

# Chemical composition and antioxidant activity of ‘Nufar’ basil (*Ocimum basilicum* L.) essential oil from three municipalities of Tolima, Colombia

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## ABSTRACT

Essential oils are natural compounds with great potential to be used as products with high antioxidant activity. The aim of this research was to determine the chemical composition and antioxidant activity of the Nufar variety basil essential oil from three municipalities in Tolima, Colombia. The essential oil extracted from the leaves of this basil variety using microwave-assisted extraction predominantly comprises linalool (37.9–41.1%), estragole (24.5–33.6%), and 1,8-cineole (5.2–7.6%). The quantities of these compounds are influenced by the plant's place of origin. Regarding antioxidant activity, the basil essential oil cultivated in the municipality of Honda shows higher antioxidant activity in the DPPH and ABTS+ tests ( $29.89 \pm 4.03$  and  $9.60 \pm 1.74$  mg/mL, respectively), compared to the values from the municipalities of Mariquita and Espinal. The Principal Component Analysis applied to the data generated biplots that explain 99.8% of the total variation. These indicate that the essential oils from basil grown in the municipality of Espinal are characterized by having a high IC<sub>50</sub> (mg/mL) in the DPPH test. On the other hand, the essential oils of basil cultivated in the municipalities of Honda and Mariquita are distinguished by their high content of monoterpenes and total terpenes. The study concludes that the geographical location significantly impacts the chemical composition and antioxidant activity of basil plants.

## 1. Introduction

Basil (*Ocimum basilicum* L.) is an easy-to-cultivate medicinal plant and profitable; therefore, it is extensively available in tropical, sub-tropical, and temperate climate regions (Shehata & Nosir, 2019). In Colombia, basil is considered a promising crop due to its economic potential. In 2022, the export of aromatic herbs reached a record number of exports, with external sales of USD 49.5 million and a growth of 15.8% compared to 2021. For its part, the weight of sales to external markets in 2022 registered 12,281, 9 net tons, with an increase of 13.2% compared to 2021 (Analdex, 2023). According to the Municipal Agricultural Evaluation (EVA, for its acronym in Spanish), in 2022, there was a production of 1040.3 t (Agronet, 2022). Tolima (65.87%) and Cundinamarca (32.08%) are the main producing departments. Currently, basil in Colombia is sold and exported mainly as two products: fresh and dried basil leaves. Essential Oils (EO) extracted from basil represent one of the main potential products of industrial interest. However, their composition may be influenced by the planting site, climatic conditions

during production, genetic origin, agronomic management, and processing method, among others (Damalas, 2019; Milenković et al., 2019). Different studies have reported that the main components of EO are terpenes, phenylpropanoids, alcohols, and aldehydes (Ilić et al., 2019). Other works have determined that the content of individual compounds such as (poly)phenolic acids and flavonoids varies depending on the variety and the part of the plant analyzed, which usually are leaves and flowers. Prinsi et al. (2020) found that anthocyanins present in purple basil leaves are mostly acetylated in nature compared to those in flowers. Furthermore, the application of basil EO has been reported as having antifungal (Saggiorato et al., 2012) and antibacterial activities in foods (Gaio et al., 2015) and antiparasitic activity (de Almeida et al., 2007), anti-inflammatory activity and antiedematogenic activity in animals (Rodrigues et al., 2016).

Accordingly, the aim of this research was to determine the chemical composition and antioxidant activity of the EO obtained from leaves of the Nufar basil variety from three municipalities of Tolima in Colombia.

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2. Material and methods

2.1. Plant material

‘Nufar’ basil (*Ocimum basilicum* L.) was selected as it is the outstanding variety cultivated in the study area. The collection was carried out in three municipalities in the department of Tolima, including Honda, Mariquita, and Espinal, between October and November 2023. The climatic and soil characteristics and agro-technical detail of the municipalities included in this study are listed in [Tables 1, 2 and 3](#). Plants were harvested 10 cm above the soil surface. They were immediately taken to the postharvest laboratory of Universidad del Tolima, located in Ibagué, Tolima Department, Colombia, and stored at 4 °C until use.

2.2. Essential oil (EO) extraction

Leaves and flowers were separated from the stem, and size reduction was performed to expand the exposure area during EO extraction. Microwave-assisted extraction was performed using the advanced Milestone ETHOS X device (Sorisole, Italy). The extraction started at atmospheric pressure in a built-in glass container with a 2 L capacity and a glass lid. The power was set at 1800 W. The instrument software controlled time, pressure, power, and temperature parameters. The Fragrances configuration was used for the system, which includes a glass Clevenger apparatus above the oven, allowing continuous condensation of volatile substances and water recirculation to the reactor. The collected EOs were dried with anhydrous Na<sub>2</sub>SO<sub>4</sub>. They were stored in amber glass containers and refrigerated (4 °C) until use.

2.3. EO composition analysis

Essential oil composition was determined following the methodology proposed by [Duque et al. \(2023\)](#), which is described as follows: A chromatographic analysis was performed on a 6890 Plus gas chromatograph (Agilent Technologies, AT, Palo Alto, CA, USA) equipped with an MS 5973 mass detector. The extracts were separated into two capillary columns, one with a 60 m × 0.25 mm (id) × 0.25 μm poly (ethylene glycol) polar stationary phase and polyethylene glycol PEG (DB-WAX, J & W Scientific, Folsom, CA, USA), and the other is a non-polar column with 5%-phenyl-poly(methyl siloxane) and 5%-pH-PDMS (DB-5MS, J & W Scientific, Folsom, CA, USA); both have the exact dimensions.

