Biological Activities and Cytotoxicity of *Eperua oleifera* Ducke Oil-resin

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**ABSTRACT**

**Background**: The oil-resin of *Eperua oleifera* Ducke has been used in popular medicine similarly to the copaiba oil (*Copaifera* spp.).

**Objective**: This study aimed to investigate the effects of the acid fraction of *E. oleifera* oil-resin (AFEOR) on cell proliferation, collagen production in human fibroblasts, inhibition of metalloproteinases, and cytotoxicity against tumor cell lines.

**Materials and Methods**: Acid fraction of *E. oleifera* was fractionated in the ion exchange column chromatography. Cytotoxicity and genotoxicity were evaluated by Alamar Blue® and Comet assay. The inhibition of metalloproteinases was performed by zymography and Western blotting.

**Results**: The predominant acidic diterpenes in the AFEOR were copalic and hardwickiic acids. AFEOR caused morphology alteration and decrease of proliferation at concentrations higher than 5 μg/mL. It also caused significant collagen proliferation in fibroblasts. It showed cytotoxicity against tumoral and nontumoral cell lines, with IC_{50} values ranging from 13 to 50 μg/mL, and a hemolytic activity with an IC_{50} value of 36.29 μg/mL. AFEOR inhibited collagenase activity, with an IC_{50} value of 46.64 μg/mL, and matrix metalloproteinase-2 (MMP)-2 and MMP-9 in HaCaT cells or MMP-1 expression in MRC-5 cells. AFEOR induced genotoxicity in MRC-5 cells with a DNA damage index between 40% and 60% when compared to the negative controls (0%–20%).

**Conclusion**: For the first time, biological activities from oil-resin *E. oleifera* demonstrated ratifying somehow its popular use.

**Key words**: collagenase, *Eperua*, metalloproteinases, tumoral cell

**SUMMARY**

- Analysis of crude oil-resin and fractionation of diterpenic fraction was performance using selective ion-exchange column chromatography
- Cytotoxicity analysis and morphology were performed with different cell lines
- Collagen production in human fibroblasts, inhibition of metalloproteinases were demonstrated by zymography and Western blotting.

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**INTRODUCTION**

Plants produce an important structural diversity of metabolites and represent the largest source of bioactive compounds. They are used worldwide as self-prescribed home medicines and in the pharmaceutical industry.[1–3] Since compounds isolated from plants have many biological activities, the invaluable biodiversity among Brazilian plants presents a great asset in the development of novel drugs.[4] The identification of any potential toxicological activity in plant bioactive substances is primordial to evaluate their risks and potential uses.[5]

*Eperua* genus is found in the Central Amazon, with 14 species described in the literature, and is distributed in the North and Western Amazonia from Ecuador to Guyana and Venezuela. *Eperua* trees have similar biological properties as the genus *Copaifera*, which is also from Fabaceae-Caesalpinioideae family and is commonly known as copaiba oil. These species produce oil-resins used for therapeutic purposes in folk medicine. Some of these *Eperua* oil-resins have similar names to *Copaifera* species such as *Eperua oleifera* and *E. purpurea*, known as “copaiba-jacare” and “copaibaranã,” respectively.[6,7] *Eperua* oil-resin is obtained by exudation of the trunk trees and has been used as skin healing, antibacterial and antifungal agents in Amazonian folk medicine.[8–10]

Chromatographic analysis of copaiba oil-resin has revealed that it contains sesqui- and diterpenes as the major compounds described as kaurane-, labdane-, and clerodane-type diterpenes. Some of the identified diterpenes, such as kaurenoic and hardwickiacids, have been reported to possess antitumor activity[11,12] and eperuic acid as an accelerator of collagen production.[13] Even considering its wide use in folk medicine, there are no in vitro studies on the biological activities of *E. oleifera* oil-resin.

Matrix metalloproteinases (MMPs) are proteinases that are involved in the breakdown and remodeling of the extracellular matrix (ECM). It plays critical roles in cell growth, angiogenesis, invasion, and metastasis. This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.

