mg Vit C g^{-1} fresh weight of sample		
Types of plant	Petroleum ether	Dichloromethane	Ethanol	Petroleum ether	Dichloromethane	Ethanol
Turmeric	72.56 ± 0.88	85.30 ± 1.02	81.29 ± 0.58	23.00 ± 0.29	27.14 ± 0.33	25.84 ± 0.19
Asiatic pennywort	2.52 ± 0.58	4.13 ± 0.90	7.83 ± 0.71	0.22 ± 0.19	0.74 ± 0.29	1.95 ± 0.23
Ginger	46.33 ± 3.76	85.21 ± 0.18	62.98 ± 0.44	14.47 ± 1.22	27.11 ± 0.06	19.88 ± 0.14
Ivy gourd	NA	8.35 ± 0.17	14.20 ± 1.24	NA	2.11 ± 0.06	4.02 ± 0.40
Shallot	2.82 ± 1.14	21.32 ± 0.55	10.07 ± 1.56	0.32 ± 0.37	6.33 ± 0.18	2.67 ± 0.51
Common basil	3.52 ± 0.51	10.14 ± 0.31	50.40 ± 4.42	0.54 ± 0.17	2.70 ± 0.10	15.79 ± 1.44
Horseradish tree	4.17 ± 0.24	4.85 ± 1.49	14.21 ± 0.77	0.76 ± 0.08	0.98 ± 0.49	4.02 ± 0.25
Indian mulberry	6.96 ± 0.68	12.2 ± 0.45	16.09 ± 0.88	1.66 ± 0.22	3.38 ± 0.15	4.63 ± 0.29
Kitchen mint	5.51 ± 0.43	30.76 ± 1.19	50.57 ± 2.60	1.19 ± 0.14	9.40 ± 0.39	15.84 ± 0.85
Common Asiatic weed	5.19 ± 0.18	15.67 ± 3.08	12.33 ± 1.68	1.09 ± 0.06	4.49 ± 1.00	3.41 ± 0.55
Leech lime	5.85 ± 1.46	31.66 ± 0.79	27.22 ± 0.42	1.30 ± 0.48	9.70 ± 0.26	8.25 ± 0.14
Holy basil	14.56 ± 0.86	15.58 ± 0.96	41.75 ± 3.08	4.13 ± 0.28	4.46 ± 0.31	12.98 ± 1.00
Common cucumber	NA	3.80 ± 0.99	4.95 ± 1.52	NA	0.64 ± 0.32	1.01 ± 0.49
Yellow-berried nightshade	6.40 ± 0.95	3.44 ± 1.17	16.35 ± 0.94	1.48 ± 0.31	0.52 ± 0.38	4.72 ± 0.31
Aloe	4.84 ± 0.15	2.56 ± 1.02	4.05 ± 1.08	0.97 ± 0.05	0.39 ± 0.28	0.71 ± 0.35

DPPH = 2,2-diphenyl-1-picrylhydrazyl; NA = not available; Values are means ± SD of triplicate independent analyses.

turmeric extract from dichloromethane fraction had the richest antioxidant activity (85.30 ± 1.02% SC, IC_{50} = 141.78 μg mL^{-1} by the DPPH assay and 93.60 ± 0.25% SC, IC_{50} = 88.05 μg mL^{-1} by the ABTS assay), followed by ginger extract from dichloromethane fraction (85.21 ± 0.18% SC, IC_{50} = 165.18 μg mL^{-1} by the DPPH assay and 93.44 ± 0.53% SC, IC_{50} = 100.52 μg mL^{-1} by the ABTS assay). The lowest antioxidant activity was found in Asiatic pennywort (2.52 ± 0.58% SC by the DPPH assay) and yellow-berried nightshade (6.34 ± 0.94% SC by the ABTS assay). Of note, the extracts obtained from solvents with higher polarities (dichloromethane and ethanol) showed greater antioxidant activities than those of solvents with lower polarities (petroleum ether).

Correlation analyses were used to evaluate the correlations between the two assays for antioxidant activity (Fig. 1a) and between the antioxidant contents and antioxidant activities (Fig. 1b–f). We found a high correlation between the two techniques employed for evaluating antioxidant activities (R^2 = 0.9102), in agreement with a previous study (18). The highest correlation was observed between the total phenolic content and antioxidant activity with regard to the DPPH assay (R^2 = 0.8203; Fig. 1b) and the ABTS assay (R^2 = 0.7213, Fig. 1c), in agreement with a previous report (19). On the contrary, total flavonoid content showed low correlation with total phenolic content (R^2 = 0.4593), and antioxidant activity as assayed with DPPH (R^2 = 0.272) and ABTS (R^2 = 0.2027) methods (Figs. 1d–f, respectively). A similar low correlation between total flavonoid and total phenolic contents was already reported (20). This suggests that phenolic compounds are major contributor to antioxidant activities in these Thai plants. The high antioxidant activities and high levels of phenolics and flavonoids in Thai plants found in this study support the contention that they may have beneficial health effects.

UV absorption properties

The spectrum of UV reaching the earth's surface has wavelengths from 280 to 400 nm: UVA (315–400 nm) and UVB

Table 5. Antioxidant activities of 15 species of Thai plant extracts derived from different solvents by ABTS assay.

