Chemical Composition and Anticholinesterase Activity of the Essential Oil from the Ecuadorian Plant *Salvia pichinchensis* Benth.

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(Received July 19, 2019; Revised December 21, 2019; Accepted December 24, 2019)

**Abstract:** *Salvia pichinchensis* Benth was collected in the South of Ecuador and the essential oil (EO) was distilled from aerial parts and analyzed by GC-MS and GC-FID. The physical properties, chemical composition, and cholinesterase inhibitory activity were determined. Six major components, all sesquiterpenes, were identified: cis-cadina-1(6),4-diene (17.11%) (1), γ-curcumene (13.75%) (2), (E)-caryophyllene (12.58%) (3), (E,E)-α-farnesene (10.00%) (4), α-gurjunene (9.46%) (5) and allo-aromadendrene (6.96%) (6). The EO showed an interesting selective inhibitory activity against the enzyme butyrylcholinesterase (50.70 μg/mL) and only low inhibitory activity against acetylcholinesterase (117.60 μg/mL). The chemical composition and butyrylcholinesterase activity of the EO of *S. pichinchensis* Benth was reported for first time.

**Keywords:** *Salvia pichinchensis*; essential oil; GC-MS analysis; in vitro ChE inhibitory activity, cis-cadina-1(6),4-diene. © 2020 ACG Publications. All rights reserved.

**1. Introduction**

The medicinal plant *Salvia pichinchensis* Benth (*S. siphonantha* Briq.) is commonly known as "matico de cerro" or "matico grande" by the community of Saraguro who live in a few villages located on the Southern Andes of Ecuador. The family Lamiaceae or Labiatae, to which *S. pichinchensis* belongs, contains approximately 236 genera and about 7173 identified species [1]. Many of them have a great economic importance due to the presence of chemical components with insecticidal and/or bactericidal activity against different organisms [2]. Despite the wide occurrence of Lamiaceae plants occur in different parts of Ecuador, the family has little been investigated and only a limited number of...
botanical collections exist in local herbaria. 27 Genera and about 219 species of Lamiaceae have been registered to grow in Ecuador, 29 of which (13.24%) are endemic. The genus *Salvia* contains the highest number (15, 52%) of all the Lamiaceae species endemic to Ecuador [3].

Morphologically, *S. pichinchensis* is a purple-green colored shrub, approximately 1.5 to 3 m high. The leaves are 3 to 8 cm wide, oval and slightly rounded at the base, with serrated or serrated margins. The flowers are dark blue or purple, whose corolla is 31 to 35 mm long [4,5].

In some locations of Ecuador, *S. pichinchensis* is also known with the popular name of Quinde-sungana-mangapaque and is used together with *Salvia scutellaroides* Kunth and *Salvia macrophylla* Benth in the preparation of a traditional medicine, called "manga-paqui", which is used for curing kidney and liver disorders. The leaves are placed on the forehead of a person to relieve headache [6]. Saraguro people use the plant to treat the infection of external wounds. To this purpose, branches are cooked in water for about ten minutes, and the affected part of the body is washed several times with the resulting infusion. In addition, an infusion of the leaves can be drunk to promote post-surgical recovery [7] and is used as a gargle to deflate the throat.

In the rare studies on the phytochemistry and pharmacology of *Salvia* species growing in Ecuador, the chemistry of the secondary metabolites and the biological activity of the extracts or isolated compounds have not yet been reported. The fatty acid composition of *S. hispanica* seed oil, known as chia, has been determined by GC analysis. The oil contains linolenic, linoleic, oleic, stearic, and palmitic acids [6].

Essential oils (EOs) isolated from medicinal plants have an increasing interest, due to their chemical composition and the wide variety of biological activities [7-9], including the inhibitory activity against acetylcholinesterase (AChE) [10] and butyrylcholinesterase (BuChE).

Recent evidence suggests that both AChE and BuChE may have roles in the aetiology and progression of Alzheimer’s disease (AD) beyond regulation of synaptic ACh levels. Both enzymes therefore represent legitimate therapeutic targets for ameliorating the cholinergic deficit considered to be responsible for the declines in cognitive, behavioural and global functioning characteristic of AD [11,12]. Indeed, clinical studies have demonstrated that with increased inhibition of ChEs there is a linear improvement in cognitive functions as well as improvements in verbal and spatial memory tests and reaction times.

