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Byrsonima crassifolia extract and fraction prevent UVB-induced oxidative stress in keratinocytes culture and increase antioxidant activity on skin

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ABSTRACT

Solar ultraviolet radiation exposure, particularly UVB rays (280–320 nm), can lead to skin lesions, photocarcinogenesis and acceleration of skin photoaging since UVB radiation may reach both the epidermal and dermal layers of the skin. Treatments that can ameliorate UVB-induced skin damage include natural extracts, which can act as skin photochemoprotective agents. *Byrsonima crassifolia* is widely used in the folk medicine. Previously studies have shown high antioxidant activity of BC leaves extracts. In this study we described the first photochemoprotective potential of *Byrsonima crassifolia* extract (BCP) and fraction (BCF) against UVB-induced damage in keratinocytes and the ability of topical formulations with BCP or BCF to increase the antioxidant activity in pig ear skin. The results of characterization of BCP and BCF indicate that the phenolic content was increased two-fold after an enrichment process for obtaining BCF. Despite differences in the phenolic content, both BCP and BCF exhibited similar IC_{50} values for lipid peroxidation and the DPPH· method during the antioxidant activity study. However, for the chemiluminescence assay using the xanthine/luminol/XOD, BCF exhibited higher antioxidant activity than BCP. The different phenolic content in BCP and BCF did not influence their photochemoprotective activity in HaCaT cells, and both samples exhibited similar levels of protection. After treatment with BCP and BCF (1.2–5 μg/mL) and UVB irradiation exposure, the effect of lipid peroxidation *in vitro* was maintained in cell culture, and both IL-6 and TNF-α secretion and NF-κB activation were suppressed. After the development of the different formulations, BCP and BCF increased the antioxidant activity on skin and the formulation containing BCF showed higher skin retention, especially for (+) catechin, which was able to pass through the stratum corneum. Based on these findings, BCF could be topically applied to prevent/treat the damage induced by UVB radiation in the skin.

1. Introduction

Among the UV wavelengths that reach the Earth's surface, UVB rays (290–315 nm) are the most energetic and the most dangerous fraction of the solar spectrum related to the photocarcinogenesis development. In addition to skin cancer, several other harmful responses can be induced by UVB exposure, such as erythema, immunosuppression, edema, sunburn, keratinocyte hyperplasia, hyperpigmentation and premature aging (Strozyk and Kulms, 2013).

Cellular chromophores are energized when UV energy is absorbed, and they are likely to react with molecular oxygen, which generates reactive oxygen species (ROS). Although the skin has several defenses mechanisms to avoid oxidant damage, prolonged exposure to ultraviolet radiation (UVR) may increase ROS formation and cause an antioxidant/oxidant imbalance (Terra et al., 2012).

ROS can react with organic molecules (proteins, carbohydrates, nucleic acids and especially lipids) and cause loss of cellular integrity, lipid peroxidation and the release of cytokines. This process will ultimately cause skin lesions and accelerate aging and the development of malignant skin diseases (Peres et al., 2011; Sivamani and Maibach, 2009). Lipid peroxidation is usually associated with skin inflammation and carcinogenesis because it increases the production of

Abbreviations: BC, *Byrsonima crassifolia*; BCP, *Byrsonima crassifolia* purified extract; BCF, *Byrsonima crassifolia* fraction; CAT, (+)-catechin; EC, (−)-epicatechin; EGCG, epigallocatechin gallate; GA, gallic acid; QG, quercetin 3-*O*-β-D-glucopyranoside

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prostaglandins (PGE_2), which cause tissue inflammation. Inflammatory cells produce more ROS, decrease DNA repair, increase DNA lesions and gene mutation that cause cellular apoptosis and carcinogenesis (Afaq and Katiyar, 2011).

Many studies have suggested that the topical application of antioxidants can prevent or delay UVB-induced skin damage, which would provide a photochemoprotective effect and fortify the endogenous protection system and ultimately reduce the oxidative damage in the skin (Salucci et al., 2014). In this context, much attention has been paid to natural products possessing antioxidant and anti-inflammatory properties, thereby protecting the skin from photodamage. Green tea, *Garcinia brasiliensis* and *Cecropia obtusa* extracts are examples of photochemoprotective agents, which presented biological effects by protecting against oxidative damage induced by UV radiation (Afaq and Katiyar, 2011; Alves et al., 2016; Figueiredo et al., 2014).

Byrsonima crassifolia (BC) is widely used in Central America and Amazon folk medicine. Studies have shown that the antioxidant properties of BC are related to its polyphenol and flavonoid contents (Mariutti et al., 2014; Silva et al., 2006; Souza et al., 2008). Some phenolic compounds have been identified in extracts obtained from BC leaves, such as catechin, epicatechin, epigallocatechin gallate, guiaverin, and quercetin, including its 3-0-[6"-galloyl] galactoside and its 3-*O*-β-D-glucopyranoside (Bejar et al., 1995; Bejar and Malone, 1993).

Several studies have demonstrated inhibitory mechanisms against molecular processes involved in UV irradiation-induced, mainly for catechins. These compounds protect epidermal cells against damages induced by UV radiation, such as cell death, inflammation, immunosuppression, prevention of DNA damage, DNA repair and modulation of cell signaling pathways critically involved in different stages of photocarcinogenesis (Afaq and Katiyar, 2011; Wu et al., 2006).

On the other hand, it is well known that the BC compounds only will present photochemoprotective effects, modulating the inflammatory process induced by UVB radiation, if these compounds are able to pass through the skin barrier comprising the *stratum corneum* and reach the viable epidermis. Therefore, the aim of this work was (i) to evaluate the photochemoprotective potential of BC leaves extract (BCP) and their fraction (BCF) against oxidative stress induced by UVB radiation in culture of keratinocytes (HaCaT) and (ii) to evaluate the ability of topical formulation with BCP or BCF to increase the antioxidant activity in pig ear skin.