	% Scavenging activity (% SC)			mg Vit C g^{-1} fresh weight of sample		
Types of plant	Petroleum ether	Dichloromethane	Ethanol	Petroleum ether	Dichloromethane	Ethanol
Turmeric	78.44 ± 0.70	93.60 ± 0.25	92.68 ± 0.02	44.19 ± 0.42	53.37 ± 0.15	52.81 ± 0.01
Asiatic pennywort	NA	12.09 ± 0.93	17.08 ± 0.33	NA	4.01 ± 0.56	7.03 ± 0.20
Ginger	60.48 ± 1.66	93.44 ± 0.53	91.28 ± 1.36	33.31 ± 1.00	53.27 ± 0.32	51.96 ± 0.83
Ivy gourd	NA	17.36 ± 3.98	32.57 ± 1.22	NA	7.20 ± 2.41	16.41 ± 0.74
Shallot	7.23 ± 1.70	41.45 ± 1.56	32.08 ± 2.11	1.07 ± 1.03	21.79 ± 0.94	16.12 ± 1.28
Common basil	7.08 ± 1.16	22.30 ± 2.23	53.29 ± 1.89	0.98 ± 0.70	10.20 ± 1.35	28.96 ± 1.15
Horseradish tree	NA	9.02 ± 2.00	31.36 ± 2.71	NA	2.15 ± 1.21	15.68 ± 1.64
Indian mulberry	11.57 ± 2.30	31.47 ± 4.27	24.72 ± 1.33	3.69 ± 1.39	15.75 ± 2.58	11.66 ± 0.80
Kitchen mint	12.39 ± 2.25	43.75 ± 2.07	62.39 ± 5.31	4.19 ± 1.36	23.18 ± 1.25	34.47 ± 3.21
Common Asiatic weed	8.25 ± 1.55	38.87 ± 1.61	38.62 ± 2.72	1.68 ± 0.94	20.23 ± 0.97	20.08 ± 1.65
Leech lime	6.74 ± 1.63	52.04 ± 0.77	64.58 ± 2.28	0.77 ± 0.99	28.20 ± 0.47	35.80 ± 1.38
Holy basil	16.10 ± 2.38	27.34 ± 1.30	58.36 ± 1.66	6.44 ± 1.44	13.24 ± 0.79	32.03 ± 1.01
Common cucumber	NA	12.00 ± 1.27	14.84 ± 0.60	NA	3.96 ± 0.77	5.67 ± 0.36
Yellow-berried nightshade	6.34 ± 0.94	7.57 ± 2.19	24.37 ± 1.19	0.53 ± 0.57	1.27 ± 1.33	11.45 ± 0.72
Aloe	6.78 ± 0.39	12.75 ± 3.85	NA	0.80 ± 0.24	4.41 ± 2.33	NA

ABTS = 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid; NA = not available; Values are means ± SD of triplicate independent analyses.

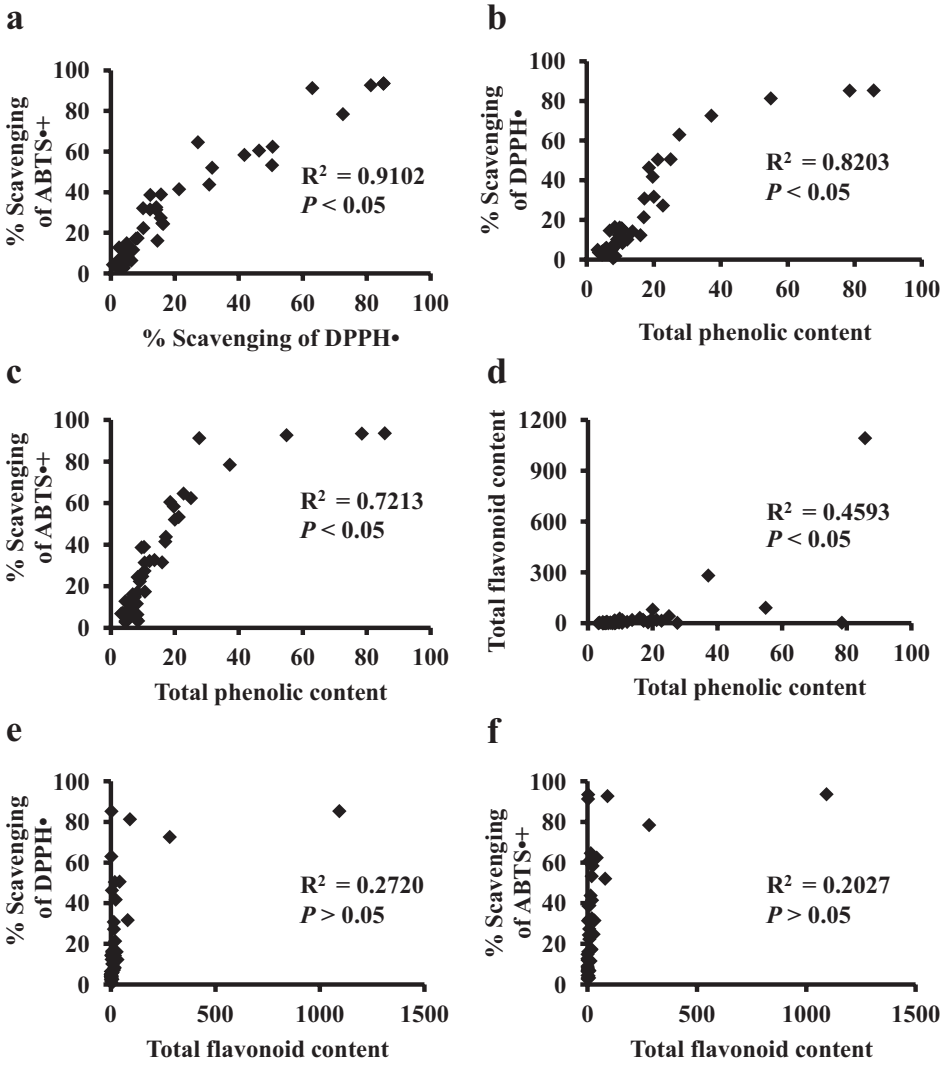

Figure 1. Correlation analyses. Correlations (R^2) between different antioxidant activity and total phenolic content (TPC) or total flavonoid content (TFC) of 15 species of Thai plant extracts. (a) 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays (R^2 = 0.9102); (b) TPC and DPPH assay (R^2 = 0.8203). (c) TPC and ABTS assay (R^2 = 0.7213); (d) TPC and TFC (R^2 = 0.4593); (e) TFC and DPPH assay (R^2 = 0.2720) (f) TFC and ABTS assay (R^2 = 0.2027). The statistical significance is indicated.

(280–315 nm). UV-absorbing properties of 15 species of Thai plant extracts are presented in Fig. 2. The maximum UVA absorption was found in turmeric extracts from the ethanol and dichloromethane fractions, whereas the maximum UVB absorption was found in ginger extract from dichloromethane fraction, followed by turmeric extract from ethanol fraction. In Fig. 2d, the comparison of UVB absorption among the three most active extracts is shown.

