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Article

Antioxidant activity and composition of *Clinopodium tomentosum* in wet and dry extraction

Miguel Angel Enríquez-Estrella[1,§]
Gabriela Joseth Serrano-Torres[2]
Silvia Hipatia Torres-Rodriguez[2]
Dennis Gabriel Cuadrado-Ayala[3]

1 Escuela de Ingeniería Agroindustrial-Facultad de Ciencias de la Tierra-Universidad Estatal Amazónica. Puyo, Ecuador.

2 Facultad de Ingeniería-Universidad Nacional de Chimborazo. Riobamba, Ecuador.

3 Consultor independiente. Ecuador.

Autor para correspondencia: menriquez@uea.edu.ec

Abstract

In ancient times, various plant species were used in traditional medicine due to their properties, being utilized in diverse processes, both in the food and pharmaceutical sectors. The purpose of this study was to characterize the bioactive components, specifically antioxidants and polyphenols, present in *Clinopodium tomentosum* in both dry and wet samples. An ultrasound-assisted extraction method was implemented to obtain the active compounds. Subsequently, the identification and quantification of the extracted compounds were performed using the FRAP (Ferric Reducing Antioxidant Power) and Folin-Ciocalteu assays. As a result of this process, it was observed that extraction from dry matter yields higher concentrations, with 1.49 mg L^{-1} of polyphenols and 1.27 mg L^{-1} of antioxidants, compared to wet extraction, which yielded concentrations of 1.39 mg L^{-1} of polyphenols and 1.04 mg L^{-1} of antioxidants. The characterization of *Clinopodium tomentosum* enabled the identification of its bioactive components, paving the way for future research on the identification of its phytochemical components and their potential application in both food and non-food industries.

Keywords:

active principle, bioactive, extracts, phytochemicals.

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Introduction

Currently, there is a growing demand for medicinal plants, with their acceptance steadily on the rise. The latest WHO Report on Traditional and Complementary Medicine (T&CM) indicates that numerous countries worldwide are formulating guidelines effectively integrate T&CM therapies into their healthcare systems (McGaw, 2005). Only a small fraction of the known biodiversity has been thoroughly examined to understand its characteristics and potential benefits for humans (Estrella, 2015).

The origin of Natural and Traditional Medicine (NTM) is closely linked to that of humanity and the history of mankind in its struggle for survival. It is regarded as a specialty that encompasses a set of therapeutic methods and techniques to restore balance between the individual and between the individual and the universe (Pulsan *et al*., 2015). It includes disciplines such as homeopathy, phytotherapy, acupuncture, ozone therapy, apitherapy and moxibustion, among others (González and Cardentey, 2016).

The significant biological and cultural diversity of Ecuador positions it as a country with great potential in the field of traditional therapy. In this context, it is crucial to explore various key aspects, such as the methods of utilization of each plant and the healing effects that traditional medicine can offer to different communities in the country (Zambrano *et al*., 2015). Throughout history, various plant species have been employed for medicinal purposes due to their bioactive properties. Since ancient times, they have been used for wound healing and treating diseases and infections, among other applications (Saltos *et al*., 2014).

Scientific research has conducted numerous studies focused on plant species, identifying biochemical components that contribute to the optimization of human metabolism (Jaric *et al*., 2018). This preference for plant-derived elements with bioactive content is attributed to their ability to enhance human metabolism, leveraging their nutritional potential (Enríquez *et al*., 2023).

The species *Clinopodium tomentosum*, derived from the Greek words klino (bed) and podion (small foot), commonly known as woolly mint, calamint, or Pumin, is a perennial herb belonging to the Lamiaceae family. It is native to Europe, including regions like the Mediterranean and the Balkans, and in Latin America, it thrives at altitudes between 3 000 and 4 000 m above sea level (Eber and Engels, 2020).

Ecuador stands out for its extensive biodiversity, with over 17 000 identified plant species, more than 3 000 of which are medicinal and widely used across various cultures for healing purposes. It is noteworthy that current legislation in this South American country promotes the integration of indigenous medicine into healthcare services (Justicia, 2007). However, most medicinal plants used in traditional medicine have not yet undergone rigorous scientific evaluations (Fitzgerald *et al*., 2020).

