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In-vitro Antioxidant and In-vivo Photoprotective Effect of Three Lyophilized Extracts of *Sedum telephium* L. Leaves

FRANCESCO BONINA, CARMELO PUGLIA, ANTONIO TOMAINO*, ANTONELLA SAIJA†, NADIA MULINACCI, ANNALISA ROMANI‡ AND FRANCO F. VINCIERI‡

*Department of Pharmaceutical Sciences, School of Pharmacy, University of Catania, *Department of Farmaco-Biologico, School of Pharmacy, University of Messina, †Department of Pharmacology of Natural Substances and General Physiology, School of Pharmacy, University of Roma "La Sapienza" and ‡Department of Pharmaceutical Sciences, School of Pharmacy, University of Firenze, Italy*

Abstract

Sedum telephium L. is a medicinal plant used in antiquity to cure many types of inflammatory skin diseases. The leaves (without the external cuticle), are used to promote healing and reduce skin inflammation and pain, and contain various components. We found two major components: flavonol glycosides and polysaccharides, with molecular weight between 13 000 and 13 500 Da. We evaluated the in-vitro antioxidant and in-vivo skin photoprotective effects of three lyophilized extracts obtained from the juice of *S. telephium* L. leaves: a total lyophilized juice, a lyophilized flavonolic fraction, and a lyophilized polysaccharidic fraction. Two in-vitro models were used: the bleaching of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) radical, and the protective effect against UV-induced peroxidation on phosphatidylcholine multilamellar vesicles, as model membranes. The antioxidant/radical scavenging activity of each lyophilized extract was also assessed in-vivo by determining their ability to reduce UVB-induced skin erythema (monitored by reflectance spectrophotometry) in healthy human volunteers.

The findings of the in-vitro experiments clearly demonstrated that, unlike the lyophilized polysaccharidic fraction, the lyophilized flavonolic fraction and total lyophilized juice possess strong antioxidant/free radical scavenging properties, which are likely due to phenolic compounds. Consistent with these findings, gel formulations of both the total lyophilized juice and, to a greater degree, the lyophilized flavonolic fraction appeared to possess a strong protective effect against UV-induced skin erythema in-vivo, whereas the lyophilized polysaccharidic fraction was completely ineffective.

The in-vitro and in-vivo results suggest that, both the total lyophilized juice and, in particular, the lyophilized flavonolic fraction, but not the lyophilized polysaccharidic fraction of *S. telephium* L. leaves, have photoprotective effects against UVB-induced skin damage.

In-vivo and in-vitro studies have shown that reactive oxygen species and free radicals are involved in both the inflammatory response elicited by acute UV skin exposure (skin erythema) (Black 1987), and in photo-ageing and carcinogenic processes induced by chronic UV skin irradiation (Griffiths et al 1998). Furthermore, the evidence that antioxidant skin systems appear impaired upon UV skin exposure suggests that the pro-oxidant/antioxidant balance can be overwhelmed by acute or chronic photo-oxidative stress (Emerit 1992). Recently, the use of antioxidants to prevent UV-induced skin damage has generated considerable interest. Topical administration of enzymatic and non-enzymatic antioxidants provides an excellent means of enriching the endogenous cutaneous protection system and therefore is an effective strategy for protecting the skin against UV-mediated oxidative damage (Duval et al 1992).

Sedum telephium L. (family Crassulaceae) has been widely used in antiquity to cure many types of inflammatory skin diseases. The leaves (without the external cuticle) or fresh juice are usually applied topically to painful wounds, burns and eczemas to

Correspondence: F. Bonina, Department of Pharmaceutical Sciences, University of Catania, Viale A. Doria n.6, 95125 Catania, Italy.
E-mail: boninaf@mbox.unict.it

promote healing and reduce inflammation and pain. *S. telephium* L. leaves contain a small amount of gallic acid and two main groups of components: flavonol glycosides (characterized as glycosylated quercetin and kaempferol), and polysaccharides (characterized as branched neutral rhamnogalacturonans), with molecular weight between 13 000 and 13 500 Da (Sendl et al 1993; Mulinacci et al 1995a,b).