In vitro biological test of protection against UV cell damages by Thai herbs extracts

The above data showed that certain Thai plant extracts particularly rich in polyphenols have UV-absorbing properties, which could protect against UV-induced cellular damage. We therefore attempted to assess the potential for these extracts to prevent DNA damage and toxicity induced by UV irradiation. Human immortalized HaCaT keratinocyte cells closely resemble normal human keratinocytes and therefore represent a valuable *in vitro* cell model for testing the effects of natural products of cosmetic interest (21). HaCaT cells are currently widely employed to study the cytotoxic effect of UV irradiation and to evaluate the potential protection by natural compounds (4,9,22–24). In a first set of experiments, we used the MTT assay to assess the toxicity of the Thai herbs in HaCaT cells exposed for 24 h to concentrations of extracts (dissolved in DMSO) ranging from 0.625 to 5 μg mL^{-1}. DMSO itself was not toxic at 0.05%, corresponding to the final content in the incubation medium with the highest concentration of extract (not shown). Most of the 45 extracts were revealed as not toxic (data not shown), with the exceptions as detailed below. Dichloromethane extract at all concentrations tested of common basil, cucumber and leech lime consistently improved by 20% the metabolic activity of HaCaT cells as measured by formazan precipitates (see Figure S1). Strikingly, the petroleum ether extract of cucumber and leech lime was by contrast very toxic at any concentration tested (Figure S1). Petroleum ether extract of common Asiatic weed (at all concentrations), holy basil (at 2.5–5.0 μg mL^{-1}), and the ethanol extract of Indian mulberry (at 5.0 μg mL^{-1}) also revealed slightly cytotoxic. We further assessed the toxicity of selected extracts at much higher concentration and for a period of incubation up to 48 h by counting the adherent viable cells. The dichloromethane extract of aloe and the ethanol extracts of Asiatic pennywort, of kitchen mint and of leech lime showed no toxicity at concentrations ranging from 5 to 100 μg mL^{-1} for up to 48 h (not shown). In fact, the latter at 5 and 50 μg mL^{-1} stimulated HaCaT cell growth by some 50% by 48 h (Figure S2). Dichloromethane extracts of turmeric at 1 μg mL^{-1} and of ginger at 5 μg mL^{-1} were not toxic, while these same extracts at concentrations of 5–10 μg mL^{-1} and 50–100 μg mL^{-1}, respectively, were shown very toxic (Figure S2).

Next, we tested under several incubation protocols the potential of some extracts to protect HaCaT cells from UVB cytotoxicity. The presence of apoptotic cells in the treated cultures was assayed by determining the amount of mono- and oligonucleosomes in the cytoplasm (see Materials and Methods section). In one set of experiments, a 30 min pretreatment of the cells with 0.625, 1.25 and 2.5 μg mL^{-1} ethanol extracts of leech lime, of Asiatic pennywort and dichloromethane extracts of aloe was sufficient to significantly reduce (by approximately 20%) the extent of apoptosis in cultures exposed to 200 mJ cm^{-2} and further incubated for 24 h in normal culture medium (Fig. 3).

Based on cytotoxic, antioxidant and UVB absorption data, for the next experiments we chose to investigate more in depth the cytoprotective potential of turmeric (at 1 μg mL^{-1}) and ginger (at 5 μg mL^{-1}) extracts (dichloromethane fraction) toward cell toxicity and DNA damage induced by UVB. In these experiments, we adopted a protocol using a dosage of UVB irradiation of 120 mJ cm^{-2}, which was shown to cause approximately 50–60% cell loss by 24–48 h post irradiation in HaCaT cultures (Fig. 4a), and a significant accumulation of CPDs in the cells (Fig. 4b). Also, 120 mJ cm^{-2} UVB irradiation caused a 3.5-fold increase in caspase-3 activity in the cells by 24 h post irradiation (not shown), and strongly limited the cloning efficiency of treated cells between 24 and 48 h (Fig. 4a; compare cell density at 24 and 48 h of UV-treated *versus* control, considering that cell density at day 0 was 25.000 $\pm$ 6.000 cells cm^{-2}). We then tested the protective activity in several protocols of preincubation time with the herbs extracts. Optimal protection was observed with a 8 h preincubation with 1 μg mL^{-1} turmeric or 5 μg mL^{-1} ginger, followed by UVB exposure and further incubation for 24 or 48 h in serum-free medium supplemented with a halved concentration of extracts. Under this condition, turmeric and ginger extracts elicited a significant reduction in the accumulation of CPDs at 24 and 48 h in live but not in dead cells (Fig. 5a), which associated with reduced activation of caspase-3 activity at 48 h (Fig. 5b) and some cell protection (not shown). A similar protocol, in which the preincubation time was increased to 24 h elicited almost a similar protection, albeit less extensive (not shown). To gain an insight on the possible mechanisms underlying such protective effect, we checked the expression of antioxidant proteins in HaCaT cells exposed to turmeric and ginger extracts. We focused on Thioredoxin-1 (TRX), a 12 kDa redox-active protein that protects the cells from oxidative stress (25) and apoptosis (26). While a 1 h incubation was inefficient (data not shown), an 8 h incubation with 1 μg mL^{-1} turmeric or 5 μg mL^{-1} ginger extract (dichloromethane fraction) was sufficient to determine a strong increase in the expression of Thioredoxin 1 (Fig. 6).

DISCUSSION

UVB (280–320 nm) reaches the earth's surface in amounts sufficient to provoke harmful biological effects on the skin that can eventually lead to skin malignancies and nonmelanoma cancers (1). UV is a complete carcinogen. UVB-induced DNA damage generates photoproducts such as CPD and pyrimidine (6-4) pyrimidone (27–29). Cells in which DNA damage is unrepaired should undergo apoptosis (30), as a mechanism to prevent the development of cancer. DNA damage and cytotoxicity occur at UVB intensity of approximately 30 mJ cm^{-2}, which corresponds to a few minutes of exposition to sunlight (31). A prolonged exposure to sunlight UVB can cause massive apoptosis in keratinocytes and consequently alters the natural barrier functions of the skin, thus predisposing to inflammation, infections and cancer. Free radicals associated with UV radiation exposure trigger skin inflammation, a condition that contributes to the development of skin cancer (2,32). In this regard, cyclooxygenase Cox-2 is one of the main player involved in inflammation and skin carcinogenesis induced by UVB exposure (33,34) and, consistently, natural products able to downregulate its expression also exert preventive activity against UV injuries (35,36).

