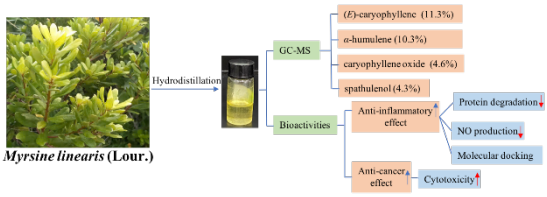
Journal of Oleo Science
Copyright ©2026 by Japan Oil Chemists' Society
doi : 10.5650/jos.ess25150
J. Oleo Sci. **75**, *(4) 477-490 (2026)*

Investigation of the Chemical Composition and Bioactivities of Essential Oil from Leaves of *Myrsine linearis* (Lour.)

Ty Viet Pham[1], Thao Xuan Hoang[2], Dang Khoa Phan Nguyen[3], Thai Hoang Truong[3], Tuong Vy Le Thi[3], Gia-Buu Tran[4], and Bich Hang Do[4]*

[1]*Faculty of Chemistry, University of Education, Hue University, 34 Le Loi, Hue City, VIETNAM*
[2]*Faculty of Biology, University of Education, Hue University, 34 Le Loi, Hue City, VIETNAM*
[3]*Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City, VIETNAM*
[4]*Research Group in Pharmaceutical and Biomedical Sciences, Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City, VIETNAM*

Abstract: ***Myrsine linearis*** **(Lour.) is a medicinal plant in Vietnam that was used to treat various diseases. However, the phytochemical and biological activities of this plant have not been extensively investigated. Therefore, this study extracted the essential oil from *Myrsine linearis* leaves (MLEO), followed by the identification of phytochemical components and the evaluation of anti-inflammatory and anticancer activities in vitro and in silico. The results showed that major constituents in MLEO were *β*-caryophyllene (11.3 %), *α*-humulene (10.3 %), caryophyllene oxide (4.6 %), and spathulenol (4.3 %). MLEO inhibited the protein denaturation (IC_{50} =34.01±5.64 μg/mL) and inhibited nitric oxide release in LPS-induced macrophage with the value of 33.19±1.78 %. The main compounds in MLEO bound to cyclooxygenase-2 and nitric oxide synthase, with binding energy values ranging from -6.1 to -7.5 kcal/mol, similar to those of positive controls. Additionally, MLEO demonstrated cytotoxicity against different cancer cells, with IC_{50} values ranging from 20.26±1.1 μg/mL to 21.11±1.41 μg/mL. Therefore, MLEO is a promising candidate for supporting the treatment of diseases relating to inflammation and cancer.**



Keywords: *Myrsine linearis* (Lour.), essential oil, anti-inflammation, anticancer

1 Introduction

The utilization of natural compounds has gained increasing attention and widespread application. Among these, essential oils (EOs) have emerged as a promising therapeutic approach due to their natural origin and diverse biological activities. EOs are derived from plants through various extraction methods, including steam distillation, hydrodistillation, and dry distillation. The main components of EOs are terpenoids, including monoterpenes, diterpenes, triterpenes, tetraterpenes, ceramides, and sesquiterpenes. Terpenoids have many beneficial properties, including antibacterial, anti-inflammatory, and antiparasitic effects [1, 2]. Numerous studies have shown that terpenoids can decrease inflammation-associated symptoms by reducing the release of proinflammatory cytokines, including nuclear transcription factor-kappa B, interleukins, tumor necrosis factor-alpha, and other inflammatory mediators [3]. Due to these remarkable effects, EOs have been extensively applied across multiple fields, including medicine, aromatherapy, food, agriculture, and cosmetics [4].

Belonging to the *Myrsine* genus, *Primulaceae* family, *Myrsine linearis* (Lour.) is a tree or shrub distributed primarily in subtropical areas. *M. linearis* grows naturally in mixed forests in Central Vietnam. The EOs extracted from several species of the *Myrsine* genus contain a large amount of sesquiterpenes such as *α*-humulene, *γ*-muurolene, *β*-

*Corresponding author: Bich Hang Do, Research Group in Pharmaceutical and Biomedical Sciences, Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City, VIETNAM
E-mail: dobichhang@tdtu.edu.vn
ORCID ID: https://orcid.org/0000-0002-6883-0823 (TVP), https://orcid.org/0000-0001-7207-8120 (GBT)
Accepted December 28, 2025 (received for review July 7, 2025)
Journal of Oleo Science ISSN 1345-8957 print / ISSN 1347-3352 online
https://www.jstage.jst.go.jp/browse/jos/ https://mc.manuscriptcentral.com/jjocs

caryophyllene, and β-selinene [5]. The extracts and compounds isolated from the *Myrsine* genus have been demonstrated to possess various biological properties, including antibacterial, antioxidant, anti-parasitic, hepatoprotective, anticancer, and anti-inflammatory effects [6, 7]. The EO isolated from leaves of *M. linearis* with significant contents of sesquiterpene hydrocarbons and oxygenated sesquiterpene showed herbicidal activity [8]. Extracts from *Myrsine parvifolia* and *Myrsine africana* were investigated for their potential to reduce inflammation by enhancing vascular permeability, promoting leukocyte migration, and inhibiting inflammatory mediators, such as 5-lipoxygenase [9, 10]. The extract from *Myrsine seguinii* showed anti-inflammatory effect via inhibition of Src/Syk/NF-κB and interleukin-1 receptor-associated kinase-1 signaling pathway [5]. High polyphenol components in *Myrsine africana* and *Myrsine umbellata* made these plants good antioxidant candidates [9, 11, 12]. Additionally, myrsininones A-B isolated from the *Myrsine* genus inhibited the proliferation of bacteria *Staphylococcus warneri*, *Streptococcus mutans*, *Streptococcus sanguis*, and *Actinomyces naeslundii* [6].

M. linearis was used traditionally to treat pimples and ulcers in Vietnam. However, no research has been conducted to investigate the anti-inflammatory and anticancer properties of this plant. This study extracted the EO from leaves of *M. linearis* and primarily identified the phytochemical components. The anti-inflammatory effect was evaluated in vitro using protein degradation and nitric oxide (NO) inhibition, while the anticancer activity was assessed using Sulforhodamine B (SRB) assays.

2 Materials and Methods

2.1 Plant materials

The fresh leaves of *M. linearis* (**Fig. 1**) were collected from Thua Thien Hue province, Vietnam, in August 2024. Their identification was confirmed by co-author Thao Xuan Hoang. A corresponding voucher specimen (MLL-2024) has been deposited at the Faculty of Chemistry, University of Education, Hue University.

2.2 Chemicals

Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were from Life Technologies (Gaithersburg, MD, USA). Trypsin-EDTA (0.05 %), sodium pyruvate, L-glutamine, penicillin/streptomycin, trichloroacetic acid (TCA), $NaHCO_3$, lipopolysaccharides (LPS) from *Escherichia coli,* ellipticine, dexamethasone, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and SRB were bought from Merck (Darmstadt, Germany). Dimethyl sulfoxide (DMSO) was bought from Thermo Fisher Scientific (Waltham, MA, USA). Griess Reagent System was supplied by Promega Cooperation (WI, USA) and diclofenac was obtained from Novartis Pharmaceutical Company (Basel, Switzerland).

Fig. 1 Myrsine linearis in its habitat.

2.3 Hydrodistillation of essential oils

The fresh leaves (1.1 kg) were thoroughly washed, chopped into small fragments, and subjected to hydrodistillation using a Clevenger-type apparatus for 4 h, following Vietnamese Pharmacopoeia guidelines [13]. The resulting MLEO was dehydrated over anhydrous Na_2SO_4 and stored at 5 °C until analysis.

