Research, Society and Development, v. 12, n. 3, e14612340536, 2023
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Antiproliferative activity from five Myrtaceae essential oils

Atividade antiproliferativa de cinco óleos essenciais de Myrtaceae

Actividad antiproliferativa de cinco aceites esenciales de Myrtaceae

Received: 02/10/2023 | Revised: 02/24/2023 | Accepted: 02/26/2023 | Published: 03/03/2023

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Abstract
The Myrtaceae plants are cultivated for their edible fruits and folk medicinal uses. Likewise, their leaves, flowers, and fruit essential oils (EOs) are described to have antiproliferative, antimicrobial, and antioxidant activities. This work analyzed the chemical compositions and evaluated the antiproliferative activities of essential oils (EO) of *Myrcia bella Cambess*, *Myrcia fallax* (Rich.) DC., *Myrcia guianensis* (Aubl.) DC., *Eugenia aurata* O. Berg, and *Eugenia punicifolia* (Kunth) DC, from the Brazilian Cerrado. EOs were obtained by leaf hydrodistillation and analyzed by Gas Chromatography-Mass Spectrometry (GC-MS) plus Gas Chromatography-Flame Ionization Detector (GC-FID). The major component in *M. bella* was α-cadinol (14.4%); in *Myrcia fallax* (Rich.) DC., guaiol acetate (14.4%); and in *M.guianensis*, (E)-iso-γ-bisabolene (17.5%). For *E. aurata* and *E. punicifolia*, bicyclogermacrene (25.3%) and (E)-caryophyllene (18%), respectively, were the most relevant chemical constituents. The EOs were tested against the human tumor cell lines UACC-62 (melanoma), MCF7 (breast), 786-0 (kidney), NCI-H460 (lung), OVCAR-3 (ovarian), HT29 (colon), and K562 (leukemia) and one non-tumor cell line (VERO). *Eugenia aurata* presented the most promising effect against HT29 (TGI = 1.5 μg/mL), K562 (TGI = 5.0 μg/mL), and no toxicity against non-tumor VERO cells (TGI> 250 μg/mL). For the other EOs, moderate to no activity was observed. So, in conclusion, *E. aurata* EOs revealed a good potential for anticancer applications.
Keywords: *Myrcia* spp. and *Eugenia* spp.*;* Essential oil, GC-MS; *in vitro* antiproliferative assay.

Resumo
As Myrtaceae são plantas cultivadas por causa seus frutos comestíveis e usos medicinais. Seus óleos essenciais de folhas, flores e frutas (OEs) são descritos como tendo atividades antiproliferativas, antimicrobianas e antioxidantes. Este trabalho analisou as composições químicas e avaliou as atividades antiproliferativas de óleos essenciais (OE) de *Myrcia bella* Cambess, *Myrcia fallax* (Rich.) DC., *M. guianensis* (Aubl.) DC., *Eugenia aurata* O. Berg e *Eugenia punicifolia* (Kunth) DC, do Cerrado brasileiro. Os OEs das folhas foram obtidos por hidrodestilação e analisados por cromatografia gasosa-espectrometria de massas (GC-MS) e cromatografia gasosa-detector de ionização de chama (GC-FID). O componente majoritário em *M. bella* foi o α-cadinol (14,4%); em *Myrcia fallax* (Rich.) DC., acetato de guaiol (14,4%); e em *M.guianensis*, (E)-iso-γ-bisaboleno (17,5%). Para *E. aurata* e E. punicifolia, biciclogermacreno (25,3%) e (E)-cariofileno (18%), foram os constituintes químicos mais relevantes. Os OEs foram testados contra as linhagens de células tumorais humanas UACC-62 (melanoma), MCF7 (mama), 786-0 (rim), NCI-H460 (pulmão), OVCAR-3 (ovário), HT29 (cólon) e K562 (leucemia) e uma linhagem celular não tumoral (VERO). *Eugenia aurata* apresentou o efeito mais promissor contra HT29 (TGI = 1,5 μg/mL), K562 (TGI = 5,0 μg/mL) e nenhuma toxicidade contra células VERO não tumorais (TGI> 250 μg/mL). Para os outros OEs, foram observadas atividades moderadas a nenhuma. Portanto, os OEs de *E. aurata* revelaram um bom potencial para aplicações anticancerígenas.
Palavras-chave: *Myrcia* spp. and *Eugenia* spp.; Óleos essenciais; Atividade antiproliferativa *in vitro*.

