Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.64921

Antioxidant and antifungal properties of essential oils of oregano (*Origanum vulgare*) and mint (*Mentha arvensis*) against *Aspergillus flavus and Penicillium commune* for use in food preservation

Priscilla de ALMEIDA[1], Núria BLANCO-PASCUAL[1], Daiane ROSOLEN[2], Julia CISILOTTO[2], Tania CRECZYNSKI-PASA[2], João LAURINDO[1]*

Abstract

This study determined the chemical composition and evaluated the antioxidant activity and antifungal properties of essential oils of oregano (*Origanum vulgare*) and mint (*Mentha arvensis*) against the fungi *Aspergillus flavus* and *Penicillium commune* as possible alternatives for food preservation. The antimicrobial activity of both oils is shown by their minimum inhibitory concentration (4 mg/mL) for oregano oil, and (8 mg/mL) for mint oil, and minimum inhibitory dose (< 110 µl/L) for oregano oil, and (< 1500 µL/L) for mint oil. In addition, both oils presented antioxidant activity superior to 70% at the concentrations of 0.5 mg/mL for oregano oil and 30 mg/mL for mint oil after 360 min of reaction. As a control, the oils were evaluated for their cytotoxic potential using cells in culture and the method based on mitochondrial activity. Both oils were cytotoxic to both cell lines tested, with cells' survival rates less than 20% when in contact with 25 µg/mL of oils concentrations. Overall, the essential oils have activity against *Aspergillus flavus* and *Penicillium commune,* and their volatile components expressed high antifungal activity that expands their use for situations in which direct contact with the liquid is undesired. However, both essential oils showed high cytotoxicity.

Keywords: essential oils; oregano; mint; antimicrobial activity; antioxidant; cytotoxicity.

Practical Application: antioxidants and antifungal additives for food preservation. Their correct use will help reduce synthetic additives, as demanded by most consumers. On the other hand, this use of the essential oils of oregano and mint can increase the agricultural production of these plants and their processing to extract their essential oils.

1. Introduction

Synthetic additives commonly used in food conservation have been essential in large-scale production. However, some substances' daily intake has been a concern to researchers and government agencies. Consumers are also concerned about the excessive use of synthetic preservatives and demand new natural preservation methods (Polônio & Peres, 2009; Ayala-Zavala et al., 2008). This concern has driven the food industry to create 'green' policies.

The consumer demand for natural, green, and preservative-free foods, along with tightened inspection legislation regarding current synthetic or chemical preservatives, has challenged the food industry to increase research into incorporating "naturally derived" antimicrobials into food packaging. As a result, recent years have witnessed an enormous increase in the number of studies focusing on utilizing essential oils (Zhang et al., 2021), whose properties such as antioxidants, antimicrobial (Yuan et al., 2016), and eco-friendly food preservatives (Falleh et al., 2020) have been reported. Furthermore, as an essential source of natural preservatives, it has been shown that essential oils have broad-spectrum antibacterial activity, further extending their application prospects (Ju et al., 2018a,b).

Products from herbs, such as essential oils, represent a feasible alternative to synthetic additives (Belletti et al., 2004; Ju et al., 2018a). Scientific reports indicate that potentially bioactive compounds present in herbs, spices, and derivatives have antimicrobial, antioxidant, and anticancer activities (Kaefer & Milner, 2008; Yuan et al., 2016). Essential oils, also called volatile or ethereal oils, are natural volatile compounds resulting from aromatic plants' secondary metabolism (Burt, 2004). They are complex mixtures characterized by two or three main compounds in relatively high concentrations (20-70%) (Bakkali et al., 2008). These oils' compositions are strongly related to the origin of the raw material, the development stage, and the plant's specific part for their extraction, growing conditions, and extraction process (Kalemba & Kunicka, 2003; Simões & Spitzer, 2003). Although the natural variability, essential oils contain 85-95% volatiles and 15% non-volatiles (Bakkali et al., 2008).

The chemical structures and the concentrations of the components present in their compositions explain the essential oils' properties (Fisher & Phillips, 2008). Some of the oil's components have shown a high antimicrobial activity and have been considered actual inhibitors of pathogenic microorganisms

Received: 24 Aug., 2021
Accepted: 19 Dec., 2021
[1]*Departamento de Engenharia Química e Engenharia de Alimentos, Universidade Federal de Santa Catarina – UFSC, Florianópolis, SC, Brasil*
[2]*Departamento de Ciências Farmacêuticas, Universidade Federal de Santa Catarina – UFSC, Florianópolis, SC, Brasil*
*Corresponding author: jb.laurindo@ufsc.br

in foods and humans (Burt, 2004; Bakkali et al., 2008; Falleh et al., 2020). As the essential oils are volatiles, their potential use is in active packaging, whose free space atmosphere will contain the oil vapor. According to Zhang et al. (2021), antimicrobial activity is closely related to the ability of essential oil-active components to be released and volatilized into the atmosphere.

Regarding fungi, the growing demand for natural antifungals results from recognizing the importance of fungal infections and difficulties to control. The development of resistance to antifungal agents, the significant drug interactions, and insufficient bioavailability of conventional antifungals have also been considered (Cavaleiro et al., 2006). Some fungi species are common food contaminants or pathogens that belong to the genus *Aspergillus* ssp. and *Penicillium* ssp. Industrially, *Aspergillus* species are applied for enzyme and organic acid production and food fermentation, but they are also frequently isolated as food contaminants. Pathogenic species are the most harmful because of their respective toxigenic secondary metabolites (Scheidegger & Payne, 2003). *Aspergillus flavus* is an aerobic fungus species that contaminate grains and other foods, producing aflatoxin B1, a carcinogenic and highly toxic mycotoxin (Espinel-Ingroff et al., 2005). Some *Penicillium* species can be used in biocontrol situations or as sources of industrial enzymes and new drugs for the pharmaceutical industry. However, further studies on food contamination are necessary because the *Penicillium* genre is among the most critical food spoilage causes, producing mycotoxins. For instance, *Penicillium commune's* species is the ancestor of *Penicillium camembert* and a producer of the mycotoxin cyclopiazonic acid (CPA) (Pitt et al., 1986).

Essential oils have been considered a good alternative for food preservation, even for fungal contamination control (Burt, 2004; Bakkali et al., 2008; Yuan et al., 2016). The consumption of natural products is based on the argument that plants and their respective derivatives are safe. Nevertheless, some plant derivatives contain potentially hazardous compounds with toxicological risks (Veiga Junior et al., 2005). The literature has linked the degree of toxicity of plant extracts to the dose and administration frequency. In some cases, even low dosages cause poisoning because of individual sensitivity (De Vincenzi et al., 2004; Veiga Junior et al., 2005). Overall, essential oils are very concentrated and have higher toxicity than the original plant, hence the need for toxicological studies to support these products' safe use (Simões & Spitzer, 2003; Veiga Junior et al., 2005). Among the several aromatic plants with antimicrobial activity, those of the family Lamiaceae, such as *Origanum vulgare* (oregano) and *Mentha arvensis* (mint), has a particular interest in food preservation. The high antimicrobial activity of the essential oil of oregano has been recognized and linked to many monoterpenes present in its composition (Lambert et al., 2001). On the other hand, *Mentha arvensis* contains 90% mint oil divided into monoterpenes, sesquiterpenes, flavonoids, phenolic acids, among others (Liest, 1998). Some Mentha species' essential oils, including *Mentha arvensis,* are potential candidates for antimicrobial, antioxidant, radical-scavenging, and anticarcinogenic activities. Such multiple biological activities might be ascribed to the presence of some chemical components, such as menthol, menthone, camphor, and linalool, among others (Gulluce et al., 2007; Pandey et al., 2003).

The objective of this study was the chemical characterization, including Gas Chromatography coupled to Mass Spectrometry (GC-MS) and Gas Chromatography with Flame Ionization Detector (GC-FID), and evaluation of biological effects by the antioxidant potential and antifungal properties of the essential oils of oregano (*Origanum vulgare*) and mint (*Mentha arvensis*) against *Aspergillus flavus* and *Penicillium commune* fungi, as well as evaluation of the oils' cytotoxicity in two cell lines.

2. Materials and methods

2.1 Materials

Essential oils of oregano (*Origanum vulgare*) and mint (*Mentha arvensis*) were purchased from Ferquima Company (São Paulo, Brazil). The oregano oil was from the Republic of Moldova (Eastern Europe), while the mint oil came from China. Both oils were extracted by steam distillation of the plants' leaves. According to the company report, the concentration of carvacrol in the essential oregano oil was about 71%, while menthol was present in the concentration of 50% in the mint essential oil.