2.4 The GC-MS analysis

GC-MS analysis was performed on a Shimadzu GC-MS-QP2010 Plus system (Shimadzu, Kyoto, Japan) equipped with an Equity-5 fused silica capillary column (30 m × 0.25 mm, 0.25 µm film thickness; Supelco, USA) [14]. Helium served as the carrier gas at a flow rate of 1.2 mL/min. The injector and interface temperatures were maintained at 280 °C. The oven temperature was programmed as follows: 60 °C (held for 2 min), increased to 240 °C at 3 °C/min (held for 10 min), then raised to 280 °C at 5 °C/min (held for 35 min). Samples (1.0 µL) were injected in split mode (30:1) at an inlet pressure of 93.2 kPa. Mass spectrometry conditions included an ionization voltage of 70 eV, a detector voltage of 0.7 kV, and a scan range of 40–450 amu at 0.5 scan/s. Retention indices (RI) were calculated relative to a C7–C40 n-alkane series co-injected with the sample. Compounds were identified by comparing their RI values with literature data and their mass spectra with NIST 11 and WILEY 7 libraries.

2.5 BSA degradation assay

The anti-inflammatory activity of MLEO was primarily evaluated by its ability to inhibit the degradation of BSA. A mixture of 0.9 mL of 0.5 % BSA (prepared in NaCl 0.9 %) and 0.1 mL of MLEO (dissolved in DMSO) to final concentration of 40, 20, 10 µg/mL was heated at 70°C in the heat block for 15 min and the turbidity of the sample was measured by using a spectrophotometer at the wavelength of 660 nm [15]. The percentage of inhibition was calculated following the equation:

$$\% \text{ inhibition} = (1 - OD_{sample} / OD_{control}) * 100$$

Diclofenac was used as a positive control, while DMSO was used as a negative control. IC_{50} values were determined using GraphPad Prism 9.0.0.121.

2.6 Nitric oxide inhibition assay

The ability of MLEO to inhibit NO release in induced macrophages was performed following a previous study [16]. RAW 264.7 cells were cultured in DMEM supplemented with 2 mM L-glutamine, 10 mM HEPES, 1 mM sodium pyruvate, and 10 % FBS at 37 °C in 5 % CO_2 incubator. Cells with a density of 2 x 10^6 cells/mL were seeded into 96-well plates and incubated for 24 h. After incubation, the culture medium was removed and replaced with FBS-free DMEM. The cells were then treated with MLEO at different concentrations (50, 10, 2, and 0.4 mg/mL) for 2 h, then incubated with LPS (1 µg/mL) for 24 h to induce NO release. Subsequently, 100 µL of culture media was mixed with 100 µL of Griess reagent, incubated for 10 min at room temperature, and the absorbance was read at 540 nm using BioTek Elx 800 spectrophotometer (Thermo, Massachusetts, USA). Dexamethasone (7.85, 1.57, and 0.31 µg/mL) was used as a positive control, while untreated cells acted as a negative control.

The inhibition of NO production was calculated following the equation:

$$\% \text{ inhibition} = (1 - OD_{sample} / OD_{control}) * 100$$

IC_{50} values were determined using TableCurve 2Dv4 software.

The viability test of treated cells was performed using the MTT assay. The cells in each well were added with 90 µL of culture medium and 10 µL of MTT (5 mg/mL). After 4 h, the medium was removed and the formazan was dissolved by 50 µL 100 % DMSO. The optical density at 540 nm was measured using a spectrophotometer, and the percentage of viable cells was calculated following the equation:

$$\% \text{ viability} = (OD_{sample} / OD_{control}) * 100$$

2.7 Docking

The main compounds of MLEO and positive control were analyzed using AutoDockTools 1.5.6 (California, USA) for their binding to inflammatory proteins through ligand docking. This model was investigated on inflammatory proteins, including cyclooxygenase-2 (COX-2) (PDB ID: 1CVU) and inducible nitric oxide synthase (iNOS) (PDB ID: 3NQS). PyMOL-3.1.3 was used as a tool to remove water molecules and optimize hydrogen bonding for protein and ligand preparation. The conversion from .pdb files to .pdbqt format was carried out using AutoDockTools-1.5.6 [17]. The ligand binding sites on both proteins were positioned at the center of the reference ligand, with a gridbox size of x = 28, y = 28, z = 28. Command Prompt facilitated the docking of ligands to target proteins and the calculation of binding energies. The 3D and 2D visualizations were processed using Discovery Studio Visualizer 2024 and PyMOL-3.1.3, respectively.

2.8 Cytotoxicity to cancer cells

Human colorectal carcinoma (HT-29) and drug-resistant human hepatocarcinoma (Huh7R) were maintained in DMEM supplemented by L-glutamine, sodium pyruvate, $NaHCO_3$, penicillin/streptomycin, and 10 % FBS at 37 °C in 5 % CO_2 incubator. After several days of culturing, the healthy cells were used for a cytotoxicity test using the SRB assay [18]. After seeding cells into the 96-well plate for 18–20 h to ensure stability, different concentrations of MLEO (100, 20, 4, and 0.8 µg/mL) were added and incubated for 48 h at 37 °C in an incubator. After 48 h, cells were fixed with TCA and then stained with 0.4 % SRB (dissolved in 1 % acetic acid) for 30 min at 37 °C. After washing with 1 % acetic acid 3 times, 200 µL of 10 mM Tris base was supplemented to dissolve SRB, and the optical density was measured at 540 nm using a spectrophotometer.

Cell growth inhibition was calculated using the formula:

% Inhibition = $(1\text{-}OD_{sample}/OD_{negative})*100$

Ellipticine (10, 2, 0.4, and 0.08 µg/mL) and 0.5 % DMSO were used as reference and negative controls, respectively. The IC_{50} values were determined using TableCurve 2Dv4 software.

2.9 Statistical analysis

The data were expressed as the mean±standard deviation of triplicate experiments. The graph was drawn and the data were analyzed using GraphPad Prism 9.0.0.121 (GraphPad Software, Inc., San Diego, CA). The difference between groups was compared using a Student's t-test, with a *p*-value of less than 0.05 considered significant.

3 Results

3.1 Chemical profile of MLEO

MLEO was obtained as a yellow liquid, yielding 0.1 % (v/w). GC-MS analysis identified 89 compounds, accounting for 99.5 % of the oil's composition (**Table 1**, **Fig. 2**). Sesquiterpene hydrocarbons were the predominant group, making up 54.0 %, followed by oxygenated sesquiterpenes (32.2 %), non-terpenic compounds (10.7 %), oxygenated monoterpenes (2.1 %), and oxygenated diterpenes (0.5 %). Notably, MLEO lacked monoterpene hydrocarbons and diterpene hydrocarbons.

The major constituents in the oil were *β*-caryophyllene (11.3 %), *α*-humulene (10.3 %), caryophyllene oxide (4.6 %), and spathulenol (4.3 %). Other compounds exceeding 1.0 % concentration were *δ*-cadinene (3.7 %), palmitic acid (3.2 %), valerianol (3.0 %), *trans*-cadina-1(6),4-diene (2.9 %), *α*-selinene (2.8 %), *δ*-selinene (2.8 %), *γ*-himachalene (2.7 %), *β*-elemene (2.7 %), germacrene D (2.4 %), humulene epoxide II (2.5 %), aromadendrene (1.9 %), eremoligenol (1.7 %), *n*-pentadecanal (1.6 %), helifolenol A (1.6 %), selin-11-en-4-*α*-ol (1.4 %), torreyol (1.3 %), *δ*-amorphene (1.2 %), *α*-copaene (1.2 %), linalool (1.2 %), germacrene B (1.1 %), *α*-calacorene (1.1 %), *n*-hexadecanol (1.0 %), *n*-heptadecane (1.0 %), and khusilol (1.0 %).

