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Chemical profile and biological properties of the *Piper corcovadense* C.DC. essential oil

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ABSTRACT

The essential oil from Piper corcovadense D.DC. (EOPc), an important plant belonging to the Piperaceae family, which is commonly found in the northern region of Brazil and poorly explored scientifically, was used in this study. Thus, the EOPc was characterized chemically by Gas Chromatography/Mass Spectrometry (GC/MS) and the antioxidant and antimicrobial activities and their potential effects on cutaneous melanoma (SK-MEL-28) and healthy peripheral blood mononuclear (PBMC) cells were determined. The major compounds identified in the EOPc were: trans-sesquisabinene hydrate, trans-caryophyllene, β-pinene, trans-β-farnesene, 14-hydroxycaryophyllene, limonene and *p*-cymene. The EOPc demonstrated antioxidant activity as evaluated by Folin-Ciocalteu reagent (FC) reducing capacity, DPPH, and ABTS methods. The values found were respectively 5.41 ± 0.17 mg GAE mL^{-1} (GAE: Gallic acid equivalent), 2.88 ± 0.17 μmol TE mL^{-1} (TE: Trolox equivalent) and 6.26 ± 0.02 μmol TE mL^{-1}. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined for different bacterial strains. The EOPc at a concentration of 2.61 μg mL^{-1} exhibited both bactericidal and bacteriostatic properties against *Escherichia coli*. The EOPc showed potential antitumor activity as it reduced the cell viability of human cutaneous melanoma cells SK-MEL-28. Besides, the EOPc did not exhibit cytotoxic activity against healthy PBMCs, indicating that it does not harm healthy cells at the tested concentrations. The EOPc increased the levels of ROS at concentrations of 250 μg mL^{-1}. The EOPc also did not stimulate the mobilization of endogenous antioxidant defenses, as assessed by total thiol (PSH) and non-protein thiols (NPSH). Thus, the study suggests that the EOPc has antioxidant and antimicrobial properties due to the presence of specific compounds. It also exhibits antitumor potential against cutaneous melanoma cells while showing no cytotoxicity to healthy PBMCs. It directly influenced ROS levels at the highest tested concentration in the cells, suggesting an antitumor effect related to the intrinsic apoptosis pathway. Nevertheless, while the study has initial findings, the results are promising and indicate an attractive biological potential of *P. corcovadense*, mainly in human cutaneous melanoma cells.

1. Introduction

Natural products come from the specialized metabolism of plants, and they are biosynthesized according to the need for adaptations to biotic and abiotic stress conditions (Marone et al., 2022). Essential oils (EO) are compounds derived from the specialized metabolism of plants, and their composition is quite diverse and includes monoterpenes, diterpenes, and sesquiterpenes, in addition to phenylpropanoids (Calo et al., 2015).

There are studies in the literature that relate the antioxidant (Mat

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Saad et al., 2021), antimicrobial (Meenu et al., 2023; Terezinha de Oliveira et al., 2019), antitumoral (Yi et al., 2022), insecticide (Li et al., 2021), antifungal and antidiabetic activities (Chander et al., 2022) with the essential oils present in the plant. The use of health treatments and therapeutics in folk medicine is also reported (Al-Abri et al., 2022). The bioactivity properties of plants have been attributed to their terpenoid, carotenoid and phenolic compounds (Balahbib et al., 2021; Braga et al., 2021; Perin et al., 2020).

Piper corcovadense D.DC. is a plant native to Brazil and South American countries under the cover of the Amazon rainforest. It occurs in tropical regions, a small plant belonging to the Piperaceae family. In Brazil, this family is represented by around five hundred species from six genera: Ottonia, Piper, Peperomia, Manekia, Pothomorphe and Sarcorhachis (Do Nascimento et al., 2012; Fernandez et al., 2020; Herrera et al., 2022). *P. corcovadense* is popularly known as João Brandinho and is used to treat colds, flu, toothaches, and treatment of tuberculosis by Amazonia's riverside community (Facundo et al., 2004; Parmar et al., 1997). Furthermore, this plant has been used as an anti-inflammatory and anti-larvicide against *Aedes aegypti* (Albuquerque et al., 2022). The main chemical constituents of *Piper* species essential oil comprise monoterpenes, sesquiterpenes, and oxygenated monoterpenes (Do Nascimento et al., 2012) with biological activities (Braga et al., 2021; Da Silva et al., 2016).

Cutaneous melanoma is a very aggressive skin cancer that can metastasize, that is, spread to other organs and be difficult to treat (Cummins et al., 2006). However, the compounds derived from the specialized metabolism of plants can be used as therapeutic agents, helping to combat tumor cells. Alternatives treatments can be less aggressive coadjuvants than conventional cancer treatments such as chemotherapy and immunotherapy (Da Silva et al., 2023).

In this work, the potential of the EOPc was explored as a source of therapeutic compounds to combat cutaneous melanoma, a highly aggressive form of skin cancer. In this way, the chemical composition of the essential oil is investigated using gas chromatography-mass spectrometry (GC-MS). The study aims to assess whether the essential oil of this plant has antioxidant and antimicrobial properties, which could help protect healthy cells and fight oxidative stress, in addition to strengthening the immune system weakened by the disease. Biochemical parameters and cell viability assay were used to assess the safety and potential efficacy of the essential oil.

2. Materials and methods

2.1. Chemical reagents

Folin-Ciocalteu phenol reagents, gallic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2-azino-bis-(3-etil-benzoatiazolina-6-acid sulfonic) (ABTS) and Trolox were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Ethanol was purchased from (Êxodo cientifica Ltda, Sumaré, SP, Brazil), BHI (Braind Heart Infusion Broth) (IonLab, Araucária, PR, Brazil), Nutrient Agar (Himedia, Curitiba, PR, Brazil), Chloranphenicol (Vetec Química Fina, Duque de Caxias, RJ, Brazil), Resazurin sodium salt (Sigma-Aldrich Chemical Co., St. Louis, MO, USA). Cell culture plates and flasks used for culture procedures were obtained from Gibco™ Thermo Fisher Scientific (Grand Island, NY, USA). Cell culture plates and flasks used for culture procedures were obtained from Gibco™ Thermo Fisher Scientific (Grand Island, NY, USA) and Invitrogen Life Technologies (Carlsbad, CA, USA).

2.2. Plant material

The plant material was obtained in November 2020 in the municipality of Cruzeiro do Sul (AC), Brazil (7° 39′ 54″ S; 72° 39′ 1″ W; at 193 m altitude). With part of the plant material, a specimen was taken for identification purposes at the State University of the Midwest – campus Guarapuava (PR), Brazil. The plant material was identified as *Piper corcovadense* D.DC. and deposited under code: ARAUCA 1154.

