

***Cantinoa althaeifolia* essential oil: chemical composition and biological, antioxidant, antimicrobial, and antitumor activities**

Composição química e atividades biológica, antioxidante, antimicrobiana e antitumoral do óleo essencial de *Cantinoa althaeifolia*

Composición química y actividades biológicas, antioxidantes, antimicrobianas y antitumorales del aceite esencial de *Cantinoa althaeifolia*

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Abstract

The species of the Lamiaceae family produce essential oils in the glandular trichomes, leaf surfaces, and in inflorescences, and can also produce many secondary compounds, which indicates great potential for study. The aim of this paper was to describe the chemical composition and the biological, antioxidant, antimicrobial, and antitumor activities of the essential oil of *Cantinoa althaeifolia* for the first time. The essential oil was obtained from the leaves by hydrodistillation and analyzed by gas chromatography mass spectrometry (GC/MS). The antioxidant activity was established by the phosphomolybdenum method. The minimum inhibitory concentration against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida albicans* was determined. The lethality test of *Artemia salina* was performed. The cytotoxicity against murine fibroblasts (3T3), non-tumor cells (McCoy), murine melanoma (B16F10), and human breast carcinoma (MCF-7) cells was assessed. Five major compounds were isolated: himachalene (11.62%), spathulenol (10.08%), caryophylline oxide (9.93%), (E)-caryophyllene (9.21%), and pogostol (6.31%). The phosphomolybdenum complex method showed positive results. The minimum inhibitory concentration was greater than 1000 µg/mL for the tested microorganisms. The mortality rate of *Artemia salina* was low. The tested

cancer cell lines showed an IC₅₀ of 12.5 and 25 µg/mL. These findings indicate that the essential oil of *Cantinoa althaeifolia* has antioxidant, antimicrobial, and antitumor activities.

Keywords: Lamiaceae; Plant oils; Microbial sensitivity tests; Phytogetic antineoplastic agents; Antioxidants.

Resumo

As espécies da família Lamiaceae produzem óleos essenciais nos tricomas glandulares, superfícies foliares e em inflorescências, podendo também produzir diversos compostos secundários, o que indica grande potencial de estudo. O objetivo deste trabalho foi descrever a composição química e as atividades biológica, antioxidante, antimicrobiana e antitumoral do óleo essencial de *Cantinoa althaeifolia* pela primeira vez. O óleo essencial foi obtido das folhas por hidrodestilação e analisado por cromatografia gasosa e espectrometria de massas (CG/EM). A atividade antioxidante foi determinada pelo método do fosfomolibdênio. A concentração inibitória mínima contra *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* e *Candida albicans* foi determinada. Foi realizado o teste de letalidade de *Artemia salina*. A citotoxicidade contra fibroblastos murinos (3T3), células não tumorais (McCoy), melanoma murino (B16F10) e células de carcinoma de mama humano (MCF-7) também foi avaliada. Cinco compostos principais foram isolados: himachaleno (11,62%), espatulenol (10,08%), óxido de cariofileno (9,93%), (E)-cariofileno (9,21%) e pogostol (6,31%). O método do complexo de fosfomolibdênio apresentou resultados positivos. A concentração inibitória mínima foi superior a 1000 µg/mL para os microrganismos testados. A taxa de mortalidade de *Artemia salina* foi baixa. As linhas de células cancerosas testadas mostraram uma CI₅₀ de 12,5 e 25 µg/mL. Esses achados indicam que o óleo essencial de *Cantinoa althaeifolia* possui atividades antioxidante, antimicrobiana e antitumoral.

Palavras-chave: Lamiaceae; Óleos vegetais; Testes de sensibilidade microbiana; Antineoplásicos fitogênicos; Antioxidantes.