In this context, many natural products have shown high anticholinesterase activity and their use for Alzheimer’s disease therapy has been proposed [13]. Desirable properties of botanical extracts or natural products include a comparatively better penetration of the blood–brain barrier than the pharmaceutical options and better specificity for human type cholinesterase’s (ChE). *In vitro* assays testing the anti-ChE of essential oils and extracts from tropical plants are limited (see, as typical examples, references 14 and 15).

The physical properties, chemical composition and anticholinesterase activity of the EO isolated from *S. pichinchensis* Benth are reported in this paper for the first time. This research is part of our ongoing program on the study and valorization of Saraguro medicinal plants.

2. Materials and Methods

2.1. Plant Material

Aerial parts of *S. pichinchensis* Benth were collected in June 2018, in Ingapirka (9593668N, 17696838E) of the San Lucas parish, in the Loja province of Southern Ecuador, at an altitude of 2800 m a.s.l. The plant collection was authorized by a governmental permission (MAE-DNB-CN-2016-0048). Dr. Fani Tinitana of the Herbarium of the Universidad Técnica Particular de Loja (HUTPL) identified the plant; a voucher sample was deposited at the Herbarium of the Universidad Técnica Particular de Loja, with the accession number PPN-la-014.

2.2. Isolation of Essential Oil

Fresh aerial parts (branches, leaves and flowers) of *S. pichinchensis* (1kg) were hydrodistilled immediately after collection for 3 hours at atmospheric pressure using a Clevenger-type apparatus.
The distilled EO was then separated from the aqueous phase and dried over anhydrous sodium sulphate, filtered and stored in brown vials at 4 °C until analysis. This procedure was repeated three times.

2.3. Physical Properties of the Essential Oil

The relative density of the oil was determined at 20 °C according to the international standard method AFNOR NF T 75-111 (ISO 279: 1998). The refractive index was measured at 20 °C on an ABBE refractometer according to the AFNOR NF 75-112 (ISO 280: 1998) international standard method. The specific rotation was determined on an automatic polarimeter Hanon P-810, according to the international standard ISO 592-1998 guidelines. Each test was performed in triplicate and an average value was calculated.

2.4. Chemical Composition of the Essential Oil

2.4.1. Gas Chromatography Coupled Mass Spectrometry (GC-MS)

Oil components were identified by GC-MS analysis performed on an Agilent Technologies Chromatograph 6890N series, coupled to a mass spectrometer-detector Agilent, series 5973, operated in electron-ionization mode at 70 eV. Two types of chromatographic columns were used; a non-polar capillary column (DB5-MS, 5%-phenyl-methylpolysiloxane stationary phase (30 m × 0.25 mm i.d. × 0.25 μm of film thickness) and a polar capillary column (HP-Innowax, 30 m × 0.25 mm i.d. × 0.25 μm of film thickness), both using helium as carrier gas (1.00 mL/min in constant flow mode). The injection system operated in split mode (40: 1) at 220 °C. The GC oven temperature was kept at 60 °C, then increased to 250 °C with a gradient rate of 3 °C/min. The ion source temperature was 250 °C. 1 μL of a solution of the oil in CH₂Cl₂ (1: 100 v/v) was injected.

2.4.2. Gas Chromatography Coupled Flame Ionization Detector (GC-FID)

Analyses were performed using an Agilent Technologies chromatograph (6890 series) coupled to a FID detector, using the DB5-MS and HP-Innowax columns. Quantification (expressed as a percentage) of each identified compound was done by comparing the area of the corresponding GC peak to the total area of identified peaks (Table 1) without applying any correction factor. Average values and standard deviations were calculated from the results of three injections. EO samples were prepared and analyzed under the same conditions as the GC-MS analysis (see supporting information for the chromatogram).