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metastasis of cancer cells through degradation of the ECM.\[14,15]\] Numerous studies have focused on compounds from plant species as potential inhibitors of MMPs with anticancer effects.\[16,17]\] MMPs are also responsible for changes in skin collagenous tissues by breakdown of collagen, i.e., a major in the ECM, especially MMP-1 (interstitial collagenase-1).\[18]\]

This study aimed to investigate the effects of the acid fraction of *E. oleifera* oil-resin (AFEOR) on cytotoxicity of normal and malignant cell lines, genotoxicity, and inhibition of metalloproteinases, cell proliferation, and collagen production.

**MATERIALS AND METHODS**

**Plant material**

Samples of the *E. oleifera* oil-resin were commercially available at the municipal market of Manacapuru, Amazonas, Brazil.

**Acid fraction procedure**

From crude oil-resin, a fractionation was performed to separate diterpenic fraction from the apolar sesquiterpenes. The usual column chromatography is not effective to separate the sesquiterpenes from diterpenes with an acid function. Therefore, a selective ion-exchange column chromatography fractionation was performed using silica impregnated with KOH, as previously used to copaiba oils. After adding the modified silica and AFEOR, the column was submitted to dichloromethane elution to obtain the sesquiterpenes and the nonacid components separated from the diterpenic carboxylic acids. The diterpenic acids remain retained by silica impregnated with KOH during dichloromethane elution. Sequentially, methanol was used to elute the diterpenic acids as potassium salts. The methanol fraction was concentrated under low pressure and immediately acidified with HCl until pH 4–5. Dichloromethane was added and the diterpenic acids were recovered from the organic phase (dichloromethane) in a separation vessel. The solvent was evaporated in a rotary evaporator and the acid fraction obtained was stored under low temperature until the analysis. After derivatization with freshly prepared diazomethane, AFEOR was analyzed by gas chromatography using flame ionization and mass spectrometry detector. Copalic and hardwickiic acids were identified as their respective methyl esters by comparison with standards obtained previously from copaiba oils.

**Cell culture**

Human primary fibroblast (HPF) and human keratinocytes (HaCaT) cells were kindly provided by Dr. Silvya Stuchi Maria-Engler, from the Department of Pharmaceutical Sciences, University of São Paulo. Human fibroblasts (MRC-5) and tumor cells of melanoma (SKMEL-19), human colorectal carcinoma (HCT116), breast adenocarcinoma (MCF-7), gastric carcinoma (A549-02), and ovarian adenocarcinoma (ES-2 and NHOVCAR) were provided by human Cytogenetics Laboratory of the Federal University of Para. The cells were grown in culture flasks of 75 cm² in Dulbecco’s Modified Eagle’s Medium (DMEM), supplemented with 10% (v/v) bovine serum (GIBCO), penicillin (100 U/ml), and streptomycin (100 U/ml) and kept in an incubator at 5% CO₂ and 37°C. After reaching 70%–80% confluence, the cells were transferred to the walls of the wells, the experiment began. AFEOR was dissolved in serum-free DMEM, yielding different concentrations ranging from 1.25 to 5.0 μg/mL. In the treated wells, the different concentrations of AFEOR were added and incubated for 24, 48, and 72 h. As control, wells were filled with DMEM and dimethyl sulfoxide (DMSO) 0.5% for the same period. The wells were then rinsed with PBS and filled with culture media. Cell counts were performed in triplicate and growth curves of the fibroblasts were determined by Trypan Blue exclusion method. Cell morphology was observed by inverted microscope.

**Matrix metalloproteinase-2 and matrix metalloproteinase-9 inhibition**

To study the effect of AFEOR on MMP-2 and MMP-9 inhibition, MRC-5 and HaCaT cells were seeded in a 12-well plate on reaching confluence, washed twice with PBS, and then treated with or without AFEOR (1.25, 2.5 and 5 μg/mL). After 24 h, the media were collected, centrifuged to avoid cellular debris, mixed with 4X sample buffer containing 62.5 mM Tris-HCl, pH 6.8, 10% glycerol, 1% sodium dodecyl sulfate (SDS), and 0.00625% bromophenol blue, and then loaded for electrophoresis on a 10% SDS-polyacrylamide gel for zymography studies.

