

## RESEARCH ARTICLE

# Characterization of volatile and odor-active compounds of the essential oil from *Bidens graveolens* Mart. (Asteraceae)\*

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## Abstract

During a bioprospection study in the Brazilian Cerrado, samples of *Bidens graveolens* Mart., an endemic species, were investigated. Chemical and olfactive analyses were performed on the essential oil obtained from fresh leaves of *B. graveolens* collected in November 2013 (BG1) and May 2014 (BG2). A total of 60 compounds were identified in the essential oil extracted from fresh leaves, by hydrodistillation. The main constituents were identified as monoterpene hydrocarbons, ranging from 86.5% to 89.6%. Limonene was the major compound, accounting for 22.8% in BG1 and 47.7% in BG2. Thirty-three oxygenated monoterpenes were identified, comprising about 9% in both essential oils (EO). The attributes described by the panelists are in accordance to the sensorial profile. Myrcene, limonene, epoxy-myrcene, and limonene dioxide were rated by all panelists. Present at a frequency of 83.3%,  $\alpha$ -pinene,  $\beta$ -pinene,  $\alpha$ -pinene oxide, and carvone were picked out by five panelists. It is noteworthy that most compounds rated with the highest intensity score belonged to the oxygenated monoterpene class, considered the most expressive terpene class used in perfumery. In general, these compounds were described as presenting green, herb, spicy, fresh, and camphoraceous odors.

## KEYWORDS

*B. graveolens*, chemical composition, essential oil, GC-O, odor-active compounds

## 1 | INTRODUCTION

The Cerrado is the second major Brazilian biome, covering near 25% of the country's national territory. It is considered a biodiversity hotspot, *that is*, presenting high concentrations of endemic species under strong anthropic pressure.<sup>1,2</sup> Although over 12 000 plant species have been cataloged in this biome, studies regarding the identification of their volatile chemical compounds are still lacking.<sup>3</sup> To the best of our knowledge, except for *Eremanthus erythropappus* DC.

MacLeish (syn *Vanillosmopsis erythropappa*, Asteraceae), no other native Cerrado species are currently used for essential oil production on an industrial scale.

The *Bidens* genus belongs to the Asteraceae family and comprises approximately 240 species distributed throughout the Americas, Africa, Europe, and Asia.<sup>4–6</sup> Nineteen *Bidens* species are estimated to exist in Brazil, and at least six are endemic to the Cerrado biome.<sup>6,7</sup> The biological properties of essential oils from some *Bidens* have been investigated, including antimicrobial, antioxidant, antifungal, and cytotoxic activities.<sup>4,5,8–13</sup> The essential oils from *B. pilosa*, *B. frondosa*, and *B. tripartite* have been tested as

\*Part 9 in the series: Scents from the Brazilian Cerrado.

natural preservatives, intended to extend the shelf life of certain food products.<sup>4,5,8,13</sup>

A large bioprospection project concerning aromatic Cerrado species was conducted by Embrapa from 2012 to 2018. Over 300 samples from 134 different plant species were collected, and their essential oils were distilled and chemically and sensorially analyzed. The sensorial analyses were performed by a senior perfumist from Givaudan, an Embrapa partner in this enterprise, and some of the results have been published.<sup>3,14,15</sup> One of the assessed species in the project was *Bidens graveolens*. The first report concerning this plant appeared in 1983, in which a petroleum ether extract was investigated and sesquiterpenoids, including germacrene D and spathulenol, were the major detected compounds.<sup>16</sup> Regarding essential oils, a preliminary report was presented in a conference abstract by our research group regarding a sample collected in April 2012, in which  $\alpha$ -pinene and limonene were the major compounds identified.<sup>17</sup> To the best of our knowledge, no other reports on the chemical composition or odor characteristics of *B. graveolens* have been published. In this context, the aim of this study was to identify and quantify volatile compounds present in the essential oil (EO) obtained from *B. graveolens* leaves as well as carry out an odor analysis by olfactometry (GC-O).

