Chemical Composition and Cytotoxic Activity of the Essential Oils of *Cantinoa stricta* (Benth.) Harley & J.F.B. Pastore (Lamiaceae)

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**Abstract:** The essential oils from the leaves and flowers of *Cantinoa stricta* (Benth.) Harley & J.F.B. Pastore (Lamiaceae) were obtained by hydrodistillation and analyzed by GC/FID and GC/MS. The major components of both oils were caryophyllene oxide (leaf – 31.6%; flower – 21.7%) and cis-pinane (leaf – 15.4%; flower – 9.7%). The flower oil also contained significant amounts of α-pinene (9.4%) and β-pinene (9.1%). The oils were tested in vitro against U251 (glioma), UACC-62 (melanoma), MCF-7 (breast), NCI-H460 (lung), PC-3 (prostate), K-562 (leukemia) human cancer cell lines and against HaCat (no cancer cell), using the sulforhodamine B method. Both oils showed antiproliferative activity against all tested cells lines (TGI < 50 \(\mu\)g/mL), with exception of K562 cells. The highest activity was observed against MCF-7 cell lines (TGI = 4.54-10.36 \(\mu\)g/mL).

**Keywords:** Lamiaceae; *Cantinoa stricta*; essential oil; caryophyllene oxide; cytotoxic activity. © 2015 ACG Publications. All rights reserved.

1. **Plant Source**

Lamiaceae comprises ca. 240 genera and 7200 species, distributed through the tropical and temperate regions of the world. In the last years, studies of molecular phylogeny have led to a revision on the limits of several genera; in particular, the large genus *Hyptis* (280 species) was recently reorganized. The new classification recognized only 144 species in *Hyptis (sensu stricto)*, dividing the remaining species into several genera. A total of 23 species were assigned to the new genus *Cantinoa*, 21 of which occur in Brazil [1, 2].

*Cantinoa stricta* (Benth.) Harley & J.F.B. Pastore (formerly *Hyptis stricta* Benth.) is an aromatic herb native from Brazil and distributed over the Southern Region of the country [2]. Flowering aerial parts of *C. stricta* were collected in Curitiba, Paraná State, Brazil (25°30’44.6’S

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49°18.7’13”W) in March 2011. The plant was identified by Élide P. Santos (plant taxonomist), who deposited voucher specimens (EPSantos 1255) in the UPCB herbarium of the Federal University of Paraná (UFPR). In the present work we describe the chemical composition of essential oils from the leaves and flowers of C. stricta, and the screening of antiproliferative activity against six human tumor cell lines.

2. Previous Studies

The ethanolic extract of the leaves of C. stricta exhibited antitumor activity in vitro against HT-29 and NCI-H460 human cell lines [3]. To our knowledge, there are no chemical reports on this species.

3. Present Study

Fresh leaves and flowers were separated from the stems of several specimens of C. stricta, and subjected to hydro-distillation in a Clevenger apparatus for 2 h (100 g each part). The oils were recovered with diethyl ether and dried over anhydrous sodium sulfate. The solvent was removed under vacuum and the oils were kept in a refrigerator at -4°C until analysis. The extraction was made in duplicate. The yields were 0.17±0.01% (leaves) and 0.25±0.03% (flowers) based on fresh weight (w/w).

The GC/FID analyses were performed on a Shimadzu GC-17A gas chromatograph, equipped with a DB-5 fused silica capillary column (30 m x 0.25 mm, 0.25 µm film thickness), and the temperature was programmed as described: 50°C for 3 min, followed by a gradual increase from 50-240°C at 3°C/min, followed by isothermal keeping at 240°C for 5 min. Helium was used as the carrier gas, with a flow rate of 1.2 mL/min. Injector port and detector temperature were set at 240°C and 250°C, respectively. Samples were injected in split mode (1:20). The relative percentage of components was calculated based on the peak areas obtained by electronic integration without FID response factor correction. The results are the average of three analyses. The GC/EIMS (70 eV) analyses were performed on a Varian Saturn 2000 apparatus equipped with a CP-Sil-8CB fused silica capillary column (30 m x 0.25 mm, 0.25 µm film thickness) in the same conditions described above. Compounds were identified by comparison of their retention indices (relative to n-alkanes C_{n}-C_{24}) and mass spectra with those found in the literature [4] and in NIST 2002 library. In addition, the sesquiterpene spathulenol, previously isolated from Magnolia ovata [5] was used as external standard. The antiproliferative activity screening of the oils was performed using U251 (glioma, CNS), UACC-62 (melanoma), MCF-7 (breast), NCI-H460 (lung, no small cells), PC-3 (prostate), K562 (leukemia) human tumor cell lines and HaCat cells. The assays followed the procedure previously described [6] (see Supporting Information).

