Mar Biotechnol
DOI 10.1007/s10126-008-9123-8

ORIGINAL ARTICLE

Repair of UVB-Damaged Skin by the Antioxidant Sulphated Flavone Glycoside Thalassiolin B Isolated from the Marine Plant *Thalassia testudinum* Banks *ex* König

Erik L. Regalado · María Rodríguez · Roberto Menéndez · Ángel A. Concepción · Clara Nogueiras · Abilio Laguna · Armando A. Rodríguez · David E. Williams · Patricia Lorenzo-Luaces · Olga Valdés · Yasnay Hernandez

Received: 30 April 2008 / Accepted: 5 June 2008

Abstract Daily topical application of the aqueous ethanolic extract of the marine sea grass, *Thalassia testudinum*, on mice skin exposed to UVB radiation resulted in a dose-dependent recovery of the skin macroscopic alterations over a 6-day period. Maximal effect (90%) occurred at a dose of 240 μg/cm^2, with no additional effects at higher doses. Bioassay-guided fractionation of the plant extract resulted in the isolation of thalassiolin B (1). Topical application of 1 (240 μg/cm^2) markedly reduces skin UVB-induced damage. In addition, thalassiolin B scavenged 2,2-diphenyl-2-picrylhydrazyl radical with an EC_{50}=100 μg/ml. These results suggest that thalassiolin B is responsible for the skin-regenerating effects of the crude extract of *T. testudinum*.

Keywords Flavonoids · Thalassiolin B · DPPH scavenged · Antioxidant activity · Skin regenerating activity · *Thalassia testudinum*

Erik L. Regalado and María Rodríguez have contributed equally to this work and should be considered as first authors.

E. L. Regalado (✉) · A. Laguna · A. A. Rodríguez · O. Valdés · Y. Hernandez
Department of Chemistry, Center of Marine Bioproducts, Loma y 37, Alturas del Vedado, C.P. 10400 Havana, Cuba
e-mail: erikluis18@gmail.com

M. Rodríguez (✉) · P. Lorenzo-Luaces
Department of Chemistry, Laboratory of Cosmetics, Center of Marine Bioproducts, Havana, Cuba
e-mail: merchy@infomed.sld.cu

R. Menéndez
Department of Pharmacology, Center of Marine Bioproducts, Havana, Cuba

Á. A. Concepción
Department of Histology, National Center of Medical Genetic, Havana, Cuba

C. Nogueiras
Department of Natural Products, Faculty of Chemistry, University of Havana, Havana, Cuba

D. E. Williams
Department of Chemistry, University of British Columbia, Vancouver V6T 1Z4, Canada

Introduction

Considerable exposure to ultraviolet (UV) light can have extremely noxious effects on skin. Specifically, UVB acute exposure of skin induces direct and indirect adverse biological effects. By impairing oxidative balance, the oxidative stress (Inal et al. 2001) results in actinic erythema, sunburn, photo-induced diseases, photo-worsened diseases, actinic aging, and skin cancers (Kligman et al. 1984; Saliou et al. 2001; Hwang et al. 2006; Svobodova et al. 2006). One approach to protecting humans from the harmful effects of UV irradiation is to use antioxidative phenolic compounds (Svobodova et al. 2003). Several lines of evidence have shown that phenolic plant compounds such as caffeic and ferulic acids, quercetin, and apigenin can reduce the harmful effects of UV radiation (Birt et al. 1997; Saija et al. 2000; Inal et al. 2001). Although

investigations are scarce, some authors have demonstrated that seaweed extracts can help to protect skin from UV radiation (Fujimura et al. 2002; Bulteau et al. 2006).

Thalassia testudinum a sea grass, commonly known as turtle grass (Littler and Littler 2000), plays an important role in marine ecosystems (Durako and Kunzelman 2002), supplying nursery grounds for many invertebrate and fish species, stabilizing the sediments of near shore coast (Corlett and Jones 2007), etc. Previous studies have shown that thalassiolin A (luteolin 7-β-D-glucopyranosyl-2''-sulphate), isolated from *T. testudinum* (collected in the Bahamas), has antibiotic activity against zoosporic fungi (Jensen et al. 1998). Moreover, another study demonstrated that thalassiolins A–C represent a new series of HIV integrase inhibitors (Rowley et al. 2002).

