Orbital
The electronic journal of chemistry

the electronic journal of chemistry

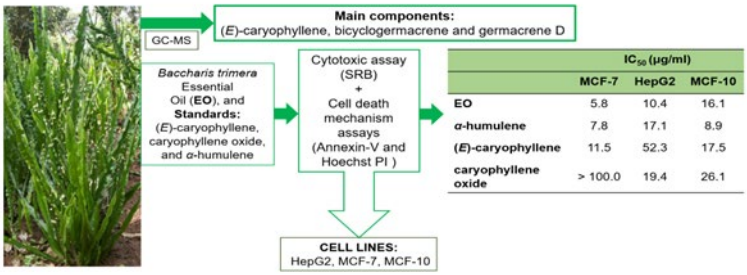
Full Paper | http://dx.doi.org/10.17807/orbital.v15i1.17021

Cytotoxic Activity of *Baccharis trimera* (Less.) DC. Essential Oil in Tumor Cell Lines and its Role in Associated Death Mechanisms

Isabela Jacob Moro [a], Flavio Alexandre Carvalho [a], Thais Fernanda Moreira [b], Felipe de Oliveira Souza [b], Alexander Alves da Silva [c], Flávio Politi [c], Christiane Pienna Soares [b], André Gonzaga dos Santos* [a]

This study evaluated the potential of *Baccharis trimera* essential oil (EO) and its components in cancer therapy through the application of cytotoxicity and cell death assays. Using gas chromatography analysis, the major components of the aerial parts of the essential oil were identified as (*E*)-caryophyllene (18.9%), bicyclogermacrene (15.6%), and germacrene D (10.5%). *Baccharis trimera* essential oil (5.8 µg/mL) and *α*-humulene (7.8 µg/mL) presented strong cytotoxic activity, while (*E*)-caryophyllene (11.5 µg/mL) and caryophyllene oxide (> 100.0 µg/mL) showed moderate and low activities, respectively, against MCF-7 cell lines. Against HepG2 cell lines, *B. trimera* essential oil (10.4 µg/mL), *α*-humulene (17.1 µg/mL), and caryophyllene oxide (19.4 µg/mL) exhibited moderate activity, while (*E*)-caryophyllene (52.3 µg/mL) displayed low activity against HepG2 cell lines. The selectivity index values of EO (MCF-7 and HepG2), *α*-humulene and (*E*)-caryophyllene (MCF-7), and caryophyllene oxide (HepG2) were found between 1.1 and 2.8, compared with MCF-10A cells. The annexin-V and Hoechst / propidium iodide assays performed with essential oil, (*E*)-caryophyllene, *α*-humulene, and caryophyllene oxide showed apoptosis and necrosis mechanisms for all cell lines. Based on these findings, *B. trimera* essential oil and its components can be considered as potential therapeutic agents against cancer.

Graphical abstract



Keywords

Baccharis trimera
cancer
cytotoxic
essential oil

Article history

Received 25 Oct 2022
Revised 22 Feb 2023
Accepted 20 Mar 2023
Available online 06 Apr 2023

Handling Editor: Fernanda R. Garcez

[a] São Paulo State University (UNESP), School of Pharmaceutical Sciences, Department of Drugs and Medicines, Rod. Araraquara-Jaú Km 01 s/n, 14800-903, Araraquara, São Paulo, Brazil. [b] São Paulo State University (UNESP), School of Pharmaceutical Sciences, Department of Clinical Analysis, Rod. Araraquara-Jaú Km 01 s/n, 14800-903, Araraquara, São Paulo, Brazil. [c] São Paulo State University (UNESP), Chemistry Institute, Department of Organic Chemistry, Rue Prof. Francisco Degni n. 55, 14.800-900, Araraquara, São Paulo, Brazil. *Corresponding author. E-mail: **andre.gonzaga@unesp.br**

1. Introduction

Over 19 million new cancer cases were recorded in 2020. Breast cancer incidence was the highest recorded, while lung cancer caused the highest amount of deaths among cancer patients (1.8 million) [1]. Cancer therapy is mainly based on surgical procedures, radiotherapy, and chemotherapy [2]; however, these therapies have been found to affect both tumor cells and healthy cells. To help tackle this underlying problem, over the past few years new therapies which are selective to tumor cells have been developed, such as natural antioxidants, gene, targeted, and nanoparticles-based therapies [3].

