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Synergistic effect of green coffee oil and synthetic sunscreen for health care application

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ABSTRACT

Green coffee oil (GCO) is a potential natural product for application in health care products. In this study, the properties of GCO from spent coffee grains were evaluated aiming its application in sunscreens. GCO showed an intense synergistic effect in SPF value when it was associated with the synthetic sunscreen ethylhexylmethoxycinnamate, leading to an increase of 20% in SPF. Additionally, the antioxidant activity, the cytotoxicity and the sun protection factor (SPF) of GCO were determined. The results show no cytotoxic effect for skin and liver cells *in vitro*, even at high concentrations. The results suggest the use of GCO as a potential natural product for sunscreens formulations to improve the efficiency and protection against skin damage.

1. Introduction

Products for skin protection, such as sunscreens and antioxidant compounds, should be highlighted in the healthy field since they could be able to prevent damages and harmful effects to the skin, including cancers caused by UV and by the intracellular imbalance between free radicals (which role in the etiology of human diseases and aging is well known) (Devasagayam et al., 2004; Harman, 1956; Matsui et al., 2009). The chronic exposure to UV radiation is the most important factor for skin disorders such as wrinkling, scaling, dryness, mottled pigment abnormalities (Degruijl and Vanderleun, 1994; Mukhtar and Elmets, 1996; Ichihashi et al., 2003; Nichols and Katiyar, 2010). Thus, the role of antioxidants based on scavenging the reactive oxygen species and suppressing the rate of oxidative reactions (Devasagayam et al., 2004), is an important property for health care products.

Sunscreens are normally based on synthetic chemicals with high capacity of absorb sun light at the region of UVB (320–290 nm) and UVA (400–320 nm) spectrum. The reduction of the concentration of such chemicals in cosmetics formulations is a strategy to improve their quality, without affects their properties. The association of natural products with antioxidant activity in sunscreens can help to improve their photoprotective activity. In addition to bioactivity, natural products are, in general, no harmful for human, no expensive, feasible to be used in a wide range of products, and it is obtained from renewable sources. However, few data are found on literature about clinical research, functional substances, concentration and the combined effect of these antioxidants and synthetic sunscreens (Matsui et al., 2009). Indeed, byproducts from the UV photodegradation of synthetic sunscreens are a matter of concern for more than a decade (Butt and Christensen, 2000). Thus, the interest for natural products as active agents in sunscreens is growing (Matsui et al., 2009). For instance, some plant extracts such as tea extracts, lutein, tamarind (Matsui et al., 2009), propolis (Gregoris et al., 2011), *Passiflora incarnate* L. and *Plantago lanceolata* extracts (Velasco et al., 2008) and others (Matsui et al., 2009), have been reported to protect the skin against UV radiation-induced damage end points. In this context, green coffee oil (GCO) has arisen as potential candidate for replace synthetic chemicals in sunscreens, as a rich source of antioxidants and polyphenols (Iwai et al., 2004; Esquivel and Jimenez, 2012).

Besides healthy, beauty has been powering the cosmetic industry to explore natural products from renewable source for bioactive and innovative formulations (Devasagayam et al., 2004; Khalaf et al., 2008).

Coffee represents one of the most important commodities in the global economy (Naidu et al., 2008; Kondamudi et al., 2008). The selection of grains for production of beverage is based only on those of high quality. A large amount, around 15 wt% presents low quality

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for beverage, representing a decrease in the income. Nevertheless, these grains are a rich source of green coffee oil which, if extracted even by mechanical pressing can be used for new applications. If so, this could lead to a valorization of this raw material, increasing a sustained economic return in the production of coffee crops.

In this study, GCO was systematically evaluated in terms of its properties for application in sunscreens and health care products. Their behavior as a synergistic agent to increase the sun protection factor (SPF) of a pure synthetic solar filter was the first time studied. Its antioxidant activity and cytotoxicity were also tested.

2. Materials and methods

2.1. Materials

The reagents 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 3-4(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT), penicillin 100 U/mL; streptomycin 0.1 mg/mL were purchased from Sigma–Aldrich. The remains reagents were supplied by different companies, as follow: Minimum Essential Media (MEM, Inlab), Fetal Calf Serum (FCS) (Cultilab), trypsin (Gibco), dimethyl sulfoxide (Synth), phosphate buffer saline (PBS), isopropyl alcohol (Synth), Transpore®tape (3 M); ethylhexyl-methoxycinnamate. All reagents and solvents were of analytical grade. Crude GCO was kindly provided by Cooxupé – Brazil and by AGTTEC Ltda – Brazil. The samples were extracted by mechanical pressing and used as received.

