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Evaluation of the Antioxidant Activity of Three Formulations of Hair Cosmetic Products Containing the Essential Oil of *Clinopodium bolivianum* (Benth.) Kuntze "inca muña"

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Abstract: *Clinopodium bolivianum* (Benth.) Kuntze, commonly known as muña, inca muña or koa, has traditionally been used for its medicinal properties in digestive disorders. Some studies have revealed its antioxidant potential and antibacterial activity. This study determined the volatile components, evaluated the antioxidant capacity of *C. bolivianum* essential oil and its incorporation into three hair cosmetic formulations: shampoo, combing cream, and capillary lotion. Gas Chromatography–Mass Spectrometry (GC–MS) confirmed pulegone as the main component, accounting for 66.85% of the essential oil. The antioxidant activity was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays, with Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) as a reference compound. The essential oil exhibited significant radical scavenging activity, with IC_{50} values of 1344.0 ± 12.23 μg/mL for DPPH and 40.125 ± 1.25 μg/mL for ABTS. Among the formulated cosmetic products, the combing cream containing 0.5% of the essential oil demonstrated the highest antioxidant activity, with IC_{50} values of 0.72 μg/mL (DPPH) and 0.068 μg/mL (ABTS). In contrast, the shampoo and capillary lotion showed lower antioxidant potential. The stability evaluation confirmed that all formulations maintained their physicochemical properties under accelerated conditions. These findings highlight the potential application of *C. bolivianum* essential oil as a natural antioxidant in cosmetic formulations, contributing to its protective and functional properties.

Keywords: antioxidant; muña; volatile components; natural products; cosmetics; in vitro

1. Introduction

Clinopodium bolivianum (Benth.) Kuntze is commonly known as muña, chuña muña, koa or inca muña by the inhabitants of Peru. This plant species is a small shrub that grows abundantly in the Andean region of Peru from January to March. It is characterized by a height of 60–80 cm, petiolate leaves (1–1.5 mm), obovate to elliptical, sub-oblong, with entire or serrated margins and 1–2 cm in length. This herb is considered the oregano of the Incas and is an aromatic plant belonging to the Lamiaceae family, which thrives at altitudes between 3200 and 4500 m (Figure 1) [1,2]. The traditional uses of *C. bolivianum* encompasses the treatment of various physiological conditions. In internal applications, muña is consumed as an infusion to address ailments such as nausea, indigestion, diarrhea, and altitude sickness [3,4]. Additionally, some medicinal properties have been studied such as antibacterial, antioxidant, anti-inflammatory, and anti-biofilm [3].

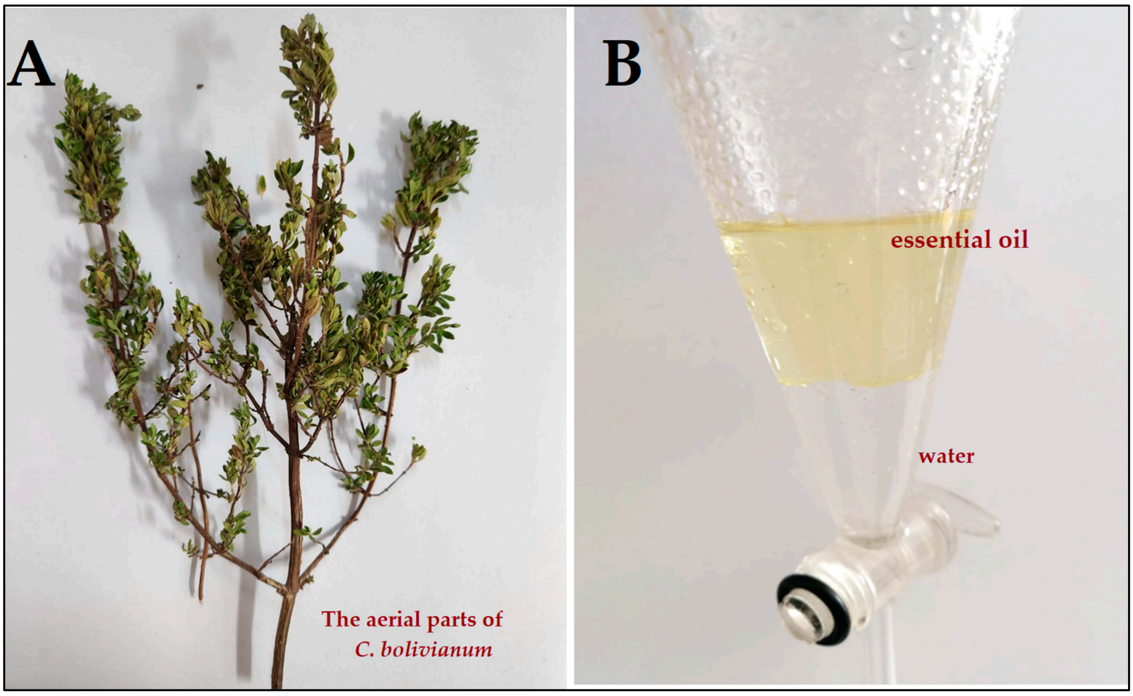

Figure 1. (**A**) The aerial parts of *C. bolivianum* from Puno, Peru. (**B**) The essential oil obtained with steam distillation.