Plants are a great source for the discovery of compounds which are effectively useful for preventing or reversing carcinogenic processes [4]. *Baccharis trimera* (Less.) DC. (Asteraceae) or "carqueja" is a Brazilian native shrub that is widely used in folk medicine for the treatment of digestive disorders, liver diseases, inflammation, rheumatism, and diabetes [5]. Pharmacological studies have shown that *B. trimera* leaves possess anti-inflammatory, antiulcerogenic, antioxidant, hepatoprotective, and antileishmanial properties [6, 7]. The main secondary metabolites which have been identified in *B. trimera* leaves include flavonoids (e. g. eupatorin, quercetin, rutin, hispidulin, and apigenin), clerodane diterpenes, quinic acid and gallic acid derivatives, as well as saponins. In addition, *B. trimera* essential oil (EO) has also been found to contain mono- and sesquiterpenes, including carquejyl acetate, *β*-pinene, *α*-cadinene, *α*-cadinol, (*E*)-caryophyllene, caryophyllene oxide, germacrene D, and bicyclogermacrene [6, 8, 9, 10].

Here, we investigated the potential of *B. trimera* EO components in cancer therapy by evaluating their cytotoxic activity in tumor (MCF-7 and HepG2) and normal (MCF-10A) human cell lines and their role in related cell death mechanisms.

2. Results and Discussion

The main components identified in the EO of *B. trimera* leaves included the following: (*E*)-caryophyllene (18.9%), bicyclogermacrene (15.6%), germacrene D (10.6%), and *δ*-cadinene (6.7%) (Table 1). According to reports in the literature, the main components of *B. trimera* leaves (CPQBA1 cultivar, Campinas State University) are germacrene D (15.3%), (*E*)-caryophyllene (14.8%), and bicyclogermacrene (14.7%) [11]; interestingly, a study conducted by Suzuki et al. [10] also showed that the main components of *B. trimera* leaves include *β*-pinene (23.4%), carquejyl acetate (19.0%), (*E*)-caryophyllene (6.4–16.1%), *α*-cadinene (11.6%), *α*-cadinol (8.5%), bicyclogermacrene (7.8%), caryophyllene oxide (6.1-7.5%), and germacrene D (5.0%).

Table 1. Chemical composition of the essential oil of the *B. trimera* aerial parts identified by GC-MS (mass spectra) and GC-FID (retention index and standards).

Components	RT (min)[a]	RI_{exp}[b]	RI_{lit}[c]	Content (%)
ni	6.72	977	--	1.7
ni	6.99	987	--	1.2
ni	8.88	1043	--	1.1
α-copaene	22.55	1377	1377	1.6
β-cubebene	23.14	1391	1390	1.6
(*E*)-caryophyllene[d]	24.52	1424	1420	18.9
ni	25.07	1438	--	1.2
α-humulene[d]	25.20	1441	1448	2.3
aromadendrene	25.81	1456	1455	2.4
germacrene D	26.99	1485	1485	10.6
ni	27.18	1489	--	0.8
bicyclogermacrene	27.63	1501	1500	15.6
α-gurjunene	28.17	1514	1519	1.2
δ-cadinene	28.51	1523	1522	6.7
ni	30.70	1579	--	3.0
ni	30.79	1582	--	1.0
caryophyllene oxide[d]	31.09	1589	1592	5.5
viridiflorol	31.37	1597	1594	3.7
rosifoliol	31.72	1606	1603	1.7
cubenol	32.52	1629	1623	0.8
ni	33.11	1643	--	1.6
ni	33.21	1646	--	1.8
α-cadinol	33.68	1659	1656	4.0
Sesquiterpene hydrocarbons				60.9
Oxygenated sesquiterpenes				15.7
ni				13.4

[a]Retention time evaluated using the Rtx-5MS capillary column; [b]Retention index evaluated relative to *n*-alkanes (C_8-C_{40}); [c]Retention index based on a comparative analysis of reports in the literature [12, 13]. [d]Compounds identified through a comparative analysis of the retention times of the standards. *ni*: not identified.

