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Essential oil from the leaves of *Croton thorelii* Gagnep.: chemical composition and biological activities

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Abstract

Aim: *Croton thorelii* Gagnep. is a lesser-known plant with ethnobotanical relevance. This study investigates the essential oil extracted from its leaves for potential use in natural preservatives and functional foods. Although traditionally used in folk medicine, information on its chemical composition and biological properties remains limited.

Methods: Essential oil was obtained by hydrodistillation using a Clevenger-type apparatus from fresh leaves collected in Central Vietnam. Chemical composition was determined by gas chromatography-mass spectrometry. Biological activities were assessed through cytotoxicity (SRB assay on SK-LU-1 and HepG2 cells), antioxidant assays (DPPH and ABTS), and anti-inflammatory testing (nitric oxide inhibition in LPS-stimulated RAW 264.7 macrophages). All tests were performed in triplicate.

Results: A total of 59 compounds were identified, accounting for 99.5% of the oil. Major groups included sesquiterpene hydrocarbons (44.5%), oxygenated monoterpenes (41.1%), and oxygenated sesquiterpenes (9.4%). The principal components were β-selinene (22.0%), 1,8-cineole (20.7%), linalool (11.2%), and (*E*)-caryophyllene (9.5%). The essential oil showed strong cytotoxicity with IC_{50} values of 54.52 ± 1.40 μg/mL (SK-LU-1) and 48.29 ± 2.09 μg/mL (HepG2), and over 90% inhibition at 100 μg/mL. Antioxidant activity was weak, with IC_{50} values above 500 μg/mL in the DPPH assay and 453.85 ± 15.87 μg/mL in the ABTS assay. In macrophages, nitric oxide inhibition exceeded 61% at 100 μg/mL, though cell viability was reduced to 57.91 ± 2.98%.

Conclusions: Essential oil from *C. thorelii* displays promising cytotoxic and anti-inflammatory activities for potential use in functional foods, but further studies are needed to address safety and optimize application.

Keywords

Euphorbiaceae, *Croton thorelii*, essential oil, biological activity

Introduction

Essential oils, primarily composed of terpenes and phenylpropanoids, are volatile secondary metabolites derived from plants with diverse biological and ecological roles [1]. These compounds serve as plant hormones, pheromones, phytoalexins, pollinator attractants, insect repellents, and defense agents against pathogens and herbivores [2]. Their complex phytochemical profiles endow essential oils with a wide range of pharmacological properties, including antiviral, anticancer, antibacterial, antinociceptive, and antiphlogistic activities [3]. Historically, essential oils have been integral to traditional medicine, perfumery, cosmetics, and flavoring due to their natural origin, biodegradability, low toxicity, and multitarget pharmacological interactions, making them promising candidates for novel drug development [4, 5]. Additionally, their herbicidal and antimicrobial attributes have garnered increasing global interest for agricultural and industrial applications [6].

The genus *Croton* L. (Euphorbiaceae), comprising approximately 1,200–1,300 species, is widely distributed across tropical and subtropical regions and is renowned for its phytochemical diversity [7]. *Croton* species produce a variety of secondary metabolites, including terpenoids, phenolic compounds, alkaloids, and phenylpropanoids, which contribute to their pharmacological potential [8]. Essential oils from *Croton* species exhibit anti-inflammatory, antibacterial, antifungal, antioxidant, cytotoxic, anticancer, insecticidal, and antiparasitic activities, as demonstrated in studies of species such as *C. delpyi*, *C. tonkinensis*, and *C. zehntneri* [9, 10]. However, while these species have been extensively studied for their chemical composition and bioactivities, *C. thorelii*, a heliophilous shrub native to Southeast Asia (including Laos, Cambodia, Thailand, and Vietnam), remains significantly understudied despite its ethnobotanical significance [11, 12].

In Vietnam, *C. thorelii*, locally known as “Cù đèn thorel”, thrives in sparse forests on arid, rocky soils at elevations of 100–500 m, particularly in provinces such as Kon Tum, Gia Lai, Binh Duong, Dong Nai, and Ba Ria-Vung Tau [11]. Ethnopharmacological records document its traditional uses, with roots employed to alleviate dysmenorrhea, leaves applied for scabies treatment, and both leaves and seeds used for insect control [12]. Previous phytochemical studies on *C. thorelii* have identified notable bioactivities in its ethyl acetate extracts, including strong HIV1-RT inhibitory activity and high cytotoxic activity against human intrahepatic cholangiocarcinoma (KKU-M213) and human mammary gland/breast adenocarcinoma (MDA-MB-231) cell lines. Additionally, the compound 5-hydroxy-7,4'-dimethoxyflavone was isolated for the first time from this species [8]. Unlike well-documented *Croton* species, such as *C. delpyi* with its high (*E*)-caryophyllene content and potent cytotoxic activity [9], or *C. zehntneri* characterized by phenylpropanoid-rich oils like estragole [10], comprehensive data on *C. thorelii*’s essential oil composition and biological activities, including cytotoxic, antioxidant, and anti-inflammatory properties, remain scarce, with existing literature primarily limited to its traditional applications and limited phytochemical analyses [12, 13]. This knowledge gap, combined with *C. thorelii*’s ecological adaptability and regional prevalence in Vietnam, justifies its selection for this study to elucidate its phytochemical profile and therapeutic potential.