2.2 Chemical composition

The chemical compositions of oregano and mint essential oils were determined by Gas Chromatography coupled to Mass Spectrometry (GC-MS) and Gas Chromatography with Flame Ionization Detector (GC-FID). GC-MS analysis was carried out with a QP2010 Plus Shimadzu gas chromatograph equipped with an RTx-5MS capillary column (30 m × 0.25 mm × 0.25 μm). Helium gas (He) was used as carrier gas at a constant flow rate of 1 mL min^{-1}. The initial oven temperature was set at 60 °C (held for 5 min), raised to 240 °C at 3 °C $min^{-1,}$ and kept for 5 min. Diluted samples (1/100 v/v, in ethyl ether) of 1 μL were injected at 250 °C. The constituents' identification was made by comparing the recorded mass spectral fragmentation patterns with data published in the literature and reference spectra in the computer libraries (NIST - National Institute of Standards and Technology - 2008). The GC-FID analysis was performed at a Shimadzu GC-FID 2010 chromatograph equipped with an OV-5 capillary column (30 m × 0.25 mm × 0.25 μm) under the same conditions of GC-MS analysis. Quantification was computed by the electronic integration of the FID peak areas.

2.3 Antimicrobial activity

The antimicrobial activity of oregano and mint essential oils was determined by the minimum inhibitory concentration (MIC) and the minimum inhibitory dose (MID), according to NCCLS (National Committee Clinical Laboratory Standards, 2002) and López et al. (2005) methods, respectively. Pure and active cultures of *Aspergillus flavus* and *Penicillium commune* were used to perform the evaluation. The strains were purchased from the Tropical Research and Technology Foundation André Tosello (Campinas – SP, Brazil).

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentrations (MIC) of the essential oils of oregano and mint were assessed by the microdilution broth method, based on M38-A standard (National Committee Clinical Laboratory Standards, 2002), with modifications. The 96-well microtiter plates containing the samples and controls were composed of Potato Dextrose Broth (PDB) (Difco™ - USA), oregano and mint essential oils dilutions, and the inoculum suspension of *Aspergillus flavus* and *Penicillium commune* (10^4 CFU/well). The oils were diluted in DMSO, and the tested concentrations ranged from 0.1 µg/mL - 4.0 mg/mL to oregano oil and 0.1 µg/mL - 8.0 mg/mL to mint oil. The oregano oil tested concentrations were specifically 0.1 µg/mL; 0.5 µg/mL; 1.0 µg/mL; 5.0 µg/mL; 10 µg/mL; 25 µg/mL; 50 µg/mL; 100 µg/mL ; 200 µg/mL; 300 µg/mL; 400 µg/mL; 500 µg/mL; 750 µg/mL; 1.0 mg/mL; 2.0 mg/mL; 3.0 mg/mL and 4.0 mg/mL. The mint oil tested concentrations were the same, besides 5.0 mg/mL; 6.0 mg/mL; 7.0 mg/mL and 8.0 mg/mL. The microplates were incubated at 28 °C in a BOD incubator chamber (ET-371 Tecnal - SP - Brazil) for 24, 48, and 72 hours. A plate reader (BioTek EL 800 - USA) at a wavelength of 660 nm was used to determine the absorbances. The MIC was considered the lowest concentration able to inhibit 100% of the microorganism's growth. The minimum concentration capable of inhibiting 50% (IC_{50}), 70% (IC_{70}), and 90% (IC_{90}) of the expected growths were calculated using the GraphPad Prism 5 software (La Jolla, CA, USA).

Determination of Minimum Inhibitory Dose (MID)

The disk volatilization test assessed the minimum inhibitory doses (MID) of the essential oils of oregano and mint (López et al., 2005). MID was considered the concentration in µL oil/L headspace (microliters of essential oil per volume unit of air above the microorganisms growing on the agar surface) to inhibit the fungal growth completely.

Sterile Potato Dextrose Agar (PDA) (Difco™ - USA) culture medium was increased by 0.1% of a 5% (w/v) Rose Bengal solution and added to sterile Petri dishes (90 mm). The solidified medium was inoculated with 100 µL of the inoculum suspension of *Aspergillus flavus* and *Penicillium commune* (10^6 CFU/mL, approximately). Each pure essential oil was diluted in ethyl acetate and added to sterile blank filter disks placed on the Petri dishes' cover. The Petri dishes were sealed with sealing film (Parafilm M - WI, USA) and incubated in a BOD incubator chamber (TE 391-1 Tecnal – SP - Brazil) at 30 °C for 96 hours. According to the literature and considering fungi growth time, a time of incubation of 48 hs minimum is recommended. Blanks were prepared by adding ethyl acetate to the filter disks. After the incubation period, the MID that caused apparent growth inhibition by comparison with the control was measured.

Following these measurements, the dishes were incubated for a further 21 days under the same temperature. Unchanged Petri dishes (without removing the antimicrobial atmosphere generated) were followed to check whether the protective effects were temporary or prolonged. Besides, the antimicrobial atmosphere was broken for other Petri dishes by removing the filter disk to check whether the antimicrobial effects were static or cidal. If microorganisms start to grow after removal, there is a static effect, whereas if no growth occurs, the effect is cidal. The plates were checked every 7 days.

2.4 Antioxidant activity

The antioxidant activity of the oregano and mint essential oils was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl), as described by Brand-Williams et al. (1995). The traditional method has been adapted to 96-well microplates. The essential oils were tested in concentrations ranging from 0.05 to 10 mg/mL to oregano oil and 5 to 500 mg/mL for mint oil. An ethanolic solution of DPPH was added to the samples, and the solutions were gently mixed and incubated in the dark for 30, 180, and 360 min at room temperature. The absorbance readings were performed in a microplate reader (Infinite M200 TECAN - Switzerland) at 517 nm. The antioxidant activities of the compounds (DDPH scavenging activity (%) or AA (%)) were expressed according to Equation 1, in which Abs_{Am} is the absorbance of the sample and Abs_{Cr} is the absorbance of the control.

$$AA(\%) = \frac{(Abs_{Cr} - Abs_{Am})}{Abs_{Cr}} \cdot 100 \quad (1)$$

The IC_{50} values, defined as the amount of essential oil required to reduce the initial concentration of DPPH to 50%, were calculated with the GraphPad Prism 5 software (La Jolla, CA, USA).

2.5 Cytotoxicity

Cell viability assays assessed the cytotoxicity of oregano and mint essential oils. Human melanocytes and murine fibroblasts were selected according to the available cells and the study's objectives.

Cell culture

Murine fibroblast (NIH-3T3) and human melanocyte (NGM) cells were obtained from the Rio de Janeiro Cell Bank (Rio de Janeiro-RJ, Brazil). The NIH-3T3 cells were cultured in DMEM (Dulbecco's Modified Eagle's medium) supplemented with 10% of fetal bovine serum, 100 U/mL of penicillin, 100 µg/mL of streptomycin, and 10 mM of HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid). The NGM cells were cultured in HAM-F12/DMEM (1:1) supplemented with 20% of fetal bovine serum, 100 U/mL of penicillin, 100 µg/mL of streptomycin, 10 mM of HEPES, 1.4 µM of hydrocortisone, 1 nM of triiodothyronine, 10 µg/mL of insulin, 10 µg/mL of transferring and 10 ng/mL of epidermal growth factor (EGF). Cells were maintained at 37 °C in a 5% CO_2 humidified atmosphere and pH 7.4. Cell stocks were maintained in DMEM with 10% of dimethyl sulfoxide (DMSO) at -180 °C in a liquid nitrogen reservoir.

Cell viability

The cytotoxicity of oregano and mint essential oils was evaluated by the colorimetric MTT (3- (4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide) method. MTT is a tetrazolium salt, reduced by metabolically active cells (Mosmann,

1983). Briefly, the oregano and mint essential oils were previously dissolved in DMSO in concentrations ranging from 0.1 to 200 μg/mL. The cells NIH-3T3 and NGM were seeded in 96-well microplates and incubated with the oil samples for 24, 48, and 72 h. The supernatant was replaced by the MTT solution (5 mg/mL), and the plates were incubated at 37 °C for 2 h. DMSO then replaced the supernatant to dissolve the formazan crystals. The absorbance was read on a microplate reader (BIOTEK EL800) using a 540 nm wavelength. The concentration required to reduce the cell number by 50% (IC_{50}) was calculated using the GraphPad Prism 5 software (La Jolla, CA, USA).

2.6 Statistical analysis

The statistical analysis was performed by analyzing variance (ANOVA) and Tukey's multiple comparison test with the significance of the difference set at $P \leq 0.05$, using Statistica 8 software (STATSOFT, Inc., USA).

