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Chemical evaluation and *in vitro* cytotoxic activity of *Copaifera reticulata* Ducke oleoresin in cancer cell lines

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ABSTRACT

Copaiba oleoresin is a natural product widely used in traditional Amazonian medicine in the treatment and cure of various ailments, having various biological activities such as anti-inflammatory, cicatrizant, antiseptic and antimicrobial. Cancer is one of the main public health problems, being the cause of thousands of deaths around the world. In view of the pharmacological properties of copaiba oleoresin, the investigation of its anticancer potential may be an alternative for obtaining new anticancer phytopharmaceuticals. The objective of this work was to evaluate the chemical composition and *in vitro* cytotoxic activity of oleoresin (OR) from *Copaifera reticulata* Ducke in cancer cell lines. The OR was distilled generating the volatile (VF) and resinous (RF) fractions, and the chemical composition was analyzed by GC-MS. In the *in vitro* cytotoxic evaluation, tumor and leukemic cell lines were tested by the MTT assay. The quantification of cells in apoptosis was analyzed by flow cytometry. Twenty -four sesquiterpenes and four diterpenes were identified. RF showed the lowest IC_{50} in strains Jurkat, Nalm 6, HOS, PC3 and H1299. The MCF7 strain was more sensitive to VF. The oleoresin and its fractions induced more than 80% of the H1299 cell line to apoptosis, especially the RF. The results confirmed the *in vitro* cytotoxic activity of oleoresin from *C. reticulata* against the tested cell lines and evidenced its potential in the production of new anticancer phytopharmaceuticals.

KEYWORDS: natural products, copaiba, anticancer, MTT assay, sesquiterpenes, diterpenes

Avaliação química e atividade citotóxica *in vitro* da oleorresina de *Copaifera reticulata* Ducke em linhagens de células cancerígenas

RESUMO

A oleorresina de copaíba é um produto natural amplamente utilizado na medicina tradicional amazônica no tratamento e cura de diversos males, possuindo várias atividades biológicas, como anti-inflamatória, cicatrizante, antisséptica e antimicrobiana. Câncer é um dos principais problemas de saúde pública, sendo a causa de milhares de mortes em todo o mundo. Tendo em vista as propriedades farmacológicas da oleorresina de copaíba, a investigação do seu potencial anticancerígeno pode ser uma alternativa para a obtenção de novos fitofármacos anticâncer. O objetivo deste trabalho foi avaliar a composição química e a atividade citotóxica *in vitro* da oleorresina (OR) de *Copaifera reticulata* Ducke em linhagens de células cancerígenas. A OR foi destilada gerando as frações volátil (FV) e resinosa (FR) e a composição química analisada por GC-MS. Na avaliação citotóxica *in vitro* foram testadas linhagens de células tumorais e leucêmicas pelo ensaio do MTT. A quantificação das células em apoptose foi analisada por citometria de fluxo. Foram identificados vinte e quatro sesquiterpenos e quatro diterpenos. A FR apresentou o menor IC_{50} nas linhagens Jurkat, Nalm 6, HOS, PC3 e H1299. A linhagem MCF7 foi mais sensível à FV. A oleorresina e suas frações induziram mais de 80% das células da linhagem H1299 à apoptose, com destaque para a FR. Os resultados confirmaram a atividade citotóxica *in vitro* da oleorresina de *C. reticulata* frente às linhagens de células testadas, e evidenciam seu potencial na produção de novos fitofármacos anticâncer.

PALAVRAS-CHAVE: produtos naturais, copaíba, anticâncer, ensaio do MTT, sesquiterpenos, diterpenos

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INTRODUCTION

Cancer is one of the main causes of premature deaths in the world population (Sung et al. 2021). High mortality rates and the emergence of resistance to conventional chemotherapy treatments make it urgent to find new molecules and effective therapies with minimal side effects (Gutiérrez-Rodríguez et al. 2018; Sung et al. 2021). It is estimated that more than 60% of existing drugs come, directly or indirectly, from natural products (Newman and Cragg 2020). The chemical complexity of natural products makes them a promising source in the development of new anticancer agents, with pharmacological screening studies being fundamental in the discovery process (Dutta et al. 2019; Naeem et al. 2022).

