A simple method was developed to obtain and fractionate essential oil simultaneously by hydrodistillation. With this method it was possible to obtain essential oils from the leaves of *Piper arboreum* with the cadinol content ranging from 17.7 to 57.9%. In the essential oils from the leaves of *P. aduncum* was identified dillapiole with content ranging from 4.6 to 96.9%; and further, essential oil from *P. marginatum* with the presence of phenylpropanoids as minor compounds. The essential oils of the three *Piper* species varied in antimicrobial activity when fractionated, with *P. marginatum* oil exhibiting the lowest minimum inhibitory concentration at 19.5 µg mL⁻¹ for the *Pseudomonas aeruginosa* bacterium. The method was efficient for the separation and concentration of chemical constituents of essential oils from the same plant, able to distinguish the different chemical profiles, both qualitatively and quantitatively.

**Keywords:** hydrodistillation, liquid-liquid extraction, essential oil, *Piper*, Piperaceae, antimicrobial

**INTRODUCTION**

Essential oils obtained from plants are generally constituted of complex mixtures of volatile compounds, mainly terpenes and their oxygenated derivatives such as alcohols, aldehydes, ketones, carboxylic acids, phenols, ethers, and esters as well as phenylpropanoids.¹,² Essential oils are obtained by various types of analytical methods, most often by hydrodistillation in the research laboratory.³ Care with the essential oil purification steps is important, due to the oil’s high volatility, complex mixtures, chemical instability and low yield. The fractionation of essential oils is generally carried out by supercritical fluid extraction using...
CO₂, liquid–liquid extraction and vacuum fractionating column. Orange essential oil was fractioned by liquid–liquid extraction using a perforated rotating disc contactor. This is an efficient extraction method but complex apparatus is needed to control the many variables. Most essential oil fractionation is not performed during the extraction process, as in the case of the fractionation of acid lime essential oil containing different systems of solvent. The simultaneous obtainment and fractionation of essential oils has been the subject of some studies with positive results, but requires specific equipment, which is not commercialization available. Simultaneous distillation-extraction of Artemisia argyi and Xylopia aromatica essential oils were carried out with a specific apparatus.

The present study was directed to the simultaneous obtainment and fractionation of essential oils from plants via hydrodistillation without the distillation of organic solvents, using only the Clevenger apparatus, which is widely available and inexpensive. The species of Piper aduncum, Piper marginatum and Piper arboreum were chosen because they belong to the Piperaceae family, which was the target of our systematic study of plant bioactive compounds. A further reason was because their essential oils present well-differentiated chemical profiles. The essential oil of P. aduncum is basically constituted of one major compound. Essential oil of P. arboreum consists of several chemical constituents; and the essential oil of P. marginatum is predominantly from one chemical class, the phenylpropanoids. The essential oil of P. aduncum is a natural source of phenylpropanoid dilapiol and has several biological activities, mainly as an insecticide, given its high content of dilapiol. Essential oil of P. arboreum showed antimicrobial activity and sesquiterpene curcumene was identified as the major compound. P. marginatum is a medicinal plant commonly used in the form of tea against diseases of the gastrointestinal tract; the phenylpropanoids E-asarone and Z-asarone are considered one of the chemical markers of their essential oil. Additionally, the antimicrobial activity oils and their organic fractions obtained were evaluated against the microorganisms Enterococcus faecalis, Bacillus subtilis, Staphylococcus aureus, Klesiella pneumoniae, Pseudomonas aeruginosa and Microsporum gypseum.

MATERIALS AND METHODS

Botanical material

For the proposed study, were selected three Piper species: P. arboreum, P. marginatum and P. aduncum. Leaves from the Piper species were collected from a fragment of forest located in the city of Recife, state of Pernambuco, Brazil. These species were identified by Dr. Margareth F. de Sales of the Department of Biology of the Federal Rural University of Pernambuco. A voucher of each species had been previously deposited in the Vasconcelos Sobrinho Herbarium of UFRPE with the numbers 18179, 48210 and 49250.