The IC_{50} values (Table 2) for MCF-7 and HepG2 cell lines were, respectively, 5.8 and 10.4 µg/mL (EO), 7.8 and 17.1 µg/mL (*α*-humulene), 11.5 and 52.3 µg/mL ((*E*)-caryophyllene), and >100.0 and 19.4 µg/mL (caryophyllene oxide). Based on the National Cancer Institute (USA) guideline protocol, which considers IC_{50} values ≤ 30.0 µg/mL as considerably relevant for plant derivatives, the results obtained for the compounds indicate a highly promising cytotoxic activity against MCF7 and HepG2 cell lines [14]. It should also be noted that, considering that the IC_{50} values for

cytotoxicity are commonly classified as strong (< 10 µg/mL), moderate (10 to 50 µg/mL), low (50 to 100 µg/mL), and inactive (> 100 µg/mL) [15]. The cytotoxicity observed for the aforementioned compounds ranged from strong to inactive. Although the selectivity indices (Table 2) for healthy (MCF-10A) and tumor cell lines were higher than 1.0 for (*E*)-caryophyllene and *α*-humulene (MCF-7), caryophyllene oxide (HepG2), and EO (MCF-7 and HepG2), with special reference to the EO (MCF-7) index (2.8), *α*-humulene and (*E*)-caryophyllene were found to be more toxic to non-neoplastic MCF-10A cell line than to HepG2 cells (SI ≤ 0.5). In a related study reported in the literature, the authors showed that (*E*)-caryophyllene increased the cytotoxic effect of other EO components and contributed to the anti-cancer activity of paclitaxel [16].

Table 2. Cytotoxic action (IC_{50} and 95% CI) of *B. trimera* EO and its components against MCF-7, MCFA-10A, and HepG2 cell lines.

Samples	MCF-7		HepG2		MCF-10A		SI[a]	SI[b]
	IC_{50} (µg/mL)	95% CI	IC_{50} (µg/mL)	95% CI	IC_{50} (µg/mL)	95% CI		
EO	5.8	14.0 - 18.6	10.4	9.0 – 12.0	16.1	14.0 - 18.6	2.8	1.6
α-humulene	7.8	6.3 - 9.1	17.1	12.9 - 22.6	8.9	6.9 - 11.4	1.1	0.5
(*E*)-caryophyllene	11.5	9.6 - 13.9	52.3	36.0 - 76.0	17.5	13.7 - 21.2	1.5	0.3
caryophyllene oxide	> 100.0	nd	19.4	16.4 – 23.0	26.1	13.6 - 50.4	nd	1.3

Positive control: 20.0 µg/mL doxorubicin; nd: not determined. [a]Selectivity index between the IC_{50} against MCF-10A *versus* MCF-7; [b]Selectivity index between the IC_{50} against MCF-10A *versus* HepG2.

The results from the HepG2 annexin-V assay (Fig. 1a) showed a higher percentage of early apoptotic cells for caryophyllene oxide treatment at 12.5 and 50.3 µg/mL, and necrotic cells for *α*-humulene at 15.0 and 30.0 µg/mL, (*E*)-caryophyllene at 50.0 and 100.0 µg/mL, and high incidence of necrosis for EO at 7.5 µg/mL. Necrosis was observed for caryophyllene oxide at 50.0, 100.0, and 200.0 µg/mL, and for (*E*)-caryophyllene at 4.0 and 16.8 µg/mL MCF-7 cell line treatments (Fig. 1b), while *α*-humulene and EO presented necrosis at 10.0, 5.0, and 15.0 µg/mL, respectively. We also observed the prevalence of early apoptosis for caryophyllene oxide at 37.5 and 150.8 µg/mL and *α*-humulene at 5.0, 10.0, and 20.0 µg/mL against the MCF-10A cell line (Fig. 1c). The earlier the occurrence of apoptosis, the lower the damages the adjacent tissues will experience. On the other hand, necrosis causes an intense inflammatory process and tissue destruction.