Resumen
Las plantas de Myrtaceae se cultivan por sus frutos comestibles y usos medicinales populares. Asimismo, se describe que sus aceites esenciales (AE) de hojas, flores y frutos tienen actividades antiproliferativas, antimicrobianas y antioxidantes. Este trabajo analizó las composiciones químicas y evaluó las actividades antiproliferativas de los aceites esenciales (AE) de *Myrcia bella* Cambess, *Myrcia fallax* (Rich.) DC., *Myrcia guianensis* (Aubl.) DC., *Eugenia aurata* O. Berg, y *Eugenia punicifolia* (Kunth) DC, del Cerrado brasileño. Los EO se obtuvieron por hidrodestilación de hojas y se analizaron por cromatografía de gases-espectrometría de masas (GC-MS) más cromatografía de gases-detector de ionización de llama (GC-FID). El componente mayoritario en *M. bella* fue α-cadinol (14,4%); en *Myrcia fallax* (Rich.) DC., acetato de guaiol (14,4%); y en M.guianensis, (E)-iso-γ-bisaboleno (17,5%). Para *E. aurata* y *E. punicifolia*, el biciclogermacreno (25,3 %) y el (E)-cariofileno (18 %), respectivamente, fueron los constituyentes químicos más relevantes. Los EO se probaron frente a las líneas de células tumorales humanas UACC-62 (melanoma), MCF7 (mama), 786-0 (riñón), NCI-H460 (pulmón), OVCAR-3 (ovario), HT29 (colon) y K562. (leucemia) y una línea celular no tumoral (VERO). Eugenia aurata presentó el efecto más prometedor contra HT29 (TGI = 1.5 μg/mL), K562 (TGI = 5.0 μg/mL) y no presentó toxicidad contra células VERO no tumorales (TGI > 250 μg/mL). Para los otros AE, se observó actividad de moderada a nula. Entonces, en conclusión, los AE de *E. aurata* revelaron un buen potencial para aplicaciones anticancerígenas.
Palabras clave: Myrcia spp. y Eugenia spp., Aceite esencial, Ensayo antiproliferativo in vitro.

1. Introduction

The Brazilian Cerrado biome is a valuable source of medicinal plants. Some of these plants, used in folk medicine, possess valuable bioactive compounds and have a wide range of uses. They have been used to improve human health, cosmetics, and food additives. Most drugs currently used in clinical trials for pharmaceutical product development are derived from plants. For this purpose, searching for new drugs is still one of the most appealing subjects in plant-natural product studies (de Melo et al., 2011; Alves et al., 2020; Jerônimo et al., 2021).

Many cancer patients have resorted to using plant complementary and alternative therapies as adjuvant treatments since this illness is considered one of the most challenging treatments in medicine. Cancer treatments encompass radiation, chemotherapy, and surgery. Chemotherapy encounters problems such as poor bioavailability, toxicity, adverse side effects, and high dose requirements (Senapati et al., 2018). Chemotherapy could be toxic because many antineoplastic agents are not specific to cancer cells and can also damage healthy cells. Moreover, solid tumors are generally resistant to chemotherapy due to the inability of the drugs to access hypoxic cells. So, the high toxicity and the lack of selectivity of conventional anticancer therapies make the search for alternative treatments a priority. So, research into new drugs with more effective anticancer activities and with no or minimal side effects could be interesting. Also, combinations of various therapies using EOs may minimize the side effects associated with chemotherapeutic treatments and ensure efficient clinical results in anticancer treatments (Abu-Izneid et al., 2020).

Myrtaceae EOs (essential oils) are described to have insecticidal, antimicrobial, antioxidant, and antiproliferative activities (Cascaes et al., 2015; Durazzini et al., 2019; Franco et al., 2021). The Myrtaceae are a group of woody shrubs or tree species distributed in tropical and subtropical regions in South America, Australia, and tropical Asia. In Brazil, the Myrtaceae family [*Myrcia*, *Eugenia*, *Psidium*, *Campomanesia*, and *Eucalyptus* genera] contains 1026 species scattered in the Brazilian forests. These species are economically relevant since they are cultivated for their edible fruits, ornamental purposes, and as a source of timber (Jerônimo et al., 2021).

