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Antioxidant and antitumoral potential of terpenes and phenylpropanoids against MCF7, A549 and HT144 cancer cell lines

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Abstract

Essential oils are a complex mixture of terpenes and phenylpropanoids that have several biological activities, including antioxidant and antitumoral activities. The present study sought to evaluate the antioxidant potential of monoterpenes and phenylpropanoids by different methods (stabilization of free radicals, inhibition of lipid peroxidation, and complexation of metals). In addition, the antitumor potentials of these compounds were evaluated on human estrogen-positive breast cancer (MCF-7), non-small cell lung cancer (A-549), and melanoma cancer (HT-144) cell lines. Cisplatin (6 μg mL^{-1}) was used as a positive control. The cell viability was studied and the quantification of DNA was performed. The antioxidant activity results were analyzed by linear regression and antitumor activity were analyzed via analysis of variance and the Dunnett test. A greater antioxidant activity was observed for the phenolic compounds carvacrol, thymol and eugenol than for the standards (BHT, mannitol and ascorbic acid) by the methods tested. A dose-response effect was observed for the less active compounds, revealing the influence of the concentration of the constituents. Carvacrol displayed an important cytotoxic activity against the estrogen-positive MCF-7 cell line, and citral was more active against A549 cells and HT144 melanoma cells. The positive control Cisplatin showed low cell viability over the A549 and HT44 cells, and showed high cell viability over the MCF-7 cell. The antitumor activity of citral observed against HT144 cells is associated with its ability to promote the cell cycle at the G1/S transition, at least in part.

Keywords: Terpenes; phenylpropanoids; essential oil; anticancer agents; antioxidant activity.
Abbreviations: A549_lung adenocarcinoma; MCF-7_breast adenocarcinoma; HT- 144_melanoma; CCD-1059Sk_fibroblasts derived from normal human skin.

Introduction

Essential oils are a complex mixture of terpenes and phenylpropanoids extracted from plants, which exhibit antioxidant, antibacterial, antifungal, antitumoral activities, among others (Iso, 2013; Wang et al., 2018; Camargo et al., 2019; Ferreira et al., 2019; Pavithra et al., 2019; Caetano et al., 2020). In recent years, with the increase in cancer cases, there has been a demand for products and medicines of natural origin that present lower long-term toxicity for living organisms (Yu et al., 2011; De Lima et al., 2014; Sokmen et al., 2020). The search for non-toxic substances has led to a large number of studies regarding the antioxidant potential of essential oils and their isolated components because they have the ability to protect certain substrates from oxidation (Yu et al., 2011). They prevent the ramification and deterioration process in food and many patholgens in the body that are caused by oxidative stress (Amorati et al., 2013; Olszowy, 2019). The evaluation of antioxidant activity is one of the most widely discussed topics regarding research involving essential oils (Lima et al., 2012; Amorati et al., 2013; Andrade et al., 2013; Ferreira et al., 2019; Fallah et al., 2020; Sokmen et al., 2020). These compounds are classified as GRAS and represent potential sources of bioactive natural molecules, and they are the object of study for possible uses as preservatives. Bioactive foods are alternative medicines in the treatment of cancer (Manjamalai and Grace 2012; Sharifi-Rad et al., 2017; FDA, 2020).

Cancer is one of the most alarming diseases in the world, and it is the result of multiple steps that include genetic and epigenetic alterations leading to uncontrolled proliferation and changes in cellular physiology. Oxidative stress seems to be associated with tumor initiation and progression, as well as tumor resistance to chemotherapy (Hanahan, 2011; Wu and Ni, 2015; Saha, 2017). According data from the World Health Organization, cancer is the second most common cause of death globally and 9.6 million deaths were

estimated for 2018 (Bray et al, 2018; WHO, 2018). Thus, measures to increase the therapeutic arsenal for cancer therapy are relevant. Natural products have been considered to be the principal source of novel bioactive substances with pharmacological proprieties, and many antineoplastic drugs were originally identified in plants (Comşa et al., 2015; Newman and Cragg, 2016; Cragg and Newman, 2018).

Several studies suggest that essential oils could serve as anticancer agents by exerting a chemopreventive action for non-tumor tissue agents and with the potential for use in the pharmaceutical industry (Manjamalai and Grace, 2012; Blowman et al., 2018; Sokmen et al., 2020). The complex chemical composition of essential oils makes it difficult to identify objectively the main active principles with therapeutic potential in the mixture.

