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# Antioxidant activity of six essential oils and its molecules in Ecuadorian Andean medicinal plants

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***Author contributions***

Paco Noriega responsible for the direction of the investigation. Edison Díaz analytical and instrumental support. Ivana Villegas data processing support. Karla Pozo, Priscila Guerrero and Pablo Guerra responsible for experimental trials. Christian Larenas support in the treatment and statistics of the results.

***Competing interests***

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***Abbreviations***

*M. Mollis*, *Minthostachys mollis*; *A. glutinosa*, *Aristeguietia glutinosa*; *B. latifolia*, *Baccharis latifolia*; DPPH, 2,2-diphenyl-1-picrylhydrazyl; ABTS, 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); GC/MS, gas chromatography coupled to mass spectrometry.

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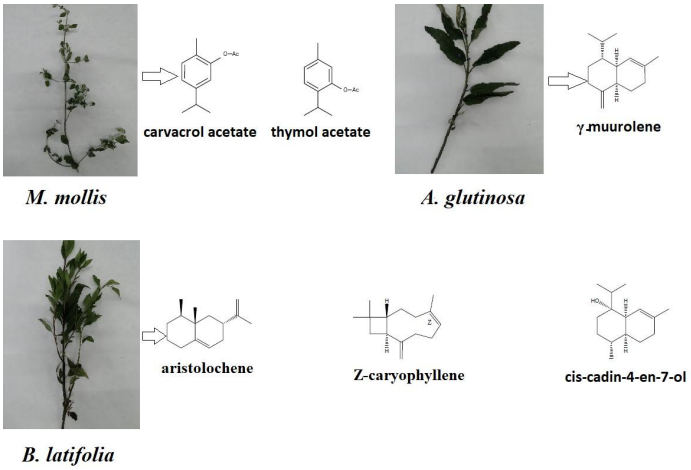
***Abstract***

**Background**: This research values the antioxidant activity and its responsible molecules in six essential oils from medicinal plants in the Ecuadorian Andes. **Methods**: The chemical composition of essential oils was determined using gas chromatography coupled mass spectrometry. For evaluated the antioxidant activity of essential oils was use tree spectrophotometric methods: diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and β-Carotene bleaching test. The essential oils with good activity were determined the responsible molecules using the Bioautographic HP-TLC-DPPH method. **Results**: The scavenging capacity of the radicals was assessed with DPPH and ABTS methods, the best results were found in the oils of *M. mollis* $IC_{50}$ DPPH 2.80 mg/ml and $IC_{50}$ ABTS 0.205 mg/mL and in *A. glutinosa* $IC_{50}$ DPPH 12.972 mg/mL and $IC_{50}$ ABTS 0.321 mg/mL, the results were compared with a pattern of natural reference in this case, the essential oil of *T. vulgaris* $IC_{50}$ DPPH 0.474 mg/mL and $IC_{50}$ ABTS 0.272 mg/mL. The evaluation of the antioxidant activity was determined by the β-carotene bleaching test, the most notable activity results were from *M. mollis* $IC_{50}$ 0.119 mg/mL, *A. glutinosa* $IC_{50}$ 0.062 mg/mL and *B. latifolia* $IC_{50}$ 0,064 mg/mL. DPPH bioautography revealed the active molecules antioxidants in oils for *M. mollis* were thymol acetate (7.73%) and carvacrol acetate (24.52%), for *A. glutinosa* was γ-muurolene (2.68%), and for *B. latifolia* Z-caryophyllene (2.99%), aristolochene (0.11%) and cis-cadin-4-en-7-ol (4.11%). **Conclusion**: The results of antioxidant activity shown in descending order that the essential oils of: *M. mollis*, *A. glutinosa* and *B. latifolia*, are those with the highest activity using the DPPH and ABTS methods. The β-Carotene bleaching test method confirms the 3 oils as the most active in the following order: *A. glutinosa*, *B. latifolia* and *M. mollis*. An antioxidant bioautographic study identified the molecules responsible for the activity in three essential oils with good activity.

**Keywords:** Andean essential oils; Minthostachys mollis; antioxidant activity; bioautography antioxidant



***Tradition***

The Ecuadorian Andes have a wide variety of medicinal plants used for the treatment of various diseases and symptoms. Several of these plants are characterized by their content of essential oils containing potentially antioxidant molecules.

Native species such as Ambrosia arborescent (marco), Aristeguietia glutinosa (matico), Baccharis latifolia (chilca), Lantana camara (supirosa), Minthostachys mollis (tipo) and Myrcianthes rhopaloides (arrayán), which are characterized by containing essential oils, could be a source of antioxidants and be used industrially as medicines or for the preservation of food and drugs, giving an added value to the properties known by the ancestral knowledge of the peoples of Ecuador.