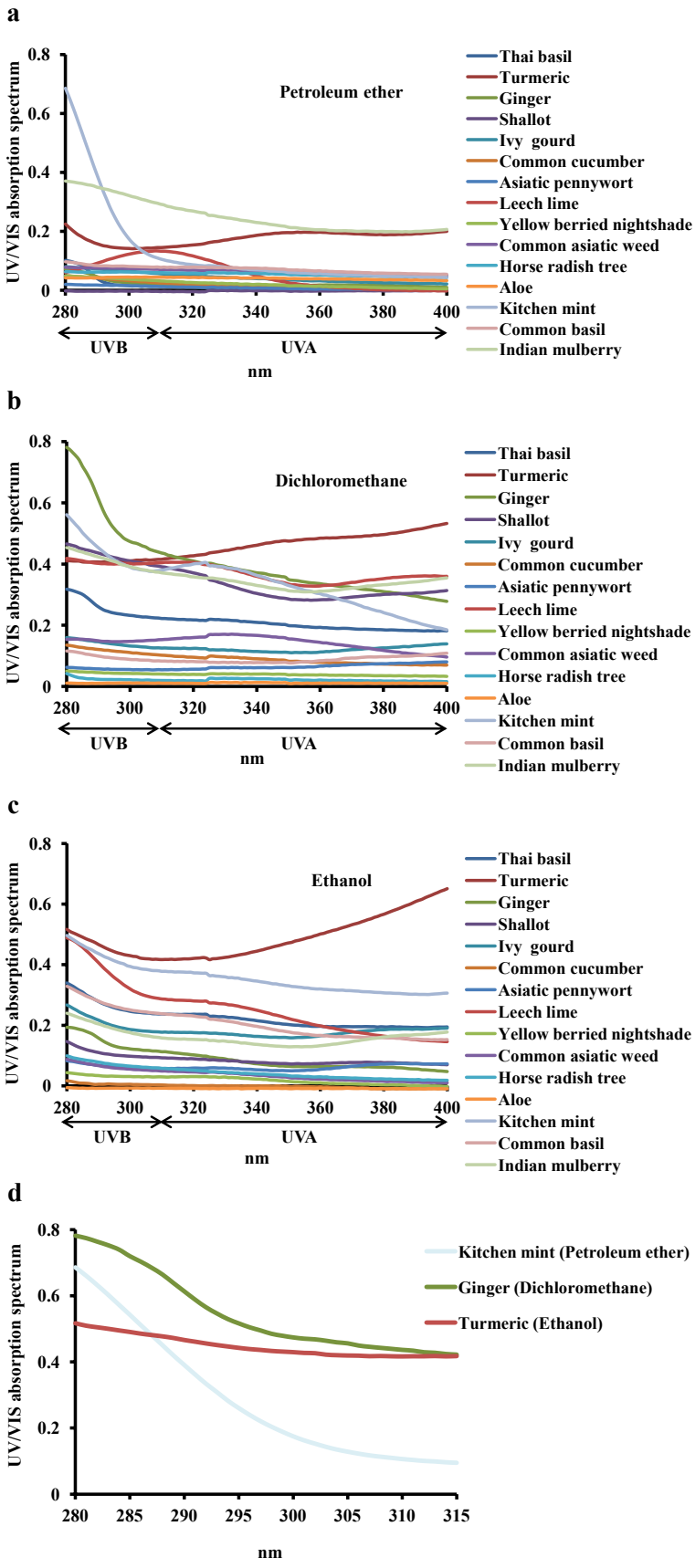

Figure 2. UV-absorbing properties of 15 species of Thai plant extracts. UVA-and UVB-absorbing properties of the 15 plant extracts: (a) petroleum ether fraction; (b) dichloromethane fraction; (c) ethanol fraction. (d) Comparison of UVB absorption of the three most active extracts.

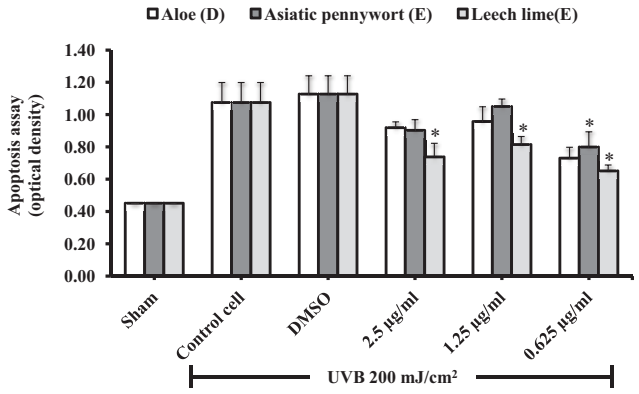

Figure 3. Apoptosis assay after UVB exposure in cells pretreated with herb extracts. The cells were pretreated with plant extracts for 30 min followed by exposition to 200 mJ cm^{-2} UVB and further incubated for 24 h. At the end, the extent of apoptosis was assessed with the cell death detection ELISA plus kit, which measure the production of mono- and oligonucleosomes. The cultures pretreated with the ethanol (E) extracts of leech lime or of Asiatic pennywort or with dichloromethane extract (D) of aloe showed a reduced number of apoptotic cells compared to control (*i.e.* not pretreated with herb extracts) culture (statistical significance $*P < 0.05$). Sham refers to UVB unexposed cells.

