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Article

Gas Chromatography-Mass Spectrometry (GC-MS) and evaluation of antioxidant and antimicrobial activities of essential oil of *Campomanesia adamantium* (Cambess.) O. Berg (Guavira)

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The essential oils from *Campomanesia adamantium* (Cambess.) O. Berg leaves, collected in the reproductive (flowering and fruit-bearing) and vegetative stages, were characterized by GC-MS (Gas Chromatography-Mass Spectrometry). A total of 95 compounds of the essential oils were identified. In the reproductive stage (flowering) the major constituents were monoterpenes (limonene, α-pinene and β-pinene) while during the vegetative stage the major constituents were the sesquiterpenes (bicyclogermacrene and globulol). The essential oil of the reproductive stage shows high antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*, and all show moderate activity against *Escherichia coli*. The essential oils were also evaluated for their radical-scavenging activity by DPPH. The chemogeographical variations of the oil composition from the four distinct localities studied all contained α-pinene, β-pinene, limonene, linalool, β-caryophyllene, germacrene D and bicyclogermacrene, however the samples from Jardim city contained neither limonene nor linalool.

Uniterms: *Campomanesia adamantium*/antioxidant activity. *Campomanesia adamantium*/antimicrobial activity. Guavira. Essential oil/caracterization.

Os óleos essenciais obtidos das folhas de *Campomanesia adamantium* foram caracterizados através da combinação de CG-EM e índice de retenção, sendo identificado um total de 95 compostos. Na floração as substâncias majoritárias foram monoterpenos (limoneno, α-pineno e β-pineno) e durante o estágio vegetativo as substâncias majoritárias foram sesquiterpenos (biciclogermacreno e globulol). Os óleos essenciais obtidos da floração e frutificação mostraram alta atividade contra *Staphylococcus aureus*, *Pseudomonas aeruginosa* e *Candida albicans* e moderada contra *Escherichia coli* em todos os estágios. Foi avaliada a atividade antioxidante dos óleos essenciais usando o método do DPPH. O óleo essencial das 4 cidades mostrou a presença de α-pineno, β-pineno, limoneno, linalol, β-cariofileno, germacreno D e biciclogermacreno, mas a amostra da cidade de Jardim não apresentou limoneno e linalol.

Unitermos: *Campomanesia adamantium*/atividade antioxidante. *Campomanesia adamantium*/atividade antimicrobiana. Guavira. Óleo essencial/caracterização.

INTRODUCTION

Essential oils are complex mixtures of isomers such as monoterpenes, sesquiterpenes, aromatic compounds and aliphatic compounds (Zhao *et al.*, 2005). Plants rich in aromatic compounds can have ecological functions, besides those that are used as alternative remedies for the treatment of many infectious diseases or the preservation of food from the toxic effects of oxidants (Tepe *et al.*, 2004).

Quantitative and qualitative differences in the terpene compositions of some plants might be influenced by

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different phenological stages as well as environmental factors, as shown in the studies of *Thymus vulgaris* (Hudaib *et al.*, 2002). For example, variations in the chemical composition at different phenological stages have been associated with the alteration of the chemical composition in antimicrobial activities, e.g., studies of the essential oils of *Salvia sahendica* (Salehi *et al.*, 2007).

Nowadays, studies of the variability of compounds in plants associated with the evaluation of antioxidant and antimicrobial activities are important. The prevention of cancer and cardiovascular diseases has been associated with the ingestion of fresh fruit, vegetables or teas rich in natural antioxidants (Ramalho, Jorge, 2006). Substances with antimicrobial activities are used in the treatment of infectious diseases, as well as antifungal agents in plants that assist in the treatment of opportunistic systemic mycoses (Rahalison *et al.*, 1994).

Campomanesia adamantium (Cambess.) O. Berg (Myrtaceae) is a small tree with edible fruit, commonly known as guavira or guabiroba. A species native to the Brazilian Cerrado bioma (Lorenzi *et al.*, 2006), the fruit is widely used to make liqueurs, juices and sweets. There are few studies published about the chemical composition of leaves of this genus.