The primary objective of this study is to characterize the chemical composition and biological activities of the essential oil distilled from *C. thorelii* leaves, collected in Central Vietnam, using gas chromatography-mass spectrometry (GC-MS) analysis. This investigation addresses a knowledge gap regarding this understudied species, which holds ethnobotanical significance and potential applications in functional foods and natural preservatives. The focus on biological activities (cytotoxic, antioxidant, and anti-inflammatory) aims to explore its safety and efficacy for food-related applications, bridging traditional medicine and modern food science.

Materials and methods

Chemicals

All reagents used in this study were of analytical grade. Anhydrous sodium sulfate (Na_2SO_4), methanol, dimethyl sulfoxide (DMSO), 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, acetate buffer, Trolox, ascorbic acid, ellipticine, and dexamethasone were purchased from Sigma-Aldrich (USA). Sulforhodamine B (SRB), *N*-(1-naphthyl)ethylenediamine dihydrochloride, sulfanilamide, phosphoric acid, and lipopolysaccharide (LPS) were obtained from REAGEN LLC (USA). Dulbecco's modified Eagle's medium (DMEM) was sourced from Gibco (USA). The SK-LU-1 (human lung carcinoma), HepG2 (hepatocellular carcinoma), and RAW 264.7 macrophage cell lines were kindly provided by Prof. Domenico Delfino, University of Perugia, Italy. All other chemicals and solvents were procured from standard commercial suppliers.

Plant materials

Fresh leaves of *C. thorelii* were collected in February 2025 from Ninh Van, Ninh Hoa, Khanh Hoa, Vietnam (12°21'48.9"N, 109°16'07.8"E). The collection was conducted during the spring season in Central Vietnam, when *C. thorelii* exhibits robust vegetative growth and optimal essential oil accumulation. The botanical identification was performed by Dr. Anh Tuan Le of the Vietnam National Museum of Nature, Mien Trung Institute for Scientific Research. A voucher specimen, assigned the code CT-22025, was deposited at the herbarium of the University of Education, Hue University. The collected leaves were mature, characterized by their fully expanded, dark green appearance, indicating peak metabolic activity suitable for essential oil extraction. Based on local ecological observations, the flowering period of *C. thorelii* in this region typically occurs, suggesting that the February collection preceded the flowering phase to maximize leaf biomass and essential oil yield.

Hydro-distillation

A Clevenger-type apparatus was used to distill 350 g of fresh *C. thorelii* leaves for four hours, following the protocol outlined in the Vietnamese Pharmacopoeia V (Ministry of Health, 2019). The entire 350 g of fresh material was processed in a single run to ensure consistency. Fresh leaves were chosen to preserve volatile terpenoid compounds, such as β-selinene and 1,8-cineole, which are prone to degradation during drying, as supported by studies on *Croton* species indicating higher essential oil quality from fresh material. The extracted essential oil was dried over anhydrous Na_2SO_4 at 5°C prior to further analysis. The yield was calculated based on the dry weight of the material to standardize comparisons with literature data, which typically report yields on a dry weight basis. To determine the dry weight, a separate 50 g sample of fresh leaves was oven-dried at 50°C until constant weight, revealing a moisture content of approximately 70%. Using this moisture content, the dry weight equivalent of the 350 g of fresh leaves was estimated to be 105 g.

The GC-MS analysis

GC-MS was employed to study the essential oils' chemical constituents. The Shimadzu GCMS-QP2010 Plus system (Japan) had a fused silica Equity-5 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness, USA) [13, 14]. The chromatographic parameters were set as follows: injector and interface temperatures were maintained at 270°C, and helium was utilized as the carrier gas at a constant flow rate of 1.2 mL/min. From 50°C (held for 3 min) to 250°C (held for 12 min) and finally to 270°C (held for 20 min), the oven temperature was programmed to rise at a rate of 4°C/min. At an intake pressure of 90.0 kPa, 1.0 μL of each sample was injected with a split ratio of 30:1. With an electron ionization voltage of 70 eV, a detection voltage of 0.80 kV, and a scan rate of 0.5 scans per second, the mass spectrometer was able to measure masses between 40 and 500 amu. Retention indices (RIs), which were determined using a homologous sequence of *n*-alkanes (C_7–C_{40}), were compared with reference data from Adams' library in order to identify the compounds [15]. The relative abundance of each component was estimated based on its peak area percentage. In order to guarantee reproducibility, every analysis was carried out in triplicate.

Cell culture

Mouse macrophage RAW 264.7 cells were used for anti-inflammatory evaluation, while human lung carcinoma (SK-LU-1) and hepatocellular carcinoma (HepG2) cell lines were employed for cytotoxicity assays. The SK-LU-1 and HepG2 cell lines were obtained from a certified cell bank and had been authenticated by short tandem repeat (STR) profiling by the supplier prior to distribution. All cells were cultured in DMEM supplemented with appropriate nutrients and 10% fetal bovine serum (FBS), and maintained at 37°C in a humidified atmosphere containing 5% CO_2. Cells were subcultured for at least two passages before being used in subsequent experiments.