In Ecuadorian folk medicine, over 50 species from this family are utilized to treat various ailments (Gachet, 2010). Within this family, the genus *Clinopodium* is widely distributed in southern and southeastern Europe, Asia, and the Americas, leading to the utilization of many of its species as medicinal plants (Drew and Sytsma, 2012). Seven *Clinopodium* species are endemic to Ecuador and are distributed in the central region of the country, where they are used, although not exclusively, for their medicinal properties (Zieliñska and Matkowski, 2014). Currently, no research has been conducted on the anti-inflammatory activity of *Clinopodium tomentosum.*

The chemical composition and antioxidant activity of *Clinopodium tomentosum* will vary significantly between wet and dry extractions. Wet extraction is expected to facilitate better identification and characterization of bioactive components due to its ability to extract water-soluble phytochemicals, while dry extraction may concentrate specific compounds, potentially revealing distinct antioxidant activity profiles.

Despite the traditional use of *Clinopodium tomentosum* for its medicinal and aromatic properties, there is a lack of specific information on the identification and characterization of the bioactive components present in this plant, which is essential for understanding its healing potential and

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conducting further studies. The knowledge of bioactive components has allowed the identification of phytochemical compounds and their benefits in human metabolism, considering the traditional and ancestral uses of this plant species, given this background, the aim of the study was to identify the chemical composition and antioxidants of *Clinopodium tomentosum* through wet and dry extraction

Materials and methods

Location

This study was conducted in the chemistry and bromatology laboratories of the Universidad Estatal Amazónica, located in the city of Puyo, Ecuador, at the following coordinates: latitude: -1.5003, longitude: -77.9961.

Type of research

This article presents an experimental investigation aimed at verifying the limited scientific information available regarding plant species. A quantitative analysis of polyphenolic and antioxidant behavior was conducted using an exploratory approach.

Materials

The plant species was collected in the tropical zone at an altitude of 3 000-4 000 m above sea level in the Chimborazo province, Riobamba city.

Method

Collection

The plant species was collected in the Lican Parish, Riobamba Canton, Chimborazo Province, at coordinates -1.6857, -78.6815. Lanceolate leaves were extracted in their wet state, measuring approximately 1 cm in length and exhibiting a grayish-green color. These leaves emit an aroma like mint and are utilized in the preparation of aromatic waters. The species is illustrated in Figure 1.

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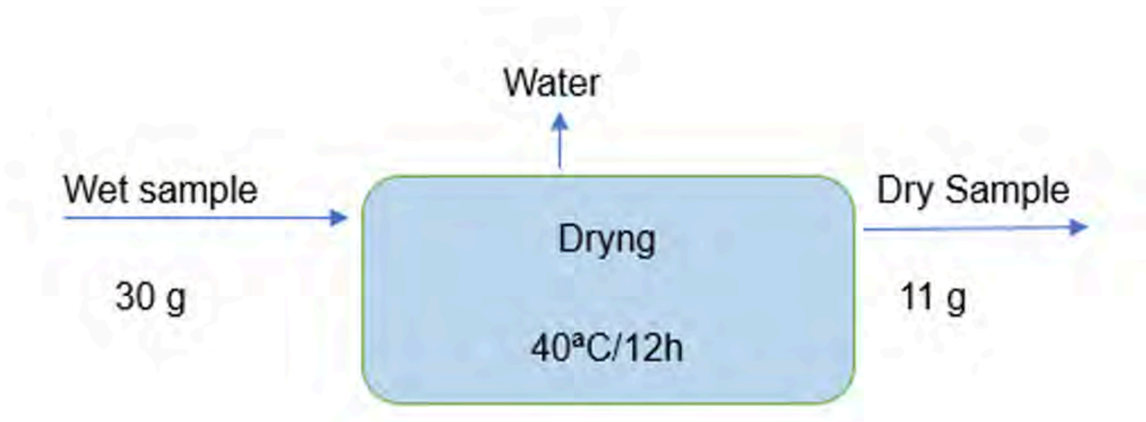
Figure 1. ***Clinopodium tormentosum*.**

Sample preparation

Two types of samples were utilized: fresh and dry. Fresh samples were processed rapidly to preserve their bioactive components, while dry samples were dehydrated according to specific temperature and time parameters. Once dried, the samples were ground to a fine powder to facilitate the extraction of the active principles, Figure 2 illustrates the drying balance.