Generally, the biological activities of essential oils are attributedheto the principal components, synergism or even antagonism between them (Amorati et al., 2013; De Lima et al., 2014; Ferreira et at., 2020). Knowing the individual potentials of the chemical constituents present in essential oils can facilitate the elucidation of mechanisms of biological action; as well as predict possible results when these components are present in the essential oils. The antioxidant potential of monoterpenes and phenylpropanoids were evaluated by different methods (stabilization of free radicals, inhibition of lipid peroxidation, and complexation of metals). In addition, the antitumor potentials of these compounds were evaluated on human estrogen-positive breast cancer (MCF-7), non-small-cell lung cancer (A549), and melanoma cancer (HT144) cell lines.

Results and Discussion

Antioxidant Activity of Terpenes and Phenylpropanoids: free radical stabilization and inhibition of lipid peroxidation

The results obtained in the tests based on free radical stabilization and inhibition of lipid peroxidation is presented in Figure 1.

Important effects can be observed. A dose-dependent behavior was observed for the standard compounds with respect to antioxidant activity. Considering the methods that involve the stabilization of radicals, a higher sensitivity of the $ABTS^{\bullet+}$ assay can be seen when compared to the $DPPH^{\bullet}$ radical sequestration method (Figure 1). This result was observed because the $ABTS^{\bullet+}$ radical is less sterically hindered than the $DPPH^{\bullet}$ radical so it is more reactive (Mareček et al., 2017). However, stabilization of the free radical only occurs with the phenolic compounds carvacrol (8), thymol (13) and eugenol (28). Methods for assessing antioxidant capacity through radical sequestration involve reactions based on transference of the hydrogen atom or an electron. Eugenol, carvacrol and thymol are phenolic compounds that are able to neutralize the free radicals through this mechanism to form phenoxide ions stabilized by resonance. Phenolic compounds are substances with high antioxidant activity because they are able to stabilize free radicals by the formation of quinones and dimers (Tonello et al., 2016; Yildiz et al., 2017; Ferreira et al., 2019). Among the compounds with antioxidant activity that can be measured by the $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical stabilization methods, the greatest activity was observed for eugenol. It was as effective as the positive control, BHT. The other compounds did not exhibit activity by these methods because they do not have structures that are propitious for the donation of hydrogen atoms or electrons to stabilize the radical formed.

A better antioxidant response was observed when the standard compounds were evaluated by the methods that involve the inhibition of lipid peroxidation. Of the 29 compounds evaluated, 16 and 20 of them had low, medium or high antioxidant activity in the desoxyrribose degradation and β-carotene bleaching assays, respectively. The deoxyribose degradation and β-carotene bleaching tests evaluate the potential for preventive and direct chain-breaking antioxidant activity. The compounds that have antioxidant potential in these tests, are able to prevent or interrupt the chain oxidation process of lipid substrates.

Activity in both assays was observed for the compounds 5, 6, 9, 11, 13, 15, 18, 19, 20, 22, 23, 26 and 28 (Figure 1). Activity was observed only in the deoxyribose degradation assay for *trans*-caryophyllene (3), cineol (24) and precocene (26), whereas activity only in the β-carotene bleaching assay was observed for methyl-eugenol (4), carvacrol (8), limonene (12), 4-allylanisol (14), *p*- cimene (17), citronelal (21) and α-terpineol (25). The mechanism of protection of lipid substrates is still not well understood. However, this observation is in agreement with the study by Kulisic et al., (2004), which cites the protection of β-carotene in the presence of the essential oils and their different fractions or individual constituents. The authors observed that the fractions containing the aldol group was the most effective and suggested that the antioxidant activity of the oregano essential oil is probably due to the more polar constituents in the mixture. Despite this observation, according to Affonso et al., (2012), phenolic compounds inhibit lipid peroxidation by sequestering reactive oxygen species (chelating effect) and stabilizing free radicals to inhibit the propagation of the chain reaction.

Reduction power and Antioxidant capacity

The compounds and the concentrations at which they presented activity in the iron reducing power and the reduction of molybdenum assays are presented in Figure 2. The values are expressed in terms of absorbance because the greater the potential of the substance to reduce the metal ions, the greater was the absorbance because the tests are based on the color change that occurs upon reduction.

The compounds with the greatest reducing power in the Fe^{3+} reduction test were carvacrol (8), thymol (13) and eugenol (28), the last being more effective than the control itself. The presence of antioxidants causes reduction of the ferricyanide (Fe^{3+}) complex to the ferrous (Fe^{2+}) form. The Fe^{2+} concentration was monitored by the formation of Prussian blue. In the phosphomolybdate test, the most active compounds were citronellal (21) and eugenol (28), both of which exhibited greater reducing power than the control. This test is based on the fact that Mo^{6+} is reduced to Mo^{5+} in the presence of a reducing agent (antioxidant) to form a green phosphomolybdate complex. It can be seen that the reducing power of the antioxidants increases with increasing concentration in both assays. The absorbance and the antioxidant capacity of the sample were directly proportional to the concentration.

