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Screening of UVA/UVB Absorption and *in vitro* Antioxidant Capacity of *Bejaria aestuans*, *Cavendishia pubescens* and *Cavendishia bracteata* Leaf Extracts

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ABSTRACT

The UV solar radiation accelerates skin aging, leading to loss of skin elasticity and decreasing the capacity of the skin to hold water, which are implicated in the formation of the most obvious sign of aging, skin wrinkling and related health problems in humans. Usually, synthetic sunscreens have been used to minimize these effects. However, some phenolic compounds present in plants play an important role as photoprotectors. The aim of this study was to explore the potential of three plants from Ericaceae family as UVA-UVB filters and its antioxidant capacity. Leaf extracts of *B. aestuans*, *C. bracteata* and *C. pubescence* showed an interesting broad-spectrum UVB/UVA absorption that was correlated with the antioxidant capacity (from 1.96E-04-3.75E-04 mg μmol^{-1} DPPH), the TPC value (from 15.29-27.35 mg GAE g^{-1} DS) and TAC value (0.36-3.31 mg C3GE g^{-1} DS). According to these results, the three plants evaluated in this research could be considered as a potential source of natural compounds with UV absorption and antioxidant capacity.

Key words: Photoprotection, UVA/UVB Broad-spectrum, ericaceae plants, andean mountains

INTRODUCTION

Direct exposure to ultraviolet radiation (UVR) can cause cutaneous alterations such as erythema, skin cancer and photoaging that may stimulate many processes associated to premature aging such as depigmentation, wrinkles and malignancies (Damiani *et al*., 2006; Ponzo and Silvia, 2013). Skin care cosmetic products that contain UV absorbers are currently a very common practice to deliver protection against the adverse effects of UVR. Although, there are numerous synthetic sunscreens that efficiently protect skin against erythema and edema and probably prevent squamous cell carcinoma, they also seem to provide much less protection than expected. Thus, the use of botanicals as an essential component of sunscreens has been gaining attention in recent times. Natural substances extracted from herbs may act as potential photoprotective resources thanks to their UV absorbing properties in the UVR region. In addition, they exhibit antioxidant, antimutagenic, anti-inflammatory and anticarcinogenic activities. Therefore, the use of botanicals is an approach to reduce the UV-generated ROS-mediated photodamage, immune-suppression and skin cancer in patients (Katiyar *et al*., 2000, 1999; Morales *et al*., 2011; Stahl *et al*., 2000; Zhou *et al*., 2009).

On the other hand, plant extracts are gaining the consumer attention as active ingredients for sunscreen preparations due to the presence of high contents of bioactive compounds with functional

properties and the possibility of using them as additives on cosmetic formulations (Duraisamy *et al*., 2011; F'guyer *et al*., 2003; Jarzycka *et al*., 2013). Plants from high altitude habitats, above 2500 m above sea level, are exposed to high levels of ultraviolet radiation and extreme environmental conditions such as fluctuations of raining and temperature, high humidity and low temperature, extraordinary UV radiation exposure, dry air, soils without enough nutrients and low atmospheric pressure. All of them induce plants to produce natural defenses against these conditions and have an important role in the evolution of specific plants. The most common characteristics of these plants are small and thick leaves, sometimes hairy, flowers growing close to the ground, a deep soil-penetrating root system and very distinct compact cushion plants. Most of these flowering plants belong to the Ericaceae family (Villota *et al*., 2012).

The Ericaceae is a relatively large family of flowering plants with almost 128 genera and about 4000 species. Members of this family are mainly found in Tropical Mountains, Mediterranean and temperate to artic climates. They prefer acidic habitats, well-lit conditions and a temperate climate. Most of them live in close association with fungi, which inhabit the roots without penetrating the cells. In fact, fungi facilitate the uptake of nutrients, especially nitrogen and elements (Hebert *et al*., 2010; Kjoller *et al*., 2010). Colombia has 22 genera and almost 270 species. These genera have therapeutic applications on arthritis and rheumatism, as well as ornamental, food, religious (Marc *et al*., 2008) and ethnobotanical uses (Tene *et al*., 2007).

