

# 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,  $\alpha$ -pinene and  $\beta$ -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  $\alpha$ -pinene,  $\beta$ -pinene, limonene, linalool,  $\beta$ -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/characterization.

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,  $\alpha$ -pineno e  $\beta$ -pineno) e durante o estágio vegetativo as substâncias majoritárias foram sesquiterpenos (bicyclogermacreno 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  $\alpha$ -pineno,  $\beta$ -pineno, limoneno, linalol,  $\beta$ -cariofileno, germacreno D e bicyclogermacreno, 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<sub>10</sub> to C<sub>21</sub>) 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 (Nitrofurantoin, Imipenen, Tetracilin, Fluconazole) discs were acquired from Cecon®/Brazil, namely, nitrofurantoin (300 µg) for *S. aureus*, imipenen (10 µg) for *P. aeruginosa*, tetracilin (30 µg) for *E. coli* and fluconazole (50 µg) for *C. albicans*.

### Plant Material

The leaves of the *C. adamantium* were collected in the state of Mato Grosso do Sul, Brazil, in the cities of Dourados (**Ddos**; latitude 22° 11' 813" S and longitude 054° 55' 801" W) during the reproductive and vegetative stages, Bela Vista (**BV**; latitude 22° 06' 35.8" S and longitude 056° 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<sub>8</sub>-C<sub>21</sub>) 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; 1 µL injection volume, split at a ratio of (1:20), with initial oven temperature of 50 °C with heating from 50 °C to 250 °C at 3 °C min<sup>-1</sup>. The injector and ion trap detector temperatures were 240 °C and 200 °C, respectively, and manifold at 70 °C with line transfer at 240 °C. The MS scan parameters included an electron impact ionization voltage of 70 eV, a mass range of 40-380 m.z<sup>-1</sup> 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 µg.mL<sup>-1</sup> (flowering), 2320 µg.mL<sup>-1</sup> (vegetative) and 2390 µg.mL<sup>-1</sup> (fruit-bearing). After incubation at 25 °C for 30 minutes, the absorbance of each solution was determined at 517 nm. The antioxidant activity (%) of radical-scavengers was calculated as  $(A_0 - A_s/A_0) \times 100$ , where A<sub>s</sub> and A<sub>0</sub> 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 µg.mL<sup>-1</sup>. *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<sup>8</sup> 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 °C 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 nitrofurantoin (300 µg) for *Staphylococcus aureus*, imipenen (10 µg) for *Pseudomonas aeruginosa*, tetracyclin (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 Douros, 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 <sup>a</sup>              | RI <sup>b</sup> | RI <sup>c</sup> | Flowering         | Fruit bearing | Vegetative |
|-------------------------------------|-----------------|-----------------|-------------------|---------------|------------|
|                                     |                 |                 | Relative area (%) |               |            |
| $\alpha$ -thujene                   | 925             | 926             | 0.61              | tr            | -          |
| $\alpha$ -pinene                    | 939             | 934             | 13.23             | 7.45          | 0.07       |
| $\alpha$ -fenchene                  | 944             | 951             | 0.50              | -             | 0.02       |
| $\beta$ -pinene                     | 976             | 977             | 8.99              | 6.69          | 0.06       |
| myrcene                             | 990             | 991             | 0.88              | 0.21          | -          |
| mesitylene                          | 993             | 994             | tr                | -             | -          |
| $\alpha$ -phellandrene              | 1004            | 1005            | 0.32              | -             | -          |
| $\delta$ -3-carene                  | 1010            | 1011            | 0.19              | -             | -          |