An amount of 50 mg of the EO from each of the municipalities evaluated was diluted in 1 mL of methylene chloride. The sample

**Table 1**  
Climatic variables of the three study area municipalities of Tolima, Department, Colombia.

| Climatic variables              | Honda        | Mariquita    | Espinal      |
|---------------------------------|--------------|--------------|--------------|
| Daily precipitation (mm)        | 3.6          | 7.1          | 4.2          |
| Monthly precipitation (mm)      | 109.2        | 214.1        | 128.4        |
| Monthly temperature (°C)        | 28.1         | 26.7         | 27.7         |
| Daily evapotranspiration (mm)   | 5.7          | 5.4          | 5.5          |
| Monthly evapotranspiration (mm) | 172.9        | 163.0        | 168.8        |
| Monthly relative humidity (%)   | 76.5         | 77.4         | 77.6         |
| Elevation (m a.s.l.)            | 337          | 355          | 431          |
| Latitude                        | 5°13'54.1"N  | 5°13'11.8"N  | 4°11'50.3"N  |
| Longitude                       | 74°47'25.1"W | 74°48'32.7"W | 74°57'56.4"W |

**Table 2**  
Physicochemical properties of the soils of the three study area municipalities of Tolima Department, Colombia.

|                        | Honda      | Mariquita  | Espinal     |
|------------------------|------------|------------|-------------|
| N <sub>total</sub> (%) | 0.08       | 0.37       | 0.01        |
| Organic matter (%)     | 1.76       | 4.01       | 1.19        |
| pH                     | 5.59       | 6.03       | 6.63        |
| K (cmol(+)/kg)         | 0.49       | 0.52       | 0.16        |
| Ca (cmol(+)/kg)        | 3.63       | 9.80       | 4.45        |
| Mn (mg/kg)             | 3.87       | 4.93       | 6.86        |
| Fe (mg/kg)             | 168.15     | 109.25     | 107.05      |
| Cu (mg/kg)             | 2.20       | 4.50       | 3.71        |
| Zn (mg/kg)             | 4.77       | 20.10      | 2.50        |
| EC (ds/m)              | 0.25       | 0.33       | 0.21        |
| Texture                | Sandy loam | Sandy loam | Loamy sandy |

EC: electrical conductivity.

**Table 3**  
Agro-technical detail of the three study area municipalities of Tolima Department, Colombia.

| Observation         | Honda                                                                                                                                                                                                                                                                                                                 | Mariquita    | Espinal      |
|---------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|--------------|
| Production method   | Conventional                                                                                                                                                                                                                                                                                                          | Conventional | Conventional |
| Seedling production | Production in semi-controlled conditions with a planting process in plastic trays with 200 cells using black peat as a substrate, disinfected and with a screening size of 0–10 mm and pH of 5.5/6.0.–10 mm. Use of two seeds of the Nufar variety certified by alveolus.                                             |              |              |
| Sowing date         | 08/08/2023                                                                                                                                                                                                                                                                                                            | 08/08/2023   | 07/09/2023   |
| Transplant date     | 02/09/2023                                                                                                                                                                                                                                                                                                            | 02/09/2023   | 03/10/2023   |
| Fertilization       | Via Fertirrigation, using soluble fertilizers in which fertilization is carried out daily according to the fertilization plan of each farm.                                                                                                                                                                           |              |              |
| Irrigation          | Drip irrigation using irrigation tapes with holes every 20 cm, 6 mm caliber and a discharge of 0.98 liters per hour. Two irrigations are carried out per day (morning and afternoon) to maintain the soils at field capacity.                                                                                         |              |              |
| Plant protection    | Use of insecticides to control pests and fungicides to control diseases. According to recommendations of the technical assistant, level of damage, incidence and severity of diseases. Active ingredients with ICA (Instituto Colombiano Agropecuario) registration and accepted by the FDA and organic seal are use. |              |              |
| Harvest date        | 23/10/2023                                                                                                                                                                                                                                                                                                            | 23/10/2023   | 23/11/2023   |

injection volume was 2 μL. Split injection (split ratio 30:1) was used, and the injection temperature was maintained at 250 °C. Helium (99.995%, AP gas, Messer, Bogotá, Colombia) was employed as carrier gas at a 1 mL/min flow rate. The GC/MS transfer line temperature was set at 230 °C when the polar column was used and 300 °C for the non-polar column. The ionization chamber and quadrupole temperatures were 250 °C and 150 °C, respectively. The mass spectrophotometer was used in electron impact ionization (EI) mode (70 eV). The mass range for acquiring ionic currents was m/z 45–450 u. The MSDChemStation G1701DA software (AT, Palo Alto, CA, USA) was used for data acquisition and processing. The identification of individual compounds was based on the calculated retention indices (RI) and on comparisons of the mass spectra obtained from the reference compounds that are available in accessible databases from the literature, including [Adams \(2017\)](#), [Wiley \(2006\)](#), and [NIST \(2014\)](#). Retention indices were determined against C<sub>6</sub>–C<sub>25</sub> and C<sub>8</sub>–C<sub>40</sub> n-alkanes (Sigma-Aldrich, St. Louis, MO, USA).

2.4. Antioxidant activity

The antioxidant activity of the EOs was evaluated using the DPPH and ABTS assays. The DPPH assay was carried out using the methodology proposed by [Brand-Williams et al. \(1995\)](#) with some modifications. A 0.1 mM solution of DPPH (2,2-diphenyl-1-picrylhydrazyl (Sigma-Aldrich, Co. LLC, St. Louis, MO, USA)) in ethanol was used with

an absorbance of  $0.95 \pm 0.03$  at 517 nm. A sample of 10  $\mu\text{L}$  at concentrations of 1, 2, 4, and 8 mg/mL was added to 140  $\mu\text{L}$  of the DPPH solution. The absorption was measured after 15, 30, and 45 min from sample addition at 517 nm.

The ABTS test was carried out following the methodology proposed by Re et al. (1999) with the following modifications. A sample of 10  $\mu\text{L}$  at concentrations of 1, 2, 4, and 8 mg/mL was added to 140  $\mu\text{L}$  of an ABTS solution. The ABTS radical cations (ABTS $^+$ ) were produced by reacting a 7 mM ABTS + solution with 2.45 mM potassium persulfate and allowing the mixture to stand in the dark for at least 6 h at room temperature before use. The ABTS $^+$  solution was diluted until an absorbance of  $0.7 \pm 0.05$  at 734 nm. Then, the absorbance was measured at 734 nm. The absorption was measured after 15, 30, and 45 min from sample addition at 734 nm. Trolox (Sigma-Aldrich, Co. LLC, St. Louis,

MO, USA) was used as a positive control. The percentage of inhibition was calculated using Eq. (1):

$$\% \text{ inhibition} = \frac{(A_{(\text{DPPH}/\text{ABTS}^+)} - A_{(\text{sample})})}{A_{(\text{DPPH}/\text{ABTS}^+)}} * 100 \quad (1)$$