The oils were characterized by presence of monoterpenes and sesquiterpenes. Phenylpropanoids and aliphatic compounds were not detected. We found a predominance of oxygenated sesquiterpenes (62.3%) in the leaf oil, while the flower oil contained similar amounts of monoterpene hydrocarbons and oxygenated sesquiterpenes (41.2% and 40.6%, respectively). Forty-seven compounds were identified, corresponding to around 90% of the oils (Table 1). Caryophyllene oxide and cis-pinenes were the major components in both oils, followed by spathulenol in the leaf oil and α-pinene and β-pinene in the flower oil. The monoterpenes limonene and β-phellandrene co-eluted in the analytical conditions employed and, therefore, they were integrated together. Previous studies on essential oils of genus Cantinoa are restricted to two species. The oils of C. americana (Hyptis spicigera) present considerable chemical variability, with some samples dominated by monoterpenes (mainly α-pinene, β-pinene and, 1,8-cineol) and others being rich in E-caryophyllene [7-17]. C. mutabilis (Hyptis mutabilis) showed a similar pattern, with four samples rich in sesquiterpenes (mainly E-caryophyllene, spathulenol, and germacrone D) [18-21] and two with predominance of monoterpenes (α-pinene, β-phellandrene, and limonene) [22]. Therefore, the oils of C. stricta differ from those of other known Cantinoa by their high content of caryophyllene oxide and cis-pinenes.
The oils showed antiproliferative activity against almost all cell lines tested, with TGIIs of 4.54-33.93 μg/mL. The exception was K562 cell line, for which the TGI value was higher than 100 μg/mL (Table 2). The flower oil was more active against MCF-7 and PC-3 cell lines, while the leaf oil
exhibited antiproliferative effects mainly against NCI-H460 and U251 cell lines. These results are partially in accordance with previous screening that point out antiproliferative activity of the leaves extracts of *C. stricta* against NCI-H460 human cell line [3]. In addition, our results showed that the oils of *C. stricta* inhibit the growth of several tumor cell lines. In comparison with the positive control (doxorubicin), the oils were less active, but also less toxic (Table 2). The bioactivity of the oils can be partially explained by the high content of caryophyllene oxide, a sesquiterpene with recognized antitumor activity [23]. In addition, the higher percentage of monoterpenes, such as α-pinene, possibly increased the potency of the flower oil against MCF-7 and PC-3 cell lines. In fact, α-pinene exhibited cytotoxic activity toward several tumor cell lines, including MCF-7 [24, 25].

Table 2. Cytotoxic activity of the essential oils of *C. stricta*.

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>leaves</th>
<th>flowers</th>
<th>doxorubicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>U251</td>
<td>12.19</td>
<td>15.54</td>
<td>1.40</td>
</tr>
<tr>
<td>UACC-62</td>
<td>33.93</td>
<td>25.19</td>
<td>0.28</td>
</tr>
<tr>
<td>MCF-7</td>
<td>10.36</td>
<td>4.54</td>
<td>0.95</td>
</tr>
<tr>
<td>NCI-H460</td>
<td>11.86</td>
<td>18.55</td>
<td>0.08</td>
</tr>
<tr>
<td>PC-3</td>
<td>13.65</td>
<td>10.58</td>
<td>1.30</td>
</tr>
<tr>
<td>K-562</td>
<td>&gt;250</td>
<td>137.90</td>
<td>55.92</td>
</tr>
<tr>
<td>HaCat</td>
<td>19.95</td>
<td>19.11</td>
<td>0.37</td>
</tr>
</tbody>
</table>

*TGI: Total Growth inhibition – concentration that inhibited cell growth by 100%. The coefficients of variation obtained were below to 5%.

Supporting Information

Supporting Information accompanies this paper on [http://www.acgpubs.org/RNP](http://www.acgpubs.org/RNP)

References