The objective of this study was to evaluate the in vitro UV-radiation absorption capacity of extracts from three plants belonging to Ericaceae family and compare them with their antioxidant capacity.

MATERIALS AND METHODS

Plant materials: Leaves of *Bejaria aestuans* Mutis ex L. (named BA), *Cavendishia pubescens* (Kunth) Hemsl. (named CP) and *Cavendishia bracteata* (Ruiz and Pav. Ex J. St.-Hil) Hoerold, (named CB) were collected at Llanos de Cuivá, Santa Rosa de Osos, Antioquia, Colombia, at 2730 m above sea level (Geographical coordinate: 6°49'50.6" N; 75°29'29.9" W). Botanical identification for BA, CP and CB was performed in the Herbarium of the University of Antioquia (HUA) and a voucher specimen was deposited under registry code HUA190418, HUA190421 and HUA190424, respectively.

Dry and powdered leaves of the *B. aestuans*, *C. pubescens* and *C. bracteata* (c.a. 1.0 g) were mixed with 50.0 mL of acetone/HCl (99.5:0.5 v/v) for 24 h at room temperature and magnetically stirred at 900 rpm. The resultant solution, previously filtered, was concentrated to dryness in a rotary evaporator under reduced pressure at 40°C to get rid of any residual solvent. Then, extracts of BA, CP and CE were redisolved in ethanol and keep at 4°C before analysis.

Phytochemical screening: Dry leaves of BA, CP and CB were powdered and then dissolved in ethanol:water (1:1) for determining the presence of chemical compounds like phenols, flavonoids, anthocyanin and tannins (Dahou *et al*., 2003).

Total Polyphenol Contents (TPC): The total polyphenol content in BA, CP and CB extracts was determined using the Folin-Ciocalteau colorimetric technique with some modifications (Singleton *et al*., 1999). Briefly, the BA, CP and CB extract solutions (c.a. 0.5 mL), were added to 0.125 mL of the Folin-Ciocalteau reagent and 1.25 mL of 20% Na_2CO_3. After incubation for 90 min at room temperature, the absorbance was read at 760 nm using an Evolution 60S

spectrophotometer (Thermo Fisher Scientific, Inc., Shanghai, China). The results were expressed as Gallic acid equivalents per gram of dry extract (GAE g^{-1} dry extract).

Total Anthocyanin Content (TAC): Total anthocyanins were estimated by a pH differential method (Lee *et al*., 2005) with some modifications. An aliquot (c.a. 1.0 mL) of solution of extract was mixed with 3.0 mL of buffer at pH 1.0 (HCl/KCl; 0.025 M) and another at pH 4.5 (acetic acid/sodium acetate; 0.4 M). After that, absorbance (A) was measured in a Thermo Scientific Evolution 60S UV-Visible spectrophotometer, at 510 and at 700 nm. The anthocyanin pigment concentration was expressed as cyanidin-3-glucoside equivalents using the following equation:

$$\text{Total anthocyanin content (mg L}^{-1}) = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times l}$$

where, A= absorbance = $(A_{520}\text{-}A_{700\ nm})_{pH1.0}\text{-}(A_{520}\text{-}A_{700\ nm})_{pH\ 4.5}$.

The MW is the molecular weight for cyanidin-3-glucoside (cyd-3-glu) = 449.2 g mol^{-1}, DF is the dilution factor established, l is the path length of the cell in cm; ε is the molar extinction coefficient of cyd-3-glu = 26,900 L mol^{-1} cm^{-1} and 10^3 is the conversion factor.

***In vitro* DPPH radical assay:** The radical scavenging capacity for each fraction in different concentrations was estimated according to the method described previously (Brand-Williams *et al*., 1995) with some modifications; the effective relative concentration (EC_{50}) at which 50% of DPPH has been removed was expressed as mg of extract μmol^{-1} of DPPH radical, based on the following equation:

$$EC_{50} = \frac{\text{Concentration of sample at steady state}}{\text{Concentration of DPPHt} = 0}$$

The exact initial DPPH concentration (92.18 μmol L^{-1}) in the reaction system was calculated according to a calibration curve (y = 1.145 E-2× -4.192 E-3, r = 0.9999, where y = absorbance and x = concentration of DPPH) at 514 nm). All experiments were performed in triplicate. All spectrophotometric data were obtained using a Thermo Scientific Evolution 60S UV-Visible spectrophotometer. Disposable cuvettes (1×1×4.5 cm) were used for visible absorbance measurements for analyses.