Obtaining of essential oils

To obtain each oil sample, it was used 500 g of fresh leaves from P. arboreum, P. marginatum and P. aduncum. Leaves were crushed and submitted to hydrodistillation in a modified Clevenger apparatus for 2 hours with addition of 5 mL of each of the following organic solvents: hexane, chloroform, ethyl acetate, and ethyl ether in Clevenger apparatus as illustrated in Figure 1. The samples of essential oils were treated with anhydrous sodium sulfate and stored at around 8 °C for further analysis. The organic fractions collected were dried under vacuum at 40 °C.
Figure 1. Design of the modified Clevenger device used to obtain and fractionate essential oils simultaneously. Water decoction (A); hexane or ethyl acetate or ethyl ether (B); hydrolate (C); chloroform or dichloromethane (D).

**GC Analysis**

Oil samples were analyzed using a Hewlett-Packard 5890 Series II GC apparatus equipped with a flame ionization detector (FID) and a J & W Scientific DB-5 fused silica capillary column (30 m × 0.25 mm i.d.), with a programmed temperature from 60 to 246 ºC at 3 ºC/min. The injector and detector temperatures were 260 and 280 ºC, respectively. Hydrogen was used as the carrier gas at a flow rate of 1.0 mL/min; injection was in split mode (1:30) and the injection volume was 1.0 µL.

**Chemical analysis**

Oil samples were analyzed using a Varian GC/MS (GC: Varian 431/GC-MS: Varian 220-MS, ionic trap detector) system operating in the EI mode at 70 eV, equipped with a J & W Scientific DB-5 fused silica capillary column (30 m × 0.25 mm i.d.), with programmed temperatures from 60 to 246 ºC by 3 ºC/min. The injector and detector temperatures were 260 and 280 ºC, respectively. The carrier gas was helium, 1 mL/min flow rate, split mode (1:30), with an injected volume of 1.0 µL of a solution containing 3 mg mL⁻¹ of oil in hexane. The initial identification of the separated components of the essential oil was carried out by comparison with previously reported values of retention indices, obtained by co-injection of oil samples and C11–C24 linear hydrocarbons and calculated using the Van den Dool & Kratz equation. Subsequently, the MS acquired for each component was matched with those stored in the Wiley/NBS mass spectral library of the GC–MS system and with other published mass spectral data. All the analyses were carried out in triplicate.

**Antimicrobial activity**

Essential oil samples were used to evaluate the antimicrobial activity for the bacteria *Staphylococcus aureus* (02), *Enterococcus faecalis* (138), *Bacillus subtilis* (86), *Klesiella pneumoniae* (396), *Pseudomonas aeruginosa* (416) and *Microsporum gypseum*. Saubouraud liquid culture media were used for fungi and a Mueller Hinton liquid medium for bacteria. The microplates were grown at 37 ºC for 24 h for bacteria and at 30 ºC for 72 h for fungi. The microplates were developed with the addition of 10 µL of a 0.01% resazurin solution and incubated for 3 h. The minimum inhibitory concentration (MIC) values of each sample were determined according to the previously reported protocol. Metronidazole and Fluconazole were used as the positive control and organic solvents as the negative control.
RESULTS AND DISCUSSION

With the proposed method, essential oils were obtained from the leaves of *P. aduncum* with different chemical profiles without performing additional steps to obtain the oil. Simultaneous extraction and fractionation allowed obtaining the essential oil from *P. aduncum* leaves with only two chemical constituents: dillapiole (96.9%) and myristicin (0.9%) when hexane (AdHex) was used for fractionation (Table I). Essential oil obtained by fractionation with chloroform (AdChl) showed, in addition to the dillapiole, the compounds δ-cadinene (1.3%), α-amorphene (4.0%), myristicin (1.2%), germacrene B (0.8%) and 9-epi-(E)-caryophyllene (2.2%). A surprising result was observed for the oil fractionated with ethyl ether (AdEte), where oleic acid was identified as the major compound (64.4%) and the dillapiole content only 4.6%. The essential oil that presented the highest number of compounds had been fractionated with ethyl acetate (AdOEt). In this fraction were identified nine chemical constituents and dillapiole (68.2%) was obtained as the major compound. Previous studies with the essential oil of *P. aduncum* leaves revealed the presence of dillapiole to a maximum content of 90%. Variation from 4.6 to 96.9% in dillapiole content in essential oils of *P. aduncum* species collected at the same location has not been reported, not even in studies of seasonality and circadian rhythm.