## 2.5. Statistical analysis

All measurements were performed in triplicate, and results were reported as the mean  $\pm$  standard deviation. An analysis of variance (ANOVA) and comparison of means with the Tukey test ( $p < 0.05$ ) were used. Furthermore, a principal component analysis (PCA) was carried out, where the descriptors that provide the highest percentage of variability were identified. The dispersion of the populations was plotted on

**Table 4**

Chemical components of the essential oils obtained from 'Nufar' basil plants from three municipalities: Honda, Mariquita, and Espinal, Tolima Department, Colombia.

| No.   | RT* (min) | Component                             | Chemical classification    | Composition (%) |               |             |
|-------|-----------|---------------------------------------|----------------------------|-----------------|---------------|-------------|
|       |           |                                       |                            | Honda           | Mariquita     | Espinal     |
| 1     | 11.5      | <i>trans</i> -2-hexen-1-al            | Medium-chain aldehyde      | 0.2             | 0.2           | –           |
| 2     | 11.6      | 3-hexen-1-ol                          | Fatty alcohol              | 0.2             | 0.1           | 0.3         |
| 3     | 14.9      | $\alpha$ -pinene                      | Bicyclic monoterpene       | 0.4             | 0.4           | 0.2         |
| 4     | 16.7      | sabinene                              | Bicyclic monoterpene       | 0.4             | 0.4           | 0.2         |
| 5     | 16.9      | $\beta$ -pinene                       | Bicyclic monoterpene       | 0.8             | 0.8           | 0.5         |
| 6     | 17.0      | 1-octen-3-ol                          | Fatty alcohol              | 0.3             | 0.2           | 0.3         |
| 7     | 17.3      | $\beta$ -myrcene                      | Acyclic monoterpene        | 0.6             | 0.6           | 0.4         |
| 8     | 18.6      | $\alpha$ -terpinene                   | Menthane monoterpene       | 0.1             | 0.1           | –**         |
| 9     | 18.9      | <i>p</i> -cymene                      | Monoterpene                | 0.1             | <i>tr</i> *** | –           |
| 10    | 19.1      | limonene                              | Monoterpene                | 0.2             | 0.2           | 0.2         |
| 11    | 19.3      | <b>1,8-cineole</b>                    | <b>Monoterpene</b>         | <b>7.6</b>      | <b>7.3</b>    | <b>5.2</b>  |
| 12    | 19.8      | <i>trans</i> - $\beta$ -ocimene       | Acyclic monoterpene        | 1.1             | 1.2           | 0.6         |
| 13    | 20.3      | $\gamma$ -terpinene                   | Monoterpene                | 0.2             | 0.1           | –           |
| 14    | 20.9      | <i>trans</i> -sabinene hydrate        | Bicyclic monoterpene       | 0.1             | 0.1           | 0.1         |
| 15    | 21.0      | octanol                               | Fatty alcohol              | 0.1             | 0.1           | –           |
| 16    | 21.4      | terpinolene                           | Menthane monoterpene       | 0.1             | 0.1           | –           |
| 17    | 22.4      | <b>linalool</b>                       | <b>Acyclic monoterpene</b> | <b>41.1</b>     | <b>37.9</b>   | <b>38.5</b> |
| 18    | 24.1      | camphor                               | Bicyclic monoterpene       | 0.2             | 0.2           | 0.1         |
| 19    | 25.0      | $\delta$ -terpineol                   | Menthane monoterpene       | 0.2             | 0.2           | 0.1         |
| 20    | 25.4      | terpinen-4-ol                         | Monoterpene                | 0.2             | 0.2           | 0.1         |
| 21    | 26.2      | <b>estragole</b>                      | <b>Phenylpropanoid</b>     | <b>24.5</b>     | <b>31.3</b>   | <b>33.6</b> |
| 22    | 26.2      | octyl acetate                         | Esters                     | 0.5             | –             | –           |
| 23    | 26.8      | nerol                                 | Monoterpene alcohol        | 0.1             | <i>tr</i>     | –           |
| 24    | 27.7      | geraniol                              | Acyclic monoterpene        | 0.1             | 0.1           | –           |
| 25    | 27.8      | chavicol                              | Phenol                     | 0.1             | 0.2           | –           |
| 26    | 29.1      | bornyl acetate                        | Bicyclic monoterpene       | 0.1             | 0.1           | 0.1         |
| 27    | 29.5      | carvacrol                             | Aromatic monoterpene       | 1.5             | 0.4           | –           |
| 28    | 31.5      | eugenol                               | Phenol                     | 4.7             | 3.2           | 1.4         |
| 29    | 32.3      | $\alpha$ -copaene                     | Sesquiterpene              | 0.1             | 0.1           | 0.2         |
| 30    | 32.7      | $\beta$ -bourbonene                   | Sesquiterpene              | 0.1             | 0.1           | –           |
| 31    | 32.8      | $\beta$ -elemene                      | Sesquiterpene              | 1               | 1             | 1.4         |
| 32    | 33.0      | methyl eugenol                        | Phenylpropanoid            | 0.3             | 0.3           | 1.3         |
| 33    | 34.1      | <i>trans</i> - $\beta$ -caryophyllene | Sesquiterpene              | 0.4             | 0.3           | 0.6         |
| 34    | 34.5      | <i>trans</i> - $\alpha$ -bergamotene  | Sesquiterpene              | 0.3             | 0.2           | 0.4         |
| 35    | 34.6      | $\alpha$ -guaiane                     | Sesquiterpene              | 0.2             | 0.2           | 0.3         |
| 36    | 35.0      | <i>trans</i> -muurola-3,5-diene       | Sesquiterpene              | 0.2             | 0.2           | –           |
| 37    | 35.4      | $\alpha$ -humulene                    | Monocyclic sesquiterpene   | 1.3             | 1.2           | 1.6         |
| 38    | 35.6      | muurola-4,14(5)-diene                 | Sesquiterpene              | 0.2             | 0.3           | –           |
| 39    | 35.6      | bicyclosesquiphellandrene             | Sesquiterpene              | –               | –             | 0.2         |
| 40    | 36.3      | germacrene D                          | Sesquiterpene              | 2.5             | 2.7           | 2.7         |
| 41    | 36.7      | bicyclogermacrene                     | Sesquiterpene              | 1.5             | 1.5           | 1.7         |
| 42    | 36.9      | $\alpha$ -bulnesene                   | Sesquiterpene              | 0.3             | 0.3           | 0.4         |
| 43    | 37.3      | $\gamma$ -cadinene                    | Sesquiterpene              | 1               | 1.1           | 1.2         |
| 44    | 37.4      | $\delta$ -cadinene                    | Sesquiterpene              | 0.1             | 0.1           | –           |
| 45    | 37.6      | <i>cis</i> -calamenene                | Sesquiterpene              | –               | –             | 0.1         |
| 46    | 37.8      | 10- <i>epi</i> -cubenol               | Sesquiterpene              | 0.1             | 0.1           | –           |
| 47    | 38.6      | <i>trans</i> -nerolidol               | Sesquiterpene              | 0.5             | 0.5           | 0.5         |
| 48    | 39.3      | spathulenol                           | Tricyclic sesquiterpene    | 0.1             | 0.1           | 0.2         |
| 49    | 40.4      | 1,10-di- <i>epi</i> -cubenol          | Sesquiterpene              | 0.5             | 0.1           | 0.6         |
| 50    | 41.2      | <i>epi</i> - $\alpha$ -cadinol        | Sesquiterpene              | 3.1             | 0.5           | 3.7         |
| 51    | 41.5      | $\alpha$ -cadinol                     | Sesquiterpene              | 0.1             | 3.1           | 0.1         |
| 52    | 41.6      | $\beta$ -cudesmol                     | Oxygenated sesquiterpene   | 0.1             | 0.1           | 0.2         |
| 53    | 43.9      | mint sulfide                          | Sesquiterpene              | –               | –             | 0.3         |
| Total |           |                                       |                            | 99.8            | 99.8          | 99.6        |