Many studies have focussed on the biological properties of quercetin and kaempferol glycosides, and, in particular, on their antioxidant and free radical scavenging activity (Rice-Evans et al 1996; Plumb et al 1999). The antioxidant activity of gallic acid is also well recognized (Rice-Evans et al 1996). Moreover, the polysaccharides isolated from *S. telephium* L. have been shown in in-vitro and in-vivo experiments to exert an anti-complementary and anti-inflammatory effect, to induce α-TNF production and to enhance phagocytosis (Sendl et al 1993).

We evaluated the in-vitro antioxidant and in-vivo skin photoprotective effect of three lyophilized extracts obtained from the juice of *S. telephium* L. leaves: a total lyophilized juice, a lyophilized flavonolic fraction, and a lyophilized polysaccharidic fraction. The antiradical and antioxidant properties of these lyophilized extracts, and of some representative biophenols (quercitin, kaempferol and gallic acid) contained in *S. telephium* L. leaves, were evaluated in two in-vitro models: the bleaching of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical, and the protective effect against the UV-induced peroxidation on phosphatidylcholine multilamellar vesicles, as model membranes. The use of two different tests, in an homogenous solution and in a membranous system, is necessary to determine the actual effectiveness and suitability of a potential antioxidant, in terms of its hydrogen-donating properties and also its partitioning ability between the aqueous medium and the lipophilic environment. In addition, the latter test is largely used to investigate the potential photoprotective effectiveness of topically-applied antioxidant agents (Bonina et al 1996; Saija et al 1999). We also assessed the antioxidant/radical scavenging activity of the three lyophilized extracts in-vivo, by determining their ability to reduce UVB-induced skin erythema (monitored by reflectance spectrophotometry) in healthy human volunteers. This test is regarded as one of the most suitable models for studying in-vivo skin damage after acute UV exposure (Montenegro et al 1995, 1997; Bonina et al 1996, 1998). Four gel formulations containing one of the three lyophilized extracts or tocopheryl acetate (used as reference), were topically applied after skin exposure to UVB irradiation.

Materials and Methods

Materials

Fresh leaves of *S. telephium* L. were collected in August 1996 from an experimental field near Florence and stored at −30°C. Carbopol 934 was a gift from Biochim (Milan, Italy). Tocopheryl acetate, triethanolamine, DPPH• radical, phosphatidylcholine, gallic acid and ethanol were all purchased from Sigma-Aldrich (Milan, Italy). Quercetin and kaempferol were purchased from Extrasynthèse (Genay, France).

Preparation of lyophilized extracts of S. telephium *L.*

S. telephium L. fresh leaves were collected and immediately frozen in liquid nitrogen. The sample was then ground, centrifuged at 5800 *g* for 10 min, and different fractions of the supernatant were used to prepare the lyophilized extracts. One fraction (100 mL) was frozen and lyophilized to obtain the total lyophilized juice. Another fraction (400 mL) was placed at 4°C for 12 h to induce the precipitation of the less soluble polysaccharide. The residual supernatant was diluted with three volumes of ethanol and the suspension obtained stirred for 12 h and left standing at 4°C for 8 h. After centrifugation at 5800 *g* for 10 min, another polysaccharide fraction was collected, washed with ethanol, frozen and lyophilized to give the lyophilized polysaccharidic fraction extract. The corresponding clear supernatant was frozen and lyophilized to obtain the lyophilized flavonolic fraction. For the biological tests, all the freeze-dried fractions were used after reconstitution in appropriate medium.

HPLC/DAD analysis

The qualitative–quantitative evaluation of the polyphenol content in the lyophilized flavonolic fraction was carried out according to the previously reported HPLC/DAD method (Mulinacci et al 1995a). Calibration curves at 350 nm obtained with previously-isolated kaempferol 3,7-*O*-dirhamnoside and quercetin 3,7-*O*-dirhamnoside (Mulinacci et al 1995b), were used to quantify the flavonol content. To evaluate the content of gallic acid in the lyophilized flavonolic fraction, a specific calibration curve at 280 nm obtained using the pure commercial standard was used.