## 2 | EXPERIMENTAL

### 2.1 | Standards

A mix of hydrocarbons ranging from  $n$ -C<sub>8</sub>-C<sub>28</sub>, obtained from Sigma-Aldrich (USA), was used to calculate the retention indices. The following compounds were used to measure the response factors:  $\alpha$ -pinene,  $\gamma$ -terpinene,  $p$ -cymene for monoterpene hydrocarbons; linalool, menthol, nerol, carvone, linalool oxide for oxygenated monoterpenes; benzyl alcohol for aromatic alcohols; geranyl acetate, menthyl acetate, for esters; (*E*)- $\beta$ -caryophyllene for sesquiterpene hydrocarbons and  $\alpha$ -bisabolol for oxygenated sesquiterpenes. All were purchased from Sigma-Aldrich and Fluka, at a purity degree of at least 95%. Analytical-grade hexane and dichloromethane were purchased from Tedia (Brazil) and Vetec Quimica Fina (Brazil).

### 2.2 | Plant material

*B. graveolens* Mart. leaves were collected from two populations ( $n > 5$  for each population) at Chapada dos Veadeiros, Goiás, Brazil, in November 2013 (BG1, 14°06'45"S, 47°38'53"W, 1279 m) and May 2014 (BG2, 13°57'04"S, 47°30'05"W, 1010 m). Both samples were collected during the flowering phase. Botanical identifications were performed by Dr. João Bernardo Bringel Junior, and voucher specimens were deposited at the Embrapa Genetic Resources and Biotechnology herbarium (CEN 88280 for BG1, and 88409 for BG2) in Brasília, DF, Brazil. All collection and analyses were authorized by the Brazilian Ministry of Environment (IBAMA process 02001.003166/2013-26).

### 2.3 | Hydrodistillation

Fresh BG1 and BG2 leaves (208.0 and 200.0 g, respectively) were subjected to hydrodistillation in the same day of the collection, using a modified Clevenger-type apparatus for 2 hours. After distillation, the EOs were collected, dried with anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>), and maintained at 8–10°C until analyses.

### 2.4 | Sensory analyses

The essential oil BG2 (2% in ethanol) was randomly evaluated on smelling paper strips by ten trained panelists (three men and seven women) during three consecutive days. The panelists were trained during 3 days to evaluate extracts and pure compounds, providing relevant vocabulary and correct olfactive families, defined internally. Panelists were asked to give an intensity score from 0 (no odor) to 4 (maximum intensity) for ten olfactive descriptors previously defined with the aid of perfumists: camphor, green, fruity, fresh, floral, citric, spicy, amber, and musk. These panelists also participated in the GC-O session.<sup>18,19</sup>

### 2.5 | Gas chromatography

#### 2.5.1 | Gas Chromatography GC(FID)

Analyses were performed using a GC(FID) 6890 (Agilent Technologies, Wilmington, NC, USA), with a double injection port, equipped with a DB-5 nonpolar column (30 m  $\times$  0.25 mm internal diameter, 0.25  $\mu$ m film thickness; Agilent). Next, 1.0  $\mu$ L of the diluted essential oils was injected in split mode (1:20), with the injector temperature maintained at 220°C. The oven temperature was programmed from 38 (3 min) to 180°C at 3°C/min and from 180 to 240°C at 20°C/min. Hydrogen was used as the carrier gas at a flow rate of 1.0 mL/min. The detector temperature was maintained at 280°C. The H<sub>2</sub>/air ratio was of 10%, and the airflow rate was of 400 mL/min.

Quantification was performed by internal standardization with the use of experimental relative response factors (RRF) and  $n$ -octadecane as an internal standard. Authentic standards, each representing a chemical class to be determined, were selected among those available at the laboratory. Three replicates of mixtures containing equal amounts of the internal standard ( $n$ -octadecane, 0.1 mg/mL) and the representative compounds diluted in dichloromethane were analyzed, at five concentrations. For each representative compound within a chemical class, a calibration curve, based on the internal standard (IS) to the reference analyte (C) peak area ratio vs. the internal standard (IS) to the reference analyte (C) concentration ratio was plotted. Whenever possible, more than one standard per class was used, and the RRF was calculated by the means of the slopes of all representative compounds.<sup>20–22</sup>

#### 2.5.2 | Gas Chromatography-Mass Spectrometry (GC-qMS)

Analyses were performed using a GC 6890 hyphenated to a MSD 5073N (Agilent Technologies) using the same column described in

the GC-FID section. Next, 1.0  $\mu\text{L}$  of the diluted EO was injected in split mode (1:20) and the injector temperature was maintained at 220°C. Oven temperature program varied from 60 to 180°C at 3°C/min and 180 to 240°C at 20 °C/min. The MS detection was performed in scan mode in the 40-450 Da range with temperatures set at 150°C and 230°C for the quadrupole and the EI source, respectively, with an electron multiplier voltage of 1145 V.