SRB assay

The cytotoxic activity of essential oils extracted from the leaves of *C. thorelii* was evaluated using the SRB assay, following a spectrophotometric method [16]. Two cancer cell lines were tested: lung carcinoma (SK-LU-1) and hepatocellular carcinoma (HepG2), kindly provided by Prof. Domenico Delfino, University of Perugia, Italy. The cells were cultured in DMEM supplemented with *L*-glutamine, sodium pyruvate, $NaHCO_3$, 10% FBS, and 1% penicillin-streptomycin (100 U/mL penicillin and 100 µg/mL streptomycin) under conditions of 37°C and 5% CO_2. Subculturing was performed at a 1:3 ratio every 3–5 days, depending on cell growth. For the assay, cells were seeded in 96-well plates at a density of 5×10^4 cells per well in 200 µL of culture medium and incubated for 24 h to allow attachment. The medium was then replaced with fresh medium containing essential oil samples at concentrations of 0.8, 4, 20, and 100 µg/mL. After 72 h of incubation at 37°C in a humidified 5% CO_2 atmosphere, the cells were fixed with 20% trichloroacetic acid (TCA) for 1 h at 4°C, washed with distilled water, and stained with 0.4% (w/v) SRB dye in 1% acetic acid for 30 min. Excess dye was removed by washing with 1% acetic acid, and bound dye was dissolved in 10 mM Tris base (pH 10.5). Optical density (OD) was measured at 540 nm using an ELISA plate reader. DMSO was used as the negative control at concentrations of 0.08, 0.4, 2, and 10 µg/mL, matching the sample concentration range. Ellipticine, used as the positive control, was tested at concentrations ranging from 0.1 to 10 µg/mL. The percentage of cell growth inhibition was calculated using the formula:

$$\text{Inhibition } (\%) = 100 - \left[\frac{OD_{sa} - OD_{d0}}{OD_{neg} - OD_{d0}}\right] \times 100$$

Where OD_{sa} represents $OD_{540\ nm}$ of the samples (the oils or reference) treated wells after 72 h incubation, OD_{neg} refers to $OD_{540\ nm}$ of the DMSO negative control wells after 72 h incubation, OD_{d0} refers to $OD_{540\ nm}$ of the control for the initial time point (Day 0). IC_{50} values, representing the concentration required to inhibit 50% of cell growth, were determined using a nonlinear regression model (four-parameter logistic curve) in GraphPad Prism software (GraphPad Software, USA). All experiments were performed in triplicate to ensure reproducibility and accuracy.

Method for assessing antioxidant activity via the DPPH assay

To evaluate the essential oils' antioxidant capacity, the DPPH (Sigma-Aldrich, USA) radical scavenging assay was employed [17]. A 0.25 mM DPPH solution was prepared in methanol, and 100 µL of this solution was combined with 1.4 µL of the essential oil sample (dissolved in DMSO) at concentrations ranging from 4 to 500 µg/mL. The mixtures were incubated in a 96-well microplate at 25°C for 30 min (in the dark). After incubation, the absorbance was recorded at 517 nm using a microplate reader (BioTek, USA). The following formula was used to determine the percentage of DPPH radical inhibition:

$$\text{Inhibition } (\%) = \frac{X - Y}{X} \times 100$$

Where X is the absorbance of the control (without sample) and Y is the absorbance in the presence of the test or reference sample. All measurements were conducted in triplicate. Ascorbic acid was used as the positive control, and IC_{50} values were calculated using GraphPad Prism software.

Method for assessing antioxidant activity via the ABTS assay

The ABTS assay (Sigma-Aldrich, USA), which was modified slightly from the Saeed et al. [18] procedure, was used to assess the test substance's antioxidant capacity. The following is how the methodology went:

deionized water was used to dilute the sample until it reached concentrations of 10,000, 2,000, 400, and 80 µg/mL. The reference standard, Trolox, was produced similarly in deionized water at concentrations of 2,000, 400, 80, and 16 µg/mL. An ABTS solution (7 mM) was combined with potassium persulfate (2.45 mM) and allowed to stand at room temperature in the dark for 16 h. To get an OD of 0.70 ± 0.02 at 734 nm, the resultant $ABTS^{\bullet+}$ solution was diluted with acetate buffer before analysis. Following that, 100 µL of each diluted sample was combined with 1,900 µL of the $ABTS^{\bullet+}$ solution, resulting in final well concentrations of 500, 100, 20, and 4 µg/mL. A 1% DMSO solution served as the negative control, while wells containing solely deionized water functioned as blanks. A BioTek 96-well plate reader was used to capture absorbance measurements at 734 nm (BioTek, USA). The proportion of $ABTS^{\bullet+}$ radical scavenging activity was determined using the formula below: A is the OD of the sample well minus the OD of the blank, and A_0 is the OD of the control well minus the OD of the blank.

$$\text{Inhibition } (\%) = \frac{A_0 - A}{A_0} \times 100$$

The IC_{50} value, which is the concentration required to block 50% of the $ABTS^{\bullet+}$ radicals, was used to express the samples' antioxidant capacity. All measurements were performed in triplicate (n = 3) to ensure statistical robustness, and results are reported as mean ± standard deviation (SD).

In this study, we combined the two methods (DPPH and ABTS assays) to provide a more comprehensive assessment and a more complete picture of the essential oil's antioxidant activity by accounting for different reaction mechanisms and sample solubilities.