Copaiba (*Copaifera* L.) oleoresin is widely used in traditional Amazonian medicine, and has anti-inflammatory, antimicrobial, antiseptic, healing, apoptotic and antitumor properties (Azani et al. 2017; Barbosa et al. 2019; Urasaki et al. 2020). *In vitro* and *in vivo* studies with oleoresin from different copaiba species have demonstrated cytotoxic activity in a variety of tumor cell lines, such as gastric carcinoma, lung adenocarcinoma, cervical cancer, and colon adenocarcinoma (Alves et al. 2017; Cardoso et al. 2017; Carneiro et al. 2020). Furthermore, constituents present in copaiba oleoresin, sesquiterpenes and diterpenes, have been reported as modulators in important signaling pathways of cancer cells, acting, for example, in the induction of cell apoptosis, cell cycle arrest, inhibition of cell proliferation, regulation of cell survival factors related to angiogenesis and tumor metastasis (Yeo et al. 2016; Hanušová et al. 2017; Zhai et al. 2019; Abu-Izneid et al. 2020; Frost et al. 2023).

Despite the evident pharmacological application of copaiba oleoresin, there are still few studies on its anticancer potential, not only of crude oleoresin, but also of the isolated resinous and volatile fractions of copaiba species endemic to the Amazon region. Therefore, the present work aimed to evaluate the chemical composition and *in vitro* cytotoxic activity of *Copaifera reticulata* Ducke oleoresin in human cancer cell lines.

MATERIAL AND METHODS

Collection and fractionation of oleoresin

The *C. reticulata* oleoresin (OR) was collected in the Tapajós National Forest at km 117 of the BR 163 highway (03°20'43.5''S, 055°01'09.1''W) municipality of Belterra, state of Pará, Brazil. The collection was carried out according to Oliveira et al. (2006) with authorization from Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio (SISBIO license # 45915-3). The botanical identification was carried out at Embrapa Amazônia Oriental (Belém, Pará), and the exsicata deposited in the Herbarium IAN of this institution with the identification number NID 58/2016.

The OR was stored in the oil bank of the Medicinal Plant Biotechnology Laboratory at Universidade Federal do Oeste do Pará (LBPM/UFOPA), code nº 584 UPA 10 - UT13. The volatile (VF) and resinous (RF) fractions of the oleoresin were obtained through simple distillation, according to Deus et al. (2011) with adaptations in water volume and distillation time. An amount of 100 ml of OR was used in 1.8 L of ultrapure water, with a distillation time of 18 hours at the boiling temperature of the water. After distillation, the VF, RF and the hydrolate were separated.

Chemical analysis of oleoresin

The chemical composition of the OR was analyzed by gas chromatography coupled to mass spectrometry (GC-MS). The analysis of volatiles was carried out on an Agilent gas chromatograph, model HP-6890 coupled to an Agilent mass selective detector, model HP-5975, HP5-MS capillary column (30 mt x 0.25 mm x 0.25 µm), under the following chromatographic conditions: injector temperature: 220 °C, column: 60 °C, 3 °C min^{-1}, 240 °C and detector: 250 °C, carrier gas flow rate (He): 1.0 ml min^{-1}, split ratio : 1:40, mass detector operating at 70 eV , ion source temperature: 230 °C, and quadrupole temperature: 150 °C. A volume of 1 µL was injected into the GC-MS for analysis. The identification of chemical constituents was carried out by comparison with the NIST spectral library (2012), calculation of the retention index, determined by the injection of a mixture of hydrocarbon standards and samples under the same chromatographic conditions and, by comparison with literature data (Adams 2007).

