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# Fruits of selected wild and cultivated Andean plants as sources of potential compounds with antioxidant and anti-aging activity

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ABSTRACT

It is known that most of the species growing in high-elevation environments in tropical mountains remain largely unexplored. In particular, little is known from a chemical and biological standpoint. The aim of this study was to conduct a preliminary screening of the effects of selected wild and cultivated Andean plants characterized by small and colorful fruits on collagenase, elastase, hyaluronidase and tyrosinase, along with antioxidant capacity, to identify possible sources of potential anti-aging substances for the development of natural cosmetic products. Sixty-five samples consisting of methanol extracts of the fruits of thirty-five Andean plants at different stages of growth were used in the study. The anti-collagenase, anti-elastase, anti-hyaluronidase and anti-tyrosinase activities were determined by spectrophotometric and fluorometric assays. The antioxidant capacity was measured by oxygen radical absorbance capacity (ORAC) and trolox equivalent anti-oxidant capacity (TEAC) assays. The total phenolic content was determined by the Folin–Ciocalteu method. Liquid–liquid partitions of extracts of interest were performed, and each fraction was tested. The inhibitory activity against skin aging-related enzymes and antioxidant properties has provided evidence for the potential utility of *Alchornea triplinervia*, *Gaultheria erecta*, *Rubus compactus* and *Ugni myricoides*, which were selected as plants of interest. In addition, the maturity state of fruits alters the properties of extracts in a statistically significant manner. In the vast majority of cases, the immature fruits presented a higher antioxidant capacity and enzymatic inhibitory activity. The extracts, fractions and sub-fractions of *G. erecta* and *U. myricoides* fruits showed effective inhibitory activity against skin aging-related enzymes and antioxidant properties, which supports the need to more extensively examine extracts from this plant material. The results of this study illuminated possible ways in which to add value to fruits of thirty-five wild and cultivated Andean plants based on their potential use as anti-aging ingredients in cosmetic formulations. After additional studies of the security and isolation of the active compounds, new cosmetic ingredients useful for anti-aging formulations could be obtained.


## 1. Introduction

The skin is the largest organ in the body, and its main functions include protection, thermal regulation, and sensory detection. It is divided into three layers: the epidermis, the dermis and subcutaneous tissue (Rittié and Fisher, 2002). The extracellular matrix (ECM) is the largest component of the dermis and provides a structural framework essential for the growth and elasticity of the skin. The ECM consists of proteoglycans interwoven with matrix metalloproteins, such as collagen, elastin and fibronectin, produced by fibroblasts. Collagen is the most abundant protein in the ECM and is responsible for the elasticity and strength of the skin, maintaining its flexibility (Ndlovu et al., 2013). Elastin is an important protein with the unique property of elastic recoil, vital to maintaining skin elasticity and resilience (Oikarinen, 2004). Hyaluronic acid (HA), a glycosaminoglycan, plays a role in retaining the moisture of the skin and its structure and elasticity. However, enzymes involved in the destruction of such components have been directly linked to the skin aging process (Mukherjee et al., 2011).

Photoaging is skin aging due to environmental aggressors as UV radiation, which is absorbed by the skin, leading to an increase in the reactive oxygen species (ROS) (Rittié and Fisher, 2002). ROS act as cell-signaling molecules and can cause lipid peroxidation, mitochondrial and DNA damage, and protein and gene modification

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(Irshad and Chaudhuri, 2002). In addition, ROS can initiate complex molecular pathways, including the activation of enzymes such as the metalloproteinase collagenase, the serine-protease elastase and the mucopolysaccharase hyaluronidase, which degrade ECM components (Mukherjee et al., 2011; Rittié and Fisher, 2002), resulting in visible changes in the skin such as wrinkles and variations in thickness (Jenkins, 2002). ROS can also accelerate skin pigmentation by its action on keratinocytes, adjacent to melanocytes, to induce melanogenesis by increasing the amounts of the melanogenic factors tyrosinase and tyrosinase-related protein 1 (TRP 1) (Sasaki et al., 2000).

Although there are several synthetic skincare products containing ingredients active against skin aging, they can produce adverse reactions such as allergic contact dermatitis, irritant contact dermatitis, phototoxic and photoallergic reactions (Mukherjee et al., 2011). Therefore, it is necessary to seek out new safe and effective skin-care cosmetic ingredients from natural resources. Plants and their secondary metabolites have been widely used in the cosmetic industry as antioxidants, skin lighteners and sunscreen agents, among other functions. *In vitro* scientific studies have shown that plant extracts and secondary metabolites such as phenolic compounds can reduce oxidant levels and inhibit collagenase, elastase, hyaluronidase and tyrosinase enzymes (Mukherjee et al., 2011; Ndlovu et al., 2013; Osorio et al., 2013; Wittenauer et al., 2015). For these reasons, many herbs, vegetables, fruits and whole grains are currently in demand worldwide due to their significant impact on skin aging. Notably, fruits constitute an important source in the search for metabolites active against skin aging due to high their antioxidant capacity and their richness in phenolic compounds, carotenoids and ascorbic acid.

