Quercetin and Rutin as Potential Sunscreen Agents: Determination of Efficacy by an in Vitro Method

Benjamin Choquenet, Céline Couteau, Eva Paparis, and Laurence J. M. Coiffard*

Faculty of Pharmacy, Université de Nantes, Nantes Atlantique Universités, LPiC, SMAB, EA2160, 1 Rue G. Veil–BP 53508, Nantes, F-44000 France

Received December 18, 2007

Given that flavonoids are known for their ultraviolet (UV)B photoprotective properties in plants that contain them, we chose to study quercetin (**1**) and rutin (**2**) as agents that could potentially be used in sunscreen products. These two substances proved to behave in similar ways. When incorporated in oil-in-water emulsions, at a concentration of 10% (w/w), **1** and **2** give sun protection factor (SPF) values similar to that of homosalate, a standard substance. These two flavonoids also provided a non-negligible level of photoprotection in the UVA range. When used in association with titanium dioxide, the SPF obtained was around 30.

Overexposure to ultraviolet radiation can cause skin damage. This can be immediate and long-term, with effects ranging from sunburn and premature wrinkling to carcinogenesis.[1–3] Sunscreens have been used for many years on exposed areas to protect the skin from the damaging effects of ultraviolet light. Although sunscreens are essential, some have adverse effects such as estrogenic activity[4,5] and photoallergenicity.[6] Hence, the present work was carried out to test the hypothesis that quercetin or 3′,4′-dihydroxyflavonol (**1**) and rutin or quercetin-3-*O*-β-rutinoside (**2**) might possess photoprotective activity. Flavonoids constitute an important class of natural compounds well-known to have antioxidant,[7,8] antimalarial,[9] anti-inflammatory,[10] and antibacterial[11] activities. The aim of this paper was to investigate the characteristics of **1** and **2** and to determine the effects of their association with inorganic UV filters, on both the sun protection factor (SPF) and the protection factor UVA (PF-UVA) values of topically applied sunscreen formulations, using an in vitro method. Spectra measured for compounds **1** and **2** in this study were very similar and gave peaks of absorption at 373 and 341 nm, respectively. For each substance, we studied the influence of concentration on their effectiveness in the UVB and UVA range (Figure 1). If **1** and **2** are compared with UVB filters currently authorized by the European Union, it can be noted that when used at a concentration of 10% (w/w), they would be ranked in the ninth position (9/18), with an efficacy comparable to that obtained with homosalate (a reference filter used to establish FDA standards). As far as their efficacy against UVA is concerned, they are also both of interest (ranked 5th out of 7 filters authorized), all the more so since they have similar levels of protection against both UVB and UVA, rendering a SPF/PF-UVA ratio of less than 3. These results confirm the attraction of using flavonoids as photoprotective agents by plants.[12] After 2 h of irradiation at 650 W/m^2, **1** and **2** both proved to be photostable. Indeed, in the case of a **1**- or **2**-based sun product, more than 90% efficacy was conserved (Table 1). In order to formulate sun products that contain no regulated organic filters, various combinations were carried out. Three series of combinations were made using the same percentage of each ingredient, (10% w/w): first only flavonoids, then combinations with titanium dioxide, and finally with zinc oxide. In this way each combination was assessed for additive effect, synergy, or incompatibility. Table 2 shows the synergistic effect obtained from the combinations with **1** and **2** in the field of both UVB and UVA. The combination of **1** with titanium dioxide was also synergistic (Table 2). This gave a product whose SPF was about 30 (with a SPF/PF-UVA ratio of 1.81), an indicator of good protection for the whole range of UV likely to cause unwanted effects. In the same way, the combination of **2** with titanium dioxide also gave interesting results (Table 2). The combinations carried out with zinc oxide showed a purely additive effect (Table 2). However, the SPF values reached (10 for **1** and 11 for **2**) enabled only a moderate level of protection to be obtained. Given that a product is only judged effective at an SPF of 15 and above, these combinations are not considered worthy of follow-up, all the more so since it is more difficult to make use of zinc oxide than titanium dioxide.

