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Propolis as potential cosmeceutical sunscreen agent for its combined photoprotective and antioxidant properties

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ABSTRACT

Propolis, bee glue, and its main polyphenolic components show high antioxidant activity as found measuring their inhibitory action on lipid peroxidation of linoleic acid (LA) in sodium dodecyl sulfate (SDS) micelles. Furthermore, these substances evidence effectiveness as broad spectrum UVB and UVA photoprotection sunscreens, as it results by measurements of sun protection factor (SPF), the universal indicator related primarily to UVB radiations, and of the two parameters giving an indication of the UVA absorbance properties, i.e. UVA/UVB ratio and critical wavelength.

The combination of these characteristics moves up propolis and its main polyphenolic components to the class of cosmeceuticals, as possible active ingredient of sunscreen commercial formulations for their protective and preventive properties.

1. Introduction

Epidemiological, clinical and *in vitro* studies show that the exposure to ultraviolet (UV) light is responsible for various skin diseases including premature aging of the skin (wrinkling, scaling, dryness, dilatation of blood vessel and loss of collagen) and melanoma and non-melanoma skin cancer (Nichols and Katiyar, 2010).

UVB radiation (290–320 nm) can penetrate the skin to a depth of 160–180 μm and cause erythema and sunburns, trigger off the induction of oxidative stress, DNA damage and premature aging of skin (De Gruijl and Van der Leun, 1994; Mukhtar and Elmets, 1996; Ichihashi et al., 2003); it is presumed that UVB rays directly impair the DNA, leading to the formation of cyclobutane pyrimidine dimmers (CPD), formed between adjacent thymine bases (Vink and Roza, 2001), liable for apoptosis, immune suppression (Vink and Roza, 2001; Taylor, 1994; Melnikova and Ananthaswarmy, 2005) and initiation of photocarcinogenesis (Kripke et al., 1992; Yarosh et al., 1992). In the DNA, indeed, the pyrimidine bases are the most sensitive to UV and they are subjected to a lot of modification due to direct absorption of photons or free-radicals generated by other chromophores (Schaefer et al., 2000).

UVA radiation (320–400 nm) can penetrate deeper into the epidermis and dermis of the skin (around 1 mm) and advance the generation of singlet oxygen and hydroxyl free radicals, which can harm proteins, lipids and DNA (Di Giovanni, 1992). These rays indirectly impair the DNA via the production of radical oxygen species (ROS) (De Gruijl, 2000; Ranger, 1999). UVA is 10 times more efficient than UVB at causing lipid peroxidation (Morliere et al., 1995).

The body is able to protect itself against UV damages by virtue of the action of enzymatic and nonenzymatic antioxidants (AOs), but sometimes excessive and chronic exposition to UV radiations and other free-radicals, generated for example by smoking, drugs and pollution, make these defences inadequate. For this reason it appears important to introduce hexogen AOs, also as sunscreens, able to inhibit or retard these damages.

In the last few years the use of natural products in pharmaceutical and cosmetic field such as sunscreens for the prevention of skin diseases has kindled widespread interest (Nichols and Katiyar, 2010). In cosmetic field polyphenols appear particularly promising because they are characterized by an absorption spectrum which can filter UV radiations so reducing the penetration of the radiations into the skin and consequently lowering inflammation, oxidative stress and DNA damaging effects (Nichols and Katiyar, 2010). Moreover, they have anti-inflammatory, immunomodulatory and antioxidant properties, so they can react with free radicals produced by UV radiation (singlet oxygen and hydroxyl free-radicals) and inhibit or retard their harmful effects (Bravo, 1998).

