

# Chemical Composition of Essential Oils of Three Species of the Genus *Bocageopsis* (Annonaceae) Amazon Region

## *Composição Química dos Óleos Essenciais de Três Espécies Do Gênero Bocageopsis (Annonaceae) da Região Amazônica*

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The essential oils (EO) of three species of *Bocageopsis* (*B. canescens*, *B. pleiosperma*, and *B. multiflora*) were investigated by gas chromatography coupled to mass spectrometry (GC/MS), revealing the presence of sesquiterpenes and their derivatives. The leaves of two individuals with different stages of development of *B. canescens* species were analyzed, being identified in the bigger and, consequently, older individual, germacrene D (19.5%), (*E*)-caryophyllene (14.8%), bicyclogermacrene (9.7%),  $\delta$ -elemene (8.5%), and  $\beta$ -elemene (8.3%) as main constituents. In the younger and smaller individual, oxygenated sesquiterpenes were predominant: spathulenol (24.1%), caryophyllene oxide (9.8%), and isospathulenol (8.5%), besides the sesquiterpene (*E*)-caryophyllene (13.6%). The main constituents of the twig from the older individual were sesquiterpenes:  $\alpha$ -muurolol (40.0%) and  $\beta$ -acorenol (16.0%). On the other hand, in the species *B. multiflora*, the main constituents in the EO extracted from the leaves were spathulenol (35.14%) and  $\alpha$ -*trans*-bergamotene (31.23%). The main compounds in the branches were  $\alpha$ -*trans*-bergamotene (25.32%),  $\beta$ -selinene (19.57%), and  $\alpha$ -gurjunene (15.42%). The sesquiterpenes  $\beta$ -selinene (28.16%) and  $\alpha$ -*trans*-bergamotene (21.92%) were predominant in the twig  $\beta$ -bisabolene was the main constituent in the leaves (89.64%) and twig (33.75%) of *B. pleiosperma* species, being also observed a significant presence of bisabolene derivative cryptomerione (25.76%) in the twig. These results represent the first report on the chemical composition of the species *B. canescens* from the Amazon region.

**Keywords:** *Bocageopsis canescens*, gas chromatography, sesquiterpenes, spathulenol.

## 1. Introduction

The Amazon region represents about 30% of all tropical areas in the world. Its importance is recognized nationally and internationally since it is considered the largest reserve of forest biodiversity on the planet. This is mainly due to its large extension of 4.2 million km<sup>2</sup>, corresponding to 49.3% of the national territory and 5% of the terrestrial surface. It has more than 600 different types of terrestrial and freshwater habitat, dictated in a broad ecosystem, with about 45.000 species of plants and vertebrates. Among the different plant families, Annonaceae stands out, being the broadest family of the Magnoliales order.<sup>1-4</sup>

The Annonaceae family is pantropical and composed of about 2.430 species, distributed in 110 genera.<sup>3-5</sup> It has a geographic distribution of the pantropical type, with approximately 900 species (40 genera) in the neotropics region.<sup>6-8</sup> In Brazil, among the 32.000 species of angiosperms cataloged, approximately 372 species are Annonaceae, consisting of 29 genera widely dispersed throughout the national territory. In the Amazon region, there are 27 genera and around 265 species of Annonaceae, where 158 are endemic.<sup>8,9</sup>

Despite being numerous, few genera of Annonaceae have been explored from a phytochemical point of view. One of these genera has not yet been well studied, the Neotropical genus *Bocageopsis*, which consists of four species, (*B. canescens* (Benth.) R. E. Fries, *B. mattogrossensis* (R. E. Fries) R. E. Fries, *B. multiflora* (Mart.) R. E. Fries, and *B. pleiosperma* Maas), all restricted to tropical South America and east of the Andes, and only the species *B. mattogrossensis* has not been subjected to any phytochemical investigation.<sup>10-17</sup>

The Neotropical genus *Bocageopsis* belongs to the subfamily Malmeeoideae, represented in Brazil by the tribe Malmeeae.<sup>5,18</sup> The species with wide distribution in the Amazon region are the species *B. canescens* (popularly known as envireira), *B. multiflora* (envira or envira

surucucu), and *B. pleiosperma* (envira preta or surueira sangue).<sup>2,5,19-21 20</sup> The biological potential of essential oils (EOs) obtained from *B. multiflora* and *B. pleiosperma* species were investigated and showed leishmanicidal activity<sup>10</sup> and antimicrobial activity,<sup>12</sup> respectively. Although the species *B. canescens* have previous phytochemical reports - confirming the presence of a new flavonoid, besides alkaloids derived from the isoquinolinic skeleton - data on the composition of essential oils are still lacking.<sup>16</sup>

Thus, in the present study, the EOs of the leaves, twig, and branches of three species of the genus *Bocageopsis* at different stages of development were investigated by GC/MS analysis. In addition, this article portrays a discussion on the variation in the content of essential oils in the individual with the highest biomass of the species *B. canescens*, i.e., in the older sample, where the concentrations of major constituents may be associated with the difference in age of the individuals studied.

