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Genotoxic, cytotoxic and fungicidal activity of the essential oil extracted from the leaves and fruits of the pink pepper (*Schinus terebinthifolius* Raddi)

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Abstract

Schinus terebinthifolius Raddi is a tree present in Latin America, mainly in Brazil. The essential oils obtained from its leaves (LEO) and fruits (FEO) were evaluated for chemical composition cytotoxic, genotoxic and antifungal activities. The extraction of the essential oils was accomplished by the hydrodistillation technique. The characterization and quantification of the constituents were performed by gas chromatography coupled to a mass spectrometry detector and gas chromatography coupled to a flame ionization detector, respectively. The cytotoxic assay using tumor cells (lung adenocarcinoma, breast carcinoma, and melanoma) and normal cells was determined by the MTS assay. Genotoxic potential on normal cells was evaluated by Cometa assay. The analysis of antifungal activity was performed by evaluating the inhibitory effect on the growth of the *Aspergillys carbonarius* and *Aspergillus flavus* filamentous fungi using the disc diffusion test. The LEO contains limonene, D-germacrene, β-trans-caryophyllene, bicyclogermacrene, α-epi-murolol and α-copaene as the principal constituents, whereas the major constituents of FEO are myrcene, Δ-3-carene, β-eudesmol and α-phellandrene. Regarding cytotoxic activity on tumor cells, FEO was more effective than LEO. The IC50 values found for FEO on tumor cells varied between 244-302 $\mu g.mL^{-1}$ compared to 653.4 ± 1.11 for normal cells. LEO was more cytotoxic against normal cell than tumor. Neither FEO nor LEO induced DNA damage at tested experimental conditions. A Minimum Inhibitory Concentration of 125 $\mu L.mL^{-1}$ of both essential oils was determined for the fungi analyzed. It is inferred that these essential oils can be used in drug formulations when used in the correct concentration.

Keywords: Natural products; Cancer; Cytotoxicity; Antifungal activity; Aroeira; Secondary metabolites.
Abbreviations: FID_flame ionization detector; GC_gas chromatography; MS_mass spectrometer; A549_lung adenocarcinoma; MCF-7_breast adenocarcinoma; HT-144_melanoma; CCD-1059Sk_fibroblasts derived from normal human skin; essential oil of leaves_LEO; essential oil of fruits_FEO.

Introduction

Brazil has one of the largest biodiversities in the world, being a rich source of bioactive compounds of commercial value. Therefore, there has been a marked increase in the use of medicinal plants in the country in recent years involving billions of dollars per year. Among several Brazilian native plants, the aroeira (*Schinus terebinthifolius* Raddi), also known as the pink pepper, stands out because of its great potential for exploration and commercial use. All the parts of *S. terebinthifolius* are used in folk medicine for the treatment of various pathologies. The nuts are widely used as a culinary seasoning, and they can be marketed in natura or as an essential oil (Silva et al., 2015).

The main components responsible for the therapeutic action of the pink pepper are those found in its essential oil (Simões et al., 2007). Because they have ample biological activity, these oils have great industrial importance, and they have been used in different sectors, such as in the food, pharmaceutical and cosmetic industries. They stand out mainly for their antibacterial, antifungal, insecticide, allelopathic, antiparasitic, antioxidant, anti-inflammatory, antitumor and anti-ophidic properties.

Because they have a wide biological activity, these oils have great industrial importance and can be used in different areas. Because of their antifungal activity attributed to the high concentration of monoterpenes, they are already used in the pharmaceutical industry in the manufacture of medicines for the treatment of mycoses and candidiasis, and they can also be used in the manufacture of food as a natural preservative instead of using synthetic preservatives (Costa et al., 2010).

Several phytochemical studies have demonstrated the biological activity of the essential oil from *Schinus terebinthifolius* against the fungi *Aspergillus niger*, *Candida albican*, *Bipolaris oryzae*, *Fusarium oxysporum, Fusarium solani, Rhizoctonia solani* and *Colletotrichum gloeosporioides* (Gundidza et al., 2009; Junior et al., 2013; Mohamed et al., 2020). There are genotoxic studies of *Schinus terebinthifolius* that indicate that the aqueous extract of its leaves does not cause chromosomal alterations in Wistar rats (Junior et al., 2015). Positive results against human breast carcinoma have been observed with the essential oil from *Schinus terebinthifolius* (Bendaoud et al., 2010).

