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Original Article

Cosmetic Antioxidant Potential of Extracts from Species of the *Cinchona Pubescens* (Vahl)

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Abstract

The present study was aimed at assessing the dry extracts from *Cinchona Pubescens* Vahl species, as a potential antioxidant raw material for the cosmetics industry. The phenolic compound and flavonoids concentration in the extract were 30 (mg GAE = mg Gallic acid equivalent)/g mg Hyp = mg hyper oxide 2 mg/g. The following flavonoids were identified in the extract: apigenin, quercetin, catechin and kaempherol. The antioxidant activity was determined by spectrophotometric methods ABTS and 1-diphenyl-2-picrylhydrazyl (DPPH), expressed as IC50 (50% inhibition of radical oxidation) were 42.00 ± 0.2 µg/ml (DPPH) and 88.00 ± 0.2 IC50 µg/ml ABTS. In cosmetic formulations (gels and creams) evaluated through photochemiluminescence, a significantly increased antioxidant potential was observed when compared to the reference formula. The potential was up to 15 times when compared to a dry extract of *Camellia sinensis.*

Keywords: *Cinchona pubescens* Vahl, antioxidant activity, flavonoids, photochemiluminescence.

Introduction

Cinchona pubescens (Vahl) is a renowned native medicinal plant species from Ecuador. It is traditionally known as "Cascarilla Roja",[1] and is native to the Andean regions, and it is distributed widely in countries like Bolivia,[2] Colombia[3] and Peru.[4]

There are several benefits described for the plant, the most important being antimalarial,[5,6] antiarrhythmic,[7,8,9] appetite stimulant,[5,10] and antipyretic;[10] recent studies also indicate that it has anti-cancer [11] and hepatoprotectives[12] properties.

Pelletier & Carentouaislaz were the first to isolate its alkaloids, the most prominent being the presence of quinine and quinidine stereoisomer. Other compounds that have been isolated are cinonine, cinchonidine, and a variety of quinoline derivatives.[5] Alkaloids are undoubtedly the main component, approximately 6.5% total alkaloids (approximately 20) among which the majority is quinine representing 70–90% of the pair of stereoisomers, 1% corresponds to quinidine,[13] along with along with its 6-dimethoxy analogs as cinchonine and cinchonidine which are useful as antimalarial and used entirely for the preparation of quina,[14] another group of compounds found in *C. pubescens* are the anthroquinones.[15] Essential oils are also an important group and represent a 0.02 to 0.08%.[16] A variety of a stringent components have been identified (dimers and trimers proantocianidoles tannins, catechin tannins 8% of total tannins) and other compounds such as flavonoids, catechin, kaempferol, apigenin and quercetin; glycosides, organic acids (quinotanic acid, cinconic red), monoglycosides such as quinovic acid (3β-hydroxyurea-droxibenzoico acid) and terpene compounds. [14]

Despite the chemical richness of these species, the compounds of a great importance, historically, have been the alkaloids that have been a cornerstone in the fight against malaria. Centuries before the advent of synthetic medicine, it was the alkaloids of cinchona that, on a global scale became pharmaceutically important, with the introduction of the plant in various parts of the world. This generates various environmental problems, *C. pubescens* is regarded as one of the 100 most damaging invasive plants in the world;[17,18] one example in Ecuador is the adverse ecological effects evidenced in the Galapagos Islands. [19]

This conjecture demands a new assessment of the plant, the presence of phenolic compounds,[14] presumably, made possible by the antioxidant effect that has been widely described in the literature. [20,21,22] There is currently strong interest in incorporating components with antioxidant activity in products for skin protection, which undergoes oxidative processes mainly due to solar radiation, poor nutrition and pollution. [23, 24] This research aims to evaluate the antioxidant activity of in vitro dry extract and cosmetic formulas.

Materials and Methods

Plant Material

Bark of *Cinchona pubescens* (Vahl) material was collected in a place known as "El Corazon" located at: Latitude: 0 ° 16'50.5 "S. Longitude 78 ° 41'25.5 "W at an altitude of 615 meters above sea level, in the province of Pichincha, Ecuador. The species identification was carried out at the herbarium of the Pontificia Universidad Catolica del Ecuador botanist Alvaro. J. Perez.

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The material was dried in an oven with re-circulating air at 30 ° C for 96 hours; it was finely ground and passed through a 500 µm mesh. The material was extracted with 96% ethanol with 250 ml capacity percolators. The extract was evaporated under vacuum to obtain the dry residue.

Evaluation of the Phenolic Content

Plant extracts were prepared for analysis using the colorimetric method of Folin Ciocalteu, as proposed by Singleton.[25] Plant extracts were left for 1.5 minutes in 20% by volume Na_2CO_3 solution. The absorbance was read at 765 nm using a spectrophotometer (UV1240 mini-Shimadzu). The phenolic content concentrations were calculated by comparing the absorbance of each sample with a series of standards (gallic acid treated under the same conditions).