***Highlights***

The study aims to assess essential oils in aromatic medicinal plants from the Ecuadorian Andes as antioxidants.

## *Background*

The plants evaluated in this research are traditionally used by the people of the Ecuadorian Andes for the treatment of various diseases. The selection of medicinal plants is related to the medicinally frequency used by the population and their common distribution in all Ecuadorian Andes.

*Ambrosia arborescens*, commonly known as “marco”, is used as an analgesic, antirheumatic, insecticide and for hot baths [1]. It is also used for its abortive qualities and to delay menstruation [2]. The plant contains lactones, sesquiterpenes, and alkaloids [3, 4].

*Aristeguietia glutinosa*, known as “matico”, is used for its anti-inflammatory qualities, against influenza and for post-partum bathing. The plant has shown the presence of friedelin, dammaradienyl acetate and amrinone acetate; its essential oil includes β-himachalene and trans-β-guaiene, which confirms its antimicrobial and antifungal activity [5, 6].

*Baccharis latifolia,* known as “chilca”, is used for its analgesic properties, it is hepatoprotective and antirheumatic [7], it also has anti-inflammatory effects for asthmatic conditions, antidiarrheal and sedative. Its essential oil contains limonene, germacrene D, δ-cadinene, β-cubebene, epi-α-bisabolol [8], various extracts have shown anti-inflammatory effects [9], antihelmintic potential [10] and antioxidant [11].

*Lantana camara,* known as “supirosa”, is an analgesic, antipyretic and skin healer [3]. Several studies confirm its insecticidal activity [12, 13], and highlight its potential as an antibacterial [14]. Research carried out in India confirms the presence of (E)-β-caryophyllene, α-humulene, 1,8-cineole, and germacrene [15], similarly, a different investigation from Cuba found mostly (E)-nerolidol, γ-cadinene, α-humulene, and (E)-β-caryophyllene [16].

*Minthostachys mollis*, known as “tipo” is a species that is appreciated for its digestive, antispasmodic, antidiarrheal, emetic, emmenagogue and tranquilizing qualities [3]. In Ecuador, this plant has been traditionally used for the treatment of diseases of the respiratory system due to its antitussive and expectorant qualities [1]. The essential oil has shown antifungal properties and is mainly and is composed of pulegone, menthone, and limonene [17]. Other chemical evaluations of oils of *M. mollis*, carried out in Argentina, have highlighted the presence of limonene, α-terpinene, linalool, menthone, isomenthone, dihydrocarcone, pulegone, carvone, carvacrol, piperitone, carvacryl acetate [18]. Furthermore, it shows an important radical scavenging activity in the oil and the presence of α-eudesmyl acetate, isolongifolol acetate, germacrene-D, 1,8-cineole and pulegone [19].

*Myrcianthes rhopaloides*, commonly known as “arrayán”, is used for its antidiarrheal, stomachache, anti-influenza, and skin-healing properties [3]. Compounds such as linalool, 1,8-cineole, limonene, and α-terpineol, highlight the plant’s antimicrobial qualities, particularly toward strains of *Streptococcus* [20].

Reactive oxygen species have the ability to produce oxidative damage to biological molecules such as DNA, lipids, carbohydrates and proteins. In addition, they cause pathophysiological damage that produces diseases such as cancer, diabetes, cardiovascular pathologies, and inflammatory pathologies, among others [21].

All the plants researched in this work present an important medicinal use, the assays seek to assess the antioxidant potential of the essential oils by several tests to determine which of these plants have molecules with qualities that will stop the oxidative processes and can serve as medicines for various diseases.

## *Experimental*

### Plant material

The plant material of *Ambrosia arborescens* and *Aristeguietia glutinosa* (Asteraceae), *Minthostachys mollis* (Lamiaceae), and *Myrcianthes rhopaloides* (Myrtaceae) were provided by the Jambi Kiwa Organization, from the town of Licto in the Province of Chimborazo, 78.6 longitudes and 1.78333 latitudes at an altitude of 2900 m.a.s.l. *Lantana camara* (Verbenaceae) and *Baccharis latifolia* (Asteraceae) were collected in the vicinity of the city of Quito, 78.4667 longitude and 0.28333 latitude, an altitude of 2600 m.a.s.l. Plants were deposited in the herbarium of Salesian Polytechnic University with the voucher name: *A. arborescens* (HUPS-as-009), *A. glutinosa* (HUPS-as-010), *M. mollis* (HUPS-lm-003), *M. rhopaloides* (HUPS-mt-008), *L. camara* (HUPS-ve-005) and *B. latifolia* (HUPS-as-011). The recognition was made by the biologist Marco Cerna.