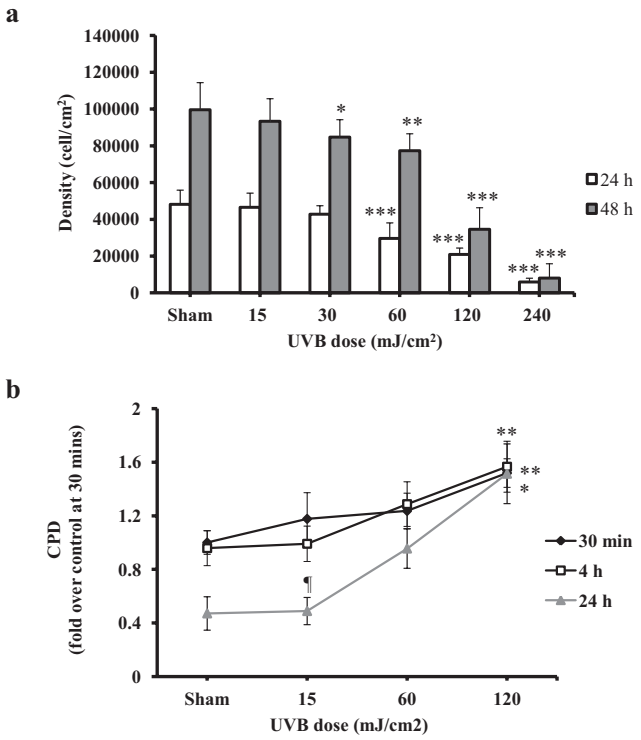

Figure 4. Dose-dependent cytotoxic effects of UVB in HaCaT cells. HaCaT cells were plated on petri dishes and let adhere for at least 24 h before starting the treatment. At day 0, cell density was 25 000 ± 6000 cells cm^{-2}. (a) In UVB unexposed cultures (sham), cells duplicated every 24 h. UVB-induced cell loss in a dose-dependent fashion as determined by cell counting of adherent viable cells at 24 and 48 h post-UVB irradiation. Cloning efficiency of irradiated cultures was largely compromised at doses of 120 and 240 mJ cm^{-2}. (b) UVB-induced DNA damage as assessed by ELISA evaluation of cyclobutane pyrimidine dimers formation at 30 min, 4 and 24 h post-UVB (15, 60 or 120 mJ cm^{-2}) irradiation. Sham indicates UVB unexposed cells. Statistical significance: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$, significant difference between comparison groups; $¶P < 0.05$, significant difference between UVB-treated cells at 30 min and at 24 h post-UVB (15 mJ cm^{-2}) using Student's *t*-test.

This study demonstrates that extracts of 15 species of Thai plant possess varying degrees of total phenolic and total flavonoid contents as well as antioxidant activities, depending on extraction solvents. In particular, extracts derived from ethanol and dichloromethane fractions were found to possess higher total phenolic and total flavonoid contents than those from petroleum ether. A high correlation between total phenolic content and antioxidant activity was found. The highest levels of total flavonoid content were detected in turmeric extract from dichloromethane fraction, followed by turmeric extract from petroleum ether fraction. In addition, turmeric and ginger extracts were shown to possess the highest content of antioxidants and the highest UV-absorbing ability. Thus, one-first conclusion of this study is that the method and solvent used for the preparation of the extract greatly affect the physical-chemical properties, and possibly also the biological activities, of the herb extract. Next, we tested the ability of the extracts to prevent UVB-induced DNA damage and cell death in keratinocytes. In general, low doses of UVB cause DNA mutations that most likely lead to tumor initiation, whereas high doses of UVB directly induce apoptosis as a consequence of oxidative stress (37). Keratinocytes undergoing apoptosis are known as "sunburn cells," and their presence is assumed as indicator of the severity of UVB-induced DNA damage (38). In this study, we exposed keratinocytes to a relatively high dose of UVB (120 mJ cm^{-2}), which in fact caused DNA damage (as indicated by a three-fold increase in CPDs) and apoptosis (approximately 50% in the culture at 24 h post irradiation). Remarkably, if prior to exposure to UVB the keratinocytes were incubated with turmeric or ginger extracts (dichloromethane fraction), CPDs were found in dead cells but not in living cells. Also, preincubation with these extracts protected to some extent from caspase-dependent apoptosis induced by UVB. Taken together, these results suggest that turmeric and ginger extracts could save those cells in which UVB-induced DNA damage was prevented, although allowing cell death in those ones bearing DNA mutations. It is conceivable that protection from UVB damage was linked to the antioxidant properties of the herbs extracts. In fact, Gingerol, the major component of ginger, was shown to reduce the UVB induction of ROS, caspases and Cox-2 in HaCaT cells (39), and curcumin, the major component of turmeric, also was shown to downregulate Cox-2 expression (40). In this study, we show that the incubation of HaCaT cells with turmeric and ginger extracts increased the expression of TRX, a protein that protects mitochondria from excessive oxidative stress. The primary cytotoxic event in human epidermis associated with UV exposure is the excessive production and accumulation of ROS, which is the consequence of UV inactivation of the thioredoxin system (41). Therefore, the increased expression of this antioxidant protein achieved by the preincubation with the turmeric and ginger extracts is likely to confer a protection to keratinocytes exposed to UVB. In conclusion, we propose that the pretreatment with turmeric or ginger extract renders skin cells more competent to face the oxidative stress induced by UVB through the upregulation of diverse antioxidant systems, including thioredoxin 1.

Cosmetic pharmaceuticals are demanded for chemoprevention of damaging effects of UVB exposure. A plethora of compounds naturally found in edible and nonedible vegetables and fruits have been shown to exert a protective action against UV skin damages,

