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Silymarin, a molecule of interest for topical photoprotection

Celine Couteau [a], Clotilde Cheignon [a], Eva Paparis [a] & Laurence J.M. Coiffard [a]

[a] Faculty of Pharmacy, Université de Nantes, Nantes Atlantique Universités, LPiC, MMS, EA2160, 1 rue G. Veil - BP 53508, Nantes, F-44000 France

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Celine Couteau, Clotilde Cheignon, Eva Paparis and Laurence J.M. Coiffard*

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

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Some UV-filters have side effects. For example, oestrogenic effect was demonstrated for 4-methylbenzylidene camphor. Given that secondary metabolites are known for their UVB photoprotective properties in plants that contain them, we chose to study silymarin as an agent which could potentially be used in sun products. This determination is based on the physical determination of the reduction of the energy in the UV range, through a film of product which has previously been spread on an adequate substrate. About 15 mg of O/W emulsion containing silymarin at various concentrations was applied on roughened PMMA plates and the transmission measurements were carried out using a spectrophotometer equipped with integrating sphere. Incorporated in O/W creams, at a concentration of 10% (w/w), silymarin gives a Sun Protection Factor similar to that of octylmethoxicinnamate, which is why it is predominantly used in Europe. Overall, these results demonstrate that silymarin is a promising new sunscreen agent.

Keywords: silymarin; SPF; PF-UVA; *in vitro* method

1. Introduction

There are different ways of protecting the skin against the negative effects of the sun and they are complementary. These complementary ways are the photoprotection coming from clothes and the topical formulations (Ghazi, Couteau, & Coiffard, 2010, 2011).

Topical products are formulated with organic and/or inorganic sun filters. Their efficacy is known, but they can lead to unwanted effects on humans and/or on the environment (Danovaro et al., 2008; Knobler et al., 1989; Schlumpf, Jarry, Wuttke, Ma, & Lichtensteiger, 2004; Soeborg, Ganderup, Kristensen, Bjerregaad, & Pedersen, 2006). In this context, finding alternatives would be relevant. Plants exposed to sunlight produce secondary metabolites to protect their genome. Some already appear as interesting sources of new photoprotective molecules for topical application. For example, boldine, which is an alkaloid coming from the boldo tree, is well known for its antioxidant properties (Hidalgo, Farah, Carrasco, & Fernández, 2005; O'Brien, Carrasco-Pozo, & Speisky, 2006). Aromatic compounds from certain lichens (1′-chloropannarine, epiphorelic acids I and II, calicine) demonstrate, *in vitro*, a comparable efficacy to octylmethoxicinnamate, the UVB filter which is mostly used within Europe (Couteau, Faure, Fortin, Paparis, & Coiffard, 2007a; Couteau, Pommier, Paparis, & Coiffard, 2007b). The flavonoids, natural colourants of plants, are also an interesting family, in particular chlorogenic acid, baicalin,

*Corresponding author. Email: laurence.coiffard@univ-nantes.fr

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luteolin, apigenin and pueranin (Choquenet, Couteau, Paparis, & Coiffard, 2009). From the marine environment, mycosporins such as amino acids would provide a photoprotection in the UVA range (Conde, Churio, & Previtali, 2000; Vaid & Katiyar, 2010; Zhang & Wu, 2007). We took particular interest in the silymarin, coming from the *Silybum marianum*, known for its antioxidant properties (Gazak, Svobodova, & Psotova, 2004; Katiyar, 2005).

2. Results and discussion

The silymarin spectrum presents a peak of absorption at 286 nm. The influence of different concentrations of silymarin on the efficacy in the UVB and UVA ranges was studied. The results obtained in terms of efficiency are extremely promising, as the use level of 10% (w/w) of silymarin allows a Sun Protection Factor (SPF) close to 9 (Figure 1). Thanks to this value measured, the silymarin can be compared to a UV filter such as the octylmethoxycinnamate (Couteau et al., 2007a), which is a very efficient sun filter and the reason why it is predominantly used in Europe. The silymarin positions itself as a UVB filter. It is less efficient in the UVA range (Figure 1), as confirmed by the spectrum measured with the aqueous solution. The combinations with the zinc oxide and the titanium dioxide give interesting results. The measured SPF values of the preparations, formulated with filters according to their respective regulations, are higher than 10 (Table 1). After 2 h of irradiation at 650 W m^{-2}, the silymarin proved to be photostable. Indeed, in the case of a silymarin-based sun product, more than 90% efficacy is kept. Therefore, the silymarin shows a photostability higher than most of the organic UV filters