The above-mentioned results emphasize the importance of performing antioxidant tests by different methods because different methods provide different results. It can be inferred that the difference between the tests is due to

factors such as the physical structure of the system, the nature of the oxidizable substrate, and the interaction of the compounds with the reaction medium, among others. Therefore, no single method is capable of representing, in a safe and precise way, the true antioxidant activity of a substance because of the different types of radicals and the different sites of action. The behavior of the compounds and the manner in which the concentration influences the response can be analyzed separately because even those compounds that have lower antioxidant activities still exhibit a dose-response relationship. This fact can be of interest in the search for bioactive compounds and for other tests in different concentrations and in conjunction with other substances might be performed to verify the possibility of synergistic effects.

Antitumor potential

In *vitro* studies using cell lines derived from different human cancers have been widely used in cancer research to identify promising antitumor agents (Ionta et al., 2015). A total of 28 compounds, which are commonly found in essential oils, were tested on three tumor cell lines (MCF-7, A549, and HT144). The substances were initially assayed at 100 µg mL^{-1} for 48 h, and those whose application resulted in a decrease in survival of 50% or lower were considered to be active. In addition, IC_{50} values were determined for more active compounds (Figure 3; Table 1).

The estrogen-positive MCF-7 cell line is derived from breast cancer (BC) and represents the luminal A subtype, which is the most frequently diagnosed BC subtype (ref). Luminal patients are treated with hormone therapy; however, some patients are refractory to this type of therapeutic approach. Thus, the resistance to hormone therapy still represents a clinical challenge to be overcome (Osborne and Schiff, 2011; Al Fakeeh and Brezden-Masley, 2018). The substances carvacrol (7), limonene (11), thymol (12), citral (14), and eugenol (27) significantly reduced cell viability in MCF-7 cultures, carvacrol being the most active among of them (Figure 3).

A549 cells are derived from non-small-cell lung cancer (NSCLC), and harbor mutation in *KRAS* (Lewandowska et al., 2013; Li et al., 2016; Schneider et al., 2017). NSCLC patients with the *KRAS* mutation appear to be refractory to the majority of systemic therapies. Therefore, it is of great interesting for identifying substances that could contribute to improve therapeutic applications for NSCLC.[45] We observed that the substances carvacrol (7), alpha-bisabolol (9), thymol (12), and citral (14) were effective in reducing the viability of A549 cells (Figure 3), and (14) was more active than (7), (9) or the RAF/MEK/ERK pathway, and the BRAF mutation in melanoma cells is associated with increased cell proliferation (Smalley, 2003). The substances carvacrol (7), thymol (12), allylanisol (13), citral (14), and eugenol (27) significantly reduced the viability of HT144 cells, and (14) was the most potent among the tested substances (Figure 3).

Considering the cytotoxic profiles of carvacrol (7) against MCF-7 cells and that of citral (14) against A549 and HT144 cells, dose-response curves were constructed to determine their IC_{50} values (Figure 4; Table 1). The lowest IC_{50} values were observed for carvacrol against the MCF-7 cell line and citral against the HT144 cell line.

There are few studies concerning the antitumor activity of carvacrol or citral against cancer cells. Cytotoxic or antiproliferative activity, or both, of carvacrol have been described for NSCLC, triple negative breast cancer and colon cancer (Koparal and Zeytinoglu, 2003; Arunasree, 2010; Fank et al., 2015). Patel et al., (2015) reported a pro-apoptotic effect on breast cancer cells (MCF-7 and MDA MB 231) by the acyclic monoterpene citral, which consists of an isomeric mixture of geranial and neral, without disturbing normal breast epithelial cells. These findings indicate a high selectivity of citral toward tumor cells, which indicates that it is a promising antitumor agent. In this study, we decided to better evaluate the effects induced by citral on HT144 melanoma cells.

Cell cycle analysis showed that citral did not alter the cell cycle progression of HT144 cells at a concentration of 20 µg mL^{-1}. By contrast, citral at 40 µg mL^{-1} induced cell cycle arrest at G1/S transition. There was an increase of the G0/G1 population with the reduction of the S and G2/M populations in cultures treated with citral at 40 µg mL^{-1} when compared to the control groups (Figure 5; Table 2).