Figura 2. Mass balance.



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Quality control of raw drugs

The evaluation of raw medicinal substances adheres to established methods detailed in the Pharmacognosy Laboratory Practices Manual of 1986, authored by Miranda. This assessment involves various procedures such as determining moisture content using the Gravimetric Technique, evaluating total ash, and examining ash solubility in both water and hydrochloric acid.

Extraction of active principles

For the acquisition of extracts, *Clinopodium tomentosum* leaves were used in two types of samples (wet and dry). The leaves of the first sample were dried by placing them in an oven at a temperature of 40 °C for 12 h. This process aimed to facilitate the subsequent determination of antioxidant and polyphenolic behavior.

The extract of *Clinopodium tomentosum* was obtained using the ultrasound-assisted extraction (UAE) method (Branson Sonics, USA). A mixture of methanol (Fisher Scientific) and water in a 9:1 ratio was used to extract the active compounds, with a solvent-to-sample ratio of 100 ml per 10 g of sample obtained. The process was conducted at 50 °C for 30 min, followed by filtration of the mixture. The resulting aqueous extract was protected from light using aluminum foil.

Determination of total polyphenols

The total polyphenol content was determined using a gallic acid calibration curve, and the concentration of total polyphenols was expressed in milligrams of gallic acid equivalents per 10 g of *Clinopodium tomentosum* leaves on a dry weight basis.

From this solution, 1 ml was taken into a 10 ml volumetric flask, and 0.5 ml of Folin-Ciocalteu reagent, diluted by half with distilled water, was added. The mixture was left to stand for 10 min. Subsequently, 0.5 ml of 20% sodium carbonate was added, and the flask was filled to the mark with distilled water. The solution was stirred, protected from light with aluminum foil, and left to rest for 2 h at room temperature (20 °C). Finally, absorbance was measured at 765 nm using a UV-Vis spectrophotometer UV-Vis. Model: 759 S. Brand: Lengguang Technology.

Determination of antioxidant activity

Método FRAP (ferric ion reducing antioxidant power)

The antioxidant activity assessment was performed using the FRAP method, following the procedure proposed by Benzi and Strain (1996). An aqueous extract was prepared from 1 g of dry sample, from which 500 µl was taken and added to a 10 ml volumetric flask, followed by the addition of 5 ml of freshly prepared FRAP solution. After adding the reagent, the flask volume was completed with distilled water up to 10 ml, and the mixture was left to rest in an oven at 37 °C for 30 min.

The absorbance was measured at a wavelength of 593 nm compared to the control solution. A Genesys 10uv Scanning spectrophotometer was used for this procedure. The concentration was calculated using the mathematical model of the calibration curve, according to equation following

$$C=\frac{A}{0.1879}$$

1. Where: C= concentration in mg L^{-1}; A= absorbance of the test solution measured at 593 nm.

Gas chromatography-mass spectrometry analysis (GC/MS

The sample analyzed by GC/MS was prepared by dissolving 10 µl of essential oil in 1 ml of dichloromethane and the injection volume was 2 µl. The analysis was performed on a chromatograph using helium as the carrier gas, with a flow rate of 1 ml min-1. The oven program detailing the temperature changes is shown in Table 1.

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Table 1. GC-MS oven program.

Initial temperature (°C)	Increase (°C min^{-1})	Time (min)	Final temperature (°C)
50	1	50	100
100	5	30	250
250	-	10	250

Given that the study aimed to determine the chemical composition and antioxidant content, including polyphenols, two equations were applied to calculate the concentration in both dry and wet samples.