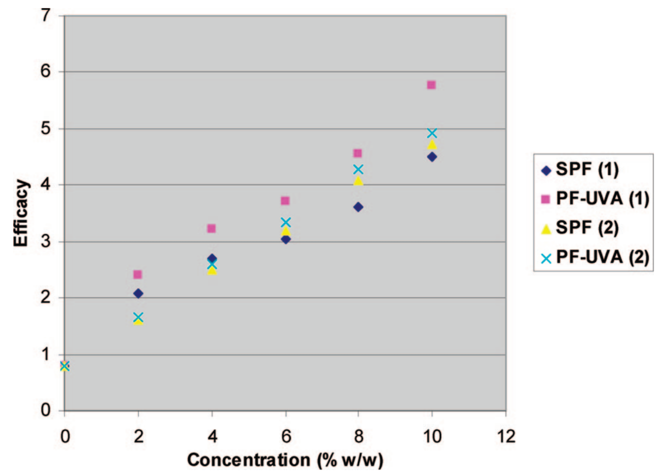


Figure 1. Influence of compounds **1** and **2** on SPF and PF-UVA.

Table 1. Photostability of **1** and **2**

	compound **1** (mean ± SD)	compound **2** (mean ± SD)
SPF(t_0)	4.52 ± 0.38	4.72 ± 0.20
SPF ($t_{120'}$)	5.64 ± 0.46	4.42 ± 0.13
PF-UVA (t_0)	5.77 ± 0.55	4.92 ± 0.20
PF-UVA ($t_{120'}$)	6.15 ± 0.58	4.59 ± 0.13

Experimental Section

General Experimental Procedures. Aqueous solutions were scanned at wavelengths between 200 and 400 nm using a double-beam spectrophotometer (Hitachi UV–visible, model U-2000). The spectra were measured against a pure water sample in quartz cells with a 1 cm optical path length.

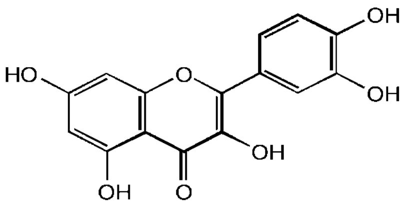
Chemicals. Quercetin (**1**) and rutin (**2**) were obtained from Fisher Bioblock (Illkirch, Germany). Dimethicone (Abil WE 09) was obtained from Goldschmidt (Montigny-le-Bretonneux, France). Cetiol HE, stearic

* Corresponding author. Tel: + 33 2 40 41 28 73. Fax: + 33 2 40 41 29 87. E-mail: laurence.coiffard@univ-nantes.fr.

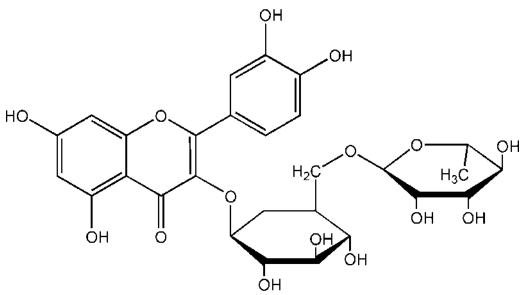
10.1021/np7007297 CCC: $40.75
Published on Web 05/31/2008

Table 2. Effect of Combinations on SPF and PF-UVA

	compound **1** (10% w/w)	compound **2** (10% w/w)	TiO_2 (10% w/w)	ZnO (10% w/w)
compound **1** (10% w/w)		SPF = 12.34 ± 2.08 PF-UVA = 14.61 ± 2.57	SPF = 29.70 ± 4.96 PF-UVA = 16.42 ± 2.34	SPF = 9.97 ± 1.67 PF-UVA = 10.28 ± 1.63
compound **2** (10% w/w)	SPF = 12.34 ± 2.08 PF-UVA = 14.61 ± 2.57		SPF = 34.29 ± 8.31 PF-UVA = 16.25 ± 2.71	SPF = 11.25 ± 3.31 PF-UVA = 9.75 ± 2.81



1



2

acid, glycerin, parabens, and triethanolamine (TEA) were purchased from Cooper (Melun, France). Xanthan gum (Keltrol BT) was obtained from Kelco (Lille Skensved, Denmark). Tayca MT-100TV (titanium dioxide, aluminum hydroxide, stearic acid) and Z-Cote Max (zinc oxide, diphenyl capryl methicone) were obtained, respectively, from Unipex (St. Ouen l'Aumône, France) and BASF (Levallois Perret, France).