### Isolation of essential oils

The essential oils were extracted by steam distillation in a 100 liters capacity stainless steel distiller belonging to the laboratories of Life Sciences of the Universidad Politécnica Salesiana.

### GC/MS analysis

The chemical composition of the oils was determined by gas chromatography coupled with mass spectrometry. A Varian gas chromatograph, model 3900, coupled to a Varian Saturn 2100 mass spectrometer was used. The column used was a VF5 (5%-phenyl-95% dimethylpolysiloxane, 30 meters long, internal diameter of 0.25 mm and 0.25 µm film thickness. The transport gas was helium at a flow rate of 1 mL/min with a split ratio of 1/50. The analysis started at 45 °C, reaching 100 °C at a rate of 1 °C/min, it then reached 250 °C at a rate of 5 °C/min and was kept at this temperature for 15 min. The conditions of the mass spectrometer were: ionization energy 70 eV and a mass range of 40-350 m/z. The components were identified using the NIST 2001 database, evaluation of theoretical retention indexes compared to the Adams 2009 database [22] and determination of the experimental retention indexes using an alkane series C8-C30 [22].

### Evaluation of the Antioxidant Potential of the essential oils.

For the evaluation of the antioxidant potential, four treatments were carried out in the essential oils. Firstly the radical-scavenging ability was determined by spectrophotometric methods of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the 2,2’-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), commonly used in research with essential oils [23, 24]. Subsequently, the antioxidant activity was evaluated through the β-carotene bleaching test [25], and finally, the bioautography test was carried out to understand the types of molecules responsible for the antioxidant activity through the methodology of HPTLC-DPPH [26].

### Free radical scavenging activity

Both tests used some volumes of essential oil diluted in dimethylsulfoxide to a volume of 100 µL. These were subsequently combined with a constant volume of 2.9 mL of DPPH 1 10-4 M and 0.90 mL of ABTS 1 10-3 M ABTS previously radicalized with a solution of 7 10-2 M K2S2O8.

In the case of DPPH, the solutions were left stirring for 30 min, and the absorbance was measured at 517 nm. For ABTS, the solutions were read in the spectrophotometer after 1 minute at 754 nm, in both cases, we used a Shimadzu UV mini 1240 spectrophotometer.

Once the measurements were obtained, the inhibition percentual of each concentration was calculated using the following equation:

$$\%\ \text{of inhibition DPPH or ABTS} = \left(1 - \frac{AM}{BM}\right) \text{x } 100$$

Where AM is the absorbance of the sample and BM the absorbance of the blank.

Finally, a graph was plotted for concentration as a function of percentage inhibition, and the $IC_{50}$ was calculated for each essential oil.

Because of its well known activity, the oil of *Thymus vulgaris* was used as a positive control for this test [27, 28]. The synthetic positive control used was butylated hydroxyanisole (BHA).

### β-Carotene bleaching test

Using the methodology proposed by Miller [29], which is based on the antioxidant capacity of a natural component immersed in a system composed of linoleic acid and β-carotene, we evaluated the ability to prevent lipid peroxidation. Using several solutions of essential oils in a Shimadzu UV mini 1240 spectrophotometer at a wavelength of 470 nm, we calculated the antioxidant activity according to the following expression.

$$AA = 100 \text{ x } \frac{(DRc - DRs)}{DRc}$$

AA = antioxidant activity

DRc = ln (a/b)/60, where a is the initial absorbance and b is the absorbance at 60 minutes in the negative control

DRs = ln (a/b)/60, where a is the initial absorbance and b is the absorbance at 60 minutes in the samples of essential oil.

### Bioautographic HP-TLC-DPPH

Bioautographic aims to identify the molecules that contribute to biological activity, it uses as its tool the separation of the components on an HP-TLC plate.

The sample of essential oils is prepared by diluting 30 µL of essential oil in 1mL of methanol. With the aid of a Camag IV dispositive, the oil samples were planted in a volume of 15 µL in HP-TLC plates Merk, silica gel 60. The solvent system was toluene/ethyl acetate/petroleum ether (97/7/20).

The plate was developed with a solution of DPPH at 0.5%, the yellow coloration of the fractions reveals the presence of activity. Active fractions were extracted from the plate by dilution with dichloromethane and injected into the system of gas chromatography coupled to masses to the same instrumental conditions of the analysis of the essential oils.

### Statistics

With the purpose of grouping the essential oils depending on their radical scavenging activities (through DPPH and ABTS), a Kruskal-Wallis test was carried out, chi-square = 16, 5789, df = 5, and a value of $P < 0.005$, the software used was Past version 2.17 c.