Results and discussions

The results presented in Table 2 correspond to the chemical analysis of the species, which allowed for the identification of purity and active principles, thereby ensuring the absence of foreign substances.

Table 2. Chemical analysis of *Clinopodium tormentosum*.

Parameters	Percentage of *Clinopodium tormentosum* raw drug	Reference values (%)
Moisture determination	11.2	14
Total ash determination	3.45	5
Ash soluble in water determination	1.82	2
Ash soluble in HCl determination	0.82	1

Table 3 displays the absorbance results obtained using the Folin method to assess polyphenolic activity and the FRAP method to calculate antioxidant activity in the dry and wet leaves of (*Clinopodium tomentosum*). A minimal difference in absorbance is observed between the two samples, attributed to moisture loss during thermal treatment.

Table 3. absorbance according to folin and frap in wet and dry.

Tratament	Absorbance
Folin Dry	0.1071
Folin Wet	0.0993
FRAP Dry	0.2395
FRAP Wet	0.1958

The calculation of antioxidant activity using the FRAP method in samples of Pumin (*Clinopodium tomentosum*), both in the wet and dry states, was carried out utilizing the Trolox formula (antioxidant standard). The results indicate that both wet and dry extractions yielded antioxidant and polyphenolic compounds. However, differences in the concentration and composition of these compounds were observed, with higher levels detected in the wet extraction. This difference is attributed to the aqueous content of the leaves, which facilitates the release and solubilization of phytochemicals during the extraction process. Table 4 illustrates these variations.

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Table 4. Concentration according to folin and frap in wet and dry extract.

Tratament	Concentration
Folin dry	1.49 mg L^{-1}
Folin wet	1.39 mg L^{-1}
Frap dry	1.27 mg L^{-1}
Frap wet	1.04 mg L^{-1}

In dry extraction, a higher concentration of certain polyphenols was observed. The recovery of bioactive compounds is influenced by the type of phenolic compound and the polarity of the extraction solvent (Nazck and Shahidi, 2006). This aspect is crucial, as the yield and content of polyphenols depend on the polarity of the solvent used (Turkmen *et al*., 2006). Therefore, the use of solvents such as methanol, ethanol, acetone, and water for these processes is recommended (Antolovich, 2000).

This relates to the study by Enríquez (2021), which identified an optimal parameter for the extraction of polyphenols and antioxidants in ultrasound-assisted dry samples. Methanol has been observed to achieve a higher concentration of polyphenols in its extracts compared to other solvents, resulting in a greater antioxidant capacity. This efficacy makes methanol one of the most used solvents for extracting and maximizing bioactive compounds (Teng *et al*., 2009).

Plants containing beneficial phytochemicals can meet the needs of the human body by acting as natural antioxidants (Boots *et al*., 2008). Phenolic compounds such as beta-carotene and ascorbic acid play roles in slowing aging, reducing inflammation, and decreasing certain types of cancer (Boots *et al*., 2008). In this experimentation, the polyphenolic compounds and antioxidant capacity of *Clinopodium tomentosum* leaves were characterized.

Wet extraction may result in extracts with lower antioxidants and polyphenolic activity compared to dry extraction, which provides a higher extraction yield. It is recommended to extract bioactive principles from the dehydrated sample, as its concentration of polyphenols is 1.49 mg L^{-1} and the antioxidant content is 1.27 mg L^{-1}, both of which are higher than those of the wet sample.

Chemical composition of *Clinopodium tomentosum*

After conducting the analysis using the gas chromatography-mass spectrometry technique with the essential oil extracted from the dried leaves of *Clinopodium tomentosum*, its chemical composition was successfully identified. The detailed results of this analysis are presented in Table 5.

Table 5. Chemical composition of *Clinopodium tomentosum* Oil.