## 2. Materials and Methods

### 2.1. Plant material

The collection of botanical material was carried out upon approval by the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado – SISGEN (AA42598 and AF4CFA7).

The botanical material (leaves and twig) of *B. canescens* (Beth.) R.E.Fr was collected in Manaus-AM, North of Brazil, in January 2016, in the Good Friends Site (BR 174 – km 19 / 2°49'55.2 S, 60°05'13.1 W). The plant material was identified by Prof. Dr. Antônio Carlos Webber (Federal University of Amazonas-UFAM), and a voucher specimen (number 10234) was deposited at UFAM Herbarium. It was collected the leaves of two individuals in different stages of development (with different heights and trunk diameters), where one was younger and smaller (Bc02) and the other bigger and older (Bc01), also presenting fruits.

The leaves, twig, and branches of *B. multiflora* were also collected in Manaus-AM, North of Brazil, in January 2017, in UFAM campus (3°05'48.6 S, 59°58'33.2 W) of previously marked individual, being a voucher specimen (number 5987) deposited at UFAM Herbarium.

The botanical material (leaves and twig) of *B. pleiosperma* was collected in Manaus-AM, North of Brazil, in February 2018, in the Forest Reserve Adolpho Ducke (AM 10 – km 26 / 2°88'33.33 S, 96°66'67 W) of previously marked individual, being a voucher specimen (number 183127) deposited at INPA Herbarium.

### 2.2. Essential oil extraction

After collection, the botanical material (leaves, twig, and branches) of *Bocageopsis* (100 g), where the twig and branches were dried for 24 hours at room temperature

(26 °C), and the leaves for 26 hours. The material was pulverized and extracted by hydrodistillation for 4 h using a Clevenger-type apparatus. The essential oils (EO) were obtained using CH<sub>2</sub>Cl<sub>2</sub>, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, filtered, and the yield was calculated based on the dry weight of the plant. The oils were stored at -15 °C until analysis.<sup>12</sup>

### 2.3. Gas chromatography coupled to mass spectrometry (GC-MS) analysis

The EOs analysis were performed in GC-MS equipment, Model Trace Ultra gas chromatograph coupled to an ISQ single quadrupole mass spectrometer (Thermo Scientific), equipped with a capillary column Rtx-5 MS (30 m x 0.25 mm x 0.25 mm). Helium gas was used as a carrier (flow of 1.0 mL/min). The injection solution was prepared by dissolving about 15 mg of oil in 1 mL of ethyl acetate, and 1 µL of the solution was injected using a split ratio of 1:20. The column temperature was increased from 60 to 240 °C at 3 °C/min. The temperatures of the injector and the ion source were 220 and 260 °C, respectively. A mixture of linear hydrocarbons (C<sub>7</sub> to C<sub>30</sub> alkanes) was injected under the same experimental conditions and the percentage composition was calculated according to the Van den Dool and Kratz equation.<sup>22</sup> The identification of the constituents was done by comparing the obtained spectra with those stored in the National Institute of Standards and Technology – NIST library and also comparing the retention index with literature data.<sup>23</sup>

## 3. Results and Discussion

### 3.1. Essential oil analysis

Essential oils (EOs) from two individuals of *B. canescens* (named as Bc01-Leaves, Bc01-Twig, and Bc02-Leaves) were obtained with yield of 0.28, 0.20, and 0.15% (in relation to the weight of the dry matter), respectively, and presenting a green-yellow color. Thirty-seven constituents were identified, corresponding to 98.8, 96.5, and 96.0% of the total oil for Bc01-Leaves, Bc02-Leaves, and Bc01-Twig, respectively (Table 1 and Figures S1-2 of Supplementary Material). It was observed the predominance of sesquiterpenic hydrocarbons and their oxygenated derivatives according to literature reports of Annonaceae.<sup>24</sup> Monoterpenes were not detected, and this result may be related to the drying time or pulverization. Perhaps these compounds could be present in fresh material. However, the hydrodistillation was not performed with fresh material due to a small amount of botanical material; therefore, it was not possible to compare the results between the fresh material and dried one. From the Bc01-Leaves, it was possible to identify germacrene D (19.5%), (*E*)-caryophyllene (14.8%), bicyclogermacrene (9.7%),  $\delta$ -elemene (8.5%), and  $\beta$ -elemene (8.3), representing 60.8% of the EO constituents (Table 1). From the Bc02-