In the light of this, we kept our mind on propolis (bee glue, CAS No. 9009-62-5), a resinous natural product with antiseptic, antimycotic, bacteriostatic, astringent, spasmolytic, anti-inflammatory, anaesthetic and antioxidant properties (Marcucci, 1995; Burdock, 1998; Banskota et al., 2001) as possible component of sunscreen

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formulations. To this purpose we studied the antioxidant activity of a propolis sample, picked up from a hood of the Veneto region (Italy), and their main polyphenolic components (flavonoids and caffeic acid derivatives), expressed as inhibitory capacity of lipid peroxidation of linoleic acid (LA) in sodium dodecyl sulfate (SDS) micelles. Moreover, we investigated its efficacy as broad spectrum UVB and UVA photoprotection sunscreens measuring: (1) the sun protection factor (SPF), the universal indicator related to UVB and short UVA radiations; (2) the UVA/UVB ratio and the critical wavelength (λ_c), two parameters giving an indication of the UVA absorbance properties.

The results here reported show that Veneto propolis and some of its components can be effectively used in natural sunscreens formulation for their remarkable antioxidant properties combined with their good broad spectrum UVB and UVA photoprotection.

2. Materials and methods

2.1. Materials

All chemicals were reagent grade and were supplied from Sigma Chemical Co. (USA). ABIP was a kind gift of Wako Chemicals USA. The aqueous solutions were prepared with quality milliQ water.

Spectrophotometric measurements were recorded on a double-beam UV–vis (Shimadzu UV-1800) instrument.

2.2. HPLC measurements

High performance liquid chromatography (HPLC) with triple quadrupole mass spectrometry detection was used to identify the constituents of propolis. Ethanolic extract of propolis (EEP) was diluted with methanol and filtered with a 0.45 μm filter. HPLC analyses were carried out by an Agilent 1100 HPLC system (Agilent Technologies, USA). For the chromatographic analysis, 5 μl of the sample was injected onto a C18 Synergy Hydro-RP 80A column (50 mm × 2 mm, 4 μm particle size) using an Aqua C18 125A pre-column (2 mm i.d. × 4 mm length). The mobile phase was acetic acid 0.1% (A) and MeOH (B). The gradient was: 10–90% B (2 min), 90–97% B (2–9 min), 97–100% B (9–10 min), 100% B (10–15 min) at a flow rate of 250 μl/min. An API 4000 triple quadrupole mass spectrometer (Applied Biosystems/MDS SCIEX, Toronto, Canada) was used to detect polyphenols in propolis. Calibration curves of peak area versus analyte concentration were plotted for the studied polyphenols using the standard addition technique. All data were acquired in negative ionization mode by multiple reaction monitoring (MRM).

2.3. Propolis

Raw native propolis, came from Cansiglio hill hood, located in the Veneto region, obtained directly from beekeepers and conserved in closed vessels at 3 °C to prevent natural oxidation, was used in this work. Ethanolic extract of propolis (EEP) was obtained dissolving raw propolis overnight under vigorous agitation at 3 °C. After filtration through a strainer to remove insoluble residual beehive products, i.e. wood fragments, bee bodies, etc., the suspension was left to sediment and the supernatant was centrifuged for 30 min at 2000 rpm. Limpid solution, without further purifications, was used for successive analyses. Solution concentration was calculated weighting dry residue after complete evaporation of all solvent until dryness.

2.4. Inhibition of lipid peroxidation

The antioxidant activity was studied in terms of inhibitory action of propolis, or its most important components, on peroxidation of linoleic acid in SDS micelles because, in our opinion, this method, more than others, mimes the efficacy of an antioxidant compound to prevent oxidative damage on lipoproteins or cell membrane by ROS injures.

The procedure has been the same followed in Fabris et al. (2008) and the antioxidant capacity was calculated as the 50% inhibitory concentration (IC_{50}), the antioxidant concentration (mg/L) that halves the rate of oxygen consumption due to the peroxidation process.

2.5. SPF determination

Sun protection factor (SPF) is the universal indicator introduced by Sayre et al. (1979) for describing the efficiency of sunscreen products against sunburn. It is a ratio calculated from the energies required to induce a minimal erythema dose for protected skin, after application of 2 mg/cm^2 of sunscreen product, and unprotected skin of human volunteers, using ultraviolet radiation usually from artificial source (FDA, 2001).