The chemical profiles of essential oils from *P. arboreum* leaves fractionated with the solvent chloroform (ArChl) and hexane (ArHex) were qualitatively and quantitatively different when compared with the unfraccionated oil (Figure 2). Cadinol sesquiterpenoid was identified as the major compound in the ArChl and ArHex oils in amounts of 57.9% and 55.0%, respectively (Table I). Cadinol content was identified in the unfraccionated oil at 17.7%; the major compound was γ-cadinene, with 23.4%. In previous studies with essential oil from *P. arboreum* leaves, the major compounds identified were the terpenes curcumene, δ-cadinene, β-caryophyllene, germacrene D and bicyclogermacrene, with cadinol identified only as a minor compound.
### Table I. Chemical constitutents of essential oils fractionated and unfractionated from *P. arboreum, P. aduncum* and *P. marginatum* leaves

| Compounds         | IR1 | IR2 | *P. arboreum oils* | *P. aduncum oils* | *P. marginatum oils* |
|-------------------|-----|-----|---------------------|-------------------|----------------------|
|                   |     |     | Ar     | ArChl | ArHex | Ad  | AdChl | AdHex | AdEte | AdOEt | Ma  | MaHex | MaDic1 | MaEte | MaOEt | MaDic |
| α-Pinene          | 932 | 925 | -      | -     | -     | -   | -     | -     | -     | -     | 3.4 | -      | -       | -     | -     | -     |
| β-Pinene          | 974 | 975 | -      | -     | -     | -   | -     | -     | -     | -     | 3.8 | -      | -       | -     | -     | -     |
| δ-3-Carene        | 1008| 1008| -      | 7.4   | -     | -   | -     | -     | -     | -     | -   | -      | -       | -     | -     | -     |
| 1.4-Cineole       | 1014| 1014| -      | 5.8   | -     | -   | -     | -     | -     | -     | 6.8 | -      | -       | -     | -     | -     |
| β-Phellandrene    | 1029| 1029| -      | -     | -     | -   | -     | -     | -     | -     | 6.8 | -      | -       | -     | -     | -     |
| E-ocimene         | 1049| 1049| -      | -     | -     | -   | -     | -     | -     | -     | 2.6 | -      | -       | -     | -     | -     |
| β-Z-ocimene       | 1226| 1226| -      | -     | -     | -   | -     | -     | -     | -     | 0.9 | -      | -       | -     | -     | -     |
| Carvona           | 1238| 1238| -      | -     | -     | -   | -     | -     | -     | -     | 4.3 | -      | -       | -     | -     | -     |
| β-E-ocimene       | 1235| 1235| -      | -     | -     | -   | -     | -     | -     | -     | 0.2 | -      | -       | -     | -     | -     |
| Phenylacetaldehyde| 1036| 1036| -      | -     | -     | -   | -     | -     | -     | -     | 2.4 | -      | -       | -     | -     | -     |
| δ-Elemene         | 1335| 1338| -      | -     | -     | -   | -     | -     | -     | -     | 1   | -      | -       | -     | -     | -     |
| isocededene       | 1374| 1374| 4.2    | -     | -     | -   | -     | -     | -     | -     | -   | -      | -       | -     | -     | -     |
| α-Copaene         | 1374| 1375| 3.1    | -     | -     | -   | -     | -     | -     | -     | -   | -      | -       | -     | -     | -     |
| Z-Caryophyllene   | 1408| 1409| 9.3    | -     | -     | -   | -     | -     | -     | -     | 2.3 | -      | -       | -     | -     | -     |
| Aromadendrene     | 1439| 1439| -      | -     | -     | -   | -     | -     | -     | -     | 2.1 | 13.1   | 7       | 1     | 1.2   | 0.7   |
| cis-Prenyl limonene| 1443| 1445| -      | -     | -     | -   | -     | -     | -     | -     | 0.8 | -      | -       | -     | -     | -     |
| α-Himachalene;    | 1449| 1450| 4.17   | -     | -     | -   | -     | -     | -     | -     | 2.1 | -      | -       | -     | -     | -     |
| α-Humulene        | 1452| 1452| 4.6    | -     | -     | -   | -     | -     | -     | -     | 3.5 | 17.5   | -       | 2.6   | 1.2   | -     |
| Croweacin         | 1457| 1458| -      | -     | -     | -   | -     | -     | -     | -     | 3.5 | 17.5   | -       | 2.6   | 1.2   | -     |
| 9-epi-(E)-Caryophyllene | 1464| 1465| 5.6    | -     | -     | -   | 2.2   | -     | -     | -     | 0.8 | 7.8    | 3.6     | -     | -     | -     |