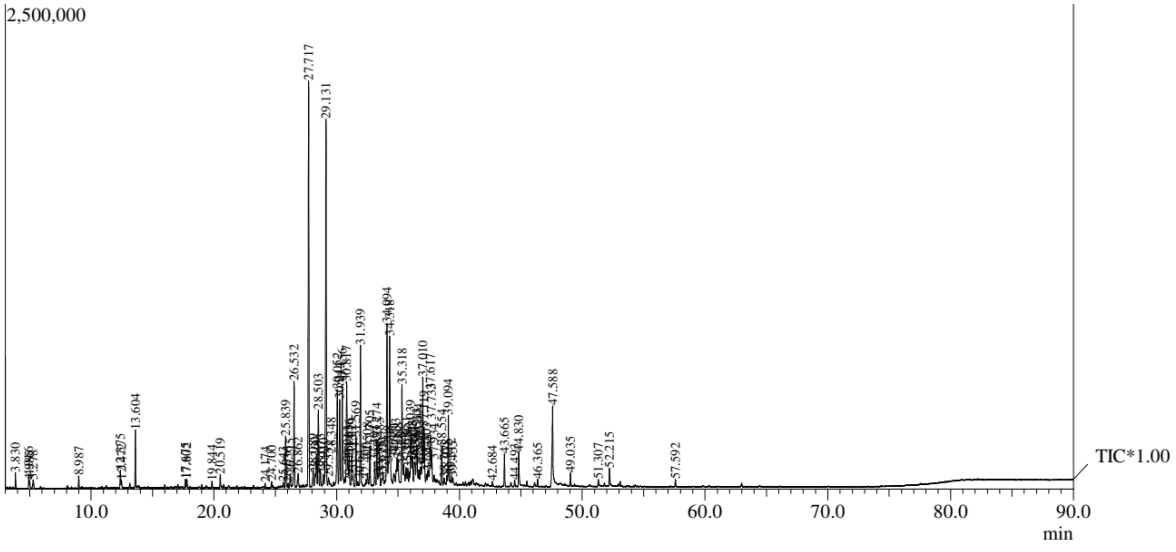


Fig. 2 The GC chromatogram of MLEO.

Table 1 Chemical composition of MLEO.

No.	RT	Compound[a]	RI[b]	RI[c]	Concentration (%)
1	3.83	(3*Z*)-Hexenal	800	797	0.3
2	4.92	(2*E*)-Hexenal	848	846	0.2
3	4.99	(3*Z*)-Hexenol	851	850	0.2
4	5.28	*n*-Hexanol	864	863	0.2
5	8.99	6-Methyl-5-hepten-2-one	986	981	0.2
6	12.38	*n*-Octanol	1070	1063	0.4
7	12.47	*cis*-Linalool oxide (furanoid)	1073	1067	0.2
8	13.60	Linalool	1100	1095	1.2
9	17.68	*α*-Terpineol	1191	1186	0.2
10	17.80	Methyl salicylate	1194	1190	0.2
11	19.84	Citronet	1240	1234	0.2
12	20.52	Geraniol	1255	1249	0.3
13	24.17	*δ*-Elemene	1338	1335	0.1
14	24.70	*α*-Cubebene	1351	1348	0.2
15	25.64	*α*-ylangene	1372	1373	0.1
16	25.84	α-Copaene	1377	1374	1.2
17	26.04	Hexyl caproate	1382	1382	0.1
18	26.22	Isobornyl acetate	1386	1383	0.4
19	26.53	*β*-Elemene	1393	1389	2.7
20	26.86	Cyperene	1401	1398	0.5
21	27.72	*β*-Caryophyllene	1422	1417	11.3
22	28.09	*β*-Copaene	1431	1430	0.5
23	28.26	*α*-*trans*-Bergamotene	1435	1432	0.4
24	28.35	*γ*-Elemene	1437	1434	0.8
25	28.50	Aromadendrene	1441	1439	1.9
26	28.67	6,9-Guaiadiene	1445	1442	0.4
27	28.91	*cis*-Muurola-3,5-diene	1450	1448	0.2
28	29.13	*α*-Humulene	1456	1452	10.3
29	29.33	*α*-Patchoulene	1461	1454	0.2
30	30.05	*trans*-Cadina-1(6),4-diene	1478	1475	2.9
31	30.24	Germacrene D	1483	1480	2.4
32	30.46	*γ*-Himachalene	1488	1481	2.7
33	30.82	*δ*-Selinene	1493	1493	2.8
34	30.82	*α*-Selinene	1497	1498	2.8
35	30.93	*n*-Pentadecane	1499	1500	0.4
36	31.02	*α*-Muurolene	1502	1500	0.7
37	31.23	(*E*,*E*)-*α*-Farnesene	1507	1505	0.5
38	31.33	*β*-Bisabolene	1510	1508	0.3
39	31.57	*δ*-Amorphene	1516	1511	1.2
40	31.94	*δ*-Cadinene	1525	1522	3.7

No.	RT	Compound[a]	RI[b]	RI[c]	Concentration (%)
41	32.4	10-*epi*-Cubebol	1537	1533	0.1
42	32.51	*α*-Cadinene	1540	1537	0.4
43	32.71	*α*-Calacorene	1545	1544	1.1
44	33.10	*β*-Vetivenene	1555	1554	0.6
45	33.27	Germacrene B	1559	1559	1.1
46	33.38	1,5-epoxysalvial-4(14)-ene	1562	1561	0.3
47	33.49	(*E*)-Nerolidol	1565	1561	0.8
48	33.49	Maaliol	1569	1566	0.8
49	33.77	Caryophyllenyl alcohol	1572	1570	0.3
50	34.09	Spathulenol	1580	1577	4.3
51	34.21	Himachalene epoxide	1583	1578	0.5
52	34.32	Caryophyllene oxide	1586	1582	4.6
53	34.65	Viridiflorol	1594	1592	0.4
54	34.84	*n*-Hexadecane	1599	1600	0.3
55	34.91	Guaiol	1601	1600	0.6
56	35.01	Ledol	1604	1602	0.6
57	35.32	Humulene epoxide II	1612	1608	2.5
58	35.45	*cis*-Isolongifolanone	1615	1612	0.6
59	35.65	Humulane-1,6-dien-3-ol	1621	1619	0.5
60	35.80	Citronellyl pentanoate	1625	1624	0.3
61	35.92	1-*epi*-Cubenol	1628	1627	0.2
62	36.04	Eremoligenol	1631	1629	1.7
63	36.17	*γ*-Eudesmol	1635	1630	0.6
64	36.33	*cis*-Cadin-4-en-7-ol	1639	1635	0.8
65	36.33	*epi*-*α*-Cadinol	1640	1638	0.8
66	36.53	Torreyol	1644	1644	1.3
67	36.69	Cubenol	1649	1645	0.2
68	36.85	*α*-Eudesmol	1653	1652	0.3
69	37.01	Valerianol	1657	1656	3.0
70	37.12	Selin-11-en-4-*α*-ol	1660	1658	1.4
71	37.43	Aromadendrene oxide	1665	1662	0.2
72	37.43	Bulnesol	1669	1670	0.4
73	37.62	Helifolenol A	1674	1674	1.6
74	37.73	Khusilol	1677	1675	1
75	37.93	Mustakone	1682	1680	0.2
76	38.55	*n*-Heptadecane	1699	1700	1.0
77	38.76	Juniper camphor	1705	1700	0.2
78	38.98	14-hydroxy-*α*-Humulene	1710	1713	0.2
79	39.09	*n*-Pentadecanal	1714	1715	1.6
80	39.31	(*E*)-Nerolidyl acetate	1720	1716	0.2
81	39.46	(2*Z*,6*E*)-Farnesol	1724	1722	0.2