2.3. Essential oil extraction

The essential oil was obtained from the roots, stems and leaves of *P. corcovadense* and was extracted in a semi-industrial plant at the company Fonte Clara Industrial e Comércio de Produtos Naturais LTDA (CNPJ 09.022.193/0001-56) headquartered on Bom Sucesso do Sul city, Paraná, Brazil (26° 6′ 36″ S; 52° 50′ 1″ W, at 543 m altitude). The essential oil was carried out by steam dragging with a yield of 1000 mL T^{-1}. After extraction, it was stored in an amber bottle in a freezer at −12° C.

2.4. Chemical characterization by GC-MS

GC-MS brand Shimadzu model GC-2010 Plus coupled to a triple quadrupole mass detector model TQ8040, automatic injector model AOC-5000 Plus and 70 eV ionization system. A solvent delay of 3.5 min and an interface temperature of 300 °C was used. The MS scan parameters included a mass range of *m/z* 40–350, a scan interval of 0.2 s and a scanning speed of 1666 amu s^{-1}.

The capillary column comprised of fused silica Rtx-5MS (5 % diphenyl + 95 % dimethyl polysiloxane) with dimensions 30 m x 0.25 mm x 0.25 μm, and the inert gas was helium at 1.02 mL min^{-1}. The volume of 1 μL was injected with an injection temperature of 250 °C and using a heating ramp from an initial temperature of 60 °C to 250 °C with heating of 3 °C min^{-1}. All samples were performed in triplicate.

Tentative identifications of the main compounds were achieved by comparison of the mass spectra obtained from the analyses. The components were identified based on the arithmetic index (AI) that occurs by the co-injection (under the same conditions of the sample) of a homologous series of saturated linear hydrocarbons containing C_8-C_{25} carbon atoms and analysis of their time of retention. Later, the AI value obtained for the component was compared with the literature and their mass spectrum with the reference mass spectra and experimental retention indices. The percentage composition of the components was calculated from the GC peak areas.

2.5. Antioxidant activity (AA)

The reducing capacity of the Folin-Ciocalteu reagent (FC) was evaluated according to Singleton et al. (1999). In this test, 0.50 mL of the EOPc was mixed with 2.5 mL of Folin-Ciocalteu and 2 mL of sodium carbonate 40 g/L (v/v). After two hours in darkness at room temperature, the absorbance was measured at 740 nm, and the results were expressed as mg GAE mL^{-1} (GAE: gallic acid equivalent) using a spectrophotometer (UV–VIS K37, Kasvi, São José dos Pinhais, Brazil). The radical scavenging ABTS (2,2-azino-bis-(3-ethyl-benzoathiazoline-6--sulfonic acid)) was performed according to Re et al. (1999). The stock solutions included 7.4 mM ABTS and 2.6 mM potassium persulfate. The solution was prepared by mixing 1 mL of the ABTS solution with 60 mL of ethanol to obtain an absorbance of 0.70 at 734 nm. Meanwhile, the DPPH free radical scavenging activity is described by Brand-Williams et al. (1995). The reaction medium consisted of 0.5 mL of EOPc, 3.0 mL of ethanol and 0.3 mL of 0.5 mM DPPH• solution. The mixture was incubated at room temperature in the dark for 45 min, and the absorbances were read at 517 nm using a spectrophotometer (Bel Photonics 2000, Piracicaba, Brazil). Both methods expressed the results in mM TE mL^{-1} (TE: Trolox equivalent). The assays were carried out in triplicate.

2.6. Antibacterial activity

The antibacterial activity of the essential oil was evaluated using *Bacillus subtilis* (ATCC 6051), *Escherichia coli* (ATCC 25922), *Listeria monocytogenes* (ATCC 19111) and *Salmonella* Typhimurium (ATCC 14028). Minimum inhibitory concentration (MIC) was determined by

triplicate microdilution method using 96 microwell plates containing BHI (Brain Hearth Infusion) and incubated on a microplate-shaker at 37 °C for 24 h (Carpes et al., 2021). The tested concentrations of the essential oil ranged from 10.00 to 0.16 μg mL^{-1}. Chloranphenicol (1.2 μg mL^{-1}) was used as the positive control. Additionally, the culture broth without antimicrobial substance was used as a growth control. To stop the reaction, 30 μL of resazurin (0.1 mg mL^{-1}) was added. MIC values were determined as no change in colour (blue), or that is the lowest concentration of each diluted, which completely inhibited microbial growth. Minimal bactericidal concentration (MBC) was evaluated on MIC positive results, carried out in Petri dishes containing BHI agar, according to the protocol of CLSI (2015). The analysis was performed in triplicate.

2.7. Biochemical parameters and cell viability assay

2.7.1. Cell culture and exposure to P. corcovadense essential oil

The human melanoma cell line SK-MEL-28 was purchased from the Cell Bank of Rio de Janeiro (BCRJ, Rio de Janeiro, Brazil). The cell culture was grown in a basal medium (DMEM - Dulbecco Modified Eagle Medium) manufactured by Vitrocell, Campinas, Brazil. The medium was supplemented with 10 % fetal bovine serum, antibiotic and antifungal 1 % (penicillin/streptomycin). The cells were grown in conditions based on a previous study performed by Da Silva et al. (2023). EOPc was dissolved in 0.2 % dimethylsulfoxide (DMSO) with the culture medium to obtain different concentrations. The cells were treated 24 h with 15.63, 62.50 and 250 μg mL^{-1} solutions. The negative control group (CT) cells received only the culture medium.

2.7.2. Peripheral blood mononuclear cells (PBMCS)

PBMCS were used to evaluate the cytotoxicity of compounds in non-tumour cells. Peripheral blood was collected by venipuncture from a healthy individual using BD Vacutainer® BD Biosciences blood collection tubes (San Diego, CA, USA) with anticoagulant K2-EDTA (1.8 mg mL^{-1}). For the isolation of PBMCS, 20 mL of blood samples were transferred and separated into 15 mL tubes containing Histopaque®-1077 Cell Density Gradient Medium (1.077 g mL^{-1}) Sigma-Aldrich (St. Louis, MO, USA) (2:1). For interphase collection, the tubes were centrifuged for 30 min at 1800 rpm. The PBMCS were transferred to a new tube and washed with saline solution (0.9 %). Further centrifugation was done for 10 min at 1500 rpm. The supernatant was discarded, and 5 mL of hemolytic buffer was added to remove remnants of red blood cells. The cells were centrifuged again for 10 min at 1500 rpm. The supernatant was discarded and washed with saline solution (0.9 %). After centrifugation, cells were resuspended in RPMI 1640 Gibco™ Thermo Fisher Scientific (Grand Island, NY, USA) containing 10 % FBS supplemented with penicillin/streptomycin/amphotericin B 0.1 %. The cells were cultured in 96-well plates at $1x10^6$ cell density and maintained in a modified atmosphere oven at 37 °C with 5 % CO_2 using a CO_2 incubator.