The essential oils of the *C. guazumifolia*, *C. rhombea* and *C. xanthocarpa* leaves are the most studied species (Limberger *et al.*, 2001), while only the essential oil from the *C. adamantium* (Vallilo *et al.*, 2004) fruit has been studied. Studies reported the isolation of three yellow pigments of the *C. lineatifolia* seeds, named champanones. Terpenes were identified in volatile extracts of pulp, peel, leaves and seeds in the same species (Bonilla *et al.*, 2005; Osorio *et al.*, 2006). Chemical studies of *Campomanesia* genus have identified quercetin, myricetin and rutin by HPLC (Schmeda-Hirschmann, 1994).

This paper describes the identification of essential oil constituents obtained at the three different phenological stages associated with the evaluation of the antioxidant and antimicrobial activities and variability of the chemical composition of four samples collected from different geographical regions.

MATERIAL AND METHODS

Chemical analysis

The solvents employed in CG-MS (Gas Chromatography-Mass Spectrometry) analysis were nanopure grade purchased from Merck (Darmstadt, Germany), whereas *n*-Alkane (C_{10} to C_{21}) solvents were obtained from Sigma Chemical Company (St Louis, MO, USA). The solvents employed in other analyses were of analytic grade. DPPH was purchased from the Sigma Chemical Co., USA, while quercetin was obtained from Sigma-Aldrich.

Antimicrobial analysis

The materials used for antimicrobial activity were obtained from Mueller-Hinton Agar (Oxoid®/Brazil). The microorganisms (*Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231) were obtained from the American Type Culture Collection (ATCC, Reston, VA acquired from *Newprov*®/Brazil) and antibiotic (Nitrofurantoín, Imipenen, Tetraciclin, Fluconazol) discs were acquired from Cecon®/Brazil, namely, nitrofurantoín (300 μg) for *S. aureus*, imipenen (10 μg) for *P. aeruginosa*, tetraciclin (30 μg) for *E.coli* and fluconazole (50 μg) for *C. albicans*.

Plant Material

The leaves of the *C. adamantium* were colleted in the state of Mato Grosso do Sul, Brazil, in the cities of Dourados (**Ddos**; latitude 22º 11' 813'' S and longitude 054º 55' 801'' W) during the reproductive and vegetative stages, Bela Vista (**BV**; latitude 22º 06' 35.8'' S and longitude 056º 33' 00.8'' W), Bonito (**BO**; latitude 21° 07' 50.0'' S and longitude 056° 24' 68.0'' W) and Jardim (**Jd**; latitude 21° 25' 02.0'' S and longitude 056° 13'' 77.0'' W) in 2005 during the only fruit-bearing stage. The species were identified by Marcos Sobral (UFMG) and voucher specimens 5196 (Dourados), 5198 (Bela Vista), 5197(Bonito) and 5195 (Jardim)) have been deposited in the Mato Grosso do Sul Herbarium-HMS, Campo Grande, MS, Brazil.

Essential Oil Isolation

The oils were isolated from a 400 g quantity of fresh *Campomanesia adamantium* leaves collected during the flowering, fruit-bearing and vegetative stages and were subjected to hydrodistillation in a Clevenger-type apparatus for 4 hours. The oil percentages were expressed as w/w in relation to fresh weight of the initial material.

Identification of Essential Oil Constituents

Oil samples of *C. adamantium* were diluted in hexane and analyzed. Retention indices were calculated according to Zhao *et al.* (2005) and Isidorov *et al.* (1998) using a quasi-linear equation at linear temperature programmed GC operating conditions and a mixture of normal paraffin

(C_8-C_{21}) as external references. The identification of oil components was performed by comparing the spectra with those of Nist 2.0 and Saturn Libraries as well as comparison of their temperature-programmed retention indices and mass spectra with those described by Adams (1995).