Other essential oils have already been studied for their anticarcinogenic activity, and positive results have been obtained. Anticarcinogenic activities agianst human lung carcinoma cells and on colon adenocarcinoma cells have been observed for the essential oil extracted from the leaf of *Croton flavens* L. (Sylvestre et al., 2006). Studies of the anticancer activity of the essential oil from the orange peel have already been reported and demonstrate inhibitory activity of this essential oil on the proliferation of human prostate tumor cells and on lung carcinoma cells (Yang et al., 2017).

Because of the increase in its share in the market, the essential oil from the pink pepper is considered to represent an important source of income, especially because of its biological properties, which have not yet been extensively studied, and because of the abundant popular use of this plant. With the purpose of broadening the characterization to better understand its action in biological systems and to permit the indication of safe applications in health and in human nutrition, the objectives of this work were to extract the essential oil from the leaves and fruits of the pink pepper, to characterize its constituents and to evaluate the cytotoxic, genotoxic, antitumor and antifungal activities.

Results and Discussions

Chemical characterization of essential oils

The chemical constitution of the essential oils obtained from the leaves and fruits of the pink pepper are presented in Tables 1 and 2. The main constituents found in the essential oil from the leaves were limonene (31.13%), D-germacrene (16.14%), β-*trans*-caryophyllene (8.75%), bicyclogermacrene (7.92%), epi-murolol (4.53%) and α-copaene (3.75%). The constituents found in the essential oil from the fruits were mirceno (33.76%), δ-3-carene (22.74%), β-eudesmol (9.65%) and α-phellandrene (8,67%).

Phytochemical studies have revealed the presence of several chemical compounds, including alcohols, ketones, acids, monoterpenes, sesquiterpenes and triterpenes, in the stem, leaves and fruits of the *aroeira da praia* (*Schinus terebinthifolius*) (Santos et al., 2006). Santos et al. (2013) studied the insecticidal activity of the essential oil from the leaves of *Schinus terebinthifolius* Raddi against the coffee borer (*Hypothenemus hampei* Ferrari) and found germacrene D (25.0%), (E)-β-caryophyllene (17.5%) and d-elemene (10.5%) as the main components. Ibrahim et al. (2010) analyzed the chemical composition of the essential oil from the fruits of the *aroeira da praia* and also observed the predominance of the terpenes α-pinene, germacrene D, camphene, β-phellandren, γ-terpinene, α-phellandren and δ-3-carene.

When determining the composition of the essential oil of the pink pepper fruits, Cole et al. (2014) identified seventeen compounds, but the principal compounds found δ-3-carene and α-phellandrene were the same as those found in the present study. This variation can be related to several environmental factors, such as seasonality, precipitation, circadian rhythm, altitude, temperature, vegetative cycle of the plants, soil type, and harvesting season, among others (Gobbo-Neto and Lopes, 2007).

Cytotoxic and genotoxic activities of essential oils against normal cells

The cell viability was assessed after 48 h of treatment with essential oils derived from fruit and leaves (Figure 1). The results showed that essential oil derived from the leaves had significant cytotoxic activity on CCD-1059Sk cells (IC_{50} = 208.0 ± 1.99). Already essential oil derived from the fruits displayed low cytotoxic activity on normal cells (IC_{50} = 653.4 ± 1.11).

The possible cytotoxicity found for the essential oil of the leaves of the pink pepper is quite controversial because the literature contains studies that show that these species of plants are completely safe for internal and external use, but there are studies showing that the cytotoxicity is associated with different administration routes (oral and intraperitoneal). Lima et al. (2009), using rats as a study model (males/females), reported that oral administration of 1.56 g/kg/day of *S. terebinthifolius* for up to 45 days did not cause significant effects. However, Bras et al. (2011) observed mild skin irritation in a clinical trial after acute dermal exposure of the ethanolic and hexane extracts of *Schinus molle* leaves.

Although the essential oil of the leaves of the pink pepper has been observed to be cytotoxic, no significant reduction in cell viability was observed in samples treated with concentrations downward 125 $\mu g.mL^{-1}$. For the use of higher concentrations, a better assessment of the risks and benefits of oral administration of this essential oil is required.

The cytotoxic effect might be directly related to the presence of toxic compounds present in the essential oil or the leaves of the pink pepper. However, it is difficult to explain the origin because of the diversity of compounds. Various biological events may be associated to cytotoxicity caused essential oils including cell membrane damage, specific alterations in lipids and proteins metabolic pathways, and depolarization of the mitochondrial membranes, which can lead to cell necrosis or apoptosis (Santiago et al., 2017).