No.	RT	Compound[a]	RI[b]	RI[c]	Concentration (%)
82	42.68	*n*-Hexadecanal	1816	1811	0.1
83	43.67	Hexahydrofarnesyl acetone	1845	1845	0.8
84	44.49	Benzyl salicylate	1869	1864	0.2
85	44.83	*n*-Hexadecanol	1879	1874	1.0
86	46.37	Methyl palmitate	1926	1921	0.2
87	47.59	Palmitic acid	1964	1959	3.2
88	52.22	*cis*-Phytol	2112	2112	0.5
89	57.59	*n*-Tricosane	2298	2300	0.2
Total					99.5
Oxygenated monoterpenes (OM)					2.1
Sesquiterpene hydrocarbons (SH)					54.0
Oxygenated sesquiterpenes (OS)					32.2
Oxygenated diterpenenes (OD)					0.5
Non-terpenic compounds					10.7

[a] Elution order on Equity-5 column; [b] Retention indices on Equity-5 column; [c] Literature retention indices (see references)

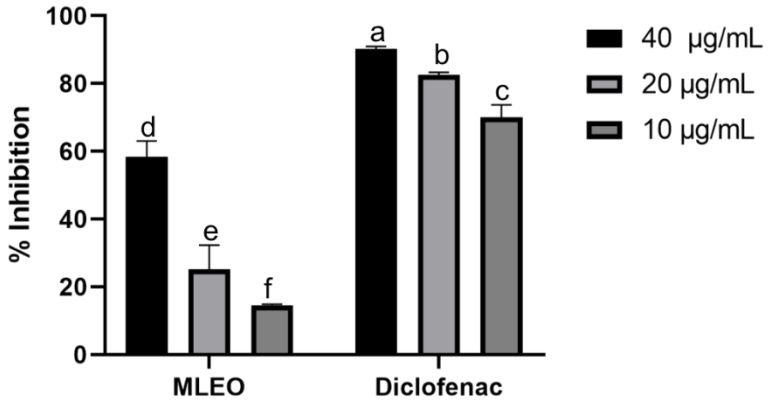


Fig. 3 The inhibition percentage of protein denaturation. Different concentrations of MLEO and diclofenac (40, 20, 10 µg/mL) were incubated with BSA, heated, and the turbidity was examined using a spectrophotometer. Tukey's HSD test indicates that bars with the same letters are not significantly different ($p < 0.05$).

3.2 BSA inhibition assay

To examine whether MLEO reduces protein denaturation, a mixture of BSA and varying concentrations of MLEO was prepared and heated at 70 °C for 15 min. The turbidity of each tube was measured at 660 nm using a spectrophotometer. The results showed that MLEO reduced the aggregation of BSA by 58.37±4.65 % at a concentration of 40 µg/mL, with the IC_{50} of 34.01±5.64 µg/mL. Positive control, diclofenac, exhibited excellent protein denaturation inhibitory activity with an IC_{50} of 4.28±0.89 µg/mL (**Fig. 3**).

3.3 NO inhibition assay

MLEO's ability to reduce inflammation by preventing macrophages from producing NO was assessed. The RAW 264.7 cells were incubated with different concentrations of MLEO, followed by treatment with LPS, and assessed for NO release by Griess assay. The results showed that at a concentration of 50 mg/mL, MLEO was toxic to cells, with the cell viability being 2.48±0.34 %. Cell viability exceeded 80 % at doses of 10 mg/mL and below, which was suitable for NO inhibition tests. However, MLEO showed moderate NO inhibition with a value of 33.19±1.78 % at 10 mg/mL, while dexamethasone effectively inhibited NO release up to 53.4±1.15 % at the concentration of 7.85 µg/mL (IC_{50} was calculated at 5.44±0.57 µg/mL) (**Fig. 4**).

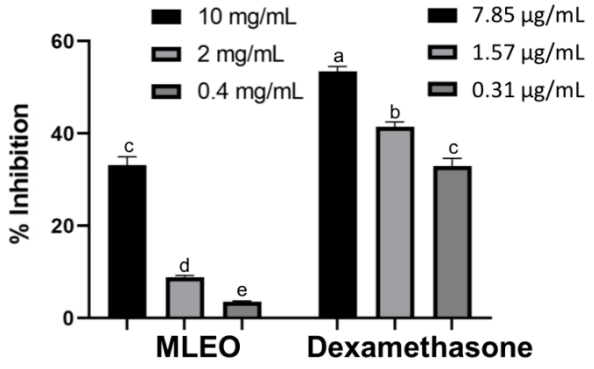


Fig. 4. The inhibition percentage of NO release. Different concentrations of MLEO and positive control, dexamethasone, were applied to LPS-induced macrophages. The NO release was measured using the Griess assay. Tukey's HSD test indicates that bars with the same letters are not significantly different ($p < 0.05$).

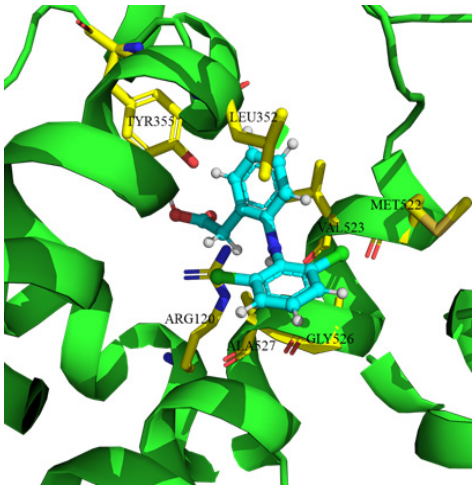
Table 2 Docking results of the main compounds of MLEO with COX-2 and iNOS.

Compounds	Binding energy (kcal/mol)	No. of H-bonds	Residues	Other interactions
Binding of compounds to COX-2 (PDB ID: 1CVU)				
Diclofenac	-8.4	2	Arg120, Tyr355	Leu352, Met522, Val523, Gly526, Ala527
β-Caryophyllene	-7.3	0	-	Val349, Leu352, Val523, Ala527
Caryophyllene oxide	-7.5	0	-	Val349, Ala527
α-Humulene	-7.0	0	-	Trp387, Val523, Ala527
Spathulenol	-7.2	0	-	Val349, Ala527
Binding of compounds to iNOS (PDB ID: 3NQS)				
Dexamethasone	-8.3	2	Gly365, Trp457	Ile195, Glu371
β-Caryophyllene	-6.3	0	-	Trp457
Caryophyllene oxide	-6.4	1	Gly365	Cys194
α-Humulene	-6.1	0	-	Ala191, Cys194, Val346
Spathulenol	-7.4	0	-	Trp188, Ala191, Cys194, Phe363