Disregulation of the cell cycle is a key feature of tumor cells, including melanoma, and targeting the cell cycle is an important approach in cancer therapy. Cyclin D1 is the critical activator of cyclin-dependent kinases 4 and 6 (CDK4/6), which promote the phosphorylation of retinoblastoma protein (pRb) and activate the transition from the G1 to the S phase of the cell cycle (Lapenna and Giordano, 2009). It has been reported that the reduction of the cyclin D expression levels in melanoma cells suppress cell proliferation (Zhu et al., 2018). It has also been reported that citral caused cell cycle arrest at the G1/S transition in ovarian carcinoma (OVCAR-3). However, the influence of citral on cell cycle progression of melanoma cells has been poorly explored (Kapur et al., 2016). A recent study reported only that citral might be useful in clinical applications to treat melanoma because it interferes with signaling pathways related to cell proliferation, such as the RAF/MEK/ERK signaling pathway (Sanches et al., 2017).

Our findings showed that citral represents a promising antitumor agent that acts against HT144 melanoma cells by inhibiting cell cycle progression at the G1/S transition. Further studies should be performed to identify the molecular mechanism underlying with antitumor activity of citral and its potential for clinical applications.

Material and Methods

Monoterpenes and phenylpropanoids studied

Monoterpenes and phenylpropanoides studied: α-Terpinene, 1,8-cineole, citronelal, L-limonene, L-borneol, mircene, p-anisaldehyde, p-cymene, thymol, α- pinene, α-bisabolol, citral, camphor, α-tocopherol, eugenol, geraniol, L-menthol, linalool, precocene, trans-caryophyllene, trans-farnesol, trans-anethole, α-terpineol, β- citronellol, β-pinene, metileugenol, 4-allylanisol and carvacrol, were obtained by Sigma Chemical, Fluka AG, AldrichChemical and Acros.

Antioxidant activity

The assays were performed with stock solutions containing 500 µg mL^{-1} of the synthetic monoterpene and phenylpropanoid standards and the positive controls (BHT,

Table 1. IC_{50} (µg.mL^{-1}) values determined after 48 h of treatment from resazurin assay data.

	Carvacrol (7)	Citral (14)
A549	91.17 ± 1.91	43.98 ± 1.72
MCF-7	64.47 ± 3.41	107.40 ± 3.11
HT144	115.40 ± 2.89	39.63 ± 1.61