For all the flavonol glycosides, the data were expressed using 600 g mol^{-1} as the median molecular weight. Quantitative analyses were also carried out on the total lyophilized juice and the lyophilized polysaccharidic fraction under the same experimental conditions.

In-vitro free radical scavenging and antioxidant activity

Free radical scavenging effect on the DPPH$^{\bullet}$ radical. The antiradical activity of the total lyophilized juice, lyophilized flavonolic fraction and lyophilized polysaccharidic fraction, and that of quercetin, kaempferol and gallic acid, was determined using the stable DPPH$^{\bullet}$ radical, as previously described (Brand-Williams et al 1995; Sánchez-Moreno et al 1998). In its radical form, DPPH$^{\bullet}$ has an absorption band at 515 nm which disappears upon reduction by an antiradical compound. Methanol solution (37·5 μL) containing different concentrations of each lyophilized extract or biophenol under investigation was added to 1·5 mL DPPH$^{\bullet}$ solution (0·025 g L^{-1} in methanol prepared daily); for the lyophilized extracts the maximum concentration used was 200 μg mL^{-1}. Absorbance at 515 nm was measured on a Shimadzu UV-1601 UV-vis spectrophotometer until the reaction reached steady state. All experiments were carried out in triplicate and repeated at least three times. The DPPH$^{\bullet}$ concentration in the reaction medium was calculated from a calibration curve analysed by linear regression. The percentage of DPPH$^{\bullet}$ remaining at steady state was calculated as follows:

$$\%\mathrm{DPPH}^{\bullet}\ \text{remaining} = [\mathrm{DPPH}^{\bullet}]_t/[\mathrm{DPPH}^{\bullet}]_0 \times 100$$

where t is the time needed to reach steady state. The percentage of DPPH$^{\bullet}$ remaining at steady state was plotted against the antioxidant concentration to obtain the concentration of antioxidant necessary to decrease the initial concentration by 50% (EC50). Based on the time needed to reach steady state at the concentration corresponding to EC50, the kinetic behaviour of the antioxidant compounds and extracts tested was classified as follows: <5 min (rapid), 5–30 min (intermediate), >30 min (slow).

Protective effect against UV-induced peroxidation in liposomal membranes

The protective effect of the total lyophilized juice, lyophilized flavonolic fraction and lyophilized polysaccharidic fraction, and that of quercetin, kaempferol and gallic acid, against UV-induced peroxidation was evaluated on phosphatidylcholine multilamellar vesicles (Bonina et al 1996; Saija et al 1999). Briefly 1·0 mL liposome suspension (in a glass flask with a 3-cm^2 exposure surface area) was exposed to UV-irradiation from a 15 watt Philips germicidal lamp (254 nm) for 1·5 h. Exposure was at 10 cm from the lamp, at room temperature. Different concentrations of each lyophilized extract (up to 200 μg mL^{-1}) or each biophenol under investigation, were added to the system. An equal volume (50 μL) of vehicle alone was added to control tubes. The malondialdehyde concentration in the mixture was measured using a colorimetric assay kit (Calbiochem-Novabiochem Corp., La Jolla, CA). All experiments were carried out in triplicate and repeated at least three times. Results are expressed as the percentage decrease with respect to control values and mean inhibitory concentrations (IC50) were calculated using the Litchfield & Wilcoxon test.

In-vivo photoprotective effect

Preparation of aqueous gels containing extracts. Carbomer gels were prepared by dispersing (with constant stirring) Carbopol 934 (0·8% w/w final concn) and triethanolamine (0·9% w/w final concn) in a solution of water–ethanol (60 : 40) containing a suitable concentration of the total lyophilized juice, lyophilized flavonolic fraction, lyophilized polysaccharidic fraction or tocopheryl acetate (used as reference), to give a final concentration of 2% active ingredients. The resulting gels were stored at room temperature for 24 h under air-tight conditions before use. A gel formulation without active compounds was used as control.