Resumen

Las especies de la familia Lamiaceae producen aceites esenciales en los tricomas glandulares, la superficie de las hojas y las inflorescencias, y también pueden producir muchos compuestos secundarios, lo que indica un gran potencial de estudio. El objetivo de este trabajo fue describir la composición química y las actividades biológicas, antioxidantes, antimicrobianas y antitumorales del aceite esencial de *Cantinoa althaeifolia* por primera vez. El aceite esencial se obtuvo de las hojas por hidrodestilación y se analizó mediante cromatografía de gases y espectrometría de masas (CG/EM). La actividad antioxidante se estableció mediante el método del fosfomolibdeno. Se determinó la concentración mínima inhibitoria contra *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* y *Candida albicans*. Se realizó la prueba de letalidad de *Artemia salina*. Se evaluó la citotoxicidad contra fibroblastos murinos (3T3), células no tumorales (McCoy), melanoma murino (B16F10) y carcinoma de mama humano (MCF-7). Se aislaron cinco compuestos principales: himachaleno (11,62%), espatulenol (10,08%), óxido de cariofileno (9,93%), (E)-cariofileno (9,21%) y pogostol (6,31%). El método del complejo de fosfomolibdeno mostró resultados positivos. La concentración inhibitoria mínima fue superior a 1000 µg/mL para los microorganismos probados. La tasa de mortalidad de *Artemia salina* fue baja. Las líneas celulares de cáncer probadas mostraron una CI₅₀ de 12,5 y 25 µg/mL. Estos hallazgos indican que el aceite esencial de *Cantinoa althaeifolia* tiene actividades antioxidantes, antimicrobianas y antitumorales.

Palabras clave: Lamiaceae; Aceites vegetales; Pruebas de sensibilidad microbiana; Antineoplásicos fitogênicos; Antioxidantes.

1. Introduction

The Lamiaceae family comprises about 240 genera and 7200 species, with a cosmopolitan distribution (Raymond Harley & Pastore, 2012). These species grow mainly in open places, in almost all types of habitats and at all altitudes, from the Arctic to the Himalayas, from Southeast Asia to Hawaii, from north to south in the New World, and in Australia and Africa as well. The Mediterranean and Central Asia are the regions with the highest concentration of species (Heywood & Royal Botanic Gardens, 2007). In Brazil, there are 34 native genera and about 496 species (Forzza et al., 2010).

The Lamiaceae family stands out for its economic importance, as it can be used for ornaments, cooking, or even as wood (RM Harley et al., 2009). Some plants can be used for culinary purposes, such as *Ocimum Basilicum* L. (lavender), *Orinagum vulvare* L. (oregano), *Mentha x piperita* L. (peppermint), *Mentha pulegium* L. (pennyroyal), *Rosmarinus officinalis* L. (rosemary), *Salvia officinalis* L. (sage), and *Thymus vulgaris* L. (thyme); others can be used for making drinks, refreshments, or teas, including *Melissa officinalis* L. (lemongrass), *Mentha* spp. (mint), *Salvia hispanica* L. (blue sage), and *Salvia splendens* Sellow ex Roem. & Schult. (Adam's blood); or for perfumery like *Lavandula angustifolia* Mill. (lavender) (Raymond Harley et al., 2004).

Recently, essential oils have been receiving great interest due to their bioactive compounds, especially because of their antimicrobial, medicinal, antioxidant, cytostatic, and insecticidal properties (Tongnuanchan & Benjakul, 2014). These oils are widely used as natural additives in foods and food products, for embalming and preserving food, and in the production of local anesthetics and antimicrobial, analgesic, sedative, anti-inflammatory, and spasmolytic remedies (Bakkali, Averbeck, Averbeck, & Idaomar, 2008).

The species of the Lamiaceae family produce essential oils in the glandular trichomes, leaf surfaces, and in inflorescences, and can also produce many secondary compounds (Raymond Harley et al., 2004), which indicates great potential for study. The aim of this study was to describe the chemical composition of the essential oil of *Cantinoa althaeifolia*, and to evaluate its antimicrobial, antioxidant, and antitumor activities.

2. Methodology

This research was organized following the Scientific Research Methodology recommendations (Pereira et al., 2018).

2.1 Plant material

The botanical material was collected at the district of Boqueirão (coordinates 25°32'43" S, 49°14'11" W), located in Curitiba, Paraná, Brazil, at 900 m of altitude, in February of 2017, 2018, and 2019. A specimen voucher was made and identified by the taxonomist Eraldo Barboza. The voucher was deposited in the Herbarium of the Botanical Museum of Curitiba, under registration n° MBM 408166. This study was authorized by the Brazilian Institute of the Environment and Renewable Natural Resources (IBAMA) and registered at the National System for the Management of Genetic Heritage and Associated Traditional Knowledge (SisGen).

2.2 Essential oil obtention

The essential oil was obtained from 600 g of dried leaves, ground in a knife mill, hydrodistilled for 6 h using modified Clevenger type apparatus. This process was repeated 3 times. The essential oil was stored in a sealed amber glass at -18°C until subsequent analysis. The yield calculation was performed in milliliters (mL/%) of essential oil per 100 g of the drug (ANVISA, 2010).

