SHORT REPORT

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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 μg/mL), with exception of K562 cells. The highest activity was observed against MCF-7 cell lines (TGI = 4.54-10.36 μg/mL).

Keywords: Lamiaceae; *Cantinoa stricta*; essential oil; caryophyllene oxide; cytotoxic activity.

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 (formely *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_9-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*-pinane 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 germacrene 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*-pinane.

Table 1. Chemical composition (%) of the oils of *C. stricta*.

Compound[a]	RRI (calc.)[b]	RRI (lit.)	%[c]	
			leaves	flowers
E-Salvene	865	854	t	0.2±0.0
α-Pinene	929	932	0.7±0.2	9.4±1.0
Sabinene	969	969	t	1.9±0.2
β-Pinene	973	974	3.2±0.2	9.1±1.2
Cis-pinane	980	982	15.4±0.3	9.7±0.6
Myrcene	988	988	-	1.7±0.1
p-Cymene	1022	1020	-	0.3±0.0
Limonene + β-Phellandrene	1026	1024/1025	3.8±0.2	8.4±0.6
1,8-Cineol	1029	1028	t	1.9±0.1
E-β-Ocimene	1044	1044	t	0.5±0.0
Terpinen-4-ol	1177	1177	t	0.4±0.1
Dihydrocarveol	1193	1192	t	0.3±0.0
Verbenone	1206	1204	0.1±0.0	-
Silphiperfol-5-ene	1318	1326	0.1±0.1	-
7-*epi*-silphiperfol-5-ene	1336	1345	1.5±0.0	0.4±0.0
α-Cubebene	1342	1345	0.4±0.0	0.4±0.1
Silphiperfol-4,7(14)-diene	1356	1358	1.1±0.0	t
Cyclosativene	1367	1369	0.4±0.0	0.6±0.2
β-Cubebene	1380	1387	0.6±0.0	1.1±0.1
β-Elemene	1385	1389	0.3±0.0	0.3±0.0
E-Caryophyllene	1423	1417	t	2.6±0.1
β-Copaene	1429	1430	t	0.3±0.1
α-Guaiene	1428	1437	t	0.3±0.1
Cis-muurola-4(14)-5-diene	1466	1465	t	-
Trans-cadina1(6)-4-diene	1470	1475	t	0.3±0.0
γ-Muurolene	1474	1478	t	t
Germacrene D	1484	1484	t	0.4±0.1
Bicyclogermacrene	1498	1500	t	0.2±0.0
α-Muurolene	1500	1500	t	t
Cameroonan-7α-ol	1508	1510	0.7±0.0	0.3±0.0
α-Bulnesene	1510	1509	0.4±0.0	t
γ-Amorphene	1511	1511	0.5±0.0	1.0±0.2
Spathulenol[d]	1570	1577	7.9±0.1	6.3±0.3
Caryophyllene oxide	1580	1581	31.6±0.4	21.7±1.0
Globulol	1586	1590	2.0±0.1	1.1±0.0
Guaiol	1593	1600	0.5±0.0	0.3±0.0
Humulene epoxide II	1602	1608	3.8±0.0	1.4±0.3
10-*Epi*-γ-Eudesmol	1625	1622	3.4±0.1	2.7±0.0
Epi-α-Cadinol	1633	1638	t	t
Allo-aromadendrene epoxide	1638	1639	t	0.8±0.0
Cubenol	1648	1645	2.1±0.1	0.4±0.1
14-Hydroxy-*Z*-caryophyllene	1658	1666	3.9±0.2	1.0±0.2
Khusinol	1681	1679	-	0.8±0.0
14-Hydroxy-4,5-dihydrocaryophyllene	1720		3.9±0.2	2.6±0.6
Isobicyclogermacrenal	1733	1733	2.5±0.2	0.2±0.0
α-Chenopodial	1855	1855	t	1.0±0.1
Total identified			90.8	92.3
Monoterpene hydrocarbons			23.1	41.2
Oxygenated monoterpenes			0.1	2.6
Sesquiterpene hydrocarbons			5.3	7.9
Oxygenated sesquiterpenes			62.3	40.6

Compounds are listed in order of their elution in a CP-Sil-8CB column; - : not detected; t: trace (< 0.05%); [a]Identification based on mass spectra and RRI published (Adams, 2007), and NIST 2002 library; [b]relative retention index experimental on a CP-Sil-8CB column; [c]average and standard deviation of three analyses; [d]identification confirmed by comparison with authentic sample.