Myrcia spp. leaves are employed in South American traditional medicine to treat diabetes, hypertension, diarrhea, inflammation, and skin infections (Franco et al., 2021). Also, *Myrcia* spp. are a rich source of EOs, mainly mono- and sesquiterpenes (Ramos et al., 2010; Stefanello et al., 2011; Cascaes et al., 2015; Scalvenzi et al., 2017). *Myrcia bella* Cambess is widely distributed in the Brazilian Cerrado, and their leaves are used in traditional medicine to treat gastrointestinal disorders and diabetes (Saldanha et al., 2020; Santos et al., 2018). Pharmacological studies have already revealed that its hydroalcoholic leaf extracts have antiproliferative, antimicrobial, and antidiabetic activities (de Souza et al., 2018). *Myrcia fallax* (Rich.) DC is

a tree distributed from eastern Mexico to the southeastern Brazilian coastal forests, and its cytotoxic activities extracts have already been established (Stefanello et al. 2011; Scalvenzi et al. 2017). *Myrcia guianensis* (Aubl.) DC., the most abundant Cerrado species, is popularly used as a tea to treat diabetes, and their macerated leaves have been used to neutralize the effects of different snake venoms (Bernardes et al., 2018).

Eugenia spp. extracts and EOs are used as antidiabetic, antirheumatic, antipyretic, anti-inflammatory, antidiarrheal, antifungal, and antibacterial, among other activities (de Souza et al., 2018). *Eugenia* spp. EOs chemical studies have demonstrated antitumor effects were correlated to their sesquiterpenes (Aranha et al., 2019; Fernandes et al., 2021). *E. punicifolia* leaves extracts are used in treatments for diabetes, fever, and influenza (Périco et al., 2019), and the chemical composition of their EOs is well-studied (Mazutti da Silva et al., 2018). These EOs are also described as adjuvants in treating *Diabetes mellitus* (Sales et al., 2014). *Eugenia aurata*, an endangered Brazilian Cerrado specie, has a described anti-inflammatory activity (Costa et al., 2016).

Due to the ongoing Myrtaceae phytotherapeutic use in traditional medicine, this study aims to correlate the chemical composition and antiproliferative activities of essential oils from the fresh leaves of *Myrcia bella* Cambess, *Myrcia fallax* (Rich.) DC., *Myrcia guianensis* (Aubl.) DC., *Eugenia aurata* O. Berg, and *Eugenia punicifolia* (Kunth) DC, from the Cerrado, in the northwest of São Paulo State, Brazil.

2. Methodology

Collections and extraction

Leaves of *Myrcia bella* Cambess (0559034/7499949 (+/-4m) UTM), *Myrcia fallax* (Rich.) DC. (0561614/7500892(+/-3m) UTM), *Myrcia guianensis* (Aubl.) DC (0561747/7501001 (+/-3m) UTM), *Eugenia aurata* O. Berg (0559055/7499970 (+/-4m) UTM) and *Eugenia* punicifolia Humb. Benpl. & Kunth (0561750/7500935 (+/-3m) were collected in the wet season (December) at the Instituto Florestal e Estações Experimentais – Floresta Estadual de Assis, in Assis, São Paulo State, Brazil. *Myrcia fallax, M. guianensis, E. aurata* O. Berg, and *E. punicifolia* were identified by Dr. Antônio C.G. Melo, and their voucher specimens (nos. 43.522, 43.523, 43.520, and 43.521) deposited in the Herbarium D. Bento Pickel, Assis, SP, Brazil. *Myrcia bella* was identified by Ms. Jorge Tamashiro, and the voucher specimen (UEC 157583) was deposited at the University of Campinas (UNICAMP). Those collections were registered at Cotec (Comissão Técnico Científica da Secretaria do Meio Ambiente-Instituto Florestal, Technical Scientific Committee of Environment Secretariat-Forestry Institute, number 206108-005.298/2009). They were also registered in Sisgen (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado, National System for the Management of Genetic Heritage and Associates Traditional Knowledge, number A9D1538). These collections were done in December 2009 before 10 pm.