The analytes were identified by comparing of their mass spectra and retention indices ( $\pm 10$  units) to the Wiley Registry of Mass Spectral Data<sup>®</sup> (1994) or the NIST<sup>®</sup> database (2013), as well as literature data.<sup>23</sup>

### 2.5.3 | Gas Chromatography-Olfactometry (GC-O)

The EO BG2 was diluted to 10% (v/v) in hexane and injected into an Agilent 6890-5973N GC-MS system, fitted with a Gerstel ODP 2 olfactometric port. The analytical column and chromatographic conditions were the same as stated above. For the GC-O analysis, a Deans Switch (DS) G2855A (Agilent Technologies) was used to direct the flow to both the olfactometric port and the mass detector, using capillary columns without a stationary phase (0.90 m  $\times$  0.2 mm internal diameter and 1.16 m  $\times$  0.15 mm internal diameter, Agilent). The detection frequency method was employed and six trained panelists (three women and three men) participated in the olfactometry experiments. These panelists were familiar with sensorial analysis techniques and were previously trained using reference aromatic compounds. The EOs were randomly evaluated three times to ensure intermediate precision of the panel. The evaluation consisted of the detection of odor-active compounds by the assessors, accompanied by qualitative descriptions. The answers were only considered when given by the same panelist twice out of the three runs. Substances with over 50% of detection (more than three perceptions) were considered odorant compounds for the EO.<sup>18,19</sup> The sniffing procedure was divided into 12 min sessions with 10 min intervals, in order to avoid lassitude.

## 3 | RESULTS AND DISCUSSION

### 3.1 | Volatile composition

The essential oil yields, calculated on a dry basis, were 0.25% for both BG1 and BG2, similar to those reported in studies carried out with other plants of this genus.<sup>4,5,24,25</sup>

The EOs were analyzed by GC-MS and quantified by GC-FID using internal standards and RFFs. The RFFs values are presented in Table 1, while the volatile constituents identified and quantified in fresh leaf BG1 and BG2 EOs are displayed in Table 2, alongside the components listed according to their order of elution on a DB-5 column. The chromatogram profiles of the two EO are presented in Figure 1.

Sixty components were identified, representing 99.7% of the EO compositions, which were richer in monoterpene hydrocarbons, especially BG2, representing almost 90%, and limonene, which appeared as the major compound (47.7%). Other major compounds were identified as  $\alpha$ -pinene (21.0 and 18.3% in BG2 and BG1, respectively),  $\beta$ -pinene (14.2 and 14.9%, respectively) and (Z)- $\beta$ -ocimene (13.2%, only in BG1).

The main compounds detected in an EO from dried *B graveolens* leaves assessed by Silva et al (2012) were  $\alpha$ -pinene (16.6%), sabinene/ $\beta$ -pinene (11.1%),  $\alpha$ -phellandrene (5.9%), and limonene (25.0%).<sup>17</sup> The volatile composition of an EO is determined by the genetics of each plant, and is specific to each organ and plant development stage. In addition, environmental conditions may also significantly alter EO composition.<sup>26</sup> Therefore, the major cause for the difference in the *B graveolens* EO composition assessed by Silva et al and the EOs of the present study may be due to differences in the plant sampling dates. In the present study, leaves were collected in May 2014 (BG2) and November 2013 (BG1), while Silva et al samples the assessed plant in April 2012. In other studies carried out with *Bidens* plants, monoterpene hydrocarbons accounted for less than 20% of the total leaf EO composition.<sup>4,5</sup>

**TABLE 1** Experimental relative response factors (RFFs), expressed as the slope of the regression line, for different volatile classes in *B graveolens* leaf EOs