Leaves, spathulenol (24.1%), caryophyllene oxide (9.8%), and *iso*-spathulenol (8.5%) were predominant, in addition to (*E*)-caryophyllene sesquiterpene (13.6%), resulting in 56% of oxygenated sesquiterpenes in the EO (Table 1). It is worth mentioning that spathulenol and caryophyllene oxide have been considered as chemophene markers in some genera of Annonaceae, such as *Xylopi*a, *Annona*, *Duguetia*,

and *Guatteria*.<sup>25-31</sup> In studies carried out with *Unonopsis* and *Onychopetalum* species, genera close to *Bocageopsis*, also identified spathulenol in their composition.<sup>11,32</sup> The compounds spathulenol and caryophyllene oxide were also reported in the species *Guatteria pogonopus*,<sup>33</sup> ratifying the frequency of these sesquiterpenoids in the Annonaceae family.

**Table 1.** The botanical material (leaves, thin and thick branches) essential oil compositions of *Bocageopsis*

RI <sup>a</sup>	RI <sup>b</sup>	Compounds	<i>B. canescens</i>			<i>B. multiflora</i>			<i>B. pleiosperma</i>	
			Bc01- Leaves	Bc02- Leaves	Bc01- Twig	Leaves	Twig	Branches	Leaves	Twig
1335	1333	δ-elemene	8.5	4.0	1.6	0.34	-	-	-	-
1348	1345	α-cubebene	0.8	1.0	-	-	-	-	-	-
1373	1371	α-ylangene	-	-	-	-	-	-	0.27	-
1374	1377	α-copaene	1.5	2.2	0.3	-	-	-	-	-
1387	1387	β-bourbonene	3.1	4.5	2.2	2.23	-	-	-	-
1389	1393	β-elemene	8.3	2.5	2.0	-	1.00	2.07	-	-
1390	1387	7-epi-sesquithujene	-	-	-	-	1.00	2.07	-	-
1409	1404	α-gurjunene	-	-	-	-	3.00	15.42	-	-
1417	1421	( <i>E</i> )-caryophyllene	14.8	13.6	3.4	2.69	1.47	0.43	-	-
1430	1434	β-copaene	0.5	-	0.2	-	-	-	-	-
1432	1431	α-trans-bergamotene	-	-	-	31.23	21.92	25.32	2.50	-
1434	1435	γ-elemene	1.9	-	-	-	-	-	-	-
1437	1446	α-guaiene	0.2	-	-	-	-	-	-	-
1439	1441	aromadendrene	-	0.6	-	-	-	-	-	-
1440	1439	( <i>Z</i> )-β-farnesene	-	-	-	-	-	-	0.58	-
1449	1440	α-himachalene	-	-	-	0.38	-	-	-	-
1452	1456	α-caryophyllene	4.1	2.9	1.3	-	-	-	-	-
1453	1448	geranyl acetone	-	-	-	-	-	-	0.13	-
1454	1454	( <i>E</i> )-β-farnesene	-	-	-	-	-	-	1.26	-
1458	1455	allo-aromadendrene	0.5	-	0.1	-	-	1.23	-	-
1479	1471	ar-curcumene	-	-	-	0.62	-	-	-	-
1480	1479	germacrene D	19.5	4.5	0.4	-	-	-	-	-
1489	1488	β-selinene	-	-	-	1.00	28.16	19.57	-	-
1495	1497	γ-amorfene	0.5	-	0.5	-	-	8.15	-	-
1500	1499	bicyclogermacrene	9.7	4.8	3.2	-	-	-	-	-
1500	1502	α-murolene	-	0.4	-	-	-	-	-	-
1505	1505	β-bisabolene	-	-	-	1.00	1.63	1.17	89.64	33.75
1511	1510	δ-amorfene	0.4	0.1	0.2	-	-	0.67	-	-
1513	1509	γ-cadinene	-	-	-	-	0.62	1.43	-	-
1522	1526	δ-cadinene	3.8	5.2	1.8	-	-	-	-	-
1528	1523	<i>E-iso</i> -γ-bisabolene	-	-	-	-	-	1.57	-	-
1533	1535	trans-cadina 1,4-diene	0.1	-	-	-	-	-	-	-
1532	1540	γ-cuprenene	-	-	-	-	1.02	-	-	-
1544	1543	α-calacorene	-	-	-	-	1.00	1.02	-	-
1546	1541	hedicariol	-	-	-	1.21	1.00	-	-	-
1548	1553	Elemol	-	-	1.4	-	2.88	-	-	-
1559	1560	germacrene B	6.0	3.3	1.2	-	-	-	-	-
1561	1559	<i>E</i> -nerolidol	-	-	-	1.09	1.00	-	-	-

**Table 1.** The botanical material (leaves, thin and thick branches) essential oil compositions of *Bocageopsis* (cont.)