Instruments. UVB-induced skin erythema was monitored as previously reported (Montenegro et al 1995, 1997; Bonina et al 1996, 1998), using a reflectance visible spectrophotometer (X-Rite model 968; X-Rite Inc., Grandville, MI) with 0° illumination and a 45° viewing angle. The instrument was calibrated with the supplied white standard traceable by the National Bureau of Standard's perfect white diffuser. The spectrophotometer was controlled by a computer, which performed all colour calculations from the spectral data by means of menu-driven programs (Spectrostart; X-Rite Inc., Grandville, MI) supplied with the instrument. Reflectance spectra were obtained over the wavelength range 400–700 nm using illuminant C and 2° standard observer.

Protocol. In-vivo experiments were performed on six volunteers, aged 25–35 years, of both sexes. The subjects were fully informed about the nature of the study and the procedures involved and they all gave their written consent. No subject was known to exhibit abnormal sensitivity to sunlight, or was taking any medication at the time of the study. The experiments were performed under standardized room conditions ($22 \pm 2^{\circ}$C and 40–50% r.h.) after a resting time of 15 min. Skin erythema was induced by UVB irradiation using an ultraviolet lamp (model UVM-57; UVP, San Gabriel, CA). This source emits over the range of 290–320 nm with an output peak at 302 nm. The flux rate measured at the skin surface was $0{\cdot}80\,\mathrm{mW\,cm^{-2}}$. For each subject, the minimal erythemal dose was preliminarily determined and an irradiation dose corresponding to double this dose was used throughout the study. For each subject, ten sites on the ventral surface of the forearms were defined using a circular template ($1\,\mathrm{cm^2}$) and demarcated with permanent ink. Skin sites were exposed to UVB irradiation and then the test gel formulations (100 mg) were spread uniformly on eight sites (each gel formulation on two sites) using a solid glass rod. For each volunteer, two controls were used by applying the gel formulation without active compounds on two sites. After gel application, each skin site was occluded for 3 h using Hill Top chambers (Hill Top Research, Inc., Cincinnati, OH), to prevent any loss of material from the skin surface. After the occlusion period, the chambers were removed and the skin surfaces were washed to remove the gel and allowed to dry for 15 min. The induced erythema was monitored for 58 h by reflectance spectrophotometry. From the reflectance spectral data obtained, the erythema index (EI) was calculated using an equation similar to that reported by Dawson et al (1980):

$$\begin{aligned} \mathrm{EI} = 100 \times (((\log 1/R_{560} + 1{\cdot}5) \\ \times (\log 1/R_{540} + \log 1/R_{580})) \\ - 2(\log 1/R_{510} + \log 1/R_{610})) \end{aligned} \quad (1)$$

where 1/R is the inverse reflectance at a specific wavelength (560, 540, 580, 510 and 610 nm). EI baseline values were taken at each designated site before UVB irradiation of gel formulations and subtracted from the EI values obtained after UVB exposure at each time point, to obtain ΔEI values. For each site, ΔEI was plotted against time and the area under the curve (AUC_{0-58}) was computed using the trapezoidal rule.

To better compare the efficacy of the different gel formulations tested, the percentage inhibition of UVB skin erythema was calculated from AUC_{0-58} values using the following equation:

$$\%\ \text{Inhibition} = ((AUC_c - AUC_t)/AUC_c) \times 100 \quad (2)$$

where AUC_c is the area under the response–time curve of the sites treated with the control gel formulation and AUC_t is the area under the response-time curve of the sites treated with test lyophilized extract or tocopheryl acetate gel formulations. Statistical analysis was performed using Student's *t*-test.

Results and Discussion

Chemical composition of S. telephium *L. extracts*

The findings of the elemental analyses and yields of the lyophilized fractions expressed as percentage (w/w) with respect to fresh weight of the leaf sample, are summarized in Table 1. The elemental analysis on the lyophilized polysaccharidic fraction showed the absence of nitrogen, thus excluding the presence of proteins in this extract. The reverse phase HPLC/DAD analyses were carried out on the total lyophilized juice and lyophilized flavonolic fractions to evaluate the concentration of the polyphenolic compounds. The residual content of flavonols in the lyophilized polysaccharidic fraction was not greater than $1 \times 10^{-5}\,\mathrm{mmol\,g^{-1}}$ and the gallic acid content was not greater than $1 \times 10^{-4}\,\mathrm{mmol\,g^{-1}}$. The highest flavonol content was revealed for the lyophilized flavonolic fraction with 5·71% glycosides with respect to the total dried weight of the sample.