On the other hand, the essential oils are complex mixtures of volatile compounds, including monoterpenes, sesquiterpenes, aldehydes, ketones, esters, among others [5]. Their extraction from aromatic plants is predominantly conducted via hydrodistillation and steam distillation, although other alternative processes such as supercritical fluid extraction with CO_2, microwave-assisted extraction, solvent-free microwave extraction, and lately the solar energy-based extraction are also considered eco-friendly improving the productivity during the extraction [6]. Furthermore, these compounds typically have low molecular weights and, in conjunction with their lipophilicity, readily permeate cell membranes [7]. Hence, modern skincare products frequently incorporate essential oils because of their complex active compounds, potent aromatic qualities, and association with natural ingredients in marketing [8]. Some characteristics of essential oils with antioxidant activity are attributed to a range of chemical compounds, including alcohols, ethers, ketones, aldehydes, monoterpenes and sesquiterpenes [9]. Some of these compounds studied as antioxidant potential are carvacrol, linalool, 1,8-cineoIe, geranial/neral, citronellal, isomenthone, and menthone [10]. Furthermore, the antioxidant activity of some essential oils was enhanced due to the presence of specific monoterpenes, notably α-terpinene, β-terpinene, and α-terpinolene [11].

Antioxidants present in cosmetic products can serve as a strategy to prevent photoaging and oxidative stress. These compounds can neutralize free radicals, thereby inhibiting the accelerated synthesis of metalloproteinases induced by ultraviolet radiation which would protect the collagen and other proteins in the skin's connective tissue [12]. Previous studies have demonstrated that antioxidants exert positive effects on both the dermis and hair, with evidence observed in the scalp and hair fiber [13,14]. Antioxidant active ingredients strengthen and maintain healthy hair follicles, resulting in robust and resilient hair [15]. These antioxidant active ingredients act by facilitating the stimulation and enhancement of hair follicles, while also contributing to the provision of nutrients to the scalp, thus preventing follicular weakening over time [16]. The use of antioxidant products is increasing continuously, and maintaining healthy hair is crucial for personal appearance and positively impacts an individual's self-esteem. Antioxidants work through various mechanisms, such as reducing agents, oxygen scavengers, and synergistic agents [17]. Hair loss may be influenced by the damaging effects of oxidative stress resulting from impaired mitochondrial function [18]. Consequently, the antioxidant activity of hair products can prevent and delay damage to the exposed hair, preserving its structural integrity and appearance over time.

Given this context, this study aimed to determine the volatile components of the essential oil of *C. bolivianum* with GC–MS analysis and evaluate the antioxidant capacity of the essential oil and hair cosmetic formulations (shampoo, combing cream, and capillary lotion) containing this essential oil using two antioxidant methods in vitro DPPH and ABTS. The present study is the first to evaluate the antioxidant activity of hair cosmetic formulations containing the essential oil of *C. bolivianum*. By exploring the potential of an underutilized Andean plant species, the research also contributes to the development of natural antioxidant-based hair care products.

2. Materials and Methods

2.1. Collection of Plant Species

The material plant was collected in the district of Ollaraya, province of Yunguyo, department of Puno, at an altitude of 3827 m, during August–September 2022. The plant material was authenticated at the Herbarium of the Natural History Museum of the Universidad Nacional Mayor de San Marcos (Id. 012-USM-MHN-2022).

2.2. Obtention of the Essential Oil

The aerial parts of *C. bolivianum* were selected and washed with copious amounts of distilled water. Subsequently, it was desiccated at ambient temperature, and the dehydrated sample was placed in a Clevenger apparatus in order to obtain the essential oil by steam distillation for three hours. A glass separating funnel was used to separate the essential oil (Figure 1). Anhydrous Na_2SO_4 was employed for the elimination of water residues, and the solution was passed through 0.25 µm filters. The resulting essential oil was preserved in an amber glass container at 4 °C until further use [19].

2.3. Chemical Composition of the Essential Oil of the Leaves C. bolivianum by Gas Chromatography–Mass Spectrometry

The volatile components of the essential oil were identified by an equipment of gas chromatography–mass spectrometry supplied by Thermo Fisher Scientific (Waltham, MA, USA) with a non-polar 5% phenyl-methylpolysiloxane-based DB-5ms column capillary column (30 m × 0.25 mm, 0.20 µm film thickness, Agilent Technologies (Santa Clara, CA, USA). The essential oil was diluted with cyclohexane solution (1:100), total 1000 µL. Volatile components were identified by comparing the relative retention indices (RI) and mass spectral data (NIST-5 library). Each retention index was calculated and compared with a homologous series of n-alkanes C9-C25 (Sigma–Aldrich, St. Louis, MO, USA) [20]. The quantitative analysis was performed using a flame ionization detector (GC-FID), in the same equipment used for GC–MS analysis, and the analytical gas chromatographic conditions were the same as those described above.

2.4. Antioxidant Activity Using the DPPH (2,2-diphenyl-1-picrylhydrazyl) Radical

The DPPH (Sigma Chemical Co., St. Louis, MO, USA) radical has a purple coloration, which changes to a yellow color owing to the presence of an antioxidant component with an unpaired electron. To perform this assay, a working DPPH solution was obtained (10 mg DPPH in 100 mL methanol). The final absorbance was measured at 517 nm using a spectrophotometer (Genesys 10) (Thermo Scientific, Waltham, MA, USA). Then, the essential oil was diluted with methanol (0–12,000 µg/mL). Next, 200 µL of each dilution was mixed with 1800 µL of DPPH. The reaction lasted for 30 min and the absorbance was measured at 517 nm. A Trolox standard curve was plotted with Trolox concentrations ranging from 0 to 1 mM [21].