Apparatus

The GC/MS system consisted of a gas chromatograph (GC 3900) equipped with an ion-trap mass spectrometer detector (Varian Saturn 2100), using a ZB-5 (5% of phenyl-dimethylpolysiloxane), fused-silica capillary column (30 m x 0.25 mm i.d., 0.25 μm film thickness), under the following conditions: carrier gas helium; 1μL injection volume, split at a ratio of (1:20), with initial oven temperature of 50 º C with heating from 50 ºC to 250 ºC at 3 ºC min^{-1}. The injector and ion trap detector temperatures were 240 ºC and 200 ºC, respectively, and manifold at 70 ºC with line transfer at 240 ºC. The MS scan parameters included an electron impact ionization voltage of 70 eV, a mass range of 40-380 $m.z^{-1}$ and a scan interval of 0.5 s. The antioxidant assay activity was recorded in methanol, employing a 700 S Femto UV Spectrophotometer at a wavelength of 517 nm.

Determination of DPPH (2,2′-diphenyl-1-picrylhydrazyl) Radical-Scavengers of Essential Oil Samples

The free radical scavenging activity of essential oils and quercetin standard solutions were determined based on their ability to react with the stable DPPH free radical. Two milliliters of DPPH (0.004% in methanol) was added to the essential oil solution in methanol at a concentration of 2270 $\mu g.mL^{-1}$ (flowering), 2320 $\mu g.mL^{-1}$ (vegetative) and 2390 $\mu g.mL^{-1}$ (fruit-bearing). After incubation at 25 ^oC for 30 minutes, the absorbance of each solution was determined at 517 nm. The antioxidant activity (%) of radical-scavengers was calculated as $(A_o\text{-}A_s/A_o) \times 100$, where A_s and A_o are the absorbance of the sample and control, respectively, at 517 nm.

Determination of Antimicrobial activity

The essential oils from *Campomanesia adamantium* leaves, collected during the flowering, fruit-bearing and vegetative stages, were individually tested against a microorganism panel, including *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Candida albicans* (ATCC 10231) in accordance with the Agar diffusion disc method (Brasileiro *et al.*, 2006). Briefly, the filter paper discs Whatman No. 1 (6 mm in diameter) were impregnated with 20 μL of the essential oil ethanolic solution at 2000 $\mu g.mL^{-1}$. *In vitro* antimicrobial activity was determined using Müeller Hinton Agar and then after Agar to solidify, the plates were inoculated with a suspension of the tested microorganism (0.1 mL of 1 x 10^8 UFC/mL) (turbidity based McFarland - Probac® - barium sulfate standard 0.5) and uniformly spread with a sterile swab. The discs were then applied and plates incubated at 37 ºC for 24 hours. The negative control assay was performed using only organisms and not the plant extract. The positive control used antibiotic discs (Cecon®) for each strain assay, with the nitrofurantoín (300 μg) for *Staphylococcus aureus*, imipenen (10 μg) for *Pseudomonas aeruginosa*, tetraciclin (30 μg) for *Escherichia coli* and fluconazole (50 μg) for *Candida albicans.* The diameters of the inhibition zones were measured in millimeters. All the assays were performed in triplicate.

RESULTS AND DISCUSSION

Characterization of the essential oils

Gas chromatography-mass spectrometry (GC-MS) has been used in the separation, identification and quantification of complex mixtures, such as essential oils. As a general rule, the identification of these compounds is not precise, because the mass spectra of these compounds are very similar and determination with the standard MS library is very difficult. For this reason the retention index –IR was used as a parameter for the GC qualitative analysis of the complex mixtures of isomers.

The *C. adamantium* leaves were collected in Dourados, Mato Grosso do Sul State during the reproductive and vegetative stages of the plant and submitted to hydrodistillation. The yields were 0.32% (flowering), 0.39% (fruit-bearing) and 0.19% (vegetative). These oil samples were then analyzed by GC-MS using a temperature program with a DB-5 capillary column.

A total of 95 compounds from the different stages of *C. adamantium* were identified, including the presence of terpenic hydrocarbons, ether, alcohol, aldehydes, ketones, esters, phenols and epoxides. Alcohol and hydrocarbons were the predominant class. Due to the complexity of the results the components were listed in order of elution on a DB-5 column, and their retention index and percentage composition are described in Table I.