## *Results*

### Isolation of essential oils

The yields (w/w) for only essential oil were: *Ambrosia arborescens* (0.30%), *Aristeguietia glutinosa* (0.97%), *Baccharis latifolia* (0.13%), *Lantana camara* (0.04%), *Minthostachys mollis* (0.44%) and *Myrcianthes rhopaloides* (0.40%).

### Chemical Composition

The evaluation of the chemical composition reveals the presence, in a majority, of carvacrol acetate 24.52%, trans-piperitone epoxide 18.57%, α-terpinene 6.41% and menthone 6.26% in *M. mollis;* dillapiole 59%, (E)-β-ocimene 11.65% and (Z)-β-ocimene 4.02% in *A. glutinosa*; γ-gurjunene 77.53%, longifolene 3.84% and ar-curcumene 2.87% in *A. arborescens*; limonene 59.77%, α-pinene 10.66%, β-pinene 7.25% and myrcene 5.04 % in *M. rhopaloides*; γ-murolene 22.23%, (E)-β-caryophyllene 17.07%, α-humulene 12.61% and bicyclo germacrene 6.22% in *L. camara* and α-phellandrene 18.11%, and limonene with 17.04% in *B. latifolia*. The chemical composition of the six essential oils is visible in Table 1; Figure 1 shows the GC analysis.

### Free radical scavenging activity

This test aims to analyze the activity through a comparison of the $IC_{50}$ values obtained for each essential oil in the methods ABTS and DPPH. The results are shown in Table 2.

The Kruskal-Wallis statistic test compares the average values of free-radical scavenging with the purpose of grouping them according to their potential and proximity to the natural reference standard oil of *T. vulgaris* (Figure 1). The data grouped in the same category three of the oils: *M. mollis*, *B. latifolia*, and *A. glutinosa*, which come close and match the natural standard. The oils of *M. rhopaloides*, *L. camara*, and *A. arborescens* move away from the natural standard, therefore it can be said that they present a low activity. The oil with the best activity is that from *M. mollis*.

**Table 1 Chemical composition of the six essential oils from plants of the Andes of Ecuador**

| Compuesto | RI[a] | RI[b] | *M. mollis* | *A. glutinosa* | *A. arborescens* | *M. rhopaloides* | *L. camara* | *B. latifolia* |
|---|---|---|---|---|---|---|---|---|
| santolina triene | 908 | 902 | | | 1.20 ±0.20 | | | |
| α-thujene | 930 | 922 | 0.58 ± 0.25 | | | | | 0.25 ± 0.02 |
| α-pinene | 939 | 929 | 0.16 ± 0.09 | 1.61 ± 0.51 | | 10.66 ± 2.47 | | 2.74 ± 0.21 |
| camphene | 954 | 945 | | | | | | 0.27 ± 0.03 |
| sabinene | 975 | 969 | 0.50 ± 0.12 | | | | | 0.66 ± 0.05 |
| β-pinene | 979 | 974 | 0.25 ± 0.20 | 1.02 ± 0.3 | 2.15 ±0. 51 | 7.25 ± 1.55 | | 1.84 ± 0.13 |
| myrcene | 990 | 988 | | | | 5.04 ± 0,18 | | 0.93 ± 0.06 |
| α-phellandrene | 1002 | 1006 | | 0.80 ± 0.11 | | | | 18.11 ± 1.16 |
| δ-3-carene | 1011 | 1007 | | | | | | 1.85 ± 0.02 |
| α-terpinene | 1017 | 1015 | | 1.00 ± 0,32 | | | | |
| p-cymene | 1024 | 1015 | 3.09 ± 0.74 | | | | | 0.87 ± 0.05 |