2.3 Identification of essential oil constituents

The characterization of chemical constituents of the essential oil of leaves of *Cantinoa althaeifolia* was carried out using gas chromatography, composed of gas chromatograph coupled to a Shimadzu® CGEM-QP 2010 Plus mass spectrometer (Shimadzu, Kyoto, Japan), with a Rtx-5MS capillary column (30m x 0.25 mm x 0.25 µm; Bellefonte, PA, USA).

The injector was set in splitless mode at 250°C, and the interface and source of ions were set at 300°C. The mass window was set between m/z 40 and m/z 350, using helium as the entrainment gas with a column pressure of 20 psi. The injection ramp started at 50°C and was kept for 5 min. Then, the temperature was increased at a rate of 5°C/min to a final temperature of 200°C.

The chemical components of the oil were identified by comparing their mass spectra with the reference spectra, and by comparing their Kovat's index with those described in the literature (Adams & Adams, 2001).

2.4 Formation of the phosphomolybdenum complex method

The formation of the phosphomolybdenum complex was performed (Prieto, Pineda, & Aguilar, 1999). Solutions of butylated hydroxytoluene (BHT), ascorbic acid, and rutin at a concentration of 200 µg/mL in methanol and Tween 80 0.5%

were used as control. Aliquots of 0.3 mL were added to 3 mL of the phosphomolybdenum reagent (0.1 M tribasic sodium phosphate (28 mL), 0.03 M ammonium solution of tetrahydrate molybdate (12 mL), 3 M sulfuric acid (20 mL) and water to make 100 mL). The tubes were closed and taken to a water bath at 95°C for 90 min. After cooling, the absorbances were obtained in 96-well microplates with round bottoms (TPP®; Techno Plastic Prod., Trasadingen, SH, Switzerland) by reading in the Multiskan FC spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at 695 nm. The antioxidant capacity of samples was expressed in relative antioxidant activity (AAR%), as compared to the standards, using the equation: $AAR\% = [(Sample\ Absorbance - White\ Absorption) / (Standard\ Absorbance - White\ Absorbance)] \times 100$.

2.5 Minimum inhibitory concentration (MIC)

2.5.1 Microorganisms

The antibacterial and antifungal activity was determined against the following microorganisms from the American Type Culture Collection (ATCC; Rockville, MD, USA): *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 40175).

2.5.2 Minimum inhibitory concentration in broth microdilution

Microorganisms were seeded in potato medium and incubated at 37°C for 48 h. The suspension of *Candida albicans* was prepared in physiological saline at a concentration of 1 - 5.0 x 10⁶ CFU/mL. Then, a 1:100 dilution was made in RPMI-1640 with glucose 2% (Gibco/Invitrogen, Carlsbad, CA, USA) in order to achieve a concentration of 10³ CFU/mL, as recommended by The Clinical & Laboratory Standards Institute (CLSI).

A stock solution of all the extracts, fractions, and sub-fractions was prepared at a concentration of 16,000 µg/5 mL in polysorbate. Aliquots of 100 µL of RPMI-1640 with glucose 2% were added to the 96-well microplates. The following concentrations of the extracts, fractions, and sub-fractions were evaluated: 2000 µg/mL, 1000 µg/mL, 500 µg/mL, 250 µg/mL, 125 µg/mL, and 62.5 µg/mL. The plates were incubated at 37°C for 24 h.

2.6 Lethality test of *Artemia salina*

The toxicity assay designed for the lethality tests uses the brine shrimp *Artemia salina*. A saline solution at the concentration of 30 g/L of sea salt at a pH of 9 was used for the hatching of *Artemia salina* cysts and for the preparation of dilutions (Meyer et al., 1982). The cysts were placed in the saline solution for 48 h, under continuous aeration and exposure to daylight. The temperature was maintained between 27 and 30°C and the pH between 8 and 9. The recipient was kept under illumination during the first hour.

After the hatching of the cysts, about 10 nauplii of *Artemia salina* were transferred to tubes containing the essential oil diluted in 0.5% of polysorbate 80 and saline solution at the concentrations of 75, 200, 500, 750 or 1000 µg/mL. A positive control was prepared with saline solution and quinidine sulphate, and a saline solution with polysorbate 80 was used as a negative control. The assay was performed in triplicate. The tubes were incubated at 27-30°C for 24 h, and the live and dead nauplii were counted by visual inspection.