2.5. Antioxidant Activity Using the ABTS (2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic Acid)) Radical

A stock ethanolic solution of 7 mM ABTS (Sigma Chemical Co., St. Louis, MO, USA) was prepared, and then potassium persulfate was added to the final stock solution (2.45 mM and allowed to react for 12–16 h at room temperature in the dark. To evaluate the antioxidant capacity of the ABTS radical, 40 µL of the essential oil (0–9370 µg/mL) was mixed with 1960 µL of ABTS radical with an absorbance of 0.7 ± 0.02 nm and incubated in the dark for 7 min, and absorbance was read at 734 nm. A standard curve was prepared using Trolox at concentrations ranging from 0 to 1000 µM. The Trolox equivalent antioxidant capacity (TEAC) was expressed as µmol Trolox Equivalent (TE)/g of essential oil [21].

2.6. Preparation of Hair Cosmetic Formulations

2.6.1. Shampoo Elaboration

The shampoo was manufactured in the laboratory containing 0.5% of the essential oil, according to Table 1, and consisted of three phases:

- Phase A: The ingredients of the aqueous phase were weighed and phase A was heated to 80 °C.
- Phase B: The ingredients of phase B were weighed and phase B was heated to 80 °C. Phase B was then added to phase A, and the mixture was stirred at 350 RPM. Phase A/B was cooled to 40 °C.
- Phase C: The ingredients of phase C were added to Phase A/B and stirred until homogenous. All at 40 °C. Finally, the appearance, color, odor, viscosity, and pH were determined at 25 °C.

The concentration of 0.5% was selected based on preliminary formulation tests to ensure a balance between antioxidant efficacy, formulation stability, and acceptable sensory characteristics such as texture and aroma.

Table 1. List of components of shampoo.

Phase	Component (INCI Name)	Function
A	Aqua Polyquaternium-10 Glycerin Propylene glycol Tetrasodium EDTA	Solvent Film-forming Humectant Humectant Chelating agent
B	Sodium C14-16 Olefin Sulfonate Cocamidopropyl Betaine Disodium laureth sulfosuccinate Cocamide MIPA Dimethyl lauramide/myristamide	Surfactant Amphoteric surfactant Mild anionic surfactant Foam booster and thickener Viscosity builder and foam stabilizer
C	Citric acid Methylisothiazolinone and methylchloroisothiazolinone Essential oil of *C. bolivianum*	pH adjuster Preservatives Active ingredient

2.6.2. Preparation of Combing Cream with a 0.5% Concentration Within the Formula

First, the raw material was obtained, and then we proceeded with the manufacture of the combing cream in the laboratory, which consisted of three phases, according to Table 2:

- Phase A: In the auxiliary container, the ingredients of the aqueous phase were weighed and phase A was heated to 80 °C.
- Phase B: In the main container, the ingredients of phase B were weighed and phase B was heated to 80 °C. Phase B was then added to phase A and stirred at 350 RPM using a homogenizer. Cool Phase A/B at 40 °C.
- Phase C: The ingredients of phase C were added to the main container and stirred until homogenization. All this at 40 °C. Finally, the appearance, color, odor, viscosity, and pH were determined at 25 °C.

Table 2. List of components of combing cream.

Phase	Component (INCI Name)	Function
A	Aqua Propylene glycol Tetrasodium EDTA Polyquaternium-55	Solvent Humectant Chelating agent Film-forming and conditioning agent
B	Sorbitan caprylate (and) propanediol (and) benzoic acid Cetyl alcohol Stearyl alcohol Cetearyl alcohol Cetrimonium chloride 50% Butyrospermum parkii (shea) butter Behentrimonium methosulfate and cetearyl alcohol Dimethicone (and) dimethiconol Amodimethicone and cetrimonium chloride and trideceth-12	Preservative, emollient, and mild humectant. Emollient and thickener Emollient and thickener Emulsifying wax Cationic surfactant Natural emollient Conditioning agent and emulsifier Silicones; thermal protection Silicone complex; shine and conditioning

Table 2. *Cont.*

Phase	Component (INCI Name)	Function
C	Essential oil of *C. bolivianum*	Active ingredient
	Citric acid	pH adjuster
	Metilisotiazolinona and metilcloroisotiazolinona	Preservatives

2.6.3. Preparation of Capillary Lotion with a 1% Concentration Within the Formula

The ingredients were weighed and mixed at room temperature in an auxiliary container. All ingredients were added and stirred until homogenization at a temperature of 25 °C (Table 3). Finally, the appearance, color, odor, viscosity, and pH were determined at 25 °C.

Table 3. List of components of capillary lotion.

Component (INCI Name)	Function
Sodium hydroxide	pH adjuster
Essential oil of *C. bolivianum*	Active ingredient
Glycerine	Humectant
Alcohol	Solvent and penetration enhancer
Polysorbate 20	Solubilizer and emulsifier
Sorbitan Caprylate (and) Propanediol (and) Benzoic Acid	Multifunctional preservative
Aqua	Solvent
DMDM hydantoin	Preservative

2.7. Antioxidant Activity of the Cosmetic Formulations

For semi-solid formulations such as the combing cream, 1 g of sample was homogenized in 10 mL of methanol and subjected to sonication for 10 min, followed by centrifugation at 3000 rpm for 10 min. The clear supernatant was collected and used for antioxidant evaluation using DPPH and ABTS assays, following the same procedure applied to the essential oil.