**Table 1 Chemical composition of the six essential oils from plants of the Andes of Ecuador *(continued)***

| Compuesto | RI[a] | RI[b] | *M. mollis* | *A. glutinosa* | *A. arborescens* | *M. rhopaloides* | *L. camara* | *B. latifolia* |
|---|---|---|---|---|---|---|---|---|
| limonene | 1029 | 1026 | | 1.68 ± 0.50 | | 59.77 ± 2.71 | | 17.04 ± 0.90 |
| β-phellandrene | 1029 | 1027 | | 1.32 ± 0,56 | | | | |
| 1,8cineole | 1031 | 1029 | | | | 8.11 ± 0.14 | | 0.15 ± 0.02 |
| (Z)-β-ocimene | 1037 | 1033 | | 4.02 ± 1.28 | | | | 2.07 ± 0.11 |
| (E)-β-ocimene | 1050 | 1043 | 0.51 ± 0.08 | 11.65 ± 3.89 | | | | |
| γ-terpinene | 1059 | 1054 | 6.41 ± 2.03 | 3.30 ± 1,09 | | | 0.79 ± 0.05 | |
| terpinolene | 1088 | 1083 | | | | | | 0.22 ± 0.02 |
| linalool | 1096 | 1103 | | | | 2.27 ± 0.30 | | |
| menthone | 1152 | 1153 | 6.36 ± 1.00 | | | | | |
| neo-menthol | 1165 | 1168 | 1.07 ±0 .37 | | | | | |
| carvone | 1243 | 1243 | 3.39 ± 0.35 | | | | | |
| piperitone | 1252 | 1252 | | 2.29 ± 0.35 | | | 1.7 ± 0.12 | |
| cis-piperitone epoxide | 1254 | 1250 | 1.63 ± 0.41 | | | | | |
| trans-piperitone epoxide | 1256 | 1254 | 18.57 ±0.65 | | | | | |
| thymol | 1290 | 1298 | 2.08 ± 1.21 | | | | | |
| carvacrol | 1299 | 1308 | 5.05 ± 1.87 | | | | | |
| bicycloelemene | 1336 | 1333 | | | | | 3.19 ± 0.06 | |
| δ-elemene | 1338 | 1338 | 1.75 ± 0.47 | | | | 3.83 ± 0.02 | |
| thymyl acetate | 1352 | 1359 | 7.73 ± 0.32 | | | | | |
| piperitone oxide | 1368 | 1373 | 0.52 ± 0.10 | | | | | |
| carvacryl acetate | 1372 | 1377 | 24.52 ±0.26 | | | | | |
| α-copaene | 1376 | 1377 | | 0.53 ± 0.16 | | | 1.97 ± 0.02 | 0.16 ± 0.003 |
| β-bourbonene | 1388 | 1382 | | | | | 0.30 ± 0.00 | |
| β-cubebene | 1388 | 1387 | | | | | 1.48 ±0.04 | |
| β-elemene | 1390 | 1389 | 0.55 ± 0.20 | | 1.81 ± 0.14 | | 5.79 ± 2.84 | 0.21 ± 0.05 |
| cyperene | 1398 | 1398 | | 0.36 ± 0.11 | | | | 0.13 ± 0.02 |
| methyl eugenol | 1403 | 1408 | | | | | | 2.94 ± 0.5 |
| longifolene | 1407 | 1411 | | | 3.84 ± 0.30 | | | |
| (Z)-β-caryophyllene | 1408 | 1412 | 6.29 ± 0.34 | 3.63 ± 1.10 | | 1.45 ± 0.34 | | 2.99 ± 0.16 |
| (E)-β-caryophyllene | 1419 | 1427 | | 0.43 ± 0.10 | | | 17.07 ± 3.97 | |
| β-cedrene | 1420 | 1425 | | | 1.46 ± 0.12 | | | |
| β-copaene | 1432 | 1425 | | | | | 4.63 ± 0.12 | |
| γ-elemene | 1436 | 1428 | | | | | 9.93 ±0 .21 | 0.21 ± 0.01 |
| α-guaiene | 1439 | 1432 | | | | | 0.37 ± 0.00 | |
| aromadendrene | 1441 | 1440 | | | | | 0.45 ± 0.01 | |
| α-humulene | 1454 | 1452 | 1.35 ± 0.01 | 1.46 ± 0.41 | | | 12.61 ± 0.23 | 0.47 ± 0.01 |
| (E)-β-farnesene | 1456 | 1457 | | | 0.43 ± 0.10 | | | |
| allo-aromandrene | 1460 | 1457 | | | | | | 0.20 ± 0.01 |
| trans-cadina-1(6),4-diene | 1476 | 1476 | | | | | | 0.20 ± 0.01 |