All the samples of essential oil predominantly demonstrated compounds of the cyclic series. The principal pathway of observed cyclization from the monoterpenic

TABLE I - Volatile compounds identified in the essential oil of *Campomanesia adamantium* leaves at different phenological stages

Compounds[a]	RI[b]	RI[c]	Flowering	Fruit bearing	Vegetative
			Relative area (%)		
α-thujene	925	926	0.61	tr	-
α-pinene	939	934	13.23	7.45	0.07
α-fenchene	944	951	0.50	-	0.02
β-pinene	976	977	8.99	6.69	0.06
myrcene	990	991	0.88	0.21	-
mesitylene	993	994	tr	-	-
α-phellandrene	1004	1005	0.32	-	-
δ-3-carene	1010	1011	0.19	-	-
α-terpinene	1016	1018	0.24	-	-
o-cymene	1024	1022	1.49	0.15	0.09
limonene	1031	1031	22.24	0.99	0.66
1,8-cineole	1030	1033	0.87	0.44	-
(Z)-β-ocimene	1037	1040	0.03	-	-
(E)-β-ocimene	1047	1050	0.28	-	-
γ-terpinene	1058	1062	0.83	0.14	0.03
terpinolene	1087	1088	-	0.41	-
p-mentha-2,4(8)-diene	1088	1086	1.75	-	0.10
linalool	1100	1098	-	4.97	0.53
α-fenchol	1113	1112	0.34	tr	0.27
cis-*p*-menth-2-en-1-ol	1121	1121	0.06	-	0.09
α-camphonelal	1125	1125	tr	-	0.11
cis-limonene oxide	1132	1134	tr	-	-
trans-sabinol	1138	1140	0.06	-	0.03
camphor	1143	1143	-	-	0.02
camphene hydrate	1146	1148	0.11	-	0.02
isoborneol	1155	1156	0.01	-	0.01
borneol	1164	1165	0.45	0.24	0.37
3-thujy'alcohol	1167	1166	tr	-	-
terpin-4-ol	1176	1177	0.57	0.26	0.04
p-cymen-8-ol	1184	1183	0.09	-	-
(Z)-3-hexenyl butyrate	1186	1186	0.01	-	-
α-terpineol	1190	1189	1.40	0.58	0.37
myrtenol	1195	1194	0.07	-	0.04
trans-piperitol	1206	1205	tr	-	-
trans-carveol	1217	1217	0.03	-	0.03
nerol	1227	1228	0.02	-	-
cis-carveol	1229	1229	0.01	-	-
cumin aldehyde	1238	1339	tr	-	-
carvone	1242	1242	0.01	-	-
geraniol	1254	1255	0.01	-	0.02
perilla aldehyde	1272	1271	0.09	-	-
α-terpinen-7-al	1282	1282	0.02	-	-
p-cymen-7-ol	1286	1287	-	-	0.01
trans-sabinyl acetate	1290	1291	tr	-	-
carvacrol	1300	1298	-	-	0.02
neo-dihydro carveol acetate	1303	1303	tr	-	-
methyl geranate	1323	1323	0.02	-	-
δ-elemene	1337	1339	0.26	0.29	0.63
α-cubebene	1349	1351	0.04	-	0.06
cyclosativene	1371	1368	0.07	-	0.14
α-ylangene	1372	1372	0.05	0.17	0.10
α-copaene	1375	1376	0.37	-	1.40
isoledene	1376	1373	-	1.57	-

TABLE I - Volatile compounds identified in the essential oil of *Campomanesia adamantium* leaves at different phenological stages (cont.)