Anti-inflammatory assay

RAW 264.7 macrophage cells were plated at a density of 2×10^5 cells per well in 96-well plates [19]. The culture medium was replaced with DMEM, which is devoid of FBS, during a 24-hour incubation period. Following that, the cells were exposed to LPS (1.0 µg/mL) and essential oils at concentrations ranging from 5 to 100 µg/mL for 24 h in order to stimulate the generation of nitric oxide (NO). To evaluate NO levels, nitrite (NO_2^-) buildup was quantified using the Griess reagent system (USA). 50 µL of 0.1% (w/v) *N*-(1-naphthyl)ethylenediamine dihydrochloride and 50 µL of 1% (w/v) sulfanilamide in 5% (v/v) phosphoric acid were mixed with 100 µL of culture supernatant in a 96-well plate. After 10 min of incubation at 25°C, absorbance at 540 nm was measured using an RNE-9002 ELISA reader (REAGEN LLC, USA). A standard curve was used to calculate the nitrite levels.

$$\text{Inhibition } (\%) = 100 - \left[\frac{NO(\textit{concentration in sample})}{NO(\textit{concentration in negative control})}\right] \times 100$$

Inhibition (%) was the formula used to determine the percentage of NO inhibition. The positive control used was dexamethasone (Sigma-Aldrich, USA). GraphPad Prism software was used to determine the IC_{50} values for each experiment, which were carried out in triplicate.

Statistical analysis

Experimental data are presented as the mean ± SD, calculated from the number of replicates. IC_{50} values were calculated using nonlinear regression analysis based on dose-response curves. These curves were generated by plotting the percentage of inhibition versus the logarithm of sample concentrations, with the IC_{50} defined as the concentration that produced 50% inhibition.

Results

Phytochemical analysis

Hydrodistillation of fresh *C. thorelii* leaves produced a dark yellow essential oil with a yield of 0.21% (v/w). GC-MS analysis identified 59 compounds, representing 99.5% of the total composition (see Table 1 and Figure S1). The oil was mainly composed of sesquiterpene hydrocarbons (44.5%), oxygenated monoterpenes (41.1%), and oxygenated sesquiterpenes (9.4%). Minor components included monoterpene hydrocarbons (3.9%), oxygenated diterpenes (0.4%), and non-terpenic compounds (0.2%). The major constituents were β-selinene (22.0%), 1,8-cineole (20.7%), linalool (11.2%), and (*E*)-caryophyllene (9.5%).

Other significant compounds (≥ 1.0%) included α-terpineol, bicyclogermacrene, α-humulene, borneol, caryophyllene oxide, spathulenol, β-bourbonene, δ-cadinene, and terpinen-4-ol.

Table 1. Chemical compositions in *Croton thorelii* Gagnep. essential oil.

No.	RT	Compound[a]	RI_E	RI_L	Concentration (%)	Class.
1	7.15	α-Pinene	932	932	0.2	mh
2	7.65	Camphene	947	946	0.2	mh
3	8.49	Sabinene	971	969	0.3	mh
4	9.10	β-Pinene	989	988	0.1	mh
5	10.42	p-Cymene	1023	1020	0.5	mh
6	10.61	Limonene	1028	1024	0.6	mh
7	10.75	1,8-Cineole	1031	1026	20.7	om
8	10.94	(*Z*)-β-Ocimene	1036	1032	0.5	mh
9	11.37	(*E*)-β-Ocimene	1046	1044	0.7	mh
10	11.83	γ-Terpinene	1057	1054	0.2	mh
11	12.20	*cis*-Sabinene hydrate	1066	1065	0.2	om
12	12.72	*cis*-Linalool oxide (furanoid)	1079	1067	0.6	om
13	13.01	*trans*-Linalool oxide (furanoid)	1086	1084	0.3	om
14	13.09	Terpinolene	1088	1086	0.6	mh
15	13.66	Linalool	1101	1095	11.2	om
16	15.61	Camphor	1145	1141	0.3	om
17	16.55	Borneol	1166	1165	2.5	om
18	17.05	Terpinen-4-ol	1177	1174	1.2	om
19	17.40	p-Cymen-8-ol	1185	1179	0.2	om
20	17.69	α-Terpineol	1192	1186	3.8	om
21	18.43	Verbenone	1208	1205	0.1	om
22	24.16	Bicycloelemene	1338	1338	0.3	sh
23	24.69	α-Cubebene	1350	1348	0.7	sh
24	25.39	Cyclosativene	1367	1369	0.7	sh
25	25.85	α-Copaene	1377	1374	0.7	sh
26	26.24	β-Bourbonene	1386	1387	1.6	sh
27	26.45	β-Cubebene	1391	1387	0.3	sh
28	26.53	β-Elemene	1393	1389	0.3	sh
29	27.76	(*E*)-Caryophyllene	1423	1417	9.5	sh
30	28.10	β-Copaene	1431	1430	0.4	sh
31	28.73	Aromadendrene	1446	1439	0.2	sh
32	29.13	α-Humulene	1456	1454	2.7	sh
33	30.13	*trans*-Cadina-1(6),4-diene	1480	1475	0.2	sh
34	30.37	β-Selinene	1486	1489	22.0	sh
35	30.92	Bicyclogermacrene	1499	1500	3.0	sh
36	31.03	α-Muurolene	1502	1500	0.1	sh
37	31.64	γ-Cadinene	1518	1513	0.5	sh
38	31.96	δ-Cadinene	1526	1522	1.3	sh
39	32.97	Elemol	1551	1548	0.3	os
40	33.16	Elemicin	1556	1555	0.2	nt
41	34.12	Spathulenol	1581	1577	1.7	os
42	34.34	Caryophyllene oxide	1586	1582	2.3	os
43	34.73	Salvial-4(14)-en-1-one	1596	1594	0.1	os
44	34.88	Guaiol	1600	1600	0.5	os
45	35.15	Khusimone	1607	1604	0.1	os
46	35.34	Humulene epoxide II	1612	1608	0.5	os
47	35.41	*cis*-Isolongifolanone	1614	1612	0.3	os