After collection, 500 g of fresh Myrtaceae leaves were subjected to hydrodistillation in a Clevenger-type apparatus for approximately 4 h to obtain the EOs, according to the procedure described in the Brazilian Pharmacopoeia. After filtration, the samples were dried over anhydrous sodium sulfate and stored at −4 °C until further chemical analysis and biological tests were conducted. Total oil yield was expressed as a percentage (g/100 g fresh plant material).

GC-MS analysis

The chemical composition of EOs was determined by an HP 6890 gas chromatograph equipped with a mass selective detector (MSD HP 5975) and an HP-5MS column (30 m × 0.25 mm × 0.24 mm). The carrier gas was He at 57.4 kPa and a 1.0 mL/min flow rate. The injector temperature was 220 °C, with a split ratio of 1/30 and injected volume of 0.1 µL. The detector temperature was 250 °C, and for the column, the temperature was increased by 3 °C/min from 60 to 240 °C. Spectra

were recorded in the electron ionization (EI) mode, with an ionization energy of 70 eV (2 scan/s). A sample of essential oil was dissolved in ethyl acetate (20 mg /mL) for analysis. The retention index was calculated using a mixture of linear alkanes (C_8–C_{30}) as an external reference (Adams, 2007). The identification of the analytes was carried out by comparing the retention indices (IR) obtained by the injection of hydrocarbon standards (C-8 to C-24) with the equipment's database (NIST-11 library) and with data from the literature (Adams 2007). Meanwhile, a GC coupled with a flame ionization detector (FID) was used for compound quantification in the same conditions described above, except for the carrier gas (hydrogen, at 1.5 mL/min). The percentage composition, in turn, was obtained by the electronic integration of the FID signal by dividing the area of each component by the total area (%).

Antiproliferative studies

To evaluate the antiproliferative activity of the five EOs, we followed the protocol developed by National Cancer Institute (Monks et al., 1991; Skehan et al., 1990). EOs were tested against seven human tumor lines: UACC-62 (melanoma), MCF-7 (breast), 786-0 (kidney), NCI-H460 (lung), OVCAR-3 (ovarian), HT-29 (colon), and K562 (leukemia) and non-tumor cells (VERO normal epithelial renal cell line, green monkey). Stock and experimental cultures were grown in media containing RPMI-1640 supplemented with 5% fetal bovine serum (FBS; Gibco-BRL, Grand Island, NY, USA). A penicillin-streptomycin mixture (100 μg mL^{-1}/1 μg mL^{-1} RPMI) was added to the experimental cultures. Cell cultures in 96-well plates (100 μL cells/well, inoculation density $(3.5–6) \times 10^4$ cell/mL were exposed to different concentrations (0.25–250 μg/mL) of DMSO/RPMI 1640 at 37º C, 5% CO_2 in the air for 48 h. The final DMSO concentration did not affect cell viability. Afterward, the cells were fixed with 50% trichloroacetic acid and incubated for 1 h at 4 ºC. The cell proliferation was determined by OD540 readings of cellular protein content using sulforhodamine B assay (Monks et al. 1991). The chemotherapeutic agent doxorubicin (DOX; range 0.025–25 μg/mL) was used as a positive control. Cell proliferation was determined by a concentration-response curve for each cell line (Medina et al. 2018). The TGI. (Total Growing Inhibition, cytostatic activity) was obtained through the nonlinear regression analysis using the Origin software 8.6 (USA). The cytostatic activity was classified following the Council for Scientific and Industrial Research (CSIR, South Africa) criteria (Fouche et al., 2008).

Data analysis

The parameter TGI was obtained from the values observed in the concentration-response curve for each cell line and determined through nonlinear regression analysis using Origin 8.6 software.

3. Results and Discussion

The Myrtaceae family is a rich source of essential oils from their aerial parts (leaves, flowers, branches, and fruits) with well-known biological activities (Cascaes et al., 2015). The terpene diversity of this family has been described in species of different genera, such as *Eugenia*, *Myrcia*, *Psidium*, and others (Oliveira & Souza Filho, 2022). In all samples tested, 62 compounds represented an average of 99.1% of the total composition. The oil yields of *M. bella* (0.10%), *M. fallax* (0.20%), *M. guianensis* (0.10%), *E. aurata* (0.20%), and *E. punicifolia* (0.40%) are analogous to the presented to the literature (Oliveira & Souza Filho, 2022). The main volatile compounds detected in EOs were mostly sesquiterpenes with caryophyllene and germacrene skeletons. For monoterpenes, mostly linalool and terpinene types were detected (Table 1).