In the present work, we describe the effect of the topical application of a cream containing the aqueous ethanolic extract of *T. testudinum* leaves (60 μg/cm^2–240 mg/cm^2) on mice that have undergone acute UVB skin damage. By means of bioassay-guided fractionation of the active extract, we isolated thalassiolin B (1). In addition, 4-hydroxybenzoic acid (2) was isolated for the first time from this plant.

Results

Repair of Acute UVB-Damaged Skin by the Extract

We have previously shown that there was no evidence of skin irritation or toxicity from repeated crude extract cream applications. Exposure to UVB radiation results in the development of macroscopic alterations such as: erythema, scabs, roughness, and wrinkling of the skin in irradiated control animals. The peak of reaction occurred between 48 and 72 h in all animals. However, topical application of a crude extract of *T. testudinum* leaves resulted in dose-dependent reduction of the macroscopic alterations (Fig. 1). The maximal effects occurred at 240 μg/cm^2, with no additional effect observed at higher concentrations (up to 480 μg/cm^2). Skin damage was significantly suppressed by 90% (mean value) at the end of the application period. The significant inhibitory effect of the crude extract at the maximal inhibitory dose on skin damage was further confirmed by histopathological studies (Figs. 2 and 3). As observed, nontreated skin showed severe histopathological alterations such as acanthosis, hyperkeratosis, infiltrating inflammatory cells, and collagen and elastic fiber degradation. In addition, macrophages were found in higher numbers in the photodamaged skin of untreated animals. However, for animals treated topically with a cream containing crude extract, the erythema was eliminated, collagens and elastics fibers were reorganized, acanthosis and hyperkeratosis decreased, and vascular damage was reduced.

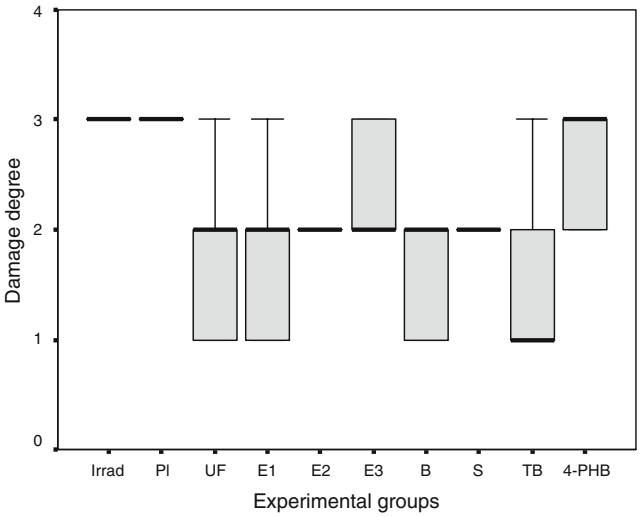

Fig. 1 Effects of the samples in photodamaged skins. *Irrad* (irradiated), *Pl* (vehicle), *UF* (antiaging cream), *E1* (crude extract, 240 μg/cm^2), *E2* (crude extract, 120 μg/cm^2), *E3* (crude extract, 60 μg/cm^2), *B* (fraction B), *S* (fraction S), *TB* (Thalassiolin B) and *4-PHB* (4-hydroxybenzoic acid; B, S, TB, and 4-PHB, 240 μg/cm^2)

Bioassay-Guided Fractionation and Identification of Active Compound

The crude extract (48 g) was dissolved in water and successively partitioned with *n*-hexane (fraction H, 50 mg), chloroform (fraction C, 240 mg), and water-saturated *n*-butanol (fraction B, 800 mg). The residual aqueous fraction was lyophilized (fraction A, 46 g) and further fractionated by precipitation with EtOH (95%); the soluble fraction was designated fraction S (10 g) and the residue as fraction N (35 g). All fractions were dried and tested for skin-regenerating activity. As shown in Fig. 1, fractions B and S exhibited skin-regenerating activity as judged by significant reduction of macroscopic signs. Fraction B showed the most potent activity, reducing macroscopic skin alterations to the same extent as the crude extract.

