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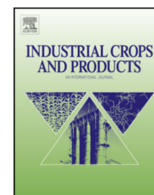

A holistic anti-aging approach applied in selected cultivated medicinal plants: A view of photoprotection of the skin by different mechanisms

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ABSTRACT

Medicinal plants are recognized by their large number of bioactive secondary metabolites. They have been used to treat several human ailments and are believed to possess many biological activities. However, little is known about the photoprotective and anti-aging properties of important medicinal plants as assessed by integrative studies. To find new bioactive ingredients of cosmetic interest, seven cultivated medicinal plants were systemically evaluated in an integrated manner. Extracts obtained from each species using solvents of diverse dielectric constants were tested on different molecular targets related to skin aging, with a focus on the protection of the main components of the extracellular matrix (ECM). Thus, the inhibition of the enzymes collagenase, elastase and hyaluronidase was determined, and antioxidant properties were evaluated. Subsequently, a photoprotective approach was taken in human dermal fibroblasts (HDF) stimulated with ultraviolet B (UVB)-radiation through the measurement of matrix-metalloproteinases (MMP-1) and procollagen production, as well as intracellular reactive oxygen species (ROS) levels, in pretreatment with bioactive extracts. The species with higher biological activity were chemically characterized through fingerprint analysis by HPLC-DAD and comparison with reference standards. The results showed the ability of some extracts from Rosmarinus officinalis, Thymus vulgaris and Smallanthus sonchifolius to protect the major components of the ECM from UVB irradiation-induced damage. Additionally, useful information, including various bioactivities and the chemical diversity of the extracts of medicinal plants, which could not be acquired from individual assessments of anti-aging activity, was obtained using a holistic anti-aging approach.

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1. Introduction

The extracellular matrix (ECM) is the structural basis of the dermis and therefore of the skin (Rittié and Fisher, 2002). The ECM is composed of proteoglycans interwoven with macromolecules such as collagen, elastin and fibronectin, which are produced by the fibroblasts of the dermis (Sahasrabudhe and Deodhar, 2010). Collagen is the main structural protein; it provides a supportive framework to cells and is responsible for the tensile strength of the skin (Thring et al., 2009). Elastin, due to its unique elastic recoil properties, is vital for providing elasticity to the skin, while hyaluronic acid, a major glycosaminoglycan (GAG), is involved in the hydration of the skin, holding water and keeping the body smooth, moist and lubricated. These connective tissue macromolecules are constantly attacked by enzymes such as matrix-metalloproteinases (MMPs; e.g., collagenase), the serine protease elastase and the mucopolysaccharase hyaluronidase, which are highly related to the aging process of the skin (Satardekar and Deodhar, 2010). The extrinsic skin aging process (also called skin photoaging) occurs as a result of exposure to environmental factors, particularly solar radiation (UV radiation), which affects human skin in different ways. UV exposure causes physical changes to the skin due to alterations that occur in the connective tissue through the formation of lipid peroxides, cell contents and enzymes (Thring et al., 2009). Furthermore, UV irradiation results in important molecular changes, including altered signal transduction pathways that promote matrix-metalloproteinase expression, decreased procollagen synthesis, connective tissue damage (Rittié and Fisher, 2002) and significantly increased reactive oxygen species (ROS). ROS act as cell signaling molecules and can cause lipid peroxidation, mitochondrial and DNA damage, and protein and gene modifications. In addition, ROS can initiate complex

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molecular pathways, including the activation of enzymes such as collagenase, elastase and hyaluronidase (Bravo et al., 2016). Therefore, skin aging is a multifactorial and complex process; strategies exploited to combat the devastating effects of photoaging should focus on the different mechanisms involved in its pathogenesis.

Natural extracts have been one of the main strategies used to decelerate and prevent the effects of skin aging (Kumar et al., 2013; Mukherjee et al., 2015). Many herbs have been reported to contain bioactive metabolites, mainly polyphenolic compounds with photoprotective and antioxidant effects, which interact with cellular signaling pathways that are directly involved in the skin. Some natural compounds are rapidly absorbed into the skin when they are incorporated into cosmetic formulations (Mukherjee et al., 2015, 2011). However, an integrated strategy is required in the search for promising raw ingredients for the cosmetic industry. There are numerous biochemical and physiological points at which it may be possible to intervene in the skin aging process; they could be targeted simultaneously through the diverse chemistry of medicinal plants. This study is based on an evaluation of seven species of medicinal plants: *Rosmarinus officinalis* L., *Thymus vulgaris* L., *Artemisia dracunculus* L., *Calendula officinalis* L., *Matricaria recutita* L., *Vaccinium meridionale* Sw., and *Smallanthus sonchifolius* (Poepp.) H. Rob., which were systemically evaluated in an integrated manner. Antioxidant properties and the effects on collagenase, elastase and hyaluronidase enzymes were determined in selected species. In addition, photoprotective properties were assessed by determining the effect of some promising extracts in human dermal fibroblasts (HDF) regarding suppression of MMP-1, enhancement of procollagen type I production and modulation of intracellular ROS. A chemical profile was constructed to find potential bioactive metabolites with anti-aging and photoprotective properties to develop standardized natural ingredients for use in cosmetic formulations.