2.7 Cell culture

Murine melanoma (B16F10), human breast carcinoma (MCF-7), murine fibroblast (3T3), and non-tumor cells (McCoy) were cultivated in RPMI medium 1640® supplemented with fetal bovine serum 10% (Gibco/Invitrogen) and antibiotics (1% of penicillin/streptazine) in a 5% CO₂, 95% humidity, 37°C incubator.

2.7.1 Cytotoxicity assay

The cytotoxicity of the essential oil was measured using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric assay to determine cell viability through mitochondrial activity. MTT is a water-soluble tetrazolium salt, which is converted to an insoluble purple compound known as formazan after cleavage of the tetrazolium salt by mitochondrial dehydrogenases (Mosmann, 1983).

McCoy, B16F10, and MCF7 cells (3×10^3 cells/well) were plated 24 h prior to exposure to *Cantinoa althaeifolia* oil for adherence, scattering, and stabilization of the cells on the plaque. The cells were then exposed to increasing oil concentrations (0.84 $\mu\text{g/mL}$ to 8.4 mg/mL in RPMI medium, supplemented with fetal bovine serum 10% and antibiotics for 72 h). Immediately prior to treatment, the solutions containing the oil and the culture medium were vigorously shaken using a vortex-type stirrer for at least 1 min.

After the treatment period, the medium was replaced by a solution of MTT (0.5 $\mu\text{g}/\mu\text{L}$) for 2 h at 37°C. The MTT solution was then replaced by isopropyl alcohol (0.1 M HCl) for solubilization of the formazan crystals, and the absorbance was read at 570 nm. A sample with no treatment was used as a control and the cell viability of other groups was expressed as the percentages of the control.

2.8 Statistical analysis

The MIC values were submitted to ANOVA and Tukey's test at the 5% significance level ($p < 0.05$). The results of the lethality test of *Artemia salina* were analyzed using the PROBIT test, which provided LC50 values with reliability of 95%. The degree of toxicity was based on three classifications: low toxicity, $\text{LC}_{50} > 500 \mu\text{g/mL}$; moderate toxicity, LC_{50} between 100 $\mu\text{g/mL}$ and 500 $\mu\text{g/mL}$; and high toxicity, LC_{50} .

The concentration that inhibited the mitochondrial dehydrogenases activity by 50% (IC_{50}) was calculated using the software GraphPad Prism, version 7.0 for Windows (GraphPad Software, La Jolla, CA, USA, www.graphpad.com), following a tutorial provided by GraphPad Software, Inc, accessed May 19, 2018. (<https://www.graphpad.com/support/faq/how-to-determine-an-icsub50sub/>).

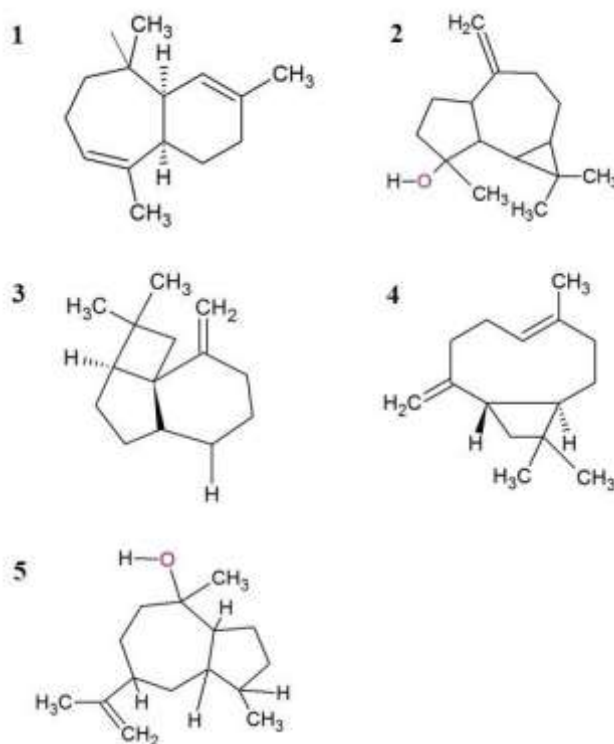
The selectivity index (SI) of the essential oil was calculated by the IC_{50} ratio between the non-tumor (McCoy) and tumor (MCF-7 and B16F10) lines according to the formula: $\text{IC}_{50} = (\text{IC}_{50} \text{ non-tumor cell line}) / (\text{IC}_{50} \text{ tumor cell line})$.