Results of the antioxidant activity and phenolic content of the crude extract and fractions B and S are shown in Table 1. The results indicate that each of the fractions exhibit radical trapping properties against DPPH radical. Fraction B exhibited the strongest scavenging activity with an EC_{50} of 97 μg/mL. In addition, fraction B showed the highest phenolic content. Extract B was then fractionated by size exclusion column chromatography (Sephadex LH-20, $CHCl_3$–MeOH, 5:1) to give a late eluting yellow band that was further fractionated via silica gel column chromatography (using 7:3:0.25 $CHCl_3$–MeOH–H_2O as eluent). This was followed by C18 reversed-phase high-performance liquid chromatography (HPLC) purification (linear gradient 5% to 30% MeOH–H_2O in 40 min) to give pure thalassiolin B (1; 40 mg). In addition, a 15 mg fraction containing 4-

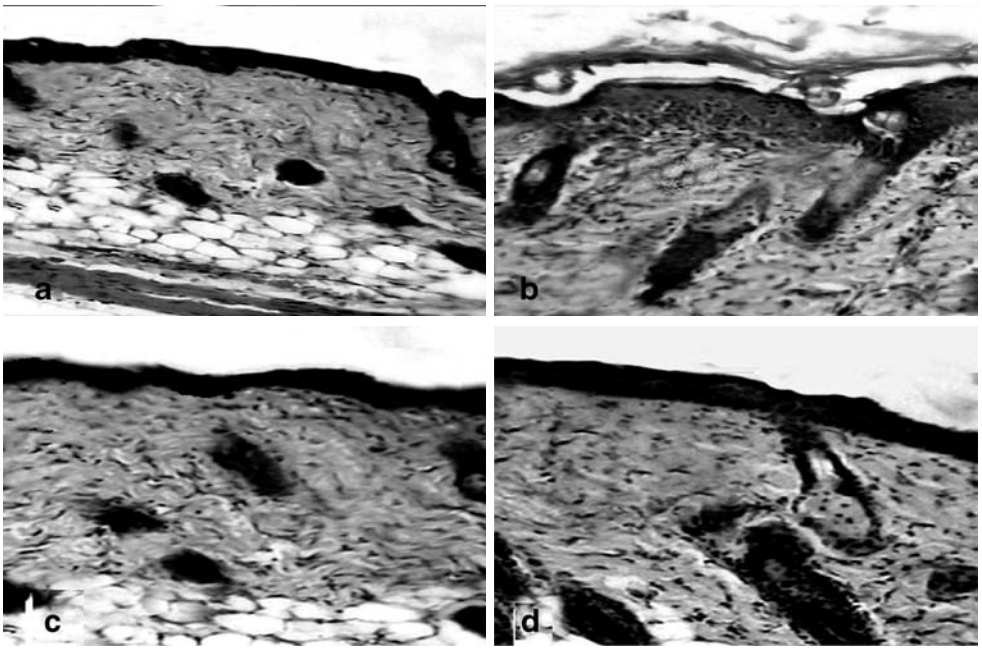

Fig. 2 **a** Untreated skin: the normal epidermis. **b** Photodamaged skin: acanthosis marked, hyperkeratoses, infiltrating inflammatory cells. **c** Topically treated with crude extract and **d** topically treated with Thalassiolin B: the skin is comparable to **a**, there is an increment of fibroblast and epidermal regeneration (H & E stain, ×10 magnification)

hydroxybenzoic acid (2) was also isolated from the LH20 chromatography. Pure 2 was obtained after silica gel column chromatography using 2:1 $CHCl_3$–AcOEt as eluent.

Thalassiolin B (1; chrysoeriol 7-β-D-glucopyranosyl-2″-sulphate) was isolated as a yellow amorphous solid with an $[M–H]^-$ ion at m/z=541 in the electrospray ionization mass spectrometry (ESIMS). The structure was confirmed by spectroscopic analysis (^1H and ^{13}C nuclear magnetic resonance (NMR), gCOSY60, gHSQC, gHMBC, and ROESY experiments) and compared to the literature (Jensen et al. 1998; Rowley et al. 2002). 4-Hydroxybenzoic acid (2) was isolated as a white crystalline solid and with an $[M–H]^-$ ion at m/z 139 in the ESIMS. The structure was confirmed by analysis of the ^{13}C and ^1H NMR.