**Table 1 Chemical composition of the six essential oils from plants of the Andes of Ecuador *(continued)***

| Compuesto | RI[a] | RI[b] | *M. mollis* | *A. glutinosa* | *A. arborescens* | *M. rhopaloides* | *L. camara* | *B. latifolia* |
|---|---|---|---|---|---|---|---|---|
| γ-gurjunene | 1477 | 1479 | | | 77.53 ± 4.03 | | | |
| γ-murolene | 1479 | 1479 | 2.44 ± 0.07 | 2.68 ± 1.16 | | | 22.23 ± 0,05 | 1.20 ± 0.04 |
| ar-curcumene | 1480 | 1482 | | | 2.87 ± 0.12 | | | |
| aristolochene | 1488 | 1486 | | | | | | 0.11 ± 0.02 |
| β-selinene | 1490 | 1485 | | | | 0.67 ± 0.05 | | |
| trans-muurola-4(14),5-diene | 1493 | 1488 | | | | | | 0.2 ± 0.04 |
| valencene | 1496 | 1490 | | 0.41 ± 0.12 | | | | |
| α-selinene | 1498 | 1492 | | | | 0.79 ± 0.09 | | |
| bicyclogermacrene | 1500 | 1492 | 2.76 ± 0.37 | 0.81 ± 0.27 | 0.59 ± 0.20 | | 6.22 ± 0.14 | 1.42 ± 0.05 |
| α-muurolene | 1500 | 1496 | | | | | 2.02 ± 0.26 | 0.15 ± 0.01 |
| β-dihydroagarofuran | 1503 | 1499 | | | | | | 0.23 ± 0.01 |
| (E,E)-α-farnesene | 1505 | 1506 | | | 1.22 ± 0.51 | | | |
| β-bisabolene | 1505 | 1507 | | | | | | 0.13 ± 0.01 |
| δ-cadinene | 1523 | 1517 | 0.29 ± 0.03 | 0.91 ± 0.29 | 1.66 ± 0.14 | 0.92 ± 0.22 | 2.11 ± 0.21 | 0.41 ± 0.08 |
| epiglobulol | | 1527 | | | | | | 2.25 ± 0.05 |
| liguloxide | 1536 | 1535 | | | | | | 6.55 ± 0.28 |
| elemol | 1549 | 1552 | | | | | | 1.49 ± 0.11 |
| germacrene B | 1561 | 1558 | | | | | 2.76 ± 0.13 | 0.37 ± 0.02 |
| spathulenol | 1578 | 1580 | | | | 0.73 ± 0.11 | | |
| caryophyllene oxide | 1583 | 1584 | | | | 1.26 ± 0.42 | | |
| guaiol | 1600 | 1604 | | | 0.98 ± 0.15 | | | |
| dillapiole | 1620 | 1627 | | 59.59 ± 15.04 | | | | |
| cis-cadin-4-en-7-ol | 1636 | 1640 | | | | | | 4.11 ± 0.23 |
| α-cadinol | 1652 | 1656 | | | | | | 0.54 ± 0.05 |
| valerianol | 1658 | 1658 | | | | | | 0.27 ± 0.05 |
| 7-epi-α-eudesmol | 1663 | 1662 | | | | | | 0.66 ± 0.10 |
| andro encecalinol | 1676 | 1672 | | | 0.84 ± 0.08 | | | |
| α-bisabolol | 1685 | 1694 | | | | | | 0.86 ± 0.06 |
| cyperotundone | 1695 | 1701 | | | | | | 1.34 ± 0.1 |
| norhalkendin | | 1758 | | | | | | 1.34 ± 0.10 |

RI[a]: theorical retention index by Adams 2009; RI[b]: experimental retention index calculated by comparing the retention rates of a homologous series of hydrocarbons C8-C30.

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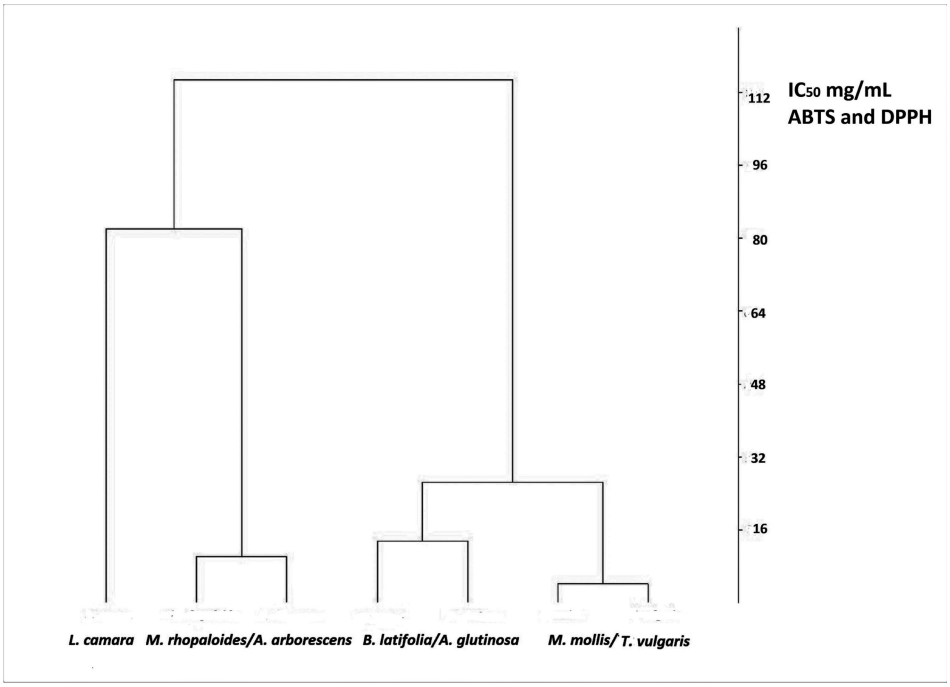