Table 1. Chemical compositions in *Croton thorelii* Gagnep. essential oil. (*continued*)

No.	RT	Compound[a]	RI_E	RI_L	Concentration (%)	Class.
48	36.04	γ-Eudesmol	1631	1630	0.2	os
49	36.35	Caryophylla-4(12),8(13)-dien-5α-ol	1640	1639	0.2	os
50	36.51	*epi*-α-Cadinol	1644	1638	0.4	os
51	36.72	Torreyol	1649	1644	0.5	os
52	36.86	α-Cadinol	1653	1652	0.1	os
53	37.03	Valerianol	1658	1656	0.9	os
54	37.20	Allohimachalol	1662	1661	0.1	os
55	37.43	Aromadendrene oxide	1669	1662	0.5	os
56	37.50	Bulnesol	1670	1670	0.2	os
57	37.64	Khusilol	1674	1675	0.1	os
58	38.20	*epi*-α-Bisabolol	1689	1683	0.4	os
59	52.28	*cis*-Phytol	2114	2112	0.4	od
Total					99.5	
Monoterpene hydrocarbons (mh)					3.9	
Oxygenated monoterpenes (om)					41.1	
Sesquiterpene hydrocarbons (sh)					44.5	
Oxygenated sesquiterpenes (os)					9.4	
Oxygenated diterpenes (od)					0.4	
Non-terpenic compounds (nt)					0.2	

[a] Elution order on the Equity-5 column. RT: retention time; RI_E: experimental retention index; RI_L: literature retention index; Class.: classification.

Cytotoxic activity

This study investigated the cytotoxic effects of *C. thorelii* leaf essential oil on SK-LU-1 and HepG2 cell lines using the SRB assay. The results demonstrated notable inhibitory activity, with IC_{50} values of 54.52 ± 1.40 µg/mL for SK-LU-1 and 48.29 ± 2.09 µg/mL for HepG2.

Antioxidant activity

The antioxidant activity of *C. thorelii* essential oil was assessed using DPPH and ABTS assays. It showed no significant activity in the DPPH assay (IC_{50} > 500 µg/mL) and weak activity in the ABTS assay (IC_{50} = 453.85 ± 15.87 µg/mL).

Anti-inflammatory properties

In LPS-stimulated RAW 264.7 macrophages, the essential oil from *C. thorelii* leaves exhibited moderate anti-inflammatory activity in the nitric oxide (NO) production assay. At 100 µg/mL, the essential oil suppressed NO generation by 61.46 ± 2.37%; however, this effect was associated with reduced cell viability (57.91 ± 2.98%), indicating possible cytotoxicity at higher concentrations. In contrast, the positive control, dexamethasone, demonstrated stronger inhibition (86.59 ± 1.12%) while maintaining higher cell viability (92.71 ± 2.45%). The inhibitory effect of *C. thorelii* essential oil decreased in a dose-dependent manner, with NO inhibition rates of 10.57 ± 1.01%, 5.85 ± 0.61%, and 1.81 ± 0.12% at 50, 25, and 12.5 µg/mL, respectively. At 50 µg/mL, cell viability improved to 83.47 ± 2.20%, suggesting reduced cytotoxic effects at lower concentrations. The IC_{50} value of the essential oil could not be determined within the tested concentration range, as 50% inhibition was not achieved under non-cytotoxic conditions. Conversely, dexamethasone exhibited an IC_{50} value of 13.24 ± 1.23 µg/mL. Although the anti-inflammatory potency of *C. thorelii* essential oil was lower than that of the standard drug, its activity may be attributed to the synergistic effects of its bioactive constituents.

Discussion

The present study aimed to investigate the chemical composition of the essential oil extracted from *C. thorelii* leaves collected in Central Vietnam using GC-MS analysis, and to assess its biological activities,

including cytotoxic, antioxidant, and anti-inflammatory properties, to explore its potential as a natural therapeutic agent. This focus addresses a gap in the literature regarding the characterization of *C. thorelii* essential oil, particularly from this region, and its comparison with other *Croton* species.

Chemical analysis

A complex phytochemical profile that distinguishes this essential oil from those of other *Croton* species reported in the literature and reflects both species-specific variability and regional impacts within Vietnam was found in the essential oil isolated from the fresh leaves of *C. thorelii* in this investigation. Comparatively, the essential oil of *C. tonkinensis* leaves from Vietnam, as reported by [20], contained significant amounts of (*E*)-caryophyllene (10.1%), linalool (7.8%), and α-humulene (7.1%), alongside bicycloelemene (8.0%) and β-bisabolene (9.6%). While (*E*)-caryophyllene and linalool are common to both species, *C. thorelii* exhibits a higher prevalence of oxygenated monoterpenes, such as 1,8-cineole (20.7%), which is absent or minimal in *C. tonkinensis*. Similarly, *C. cascarilloides* from the same study was dominated by hydrocarbon compounds like (*E*)-caryophyllene (13.5%) and α-humulene (5.9%), with β-selinene (6.7%) present but at a much lower concentration than in *C. thorelii* (22.0%) [20]. In contrast, *C. chevalieri* showed a distinct profile with non-terpenoid compounds like cyclohexanone (6.8%) and benzyl benzoate (18.8%), which are negligible in *C. thorelii* (0.2% non-terpenoids) [20].