Myrcia spp. EOs compositions mainly comprise monoterpenes and sesquiterpenes (Cascaes et al., 2015; Gatto et al., 2020; Oliveira & Souza Filho, 2022). *Myrcia bella* and *M. fallax* EOs are composed of oxygenated sesquiterpenes (67.4% and 57.0%, respectively) and *M. guianensis* sesquiterpene hydrocarbons (74.3%). Monoterpene hydrocarbons were found mainly

in *M. fallax* (3.7%) and lower proportions in *M. bella* (1.7%). Oxygenated monoterpenes were found in all *Myrcia* spp. (*M. bella* 0.4%; *M. fallax* 0.6%, and *M. guianensis* 10.5%) (Table 1).

In *Eugenia* spp. EOs compositions predominate cyclic sesquiterpenes and monoterpenes in a minor fraction (Farag et al., 2018). In this manuscript, *E. aurata* and *E.punicifolia* presented mainly sesquiterpene hydrocarbons on their EO compositions (75.0% and 62.5%, respectively). Monoterpene hydrocarbons were found in *E. punicifolia* (6.6%), and oxygenated monoterpenes were found in *E. punicifolia* (2.1%). There are no monoterpenoids detected in *E. aurata* (Table 1).

Table 1 - Relative percentage compositions and retention indexes of the components of the Myrtaceae essential oils.

	Percentage compositions					RI	
Compound	**MB**	**MF**	**MG**	**EA**	**EP**	**Exp**	**Lit**
α-pinene		1.0			2.0	931	932
β-pinene					4.0	973	974
sabinene		1.0				973	969
limonene					0.7	1027	1024
linalool oxide (furanoid)			4.8			1072	1067
linalool	0.4	0,6	3.5		1.3	1102	1095
terpinen-4-ol					0.8	1178	1174
(3Z) hexenyl butanoate					0.8	1186	1184
α-terpinene	1.7	1,7				1192	1186
α-terpineol			2.2		2.7	1192	1186
δ-elemene		4,7			0.9	1336	1335
α-copaene			2.5	0.8	2.2	1373	1374
(3Z) hexenyl hexanoate			2.0			1381	1378
β-elemene	0.7	0,9		3.8	2.3	1390	1389
(E) caryophyllene	1.5	7,4	3.4	5.3	18.0	1416	1417
β-copaene				0.8	1,2	1426	1430
aromadendrene	0.4	2,0				1435	1439
α-guaiene				2.3		1436	1437
α-humulene	0.7			4.4	6,0	1450	1452
Amorpha-4,7(11)diene				19.8		1480	1479
sesquisabinene			9.2			1456	1457
allo-aromadendrene				0.7		1457	1458
γ-muurolene	1.1	6,6			8,7	1474	1478
γ-muurolol				1.0		1475	1478
germacrene D	11.8					1478	1484
γ-himachalene		1,2	16.2			1483	1481
δ-selinene		5.7				1488	1492
cis-β-guaiene		2.2	8.5			1490	1492
β-selinene	7.4			0.5	2.2	1493	1489
bicyclogermacrene				25.3		1495	1500
α-muurolene	1.1	1.5	2.0		2.1	1498	1500
isodaucene					1.1	1501	1500
premnaspirodiene				6.5		1503	1505
β-bisabolene	0.4		5.7			1507	1505
γ-cadiene	0.6	0.7		0.8	1.4	1511	1513
7-epi-α-selinene			5.6			1514	1520
δ-cadinene	2.6	4.2	3.7	4.0	3.2	1521	1522
(E)-iso-γ–bisabolene			17.5			1541	1528
elemol	0.9					1549	1548
germacrene B	1.2	1.2			13.1	1553	1559
longipinanol	0.6					1558	1569
maaliol	2.9			1.2		1564	1566
spathulenol	2.4				2.5	1576	1577
caryophyllene oxide					6.0	1579	1582
Thujopsan-2-α-ol				2.5		1582	1586
ledol	12.2					1582	NIST
globulol	6.3	2.0		3.4		1582	1590
guaiol	3.7	8.0				1592	1600
rosifoliol	4.3	1.2		1.6		1600	1600