Despite the cytotoxic activity of the samples, we observed the prevalence of early apoptosis for caryophyllene oxide when this compound was tested against HepG2 (Fig. 2a) and MCF10, resulting in cell death and minor secondary damage effects to adjacent tissues. Some studies reported in the literature have shown that some sesquiterpenes are apoptosis inducers in several types of tumors through various hyperactive pathways in cancer [17]; these sesquiterpenes are able to inhibit cell proliferation by interfering in the cycle stages, as well as in the genetic material mechanisms, or via metastasis damage, thus, playing an influential role in angiogenesis or in drug resistance [18].

Hoechst / propidium iodide assays conducted against MCF-7 cell line (Fig. 2b) showed higher necrotic cells after treatment with caryophyllene oxide at 100.0 and 200.0 µg/mL, and (*E*)-caryophyllene at 16.0 µg/mL; in addition, we noted the prevalence of early apoptotic cells following the treatment with α-humulene at 2.5 and 5.0 µg/mL, and EO at 3.8, 7.5 and 15.0 µg/mL (though, no statistical significance was presented). For the MCF-10A cell line (Fig. 2c), we noted the predominance of early apoptosis, especially following the treatments with caryophyllene oxide (37.5 µg/mL) and (*E*)-caryophyllene (7.5 µg/mL), as well as with *α*-humulene at 5.0, 10.0, and 20.0 µg/mL and EO at 5.0 and 10.0 µg/mL.

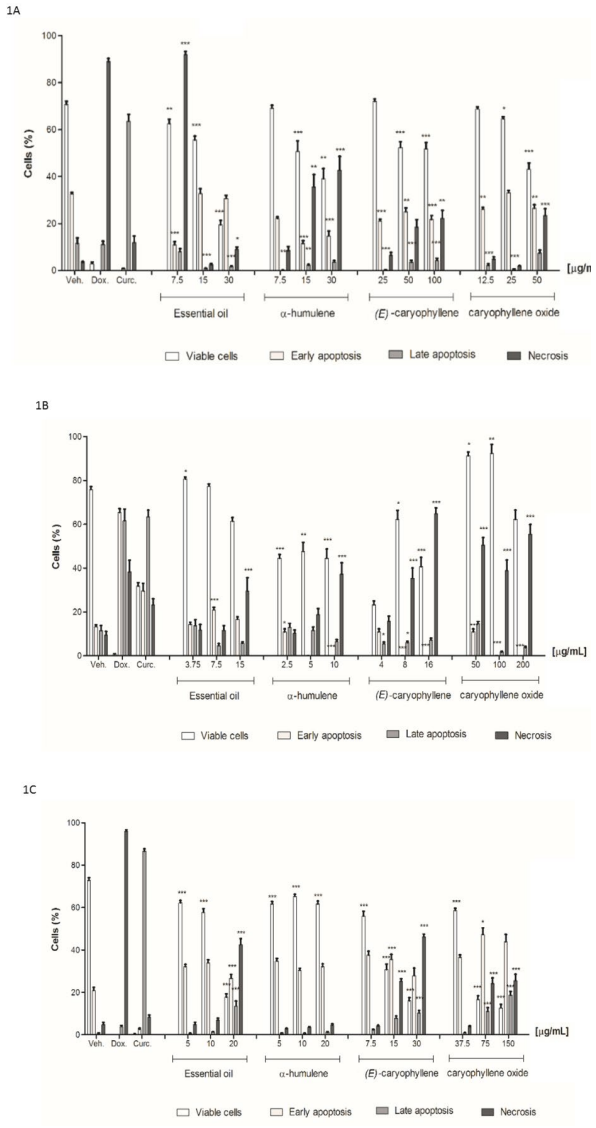


Fig. 1. Annexin-V assay: An outline of viable cells, early apoptosis, late apoptosis, and necrosis based on the application of Annexin-V assays after using EO, *α*-humulene, (*E*)-caryophyllene, and caryophyllene oxide for the treatment of HepG2. 1a. MCF-7, 1b. MCF-10A, and 1c. cell lines. Controls evaluated: vehicle control (DMSO 1%); necrosis positive control (doxorubicin 20.0 µg/mL); and apoptosis-positive control (curcumin 53.0 µg/mL).