The prominence of (*E*)-caryophyllene in *Croton* species is a recurring theme across studies. For instance, *C. delpyi* leaves from Vietnam yielded (*E*)-caryophyllene (54.34%) and α-humulene (18.19%) as the major components [21], far exceeding the 9.5% and 2.7% observed in *C. thorelii*, respectively. This suggests a stronger sesquiterpene hydrocarbon focus in *C. delpyi* compared to the balanced sesquiterpene-monoterpene profile of *C. thorelii*. Likewise, *C. hirtus* aerial parts from Vietnam contained α-humulene (8.5%), germacrene D (11.6%), and (*E*)-caryophyllene (32.8%) [10], aligning with *C. thorelii* in terms of sesquiterpene presence but lacking the high oxygenated monoterpene content seen in our study.

Its constituents, such as linalool (15.05% vs. 11.2%), (*E*)-caryophyllene (7.91% vs. 9.5%), bicyclogermacrene (7.36% vs. 3.0%), and 1,8-cineole (6.53% vs. 20.7%), are rather similar to those of *C. thorelii* [22]. However, the higher 1,8-cineole content in *C. thorelii* underscores a greater oxygenated monoterpene contribution, possibly linked to differences in plant parts (leaves vs. stems) or environmental factors. In contrast, *C. zehntneri* leaves from Brazil exhibited a markedly different composition, with estragole dominating at 84.7% or 76.8%, alongside minor amounts of 1,8-cineole (7.0%) and spathulenol (5.6%) [23, 24]. This phenylpropanoid-heavy profile contrasts sharply with the terpene-rich essential oil of *C. thorelii.*

Further afield, *C. matourensis* leaves from Brazil contained (*E*)-caryophyllene (12.41%) and thunbergol (11.74%) [25], while *C. piauhiensis* leaves featured (*E*)-caryophyllene (21.58%) and *D*-limonene (13.47%) [26]. Neither species showed the high levels of β-selinene or 1,8-cineole found in *C. thorelii*. Similarly, *C. hirtus* leaves from Brazil varied by location, with spathulenol (26.7%) or (*E*)-caryophyllene (27.9–37.3%) as major constituents [27], compared to 1.7% and 9.5% in *C. thorelii*, respectively. Both *C. monteverdensis* and *C. niveus* bark essential oils from Costa Rica were high in α-pinene (17.1% and 14.4%) and 1,8-cineole (11.6% in *C. niveus*) [28], which is consistent with the occurrence of 1,8-cineole in *C. thorelii* but differs in terms of sesquiterpene dominance.

The chemical diversity among *Croton* species is further exemplified by several notable cases. For example, linalool accounted for 34.9% of *C. micradenus* leaf essential oil from Cuba [29], which is significantly more than the 11.2% observed in *C. thorelii*. In contrast, methyl eugenol, which was completely missing from our analysis, was abundant in *C. malambo* leaves from Venezuela (94.2%) [30]. Similarly, *C. huberi*, also from Venezuela, contained substantial amounts of germacrene D (16.1%) and (*E*)-caryophyllene (18.3%) [31]. Meanwhile, genotypes of *C. tetradenius* from Brazil exhibited either camphor (13.95%) or p-cymene (17.55%) as dominant components [32]. None of these major constituents were significant in *C. thorelii*, where their presence did not exceed 1.0%.

The elevated β-selinene (22.0%) in *C. thorelii* is particularly notable, as it exceeds levels reported in other *Croton* species, such as 6.7% in *C. cascarilloides* [20]. This, combined with substantial 1,8-cineole and linalool, suggests a unique chemotype influenced by genetic factors, soil conditions, or climate in Central Vietnam. Variations across *Croton* species may also reflect differences in harvest time, plant part, or extraction methods (e.g., hydro-distillation vs. steam distillation). These findings underscore the chemical diversity within the genus *Croton* and highlight *C. thorelii* as a distinct source of terpene-rich essential oil with potential pharmacological applications, warranting further investigation into its biosynthetic pathways and ecological roles.

Cytotoxic activity

SK-LU-1 and HepG2 cell lines were both significantly cytotoxically affected by the essential oil extracted from *C. thorelii* leaves, with IC_{50} values of 54.52 ± 1.40 μg/mL and 48.29 ± 2.09 μg/mL, respectively (Table 2). The essential oil showed substantial cytotoxic potential, inhibiting cell proliferation in both cell lines by over 90% at the highest tested concentration of 100 μg/mL. However, at lower concentrations (20, 4, and 0.8 μg/mL), the inhibition decreased significantly, ranging from 7% to 25%, indicating a dose-dependent response. The chemical composition of *C. thorelii* essential oil, dominated by sesquiterpene hydrocarbons, oxygenated monoterpenes, and oxygenated sesquiterpenes, with major constituents such as β-selinene (22.0%), 1,8-cineole (20.7%), linalool (11.2%), and (*E*)-caryophyllene (9.5%), likely contributes to its bioactivity. These findings align with the broader literature on *Croton* species, though the potency and specificity of cytotoxic effects vary across species and cell lines.