2.8. Accelerated Stability of the Three Formulations with the Essential Oil of C. bolivianum

The physical and chemical stabilities of hair cosmetic formulations (Figure 2) were analyzed, both with the essential oil of *C. bolivianum* in the absence of the essential oil. Likewise, the temperature was set at 30 °C, and 40 °C, as well as data collection was collected for 15 days, 1 month, 2 months, and 3 months, respectively. Among the acceptance criteria for the stability study, the sample must not present changes in texture or phase separation, nor should there be any degradation of the ingredients [22].

2.9. Statistical Analysis

Data analysis was performed using Graph Pad Prism v4.0 program. Trolox equivalent antioxidant capacity (TEAC) and mean inhibitory concentration (IC_{50}) were determined using linear regression analysis in Microsoft Excel. The antioxidant activities of the three hair cosmetic formulations were analyzed in triplicate.

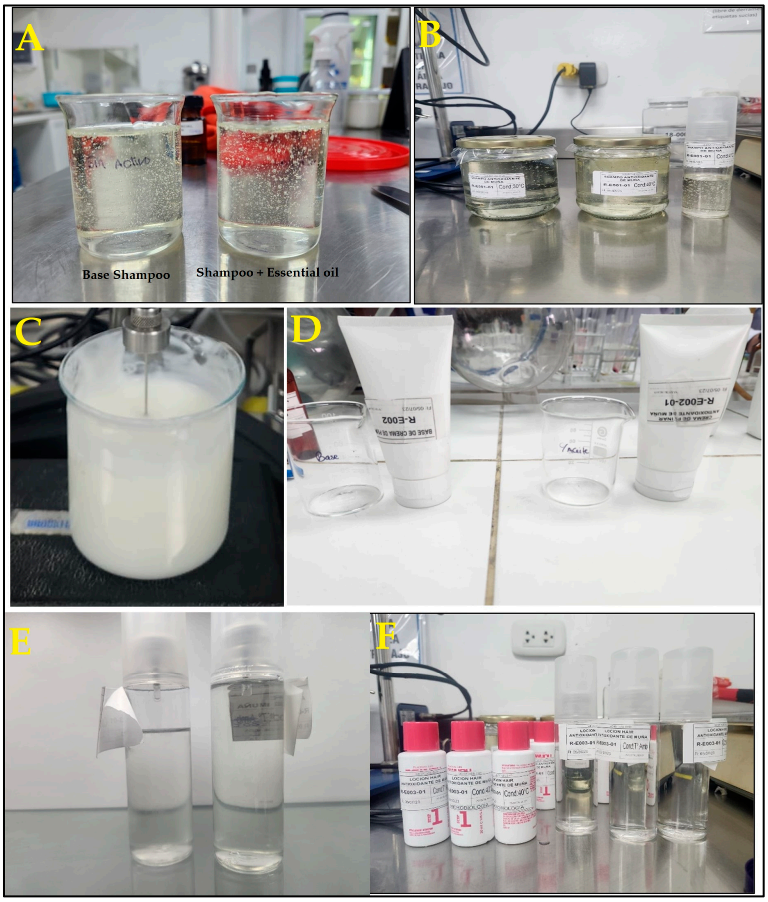

Figure 2. Elaborated cosmetic preparations: (**A**) comparison between base shampoo and shampoo enriched with essential oil; (**B**) shampoo formulations subjected to accelerated stability testing under different conditions; (**C**) preparation of combing cream incorporating essential oil; (**D**) combing cream formulations during the stability study; (**E**) capillary lotion formulated with essential oil; (**F**) capillary lotion samples evaluated under stability testing conditions.

3. Results

3.1. GC–MS Analysis of the Volatile Components of the Essential Oil of C. bolivianum

Table 4 reveals a complex mixture of compounds, with a notable predominance of certain components. Although monoterpene hydrocarbons are present in a moderate amount (4.39% in total), other compounds belonging to the oxygenated monoterpenes represent the 90.65%. On the other hand, sesquiterpenes, which include compounds such as caryophyllene, γ-muurolene, bicyclogermacrene, caryophyllene oxide and spathulenol, represent only 4.05% of the mixture, indicating a lower proportion of these compared to monoterpenes. However, the most notable was the presence of pulegone, which with 66.85% constitutes the major component of the essential oil. In addition, other compounds, such as octen-3-yl acetate (3.29%), carvacrol (4.30%), isocitral (4.57%), and 1,8-Cineole (6.47%), contributed to the diversity of the composition.

Table 4. Volatile components of the essential oil of *C. bolivianum*. RT = Retention time; LRI [a] = calculated linear retention index; LRI [b] = linear retention index from reference; % ± SD = area percentage and standard deviation of triplicate injections.