In a next step, the ability of the essential oils to induce DNA damage was evaluated by comet assay. The results showed that the essential oils at 200 $\mu g.mL^{-1}$ did not induce DNA damage in CCD-1059Sk after 48 hours of treatment (Figure 2). These findings indicate that essential oil of the fruits can be used safely as a therapeutic agent because the results obtained in this study suggest an absence of genotoxic potential at the concentration evaluated, and the use of low concentrations may be recommended because of the cytotoxicity was only observed in the essential oil from the leaves at high concentration.

Other studies with essential oils using the Comet assay were performed in an attempt to verify the genotoxic potential of these metabolites. No cytotoxicity or genotoxicity in human leukocytes at concentrations of 50-300 $\mu g.mL^{-1}$ were observed when the essential oil from the leaves of *Alpinia zerumbet* was used, which contains 98.3% of monoterpenes.

Table 1. Composition of the essential oil from the leaves of the pink pepper.

Peak	RI	Compoud	%
1	933	α-Pinene	2.32
2	972	Sabinene	5.02
3	978	β-Pinene	0.85
4	989	Mircene	0.35
5	1025	p-Cymene	0.46
6	1029	Limonene	31.13
7	1182	α-Terpineol	1.25
8	1375	α-Cupene	3.75
9	1398	β-Elemene	1.94
10	1419	β-*Trans*-caryophylene	8.75
11	1449	α-Himachalene	0.43
12	1454	α-Humulene	0.82
13	1459	Aromadendrene	0.76
14	1480	D-Germacrene	16.19
15	1495	Bicyclogermacrene	7.97
16	1513	Cadinene	2.07
17	1518	Δ-Cadinene	2.76
18	1558	β-Germacrene	0.41
19	1578	Espatulenol	1.61
20	1595	Viridiflorol	0.89
21	1643	α-epi Muurolol	4.53
		Total identified	94.26

RI = Retention index. % = Percentage of each compound in the essential oils.

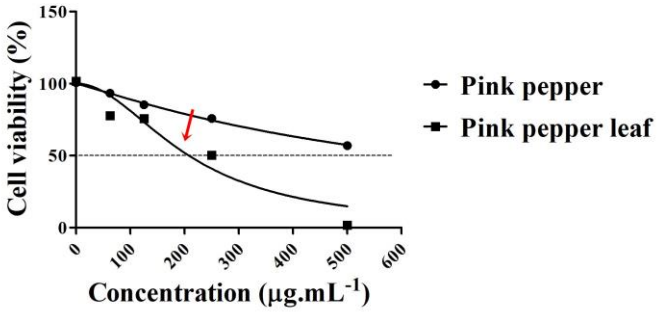

Figure 1. Cytotoxicity study against the CCD-1059Sk line after treatment for 48 hours with different concentrations of the essential oils. Cytotoxic effect observed at a concentration of approximately 200 µg mL-1 for the essential oil from the pink pepper leaves.

Table 2. Composition of the essential oil from the fruit of the pink pepper.

Peak	RI	Compound	%
1	931.93	*α*-Pinene	3.18
2	977.53	*β*-Pinene	0.27
3	987.57	Mircene	33.76
4	1006.65	*α*-Phellandrene	8.67
5	1010.22	Δ-3-Carene	22.74
6	1024.09	*p*-Cymene	1.76
7	1029	Limonene	3.62
8	1481.09	Δ-Germacrene	2.35
9	1518.34	Δ-Cadinene	0.75
10	1548.68	*α*-Elemol	5.78
11	1581.76	Caryophylene oxide	0.78
12	1632.52	γ-Eudesmol	5.7
13	1654.47	*β*-Eudesmol	9.65
		Total identified	99.02

RI= Retention index. % = percentage of each compound in the essential oil from the pink pepper.