Sunscreen products are classified in conformity with their SPF values as it follows: from 2 to under 12 are defined “minimal sun protection products”; from 12 to under 30 “moderate sun protection products” while sunscreens with SPF values of 30 or above are defined “high sun protection products” (FDA, 2001).

For economical, practical and ethical reasons, *in vitro* methods have been introduced (Diffey and Robson, 1989; Reece et al., 1992; Springsteen et al., 1999) based on the assumption that the UV protection of sunscreens depends on their absorption characteristics and concentrations. *In vitro* SPF is calculated as follows (1) (Diffey and Robson, 1989):

$$\mathrm{SPF} = \frac{\sum_{\lambda=290\,\mathrm{nm}}^{400\,\mathrm{nm}} S(\lambda)EA(\lambda)}{\sum_{\lambda=290\,\mathrm{nm}}^{400\,\mathrm{nm}} S(\lambda)EA(\lambda)T(\lambda)} \quad (1)$$

where S is the solar spectral irradiance, EA is the erythemal action spectrum and T is the spectral transmittance. S and EA are given by literature (Diffey and Robson, 1989) while T is measured for every sunscreen.

All *in vitro* methods use substrates with inhomogeneous surfaces to mime the inhomogeneous surface structure of the human skin, but reproducible distribution of the sample on such substrates is difficult. So, for SPF determination we used the calibrated step film model of Herzog et al. (2004), used also in Ciba Sunscreen Simulator (www.ciba.com/SUNSCREENSIMULATOR/), where the inhomogeneity of the film is introduced mathematically. In this case T is given by (2):

$$T(\lambda) = g \times 10^{-\varepsilon(\lambda)cd(1-f)} + (1-g) \times 10^{-\varepsilon(\lambda)cd[gf/(1-g)+1]} \quad (2)$$

where d is the average thickness of the step film and it coincides with 20 μm (corresponding to an application in vivo of 2 mg/cm^2); $\varepsilon(\lambda)$ is the molar extinction coefficient, c is the molar concentration of the sunscreen; g and f are two parameters describing the structure of the step film. Herzog (2002) found that for all oil/water emulsions $g = 0.269$ and $f = 0.935$.

2.6. UVA/UVB ratio and critical wavelength measurements

SPF provides an index of protection against UV-induced erythema that is caused by UVB and short wavelength UVA (320–340 nm). Because this action spectrum is similar to that for DNA damage and inducting skin tumours, SPF has been considered a good parameter to valuate the protection against UV rays. Recently it has become evident that also longer wavelengths of solar UV can contribute to skin damage (Lavker et al., 1995a, 1995b; Lavker and Kaidbey, 1997), so it is necessary to introduce another parameter

Table 1
Polyphenols concentration of Cansiglio EEP.

Polyphenols	Abbr.	mg/g propolis
Pinocembrin	P	45.6
Chrysin	CH	41.4
Caffeic acid phenethyl ester	CP	18.3
Galangin	G	17.3
Caffeic acid	CA	9.9
1,1-Dimethylallylcaffeate	D	7.3
Naringenin	N	3.4
Apigenin	A	1.9
Kaempferol	K	1.5
Quercetin	Q	1.3

regarding UVA photoprotection. Unfortunately there is not agreement upon method to measure the protection against UVA (Boots the Chemist Ltd., 1992), so we used the UVA/UVB ratio (Boots the Chemist Ltd., 1992) and the critical wavelength (Diffey, 1994), the most used methods. The first one is the ratio of the average extinctions in the UVA and UVB range and it is given by the following expression (3):

$$\frac{\mathrm{UVA}}{\mathrm{UVB}} = \frac{\int_{320}^{400} lg[1/T(\lambda)]d(\lambda) / \int_{320}^{400} d(\lambda)}{\int_{290}^{320} lg[1/T(\lambda)]d(\lambda) / \int_{290}^{320} d(\lambda)} \quad (3)$$