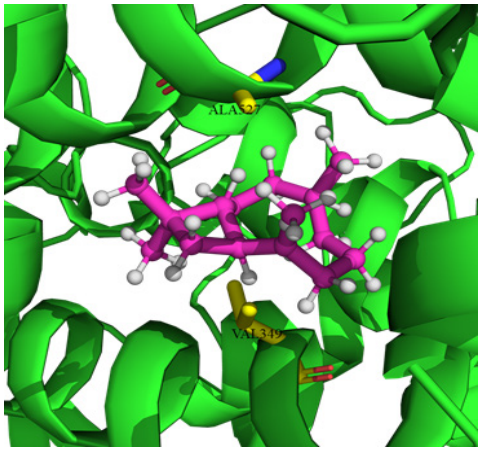
3.4 Docking

The anti-inflammatory activity of MLEO was considered through molecular docking and binding energy calculations using the Command Prompt software. The binding of main compounds in MLEO (*β*-caryophyllene, *α*-humulene, caryophyllene oxide, and spathulenol) to COX-2 and iNOS was compared to that of diclofenac and dexamethasone, which specifically bound to COX-2 and iNOS proteins, respectively (**Table 2**, **Fig. 5**). **Table 2** revealed that all 4 tested compounds interact with both COX-2 and iNOS at high binding energy levels, with the binding energy values ranging from -6.1 to -7.5 kcal/mol. Notably, these compounds formed hydrogen bonds and interactions at active sites with the tested inflammatory proteins. Diclofenac and dexamethasone exhibited strong binding affinities to COX-2 (-8.4 kcal/mol) and iNOS (-8.3 kcal/mol), respectively.

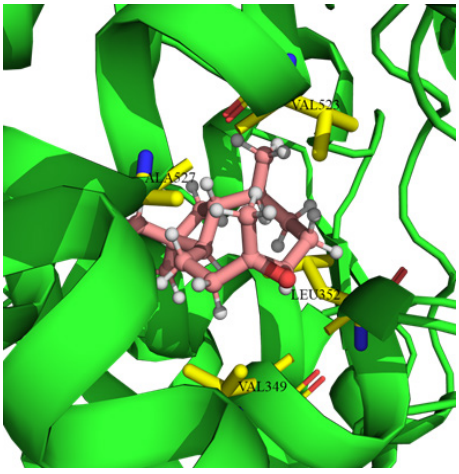
COX-2 and Diclofenac



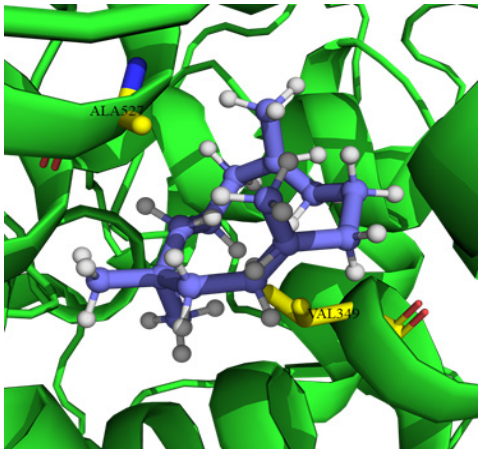
COX-2 and β-Caryophyllene



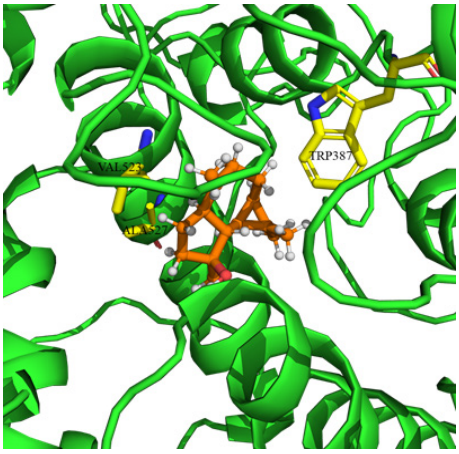
COX-2 and Caryophyllene oxide



COX-2 and α-Humulene



COX-2 and Spathulenol



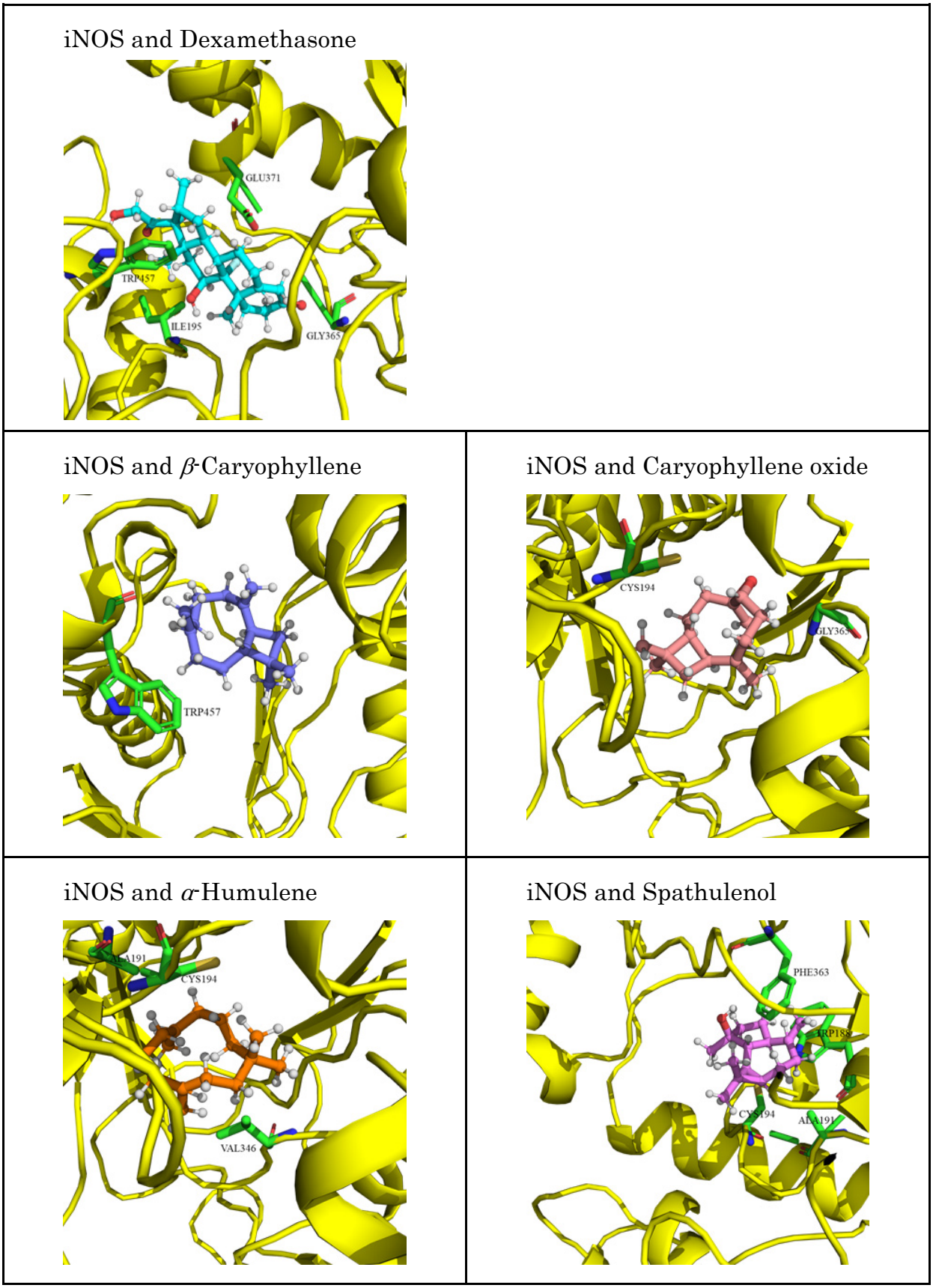


Fig. 5 3D visualization of the interaction between main components in MLEO with COX-2 and iNOS.