Fig. 1

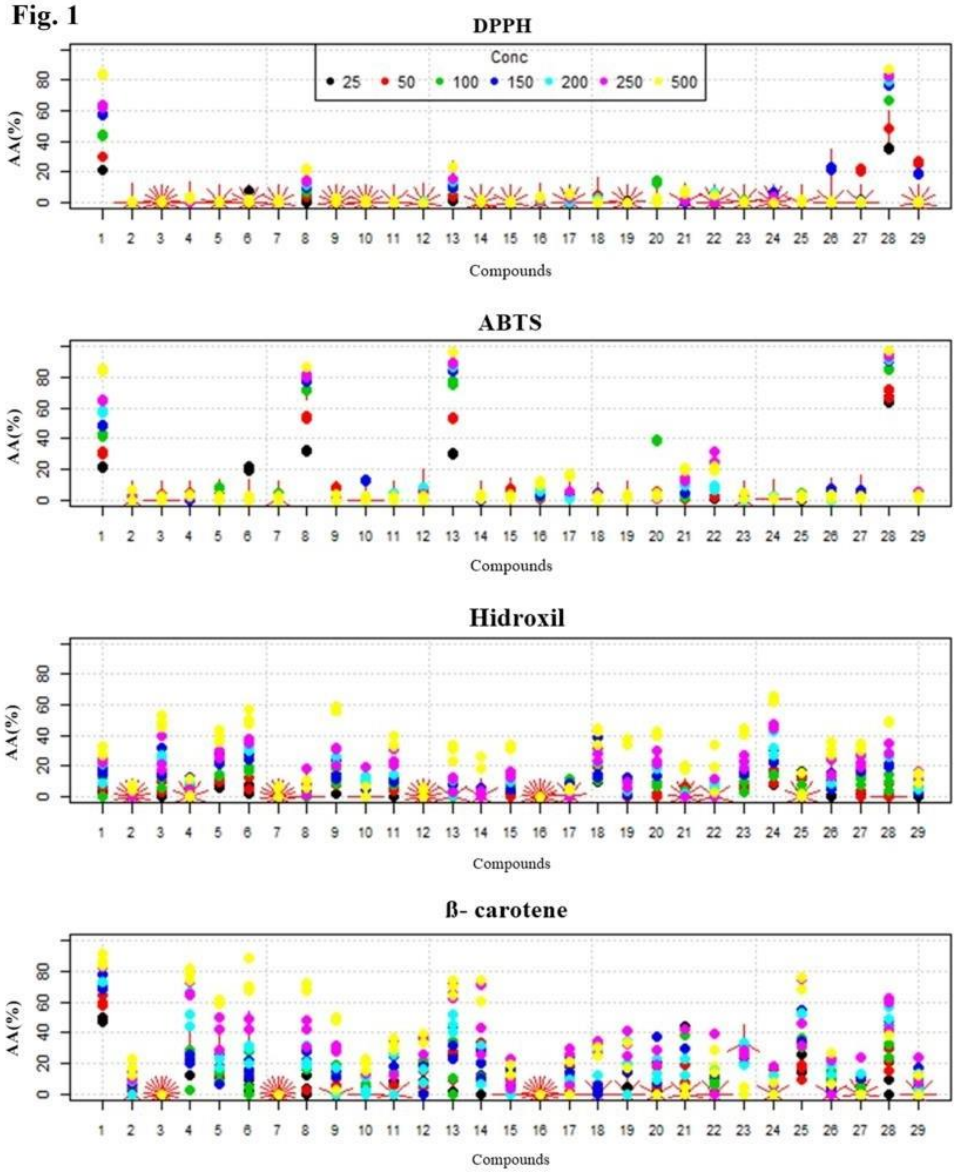

Figure 1. Absorbance values (nm^{-1}) determined by the methods involving DPPH, ABTS, Hidroxil and β-carotene, together with the respective concentrations of the compounds (µg mL^{-1}). 1 - Positive control; 2 - β-pinene; 3 - *trans*-caryophyllene; 4- methyleugenol; 5- 1,8-cineole; 6 - geraniol; 7 - α-pinene; 8 - carvacrol; 9 - borneol; 10- α-bisabolol; 11 - linalool; 12 - limonene; 13 - thymol; 14 - 4-allylanisol; 15 - citral; 16- mircene; 17 - *p*-cymene; 18 - β-citronellol; 19 - menthol; 20 - carvona; 21 - citronelal; 22 - *trans*-anethole; 23 - camphor; 24 - cineol; 25 - α-terpineol; 26 - precocene; 27- *p*-anisaldehyde; 28 - eugenol; 29 - *trans*-farnesol.

Table 2. Cell cycle analysis of HT144 cell cultures after treatment with citral (20 and 40 µg mL^{-1}) for 48 h.

	Sub-G1	G0/G1	S	G2/M
Control	1.16 ± 0.37	48.11 ± 0.61	20.93 ± 0.42	29.80 ± 0.28
20	1.47 ± 0.47	51.83 ± 1.27	19.68 ± 0.36	27.02 ± 0.36
40	0.89 ± 0.12	60.39 ± 0.74***	17.94 ± 0.88***	20.77 ± 0.36***