To identify diterpenes, the OR was diluted in ethyl acetate at a concentration of 20 mg ml^{-1}, and 10 µL of onpot trimethylsilyldiazomethane (TMSD) reagent was added, a derivatizer to esterify diterpenic acids, and a volume of 1 µL was injected into the GC-MS for analysis. The analysis of the chemical composition of the resinous constituents, diterpenic acids, present in both the OR and RF was carried out using the mass spectrometry data set compared with data from standards available in the laboratory, previously isolated and characterized by GC-MS and nuclear magnetic resonance (NMR), and from the NIST spectrum library.

Cell cultivation

The *in vitro* cytotoxic activity was evaluated using the human tumor cell lines MCF7 (breast cancer), HOS (osteosarcoma), PC3 (prostate adenocarcinoma) and H1299 (non-small cell lung carcinoma) and the leukemic cell lines Nalm 6 (B leukemia) and Jurkat (T leukemia), all being ATCC (American Type Culture Collection) cells. The cells were cultivated in RPMI 1640 medium and incubated at

37 °C, 5% CO_2 saturation, for 48 hours, with medium renewal every 24 hours. The tumor cells were washed with 5 ml of saline, followed by the addition of 4 ml of trypsin to the 75 cm^2 culture flasks and 8 ml to the 175 cm^2 flasks, which were incubated for 10 min. Trypsin was removed by adding 5 ml of washing medium, centrifugation and discarding the supernatant. The cell density used was 1.8 x 10^{-6} cells from each tumor lineage and 3.6 x 10^{-6} cells from each leukemic lineage.

Standardization of treatments (OR, VF and RF)

100µL of each sample were weighed, obtaining the following results: OR = 26.8 mg ml^{-1}, VF = 26.3 mg ml^{-1} and RF = 23.7 mg ml^{-1}. The samples were diluted in 1 ml of dimethylsulfoxide (DMSO), and the RF remained with the reagent for 24 hours before testing for greater dissolution. A volume of 224 µl of each sample was added to 5 ml of RPMI 1640 medium minus each test concentration.

In vitro cytotoxic activity

In the cytotoxicity test *in vitro* we used OR, VF, RF, and Paclitaxel (PX) and Vincristine (VCR) as positive controls for tumor and leukemic lines, respectively. 100 µl of each cell line were cultivated in a 96-well plate, in triplicate, as well as the Imput plate (control plate). The plates were incubated at 37 °C**,** 5% CO_2 saturation for 24 hours. Samples and positive controls were used at final concentrations of 5 µg ml^{-1}, 25 µg ml^{-1}, 50 µg ml^{-1}, 125 µg ml^{-1}, 200 µg ml^{-1}, 400 µg ml^{-1}, and 600 µg ml^{-1}, followed by incubation for 48 hours under the conditions described above. After this, the cells were washed with 200 µl of saline, preceded by discarding the cell culture medium.

In vitro cytotoxic activity was evaluated using the MTT assay (Mosmann 1983; Liu et al. 2011; Elbe et al. 2022; Jin et al. 2023; Yildirim et al. 2024). 25 µL of MTT at a concentration of 5 mg ml^{-1} were added to each well of the culture plates, followed by incubation at 37 °C, 5% CO_2 saturation for 4 hours. After this period, the cells were treated with 175 µL of isopropyl alcohol (PA – ACS). After evaporation of the alcohol, both the control plate (100% viable cells) and the plates with tumor and leukemic lines were read using a plate spectrophotometer at a wavelength of 560 nm. The cytotoxicity index (IC_{50}) was calculated by non-linear regression using the Origin 8.0 software and the graphs generated by the GraphPad Prism 2007 Software.

Quantification of apoptosis by flow cytometry

Only the strain that demonstrated greater sensitivity to treatments (OR, VF and RF) was used in this assay. After culturing the cells, the plates were incubated at 37 °C, 5% CO_2 saturation for 24 hours. A concentration of 0.5 µg ml^{-1} of OR, VF and RF was added to each well, followed by incubation. The Apoptosis Detection Kit (BD Pharmigen®) was used, following the manufacturer's standard protocol.