2.7.3. Cell viability by MTT assay

The cytotoxicity assay of EOPc on SK-MEL-28 and CCD-059sk was made with 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Sigma Aldrich, MO, USA) according to Mosmann (1983). Briefly, both cells were separately seeded in 96-well plates, in 4 replicates, at densities of 1×10^5 cells/well. The cells were treated with different concentrations of EOPc. After the exposure times, the supernatant was removed, and the cells were washed once with phosphate-buffered saline (PBS) (0.1 mol/L, pH 7.4) to avoid any interference from the compound used in the treatment. The MTT reagent (5 mg mL^{-1}) dissolved in PBS was added, and plates were incubated for 2 h at 37 °C. Subsequently, the supernatant was discarded, and 200 μL of DMSO was added to dissolve the formazan crystals generated by reduction of tetrazolium salts by dehydrogenases and reductases. The absorbance was measured at 570 nm using a SpectraMax® i3 Multimode Plate Reader (Molecular Devices, San Jose, CA, USA) and the results were expressed as percentage (%) of cell viability relative to control.

2.7.4. Cell viability by fluorescence microscopy

The fluorescence microscopy assay was performed according to Da Silva et al. (2023). This technique is based on marking nuclei acids using the fluorophore acridine orange (AO) (McGahon et al., 1995). After treatment with EOPc for 24 h, cells were seeded at a density of approximately 1×10^5 cells/well in 96-well plates, washed twice with phosphate-buffered saline (PBS) and stained with AO (1 μg mL^{-1}). Then, fluorescence micrographics were taken using an inverted microscope Nikon® Eclipse TS2-FL (magnification of 10x) in the excitation of 480 nm and emission of 490 nm. The final images were adjusted for brightness and contrast linearly by the software Imagej®. The results were expressed as a percentage (%) of fluorescence intensity relative to the control. All experiments were done at least three times in triplicates.

2.7.5. Apoptotic bodies

Detection of apoptotic body formation was made following the protocol established by Da Silva et al. (2023). For this time elapsed of treatment with EOPc, cells were washed twice with PBS, and stained with the AO (1 μg mL^{-1}) for 10 s. Afterwards, AO solution was removed by twice washing with phosphate-buffered saline PBS and fluorescence micrographics were made under the same condition employed to the cell viability by fluorescence microscopy. The photomicrographs were converted into grayscale to make clearer observations of apoptotic bodies' morphotypes and DNA fragmentations. The images were auto-adjusted for brightness and contrast by the software Imagej®, and the visual scan was made. All experiments were done at least three times in triplicates.

2.7.6. Oxidative stress test

The melanoma cells were seeded in 6-well plates at 1×10^6 cells/well densities and treated with different concentrations of EOPc, as previously described. After that, the supernatant was collected, and the detection of intracellular reactive oxygen species (ROS), nitric oxide (NOx) and levels of total thiol (PSH) and non-protein thiol (NPSH) were determined. All analyses were done in triplicate.

2.7.6.1. Intracellular reactive oxygen species (ROS). A commercial Fluorometric Intracellular ROS kit assay (Sigma Aldrich, Germany) was used. In the presence of ROS, the 2′,7′-dichlorofluorescein diacetate (H_2DCF-DA), a cell-permeable non-fluorescent substance, is oxidized into a fluorescent molecule, the 2′,7′-dichlorofluorescein (DCF). The cells received a solution containing H_2DCF-DA for 30 min at 37 °C. After, they were washed with PBS, and the product of the fluorimetric reaction proportional to the amount of ROS present was measured at excitation = 490 and emission = 520 nm in a microplate reader Varioskan™ LUX model (Thermo Scientific™, Waltham, MA USA).

2.7.6.2. Nitric oxide (NOx). According to Tatsch et al. (2011), nitric oxide is a reactive nitrogen species, and its degradation products as the nitrate/nitrite are more stable than nitric oxide. They can be easily detected by the Griess method with a microplate reader. The Griess reagent was prepared by combining 2 % sulphanilamide and 0.2 % N-1-naftil-etilendiamina-diclorhidrato (NED) in a 5 % orthophosphoric acid (H_3PO_4) medium. In this analysis, 50 μL of each sample was mixed with 50 μL of a solution containing 0.08 % vanadium chloride (VCl_3) and 50 μL of the Griess reagent. The mixture was then incubated at 37 °C for 20 min. The diazonium salt subsequently reacted with NED, resulting in the production of a purple-azo-dye product. The absorbance of this purple product formed in the reaction was measured at 540 nm using a spectrophotometer. A calibration curve was prepared, and results were expressed as μmol/L.

2.7.6.3. Total thiol (PSH) and non-protein thiol (NPSH). The levels of both total thiols and non-protein thiols were determined following the method of Ellman (1959). This method involves the reduction of *5,5'-dithiobis(2-nitrobenzoic acid)* (DTNB). For the total thiol assay, 40 µL of the sample supernatant was added to a 96-well plate with 200 µL of potassium phosphate buffer (PPB) at 1 mol/L, pH 6.8, and 20 µL of DTNB. The absorbance was immediately read at 412 nm using a spectrophotometer. However, for the non-protein thiol assay, the samples were deproteinized by adding an equal volume of 10 % trichloroacetic acid (TCA) before analysis. The remaining supernatant was then used for the assay. A standard curve with cysteine was used to determine the concentrations of total thiols and non-protein thiols in the samples. The results were expressed in µmol/L.

3. Results

3.1. Gas chromatography of Piper corcovadense essencial oil

A total of 38 compounds (Table 1) from the EOPc were identified by GC/MS assay. The main classes found in the EOPc were sesquiterpenes (30.57 %), oxygenated sesquiterpenes (38.97 %), and monoterpenes (16.37 %). The major compounds found in the essential oil were trans-sesquisabinene hydrate (24.91 %), trans-caryophyllene (10.75 %), ß-pinene (5.61 %), trans-ß-farnesene (5.22 %), 14-hydroxycaryophyllene (4.63 %), limonene (3.76 %), and *p*-cymene (3.62 %). The chromatogram has been inserted in Fig. S1 (Supplementary material).

Table 1
Chemical constituents identified in *Piper corcovadense* D.DC.