2.4.3. Identification of Chemical Compounds

The chemical components of the EO (Table 1) were identified by comparing their calculated Linear Retention Indices (LRI) and EIMS spectra with the spectra of compounds having close retention indices reported in the literature. The comparison of the indices was considered reasonable in a range of ± 20 units. Linear retention indices (LRI) were determined, according to Van Den Dool and Kratz [17], on the basis of a homologous series of n-alkanes from C9 to C24 (C9 from BDH, purity 99%, and C10–C24 from Fluka, purity 99%), which were injected on the DB5-MS and HP-Innowax columns after the EO, under identical conditions.

2.5. Cholinesterase (ChE) Inhibition Assay

Cholinesterase inhibitory activity of EO was determined against acetylcholinesterase (AChE, from Electrophorus electricus, Sigma-Aldrich, C3389, St Louis MO.) and butyrylcholinesterase (BuChE, from equine serum, Sigma-Aldrich, SRE020, St Louis MO.), according to Ellman et al. [18]. A typical 200 μL inhibition assay volume contained phosphate buffered saline solution (pH 7.4),
DTNB (1.5 mM), tested sample in DMSO (1% v/v final). Both AChE (Type V-S, lyophilized powder, 744 U/mg solid, 1 272 U/mg protein) and BuChE (lyophilized powder, _900 U/mg protein) were dissolved in PBS pH 7.4 and used at 25 mU/mL for the assay. After 10 min of pre-incubation, the substrate acetylthiocholine iodide (1.5 mM) was added to start the reaction. During 1 h of incubation at 30 °C, 96-well microtiter multiplates were read on a PherastarFS (BMG Labtech) detection system. Enzymatic activities were tested in the presence of 0.05 to 250 µg/mL of EO dissolved in DMSO, whose concentration was kept constant. Donepezil was used a reference ChE inhibitor for both enzymes [12]. The results were expressed as the mean ± SD of three replicates. IC₅₀ values were determined from nonlinear regression model by using the online GnuPlot package (www.ic50.tk, www.gnuplot.info).

3. Results and Discussion

3.1. Physical Properties

The S. pichinchensis fresh aerial parts produced a clear yellow oil and the yield of the hydrodistilled EO was 0.04±0.01% (w/w); the relative density, refraction index and specific optical rotation of the EO were d = 0.9 ± 0.004 g/L, n = 1.50 ± 0.02, and [α]D²° = −59.64 ± 0.26 (c 0.1 in CH₂Cl₂), respectively.

3.2. Chemical Composition

The constituents of the S. pichinchensis EO (Table 1) were identified by GC-MS and GC-FID. 44 Compounds were identified, that represented 98.74% of the total mixture on a DB5-MS chromatographic column and 98.13% on a HP-Innowax column, respectively. The most important class of compounds were sesquiterpene hydrocarbons that accounted for 90.02 % of the total mixture analyzed on the DB5-MS column. Six compounds represented about 70% of the EO (see Table 1 and Figure 1 in supporting information): cis-cadina-1(6),4-diene (1) (17.11%), γ-curcumene (2) (13.75%), (E)-caryophyllene (3) (12.58%), (E,E)-α-farnesene (4) (10.00%), α-gurjunene (5) (9.46%), and allo-aromadendrene (6) (6.96%) (see supporting information).