**Zymography of matrix metalloproteinase-2 and matrix metalloproteinase-9 inhibition**

In the zymography assay, gelatine was used as a substrate for MMP-2 and MMP-9.\[20]\] Gelatine at a concentration of 0.1% was incorporated into 10% polyacrylamide gel containing 0.4% SDS. Electrophoresis under nonreducing conditions was performed using a Bio-Rad mini-gel system at 120 V for 90–120 min. After electrophoresis, the gels were washed twice for 30 min in 2.5% Triton X-100 (v/v) to remove the SDS, and then incubated overnight in the developing buffer (50 mM Tris-HCl, pH 7.6, 200 mM NaCl, 5 mM CaCl₂, and 10 mM ZnCl₂) at 37°C. Digestion bands were quantified by Quantity One (Bio-Rad Laboratories, Hercules, CA, USA).
Western blot analysis of matrix metalloproteinase-2 and matrix metalloproteinase-9 inhibition

MRC-5 and HaCaT cells were cultured in 6-well plates (1 × 10^6 cells per well) and incubated with AFEOR in concentrations of 2.5, 5, and 10 μg/mL and incubated for 24 h. After incubation, cells were washed with PBS and lysed with lysis buffer consisting of Tris-HCl (50 mM, pH 7.5), 150 mM NaCl, 0.5% Nonidet P-40, 1 mM EDTA, 1 mM MgCl₂, 10% glycerol, and proteases inhibitors (Cocktail of proteases inhibitors EDTA-free, Roche; 1 mM phenylmethyleneasulfonyl fluoride). After 1 h at 40°C, cells lysates were obtained by centrifugation at 10,000 g for 10 min. The total protein concentration in the lysates was measured by the Bradford method with bovine serum albumin as standard. Samples containing equal amounts of protein concentration were separated by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes. Nonspecific binding was blocked with Tris-buffered saline with Tween 20 (1 M Tris-HCl [pH 7.5], 2.5 M NaCl, and 0.5% Tween 20) containing 5% nonfat milk for 2 h at room temperature. The membranes were incubated overnight with the primary antibody (MMP-1 and β-actin) diluted in Tris-buffered saline with Tween 20 (1:1000 and 1:2000, respectively), then washed with the same buffer, and incubated with horseradish peroxidase-conjugated anti-immunoglobulin G antibody (goat anti-rabbit immunoglobulin G) as secondary antibody for 1 h at room temperature. The immunoblots were visualized with chemiluminescence detection kit according to the manufacturer’s recommendations (kit Pierce). Western blots are representative of three independent experiments.

Hemolysis test

The hemolytic activity of the AFEOR was evaluated according to Fischer et al., with modifications. Blood from Swiss mice was collected in heparinized tubes and centrifuged at 700 g for 10 min. The pellet was washed three times with cold PBS pH 7.4 by centrifugation at 700 g for 10 min and resuspended in the same buffer. This suspension of red blood cells was always freshly prepared and used within 24 h after collection. Different concentrations of AFEOR were prepared in PBS buffer, added to the erythrocytes, and incubated for 60 min at 37°C in a shaking water bath. The release of hemoglobin was determined after centrifugation (700 g for 10 min) by spectrophotometric analysis of the supernatant at 540 nm. Complete hemolysis was achieved using 0.2% Triton X-100 yielding the 100% control value. Less than 10% hemolysis was considered as nontoxic effect level in our experiments. The experiments were run in triplicate.