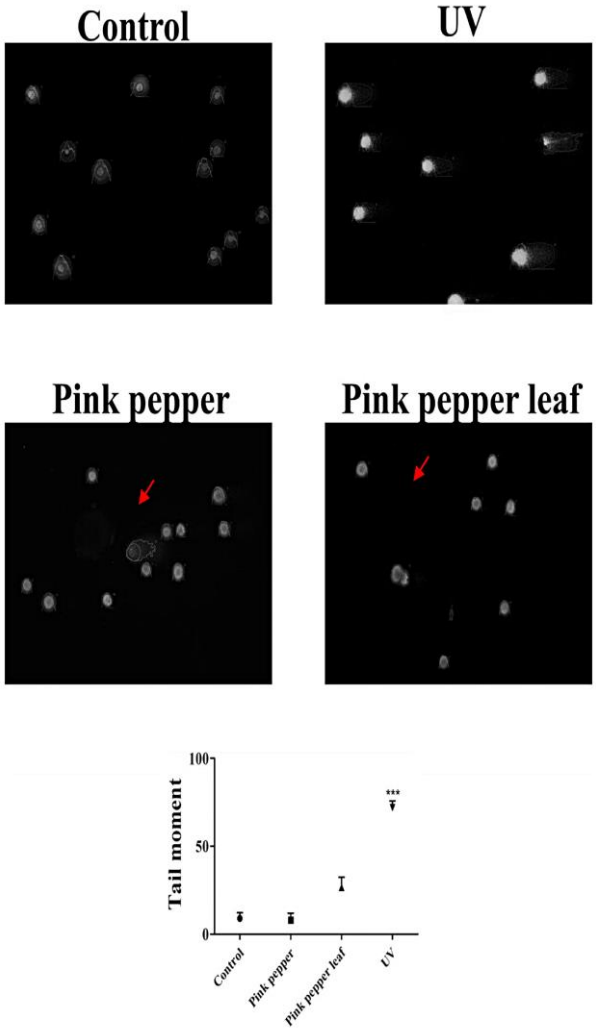

Figure 2. Genotoxicity study of the CCD-1059Sk line after a 48-hour treatment with 200 µg mL^{-1} of the essential oils. Absence of genotoxic potential in the essential oil from the leaves and fruits of the pink pepper at a high concentration (200 µg mL-1) in cells of the CCD-1059Sk. Notes: (A) Illustrative images of comets in samples viewed under fluorescence microscopy after staining with SybrGreen. (B) Analysis performed from 50 comets per slide using OpenComet software. The plot data refer to the tail movement parameter, and they are represented as the average of two independent experiments performed in duplicate. The error bar represents the standard deviation. ST-no treatment; UV-ultraviolet * p <0.05 according to ANOVA analysis of variance and post-test Tukey.

Table 3. IC_{50} values determined after 48 hours of treatment with essential oils at different concentrations.

	A549	MCF-7	HT-144
Pink pepper	302.0 ± 3.42	244.3 ± 1.99	272.8 ± 2.99
Pink pepper leaf	407.5 ± 8.03	394.2 ± 6.95	363.8 ± 5.45

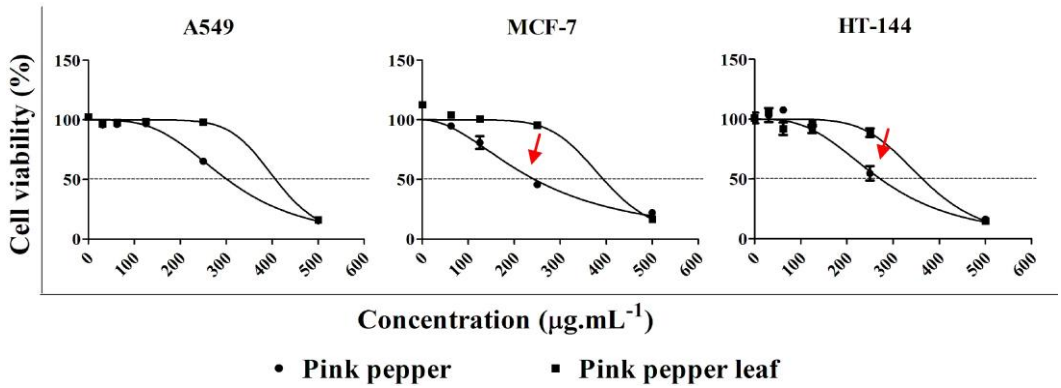

Figure 3. Cell viability of the A549, MCF-7 and HT-144 lines after the 48 hour treatment with the essential oils. Best results: MCF-7 - 50% reduction in cell viability at a concentration of 244.3 µg mL^{-1} of the essential oil from the fruits; HT-144 - 50% reduction in cell viability at a concentration of 363.8 µg mL^{-1} of leaf essential oil.