Compound Class	Compounds	RFF	Mean RF $\pm$ STD
Hydrocarbon monoterpenes	$\gamma$ -terpinene	1.06	
	$\alpha$ -pinene	1.07	1.05 $\pm$ 0.03
	<i>p</i> -cymene	1.02	
Oxygenated monoterpenes	linalool	1.48	
	menthol	1.28	
	nerol	1.44	1.48 $\pm$ 0.13
	carvone	1.64	
	linalool oxide	1.54	
Aromatic alcohols	benzyl alcohol	1.76	1.76
Monoterpene esters	menthyl acetate	1.10	1.24 $\pm$ 0.21
	geranyl acetate	1.39	
Sesquiterpene Hydrocarbon	( <i>E</i> )-caryophyllene	1.01	1.01
Oxygenated sesquiterpene	$\alpha$ -bisabolol	1.35	1.35

**TABLE 2** Chemical composition of *B graveolens* leaf essential oils

Peak	LRI <sup>a</sup> calc	LRI <sup>b</sup> lit	Substance	RFF <sup>c</sup>	BG1		BG2	
					mg/g <sup>d</sup>	%	mg/g <sup>d</sup>	%
1	921	921	tricyclene	1.05	0.4 ± 0.00	tr	0.5 ± 0.04	tr
2	925	924	α-thujene	1.05	0.4 ± 0.06	tr	2.1 ± 0.16	0.2 ± 0.01
3	932	932	α-pinene	1.05	156.3 ± 7.09	21.0 ± 0.07	202.1 ± 1.33	18.3 ± 0.1
4	946	946	camphene	1.05	6.5 ± 0.18	0.9 ± 0.06	9.6 ± 0.56	0.9 ± 0.05
5	970	969	sabinene	1.05	15.9 ± 1.37	2.1 ± 0.09	22.1 ± 0.8	2.0 ± 0.08
6	975	974	β-pinene	1.05	105.6 ± 4.83	14.2 ± 0.05	164.9 ± 1.35	14.9 ± 0.16
7	989	988	myrcene	1.05	50.2 ± 2.16	6.7 ± 0.01	24.8 ± 0.07	2.2 ± 0.01
8	1004	1003	<i>p</i> -mentha-1(7),8-diene	1.05	2.5 ± 0.05	0.3 ± 0.02	11.2 ± 0.47	1.0 ± 0.04
9	1009	1008	δ-3-carene	1.05	12.8 ± 2.48	1.7 ± 0.01	2.4 ± 0.03	0.2 ± 0.00
10	1019	1022	<i>o</i> -cymene	1.05	1.5 ± 0.12	0.2 ± 0.02	22.4 ± 1.71	2.0 ± 0.15
11	1022	1020	<i>p</i> -cymene	1.05	8.7 ± 0.17	1.2 ± 0.03	-	-
12	1026	1024	limonene	1.05	169.6 ± 6.65	22.8 ± 0.07	526.5 ± 10.43	47.7 ± 0.71
13	1034	1032	( <i>Z</i> )-β-ocimene	1.05	98.4 ± 5.61	13.2 ± 0.20	0.8 ± 0.06	tr
14	1045	1044	( <i>E</i> )-β-ocimene	1.05	6.2 ± 0.19	0.8 ± 0.01	-	-
15	1048	1046	dihydro-tagetone	1.48	3.1 ± 0.13	0.5 ± 0.09	-	-
16	1067	1065	<i>cis</i> -sabinene hydrate	1.48	-	-	0.6 ± 0.04	tr
17	1088	1089	<i>p</i> -cymenene	1.05	-	-	0.8 ± 0.04	tr
18	1092	1090	epoxy-myrcene	1.48	-	-	0.5 ± 0.02	tr
19	1096	1099	α-pinene oxide	1.48	4.7 ± 0.41	0.6 ± 0.03	9.4 ± 0.12	0.8 ± 0.11
20	1100	1102	perillene	1.05	-	-	0.5 ± 0.04	tr
21	1119	1119	<i>trans-p</i> -mentha-2,8-dien-1-ol	1.48	-	-	1.9 ± 0.06	0.2 ± 0.01
22	1124	1122	α-campholenal	1.48	-	-	2.5 ± 0.22	0.2 ± 0.02
23	1129	1131	( <i>Z</i> )-myroxide	1.48	45.2 ± 0.11	6.2 ± 0.25	-	-
24	1131	1132	<i>cis</i> -limonene oxide	1.48	-	-	5.2 ± 0.30	0.5 ± 0.03
25	1133	1133	<i>cis-p</i> -mentha-2,8-dien-1-ol	1.48	-	-	3.1 ± 0.13	0.3 ± 0.01
26	1134	1135	<i>trans</i> -pinocarveol/ <i>trans</i> -limonene oxide	1.48	2.5 ± 0.24	0.3 ± 0.02	10.9 ± 0.12	1.0 ± 0.01
27	1138	1140	( <i>E</i> )-myroxide	1.48	2.2 ± 0.17	0.3 ± 0.01	-	-
28	1143	1140	<i>trans</i> -verbenol	1.48	-	-	2.3 ± 0.10	0.2 ± 0.01
29	1157	1160	pinocarvone	1.48	0.7 ± 0.01	0.1 ± 0.01	5.5 ± 0.29	0.5 ± 0.03
30	1160	1165	borneol	1.48	1.2 ± 0.10	0.2 ± 0.01	-	-
31	1173	1173/1174	rosefurane epoxide/ terpinen-4-ol	1.48	2.1 ± 0.10	0.3 ± 0.00	3.9 ± 0.16	0.4 ± 0.02
32	1181	1183	cryptone	1.48	0.9 ± 0.04	0.1 ± 0.00	12.0 ± 0.13	1.1 ± 0.01
33	1186	1186	α-terpineol	1.48	0.4 ± 0.02	tr	1.1 ± 0.05	tr
34	1191	1194	myrtenol	1.48	0.5 ± 0.03	tr	6.1 ± 0.13	0.6 ± 0.01
35	1192	1195	myrtenal	1.48	0.6 ± 0.01	0.1 ± 0.00	6.0 ± 0.26	0.5 ± 0.02
36	1206	1204	verbenone	1.48	-	-	1.8 ± 0.05	0.2 ± 0.00
37	1217	1215	<i>trans</i> -carveol	1.48	-	-	6.8 ± 0.12	0.6 ± 0.01
38	1229	1226	<i>cis</i> -carveol	1.48	-	-	2.7 ± 0.03	0.2 ± 0.00
39	1237	1238	cumin aldehyde	1.48	-	-	2.4 ± 0.01	0.2 ± 0.02