Comet assay

The Comet assay with MRC-5 cells was used to detect DNA damage. Before each experiment, frosted microscope slides were precoated with two layers (100 μl) of normal agarose (1% in Milli-Q water) and left at room temperature to allow agarose to dry. The cells were treated during 3 h with different concentrations of the test samples. The cell dilution (5 × 10^6 cells in 60 μl) was mixed with an equal volume of low-melting point agarose (1.2% in PBS). This agarose cell suspension (120 μl) was spread onto each precoated slide and covered with a coverslip. After 10 min on ice, the cover slip was gently removed, and the slides were placed in a tank filled with the lysate buffer (2.5 M NaCl, 100 mM EDTA, 10 mM Tris-HCl, 1% sodium sarcosinate pH 10, 1% of Triton X-100, and 10% DMSO). They were immersed for 1 h in this buffer (4°C, in the dark). The slides were then transferred into the electrophoresis buffer (NaOH 10 N, EDTA 200 mmol/l, and pH 13 in distilled water) for 20 min at room temperature in darkness. Electrophoresis was carried out for 15 min at 25 V, 300 mA. Finally, the slides were gently rinsed with neutralization solution (0.4 M Tris-HCl, pH 7.5) 3 times for 5 min each. Staining of DNA was accomplished using 50 μl of ethidium bromide solution (20 μg/mL in PBS) per slide. The slides were examined using an epifluorescence microscope.

Quantification of the comet assay

A total of 100 comets on each scored slide for each sample concentration were visually scored according to the relative intensity of fluorescence in the tail and classified as belonging to one of five classes. We utilized three slides for each extract concentration, and the experiments were performed in triplicate. Each comet class was given a value of 0, 1, 2, 3, or 4 (from undamaged, 0 to maximally damaged, 4) as described by Cavalcanti et al.[1] The total score of DNA damage was calculated by the following equation: Total DNA damage = (number of cells in class 0 × 0) + (number of cells in class 1 × 1) + (number of cells in class 2 × 2) + (number of cells in class 3 × 3) + (number of cells in class 4 × 4).

Statistical analysis

Results are expressed as the means and standard deviations of triplicate measurements. Each experiment was performed at least three times. Differences between groups were assessed by one-way analysis of variance followed by the Bonferroni, Dunnett’s, and Tukey’s posttest. P < 0.05 indicated significance.

RESULTS

Chemical composition of Eperua oleifera oil-resin

A selective fractionation was performed to separate an acidic diterpenic fraction from the apolar sesquiterpenes, all naturally present in the E. oleifera oil-resin and observed after derivatization with diazomethane by gas chromatography–mass spectrometry. In this analysis, two main diterpenes acids were identified: copalic and hardwickiic acids [Figure 1]. Unfortunately, it was not possible to perform the quantification of these in the fraction obtained yet their presence as major components can partly explain the biological activity observed in this study.

Effect of Eperua oleifera oil-resin on cell viability

To investigate the cytotoxicity of AFEOR on human HaCaT keratinocytes, normal FPH and MRC-5 fibroblasts, and the malignant cells lines MCF-7, HCT116, ACP-02, ES-2, SKMELL 19, and NHOVCAR, the cells were seeded into wells of 96-well culture plates at a density of 0.5 × 10^4 cells/
The influence of AFEOR at various concentrations on different cellular toxicity was analyzed using the Alamar Blue® assay. Together with the increase in incubation time, we observed a concentration-dependent reduction in the cell viability after 72 h. A decrease of FPH cell viability was only observed at the highest concentration (50 μg/mL). However, the HaCaT cells showed a significant loss in viability of about 56%–86% observed at concentrations ≥25 μg/mL. After 72 h, we determined >85% of viable cells at a concentration of 12.5 μg/mL. Below this concentration, the cell viability did not change in comparison with control cells. Independent from the time of exposure, incubation with 3.12 μg/mL of AFEOR, all cells were viable. As a negative control, we considered cells that were not treated with AFEOR, and as a positive control, the cells that were treated with doxorubicin.

The cytotoxicity effects of AFEOR on MRC-5 cell, after 72 h, are described in Figure 2a-f. Results showed a significant loss in viability of about 40%–80% observed at concentrations higher than 25 μg/mL, with several cell deaths at a maximum concentration of 50 μg/mL and IC₅₀ value of ~25 μg/mL. On the other hand, below this concentration, cells were 100% viable, as well as control cells.