However, there was a decrease in cell proliferation and viability, and an increase in DNA damage when a concentration of 500 μg.mL^{-1} was employed. On the other hand, no mutagenicity in peripheral blood and bone marrow cells in rats was observed in in-vivo experiments with a 400 mg/kg dose of the essential oil (Cavalcanti et al., 2012).

Cytotoxic activity of essential oils on tumor cells

The essential oil derived from fruit was more cytotoxic on tumor cells than oil derived from leaf. The IC_{50} values found for essential oils on tumor cells are shown in Table 3. The MCF-7 cells (IC_{50} = 244.30 μg.mL^{-1}) were more responsive to essential oil derived from the fruits than A549 (IC_{50} = 302.0 ± 3.42μg.mL^{-1}) and HT-144 (272.8 ± 2.99) cells (Figure 3). Further studies will be addressed to evaluate the molecular mechanism associated to cytotoxicity of this essential oil on MCF-7 cells, which was effective in reducing cell viability in tumor cells, especially in MCF-7 cell, but had no significant cytotoxic activity on normal cells. These finding indicate promising antitumor potential of essential oil derived from fruit.

Bendaoud et al. (2010) studied the antitumor activity of the essential oils from the fruits of *Schinus terebinthifolius* Raddi and *Schinusmolle* L. originating in southern Tunisia. It was found that the essential oil from *Schinus terebinthifolius* Raddi was more effective against human breast cancer (MCF-7) lines. Its IC_{50} was 47 mg.L^{-1}. These results corroborate those observed in the present research, and the higher inhibition rates were probably due to the difference in the chemical compositions of the oils.

Lesgards et al. (2014) studied the effects of essential oils on tumor cells and determined that the constituents seem to act synergistically with chemotherapy and conventional radiotherapy. For those authors, the antitumor activity of the essential oils is related to the activation of cell death (apoptosis) induced by the caspase proteins in cancer cells, with minimal impact on healthy cells. The phenomena that appear to occur are overexpression and regulation of liver detoxification enzymes, changes in the membrane potential of cancer cells and mitochondria, production of free radicals in cancer cells, inhibition of angiogenesis, and modification of induction of tumor genes.

Conventional treatments against cancer are mainly based on the induction of an overproduction of reactive oxygen species in tumor cells. Different anticancer drugs used in cancer therapy induce the production of free radicals. However, many side effects have been observed because it has not yet been possible to locate the specific characteristics of the malignant cells that would help one to selectively treat them. This fact implies that other rapidly dividing healthy cells are also affected by chemotherapy.

Consequently, side effects such as hair loss, infections, anemia and bleeding commonly occur (Gilliam and Daret 2011; Lamberti et al., 2012).

Few studies have been reported concerning to antitumor activity of essential oils, which display several constituents. The complex mixture of essential oils difficult the study on their mechanism of action on tumor cells once antitumor activity may be due to individual component or by association of their components.

Antifungus activities of the essential oils

The MIC of the essential oils obtained from the leaves and fruits of the pink pepper were both equal to 125 μg.mL^{-1}. No inhibition was observed for the negative control (DMSO), whereas the MIC for the positive control (fluodioxonl) was 2 μg.mL^{-1}. The antifungal activity of the essential oils might be due to their lipophilicity and the variety of chemical constituents that exhibit various organic functions. Essential oils can act in two ways to inhibit the growth of fungi. The first manner involves the direct contact with the microorganism, interacting with the cell membrane of fungi and inhibiting the synthesis of ergosterol, the hormone responsible for cellular vitality. The second manner occurs when the essential oil crosses the cell membrane of the fungus and causes damage to several organelles, such as mitochondria, leading to the leakage of enzymatic cofactors such as K^+ and Mg^{2+} and causing cell death (Kedia et al., 2015). Works by Piras et al. (2017) showed that the essential oil of the pink pepper exhibits antifungal activity against other fungi in additon to the *Aspergillus* genus. The authors investigated the essential oil from the pink pepper fruits at a concentration of 0.32 mg.mL^{-1} and found 70% inhibition of the growth of *Candida albicans* germ tubes. Twenty-one and thirteen constituents were identified in the essential oils from the leaves and fruits, respectively. The essential oil obtained from the fruits significantly reduced cell viability in MCF-7 samples. The essential oils did not have a genotoxic effect, but a cytotoxic effect was observed for the essential oil extracted from the leaves. Antifungal activity against *Aspergillus carbonarius* and *Aspergillus flavus* was observed for both essential oils. The best results were obtained with the essential oil from the fruits.