| Table 1. Chemical composition of Salvia pichinchensis essential oil |
|---------------------------------------------------------------|
| **Compound** | **DB5-MS column** | | **HP-Innowax column** | |
| | **LRIexp** | **LRIref** | **% ± S.D.** | **LRIexp** | **LRIref** | **% ± S.D.** |
| α-Pinene | 923 | 932 | 0.09 ± 0.00 | 1066 | 1075 | 0.51 ± 0.06 |
| Camphene | 938 | 946 | 0.43 ± 0.00 | 1084 | 1076 | 0.06 ± 0.00 |
| Limonene | 1024 | 1024 | 0.11 ± 0.01 | 1199 | 1194 | 0.13 ± 0.01 |
| 1,8-cineole | - | - | - | 1205 | 1202 | 0.13 ± 0.00 |
| (Z)-β-Ocimene | 1032 | 1032 | 0.34 ± 0.03 | 1236 | 1235 | 0.39 ± 0.00 |
| (E)-β-Ocimene | 1042 | 1044 | 1.59 ± 0.07 | 1253 | 1248 | 1.81 ± 0.01 |
| Borneol | 1166 | 1165 | 0.20 ± 0.01 | - | - | - |
| (Z)-Ocimenone | 1225 | 1226 | 0.30 ± 0.01 | 1571 | 1570 | 0.35 ± 0.00 |
| Bornyl acetate | 1277 | 1284 | 0.86 ± 0.00 | 1575 | 1570 | 0.30 ± 0.00 |
| α-Cubebene | - | - | - | 1483 | 1474 | 0.94 ± 0.00 |
| α-Copaene | 1363 | 1374 | 0.73 ± 0.00 | 1483 | 1488 | 0.69 ± 0.02 |
| β-Bourbonene | 1369 | 1387 | 0.53 ± 0.04 | 1508 | 1507 | 0.61 ± 0.00 |
| β-Cubebene | 1377 | 1387 | 0.12 ± 0.06 | 1531 | 1543 | 0.34 ± 0.00 |
| β-Elemene | 1380 | 1389 | 0.52 ± 0.03 | - | - | - |
| α-Gurjunene | 1393 | 1409 | 9.46 ± 0.02 | 1520 | 1538 | 10.18 ± 0.01 |
| α-Cedrene | 1398 | 1410 | 0.55 ± 0.03 | 1553 | 1568 | 0.33 ± 0.02 |
| Unidentified | - | - | - | 1563 | - | 0.14 ± 0.00 |
| Caryophyllene* | - | - | - | 1585 | 1588 | 2.07 ± 0.03 |
| (E)-Caryophyllene | 1405 | 1417 | 12.58 ± 0.08 | 1586 | 1598 | 12.71 ± 0.00 |
| β-Copaene | 1415 | 1430 | 0.24 ± 0.01 | - | - | - |
| Compound                  | Retention Time (min) | Percentage ± S.D. |
|---------------------------|----------------------|-------------------|
| β-Gurjunene               | 1432                 | 0.22 ± 0.02       |
| Allo-Aromadendrene        | 1444                 | 6.96 ± 0.04       |
| γ-Elemene                 | -                    | -                 |
| α-Humulene                | 1439                 | 0.43 ± 0.00       |
| Aromadendrene             | 1446                 | 1.20 ± 0.04       |
| Unidentified              | -                    | -                 |
| Sesquisabinine            | 1451                 | 0.54 ± 0.00       |
| (E)-β-Farnesene           | 1467                 | 17.11 ± 1.12      |
| γ-Gurjunene               | 1457                 | 0.51 ± 0.00       |
| Unidentified              | -                    | -                 |
| cis-Cadin-1(6),4-diene    | 1467                 | 17.11 ± 1.12      |
| γ-Curcumene               | 1472                 | 13.75 ± 0.33      |
| ar-Curcumene              | 1474                 | 2.04 ± 0.07       |
| α-Muuroleone              | -                    | 1712              |
| Bicyclogermacrene         | 1481                 | 5.75 ± 0.19       |
| (E,E)-α-Farnesene         | 1490                 | 10.00 ± 0.18      |
| α-Zingiberene             | 1500                 | 4.88 ± 0.15       |
| δ-Amorphene               | 1507                 | 1.60 ± 0.22       |
| α-Cadinene                | 1523                 | 0.19 ± 0.01       |
| (E)-γ-Bisabolene          | 1538                 | 0.11 ± 0.00       |
| cis-Muurol-5-en-4-β-ol    | 1552                 | 0.31 ± 0.01       |
| cis-Muurol-5-en-4-α-ol    | 1562                 | 0.51 ± 0.07       |
| Unidentified              | -                    | -                 |
| Zierone                   | 1578                 | 0.24 ± 0.03       |
| Unidentified              | -                    | 0.19 ± 0.04       |
| Spathulenol               | 1587                 | 1.44 ± 0.47       |
| Unidentified              | -                    | 1889              |
| Muurola-4,10(14)-dien-1-β-ol | 1618             | 0.15 ± 0.05       |
| Palustrol                 | -                    | 1915              |
| Caryophyllene oxide       | -                    | 1967              |
| Unidentified              | -                    | 2011              |
| Ledol                     | -                    | 2017              |
| Germacrene D-4-ol         | -                    | 2044              |
| Viridiflorol              | -                    | 2075              |
| εpi-α-Cadinol             | 1629                 | 0.26 ± 0.01       |
| Hinesol                   | 1631                 | 0.23 ± 0.00       |
| γ-Eudesmol                | 1634                 | 0.16 ± 0.00       |
| α-Muurolole               | 1642                 | 0.56 ± 0.02       |
| Unidentified              | -                    | 2182              |
| α-Cadinol                 | -                    | 2230              |
| 7-epi-α-Eudesmol          | 1676                 | 0.91 ± 0.16       |