Compounds 1 and 2 were tested for skin-regenerating activity (Fig. 4). The results (Fig. 1) show that serial administration of thalassiolin B (1) markedly restored skin damage induced by acute UVB radiation exposure by significantly reducing macroscopic alterations. Thalassiolin B (1) exhibits stronger activity than the crude extract. By the end of the experiment (6 days), almost all of the topically treated UVB-induced damaged skin (99%, N=10) had recovered, according to the macroscopic parameters examined, to the extent that there were no significant differences between the control

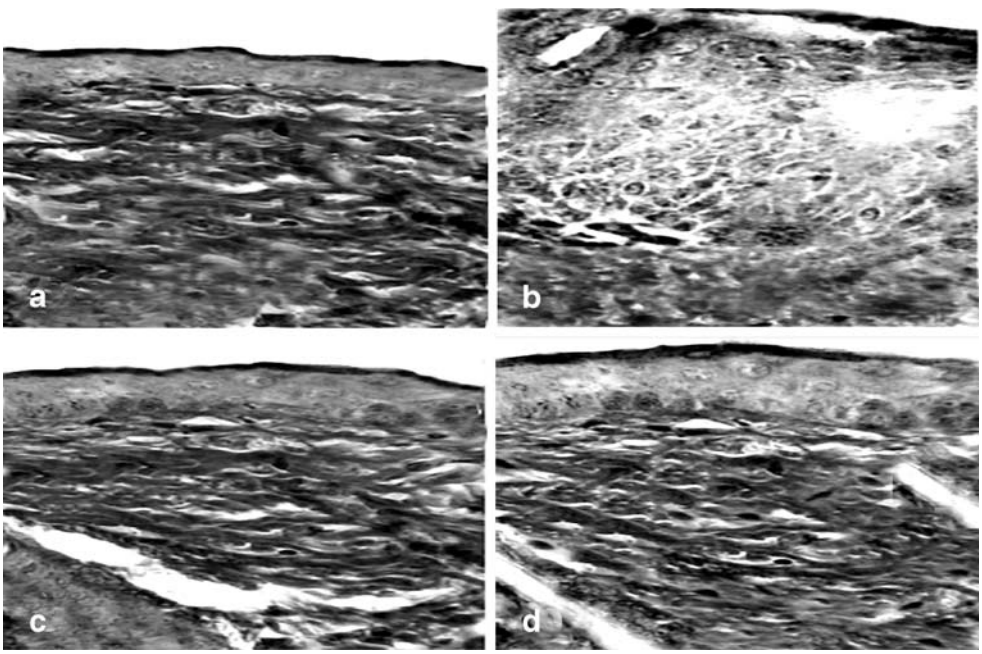

Fig. 3 **a** Untreated skin: the normal epidermis. **b** Photodamaged skin: acanthosis marked, hyperkeratoses, infiltrating inflammatory cells, collagen alterations. **c** Topically treated with crude extract and **d** topically treated with Thalassiolin B: epidermal regeneration, reorganized collagen fibers (Van Geison's stain, ×25 magnification)

Table 1 Scavenging activity (EC_{50}) on DPPH radicals and phenolic content (%) of samples from *T. testudinum*

Samples	DPPH Scavenged (EC_{50})[a], μg/ml	Total phenolic content[b], %
Crude extract	158	18±1.5
Fraction B	97	39±1.5
Fraction S	360	15±2.1
4-Hydroxybenzoic acid	>400	–
Thalassiolin B	100	–

[a] Values are expressed as mean±standard deviation (n=3). Coefficients of variance (CV) are less than 20%

[b] Results are expressed as pyrogallol equivalents. Values are also expressed as mean±standard deviation (n=3)

and the thalassiolin B (1) treated group. Histopathological examination (Figs. 2 and 3) confirmed the macroscopic observations. Thalassiolin B (1) treated skin exhibited little difference compared to nonirradiated skin and suggested that the skin of mice, treated with this compound, recovered to within the normal range over the treatment period. The elastic fiber content was within normal range; no detectable loss of collagen fiber was observed and no acanthosis and inflammatory infiltrates were observed. When 4-hydroxybenzoic (2) was applied topically no skin-regenerating activity was seen (Fig. 1).