UVA-UVB absorption capacity: Briefly, an aliquot (c.a. 50 μL) of crude extract was added to 2000 mL of methanol in a quartz cuvette and its absorption spectra (wavelengths 200-400 nm) were acquired using a Thermo Scientific Evolution 60S UV-Visible spectrophotometer against a blank containing methanol. The UVA-UVB absorption capacity was determined using the absorption coefficient (absorbance DM mg^{-1} mL^{-1}) measured at 290, 310, 340 and 380 nm. Benzophenone 3 (B3), butyl methoxydibenzoylmethane (BMDM) and ethylhexyl methoxycinnamate (EHMC) were used as conventional sunscreen standards.

Statistical analysis: Results were expressed as Mean±Standard Deviation (at least three replicates). Analysis of variance and significant differences among means and correlation analysis were performed with one-way ANOVA. The experimental data was analyzed using SPSS V.21.0 software.

RESULTS AND DISCUSSION

The UV radiation accelerates skin aging leading to loss of skin elasticity and decreasing the capacity of the skin to hold water, which are implicated in the formation of the most obvious sign of aging, skin wrinkling. Some promising topical treatments of skin aging include herbal extracts, vitamin and antioxidant supplements, which have been widely accepted to scavenge free radicals from skin cells and to restore skin elasticity.

In this study, the phytochemical study showed the presence of phenols, flavonoids, anthocyanins, tannins and quinones in all three extracts (Table 1). Data from Ericaceae species is difficult to compare due to its environmental conditions. However, the results of phytochemical analysis obtained are in good agreement with data reported by some authors (Akkol *et al*., 2008; Harnafi *et al*., 2007), which have detected the presence of flavonoids, tannins, proanthocyanidins, coumarins on Ericaceae species.

A suitable mix of conventional sunscreens must absorb in UVA-UVB range and provide a broad-spectrum UVB/UVA protection. Extracts of *B. aestuans, C. bracteata* and *C. pubescence* showed an interesting broad-spectrum UVB/UVA absorption. However, *B. aestuans* and *C. pubescence* extracts displayed a higher ultraviolet radiation absorption in the range of UVB than *C. bracteata* extracts. Nevertheless, all of them showed a broad-spectrum range similar to the standards (Fig. 1). This result has been correlated with the presence of phenolic compounds in the extracts that have demonstrated good absorption of UV radiation (Stevanato *et al*., 2014). On the other hand, the absorption coefficients of the extracts was calculated in order to obtain comparative data at wavelength 290, 310, 340 and 380 nm, typical regions for UVA and UVB solar spectrum (Fig. 2). Here, we can observe that all of them have broad-spectrum coverage, but this was much higher in

Table 1: Phytochemical screening of *Bejaria aestuans, Cavendishia pubescence* and *Cavendishia bracteata* leaf extracts

Phytochemicals	*B. aestuans*	*C. pubescence*	*C. bracteata*
Phenols	+	+	+
Flavonoids	+	+	+
Anthocyanins	+	+	+
Tannins	+	+	+
Quinones	+	+	+

+: Detected, According to these results, the UV absorption spectra of the BA, CP and CB extracts and three common UV filters, Benzophenone-3 (B3), EHMC: Ethylhexyl methoxycinnamate and BMDM Butyl methoxydibenzoylmethane were recorded (Fig. 2)