(continues on the next page)
Table I. Chemical constituents of essential oils fractionated and unfractionated from *P. arboreum*, *P. aduncum* and *P. marginatum* leaves (cont.)

| Compounds          | IR¹   | IR²   | *P. arboreum oils* | *P. aduncum oils* | *P. marginatum oils* |
|--------------------|-------|-------|--------------------|-------------------|----------------------|
|                    |       |       | Ar     | Chl   | Hex | Ar     | Chl   | Hex | Ete | OEt | Ma | Hex | Dic1 | Ete | OEt | Dic |
| γ-Muurolene        | 1478  | 1479  | 1.3    | -     | -   | -     | -     | -   | -   | -   | -  | -   | -    | -   | -   | -   |
| γ-Himachalene      | 1481  | 1482  | 3.1    | 5.9   | -   | -     | -     | -   | -   | -   | -  | -   | -    | -   | -   | -   |
| α-Amophene         | 1483  | 1487  | 4.0    | -     | -   | -     | -     | -   | -   | -   | -  | -   | -    | -   | -   | -   |
| Methyl isoeugenol  | 1491  | 1493  | 4.9    | 8.19  | -   | -     | -     | -   | -   | -   | -  | -   | -    | -   | -   | -   |
| cis-cadina-1,4-diene| 1495  | 1495  | 4.9    | -     | -   | -     | -     | -   | -   | -   | -  | -   | -    | -   | -   | -   |
| Valencene          | 1496  | 1497  | 4.9    | 8.19  | -   | -     | -     | -   | -   | -   | -  | -   | -    | -   | -   | -   |
| Viridiflorene      | 1496  | 1496  | 4.9    | 8.19  | -   | -     | -     | -   | -   | -   | -  | -   | -    | -   | -   | -   |
| Epizonarene        | 1501  | 1502  | 4.9    | 8.19  | -   | -     | -     | -   | -   | -   | -  | -   | -    | -   | -   | -   |
| γ-Cadinene         | 1513  | 1513  | 23.4   | 0.5   | 2.0| -     | -     | -   | -   | -   | -  | -   | -    | -   | -   | -   |
| Myristicin         | 1517  | 1518  | 1.0    | 1.2   | 0.9| -     | -     | -   | -   | -   | -  | -   | -    | -   | -   | -   |
| δ-Cadinene         | 1522  | 1522  | 1.2    | 1.3   | -   | -     | -     | -   | -   | -   | -  | -   | -    | -   | -   | -   |
| Nerolidol          | 1561  | 1561  | 4.7    | 1.8   | 1.2| 20.1  | 2.2   | -   | -   | -   | -  | 0.3 | -    | -   | -   | -   |
| Spathulenol        | 1577  | 1579  | 8.0    | 14.0  | -   | -     | -     | -   | -   | -   | -  | -   | -    | -   | -   | -   |
| Germacrene B       | 1559  | 1560  | 0.8    | -     | -   | -     | -     | -   | -   | -   | -  | -   | -    | -   | -   | -   |
| Carotol            | 1594  | 1597  | 1.9    | -     | -   | -     | -     | -   | -   | -   | -  | -   | -    | -   | -   | -   |
| Guaiol             | 1600  | 1601  | 0.5    | -     | -   | -     | -     | -   | -   | -   | -  | 10.0| 1.7  | -   | -   | -   |
| Z-asarone          | 1616  | 1616  | 18.2   | 19.3  | 25.4| 22.4  | 26.4  | 1.2 | -   | -   | -  | -   | -    | -   | -   | -   |
| Dillapiole         | 1620  | 1622  | 13.0   | 14.8  | 19.5| 17.3  | 9.1   | 0.6 | -   | -   | -  | -   | -    | -   | -   | -   |
| Cadinol            | 1652  | 1651  | 17.7   | 57.9  | 55.0| -     | -     | -   | -   | -   | -  | -   | -    | -   | -   | -   |
| E-asarone          | 1675  | 1676  | 4.7    | 4.7   | 11.5| 10.3  | 7.5   | 0.5 | -   | -   | -  | -   | -    | -   | -   | -   |

(continues on the next page)
Table I. Chemical constituents of essential oils fractionated and unfracti onated from *P. arboreum*, *P. aduncum* and *P. marginatum* leaves (cont.)

