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*Research Article*

# Composition of *Clinopodium acutifolium* essential oil from Peru

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## Abstract

Research on the *Clinopodium* genus has highlighted the varied chemical compositions and possible applications of its essential oils, yet the oil profiles of certain species remain uncharted. Specifically, the essential oil of *Clinopodium acutifolium* has not been thoroughly investigated until now. This study marks the first comprehensive analysis of *C. acutifolium*'s essential oil, employing steam distillation for extraction followed by GC/MS and GC/FID techniques to identify and quantify its chemical constituents. Its chemical profile revealed a high content of sesquiterpene hydrocarbons (22.0%) and oxygenated monoterpenes (52.9%), with piperitone oxide (29.1%), germacrene-D (12.4%), linalool (7.3%), and piperitenone oxide (6.8%) comprising the major compounds of the essential oil. These results provide fundamental data for future investigations into the ethnobotanical or biological properties of this species.

## 1. Introduction

*Clinopodium acutifolium* (Benth.) Govaerts is a species belonging to the Lamiaceae family, is indigenous to South America, and has a notable prevalence in Ecuador and Peru [1]. *C. acutifolium* exhibits soft and silky branches, and its leaves vary in size, typically being elongated and narrow, with smooth margins that exhibit a slight downward curvature [1]. The plant generally bears three flowers at the base of the upper leaves, and these flowers are borne on short pedicels [1].

Species of the *Clinopodium* genus are known for their characteristic aroma and value in medicinal applications [2-6]. Various studies have highlighted the analgesic, anti-inflammatory, antioxidant, and tranquillizing properties of several species within this genus [2-6]. The essential oil of *C. vulgare* is characterized by its high content of thymol (38.9%) and γ-terpinene (29.6%) [7]. The essential oil of *C. nepeta* is characterized by the content of piperitone oxide (51.7%), and piperitenone oxide (23.4%) [8], while the essential oil of *C. macrostemum* features a rich composition of menthone (35.3%) and piperitone oxide (31.2%) [9]. Finally, *C. nubigenum* is notable for its concentration of carvacrol (32.9%) and pulegone (25.4%) [10].

Although these major compounds exhibit a variety of bioactive effects, in general, the essential oils from the *Clinopodium* genus have been associated with these properties underscoring the therapeutic potential of *Clinopodium* essential oils, encouraging further research to explore their bioactive applications and expanding knowledge on their health benefits.

*Clinopodium acutifolium* is one of the members of the *Clinopodium* genus that largely remains unexplored.

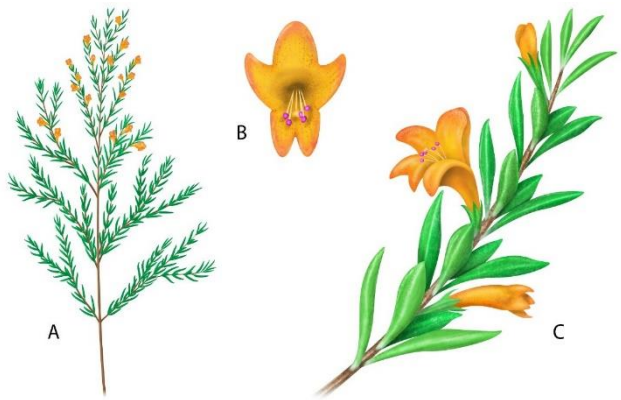


**Figure 1.** Botanical illustration of *Clinopodium actufolium* species used in the study. Illustrated by Rick Simonson, Science Lab Studios, Inc. (Kearney, NE, USA).

Personal communication with a local traditional healer from Pomacocha-Peru shared the ethnobotanical uses that *C. acutifolium* is credited, specifically with infusions of the plant. Infusions of this species are used to treat hair loss, and to treat dizziness, vomiting, and digestive problems [11]. Despite being part of a family known for its potential in essential oils, to the best of the authors' knowledge, there is no substantial information on its ethnobotanical characteristics, bioactivity studies, or further detailed information on the species in the existing scientific literature, which highlights the importance of expanding scientific knowledge on this species. In this study, we focus on characterizing the chemical composition of the essential oil extracted from *Clinopodium acutifolium* from Peru. Through GC/MS and GC/FID, we aim to provide a detailed profile of the constituents present in this oil providing a fundamental base for the research of *C. acutifolium* and for future studies that allow understanding of the potential of this understudied species.

## 2. Materials and methods

*Clinopodium acutifolium* plant material (Fig. 1) was collected in December 2022 from cultivated populations in Pomacochas, Peru (5°49'36.8" S 77°57'52.5" W). Branches and leaves of the species were harvested and immediately distilled. A representative voucher sample of the species is held at the Universidad Nacional de Cajamarca (Herbario Isidoro Sánchez Vega_UNC; herbarium code CPUN). Distillation was carried out in a 250 L distillation chamber (Albrigi Luigi S.R.L., Italy). Distillation was carried out by steam distillation for 2 hours. The essential oil obtained was separated by a cooled condenser, collected, filtered, and stored in sealed amber vials at room temperature (25 °C) until analysis. The essential oil yield was calculated as the ratio of the essential oil volume (mL) to the plant material mass (kg) before the distillation process.