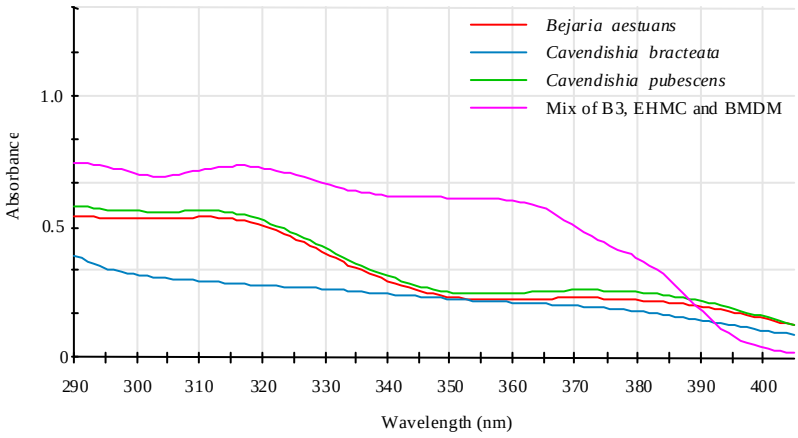

Fig. 1: UVA-UVB absorption coefficient assays (absorbance DM mg $^{-1}$ mL^{-1}) at 290, 310, 340 and 380 nm

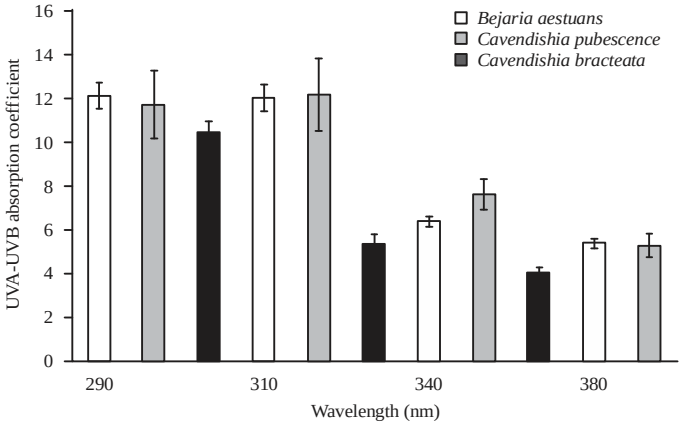

Fig. 2: UV spectrum of *Bejaria aestuans, Cavendishia pubescence* and *Cavendishia bracteata* leaf extracts (at 0.015 mg mL^{-1}, all of them) and a mixed sample of benzophenone 3, butyl methoxydibenzoylmethane, ethylhexyl methoxycinnamate (at 0.017 mg mL^{-1}, all of them)

Table 2: *In vitro* antioxidant capacity, total polyphenol and anthocyanin contents

Samples	Yield (dry extract%)	TPC (mg GAE g^{-1} DS)*	EC_{50} (mg ext μmol^{-1} DPPH)**	TAC (mg C3GE g^{-1} DS)***
B. aestuans	24.12±0.84^a	21.82±0.39^a	2.64E-04±3.5E-05^a	1.61±0.15^a
C. pubescens	20.40±1.95^b	27.35±1.95^b	1.96E-04±2.8E-05^b	3.31±0.55^b
C. bracteata	16.32±1.42^c	15.29±1.39^c	3.75E-04±3.7E-05^c	0.36±0.12^c

*Total phenolic content. **Effective concentration at 50%. ***Total anthocyanin content, Means in the same column followed by the same letter do not differ significantly ($p<0.05$)

the UVB range. This increase could be attributed to the inherent presence of polyphenols and anthocyanins in the extracts (Table 2), which have demonstrated the ability to act as broad-spectrum sunscreens, covering the UVA and UVB range (Velasco *et al*., 2008).

Polyphenolic compounds from plants exert their beneficial health effects mainly through their antioxidant capacity due to their property of scavenging free radicals, chelating metal catalysts, activating antioxidant enzymes and so on. Total Phenolic Content (expressed in GAE g^{-1} DS) of the antioxidant extracts from selected plants are presented in Table 2. The highest concentration of TPC (27.35 mg GAE g^{-1} DS) was obtained from *C. pubescens*, followed by *B. aestuans* and *C. bractaeata*. Difference analysis between the three cultivars of Ericaceae species showed significant differences ($p<0.05$) in TPC. The average TPC value (c.a 21.48 mg GAE g^{-1} DS) of all selected Andean Colombian plants was higher than that reported for common cultivars, like strawberries (0.844 mg GAE g^{-1}) (Kajdzanoska *et al*., 2011), common legumes (0.57-9.60 mg GAE g^{-1}) (Xu *et al*., 2007) and *Arbutusandrachne* L. (Fam. Ericaceae) fruits from Turkey (2.4-4.1 mg GAE g^{-1}) (Serce *et al*., 2010). Concerning the anthocyanin contents, the results were similar to those obtained with TPC and ranged from 0.36-3.31 mg C3GE g^{-1} DS, with the highest content in *C. pubescens* extract, followed by *B. aestuans* and *C. bractaeata* (Table 2).