The inhibition of the activity of ECM-degrading enzymes may be a useful approach to prevent photoaging. Additionally, the scavenging of ROS by natural antioxidants might be an option in the prevention of enzyme activation and macromolecular damage associated with ROS. Therefore, this study evaluated the antioxidant properties and the effect on collagenase, elastase, hyaluronidase and tyrosinase of the methanolic extracts of fruits belonging to thirty-five plant species found in the Andean lands of Colombia at different stages of growth. It is known that most of the species growing in high-elevation environments in tropical mountains remain largely unexplored. In particular, little is known from a chemical and biological standpoint (Carrillo-Hormaza et al., 2015). Additionally, the best extraction solvents for the potential species were obtained, and liquid–liquid partitions were performed to obtain rich fractions with greater anti-aging effects.

## 2. Materials and methods

### 2.1. Plant collection and preparation of extracts

Fruits from thirty-five Andean species belonging to 13 families were collected and identified in rural areas of Antioquia and Nariño Departments in Colombia (Table 1). The fruits of the wild species *Cavendishia pubescens* (Kunth) Hemsl., *Cestrum nocturnum* L., *Coccocypselum lanceolatum* (Ruiz & Pav.) Pers., *Faramea oblongifolia* Standl., *Galium hypocarpium* (L.) Endl. ex Griseb., *Lycianthes radiata* (Sendtn.) Bitter., *Monnina speciosa* Triana & Planch., *Palicourea garciae* Steyerm., *Psidium guineense* Sw., *Solanum nutans* Ruiz & Pav., *Solanum ovalifolium* Dunal., *Syzygium paniculatum* Gaertn., and *Vaccinium meridionale* Sw., were collected in Rionegro—Antioquia (altitude 2120 m); the fruits of *Alchornea triplinervia* (Spreng.) Müll. Arg., *Gaultheria erecta* Vent., *Hedyosmum goudotianum* Solms., *Miconia myrtillifolia* Naudin., *Morella parvifolia* (Benth.) Parra-Os., *Muehlenbeckia tamnifolian* (Kunth) Meisn., *Nertera granadensis* (Mutis ex L.f.) Druce., *Palicourea aschersonianoides* (Wernham) Steyerm., *Palicourea zarucchi* C.M. Taylor., *Passiflora cumbalensis* (H. Karst.) Harms., *Rubus compactus* Benth., *Solanum nigrescens* M. Martens & Galeotti., and *Ugni myricoides* (Kunth) O. Berg., were collected in the Páramo of San Felix, Bello—Antioquia (altitude 3060 m); and the fruits of *Hieronyma antioquensis* Cuatrec., were collected in San Pablo—Nariño (altitude 2500 m). The fruits of the cultivated species *Fragaria vesca* L., *Passiflora edulis* Sims., *Passiflora tarminiana* Coppens & V.E. Barney., *Physalis peruviana* L., *Rubus glaucus* Benth., *Rubus robustus* C. Presl., *Solanum betaceum* Cav. (purple), *S. betaceum* (yellow) and *Vaccinium floribundum* Kunth., were obtained from local farmers in Santa Elena—Antioquia (altitude 2600 m). When possible, the fruits were classified according to maturation state as immature (S1), intermediate (S2) or mature (S3).

The materials (whole fruits) were dried at 40 °C for 72 h and then powdered using an electric blender. The powdered fruits were extracted twice with methanol and hexane by sonication for 45 min. Afterward, the supernatants were combined, filtrated with filter paper and centrifuged at 4000 rpm for 40 min. The resulting filtrates were evaporated under vacuum at 40 °C and stored at 4 °C until use. The property measurements of these species were compared with a commercial extract of *Camellia sinensis* (Theaceae), the well-known green tea, which was used as a positive control in this study.

### 2.2. In-vitro *determination of anti-aging properties*

The anti-aging properties of extracts and fractions were measured by their capacity to inhibit the activity of skin aging-related enzymes.

#### 2.2.1. Anti-collagenase activity

The inhibition of collagenase enzyme was measured using the EnzCheck® Gelatinase/Collagenase assay kit (Molecular Probes Inc.). Briefly, 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-gelatin or DQ-collagen type IV substrate followed by 100 μL of active enzyme were added, and the fluorescence intensity was measured by a Synergy HT Multi-Mode Microplate Reader (BioTek Instruments, Inc.; Winooski, USA) for excitation at 485 nm and emission detection at 515 nm at each minute for 20 min. The increase in fluorescence is proportional to the proteolytic activity. Therefore, the decrease in fluorescence compared with the enzyme activity alone (control) was observed to identify a potential gelatinase/collagenase inhibitor. Oleanolic acid (250 μM) was used as a reference inhibitor. Each reaction was performed in triplicate. The percent inhibition of collagenase reaction was calculated as follows:

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

where $M_{\text{Control}}$ and $M_{\text{Sample}}$ are the slopes of the graph fluorescence *vs* time of the control and sample, respectively.

#### 2.2.2. Anti-elastase activity

The effect on elastase enzyme was measured using the EnzCheck® Elastase assay kit (Molecular Probes Inc.). Briefly, 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-elastine substrate and 100 μL of active enzyme were added. The fluorescence intensity was measured under the conditions described for the previous assay. Oleanolic acid (500 μM) was used as a reference inhibitor. Each reaction was performed in triplicate. The percent inhibition of elastase reaction was calculated using Eq. (1).