3. Results and discussion

3.1 Chemical composition

The chemical composition of the essential oils of oregano and mint are presented in Tables 1 and 2, respectively. The oregano oil composition analysis identified and quantified more than 20 compounds, amounting to 98.5% and 94.9% of the total oil components obtained by GC-FID and GC-MS analysis, respectively. Despite differences in relative concentrations determined by both analytical techniques, Carvacrol was the main compound. Carvacrol is presented in Figure 1A as a phenolic monoterpene, common in essential oils of different oregano species. The antimicrobial activity of oregano oil is related to carvacrol's mechanism, which produces harmful effects on the microbial cell membrane (Lambert et al., 2001). With a hydroxyl group attached to the phenolic ring, its structure is probably responsible for the component's activity (Dorman & Deans, 2000).

The mint oil composition analysis allowed the identification and quantification of 15 compounds, amounting to 99.2% and 96.7% of total oil components, obtained by GC-FID and GC-MS analysis, respectively. The antibacterial, antiviral, and antifungal activities of the essential oil of *Mentha arvensis* are associated with the majority compounds menthol, menthone, and isomenthone, mainly (Singh et al., 2011). The major compound, menthol, presented in Figure 1B, is cyclic terpene alcohol of high volatility and partially soluble in water (Soottitantawat et al., 2005). Widely used in food and pharmaceutical industries, menthol is considered the main antifungal compound of mint species (Pandey et al., 2003). The menthone exists as two isomers: menthone and isomenthone, the second and third main compounds.

Table 1. Composition of the essential oil of oregano (*Origanum vulgare*), retention time (min) and relative concentration (%).

Compound	Retention time (min)	Relative concentration (%) (GC-ME)	Relative concentration (%) (GC-FID)
α-tujeno	6.67	0.40	0.19
α-pinene	6.93	0.69	0.33
canfeno	7.49	0.33	0.17
β-pinene	8.62	1.73	1.00
myrcene	9.25	0.70	0.42
α-phellandrene	9.81	0.38	0.14
α-terpinene	10.36	1.28	0.47
o-cymene	10.73	5.55	3.82
limonene	10.91	1.75	0.78
1,8 cineole	11.01	1.84	0.85
γ-terpinene	12.33	5.25	3.60
linalool	14.31	3.21	1.93
camphor	16.34	1.53	0.38
borneol	17.40	2.13	0.88
4-terpineol	17.96	1.68	0.67
α-terpineol	18.63	0.51	0.15
thymol	23.50	5.66	2.32
carvacrol	24.22	53.76	76.10
β-caryophyllene	28.83	6.32	3.83
β-bisabolene	32.53	0.24	0.10
caryophyllene oxide	35.43	0.85	0.35

Table 2. Composition of the essential oil of mint (*Mentha arvensis*), retention time (min), and relative concentration (%).

Compound	Retention time (min)	Relative concentration (%) (GC-ME)	Relative concentration (%) (GC-FID)
α-pinene	6.93	2.67	1.85
sabinene	8.49	0.73	0.36
β-pinene	8.62	2.38	1.63
3-octanol	9.47	0.82	0.44
limonene	10.92	4.98	4.25
isopulegol	16.48	3.46	5.33
menthone	16.98	18.18	18.56
isomentona	17.45	15.47	16.25
menthol	18.09	35.15	44.65
isomenthol	18.36	2.03	0.53
α-terpineol	18.67	0.97	0.06
pulegone	20.89	2.46	1.54
menthyl acetate	23.43	5.59	3.44
α-ylangene	26.75	0.58	0.19
α-copaene	27.03	1.24	0.10

3.2 Antimicrobial activity

Determination of the Minimum Inhibitory Concentration (MIC)

Figure 2 presents the concentration-response of each essential oil in the inhibition of fungal growth. According to the preliminary tests results and the limitations and requirements of the reference method (microdilution broth method) used, both essential oils were tested in different concentrations for 24 hs of incubation. Considering the MIC as the lowest concentration capable of inhibiting 100% of the fungal growth, the MIC values found for the oregano oil were 1 mg/mL for *A. flavus* and 4 mg/mL for *P. commune* (Figure 2A). The MIC values were

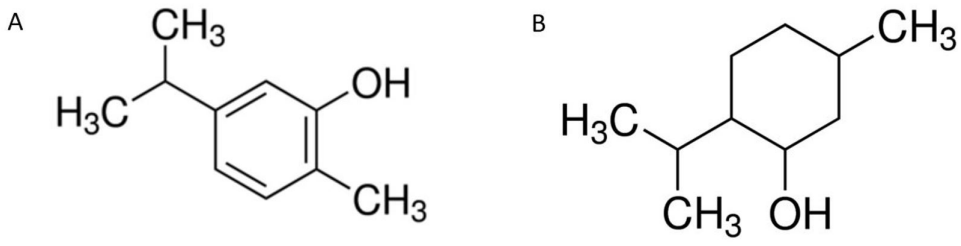

Figure 1. Chemical structure of carvacrol (A) and menthol (B).

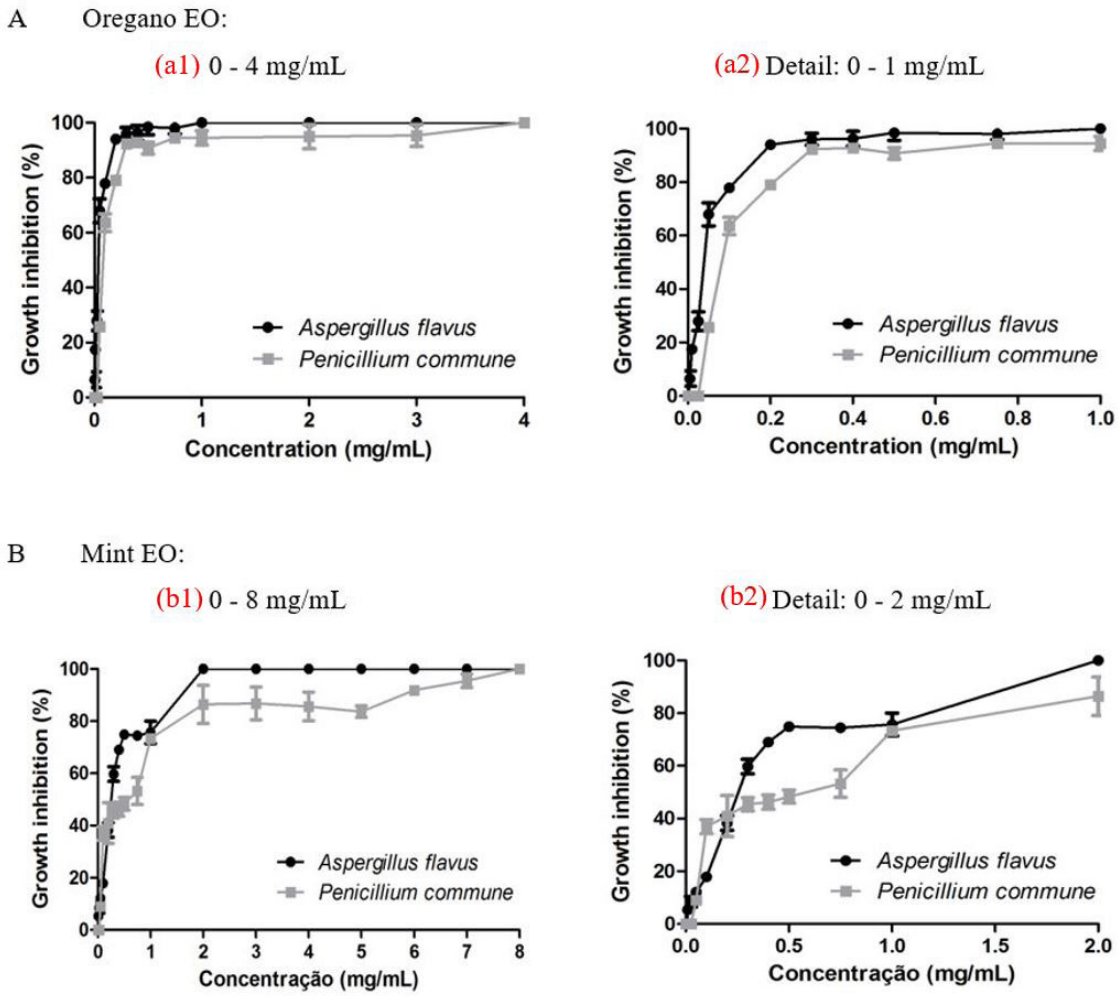

Figure 2. Growth inhibition (%) of *Aspergillus flavus* and *Penicillium commune* by essential oils (EO) of oregano (A) and mint (B).

two-fold higher for mint oil, 2 mg/mL for *A. flavus,* and 8 mg/mL for *P. commune* (Figure 2B). With a higher MIC we concluded that *P. commune* is more resistant to the effects of the essential oils than the *A. flavus*. The inhibition profile of the microbial growth is shown in Figures a1 and b1. The results demonstrate that 1 mg/ml of oregano oil concentration inhibited 100% of the *A. flavus* growth and 94.5% of the *P. commune* growth. Likewise, 2 mg/mL of mint oil inhibited 100% of the *A. flavus* growth and 86.4% of the *P. commune* growth. These results confirm that oregano oil is more effective against fungal growth than mint oil. According to Kocić-Tanackov et al. (2012), numerous studies have shown that the antifungal activity of the oregano's plant, extracts, and essential oils active molecules is considered potent inhibitors of mold growth and mycotoxin biosynthesis.