Table 2. The cytotoxic activity of *Croton thorelii* Gagnep. essential oil.

Concentration (μg/mL)	*Croton thorelii* (% inhibition)		Concentration (μg/mL)	Ellipticine[a] (% inhibition)	
	SK-LU-1	HepG2		SK-LU-1	HepG2
100	93.38 ± 1.74	99.64 ± 2.37	10	90.92 ± 0.55	95.07 ± 2.16
20	20.78 ± 0.99	24.43 ± 1.42	2	75.07 ± 1.36	81.04 ± 1.15
4	11.20 ± 1.12	14.41 ± 1.23	0.4	49.81 ± 1.43	51.74 ± 1.24
0.8	7.58 ± 0.80	8.71 ± 0.88	0.08	21.74 ± 1.11	23.09 ± 0.92
IC_{50}[b]	54.52 ± 1.40	48.29 ± 2.09	IC_{50}	0.39 ± 0.03	0.33 ± 0.02

[a] Positive control. SK-LU-1: human lung adenocarcinoma cell line; HepG2: human hepatocellular carcinoma cell line. [b] The half-maximal inhibitory concentration. Values are expressed as mean ± standard deviation (SD) (*n* = 3).

Comparatively, the essential oil from *C. delpyi* leaves, also sourced from Vietnam, displayed significantly higher cytotoxicity against HepG2 cells (IC_{50} = 2.09 ± 0.11 μg/mL) and HeLa cells (IC_{50} = 3.51 ± 0.15 μg/mL) [21]. The *C. delpyi* essential oil was rich in (*E*)-caryophyllene (54.34%) and α-humulene (18.19%), with smaller amounts of linalool (3.22%), contrasting with the more balanced sesquiterpene-monoterpene profile of *C. thorelii*. The increased (*E*)-caryophyllene concentration of *C. delpyi* essential oil, a substance known for its cytotoxic qualities through mechanisms like apoptosis induction and cell cycle arrest, may be the cause of its greater potency. Although *C. thorelii* contains (*E*)-caryophyllene (9.5%), its lower concentration, combined with the presence of 1,8-cineole and β-selinene, may result in a more moderate cytotoxic effect.

In contrast, estragole, the primary component (84.7%) of *C. zehntneri* essential oil from Brazil, showed no significant inhibition against MCF-7, HEP-2, and NCI-H292 cell lines, despite exhibiting toxicity against *Artemia salina* (LC_{50} = 4.54 μg/mL) [23]. The lack of cytotoxicity of *C. zehntneri* essential oil in human cell lines, despite its high estragole content, suggests that phenylpropanoids like estragole may not be as effective against cancer cells as terpenoids, which dominate *C. thorelii* essential oil. This highlights the role of terpenoid constituents, such as linalool and (*E*)-caryophyllene, in *C. thorelii*, in driving its cytotoxic activity.

The essential oil from *C. matourensis* leaves exhibited IC_{50} values of 28.5 μg/mL (HepG2), 23.3 μg/mL (MCF-7), 28.9 μg/mL (HCT116), 17.8 μg/mL (HL-60), and 25.8 μg/mL (MRC-5) against various cell lines [25]. The *C. matourensis* essential oil contained thunbergol (11.74%) and (*E*)-caryophyllene (12.41%), with a higher (*E*)-caryophyllene content than *C. thorelii* but lacking significant oxygenated monoterpenes like 1,8-cineole. *C. matourensis* has a lower IC_{50} value (28.5 μg/mL) against HepG2 than *C. thorelii* (48.29 μg/mL), indicating increased efficacy, maybe as a result of the synergistic actions of its sesquiterpene and diterpenoid (thunbergol) components. However, *C. thorelii*'s broader terpenoid profile may contribute to its consistent activity across both SK-LU-1 and HepG2 cell lines.

Likewise, the leaf essential oil of *C. malambo* from Venezuela, which is rich in methyl eugenol (94.2%), demonstrated cytotoxic effects against MCF-7 cells (IC_{50} = 72.84 μg/mL), while displaying lower activity against PC-3 and LoVo cell lines [30]. The IC_{50} value for MCF-7 is higher than the IC_{50} values of *C. thorelii* against SK-LU-1 and HepG2, indicating that *C. thorelii* essential oil is more potent against the tested cell lines. The high methyl eugenol content in *C. malambo* essential oil, a phenylpropanoid, contrasts with the terpenoid-rich composition of *C. thorelii*, further supporting the hypothesis that terpenoids like linalool, 1,8-cineole, and (*E*)-caryophyllene may be more effective cytotoxic agents in this context.

The cytotoxic activity of *C. thorelii* essential oil, while moderate compared to *C. delpyi* and *C. matourensis*, demonstrates its potential as an anticancer agent, particularly given its efficacy at higher concentrations (> 90% inhibition at 100 μg/mL). The presence of linalool and 1,8-cineole, known for their antiproliferative and apoptotic effects, likely contributes to this activity, alongside (*E*)-caryophyllene's established role in cancer cell inhibition. The variability in potency across *Croton* species underscores the influence of chemical composition, which is shaped by genetic, environmental, and geographical factors. To help create new therapeutic treatments, more research is required to determine the precise mechanisms of action of *C. thorelii* essential oil and evaluate its possible effectiveness against a wider variety of cancer cell lines.