(Continues)

TABLE 2 (Continued)

Peak	LRI <sup>a</sup> calc	LRI <sup>b</sup> lit	Substance	RFF <sup>c</sup>	BG1		BG2	
					mg/g <sup>d</sup>	%	mg/g <sup>d</sup>	%
40	1242	1239	carvone	1.48	-	-	11.3 ± 0.17	1.0 ± 0.01
41	1281	1283	bornyl acetate	1.24	0.7 ± 0.03	0.1 ± 0.00	0.9 ± 0.02	0.1 ± 0.00
42	1291	1289	<i>p</i> -cymen-7-ol	1.48	-	-	0.7 ± 0.09	tr
43	1299	1294	limonene dioxide	1.48	-	-	1.6 ± 0.06	0.1 ± 0.01
44	1323	1325	<i>p</i> -mentha-1,4-dien-7-ol	1.48	-	-	1.4 ± 0.04	0.1 ± 0.00
45	1340	1342	limonen-1,2-diol	1.48	-	-	0.7 ± 0.02	tr
46	1387	1389	β-elemene	1.01	0.8 ± 0.04	0.1 ± 0.00	-	-
47	1412	1417	( <i>E</i> )-β-caryophyllene	1.01	4.6 ± 0.12	0.6 ± 0.01	0.5 ± 0.07	tr
48	1432	1430	β-copaene	1.01	-	-	0.5 ± 0.06	tr
49	1446	1452	α-humulene	1.01	1.7 ± 0.06	0.2 ± 0.00	-	-
50	1472	1471	4,5-di- <i>epi</i> -aristolochene	1.01	0.8 ± 0.04	0.1 ± 0.00	-	-
51	1477	1480	germacrene D	1.01	3.8 ± 0.20	0.5 ± 0.01	-	-
52	1494	1494	bicyclogermacrene	1.01	0.2 ± 0.01	tr	-	-
53	1504	1505	β-bisabolene	1.01	0.2 ± 0.04	tr	-	-
54	1517	1522	δ-cadinene	1.01	1.2 ± 0.03	0.2 ± 0.00	-	-
55	1558	1561	( <i>E</i> )-nerolidol	1.35	0.5 ± 0.02	tr	-	-
56	1574	1577	spathulenol	1.35	13.1 ± 0.19	1.8 ± 0.05	6.7 ± 0.76	0.6 ± 0.01
57	1579	1582	caryophyllene oxide	1.35	4.6 ± 0.10	0.6 ± 0.01	9.0 ± 1.13	0.8 ± 0.03
58	1636	1638	<i>epi</i> -α-cadinol	1.35	1.1 ± 0.01	0.2 ± 0.01	-	-
59	1651	1652	α-cadinol	1.35	3.6 ± 0.35	0.5 ± 0.03	-	-
60	1683	1685	4(15),5,10(14)germacra-1β-trienol	1.35	2.3 ± 0.22	0.3 ± 0.02	-	-
					Total	99.7	Total	99.7
					Monoterpene hydrocarbons	86.5	Monoterpene hydrocarbons	89.6
					Oxygenated monoterpenes	9.0	Oxygenated monoterpenes	8.8
					Sesquiterpene hydrocarbons	1.8	Sesquiterpene hydrocarbons	tr
					Oxygenated sesquiterpenes	2.5	Oxygenated sesquiterpenes	1.3

