UFSM

CIÊNCIA e NATURA

ISSN 2179-460X
OPEN ACCESS

Ci. e Nat., Santa Maria, e87996, v. 47, 2025 • https://doi.org/10.5902/2179460X87996
Submitted: 24/06/2024 • Approved: 01/04/2025 • Published: 17/04/2025

Chemistry

Anticancer activity of *Citrus limon* (L.) Burm. f. and *Citrus sinensis* (L.) Osbeck essential oil and their nanoemulsions

Atividade anticâncer do óleo essencial de *Citrus limon* (L.) Burm. f. e *Citrus sinensis* (L.) Osbeck e suas nanoemulsões

Gustavo Oliveira Everton[I], Brendha Araújo de Sousa[I], Victor Elias Mouchrek Filho[I], Odair dos Santos Monteiro[I]

[I] Universidade Federal do Maranhão, São Luís, MA, Brazil

ABSTRACT

This article presents the chemical composition, antioxidant, and anticancer activity in vitro of *Citrus sinensis* and *Citrus limon* essential oil and their nanoemulsions (NEOs). Leaves of *C. sinensis* and bark of *C. limon* were collected in the state of Maranhão, and their essential oils were extracted by hydrodistillation. The compounds present in the EOs and NEOs were identified and quantified by GC-MS. The NEOs were formulated by the phase inversion method. The antioxidant activity was performed by the elimination assays of radicals ABTS, DPPH, superoxide, hydrogen peroxide, and hydroxyl. The anticancer activity in vitro was evaluated against tissue cells: colon, lung, liver, cervix, prostate, oral, and neuroblastoma. In the EO of *C. sinensis*, limonene was identified as the major compound, and in the EO of *C. limon* also through the GC-MS assay. The NEOs of *C. sinensis* and *C. limon* obtained were stable, respectively, with a droplet size of 69.12; 71.66 nm, zeta potential -20.11; -21.55 mV, polydispersion index of 0.26; 0.28 and pH 4.88; 4,12. EOs and NEOs showed strong antioxidant activity with IC_{50} ranging from 6.23 to 159.02 µg/mL. They also showed significant cytotoxic activity against all cell lines used in the study, with the greatest effects against the cell line 502713 (colon), IMR-32 (neuroblastoma), Hep-2 (liver) and SiHa (cervix) with IC_{50} values of 1.59; 1,75; 1.91 and 2.54 µg/mL, respectively. These findings suggest that the EOs and NEOs of *C. sinensis* and *C. limon* may be alternatives for innovative therapies.

Keywords: Cells; Chemical compounds; Free radicals

RESUMO

Este artigo apresenta a composição química, atividade antioxidante e anticâncer in vitro do óleo essencial de *Citrus sinensis* e *Citrus limon* e suas nanoemulsões (NOEs). Folhas de *C. sinensis* e cascas de *C. limon* foram coletadas no estado do Maranhão e seus óleos essenciais foram extraídos por hidrodestilação. Os compostos presentes nos OEs e NOEs foram identificados e quantificados por CG-

EM. As NOEs foram formuladas por método de inversão de fases. A atividade antioxidante foi executada pelos ensaios de eliminação de radiciais ABTS, DPPH, superóxido, peroxido de hidrogênio e hidroxila. A atividade anticâncer in vitro foi avaliada frente a células de tecido: cólon, pulmão, fígado, colo do útero, próstata, oral e neuroblastoma. No OE de *C. sinensis* foi identificado o limoneno como composto majoritário e no OE de *C. limon* também através do ensaio de CG-EM. As NOEs de *C. sinensis* e *C. limon* obtidas foram estáveis, respectivamente, com tamanho de gota de 69,12; 71,66 nm, potencial zeta -20,11; -21,55 mV, índice de polidispersão de 0,26; 0,28 e pH 4,88; 4,12. Os OEs e NEOs apresentaram atividade antioxidante forte com IC_{50} variando de 6,23 a 159,02 μg/mL. Eles também apresentaram atividade citotóxica significativa frente a todas as linhagens celulares utilizadas no estudo, sendo os maiores efeitos frente a linhagem celular 502713 (cólon), IMR-32 (neuroblastoma), Hep-2 (fígado) e SiHa (colo do útero) com valores de IC_{50} de 1,59; 1,75; 1,91 e 2,54 μg/mL, respectivamente. Estas descobertas sugerem que os OEs e NEOs de *C. sinensis* e *C. limon* podem ser alternativas para terapias inovadoras.

Palavras-chave: Células; Compostos químicos; Radicais livres

1 INTRODUCTION

Cancer is the second leading cause of death worldwide, accounting for 9.6 million deaths in 2018, making it a significant health concern worldwide (Siegel et al., 2020). As one of the world's deadliest diseases, cancer arises primarily from genetic mutations, which can be exacerbated by other carcinogens. These genetic and cancerous mutations disrupt cellular functions and metabolism, leading to uncontrolled replication and spread of cancer cells. Cancer cells grow and multiply rapidly, forming clumps that invade surrounding normal cells (Hassanpour & Dehghani, 2017).

The primary goal of cancer treatment is to selectively target and kill tumor cells while sparing normal cells. To achieve this, researchers focus on increasing drug efficacy, improving target specificity, and reducing immunosuppression and drug resistance. Cancer treatments can be broadly categorized into two approaches: directly targeting tumor cells and targeting immune cells (Ju et al., 2023).

Natural products of various terrestrial and marine microorganisms and macroorganisms continue to play a crucial role in drug discovery, including the development of new oncological agents. Notably, approximately 50% of the 175 small molecule anticancer drugs approved in Western medicine between 1940 and 2014

were derived directly from micro- and macroorganisms or synthesized from natural molecules (Newman & Cragg, 2016).

Phytochemical screening of plant species revealed important bioactive compounds that contribute to their therapeutic potential. Essential oils and flavonoids are among the most significant compounds, exhibiting pronounced therapeutic and pharmacological activities (Roriz et al., 2014; Zannou et al., 2015). Qualitative and quantitative analysis of these compounds can provide valuable insights into the therapeutic potency of the plant (Oladeji, Odelade, & Oloke, 2020). Frutas cítricas, incluindo laranjas doces e limões, têm atividade anticancerígena (Chidambara Murthy et al., 2012).