The cells were resuspended in binding buffer at a concentration of 1x10^{6} cells ml^{-1}, 100µl of the solution were transferred to a 5 ml crystal tube, and 5 µl of annexin V FITC and 5 µL propidium iodide (PI) were added. The tubes with the cells were gently shaken and incubated for 15 min at 25 ºC. 400µl of binding buffer was added to each tube and the analysis continued in a flow cytometer (excitation at 488 nm with 530 nm). Quantification of cells undergoing apoptosis was performed using the Facs DIVA software (Liu et al. 2011; Elbe et al. 2022; Jin et al. 2023; Yildirim et al. 2024).

Data analysis

The results were subjected to ANOVA and the means were compared pairwise using the *post-hoc* Tukey test at a significance level of 95% ($p < 0.05$) with the GraphPad Prism 6 software.

RESULTS

Chemical composition of the OR

We identified 24 sesquiterpenes and four diterpenes, with the majority of constituents being β-caryophyllene (44.82%), β-bisabolene (8.57%), α-humulene (7.01%), β-selinene (6 .58%) and trans-α-bergamotene (5.24%) (Table 1). The analysis of diterpenic acids is carried out using mass spectrometry coupled to gas chromatography, for which the samples need to undergo derivatization with TMSD and, consequently, the diterpenic acids are identified as their derivative methyl esters. Thus, like their respective methyl esters, five diterpenes were detected, and four of them were identified: cativic, kaur-16-en-18-oic, kauran-19-oic and daniellic acids (Table 2).

In vitro cytotoxic activity

The OR, VF and RF showed cytotoxic activity against the tested cancer cell lines, mainly the RF, which presented the lowest IC_{50} against the Jurkat, Nalm 6, HOS, PC3 and H1299 lines (Figure 1, 2 and 3). RF had greater activity in H1299 cells with an IC_{50} of 0.6 µg ml^{-1} (Figure 3A). The MCF7 cell line was more sensitive to the VF with an IC_{50} of 13.7 µg ml^{-1} (Figure 3B).

Quantification of apoptosis

Quantification of apoptosis was carried out only in the H1299 cell line in which the tested samples showed greater cytotoxicity, at a concentration of 0.5 µg ml^{-1}, a concentration close to the lowest IC_{50} calculated for this line in the cytotoxicity assay *in vitro*. OR, VF and RF induced more than 80% of the cells of the H1299 line to death, mainly RF, which induced more than 95% of the cells to apoptosis (Table 3).

Table 1. Sesquiterpene constituents identified in *Copaifera reticulata* Ducke oleoresin (OR) from the Tapajós National Forest, Pará state, Brazil.

Constituent	RI	R_t (min)	(%)
α-copaene	1373	22.085	0.30
β-elemene	1391	22.818	2.64
Cyperene	1397	23.060	0.25
β-caryophyllene	**1426**	**24.229**	**44.82**
trans-α-bergamotene	**1436**	**24.661**	**5.24**
cis-βfarnesene	1443	24.937	0.13
α- humulene	**1454**	**25.379**	**7.01**
4,5-di-epi-aristolochene	1467	25.895	0.12
β-chamigrene	1473	26.113	0.20
Dauca-5,8-diene	1475	26.230	0.29
NI (M = 204)	1479	26.382	0.54
β-selinene	**1486**	**26.668**	**6.58**
α-selinene	1495	26.998	3.75
α-muurolene	1498	27.139	0.11
cis-α-bisabolene	1502	27.291	0.68
β-bisabolene	**1511**	**27.62**	**8.57**
7-epi-α-selinene	1515	27.799	0.23
δ-cadinene	1523	28.086	1.14
β-macrocarpene	1541	28.814	0.77
Cariolan-8-ol	1566	29.779	0.17
caryophyllene oxide	1579	30.277	0.15
Widrol	1614	31.616	1.89
NI (M = 206)	1633	32.298	0.55
β-acorenol	1642	32.656	0.49
Selin-11-en-4α- ol	1651	32.959	0.59
α-epi-bisabolol	1680	34.037	0.14
Total compounds identified			86.26

RI = constituent retention index; R_t (min) = retention time of the constituent in the chromatograph column given in minutes; (%) = relative percentage concentration of the compound in the crude oleoresin. Components in bold are majority constituents (concentration ≥ 5%). NI = constituent not identified; M = molar mass of the compound.