Thalassiolin B (1) and 4-hydroxybenzoic acid (2) were assayed for radical trapping activity. Thalassiolin B (1) was found to scavenge DPPH radicals with an EC_{50}=100.0 μg/ml. However, 4-hydroxybenzoic acid (2) was found not to scavenge this radical at concentrations >400 μg/ml (Table 1).

Discussion

Several lines of evidence have shown that UVB-induced acute photodamage is characterized by degradation of collagen and accumulation of abnormal elastin in the superficial dermis. UV radiation causes collagen to break down at a higher rate than with just chronologic aging (Fisher et al. 1996, 2002). Mast cells and macrophages, which are found in higher numbers in photoaged skin, have been implicated in this process. Skin inflammation further traumatizes the damaged skin, which causes bleeding into the affected tissues, such that hemoglobin is released from the red blood cells (Kaminer 1995; Trautinger 2001). Our results show that UVB-damaged skin, which appears over a 48–72-h period in all animals, was gradually repaired upon topical application of thalassiolin B (1). Over the treatment period, histopathological studies revealed the presence of new connective tissue with parallel collagen bundles and a network of fine elastic fibers along with a decrease in mononuclear cells. We also observed that for treatment with both crude extract and thalassiolin B (1) the new collagen formed in the repair zone resembled that of the normal nonirradiated tissue with respect to the staining properties. The presence of fibroblasts, the major collagen-producing cells in skin, was also increased and had morphological features characteristic of high metabolic rate. These results suggest that the thalassiolin B (1) contributes to skin repair by allowing for increased synthesis of collagen fibers utilizing hyperactive fibroblasts.

Thalassiolin B (1) exhibits a strong radical scavenging effect against DPPH. Previous studies have demonstrated the radical trapping properties and the antilipoperoxidative action of phenolic containing compounds (Rice-Evans et al. 1996; Rice-Evans 2001; Nakai et al. 2006). Prooxidant states may well contribute in initiating UV-induced damage (Inal et al. 2001; Fuchs et al. 2001). UV acute exposure depletes antioxidant enzymes and induces protein oxidation in skin (Sander et al. 2002; Pillai et al. 2005; Sin and Kim 2005) which in so doing impairs the skins ability to protect itself against the free radicals generated after exposure to UVB radiation. This suggests that the skin-regenerating effects of thalassiolin B (1) may in part be due to its antioxidant effect.

Previous reports have shown that thalassiolin A has antibiotic activity against zoosporic fungi (Jensen et al. 1998). Also thalassiolins A, B, and C inhibit the HIV integrase protein (Rowley et al. 2002). In our study, we have demonstrated, for the first time, the antioxidant and skin-regenerating activity of thalassiolin B (1).

Experimental

General Experimental Procedures

Column chromatography was carried out using Silica gel 60 *Merck* (35–70 and 70–230 mesh), Sephadex LH-20 (Pharmacia, Sweden). Silica gel 60F254 TLC plates were used to check the purity of compound 2. TLC plates were visualized