Driven by consumer demand for their health benefits, refreshing aroma, and appealing taste (Vashisth & Kadyampakeni, 2020), citrus fruits are now cultivated in over 80 countries according to the Food and Agriculture Organization of the United Nations. The most prevalent variety is *Citrus sinensis* – the sweet orange – which accounts for about 70% of all citrus production (Favela-Hernández et al., 2016). *Citrus limon*, the lemon, is third in terms of cultivation and is a common ingredient in both food and beverages for flavoring, and also for its preservative qualities (Di Matteo et al., 2021).

Naturally-derived antioxidants from plant sources offer significant health benefits by mitigating the damage caused by oxidative species (Guo et al., 2020). While synthetic antioxidants were favored for a period due to their greater availability, lower cost, and improved stability and performance, the long-term consumption of these synthetic compounds has been associated with health concerns. These include an elevated risk of cancer, gastrointestinal issues, and skin allergies. Furthermore, the environmental impact of these synthetic chemicals remains poorly understood (Lourenço et al., 2019). As a result, the exploration of natural antioxidant sources has become a focus of considerable research.

This study offers a novel examination of the biotechnological profile of nanoemulsions formulated using the low-energy method with essential oils from *C.*

limon and *C. sinensis*, focusing on their chemical and biological activities, including antioxidant and anticancer properties.

2 METHODOLOGY

2.1 Collection and identification of plant material

Bark from *C. limon* and leaves from *C. sinensis* were collected in São Luís, MA, Brazil, during the morning shift in July 2022. All species were identified by the Herbarium of Maranhão (UFMA). The plant materials were then transported to the Laboratory for Research and Application of Essential Oils (LOEPAV/UFMA) at the Federal University of Maranhão (UFMA). Subsequently, they were crushed, and their mass was measured for yield calculations (Farmacopeia, 2019).

2.2 Extraction of essential oils

Hydrodistillation was employed to extract the essential oil using a glass Clevenger extractor connected to a round-bottom flask, which was heated with an electric blanket. Distilled water was used as the solvent in a 1:10 ratio. The hydrodistillation was carried out at 100°C for 2 hours and 30 minutes. The extracted essential oil was then collected, dried using anhydrous sodium sulfate (Na_2SO_4) through percolation, and centrifuged. The samples will be stored in amber glass vials in a refrigerator at 4°C (Farmacopeia, 2019). Triplicate assays were conducted.

2.3 GC-FID-MS analysis and identification of bioactive compounds

Chemical constituents were identified using Gas Chromatography-Mass Spectrometry (GC-MS) with a QP 2010 Plus instrument (Shimadzu, Kyoto, Japan) on a fused silica capillary column (30 m × 0.25 mm) with a DB-5 phase (0.25 µm thickness). Helium served as carrier gas at a flow rate of 1.0 mL/min. The injector and detector were set at 220°C and 20°C, respectively. The sample injection volume was 0.5 µL,

diluted in hexane (1%), with a split ratio of 1:100. The temperature ramp started at 60°C, increasing 3°C/min to 20°C, then 10°C/min until reaching 300°C, held for 7 minutes. Column pressure was about 71.0 kPa.

The mass spectrometer operated at 70 eV ionization potential and 200°C ion source temperature. Mass analysis was conducted in full scan mode (5-500 Da) with a scan rate of 1000 Da/s and 0.5 fragments/s interval. Data were processed using Lab Solutions LC/GC Workstation 2.72 (Shimadzu, Kyoto, Japan).

Retention indices for the compounds were calculated based on n-alkanes (nC9-nC18) using the Van den Dool and Kratz (1963) equation. Compound identification was based on calculated retention rates compared to literature values (Adams, 2017) and mass spectra compared with libraries FFNSC 1.2, NIST107, and NIST21.

Quantitative analysis was performed using Gas Chromatography with a Flame Ionization Detector (GC-FID) on a GC-2010 instrument (Shimadzu, Kyoto, Japan), maintaining the same conditions as the qualitative analysis, except for a detector temperature of 300°C. Relative percentages of each compound were determined by the area normalization method.

2.4 Preparation and characterization of the droplet size of nanoemulsions

Oil-in-water (O/W) nanoemulsions were prepared using an adapted method based on the work of Sugumar et al. (2014), Costa et al. (2014), and Rodrigues et al. (2014), employing a low-energy phase inversion technique. The formulations comprised essential oils (EOs), nonionic surfactants, and distilled water. A stable composition was achieved, containing EOs at concentrations of 2-5% and active surfactants (Tween 20 and Tween 80) at 1-3%, through a factorial design resulting in a total of 156 formulations for each essential oil.

Final homogenization was conducted under constant agitation at 6,000 rpm until the temperature decreased to 25°C ± 2°C (with sonication for 10 minutes). To assess stability, the formulations underwent various stress tests, including centrifugation,

thermal cycling, and freeze-thaw cycles, in accordance with the method established by Shafiq et al. (2007).

The particle size distributions and polydispersity indices of the nanoemulsions were analyzed using a dynamic light scattering instrument (Zetasizer Nano ZS). This device determines particle size by measuring intensity fluctuations of a 632.8 nm laser beam scattered at a 90° angle across the sample. Each measurement was an average of 13 individual runs. To minimize multiple scattering effects, samples were diluted with distilled water in a 1:100 ratio prior to analysis. Measurements were conducted at regular intervals: immediately following emulsion synthesis and again after 14, 21, and 28 days of storage.