	Compounds	AI (Determined) [a]	AI (Literature) [b]	Content (as % of total oil) *	Chemical formula[b]	Fragmentations – MS (*m/z*)
1	α-pinene	923	932	2.52	$C_{10}H_{16}$	M:136 [121; 105; 93 (100 %); 77]
2	ß-pinene	967	974	5.61	$C_{10}H_{16}$	M:136 [121; 107; 93 (100 %); 79; 69]
3	myrcene	978	988	0.13	$C_{10}H_{16}$	M:136 [121; 107; 93 (100 %); 79; 69; 57]
4	α-phellandrene	997	1002	0.33	$C_{10}H_{16}$	M:136 [93 (100 %); 77; 57]
5	δ-3-Carene	1000	1008	0.40	$C_{10}H_{16}$	M: 136 [121; 105; 93 (100 %); 79]
6	*p*-cymene	1015	1020	3.62	$C_{10}H_{14}$	M: 134 [119 (100 %); 91]
7	limonene	1019	1024	3.76	$C_{10}H_{16}$	M: 136 [121; 107; 93; 79; 68 (100 %)]
8	*cis*-piperitol acetate	1323	1332	0.28	$C_{12}H_{20}O_2$	M: 196 [161; 136; 121 (100 %); 93]
9	α-cubebene	1335	1348	1.32	$C_{15}H_{24}$	M: 204 [161; 119; 105 (100 %); 91]
10	cyclosativene	1358	1369	0.39	$C_{15}H_{24}$	M: 204 [161; 119; 105 (100 %); 93]
11	α-ylangene	1364	1373	3.98	$C_{15}H_{24}$	M: 204 [161; 119; 105 (100 %); 93]
12	ß-elemene	1378	1389	0.51	$C_{15}H_{24}$	M: 204 [189; 161; 147; 119; 107; 93 (100 %); 81; 68]
13	3-dodecanone	1387	1389	0.15	$C_{12}H_{24}O$	M: 184 [85; 71; 57 (100 %); 43]
14	7-*epi*-sesquithujene	1390	1390	0.12	$C_{15}H_{24}$	M: 204 [119 (100 %); 93; 71; 57; 41]
15	siberene	1395	1400	0.27	$C_{15}H_{24}$	M: 204 [189; 161; 147; 133; 119; 105 (100 %); 91; 81; 69]
16	sesquithujene	1400	1405	0.17	$C_{15}H_{24}$	M: 204 [161; 119 (100 %); 107; 93; 81; 69]
17	trans-caryophyllene	1411	1417	10.75	$C_{15}H_{24}$	M: 204 [161; 133; 120; 105; 93 (100 %); 79; 69]
18	ß-copaene	1426	1430	0.92	$C_{15}H_{24}$	M: 204 [161; (100 %); 147; 133; 105; 91]
19	*trans*-α-bergamotene	1430	1432	2.29	$C_{15}H_{24}$	M: 204 [119; 107; 93 (100 %); 69]
20	aromadendrene	1439	1439	0.87	$C_{15}H_{24}$	M: 204 [189; 161; 133; 119; 105; 93 (100 %); 79; 69]
21	trans-ß-farnesene	1457	1454	5.22	$C_{15}H_{24}$	M: 204 [133; 120; 93; 79; 69 (100 %)]
22	linalool isovalerate	1463	1466	1.10	$C_{15}H_{26}O_2$	M: 238; 147; 121; 107; 93 (100 %); 80]
23	γ-gurjunene	1470	1475	0.40	$C_{15}H_{24}$	M: 204 [189; 161 (100 %); 147; 133; 119; 105; 93; 81; 67]
24	α-amorphene	1491	1495	1.09	$C_{15}H_{24}$	M: 204 [161; (100 %); 133; 119; 105; 93; 81]
25	ß-himachalene	1499	1500	2.61	$C_{15}H_{24}$	M: 204 [161; 145; 132; 119 (100 %); 105; 91]
26	ß-bisabolene	1506	1505	0.26	$C_{15}H_{24}$	M: 204 [161; 133; 120; 105; 93; 69 (100 %);]
27	10-*epi*-cubebol	1534	1533	0.53	$C_{15}H_{26}O$	M: 222 [161; (100 %); 147; 133; 119; 105; 93; 79; 69]
28	trans-sesquisabinene hydrate	1583	1577	24.91	$C_{15}H_{26}O$	M: 222 [161; 119; 109; 93; 79; 69 (100 %)]
29	guaiol	1601	1600	1.13	$C_{15}H_{26}O$	M: 222 [161; (100 %); 133; 119; 105; 93; 81]
30	2,(7*Z*)-bisaboladien-4-ol	1614	1618	0.67	$C_{15}H_{26}O$	M: 222 [135; 119; 107; 93 (100 %); 79]
31	2-*epi*-ß-cedren-3-one	1647	1643	0.97	$C_{15}H_{22}O$	M: 218 [161; 121 (100 %); 105; 93; 81]
32	14-hydroxycaryophyllene	1667	1666	4.63	$C_{15}H_{24}O$	M: 220 [205; 187; 159; 147; 131; 119 (100 %); 105; 91; 79; 69]
33	*epi*-zizanone	1671	1668	2.99	$C_{15}H_{22}O$	M: 218 [121; 105; 93; 79 (100 %); 69]
34	5-*neo*-cedranol	1676	1684	0.17	$C_{15}H_{26}O$	M: 222 [161; 147; 133; 121; 107; 93; 81; 69; 57; 43 (100 %)]
35	eudesm-7(11)-en-4-ol	1704	1700	0.37	$C_{15}H_{26}O$	M: 222 [179; 161 (100 %); 119; 105; 93; 81; 69]
36	curcuphenol	1727	1717	0.22	$C_{15}H_{22}O$	M: 218 [157; 135; 43 (100 %)]
37	guaiol acetate	1731	1725	0.15	$C_{17}H_{28}O_2$	M:264 [204; 161 (100 %); 119; 105; 81]
38	8-α-11-elemodiol	1746	1746	1.13	$C_{15}H_{26}O_2$	M: 238 [189; 161; 157; 121; 107 (100 %); 93; 81; 71]
	Monoterpens	16.37 % (7)				
	Oxygenate Monoterpenes	0.28 % (1)				
	Sesquiterpenes	30.57 % (16)				
	Oxygenated sesquiterpenes	38.97 % (13)				
	Others	0.15 % (1)				
	Total	**86.35 % (38)**				

* Peaks listed in order of elution from a 30 m Rtx-5MS capillary column, TQ8040 detector;
[a] Arithmetic Indices (AI) calculated in relation to a series of C_8-C_{30} n-alkanes;
[b] Arithmetic indices (AI) and chemical formula found in the literature (ADAMS, 2007).
m/z – mass-to-charge ratio
M –Molecular peak (100%) – Base íon.

3.2. Antioxidant and antibacterial activity of P. corcovadense essential oil (EOPc)

Various methods were employed to assess the antioxidant activity of the essential oil, considering that each method targets specific mechanisms of action. Thus, we can gain a comprehensive understanding of the antioxidant activity of essential oil. The combination of electron transfer and hydrogen atom transfer-based assays provides insights into different aspects of the oil's antioxidative potential in both lipophilic and hydrophilic environments. This information is valuable for studying the oil's potential health benefits and its suitability for various applications. Therefore, the methods of the reducing capacity of the Folin-Ciocalteu reagent (FC) and the radical scavenging methods ABTS and DPPH were used.