\* RT: retention times; \*\*: Not detected; \*\*\**tr*: traces ( $< 0.1\%$ ).

the plane determined by the first two principal components. All information was processed using the Statgraphic 19 statistical software.

### 3. Results and discussion

#### 3.1. Chemical composition of EOs

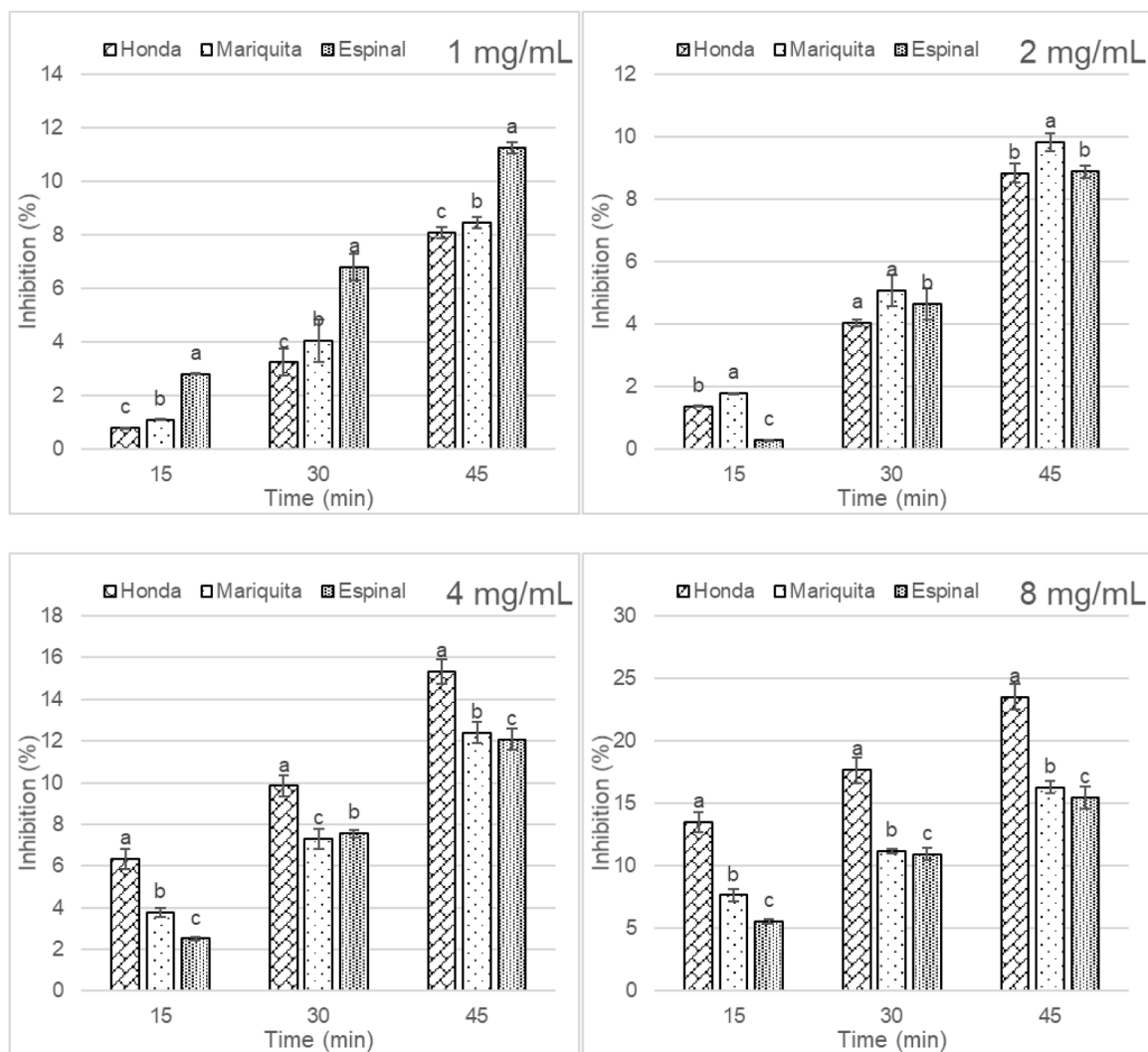
Table 4 presents the chemical components of the essential oils (EOs) evaluated from three municipalities in the department of Tolima. A total of 53 compounds were identified in the EOs. The highest number of components was found in the EO from plants harvested in Honda (50 components), followed closely by Mariquita (49 components), and a relatively smaller number from Espinal (37 components). Generally, the EO of 'Nufar' basil leaves primarily contains linalool (41.1% in Honda, 37.9% in Mariquita, and 38.5% in Espinal), estragole (24.5% in Honda, 31.3% in Mariquita, and 33.6% in Espinal), and 1,8-cineole (7.6% in Honda, 7.3% in Mariquita, and 5.2% in Espinal). Compounds such as *trans*-2-hexen-1-al,  $\alpha$ -terpinene,  $p$ -cymene,  $\gamma$ -terpinene, octanol, terpinolene, octyl acetate, nerol, geraniol, chavicol, carvacrol,  $\beta$ -bourbonene, *trans*-muurola-3,5-diene, muurola-4,14(5)-diene,  $\delta$ -cadinene, and 10-epi-cubenol were exclusively found in the EOs from basil harvested in Honda and Mariquita. Conversely, bicyclosesquiphellandrene, cis-calamenene, and mint sulfide were identified only in samples from Espinal. These findings indicate that the chemical composition of the EO