2.5 DPPH Radical Scavenging Antioxidant Activity (2,2-Diphenyl-1-picrylhydrazyl)

The method used to evaluate the antioxidant capacity of essential oils (EOs) and NEOs was adapted from the protocol established by Brand-Williams, Cuvelier and Berset (1995). Specifically, 50 µL of essential oil solutions and nanoemulsions were combined with 950 µL of ethanol and 2 mL of DPPH radical solution, resulting in a total volume of 3 mL. This mixture was homogenized and allowed to react in the dark for 30 minutes. The absorbance of the samples was subsequently measured using a UV-Vis spectrophotometer at 540 nm. The reduction of the DPPH radical was expressed as a percentage, and the 50% inhibitory concentration (IC_{50}), indicating the concentration required to inhibit 50% of the radical, was reported in µg/mL.

2.6 ABTS Radical Scavenging Antioxidant Activity (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)

The determination of antioxidant activity by the ABTS method [2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] was adapted from Re et al. (1999). Based on the concentrations of EOs and NEOs (5-150 µg/mL), the reaction mixture was

prepared with ABTS radical cation. In a dark environment, a 30 µL aliquot containing 3.0 mL of ABTS radical cation was transferred from each sample concentration and homogenized in a stirrer tube, and after 6 minutes the absorbance of the reaction mixture was read together with a spectrophotometer. 760nm. Tests were performed in triplicate. The elimination of the ABTS radical was expressed as a percentage and the inhibitory concentration of 50% (IC_{50}) capable of preventing the elimination of 50% was expressed in µg/mL.

2.7 Hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of EOs and NEOs was performed by measuring the hydroxyl radicals generated from the Fe^{3+}/ascorbate/EDTA/H_2O_2 system (Kunchandy & Rao, 1990). The attack of the hydroxyl radical on deoxyribose leads to the formation of thiobarbituric acid reactive substances (TBARS) (Ohkawa; Ohishi; Yagi, 1979). Concentrations of EOs and NOEs of 5-150 µg/mL (in n-hexene) were added to a reaction mixture containing 3.0 mM deoxyribose, 0.1 mM FeCl3, 0.1 mM EDTA, 0.1 mM ascorbic acid, 1 mM H_2O_2 and 20 mM phosphate buffer (pH 7.4), in a final volume of 3.0 mL. The reaction mixture was incubated at 37 °C for 1h. Then, 1 mL of thiobarbituric acid (TBA, 1%) and 1.0 mL of trichloroacetic acid (TCA, 2.8%) were added to the test tubes, where they were incubated at 100 °C for 20 min. After cooling the mixtures, absorbance was measured at 532 nm. The scavenging activity of hydroxyl radicals was expressed as a percentage and the inhibitory concentration of 50% (IC_{50}) capable of preventing the elimination of 50% was expressed in µg/mL.

2.8 Superoxide radical inhibition activity

The superoxide radical inhibition activity of EOs and NEOS was carried out from the generation of the superoxide radical by the xanthine/xanthine oxidase system, determined spectrophotometrically by monitoring the production of nitrotetrazolium

blue (NBT) (Robak; Gryglewski, 1988). Concentrations of EOs and NOEs of 5-150 µg/mL (in n-hexene) were added to a reaction mixture containing 2 nM xanthine, 12 nM NBT, 1.0 µg/mL xanthine oxidase and 0.1 M phosphate buffer (pH 7.4), making a final volume of 2.0 mL. After incubation of the mixture at 25 °C for 10 min, the absorbance was read at 560 nm and compared with the control samples in which the enzyme was not included.

2.9 Hydrogen peroxide scavenging activity

The ability of EOs and NEOS to sequester hydrogen peroxide has been determined spectrophotometrically as described by Ruch (1989). For this, a hydrogen peroxide solution (2 mM) was prepared in 0.17 M phosphate buffer (pH 7.4). Various concentrations of the samples (in methanol) were added to the reaction mixture containing 2 mM hydrogen peroxide. After 10 min of incubation at room temperature, the absorbance was read against a blank at 230 nm.

2.10 Anticancer activity

The human cancer cell lines used in this study were: colon (HT-29, HCT-15, SW-620, 502713), lung (A549, HOP-62, H-226), cervical cells (SiHa) and oral cells (KB), prostate (DU-145), cultured and maintained in RPMI-1640 medium (pH 7.4), while MEM for neuroblastoma (IMR-32) and liver (Hep-g-2). The medium was supplemented with 10% fetal calf serum, glutamine (2 mM), penicillin (100 units/mL) and streptomycin (100 µg/mL). Cell cultures were grown in a CO_2 incubator (Heraeus, GmbH, Germany) at 37°C, 90% moisture and 5% CO_2 as described (Samanta et al., 2005; Sharma et al., 2009).

A stock solution of the essential oils (10%, v/v) was prepared in DMSO and the nanoemulsions were serially diluted in culture medium to obtain the desired concentration. Cytotoxicity at concentrations ranging from 0.89 to 890 µg/mL was determined by a semi-automated assay (Skehan, 1990) using sulforodamine-B (SRB)

as previously described (Samanta et al., 2005). Untreated control cultures received only vehicle (DMSO, <0.1%). Results are reported as IC_{50} values.

3 RESULTS AND DISCUSSION

Table 1 presents the chemical constituents identified by GC-FID-MS in the EO of *C. sinensis* and its nanoemulsion.

According to Table 1, 20 components were identified in the EO of *C. sinensis* and 15 components in its nanoemulsion. Significant amounts of monoterpene hydrocarbons were found in C*. sinensis* EO and its nanoemulsion. Limonene was obtained as the major component of the composition of the essential oil and its nanoemulsion.

Similar to the results of Ferronato & Rossi (2018), who identified 12 components, the *C. sinensis* essential oil was found to be predominantly composed of limonene (91.4%), followed by β-myrcene (2.47%) and linalool (1.58%). Matuka et al. (2020) identified sabinene (20.4%), terpinen-4-ol (13.2%), linalool (7.6%), limonene (7.5%) and δ-3-Carene (7.5%) as the most abundant components in essential oils collected in South Africa.