Compound	RT	LRI [a]	LRI [b]	Chemical Compound	Molecular Formula	% ± SD
1	8.767	936	923	α-Pinene	$C_{10}H_{16}$	0.61 ± 0.15
2	10.515	977	969	Sabinene	$C_{10}H_{16}$	1.22 ± 0.08
3	10.736	983	974	β-Pinene	$C_{10}H_{16}$	0.52 ± 0.04
4	11.185	989	979	1-Octen-3-ol	$C_8H_{16}O$	0.32 ± 0.01

Table 4. *Cont.*

Compound	RT	LRI [a]	LRI [b]	Chemical Compound	Molecular Formula	% ± SD
5	13.018	1022	1020	ρ-Cymene	$C_{10}H_{14}$	1.33 ± 0.07
6	13.355	1030	1026	1,8-Cineole	$C_{10}H_{18}O$	6.47 ± 0.32
7	13.954	1042	1044	(*E*)-β-Ocimene	$C_{10}H_{16}$	0.71 ± 0.03
8	16.787	1102	1095	Linalool	$C_{10}H_{18}O$	1.38 ± 0.09
9	16.998	1106	1110	1-Octen-3-yl acetate	$C_{10}H_{18}O_2$	3.29 ± 0.23
10	20.008	1169	1158	Iso-Menthone	$C_{10}H_{18}O$	1.58 ± 0.15
11	20.623	1182	1177	(*E*)-Isocitral	$C_{10}H_{16}O$	4.57 ± 0.25
12	21.575	1202	1186	α-Terpineol	$C_{10}H_{18}O$	0.83 ± 0.09
13	22.579	1234	1234	(*E*)-Decen-2-one	$C_{10}H_{18}O$	0.42 ± 0.03
14	23.701	1248	1233	Pulegone	$C_{10}H_{16}O$	66.85 ± 0.76
15	23.953	1243	1239	Carvone	$C_{10}H_{14}O$	0.52 ± 0.05
16	25.422	1285	1283	Isobornyl acetate	$C_{12}H_{20}O_2$	0.54 ± 0.03
17	26.463	1308	1298	Carvacrol	$C_{10}H_{14}O$	4.30 ± 0.24
18	26.820	1316	-	Unidentified	-	0.50 ± 0.07
19	31.197	1417	1417	(*E*)-Caryophyllene	$C_{15}H_{24}$	1.32 ± 0.07
20	33.853	1480	1478	γ-Muurolene	$C_{15}H_{24}$	0.93 ± 0.05
21	34.476	1505	1500	Bicyclogermacrene	$C_{15}H_{24}$	0.64 ± 0.06
22	38.026	1584	1577	Spathulenol	$C_{15}H_{24}O$	0.87 ± 0.03
23	38.159	1588	1582	Caryophyllene oxide	$C_{15}H_{24}O$	0.29 ± 0.01
			Monoterpene hydrocarbons (%)			4.39
			Oxygenated monoterpenes (%)			90.65
			Sesquiterpene hydrocarbons (%)			2.89
			Oxygenated sesquiterpenes (%)			1.16
			Other compounds %)			0.92
			Total (%)			100.00

3.2. Antioxidant Capacity of the Essential Oil

The antioxidant activity of the essential oil was evaluated using the DPPH and ABTS assays (Table 5). The results indicate that the essential oil exhibited a TEAC-DPPH value of 2.96 ± 0.05 µmol TE/g and an IC_{50} of 1344.0 ± 12.23 µg/mL. In comparison, Trolox, used as a reference antioxidant, showed a significantly lower IC_{50} value (3.97 ± 0.05 µg/mL), highlighting the relatively lower antioxidant potency of the essential oil in the DPPH assay ($p < 0.0001$). Similarly, the ABTS assay results demonstrated a TEAC-ABTS value of 43.13 ± 2.60 µmol TE/g for the essential oil, with an IC_{50} of 40.125 ± 1.25 µg/mL. Trolox exhibited a significantly lower IC_{50} value (1.73 ± 0.002 µg/mL), confirming the lower antioxidant efficiency of the essential oil in this assay ($p < 0.0001$).

Table 5. Antioxidant capacity of the essential oil assessed by DPPH, and ABTS methods. Mean ± SD (n = 3).

Antioxidant Assay		Essential Oil	Trolox	*p* Value (*T*-Student's Test)
DPPH	µmol TE/g	2.96 ± 0.05	-	
	IC_{50} (µg/mL)	1344.0 ± 12.23	3.97 ± 0.05	$p < 0.0001$
ABTS	µmol TE/g	43.13 ± 2.60	-	
	IC_{50} (µg/mL)	40.125 ± 1.25	1.73 ± 0.002	$p < 0.0001$

3.3. Antioxidant Capacity of Cosmetic Formulations

The antioxidant potential of various cosmetic formulations was assessed using DPPH and ABTS assays, and the results are summarized in Table 6. The combing cream exhibited the highest antioxidant activity among all formulations, with a TEAC-DPPH value of 5.537 ± 0.216 µmol TE/g and a TEAC-ABTS value of 25.34 ± 1.460 µmol TE/g. This high antioxidant capacity translated into the lowest IC_{50} values of 0.72 µg/mL (DPPH) and 0.068 µg/mL (ABTS), indicating a strong radical scavenging ability. The combing cream base also demonstrated notable antioxidant activity, with IC_{50} values of 2.17 µg/mL (DPPH) and 1.95 µg/mL (ABTS), which were significantly lower than those of other formulations. In contrast, the shampoo and capillary lotion formulations exhibited relatively weak antioxidant activities, with IC_{50} values ranging from 34.52 to 152.69 µg/mL for DPPH and from 61.79 to 82.38 µg/mL for ABTS. Their corresponding TEAC values were also lower, indicating a limited radical scavenging potential.