The second one is the wavelength (λ_c) which determines, from 290 nm to λ_c, 90% of the integral of the absorption spectrum from 290 nm to 400 nm, that is (4):

$$\int_{290}^{\lambda_c} lg\left[\frac{1}{T(\lambda)}\right] d\lambda = 0.9 \int_{290}^{400} lg\frac{1}{T(\lambda)} d\lambda \quad (4)$$

Based on the level of the UVA/UVB ratio and the critical wavelength (λ_c), a classification into five categories had been proposed (Boots the Chemist Ltd., 1992; Diffey, 1994): a good UVA protection is obtained with UVA/UVB ratio between 0.41 and 0.6, a superior protection with a ratio between 0.61 and 0.8 and a maximum one with a ratio >0.8.

With respect to the critical wavelength, the maximum UVA protection is achieved with $\lambda_c \geq 370$ nm.

Furthermore, a substance to be a good sunscreen should be a broad spectrum UVB and UVA photoprotection product.

3. Results and discussion

3.1. HPLC/MS and UV measurements

HPLC/MS analysis of EEP (Table 1) shows significant presence of polyphenolic structures, such as caffeic acid (CA) and its derivative caffeic acid phenethyl ester (CP) and 1,1-dimethylallylcaffeate (D), apart from kaempferol (K), quercetin (Q) and galangin, all known as effective antioxidants. The UV spectrum of the propolis sample (Fig. 1) evidences high specific absorbance values in all UV region, with an absorption maximum at 290 nm ($\varepsilon^{1\%}_{1\,cm} = 450$). Furthermore, a shoulder at about 320 nm and significative absorbance values at higher wavelength ($\lambda \geq 350$ nm) are also noticed. Chemical composition, such as the UV spectrum, appear very similar to those referred to other propolis samples collected from different areas of the Veneto region, characterized by different orography, natural environment and habitative density, so indicating a common origin of the matter to this purpose sucked from the bees (Gregoris and Stevanato, 2010).

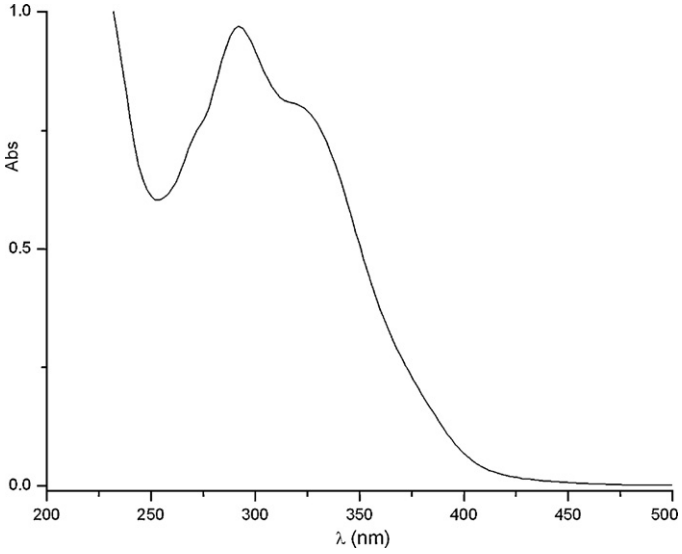

Fig. 1. UV–VIS spectrum of EEP.

3.2. Inhibition of lipid peroxidation

IC_{50} values, i.e. the antioxidant capacity, of EEP, its main polyphenolic components and catechin (CT) taken as reference, are reported in Fig. 2. The graph evidences strong antioxidant properties of EEP and major part of its components. In fact, EEP, caffeic acid and its derivatives, kaempferol, quercetin and galangin evidence an $IC_{50} < 1$ mg/L, similar to catechin. In particular, EEP shows an effectiveness two times higher than catechin, while caffeic acid and derivatives are on an average five times more effective. On the contrary, pinocembrin, chrysin, naringenin and apigenin show very low antioxidant activity, about 15 times less effective ($IC_{50} > 15$ mg/L) than catechin.