Compounds[a]	RI[b]	RI[c]	Flowering	Fruit bearing	Vegetative
			Relative area (%)		
β-elemene	1391	1391	0.60	0.50	1.21
α-gurjunene	1409	1409	0.15	0.26	0.24
β-caryophyllene	1419	1418	3.23	8.97	6.12
β-gurjunene	1428	1432	0.21	0.36	0.35
aromadendrene	1438	1439	0.79	1.38	2.48
α-humullene	1453	1454	1.12	4.67	2.60
seychellene	1460	1460	0.43	1.01	-
cis-muurola-4(14)-5-diene	1462	1460	0.05	-	1.28
drima-7,9(11)-diene	1469	1469	tr	-	-
γ-gurjunene	1472	1473	-	-	0.09
γ-muurolene	1476	1477	0.68	1.00	1.15
germacrene D	1481	1480	2.66	11.82	5.87
β-selinene	1485	1485	0.34	0.22	0.47
cis-β-guaiene	1491	1490	0.21	-	0.23
bicyclogermacrene	1496	1494	4.48	18.95	16.17
trans-β-guaiene	1500	1500	-	-	0.52
α-bulnesene	1504	1505	0.14	-	0.18
germacrene A	1505	1503	-	0.42	-
γ-cadinene	1513	1513	0.47	0.60	0.97
δ-cadinene	1523	1524	1.67	3.63	2.82
cadina-1,4-diene	1532	1532	0.04	-	0.08
α-cadinene	1537	1538	0.13	-	0.18
selina-3,7(11)-diene	1541	1542	0.06	-	-
α-calacorene	1542	1542	0.04	-	-
germacrene B	1556	1556	0.36	0.27	0.30
epi-longipinanol	1559	1561	-	0.42	-
(E)-nerolidol	1564	1564	1.07	-	0.15
ledol	1566	1565	-	1.06	-
spathulenol	1577	1576	2.08	1.62	7.34
globulol	1584	1583	3.91	4.64	11.05
viridiflorol	1591	1590	-	2.54	-
guaiol	1593	1595	0.19	1.10	-
humulene epoxide II	1608	1606	0.24	tr	1.64
epi-1,10-di-cubenol	1614	1614	0.26	tr	0.29
epi-1-cubenol	1627	1627	0.64	-	1.33
γ-eudesmol	1631	1630	0.40	0.21	0.86
epi-α-cadinol	1640	1640	1.00	1.99	1.17
α-muurolol	1645	1645	0.50	0.56	0.85
α-cadinol	1654	1653	2.63	2.18	2.98
cadalene	1675	1674	-	-	0.19
juniper camphor	1693	1691	0.07	-	-

[a] Constituents listed in order of elution in DB-5 column. [b] RI= Retention index calculation using a temperature program according to n-alkanes. [c] RI= Retention index described by Adams17. tr = traces (%< 0.01)

compounds were mentane and pinane, best represented by limonene and α-pinene. The principal pathway cyclization from the sesquiterpenic compounds was germacrane, represented by the main compounds bicyclogermacrene, germacrene D and globulol.

A total of 82 compounds were identified in the essential oil from the flowering stage, where there were 48.78% of both monoterpenes and sesquiterpenes. The main compounds identified in this essential oil were limonene (22.24%), α-pinene (13.23%) and β-pinene (8.99%). During the fruit-bearing stage there were 44 compounds identified , consisting of 31.82% monoterpenes and 68.19% sesquiterpenes, the main compounds of which were bicyclogermacrene (18.95%), germacrene

D (11.82%), β-caryophyllene (8.97%), α-pinene (7.45%) and β-pinene (6.69%). In the essential oil composition collected from the vegetative stage of the same plant, 60 compounds were identified. This consisted of 38.33% monoterpenes and 61.67% sesquiterpenes, with the main compounds being bicyclogermacrene (16.17%), globulol (11.05%), β-caryophyllene (6.12%) and germacrene D (5.87%).

The chemical composition of the essential oils from different reproductive and vegetative stages was similar in relation to major components, however the composition percentage of these was very different. This is because the samples from the vegetative stage showed a higher amount of sesquiterpenes (relative area), while the flowering stage samples showed the opposite, in which the major compounds were monoterpenes.

Studies reporting on *C. xanthocarpa* (Limberger *et al.*, 2004) and *C. phaea* (Adati, Ferro, 2006) leaves, both collected in the vegetative stage, showed predominance in the sesquiterpenes, while in the *C. lineatifolia* (Osorio *et al.*, 2006) leaves studied during the fruit-bearing stage the major compounds were 1,8-cineol, α-pinene and β-caryophyllene.