Monoterpene hydrocarbons (%): 2.57
Oxygenated monoterpenes (%): 0.51
Sesquiterpene hydrocarbons (%): 90.02
Oxygenated sesquiterpenes (%): 4.78
Others (%): 0.86

Total Identified (%) 98.74 98.13

*Unidentified isomer; LRIexp, Linear Retention Index calculated against n-alkanes C9-C24; LRIref, Linear Retention Index obtained from the literature: * [19], † [20], ‡ [21], § [22], †* [23], †‡ [24], †§ [25], †† [26], †‡* [27], †§* [28], †‡[29], †‡[30], †* [31], †* [32], †* [33], †§ [34], †‡ [35], †‡* [36], †* [37], †*[38], †* [39], †* [40], †[41], †* [42], †*[43], †‡ [44], †* [45]; % ± S.D.: percentage and standard deviation of each compound determined from the GC-FID chromatogram.
The presence of high amount of cis-cadina-1(6),4-diene is a special characteristic of the EO of *S. pichinchensis* from Ecuador, because this finding differs markedly from the data reported previously for the EOs of *Salvia* species. For example, the presence of this sesquiterpene was not reported in different studies carried out with several species from Turkey, such as *S. adenocaulon*, *S. adenophylla*, *S. aethiopis*, *S. aramiensis*, *S. atropatana*, *S. atherci var. atherci*, *S. blepharochlaena*, *S. bracteata*, *S. camdica*, *S. caespitosa*, *S. candidissima*, *S. chionantha*, *S. cilicica*, *S. cryptantha*, *S. divaricata*, *S. euphratica* var. *leiocalyxina*, *S. frigida*, *S. glutinosa*, *S. heldreichiana*, *S. huberi*, *S. hydrangea*, *S. hypargeia*, *S. kronenburgii*, *S. limbata*, *S. macrochlamys*, *S. microstegia*, *S. modesta*, *S. multicaulis*, *S. nemorosa*, *S. pachystachys*, *S. palestina*, *S. pisidica*, *S. poculata*, *S. potentillifolia*, *S. recognita*, *S. rosifolia*, *S. russelli*, *S. sclarea*, *S. staminea*, *S. suffruticosa*, *S. syriaca*, *S. tomentosa*, *S. trichoclada*, *S. verticillata* subsp. *amasiaca* and *S. virgata* [46], *S. adenophylla*, *Salvia pilifera*, *Salvia viscosa* [30], *Salvia montbretii* [31], *S. ballisiana*, *S. cyanescens*, *S. divaricata*, *S. hydrangea*, *S. kronenburgii*, *S. macrochlamys*, *S. nydeggeri*, *S. pachystachys*, *S. pseudoeuphratica* and *S. russellii* [40]. Analogously, the EOs of *T. tebesana* [47], *S. reuterana* [48], *S. nemorosa*, *S. sclarea*, *S. macrocephon*, *S. verticillata*, *S. eremophila*, *S. aethiopis*, *S. virgata*, *S. reuterana*, *S. limbata*, collected in Iran [49], and *S. officinalis* from Albania [50] did not contain this sesquiterpene.