**Morphology of human fibroblasts and keratinocytes incubated with acid fraction of Eperua oleifera oil-resin**

Substantial changes in MRC-5 cell morphology were detected microscopically after 24 h and exposure with the oil-resin and becoming more prominent after 72 h of incubation. The AFEOR at 3.12 μg/mL...
Effect of *Eperua oleifera* on proliferation and synthesis of collagen of FPH and MRC-5 cells

In this study, the effect of AFEOR on cell growth was investigated for two different fibroblast cell lines. After treatment with AFEOR, FPH and MRC-5 cell proliferation [Figure 5a and b] decreased when cells were incubated at a concentration of 5 μg/mL.

The production of collagen by MRC-5 and FPH cells was also investigated, using the Sirius red assay [Figure 5c]. To measure the effects of AFEOR on collagen synthesis, cells were incubated for 24, 48, and 72 h with AFEOR [Figure 5d and e]. Results of MRC-5 cells showed that collagen synthesis increased significantly after 24 h when incubated with 1.25 μg/mL AFEOR. Thus, on FPH cell line, the collagen production was increased at about 50%, after 24 h of treatment, and at 25% after 48 h of treatment with 5 μg/mL of AFEOR, in comparison with nontreated cells.

Determination of the activity and expression of metalloproteinases

To determine the inhibitory effects of AFEOR on collagenases activities, different concentrations of the oil-resin were incubated with 25 μg/mL of collagenase from *Clostridium histolyticum* and applied to gelatin zymography. Negative controls contain DMSO (0.1%). As shown in Figure 6a-e, treatment with AFEOR was capable of inhibiting collagenase activity in 88% at the maximum concentration of 200 μg/mL. It exhibited an IC₅₀ value of 46.64 μg/mL.

To determine if the AFEOR could inhibit the MMP-2 and MMP-9 enzymatic activities, HaCaT and MRC-5 cells were treated with 5, 2.5 and 1.25 μg/mL of AFEOR in serum-free medium and incubated for 24 h. Then, the conditioned media of the cells were collected, and their MMP-2 and MMP-9 enzymatic activities were assessed using the gelatin zymography method. As shown in Figure 6b and f, MMP-2 and -9 activities were detected in the conditioned media and zymography quantitative analysis did not show a significant decrease of MMP-9 activity compared with the control. In addition, there was only a small decrease in MMP-2 activity when the cells were treated with AFEOR.

To evaluate the effects of the AFEOR on MMP-1 protein expression, MRC-5 cells were exposed to the oil-resin treatment (1.25–5 μg/mL) for 24 h. Levels of MMP-1 expression were measured by Western blot assay [Figure 6c] after protein dosage of total cell lysates. AFEOR treatment decreased protein expression in a dose-dependent manner, i.e. 39%, 47%, and 86% at, respectively, 1.25 μg/mL, 2.5 μg/mL, and 5 μg/mL [Figure 6d].

**Figure 3:** Representative photograph of human fibroblast (HPF) and Keratinocyte morphology (HaCat). The HPF (a-c) and HaCat (d-f) cells were incubated with two different concentrations of *E. oleifera* oil-resin in DMEM media for 24 h. Morphological changes were observed and compared with nontreated cells. The cell culture was examined and photographed using an inverted microscope (×40)

**Figure 4:** Representative photograph of human fibroblast and keratinocyte morphology. The FPH (a-c) and HaCat (d-f) cells were incubated with acid fraction of *Eperua oleifera* oil-resin in Dulbecco’s modified Eagle medium media for 24 h. Morphological changes were observed and compared with nontreated cells. The cell culture was examined and photographed using an inverted microscope (×40)
In addition, other three malignant cell lines were also used in this assay, i.e., ES-02, NH0VCAR, and ACP-02. AFEOR at 50 μg/mL exhibited a high cytotoxicity against these tested malignant cell lines, in comparison with doxorubicin at 5 μg/mL. The only exception was that ACP-02, ES-2, and NH0VCAR malignant cell lines that showed more resistant toward AFEOR treatment.