Materials and Methods

Collection of plant material

The leaves and fruits of the pink pepper were collected at the Fazenda Campigado, municipality of Bambuí, MG, Brazil, at a latitude of -19.8875 and longitude of -46.0197 19° 53' 15" south and 46° 1' 11'' west, and at an altitude of 662 meters. The collections were accomplished in the morning on sunny days and in the absence of rainfall.

Extraction of essential oil

The essential oils were extracted in the Laboratory of Organic Chemistry of the Department of Chemistry of the Federal University of Lavras (UFLA). The extraction occurred by hydrodistillation over a period of 2 hours using a modified Clevenger apparatus coupled to a 5-L flask. The hydrolate was centrifuged at 965.36*g* for 15 minutes, and the essential oils were separated using a Pasteur pipette. They were then packed in amber glass vials and stored under refrigeration in the absence of light (Pimentel et al., 2008; Brasil, 2010).

Chemical characterization of essential oils

The chemical analysis of the constituents of the essential oils was performed at the Center of Analysis and Chemical Prospection (CAPQ) at the Department of Chemistry of UFLA. The compounds were identified by gas chromatography coupled to mass spectrometry (GC-MS) (Shimadzu, model QP 2010 Plus). A fused-silica capillary column (30 m x 0.25 mm) with a DB5 stationary phase (5% phenyl, 95% dimethylpolysiloxane) was employed. The entrainment gas was helium (Pró-service), with a flow rate of 1.0 mL.min^{-1}. The temperature was programmed, starting at 60 °C and increasing at 3 °C per minute to 240 °C, and then at 10 °C min^{-1} to 300 °C, where it was maintained for 7 minutes. The injector and detector temperatures were 220 °C and 240 °C, respectively. A 0.5 μL.mL^{-1} aliquot of the

sample dissolved in hexane (Sigma-Aldrich, Steinheim, Alemanha) was injected, the split ratio was 1:100, and the column pressure was 71.0 kPa. A 1000 scan detector was used in the mass spectrometer, with a scanning interval of 0.50 fragments detected in the range of 45 to 500 Da. The compounds were identified on the basis of the comparison of the retention indices from the literature and by comparison with the NIST107 and NIST21 equipment libraries (Adams, 2014).

Quantification of the constituents was achieved by gas chromatography (Shimadzu GC model 2010) using a flame ionization detector (GC-FID) under the same experimental conditions as those used in the identification procedure. For the calculation of the retention index, the Van den Dool and Kratz equation (1963) based on the homologous series of alkanes (C8-C18) was used, with extrapolation for C19 and C20.

Cytotoxic and genotoxic activity of essential oils

Cell line and culture conditions

Tumor cells (A549, lung adenocarcinoma; MCF-7, breast adenocarcinoma; and HT-144, melanoma) and normal cells (fibroblasts derived from normal human skin, CCD-1059Sk) were used in the present study. The A549, MCF-7, and CCD-1059Sk cell lines were purchased from Rio de Janeiro Cell Bank, and HT-144 cell line was kindly provided by Dr Glaucia Maria Machado-Santelli from Institute of Biomedical Sciences (University of Sao Paulo). The cells were cultured in DMEM (Dulbecco's Modified Eagle Medium Sigma, Sigma, CA, USA) supplemented with 10% fetal bovine serum (FBS, Cultilab, SP, Brazil). The cultures were kept in an oven at 37 °C under controlled atmosphere (95% air and 5% CO_2), and subcultures were performed regularly every two or three days.

Cell viability assay

Cytotoxic activity was determined by MTS colorimetric assay using the CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay Kit (Promega Corporation, Madison, WI, USA). The cells were seeded into 96-well plates at the density of $1x10^4$ cells/well (MCF-7, HT-144, and CCD-1059Sk) or $5x10^3$ cell/well (A549). After adhesion (24 hours), the cells were treated for 48 hours with the essential oil at different concentrations (62.5; 125; 200; 250 and 500 $\mu g.mL^{-1}$). Cell viability was assessed by MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-4-sulfophenyl)-2H-tetrazolium) using an Elisa reader at 490 nm (Cory et al., 1991). IC_{50} values were determined using the GraphPadPrism® program (GraphPad Software, Inc., San Diego, CA, USA). The values represent the average of three independent experiments performed in triplicate ± standard deviation.