Table 2. Diterpene constituents identified in the resinous fraction (RF) of *Copaifera reticulata* Ducke oleoresin from the Tapajós National Forest, Pará state, Brazil.

Constituent	R_t (min)	(%)
Cativic acid methyl ester	19.471	7.06
Kaur-16-en-18-oic acid methyl ester	19.762	31.54
Kauran-19-oic acid methyl ester	**19.949**	**41.38**
NI	20.498	13.3
Daniellic acid methyl ester	20.557	6.73
Total compounds identified		86.71

R_t (min) = retention time of the constituent in the chromatograph column given in minutes; (%) relative percentage concentration of the compound in the resinous fraction. Components in bold are majority constituents. NI = constituent not identified.

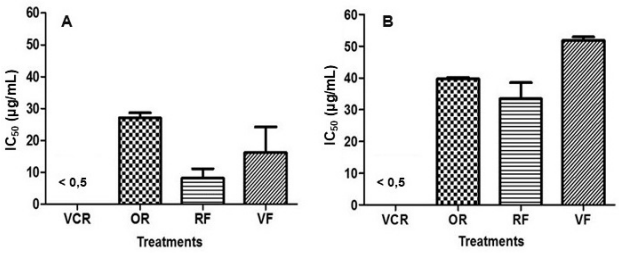


Figure 1. Cytotoxicity index (IC_{50}) of the crude oleoresin (OR), resinous fraction (RF) and volatile fraction (VF) of *Copaifera reticulata* Ducke from the Tapajós National Forest (Pará, Brazil) against the strains Jurkat (T leukemia) (**A**) and Nalm 6 (B leukemia) (**B**). Vincristine (VCR) = positive control. The columns are the mean and the bars the standard deviation (N = 3).

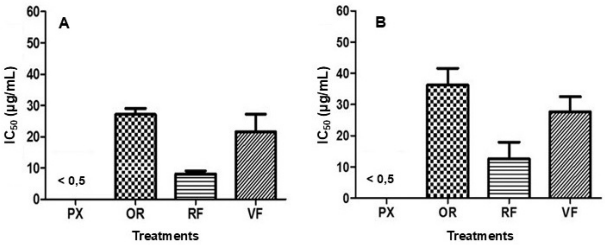


Figure 2. Cytotoxicity index (IC_{50}) of the crude oleoresin (OR), resinous fraction (RF) and volatile fraction (VF) of *Copaifera reticulata* Ducke from the Tapajós National Forest (Pará, Brazil) against lines HOS (osteosarcoma) (**A**) and PC3 (prostate adenocarcinoma) (**B**). Paclitaxel (PX) = positive control. The columns are the mean and the bars the standard deviation (N = 3).

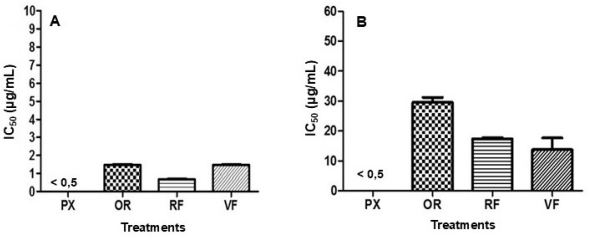


Figure 3. Cytotoxicity index (IC_{50}) of the crude oleoresin (OR), resinous fraction (RF) and volatile fraction (VF) of *Copaifera reticulata* Ducke from the Tapajós National Forest (Pará, Brazil) against lines H1299 (non-small cell lung carcinoma) (**A**) and MCF7 (breast cancer), (**B**). Paclitaxel (PX) = positive control. The columns are the mean and the bars the standard deviation (N = 3).