from 'Nufar' basil could be influenced by the edaphoclimatic conditions of the planting site.

The results of this study align with previous research that identified monoterpenes, such as linalool and 1,8-cineole, and phenylpropanoids, such as estragole (also known as methyl chavicol), as the primary components of basil EOs (Dudai et al., 2020; Kanmaz et al., 2023; Farsaei et al., 2020). Similarly, earlier studies have recognized linalool as the main component of basil essential oil across various regions and cultivars (Talebi et al., 2018). It is well-established that linalool levels in basil cultivars vary due to several factors, including plant origin, environmental conditions, geographical distribution, irrigation, fertilization, and the timing and season of harvest (Muráriková et al., 2017). The formation of monoterpenes is driven by terpene synthesis, which is influenced by developmental and stress-related factors (Tholl, 2006).

#### 3.2. Antioxidant activity

Antioxidants interact with free radicals through several methods, including hydrogen atom transfer and electron transfer processes. In most of these methods, both reactions occur at the same time. The specific reaction mechanism is determined by the structure and solubility of the antioxidant (Aebischer et al., 2021). Figs. 2 and 3 show the results obtained from the ABTS and DPPH tests of the evaluated EOs obtained from 'Nufar' basil. In these results, the capacity of the tested



**Fig. 1.** Antioxidant capacity (as % of inhibition) of essential oils against the DPPH+ radical; 1, 2, 4, and 8 mg/mL are concentrations of essential oils from the reaction. Bars with different letters are statistically different ( $p < 0.05$ ).

EOs to eliminate the ABTS $\cdot$ + radical was notably higher than that of DPPH, agreeing with the results obtained by previous research (Gil et al., 2000). Likewise, Floegel et al. (2011) found that in diverse food samples, the results of the ABTS assay generally exceed those of the DPPH assay. This indicates that the ABTS $\cdot$ + assay interacts with a higher number of components and in more varied ways than the DPPH assay. Despite this, these results should be understood as corroboration of the DPPH trial. Fig. 1

Within the EOs evaluated, it can be observed that, despite being obtained from the same variety, the origin significantly affected the antioxidant activity. The highest percentage of inhibition came from the EO of basil planted in Honda, followed by Mariquita, and finally the one coming from Espinal. The removal capacity gradually increased over time, reaching the highest level after 45 min. Furthermore, the increase in antioxidant activity was not proportional to the increase in EO concentration. For example, the EO from Honda showed an 8% removal of DPPH after 45 min with a concentration of 1 mg/mL. However, by increasing the concentration to 8 mg/mL, which is an 800% increase, the removal of DPPH only increased to 24%, representing a 300% increase. This suggests that antioxidant activity does not increase linearly with EO concentration. The analysis carried out with the ABTS $\cdot$ + radical coincided with the DPPH assay, although a higher antioxidant capacity was observed (53%). In general, a prolonged reaction to DPPH and ABTS $\cdot$ + was evident.

The half-maximal inhibitory (IC<sub>50</sub>) value was calculated and can be

observed in Fig. 3. This value represents the EO concentration of the 'Nufar' basil necessary to neutralize 50% of the initial concentration of the DPPH and ABTS $\cdot$ + radical. Results indicate that the EO from Honda has a higher antioxidant activity in the DPPH and ABTS $\cdot$ + tests ( $29.89 \pm 4.03$  and  $9.60 \pm 1.74$  mg/mL, respectively) than the EO from Mariquita and Espinal. The results obtained are similar to previous research, where IC<sub>50</sub> values in DPPH tests of 17.52 and 55.15 mg/mL are reported for EO of sweet basil from Assiut and BeniSuef in Egypt (Ahmed et al., 2019), corroborating the effect of edaphoclimatic conditions on EO characteristics. In accordance with the above, the visual analysis of the effect of the collection site on the characteristics of the EOs evaluated is carried out in the form of bidirectional data, which will allow a more accurate assessment of the results (Fig. 4). Biplots created using principal component analysis (PCA) are one of the most widely used approaches for the visual evaluation of bidirectional data (Akçura, 2023). The Biplot explained 99.8% of the total variation. The EO from the municipality of Espinal was characterized by having a high IC<sub>50</sub> (mg/mL) in the DPPH test and high phenylpropanoid content. In contrast, the EOs from Honda and Mariquita were characterized by their high content of monoterpenes and total terpenes, which confer excellent antioxidant properties and antibacterial, antifungal, anti-inflammatory, antiviral, anticancerous, and enzyme inhibitory activities (Z. S. Ilić et al., 2022). Likewise, soil and climate characteristic variables were included, showing that, in the town of Espinal, some soil characteristics (e.g., pH, conductivity, organic matter, and nutrients such as N, Ca, K, Cu, Mn, and Zn) and climate