Table 3 shows the IC_{50}, $IC_{70,}$ and IC_{90} values for oregano and mint essential oils. These results corroborate the higher antifungal activity of the oregano oil.

Some compounds' action mechanisms are determined by the morphological changes caused in the treated cells (de Billerberk et al., 2001; Rasooli & Abyaneh, 2004). The amphiphilic character of certain compounds allows the molecules to migrate through an aqueous extracellular environment, causing damage to microbial lipid membranes, whose barrier functions are damaged (Turina et al., 2006; Ju et al., 2018b; Khaneghah et al., 2018; Guo et al., 2020). The lipophilic compounds accumulate in the cell membrane and affect its physicochemical properties and operation (Weber & De Bont, 1996; Salehi et al., 2020).

Results show that oregano and mint essential oils achieved 100% fungi inhibition growth, depending on the concentration. In their study, Sánchez-González et al. (2010) showed that chitosan-bergamot essential oil films significantly inhibited the growth of *Penicillium italicum* according to the essential oil concentration. The antimicrobial mechanism of essential oils depends mainly on the type and concentration of their chemicals. Different chemical components can act through different mechanisms, and the same chemical composition may also have different effects when applied to different types of microorganisms because the composition and thickness of the cell membranes of different microbial species are different (Ju et al., 2019). Despite that, regarding future applications, analyses of different concentrations' effects should be time-tested to use essential oils in lower concentrations.

The dilution methods used to determine MIC are of frequent use, but the expression of the results of antimicrobial tests of natural products does not follow a pattern (Ostrosky et al., 2008). These results are influenced by the culture medium composition, pH, inoculum size, time and temperature of incubation (Barchiesi et al., 1993), the method of analysis, microorganisms, and selected samples. In terms of essential oils, physical and chemical properties such as solubility and volatility have a considerable effect on these compounds' in vitro antimicrobial activity (Dorman & Deans, 2000; Inouye et al., 2001). This fact justifies the importance of evaluating the antimicrobial properties of essential oils by both methods (MIC and MID).

Determination of Minimum Inhibitory Dose (MID)

The MID of oregano and mint essential oils was considered the lowest concentration of volatile compounds in the headspace capable of completely inhibiting the fungal growth after 96 h of incubation. Figure 3 presents the plating result to determine MID (presence or absence of fungal growth), and Table 4 presents the MID values for oregano and mint essential oils. These results have shown a potent antimicrobial activity of the volatile compounds of the essential oils. The MID analysis results suggested that *A. flavus* is more resistant to the effect of the oils' volatile compounds than *P. commune*. Again, the oregano oil compounds showed a higher inhibitory effect, as observed in the MIC analysis.

Kloucek et al. (2012) evaluated the antimicrobial activity of the volatile compounds of the essential oils of *Origanum vulgare* and

Table 3. ICs values (μg/mL) of the oregano and mint essential oils for *Aspergillus flavus* and *Penicillium commune*.

Oregano oil			
IC (μg/mL)	IC_{50}	IC_{70}	IC_{90}
A. flavus	35.7 ± 3.8[a]	77.5 ± 2.6[a]	235.7 ± 1.9[a]
P.commune	81.6 ± 2.7[b]	175.8 ± 2.1[b]	523.0 ± 2.0[b]
Mint oil			
IC (μg/mL)	IC_{50}	IC_{70}	IC_{90}
A. flavus	261.2 ± 2.8[a]	535.8 ± 1.2[a]	1305.3 ± 0.5[a]
P.commune	399.9 ± 2.7[b]	972.6 ± 0.1[b]	4776.9 ± 3.1[b]

Different letters in the same column indicate significant differences ($p < 0.05$).

Table 4. MID values (μL/L) for oregano and mint essential oils against *Aspergillus flavus* and *Penicillium commune*.

Microorganism	MID (μL/L)	
	Oregano oil	Mint oil
Aspergillus flavus	107.3	1418.0
Penicillium commune	53.7	1134.4

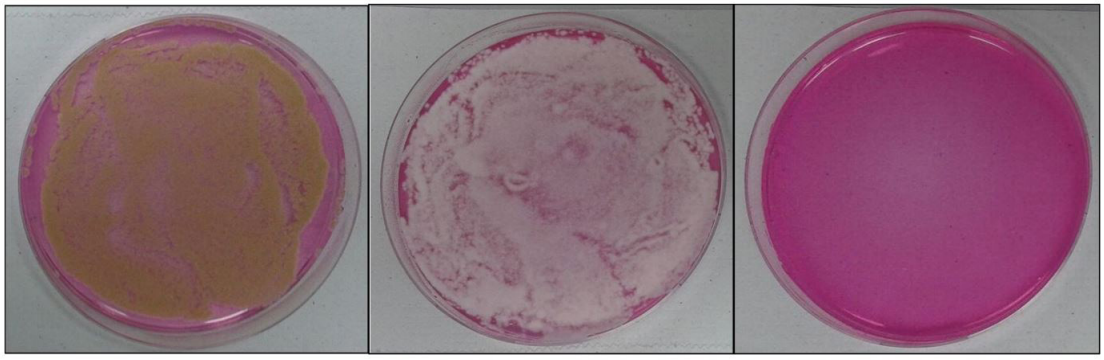

Figure 3. Visual evaluation of MID of essential oils of oregano and mint against *A. flavus* and *P. commune* fungi.

Mentha spicata against *Aspergillus niger* and *Penicillium digitatum*. They also reported that the oregano oil showed better potential for inhibiting fungal growth than the mint oil. However, the *Penicillium* appeared more resistant to the treatment with the volatiles than the *Aspergillus*, unlike the present study.

The mechanism of action of the essential oils' volatile compounds is related to the induced effects on the fungal life cycle's different stages. Dao & Dantigny (2011) studied ethanol's effect on germination and fungal growth and reported that the culture medium absorbed part of the ethanol present in the headspace. According to the authors, the effect of ethanol vapor in inactivating spores depends upon the temperature, the compound concentration, and water activity. Regarding the essential oils, the volatile compounds' vapor pressure hinders the spores' breathing (Inouye et al., 1998). Therefore, when vegetative hyphae are exposed to these essential oil vapors, they undergo a segmentation to a more stable situation, probably as a survival strategy (Inouye, 2003).

The mode of action of volatile compounds in essential oils is represented by the deleterious effects caused to the fungi. According to Dao & Dantigny (2011), fungi can grow on a wide variety of substrates and over a wide pH range, a_w and temperature. In one of their studies, Inouye et al. (1998) demonstrated that the formation of spores of four species of filamentous fungi, including the genera *Aspergillus* and *Penicillium* were suppressed by some essential oils. The differences in fungi behavior and composition suggest the reason for their differences in resistances to the essential oils, according to the evaluating method used; that is why it is essential to use complementary evaluating methods of antimicrobial activity. Furthermore, the antimicrobial capacity of essential oils depends on the source, composition, structure, and concentration (Barrera-Ruiz et al., 2020).

The effects of the essential oil vapors showed that the oregano oil has a prolonged impact on both fungi, unlike the mint oil, which had a temporary effect on the same microorganisms. Moreover, both oils had a static effect for both organisms, i.e., both essential oils' volatile compounds have a fungistatic impact on oil concentration and inoculum size. However, other factors interfere with antifungal activity. According to Inouye (2003), the actual vapor concentration is much less than the nominal concentration calculated from the MID values, suggesting that only a part of the vaporized oil may be effective against microorganisms.

The antimicrobial mechanism of essential oils has been widely investigated, although it remains controversial and not completely understood (Zhang et al., 2021). According to some authors, the hydrophobic essential oils could improve the cell membranes' permeability by disrupting them, leading to the leakage of cellular substances and eventually cell death (Guo et al., 2020), besides DNA damage (Salehi et al., 2020) and the release of lipopolysaccharides, inhibition of protein expression, or interfering with glucose uptake (Cho et al., 2020). On the other hand, their hydrophobicity allows them to interact with the lipids of the microbial cell membrane and mitochondria, making the structures less organized and thus more permeable, causing the outflow of ions and other cell contents, leading to cell death (Ju et al., 2018b; Khaneghah et al., 2018). The action route of the essential oil antimicrobial mechanism is not single, and two or more routes exist simultaneously (Ju et al., 2019).