| Compounds          | IR\(^a\) | IR\(^b\) | \(P. arboreum\) oils | \(P. aduncum\) oils | \(P. marginatum\) oils |
|--------------------|----------|----------|------------------------|----------------------|------------------------|
|                    |          |          | Ar  | ArChl | ArHex | Ad  | AdChl | AdHex | AdEte | AdOEt | Ma  | MaHex | MaDic1 | MaEte | MaOEt | MaDic |
| Geranyl linalol     | 1960     | 1963     | -   | -     | 0.7   | -   | -     | 0.4   | -     | -     | -   | -     | -      | -     | -     | -     |
| Octadecanol        | 2077     | 2074     | -   | -     | 0.6   | -   | -     | 2.5   | -     | -     | -   | -     | -      | -     | -     | -     |
| Methyl linoleate    | 2095     | 2094     | -   | -     | 64.4  | -   | -     | 11.4  | -     | -     | -   | -     | -      | -     | -     | -     |
| Linoleic acid      | 2132     | 2130     | -   | -     | 9.1   | -   | -     | 11.1  | -     | -     | -   | -     | -      | -     | -     | -     |
| Oleic acid         | 2141     | 2144     | -   | -     | -     | -   | -     | 3.8   | -     | -     | -   | -     | -      | -     | -     | -     |
| Tetracosane        | 2400     | 2404     | -   | -     | -     | -   | -     | -     | -     | -     | -   | -     | -      | -     | -     | -     |
| Pentacosane        | 2500     | 2509     | -   | -     | -     | -   | -     | -     | -     | -     | -   | -     | -      | -     | -     | -     |
| Fatty acids        | -        | -        | -   | -     | -     | -   | -     | -     | -     | -     | -   | -     | -      | -     | -     | -     |
| Total (%)          | 91.1     | 91.0     | 87.1| 93.2  | 90.2  | 97.8| 93.7  | 90.4  | 76.7  | 78.1  | 85.3| 70.6  | 80.7   | 71.9  |        |        |
| Oil yields (%)     | 0.02     | 0.01     | 0.03| 0.20  | 0.09  | 0.11| 0.12  | 0.12  | 0.33  | 0.23  | 0.11| 0.31  | 0.14   | 0.08  |        |        |

\(^a\)Retention indices from the literature; \(^b\)Retention indices calculated; *P. arboreum* essential oil unfracionated, (Ar) chloroform (ArChl), hexane (ArHex) fractions; *P. aduncum* essential oil unfracionated (Ad), chloroform (AdChl), hexane (AdHex), ethyl (AdEte), ethyl acetate (AdOEt) fractions; *P. marginatum* essential oil unfracionated (Ma), dichloromethane (MaDic1) hexane (MaHex), ethyl (MaEte), ethyl acetate-dichloromethane (MaOEt-MaDic) fractions.
Obtaining and fractioning the essential oil from *P. marginatum* leaves was carried out with a three-phase solvent system composed of dichloromethane-water-ethyl acetate, with two chemical profiles of the oil obtained simultaneously (Figure 3). In the ethyl acetate fraction were identified four major compounds: phenylpropanoids Z-asarone (26.4%) and dillapiole (9.1%) as well as sesquiterpenes nerolidol (20.1%) and guaiol (10.0%). The dichloromethane fraction showed major peaks related to hydrocarbons and long chain fatty acids. The fractionation of the essential oil from the leaves of *P. marginatum* using ethyl ether, hexane or dichloromethane separately were also obtained, enabling identification, in addition to the phenylpropanoids Z-asarone, dillapiole, croweacin and *E*-asarone, the sesquiterpenes aromadendrene and himachalene as the major constituents. The chemistry of *P. marginatum* essential oil is well known and more than seven different chemotypes have been reported for the plant. Previous studies with essential oil obtained from the leaves of allopatric species of *P. marginatum* have revealed a great variation in chemical composition: anethole (45.9%), isosafrole (37.3%), *E*-asarone (32.6%), Z-asarone (30.4%), anisaldehyde (22.0%) and notosirnol (22.7%).
In general, the method allowed obtaining different chemical profiles of the essential oils of the leaves of *P. aduncum*, *P. arboreum*, and *P. marginatum*, using only the Clevenger apparatus. This is the main difference between our method and those previously reported: organic solvents were added to the Clevenger apparatus as the stationary phase of a chromatographic column and condensed water together with the plant volatiles, as the mobile phase. Additionally, all fractionated and unfractionated essential oils showed antimicrobial activity against the six microorganisms tested with MIC values ranging from 19.5 to 2500 µg mL\(^{-1}\) (Table II). The best results were observed for *P. marginatum* oils fractionated with ethyl acetate (MaOEt) and ethyl ether (MaEte) which presented MIC of 19.5 and 78.1 µg mL\(^{-1}\) against *P. aeruginosa* bacteria. However, *P. marginatum* oil fractionated with dichloromethane exhibited an MIC of 2500 µg mL\(^{-1}\); the phenylpropanoids present in MaOEt and MaEte oils were identified in the dichloromethane fraction as minor compounds. The essential oils of *P. aduncum* were the least active against the microorganisms tested. Essential oil containing dillapiole (68.4%) exhibited lower MIC of 625, 625 and 312.5 µg mL\(^{-1}\) against the microorganism *E. faecalis*, *K. pneumoniae* and *M. gypseum*, respectively, indicating the influence of dillapiole content on the antimicrobial activity of the oil. The antimicrobial activity of the essential oil of *P. arboreum* also varied when the oil was fractionated. ArChl oil had the highest cadinol content, being the least active when compared to the Ar and ArHex oils, except for the bacteria *S. aureus* where ArChl oil exhibited a MIC of 312.5 µg mL\(^{-1}\).
### Table II. Antimicrobial activity with MIC values in µg mL⁻¹ of fractionated and unfractionated essential oils from leaves of *P. arboreum*, *P. aduncum* and *P. marginatum*