3. Results and Discussion

The extract of the essential oil of *Cantinoa althaeifolia* was made from the leaves of the dried and crushed plant. The oil obtained had a yellowish color and a strong odor, similar to other species of the Lamiaceae family. The calculated yield on the dry material mass was 2.21% (v/m), with a final volume of 3.2 mL of essential oil.

The chemical composition of the essential oil of the leaves is shown in Table 1. The major chemical constituents in order of abundance were himachalene (11.62%), spathulenol (10.08%), caryophyllene oxide (9.93%), (E)-caryophyllene (9.21%), and pogostol (6.31%), as presented in Figure 1. Himachalene is of extreme interest because it has not yet been identified in the Lamiaceae family, and there is little information about this compound in the literature. Spathulenol is found in alcoholic beverages and reported in several families, especially in Lamiaceae. It has antimicrobial (Tadić et al., 2017), toxicity and antitumor (Rolim et al., 2017), antinociceptive and anti-inflammatory activities (Barreto et al., 2016). Caryophyllene oxide is widely used as a flavoring agent and is found in several families, including the one of interest. This compound has anti-inflammatory, antioxidant, antibiotic, cytotoxic (Côté et al., 2017), and anti-parasitic properties (Abd El-Gawad, El-Amier, & Bonanomi, 2018). (E)-caryophyllene has proven anticancer, antioxidant and antimicrobial actions (Estevam et al., 2017). Finally, pogostol presents antimicrobial activity (Côté et al., 2017).

Figure 1. Major components of the *Cantinoa althaeifolia* essential oil.



* (1) himachalene, (2) spathulenol, (3) caryophyllene oxide, (4) (E)-caryophyllene, and (5) pogostol, Source: Authors (2020).

Table 1. Chemical constitution of the essential oil from the leaves of *Cantinoa althaeifolia*.

AI	AI_{theoretical}	Compound	[] %
1020	1024	Limonene	1.94
1036	1044	(E)- β -Ocimene	0.60
1158	1174	Terpinen-4-ol	0.85
1196	1215	Carveol	0.38
1212	1239	Carvone	0.40
1343	1350	α - Longipinene	1.10
1364	1373	α -Ylangene	0.33
1369	1374	α -Copaene	0.36
1375	1387	β -Bourbonene	0.17
1382	1389	β -Elemene	0.45
1392	1400	Sibirene	0.43
1402	1409	α -Gurjunene	0.33
1409	1417	(E)- Caryophyllene	9.21
428	1439	Aromadendrene	1.73
1436	1442	6,9-guaiadiene	0.50
1441	1452	α -Humulene	2.45
1465	1478	γ -Muurolene	0.64
1471	1481	γ- Himachalene	11.62
1473	1489	β -Selinene	0.48
1483	1500	Bicyclogermacrene	3.48
1488	1502	Trans- β -Guaiene	0.91
1498	1500	α -Muurolene	0.94
1509	1522	δ -Cadinene	1.94
1520	1544	α -Calacorene	0.32
1557	1577	Spathulenol	10.08
1561	1582	Caryophyllene Oxide	9.93
1583	1608	Humulene epoxide	1.65
1609	1627	1-epi-cubenol	1.21
1610	1632	α -acorenol	0.38
1613	1639	Aromadendrene epoxide	0.54
1618	1635	cis-Cadin-4-en-7-ol	2.49
1622	1636	β -acorenol	2.80
1630	1651	Pogostol	6.31
1645	1666	14-hidroxi-(Z) Caryophyllene	3.43
1657	1685	Germacre-4(15),5,10(14)-trien-1- α -ol	0.91
1690	1690	6-isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naftalen-2-ol	0.54

Source: Authors (2020).

The antioxidant activity of the essential oil was calculated in relation to BHT, ascorbic acid, and rutin. Table 2 presents the results of the antioxidant activity by the phosphomolybdenum complexation method. The sample showed a characteristic green color, which demonstrates its antioxidant activity.

Table 2. Antioxidant activity of the *Cantinoa althaeifolia* essential oil.

Sample	Antioxidant activity in relation to BHT - AA (%) ± SD	Antioxidant activity in relation to AAC - AA (%) ± SD	Antioxidant activity in relation to rutin - AA (%) ± SD
BHT	100 ^a	-----	-----
AAC	-----	100 ^a	-----
RUTIN	-----	-----	100 ^a
EO	472.66 ± 3.36 ^h	243.93 ± 1.14 ^g	524.98 ± 2.46 ^g

BHT – butylated hydroxytoluene; AA – antioxidant activity; SD - standard deviation; AAC – ascorbic acid; and EO – essential oil. Data followed by same lower-case letters indicate not significant differences within columns (p>0.05, ANOVA and Tukey's test). Source: Authors (2020).