| $\alpha$ -terpinene                 | 1016            | 1018            | 0.24              | -             | -          |
| <i>o</i> -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          | -          |
| ( <i>Z</i> )- $\beta$ -ocimene      | 1037            | 1040            | 0.03              | -             | -          |
| ( <i>E</i> )- $\beta$ -ocimene      | 1047            | 1050            | 0.28              | -             | -          |
| $\gamma$ -terpinene                 | 1058            | 1062            | 0.83              | 0.14          | 0.03       |
| terpinolene                         | 1087            | 1088            | -                 | 0.41          | -          |
| <i>p</i> -mentha-2,4(8)-diene       | 1088            | 1086            | 1.75              | -             | 0.10       |
| linalool                            | 1100            | 1098            | -                 | 4.97          | 0.53       |
| $\alpha$ -fenchol                   | 1113            | 1112            | 0.34              | tr            | 0.27       |
| <i>cis-p</i> -menth-2-en-1-ol       | 1121            | 1121            | 0.06              | -             | 0.09       |
| $\alpha$ -camphonelal               | 1125            | 1125            | tr                | -             | 0.11       |
| <i>cis</i> -limonene oxide          | 1132            | 1134            | tr                | -             | -          |
| <i>trans</i> -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       |
| <i>p</i> -cymen-8-ol                | 1184            | 1183            | 0.09              | -             | -          |
| ( <i>Z</i> )-3-hexenyl butyrate     | 1186            | 1186            | 0.01              | -             | -          |
| $\alpha$ -terpineol                 | 1190            | 1189            | 1.40              | 0.58          | 0.37       |
| myrtenol                            | 1195            | 1194            | 0.07              | -             | 0.04       |
| <i>trans</i> -piperitol             | 1206            | 1205            | tr                | -             | -          |
| <i>trans</i> -carveol               | 1217            | 1217            | 0.03              | -             | 0.03       |
| nerol                               | 1227            | 1228            | 0.02              | -             | -          |
| <i>cis</i> -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              | -             | -          |
| $\alpha$ -terpinen-7-al             | 1282            | 1282            | 0.02              | -             | -          |
| <i>p</i> -cymen-7-ol                | 1286            | 1287            | -                 | -             | 0.01       |
| <i>trans</i> -sabinyl acetate       | 1290            | 1291            | tr                | -             | -          |
| carvacrol                           | 1300            | 1298            | -                 | -             | 0.02       |
| <i>neo</i> -dihydro carveol acetate | 1303            | 1303            | tr                | -             | -          |
| methyl geranate                     | 1323            | 1323            | 0.02              | -             | -          |
| $\delta$ -elemene                   | 1337            | 1339            | 0.26              | 0.29          | 0.63       |
| $\alpha$ -cubebene                  | 1349            | 1351            | 0.04              | -             | 0.06       |
| cyclosativene                       | 1371            | 1368            | 0.07              | -             | 0.14       |
| $\alpha$ -ylangene                  | 1372            | 1372            | 0.05              | 0.17          | 0.10       |
| $\alpha$ -copaene                   | 1375            | 1376            | 0.37              | -             | 1.40       |
| isolede                             | 1376            | 1373            | -                 | 1.57          | -          |