<sup>a</sup>LRI<sub>calc</sub> = On DB-5 column.

<sup>b</sup>LRI<sub>lit</sub> = from reference 18.

<sup>c</sup>relative response factor from 1.

<sup>d</sup>mg of the compound per gram of essential oil; tr = trace (<0.1%).

A total of 25 oxygenated monoterpenes were identified in BG2 and 12 in BG1, representing about 9% of the relative EO area. α-Pinene oxide, pinocarvone, *trans*-pinocarveol, *trans*-limonene oxide, rose furane epoxide, cryptone, myrtenol, and myrtenal were found in both EOs and described for the first time in an EO from this genus. Oxygenated monoterpenes such as epoxy-myrcene, α-pinene oxide, *trans*-pinocarveol, *trans*-verbenol, *cis* and *trans*-carveol, carvone, and limonene dioxide were observed only in BG2. Since the oil was distilled in the same day of plant collection, kept in the dark under

reduced temperature and analyzed no more than a week later, it does not seem feasible these epoxides were present due to aging of the essential oil after distillation.

Sesquiterpene hydrocarbons have been reported as the major compounds in other studies carried out with *Bidens* EOs.<sup>4,5,27</sup> In the present study, this class comprised about 2% of the chemical composition of the BG1 EO. In essential oils from fresh *B. sulfurea* and *B. pilosa* leaves, sesquiterpenes represent 95.8% and 35.9%, respectively of total EO composition.<sup>4,25</sup>

The findings demonstrate differences between the chemical EO compositions, especially in the sesquiterpene group. Concerning plant collection dates, a distinct difference between climate and rainfall regime in the cultivation area is noted, and environmental factors have been reported as influencing the storage of volatile compounds in secretory plant structures.<sup>26,28</sup>