*** $p < 0.001$ according to ANOVA followed by Dunnett post-test.

Fig. 2

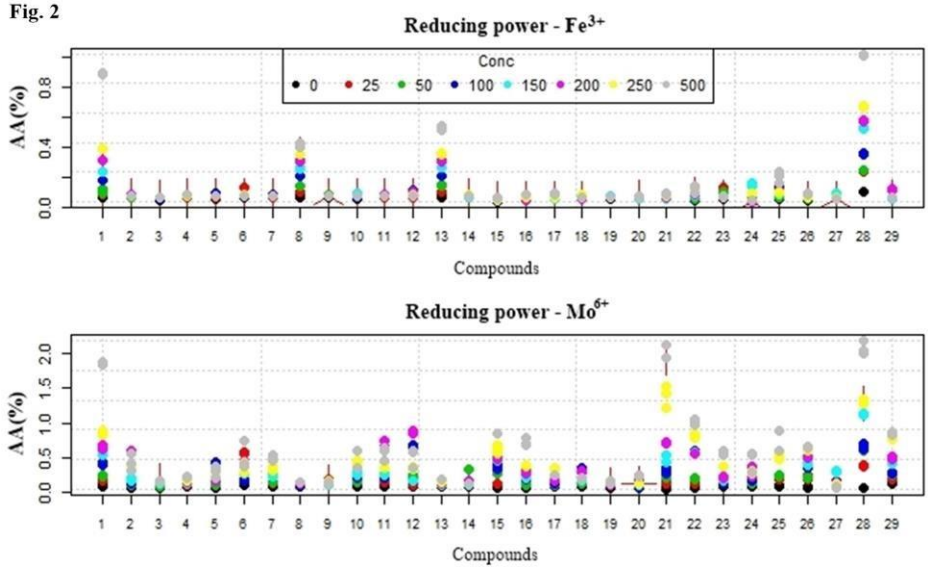

Figure 2. Absorbance values (nm^{-1}) determined by the methods involving the reduction of metal ions, together with the respective concentrations of the compounds (µg mL^{-1}). 1 - Positive control; 2 - β-pinene; 3 - *trans*-caryophyllene; 4 - methyleugenol; 5- 1,8-cineole; 6 - geraniol; 7 - α-pinene; 8 - carvacrol; 9 - borneol; 10 - α-bisabolol; 11 linalool; 12 - limonene; 13 - thymol; 14 - 4-allylanisol; 15 - citral; 16 - mircene; 17 - *p*- cymene; 18 - β-citronellol; 19 - menthol; 20 - carvona; 21 - citronelal; 22 - *trans*-anethole; 23 - camphor; 24 - cineol; 25 - α-terpineol; 26 - precocene; 27- *p*- anisaldehyde; 28 - eugenol; 29 - *trans*-farnesol.

Fig. 3

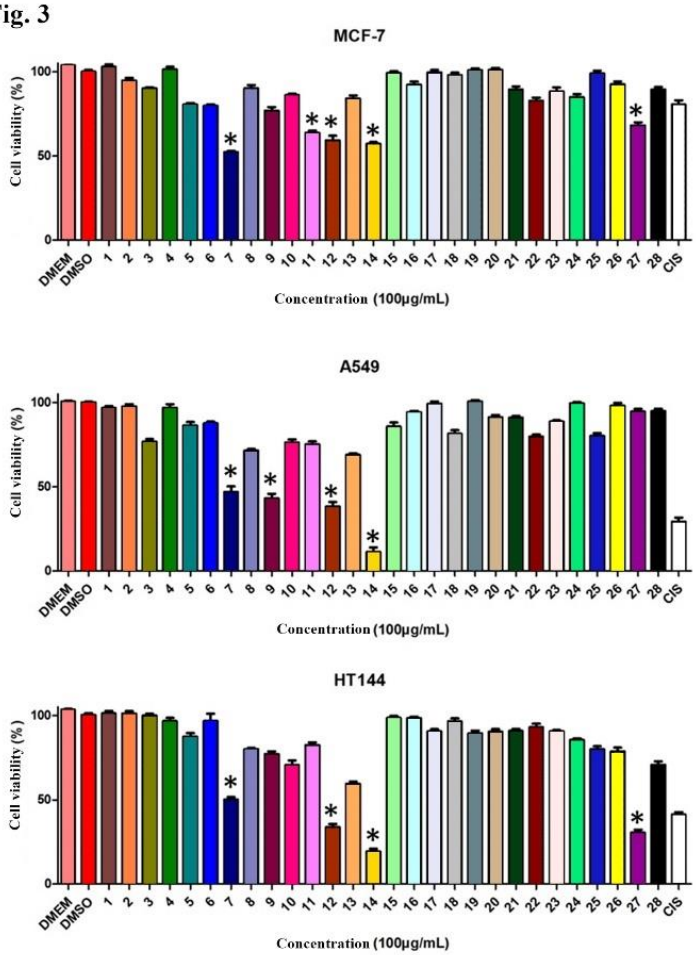

Figure 3: Cell viability (%) determined by resazurin colorimetric assay after treatment with different compounds at 100 µg mL^{-1} for 48 h DMEM - no treatment; DMSO; vehicle; 1 - β-pinene; 2 - methyleugenol; 3 - *trans*-caryophyllene; 4 - 1,8-cineole; 5 - geraniol; 6 - α-pinene; 7 - carvacrol; 8 - borneol; 9 - α-bisabolol; 10 - linalool; 11 - limonene; 12 - thymol; 13 - 4-allylanisol; 14 - citral; 15 - mircene; 16 - *p*-cymene; 17 - β-citronellol; 18 - menthol; 19 - carvone; 20 - citronelal; 21- *trans*-anethol; 22 - camphor; 23 - cineol; 24 - α-terpineol; 25 - precocene; 26 - *p*-anisaldehyde; 27 - eugenol; 28 - *trans*-farnesol; CIS – cisplatin at 6 µg.mL^{-1}.