In-vitro free radical scavenging and antioxidant activity

The results of the in-vitro tests are reported in Tables 2 and 3 ($DPPH^{\bullet}$ test) and Table 4 (UV-induced peroxidation). For the $DPPH^{\bullet}$ test, the %$DPPH^{\bullet}$ remaining (evaluated at a concn of $95\,\mu\mathrm{g\,mL^{-1}}$ for all extracts) gave the following effectiveness order: lyophilized flavonolic fraction $\gg$ total lyophilized juice $\gg$ lyophilized polysaccharidic fraction (Table 3). In addition, the scavenging effect elicited by the lyophilized flavonolic fraction and the total lyophilized juice was concentration-dependent, and the respective EC50 values were calculated (Table 2). The kinetic behaviour of the total lyophilized juice and the lyophilized flavonolic fraction appeared consistent with that observed for the reference drugs (quercetin, kaempferol and gallic acid), indicating that the antioxidant activity of these extracts is related mainly to their biophenol content (Table 3).

Table 1. Concentrations of gallic acid, quercetin and kaempferol glycosides in the *Sedum telephium* L. lyophilized extracts.

Extract	Quercetin glycosides (%)	Kaempferol glycosides (%)	Gallic acid (%)	Yield (w/w)
Total lyophilized juice	1·16	2·16	0·24	2–2·1
Lyophilized flavonolic fraction	1·98	3·73	0·42	1·2–1·3
Lyophilized polysaccharidic fraction	< 0·006	< 0·006	< 0·002	0·8–0·82

Data are expressed as percentage values with respect to the dried weight of each sample. The yields of the lyophilized fractions with respect to the fresh weight of the leaf sample are also given.

Table 2. Scavenging activity of the three *Sedum telephium* L. extracts and three representative biophenols against the free radical DPPH$^{\bullet}$, according to their kinetic behaviour.

Compounds	EC50 (μg mL^{-1})	Kinetic behaviour
Total lyophilized juice	94·33 ± 3·24	Slow
Lyophilized flavonolic fraction	25·02 ± 0·82	Slow
Lyophilized polysaccharidic fraction	>200	
Quercetin	1·73 ± 0·09	Slow
Kaempferol	4·23 ± 0·37	Intermediate
Gallic acid	0·92 ± 0·06	Intermediate/ slow

Kinetic behaviour: < 5 min = rapid, 5–30 min = intermediate and >30 min = slow. Data are expressed as mean ± s.d. of three experiments. EC50 = drug concentration required to decrease the initial concentration by 50%.

Table 3. Kinetic behaviour of three *Sedum telephium* L. extracts in the reaction with the free radical DPPH$^{\bullet}$.

Extract	Concentration (μg mL^{-1})	DPPH$^{\bullet}$ remaining (%)
Total lyophilized juice	94·33	49·73 ± 1·03
Lyophilized flavonolic fraction	94·33	14·02 ± 0·29
Lyophilized polysaccharidic fraction	94·33	91·38 ± 2·55

Values of DPPH$^{\bullet}$ remaining at steady state are expressed as mean ± s.d. of three experiments.

Table 4. Inhibition of UV-induced peroxidation on phosphatidylcholine liposomes by increasing concentrations of the three *Sedum telephium* L. extracts and three representative biophenols.