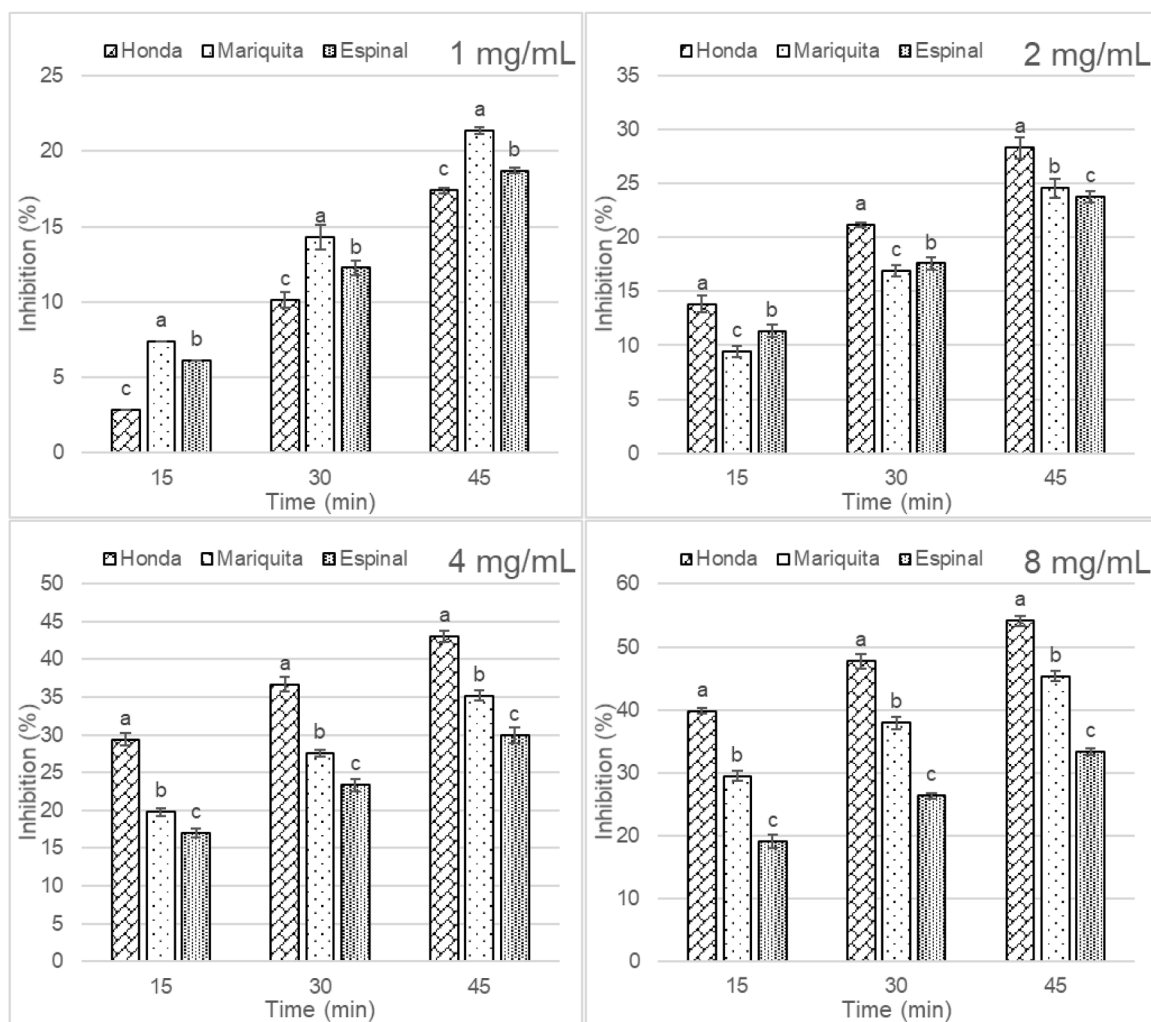
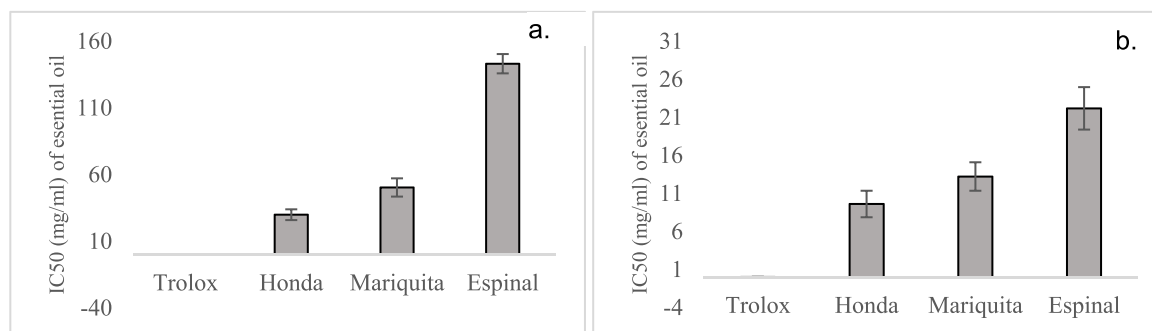
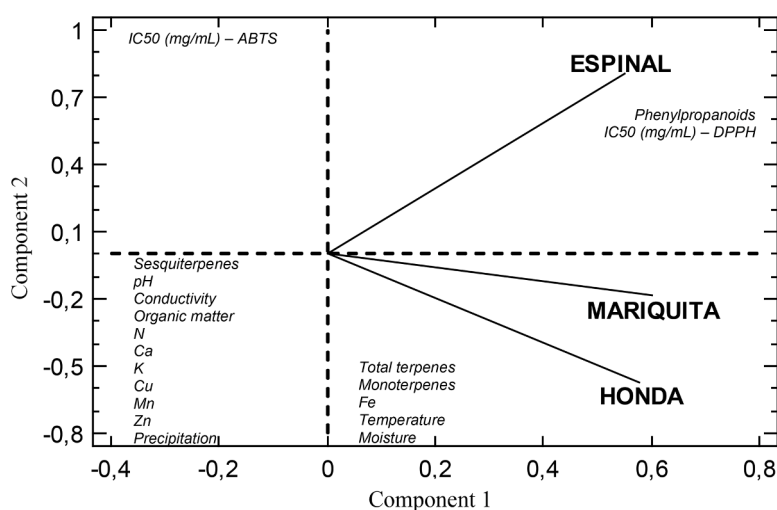


Fig. 2. Antioxidant capacity (as % of inhibition) of essential oils against the ABTS $\cdot$ + radical; 1, 2, 4, and 8 mg/mL are concentrations of essential oils from the reaction. Bars with different letters are statistically different ( $p < 0.05$ ).