The essential oil of *Cantinoa althaeifolia* showed potential antimicrobial activity for the tested microorganisms. *S. aureus*, *P. aeruginosa* and *C. albicans* presented a MIC of 1000 mg/mL, while *E. coli* presented a MIC of 500 mg/mL. The bactericidal activity of sesquiterpenes like δ -cadinene, α -humulene, and karyophyllene has been previously described (Pereira et al., 2017). However, when these substances were tested together, this activity were either reduced by competitive inhibition or improved because of the synergistic effect (Damasceno et al., 2018).

Artemia salina is a micro-crustacean easily found in specialized aquarium stores, that can used as live food for fish and to estimate toxicity by the average lethal concentration (LC50). This bioassay has been used in previous studies of plant extracts with possible antitumor activity and can be used for the initial assessment of a large number of samples for the evaluation of toxicity and phototoxicity. Several studies have attempted to correlate crustacean toxicity with antifungal, antimicrobial, parasitocidal, virucidal, and trypanosomicidal activities (Meyer et al., 1982). For the calculation of the LC₅₀, the results were subjected to the Probit statistical test (Finney, 1971) and are presented in Table 3.

Table 3. Mortality rate of *Artemia salina*.

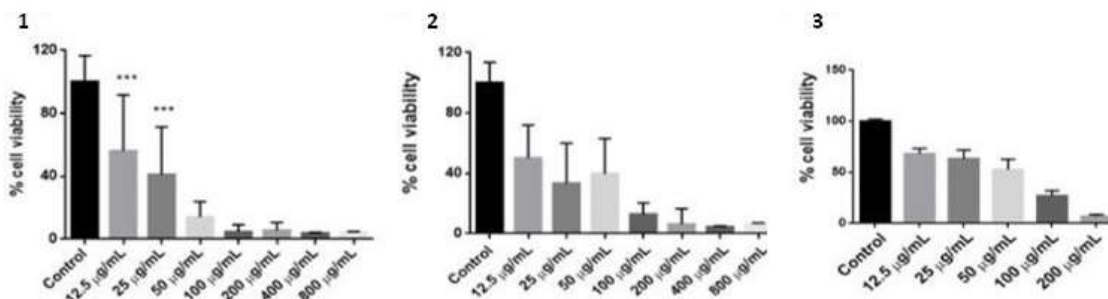
	Concentration (µg/mL) / mortality (dead nauplii)									LC ₅₀ (µg/mL)	IC 95% (µg/mL)
	50	75	100	125	150	200	500	750	1000		
Essential oil	-	1	-	-	-	1	3	3	4	>1000	-
Quinidine	13	16	25	26	28	-	-	-	-	142.27	122.48- 186.91

LC – lethal concentration; and IC – inhibitory concentration. (PROBIT test). Source: Authors (2020).

The cytotoxicity test results are presented in Figure 2. As shown in the first graph (1), the two initial concentrations tested (12.5 and 25 µg/mL) for the MCF7 cell line were not statistically significant, and the IC₅₀ value was 31.40. For the B16F10 cell line (2), the IC₅₀ was detected in the first concentration tested (12.5 µg/mL), with an IC₅₀ value of 99.39. The 3T3 cell line (3) was more resistant to the essential oil, showing an IC₅₀ from the concentration of 50 µg/mL, with an IC₅₀

value of 83.62. This cell line could be considered more selective in relation to this sample. The SI for the essential oil in the MCF7 and B16F10 cell lines was 2.66 and 0.84, respectively.