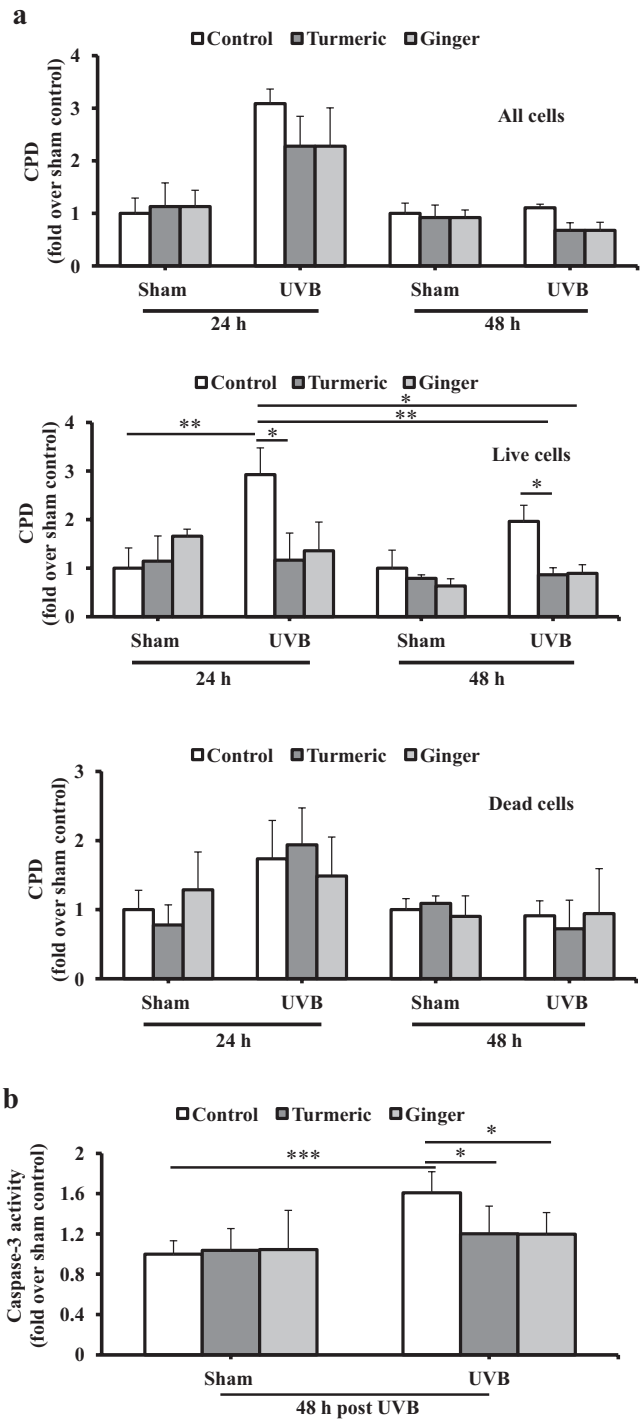

Figure 5. Protective activity of turmeric and ginger extracts against UVB cytotoxicity. The cells were pretreated for 8 h with the dichloromethane extracts of turmeric (at 1 μg mL^{-1}) or ginger (at 5 μg mL^{-1}) and then exposed to UVB (120 mJ cm^{-2}), followed by incubation in medium contained halved concentration of the herb extract. (a) 24 and 48 h post-UVB, cyclobutane pyrimidine dimers formation was evaluated in pooled live and dead cells (upper panel), in live cells only (middle panel), and in dead cells only (lower panel). (b) Caspase-3 activity determined in the culture at 48 h post-UVB irradiation. Statistical significance: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, significant difference between comparison groups.

including inflammation and cancer (9,35,36,42–44), and many of these constitute the active pharmacologic ingredient of cosmetic formulations that can prevent skin injuries (45) or improve skin repair

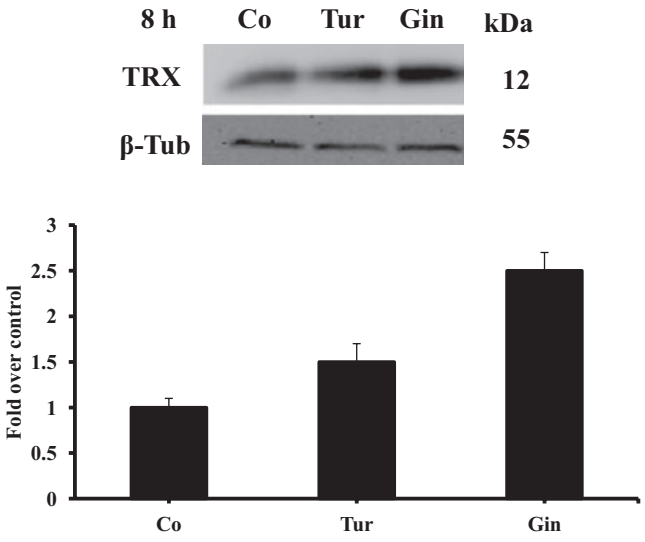

Figure 6. Expression of Thioredoxin 1 in HaCaT cells pretreated with turmeric and ginger extracts. The cells were plated on petri dishes, let adhere for 24 h and then incubated for 8 h with the dichloromethane extracts of turmeric (at 1 μg mL^{-1}) or ginger (at 5 μg mL^{-1}). The cell homogenate was analyzed by Western blotting first for the expression of Thioredoxin 1, then it was stripped and reprobed with anti-β-tubulin antibody for control loading of cell proteins. The experiment was replicated three times (one representative gel is shown). The intensity of bands was evaluated and the relative intensity of Thioredoxin 1 *versus* β-tubulin was calculated. Data of the three experiments are given as fold increases (average $\pm$ SD) in treated *versus* control cells (assumed as value 1).

(21,46). The present data showing that turmeric and ginger extracts could prevent UV-induced DNA and cellular toxicity in keratinocytes support their utilization in cosmetic biopharmaceuticals.

Acknowledgements—This study was financially supported by the National Research Council of Thailand and Chulalongkorn University Centenary Academic Development Project and the 90th anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund). VT is recipient of a scholarship from the Royal Golden Jubilee Ph.D. Program of Thailand Research Fund (Ph.D. Program in Clinical Biochemistry and Molecular Medicine) and the Graduate School, Chulalongkorn University. VT also received a research assistant fellowship from Chulalongkorn University Centenary Academic Development Project. Research in the laboratory of CI was supported by Comoli, Ferrari & SpA (Novara, Italy). The authors are grateful to Asst. Prof. Dr. Siriporn Chuchawankul, head of the Innovation Center for Research and Development of Medical Diagnostic Technology Project (Chulalongkorn University) for providing useful comments and supporting certain laboratory instruments.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Effect of Thai herbs extracts in HaCaT cells as determined by MTT assay.

Figure S2. The effect of Leech lime, Turmeric and Ginger on cell growth in HaCaT cells.