junenol					1.2	1615	1618
1-epi-cubenol	1.7					1626	1627
guaiol acetate		24.7				1726	1725
γ-eudesmol	4.3		5.9			1631	1630
τ-muurolol		6.1				1640	1631
epi-α–muurolol	9.4		2.0	3.0	5.5	1642	1642
α-muurolol				0.8	2.5	1646	1644
cubenol	3.4		1.6			1646	1645
β-eudesmol			3.2			1649	1649
α-cadinol	14.4	10.5	4.8	11.3	6.3	1655	1652
germacra-4(15),5,10(14)-trien-1-α-ol	0.7					1685	1685
eudesm-7(11)-em-4-ol	0.4	2.0			1.0	1704	1700
Monoterpene hydrocarbons	1.7	3.7			6.6		
Oxigenated monoterpenes	0.4	0.6	10.5		2.1		
Monoterpenes: total	2.1	4.4	10.5		8.7		
Sesquiterpene hydrocarbons	29.5	36.0	74.3	75.0	62.5		
Oxigenated sesquiterpenes	67.4	57.0	12.8	24.8	27.7		
Sesquiterpenes: total	96.9	93.0	87.1	99.8	90.3		
Others			2.0		0.8		
Total identified	99.0	97.4	99.6	99.8	99,8		
% Yield (w/w)	0.10	0.20	0.10	0.20	0.40		

MB (*Myrcia bella*); **MF** (*M. fallax*); **MG** (*M.guianensis*); **EA** (*Eugenia aurata*); **EP** (*E. punicifolia*).**RI EXP:** Retention index relative to *n*-alkanes (C8-C20) on the DB5-ms (30 m X 0.25 mm; 0.250 µm) column; **RI LIT:** Retention index found in the literature. (Adams (2007). Source: Authors.

For *M. bella*, the major chemical constituents identified were the oxygenated sesquiterpenes α-cadinol (14.4%) and ledol (12.2%) and the sesquiterpene hydrocarbon germacrene D (11.8%). The α-cadinol and germacrene D are ubiquitous in the EOs of *Myrcia* spp. (Stefanello et al. 2011; Cascaes et al. 2015). In a previous study of the EOs leaves composition, *Calycolpus goetheanus* (Myrtaceae) presented α-cadinol (9.03%) (Franco et al., 2022), and germacrene D was characterized by the EOs of *M. tomentosa* (11.45%) (Franco et al., 2021). Ledol was characterized in the leaves of *M. hatschbachii* D. Legrand (2.95%), for the flowers (19.8%) of *Campomanesia pubescens* and *C. adamantium* seeds (20.9 %) (Cardoso & Ré-Poppi, 2009; Gatto et al., 2020; Stefanello et al., 2011)

Myrcia fallax Rich DC (syn. *M. splendens* (Sw.) DC) contrasted the EOs composition with the same species reported in the literature. In this manuscript, the main sesquiterpenoid compound found in *M. fallax* was guaiol acetate (24.7%), followed by α-cadinol (10.5%). From Amazonian Ecuador, the sesquiterpene alcohols trans-nerolidol (67.81%) and α-bisabolol (17.51%) were found to be as most important compounds (Scalvenzi et al. 2017). From Amazonian Venezuela, the sesquiterpenoid guaiol (31.0%) and the monoterpenes α- and β-pinene (7.7 and 6.9%) were detected as the major constituents (Alarcón et al. 2009). In Brazil's southeastern Atlantic Forest, the sesquiterpene hydrocarbon α-bisabolene (80%) was the major compound detected (Nakamura et al. 2010).

The major chemical compounds identified in *M. guianensis* (syn *Myrcia myrtillifolia* sensu McVaugh non DC.) were the sesquiterpenes (E)-iso-γ-bisabolene (17.5%) and γ–himachalene (16.2%). *M. myrtillifolia* EOs, from tropical desert-like northeastern Brazil, showed an EO profile rich in monoterpenes such as α-pinene (80.4%) and the oxygenated monoterpene α-terpineol (7.0%) (Cerqueira et al., 2007).