**Fig. 3.** Antioxidant activity measured as half-maximal inhibitory concentration (IC<sub>50</sub> in mg/mL) of the essential oil of 'Nufar' basil cultivated in different municipalities of Tolima Department, Colombia (Honda, Mariquita and Espinal), using the DPPH (a.) and ABTS (b.) assays. Trolox is used as positive control.



**Fig. 4.** Biplot graph of the principal components analysis based on the two principal components of essential oil samples of 'Nufar' basil from three municipalities in Tolima Department (Honda, Mariquita and Espinal), Colombia.

(precipitation) obtained different values compared to Mariquita and Honda, which may be reflected in the EO characteristics. The above differs from what was reported by Beltrame et al. (2014), who carried out a comparative study of the chemical composition of basil essential oils grown in different locations in southern Brazil and concluded that there were no significant differences in the chemical composition of the oil from different regions. However, Zhejzakov et al. (2008) reported that the 38 basil genotypes tested differed significantly with respect to the essential oil content and composition.

#### 4. Conclusions

Essential oils are of great interest in the industry thanks to their multiple properties. The EO of 'Nufar' basil leaves mainly contains linalool (41.1% Honda, 37.9% Mariquita, and 38.5% Espinal), estragole (24.5% Honda, 31.3% Mariquita, and 33.6% Espinal) and 1,8-cineole (7.6% Honda, 7.3% Mariquita, and 5.2% Espinal). Regarding antioxidant activity, the EO from Honda registered a higher antioxidant activity in the DPPH and ABTS+ assays ( $29.89 \pm 4.03$  and  $9.60 \pm 1.74$  mg/ML, respectively) than the EO from Mariquita and Espinal. The biplots generated through principal component analysis (PCA) explained 99.8% of the total variation in the data. This analysis revealed distinctive characteristics in EOs from different regions. The EOs from the municipality of Espinal were distinguished by having a higher IC<sub>50</sub> value (mg/mL) in the DPPH assay. On the other hand, the EOs of Honda and Mariquita stood out for their high content of monoterpenes and total terpenes. Most importantly, geographical location can influence the

chemical composition and antioxidant activity of basil plants. Future research on 'Nufar' basil essential oil in Colombia could explore a variety of bioactivities to further establish its economic potential. Investigation of its antimicrobial properties against a variety of pathogens could highlight its use in food preservation and as a natural antimicrobial agent. Additionally, examining its potential to reduce stress and anxiety could support its application in aromatherapy and mental health treatments.

#### Data availability

Data will be made available upon request.

#### Formatting of funding sources

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## CRediT authorship contribution statement

**Martha López-Hernández:** Writing – original draft, Methodology, Formal analysis, Conceptualization. **Jenifer Criollo-Núñez:** Validation, Supervision. **Jose Isidro Beltran:** Methodology, Data curation. **Ángel-ica Sandoval-Aldana:** Writing – review & editing, Validation, Supervision, Data curation, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available on request.