REFERENCES

1. Matsumura, Y. and H. N. Ananthaswamy (2004) Toxic effects of ultraviolet radiation on the skin. *Toxicol. Appl. Pharmacol.* **195**, 298–308.

2. Ridley, A. J., J. R. Whiteside, T. J. McMillan and S. L. Allinson (2009) Cellular and sub-cellular responses to UVA in relation to carcinogenesis. *Int. J. Radiat. Biol.* **85**, 177–195.
3. Adhami, V. M., D. N. Syed, N. Khan and F. Afaq (2008) Phytochemicals for prevention of solar ultraviolet radiation-induced damages. *Photochem. Photobiol.* **84**, 489–500.
4. Wang, X. Y., Y. G. Wang and Y. F. Wang (2011) Ginsenoside Rb1, Rg1 and three extracts of traditional Chinese medicine attenuate ultraviolet B-induced G1 growth arrest in HaCaT cells and dermal fibroblasts involve down-regulating the expression of p16, p21 and p53. *Photodermatol. Photoimmunol. Photomed.* **27**, 203–212.
5. Afnan, Q., M. D. Adil, A. Nissar-Ul, A. R. Rafiq, H. F. Amir, P. Kaiser, V. K. Gupta, R. Vishwakarma and S. A. Tasduq (2012) Glycyrrhizic acid (GA), a triterpenoid saponin glycoside alleviates ultraviolet-B irradiation-induced photoaging in human dermal fibroblasts. *Phytomedicine* **19**, 658–664.
6. Wölfle, U., P. R. Esser, B. Simon-Haarhaus, S. F. Martin, J. Lademann and C. M. Schempp (2011) UVB-induced DNA damage, generation of reactive oxygen species, and inflammation are effectively attenuated by the flavonoid luteolin in vitro and in vivo. *Free Radic. Biol. Med.* **50**, 1081–1093.
7. Fonseca, Y. M., C. D. Catini, F. T. Vicentini, A. Nomizo, R. F. Gerlach and M. J. Fonseca (2010) Protective effect of Calendula officinalis extract against UVB-induced oxidative stress in skin: Evaluation of reduced glutathione levels and matrix metalloproteinase secretion. *J. Ethnopharmacol.* **127**, 596–601.
8. Hwang, B. M., E. M. Noh, J. S. Kim, J. M. Kim, Y. O. You, J. K. Hwang, K. B. Kwon and Y. R. Lee (2013) Curcumin inhibits UVB-induced matrix metalloproteinase-1/3 expression by suppressing the MAPK-p38/JNK pathways in human dermal fibroblasts. *Exp. Dermatol.* **22**, 371–374.
9. Guahk, G. H., S. K. Ha, H. S. Jung, C. Kang, C. H. Kim, Y. B. Kim and S. Y. Kim (2010) Zingiber officinale protects HaCaT cells and C57BL/6 mice from ultraviolet B-induced inflammation. *J. Med. Food* **13**, 673–680.
10. Singelton, V. R., R. Orthifer and R. M. Lamuela-Raventos (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin–Ciocalteu reagent. *Meth. Enzy.* **299**, 152–178.
11. Woisky, R. G. and A. Salatino (1998) Analysis of propolis: Some parameters and procedures for chemical quality control. *J. Apic. Res.* **37**, 99–105.
12. Brand-Williams, W., M. E. Cuvelier and C. Berset (1995) Use of free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.* **28**, 25–30.
13. Arnao, M. B., A. Cano and M. Acosta (2001) The hydrophilic and lipophilic contribution to total antioxidant activity. *Food Chem.* **73**, 239–244.
14. Kunisada, M., H. Kumimoto, K. Ishizaki, K. Sakumi, Y. Nakabeppu and C. Nishigori (2007) Narrow-band UVB induces more carcinogenic skin tumors than broad-band UVB through the formation of cyclobutane pyrimidine dimer. *J. Invest. Dermatol.* **127**, 2865–2871.
15. Cagnin, M., M. Ozzano, N. Bellio, I. Fiorentino, C. Follo and C. Isidoro (2012) Dopamine induces apoptosis in APPswe-expressing Neuro2A cells following Pepstatin-sensitive proteolysis of APP in acid compartments. *Brain Res.* **1471**, 102–117.
16. MacDonald-Wicks, L. K., L. G. Wood and M. L. Garg (2006) Methodology for the determination of biological antioxidant capacity in vitro: A review. *J. Sci. Food Agric.* **86**, 2046–2056.
17. Moon, J. K. and T. Shibamoto (2009) Antioxidant assays for plant and food components. *J. Agric. Food Chem.* **57**, 1655–1666.
18. Floegel, A., D. O. Kim, S. J. Chung, S. I. Koo and O. K. Chun (2011) Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *J. Food Comp. Anal.* **24**, 1043–1048.
19. Singh, D. R., S. Singh, K. M. Salim and R. C. Srivastava (2012) Estimation of phytochemicals and antioxidant activity of underutilized fruits of Andaman Islands (India). *Int. J. Food Sci. Nutr.* **63**, 446–452.
20. Kim, I. S., M. Yang, T. H. Goo, C. Jo, D. U. Ahn, J. H. Park, O. H. Lee and S. N. Kang (2012) Radical scavenging-linked antioxidant activities of commonly used herbs and spices in Korea. *Int. J. Food Sci. Nutr.* **63**, 603–609.
21. Ranzato, E., S. Martinotti and B. Burlando (2011) Wound healing properties of jojoba liquid wax: An in vitro study. *J. Ethnopharmacol.* **134**, 443–449.
22. Williams, K. A., K. Kolappaswamy, L. J. DeTolla and I. Vucenik (2011) Protective effect of inositol hexaphosphate against UVB damage in HaCaT cells and skin carcinogenesis in SKH1 hairless mice. *Comp. Med.* **61**, 39–44.
23. Han, W., M. Ming and Y. Y. He (2011) Caffeine promotes ultraviolet B-induced apoptosis in human keratinocytes without complete DNA repair. *J. Biol. Chem.* **286**, 22825–22832.
24. Magina, S., M. A. Vieira-Coelho, M. P. Serrão, C. Kosmus, E. Moura and D. Moura (2012) Ultraviolet B radiation differentially modifies catechol-*O*-methyltransferase activity in keratinocytes and melanoma cells. *Photodermatol. Photoimmunol. Photomed.* **28**, 137–141.
25. Hanschmann, E. M., J. R. Godoy, C. Berndt, C. Hudemann and C. H. Lillig (2013) Thioredoxins, Glutaredoxins, and Peroxiredoxins-Molecular Mechanisms and Health Significance: From Cofactors to Antioxidants to Redox Signaling. *Antioxid. Redox Signal.* [Epub ahead of print].
26. Saitoh, M., H. Nishitoh, M. Fujii, K. Takeda, K. Tobiume, Y. Sawada, M. Kawabata, K. Miyazono and H. Ichijo (1998) Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J.* **17**, 2596–2606.
27. Niggli, H. J. and R. Röthlisberger (1988) Cyclobutane-type pyrimidine photodimer formation and induction of ornithine decarboxylase in human skin fibroblasts after UV irradiation. *J. Invest. Dermatol.* **91**, 579–584.
28. Vink, A. A., R. J. Berg, F. R. de Gruijl, L. Roza and R. A. Baan (1991) Induction, repair and accumulation of thymine dimers in the skin of UV-B-irradiated hairless mice. *Carcinogenesis* **12**, 861–864.
29. Katiyar, S. K., M. S. Matsui and H. Mukhtar (2000) Kinetics of UV light-induced cyclobutane pyrimidine dimers in human skin in vivo: An immunohistochemical analysis of both epidermis and dermis. *Photochem. Photobiol.* **72**, 788–793.
30. Cleaver, J. E., E. T. Lam and I. Revet (2009) Disorders of nucleotide excision repair: The genetic and molecular basis of heterogeneity. *Nat. Rev. Genet.* **10**, 756–768.
31. Wang, C. B., M. Q. Huang, G. L. Tao, G. Y. Yu, Z. W. Han, Z. H. Yang and Y. J. Wang (2004) Polypeptide from *Chlamys farreri* protects HaCaT cells from UVB-induced apoptosis. *Chem. Biol. Interact.* **147**, 119–127.
32. Bickers, D. R. and M. Athar (2006) Oxidative stress in the pathogenesis of skin disease. *J. Invest. Dermatol.* **126**, 2565–2575.
33. Buckman, S. Y., A. Gresham, P. Hale, G. Hruza, J. Anast, J. Masferrer and A. P. Pentland (1998) COX-2 expression is induced by UVB exposure in human skin: Implications for the development of skin cancer. *Carcinogenesis* **19**, 723–729.
34. Keum, Y. S., H. G. Kim, A. M. Bode, Y. J. Surh and Z. Dong (2013) UVB-induced COX-2 expression requires histone H3 phosphorylation at Ser10 and Ser28. *Oncogene* **32**, 444–452.
35. Kwon, J. Y., K. W. Lee, J. E. Kim, S. K. Jung, N. J. Kang, M. K. Hwang, Y. S. Heo, A. M. Bode, Z. Dong and H. J. Lee (2009) Delphinidin suppresses ultraviolet B-induced cyclooxygenases-2 expression through inhibition of MAPKK4 and PI-3 kinase. *Carcinogenesis* **30**, 1932–1940.
36. Rahman, M., J. K. Kundu, J. W. Shin, H. K. Na and Y. J. Surh (2011) Docosahexaenoic acid inhibits UVB-induced activation of NF-κB and expression of COX-2 and NOX-4 in HR-1 hairless mouse skin by blocking MSK1 signaling. *PLoS One* **6**. [Epub ahead of print].
37. Kulms, D. and T. Schwartz (2000) Molecular mechanisms of UV-induced apoptosis. *Photodermatol. Photoimmunol. Photomed.* **16**, 195–201.
38. Claerhout, S., A. Van Laethem, P. Agostinis and M. Garmyn (2006) Pathways involved in sunburn cell formation: Deregulation in skin cancer. *Photochem. Photobiol. Sci.* **5**, 199–207.
39. Kim, J. K., Y. Kim, K. M. Na, Y. J. Surh and T. Y. Kim (2007) [6]-Gingerol prevents UVB-induced ROS production and COX-2 expression in vitro and in vivo. *Free Radic. Res.* **41**, 603–614.
40. Goel, A., S. Jhurani and B. B. Aggarwal (2008) Multi-targeted therapy by curcumin: How spicy is it? *Mol. Nutr. Food Res.* **52**, 1010–1030.