RI <sup>a</sup>	RI <sup>b</sup>	Compounds	<i>B. canescens</i>			<i>B. multiflora</i>			<i>B. pleiosperma</i>	
			Bc01- Leaves	Bc02- Leaves	Bc01- Twig	Leaves	Twig	Branches	Leaves	Twig
1577	1581	spathulenol	5.2	24.1	2.7	35.14	4.33	-	-	-
1582	1586	caryophyllene oxide	1.9	9.8	4.6	2.00	3.00	1.94	-	-
1590	1588	globulol	0.3	-	0.2	-	-	-	-	-
1592	1595	viridiflorol	-	0.3	2.8	-	-	-	-	-
1599	1595	widdrol	-	-	-	1.00	2.00	2.44	-	-
1600	1595	Guaiol	-	-	0.1	-	-	-	-	-
1607	1600	5- <i>epi</i> -7- <i>epi</i> - $\alpha$ -eudesmol	3.0	4.0	2.0	-	-	-	-	-
1608	1612	humulene epoxide II	-	1.2	2.5	-	-	-	-	-
1608	1607	$\beta$ -atlantol	-	-	-	-	-	-	-	3.97
1633	1627	<i>iso</i> -spathulenol	3.7	8.5	2.0	-	-	-	-	-
1636	1634	$\beta$ -acorenenol	-	-	16.0	-	-	-	-	-
1638	1635	<i>epi</i> - $\alpha$ -cadinol	-	-	-	3.00	4.22	6.60	-	-
1639	1647	<i>allo</i> -epoxide aromadendreno	-	-	-	2.00	3.61	-	-	0.93
1644	1646	$\alpha$ -muurolol	-	1.0	40.0	-	-	-	-	-
1645	1645	cubenol	-	0.6	-	-	-	-	-	-
1649	1654	$\beta$ -eudesmol	-	-	0.5	-	-	-	-	0.74
1651	1652	pogostol	-	-	-	-	-	-	-	4.25
1652	1659	$\alpha$ -cadinol	0.5	0.1	1.8	-	-	-	-	-
1666	1666	14-hidroxi-(Z) caryophyllene	-	0.3	0.7	-	-	-	-	-
1668	1662	14-hidroxi-9- <i>epi</i> -(E)-caryophylleno	-	-	0.1	-	-	-	-	2.76
1698	1691	(2Z, 6Z) - farnesol	-	-	-	-	-	-	-	11.89
1733	1742	isobicyclgermacrene	-	-	-	-	3.60	3.16	-	-
1724	1722	cryptomerione	-	-	-	-	-	-	1.32	25.76
1742	1740	(2E- 6E)-farnesol	-	-	-	-	-	-	-	2.11
1775	1771	2- $\alpha$ -hidroxi-amorfa-4,7(11)-diene	-	-	0.1	-	-	-	-	-
1913	1910	<(5E,9E)>farnesil acetone	-	-	0.1	-	-	-	-	-
<b>Total identified</b>			98.80%	96.5%	96.0%	86.22%	85.46%	92.19%	95.70%	86.16%

RI<sup>a</sup> = Retention Index of the literature that was compared with RI calculated through a homologous series of normal alkanes; Compounds  $\leq 0.1\%$  were not listed. RI<sup>b</sup> = Retention Index of the calculated. (-) = not detected.

The main components in the essential oil obtained from Bc01-Twig were sesquiterpenes absent in the leaves:  $\alpha$ -muurolol (40.0%) and  $\beta$ -acorenenol (16.0%), representing 56.0% of EO constituents (Table 1). These results show a great difference in the constituents present in these two parts of the plant. The compounds  $\beta$ -acorenenol has already been reported in the essential oil extracted from the fruits of *Xylopiya sericea* St. Hill.<sup>31</sup>

The chemical composition of essential oils is affected by several factors, such as seasonality, circadian rhythm, altitude, availability of water, nutrients in the soil, temperature, organ development stage, phenology, genetics, and plant age.<sup>35,38</sup> Then, the variation in the yield and composition of essential oils of the leaves of *B. canescens* can be associated with the age difference of the individuals studied. Younger individuals generally show great biosynthetic activity, increasing the production of various compounds, including essential oils. The enzymatic arsenal

of defense and development of plants maybe enables them to perform the germacrene-bicyclgermacrene-spathulenol biosynthetic route<sup>37</sup> in the vegetative phase in such individuals; however, the final phase of this route seemed less activated in the more mature individual, what was in the period of fruiting, being observed the accumulation of sesquiterpenes in detriment of the production of oxygenated sesquiterpenes biosynthetically related to them.