As regards the occurrence of small amounts of cis-cadina-1(6),4-diene (1) in *Salvia* species, the EO from flowering parts of *S. samuelssonii* collected in Jordan contains this compound in trace quantities (less than 0.05%) [51]. Likewise, the EO of *S. fracticosa* collected in Greece contains the two isomers, cis-cadina-1(6),4-diene (1) and trans-cadina-1(6),4-diene in low percentages (0.2 and 0.4%, respectively) [50]. On the other hand, the EOs from *S. dolomitica* and *S. somalensis* collected in Italy contain only the trans isomer, although in small amounts (0.35 and 0.59%, respectively) [52]. As regards *Salvia* species growing in South America, the presence of cis-cadina-1(6),4-diene (1) has not been reported in the EOs of *S. palafolia* from Colombia [53], *S. officinalis*, *S. sclarea*, *S. lavandulifolia*, and *S. triloba* from Brazil [54], and *S. lavandulifolia* from Perú [55].

The composition of the EO distilled from *S. pichinchensis* differs from all the oils isolated from the other *Salvia* species investigated so far. The high amount of cis-cadina-1(6),4-diene (1), γ-curcumene (2), (E)-caryophyllene (3), (E,E)-α-farnesene (4), α-gurjunene (5), and allo-aromadendrene (6) suggests the existence of a new chemotype of this native plant to the Ecuadorian Andes.

* cis-Cadina-1(6),4-diene (1) has the cadinane skeleton, and belongs to a group of biologically active sesquiterpenes. In particular, they exhibit high antifungal properties, that have been evaluated against *Lenzites betulina*, *Trametes versicolor*, *Laetiporus sulphureus* [56], *Coriolus versicolor*, and *Laetiporus sulphureus* [57]. (E)-Caryophyllene (3) has shown anti-inflammatory and local anesthetic properties [58], antioxidant and neuroprotective effects [59], and a significative cytotoxicity against several types of cancer cells [60]. (E,E)-α-farnesene (4) is an alarm pheromone secreted by the termite *Prorhinotermes canalifrons* [61]. α-Gurjunene (5) occurs in other species of *Salvia*, such as *S. reuterana*, and its concentration in the EO of wild plants of this species varies between 5.43 and 13.70%, depending on the plant habitat and climatic conditions [48]. Allo-aromadendrene (6), isolated from *Eucaliptus globulus*, has defense activity against the insect *Cienartaya eucalypti* [62].

### 3.3. Cholinesterase Inhibition Assay

*S. pichinchensis* EO showed low inhibitory activity (IC$_{50}$ = 117.60 ± 13.90 μg/mL) against acetylcholinesterase (AChE). In contrast, the inhibitory activity against BuChE (IC$_{50}$ = 50.7 ± 3.10 μg/mL) was quite remarkable. For comparison, the reference ChE inhibitor donepezil exhibited an IC$_{50}$ = 0.040 ± 0.005 μg/mL against AChE and an IC$_{50}$ = 3.6 ± 0.20 μg/mL against BuChE. *S. pichinchensis* essential oil thus exhibited selective inhibition of BuChE. This finding differs from previous studies where EOs of *S. pseudoeuphratica*, *S. hydrangea* and *S. divaricata* showed a high inhibition of the AChE enzyme with IC$_{50}$ values of 26.00 ± 2.00; 40.00 ± 4.00 and 64.68 ± 4.16 μg/mL, respectively and low levels of BuChE inhibition with IC$_{50}$ values > 80 μg/mL for the three species [40].

In our study the EO from the aerial parts of *S. pichinchensis* exhibited high selective activity against BuChE, which was higher than against AChE. The inhibitory activity of the EO likely results from a complex interaction of its chemical components, ultimately producing synergistic or
antagonistic inhibitory responses [63,64]. This interesting bioactivity shows that the essential oil of S. pichinchensis is a promising source for further studies on the anti-BuChE activity and for the possible development of new drugs against neurodegenerative diseases [11,12].

Author’s contributions

C.A. and J.M A. collected the plant material. M.S. and J.C. performed the hydrodistillation. M.S., J.R., C.A. and G.V. shared the contributions to data analysis, N.B and C.L. conducted the biological assays.

Acknowledgments

We are grateful to the Universidad Técnica Particular de Loja for financial support.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

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