The oils showed antiproliferative activity against almost all cell lines tested, with TGIs 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*.

Cell lines	TGI (μg/mL)[a]		
	leaves	flowers	doxorubicin
U251	12.19	15.54	1.40
UACC-62	33.93	25.19	0.28
MCF-7	10.36	4.54	0.95
NCI-H460	11.86	18.55	0.08
PC-3	13.65	10.58	1.30
K-562	>250	137.90	55.92
HaCat	19.95	19.11	0.37

[a]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

References

[1] R. Harley and J. F. Pastore (2012). A generic revision and new combinations in the Hyptidinae (Lamiaceae), based on molecular and morphological evidence, *Phytotaxa* **58**, 1-55**.**

[2] R. Harley, F. Franca, E. P. Santos, J. S. Santos and J. F. Pastore (2013). Lamiaceae. In Lista de Espécies da Flora do Brasil, Jardim Botânico do Rio de Janeiro, RJ. http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB8183.

[3] N. R. Monks, S. A. L. Bordignon, A. Ferraz, K. R. Machado, D. H. Faria, R. M. Lopes, C. A. Mondin, I. C. C. Souza, M. F. S. Lima, A. B. Rocha and G. Schwartsmann (2002). Anti-tumour Screening of Brazilian Plants, *Pharm. Biol.* **40**, 603-606.

[4] R. P. Adams (2007). Identification of essential oil components by gas chromatography/mass spectrometry, Allured Publishing Corp., Carol Stream, IL, USA.

[5] L. F. L. Barros, A. Barison, M. J. Salvador, R. Mello-Silva, E. C. Cabral, M. N. Eberlin and M. E. A. Stefanello (2009), Constituents of the leaves of *Magnolia ovata*. *J. Nat. Prod*. **72**, 1529-1532.

[6] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J. T. Warren, H. Bokesch, S. Kenney and M. R. Boyd (1990). New colorimetric cytotoxicity assay for anticancer-drug screening, *J. Natl. Cancer Inst.* **82**, 1107-1118.

[7] G. S. Bogninou-Agbidinoukoun, H. Yedomonhan, F. Avlessi, D. Sohounhloué, P. Chalard, J.-C. Calchat, L. Delort, H. Billard, F. Caldefie-Chézet and G. Figueiredo (2013). Volatile oil composition and antiproliferative activity of *Hyptis spicigera* Lam against human breast adenocarcinoma cells MCF-7, *Res. J. Chem. Sci.* **3**, 27-31.

[8] C. Takayama, F. M. Faria, A. C. A. Almeida, D. A. O. Valim-Araújo, C.S. Rehen, R. J. Dunder, E. A. R. Socca, L. P. Manzo, A. L. Rozza, M. J. Salvador, C. H. Pellizon, C. A. Hiruma-Lima, A. L. Ferreira and A. R. M. Souza-Brito (**2011**). Gastroprotective and ulcer healing effects of essential oil from *Hyptis spicigera* Lam. (Lamiaceae), *Journal of Ethnopharmacology* **135**, 147-155.

[9] B. Conti, G. Benelli, G. Flamini, P. L. Cioni, R. Profeti, L. Ceccarini, M. Macchia and A. Canale (2012). Larvicidal and repellent activity of *Hyptis suaveolens* (Lamiaceae) essential oil against the mosquito *Aedes albopicus* Skuse (Diptera: Culicidae), *Parasitol. Res.* **110**, 2013-2021.

[10] F. Noudjou, H. Kouninki, L. S. T. Ngamo, P. M. Maponmestsem, M. Ngassoum, T. Hance, E. Haubruge, F. Malaisse, M. Marlier and G. C. Lognay (2007). Effect of site location and collecting period on the chemical composition of *Hyptis spicigera* Lam., an insecticidal essential oil from North-Cameroon, *J. Essent. Oil Res*. **19**, 597-601.

[11] M. B. Ngassoum, L. S. N. Tinkeu, I. Ngatanko, L. A. Tapondjou, G. Lognay, F. Malaisse and T. Hance (2007). Chemical composition, insecticidal effect and repellent activity of essential oils of three aromatic plants, alone and in combination, towards *Sitophilus oryzae* L. (Coleoptera: Curculionidae), *Nat. Prod. Comm.* **12**, 1229-1232.