2. Materials and methods

2.1. Plant collection and extracts

All plants were obtained from different farmers in Eastern Antioquia (Colombia) during 2014 (Table 1). The aerial parts of *R. officinalis, T. vulgaris, A. dracunculus*, as well as the flowers of *C. officinalis, M. chamomilla* and the leaves of *S. sonchifolius* were dried at 37 °C for 72 h and then powered using an electric blender. The fruits of *V. meridionale* and the tubers of *S. sonchifolius* were lyophilized to a powder. Extracts were formulated with organic solvents of a wide range of dielectric constants, including hexane, ethyl acetate, acetone, 75–25% acetone-water, 50–50% acetone-water, 25–75% acetone-water and water. The powdered plants were extracted three times with these solvents with sonication for 45 min at 30 ± 5 °C. Subsequently, the supernatants were filtrated through filter paper, combined and centrifuged at 5000 rpm for 30 min. The resulting filtrates were evaporated under vacuum at 40 °C and stored at −20 °C until use.

2.2. Determination of antioxidant capacities

2.2.1. Oxygen radical absorbance capacity (ORAC) assay

The hydrophilic ORAC assay was adapted from previously described procedures (Bravo et al., 2015) with some modifications. AAPH (2,2′-Azobis(2-amidinopropane) dihydrochloride) was used as a peroxyl radical generator, and Trolox was used as a standard; fluorescein was used as a fluorescent probe. Samples, AAPH and fluorescein were prepared in phosphate buffer (92.4 mM, pH 7.4). First, 50 μL of sample solution or Trolox standard was mixed with 150 μL of fluorescein (1.6 μM) and incubated at 37 °C for 30 min, then was added 50 μL of AAPH solution (125 mM). Fluorescence was measured every 2 min for 120 min at an excitation wavelength of 485 nm and emission wavelength of 520 nm using a Synergy HT Multi-mode-microplate reader (Biotek Instruments, Inc; Winooski, USA). The relative ORAC values were calculated using the differences in the areas under the decay curves and were expressed as μmol Trolox Equivalent per gram of extract (μmol TE/g).

2.2.2. Ferric reducing antioxidant power (FRAP)

Measurement of the reducing power of the samples was adapted from previously described procedures (Jimenez et al., 2015). The FRAP working solution contained acetate buffer (300 mM, pH 3.6), TPTZ (2, 4, 6-tripyridyl-*s*- triazine) (10 mM) in HCl solution (40 mM) and $FeCl_3 \cdot 6H_2O$ (20 mM) at a 10:1:1 ratio to provide the reducing solution, and Trolox was used as a standard. First, 10 μL of sample or Trolox was added to each well in a 96-well plate, mixed with 250 μL FRAP working solution, and incubated at 37 °C for 10 min in the dark. The absorbance was measured at 593 nm using a Synergy HT Multi-mode-microplate reader. The results were expressed as μmol Trolox Equivalent per gram of extract (μmol TE/g).

2.2.3. Total phenol content (TPC)

Total phenol content was determined using the Folin-Ciocalteu method used in Bravo et al. (2015) with some modifications. Briefly, 25 μL of extract solution diluted with distilled water was added to a 96-well plate, and 125 μL Folin-Ciocalteu reagent diluted with distilled water (1:10) was added and mixed. Next, 100 μL of sodium carbonate (7.5% p/v) was added to each well, and after 60 min of reaction at room temperature and protected from light, the absorbance was read at 765 nm using a Synergy HT Multi-mode-microplate reader. Quantifications were calculated using a gallic acid standard for the calibration curves. The results were expressed as milligrams of GA equivalents per gram of extract (mg GAE/g).

2.3. In vitro *determination of anti-aging properties*

2.3.1. Anti-collagenase activity

The anti-collagenase activity was performed according to the method proposed by Bravo et al. (2016). The inhibition of collagenase was measured using the EnzCheck® Gelatinase/Collagenase assay kit (Molecular Probes Inc.). Aliquots of 20 μL of sample solutions or buffer (control) were added to each well of a 96-well plate. Then, 80 μL of DQ-Collagen type IV substrate and 100 μL of active enzyme were added, and the fluorescence intensity was measured by a Synergy HT Multi Microplate Reader for excitation at 485 nm and emission detection at 515 nm every minute for 20 min. The increase in fluorescence is proportional to the proteolytic activity of the enzyme. Therefore, a decrease in fluorescence compared with the enzyme alone (control) would identify a potential collagenase inhibitor. Oleanolic acid (250 μM or 112.07 μg/mL) was used as a reference inhibitor. Each reaction was performed in triplicate. The percent inhibition of collagenase reaction was calculated as follows:

$$Inhibition\,(\%) = \frac{M_{control} - M_{sample}}{M_{control}} \times 100 \quad (1)$$

where $M_{control}$ and M_{sample} are the slopes of the fluorescence vs time graphs of the control and the sample, respectively.

2.3.2. Anti-elastase activity

The anti-elastase activity was assessed according to a previously proposed method (Bravo et al., 2016). The effect on the elastase enzyme was measured using the EnzCheck® Elastase assay kit (Molecular Probes Inc.). Aliquots of 50 μL of sample solutions or buffer (control) were added to each well of a 96-well plate. Then, 50 μL of DQ-elastin substrate and 100 μL of active enzyme were

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