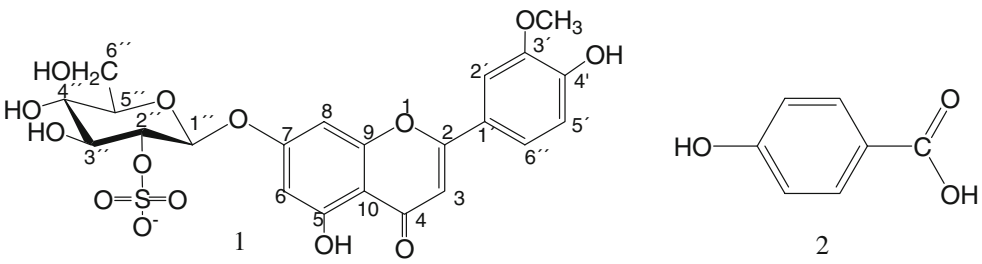

Fig. 4 Structures of compounds 1 and 2 isolated from *T. testudinum*

under UV light (254 and 365 nm) with a Vilber Lourmat lamp and subsequently sprayed with perchloric acid–vanillin (1%) on the TLC. K-1001 Knauer pump equipped with UV K-2501 detector and a Bondapack Waters C18 (10×250 mm) column were used for HPLC. All solvents used for HPLC were Fisher HPLC grade. NMR experiments were performed at ambient temperature on a Bruker AV-600 MHz spectrometer in dimethyl sulfoxide (DMSO-d_6; 1) and $CDCl_3$+CD_3OD (2). Low-resolution ESI-QIT-MS were recorded on a Bruker-Hewlett Packard 1100 Esquire-LC system mass spectrometer in negative mode (1). The EI mass spectrum of 2 was recorded on a MS Concept I/HQ mass spectrometer. UV absorption spectra were recorded on a Shimadzu UV-1201 spectrophotometer. Optical rotation was measured using a Jasco P-1010 Polarimeter with sodium light (589 nm).

Sea Grass Collection

T. testudinum (Banks and Soland ex. Koenig) was collected March 2004 from "La Concha" Beach (22° 05' 45" N 82° 27' 15" W) and identified by Dr. Areces J.A. (Institute of Oceanology, Havana, Cuba). A voucher sample (No. IdO39) has been deposited in the herbarium of the Cuban National Aquarium.

Extraction and Isolation

Whole dry and ground *T. testudinum* leaves (8 kg) were continuously extracted with EtOH–H_2O (50:50, vol/vol) over a period of 7 days at room temperature. The combined extracts were filtered and concentrated under reduced pressure and low temperature (40°C) to yield 48 g of residue. Bioassay-guided fractionation of the crude extract led to the isolation and identification of thalassiolin B (1) along with 4-hydroxybenzoic acid (2; see "Results" for details).

Measurement of Antioxidant Activity

2,2-Diphenyl-2-picrylhydrazyl Radical Scavenging Assay

The antioxidant activity of the crude extract, fractions B and S, and compounds 1 and 2 was measured in terms of radical scavenging ability according to DPPH method of Brand-Williams et al. (1995) with minor modifications. Basically, a 60-μM methanolic solution of DPPH (980 μl) [Sigma-Aldrich Co. (St. Louis, MO, USA)], prepared daily, was placed in a spectrophotometer cuvette, and different concentrations of samples in DMSO–MeOH (*v*/*v*) solution (20 μl) were added. The decrease in absorbance at 515 nm was determined at 1-min intervals, in a UV-1201 spectrophotometer (Shimadzu, Japan) with Data Capture software, until the reaction plateau step was reached; a time of 180 min was needed to arrive to this plateau step with all samples. Methanol was used to zero the spectrophotometer. EC_{50} values were determined from the plotted graph of scavenging activity against the concentration of samples. Triplicate measurements were carried out, and their scavenging effect was calculated based on the percentage of DPPH scavenged.

Total Polyphenols Content

Total phenolic content was determined according to the method described in the British Pharmacopeia (2007). The samples were extracted with 2 ml of distilled water at room temperature for 2 h and were centrifuged at 1,000×*g* for 15 min. The supernatant (5 ml) was mixed with water to 25 ml. This solution (2 ml) was mixed with 1 ml of Folin-Ciocalteu's phenol reagent, 10 ml of *water* and a 29.0% *w*/*v* solution of sodium carbonate to 25 ml. After 30 min, absorbance was read at 760 nm. Results were expressed as pyrogallol (1,2,3-Trihydroxybenzol) equivalents (Merck). The concentration used was in a range between 0.005 and 0.099 mg/ml.

Animals and Treatment

Animal care and experimental procedures were done in accordance to the Helsinki Declaration. The number of animals used in the present experiments was kept to a minimum required for meaningful interpretation of the data. Male albino mice (Balb/C, 25±1.5 g) obtained from the Center for Animal Lab Production (Havana, Cuba) were used. Before the experiments, animals were adapted to laboratory conditions with water and food ad libitum. Prior to the assay, mice were depilated in the back (2 cm^2). One day after depilation, the animals were divided into the experimental groups (ten animals each): an irradiated control, an irradiated control treated topically with vehicle, and the irradiated group treated with an antiaging cream containing natural extracts (Ultra Facial, UF, Zermat Internacional S.A). In the treated groups, crude extract, chromatographic fractions, and pure compounds isolated from the leaves of *T. testudinum* were topically applied at different concentrations. The base cream used as vehicle was a simple oil-in-water cosmetic emulsion prepared without preservatives and prepared by adding the same volume of distilled water. The crude extract, fractions, and pure compounds were dissolved in distilled water and mixed with this base cream by manual agitation to produce creams (0.008, 0.004, 0.002, and 0.001 mg of crude extract per milligram of cream (wt/wt) and 0.004 mg of the fractions per milligram of cream). Weighed aliquots of the cream were applied by spreading evenly over the dorsal area with a plastic applicator (60 mg/cm^2). Mice were held on their cage tops for approximately 15 min while the cream

was absorbed to discourage loss of cream by contact with the cage or bedding.