Table 3. Quantification of apoptosis by crude oleoresin (OR), resinous fraction (RF) and volatile fraction (VF) of *Copaifera reticulata* Ducke from the Tapajós National Forest (Pará, Brazil) in the H1299 lineage (non-small cell lung carcinoma) by flow cytometry.

Concentration (µg ml^{-1})	**Apoptosis** (%)		
	OR	**RF**	**VF**
0	0.4	0.4	0.4
0.5	92.5	95.9	86.1

DISCUSSION

Other studies on the chemical composition of *Copaifera* species, including *C. reticulata*, identified compounds also present in the oleoresin analyzed in this work, especially the majority ones, further demonstrating the similarity in chemical composition and relative concentration of major components among species of this genus. *Copaifera reticulata* oleoresin collected near Belém (Pará), presented 35 sesquiterpenes and 13 diterpenes, with β-caryophyllene (40.9%) and α-humulene (6.0%) as main sesquiterpenes, and eight diterpenes including kaurenoic acids (3.9%) (Veiga-Junior et al. 2007). The analysis of oleoresin from 24 *C. reticulata* individuals from two other areas in Tapajós National Forest, at km 72 and km 83 of the BR 163 highway, also identified β-caryophyllene, trans-α-bergamotene and β-bisabolene as the main constituents, as well as a high intrapopulational variability in the composition and concentration of sesquiterpenes, which may be comparable to interspecific variability (Herrero-Jáuregui et al. 2011). The majority β-bisabolene (29.9%), trans-α-bergamotene (25.7%) and β-caryophyllene (10.3%) were identified, in different concentrations, in samples of copaiba (*Copaifera* sp.) oleoresin purchased in local markets in Ilhéus and Itabuna, Bahia state (Fonseca et a. 2015). In *C. reticulata* oleoresin collected in the municipality of Brasil Novo, Pará, the majority of components were the sesquiterpenes β-bisabolene, trans-α-bergamotene, β-selinene, α-selinene, and the diterpenes ent-agatic-15-methyl ester, ent-copalic acid and ent-polyaltic acid (Bardají et al. 2016). In the oleoresin of three other species (*C. multijuga* Hayne, *C. pubiflora* Benth. and *C. trapezifolia* Hayne), 14 diterpenes were identified, including two not yet reported in the literature, ent-16-hydroxy-3,13 clerodadiene-15,18-dioic acid and ent-labda-5,13-dien- 15-oic (Carneiro et al. 2020).

The diterpenic acids identified in the present work (cativic, kaur-16-en-18-oic, kauran-19-oic and daniellic) are commonly reported in copaiba oils. Yet few copaiba oils are as rich in cauran skeleton diterpenes as *C. reticulata,* and several biological activities are reported for diterpenes with this structure (Arruda et al. 2019; Sarwar et al. 2020). Several studies attribute the anticancer potential of copaiba oleoresin mainly to diterpenes (Barbosa et al., 2019; Sarwar et al. 2020) which are constituents of the resinous fraction, as also observed in the present work. Kaurenoic acid induces DNA damage and increases the frequency of micronuclei in a dose-dependent manner in gastric cancer cells (Cardoso et al. 2017). *Copaifera multijuga* oleoresin and copalic acid also significantly reduced the formation of preneoplastic lesions of aberrant crypt foci and aberrant crypt in the distal colon tissue of rats treated with colon carcinogen (DMH) (Alves et al. 2017). The oleoresin of *C. multijuga, C. pubiflora* and *C. trapezifolia* showed *in vitro* cytotoxic activity against most tested tumor lines breast adenocarcinoma (MCF-7), gastric carcinoma (ACP-01), lung adenocarcinoma (A549) and human cervical cancer (HeLa), with the most active compound being the newly identified diterpene ent-labda-5,13-dien-15-oic acid, which demonstrated a high level of selectivity (Carneiro et al. 2020).