Table 6. Antioxidant capacity of cosmetic formulations assessed by DPPH and ABTS methods. Mean ± SD (n = 3).

Formulations	TEAC-DPPH μmol TE/g Mean +/− SD	TEAC-ABTS μmol TE/g Mean +/− DE	DPPH IC_{50} (μg/mL)	ABTS IC_{50} (μg/mL)
Shampoo	0.115 ± 0.018	0.028 ± 0.009	34.52	61.79
Shampoo base	0.046 ± 0.015	0.025 ± 0.008	86.30	69.20
Combing cream	5.537 ± 0.216	25.34 ± 1.460	0.72	0.068
Combing cream base	1.826 ± 0.142	0.888 ± 0.752	2.17	1.95
Capillary lotion	0.062 ± 0.051	0.025 ± 0.021	64.03	69.20
Capillary lotion base	0.026 ± 0.010	0.021 ± 0.009	152.69	82.38

3.4. Evaluation of the Physicochemical Parameters of the Three Cosmetic Formulations Containing the Essential Oil of C. bolivianum

As shown in Table 7, the shampoo exhibited a clear viscous liquid appearance with a light-yellow color and herbal fragrance. The pH was 6.39, which falls within the acceptable range of 5.5 to 7.5, indicating mild acidity suitable for hair and scalp care. The viscosity at 25 °C was 12,600 cP, within the expected range of 4000 to 20,000 cP, ensuring an adequate consistency for application and foaming. The combing cream was presented as a viscous emulsion with a slightly yellowish color and the same herbal fragrance. The pH was 4.00, aligning with the required range of 3.0 to 5.0, which is ideal for smoothing and conditioning the hair cuticle. The viscosity measured at 25 °C was 234,000 cP, fitting within the expected range of 100,000 to 250,000 cP, ensuring a thick and creamy texture suitable for detangling and hair protection. The capillary lotion appeared as a transparent liquid with a slightly yellowish hue and an herbal muña aroma. The pH was 5.10, which is within the recommended range of 4.0 to 6.0, suggesting compatibility with hair and scalp health. However, viscosity data were not available, likely because of the product's fluid nature, indicating a lightweight formulation designed for easy application and absorption. These results confirmed that all three formulations meet the expected physicochemical criteria, ensuring their stability and effectiveness in cosmetic use.

Table 7. Physicochemical parameters of the three cosmetic formulations.

Parameters	Shampoo	Combing Cream	Capillary Lotion
Aspect	Clear viscous liquid	Viscous emulsion	Transparent
Color	Light yellow	Slightly yellowish	Slightly yellowish
Odor	Herbal muña	Herbal muña	Herbal muña
pH	6.39	4.00	5.10
Viscosity (25 °C) × 1 min Brookfield Low Viscosity Digital Viscometer (LVDV)	12,600	234,000	-

3.5. Stability Study

Tables 8–10 show the stability of these formulations, which were tested for consistent organoleptic and physicochemical properties under accelerated conditions (30 °C and 40 °C, 75% RH) for three months. The formulations maintained their appearance, odor, color, and other physicochemical parameters under accelerated conditions.

Table 8. Accelerated stability study of shampoo with the essential oil of *C. bolivianum*.

Organoleptic	Temperature	0 Day	15 Days	1st Month	2nd Month	3rd Month
Appearance (25 °C)	30 °C	Viscous clear liquid	Viscous clear liquid	ID	ID	ID
	40 °C		Viscous clear liquid	ID	ID	ID
Color (25 °C)	30 °C	Light yellow	Light yellow	Light yellow	Light yellow	Light yellow
	40 °C		Light yellow	Slightly more yellow	Yellowish	Yellowish
Odor (25 °C)	30 °C	Herbal muña (+++)	Herbal muña	Herbal muña	Herbal muña	Herbal muña
			(+++)	(+++)	(++)	(+)
	40 °C		Herbal muña	Herbal muña	Herbal muña	Herbal muña
			(+++)	(+++)	(++)	(+)
Physicochemical						
pH (25 °C)	30 °C	6.39	6.46	6.29	6.29	6.29
	40 °C		6.46	6.20	6.18	6.12
Viscosity (25 °C) × 1 min	30 °C	12,600 cP	14,217 cP	13,940 cP	16,456 cP	18,336 cP
	40 °C		14,100 cP	13,740 cP	19,896 cP	Out of limit

ID: identically; cP: centipoise; (+++): strong odor; (++): moderate odor; (+): mild odor.

Table 9. Accelerated stability study of combing cream with the essential oil of *C. bolivianum*.

Organoleptic	Temperature	0 Day	15 Days	1st Month	2nd Month	3rd Month
Appearance (25 °C)	30 °C	Viscous emulsion	Viscous emulsion	Viscous emulsion	Viscous emulsion	Viscous emulsion
	40 °C		Viscous emulsion	Viscous emulsion	Viscous emulsion	Viscous emulsion
Color (25 °C)	30 °C	Slightly yellowish	Slightly yellowish	Slightly yellowish	Slightly yellowish	Yellowish
	40 °C		Slightly yellowish	Slightly yellowish	Slightly yellowish	Yellowish
Odor (25 °C)	30 °C	Herbal muña (+++)	Herbal muña (+++)	Herbal muña (+++)	Herbal muña (++)	Herbal muña (+)
	40 °C		Herbal muña	Herbal muña	Herbal muña	Herbal muña
			(+++)	(+++)	(++)	(+)
Physiochemical						
pH (25 °C)	30 °C	4.06	4.11	4.1	4.14	4.23
	40 °C		4.09	4.18	4.16	4.2
Viscosity (25 °C) × 1 min	30 °C	234,000 cP	277,200 cP	13,940 cP	239,700 cP	412,000 cP
	40 °C		250,600 cP	13,740 cP	253,800 cP	395,000 cP

ID: identically; Cp: centipoise; (+++): strong odor; (++): moderate odor; (+): mild odor.