The production and types of the terpenes can be linked to external factors, such as differences in light, temperature and water levels (Lima *et al.*, 2003). During the flowering stage, the plant was exposed to rain and high temperatures in the spring, while during the vegetative stage the plant was exposed to dryness and low temperatures in the fall. At the fruit-bearing stages the relative area is very well divided between monoterpenes and sesquiterpenes, with high temperatures and less rain during the summer. The chemical variability can also be related to an adaptation of pollination from different species of insects, due to the reproductive strategy of the plant (Stefanello *et al.*, 2006).

In addition to the aforementioned factors contributing to differences in the chemical composition of the essential oils, the differing altitudes and soil types between our sample collection areas may also be a factor. Due to these factors, the *C. adamantium* leaves were collected in the cities of Dourados, Bonito, Jardim and Bela Vista during the fruit-bearing stage and were submitted to hydrodistillation where the essential oils yielded 0.39; 0.20; 0.10 and 0.13%, respectively.

Table 2 shows the difference in the chemical composition of the samples collected from different regions, while Figure 1 shows the variation in relative areas of the major compounds identified in the four cities. The samples from Dourados and Jardim are characterized by sesquiterpene bicyclogermacrene, germacrene D and β- caryophyllene amounts, while the samples from Bela Vista and Bonito are similar, mainly in the monoterpene amounts of α-pinene, β-pinene, limonene and linalool.

TABLE II - Compounds identified in the essential oil of *Campomanesia adamantium* leaves in different localities of Mato Grosso do Sul State, Brazil, during the fruit-bearing stage

Compounds[a]	IR_{lit}[b]	IR_{cal}[c]	BV	BO	Jd	Ddos
			Relative area (%)			
α-thujene	931	925	-	Tr	-	tr
α-pinene	939	931	11.29	12.58	5.02	7.45
α-fenchene	951	946	Tr	0.44	-	-
n-heptanol	969	965	-	0.04	-	-
pentyl propanoate	972	968	-	tr	-	-
β-pinene	976	975	5.54	9.81	3.36	6.69
myrcene	991	990	0.56	0.88	Tr	-
α-phellandrene	1005	1004	Tr	0.45	0.93	-
δ-3-carene	1011	1010	tr	0.22	-	-
α-terpinene	1018	1016	0.25	0.39	-	-
o-cymene	1022	1023	0.41	0.97	5.45	0.15
limonene	1031	1027	11.06	24.00	Tr	0.99
1.8-cineole	1033	1031	3.65	1.41	-	0.44
(Z)-β-ocimene	1040	1037	-	tr	0.55	-
(E)-β-ocimene	1050	1047	0.31	0.32	0.81	-
γ-terpinene	1062	1058	0.61	1.25	-	0.14
terpinolene	1088	1088	0.91	2.49	0.94	0.41
linalool	1098	1100	7.40	3.60		4.97
α-fenchol	1112	1113	0.30	0.19	-	tr
borneol	1165	1164	0.53	0.39	-	0.24
terpin-4-ol	1177	1176	-	0.53	-	0.26

TABLE II - Compounds identified in the essential oil of *Campomanesia adamantium* leaves in different localities of Mato Grosso do Sul State, Brazil, during the fruit-bearing stage (cont.)