Comet assay

Genotoxic activity was determined by Comet test using CCD-1059Sk cells, which were seeded into 24-well plates (5×10^4 cells/well). After adhesion, the cultures were treated with 200 $\mu g.mL^{-1}$ of essential oil for 48 hours. The cell suspension was obtained by enzymatic digestion (Trypsin-EDTA solution/Sigma-Aldrich LTDA, Brazil). The samples were centrifuged at 112*g* for 5 minutes. The precipitate was dissolved in 100 $\mu L.mL^{-1}$ of culture medium, and 5×10^5 cells were homogenized in 100 $\mu L.mL^{-1}$ of low-melting agarose (Sigma Aldrich LTDA, Brazil) and distributed on a histological slide previously covered with agarose (Sigma-Aldrich). The slides were maintained at 4 °C in lysis solution.

The electrophoresis was performed in a horizontal system under alkaline conditions for 20 minutes at 20 volts and 300 mA ($1v/cm^2$). The slides were kept in neutralization buffer for 15 minutes and then dried and stained with Sybr Green (Molecular Probes SYBR Green I nucleic acid gel stain). The analysis was performed using a fluorescence microscope (Eclipse 80i, Nikon), and 50 comets per slide were analyzed using the Open Comet/ImageJ software (Wayne Rasband, National Institutes of Health, USA) (Singh et al., 1988). The results presented refer to the mean ± standard deviation (SD) of three independent experiments performed in triplicate.

Antifungal activity of essential oils

The antifungal activity of the essential oils was determined in the Laboratory of Micologia of the Department of Food Science of UFLA. Two species of pathogenic fungi carried by food were used: *Aspergillus carbonarius* (CCDCA 10507) and *Aspergillus flavus* (CCDCA 10508) were acquired from the Culture Collection of Microrganisms of the Department of Food Science of UFLA.

The analysis of the Minimum Inhibitory Concentration (MIC) of the essential oils against fungi was performed using the disc diffusion test accepted by the Food and Drug Administration and established by the National Committee for Clinical Laboratory Standards. An inoculum was used at the concentration of 10^6 spores mL^{-1}, counted in a Newbauer chamber. The inoculum was transferred to a Petri dish containing the Malt Extract Agar (MEA) medium (Merck, Darmstadt, Alemanha), followed by spreading on the medium using a drigalsk loop and the surface-scattering technique.

Sterile filter paper discs of 5-mm diameter soaked with 10 µL mL^{-1} of the oils diluted in DMSO (Synth, Diadema, SP, Brasil) at concentrations of 500, 250, 125, 62.5, 31.25, 15.62 and 7.81 $\mu L.mL^{-1}$ were placed on the culture medium. As a relative negative control, a filter paper disk embedded with 10 $\mu L.mL^{-1}$ of DMSO was used, and a 2 $\mu L.mL^{-1}$ concentration of the synthetic fungicide fluodioxonil (Sigma Aldrich, Alemanha) was used as the standard control for comparison. The plates were incubated in BOD at 25 °C for a period of 72 hours. Diametrically opposite measurements of the inhibition halos were performed. The susceptibility profile of the filamentous fungi in the various concentrations of the essential oil from each sample was evaluated using the measured diameters. The test was performed in triplicate (Andrade et al., 2015). The MIC was defined as the lowest concentration of essential oil for which there was an inhibition halo.

Statistical analysis

Quantitative data were presented as the mean ± standard deviation of three independent experiments. Statistical differences were determined according to the one-way ANOVA analysis of variance, followed by Tukey's multiple post-test comparisons using GraphPadPrism® software (GraphPad Software, Inc., San Diego, CA, USA).

Conclusion

The principal constituents found in the essential oil from the leaves were limonene, D-germacrene, β-*trans*-caryophyllene, bicyclogermacrene, α-epi-murolol and α-

copaene, and those found in the essential oil from the fruits were myrcene, Δ-3-carene, β-eudesmol and α-phellandrene. The leaf essential oil exhibited a cytotoxic effect on normal cells when used in concentration upward 125 $\mu g.mL^{-1}$, and displayed low cytotoxic activity on tested tumor cells By contrast, the fruit essential oil was effective in reducing cell viability of breast cancer MCF-7 cells and displayed low cytotoxic activity on normal cells suggesting promising antitumoral potential. The essential oils had a MIC of 125 $\mu L.mL^{-1}$ for both fungi analyzed. The best results were obtained with the essential oil from the fruits.

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