Determination of Efficacy as Sunscreen Agents. An o/w emulsion was prepared in the laboratory by adding known concentrations of substances tested into the formulation components. A detailed description of the preparation of this formula can be found in a previous paper.[13] Compounds **1** and **2** at various concentrations were incorporated alone or in association with TiO_2 or ZnO into creams. Then, 30 mg of product precisely weighed was spread across the entire surface (25 cm^2) of a PMMA plate (Helioscience, Creil, France) using a cot-coated finger. After spreading, 15 mg remained on the finger cot. PF-UVA values of the creams were then measured in vitro. Three plates were prepared for each product to be tested, and nine measurements were performed on each plate. Transmission measurements between 290 and 400 nm and between 320 and 400 nm, respectively, for SPF and PF-UVA were carried out using a spectrophotometer equipped with an integrating sphere (UV transmittance analyzer UV1000S Labsphere, North Sutton, NH). The calculations were carried out according to the following equations:

$$\mathrm{SPF} = \sum_{290}^{400} E_\lambda I_\lambda \Delta_\lambda \Big/ \sum_{290}^{400} E_\lambda I_\lambda T_\lambda \Delta_\lambda \qquad (1)$$

$$\mathrm{PF\text{-}UVA} = \sum_{290}^{400} E_\lambda I_\lambda \Delta_\lambda \Big/ \sum_{290}^{400} E_\lambda I_\lambda T_\lambda \Delta_\lambda \qquad (2)$$

where E_λ is the spectral irradiation of terrestrial sunlight at λ, I_λ is the erythemal action spectrum at λ, and T_λ is the spectral transmittance of the sample at λ.[14,15] The plates were irradiated for 2 h with a solar simulator (Suntest CPS+; Atlas, Moussy le Neuf, France) apparatus equipped with a xenon arc lamp (1500 W) and special glass filters restricting transmission of light below 290 nm. The light source emission was maintained at 650 W/m^2 in accordance with global solar spectral irradiance.[16] Before and after irradiation, the SPF and the PF-UVA data of the creams were measured in vitro.

References and Notes

(1) Newman, W. G.; Agro, A. D.; Woodruff, S. I.; Mayer, J. A. *Am. J. Prev. Med.* **1996**, *12*, 186–194.
(2) Taylor, C. R.; Sober, A. J. *Annu. Rev. Med.* **1996**, *47*, 181–191.
(3) Wentzell, J. M. *Am. Fam. Phys.* **1996**, *53*, 1713–1733.
(4) Morohoshi, K.; Yamamoto, H.; Kamata, R.; Shiraishi, F.; Koda, T.; Morita, M. *Toxicol in Vitro* **2005**, *19*, 457–469.
(5) Kunz, P. Y.; Fent, K. *Toxicol. Appl. Pharmacol.* **2006**, *217*, 86–89.
(6) Veysey, E. C.; Orton, D. I. *Contact Dermatitis* **2006**, *55*, 54.
(7) Das, N. P.; Pereira, T. A. *J. Am. Oil Chem. Soc.* **1990**, *67*, 255–258.
(8) Rios, J. L.; Nanez, S.; Paya, M.; Alcaraz, M. J. *Phytochemistry* **1992**, *31*, 1947–1950.
(9) Oliveira, F. Q.; Andrade-Neto, V.; Krettli, A. U.; Brandao, M. G. *J. Ethnopharmacol.* **2004**, *93*, 39–42.
(10) Williams, C. A.; Harborne, J. B.; Geiger, H.; Hoult, J. R. S. *Phytochemistry* **1999**, *51*, 417–423.
(11) Haraguchi, H.; Tanimoto, K.; Tamura, Y.; Mizutani, K.; Kinoshito, T. *Phytochemistry* **1998**, *48*, 125–129.
(12) Harborne, J. B.; Williams, C. A. *Phytochemistry* **2000**, *55*, 481–504.
(13) El-Boury, S.; Couteau, C.; Boulande, L.; Paparis, E.; Coiffard, L. J. M. *Int. J. Pharm.* **2007**, *340*, 1–5.
(14) Groves, G. A.; Agin, P. P.; Sayre, R. M. *Aust. J. Dermatol.* **1979**, *20*, 112.
(15) Diffey, B. L.; Robson, J. *J. Soc. Cosmet. Chem.* **1989**, *40*, 127.
(16) Couteau, C.; Faure, A.; Fortin, J.; Paparis, E.; Coiffard, L. J. M. *J. Pharm. Biomed. Anal.* **2007**, *44*, 270–273.

NP7007297