**Comet assay**

The induction of DNA damage in MRC-5 cells after exposition to different AFEOR concentrations was investigated using the Comet assay. Data are reported as total DNA damage in Figure 9a and damage frequency in Figure 9b. It was indicated that the oil-resin-induced genotoxicity (18%–60%) at concentrations of 7.5–30 μg/ml with a significant difference between the total DNA damage of the negative control (DMSO 0.2%). The frequency of DNA damage in MRC-5 cells was described as [Figure 9b] the different levels of tail comets extents increased by AFEOR treatment, compared with DMSO (0.1%) as a negative control and doxorubicin (10 μg/mL) as a positive control.

**DISCUSSION**

This research aimed to study *E. oleifera* biological activities. Here, we have explored its activity on normal and tumor cell viability, cell proliferation,
metalloproteinase activity and expression, and collagen production. The present study is the first report demonstrating biological activities of *E. oleifera* on human cell lines.

The chemical composition of *E. oleifera* oil-resin is a dispersion of diterpene acids in a mixture of mono- and sesquiterpenes. The main components found in AFEOR were labdane, clerodane, and kaurane diterpenes. Some of the diterpenes reported in *Eperua* species are eperuic, kaurenoic, and hardwickiic acids, mostly present in oil-resin of the genus *Copaifera*. Kaurenoic acid, i.e., a diterpene isolated from *Copaifera langsdorffii* oil-resin, has been reported by Costa-Lotufo et al. for its cytotoxic and embryotoxic effects. Ohsaki et al. described the antitumor activity of hardwickiic acid against IMC carcinoma in Swiss mice. Copaiba oil-resin is generally used in traditional medicine for its anti-inflammatory, antitumor, antimicrobial, wound healing, and antiseptic properties.

Diterpenes and sesquiterpenes are well known for their antitumor and anti-inflammatory properties. Paclitaxel, a known diterpene isolated from *Taxus brevifolia*, is used for treating solid tumors. It works by increasing microtubules stability and thus blocking progression of mitosis. A recent study has investigated cytotoxic, mutagenic, and genotoxic effects of copalic acid, isolated from *C. langsdorffii*. To the best of our knowledge, there are no data available on the cytotoxicity of AFEOR. In this work, we reported an in-depth analysis of its cytotoxic effects on a variety of cell lines and compared it with the known drug doxorubicin.

### Table 1: Cytotoxicity of *Elaeis oleifera* oil-resin in different cell lines compared with standard doxorubicin (IC₅₀ value µg/mL)

| Treatment cell line | *Elaeis oleifera* | Doxorubicin |
|---------------------|------------------|-------------|
| MRC-5               | 14.65 (13.79-15.55) | 3.2 (2.6-4.0) |
| FPH                 | >50              | 2.14 (1.10-4.18) |
| HaCaT               | 22.92 (18.16-28.94) | 0.58 (0.40-0.83) |
| HCT116              | 17.22 (14.60-20.30) | 0.42 (0.37-0.78) |
| MCF-7               | 19.49 (16.95-22.40) | 0.95 (0.73-1.24) |
| SK-Mel-19           | 15.99 (13.97-18.31) | 0.779 (0.57-1.03) |

*Average values for IC₅₀ (µg/mL); results are represented by the means±SD of three experiments. SD: Standard deviation.
Fibroblasts have a higher basal proliferative capacity and its proliferation is one of the early processes during dermal wound healing. Several studies on the molecular mechanisms of cellular carcinoma have revealed that deregulation of proliferation plays an important role in tumorigenesis. In addition, the normal fibroblasts derived from a primary cell culture were used together with MRC-5 fibroblasts and human keratinocytes (HaCaT) to evaluate the effects of AFEOR on MMP activity and expression, cell proliferation, and production of collagen. Morphological changes of fibroblasts corresponded well with the cytotoxicity tests. There were clear differences in cytotoxicity and morphology between cells with and without AFEOR treatment (≥10 μg/mL). However, the significant reduction on cell viability of normal fibroblasts and keratinocytes by AFEOR treatment hampers its use as a potential antitumor drug.