3.3 Antioxidant activity

The antioxidant activity of both essential oils, evaluated by the DPPH method, are shown in Figure 4. According to Hassimotto et al. (2005), antioxidant activity values above 70% indicate an excellent antioxidant effect. The results show that the minimum concentrations of oregano oil and reaction times resulting in an antioxidant activity higher than 70% were 2.5 mg/ml after 30 min, 0.75 mg/ml after 180 min, and 0.5 mg/ml after 360 min of reaction (Figure 3A). On the other hand, the minimum concentrations of mint oil and reaction times that resulted in an antioxidant activity higher than 70% were 400 mg/ml after 30 min, 50 mg/ml after 180 min, and 30 mg/ml after 360 min of reaction (Figure 3B). These results suggest that the reaction time interferes with the oil's antioxidant ability since lower concentrations for an antioxidant activity > 70% were necessary as the reaction time increased. The results also showed that oregano's essential oil presents higher antioxidant potential than the mint essential oil.

Table 5 presents the IC_{50} values of the essential oils for antioxidant activity. The results suggest once more that oregano

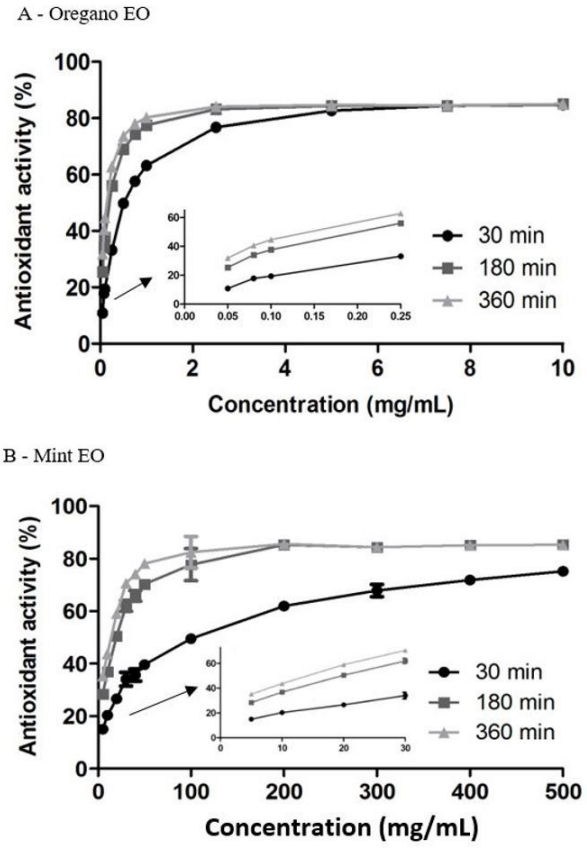

Figure 4. Antioxidant activity (%) of oregano (A) and mint (B) essential oils (EO). Data magnifications were inserted into the figures to show the almost-linear behavior of the antioxidant activity with the concentration.

oil has a higher antioxidant capacity than mint oil. Furthermore, statistical analysis ($p < 0.05$) showed that the reaction time influences the essential oil's antioxidant power.

Henn et al. (2010) evaluated the antioxidant power of the essential oil of *Origanum vulgare* by the DPPH method. They found an IC_{50} value (IC_{50} = 174,2 mg/mL) higher than the value found in this study after 30 min of reaction. According to Kaurinovic et al. (2011), antioxidant activity depends on the quantity and quality of the compounds present in the extracts.

Carvacrol and menthol found in oregano and mint essential oils, respectively, are the main compounds in both oils, according to the chemical composition evaluation. Both structures have hydroxyl (OH) groups attached to the aromatic ring, responsible for their antimicrobial and antioxidant activities (Velluti et al., 2003). However, essential oils present a high chemical complexity due to compounds with different functional groups, polarity, and chemical behavior. Thus, these essays' results may reflect only a part of its antioxidant capacity (Sacchetti et al., 2005).

Table 5. IC_{50} (mg/mL) values of essential oils of oregano and mint.

Reaction time (min)	IC_{50} (mg/mL)	
	Oregano oil	Mint oil
30	0.5 ± 0.0[a]	88.2 ± 0.1[a]
180	0.2 ± 0.0[b]	17.6 ± 0.1[b]
360	0.1 ± 0.0[c]	11.1 ± 0.0[c]

Different letters in the same column indicate significant differences ($p < 0.05$).

3.4 Cytotoxicity

The FDA (Food and Drug Administration-USA) recognizes many essential oils as GRAS (Generally Recognized as Safe). However, adverse reactions to specific compounds are commonly reported, manifested as irritations and toxic effects. Therefore, toxicological studies, including cell viability assays, are necessary (Burt, 2004). Figure 5 presents NGM and NIH-3T3 cells' viability incubated with oregano and mint essential oils for 24, 48, and 72 h.

The cytotoxicity of the essential oils of oregano and mint was investigated with a maximum concentration of 200 µg/mL. Above 25 µg/mL, the survival rate of NGM cells was ≤ 20%, the rate obtained with both oils (Figure 4A and B). For NIH-3T3 cells, the rate was <10% for both oils (Figure 5C and D). The statistical analysis ($P < 0.05$) proved that increasing sample concentrations above 25 µg/mL did not significantly differ in cell viability. Cytotoxicity results are closely related to the analysis

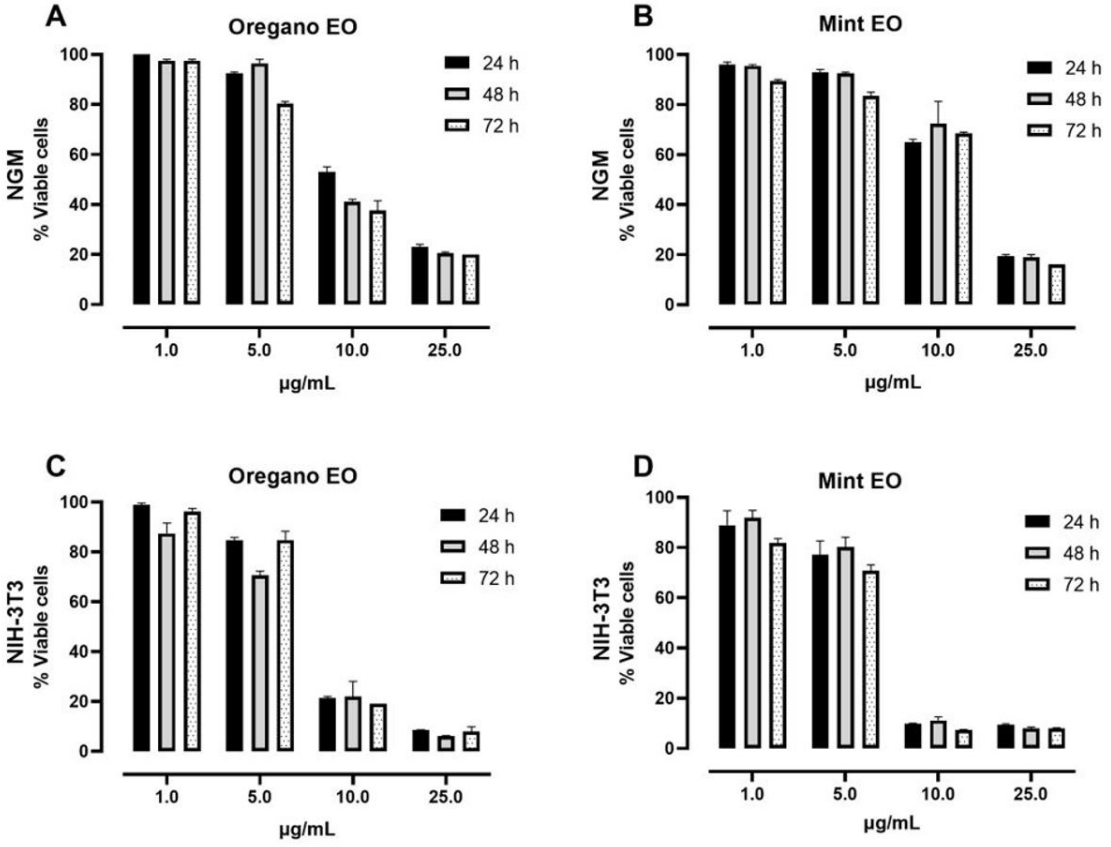

Figure 5. Effect of the concentrations (µg/mL) of the essential oils (EO) of oregano and mint and incubation periods (h) on the viability of NGM (A, B) and NIH-3T3 (C, D) cells.

method, besides the type, origin, and composition of the oil and cells selected for the study, as shown by Vimalanathan & Hudson (2012), Hussain et al. (2010a), and Yamaguchi et al. (2013).