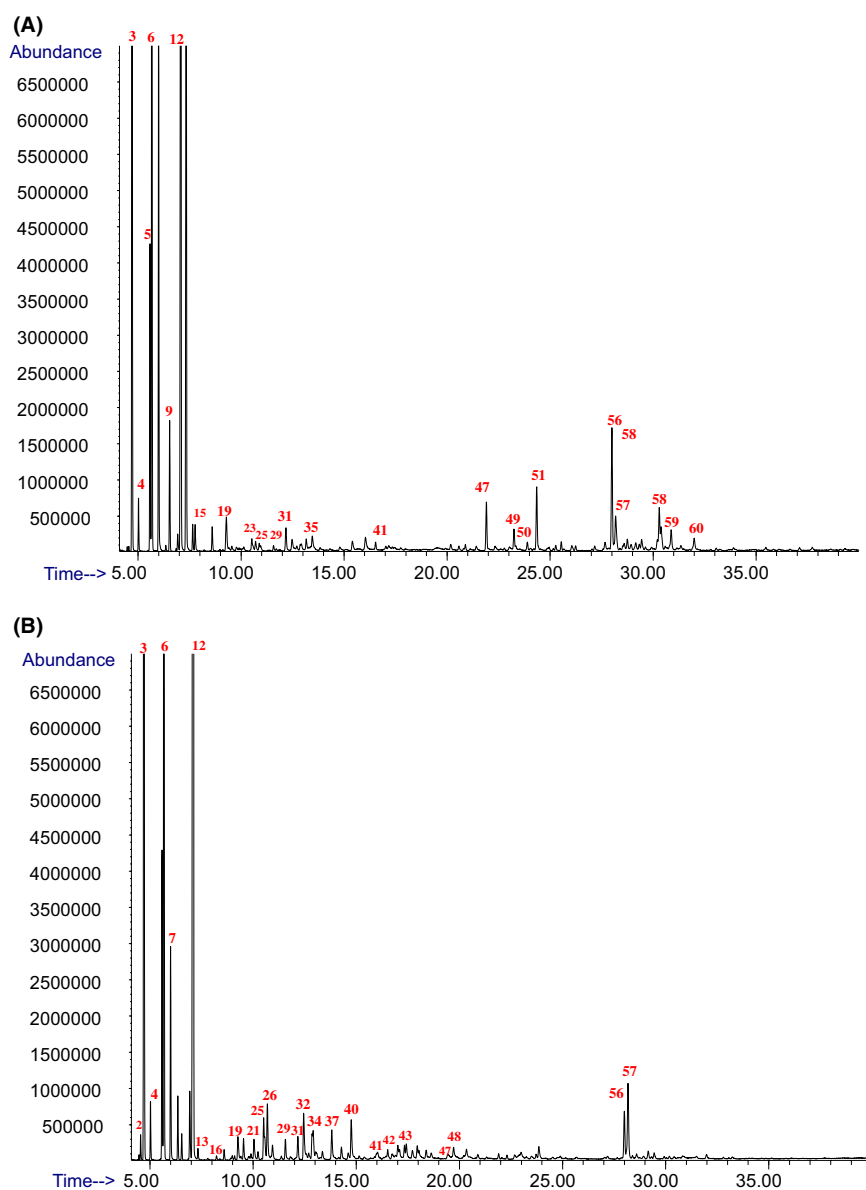
### 3.2 | Characterization of BG2 odor-active compounds

The olfactive descriptors green, fresh, spicy, camphor, fruity, and floral were used by panelists to characterize the BG2 EO in the sensory analysis. This EO was considered by the perfumer as one of the more interesting samples, with its odor described as "...green and mustard leaves, coriander, and watery fruits." The attributes described by the panelists are in accordance to the sensorial profile observed in the

perfumist evaluation. The herbal and spicy odors are related to the description of green notes that are associated to coriander, while camphoraceous refers to a camphor odor and fresh and fruity odors may refer to the fresh fruit odor.

Sensory analyses can indicate essential oil odor descriptions, but it is not possible to identify which volatile compounds are responsible for the described odor. To obtain this information, a more complex analysis is required, and one of the main techniques currently applied is GC-O.

The GC-O using detection frequency method allowed for the determination of odor-active compounds with minimum panelist time and training.<sup>29</sup> Six panelists participated in the analysis and considered as main *B graveolens* OE odor compounds those observed at 100% of frequency detection (FD, detected by all panelists). Substances presenting over 66% FD, that is, detected by at least two panelists, were accepted as odoriferous compounds.