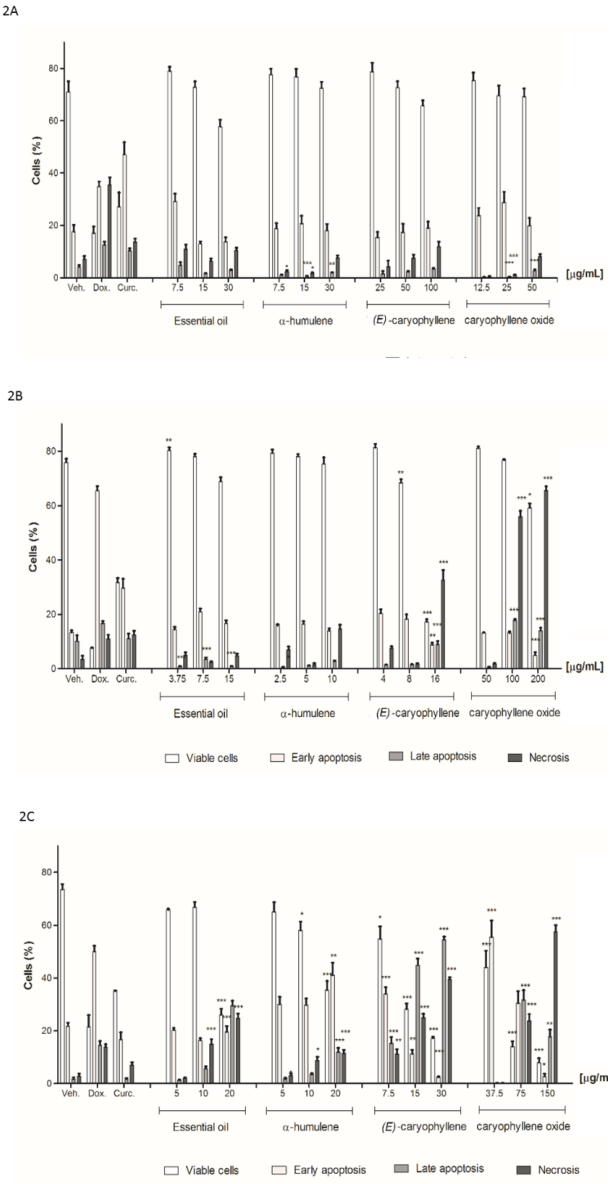


Fig. 2. Hoechst / propidium iodide assays: An outline of viable cells, early apoptosis, late apoptosis, and necrosis based on the application of Hoechst/PI assays after using EO, *α*-humulene, (*E*)-caryophyllene, and caryophyllene oxide for the treatment of HepG2. 2a. MCF-7, 2b. MCF-10A, and 2c. cell lines. Controls evaluated: vehicle control (DMSO 1%); necrosis positive control (20.0 µg/mL doxorubicin); and apoptosis-positive control (53.0 µg/mL curcumin).

The results from our analysis showed a decrease in MCF-10A necrosis death dose dependence (Fig. 1 and 2) for EO. Regarding the HepG2 cell line (Fig. 1a), the application of lower concentrations of EO led to a reduction in early apoptosis and an increase in necrosis. The MCF-7 cell line (Fig. 1b) exhibited necrosis at 15.0 µg/mL and apoptosis at 7.5 µg/mL. Reports in the literature have shown that caryophyllene oxide exhibited a prevalence of apoptosis. Sousa [17] reported anti-angiogenic activity associated with increased apoptosis relative to angiogenesis inhibition for the PI3k pathway and inhibition of the p65 of NF-κ*β*, which triggered anti-angiogenic and pro-apoptotic responses.

3. Material and Methods

3.1 Plant material

B. trimera aerial parts were collected at the Chemical, Biological and Agricultural Pluridisciplinary Research Center (CPQBA) of the Campinas State University (UNICAMP), Paulínia-SP, Brazil, on October 20th, 2014 (22°47'42.347" S, 47°6'40.061" W). The voucher specimen was deposited at the CPQBA Herbarium, Campinas, under the reference number 1286; access to the genetic heritage was registered under the reference number A742C6E (SisGen, Brazil).

3.2 Essential oil extraction

The dried aerial parts (100 g) of the *B. trimera* were subjected to hydrodistillation in a Clevenger-type apparatus for 4 h [19], and this yield 0.4% (v/w) EO.