3.5 Anticancer activity

The cytotoxicity of MLEO against various cancer cells was assessed. The cells were treated with varying concentrations of MLEO, and the surviving cells were examined using an SRB assay. **Figure 6** showed that MLEO effectively suppressed HT-29 and Huh-7R cancer cells up to 97.08±2.64 % with IC_{50} of 21.11±1.41 and 20.26±1.1 µg/mL, respectively. Positive control, ellipticine showed good cytotoxicity to cells with IC_{50} values of approximately 0.37±0.03 µg/mL (HT-29) and 0.41±0.04 µg/mL (Huh7R).

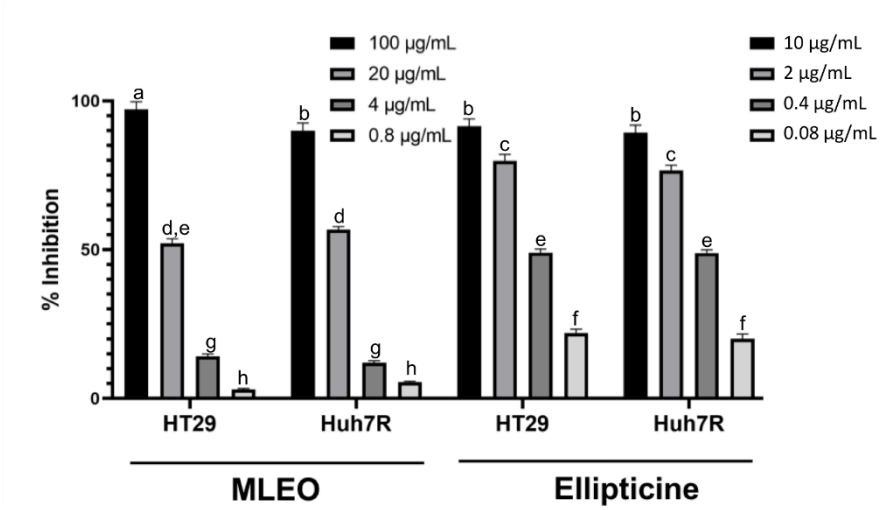


Fig. 6 The cytotoxicity of MLEO to cancer cells. Different concentrations of MLEO and positive control, ellipticine, were incubated with HT-29 and Huh7R cancer cells. The living cells were assessed using the SRB assay. Tukey's HSD test indicates that bars with the same letters are not significantly different ($p < 0.05$).

4 Discussion

Myrsine is a potential genus that exhibits various biological activities, including antioxidant, antibacterial, anti-inflammatory, and anticancer properties. This study aims to investigate the chemical composition and preliminary evaluation of the anti-inflammatory and anticancer activities of EO from the leaves of *M. linearis*.

MLEO is characterized by a predominance of sesquiterpene hydrocarbons (54.0 %) and oxygenated sesquiterpenes (32.2 %), with *β*-caryophyllene (11.3 %), *α*-humulene (10.3 %), caryophyllene oxide (4.6 %), and spathulenol (4.3 %) as the major constituents. Similar results in composition were observed in the study by Tong V. Giang et al., with 59.2 % of sesquiterpene hydrocarbons and 35 % of oxygenated sesquiterpenes [8]. However, the main compounds in this study were slightly different from those in our study. Particularly, the amount of *β*-caryophyllene, caryophyllene oxide, *α*-cadinol, and germacrene B was decreased, while that of *α*-humulene was increased. The composition of Eos varies depending on factors such as location, environment, and the time of collection. *M. parvifolia*, *M. rubra*, and *M. gardneriana* are similar to *M. linearis* in their high sesquiterpene content. Specifically, *M. parvifolia* leaf oil contains caryophyllene oxide (14.4 %), *β*-caryophyllene (12.6 %), and *γ*-muurolene (7.9 %), while its fruit oil includes *β*-caryophyllene (11.7 %) and *δ*-cadinene (7.1 %) [19]. Similarly, *M. rubra* leaf oil is dominated by *β*-caryophyllene (17.2 %), *γ*-muurolene (11.1 %), and germacrene B (10.0 %), while *M. gardneriana* leaf oil is dominated by *β*-caryophyllene (18.0 %) and *γ*-muurolene (8.4 %) [19]. The presence of *β*-caryophyllene at 11.3 % in *M. linearis* closely matches these species, although its concentration is slightly lower than in *M. rubra* and *M. gardneriana*. Additionally, caryophyllene oxide in *M. linearis* (4.6 %) is less abundant than in *M. parvifolia* (14.4 %), suggesting variations in oxidative metabolism or environmental factors influencing sesquiterpene profiles. The prominence of *α*-humulene (10.3 %) in *M. linearis* distinguishes it from many *Myrsine* species. Still, it aligns with *M. guianensis*, where *α*-humulene constitutes 7.80 % of the leaf oil, alongside isocaryophyllene (21.81 %), valencene (11.52 %), and *δ*-cadinene (11.03 %) [20]. The shared presence of *δ*-cadinene (3.7 % in *M. linearis*, 11.03 % in *M. guianensis*) further supports a chemical affinity between these species. This compound is also a recurring constituent in *M. parvifolia* fruit oil (7.1 %), *M. coriacea* (7.1 %), *M. venosa* (6.2 %), *M. leuconeura* (6.7–7.2 %), and *M. umbellata* (6.30 %), indicating its widespread occurrence within the genus [11, 19, 21, 22]. The complete absence of monoterpene hydrocarbons in *M. linearis* highlights a significant biosynthetic divergence, potentially reflecting ecological or genetic differences. In contrast, *M. lineata* deviates sharply from *M. linearis*, with its leaf oil dominated by monoterpene

hydrocarbons, including α-pinene (35.5 %), β-pinene (20.3 %), and silvestrene (12.8 %) [23]. Overall, MLEO in our study differs from that of other species in the genus, but the abundance composition remains the same.

The anti-inflammatory activity of MLEO may be derived from its major components. α-Humulene has been demonstrated to be effective in reducing inflammation by downregulating IL-6 production [24, 25]. Similarly, β-caryophyllene possessed anti-inflammatory activity via reducing pro-inflammatory proteins, including interleukin 6 (IL-6), interleukin-1ß, tumor necrosis factor-alfa, IFN-γ, COX-2, nuclear factor kappa-light-chain-enhancer of activated B cells, while increasing the level of interleukin 10, and GPx [26–28]. Caryophyllene oxide, a derivative of β-caryophyllene, can potentially inhibit several signaling pathways in the inflammatory process [29]. Spathulenol is considered to have anti-inflammatory potential, as evidenced by its ability to reduce paw edema in mice [30]. The results of in silico and protein degradation inhibition in vitro experiments showed similar findings to previous studies on the effective anti-inflammatory properties of high-concentration compounds in MLEO. Particularly, MLEO significantly reduced protein aggregation with an IC_{50} of 34.01±5.64 μg/mL (**Fig. 3**). β-Caryophyllene, α-humulene, caryophyllene oxide, and spathulenol were able to bind to COX-2 or iNOS with a good docking pose, slightly lower than that of the positive control (**Table 2**, **Fig. 5**). However, NO inhibition assay demonstrated weak activity of MLEO, caused by the toxicity of MLEO to the cells, and probably the interaction of compounds in MLEO (including inflammatory enhancing agents such as *n*-hexanal, *n*-hexadecanol, and palmitic acid) reduced the action of MLEO. Our results were in accordance with previous studies on the anti-inflammatory properties of the *Myrsine* genus [9, 31]. Therefore, these results demonstrated the potential anti-inflammatory activity of MLEO in supporting the treatment of inflammation-related diseases.