The antiradical activities of Ericacea plant extract were expressed as EC_{50} value. The EC_{50} values found were 2.64 E-04, 1.96 E-04 and 3.75 E-04 mg μmol^{-1} DPPH of reaction medium in the reduction of DPPH. These results are closely to those reported by Guendouze-Bouchefa *et al*. (2015) on methanolic extracts of three Ericaceae species (Guendouze-Bouchefa *et al*., 2015). Therefore, the extracts showed an interesting antiradical capacity that could be linked to defense mechanisms against oxidative stress in plants of high altitude and could be useful as cell protectors in our own bodies.

Present study confirms that Ericaceae extracts should be useful asa potential source of natural photoprotection and antioxidants compounds because of their capability to absorb UV radiation and scavenge free radicals. In addition, a chemical study of these plants could be performed in order to identify novel compounds that are responsible of these biological properties.

CONCLUSION

The *in vitro* test showed that these matrices in association with conventional organic and inorganic sunscreens could be useful as potential ingredients on dermatological formulations with health benefits and will be used as an additive for photoprotection and free radical scavenging.

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REFERENCES

Akkol, E.K., E. Yesilada and A. Guvenc, 2008. Valuation of anti-inflammatory and antinociceptive activities of *Erica* species native to Turkey. J. Ethnopharmacol., 116: 251-257.

Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci. Technol., 28: 25-30.

Dahou, N., K. Yamni, S. Tahrouch, L.M. Idrissi Hassani, A. Badoc and N. Gmira, 2003. Screening phytochimique d'une endemique ibero-marocaine Thymelaea lythroides. Bull. De La Societe De Pharmacie De Bordeaux, 142: 61-78.

Damiani, E., L. Rosati, R. Castagna, P. Carloni and L. Greci, 2006. Changes in ultraviolet absorbance and hence in protective efficacy against lipid peroxidation of organic sunscreens after UVA irradiation. J. Photochem. Photobiol. B: Biol., 82: 204-213.

Duraisamy, A., N. Narayanaswamy, A. Sebastian and P.K. Balakrishnan, 2011. Sun protection and anti-inflammatory activities of some medicinal plants. Int. J. Res. Cosmetic Sci., 1: 13-16.

F'guyer, S., F. Afaq and H. Mukhtar, 2003. Photochemoprevention of skin cancer by botanical agents. Photodermatol. Photoimmunol. Photomed., 19: 56-72.

Guendouze-Bouchefa, N., K. Madani, M. Chibane, L. Boulekbache-Makhlouf and D. Hauchard *et al*., 2015. Phenolic compounds, antioxidant and antibacterial activities of three Ericaceae from Algeria. Ind. Crops Prod., 70: 459-466.

Harnafi, H., N. El-Houda-Bouanani, M. Aziz, H.S. Caid, N. Ghalim and S. Amrani, 2007. The hypolipidaemic activity of aqueous Erica multiflora flowers extract in Triton WR-1339 induced hyperlipidaemic rats: A comparison with fenofibrate. J. Ethnopharmacol., 109: 156-160.

Hebert, F., N. Thiffault, J.C. Ruel and A.D. Munson, 2010. Ericaceous shrubs affect black spruce physiology independently from inherent site fertility. For. Ecol. Manage., 260: 219-228.

Jarzycka, A., A. Lewinska, R. Gancarz and K.A. Wilk, 2013. Assessment of extracts of *Helichrysum arenarium*, *Crataegus monogyna*, *Sambucus nigra* in photoprotective UVA and UVB; photostability in cosmetic emulsions. J. Photochem. Photobiol. B: Biol., 128: 50-57.