E. punicifolia, collected from in the northwest of São Paulo State (southeast Brazilian Cerrado), revealed a prevalence of sesquiterpenes, such as *E*-caryophyllene (18.0%) and germacrene B (13.1%). *E*-caryophyllene and sesquiterpenes are also found in the other *E punicifolia* collected from other parts of the country. From the Amazon, *E. punicifolia* also revealed the prevalence of (*E*)-caryophyllene (9.87%) and bicyclogermacrene (8.75%). Additionally, the Brazilian Southeast Maritime Forest (Restinga) contained sesquiterpenes, such as β-elemene (22.1%), β-caryophyllene (8.5%), and components of the

selinane (24.8%) and cadinane (14.0%) skeletal-types (Ramos et al., 2010). Sesquiterpenes are also found in *E. punicifolia* collected in the Atlantic Forest in Rio de Janeiro, which showed β-elemene (22.1%) followed by β-caryophyllene (8.5%) as major constituents (Ramos et al., 2010). In the study conducted by Franco et al. (2021), the EOs extracted from *E.punicifolia* were composed of sesquiterpenes such as α-cadinol (10.6%), 10-*epi*-γ-eudesmol (10.2%), and paradisiol (9%) (Franco et al., 2021). Although most *E. punicifolia* EOs have been described as mainly presenting sesquiterpenes, some are rich in monoterpenes, such as those found in Maranhão State (northeast Brazil). In these EOs, composition profiles abound monoterpenoids as α-pinene (53.6%), 1,8-cineole (13.8%), and α-terpineol (7.6%) (Fernandes et al. 2021).

It is the first study reporting the chemical composition of *E. aurata* EOs, where the sesquiterpenes bicyclogermacrene (25.3%) and amorpha-4,7(11)diene (19.8%) were the main compounds found. The Myrtaceae chemical constitution of essential oils exhibits considerable variability. These EOs usually show high chemodiversity, i.e., different chemical compositions in plants of the same species, due to the taxonomic, genetic variability and the geographical and environmental conditions in which the plants grow (Fernandes et al. 2021). This aspect is more evident when the species studied are from areas with high biodiversity, where the interaction among different species can induce secondary plant metabolism and differentiated biosynthetic pathways, resulting in molecular diversity (Barbosa et al., 2016; Cascaes et al., 2015; Scalvenzi et al., 2017).

The anticancer potential of Myrtaceae EOs has been investigated, seeking to apply them as therapeutic agents, whether in alternative or complementary treatments (Russo et al., 2015). The antiproliferative activity of EOs could be attributed to the synergistic effects of all terpenes in their chemical composition; even compounds present in low concentrations could be responsible for the antiproliferative effects detected (Cascaes et al., 2015; Durazzini et al., 2019; Furtado et al., 2018)

For analysis of the antiproliferative activities of EOs, we followed the criteria established by Fouche et al. (2008) to determine TGI values (Fouche et al., 2008). The TGI values are considered to indicate inactive (TGI > 50 μg/mL), weakly active (15 μg/mL < TGI < 50 μg/mL), moderately active (6.25 μg/mL < TGI < 15 μg/mL), and potent (TGI < 6.25 μg/mL) antiproliferative activities.

Following this classification, the EOs from *Myrcia* spp. weakly inhibited tumor cell proliferation for all tested lineages, including non-tumor cell proliferation (VERO, normal cell line)(Table 2). *Myrcia bella* exhibited a weak effect for 786-0 (kidney) and HT29 (colon) tumor cells; *M. fallax* showed a weak cytostatic effect against UACC-62 (melanoma), 786-0 (ovarian), and HT-29 cells; and *M. guianensis* EOs weakly inhibited OVCAR-03 and HT-29 tumor cells. *E. aurata* EOs exhibited potent inhibition of cell growth for HT29 (TGI = 1.5 μg/mL) and K562 (chronic myeloid leukemia; TGI = 5 μg/mL), as well as moderate cell growth inhibition for 786-0 (TGI = 10 μg/mL) and a low inhibition for HT29 (TGI = 22.4 μg/mL). Moreover, *E. aurata* and *E. punicifolia* EOs did not affect the cell growth of non-tumor cells (VERO, TGI> 250 μg/mL). (Table 2).

Table 2 - Antiproliferative activity (TGI[a], μg/mL) of doxorubicin and the essential oil from five Myrtaceae leaves.