Considering the concentrations of each component in propolis, the good EEP antioxidant property is due above all to caffeic acid, its derivatives and to galangin, because kaempferol and quercetin, although good antioxidants, are present in low amount in EEP (Table 1).

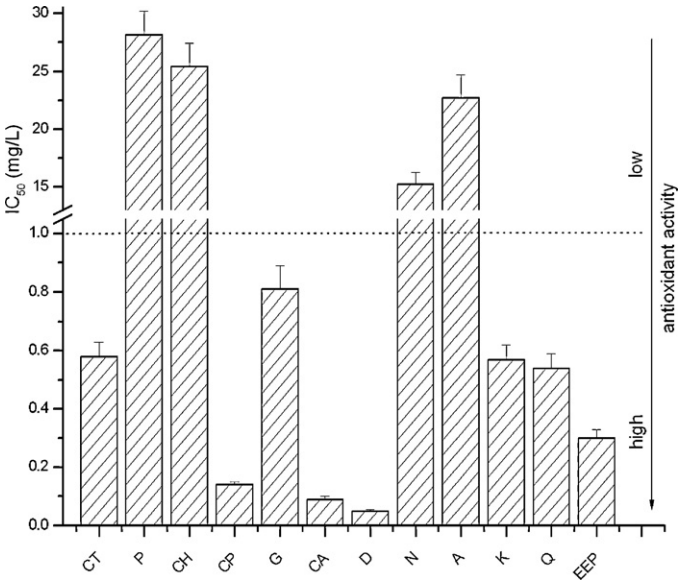

Fig. 2. Antioxidant activity of EEP, its main components and catechin (CT) taken as reference, expressed as 50% inhibitory concentration (IC_{50}) of lipid peroxidation.

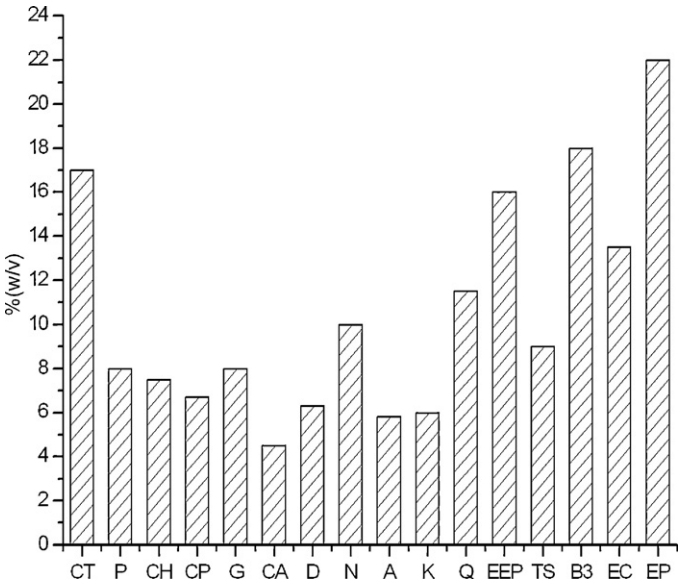

Fig. 3. Concentration (%, w/v) of each compound to obtain a SPF 20. TS: tinosorb S; B3: oxybenzone; EC: octinoxate; EP: padimate O.

3.3. SPF factor

In Fig. 3, the concentration (%, w/v) of each compound necessary to reach SPF 20, corresponding to a moderate sun protection product, is reported. As a reference, the UVB and UVA sunscreen drugs widely used in skin care market, tinosorb S (TS), oxybenzone (B3), octinoxate (EC), and padimate O (EP), are also reported (Hexsel et al., 2008). The graph shows that to have a SPF = 20, a concentration $\leq$ 8% of almost all the propolis' components is sufficient; this is a value about a factor two lower than B3, EC and EP, which require concentrations similar to that of EEP. Between the propolis' components, caffeic acid shows an extraordinary effectiveness, because only about 4% of this product to obtain a SPF = 20 is necessary.