Compounds[a]	IR_{lit} [b]	IR_{cal} [c]	BV	BO	Jd	Ddos
			Relative area (%)			
α-terpineol	1189	1189	2.38	0.17	-	0.58
Myrtenol	1194	1195	-	tr	-	-
(E)-2-decenal	1261	1261	-	tr	-	-
perilla aldehyde	1271	1273	-	tr	-	-
perillol	1295	1297	1.24	tr	-	-
δ-elemene	1339	1337	0.31	0.37	Tr	0.29
α-cubebene	1351	1350	-	tr	-	-
α-ilangene	1372	1372	-	-	-	0.17
α-copaene	1376	1376	0.20	0.27	1.42	1.57
β-elemene	1391	1392	0.81	0.27	0.93	0.50
α-gurjunene	1409	1409	0.41	tr	-	0.26
β-caryophyllene	1418	1419	3.12	3.15	8.14	8.97
β-gurjunene	1432	1429	-	0.13	Tr	0.36
aromadendrene	1439	1439	1.32	0.54	0.50	1.38
Z-β-farnesene		1443	-	0.04	-	-
α- caryophyllene	1454	1453	1.01	0.93	2.07	4.67
Seichellene	1460	1460	0.55	0.30	0.70	1.01
γ-muurolene	1477	1477	0.76	0.57	1.64	1.00
germacreno D	1480	1481	4.86	2.97	10.65	11.82
β-selinene	1485	1486	-	0.17	0.77	0.22
valencene	1491	1491	-	0.24	0.81	-
biciclogermacrene	1494	1496	9.13	5.97	20.05	18.95
α-bulnesene	1505	1505	0.31	-	-	-
germacrene A	1503	1505	-	0.15	-	0.42
γ-cadinene	1513	1514	0.49	0.39	0.82	0.60
δ-cadinene	1524	1524	1.25	1.25	4.18	3.63
cadina-1,4-diene	1532	1532	-	tr	-	-
α-cadinene	1538	1538	-	tr	-	-
selina-3,7(11)-diene	1542	1542	-	tr	-	-
elemol	1549	1552	-	tr	-	-
germacrene B	1556	1557	-	0.16	0.64	0.27
epi-longipinanol	1561	1560	-	0.12	-	0.42
(E)-nerolidol	1564	1564	5.50	0.82	Tr	-
ledol	1565	1565	-	0.13	1.93	1.06
spathulenol	1576	1576	2.64	1.15	1.15	1.62
globulol	1583	1583	6.46	3.63	5.82	4.64
viridiflorol	1590	1590	1.85	1.16	2.38	2.54
cis-β-elemonene	1594	1593	-	-	1.18	
guaiol	1595	1595	-	0.70	-	1.10
humullene epoxide II	1606	1609	0.33	0.19	Tr	tr
epi-1,10-di-cubenol	1614	1615	-	0.18	Tr	tr
epi-10-δ-eudesmol	1619	1619	-	0.23	Tr	-
epi-1-cubenol	1627	1627	0.44	0.56	0.40	-
γ-eudesmol	1630	1630	-	tr	0.25	0.21
epi-α-cadinol	1641	1641	1.70	1.92	-	1.99
α-muurolol	1645	1646	0.36	0.23	3.00	0.56
α-cadinol	1653	1654	1.89	2.35	0.84	2.18

[a] Constituents listed in order of elution in DB-5 column. [b] RI= Retention index calculation using a temperature program according to n-alkanes. [c] RI= Retention index described by Adams17. tr = traces (%< 0.01).

From these results it can be concluded that the chemical composition of the essential oil from the leaves of the *C. adamantium* is influenced during different stages (including the fruit-bearing stage) as well as by different regions.