The volatile fraction of copaiba oleoresin is made up of sesquiterpenes (Banik et al. 2023). This class of compounds has several biological activities, including anticancer action, by modulating the activity of the nuclear factor Kappa (NF-kB) in the inhibitory action against lipid peroxidation, delaying the production of reactive oxygen and reactive nitrogen species (Abu-Izneid et al. 2020; Banik et al. 2023). For example, β-bisabolene has specific cytotoxicity for human and murine mammary tumor cells *in vitro* and *in vivo* by inducing cells to apoptosis, as evidenced by the annexin V/propidium iodide method and the caspase-3/7 activity assay (Yeo et al. 2016). Likewise, β-elemene inhibits cell proliferation by inducing cells to apoptosis, regulating the expression of molecules related to angiogenesis and tumor metastasis (vascular endothelial growth factor, matrix metalloproteinases, E-cadherin, N-cadherin and vimentin), as well as the immune response by increasing the sensitivity of cancer cells to chemoradiotherapy (Zhai et al. 2019).

In our study, the breast cancer cell line MCF7 was most sensitive to the volatile fraction, which had the sesquiterpene β-caryophyllene its most predominant component. β-caryophyllene in copaiba oil was shown to have anti-inflammatory and antioxidant properties (Ames-Sibin et al. 2018), so that these properties, and possibly also its synergistic action with the other compounds of the volatile fraction, were likely responsible for the MCF7 response. At a non-cytotoxic concentration (10 μg ml^{-1}), β-caryophyllene significantly increased the anticancer activity of α-humulene and isocaryophyllene against MCF7 cells by 75 % and 90%, respectively, and potentiated the anticancer activity of paclitaxel in the MCF7, DLD-1 (colon adenocarcinoma) and L-929 (murine fibroblast) cell lines (Legault and Pichette 2007).

β-caryophyllene induces the positive regulation of the stearoyl-CoA desaturase (SCD) gene, suggesting that the compound may interfere with the lipid signature modulated by hypoxia, affecting the biosynthesis or composition of the membrane of breast cancer cells, impairing cellular replication (Frost et al. 2023).As in the analysis of *in vitro* cytotoxic activity, our results on the quantification of apoptosis pointed to a relation of the apoptotic effect mainly to the diterpenes present in the oleoresin of *C. reticulata*. As already mentioned, the copalic acid in the oleoresin of *C. multijuga* showed chemopreventive activity in the colon cancer model of rats treated with DMH (colon carcinogen) (Alves et al. 2017). NF-κB proteins, which regulate the expression of protein genes involved in apoptosis, cell adhesion, immunological and inflammatory responses, and cellular stress, may be involved in this effect (Alves et al. 2017). The kaurenoic acid

obtained from the oleoresin of *C. langsdorffii* Desf. is capable of inducing cell cycle arrest and apoptosis in gastric cancer cells in response to DNA damage (Cardoso et al. 2017). The essential oil of *Copaifera sp.* reduced the viability of human neuroblastoma cells (SH-SY5Y) through activation of the apoptosis signaling pathway in a time-dependent manner (Urasaki et al. 2020).

CONCLUSIONS

The oleoresin of *Copaifera reticulata* Ducke from Tapajós National Forest, in Pará (Brazil) had a chemical composition characteristic of the *Copaifera* genus, a mixture of sesquiterpene and diterpene constituents. The resinous fraction showed great cytotoxic potential against the H1299 lineage (non-small cell lung carcinoma), and the volatile fraction showed higher cytotoxic activity against the MCF7 breast cancer lineage. Further studies should elucidate the mechanism of action of these compounds to potentially allow for the development of new anticancer phytopharmaceuticals.

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