2.2. Methods

2.2.1. Antioxidant activity

The antioxidant activity of GCO was determined by its capability of scavenger the DPPH or ABTS radicals. The DPPH method was used to determine the radical scavenging activity (Mensor et al., 2001). Solutions of GCO in ethanol were prepared at concentrations in the range of 0–20 mg/mL. 2.5 mL of each one was mixed with 1 mL of 0.004% DPPH in ethanol, at room temperature (RT) and in the dark. The samples were kept in the dark for 30 min (RT) and just after, the absorbance was measured at 515 nm in a Hitachi U-2001 spectrophotometer. The blank solution was composed by ethanol. The negative control solution was prepared by mixing 1.0 mL of 0.004% DPPH solution with 2.5 mL of absolute ethanol. A curve of free radicals inhibition (%) *versus* concentration of GCO was plotted and used to calculate the concentration of GCO to inhibit 50% of free radicals in the solutions (IC_{50}). The experiments were repeated 3 times to confirm the reproducibility of the data. The antioxidant activity, expressed as the percentage of DPPH radical inhibition, was calculated by using Eq. (1).

$$\%\,\text{of DPPH inhibition} = \frac{\text{Abs negative control} - \text{Abs sample}}{\text{Abs negative control}} \times 100 \quad (1)$$

The IC_{50} was calculated by linear regression of the curves obtained by plotting the results of % DPPH inhibition. On these plots, the abscissa represents the concentration of GCO and the ordinate represents the antioxidant activity. For the fitting, only the linear region of the curve was used, what means that the *plateau* was not considerate for the calculations.

The same procedure was used for the data obtained from the solution of ascorbic acid (0–5 μg/mL), which was the standard antioxidant substance used in this study.

The radical scavenging activity by ABTS method was determined as follows: 7 mM ABTS solution was prepared and reacted, in the dark, with potassium persulfate solution (140 mM) over 16 h, to generate the ABTS radical. The solution was diluted with ethanol to reach the absorbance of 0.70 ± 0.05 at 734 nm at room temperature in a Hitachi U-2001 spectrophotometer. 3 mL of ABTS radical solution was added to 30 μL of GCO solution. After 6 min the absorbance was measured at 734 nm (Rufino et al., 2007).

Ethanol was used for the blank solution and the negative control was composed by 3.0 mL of ABTS radical solution and 30 μL of absolute ethanol. The analytical curve (inhibitory percentage of the free radicals by the GCO *versus* concentration of green coffee oil) was plotted and used to calculate the concentration of GCO which inhibit 50% of free radicals in solution (IC_{50}). The assays were carried out in triplicate (Rufino et al., 2007).

The antioxidant activity, expressed as percentage of ABTS radical inhibition was calculated by Eq. (1). The results obtained were also compared with the same experiment using ascorbic acid solution, as described on the DPPH method.

2.2.2. In vitro cytotoxicity assay

The cytotoxic effect of GCO was assessed by MTT assay method using human hepatoma cells (HepG2) (Chiari et al., 2012) and human keratinocytes cells (HaCaT) (Sanchez et al., 2006; Van de Sandt et al., 1999; Zanatta et al., 2008). Both cells were grown separately in MEM, supplemented with 10% of fetal calf serum and antibiotics (penicillin 100 U/mL; streptomycin 0.1 mg/mL). The cultures were maintained at 37 ± 2 °C in 5% CO_2 atmosphere. The cells in confluence of 80–90% were trypsinized, centrifuged for 3 min at 1200 rpm, and transferred to a 96-well plates (TPP). MEM with fetal calf serum was used to neutralize the trypsin. The cell density used for cytotoxicity was 1×10^6 cells/mL.

The 96-well plates were incubated for 24 h for complete cell adhesion to the plate. After, the cells were treated with 100 μL of positive control (10% of dimethyl sulfoxide), negative control (MEM) and different concentrations of GCO. Due to the low solubility of GCO in MEM, ethanol was used to help the solubilization. Therefore, for each ethanol concentration, a negative control containing the same amount of ethanol in MEM was tested. After 24 h, the solution was removed and the plates were gently washed with PBS (Brugginsser et al., 2002). 100 mL of MTT (1 mg/mL in PBS) was added to each well. The microplates were incubated at 37 ± 2 °C for 4 h, protected from light, to allowed the growing of formazan violet crystals) (Mosmann, 1983). The solubilization of formazan crystals was carried out by adding 100 μL of isopropyl alcohol for each well. The absorbance was read at 595 nm in a Bio-rad Model 550 spectrophotometer. The cytotoxicity assays were performed by at least three independent assays, and, for each one, the treatment was done in triplicate.

The percentage of dead and live cells was calculated regarding to the negative control and represents the cytotoxicity of each treatment, as proposed by Zhang et al. (2004), as follows:

$$\%\,\text{of cellular viability} = \frac{\text{Abs of negative control} - \text{Abs of treatment}}{\text{Abs of negative control}} \times 100 \quad (2)$$

The concentration that promotes the reduction of 50% in the cellular viability (IC_{50}) was calculated by linear regression of the curve dose *versus* response, for different concentrations of GCO.

2.2.3. In vitro sun protection factor (SPF)

The SPF was obtained for formulations containing a synthetic sunscreen and the association of the synthetic sunscreen and GCO. The formulations consisted of the base cream (C); the base cream plus 10 wt% of GCO (C + O); pure GCO (O); the base cream plus the synthetic sunscreen (ethylhexylmethoxycinnamate) at 7.5 wt% (C + S) and the base cream plus synthetic sunscreen