Evaluation of the Flavonoids Content

The flavonoid concentration was determined using Carnat Lamaison's protocol with $AlCl_3$. [26] An amount of extract was dissolved in methanol and 2% $AlCl_3.6H_2O$ was added 10 minutes prior to measuring absorbance at 394 nm. Sample concentrations were obtained by comparison with set of standards prepared under the same conditions.

Identification of Flavonoids by TLC

An extract solution of 30 mg/ml is prepared for analysis on a Merk silica gel 60 TLC plate, with F 254 fluorescent indicators. The total volume analysed with a CAMAG device, Linomat 5 model, was of 10 µl. The mobile phase used was ethyl acetate, formic acid, acetic acid and water (100: 11: 11: 27 v/v); the standards used are those described in the literature found of *C. pubescens* (catechin, kaempferol, apigenin and quercetin) developed from the extract, were compared to those generated by the standards.

Evaluation of Antioxidant Activity

The evaluation of antioxidant activity was conducted using DPPH spectrophotometric method, results were compared with (ABTS) [29, 30] Spectrophotometric method as well.

Spectrophotometric DPPH and ABTS

DPPH assay, varying amounts of oil from *C. pubescens* solids were collected; these were then dissolved in 96% ethanol to a volume of 100 ul. To each solution 2.9 ml of 1, 1-diphenyl-2-picrylhydrazyl (DPPH; 1 x 10 4 in ethanol) was added, and then stirred vigorously for 30 minutes in the dark at room temperature. The absorbance was measured at 517 nm in a mini UV1240 Shimadzu spectrophotometer.

Similarly, with the ABTS test, each solution was dissolved in methanol and 0.9 ml of (2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) mM radicalized with a solution of $K_2S_2O_8$ was added. The absorbance was measured at 734 nm in a mini UV1240 Shimadzu spectrophotometer. The antioxidant activity seen as the percentage inhibition of the oxidation of either the radical ABTS or DPPH was calculated according to the following formula:

$$DPPH\% = \frac{Ab - Aa}{Ab} X100$$

Blank samples are designated Ab and Aa, and the absorbance of the samples Ab, respectively. These were measured after 30 min (DPPH) and 1 minute for ABTS. The anti-radical activity of the oil is evaluated calculating 50% inhibition of oxidation of DPPH and ABTS. The calibration curves were used in determining of the concentration, percentage of inhibition based on a reference, activities of dried extracts of *C. sinensis*, *and* ascorbic acid.

Formulation of Creams and Gels

The detailed composition of the formulations is described in Table 1 (cream) and Table 2 (gel).

Antioxidant Activity of the Formulations

The various formulations (gels and creams) were evaluated through a method known as photo-chemiluminescence described as ideal for this type of testing in several studies. [31,32] The methodology for antioxidant capacity in various phases are defined as the Antioxidative Capacity in Lipid (ACL) applicable for lipid soluble substances (creams) and Antioxidative Capacity in Water-soluble (ACW) used for sample of formulation in gel. The standards for these protocols are Trolox and ascorbic acid. (PHOTOCHEM®, Analytik Jena AG, Jena, Germany was used).

Table 1. Formulations (cream) made from dried extract of *C. pubescens* and reference base *C. sinensis.*

Ingredients	% and Type of Formulation			
	Ext *C. pubescens* 0.5 %	Ext *C. pubescens* 0.75 %	Ext *C. pubescens* 1.00 %	Ext *C. sinensis* 0.50%
Cetylstearyl Alcohol (and) Polysorbate 60	8.00	8.00	8.00	8.00
Cetyl alcohol	3.00	3.00	3.00	3.00
Steareth-20	3.00	3.00	3.00	3.00
Mineral oil	20.00	20.00	20.00	20.00
Aqua	q.s.	q.s.	q.s.	q.s.
Propylene-glycol	5.00	10.00	15.00	5.00
Dry extract	0.50	0.75	1.00	0.50
Dimethicone	0.50	0.50	0.50	0.50
Phenoxyethanol, methylparaben, ethtylparaben	0.80	0.80	0.80	0.80

Table 2. Formulations (gel) made from dried extract of *C. pubescens* and reference base *C. sinensis.*

Ingredients	% and Type of Formulation			
	Ext *C. pubescens* 0.25 %	Ext *C. pubescens* 0.50 %	Ext *C. pubescens* 1.75 %	Ext *C. sinensis* 0.50 %
Acrylates Copolymer	20.00	20.00	20.00	20.00
Triethanolamine	1.00	1.00	1.00	1.00
Aqua	q.s.	q.s.	q.s.	q.s.
Propylene-glycol	5.00	7.00	10.00	5.00
Dry extract	0.25	0.50	0.75	0.50
Dimethicone	0.50	0.50	0.50	0.50
Phenoxyethanol, methylparaben, ethtylparaben	0.80	0.80	0.80	0.80

Results and Discussion

Evaluation of Total Phenolic Compounds and Total Flavonoids

The concentration of total phenolic by *C. pubecens* in dry extract compared with total phenolic in dry extract of *C. Sinensis.*

Table 3. Concentration of total phenolic compounds and total flavonoids in dried extracts of *C. pubescens* and *C. sinensis.*

Dry extract	Total phenolic compounds expressed as mg GAE/gr	Total flavonoids expressed ass mg Hyp/gr
C. pubescens	30.1	2.0
C. sinensis	78.0	10.0

Identification of Flavonoids

The graph of TLC, Figure 1, shows the presence of the 4 flavonoids mentioned in the literature: apigenin, quercetin, catechin and kaempherol.