Kajdzanoska, M., J. Petreska and M. Stefova, 2011. Comparison of different extraction solvent mixtures for characterization of phenolic compounds in strawberries. J. Agric. Food Chem., 59: 5272-5278.

Katiyar, S.K., M.S. Matsui, C.A. Elmets and H. Mukhtar, 1999. Polyphenolic antioxidant (-)-epigallocatechin-3-gallate from green tea reduces UVB-induced inflammatory responses and infiltration of leukocytes in human skin. Photobiol. Photochem., 69: 148-153.

Katiyar, S., K., A. Perez and H. Mukhtar, 2000. Green tea polyphenol treatment to human skin prevents formation of ultraviolet light B-induced pyrimidine dimers in DNA. Clin. Cancer Res., 6: 3864-3869.

Kjoller, R., M. Olsrud and A. Michelsen, 2010. Co-existing ericaceous plant species in a subarctic mire community share fungal root endophytes. Fungal Ecol., 3: 205-214.

Lee, J., R.W. Durst and R.E. Wrolstad, 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants and wines by the pH differential method: Collaborative study. J. AOAC Int., 88: 1269-1278.

Marc, E.B., E.B., A. Nelly, D.D. Annick and D. Frederic, 2008. Plants used as remedies antirheumatic and antineuralgic in the traditional medicine of Lebanon. J. Ethnopharmacol., 120: 315-334.

Morales, L.O., R. Tegelberg, M. Brosche, A. Lindfors, S. Siipola and P.J. Aphalo, 2011. Temporal variation in epidermal flavonoids due to altered solar UV radiation is moderated by the leaf position in *Betula pendula*. Physiol. Plant., 143: 261-270.

Ponzo, O.J. and C. Silvia, 2013. Evidence of reproductive disruption associated with neuroendocrine changes induced by UV-B filters, phtalates and nonylphenol during sexual maturation in rats of both gender. Toxicology, 311: 41-51.

Serce, S., M. Ozgen, A.A. Torun and S. Ercisli, 2010. Chemical composition, antioxidant activities and total phenolic content of *Arbutus andrachne*, L. (Fam. Ericaceae) (the Greek strawberry tree) fruits from Turkey. J. Food Comp. Anal., 23: 619-623.

Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos, 1999. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. In: Methods in Enzymology, Abelson, J.N., M. Simon and H. Sies (Eds.). Elsevier, London, UK.

Stahl, W., U. Heinrich, H. Jungmann, H. Sies and H. Tronnier, 2000. Carotenoids and carotenoids plus vitamin E protect against ultraviolet light-induced erythema in humans. Am. J. Clin. Nutr., 71: 795-798.

Stevanato, R., M. Bertelle and S. Fabris, 2014. Photoprotective characteristics of natural antioxidant polyphenols. Regul. Toxicol. Pharmacol., 69: 71-77.

Tene, V., O. Malagon, P. Vita Finzi, G. Vidari, C. Amijos and T. Zaragoza, 2007. An ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchipe, Ecuador. J. Enthnopharmacol., 111: 63-81.

Velasco, M.V.R., F.D. Sarruf, I.M.N. Salgado-Santos, C.A. Haroutiounian-Filho, T.M. Kaneko and A.R. Baby, 2008. Broad spectrum bioactive sunscreens. Int. J. Pharmaceut., 363: 50-57.

Villota, A., S. Leon-Yanez and H. Behling, 2012. Vegetation and environmental dynamics in the Paramo of Jimbura region in the southeastern ecuadorian andes during the late quaternary. J. South Am. Earth Sci., 40: 85-93.

Xu, B.J., S.H. Yuan and S.K.C. Chang, 2007. Comparative analyses of phenolic composition, antioxidant capacity and color of cool season legumes and other selected food legumes. J. Food Sci., 72: 167-177.

Zhou, Y.H., Y.Y. Zhang, X. Zhao, H.J. Yu, K. Shi and J.Q. Yu, 2009. Impact of light variation on development of photoprotection, antioxidants and nutritional value in *Lactuca sativa* L. J. Agric. Food Chem., 57: 5494-5500.