3.3 Gas chromatography analysis

Gas chromatography-mass spectrometer analysis was performed using Shimadzu® QP2010 Plus system equipped with an AOC-5000 autosampler and Rtx-5MS fused silica capillary column (5% diphenyl and 95% polydimethylsiloxane 30 m, 0.25 mm, and 0.25 µm film thickness). The analysis was conducted using the following conditions: i) Helium (99.9999%) was employed as carrier gas at 1.3 mL/min flow rate; ii) injection volume applied: 1.0 µl (1.0 mg/mL, hexane); and split ratio: 1:60. The injector and ion source temperatures were set at 250 and 280 °C, respectively. The oven temperature was set to rise from 60 to 240 °C (3 °C/min). Electron ionization mass spectra analysis was performed using the following conditions: potential of 70 eV, scan interval of 0.5 s, and mass range of 40 to 600 Da.

Gas chromatography-flame ionization detector analyses were performed using Varian® CP3800, equipped with Supelco® SPB-5 Rtx-5MS column (5% diphenyl and 95% polydimethylsiloxane 30 m, 0.25 mm, and 0.25 µm film thickness). The analyses were performed using the following conditions: H_2 was employed as the carrier gas at 1.0 mL/min flow rate; injection volume applied: 1.0 µl (1.0 mg/mL, hexane); and split ratio: 1:60. The injector temperature applied was 250 °C; the oven temperature was set to rise from 60 to 240 °C (3 °C/min).

The EO components were identified based on the linear retention indices relative to a homologous series of *n*-alkanes (C_8-C_{40} Sigma-Aldrich®) and the retention times of (*E*)-caryophyllene, *α*-humulene, and caryophyllene oxide standards (Sigma-Aldrich®) [12, 13]. The mass spectra were computer-matched with the NIST 08, WILEY 7, and FFNSC 1.2 spectral libraries.

3.4 Biological assays

To conduct the biological assays *(Sulphorrodamine B, Annexin-V,* and *Hoechst PI)*, the cells were cultured in Dulbecco´s low glucose modified Eagle medium (DMEM, Sigma Chemical®) supplemented with 10% fetal bovine serum (Sigma Chemical®) and kept at 37 °C, with 5% CO_2. 1% DMSO was used both as vehicle and control. The cell lines tested included the following: HepG2 (ATCC® HB-8065™), MCF-10A (ATCC® CRL-10317™), and MCF-7 (ATCC® HTB-22™). Compounds *α*-humulene, (*E*)-caryophyllene, and caryophyllene oxide were acquired from Sigma-Aldrich®.

3.5 Sulphorhodamine B (cytotoxic assay)

The cytotoxic assay was performed using 5 x 10^4 cells/well in 96 well plates. After 24 h of cell adhesion, the media were acidified with trichloroacetic acid (TCA, 100

µL/well). After keeping the media at rest for 1 h at 4 °C, TCA was removed and 0.4% Sulforhodamine B (Sigma-Aldrich®) was added to the media and bound to the amino acid protein of the cells. Absorbance was quantified in a plate reader (Synergy II™, BioTek Instruments Inc., Vermont, USA) at 570 nm [20]. The cells were treated for 24 h using EO and its components (*α*-humulene, (*E*)-caryophyllene, and caryophyllene oxide). The survival cell was calculated using the average absorbance of the blank (MAbsBl) and vehicle (MabsVC), where each concentration of the tested substances (MabsT) and positive control (MabsPC) was considered: % survival = [(MAbsT – MAbsBl) / (MAbsVC – MAbsBl)] x 100. 20.0 µg/mL Doxorubicin (Sigma-Aldrich®) was used as positive control. Cytotoxicity analysis of the cell lines was conducted using selectivity index (SI) through the application of the IC_{50} of MCF-10A and tumor cells (HepG2 and MCF-7). The concentrations employed in the death tests were determined based on the IC_{50} value through the cytotoxicity assay.