**Figure 1 Statistical grouping of essential oils depending on the radical scavenging activity as $IC_{50}$ mg/mL**

**Table 2 Radical scavenging activity of essential oils expressed as $IC_{50}$**

| Essential oils | DPPH $IC_{50}$ (mg/mL) | ABTS $IC_{50}$ (mg/mL) |
|---|---|---|
| *Minthostachys mollis* | 2.830 ± 0.002 | 0.205 ± 0.001 |
| *Aristeguietia glutinosa* | 12.972 ± 0,018 | 0.321 ± 0.001 |
| *Ambrosia arborescens* | 56.836 ± 0.204 | 0.990 ± 0.002 |
| *Myrcianthes rhopaloides* | 62.661 ± 0.325 | 0.849 ± 0.007 |
| *Lantana camara* | 107.120 ± 1.520 | 0.483 ± 0.001 |
| *Baccharis latifolia* | 20.802 ± 0.023 | 2.1853 ± 0.013 |
| *Thymus vulgaris* | 0.474 ± 0.0027 | 0.272 ± 0.001 |
| BHA | $5 \times 10^{-3}$ | $5 \times 10^{-3}$ |

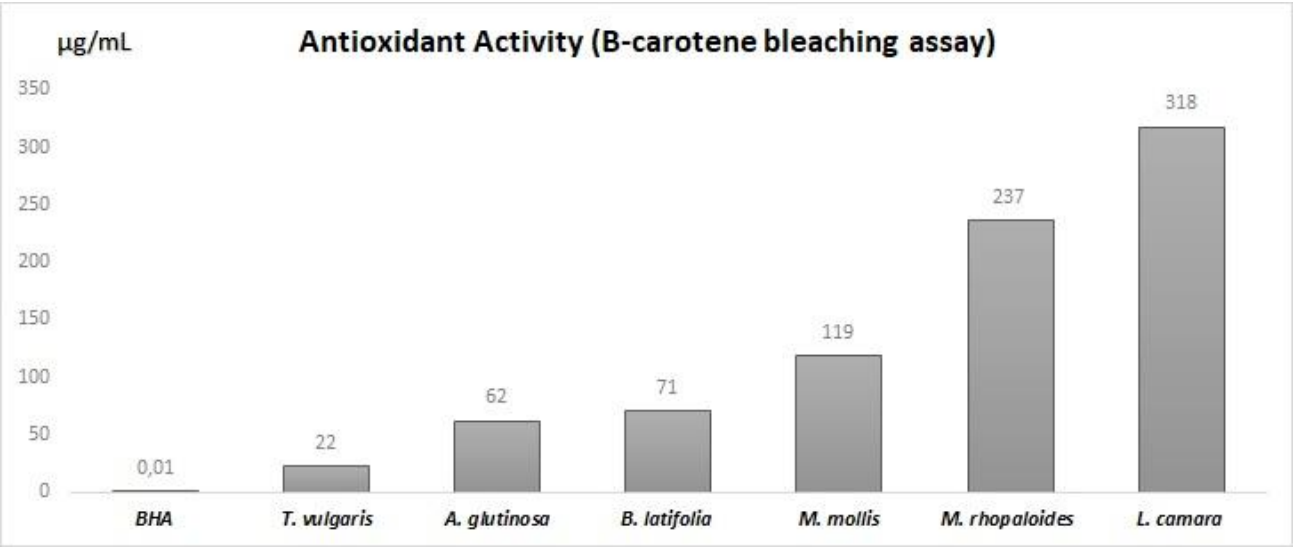


**Figure 2 Antioxidant activity from the Andean essential oils, evaluated by β-carotene bleaching test**

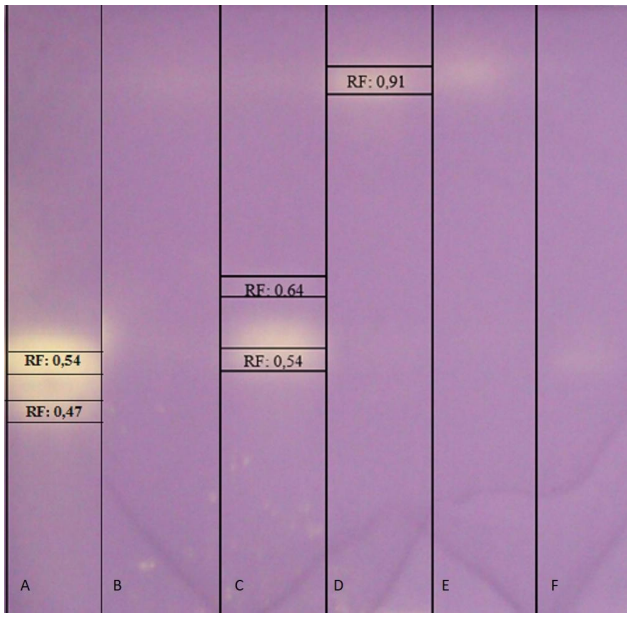


**Figure 3 Bioautography HPTLC-DPPH of Andean essential oils.** A (*B. latifolia*); B (*A. arborescens*); C (*M. mollis*); D (*A. glutinosa*); E (*L. camara*) and F (*M. rhopaloides*).