Our experiment shows that AFEOR inhibits fibroblast cells proliferation even in low concentrations (5 μg/mL). These effects of AFEOR were only detected at concentrations ≥25 μg/mL in the cell viability assays. Our studies also aimed to investigate whether AFEOR increases the stimulation of collagen production in human fibroblasts. At 5 μg/mL, AFEOR stimulated the collagen production, even when there was a small decrease in cell number. It is an important result because collagen stimulation is ideal to counteract skin aging and to improve healing repair.

A patent application has been filed for isolated compounds of *Eperua falcate* resin, as an accelerator of collagen production. This product comprises a mix of labd-8(17)-en-15-oic acid (eperuic acid) and labd-8-ene-15-oic acid, obtained by chemically treating labdenoic acid. These findings are important since the species of *E. oleifera* contain the
same compounds. It is noteworthy that, in traditional medicine, AFEOR is used for promoting wound healing.[8]

Natural products that inhibit MMPs can be beneficial in the prevention and/or treatment of cancer metastasis, skin aging, and wound repair.[32-34] In this study, zymographic analysis revealed inhibitory effects of AFEOR on collagensases activity. The possible mechanisms by which AFEOR inhibits the MMPS are presently unknown. Analysis of our data showed a significant inhibition by AFEOR of collagensases activity from C. histolyticum, with an IC₅₀ value of 46.64 μg/mL. Therefore, we analyzed in cell culture whether AFEOR could have any direct effects on MMP-2 and MMP-9 activity, which are known to play key roles in several pathological conditions.[34-36] Indeed, in low concentrations from 1.25 to 5 μg/mL, AFEOR weakly inhibited gelatinase activity of HaCaT cells. Unfortunately, the applicability of the AFEOR on cell lines at concentrations up to 10 μg/mL was hampered by its cytotoxicity.

AFEOR exhibited a significant cytotoxicity in vitro against all tested cancer cell lines, but the highest cytotoxicity was observed for SKMELL19 (IC₅₀ = 15.99 μg/mL), HCT116 (IC₅₀ = 17.22 μg/mL), and MCF-7 (IC₅₀ = 19.49 μg/mL) cell lines, but IC₅₀ is still higher than the reference drug doxorubicin. Besides the cytotoxicity, the hemolytic activity of AFEOR in Swiss mice erythrocytes was evaluated. A high hemolytic activity with an IC₅₀ value of 38.28 μg/mL was found for AFEOR after 30 min of exposure. This result suggests that the high cytotoxicity could be related to the membrane-damaging properties of AFEOR. This is confirmed by literature data, showing that diterpenes cause hemolysis and membrane damage of erythrocytes.[35,36] Cytotoxic effects of AFEOR are mediated not only by interaction of AFEOR compounds with cell membranes but also by cellular uptake and subsequent activation of necrosis or apoptosis. Therefore, more research is needed by isolating the pharmacologically active compounds from this medicinal oil. The challenge will be to isolate compounds that show almost no cytotoxicity on normal cells but a significant cytotoxic effect on tumor cells. For example, synthesis of the sesquiterpene (-)-hyrtiosal from copalic acid, i.e. a characteristic diterpene of copaiba oil-resin, led to a patent describing its selective cytotoxic activity against melanoma and leukemic cell lines.[37]
could be useful in the search for new anticancer agents since it possesses an important cytotoxicity and inhibition of metalloproteinases. Further studies are needed to isolate the pharmacologically active compounds from AFEOR and to search for compounds with a lower cytotoxicity on normal cells. Taken together, our data also suggest that AFEOR in low concentrations could be a potential agent to develop effective MMP-1 inhibitors as chemotherapeutic agents and for skin photoprotection.

CONCLUSION

Our results showed that oil-resin from *E. oleifera* have cytotoxic effects on tumor cells, hemolytic activities, and genotoxicity on fibroblasts and exhibits significant anti-collagenase activity. Further investigations, such as screening of cytotoxic effects of the isolated compounds from AFEOR, in* vivo* studies, and research on mechanisms of action will be necessary to better understand the biological effects of *E. oleifera* oil-resin.

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Conflicts of interest

There are no conflicts of interest.

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