The main components in MLEO were demonstrated to be good anticancer agents. β-Caryophyllene was reported to inhibit the growth of different cancer cells by inducing G1 phase cell cycle arrest through the downregulation of cyclin D1, and other cyclin-dependent kinases, thereby enhancing the apoptosis pathway [32, 33]. Alpha-humulene inhibited the proliferation of hepatocellular carcinoma via apoptosis, promoting the activation of caspase-3 and PARP cleavage [34]. Caryophyllene oxide exhibited cytotoxicity against cancer cells by triggering apoptosis and necrosis signaling pathways [35]. In our study, MLEO expressed high cytotoxicity against cancer cells with an IC_{50} values of (**Fig. 6**). The high anticancer activity of MLEO was similar to previous results about other species in *Myrsine* genus [36, 37]. Particularly, the extracts from *M. umbellata* showed cytotoxicity to melanoma cells with IC_{50} values of 9.51–24.95 μg/mL [37]. Compounds isolated from the Myrsine genus, such as myrseguinoside D, myrseguinoside E, and ardisiacrispin B were cytotoxic to cancer cells with IC_{50} values of 6-100.1 μg/mL [36]. IC_{50} values of MLEO for the tested cancer cells were approximately 20 μg/mL, which is within the range of other studies on this genus.

5 Conclusion

The GC-MS analysis of MLEO, sourced from a different location and exhibiting a distinct chemotype, was successfully performed, and its anti-protein denaturation, anti-inflammatory, and cytotoxic activities were evaluated for the first time. The results showed that major constituents in MLEO were β-caryophyllene (11.3 %), α-humulene (10.3 %), caryophyllene oxide (4.6 %), and spathulenol (4.3 %). MLEO inhibited the protein denaturation and slightly reduced NO release in LPS-induced RAW-264.7 macrophage via binding to COX-2 and iNOS. In addition, MLEO showed anticancer activity by reducing the proliferation of different cancer cells. However, due to the limitations of this study, further research should be conducted to elucidate the signaling pathway underlying the anti-inflammatory and anticancer activities of MLEO. To further enhance the potential of this intriguing plant, additional MLEO actions, such as antioxidant, antibacterial, anti-diabetic, etc., should be investigated.

Funding

The authors received no financial support for this research.

Conflict of Interest

The authors state no conflict of interest.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1) Zuo, X.; Gu, Y.; Wang, C.; Zhang, J.; Zhang, J. et al. A systematic review of the anti-inflammatory and immunomodulatory properties of 16 essential oils of herbs. *Evid. Based Complement. Alternat Med.* **2020**, 8878927 (2020). doi:10.1155/2020/8878927
2) Chouhan, S.; Sharma, K.; Guleria, S. Antimicrobial activity of some essential oils—Present status and future perspectives. *Medicines* **4**, 58 (2017). doi:10.3390/medicines4030058
3) Devi, M.; Bamrah, P.K.; Goyal, R.; Choudhary, M.; Chopra, H. Insights on the emerging therapeutic potential of terpenoids as anti-inflammatory agents: A scoping review. *J. BioX Res.* **7**, 0006 (2024). doi:10.34133/jbioxresearch.0006
4) Bolouri, P.; Salami, R.; Kouhi, S.; Kordi, M.; Asgari Lajayer, B. et al. Applications of Essential oils and plant extracts in different industries. *Molecules* **27**, 8999 (2022). doi:10.3390/molecules27248999
5) Oliveira, A.P.; Seibert França, H.; Guerra Santos, M. Volatile Constituents of Three Myrsine L. Species from Brazil. *Records of Natural Products* **2017**, 82-87 (2017). https://www.researchgate.net/publication/302452456
6) Thoa, N.T.; Son, N.T. The genus Myrsine: A review of phytochemistry, pharmacology, and toxicology. *Fitoterapia* **177**, 106121 (2024). doi:10.1016/j.fitote.2024.106121
7) Silva, F.L.; Paola Cristina, B.; Amanda, D.E.S.; Nayara, de O.S.; Letícia Megumi, I. et al. Exploring *Myrsine umbellata*: A comprehensive analysis of phytochemicals, antioxidant potential, antityrosinase effect, and cytotoxic activities across distinct plant parts. *J. Herbs Spices Med. Plants* **31**, 209-223 (2025). doi:10.1080/10496475.2025.2458480
8) Giang, T.V.; Chac, L.D.; Huyen, T.T. Chemical composition and herbicidal action of essential oil from myrsine linearis leaves. *Chem. Nat. Compd.* **61**, 580-582 (2025). doi:10.1007/s10600-025-04708-0
9) Fibrich, B.; Gao, X.; Puri, A.; Banga, A.K.; Lall, N. In vitro antioxidant, anti-inflammatory and skin permeation of *Myrsine africana* and its isolated compound myrsinoside B. *Front. Pharmacol.* **10**, 1410 (2019). doi:10.3389/fphar.2019.01410.
10) Corrêa, A.L.; Oliveira, A.P.; Ruppelt, B.M.; de Araújo, E.R.de A.; Santos, M.G. et al. Protective effect of *Myrsine parvifolia* plant extract against the inflammatory process induced by *Bothrops jararaca* snake venom. *Toxicon* **157**, 66-76 (2019). doi:10.1016/j.toxicon.2018.11.289
11) Laskoski, L.V.; Bandeira, D.M.; Batista, J.M.; da Costa, W.F.; Baeza, L.C. et al. Phytochemical prospection and evaluation of antimicrobial, antioxidant and antibiofilm activities of extracts and essential oil from leaves of *Myrsine umbellata* Mart. (Primulaceae). *Braz. J. Biol.* **82**, e263865 (2022). doi:10.1590/1519-6984.263865
12) Gul, H.; Raja, G.K.; Shahid, M.; Iqbal, M.; Irum, S. et al. Phytochemistry, antioxidant and antibacterial activities of fruit extracts of *Myrsine africana* L. *Pak. J. Pharm. Sci.* **34**, 507-512 (2021). doi:10.36721/PJPS.2021.34.2.REG.507-512.1
13) Pham, T.V.; Hoang, T.X.; Do, B.H.; Nguyen, K.T.; Nguyen, N.H.; Tran, G. Chemical compositions, molecular docking, anti - inflammatory, and anti - cancer effects of the leaf essential oils isolated from three species of the Rutaceae family in Vietnam. *Chem. Biodivers.* **22**, e202401466 (2025). doi:10.1002/cbdv.202401466
14) Pham, T.V.; Le, A.T.; Nguyen, N.H.; Nguyen, H.H.; Tran, G.-B.; Do, B.H. Phytochemical analysis and investigation of the anti-inflammatory and anticancerous activity of *Walsura robusta* leaf volatile compounds. *J. Essent. Oil-Bear. Plant* **28**, 83-94 (2025). doi:10.1080/0972060X.2025.2451383
15) Tran, G.B.; Pham, T.V.; Tuan Le, A.; Nguyen, N.H.; Vo, N.H.H.; Do, B.H. Chemical composition and the anti-inflammatory effect of volatile compounds from *Anaxagorea Luzonensis* A. Gray. *Zeitschrift für Naturforschung C* **79**, 329-336 (2024). doi:10.1515/znc-2023-0177
16) Tran, G.-B.; Pham, T.V.; Turkmen, M.; Nguyen, N.H.; Do, B.H. The anti-inflammatory effect and molecular docking of essential oils extracted from rhizomes of *Distichochlamys benenica* and *Distichochlamys citrea*. *J. Oleo Sci.* **73**, 1505-1514 (2024). doi:10.5650/jos.ess24148
17) Rizvi, S.M.D.; Shakil, S.; Haneef, M.A Simple click by click protocol to perform docking: Autodock 4.2 made easy for non-bioinformaticians. *EXCLI J.* **12**, 831-857 (2013).
18) Shakil, M.S.; Rana, Z.; Hanif, M.; Rosengren, R.J. Key considerations when using the sulforhodamine b assay for screening novel anticancer agents. *Anti-Cancer Drugs* **33**, 6-10 (2022). doi:10.1097/CAD.0000000000001131
19) Corrêa, A.L.; França, H.S.; Tietbohl, L.A.C.; Luna, B.N.; Santos, M.G. et al. Volatile constituents of three Myrsine L. species from Brazil. *Rec. Nat. Prod.* **11**, 82-87 (2017).
20) de Jesus, G.S.; Micheletti, A.C.; Padilha, R.G.; de Souza de Paula, J.; Alves, F.M. et al. Antimicrobial potential of essential oils from cerrado plants against multidrug-resistant foodborne microorganisms. *Molecules* **25**, 3296 (2020). doi:10.3390/molecules25143296
21) de Luna, B.N.; e Defaveri, A.C.A.; Sato, A.; Bizzo, H.R.; Freitas, M.de F.; Barros, C.F. Leaf secretory tissues in