Kammoun et al. (2021) reported differing results, finding forty-seven compounds in their study. Their study suggests differences in essential oil composition, with results varying based on the *Citrus sinensis* cultivars used. The study noted that the main monoterpene hydrocarbons identified include sabinene (8.25%–28.81%), 2-carene (11.25%–16.72%), cis-β-ocimene (10.22%–13.93%), d-limonene (6.52%–11.99%) and γ-terpinene (2%–4.54%). They found β-citronellal (0.28%–7.70%), terpinen-4-ol (2.99%–6.63%), β-myrcene (3.37–5.6%), and linalool (0.17–5.29%) as the main oxygenated monoterpenes.

Table 1 – Chemical constituents identified by GC-FID-MS in the essential oil of *C. sinensis* and its nanoemulsion

n	Chemical constituents	RIexp	RIcalc	EO C. *sinensis* (%)	NEO C. *sinensis* (%)
1	α-Thujene	924	921	0,46	-
2	α-Pinene	930	932	1,18	0,89
3	Sabinene	971	973	0,91	0,59
4	β-Myrcene	991	993	2,30	1,59
5	Octanal	1004	1006	0,76	0,74
6	Limonene	1007	1009	85,28	90,82
7	β-Ocimene	1053	1051	0,68	0,66
8	γ-Terpinene	1063	1065	1,63	1,61
9	Terpinolene	1089	1093	0,50	0,04
10	Linalool	1104	1106	1,34	1,32
11	Nonanal	1106	1110	0,50	0,01
12	Citronellal	1159	1159	0,48	0,34
13	α-Terpineol	1199	1203	0,49	0,14
14	Decanal	1208	1206	0,63	0,49
15	Neral	1244	1248	0,48	0,24
16	Geranial	1273	1271	0,53	0,51
17	Neryl acetate	1365	1367	0,44	-
18	α-Copaene	1377	1374	0,46	-
19	Dodecanal	1417	1419	0,45	-
20	Germacrene D	1482	1486	0,50	-

Note: RIexp- Retention time experimental; RIcalc; Retention time calculated; Source: Authorship (2025)

Table 2 presents the chemical constituents identified by GC-FID-MS in the essential oil of *C. limon* and its nanoemulsion.

The composition of the *C. limon* essential oil and its nanoemulsion analyzed in this study contains only monoterpene hydrocarbons and oxygenated monoterpenes. In the composition of the essential oil and nanoemulsion, limonene was detected as the major component.

As shown in Table 2, 22 compounds were identified in the EO de *C. limon* and 15 compounds in this nanoemulsion. Chromatographic analysis of the EO of *C. limon* revealed limonene, γ-terpinene and β-pinene, confirming the data obtained by Benoudjit et al., (2020), who also described limonene (64.75%), γ-terpinene (11.72%)

and β-pinene (11.24%) as the main components of the EO of *C. limon* grown in northern Algeria.

Table 2 – Chemical constituents identified by GC-FID-MS in the essential oil of *C. limon* and its nanoemulsion

n	Chemical constituents	RI exp	RI calc	Content EO C. *limon* (%)	Content NEO *C. limon* (%)
1	α-Thujene	931	934	0,28	-
2	α-Pinene	938	941	0,76	-
3	Sabinene	973	976	0,35	-
4	β -Pinene	980	983	5,90	3,22
5	Myrcene	993	996	1,06	1,41
6	α-Terpinene	1012	1015	0,33	-
7	Limonene	1032	1035	65,12	70,20
8	β-Ocimene	1038	1036	0,25	0,60
9	γ-Terpinene	1057	1055	19,17	17,11
10	Terpinolene	1086	1084	0,39	0,74
11	Linalool	1098	1096	0,31	0,66
12	Citronelal	1148	1146	0,32	0,67
13	Terpinen-4-ol	1178	1176	0,50	-
14	α-Terpineol	1189	1191	0,49	0,84
15	Citronellol	1229	1231	0,45	-
16	Nerol	1236	1238	0,29	0,64
17	Neral	1242	1244	0,43	0,78
18	Geraniol	1255	1257	0,31	0,66
19	Geranial	1275	1277	0,74	0,88
20	Perilla alcohol	1297	1299	0,35	-
21	Neryl acetate	1370	1372	1,49	0,90
22	Geranyl acetate	1388	1390	0,78	0,72

Note: RIexp- Retention time experimental; RIcalc; Retention time calculated; Source: Authorship (2025)

Jaradat et al. (2024) reported inconsistent findings when quantifying thirty-six molecules, representing 100% of the total oil. Their analysis revealed that geranial and neral, a mixture of cis and trans isomers of citral (3,7-dimethyl-2,6-octadienal), along with limonene in smaller quantities, constituted the majority of the chemical composition. Furthermore, oxygenated monoterpenoids and hydrocarbon monoterpenes were

identified as the primary phytochemical groups in *C. limon*, accounting for 80.14% and 15.75%, respectively.

Table 3 presents the characterization of the essential oil nanoemulsion of *C. sinensis* and *C. limon*.

Table 3 – Characterization of the essential oil nanoemulsion of *C. sinensis* and *C. limon*

	NEO C. *sinensis*	**NEO C. *limon***
Droplet size (nm)	69,12±0,22	71,66±0,24
Zeta potential (mV)	-20,11±0,02	-21,55±0,02
Polydispersion Index	0,26±0,01	0,28±0,02
pH	4,88±0,45	4,12±0,48

Source: Authorship (2025)

The formulations showed stability for 30, 90 and 120 days, slightly negative zeta potential and acidic pH.

The nanoemulsions exhibited an average droplet size of 69.12 nm for *C. sinensis* and 71.66 nm for *C. limon*. The small droplet size observed likely resulted from the high surfactant content in the formulations, consistent with previous research showing that nanoemulsion droplet size decreases with a lower oil-to-surfactant ratio (Wang et al., 2009; Saberi et al., 2013; Gulotta et al., 2014; Li et al., 2017). Furthermore, the sonication process likely contributed significantly to these small droplet sizes, as it is known to reduce both droplet size and PDI (Donsì & Ferrari, 2016). Specifically, brief ultrasonication (e.g., 3 minutes) has been shown to substantially decrease droplet size (Lee et al., 2019).