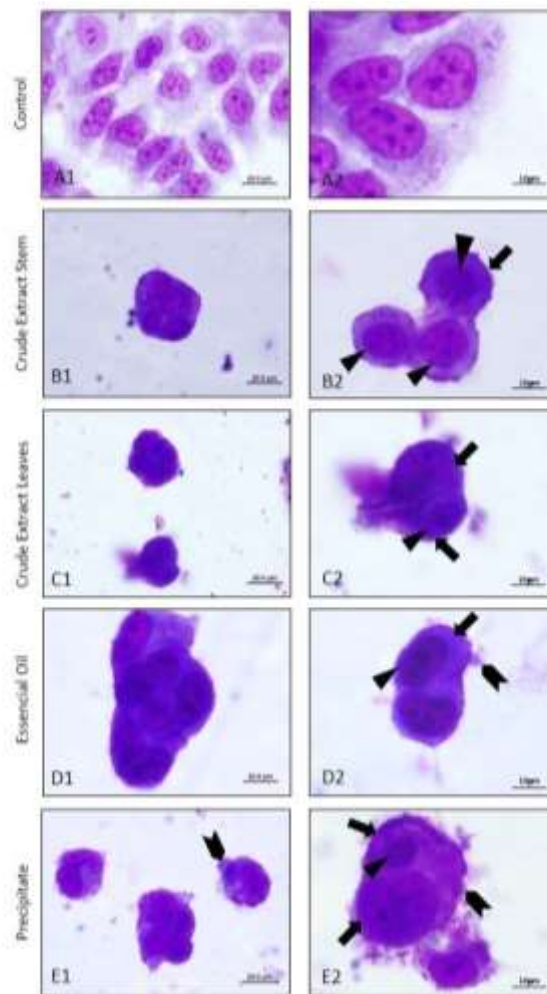
Figure 2. Viability of cell lines MCF7 (1), B16F10 (2), and 3T3 (3).



* Vertical bars represent mean (\pm SD).
Source: Authors (2020).

Figure 3 shows the morphological changes in the MCF7 cell line after being incubated with 50 $\mu\text{g/mL}$ of the *Cantinoa althaeifolia* essential oil for 24 h. It is possible to identify important morphological changes, such as chromatin condensation (\blacktriangle), cell rounding (\blackrightarrow), and bubble formation (\blacktriangleright). This is the first study to evaluate the cytotoxic activity of this essential oil. The root extract of *Prunella vulgaris*, a species also from the Lamiaceae family, reportedly has potent anticancer effects in MCF7 human BC cells both *in vitro* and *in vivo*, being able to induce apoptosis, inhibit angiogenesis, arrest cell cycle, and modulate the PI3K/AKT signaling pathway (Gao & Xu, 2019).

Figure 3. Morphological analysis of MCF7 cells.



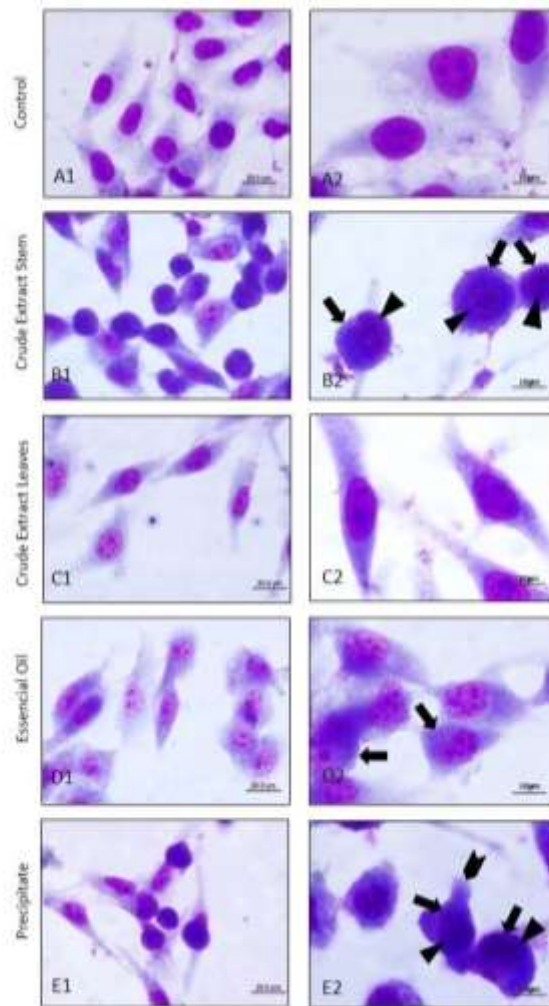
(Magnification: 1000 x (left column) and 2000 x (right column)).

*Chromatin condensation (▲), cell rounding (⇨), and bubble formation (◼).

Source: Authors (2020).

Figure 4 displays the morphological changes found in the murine melanoma tumor line, B16F10, after being incubated with 50 $\mu\text{g/mL}$ of the *Cantinoa althaeifolia* essential oil for 24 h. It is also possible to notice chromatin condensation (▲), roundness (⇨), and bubble formation (◼). Shakeri et al. (2019) tested the activity of *Stachys parviflora*, another species from the Lamiaceae family, against human ovarian carcinoma cells (A2780), human colon carcinoma cells (HCT), and mouse melanoma cells (B16F10), and also obtained a strong cytotoxic effect (Shakeri et al., 2019).