Most studies on the cytotoxicity of essential oils are developed with tumor cells due to their antitumor properties. Grbović et al. (2013), Hussain et al. (2010b), and Weecharangsan et al. (2014) evaluated the cytotoxicity of oregano and mint oils in human tumoral cells. They proved that tumoral cells are less sensitive to the treatment than non-tumoral cells. According to the authors, the high concentration of phenols, especially carvacrol, explains the cytotoxic activity of oregano oil. Vimalanathan & Hudson (2012) found that carvacrol showed high antiviral activity alone and high cytotoxicity. According to these authors, oregano essential oils with minor cytotoxicity present components with protective properties that neutralize carvacrol's substantial toxic effect. The compound responsible for the cytotoxicity in the mint oil is menthol mainly. Overall, the main components determine the biological properties of the essential oils (Bakkali et al., 2008). However, research reports have suggested that minor components may also play a considerable role in these activities. Thus, an antagonistic or synergistic effect of minority compounds must also be considered (Lambert et al., 2001).

Table 6 presents the IC_{50} values of the essential oils of oregano and mint. The statistical analysis ($P < 0.05$) showed that higher incubation times did not significantly differ in the IC_{50} values.

Substances are classified in more or less toxic potential according to the IC_{50} value: i) IC_{50} <10 μg/mL is a very toxic substance, ii) IC_{50} = 10-100 μg/mL is a toxic substance, iii) IC_{50} = 100-1000 μg /mL is a harmful substance, and iv) IC_{50} > 1000 μg/mL is a non-toxic substance (Gad-Shayne, 2009). According to this classification, oregano and mint oils might be considered potentially toxic to human cells (NGM) and potentially very toxic to murine cells (NIH-3T3) in the conditions tested.

The *in vitro* assessment of oregano and mint oils' cytotoxicity aimed to establish a reference to these compounds' use, whose primary purpose was its application as antimicrobial agents in food preservation. However, most studies have focused on the pharmaceutical and medical fields for treating tumor cells. Nowadays, little published research on essential oils' cytotoxicity for enforcement purposes as commercial antimicrobial agents (Laird & Phillips, 2012). The degree of toxicity of an extract depends on several factors, including the dose and administration frequency. In some cases, lower dosages lead to poisoning because of the individual sensitivity, resulting from sensitization and allergy to more severe problems. The dose-dependent factor of oils associated with the individual sensitivity to different components makes it difficult to set a safety limit for the compounds' applicability. Due to these factors, the use of plants derived products requires previous studies on various aspects, such as their toxic effect on animal organisms (Cleff et al., 2008).

Table 6. IC_{50} values of oregano and mint essential oils for NGM and NIH-3T3 cells in different incubation periods.

Essential oil	Incubation period (h)	IC_{50} (μg/mL)	
		NGM	NIH-3T3
oregano	24	16.3 ± 4.2[a]	7.6 ± 1.7[a]
	48	16.2 ± 1.8[a]	9.1 ± 3.8[a]
	72	17.7 ± 0.3[a]	10.4 ± 2.9[a]
mint	24	21.6 ± 4.8[a]	9.8 ± 3.1[a]
	48	22.2 ± 3.9[a]	7.5 ± 2.4[a]
	72	21.2 ± 6.7[a]	6.9 ± 0.6[a]

Different letters in the same column indicate significant differences ($p < 0.05$).

Cleff et al. (2008) developed an *in vivo* study of the cytotoxicity of the essential oil of *Origanum vulgare* in adult Wistar rats. The rats received 3% (v/v) of the oil orally and intravaginally for 30 days. The results showed no toxic effects during the observation period. None of the experimental animals showed clinical, behavioral changes or death. The addition of 1000 ppm of the essential oil in swine diets did not result in any negative changes; contrarywise, positive effects were observed in the animals' health and production. The antioxidant probably explains these results, the antibacterial and anti-inflammatory action of the essential oil of oregano with monophenols, such as carvacrol and thymol in its composition (Allan & Bilkei, 2005).

In recent years, there has been increasing realization that many essential oils from various plants have been reported to be safe and possess strong antimicrobial effects (Zhang et al., 2017). Although synthetic antimicrobial agents' antimicrobial mode of action has been reviewed to some extent, there is still a lack of determining the mode of action of plant-based natural compounds, including essential oils, against pathogenic microorganisms (Bajpai et al., 2013). In this way, the relatively low cost (given the need for small concentrations) opens exciting possibilities for using these essential oils as antimicrobial agents. The results of the *in vitro* studies, associated with the *in vivo* studies, are essential references for establishing safe limits for using essential oils in food.

4 Conclusion

Origanum vulgare and *Mentha arvensis* essential oils present great antioxidant potential as a sign of more potential bioactivity. In addition, these oils have antifungal activity against *Aspergillus flavus* and *Penicillium commune*, and the antifungal activity of the respective volatile compounds is even greater. All tests show that oregano's essential oil presents higher biological potentials than the essential oil of mint. The results of *in vitro* cytotoxicity of oregano and mint essential oils are dependent on the oil composition and cell lines, being the results presented helpful as a reference to further studies.

Overall, essential oils have potential activity against *Aspergillus flavus* and *Penicillium commune*, but further *in vivo* studies are necessary to ensure their safe use in food preservation.

Acknowledgements

The authors thank the Coordenação de Pessoal de Nível Superior (CAPES) for financial support and fellowships for Núria B-P., Daiane R., Júlia C., and CNPq for the productivity scholarship of J.B. Laurindo.

References

Allan, P., & Bilkei, G. (2005). Oregano improves reproductive performance of sows. *Theriogenology*, 63(3), 716-721. http://dx.doi.org/10.1016/j.theriogenology.2003.06.010. PMid:15629791.

Ayala-Zavala, J. F., Soto-Valdez, H., González-León, A., Álvarez-Parrilla, E., Martín-Belloso, O., & González-Aguilar, G. A. (2008). Microencapsulation of cinnamon leaf (*Cinnamomum zeylanicum*) and garlic (*Allium sativum*) oils in *b*-cyclodextrin. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, 60(3-4), 359-368. http://dx.doi.org/10.1007/s10847-007-9385-1.

Bajpai, V. K., Sharma, A., & Baek, K-H. (2013). Antibacterial mode of action of Cudrania tricuspidata fruit essential oil, affecting membrane permeability and surface characteristics of food-borne pathogens. *Food Control*, 32(2), 582-590.

Bakkali, F., Averbeck, S., Averbeck, D., & Idaomar, M. (2008). Biological effects of essential oils – A review. *Food and Chemical Toxicology*, 46(2), 446-475. http://dx.doi.org/10.1016/j.fct.2007.09.106. PMid:17996351.

Barchiesi, F., Del Poeta, M., Morbiducci, V., Ancarani, F., & Scalise, G. (1993). Turbidimetric and visual criteria for determining the *in vitro* activity of six antifungal agents against *Candida spp*. and *Cryptococcus neoformans*. *Mycopathologia*, 124(1), 19-25. http://dx.doi.org/10.1007/BF01103052. PMid:8159215.

Barrera- Ruiz, D. G., Cuestas- Rosas, G. C., Sánchez-Marinez, R. I., Álvarez-Ainza, M. L., Moreno-Ibarra, G. M., López-Meneses, A. K., Plascencia-Jatomea, M., & Cortez-Rocha, M. O. (2020). Antibacterial activity of essential oils encapsulated in chitosan nanoparticles. *Food Science and Technology (Campinas)*, 40(Suppl. 2), 568-573.

Belletti, N., Ndagijimana, M., Sisto, C., Guerzoni, M. E., Lanciotti, R., & Gardini, I. F. (2004). Evaluation of the antimicrobial activity of citrus essences on *Saccharomyces cerevisiae*. *Journal of Agricultural and Food Chemistry*, 52(23), 6932-6938. http://dx.doi.org/10.1021/jf049444v. PMid:15537299.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Food Science and Technology Lebensmittel-Wissenschaft & Technologie.*, 28(1), 25-30. http://dx.doi.org/10.1016/S0023-6438(95)80008-5.

Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods - a review. *International Journal of Food Microbiology*, 94(3), 223-253. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.03.022. PMid:15246235.

Cavaleiro, C., Pinto, E., Gonçalves, M. J., & Salgueiro, L. (2006). Antifungal activity of *Juniperus* essential oils against dermatophyte *Aspergillus* and *Candida strains*. *Journal of Applied Microbiology*, 100(6), 1333-1338. http://dx.doi.org/10.1111/j.1365-2672.2006.02862.x. PMid:16696681.

Cho, T. J., Park, S. M., Yu, H., Seo, G. H., Kim, H. W., Kim, S. A., & Rhee, M. S. (2020). Recent advances in the application of antibacterial complexes using essential oils. *Molecules (Basel, Switzerland)*, 25(7), 1752. http://dx.doi.org/10.3390/molecules25071752. PMid:32290228.