Essential oil was analyzed, and volatile compounds were identified, by GC/MS using an Agilent 7890B GC/5977B MSD (Agilent Technologies, Santa Clara, CA, USA) and Agilent J&W DB-5, 60 m × 0.25 mm, 0.25 μm film thickness, fused silica capillary column. Operating conditions: 0.2 μL of the sample, 25:1 split ratio, initial oven temperature of 60 °C with an initial hold time of 2 min, oven ramp rate of 4.0 °C per minute to 245 °C with a hold time of 5 min, helium carrier gas. The electron ionization energy was 70 eV, scan range 35–550 amu, scan rate 2.4 scans per second, source temperature 230 °C, and quadrupole temperature 150 °C. Volatile compounds were identified using the Adams volatile oil library [12] using Chemstation library search in conjunction with retention indices. Volatile compounds were quantified and are reported as a relative area percent by GC/FID using an Agilent 7890B and Agilent J&W DB-5, 60 m × 0.25 mm, 0.25 μm film thickness, fused silica capillary column. Operating conditions: 0.1 μL of sample, 25:1 split injection, initial oven temperature at 40 °C with an initial hold time of 2 min, oven ramp rate of 3.0 °C per minute to 250 °C with a hold time of 3 min, helium carrier gas. Essential oil samples were analyzed in triplicate by GC/FID to ensure repeatability (standard deviation < 1 for all compounds). Compounds were assigned using retention indices coupled with the retention time data of reference compounds (MilliporeSigma, Sig-ma-Aldrich, St. Louis, MO, USA).

## 3. Results and discussion

The essential oil yield of *Clinopodium acutifolium* was 1.25 mL/kg, and the chemical profile is detailed in Table 1, revealing this essential oil is rich in oxygenated monoterpenes and sesquiterpene hydrocarbons.

Forty-two compounds of *Clinopodium acutifolium* essential oil were identified. The primary monoterpene hydrocarbons were β-pinene (3.1%), limonene (2.9%), and sabinene (2.6%). The major oxygenated monoterpenes were piperitone oxide

**Table 1.** Chemical profile of *C. acutifolium* essential oil

| KI | Compound Name | Area percentage (%) |
|---|---|---|
| 924 | α-Thujene | 0.1 |
| 932 | α-Pinene | 1.1 |
| 946 | Camphene | 0.1 |
| 969 | Sabinene | 2.6 |
| 974 | β-Pinene | 3.1 |
| 988 | Myrcene | 0.3 |
| 1024 | Limonene | 2.9 |
| 1026 | 1-8-Cineole | 3.8 |
| 1032 | (Z)-β-Ocimene | 0.4 |
| 1086 | Terpinolene | 0.1 |
| 1095 | Linalool | 7.3 |
| 1110 | 1-Octen-3-yl acetate | 6.8 |
| 1120 | 3-Octanol acetate | 1.5 |
| 1148 | Isomenthone | 1.9 |
| 1174 | Terpinen-4-ol | 0.1 |
| 1186 | α-Terpineol | 0.6 |
| 1195 | Myrtenal | 0.4 |
| 1233 | Pulegone | 1.8 |
| 1250 | Piperitone oxide | 29.1 |
| 1254 | Linalyl acetate | 0.1 |
| 1284 | Bornyl acetate | 0.7 |
| 1340 | Piperitenone | 0.4 |
| 1366 | Piperitenone oxide | 6.8 |
| 1374 | α-Copaene | 0.9 |
| 1387 | β-Bourbonene | 1.2 |
| 1389 | β-Elemene | 0.2 |
| 1417 | (E)-caryophyllene | 1.0 |
| 1421* | Bicyclosesquiphellandrene | 0.2 |
| 1480 | Germacrene D | 12.4 |
| 1500 | Bicyclogermacrene | 5.3 |
| 1522 | δ-Cadinene | 0.7 |
| 1574 | Germacrene D-4-ol | 0.4 |
| 1577 | Spathulenol | 0.8 |
| 1640 | τ-Muurolol | 0.2 |
| ***Compound Classes*** | | |
| *Monoterpene hydrocarbons* | | 10.7 |
| *Oxygenated monoterpenes* | | 52.9 |
| *Sesquiterpene hydrocarbons* | | 22.0 |
| *Oxygenated sesquiterpenes* | | 1.4 |
| *Others* | | 8.4 |
| ***Total identified*** | | ***95.4*** |

**Note:** Essential oil sample was analyzed in triplicate to ensure repeatability (standard deviation < 1). Unidentified compounds of less than 0.5% are not included. KI is the Kovat's Index previously calculated by Robert Adams using a linear calculation on a DB-5 column [12]. *KI not previously calculated [12]. Manual calculation performed using alkane standards. Relative area percent was determined by GC/FID.