| Oil Essential Samples | Bacteria |        |        |        |        |        | Fungi       |
|-----------------------|----------|--------|--------|--------|--------|--------|-------------|
|                       |          | *E. faecalis* | *K. pneumoniae* | *S. aureus* | *B. subtilis* | *P. aeruginosa* | *M. gypseum* |
| Ma                    | 1250     | 1250   | 1250   | 2500   | 19.5   | 1250   |             |
| MaOEt                 | 312.5    | 312.5  | 2500   | 1250   | 19.5   | 625    |             |
| MaDic                 | 1250     | 1250   | 2500   | > 2500 | 2500   | 2500   |             |
| MaHex                 | 312.5    | 312.5  | 625    | 1250   | 321.5  | 312.5  |             |
| MaEte                 | 1250     | 1250   | 2500   | 2500   | 78.1   | 2500   |             |
| MaDic1                | 625      | 1259   | 1250   | 1250   | 312.5  | 1250   |             |
| Ad                    | 2500     | 2500   | 2500   | 2500   | 2500   | 2500   |             |
| AdOEt                 | 625      | 625    | 625    | 1250   | 1250   | 312.5  |             |
| AdEte                 | 2500     | 2500   | 1250   | 2500   | 2500   | 2500   |             |
| AdHex                 | 2500     | 2500   | 1250   | 2500   | 1250   | na     |             |
| AdChl                 | 2500     | 2500   | 1250   | 2500   | 2500   | na     |             |
| Ar                    | 625      | 312.5  | 625    | 625    | 1250   | na     |             |
| ArChl                 | 2500     | 2500   | 312.5  | 2500   | 1250   | na     |             |
| ArHex                 | 625      | 1250   | 625    | 625    | 1250   | na     |             |
| Metronidazole<sup>a</sup> | 156.3 | 19.5   | 78.1   | 2500   | 19.5   | na     |             |
| Fluconazole<sup>a</sup> | na<sup>b</sup> | na | na | na | na | 19.5 |             |

<sup>a</sup>Positive controller; <sup>b</sup>not evaluated

### CONCLUSIONS

The model developed made it possible to obtain essential oils with unidentified chemical constituents in unfractionated oils, with a significant variation in the contents of the major compounds. Most of the time, because essential oils consist of a complex mixture of volatile compounds, these are difficult to distinguish.

It is not always possible to carry out a liquid-liquid fractionation of essential oils, after obtaining them by hydrodistillation, due to the low concentration of the constituents in the hydrolate and the immiscibility of most of the oil in an aqueous medium. In the present study, it was possible to obtain and fractionate the essential oils simultaneously, without adding a fractionation step, using a small amount of solvent, in a quick and simple way.

The method is applicable to biomonitored chemical studies of essential oils with identification of minor compounds and for association of different chemical profiles with the biological activity of the oil.

### Conflicts of interest

No potential conflict of interest was reported by the authors.

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