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## References

- Adams, R. P. (2017). *Identification of essential oil components by gas chromatography/mass spectrometry*. Gruver, TX USA: Texensis Publishing, 5 online ed <http://www.juniper.us.org/uploads/2/2/6/3/22639912/bk4frontisbnpreface-contents5thedonline2017.pdf>.
- Aebisher, D., Cichoski, J., Szpyrka, E., Masjonis, S., & Chrzanowski, G. (2021). Essential oils of seven lamiaceae plants and their antioxidant capacity. *Molecules*, 26(13). <https://doi.org/10.3390/molecules26133793>. Article 13.
- Agronet. (2022). *Reporte: área, producción y rendimiento nacional por cultivo*. <https://www.agronet.gov.co/estadistica/Paginas/home.aspx?cod=1>.
- Ahmed, A.F., Attia, F.A.K., Liu, Z., Li, C., Wei, J., & Kang, W. (2019). Antioxidant activity and total phenolic content of essential oils and extracts of sweet basil (*Ocimum basilicum* L.) plants. *Food Science and Human Wellness*, 8(3), 299–305. <https://doi.org/10.1016/j.fshw.2019.07.004>.
- Akçura, S. (2023). Biplot analysis of monthly variations in essential oil concentration and chemical composition of *Pittosporum tobira* leaves in Mediterranean conditions. *Biochemical Systematics and Ecology*, 110, Article 104712. <https://doi.org/10.1016/j.bse.2023.104712>.
- Analdex. (2023, febrero 24). Las exportaciones de Colombia se impulsan con las hierbas aromáticas. *Analdex - Asociación Nacional de Comercio Exterior*. <https://analdex.org/2023/02/24/las-exportaciones-de-colombia-se-impulsan-con-las-hierbas-aromaticas/>.
- Beltrame, J. M., Angnes, R. A., Chiavelli, L. U. R., Costa, W. F. da, Montanher, S. F., Rosa, M. F. da, Lobo, V. da S., & Pomini, A. M. (2014). Chemical Composition of the Essential Oil Obtained from *Ocimum basilicum* (Basil) Cultivated in Two Regions from South Brazil. *Journal of Essential Oil Bearing Plants*, 17(4), 658–663. <https://doi.org/10.1080/0972060X.2014.895212>.
- Brand-Williams, W., Cuvelier, M.E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1), 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5).
- Damalas, C. A. (2019). Improving drought tolerance in sweet basil (*Ocimum basilicum*) with salicylic acid. *Scientia Horticulturae*, 246, 360–365. <https://doi.org/10.1016/j.scienta.2018.11.005>.
- de Almeida, I., Alviano, D. S., Vieira, D. P., Alves, P. B., Blank, A. F., Lopes, A. H. C., Alviano, C. S., & Rosa, M. do S. S. (2007). Antigiardial activity of *Ocimum basilicum* essential oil. *Parasitology Research*, 101, 443–452.
- Dudai, N., Nitzan, N., & Gonda, I. (2020). *Ocimum basilicum* L. (Basil). In En J. Novak, & W.-D. Blüthner (Eds.), *Medicinal, aromatic and stimulant plants* (pp. 377–405). Springer International Publishing. [https://doi.org/10.1007/978-3-030-38792-1\\_10](https://doi.org/10.1007/978-3-030-38792-1_10).
- Duque, J. E., Urbina, D. L., Vesga, L. C., Ortiz-Rodríguez, L. A., Vanegas, T. S., Stashenko, E. E., & Mendez-Sanchez, S. C. (2023). Insecticidal activity of essential oils from American native plants against *Aedes aegypti* (Diptera: Culicidae): An introduction to their possible mechanism of action. *Scientific Reports*, 13(1). <https://doi.org/10.1038/s41598-023-30046-8>. Article 1.
- Farsaraei, S., Moghaddam, M., & Pirbalouti, A. G. (2020). Changes in growth and essential oil composition of sweet basil in response of salinity stress and superabsorbents application. *Scientia Horticulturae*, 271, Article 109465. <https://doi.org/10.1016/j.scienta.2020.109465>.
- Floegel, A., Kim, D.-O., Chung, S.-J., Koo, S. I., & Chun, O. K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis*, 24(7), 1043–1048. <https://doi.org/10.1016/j.jfca.2011.01.008>.
- Gaio, I., Saggiolato, A. G., Treichel, H., Cichoski, A. J., Astolfi, V., Cardoso, R. I., Toniazzo, G., Valduga, E., Paroul, N., & Cansian, R. L. (2015). Antibacterial activity of basil essential oil (*Ocimum basilicum* L.) in Italian-type sausage. *Journal Für Verbraucherschutz Und Lebensmittelsicherheit*, 10(4), 323–329. <https://doi.org/10.1007/s00003-015-0936-x>.
- Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M., & Kader, A. A. (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *Journal of Agricultural and Food Chemistry*, 48(10), 4581–4589. <https://doi.org/10.1021/jf000404a>.
- Ilić, A. S., Antić, M. P., Jelčić, S. C., & Knudsen, T. M.Š. (2019). Chemical composition of the essential oils of three *ocimum basilicum* L. cultivars from Serbia. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 47(2). <https://doi.org/10.15835/nbha47111250>. Article 2.
- Ilić, Z. S., Milenković, L., Tmušić, N., Stanojević, L., Stanojević, J., & Cvetković, D. (2022). Essential oils content, composition and antioxidant activity of lemon balm, mint and sweet basil from Serbia. *Lwt*, 153, Article 112210.
- Kanmaz, H., Gokce, Y., & Hayaloglu, A. A. (2023). Volatiles, phenolic compounds and bioactive properties of essential oil and aqueous extract of purple basil (*Ocimum basilicum* L.) and antidiabetic activity in streptozotocin-induced diabetic Wistar rats. *Food Chemistry Advances*, 3, Article 100429. <https://doi.org/10.1016/j.focha.2023.100429>.
- Milenković, L., Stanojević, J., Cvetković, D., Stanojević, L., Lalević, D., Šunić, L., Fallik, E., & Ilić, Z. S. (2019). New technology in basil production with high essential oil yield and quality. *Industrial Crops and Products*, 140, Article 111718. <https://doi.org/10.1016/j.indcrop.2019.111718>.
- Muráriková, A., Tažký, A., Neugebauerová, J., Planková, A., Jampílek, J., Mučaji, P., & Mikuš, P. (2017). Characterization of Essential Oil Composition in Different Basil Species and Pot Cultures by a GC–MS Method. *Molecules*, 22(7). <https://doi.org/10.3390/molecules22071221>. Article 7.
- NIST, N. (2014). *EPA/NIH mass spectral library*. Gaithersburg: National Institute of Standards and Technology.
- Prinsi, B., Morgutti, S., Negrini, N., Faoro, F., & Espen, L. (2020). Insight into Composition of Bioactive Phenolic Compounds in Leaves and Flowers of Green and Purple Basil. *Plants*, 9(1), Article 1. <https://doi.org/10.3390/plants9010022>.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9), 1231–1237. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3).
- Rodrigues, L. B., Martins, A. O. B. P. B., Cesário, F. R. A. S., e Castro, F. F., de Albuquerque, T. R., Fernandes, M. N. M., da Silva, B. A. F., Júnior, L. J. Q., da Costa, J. G. M., & Coutinho, H. D. M. (2016). Anti-inflammatory and antiedematogenic activity of the *Ocimum basilicum* essential oil and its main compound estragole: In vivo mouse models. *Chemico-biological interactions*, 257, 14–25.
- Saggiolato, A. G., Gaio, I., Treichel, H., de Oliveira, D., Cichoski, A. J., & Cansian, R. L. (2012). Antifungal activity of basil essential oil (*Ocimum basilicum* L.): evaluation in vitro and on an Italian-type sausage surface. *Food and Bioprocess Technology*, 5(1), 378–384. <https://doi.org/10.1007/s11947-009-0310-z>.
- Shehata, A.M., & Nosir, W.S.E. (2019). Response of sweet basil plants (*Ocimum basilicum* L.) grown under salinity stress to spraying seaweed extract. *Future Journal of Biology*, 2(1), 16–28.
- Talebi, M., Moghaddam, M., & Pirbalouti, A. G. (2018). Methyl jasmonate effects on volatile oil compounds and antioxidant activity of leaf extract of two basil cultivars under salinity stress. *Acta Physiologiae Plantarum*, 40(2), 34. <https://doi.org/10.1016/j.pbi.2006.03.014>.
- Tholl, D. (2006). Terpene synthases and the regulation, diversity and biological roles of terpene metabolism. *Current Opinion in Plant Biology*, 9(3), 297–304. <https://doi.org/10.1016/j.pbi.2006.03.014>.
- Wiley, J. (2006). *Wiley registry of mass spectral data*. NJ: John Wiley Hoboken. <https://www.academia.edu/download/62636162/Wiley-Registry-Of-Mass-Spectral-Data-by-John-Wiley-Sons-Ltd20200331-6448-v2q0d7.pdf>.
- Zheljazzkov, V. D., Callahan, A., & Cantrell, C. L. (2008). Yield and oil composition of 38 basil (*Ocimum basilicum* L.) accessions grown in Mississippi. *Journal of Agricultural and Food Chemistry*, 56(1), 241–245. <https://doi.org/10.1021/jf072447y>.