The EOPc showed AA by the reducing capacity of the Folin-Ciocalteu reagent (FC) of 5.41 ± 0.17 mg GAE g^{-1} (Table 2). According to Torres and Chow (2017), essential oil contains monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), terpenoids (isoprenoids) and aromatic compounds such as aldehydes and alcohols that can interact with the Folin-Ciocalteu reagent. In fact, in Table 1, it can be observed that among the listed compounds, we have the oxygenated sesquiterpenes (33.76 %) that can also be classified as sesquiterpene alcohol, due to the presence of the hydroxyl group in the structure, in this classification is found the major compound identified trans-sesquisabinene hydrate (24.91 %).

The antioxidant activity by the DPPH method was 2.88 ± 0.17 μmol TE mL^{-1}, and by ABTS assay of 6.26 ± 0.02 μmol TE mL^{-1} (Table 2). The main difference between the values obtained in these two methods of evaluation of antioxidant activity can be explained by the fact that the ABTS radical has a greater affinity with hydrophilic and lipophilic compounds (affinity for lipids) while the DPPH has an affinity with hydrophobic compounds (Floegel et al., 2011; Sridhar and Charles, 2019).

Furthermore, in this study, the EOPc showed interesting antibacterial properties against *Bacillus subtilis, Listeria monocytogenes* and *Salmonella* Typhimurium, having MIC and MBC of 10.46 μg mL^{-1} (Table 3). The EOPc can be considered bacteriostatic (decreased cell activity) and bactericidal (causing cell death) in this concentration.

However, *in Escherichia coli*, the values for MIC and MBC were more promising (2.61 μg mL^{-1}) due to a greater sensitivity of the cell wall causing cell death. Thus, the oil at this concentration can be considered bacteriostatic and bactericidal.

3.3. Cell viability in healthy PBMC peripheral blood cells and SK-MEL-28 human cutaneous melanoma tumour cells

The results of cell viability assessed in healthy peripheral blood mononuclear cells (PBMCs) and human cutaneous melanoma tumour cells (SK-MEL-28) after exposure to the essential oil are depicted in Fig. 1A and B.

For the healthy PBMCs, it was observed that none of the tested concentrations of the EOPc caused a decrease in cell viability compared to the control (CT) ($p > 0.0001$) (Fig. 1A). This finding is significant as it demonstrates that EOPc compounds do not adversely affect the viability of normal human cells. This safety profile is crucial for potential future pharmaceuticals, food products, or cosmetics applications.

Table 2
Antioxidant activity of *Piper corcovadense* essential oil.

FC (mg GAE mL^{-1})	DPPH (μmol TE mL^{-1})	ABTS (μmol TE mL^{-1})
5.41 ± 0.17*	2.88 ± 0.17	6.26 ± 0.02

*Mean and standard deviation; FC: reducing capacity of the Folin-Ciocalteu reagent,
GAE: Gallic acid equivalent; TE: Trolox equivalent.

Table 3
Minimum inhibitory and minimum bactericidal concentration of *Piper corcovadense* essential oil.

Bacteria	MIC (μg mL^{-1})	MBC (μg mL^{-1})
Bacillus subtilis	10.46	10.46
Listeria monocytogenes	10.46	10.46
Escherichia coli	2.61	2.61
Salmonella Typhimurium	10.46	10.46

MIC: Minimum inhibitory concentration; MBC: minimum bactericidal concentration

On the other hand, cell viability studies against SK-MEL-28 human cutaneous melanoma cells were conducted, and the EOPc was tested at three concentrations: 15.63, 62.50, and 250 μg mL^{-1}. The results are presented in Fig. 1B. The first concentration (15.63 μg mL^{-1}) showed no significant difference in cell viability compared to the control (CT) ($p > 0.0001$). However, the two higher concentrations (62.50 and 250 μg mL^{-1}) exhibited a notable decrease in cell viability in the tumour cells when compared to the control (CT) ($p < 0.0001$).

The fluorescence microscopy assay, shown in Fig. 2, corroborate the results found in MTT assay, in which all tested concentrations of treatment with EOPc highly reduced the cell viability of SK-MEL-28 cells after 24 h compared to control ($p < 0.0001$).

Furthermore, the study revealed that as the EOPc concentration increased in the treated cells, the inhibition of cell viability also increased (Fig. 1B). This suggests that higher concentrations of the EOPc made the cancer cells more susceptible to its cytotoxic effects.

3.4. Induction of apoptotic bodies

As shown in Fig. 3, the micrographics taken in the assay were performed to assess apoptotic body induction by the treatment with EOPc. After the exposition of SK-MEL-28 cells for 24 h to EOPc, it was possible to verify that treatment induced apoptotic body formation in all tested concentrations, presenting nuclei fragmentation and changes in cell morphology.

3.5. Detection of intracellular reactive oxygen species (ROS), nitric oxide (NOX), total thiols (PSH) and non-protein thiols (NPSH)

Fig. 1C and D represent the results of the ROS and NOX analyses. It can be seen that there was a statistically significant difference in the ROS levels at 250 μg mL^{-1} ($p < 0.05$) treatment with EOPc essential oil compared to control. In addition, at concentrations of 15.63 μg mL^{-1} and 62.50 μg mL^{-1}, there is notably a tendency for ROS to increase. There was no statistical significance for the NOX levels ($p > 0.05$).

The results of the PSH and NPSH analysis are shown in Fig. 1E and 1F. In this study, the level of PSH was not altered during the analysis, meaning that the EOPc did not cause any changes in the defense cells compared to the control group (CT) ($p > 0.0001$). Moreover, for NPSH analyses was possible to verify that in the concentrations of 15.63 and 250 μg mL^{-1} there was no significant difference in NPSH levels compared to the control (CT) ($p > 0.0001$).

At the concentration of 62.5 μg mL^{-1} of the EOPc, there was a significant decrease in NPSH levels compared to the control group (CT), with a statistically significant difference ($p < 0.0001$). This indicates that at this specific concentration, the EOPc caused a notable inhibition of the NPSH endogenous antioxidant systems. It is worth noting that NPSH levels are largely composed of glutathione, accounting for approximately 90 % of the intracellular NPSH levels (Nassar et al., 2014). Therefore, the decrease in NPSH levels suggests that the EOPc might have inhibited the glutathione-based antioxidant system at the concentration of 62.5 μg mL^{-1}.