Non-zero Zeta potential values are required to avoid repulsion forces between droplets and the negative character may be related to the presence of the nonionic surfactant (Ferreira et al., 2016). It is widely assumed that zeta potential values of -30 mV a + 30 mV characterize a stable system (Jenning et al., 2002).

The nanoemulsions exhibited a negative surface charge, which is consistent with EO-based nanoemulsions prepared using nonionic surfactants. These surfactants are known to impart a negative charge, likely due to their influence on the dispersed

phase surface (Acedo-Carrillo et al., 2006; Fernandes et al., 2014; Salvia-Trujillo et al., 2015; Hashem et al., 2018; Giunti et al., 2019).

The affinity between the surfactant and the oil may also contribute, as the absorption of negative ions (−OH) at the oil-water interface can vary, leading to differing zeta potentials (Zhao et al., 2010; Martins et al., 2012; Li et al., 2016; Salvia-Trujillo et al., 2015). Consequently, the zeta potentials, like particle size, differed depending on the plant species used, as seen in the *C. sinensis* formulation.

Table 4 shows the antioxidant activity of essential oils and nanoemulsions of *C. limon* and *C. sinensis*.

Table 4 – Antioxidant activity

	Method	IC50 µg/mL	IC90 µg/mL	R2
EO C. sinensis	ABTS	55.01	103.59	0.9999
	DPPH	60.99	109.95	0.9999
	Superoxide	19.43	36.53	0.9999
	Hydrogen peroxide	67.13	126.20	0.9997
	Hydroxyl	24.25	45.59	0.9999
NEO C. sinensis	ABTS	17.63	33.20	0.9999
	DPPH	19.55	35.24	0.9999
	Superoxide	6.23	11.71	0.9999
	Hydrogen peroxide	21.52	40.45	0.9999
	Hydroxyl	7.77	14.61	0.9991
EO C. limon	ABTS	69.32	130.52	0.9999
	DPPH	76.85	138.54	0.9999
	Superoxide	24.48	46.03	0.9999
	Hydrogen peroxide	84.58	159.02	0.9999
	Hydroxyl	30.55	57.44	0.9995
NEO C. limon	ABTS	41.02	77.23	0.9999
	DPPH	45.47	81.97	0.9999
	Superoxide	14.49	27.23	0.9996
	Hydrogen peroxide	50.05	94.09	0.9999
	Hydroxyl	18.08	33.99	0.9999

Source: Authorship (2025)

Although all the samples studied showed a good reducing capacity, the nanoemulsions exhibited a higher sensitivity to all antioxidant assays. Mainly for the superoxide and hydroxyl radical scavenging assay, being 19.43 µg/mL and 24.25 µg/mL, respectively, for C*. sinensis* NEO, and 6.23 µg/mL and 7.77 µg/mL for NEO, C. *sinensis.* For *C. limon* EO at IC_{50} of 19.43 µg/mL and 24.25 µg/mL, and for NEO *C. limon* at 14.49 µg/mL and 18.08 µg/mL for the superoxide and hydroxyl assay, respectively.

Unlike our study, Othman et al., (2022) reported a low antioxidant capacity of *C. limon* essential oil with an IC_{50} of 29.14 mg/mL for the DPPH assay. Farahmandfar et al., (2019) also evaluated the oil from the fresh peels of *C. sinensis* and found an IC_{50} of 7.86 mg/mL for the DPPH assay. According to Denkova-Kostova et al. (2021), these activities can be attributed to the beta-pinene and limonene present in the essential oil.

Table 5 presents the influence of essential oils and nanoemulsions under analysis in this study on the proliferation of several human cancer cell lines.

Table 5 – Influence of essential oils and nanoemulsions on the proliferation of various human cancer cell lines

Tissue/ Cell Line	**IC50 µg/mL C. *sinensis***		**IC50 µg/mL C. *limon***	
	OE	**NEO**	**OE**	**NEO**
Colon HT-29	C. limon	16.38±0.02	48.28±0.09	25.11±0.07
Colon HCT-15	OE	NEO	OE	NEO
Colon SW-620	20.88±0.02	10.81±0.01	32.01±0.07	16.58±0.06
Colon 502713	3.12±0.01	1.59±0.01	4.78±0.01	2.44±0.01
Colon H-226	45.62±0.06	23.69±0.03	69.95±0.11	36.33±0.08
Lung A-549	36.92±0.05	19.24±0.02	56.62±0.10	29.50±0.07
Lung Hop-62	58.70±0.08	30.53±0.04	90.02±0.13	46.82±0.09
Liver Hep-2	3.56±0.01	1.91±0.01	5.46±0.06	2.93±0.06
Cervix SiHa	4.83±0.01	2.54±0.01	7.41±0.01	3.90±0.01
Prostate DU-145	30.77±0.04	16.06±0.02	47.18±0.09	24.63±0.07
Oral KB	37.75±0.05	19.56±0.02	57.89±0.10	29.99±0.07
Neuroblastoma IMR-32	3.50±0.01	1.75±0.01	5.36±0.06	2.68±0.06

Source: Authorship (2025)

Among the human cancer cell lines analyzed, treatment showed a significant concentration-dependent inhibition of cell growth. The IC_{50} values for EOs and NEOs ranged from 1.59 to 69.95 µg/mL.

The observed carcinogenic activity is attributed to the presence of limonene, as it is a key molecule in the activation of the apoptotic pathway in the tumor animal model and tumor cell lines (Jia et al., 2013; Hafidh et al., 2018; Ye et al., 2020). These results help establish limonene as a potent pro-apoptotic agent, making it an important therapeutic target.

Oral administration of limonene in humans is well tolerated at a low dose (lethal dose is estimated to be 0.5-5 g/kg) (Vigushin et al., 1998) supporting its investigation as a potential bioactive for cancer prevention at pharmacological doses, despite a risk of metabolite allergy (Mukhtar et al., 2018). Limonene has been shown to interfere with apoptosis pathways, cell cycle/proliferation, angiogenesis, and DNA damage repair (Hafidh et al., 2018), suggesting a pleiotropic pharmacological activity targeting several signaling pathways.