Fig. 4

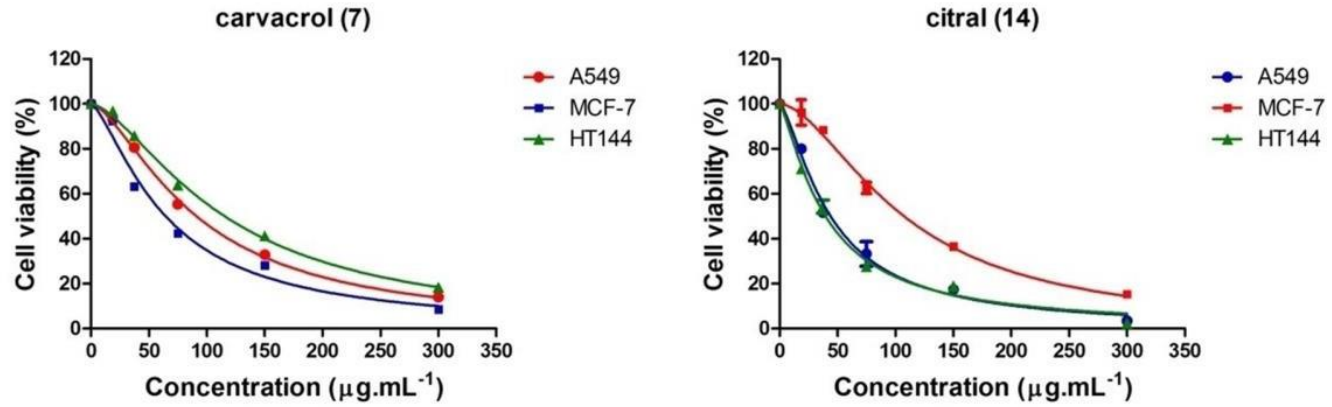

Figure 4. Dose-response curves performed after 48 h of treatment with carvacrol (7) and citral (14) in different concentrations.

Fig. 5

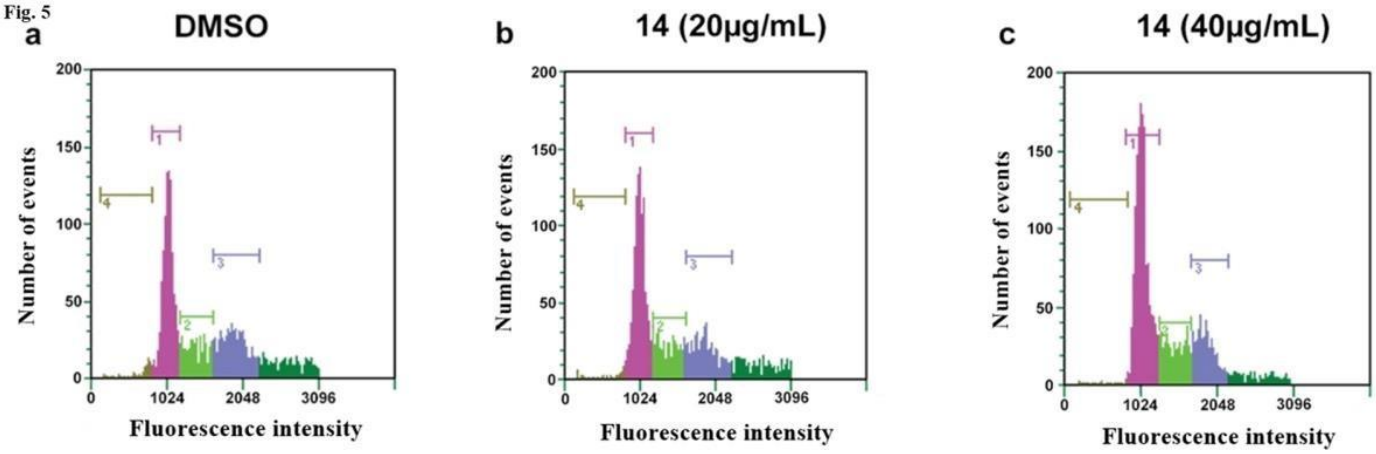

Figure 5. Illustrative histograms obtained by flow cytometry showing cell populations distributed in different phases of cell cycle. Pink (1): G0/G1 phases; green (2): S phase; blue (3): G2/M phases; brown (4): Sub-G1 population. HT144 cells were treated with citral (20 and 40 µg mL^{-1}) for 48.

mannitol and ascorbic acid) were prepared in ethanol for the antioxidant assays ($DPPH^{\bullet}$, $ABTS^{\bullet+}$, reducing power, antioxidant capacity and β-carotene bleaching assays) and in water (desoxyrribose degradation assays). The samples were evaluated at the concentrations of 25, 50, 100, 150, 200, 250 and 500 µg mL^{-1}. The readings were performed in triplicate in all the antioxidant assays.

DPPH$^{\bullet}$ radical scavenging activity

The evaluation of the antioxidant activity by means of the sequestration of the 2,2-diphenyl-1-picryl-hydrazyl free radical ($DPPH^{\bullet}$) was performed in accordance with the methods employed by Lopes-Lutz et al., (2008) and Guimarães et al., (2011).

