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Technical Report

# Characterization of the volatile fraction of Brazilian essential oil, namely *Cordia verbenaceae*

## SPME followed by GC-qMS for volatile charcaterization of *Cordia verbenaceae*

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## Abstract:

Essential oil of a Brazilian medical plant, *Cordia verbenaceae* L., was characterized by means of GC-MS technique. The volatile compounds were identified using the FFNSC MS library, a flavor and fragrance database with embedded linear retention index (LRI). Using two simultaneous filters, namely ≥ 90% MS similarity and ±5 LRI, 48 individual molecules were identified, providing a very fast and reliable peak assignment in the identification process.


**Keywords: GC-MS, Flavour and Fragrances Natural and Synthetic Compounds (FFNSC) MS spectral library**

## 1. Introduction

Essential oils (EOs) are high-value ingredients extracted from plants. EOs are widely used in cosmetics and foodstuffs since they present flavouring and preservative properties. Medical and antimicrobial effects have also been highlighted in some kind of EOs. It is well-known that plants have been palying important roles in many traditional medicines, among which the Brazilian traditional medicine. In particular the attention has been focused on a plant of the genus *Cordia*, belonging to the Boraginaceae family, for which many positive effects has been reported. The genus *Cordia* includes about 250 species, which are are mainly tree- or shrub-sized distributed in tropical and subtropical areas in Central and South America, India, Asia, and Africa. In particular, *Cordia verbenaceae* L., known popularly as "erva baleeira" is typical from the Americas and can be found in the costal region of Brazil, in Central America and Argentina (Fig. 1). The EO extracted from the leaves has important antioxidant and pharmacological activities and they are used in phytotherapy for its antimicrobial, antiinflammatory, antirheumatic, analgesic, tonic properties and in arthritis treatment [1–7]. In the volatile fraction of the EO extracted from leaves, two sesquiterpene compounds, namely $\alpha$-humulene and trans-caryophyllene, have been reported to be responsible for the main anti-inflammatory effects of *C. verbenaceae* EO [8]. The EO extracted from *Cordia verbenaceae* L. has been characterized and relative quantify using GC-MS and a MS database containing LRI information, namely the Flavour and Fragrances Natural and Synthetic Compounds (FFNSC) MS spectral library.

The intense fragmentation and the high reproducibility of the mass spectra generated with the electron impact ionization are of great support for a reliable identification of complex mixture of compounds. However, although the major EO components can be usually identified with MS spectral comparison and literature data, this procedure can fail for some substances, like sesquiterpenes, due to a high similarity in mass fragmentation, leading to misidentification of individual components. In this case, the use of the Linear Retention Index (LRI) information, associated to the MS similarity is of great support for a reliable peak assignment [9].

Fig. 1 *Cordia verbenaceae* plant.

## 2. Experimental

### 2-1. Reagents, materials, and sample preparation

The *Cordia verbenaceae* leaf EO was extracted with hydrodistillation using Clevenger apparatus. Hexane (purity > 97 %) was used to dilute the samples. For the calculation of the LRI a C7–C30 saturated n-alkane series (49451-U SUPELCO) was used. For quantification nonane was used as internal standard at 100 mg/mL.

Every sample was injected in two different concentrations three times, to maximise the number of the identifiable compounds.

### 2-2. Instrumentation (Shimadzu)

- GC-2010 gas chromatograph
- GCMS-QP2010 Ultra

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## 2-3. Chromatographic method

| | |
|---|---|
| Column | : SLB-5ms 30 m × 0.25 mm ID × 0.25 µm $d_f$ column [silphenylene polymer virtually equivalent in polarity to poly (5% diphenyl/95% methylsiloxane)] (Supelco, Milan, Italy) |
| GC oven | : from 50°C to 280°C (5 min) at 3°C/min |
| Carrier gas | : Helium |
| Linear velocity | : 30 cm/s (constant mode) |
| Injection | : split 1:50 |
| Injection temperature | : 280°C |

| MS parameters | | FID parameters | |
|---|---|---|---|
| MS ionization mode | : EI | FID temperature | : 300°C |
| Acquisition frequency | : 33 Hz | Hydrogen flow | : 40 mL/min |
| Mass range | : 45–360 *m/z* | Air flow | : 400 mL/min |
| Ion source temperature | : 220°C | Make up ($N_2$) flow | : 40 mL/min |
| Interface temperature | : 250°C | | |

## 2-4. Software

GCMSsolution software Ver. 4.20; GCsolution Ver. 2.43

MS Database: FFNSC 3

## 3. Results and discussion

Qualitative and quantitative (relative) characterization of a *C. verbenaceae* EO was carried out by means of GC-MS and GC-FID, respectively. The GC-MS volatile fraction profile of the EO samples is shown on Fig. 2.