Number	Compounds	(%)
1	3-Ethylpentane	0.03
2	Tetrahydro-2,5-dimethylfuran	0.05
3	α-Pinene	0.21
4	Sabinene	0.17
5	β-Pinene	0.26
6	Myrcene	0.19
7	Limonene	1.66
8	Cis-Piperitone epoxide	0.18
9	Trans-Piperitone epoxide	41.72
10	Piperitenone oxide	49.3
11	β-Elemene	0.18

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Number	Compounds	(%)
12	E-Caryophyllene	1.3
13	Germacrene D	1.18
14	Bicyclogermacrene	0.16
15	2,7-Cyclodecadien-1-ol, 1,7-dimethyl-4-(1-methylethyl)	0.09
16	Caryophyllene oxide	0.07
17	Shyobunol	0.08
18	9-Nonadecene	0.26
19	Tricosane	0.19
20	Tetracosane	0.1
21	Hexacosane	0.24
22	Octacosane	0.08
	Total	9.7

The chemical analysis of *Clinopodium tomentosum* revealed a moisture content of 11.2%, which is below the reference value of 14%. The total ash content was 3.45%, also under the reference value of 5%, with water-soluble and HCl-soluble ash measured at 1.82% and 0.82%, respectively. Regarding polyphenolic and antioxidant activity, the absorbance measured by the Folin method was 0.1071 for dry leaves and 0.0993 for wet leaves.

The FRAP method yielded absorbance values of 0.2395 for dry leaves and 0.1958 for wet leaves. Higher concentrations of polyphenols and antioxidants were found in wet extractions, with 1.39 mg L^{-1} (Folin) and 1.04 mg L^{-1} (FRAP), compared to dry extractions with 1.49 mg L^{-1} (Folin) and 1.27 mg L^{-1} (FRAP). The essential oil obtained from dry leaves predominantly contained trans-Piperitone epoxide (41.72%) and Piperitenone oxide (49.30%), with other significant compounds including Limonene (1.66%) and E-Caryophyllene (1.3%), totaling 97.7% of the oil composition.

These results indicate notable variability in the chemical composition and antioxidant activity of *Clinopodium tomentosum* depending on the leaf state (wet vs dry). The higher concentration of polyphenols and antioxidants in wet extractions supports Enriquez (2021), who noted that wet extraction techniques typically capture more bioactive compounds due to the aqueous nature of the process, which enhances the solubilization of phytochemicals. Conversely, dry extraction yielded higher concentrations of certain polyphenols, supporting the notion that drying can concentrate specific compounds.

This observation aligns with the research by Nazck and Shahidi (2006), who highlighted that the recovery of bioactive compounds is influenced by the polarity of the extraction solvent, suggesting that both the extraction method and solvent polarity are crucial for the efficacy of polyphenol recovery. Turkmen *et al*. (2006); Antolovich (2000) emphasize that solvent choice significantly impacts extraction yield and polyphenol content, recommending solvents such as methanol, ethanol, acetone, and water for their effectiveness in extracting a broad range of phytochemicals.

Authors as Teng *et al*. (2009) corroborate that methanol often results in higher polyphenol concentrations. Additionally, the study confirms that polyphenolic compounds, such as beta-carotene and ascorbic acid, offer significant health benefits, including anti-aging, anti-inflammatory, and anti-cancer properties (Boots *et al*., 2008). The observed variation in antioxidant activity between wet and dry extractions underscores the importance of optimizing extraction conditions to maximize bioactive compound yields. Finally, the essential oil composition, with high percentages of trans-Piperitone epoxide and Piperitenone oxide, suggests a significant presence of these bioactive compounds with therapeutic properties, reinforcing the potential benefits and applications of *Clinopodium tomentosum*.

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Conclusions

The detailed characterization of *Clinopodium tomentosum* has provided crucial insights for achieving an optimal extraction of its compounds. Upon analyzing the antioxidant and polyphenolic contents using the Folin Ciocalteau and FRAP methods, it is concluded that the application of ultrasound to the dry sample results in enhanced performance. Based on the chemical composition, two elements were identified as the most significant: trans-piperitone epoxide and piperitenone oxide, both belonging to the group of oxygenated cyclic ethers and derived from piperitone.

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