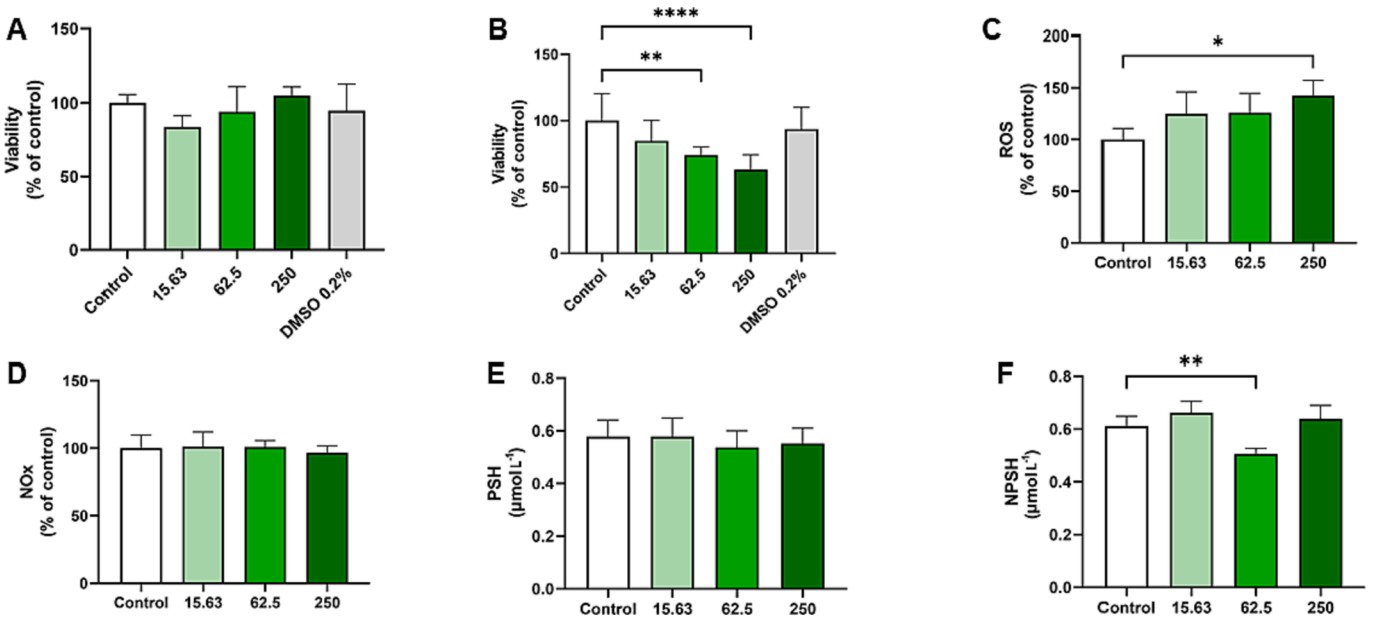

Fig. 1. (A) Cell viability in healthy peripheral blood PBMC cells, (B) cell viability in SK-MEL-28 human cutaneous melanoma cells, (C) oxidative stress marker for reactive oxygen species (ROS), (D) oxidative stress marker for nitric oxide, (E) protein thiol antioxidant defenses (PSH), and (F) non-protein thiol antioxidant defenses (NPSH) of the *Piper corcovadense* essential oil (EOPc) in different concentrations. Statistical analysis: ANOVA. Values with $p < 0.05$ were considered statistically significant ($p < 0.05$) *($p < 0.01$) *($p < 0.001$) **($p < 0.0001$).

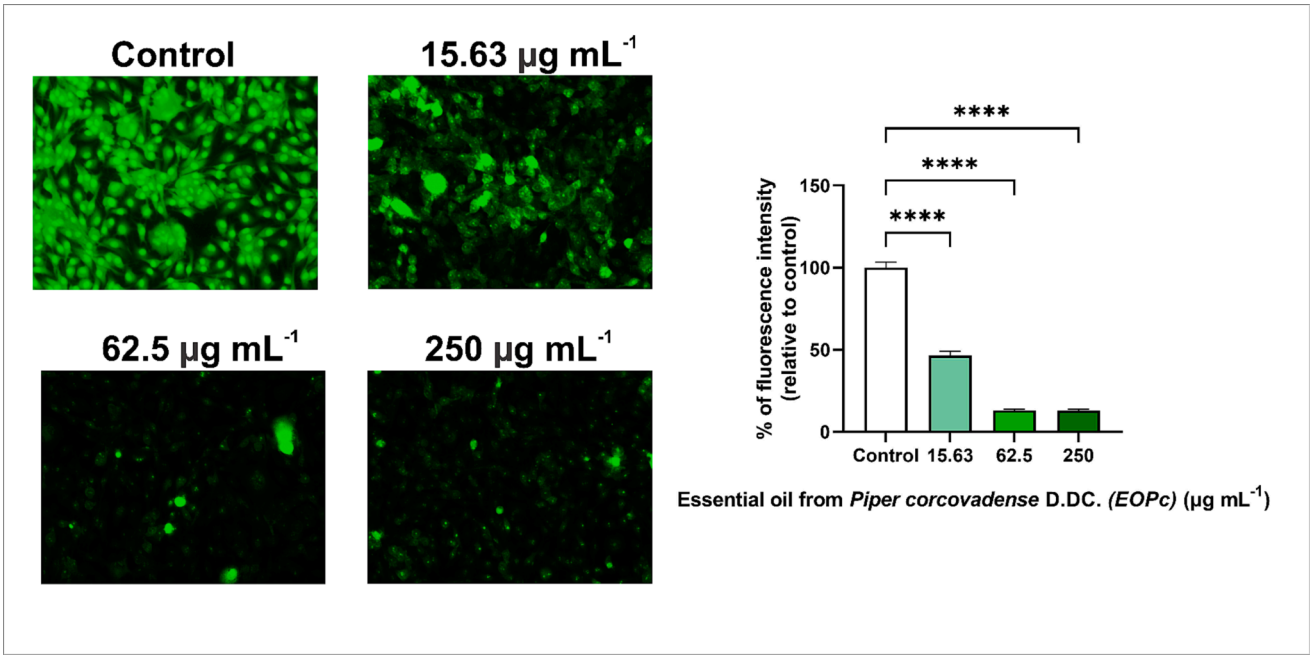

Fig. 2. Fluorescence microscopy of the control and *Piper corcovadense* essential oil (EOPc) in different concentrations at SK-MEL-28 (tumor) cells. Values with $p < 0.05$ were considered statistically significant *($p < 0.05$) **($p < 0.01$) ***($p < 0.001$) ****($p < 0.0001$).

4. Discussion

4.1. Chemical composition

To date, it is the first time that trans-sesquisabinene hydrate has been found in EOPc. However, several authors have reported the presence of sesquiterpenoids in other species of the Piperaceae family. The *Piper regnellii* essential oil has anti-inflammatory, antioxidant and antimicrobial properties (Braga et al., 2021; Terezinha de Oliveira et al., 2019). In addition, the larvicidal activities against the Dengue gnat (*Aedes aegypti*) were found in *P. corcovadense* essential oil (da Silva et al., 2016).