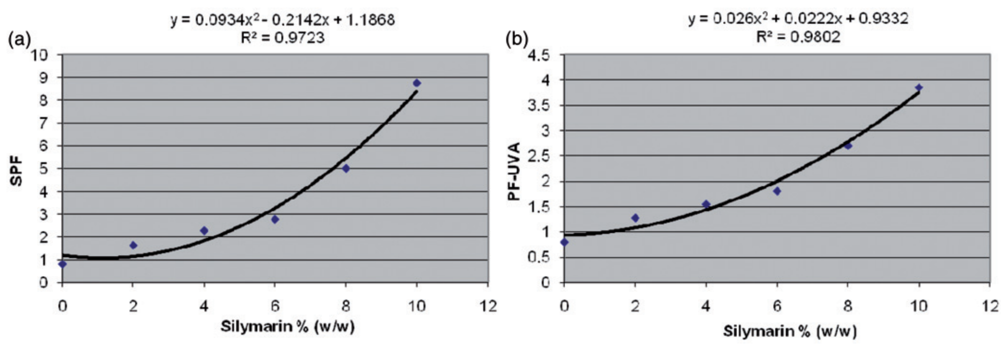

Figure 1. Efficacy of silymarin in UVB (1) and UVA (2) ranges.

Table 1. Data concerning the combination between silymarin and inorganic sun filters, before and after irradiation during 2 h at 650 W/min.

Product associated with silymarin 10% (w/w)	$SPF_0 \pm SD$	$PF\text{-}UVA_0 \pm SD$	λ_{c0} (nm)	$SPF_{2\,h} \pm SD$	$PF\text{-}UVA_{2\,h} \pm SD$	$\lambda_{c2\,h}$ (nm)
Z-Cote max (10% w/w)	12.37 ± 4.39	7.32 ± 2.34	378	11.84 ± 3.84	7.18 ± 2.06	378
Eusolex T-Oléo (10% w/w)	16.30 ± 2.98	7.61 ± 0.97	377	16.49 ± 2.95	17.00 ± 1.39	378

sold on the market at the moment (Couteau et al., 2007b). Other studies have already demonstrated that the silymarin would be interesting in the prevention of photocarcinogenesis phenomena (Jiang, Wang, Onodera, & Ikejima, 2009; Nichols & Katiyar, 2010; Rancan et al., 2002; Zudaire & Roy, 2001). By its efficacy, its photostability and the other properties already displayed, the silymarin is an ingredient to consider and to emphasise in the topical photoprotection domain.

3. Experimental

3.1. *Materials*

Silymarin, with a purity of 80% as total flavonolignans determined by HPLC, was obtained from Bioserae (Bram, France). Cetiol® HE, stearic acid, glycerin, parabens and triethanolamine (TEA) were purchased from Cooper (Melun, France). Xanthan gum (Keltrol® BT) was obtained from Kelco (Lille Skensved, Denmark). Zinc oxide (Z-Cote Max®) was obtained from BASF (Ludwigshafen, Germany). Titanium dioxide (Eusolex® T-aqua) was obtained from Merck (Damstadt, Germany). Polymethylmethacrylate (PMMA) plates were purchased from Europlast (Aubervilliers, France).

3.2. *Methods*

Aqueous solutions were scanned at wavelengths between 200 and 400 nm using a spectrophotometer double-beam (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.

An O/W emulsion was prepared in the laboratory by adding known concentrations of silymarin into the formulation components. A detailed description of the protocol is described in a previously published paper (Couteau et al., 2007b). The plates were irradiated for 2 h with a solar simulator (Suntest CPS+; Atlas, Moussy le Neuf, France) device equipped with a xenon arc lamp (1500 W) and special glass filters restricting the transmission of light below 290 nm. The light source emission was maintained at $650\,W\,m^{-2}$ in accordance to the global solar spectral irradiance (Couteau et al., 2007a). Before and after irradiation, the SPF and the PF-UVA of the creams were measured *in vitro*. These values are obtained using Diffey and Robson formulas (Diffey & Robson, 1989).

4. Conclusion

By its efficacy, its photostability and the other properties already displayed, the silymarin is an ingredient to consider and to emphasise in the topical photoprotection domain.

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