4 CONCLUSIONS

In conclusion, the compounds identified are described and confirmed in the literature for the species under study. The NEOs showed a greater effect than their individual EOs, and their characterization affirmed their stability. Antioxidant activity was duly observed with active effects for both cases. Finally, the data indicated induced differential cytotoxicity in vitro in 12 human cancer cell lines. These findings suggest that EOs and NEOs of *C. sinensis and C. limon* may be strong alternatives for innovative therapies.

ACKNOWLEDGEMENTS

Federal University of Maranhão, the Laboratory for Research and Application of Essential Oils (LOEPAV/UFMA) and FAPEMA.

REFERENCES

Acedo-Carrillo, J. I., Rosas-Durazo, A., Herrera-Urbina, R., Rinaudo, M., Goycoolea, F. M., & Valdez, M. A. (2006). Zeta potential and drop growth of oil in water emulsions stabilized with mesquite gum. *Carbohydrate Polymers*, *65*(3), 327-336.

Adams, R. P. (2017). *Identification of essential oil components by gas chromatogramphy/mass spectrometry*. 5th ed. Texensis Publishing.

Benoudjit, F., Maameri, L., & Ouared, K. (2020). Evaluation of the quality and composition of lemon (Citrus limon) peel essential oil from an Algerian fruit juice industry. *Algerian Journal of Environmental Science and Technology*, *6*(4), 1575-1581.

Brand-Williams, W., Cuvelier, M. E., & Berset, C. L. W. T. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT-Food science and Technology*, *28*(1), 25-30.

Chang, Y., McLandsborough, L., & McClements, D. J. (2013). Physicochemical properties and antimicrobial efficacy of carvacrol nanoemulsions formed by spontaneous emulsification. *Journal of agricultural and food chemistry*, *61*(37), 8906-8913.

Costa I. C., Rodrigues R. F., Almeida F. B., Favacho H. A., Falcão D. Q., Ferreira A. M., Vilhena J. C. E., Florentino A. C., Carvalho J. C. T., & Fernandes C. P. (2014). Development of jojoba oil (Simmondsia chinensis (Link) CK Schneid.) based nanoemulsions. *Lat. Am. J. Pharm*, *33*(3), 459-63.

Denkova-Kostova, R., Teneva, D., Tomova, T., Goranov, B., Denkova, Z., Shopska, V., Slavchev, A., & Hristova-Ivanova, Y. (2021). Chemical composition, antioxidant and antimicrobial activity of essential oils from tangerine (Citrus reticulata L.), grapefruit (Citrus paradisi L.), lemon (Citrus lemon L.) and cinnamon (Cinnamomum zeylanicum Blume). *Zeitschrift für Naturforschung C*, *76*(5-6), 175-185.

Matteo, A., Simeone, G. D. R., Cirillo, A., Rao, M. A., & Di Vaio, C. (2021). Morphological characteristics, ascorbic acid and antioxidant activity during fruit ripening of four lemon (Citrus limon (L.) Burm. F.) cultivars. *Scientia Horticulturae*, *276*, 109741.

Donsì, F., & Ferrari, G. (2016). Essential oil nanoemulsions as antimicrobial agents in food. *Journal of biotechnology*, *233*, 106-120.

Farhat, I., Hammami, M., Cherif, M., & Nasraoui, B. (2020). Chemometric analysis of geographic origins and compositions of Citrus sinensis (L.) Osbeck var 'Maltaise demi sanguine'essential oil. *Journal of Essential Oil Research*, *32*(3), 216-226.

Farmacopeia, C. (2019). Farmacopeia Brasileira. 6th ed. Agência Nacional de Vigilância Sanitária.

Favela-Hernández, J. M. J., González-Santiago, O., Ramírez-Cabrera, M. A., Esquivel-Ferriño, P. C., & Camacho-Corona, M. D. R. (2016). Chemistry and pharmacology of Citrus sinensis. *Molecules*, *21*(2), 247.

Fernandes, C. P., Almeida, F. B. de., Silveira, A. N., Gonzalez, M. S., Mello, C. B., Feder, D., Apolinário, R., Santos, M. G., Carvalho, J. C. T., Tietbohl, L. A. C., Rocha, L., & Falcão, D. Q. (2014). Development of an insecticidal nanoemulsion with Manilkara subsericea (Sapotaceae) extract. *Journal of Nanobiotechnology*, *12*(22), 1-9.

Ferreira, L. M., Sari, M. H. M., Cervi, V. F., Gehrcke, M., Barbieri, A. V., Zborowski, V. A., Beck, R. C. R., Nogueira, C. W., & Cruz, L. (2016). Pomegranate seed oil nanoemulsions improve the photostability and in vivo antinociceptive effect of a non-steroidal anti-inflammatory drug. *Colloids and Surfaces B: Biointerfaces*, *144*, 214-221.

Giunti, G., Palermo, D., Laudani, F., Algeri, G. M., Campolo, O., & Palmeri, V. (2019). Repellence and acute toxicity of a nano-emulsion of sweet orange essential oil toward two major stored grain insect pests. *Industrial Crops and Products*, *142*, 111869.

Gulotta, A., Saberi, A. H., Nicoli, M. C., & McClements, D. J. (2014). Nanoemulsion-based delivery systems for polyunsaturated (ω-3) oils: formation using a spontaneous emulsification method. *Journal of agricultural and food chemistry*, *62*(7), 1720-1725.

Guo, C., Shan, Y., Yang, Z., Zhang, L., Ling, W., Liang, Y., Ouyang, Z., Zhong, B., & Zhang, J. (2020). Chemical composition, antioxidant, antibacterial, and tyrosinase inhibition activity of extracts from Newhall navel orange (Citrus sinensis Osbeck cv. Newhall) peel. *Journal of the Science of Food and Agriculture*, *100*(6), 2664-2674.

Hafidh, R. R., Hussein, S. Z., MalAllah, M. Q., Abdulamir, A. S., & Abu Bakar, F. (2018). A high-throughput quantitative expression analysis of cancer-related genes in human HepG2 cells in response to limonene, a potential anticancer agent. *Current cancer drug targets*, *18*(8), 807-815.