Drugs	IC50 (μg mL^{-1})	95% Confidence limits (μg mL^{-1})
Total lyophilized juice	84·24	69·56–102·98
Lyophilized flavonolic fraction	60·31	52·83–72·15
Lyophilized polysaccharidic fraction	245·4	185·09–326·48
Quercetin	1·73	1·33–1·95
Kaempferol	5·86	4·97–6·31
Gallic acid	28·89	20·61–35·94

For UV-induced peroxidation, exposure of phosphatidylcholine liposomal membranes to UV light for 1·5 h elicited a large increase in malondialdehyde production. The addition of the lyophilized flavonolic fraction and, to a lesser extent, the total lyophilized juice, reduced the amount of malondialdehyde formed in a concentration-dependent manner which allowed calculation of the IC50 (Table 4). The lyophilized polysaccharidic fraction, tested at concentrations up to 200 μg mL^{-1}, was completely ineffective.

The in-vitro experiments clearly show that the lyophilized flavonolic fraction and total lyophilized juice possess strong antioxidant/free radical scavenging properties. From the concentration of phenolic compounds (flavonols and hydroxycinnamic acids) in lyophilized flavonolic fraction and total lyophilized juice (Table 1), and the EC50 values calculated for some representative biophenols (quercetin, kaempferol and gallic acid) (Table 2), the antioxidant effectiveness of these extracts appears to be due to their phenolic active principles. The lyophilized polysaccharidic fraction, which contains only small quantities of biophenols, appeared not to have any antioxidant activity. Furthermore, consistent with its higher phenolic content, the lyophilized flavonolic fraction showed greater antioxidant activity than the total lyophilized juice. However, the difference in the antioxidant effectiveness of the lyophilized flavonolic fraction and the total lyophilized juice was less evident in the UV-induced peroxidation test than in the DPPH$^{\bullet}$ test. It could be that the vegetable matrix present in the total lyophilized juice may influence the interaction of phenolic active principles with radicals generated in the aqueous medium or within lipid membranes differently. It is known that some flavonoids and hydroxycinnamic acids act as strong UV absorbers (Bonina et al 1996; Saija et al 1999); thus we cannot exclude that, in addition to scavenging UV-induced radicals, the active principles contained in the lyophilized flavonolic fraction and total lyophilized juice may provide a protective effect against UV-induced damage by acting as UV-absorbing screens.

In-vivo photoprotective effect

The protective effect of gel formulations containing the lyophilized extracts or tocopheryl acetate, an antioxidant commonly used in cosmetic formulations, against UVB-induced skin erythema was assessed in human volunteers using a reflectance spectrophotometry to monitor the extent of erythema. The time course of erythema for skin treated with the total lyophilized juice, lyophilized flavonolic fraction, lyophilized polysaccharidic fraction and tocopheryl acetate gel formulations is shown in Figure 1. From ΔEI plots against time, the areas under the response–time curve (AUC) were computed using the trapezoidal rule, and AUC values are reported in Table 5. The total lyophilized juice, lyophilized flavonolic fraction and tocopheryl acetate gel formulations inhibited UVB-induced skin erythema, as the AUC values for skin sites treated with these formulations were lower and significantly different from those of the control gel base formulation. Table 5 reports the percentage inhibition of erythemal calculated for the total lyophilized juice, lyophilized flavonolic fraction and tocopheryl acetate gel formulations. Both the total lyophilized juice and lyophilized flavonolic fraction gel formulations appeared to possess an protective effect against UV which was greater than that elicited by the natural antioxidant tocopheryl acetate. The lyophilized flavonolic fraction was the most effective gel formulation (inhibition = 61·7%). The lyophilized polysaccharidic fraction extract did not inhibit UVB-induced skin erythema, as the AUC values obtained for skin sites treated with the corresponding gel were not significantly different from the control values (Table 5).

The results of the in-vivo photoprotective evaluation were in good agreement with the radical scavenging/antioxidant activity observed in in-vitro experiments. Thus, the greater photoprotective effect of the lyophilized flavonolic fraction, compared with the total lyophilized juice, could be related to the higher in-vitro antiradical and antioxidant activity shown by the flavonolic fraction.