In this work, the trans-ß-farnesene and the *p*-cymene were found in more significant amounts than what was seen by Da Silva et al. (2016) in EOPc collected in Recife (PB, Brazil). The compounds trans-caryophyllene and ß-pinene were found in amounts of 4.41 % and 6.96 % on EOPc collected in Recife (PE, Brazil) (de Lira Pimentel et al., 2022). Additionally, limonene was found in EOPc from Recife (PE, Brazil) and Rio de Janeiro (RJ, Brazil) (Da Silva et al., 2016; Facundo et al., 2004). By making a deeper analysis of the terpenes found in this sample with those reported in the literature, it is possible to observe that

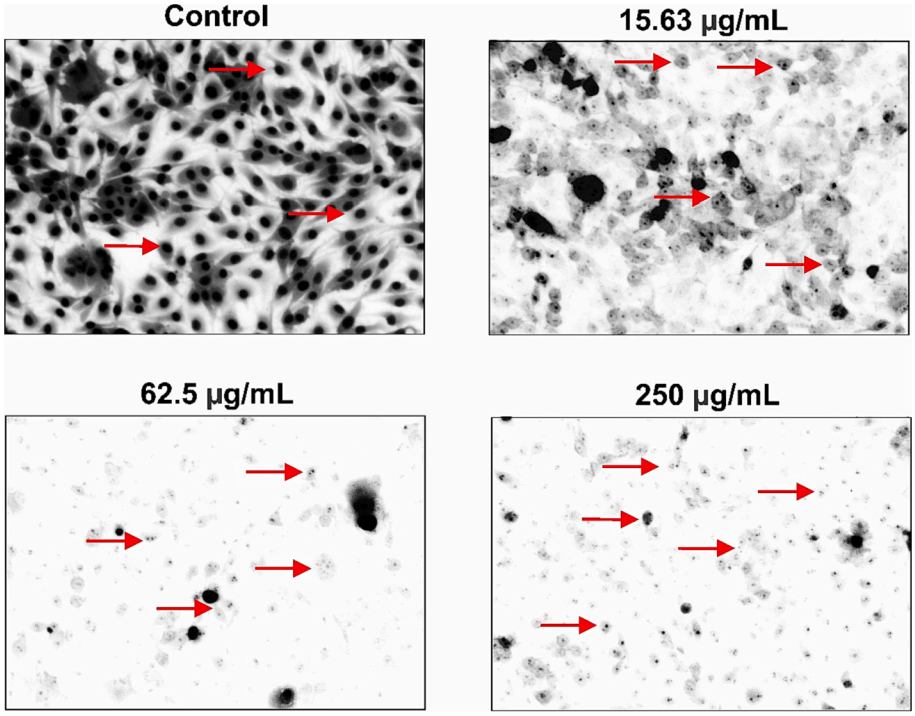

Fig. 3. Apoptotic bodies at the tested concentrations of the *Piper corcovadense* essential oil (EOPc) at SK-MEL-28 (tumor) cells.

there are similar minority compounds in the study of EOPc.

It should be taken into account that environmental factors can be decisive for the chemical composition of the metabolites produced by the plant, where it produces compounds according to its need (Sá Filho et al., 2022). The plant from which the essential oil was extracted for this work was obtained in the northern region of Brazil, a humid tropical forest region. In contrast, the others were obtained in Brazil's northeast and southeast areas, with different climates and soil, which may have influenced the profile chemical.

Another factor that can affect the chemical composition is the technique used for the extraction. In work present in the literature with *P. corcovadense*, the techniques of extraction by hydrodistillation, maceration, supercritical fluid, headspace, steam distillation and ultrasound-assisted were used. The hydrodistillation and headspace extraction techniques were the ones that had the best extraction of the compounds. In contrast, the others had low yields, with supercritical fluid extraction being the technique that obtained the lowest result (Albuquerque et al., 2022).

The works carried out with plants of the genus *Piper* showed a chemical composition similar to that found in this study with the EOPc. In fact, monoterpenoids and sesquiterpenoids have been identified in the essential oil from *Piper marginatum* (Brú & Guzman, 2016). The compounds found were ß-pinene, limonene and *p*-cymene, the majority in the present study. Additionally, minor compounds in common were also found, such as α-pinene, ß-pinene, α-phellandrene, α-cubebene, cyclosativene, ß-elemene and γ-gurjunene (Brú & Guzman, 2016). In the meantime, on *Piper nigrum*, was possible to observe the presence of limonene and *E*-caryophyllene isomers that were found as the majority in the present study, as *p*-cymene but in smaller quantities. The α-pinene, ß-pinene, myrcene, ß-elemene minor compounds were also detected and identified in essential oil from *Piper nigrum* (Bastos et al., 2020).

Trans-sesquisabinene hydrate and trans-caryophyllene were found in *Piper aduncum* (*Jaborandi* do mato) collected in southern Brazil. Both compounds were found at the highest levels that in the present study. These compounds were found in two other species *Piper arboreum* and *Piper gaudichaudianum* milar (Silva et al., 2019).

In general, the EOPc followed the composition pattern cited in the literature for the genus piper in which its composition has monoterpenes, sesquiterpenes, monoterpenes and oxygenated sesquiterpenes (Braga et al., 2021; Bastos et al., 2020). It is possible to find in the literature reports of biological activities of the major compounds identified in *Piper corcovadense*, being a starting point for future applications.

4.2. Antioxidant and antibacterial activity

In a previous study (Sharopov et al., 2015), with essential oil from celery (*Apium graveolens*), sweet wormwood (*Artemisia annua*), black cumin (Nigella sativa), and thyme (*Thymus vulgaris*) was found *p*-cymene, limonene and ß-pinene. These compounds, also identified in this work as major, were evaluated separately by the ABTS and DPPH methods and showed good antioxidant potential.

An isomer of the trans-sesquisabinene hydrate compounds was reported in works with *Cynometra cauliflora* L by Samling et al. (2021). In the essential oil of the branches of the plant, levels of 58.77 % of *trans*-sabinene were quantified, which was reported as the major compound. In this work, it is reported that the antioxidant activity of the essential oil of the branches is superior to that of the leaves and roots.

Other compounds identified in this work and their isomers are reported in the literature with antioxidant potential, as is the case of trans-caryophyllene compounds extracted from *Carum nigrum* seeds (Singh et al., 2006), ß-pinene extracted from *Hyssopus officinalis* (Gharakhani-Beni et al., 2022), trans-*β*-farnesene (isolated compound) (Lateef et al., 2013), limonene (Santa et al., 2022), a compound widely used in the cosmetic and food industry (Ravichandran et al., 2018) and finally, the *p*-cymene compound also has antioxidant activity reported in the literature (Balahbib et al., 2021). However, the levels obtained strongly depend on the species studied and on the climatic conditions and environments in which the plant was submitted.