Cleff, M. B., Meinerz, A. R., Sallis, E. S., Antunes, T. A., Mattei, A., Rodrigues, M. R., Meireles, M. C. A., & Mello, J. R. B. (2008). Toxicidade Pré-Clínica em Doses Repetidas do Óleo Essencial do *Origanum vulgare* L. (Orégano) em Ratas Wistar. *Latin American Journal of Pharmacy*, 27(5), 704-709.

Dao, T., & Dantigny, P. (2011). Control of food spoilage fungi by ethanol. *Food Control*, 22(3-4), 360-368. http://dx.doi.org/10.1016/j.foodcont.2010.09.019.

de Billerberk, V. G., Roques, C. G., Bessiere, J. M., Fonvieille, J. L., & Dargent, R. (2001). Effects of *Cymbopogon nardus* (L.) W. Watson essential oil on the growth and morphogenesis of *Aspergillus niger*. *Canadian Journal of Microbiology*, 47(1), 9-17. http://dx.doi.org/10.1139/w00-117. PMid:15049444.

De Vincenzi, M., Stammati, A., De Vincenzi, A., & Silano, M. (2004). Constituents of aromatic plants: carvacrol. *Fitoterapia*, 75(7-8), 801-804. http://dx.doi.org/10.1016/j.fitote.2004.05.002. PMid:15567271.

Dorman, H. J. D., & Deans, S. G. (2000). Antimicrobial agents from plants: antibacterial activity of plant volatile oils. *Journal of Applied Microbiology*, 88(2), 308-316. http://dx.doi.org/10.1046/j.1365-2672.2000.00969.x. PMid:10736000.

Espinel-Ingroff, A., Barchiesi, F., Cuenca-Estrella, M., Pfaller, M. A., Rinaldi, M., Rodriguez-Tudela, J. L., & Verweij, P. E. (2005). International and multicenter comparison of EUCAST and CLSI M27-A2 broth microdilution methods for testing susceptibilities of *Candida* spp. to fluconazole, itraconazole, posaconazole and voriconazole. *Journal of Clinical Microbiology*, 43(8), 3884-3889. http://dx.doi.org/10.1128/JCM.43.8.3884-3889.2005. PMid:16081926.

Falleh, H., Ben Jemaa, M., Saada, M., & Ksouri, R. (2020). Essential oils: a promising eco-friendly food preservative. *Food Chemistry*, 330: 127268. http://dx.doi.org/10.1016/j.foodchem.2020.127268. PMid:32540519.

Fisher, K., & Phillips, C. (2008). Potential antimicrobial uses of essential oils in food: is citrus the answer? *Trends in Food Science & Technology*, 19(3), 156-164. http://dx.doi.org/10.1016/j.tifs.2007.11.006.

Gad-Shayne, C. (2009). Alternatives to *in vivo* studies in toxicology. In B. Ballantyne, T. C. Marrs, & T. Syversen (Eds.), *General and applied toxicology* (Vol. 6). Hoboken: John Wiley & Sons Inc.

Grbović, F., Stankovic, M. S., Curcic, M., Dordevic, N., Seklic, D., Topuzovic, M., & Markovic, S. (2013). *In Vitro* cytotoxic activity of *Origanum vulgare* L. on HCT-116 and MDA-MB-231 cell lines. *Plants*, 2(3), 371-378. http://dx.doi.org/10.3390/plants2030371. PMid:27137381.

Gulluce, M., Sahin, F., Sokmen, M., Ozer, H., Daferera, D., Sokmen, A., Polissiou, M., Adiguzel, A., & Ozkan, H. (2007). Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. longifolia. *Food Chemistry*, 103(4), 1449-1456. http://dx.doi.org/10.1016/j.foodchem.2006.10.061.

Guo, M., Zhang, L., He, Q., Arabi, S. A., Zhao, H., Chen, W., Ye, X., & Liu, D. (2020). Synergistic antibacterial effects of ultrasound and thyme essential oils nanoemulsion against Escherichia coli O157:H7. *Ultrasonics Sonochemistry*, 66, 104988. http://dx.doi.org/10.1016/j.ultsonch.2020.104988. PMid:32222643.

Hassimotto, N. M. A., Genovese, M. I., & Lajolo, F. M. (2005). Antioxidant activity of dietary fruits, vegetables and commercial frozen fruit pulps. *Journal of Agricultural and Food Chemistry*, 53(8), 2928-2935. http://dx.doi.org/10.1021/jf047894h. PMid:15826041.

Henn, J. D., Bertol, T. M., Moura, N. F., Coldebella, A., Brum, P. A. R., & Casagrande, M. (2010). Oregano essential oil as food additive for piglets: antimicrobial and antioxidant potential. *Revista Brasileira de Zootecnia*, 39(8), 1761-1767. http://dx.doi.org/10.1590/S1516-35982010000800019.

Hussain, A. I., Anwar, F., Chatha, S. A. S., Jabbar, A., Mahboob, S., & Nigam, P. S. (2010a). *Rosmarinus officinalis* essential oil: antiproliferative, antioxidant and antibacterial activities. *Brazilian Journal of Microbiology*, 41(4), 1070-1078. http://dx.doi.org/10.1590/S1517-83822010000400027. PMid:24031588.

Hussain, A. I., Anwar, F., Nigam, P. S., Ashraf, M., & Gilani, A. H. (2010b). Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four *Mentha* species. *Journal of the Science of Food and Agriculture*, 90(11), 1827-1836. http://dx.doi.org/10.1002/jsfa.4021. PMid:20602517.

Inouye, S. (2003). Laboratory evaluation of gaseous essential oils (Part 1). *International Journal of Aromatherapy*, 13(2-3), 95-107. http://dx.doi.org/10.1016/S0962-4562(03)00081-X.

Inouye, S., Takizawa, T., & Yamaguchi, H. (2001). Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. *The Journal of Antimicrobial Chemotherapy*, 47(5), 565-573. http://dx.doi.org/10.1093/jac/47.5.565. PMid:11328766.

Inouye, S., Watanabe, M., Nishiyama, Y., Takeo, K., Akao, M., & Yamaguchi, H. (1998). Antisporulating and respiration-inhibitory effects of essential oils on filamentous fungi. *Mycoses*, 41(9-10), 403-410. http://dx.doi.org/10.1111/j.1439-0507.1998.tb00361.x. PMid:9916464.

Ju, J., Liao, L., Qiao, Y., Xiong, G., Li, D., Wang, C., Hu, J., Wang, L., Wu, W., Ding, A., Shi, L., & Li, X. (2018a). The effects of vacuum package combined with tea polyphenols (V+TP) treatment on quality enhancement of weever (Micropterus salmoides) stored at 0 °C and 4 °C. *Lebensmittel-Wissenschaft + Technologie*, 91, 484-490. http://dx.doi.org/10.1016/j.lwt.2018.01.056.

Ju, J., Xu, X., Xie, Y., Guo, Y., Cheng, Y., Qian, H., & Yao, W. (2018b). Inhibitory effects of cinnamon and clove essential oils on mold growth on baked foods. *Food Chemistry*, 240, 850-855. http://dx.doi.org/10.1016/j.foodchem.2017.07.120. PMid:28946351.

Ju, J., Xie, Y., Guo, Y., Cheng, Y., Qian, H., & Yao, W. (2019). The inhibitory effect of plant essential oils on foodborne pathogenic bacteria in food. *Critical reviews in food science and nutrition*, 59(20), 3281-3292. PMid:29902072.

Kaefer, C. M., & Milner, J. A. (2008). The role of herbs and spices in cancer prevention. *The Journal of Nutritional Biochemistry*, 19(6), 347-361. http://dx.doi.org/10.1016/j.jnutbio.2007.11.003. PMid:18499033.

Kalemba, D., & Kunicka, A. (2003). Antibacterial and Antifungal Properties of Essential Oils. *Current Medicinal Chemistry*, 10(10), 813-829. http://dx.doi.org/10.2174/0929867033457719. PMid:12678685.

Kaurinovic, B., Popovic, M., Vlaisavljevic, S., & Trivic, S. (2011). Antioxidant capacity of *Ocimum basilicum* L. and *Origanum vulgare* L. extracts. *Molecules (Basel, Switzerland)*, 16(9), 7401-7414. http://dx.doi.org/10.3390/molecules16097401. PMid:21878860.

Khaneghah, A. M., Hashemi, S. M. B., & Limbo, S. (2018). Antimicrobial agents and packaging systems in antimicrobial active food packaging: an overview of approaches and interactions. *Food and Bioproducts Processing*, 111, 1-19. http://dx.doi.org/10.1016/j.fbp.2018.05.001.