For the *B. multiflora* species were obtained yields of their dark green EOs: 0.25, 0.22, and 0.23% (relative to weights of dry material of leaves, twig, and branches, respectively, which were studied separately). From these EOs, 86.22, 85.46, and 92.19% of the constituents were identified, respectively, and these results are shown in Figures S3-S4 (Supplementary Material). The chemical profile showed a predominance of hydrogenated sesquiterpenes for these EOs; however, the sesquiterpenoid spathulenol (35.14%) was highlighted as the majority in the leaves, besides

$\alpha$ -*trans*-bergamotene (31.23%), (Table 1). Studies carried out on the EO of the leaves collected during the dry and rainy season in Manaus – AM also reported spathulenol as the majority compound, with 16.2%<sup>10</sup> and 20.3%<sup>14</sup>, respectively. The constituents  $\alpha$ -*trans*-bergamotene and  $\beta$ -selinene were predominant in the EOs obtained from twig and branches, with concentrations of 21.92 and 25.32% for the first plant part, and 28.16 and 19.57% for the other one. It was also observed a significant concentration of  $\alpha$ -gurjunene (15.42%) in the branches. These EOs differ mainly for the presence of  $\gamma$ -amorfene (8.15%) present in the branches (Table 1). A previous report<sup>17</sup> showed exo-2-norborneol acetate (10.3%) and *air*-curcumenium (10.2%) as the main compounds in the EO extracted from branches of this species, evidencing a variability in the composition of these EOs.

Many of the compounds identified in the present study have already been reported<sup>24</sup> in essential oils extracted from species of the Annonaceae family, including *Onychopetalum amazonicum*, *Onychopetalum periquino*, *Unonopsis guatterioides*, and *Unonopsis stipitata* - species close to the one studied in this work.<sup>11,31,38</sup> The high concentration of  $\alpha$ -*trans*-bergamotene, spatulenol,  $\beta$ -selinene, and  $\alpha$ -gurjunene is of great importance since the essential oils rich in these compounds have important activities already described in the literature, such as antioxidant, antimicrobial, antitumor, and larvicide.<sup>39-42</sup>

The *B. pleiosperma* species presented a yield of 0.26 and 0.24% of the EO in the leaves and twig, respectively (relative to weights of dry material), presenting a light yellow-green coloration and totalizing 95.70 and 86.16% of their constituents, respectively. It was possible to highlight the  $\beta$ -bisabolene (sesquiterpene) as the majority constituent, with concentrations of 89.64% in the leaves and 33.75% (see Figures S5-6 – Supplementary Material). The EO extracted from the branches also showed the compounds (2*Z*,6*Z*)-farnesol (11.89%) and cryptomerione (25.76%) in significant concentrations (Table 1). It is interesting to observe the decrease in the concentration of bisabolene in the twig, parallel to the appearance of (2*Z*,6*Z*)-farnesol and increase in the concentration of cryptomerione, being these sesquiterpenoids structurally related to bisabolene.<sup>43</sup> Our previous report also showed the presence of  $\beta$ -bisabolene as the main constituent in the *B. pleiosperma* EO - 55.77% in the leaves, 34.37% in the twig, and 38.53% in the bark of the trunk.<sup>12</sup> It was not registered the presence of the biosynthetic markers of Annonaceae (spathulenol and caryophyllene oxide) in these investigated parts.

#### 4. Conclusions

Chemical analysis by GC-MS of the essential oils (EOs) allowed the characterization of sixty-three substances (sesquiterpenes and their derivatives) for the studied species. Many of the structures have been reported in the

literature on species of the *Unonopsis* and *Onychopetalum* genera. Spathulenol and caryophyllene oxide are found in several genera of Annonaceae and were confirmed in all parts of the species *B. canescens* and *B. multiflora* but absent in *B. pleiosperma*. This is the first report on the terpenic constituents present in the EOs extracted from *B. canescens* and twig and branches of *B. multiflora*. This work also confirms that the chemical composition of these oils is affected by several factors, such as seasonality, organ development stage and age of the plant, considering the results obtained with those described in the literature. These variations in compositions justify and encourage further investigation of essential oils of these species collected at different times and seasons.

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