Using the FFNSC Library, as main reference database, all integrated peak of the chromatogram can be identified automatically by GCMS solution setting 2 different filters, namely the MS spectral similarity (90%) and the LRI (±5 LRI units). Applying only the MS spectral similarity, even over 90%, many candidates were listed for the target molecule, as shown Fig. 3. While, adding the LRI filter the number of possibile candidates can be reduced, in most cases to a uniqe match (Fig. 4). The use of such a tool allows a very fast and reliable peak assignment during the identification process.

Overall in the *C. verbenaceae* EO 48 individual flavour and fragrance compounds were found. Only two peaks were identified with the support of a NIST11 library, namely bergamotal <alpha-(E)-trans> and santalal <alpha-(E)>, as shown in Table 1. These compounds will be isolated and fully characterized to be included in the FFNSC Library, thus assuring a constant update of the database. The major constituent of the *C. verbenaceae* oil was the monoterpene $\alpha$-pinene (35%) besides the sesquiterpenes as $\alpha$-santalene (15%), trans-9-epi-caryophyllene (13%) and $\delta$-cadinene (4%). Other important minor components were also found between 2 and 4%, namely calarene, $\alpha$-thujene, eucalyptol, caryophyllene oxide and $\alpha$-humulene. Presumably due to the significant seasonal variation of the volatile fraction composition and the biological activity, the results only partially correspond with the literature data, where the main compounds were found $\alpha$-pinene (6–16%), $\beta$-phellandrene (3–11%), sabinene (16–70%), $\gamma$-elemene (6–13%), $\delta$-elemene (3–13%), bicyclogermacrene (3–11%), $\beta$-caryophyllene (6–25%), $\gamma$-caryophyllene (5–16%), $\delta$-cadinene (2–9%) and germacrene B (3–14%) [10, 11].