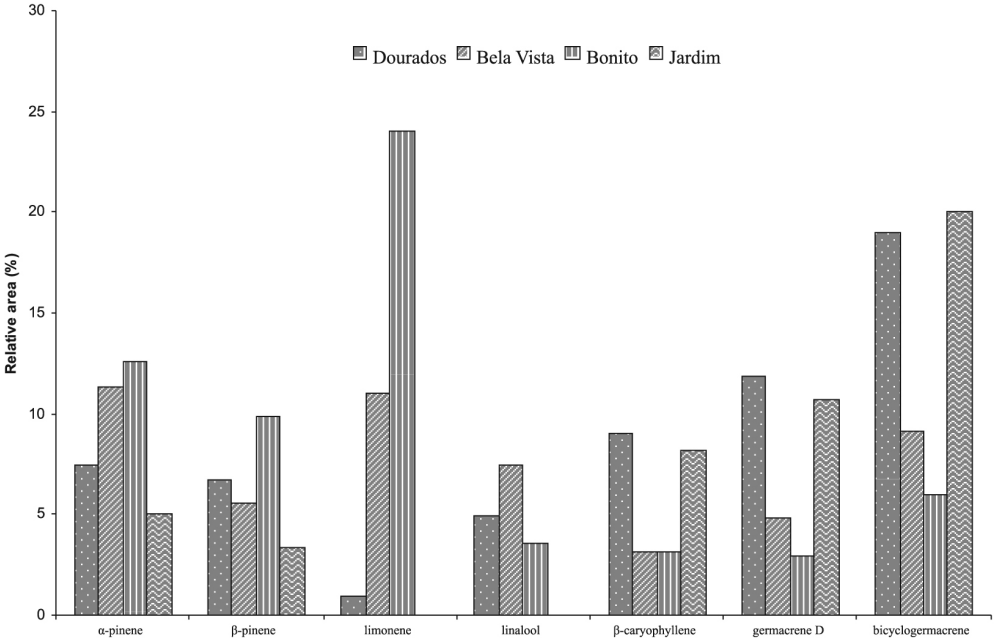

FIGURE 1 - Major compounds identified in the *Campomanesia adamantium* leaves collected in different localities of Mato Grosso of the Sul State, Brazil during the fruit-bearing stage.

Determination of DPPH Radical-Scavengers

The essential oils were screened for antioxidant activity. The use of 1,1-diphenyl-2-picryl-hydrazyl (DPPH) as a reagent for screening the antioxidant activity of small molecules has been reported (Tepe *et al.*, 2005).

The inhibition percentage of the radical-scavengers activity in the essential oil was 9.91% (2270 μg.mL^{-1}) at the flowering stage, 7.47% (2390 μg.mL^{-1}) at the fruit-bearing stage, and 6.89% (2320 μg.mL^{-1}) at the vegetative stage. The reference compound quercetin showed a scavenging effect of 90% (20 μg.mL^{-1}), 91% (40 μg.mL^{-1}), 93% (80 μg.mL^{-1}), 94% (160 μg.mL^{-1}) and 97% (320 μg.mL^{-1}).

In this test, the scavenging of the DPPH radical is followed by monitoring of the decrease in absorbance at 517 nm, which occurs due to the antioxidant reduction, and has been used to assess the ability of phenolic compounds to transfer labile H atoms to radicals (Djeridane *et al.*, 2006). The lower antioxidant activity has been attributed to the absence and/or lower amount of the donor groups of the electron in ortho position in relation to phenolic hydroxyl, and the presence of larger amounts of hydrocarbons terpenes. This result is in agreement with other studies of essential oils with similar patterns (Sacchetti *et al.*, 2005).

Assay of antimicrobial activity

Results from the assessment of antimicrobial activity using the Agar diffusion disc method are summarized in Table 3. The essential oil at all stages exhibited moderate to high activity against the tested microorganism. The essential oil in the flowering and fruit-bearing stages exhibited an even better effect than that provided by the reference antibiotics against *Staphylococcus aureus* and *Candida albicans,* and moderate effect in relation to *Pseudomonas aeruginosa* and *Escherichia coli*. Meanwhile, the samples

TABLE III - Antimicrobial activity of the essential oil from *Campomanesia adamantium* leaves

Microorganism	EOFl*	EOFr*	EOV*	Antibiotics
Staphylococcus aureus	20.00±0.40	20.00±0.60	16.00±0.20	22.00±0,60[a]
Pseudomonas aeruginosa	10.00±0,20	10.00±0,00	6.00±0,00	17.40±0.60[b]
Escherichia coli	2.00±0,00	2.00±0,00	2.00 ±0,00	4.00±0.00[c]
Candida albicans	26.00±0,60	26.00±0,40	16.00±0.20	22.00±0.40[d]

*EOFl: essential oil flowering stage; EOFr: essential oil fruit-bearing stage; EOV: essential oil vegetative stage. All analyses used 40 μg of the essential oil samples. [a] nitrofurantoin (300 μg); [b] Impenen (10 μg); [c] tetracicilin (30 μg); [d] fluconazole (50 μg).

of essential oil during the vegetative stage showed very weak activity against all tested microorganisms.

The different antimicrobial activity offered by essential oils, can be linked to their different chemical compositions, therefore the essential oils isolated from the flowering and fruit-bearing stages have larger amounts of monoterpene hydrocarbons with allylic groups and ether, alcohol, aldehydes, ketones, esters and phenols than the essential oil isolated from the vegetative stage. The biological activity of the terpenes can be seen in relation to the chemical structure, functional groups and stereochemistry of identified compounds (Henriques *et al.*, 2006).

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