3.6 Annexin-V and Hoechst PI (cell death assays)

Cell death was analyzed through the application of the Annexin-V and Hoechst IP assays. The analyses were conducted using Annexin V, FITC (fluorescein isothiocyanate), PI (propide iodate), *Hoechst* FDA (3.5 µg/mL) and propidium iodide (2.5 µg/mL) (acquired from Thermo Fisher Scientific®), and Hoechst 33342 (obtained from Invitrogen™). The Annexin V assay [21]: after cell adhesion, the plates were washed with PBS, and annexin-V binding buffer (Hepes® 10 mm, NaCl 140 mm, and $CaCl_2$ 2.5mm) containing 1.3 µg/mL of annexin-V conjugated with FITC (Life technologies®) and 1.6 µg/mL of *Hoechst* (1.0 mg/mL) were added to the mixture; subsequently the mixture was subjected to incubation for 15 min. The images related to the assay were captured by the IN Cell Analyzer 2200 (GE Healthcare Life Sciences©). Fluorochrome exclusion assay with Hoechst/Propide iodate [22]: the distinction between apoptotic and necrotic cells was made using staining cells with a solution composed of 3.5 µg/mL fluorescein diacetate, propide iodate 2.5 µg/mL, and *Hoechst* 1.5 µg/mL (HO) 33342 (Invitrogen®). After the period of treatment, the plates were centrifuged, washed, and stained (0.100 mL/well) with the fluorochromes for 10 min in the dark. After the incubation period, the images were acquired and analyzed using the IN Cell Analyzer 2200.

Regarding Annexin-V and Hoechst, the IP assays were performed in 5 x 10^4 cells/well plates, and the cells were subjected to treatment for 12 h. In the case of the controls, 53.0 µg/mL curcumin (Sigma-Aldrich®) was used as apoptosis-positive control, while 20.0 µg/mL doxorubicin (Sigma-Aldrich®) was employed as necrosis positive control. The cells were treated using the EO and its components (*α*-humulene, (*E*)-caryophyllene, and caryophyllene oxide).

3.7 Statistical analysis

One-way analysis of variance (ANOVA) and Tukey's post-test considering $p > 0.05$ were performed using GraphPad Software 5 (GraphPad Software, San Diego, California, USA). The assays were carried out in triplicate, and the results were expressed as mean plus standard error.

4. Conclusions

The EO of the aerial parts of *B. trimera* presented significant therapeutic potential against cancer, exhibiting considerable cytotoxic activity against tumor cell lines and lower cytotoxic activity against healthy cells. The major components found in the EO of *B. trimera* included the following: (*E*)-caryophyllene, bicyclogermacrene, and germacrene D. Although the EO components, namely, (*E*)-caryophyllene, caryophyllene oxide, and *α*-humulene also exhibited cytotoxic activity against the cell lines, they were found to be less cytotoxic than EO and with lower selectivity indices. Necrosis and apoptosis were observed for all cell lines treated with EO, (*E*)-caryophyllene, caryophyllene oxide, and *α*-humulene.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 and National Institute of Science and Technology on Biodiversity and Natural Products – INCT-BioNat (CNPq and FAPESP, Grant #465637/2014-0). This work was supported by FAPESP Grant #14/10552-3.

Author Contributions

Isabela Jacob Moro: Investigation, Conceptualization, Methodology, and Software. Flavio Alexandre Carvalho.: Visualization and Writing- Original draft preparation. Thais Fernanda Moreira: Data curation and Software. Felipe de Oliveira Souza: Investigation and Data curation. Alexander Alves da Silva: Investigation and Software. Flávio Politi: Methodology and Software: Christiane Pienna Soares: Conceptualization and Writing- Reviewing and Editing. André Gonzaga dos Santos: Supervision, Funding acquisition, Writing- Reviewing and Editing, and Project administration.

References and Notes

[1] Sung, H.; Ferlay, J.; Siegel, R. l.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. 2021. *CA: Cancer J. Clin*. **2021**, *71*, 209. [Crossref]

[2] Greene, R.; Harris, N. Patologia e terapêutica para farmacêuticos: bases para a prática da farmácia clínica. 3 ed. Porto Alegre: Artmed, **2012**, p. 968.