Table 10. Accelerated stability study of capillary lotion with the essential oil of *C. bolivianum*.

Organoleptic	Temperature	0 Day	15 Days	1st Month	2nd Month	3rd Month
Appearance (25 °C)	30 °C	Clear liquid without the presence of particles	Clear liquid	ID	ID	ID
	40 °C		Clear liquid	ID	ID	ID
Color (25 °C)	30 °C	Slightly yellowish	Slightly yellowish	Slightly yellowish	Clear liquid	Clear liquid
	40 °C		Slightly yellowish	Slightly yellowish	Slightly yellowish	Yellowish
Odor (25 °C)	30 °C	Intense herbal muña (+++)	Herbal muña (+++)	Herbal muña (+++)	Herbal muña (++)	Herbal muña (++)
	40 °C		Herbal muña (+++)	Herbal muña (+++)	Herbal muña (++)	Herbal muña (++)
Physiochemical						
pH (25 °C)	30 °C	5.1	5.21	5.06	4.91	4.96
	40 °C		5.22	5.02	4.97	4.92

ID: identically; (+++): strong odor; (++): moderate odor.

4. Discussion

The *Clinopodium* genus, which belongs to the Lamiaceae family, is known for its diverse array of volatile components that contribute to the aromatic properties and potential medicinal uses of plants. The composition of these volatile components can vary significantly among different *Clinopodium* species and is influenced by factors such as geographical location, environmental conditions, and plant maturity. Common volatile compounds found in *Clinopodium* species include monoterpenes such as pulegone, menthone, and limonene [23,24] and also sesquiterpenes such as caryophyllene and germacrene D [24]. The presence and relative concentrations of these compounds contribute to the distinct scents and flavors associated with various *Clinopodium* species as well as their potential therapeutic properties, including antimicrobial, antioxidant, and anti-inflammatory activities [25].

Regarding the importance of *C. bolivianum,* in our study, phytochemical analysis revealed 33 chemical compounds determined with GC–MS. The main metabolites with the highest percentages were pulegone (63.82%) and carvacrol (5.52%). Compared to a study carried out by Hospino Cosme, 107 volatile components were reported in *C. bolivianum* being cyclohexanone, 5-methyl-2-(1-methylethyl)-(2R-cis), (28.65%), linalool (15.80%), and pulegone (15.32%) the highest percentages [26]. Huanqui Franco [27] determined the presence of 38 chemical constituents in the essential oil, reporting pulegone as the major compound (38.69%), followed by isopulegone (11.94%), eucalyptol (10.63%), and isomenthone (6.65%). Zuñiga Olaguibel [28] evaluated the chemical composition being isomenthone (24.08%) the major component, followed by menthone (16.3%), thymol (15.82%), *p*-cymene (10.7%), terpinene (7.21%), and pulegone (6.55%). Solis Tito [29] determined 17 components, the major components obtained being isomenthone (24.08%), menthone (16.13%), thymol (15.82%), *p*-cymene (10.17%), γ–terpinene (6.65%), pulegone (6.55%), and eucalyptol (2.87%). The observed variability in essential oil composition across various Peruvian regions may be attributed to the geographical origin of the plant material analyzed in this study. Our results, in line with previous studies, indicate that essential oil composition is affected by several factors, including plant age, harvest time, and environmental conditions such as temperature, annual rainfall, and altitude [30].

In contrast, pulegone is abundant in several aromatic plants, particularly those belonging to the Lamiaceae family, owing to its role as a key intermediate in the biosynthesis of essential oils. In *Mentha* species, such as *Mentha suaveolens* and *Mentha longifolia*, pulegone is one of the main aromatic constituents, often occurring at high concentrations [31]. For instance, in *M. longifolia*, pulegone accounts for 26.07% of the essential oil composition [32]. Interestingly, the abundance of pulegone varies depending on environmental conditions and plant growth stages. In peppermint (*Mentha piperita*), pulegone is a central intermediate in the biosynthesis of menthol, the most significant component of peppermint essential oil [33]. The metabolic synthesis of pulegone is controlled through the transcriptional regulation of enzymes, such as menthofuran synthase and pulegone reductase. Environmental factors can influence these regulatory processes and affect the relative abundance of pulegone and its derivatives [33,34].

The antioxidant activity of *C. bolivianum* was determined by the DPPH and ABTS methods, which revealed a stronger antioxidant response against ABTS radicals, both in the pure essential oil and in the cosmetic formulations. In a study by Huanqui Franco [27], the in vitro antioxidant activity was assessed using the DPPH assay, showing an inhibition percentage of 92.74% at a concentration of 100 μg/mL. Other species of the *Clinopodium* genus, such as *C. brownei* from Ecuador [35], exhibited IC_{50} values of 1.77 mg/mL for DPPH and 0.06 mg/mL for ABTS. Similarly, a study conducted by Tepe et al. [24] reported that *C. vulgare* showed an IC_{50} of 63.0 ± 2.71 μg/mL in the DPPH assay.