Evaluation of the Antioxidant Activity of the Extract of *C. Pubescens* through Spectrophotometric Methods DPPH and ABTS

From the graphs of percent inhibition vs. concentration it was possible to calculate the IC_{50}, equivalent to 50% inhibition of oxidation of DPPH radical or the radical ABTS as results show in Table 4.

Table 4. Antioxidant activity (IC_{50}) of the extract of *C. pubescens*.

Extract/compound	IC_{50} µg/ml	
	DPPH	ABTS
Ascorbic acid	2.40 ± 0.3	4.35 ± 0.4
C. sinensis	7.20 ± 0.1	123.10 ± 0.4
C. pubescens	42.00 ± 0,2	88.00 ± 0.7

Evaluation of Antioxidant Activity in Cosmetic Formulations

The results of antioxidant activity in both cosmetic formulations (creams and gels) expressed as mole Trolox /g are shown in Table 5.

Conclusions

The extract of *C. pubescens* shows an interesting mix of compounds with antioxidant nature (phenols and flavonoids) that

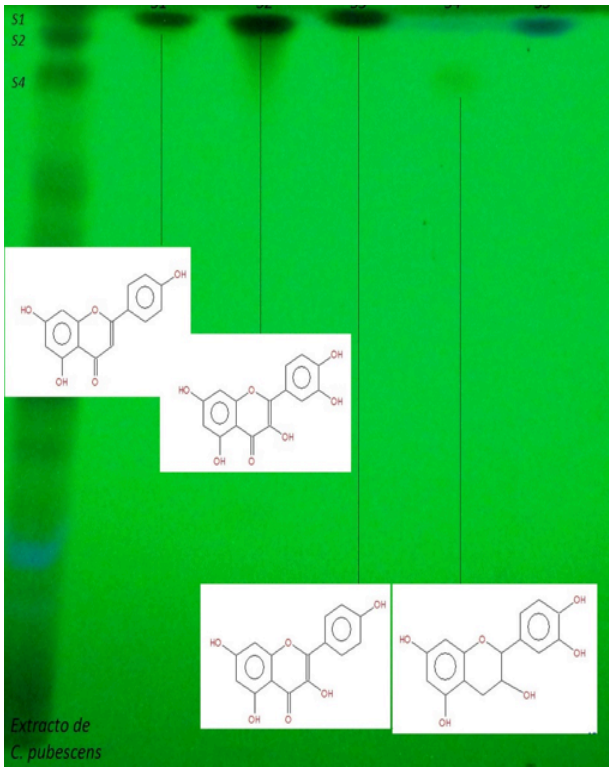

Figure 1. Identification by TLC of flavonoids present in the dry extract of *C. pubescens*: S1 apinenin, S2 Quercetin, S3 Kaempferol, S4 Catechin.

Table 5. Antioxidant activity of the formulations based on *C. pubescens*.

Formulation	Antioxidant activity µmol trolox or ascorbic acid/gr
Cream base	0.00 ± 0.0
Control cream *(C. sinensis 0,5%)*	5.4423 ± 0.1072
Cream *(C. pubescens 0,50%)*	10.3021 ± 1.2918
Cream *(C. pubescens 0,75%)*	14.2029 ± 0.1646
Cream *(C. pubescens 1,0 %)*	14.8230 ± 0.3215
Gel base	0.00 ± 0.0
Control gel *(S. sinensis 0,5%)*	10.9513 ± 0.1497
Gel *(C. pubescens 0,25%)*	7.3509 ± 0.6128
Gel *(C. pubescens 0,50%)*	12.3446 ± 0.1562
Gel *(C. pubescens 0,75%)*	13.8231 ± 0.3618

have been verified in the various references. The antioxidant properties of dry extracts from *C. pubecens* were observed at higher level than those of the natural reference (cream and gels with 0.5% extract of *C. sinensis*). Further investigation to confirm identities of substances using more rigorous methodology and technology such as HPLC is recommended. Results of those studies may also support ways to increase the antioxidant potential in formulations, which is possible using larger quantities of this type of extracts. This study also identifies a new potential for use of *C pubecens* that in turn may offer an opportunity for release of the environment stress caused by invasive growth of the plant in the protected areas of Ecuadorian Galapagos region.

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