**Antioxidant activity (β-carotene bleaching test)**

This evaluation indicates that the oil with the best antioxidant activity was *A. glutinosa* whose $IC_{50}$ was 62 ± 4 µg/mL, followed by *B. latifolia*, 71 ± 10 µg/mL and *M. mollis* 119 ± 2 µg/mL. The oils with the least activity were *M. rhopaloides* 237 ± 3 µg/mL, *L. camara* 318 ± 5 µg/mL and *A. arboresecens* 704 ± 6 µg/mL. Figure 2 shows the differences.

**Bioautographic HP-TLC-DPPH**

The tree of the six essential oils showed fractions with yellow coloring (Figure 3), which indicates the presence of molecules with antioxidant activity (Table 3).

For *M. mollis*, the molecules responsible for its antioxidant activity are carvacrol acetate 24.52% and 7.73% thymol acetate, which are the major components of the essential oil. Essential oils with these two components reflect good antioxidant activity, as is the case of *Thymus caespititius* [30] and *Mosla chinensis* [31].

For *A. glutinosa*, the molecule with the most activity is γ-muurolene, research confirms an important activity in essential oils that contain this molecule [32, 33].

In *B. latifolia* the molecules responsible for the activity are aristolochene, (*Z*)-β-caryophyllene and cis-cadin-4-en-7-ol, for these molecules, there are no references in essential oils with antioxidant activity.

*A. glutinosa* and *B. latifolia* belong to the family Asteraceae, some essential oil from this plant family have great antioxidant properties, an extraordinary example have *Achillea millefolium* essential oil [34], with DPPH $IC_{50}$ of 1.56 µg/mL. Another research in *Artemisia annua* shows an antioxidant activity is equivalent to 18% of the reference compound (α-tocopherol) [35]. Lamiaceae family has several plants with essential oils that were shown to have antioxidant activity, standing out: *Melissa officinalis* [36], *Rosmarinus officinalis*, and *Salvia officinalis* [37], known and used plants around the world.

***Discussion***

Three out of the six essential oils studied have an interesting antioxidant activity, which is verified in the spectrophotometric assays performed in this research, i.e., DPPH, ABTS and β-Carotene bleaching test. The oils are repeated and are similar in the DPPH and ABTS tests, confirming *M. mollis* as the most active (lowest $IC_{50}$ value), followed by *A. glutinosa* and *B. latifolia*. Previous studies conducted in the essential oils *M. mollis* [19] and *B. latifolia* [11], have confirmed this activity. Regarding *M. mollis*, the statistical treatment groups it at the same activity level as the natural reference of antioxidant activity used in this research, i.e., the essential oil of *T. vulgaris*.

**Table 3 Fractions with antioxidant activity according to the evaluation HPTLC-DPPH-GC/MS**

| RF | *Minthostachys mollis.* | *Aristeguietia glutinosa* | *Baccharis latifolia* |
|---|---|---|---|
| 0.91 | - | γ-muurolene | - |
| 0.64 | thymyl acetate | - | |
| 0.54 | carvacryl acetate | - | cis-cadin-4-en-7-ol |

The results shown in the β-Carotene bleaching test confirm the same three essential oils as the most active; however, the one that shows the highest activity is *A. glutinosa*, a plant known for its healing and anti-inflammatory properties, possibly because of its protective potential against oxidizing agents.

The molecules showing activity in the three oils: thymol acetate, carvacrol acetate, γ-muurolene, Z-caryophyllene, aristolochene and cis-cadin-4-en-7-ol, do not have individual previous studies highlighting their antioxidant activity.

***Conclusion***

There are three relevant results in this research. The first is that the direct activities of essential oils could be used as industrial coadjuvants for the preservation of food or pharmaceutical products, contributing to the reduction of synthetic antioxidants in these type of products. The second is related to the intrinsic properties of plants and the contribution of the antioxidant properties to enhance the medicinal effects that are already known by the traditional knowledge of Andean peoples. And finally, the possibility of to continue studying the individual effects of the antioxidant effects of the six molecules that were detected in the bioautographic studies.

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