2. Material and methods

2.1. Materials

Chemicals and the reagents 2,2-diphenyl-1-picrylhydrazyl (DPPH·), xanthine, xanthine oxidase, luminol, neutral red, tetraethoxypropane, an antibiotic solution containing 10,000 units of penicillin, 10 mg of streptomycin and 25 μg of amphotericin B per mL, gallic acid (GA), (+) catechin (CAT), (−) epicatechin (EC), epigallocatechin gallate (EGCG) and quercetin 3-*O*-β-D-glucopyranoside (QG) (isoquercetin) were purchased from Sigma-Aldrich (St Louis, MO,USA). Folin-Ciocalteu reagent was obtained from Imbralab (São Paulo, Brazil) and aluminum chloride, trichloroacetic acid, dimethyl sulfoxide (DMSO) and ethanol were obtained from Synth (Sao Paulo, Brazil). Butanol was purchased from Dinâmica (São Paulo, Brazil) and *o*-phthaldialdehyde was purchased from ACROS (Rio Grande do Sul, Brazil). Acetonitrile, methyl alcohol and formic acid of HPLC grade were obtained from J.T. Baker (Phillipsburg, NJ, USA). Enzyme-linked immunosorbent assay kits specific for mouse tumor necrosis factor (TNF)-α and interleukin-6 (IL-6) were obtained from ELISA Ready-SET-Go!®, eBioscience (San Diego, CA, USA). Roswell Park Memorial Institute Medium (RPMI) and fetal bovine serum (FBS) were purchased from Gibco (Grand Island, NY, USA). C18 column and pre-column Hypersyl GOLD was purchased from Thermo Electron Corporation (San José, CA).

2.2. Plant material

The BC extract and fraction were kindly manufactured and provided by Amazon Dreams (Belém, Brazil). Briefly, 80 kg of fresh leaves of *Byrsonima crassifolia* were cleaned, dried at 55 °C for 4 h and ground (0.5–0.8 mm^2). Crude extract was obtained after a triple extraction with ethanol solution (45%) at 60 °C for four hours each step and intermediate filtration (80 μm). After the extract was concentrated, the crude extract was partially purified by an adsorption/desorption process onto a synthetic styrene divinylbenzene macroporous resin following concentration in a vacuum-drying process to obtain the *Byrsonima crassifolia* purified extract (BCP) (Silva et al., 2013, 2007).

Then, the BCP extract was adsorbed in an acrylic macroporous resin and desorbed using hydroethanolic solutions with 0, 32, 64 and 96°GL. The sample of interest for this study comprises the fraction eluted at 32 and 64°GL hydroethanolic solutions, which contains phenolic compounds of intermediate polarity. Finally, the solvent was evaporated and the sample was lyophilized to obtain the *Byrsonima crassifolia* fraction (BCF).

2.3. Chemical characterization of BCP and BCF and antioxidant capacity

2.3.1. Total phenolic and flavonoid contents

The total phenolic and total flavonoid contents of BCP and BCF were determined by the Folin-Ciocalteu and aluminum chloride colorimetric methods, respectively, based on a previously described procedure (Kumazawa et al., 2004). The total phenolic content was expressed as mg of gallic acid equivalents (GAE) per g of dry weight of extract and the total flavonoid content was calculated as mg of quercetin equivalents (QE) per g of dry weight of extract.

2.3.2. HPLC analysis

The analyses were performed in a C18 column (250 mm × 4.6 mm, 3 μm) equipped with a C18 pre-column (10 mm × 4 mm, 3 μm) at room temperature. The instruments used included an LC-10AT Shimadzu HPLC system coupled to an SPD-10A UV/VIS detector operating at 280 nm and a Chromatopac C-R6A integrator. The gradient of elution was achieved with water:formic acid (99:1, solvent A) and acetonitrile:formic acid (99:1, solvent B): 0–7 min: 7–15% B; 7–25 min: 15–20% B; 25–37 min: 20% B; 37–42 min: 20–35% B; 42–45 min: 35–7% B; 45–50 min: 7% B, at a total flow rate 1.0 mL min^{-1} and 20-μL injection volume.

The stock solutions of the standards, BCP and BCF were prepared in 80% methanol at 1 mg/mL. The working solutions were prepared in water. The standard calibration curves were prepared in three replicates to assess linearity (1–20 μg/mL). The intermediate precision (inter-day) was assessed by analyzing five replicates on five different days. The concentration used was 125 μg/mL for BCP and BCF. To determine the phenolic compounds in BCP and BCF, the peak retention times were compared to the standard retention times and were further confirmed by the addition of a known amount of each standard in the BCP and BCF solutions.

2.3.3. Antioxidant activity

The BCP and BCF antioxidant activity was evaluated by H-donor activity using the DPPH radical (Blois, 1958), inhibition of lipid peroxidation (Rodrigues et al., 2002) and scavenging superoxide radicals produced in the chemiluminescence assay using the xanthine/luminol/xanthine-oxidase (XOD) system (Girotti et al., 2000). BCP and BCF were first solubilized with 50% ethanol following dilution with the medium of each reaction. The final concentration range for DPPH· and lipid peroxidation was 0.3–5 μg/mL, and for chemiluminescence it was 0.06–0.93 μg/mL. The antioxidant capacity is expressed as the IC_{50} values, which were calculated as the mass of BCP or BCF per mL of reaction medium that inhibited 50% of the absorbance of the DPPH· alcoholic solution, lipid peroxidation or the generation of