Hashem, A. S., Awadalla, S. S., Zayed, G. M., Maggi, F., & Benelli, G. (2018). Pimpinella anisum essential oil nanoemulsions against Tribolium castaneum—insecticidal activity and mode of action. *Environmental Science and Pollution Research*, *25*(19), 18802-18812.

Hassanpour, S. H., & Dehghani, M. (2017). Review of cancer from perspective of molecular. *Journal of cancer research and practice*, *4*(4), 127-129.

Jaradat, N., Hawash, M., Abualhasan, M., Al-Maharik, N., Qadi, M., Qabaha, R., Qassarwi, S., Issa, L., Makhamov, T., Ergasheva, N., & Sattarov, A. (2024). Chemical analysis and bioactivity evaluation of citrus limon leaves volatile oil from palestine: investigating phytochemical, anti-inflammatory, antimicrobial, and cytotoxic properties. *Journal of Herbal Medicine*, *48*, 100954.

Jenning, V., Lippacher, A., & Gohla, S. H. (2002). Medium scale production of solid lipid nanoparticles (SLN) by high pressure homogenization. *Journal of microencapsulation*, *19*(1), 1-10.

Jia, S. S., Xi, G. P., Zhang, M., Chen, Y. B., Lei, B. O., Dong, X. S., & Yang, Y. M. (2013). Induction of apoptosis by D-limonene is mediated by inactivation of Akt in LS174T human colon cancer cells. *Oncology reports*, *29*(1), 349-354.

Ju, H., Yu, C., Zhang, X. D., Liu, W., Wu, Y. C., Gong, P. X., Li, H.-H., Liu, Y., & Li, H. J. (2023). Recent trends in anti-cancer activities of terrestrial plants-based polysaccharides: A review. *Carbohydrate Polymer Technologies and Applications*, *6,* 100341.

Kammoun, A. K., Altyar, A. E., & Gad, H. A. (2021). Comparative metabolic study of Citrus sinensis leaves cultivars based on GC–MS and their cytotoxic activity. *Journal of Pharmaceutical and biomedical Analysis*, *198*, 113991.

Kunchandy, E., & Rao, M. N. A. (1990). Oxygen radical scavenging activity of curcumin. *International journal of pharmaceutics*, *58*(3), 237-240.

Lee, J. Y., Garcia, C. V., Shin, G. H., & Kim, J. T. (2019). Antibacterial and antioxidant properties of hydroxypropyl methylcellulose-based active composite films incorporating oregano essential oil nanoemulsions. *LWT*, *106*, 164-171.

Li, J., Chang, J. W., Saenger, M., & Deering, A. (2017). Thymol nanoemulsions formed via spontaneous emulsification: Physical and antimicrobial properties. *Food chemistry*, *232*, 191-197.

Li, X., Qin, Y., Liu, C., Jiang, S., Xiong, L., & Sun, Q. (2016). Size-controlled starch nanoparticles prepared by self-assembly with different green surfactant: The effect of electrostatic repulsion or steric hindrance. *Food chemistry, 199*, 356-363.

Lourenço, S. C., Moldão-Martins, M., & Alves, V. D. (2019). Antioxidants of natural plant origins: From sources to food industry applications. *Molecules*, *24*(22), 4132.

Manzur, M., Luciardi, M. C., Blázquez, M. A., Alberto, M. R., Cartagena, E., & Arena, M. E. (2023). Citrus sinensis essential oils an innovative antioxidant and antipathogenic dual strategy in food preservation against spoliage bacteria. *Antioxidants*, *12*(2), 246.

Martins, S., Tho, I., Souto, E., Ferreira, D., & Brandl, M. (2012). Multivariate design for the evaluation of lipid and surfactant composition effect for optimisation of lipid nanoparticles. *European journal of pharmaceutical sciences, 45*(5), 613-623.

Matuka, T., Oyedeji, O., Gondwe, M., & Oyedeji, A. (2020). Chemical composition and in vivo anti-inflammatory activity of essential oils from Citrus sinensis (L.) osbeck growing in South Africa. *Journal of Essential Oil Bearing Plants*, *23*(4), 638-647.

Mukhtar, Y. M., Adu-Frimpong, M., Xu, X., & Yu, J. (2018). Biochemical significance of limonene and its metabolites: future prospects for designing and developing highly potent anticancer drugs. *Bioscience Reports*, *38*(6), BSR20181253.

Newman, D. J., & Cragg, G. M. (2016). Natural products as sources of new drugs from 1981 to 2014. *Journal of natural products*, *79*(3), 629-661.

Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, *95*(2), 351-358.

Oladeji, O. S., Odelade, K. A., & Oloke, J. K. (2020). Phytochemical screening and antimicrobial investigation of Moringa oleifera leaf extracts. *African Journal of Science, Technology, Innovation and Development*, *12*(1), 79-84.

Othman, H. I. A., Alkatib, H. H., Zaid, A., Sasidharan, S., Rahiman, S. S. F., Lee, T. P., Dimitrovski, G., Althakafy, J., & Wong, Y. F. (2022). Phytochemical composition, antioxidant and antiproliferative activities of Citrus hystrix, Citrus limon, Citrus pyriformis, and Citrus microcarpa leaf essential oils against human cervical cancer cell line. *Plants*, *12*(1), 134.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, *26*(9-10), 1231-1237.

Robak, J., & Gryglewski, R. J. (1988). Flavonoids are scavengers of superoxide anions. *Biochemical pharmacology*, *37*(5), 837-841.

Rodrigues, E. D. C., Ferreira, A. M., Vilhena, J. C., Almeida, F. B., Cruz, R. A., Florentino, A. C., Souto, R, N. P., Carvalho, J. C. T., & Fernandes, C. P. (2014). Development of a larvicidal nanoemulsion with Copaiba (Copaifera duckei) oleoresin. *Revista Brasileira de Farmacognosia*, *24*(6), 699-705.