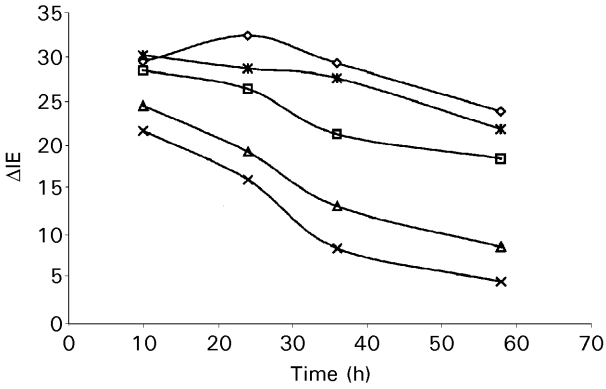

Figure 1. Typical trend of erythema index variations (ΔIE) vs time for one subject. Gel formulation containing the total lyophilized juice (△), lyophilized flavonolic fraction (×), lyophilized polysaccharidic fraction (⁎), tocopheryl acetate (□) or without active compounds (control; ◇) were applied to the skin immediately after UVB irradiation.

In addition to antioxidant properties, flavonoids possess an interesting anti-inflammatory profile related to their ability to interfere with a variety of processes involved in mediator release (e.g. arachidonic metabolism, histamine release from mast cells and basophils, Ca^{2+}-regulated events and neutrophil respiratory burst) (Kahl 1991). These properties may contribute to the observed in-vivo protective effect of the lyophilized flavonolic fraction and the total lyophilized juice extracts against UV-induced skin inflammation. Lipid peroxidation products exert acute inflammatory effects in mammalian skin (Fuchs & Packar 1991) and the usefulness of the UVB-erythema test for the evaluation of steroidal and nonsteroidal anti-inflammatory agents has been reported (Hughes-Formella et al 1998).

In this study, the lyophilized polysaccharidic fraction appeared not to protect against UVB-

Table 5. Area under the curve values obtained by applying the total lyophilized juice, lyophilized flavonolic fraction, lyophilized polysaccharidic fraction, tocopheryl acetate or control gel formulations to UVB-exposed skin sites.

Subject	Control	Tocopheryl acetate	Lyophilized flavonolic fraction	Lyophilized polysaccharidic fraction	Total lyophilized juice
1	1387·6	1108·3	532·7	1293·5	741·4
2	1563·0	873·4	473·2	1094·7	705·4
3	1125·4	1279·5	668·5	1415·6	934·7
4	1623·5	1013·4	612·7	1127·5	848·8
5	1275·8	1167·2	537·4	1343·1	975·1
6	1482·7	823·2	411·3	903·5	635·7
Mean ± s.d	1409·6 ± 86·4	1026·5 ± 172·8**	539·3 ± 92·61**,‡	1196·1 ± 189·4	806·8 ± 134·3**,†
Inhibition of erythema (%)	–	27·2	61·7	–	42·7

**$P < 0{\cdot}01$ vs control; †$P < 0{\cdot}05$ and ‡$P < 0{\cdot}01$ vs tocopheryl acetate.

induced skin erythema (Figure 1 and Table 5). These in-vivo findings are consistent with the in-vitro results, indicating that the lyophilized polysaccharidic fraction lacks antioxidant activity (Tables 2–4). Thus, the lack of in-vivo photoprotective activity might be related to its very low in-vitro antioxidant activity.

The polysaccharides isolated from *S. telephium* L. leaves were previously shown to possess anti-inflammatory activity (Sendl et al 1993). However, the anti-inflammatory potential of these polysaccharides is likely due to their anticomplementary effect and thus might not be evidenced by the UVB erythema test used in our study. Furthermore, suitable percutaneous absorption is an essential requirement for topically-applied photoprotective agents (Bonina et al 1996). The polysaccharides contained in the lyophilized polysaccharidic fraction are unlikely to permeate human skin because of their high polarity and molecular weight, further justifying their lack of in-vivo photoprotective activity.

In conclusion, the in-vitro and in-vivo findings demonstrate that the total lyophilized juice and, in particular, the lyophilized flavonolic fraction isolated from *S. telephium* L. leaves give excellent photoprotection against UVB-induced skin damage. These extracts could have important therapeutic applications in certain skin diseases caused, initiated or exacerbated by excessive free radical production.

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