[3] Pucci, C.; Martinelli, C. Ciofani, G. *Ecancermedicalscience* **2019**, 13. [Crossref]

[4] Newman, D. J.; Cragg, G. M. *J. Nat. Prod*. **2020**, *83*, 770. [Crossref]

[5] Agra, M. F.; Freitas, P. F.; Barbosa-Filho, J. *Rev. Bras. Farmacog*. **2007**, *17*, 114. [Crossref]

[6] Clementino, L. C.; Sahid, E. D. N.; Claudino, J. C.; Oda, F. B.; Carvalho, F. A.; Santos, A. G.; Graminha, M. A. S. *Nat. Prod. Res*. **2021**, 1. [Crossref]

[7] Menezes, A. P. S.; Silva, J.; Fisher, C.; Silva, F. R.; Reyes, J. m.; Picada, J. N.; Ferraz, A. G.; Corrêa, D. S.; Premoli, S. M.; Dias, J, F. *Chemosphere* **2016**, *146*, 396. [Crossref]

[8] Verdi, L. G.; Brighente, I. M. C.; Pizzolatti, M. G. *Quim. Nova* **2005**, *28*, 85. [Crossref]

[9] Struiving, S.; Hacke, A. C. M.; Simionatto, E. l.; Scharf, D. R.; Klimaczewski, C. V.; Besten, M. A.; Heiden, G.; Boligon, A. A.; Rocha, J. B. T.; Vellosa, J. C. TR. *Foods* **2020**, *9*, 1433. [Crossref]

[10] Suzuki, E. Y.; Caneschi, C. A.; Fochat, R. C.; Brandão, M.

A. F.; Raposo, N. R B. *Rev. Cuba. Plantas Med*. **2017**, *21*, 346.

[11] Oliveira, R. N.; Rehder, V. L. G.; Oliveira, A. S. S.; Júnior, Í. M.; Carvalho, J. E. Ruiz, A. L. T. G.; Jeraldo, V. L. S.; Linhares, A. X.; Allegretti, S.M. *Exp. Parasitol.* **2012**, *132*, 135. [Crossref]

[12] Adams, R. P. Identification of Essential Oil components by Gas Chromatography/Mass Spectrometry. 4th ed. USA: Allured, **2007**, p. 804.

[13] Van Den Dool, H.; Kratz, P. D. *J Chromatogr*. **1963**, *11*, 463. [Crossref]

[14] Geran, R.; Greenberg, H. H.; Macdonald, M. M.; Schumacher, A. M. *Cancer Chemother. Rep.* **1972**, *3*, 1.

[15] Indrayanto, G.; Putra, G. S.; Suhud, F. In: *Profiles of Drug Substances, Excipients and Related Methodology*, Vol. 46, Academic Press, 2021, 273. [Crossref]

[16] Legault, J.; Pichette, A. *J. Pharm. Pharmacol*, **2007**, *59*, 1643. [Crossref]

[17] Sousa, D. P. Bioactive essential oils and cancer. Springer, 2015.

[18] Gautam, N.; Mantha, A. K.; Mittal, S. *Biomed Res. Int*. **2014**, *2014*, 154106. [Crossref]

[19] Brasil. AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA. Farmacopeia Brasileira. 5 ed. Brasília: ANVISA. 2010.

[20] Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; Mcmahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst*. **1990**, *82*, 1107. [Crossref]

[21] Duarte, R. A.; Mello, E. R.; Araki, C.; Bolzani, V. S.; Silva, D. H. S.; Regasini, L. O.; Silva, T. G. A.; Morais, M. C. C.; Ximenes, V. F.; Soares, C. P. *Tumor Biol*. **2010**, *31*, 513. [Crossref]

[22] Hashimoto, Y.; Shimada, Y.; Itami, A.; Ito, T.; Kawamura, J.; Kawabe, A.; Kaganoi, J.; Maeda, M.; Watanabe, G.; Imamura, M. *Eur. J. Cancer* **2003**, *39*, 2239. [Crossref]

How to cite this article

Moro, I. J.; Carvalho, F. A.; Moreira, T. F.; Souza, F. O.; da Silva, A. A.; Politi, F.; Soares, C. P.; dos Santos, A. G. *Orbital: Electron. J. Chem.* **2022**, *15*, 21. http://dx.doi.org/10.17807/orbital.v15i1.17021