Myrsine coriacea and *Myrsine venosa* (Primulaceae): Ontogeny, morphology, and chemical composition of essential oils. *Botany* **92**, 757-766 (2014). doi:10.1139/cjb-2014-0044
22) Costa, L.F.P.B.; Taleb Contini, S.H.; Teixeira, S.P.; Freitas, M.de F.; França, S.de C. et al. Secretory structures and chemical composition of the essential oil from leaves of *Myrsine leuconeura* Mart. (Primulaceae). *Flora: Morphology, Distribution, Functional Ecology of Plants* **262**, 151496 (2020). doi:10.1016/j.flora.2019.151496
23) França, J.V.; e Defaveri, A.C.A.; de Luna, B.N.; Bizzo, H.R.; Sato, A. Composition of essential oils from the species *Ardisia humilis* and *Myrsine lineata* (Primulaceae). *8th Brazilian Symposium on Essential Oils - International Symposium on Essential Oils*. (2015).
24) Becker, L.; Holtmann, D. Anti-inflammatory effects of α-humulene on the release of pro-inflammatory cytokines in lipopolysaccharide-induced THP-1 cells. *Cell Biochem. Biophys.* **82**, 839-847 (2024). doi:10.1007/s12013-024-01235-7
25) Viveiros, M.M.H.; Costa, J.G.M.da; Oliveira, A.G.de; Rubio, C.; Padovani, C.R. et al. Anti-inflammatory effects of α-humulene and ß-caryophyllene on Pterygium fibroblasts. *Int. J. Ophthalmol.* **15**, 1903-1907 (2022). doi:10.18240/ijo.2022.12.02
26) Mazzantini, C.; El Bourji, Z.; Parisio, C.; Davolio, P.L.; Cocchi, A. et al. Anti-inflammatory properties of cannabidiol and beta-caryophyllene alone or combined in an in vitro inflammation model. *Pharmaceuticals* **17**, 467 (2024). doi:10.3390/ph17040467
27) Gushiken, L.F.S.; Beserra, F.P.; Hussni, M.F.; Gonzaga, M.T.; Ribeiro, V.P. et al. Beta-Caryophyllene as an antioxidant, anti-inflammatory and re-epithelialization activities in a rat skin wound excision model. *Oxid. Med. Cell Longev.* **2022**, 1-21 (2022). doi:10.1155/2022/9004014
28) Scandiffio, R.; Geddo, F.; Cottone, E.; Querio, G.; Antoniotti, S. et al. Protective effects of (*E*)-ß-caryophyllene (BCP) in chronic inflammation. *Nutrients* **12**, 3273 (2020). doi:10.3390/nu12113273
29) Chavan, M.J.; Wakte, P.S.; Shinde, D.B. analgesic and anti-inflammatory activity of caryophyllene oxide from *Annona squamosa* L. bark. *Phytomedicine* **17**, 149-151 (2010). doi:10.1016/j.phymed.2009.05.016
30) Dos Santos, E.; Radai, J.A.S.; do Nascimento, K.F.; Formagio, A.S.N.; de Matos Balsalobre, N. et al. Contribution of spathulenol to the anti-nociceptive effects of *Psidium guineense*. *Nutr. Neurosci.* **25**, 812-822 (2022). doi:10.1080/1028415X.2020.1815330
31) Corrêa, A.L.; Oliveira, A.P.; Ruppelt, B.M.; de Araújo, E.R.de A.; Santos, M.G. et al. Protective effect of *Myrsine parvifolia* plant extract against the inflammatory process induced by *Bothrops jararaca* snake venom. *Toxicon* **157**, 66-76 (2019). doi:10.1016/j.toxicon.2018.11.289
32) Ahmed, E.A.; Abu Zahra, H.; Ammar, R.Ben; Mohamed, M.E.; Ibrahim, H.-I.M. Beta-Caryophyllene enhances the anti-tumor activity of cisplatin in lung cancer cell lines through regulating cell cycle and apoptosis signaling molecules. *Molecules* **27**, 8354 (2022). doi:10.3390/molecules27238354
33) Chung, K.-S.; Hong, J.Y.; Lee, J.-H.; Lee, H.-J.; Park, J.Y. et al. ß-Caryophyllene in the essential oil from *Chrysanthemum boreale* induces g1 phase cell cycle arrest in human lung cancer cells. *Molecules* **24**, 3754 (2019). doi:10.3390/molecules24203754
34) Chen, H.; Yuan, J.; Hao, J.; Wen, Y.; Lv, Y. et al. α-Humulene inhibits hepatocellular carcinoma cell proliferation and induces apoptosis through the inhibition of Akt signaling. *Food Chem. Toxicol.* **134**, 110830 (2019). doi:10.1016/j.fct.2019.110830
35) Ramírez-Santos, J.; Calzada, F.; García-Hernández, N.; Barbosa, E.; Velázquez, C.; Valdes, M. Caryophyllene oxide, a bicyclic terpenoid isolated from *Annona macroprophyllata* with antitumor activity: In vivo, in vitro, and in silico studies. *Int. J. Mol. Sci.* **25**, 13355 (2024). doi:10.3390/ijms252413355
36) Thoa, N.T.; Son, N.T. The genus Myrsine: A review of phytochemistry, pharmacology, and toxicology. *Fitoterapia* **177**, 106121 (2024). doi:10.1016/j.fitote.2024.106121
37) Silva, F.L.; Branco, P.C.; Do Espírito Santo, A.; de Oliveira Santos, N.; Ishibaru, L.M. et al. Exploring *Myrsine umbellata*: A comprehensive analysis of phytochemicals, antioxidant potential, antityrosinase effect, and cytotoxic activities across distinct plant parts. *J. Herbs Spices Med. Plants* **31**, 209-223 (2025). doi:10.1080/10496475.2025.2458480