Roriz, C. L., Barros, L., Carvalho, A. M., Santos-Buelga, C., & Ferreira, I. C. (2014). Pterospartum tridentatum, Gomphrena globosa and Cymbopogon citratus: A phytochemical study focused on antioxidant compounds. *Food research international*, *62*, 684-693.

Ruch, R. J., Cheng, S. J., & Klaunig, J. E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, *10*(6), 1003-1008.

Saberi, A. H., Fang, Y., & McClements, D. J. (2013). Fabrication of vitamin E-enriched nanoemulsions: Factors affecting particle size using spontaneous emulsification. *Journal of colloid and interface science*, *391,* 95-102.

Salvia-Trujillo, L., Rojas-Graü, A., Soliva-Fortuny, R., & Martín-Belloso, O. (2015). Physicochemical characterization and antimicrobial activity of food-grade emulsions and nanoemulsions incorporating essential oils. *Food hydrocolloids*, *43*, 547-556.

Samanta, S., Pain, A., Dutta, S., Saxena, A. K., Shanmugavel, M., Pandita, R. M., Qazi, G. N., & Sanyal, U. (2005). Antitumor activity of Nitronaphthal-NU, a novel mixed-function agent. *Journal of Experimental Therapeutics & Oncology*, *5*(1), 15-22.

Shafiq, S., Shakeel, F., Talegaonkar, S., Ahmad, F. J., Khar, R. K., & Ali, M. (2007). Development and bioavailability assessment of ramipril nanoemulsion formulation. *European journal of pharmaceutics and biopharmaceutics*, *66*(2), 227-243.

Sharma, P. R., Mondhe, D. M., Muthiah, S., Pal, H. C., Shahi, A. K., Saxena, A. K., & Qazi, G. N. (2009). Anticancer activity of an essential oil from Cymbopogon flexuosus. *Chemico-biological interactions*, *179*(2-3), 160-168.

Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S., & Boyd, M. R. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. *JNCI: Journal of the National Cancer Institute*, *82*(13), 1107-1112.

Sugumar, S., Clarke, S. K., Nirmala, M. J., Tyagi, B. K., Mukherjee, A., & Chandrasekaran, N. (2014). Nanoemulsion of eucalyptus oil and its larvicidal activity against Culex quinquefasciatus. *Bulletin of entomological research*, *104*(3), 393-402.

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, *71*(3), 209-249.

Van Den Dool, H. A. N. D., & Kratz, P. D. (1963). A generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. *Journal of chromatography, 11,* 463-471.

Vashisth, T., & Kadyampakeni, D. (2020). Diagnosis and management of nutrient constraints in citrus. In Srivastava, A. K., & Hu, C., (Eds.). *Fruit crops* (pp. 723-737). Elsevier.

Vigushin, D. M., Poon, G. K., Boddy, A., English, J., Halbert, G. W., Pagonis, C., Jarman, M., & Cancer Research Campaign Phase I/II Clinical Trials Committee. (1998). Phase I and pharmacokinetic study of D-limonene in patients with advanced cancer. *Cancer chemotherapy and pharmacology*, *42*(2), 111-117.

Wang, L., Dong, J., Chen, J., Eastoe, J., & Li, X. (2009). Design and optimization of a new self-nanoemulsifying drug delivery system. *Journal of colloid and interface science*, *330*(2), 443-448.

Wang, L., Dong, J., Chen, J., Eastoe, J., & Li, X. (2009). Design and optimization of a new self-nanoemulsifying drug delivery system. *Journal of colloid and interface science*, *330*(2), 443-448.

Ye, Z., Liang, Z., Mi, Q., & Guo, Y. (2020). Limonene terpenoid obstructs human bladder cancer cell (T24 cell line) growth by inducing cellular apoptosis, caspase activation, G2/M phase cell cycle arrest and stops cancer metastasis. *J. BUON*, *25*(1), 280-285.

Zannou, A., Konfo, T. R. C., Gbaguidi, A. N. M., & Ahoussi-Dahouenon, E. (2015). Antimicrobial activity of extracts from Cymbopogon citratus L. and of Mentha spicata L. against fungal and bacterial strains isolated from peuhl's cheese (Waragashi) produced in Benin. *Int J. Adv Res*, *3*(10), 1684-1695.

Zhao, Y., Wang, C., Chow, A. H., Ren, K., Gong, T., Zhang, Z., & Zheng, Y. (2010). Self-nanoemulsifying drug delivery system (SNEDDS) for oral delivery of Zedoary essential oil: formulation and bioavailability studies. *International journal of pharmaceutics*, *383*(1-2), 170-177.

Authorship contributions

1 – Gustavo Oliveira Everton

Master's degree in Health and Environment from the Federal University of Maranhão
https://orcid.org/0000-0002-0457-914X • gustavooliveiraeverton@gmail.com
Contribution: Conceptualization, Data, Curation, Formal Analysis, Funding, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review editing

2 – Brendha Araújo de Sousa

Specialization in Environment and Sustainability from the Leonardo da Vinci University Center.
https://orcid.org/0000-0003-4504-4341 • brendha.walkiria@gmail.com
Contribution: Writing – original draft, Writing – review editing

3 – Victor Elias Mouchrek Filho

PhD in Chemistry from the University of São Paulo (2000).
https://orcid.org/0000-0003-2855-7292 • victor.mouchrek@ufma.br
Contribution: Funding acquisition. Project administration

4 – Odair dos Santos Monteiro

Post-Doctorate in Organic Chemistry from the Federal University of Pará (UFPA).
https://orcid.org/0000-0003-0607-1531 • odair.sm@ufma.br
Contribution: Funding acquisition. Project administration

How to quote this article

Everton, G. O., Sousa, B. A., Mouchrek Filho, V. E., & Monteiro, O. S. (2025). Anticancer activity of *Citrus limon* (L.) Burm. f. and *Citrus sinensis* (L.) Osbeck essential oil and their nanoemulsions. *Ciencia e Natura*, 47, e87996. DOI: https://doi.org/10.5902/2179460X87996. Available in: https://doi.org/10.5902/2179460X87996