Table 1 Identified compounds of *Cordia verbenaceae* essential oil

| | Compound | $LRI_{FFNSC}$ | $LRI_{exp}$ | ΔLRI | MS % | Area % |
|---|---|---|---|---|---|---|
| 1 | Thujene <alpha-> | 927 | 925 | 2 | 97 | 2.46 |
| 2 | Pinene <alpha-> | 933 | 934 | –1 | 97 | 35.1 |
| 3 | Camphene | 954 | 951 | 3 | 96 | 0.14 |
| 4 | Thuja-2,4(10)-diene | 953 | 954 | –1 | 95 | 0.04 |
| 5 | Sabinene | 972 | 972 | 0 | 97 | 0.55 |
| 6 | Pinene <beta-> | 979 | 978 | 1 | 95 | 0.25 |
| 7 | Myrcene | 991 | 989 | 2 | 92 | 0.12 |
| 8 | Cymene <para-> | 1025 | 1025 | 0 | 96 | 0.21 |
| 9 | Limonene | 1028 | 1030 | –2 | 92 | 0.11 |
| 10 | Eucalyptol | 1032 | 1032 | 0 | 90 | 2.3 |
| 11 | Terpinene <gamma-> | 1058 | 1059 | –1 | 93 | 0.08 |
| 12 | Sabinene hydrate <cis-> | 1069 | 1071 | –2 | 91 | 0.08 |
| 13 | Sabinene hydrate <trans-> | 1099 | 1102 | –3 | 90 | 0.09 |
| 14 | Pinocarveol <trans-> | 1141 | 1142 | –1 | 95 | 0.44 |
| 15 | Verbenol <trans-> | 1145 | 1147 | –2 | 91 | 0.17 |
| 16 | Pinocarvone | 1164 | 1164 | 0 | 91 | 0.11 |
| 17 | Terpinen-4-ol | 1184 | 1182 | 2 | 91 | 0.43 |
| 18 | Bornyl acetate | 1285 | 1285 | 0 | 95 | 0.48 |
| 19 | Cyclosativene | 1367 | 1370 | –3 | 93 | 0.07 |
| 20 | Copaene <alpha-> | 1375 | 1378 | –3 | 92 | 1.57 |
| 21 | Bourbonene <beta-> | 1382 | 1384 | –2 | 92 | 0.24 |
| 22 | Sesquithujene <7-epi-> | 1387 | 1388 | –1 | 93 | 0.53 |
| 23 | Elemene <beta-> | 1390 | 1391 | –1 | 92 | * |
| 24 | Funebrene <alpha-> | 1403 | 1403 | 0 | 98 | 0.19 |
| 25 | Bergamotene <alpha-, cis-> | 1416 | 1415 | 1 | 97 | 0.55 |
| 26 | Santalene <alpha-> | 1418 | 1421 | –3 | 95 | 13.21 |
| 27 | Caryophyllene <(E)-> | 1424 | 1422 | 2 | 94 | * |
| 28 | Calarene | 1434 | 1434 | 0 | 91 | 3.65 |
| 29 | Sesquisabinene | 1455 | 1455 | 0 | 98 | 0.25 |
| 30 | Humulene <alpha-> | 1454 | 1457 | –3 | 97 | 2.17 |
| 31 | Caryophyllene <9-epi-(E)-> | 1464 | 1464 | 0 | 97 | 14.82 |
| 32 | Germacrene D | 1480 | 1483 | –3 | 92 | 1.03 |
| 33 | Bergamotene <beta-, trans-> | 1483 | 1485 | –2 | 95 | 0.1 |
| 34 | Selinene <beta-> | 1492 | 1491 | 1 | 95 | 0.21 |
| 35 | Bicyclogermacrene | 1497 | 1498 | –1 | 95 | 0.85 |
| 36 | Muurolene <alpha-> | 1497 | 1500 | –3 | 95 | 0.15 |
| 37 | Bisabolene <beta-> | 1508 | 1509 | –1 | 95 | 0.21 |
| 38 | Cubebol | 1518 | 1519 | –1 | 93 | 0.12 |
| 39 | Cadinene <delta-> | 1518 | 1521 | –3 | 93 | 4.19 |
| 40 | Sesquiphellandrene <beta-> | 1523 | 1525 | –2 | 93 | 0.08 |
| 41 | Bisabolene <(E)-, gamma-> | 1528 | 1528 | 0 | 93 | 0.05 |
| 42 | Spathulenol | 1576 | 1579 | –3 | 90 | 1.73 |
| 43 | Caryophyllene oxide | 1587 | 1585 | 2 | 90 | 2.18 |
| 44 | Humulene epoxide II | 1613 | 1614 | –1 | 94 | 0.32 |
| 45 | Bergamotal <alpha-(E)-trans> | — | 1673 | | 93 | 0.49 |
| 46 | Santalol <(Z)-alpha-> | 1676 | 1678 | –2 | 95 | 0.17 |
| 47 | Santalal <alpha-(E)> | 1679 | 1682 | –3 | 92 | 0.63 |
| 48 | Santalol acetate<(Z)-alpha-> | 1778 | 1776 | 2 | 94 | 0.06 |

* coeluted with previous peak

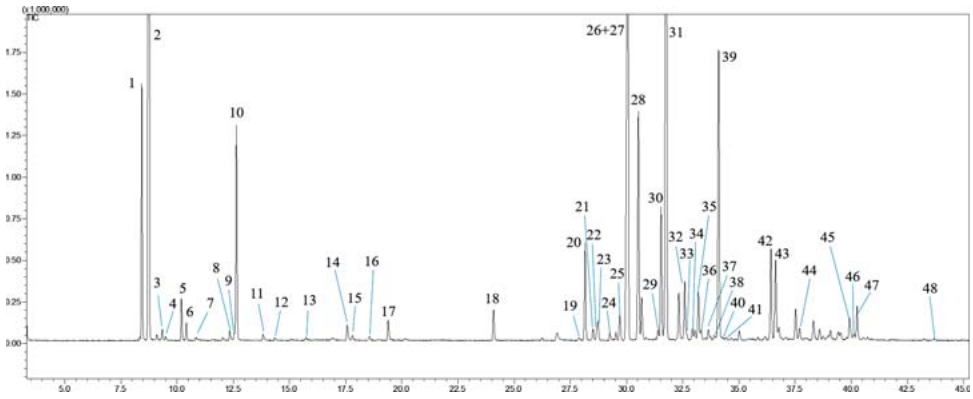

Fig. 2 GC-MS chromatogram of *Cordia verbenaceae* essential oil. Peak identity as reported in Table 1.