Kloucek, P., Smid, J., Frankova, A., Kokoska, L., Valterova, I., & Pavela, R. (2012). Fast screening method for assessment of antimicrobial activity of essential oils in vapour phase. *Food Research International*, 47(2), 161-165. http://dx.doi.org/10.1016/j.foodres.2011.04.044.

Kocić-Tanackov, S., Dimić, G., Lević, J., Tanackov, I., Tepić, A., Vujičić, B., & Gvozdanović-Varga, J. (2012). Effects of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.) essential oils on the *Aspergillus versicolor* growth and sterigmatocystin production. *Journal of Food Science*, 77(5), M278-M284. http://dx.doi.org/10.1111/j.1750-3841.2012.02662.x. PMid:22497489.

Laird, K., & Phillips, C. (2012). Vapour phase: a potential future use for essential oils as antimicrobials? *Letters in Applied Microbiology*, 54(3), 169-174. http://dx.doi.org/10.1111/j.1472-765X.2011.03190.x. PMid:22133088.

Lambert, R. J. W., Skandamis, P. N., Coote, P. J., & Nychas, G. J. (2001). A Study of the minimum inhibitory concentration and mode of action of oregano essencial oil, thymol and carvacrol. *Journal of Applied Microbiology*, 91(3), 453-462. http://dx.doi.org/10.1046/j.1365-2672.2001.01428.x. PMid:11556910.

Liest, H. (1998). Phytochemical studies of medicinal plants. *International Journal of Plant Sciences*, 68, 130-142.

López, P., Sánchez, C., Batlle, R., & Nerín, C. (2005). Solid and vapor-phase antimicrobial activities of six essential oils: susceptibility of selected foodborne bacterial and fungal strains. *Journal of Agricultural and Food Chemistry*, 53(17), 6939-6946. http://dx.doi.org/10.1021/jf050709v. PMid:16104824.

Mosmann, T. (1983). Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *Journal of Immunological Methods*, 65(1-2), 55-63. http://dx.doi.org/10.1016/0022-1759(83)90303-4. PMid:6606682.

National Committee Clinical Laboratory Standards – NCCLS (2002). *M38A Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi* (Vol. 22). Wayne, PA: USA.

Ostrosky, E. A., Mizumoto, M. K., Lima, M. E. L., Kaneko, T. M., Nishikawa, S. O., & Freitas, B. R. (2008). Métodos para avaliação da atividade antimicrobiana e determinação da concentração mínima inibitória (CMI) de plantas medicinais. *Brazilian Journal of Pharmacognosy*, 18(2), 301-307. http://dx.doi.org/10.1590/S0102-695X2008000200026.

Pandey, A. K., Rai, M. K., & Acharya, D. (2003). Chemical composition and antimycotic activity of the essential oils of corn mint (*Mentha arvensis*) and lemon grass (*Cymbopogon flexuosus*) against human pathogenic fungi. *Pharmaceutical Biology*, 41(6), 421-425. http://dx.doi.org/10.1076/phbi.41.6.421.17825.

Pitt, J. I., Cruickshank, R. H., & Leistner, L. (1986). *Penicillium commune., P. camembertii*, the origin of white cheese moulds and the production of cyclopiazonic acid. *Food Microbiology*, 3(4), 363-371. http://dx.doi.org/10.1016/0740-0020(86)90022-5.

Polônio, M. L. T., & Peres, F. (2009). Consumo de aditivos alimentares e efeitos à saúde: desafios para a saúde pública brasileira. *Cadernos de Saúde Pública*, 25(8), 1653-1666.

Rasooli, I., & Abyaneh, M. R. (2004). Inhibition effects of Thyme oils on growth and aflatoxin production by *Aspergillus parasiticus*. *Food Control*, 15(6), 479-483. http://dx.doi.org/10.1016/j.foodcont.2003.07.002.

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., Radice, M., & Bruni, R. (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals, and antimicrobials in foods. *Food Chemistry*, 91(4), 621-632. http://dx.doi.org/10.1016/j.foodchem.2004.06.031.

Salehi, F., Behboudi, H., Kavoosi, G., & Ardestani, S. K. (2020). Incorporation of Zataria multiflora essential oil into chitosan biopolymer nanoparticles: a nanoemulsion based delivery system to improve the in-vitro efficacy, stability and anticancer activity of ZEO against breast cancer cells. *International Journal of Biological Macromolecules*, 143, 382-392. http://dx.doi.org/10.1016/j.ijbiomac.2019.12.058. PMid:31830446.

Sánchez-González, L., Cháfer, M., Chiralt, A., & González-Martínez, C. (2010). Physical properties of edible chitosan films containing bergamot essential oil and their inhibitory action on Penicillium italicum. *Carbohydrate Polymers*, 82(2), 277-283. http://dx.doi.org/10.1016/j.carbpol.2010.04.047.

Scheidegger, K. A., & Payne, G. A. (2003). Unlocking the secrets behind secondary metabolism: a review of *Aspergillus flavus* from pathogenicity to functional genomics. *Toxin Reviews*, 22, 423-459.

Simões, C. M. O., & Spitzer, V. (2003). Óleos voláteis. In: C. M. O. Simões. *Farmacognosia: da planta ao medicamento* (5. ed., pp. 467-495). Porto Alegre: Editora UFRGS.

Singh, R., Shushni, M. A. M., & Belkheir, A. (2011). Antibacterial and antioxidant activity of *Mentha piperita* L. *Arabian Journal of Chemistry*, 4(1), 1-20.

Soottitantawat, A., Takayama, K., Okamura, K., Muranaka, D., Yoshii, H., Furuta, T., Ohkawara, M., & Linko, P. (2005). Microencapsulation of l-menthol by spray drying and its release characteristics. *Innovative Food Science & Emerging Technologies*, 6(2), 163-170. http://dx.doi.org/10.1016/j.ifset.2004.11.007.

Turina, A. D. V., Nolan, M. V., Zygadlo, J. A., & Perillo, M. A. (2006). Natural terpenes: self-assembly and membrane partitioning. *Biophysical Chemistry*, 122(2), 101-113. http://dx.doi.org/10.1016/j.bpc.2006.02.007. PMid:16563603.

Veiga Junior, V. F., Pinto, A. C., & Maciel, M. A. M. (2005). Plantas medicinais: cura segura? *Quimica Nova*, 28(3), 519-528. http://dx.doi.org/10.1590/S0100-40422005000300026.

Velluti, A., Sanchis, V., Ramos, A. J., Egido, J., & Marin, S. (2003). Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B1 production by *Fusarium proliferatum* in maize grain. *International Journal of Food Microbiology*, 89(2-3), 145-154. http://dx.doi.org/10.1016/S0168-1605(03)00116-8. PMid:14623380.

Vimalanathan, S., & Hudson, J. (2012). Anti-influenza virus activities of commercial oregano oils and their carriers. *Journal of Applied Pharmaceutical Science*, 02(07), 214-218. http://dx.doi.org/10.7324/JAPS.2012.2734.

Weber, F. J., & De Bont, J. A. (1996). Adaptation mechanisms of microorganisms to the toxic effects of organic solvents on membranes. *Biochimica et Biophysica Acta*, 1286(3), 225-245. http://dx.doi.org/10.1016/S0304-4157(96)00010-X. PMid:8982284.

Weecharangsan, W., Sithithaworn, W., & Siripong, P. (2014). Citototoxic activity of essential oils of *Mentha* spp. on human carcinoma cells. *J Health Res.*, 28(1), 9-12.

Yamaguchi, K. L., Veiga Junior, V. F., Pedrosa, T. N., Vasconcellos, M. C., & Lima, E. S. (2013). Atividades biológicas dos óleos essenciais de *Endlicheria citriodora*, uma Lauraceae rica em geranato de metila. *Química Nova*, 36(6), 826-830.

Yuan, G., Chen, X., & Li, D. (2016). Chitosan films and coatings containing essential oils: the antioxidant and antimicrobial activity, and application in food systems. *Food Research International*, 89(Pt 1), 117-128. http://dx.doi.org/10.1016/j.foodres.2016.10.004. PMid:28460897.

Zhang, L.-L., Zhang, L.-F., Hu, Q.-P., Hao, D.-L., & Xu, J.-G. (2017). Chemical composition, antibacterial activity of Cyperus rotundus rhizomes essential oil against Staphylococcus aureus via membrane disruption and apoptosis pathway. *Food Control*, 80, 290-296. http://dx.doi.org/10.1016/j.foodcont.2017.05.016.

Zhang, X., Ismail, B. B., Cheng, H., Jin, T. Z., Qian, M., Arabi, S. A., Liu, D., & Guo, M. (2021). Emerging chitosan-essential oil films and coatings for food preservation - A review of advances and applications. *Carbohydrate Polymers*, 273, 118616. http://dx.doi.org/10.1016/j.carbpol.2021.118616. PMid:34561014.