The chemical compounds that make up the EOPc are reported in the literature as antimicrobial agents. In fact, the trans-caryophyllene, one of the major compounds found in this work, was reported with antimicrobial potential in previous studies conducted by Sabulal et al. (2006) in the essential oil from *Zingiber nimmonii* against *Bacillus*

subtilis and *Candida albicans*.

The ß-pinene compound and its α-pinene isomer have also been reported to have antimicrobial activity against *Staphylococcus aureus*, *Candida albicans* and *Rhizopus oryzae* (da Silva et al., 2012). Additionally, the compounds trans-*β*-farnesene, *p*-cymene and limonene found in this work also have antimicrobial activity reported in the literature (Lateef et al., 2013; Tian et al., 2018; Yu et al., 2022).

4.3. Cell viability in healthy PBMC and SK-MEL-28

The EOPc demonstrated a safe profile when tested on healthy peripheral blood mononuclear cells (PBMCs). However, it exhibited cytotoxic effects on SK-MEL-28 human cutaneous melanoma cells, particularly at higher concentrations. These findings indicate that essential oil may have the potential as a therapeutic agent for cancer treatment and warrant further investigation.

This antitumor activity can be explained by the compounds that make up the essential oil, and some of the compounds that were identified in the present work also have been reported in the literature with antitumor potential (Wu et al., 2015). In fact, the compound trans-sesquisabinene hydrate, the major compound in the EOPc (24.91 %) (Table 1), has already been reported as an antitumor against MCF-7 cells (human breast cancer cell) (Samling et al., 2021).

In addition, another primary compound found in this work trans-ß-farnesene (5.22 %), showed antitumor activity against myeloma tumour cells, pancreatic cancer, breast and prostate carcinoma (Hyun et al., 2015) and also against leukemia cell lines (Hyuck et al., 2015). The compound *p*-cymene (3.62 %) also showed antitumor activity against MCF-7 cells (human breast cancer cells) (Kalu et al., 2015).

It is important to note that while certain compounds in essential oil may show promise as having antitumor potential, further research is typically required to fully understand their mechanisms of action, assess their efficacy, and determine their safety before they can be considered for therapeutic use in treating tumours or cancer. Moreover, the effects of essential oils on tumours can vary depending on the type of cancer, the concentration of the compounds, and other factors, so a cautious and comprehensive investigation is essential.

This observation strengthens the case for the EOPc possessing antitumor activity in vitro, making it a promising candidate for potential cancer treatments. However, it is important to conduct further research and experiments to understand its mechanism of action better and assess its efficacy and safety in more complex systems before considering clinical applications.

4.4. Biochemical markers

Reactive oxygen species and reactive nitrogen species are highly reactive compounds that can lead to a large accumulation of oxidative stress in the human body. Thus, important biological markers can be the starting point for various diseases (Makris et al., 2023). However, the levels of total thiols (PSH) and non-protein thiols (NPSH) are also important biochemical markers that play a crucial role in the body's antioxidant defense system. They are part of the endogenous antioxidant system, which helps neutralize the harmful effects of free radicals and oxidative stress (Sies, 2015).

In the present study, the EOPc at the tested concentrations did not exhibit effects in NOx levels. However, at higher concentrations, EOPc increased the levels of ROS, indicating oxidative stress induced in SK-MEL-28 cells. Thus, the EOPc can be considered with antitumor potential against cutaneous melanoma.

The results showed a negative effect on NPSH levels at concentrations of 62.5 μg mL^{-1} of EOPc. There was an increase in ROS levels at higher concentrations of the substance. Given the decrease in tumor cell viability, accompanied by increased ROS levels and the formation of apoptotic bodies after treatment, a possible mechanism of action of the essential oil may be related to the induction of the intrinsic apoptosis pathway (Tang et al., 2019). This effect is related to an increase in ROS levels and apoptosis, as shown in a study performed by Manica et al. (2023). This way, additional research is necessary to determine the precise cause of the observed effects.

5. Conclusions

CG-MS could identify the preliminary profile of chemical compounds present in EOPc. The presence of sesquiterpenoids and monoterpenoids such as trans-sesquisabinene hydrate (24.91 %), trans-caryophyllene (10.75 %), ß-pinene (5.61 %), trans-ß-farnesene (5.22 %), 14-hydroxy-caryophyllene (4.63 %), limonene (3.76 %), and *p*-cymene (3.62 %) can be responsible for the important biological properties of this essential oil.

The EOPc showed antioxidant activity and antimicrobial against the main microorganisms that can cause foodborne infections. Besides, EOPc could inhibit tumor cell growth mainly at 62.50 and 250 μg mL^{-1} concentrations. Additionally, the EOPc does not show cell cytotoxicity in healthy cells. That was, EOPc selectively reached tumor cells without causing significant damage to healthy cells.

Regarding ROS levels, there was a significant difference observed between the control group (cells not treated with essential oil) and the experimental group (cells treated with essential oil). This suggests that the essential oil causes oxidative stress in the tumour cells, culminating in apoptosis by the intrinsic pathway, with apoptotic bodies forming.

As for the levels of total thiols (PSH) and non-protein thiols (NPSH), there was no significant difference. However, at a lower concentration of the EOPc, there was a decrease in non-protein thiol levels (NPSH), which was subsequently normalized at a higher concentration and could not determine the specific metabolic pathway through which the tumour cells died.

While the study may provide valuable insight into the therapeutic potential of EOPc, more research is needed to translate these findings into effective clinical treatments. However, this work brings promising results and shows the attractive biological potential of EOPc.

Additionally, with the increasing demand for adjunct compounds in treating melanoma in humans, this study is a crucial starting point for future investigations in melanoma cell lines. In fact, it demonstrates the EOPc's ability as an antitumor and antimicrobial agent, highlighting its potential as a preventive agent against cutaneous melanoma and bacterial infections.

CRediT authorship contribution statement

Bruno Henrique Fontoura: Investigation, Writing – original draft, Formal analysis. **Ellen Cristina Perin:** Formal analysis. **Ana Paula Buratto:** Formal analysis. **Jucemar Francisco Schreiner:** Formal analysis. **Kamyla Menezes Cavalcante:** Supervision, Resources, Data curation. **Sirlei Dias Teixeira:** Supervision, Resources, Data curation. **Daiane Manica:** Supervision, Resources, Data curation. **Rafael Antônio Narzetti:** Supervision, Resources, Data curation. **Gilnei Bruno da Silva:** Supervision, Resources, Data curation. **Margarete Dulce Bagatini:** Supervision, Resources, Data curation. **Tatiane Luiza Cadorin Oldoni:** Supervision, Resources, Data curation. **Solange Teresinha Carpes:** Project administration, Writing – review & editing, Supervision, Resources, Data curation.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.

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