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

| Compounds <sup>a</sup>            | RI <sup>b</sup> | RI <sup>c</sup> | Flowering         | Fruit bearing | Vegetative |
|-----------------------------------|-----------------|-----------------|-------------------|---------------|------------|
|                                   |                 |                 | Relative area (%) |               |            |
| $\beta$ -elemene                  | 1391            | 1391            | 0.60              | 0.50          | 1.21       |
| $\alpha$ -gurjunene               | 1409            | 1409            | 0.15              | 0.26          | 0.24       |
| $\beta$ -caryophyllene            | 1419            | 1418            | 3.23              | 8.97          | 6.12       |
| $\beta$ -gurjunene                | 1428            | 1432            | 0.21              | 0.36          | 0.35       |
| aromadendrene                     | 1438            | 1439            | 0.79              | 1.38          | 2.48       |
| $\alpha$ -humullene               | 1453            | 1454            | 1.12              | 4.67          | 2.60       |
| seychellene                       | 1460            | 1460            | 0.43              | 1.01          | -          |
| <i>cis</i> -muurola-4(14)-5-diene | 1462            | 1460            | 0.05              | -             | 1.28       |
| drima-7,9(11)-diene               | 1469            | 1469            | tr                | -             | -          |
| $\gamma$ -gurjunene               | 1472            | 1473            | -                 | -             | 0.09       |
| $\gamma$ -muurolene               | 1476            | 1477            | 0.68              | 1.00          | 1.15       |
| germacrene D                      | 1481            | 1480            | 2.66              | 11.82         | 5.87       |
| $\beta$ -selinene                 | 1485            | 1485            | 0.34              | 0.22          | 0.47       |
| <i>cis</i> - $\beta$ -guaiene     | 1491            | 1490            | 0.21              | -             | 0.23       |
| bicyclogermacrene                 | 1496            | 1494            | 4.48              | 18.95         | 16.17      |
| <i>trans</i> - $\beta$ -guaiene   | 1500            | 1500            | -                 | -             | 0.52       |
| $\alpha$ -bulnesene               | 1504            | 1505            | 0.14              | -             | 0.18       |
| germacrene A                      | 1505            | 1503            | -                 | 0.42          | -          |
| $\gamma$ -cadinene                | 1513            | 1513            | 0.47              | 0.60          | 0.97       |
| $\delta$ -cadinene                | 1523            | 1524            | 1.67              | 3.63          | 2.82       |
| cadina-1,4-diene                  | 1532            | 1532            | 0.04              | -             | 0.08       |
| $\alpha$ -cadinene                | 1537            | 1538            | 0.13              | -             | 0.18       |
| selina-3,7(11)-diene              | 1541            | 1542            | 0.06              | -             | -          |
| $\alpha$ -calacorene              | 1542            | 1542            | 0.04              | -             | -          |
| germacrene B                      | 1556            | 1556            | 0.36              | 0.27          | 0.30       |
| <i>epi</i> -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       |
| <i>epi</i> -1,10-di-cubenol       | 1614            | 1614            | 0.26              | tr            | 0.29       |
| <i>epi</i> -1-cubenol             | 1627            | 1627            | 0.64              | -             | 1.33       |
| $\gamma$ -eudesmol                | 1631            | 1630            | 0.40              | 0.21          | 0.86       |
| <i>epi</i> - $\alpha$ -cadinol    | 1640            | 1640            | 1.00              | 1.99          | 1.17       |
| $\alpha$ -muurolol                | 1645            | 1645            | 0.50              | 0.56          | 0.85       |
| $\alpha$ -cadinol                 | 1654            | 1653            | 2.63              | 2.18          | 2.98       |
| cadalene                          | 1675            | 1674            | -                 | -             | 0.19       |
| juniper camphor                   | 1693            | 1691            | 0.07              | -             | -          |

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

compounds were mentane and pinane, best represented by limonene and  $\alpha$ -pinene. The principal pathway cyclization from the sesquiterpenic compounds was germacrene, 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%),  $\alpha$ -pinene (13.23%) and  $\beta$ -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%),  $\beta$ -caryophyllene (8.97%),  $\alpha$ -pinene (7.45%) and  $\beta$ -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%),  $\beta$ -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,  $\alpha$ -pinene and  $\beta$ -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 hydro-distillation 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  $\beta$ -caryophyllene amounts, while the samples from Bela Vista and Bonito are similar, mainly in the monoterpene amounts of  $\alpha$ -pinene,  $\beta$ -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 <sup>a</sup>         | IR <sub>lit</sub> <sup>b</sup> | IR <sub>cal</sub> <sup>c</sup> | Relative area (%) |       |      |      |
|--------------------------------|--------------------------------|--------------------------------|-------------------|-------|------|------|
|                                |                                |                                | BV                | BO    | Jd   | Ddos |
| $\alpha$ -thujene              | 931                            | 925                            | -                 | Tr    | -    | tr   |
| $\alpha$ -pinene               | 939                            | 931                            | 11.29             | 12.58 | 5.02 | 7.45 |
| $\alpha$ -fenchene             | 951                            | 946                            | Tr                | 0.44  | -    | -    |
| <i>n</i> -heptanol             | 969                            | 965                            | -                 | 0.04  | -    | -    |
| <i>pentyl</i> propanoate       | 972                            | 968                            | -                 | tr    | -    | -    |
| $\beta$ -pinene                | 976                            | 975                            | 5.54              | 9.81  | 3.36 | 6.69 |
| myrcene                        | 991                            | 990                            | 0.56              | 0.88  | Tr   | -    |
| $\alpha$ -phellandrene         | 1005                           | 1004                           | Tr                | 0.45  | 0.93 | -    |
| $\delta$ -3-carene             | 1011                           | 1010                           | tr                | 0.22  | -    | -    |
| $\alpha$ -terpinene            | 1018                           | 1016                           | 0.25              | 0.39  | -    | -    |
| <i>o</i> -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 |
| ( <i>Z</i> )- $\beta$ -ocimene | 1040                           | 1037                           | -                 | tr    | 0.55 | -    |
| ( <i>E</i> )- $\beta$ -ocimene | 1050                           | 1047                           | 0.31              | 0.32  | 0.81 | -    |
| $\gamma$ -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 |
| $\alpha$ -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 |