Experimental Design

Animals were exposed to ultraviolet radiation (UVB; 312 nm) with a Spectroline® lamp (Spectronics Corporation, New York, NY, U.S.A). Irradiance was at the back of the mice (30 cm from the lamp) for an interval of 6 min; immediately after exposure, animals were treated and, later on, at intervals of 24 h during 6 days. Daily, before each treatment, animals were observed by the same observer in order to detect the presence or not of macroscopic skin alterations. Dermal alterations, such as erythema, scabs, roughness, and wrinkling of the skin, were quantified in a 1 to 4 point value. At the end of the experiment, 24 h after the last treatment, mice were euthanized and the skins were collected for histological analysis.

Histological Analysis

Six animals per group were analyzed. Dorsal skins biopsy specimens, 2 cm, were processed for light microscopy. The samples were fixed in 10% buffered formalin for 24 h, processed routinely, and embedded in paraffin. Slices of 5 μm were used for the analyses and stained with hematoxylin and eosin and Van Gieson's for collagen. The slides were evaluated in coded manner.

Statistical Analysis

Statistical analyses were done by using the statistical package SPSS V.10.0 2000 for Windows. Comparisons between control and treated groups were done by the Mann Whitney *U* test. A two-tailed test was used and $P<0.05$ was considered to be statistically significant.

Acknowledgements The authors are grateful to Boehringer Ingelheim Fonds for financial support provided by a travel grant (E. L. R). We are also grateful to Prof. Raymond J. Andersen (Department of Chemistry, University of British Columbia, Vancouver, Canada) and Dr. Olivier P. Thomas (Department of Marine Natural Products, Faculty of Sciences, University of Nice, France) for collaborating in the results. This work was partially supported by a Grant-in-Aid for Scientific Research No. 038 in 2004–2006 from the Ministry of Science Technology and Environment of Cuba and a research grant (E. L. R) No. 4500045956 of the Intergovernmental Oceanographic Commission of the United Nations Educational, Scientific, and Cultural Organization.

References

Birt DF, Mitchell D, Gold B, Pour P, Pinch HC (1997) Inhibition of ultraviolet light induced skin carcinogenesis in SKH-1 mice by apigenin, a plant flavonoid. Anticancer Res 17:85–92

Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. Lebensm Wiss Technol 28:25–30

British Pharmacopoeia (2007) Tannins in Herbal Drugs (Method 2.8.14). Appendix XI M. Volume I & II. Version 11.0 DataÒ System Simulation Ltd. The Stationary Office (TSO), Norwich

Bulteau AL, Moreau M, Saunois A, Nizard C, Friguet B (2006) Algae extract mediated stimulation and protection of proteasome activity within human keratinocytes exposed to UVA and UVB irradiation. Antioxid Redox Signal 8:136–143

Corlett H, Jones B (2007) Epiphyte communities on *Thalassia testudinum* from Grand Cayman, British West Indies: their composition, structure, and contribution to lagoonal sediments. Sedimen Geol 194:245–262

Durako MJ, Kunzelman JI (2002) Photosynthetic characteristics of *Thalassia testudinum* measured in situ by pulse-amplitude modulated (PAM) fluorometry: methodological and scale-based considerations. Aquat Bot 73:173–185

Fisher GJ, Wang ZQ, Datta SC, Varani J, Kang S, Voorhees JJ (1996) Pathophysiology of premature skin aging induced by ultraviolet light. New Engl J Med 337:1419–1428

Fisher GJ, Datta SC, Talwar HS, Wang ZQ, Varani J, Kang S, Voorhees JJ (2002) Molecular basis of sun-induced premature skin ageing and retinoid antagonism. Nature 379:335–339