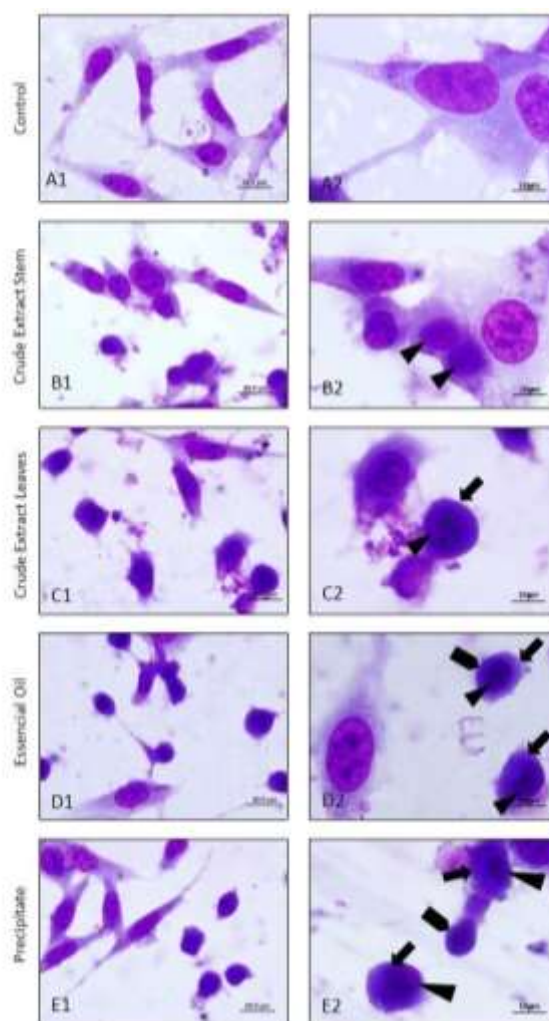
Figure 4. Morphological analysis of B16F10 cells.



(Magnification: 1000 x (left column) and 2000 x (right column)).
*Chromatin condensation (▲), cell rounding (⇨), and bubble formation (◼).
Source: Authors (2020).

Figure 5 presents the morphological changes found in non-tumor murine fibroblasts (3T3), after being incubated with 50 µg/mL of the essential oil of *Cantinoa althaeifolia* for 24 h. Once again, it was possible to detect condensation of chromatin (▲), cell rounding (⇨), and possible apoptotic body (◼).

Figure 5. Morphological analysis of murine 3T3 fibroblasts.



(Magnification: 1000 x (left column) and 2000 x (right column)).

*Chromatin condensation (▲), cell rounding (➡), and bubble formation (●).

Source: Authors (2020).

The findings from the lethality test of *Artemia salina* combined with the results from the cytotoxicity test with MTT are promising because they confirm that the oil is only toxic against the tumor cell lines. These findings corroborate with reports that oils with terpenes and sesquiterpenes in their chemical composition have antioxidant and antitumor activities (Morshedloo, Quassinti, Bramucci, Lupidi, & Maggi, 2017).

The literature reports that some constituents found in the essential oil of *Turkish Origanum onites*, also from the Lamiaceae family, have activity against parasitic diseases (Tasdemir, Kaiser, Demirci, Demirci, & Baser, 2019). According to Battistini et al. (2019), the chemical constituents of the essential oils of several fruits from the Lamiaceae family have activity against the hepatitis A virus, and several of these constituents were found in the present study (Battistini et al., 2019).

4. Conclusion

Although there is no marked bactericidal activity, the *Cantinoa althaeifolia* essential oil has shown considerable antimicrobial activity against all the tested microorganisms, indicating that this plant can be a promising treatment for various infections.

The *Cantinoa althaeifolia* essential oil has cytotoxic activity against the tested cancer cell lines, especially against

melanoma cells. The decrease in the number of cells, morphological changes, and increase in the sub-G0 phase may suggest that the mechanism involved in this effect may be related to apoptosis. The selectivity index showed that the tested samples were less toxic to blood cells, suggesting that this compound could be a promising drug in the treatment of melanoma and human breast cancer.

Further studies, especially *in vivo*, are necessary to confirm that this plant can be an alternative for enhancing antineoplastic therapy. The susceptibility of different microorganisms and different strains could also be an interesting field to explore in the future.

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