41. Sundaram, C., W. Köster and K. U. Schallreuter (1990) The effect of UV radiation and sun blockers on free radical defence in human and guinea pig epidermis. *Arch. Dermatol. Res.* **282**, 526–531.
42. Reagan-Shaw, S., H. Mukhtar and N. Ahmad (2008) Resveratrol imparts photoprotection of normal cells and enhances the efficacy of radiation therapy in cancer cells. *Photochem. Photobiol.* **84**, 415–421.
43. Li, J., M. Malakhova, M. Mottamal, K. Reddy, I. Kurinov, A. Carper, A. Langfald, N. Oi, M. O. Kim, F. Zhu, C. P. Sosa, K. Zhou, A. M. Bode and Z. Dong (2012) Norathyriol suppresses skin cancers induced by solar ultraviolet radiation by targeting ERK kinases. *Cancer Res.* **72**, 260–270.
44. Choi, J. Y., D. I. Choi, J. B. Lee, S. J. Yun, D. H. Lee, J. B. Eun and S. C. Lee (2013) Ethanol extract of peanut sprout induces Nrf2 activation and expression of antioxidant and detoxifying enzymes in human dermal fibroblasts: Implication for its protection against UVB-irradiated oxidative stress. *Photochem. Photobiol.* **89**, 453–460.
45. Anunciato, T. P. and P. A. da Rocha Filho (2012) Carotenoids and polyphenols in nutricosmetics, nutraceuticals, and cosmeceuticals. *J. Cosmet. Dermatol.* **11**, 51–54.
46. Korać, R. R. and K. M. Khambholja (2011) Potential of herbs in skin protection from ultraviolet radiation. *Pharmacogn. Rev.* **5**, 164–173.