Fuchs J, Herrling T, Groth N (2001) Detection of free radicals in skin: a review of the literature and new developments. In: Thiele J, Elsner P (eds) Oxidants and antioxidants in cutaneous biology. Current problems in dermatology. vol. 29. Karger, Basel, pp 1–17

Fujimura T, Tsukahara K, Moriwaki S, Kitahara T, Sano T, Takema Y (2002) Treatment of human skin with an extract of *Fucus vesiculosus* changes its thickness and mechanical properties. J Cosmet Sci 53:1–9

Hwang IK, Yoo KY, Kim DW, Jeong SJ, Won CK, Moon WK, Kim YS, Kwon DY, Won MH, Kim DW (2006) An extract of *Polygonum multiflorum* protects against free radical damage induced by ultraviolet B irradiation of the skin. Braz J Med Biol Res 39:1181–1188

Inal ME, Kahramant A, Kökent T (2001) Beneficial effects of quercetin on oxidative stress induced by ultraviolet A. Clin Exp Dermatol 26:536–539

Jensen PR, Jenkins KM, Porter D, Fenical W (1998) Evidence that a new antibiotic flavone glycoside chemically defends the sea grass *Thalassia testudinum* against zoosporic fungi. Appl Environ Microb 64:1490–1496

Kaminer MS (1995) Photodamage: magnitude of the problem. In: Gilchrest BA (ed) Photodamage. Blackwell Science, Cambridge, pp 1–11

Kligman LH, Chen HD, Kligman AM (1984) Topical retinoid acid enhances the repair of ultraviolet damaged connective tissue. Connect Tissue Res 17:139–150

Littler DS, Littler MS (2000) Caribbean reef plants. An identification guide to the reef plants of the Caribbean, Bahamas, Florida and Gulf of México. Offshore Graphics, London, pp 480–484

Nakai M, Kageyama N, Nakahara K, Miki W (2006) Phlorotannins as radical scavengers from the extract of *Sargassum ringgoldianum*. Mar Biotechnol 8:409–414

Pillai S, Oresajo C, Hayward J (2005) Ultraviolet radiation and skin aging: roles of reactive oxygen species, inflammation and protease activation, and strategies for prevention of inflammation-induced matrix degradation: A review. Int J Cosmet Sci 27:17–34

Rice-Evans CA (2001) Flavonoids antioxidants. Curr Med Chem 8:797–807

Rice-Evans CA, Miller NJ, Paganga G (1996) Structure-antioxidant activity relationship of flavonoids and phenolic acids. Free Rad Biol Med 20:933–956

Rowley DC, Hansen MS, Rhodes D, Sotrifer CA, Ni H, Mccammon JA, Bushman FD, Fenical W (2002) Thalassiolins A–C: new marine-derived inhibitors of HIV cDNA integrase. Biorgan Med Chem 10:3619–3625

Saija A, Tomatino A, Trombetta D, De Pasquale A, Uccella N, Barbuzzi T, Paolino D, Bonina F (2000) In vitro and in vivo evaluation of caffeic and ferulic acids as topical photoprotective agents. Int J Pharm 199:39–47

Saliou C, Rimbach G, Moini H, McLaughlin L, Hosseini S, Lee J, Watson RR, Packer L (2001) Solar ultraviolet-induced erythema in human skin and nuclear factor-kappa-B-dependent gene expression in keratinocytes are modulated by French maritime pine bark extract. Free Radical Bio Med 30:154–160

Sander CS, Chang H, Salzmann S, Muller CSL, Akanayabe-Mudiyanelage S, Elsner P, Thiele JJ (2002) Photoaging is associated with protein oxidation in human skin in vivo. J Invest Dermatol 118:618–625

Sin BY, Kim HP (2005) Inhibition of collagenase by naturally-occurring flavonoids. Arch Pharm Res 28:1152–1155

Svobodova A, Psotova J, Walterova D (2003) Natural phenolics in the prevention of UV-induced skin damage: a review. Biomed Pap Med 147:137–145

Svobodova A, Walterova D, Vostalova J (2006) Ultraviolet light induced alteration to the skin. Biomed Pap Med 150(1):25–38

Trautinger F (2001) Mechanisms of photodamage of the skin and its functional consequences for skin ageing. Clin Exp Dermatol 26:573–577