In contrast, the antioxidant activity of the essential oil of *C. bolivianum* in the formulated cosmetic products may be influenced by interactions with other ingredients present in the formulations. The results indicated that the combing cream exhibited the highest antioxidant capacity, whereas the shampoo and capillary lotions displayed significantly lower antioxidant activity. These variations suggest that certain formulation components may enhance or inhibit the effectiveness of the essential oil as antioxidants. It is known that the ABTS method has a wide pH range of stability (pH 1–8), whereas for the DPPH method, the pH ranged between 1 and 8 [36]. Emulsifiers, surfactants, and preservatives are key components of cosmetic formulations that may affect the stability and bioavailability of antioxidant compounds [37]. In the combing cream, the presence of fatty alcohols such as cetyl and stearyl alcohol, along with shea butter and cationic surfactants such as behentrimonium methosulfate, may have facilitated the solubilization [38] and sustained release of antioxidant compounds from the essential oil, enhancing its efficacy [39]. In contrast, the lower antioxidant activity observed in the shampoo and capillary lotion formulations might be attributed to the presence of surfactants, such as sodium C14-16 olefin sulfonate or cocamidopropyl betaine. Furthermore, the high aqueous content of these formulations may reduce the solubility and stability of the hydrophobic antioxidant compounds present in the essential oils, leading to diminished activity. Another factor influencing antioxidant activity is the pH of the formulations. The combing cream had a lower pH (4.0), which may have contributed to better antioxidant stability because acidic conditions can prevent the oxidation of certain bioactive compounds. Conversely, the shampoo had a pH of 6.39, which is within an acceptable range for hair care and may have affected the reactivity of the antioxidant components. Although the essential oil of *C. bolivianum* exhibited a significantly lower antioxidant capacity compared to the synthetic standard Trolox (p < 0.0001), this outcome is expected due to the nature of Trolox as a pure, highly active compound. Essential oils, being complex natural mixtures, typically demonstrate moderate radical scavenging activity. Nonetheless, their antioxidant potential remains relevant, particularly within cosmetic applications where the emphasis is placed on natural origin, multifunctionality, and compatibility with formulation matrices. Interestingly, the combing cream formulation enhanced the antioxidant activity of the essential oil, likely due to improved solubilization and stabilization of active constituents within the emulsion, highlighting its potential in practical applications.

On the other hand, the accelerated stability of the three formulations was evaluated for three months, observing the organoleptic and physicochemical changes of each formulation with the essential oil of *C. bolivianum*. It can be stated that our formulations were stable over the evaluated time; however, at an extreme temperature of 40 °C, a slight increase in the color and viscosity of the shampoo, as well as in the combing cream, occurred. In the process of manufacturing the shampoo, it was also observed that the essential oil acted quickly, increasing viscosity. This was considered when *C. bolivianum* essential oil was added. Stability studies of cosmetic products are important because the objective is to evaluate the capacity of a product to preserve its chemical, physical, and microbiological properties for a long time [40]. The stability study applied in the present investigation was based on the international guide that indicates the conditions, factors that influence, types of studies, possible alterations, and appropriate and pertinent parameters of the stability studies according to the type of product selected [22]. This study is necessary to design and manufacture cosmetic products safely, so that they last the estimated time, as well as for the storage of the product. In the present investigation, during the manufacture of the shampoo, it was observed that the essential oil of *C. bolivianum* affected the formulation by significantly increasing the viscosity. Shkreli et al. [41] evaluated the stability of a cosmetic emulsion of a synthetic preservative in comparison with that of lemongrass essential oil, which is a natural preservative. Both emulsions remained stable in terms of smell, appearance, color, and homogeneity; however, their viscosity was influenced by the concentration of the essential oil—the higher the concentration, the lower the viscosity. While the addition of an essential oil did not affect the pH of the formulation, it did have a noticeable impact on viscosity. Compared to the present study, these differences were attributed to variations in the ingredients used in each formulation.

A key limitation of this study was the use of a fixed essential oil concentration in each formulation, chosen to ensure consistency in antioxidant evaluation. However, differences in concentration among products (0.5% in shampoo and combing cream vs. 1% in capillary lotion) may have influenced the results. Future studies should assess a wider concentration range and include sensory evaluations to optimize both efficacy and consumer acceptance.

5. Conclusions

This study demonstrated that the essential oil of *C. bolivianum* exhibits notable antioxidant activity, with IC_{50} values of 1344.0 ± 12.23 μg/mL (DPPH assay) and 40.125 ± 1.25 μg/mL (ABTS assay). When incorporated into cosmetic formulations, especially the combing cream, the antioxidant activity was significantly enhanced, reaching IC_{50} values of 0.72 μg/mL (DPPH) and 0.068 μg/mL (ABTS). These findings suggest a strong synergistic effect between the essential oil and specific formulation components. Additionally, all formulations maintained their physicochemical properties under accelerated stability conditions for three months. The results support the potential application of *C. bolivianum* essential oil as a natural, functional ingredient in antioxidant-rich hair care products. Further studies are recommended to explore its long-term effects and potential synergies of the essential oil with other bioactive compounds as well as compare the formulations with commercial hair care products to better understand their relative antioxidant performance and market potential.

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