If 10% titanium dioxide (TiO_2) is added to propolis or its main antioxidant components at the concentrations reported in Fig. 3, the SPF value increases from 20 to 50–60 showing a high synergic effect (Table 2). With this combination high sun protection products can be obtained.

3.4. Critical wavelength and UVA/UVB ratio

The UVA photoprotection properties of the analyzed compounds are summarized in Fig. 4, where the critical wavelength (λ_c) and the UVA/UVB ratio values are reported.

According to the critical wavelength method (Diffey, 1994), EEP and all propolis' components give maximum protection against UVA rays, showing λ_c higher than 370 nm, similar to tinosorb S. On the basis of these results, all these compounds are characterized by 4 stars in the Broad Spectrum Rate classification (Diffey, 1994). On the contrary, oxybenzone, octinoxate and padimate O, widely used in commercial formulations, are characterized by very lower λ_c, conferring them 3, 2 and 1 stars respectively.

Table 2
Effect on SPF of the combination of antioxidants and TiO_2.

Antiox.	% antiox. (w/v)	% TiO_2 (w/v)	SPF
CA	4.5	10.0	55
CP	6.7	10.0	56
D	6.3	10.0	55
G	8.0	10.0	59
EEP	16.0	10.0	56

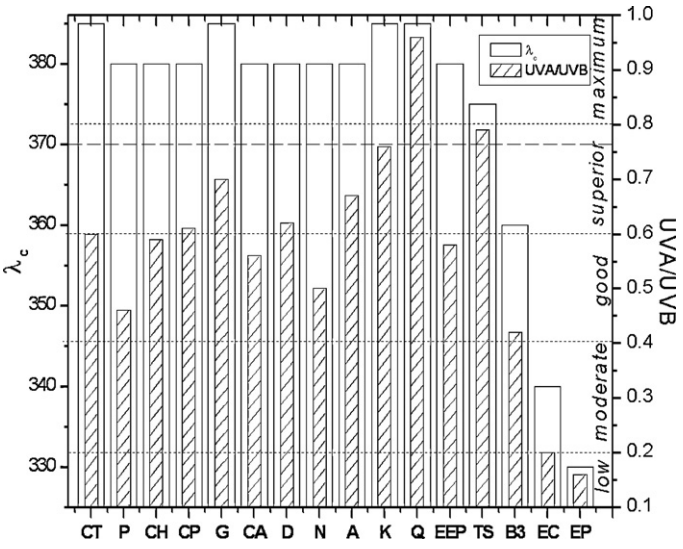

Fig. 4. Critical wavelength (λ_c) and UVA/UVB ratio values. The percentage of each compound is the same as reported in Fig. 3, i.e. that necessary to obtain SPF 20.

Applying the UVA Star Rating System to the UVA/UVB ratio (Boots the Chemist Ltd., 1992), propolis results a good almost superior UVA screen (0.58 UVA/UVB ratio, 2–3 stars), quercetin maximum (0.96, 4 stars) and all the others from good to superior (ranging between 0.46 and 0.76, 2–3 stars). In this classification tinosorb is superior (0.79, 3 stars), oxybenzone just good (0.42, 2 stars), while octinoxate and padimate O are confined at the low level (0.2 and 0.16 respectively, 0 stars).

4. Conclusion

All results of this study indicate that EEP and its components give good protection to ultraviolet radiation (UVR), certainly higher than the UVR filters widely used in skin care market, we have taken as reference.

Natural products characterized by high SPF values must be taken into careful attention considering that, according to recent studies, TiO_2, generally added to not much effective synthetic sunscreens in order to reach high SPF values, under certain conditions can generate dangerous free radicals (Gasparro et al., 1998) so adding a further risk factor to those products proposed as photoprotectives.

Furthermore, EEP and many of its components evidence strong antioxidant activity. The combination of these two characteristics moves up EEP to the class of cosmeceuticals, as possible active ingredient of sunscreen commercial formulations for its cosmetics, protective and preventive characteristics.

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