ABTS$^{\bullet+}$ radical scavenging activity

The determination of the antioxidant activity by the $ABTS^{\bullet+}$ [2,2'-azinobis- (3-ethylbenzothiazoline-6-sulfonic acid)] method was performed according to the method described by Rezende et al., (2017).

Desoxyrribose degradation assay

The assay involving the capture of the hydroxyl radical was conducted as reported by Boulanouar et al., (2013).

β-Carotene bleaching assay

The evaluation of the antioxidant activity through the inhibition of the oxidation of the β-carotene/linoleic acid system was performed according to the modified method of Kulisic et al., (2004).

Reducing power assay

The determination of the reducing power of the samples was performed according to a method described by Santos et al., (2007).

Antioxidant Capacity

The antioxidant capacity was determined by the molybdenum reduction method using the procedure described by Prieto et al., (1999). Initially, 100 µL of the ethanolic solutions of the samples were added to test tubes, followed by 2000 µL of the solution of the ammonium phosphomolybdate complex formed from 10% sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate. The tubes were incubated at 95 °C for 60 minutes. After cooling the tubes, the spectrophotometer was read at 695 nm, and the results were expressed as a function of the standard curve for ascorbic acid.

Investigation of in vitro antitumor activity

The tests were performed on three cell lines derived from human tumors: breast adenocarcinoma (MCF-7), melanoma

(HT144) and lung adenocarcinoma (A549). Cell cultures were maintained in DMEM (Dulbecco's Modified Eagle's Medium, Sigma, CA, USA) supplemented with 10% fetal bovine serum (Vitrocell, Campinas, Brazil). The cells were cultured in a humidified incubator containing 5% CO_2 at 37 °C. The compounds were dissolved in DMSO (stock solution at 100 mg mL^{-1}) and stored at 4 °C. Dilutions were performed in culture medium immediately before treatment. Untreated cells were also used as a negative control, and cells treated only with DMSO, ensuring that this compound did not have sufficient action on tumor cells.

Cell viability analysis

The tumor cells were seeded in 96-well plates ($1x10^4$ cells/well). After adherence, the samples were treated for 48 h with the constituents of the essential oils at the concentration of 100 µg mL^{-1} to screen substances with greater activity (Ferreira-Silva et al., 2017). Dose-response curves were constructed using selected substances at different concentrations (18.75, 37.5, 75, 150 and 300 µg mL^{-1}) over a 48-h period. The resazurin reduction assay was executed according to the manufacturer's instructions (Sigma-Aldrich) to assess relative cell viability. The resazurin (blue absortion peak at 600 nm) is reduced to resofurin (pink, absortion peak at 570 nm) by metabolically active cells (Rampersad, 2012). Cell viability was directly proportional to the resazurin reduction rate. The experiments were conducted in triplicate, and the data were presented as means ± standard deviation. IC_{50} values were obtained from regression curves using the GraphPad® Software. Cisplatin (6 µg mL^{-1}), a potent cytotoxic drug, was used as a positive control.

Quantification of DNA for analysis of cell cycle progression

The cells were seeded in 35-mm plates, and, after treatment for 48 h, the cells were collected by enzymatic digestion (Trypsin-EDTA solution, Sigma Aldrich LTDA, Brazil) and transferred to Falcon tubes. The cell pellet was obtained by centrifugation (5 min at 1000 rpm), and the cells were fixed with ice-cold ethanol (75% in PBSA- buffered saline phosphate) for 30 minutes. After further centrifugation, the cells were stained in a solution containing PBSA, RNAse (3 mg mL^{-1}) and propidium iodide (30 µg mL^{-1}) (Guava Technologies-Merck Millipore). Samples were analyzed in the flow cytometer (Guava Mini EasyCyte, 8HT) using GuavaSoft 2.7 software.

Statistical analysis

The dose-response relationship between the concentration of the compounds and the antioxidant activity (% AA) was analyzed by univariate linear regression (linear model), using the Rstudio®, version 4.0.2 statistical software. The results obtained for antitumor activity were analyzed via analysis of variance (ANOVA) and the Dunnett test ($p < 0.01$) using the Prism software (Prism 5.00, GraphPad Software, Inc).

Conclusion

A greater antioxidant potential was observed for the phenolic compounds carvacrol, thymol and eugenol than for the other standards compounds with the methods tested. A dose-response effect was observed for the less active compounds, revealing the influence of the concentration of the constituents on the activity. Carvacrol displayed an important cytotoxic activity against the estrogen-positive MCF-7 cell line, and citral was more active on NSCLA549 cells and HT144 melanoma cells. The antitumor activity of citral observed on HT144 cells is associated with its ability of promoting the cell cycle at the G1/S transition, at least in part.

Conflict of interest

The authors declare that no conflict of interests exists.

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