	Samples[c]					
Cell lines[b]	**DOX**	**MB**	**MF**	**MG**	**EA**	**EP**
UACC-62	<2.5 (P)	106.9±6.3 (I)	30* (W)	64.09±0.01(I)	93.6±37.1(I)	66.8±6.8 (I)
MCF7	<2.5 (P)	164.3±3.8 (I)	41.0 ± 0.1 (W)	109.9±27.3 (I)	>250 (I)	141.3±31.9 (I)
786-0	2.6 ±1.9 (P)	47.5±32.3(W)	37.3±17.8 (W)	68.5±24.1 (I)	10* (M)	60.8±27.5 (I)
NCI-H460	<2.5 (P)	>250 (I)	>250 (I)	121.9±7.7 (I)	>250 (I)	>250 (I)
OVCAR-03	<2.5 (P)	91.2±23.4 (I)	39.7± 9.6 (W)	40* (W)	18* (W)	66.3±20.3 (I)
HT29	<2.5 (P)	40* (W)	22.3±0.6 (W)	40* (W)	1.5* (P)	22.4±19.8(W)
K562	6.6±3.4 (M)	52.0±21.0 (I)	75.3±2.8 (I)	64.5±19.9 (I)	5* (P)	52.6±31.7 (I)
VERO	<2.5 (P)	>250 (I)	162.4±23.5(I)	40* (W)	>250 (I)	79.8±13.4 (I)

[a] Results expressed as the concentration required to elicit total growth inhibition (TGI) in μg/mL followed by the standard error of the mean (SEM), N=3, calculated by sigmoidal regression using Origin 8.6 software; Results classified according to CSIR's criteria: inactive (I, TGI > 50 μg/mL), weak (W, 15 μg/mL < TGI < 50 μg/mL), moderate (M, 6.25 μg/mL < TGI < 15 μg/mL) or potent (P, TGI < 6.25 μg/mL) activity (Fouche et al., 2008); * Estimated TGI value = when experimental data did not converge (standard error higher than calculated effective concentration).
[b] Cell line panel: composed of seven human tumor cell lines; UACC-62 (melanoma); MCF7 (breast, adenocarcinoma); 786-0 (kidney, adenocarcinoma); NCI-H460 (lung, large cell carcinoma); OVCAR-3 (ovarian adenocarcinoma); HT29 (colon, adenocarcinoma); K562 (chronic myeloid leukemia) and one non-tumor cell line (VERO, immortalized epithelial cell from green monkey kidney)
[c] Samples: DOX = Doxorubicin (positive control); MB = *Myrcia bella*; MS = *M. fallax*; MG = *M. guianensis* leaves; EA = *Eugenia aurata*; EP = *E. punicifolia*. Source: Authors.

Myrcia and *Eugenia* spp. were composed of sesquiterpenes such as germacrane, caryophyllane, cadinane, and similar skeleton types. For monoterpenes, the composition profile presented linalool and terpinene-type skeletons. These compounds exhibit a wide range of antiproliferative activities directly linked to their chemical variability. Antiproliferative activities are usually correlated to the cytotoxic sesquiterpenes such as β-caryophyllene and germacrene derivatives (Salvador et al., 2011;Cascaes et al., 2015; Furtado et al., 2018; Silva et al., 2019). Also, monoterpenes such as α-terpineol, α-pinene, β-pinene, and linalool have been described as having antiproliferative effects on the K-562, MCF-7, and HT29 cell lines.

E. aurata EOs, which has biciclogermacrene (25%) and sesquiterpenoid amorpha-4,7(11)diene (19,8%), has no monoterpene in its composition. So, monoterpenes appear less relevant for antiproliferative activity than sesquiterpenes (Tables 1-2). A slight synergistic effect between monoterpenes and sesquiterpenes could be observed in *M. fallax*.

4. Final Considerations

Our findings provide novel insights into the field of antiproliferative activities of some Myrtaceae EOs. The antiproliferative activities found by this study may be justified by the presence of these major constituents, the sesquiterpenes, since all of them already have their anticancerous activity well described in the literature. The results of this study highlight *E.aurata* EOs as potential sources in the search for new antiproliferative agents and reinforce the importance of further studies to evaluate their phytochemical, toxicological and pharmacological aspects.

Acknowledgments

We thank the Foundation for supporting research in the São Paulo State FAPESP (grant number 2017/15610-0).

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