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Research paper

Evaluation of the antioxidant activity of soybean extract by different in vitro methods and investigation of this activity after its incorporation in topical formulations

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Abstract

Chemoprevention by natural products is an emerging therapeutic approach for free radical-mediated diseases including cancer. This is a consequence of its wide applicability and acceptance. In the present study, the antioxidant activity of the soybean extract (Isoflavin Beta®) and of formulations added with this extract were evaluated using stable free radical 2,2-diphenyl-1-pycrylhydrazyl (DPPH·) and deoxyribose as well as the lipid peroxidation inhibition assays. For all the assays the extract showed a dose-dependent activity, and IC_{50} of 21.03 μg/mL in lipid peroxidation inhibition, 161.8 μg/mL in DPPH·, and 33.5 ng/mL in hydroxyl radical scavenging assay. The antioxidant activity of the extract added in the formulations could not be assessed using the deoxyribose assay. However, the lipid peroxidation inhibition and DPPH· scavenging assays could be successfully applied for the antioxidant activity evaluation of the formulations added with soybean extract to protect the skin against free radicals, which can be generated by the ultraviolet radiation exposure.

Keywords: Soybean extract; Antioxidant; Polyphenols; Topical formulation; DPPH·; Deoxyribose; Lipid peroxidation

1. Introduction

It is well established that the inflammatory response following acute UV light skin irradiation and the degenerative processes related to chronic UV radiation skin exposure are largely mediated by the overproduction of reactive oxygen species (ROS) and free radicals, and by impairment of antioxidant systems [1].

Furthermore, ROS are believed to be involved in many skin disorders such as cancer formation, cutaneous autoimmune diseases, phototoxicity, photosensitivity and skin aging. Two of the early cellular events following UV light exposure are the lipid peroxidation induction and the suppression of replicate DNA synthesis due to DNA damage [2].

Considering that solar UV radiation is the major environmental inducer factor of skin cancer, various efforts have been made to prevent skin cancer caused by sun exposure. For instance, application of sunscreens agents on the body surface is the most used [3]. However, the currently used sunscreens may be sometimes overwhelmed by excessive sun exposure or present several deleterious effects (i.e. allergy, inflammation) on the skin. Therefore, novel materials for chemoprevention of UV-induced skin cancer are needed. In this regard, one of the safest approaches is the prevention by anti-oxidative chemicals or plants materials [3]. Corroborating, soybean-germ oil

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possesses a remarkable protective activity against UVB-induced skin inflammation [4].

Soybeans contain isoflavones that have several known activities, including estrogenic, fungitoxic, and antioxidant [5]. The biologically active components of soy isoflavones include genistein, daidzein and biochanin A [6]. Genistein inhibits protein tyrosine kinase activity, topoisomerases I and II, ribosomal 6S kinase and alters cell proliferation [7]. It also has antioxidant properties and suppresses skin tumorigeneses. In many cases, the combined effect of soy isoflavones might be better than the effect of any single isoflavone compound [8].

Thus, considering that soybeans constituents present potential anti-cancer effects and antioxidant activity, the development of topical formulations added with soybean extracts as well as the correct evaluation of their antioxidant activity by using different methodologies would be very helpful to choose the most adequate one.

Therefore, in the present study it was evaluated the chemical composition, antioxidant and free radical scavenging activities of the soybean extract Isoflavin Beta® alone and added in different topical formulations. The antioxidant activity was evaluated by their ability to inhibit lipid peroxidation induced by Fe^{2+}, H-donor capability and scavenging hydroxyl radical effect. Furthermore, physical-chemical parameters such as centrifugation stability, pH and globule size were also addressed. Therefore, it will be possible to choose the most adequate antioxidant methodology to make the quality control and stability studies of all the formulations in which the soybean extract was added.

2. Materials and methods

2.1. Chemicals

Isoflavin Beta® extract from France and raw materials for formulations (presented in the formulation section) were obtained from Galena (Campinas, SP, Brazil). Genistein, daidzein, thiobarbituric acid (TBA) and 2,2-diphenyl-1-picrylhydrazyl (DPPH·) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). 2-Deoxy-D-ribose was obtained from Acros (New Jersey, USA). All other reagents were of the highest grade commercially available. The raw materials were dissolved in dimethylsulfoxide for the antioxidant assays. The amount of solvent used had no effects on the assays.

2.2. Total polyphenols and flavonoids contents in the Isoflavin Beta® soybean extract

One hundred milligrams of extract was stirred with 80% ethanol for 15 min. The ethanol suspensions were centrifuged at 1660*g* for 10 min and supernatant fraction collected into 25 mL of the volumetric flask. The precipitate was extracted with 5 mL of 80% ethanol. Finally, the supernatant fraction was combined and the volume adjusted to 25 mL with deionized water.

Total polyphenols contents were determined by Folin-Ciocalteau colorimetric method [9]. In this methodology, 0.5 mL sample was mixed with 0.5 mL of the Folin-Ciocalteau reagent and 0.5 mL of 10% Na_2CO_3, the absorbance was measured at 760 nm after 1 h incubation at room temperature. Total polyphenols contents were expressed as mg/g (gallic acid equivalents).

Total flavonoids contents of the soybean extract were determined using the aluminum chloride colorimetric method. To 0.5 mL of sample, 0.5 mL of 2% $AlCl_3$ ethanol solution was added. After 1 h at room temperature, the absorbance was measured at 420 nm [10]. Total flavonoids contents were calculated as quercetin (mg/g) from an analytical curve.

2.3. HPLC analysis

Genistein and daidzein contents in Isoflavin Beta® extract were determined by reversed-phase HPLC analysis. Isoflavin Beta® was dissolved in 25 mL methanol, and diluted in the mobile phase 1:2, filtered with a 0.45 μm filter, and 20 μL was injected into the HPLC system. The separation of the isoflavonoids genistein and daidzein was performed by employing the SuperPac Sephasil C18 (5 μm) column, 250 × 4 mm attached to a pre column. A mobile phase was employed; it consisted of 0.1% acetic acid in acetonitrile, 0.1% acetic acid (70:30), 1 mL/min. Eluted isoflavonoids were detected by their absorbance at 250 nm [11]. The linearity was obtained with concentrations of 0.05–10 μg/mL. Quantitative data for daidzein and genistein were obtained by comparison to known standards.

2.4. Formulations

The formulations were developed varying the content of lipidic and emulsifying agent. Formulation 1 was prepared with commercially available self-emulsifying wax Polawax® (cetostearyl alcohol + polyoxyethylene derived of a fatty acid ester of sorbitan 20E), formulation 3 with Croda® (mineral oil + petroleum + lanolin alcohol + ethoxylated fatty alcohol) and into formulations 2 and 4 anionic hydrophilic colloid (carboxypolymethylene, Carbopol®) was also added as a stabilizing agent. Macadamia nut oil was added as an emollient, and propylene glycol as a moisturizer. The preservative used was a mixture of parabens and imidazolidinyl urea. Deionized water was used for the preparation of all formulations (Table 1). Extract of soybean (Isoflavin Beta®) 2.0% was firstly solubilized in propylene glycol and then incorporated into the formulations at room temperature.

2.5. Preparation of samples

The extract Isoflavin Beta® was diluted with dimethylsulfoxide (DMSO) to final concentrations of 12.5, 25, 50, 100, 150, 200, 250 and 300 μg/mL, and 31.25, 62.5, 125,