[12] K. Koba, C. Raynaud, J. Millet, J.-P. Chaumont and K. Sanda (2007). Chemical composition of *Hyptis pectinata*, *H. lanceolata*, *H. suaveolens* and *H. spicigera* essential oils from Togo, *J. Essent. Oil-Bear. Plants.* **10**, 357-364.

[13] F. Tchoumbougnang, P. H. A. Zollo, F. F. Boyom, M. A. Nyegue, J. M. Bessière and C. Menut (2005). Aromatic plants of tropical Central Africa. XLVIII. Comparative study of the essential oils of four *Hyptis* species from Cameroon: *H. lanceolata* Poit., *H. pectinata* (L.) Poit., *H. spicigera* Lam. and *H. suaveolen*s Poit, *Flavour Fragr J.* **20**, 340-343.

[14] L. Sidibé, J. C. Chalchat, R. P. Garry and M. Harama (2001). Aromatic plants of Mali (III): Chemical composition of essential oils of two *Hyptis*: *H. suaveolens* (L.) Poit and *H. spicigera* Lam., *J. Essent. Oil Res.* **13**, 55-57.

[15] L. Jirovetz, G. Buchbauer, C. Puschmann and M. B. Ngassoum (2000). Investigations of aromatic plants from Cameroon: analysis of the essentials oils of flowers of *Hyptis spicigera* (L.) Poit by GC, GC/MS and olfactometry, *J. Essent. Oil-Bear. Plants*. **3**, 71-77.

[16] F. Kini, B. Kam, J. P. Aycard, E. M. Gaydou and I. Bombarda (1993). Chemical composition of the essential oil of *Hyptis spicigera* Lam. from Burkina Faso, *J. Essent. Oil Res.* **5**, 219-221.

[17] O. A. Onayade, A. Looman, J. J. C. Scheffer and A. B. Svendsen (1990). Composition of the herb essential oil of *Hyptis spicigera*, *Flavour Fragr. J.* ***5***, 101-105.

[18] J. S. Dambolena, M. P. Zunino, E. I. Lucini, J. A. Zygadlo, A. Rotman, O. Ahumada and F. Biurrun (2009). Essential oils of plants used in home medicine in North of Argentina, *J. Essent. Oil Res.* **21**, 405-409.

[19] Y. G. Gillij, R. M. Gleiser and J. A. Zygadlo (2008). Mosquito repellent activity of essential oils of aromatic plants growing in Argentina, *Bioresource Technol.* **99**, 2507-2515

[20] M. M. Oliva, M. S. Demo, A. G. Lopez, M. L. Lopez and J. A. Zygadlo (2006). Antimicrobial activity and composition of *Hyptis mutabilis* essential oil, *J. Herbs, Spices Med. Plants.* **11**, 57-63.

[21] P. Bailac, C. Duschatzky, M. Ponzi and N. Firpo (1999). Essential oil of *Hyptis mutabilis* (Rich.) Briq. grown in San Luis, Argentina, *J. Essent. Oil Res.* **11**, 217-219.

[22] A. I. R. Luz, M. G. B. Zoghbi, L. S. Ramos, J. G. S. Maia and M. L. Silva (1984). Essential oils of some Amazonian Labiatae. 1. Genus *Hyptis*, *J. Nat. Prod.* **47**, 745-747.

[23] K.-R. Park, D. Nam, H.-M. Yun, S.-G. Lee, H-J. Jang, G. Sethi, S. K. C. Cho and K. S. Ahn (2011). β-caryophyllene oxide inhibits growth and induces apoptosis through the suppression of PI3K/AKT/mTOR/S6K1 pathways and ROS-mediated MAPKs activation, *Cancer Lett.* **312**, 178-188.

[24] A. L. Matsuo, C. R. Figueiredo, D. C. Arruda, F. V. Pereira, J. A. B. Scutti, M. H. Massaoka, L. R. Travassos, P. Sartorelli and J. H. G. Lago (2011). α-Pinene isolated from *Schinus terebinthifolius* Raddi (Anacardiaceae) induces apoptosis and confers antimetastic protection in a melanoma model, *Biochem. Biophys. Res. Comm.* **411**, 449-454.

[25] R. A. Cole, A. Bansal, D. M. Moriarity, W. A. Habber and W. N. Setzer (2007). Chemical composition and cytotoxic activity of the leaf essential oil of *Eugenia zuchowskiae* from Monteverde, Costa Rica. *J. Nat. Med.* **61**, 414-417.