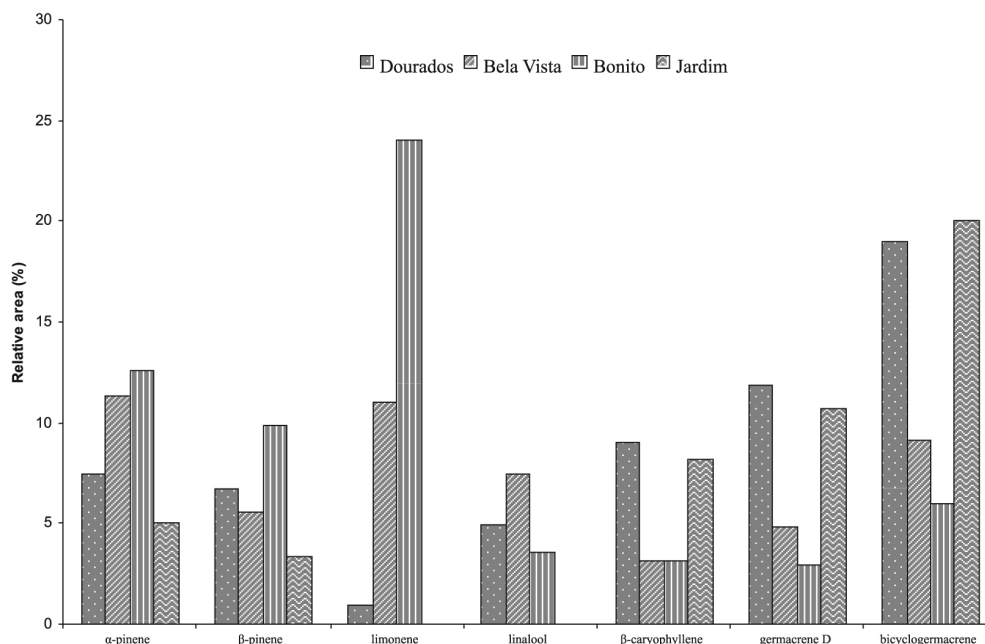
**TABLE II** - Compounds identified in the essential oil of *Campomanesia adamantium* leaves in different localities of Mato Grosso do Sul State, Brazil, during the fruit-bearing stage (cont.)