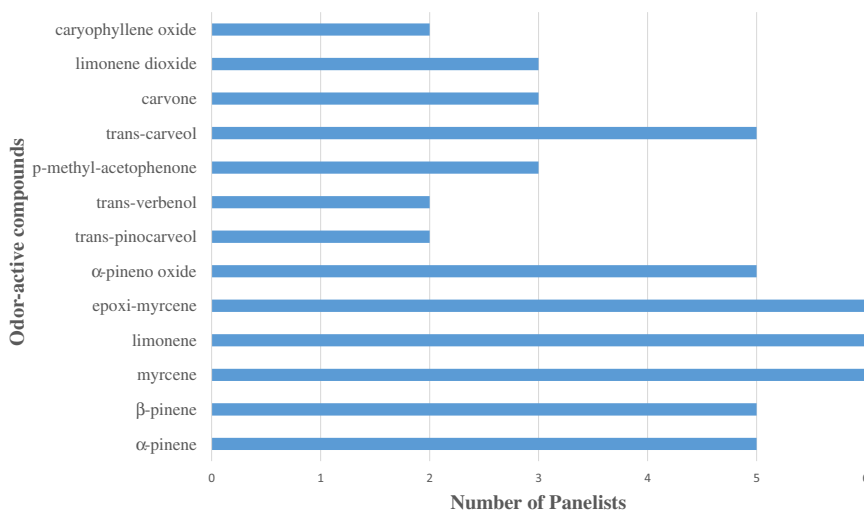


**FIGURE 1** Representative total ion chromatograms of essential oils from *B. graveolens*. (A) BG1; (B) BG2. peak numbers correspond to those listed in 2. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

**TABLE 3** Odor-active compounds in essential oil BG2 according to the frequency detection method and odor descriptors indicated by the panelists

Peak	Substance	$\Sigma Ni$	Descriptors	% FD
3	$\alpha$ -pinene	5	Grass, floral, and citrus	83.33
6	$\beta$ -pinene	5	Spicy, fresh, grass, and green	83.33
7	myrcene	6	Green, and camphor	100.00
12	Limonene	6	Floral, fruity, fresh, and green	100.00
	<LD	3	-	50.00
	N.I.	3	Herb, green, camphor, and fresh	50.00
18	epoxy-myrcene	6	Green, mint, and spicy	100.00
19	$\alpha$ -pinene oxide	5	Fruity and citrus	83.33
	N.I. MM = 154	6	Spicy and green	100.00
26	<i>trans</i> -pinocarveol/ <i>trans</i> -limonene oxide	2	Citrus and floral	33.33
28	<i>trans</i> -verbenol	2	Herb	33.33
	<LD	3	-	50.00
37	<i>trans</i> -carveol	5	-	83.33
40	carvone	3	Spicy, fresh, and citrus	50.00
	N.I.	6	-	100.00
43	Limonene dioxide	3	Spicy, fresh, and citrus	50.00
	N.I.	3	Fresh	50.00
59	caryophyllene oxide	2	-	33.33

Abbreviations: FD, frequency detection; <LD, below the limit of detection; N.I., not identified;  $\Sigma Ni$ , number of panelists.

**FIGURE 2** Aromagram of BG2 odor-active compounds obtained by the frequency detection method. [Colour figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

Five compounds were perceived by all of the panelists, while four were identified by five. Altogether, eighteen compounds were perceived and fourteen of them were identified by matching the LRI and mass spectrum, listed in Table 3. The corresponding aromagram is presented in Figure 2, indicating the odor-active compounds obtained by the frequency detection method. Two odor-active compounds were not identified, as they were present at trace levels, lower than the GC-MS and GC-FID limits of detection, and three due to the high MS background.

All panelists pointed to an unidentified substance with an IRL of 1115, suggesting an oxygenated monoterpene.

$\alpha$ -Pinene,  $\beta$ -pinene,  $\alpha$ -pinene oxide, and carvone were identified by five panelists, at 83.3% of frequency detection. It is noteworthy that most compounds rated the highest intensity score were oxygenated monoterpenes, considered to be the most expressive terpene class used in perfumery.<sup>20</sup> Furthermore, other compounds identified by the panelists, such as *trans*-pinocarveol, *trans*-limonene oxide, *trans*-verbenol, and *trans*-carveol, perceived by at least two panelists, also belong to this class, corroborating their importance. It is interesting to note that these oxygenated monoterpenes, important EO odor compounds, have not been described previously for this genus.<sup>4,5,25</sup> These compounds were associated with the green, herb,



spicy, fresh, and camphoraceous olfactory descriptors in *B graveolens* EOs.

## 4 | CONCLUSIONS

Volatile *B graveolens* essential oil constituents were identified and quantified, and monoterpene hydrocarbons were identified as the major compounds, in contrast to EOs from other species belonging to the same genus, in which sesquiterpene hydrocarbons are described as the main class. The differences in sesquiterpene composition between both EOs demonstrate the difficulty in obtaining standardized raw materials. Sensory analyses and GC-O determinations by the frequency detection method corroborate the main *B graveolens* EO odor aspects. Finally, this study has presented the first chemical and olfactory characterization results for *B graveolens* EO, which are promising and may promote the use of this EO.

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## CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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