(29.1%), linalool (7.3%), and piperitenone oxide (6.8%). The principal sesquiterpene hydrocarbons were germacrene D (12.4%), bicyclogermacrene (5.3%), and β-bourbonene (1.2%). The major oxygenated sesquiterpenes were spathulenol (0.8%), germacrene-D-4-ol (0.4%), and α-cadinol (0.2%). Figure 2 is provided for a more intuitive visual representation.

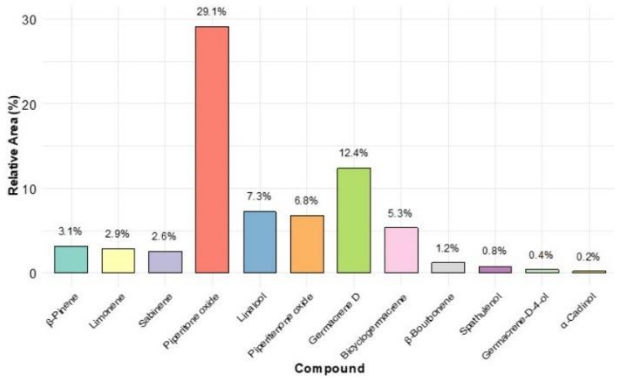


**Figure 2.** Comparison of Compound Concentrations in the Essential Oil of *Clinopodium acutifolium*.

These findings have parallels and divergences with the composition of essential oils from other species within the *Clinopodium* genus. For instance, piperitone oxide and piperitenone oxide were also identified as prominent compounds in *Clinopodium nepeta* essential oil [8]. This similarity suggests that there might be a close chemical relationship between *C. acutifolium* and *C. nepeta*. Additionally, piperitone oxide was also identified as one of the major compounds in *Clinopodium macrostemum* [9], indicating that this compound may play a significant role in various species within the genus.

On the other hand, it is noteworthy that germacrene D was identified as one of the major constituents in this study. This compound was also found as a major compound in *Clinopodium gracile* and *Clinopodium sericeum* [13,14]. This may suggest that in addition to piperitone oxide and piperitenone oxide, germacrene D could be another chemical marker within certain *Clinopodium* species. However, it is also important to recognize the diversity in the composition of essential oils within the genus, as compounds such as thymol and γ-terpinene which are prominent in *C. vulgare*, and carvacrol and pulegone which are major compounds in C. *nubigenum* [7,10], were not major compounds, or not detected in *Clinopodium acutifolium* essential oil in this study.

The principal chemical constituents of essential oils generally dictate their bioactivities [15]. Piperitone oxide (Fig. 3) isolated from extracts of *Mentha longifolia*

has demonstrated high activity in the reduction of PCSK9 expression [16], suggesting the use of it as a dietary supplement to help manage cholesterol levels.

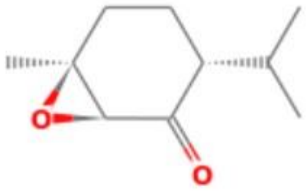

**Figure 3.** Piperitone oxide chemical structure. Obtained from NIST [17].

Piperitenone oxide (Figure 4) has been studied for various bioactivities [18]. It has been experimentally observed to have central analgesic effects, indicating its potential for pain relief [18]. This compound also exhibited toxicity against the larvae of various mosquito species in experimental settings [19-22]. Additionally, it has shown some trypanocidal activity against *Trypanosoma cruzi* [23].

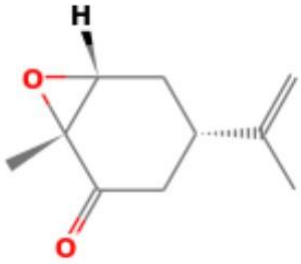

**Figure 4.** Piperitenone oxide chemical structure. Obtained from NIST [24].

## 4. Conclusions

This study provides, for the first time to the authors' best knowledge, the chemical composition of *Clinopodium acutifolium* essential oil, demonstrating the presence of piperitone oxide, Germacrene D, linalool, and piperitenone oxide as the major compounds. This information is relevant for the identification and characterization of different plant species and for the evaluation of their therapeutic or pharmacological potential, as well as for the understanding of the chemical diversity within the *Clinopodium* genus. Future research into the biological properties of the essential oil of this species is recommended, as well as expanding the research to other unexplored species within the genus.

## Authors' contributions

Conceptualization, C.P.; Methodology, C.P., and A.A.; Software, C.P., A.A., T.M.W., and T.O.; Validation, C.P.; Formal Analysis (GC/MS, GC/FID), C.P., A.A., T.M.W., T.O.; Investigation, C.P., and A.A.; Resources, C.P., E.C.; Data Curation, C.P., A.A., T.M.W.; Writing – Original Draft, C.P. and A.A.; Writing – Review & Editing, C.P., A.A., T.M.W., T.O., E.C., and O.P.

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## Availability of data and materials

All data will be made available on request according to the journal policy.

## Conflicts of interest

The authors declare no conflict of interest. The funding entity had no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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