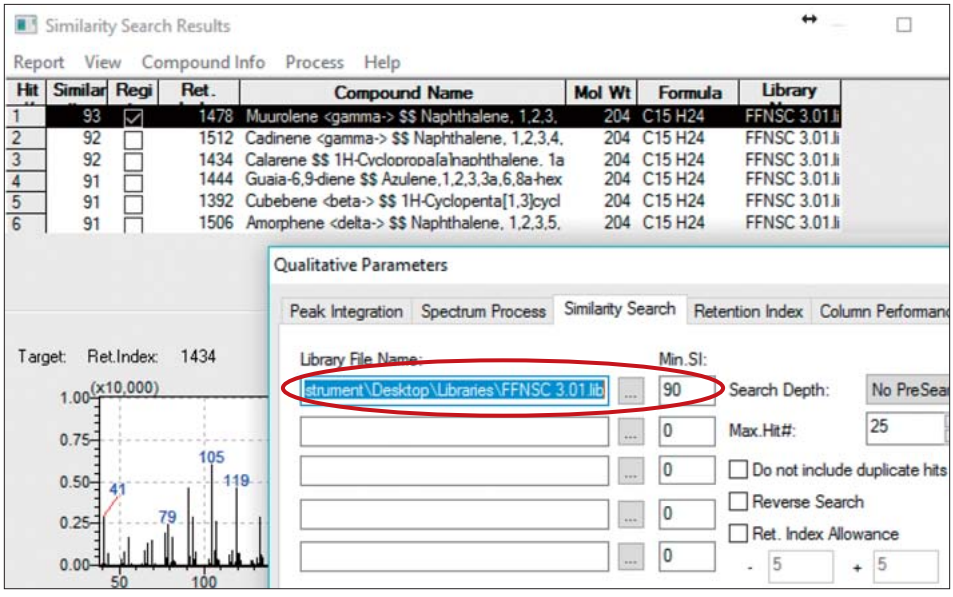


Fig. 3 Identification candidates obtained using only MS similarity filter automatically in GCMSsolution.

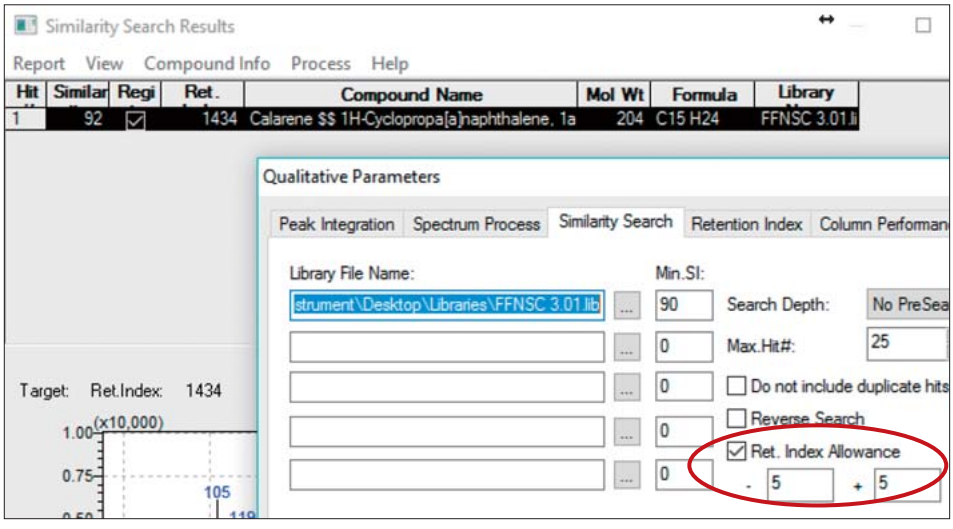


Fig. 4 Unique candidate obtained using MS similarity and LRI filters automatically in GCMSsolution.

## 4. Conclusions

Essential oil extracted from *Cordia verbenaceae* L. leaves was analysed by GC-MS. The support of an MS database (FFNSC Library), dedicated to the flavour and fragrance field, which includes LRI calculated under specific chromatographic conditions (fully reported) and using three different stationary phases, provided the rapid and reliable determination of the greater part of the main components using the MS similarity match over 90% and a ±5 LRI range, simultaneously.

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