| Compounds <sup>a</sup>             | IR <sub>lit</sub> <sup>b</sup> | IR <sub>cal</sub> <sup>c</sup> | BV   | BO   | Jd    | Ddos  |
|------------------------------------|--------------------------------|--------------------------------|------|------|-------|-------|
|                                    | Relative area (%)              |                                |      |      |       |       |
| $\alpha$ -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   | -     | -     |
| $\delta$ -elemene                  | 1339                           | 1337                           | 0.31 | 0.37 | Tr    | 0.29  |
| $\alpha$ -cubebene                 | 1351                           | 1350                           | -    | tr   | -     | -     |
| $\alpha$ -ilangene                 | 1372                           | 1372                           | -    | -    | -     | 0.17  |
| $\alpha$ -copaene                  | 1376                           | 1376                           | 0.20 | 0.27 | 1.42  | 1.57  |
| $\beta$ -elemene                   | 1391                           | 1392                           | 0.81 | 0.27 | 0.93  | 0.50  |
| $\alpha$ -gurjunene                | 1409                           | 1409                           | 0.41 | tr   | -     | 0.26  |
| $\beta$ -caryophyllene             | 1418                           | 1419                           | 3.12 | 3.15 | 8.14  | 8.97  |
| $\beta$ -gurjunene                 | 1432                           | 1429                           | -    | 0.13 | Tr    | 0.36  |
| aromadendrene                      | 1439                           | 1439                           | 1.32 | 0.54 | 0.50  | 1.38  |
| Z- $\beta$ -farnesene              |                                | 1443                           | -    | 0.04 | -     | -     |
| $\alpha$ -caryophyllene            | 1454                           | 1453                           | 1.01 | 0.93 | 2.07  | 4.67  |
| Seichellene                        | 1460                           | 1460                           | 0.55 | 0.30 | 0.70  | 1.01  |
| $\gamma$ -muurolene                | 1477                           | 1477                           | 0.76 | 0.57 | 1.64  | 1.00  |
| germacreno D                       | 1480                           | 1481                           | 4.86 | 2.97 | 10.65 | 11.82 |
| $\beta$ -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 |
| $\alpha$ -bulnesene                | 1505                           | 1505                           | 0.31 | -    | -     | -     |
| germacrene A                       | 1503                           | 1505                           | -    | 0.15 | -     | 0.42  |
| $\gamma$ -cadinene                 | 1513                           | 1514                           | 0.49 | 0.39 | 0.82  | 0.60  |
| $\delta$ -cadinene                 | 1524                           | 1524                           | 1.25 | 1.25 | 4.18  | 3.63  |
| cadina-1,4-diene                   | 1532                           | 1532                           | -    | tr   | -     | -     |
| $\alpha$ -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  |
| <i>epi</i> -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  |
| <i>cis</i> - $\beta$ -elemonene    | 1594                           | 1593                           | -    | -    | 1.18  | -     |
| guaïol                             | 1595                           | 1595                           | -    | 0.70 | -     | 1.10  |
| humullene epoxide II               | 1606                           | 1609                           | 0.33 | 0.19 | Tr    | tr    |
| <i>epi</i> -1,10-di-cubenol        | 1614                           | 1615                           | -    | 0.18 | Tr    | tr    |
| <i>epi</i> -10- $\delta$ -eudesmol | 1619                           | 1619                           | -    | 0.23 | Tr    | -     |
| <i>epi</i> -1-cubenol              | 1627                           | 1627                           | 0.44 | 0.56 | 0.40  | -     |
| $\gamma$ -eudesmol                 | 1630                           | 1630                           | -    | tr   | 0.25  | 0.21  |
| <i>epi</i> - $\alpha$ -cadinol     | 1641                           | 1641                           | 1.70 | 1.92 | -     | 1.99  |
| $\alpha$ -muurolol                 | 1645                           | 1646                           | 0.36 | 0.23 | 3.00  | 0.56  |
| $\alpha$ -cadinol                  | 1653                           | 1654                           | 1.89 | 2.35 | 0.84  | 2.18  |

<sup>a</sup> Constituents listed in order of elution in DB-5 column. <sup>b</sup> RI= Retention index calculation using a temperature program according to n-alkanes. <sup>c</sup> 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% ( $2270 \mu\text{g.mL}^{-1}$ ) at the flowering stage, 7.47% ( $2390 \mu\text{g.mL}^{-1}$ ) at the fruit-bearing stage, and 6.89% ( $2320 \mu\text{g.mL}^{-1}$ ) at the vegetative stage. The reference compound quercetin showed a scavenging effect of 90% ( $20 \mu\text{g.mL}^{-1}$ ), 91% ( $40 \mu\text{g.mL}^{-1}$ ), 93% ( $80 \mu\text{g.mL}^{-1}$ ), 94% ( $160 \mu\text{g.mL}^{-1}$ ) and 97% ( $320 \mu\text{g.mL}^{-1}$ ).

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             |
|-------------------------------|------------|------------|------------|-------------------------|
| <i>Staphylococcus aureus</i>  | 20.00±0.40 | 20.00±0.60 | 16.00±0.20 | 22.00±0.60 <sup>a</sup> |
| <i>Pseudomonas aeruginosa</i> | 10.00±0.20 | 10.00±0.00 | 6.00±0.00  | 17.40±0.60 <sup>b</sup> |
| <i>Escherichia coli</i>       | 2.00±0.00  | 2.00±0.00  | 2.00±0.00  | 4.00±0.00 <sup>c</sup>  |
| <i>Candida albicans</i>       | 26.00±0.60 | 26.00±0.40 | 16.00±0.20 | 22.00±0.40 <sup>d</sup> |

\*EOFl: essential oil flowering stage; EOFr: essential oil fruit-bearing stage; EOV: essential oil vegetative stage. All analyses used 40  $\mu\text{g}$  of the essential oil samples. <sup>a</sup> nitrofurantoin (300  $\mu\text{g}$ ); <sup>b</sup> Imipenem (10  $\mu\text{g}$ ); <sup>c</sup> tetracycline (30  $\mu\text{g}$ ); <sup>d</sup> fluconazole (50  $\mu\text{g}$ ).



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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