Antioxidant activity

With an IC_{50} value of more than 500 μg/mL in the DPPH assay (Table 3) and 453.85 ± 15.87 μg/mL in the ABTS assay (Table 4), the essential oil isolated from *C. thorelii* leaves had little antioxidant activity. The IC_{50} values of 7.31 ± 0.41 μg/mL (DPPH) and 7.76 ± 0.42 μg/mL (ABTS) for the standard *L*-ascorbic acid were significantly lower than these values, suggesting that *C. thorelii* essential oil has a weaker capacity to scavenge radicals than the reference chemical. The mild antioxidant activity of *C. thorelii* essential oil is probably influenced by its chemical composition. The results could be explained by the lack of important phenolic substances, which are recognized for their strong antioxidant qualities.

Table 3. DPPH scavenging activity of *Croton thorelii* Gagnep. essential oil.

Concentration (μg/mL)	DPPH scavenging (%)	
	Croton thorelii	***L*-ascorbic acid**[a]
500	21.95 ± 2.26	-
100	14.18 ± 1.44	91.04 ± 1.40
20	4.12 ± 0.23	75.89 ± 1.21
4	2.55 ± 0.18	35.27 ± 0.82
0.8	-	12.79 ± 0.55
IC_{50}[b]	> 500	7.31 ± 0.41

[a] Positive control; [b] scavenging concentration at 50%—concentration that neutralizes 50% of DPPH free radicals. -: not tested. Values are expressed as mean ± standard deviation (SD) (*n* = 3). DPPH: 2,2-diphenyl-1-picrylhydrazyl.

In contrast, the essential oil extracted from the leaves of *C. piauhiensis* exhibited a wide range of antioxidant activity in several tests, with IC_{50} values varying between 171.21 and 4,623.83 μg/mL [26]. Specifically, in the DPPH assay, *C. piauhiensis* essential oil had an IC_{50} of 4,623.83 μg/mL (compared to quercetin, IC_{50} = 2.71 μg/mL), which is significantly higher than *C. thorelii*'s IC_{50} (> 500 μg/mL), indicating

Table 4. Free-radical scavenging activities of *Croton thorelii* Gagnep. essential oil in ABTS assay.

Concentration (μg/mL)	$ABTS^{\bullet+}$ radical-scavenging (%)	
	Croton thorelii	***L*-ascorbic acid**[a]
500	54.18 ± 1.42	-
100	14.17 ± 0.94	90.18 ± 1.26
20	2.92 ± 0.28	71.28 ± 0.92
4	0.80 ± 0.09	34.5 ± 0.96
0.8	-	15.01 ± 0.39
IC_{50}[b]	453.85 ± 15.87	7.76 ± 0.42

[a] Positive control; [b] scavenging concentration at 50%—concentration that neutralizes 50% of ABTS free radicals. -: not tested. Values are expressed as mean ± standard deviation (SD) (*n* = 3). ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid).

poorer DPPH radical scavenging capacity. However, *C. piauhiensis* obtained an IC_{50} of 171.21 μg/mL in other tests, such as the β-carotene bleaching test (BCB), indicating greater activity in specific situations. The essential oil of *C. piauhiensis* consist of Germacrene D (9.56%), *D*-limonene (13.47%), and (*E*)-caryophyllene (21.58%). Since monoterpenes with allylic hydrogens are known to have protective effects against lipid peroxidation, the presence of monoterpene hydrocarbons like *D*-limonene, which is present in trace amounts in *C. thorelii* (0.6%), may be a factor in its improved performance in the BCB assay.

In the DPPH assay, the essential oils of *C. argyrophylloides*, *C. nepetifolius*, and *C. jacobinensis* exhibited weaker antioxidant activity, showing IC_{50} values of 12.55, 22.11, and 24.96 μg/mL, respectively, which were higher than those of the standards thymol and butylated hydroxytoluene, with IC_{50} values of 3.47 μg/mL and 5.16 μg/mL [33]. Although the chemical compositions of these essential oils were not specified, prior work by the same group highlighted that phenolic compounds, monoterpene hydrocarbons (e.g., terpinolene, α-terpinene, γ-terpinene), and allylic alcohols contribute significantly to antioxidant activity [9]. In *C. thorelii*, the low monoterpene hydrocarbon content (3.9%) and absence of phenolics likely underlie its weaker DPPH performance compared to these species.

With an IC_{50} of less than 63.59 μg/mL, the essential oil from *C. cajucara*, which has a high concentration of 7-hydroxycalamenene (28.4%–37.5%), demonstrated greater antioxidant activity in the DPPH assay [34]. The presence of 7-hydroxycalamenene, a sesquiterpene with a phenolic-like structure, likely accounts for its superior radical scavenging capacity. In contrast, *C. thorelii* lacks phenolic or phenolic-like compounds, relying instead on terpenoids like linalool (11.2%) and 1,8-cineole (20.7%), which offer only moderate antioxidant effects due to their ability to donate hydrogen atoms.

The IC_{50} of *C. ferrugineus* essential oil in the ABTS experiment was 901 ± 20 μg/mL [35], which is higher than that of *C. thorelii* (453.85 ± 15.87 μg/mL), indicating that *C. thorelii* has a relatively better ABTS radical scavenging capacity. The chemical composition of *C. ferrugineus* was not detailed, but its lower antioxidant activity suggests a possible lack of effective radical scavengers like linalool, which is present in *C. thorelii* and known to contribute to ABTS scavenging through its hydroxyl group.

Finally, the essential oil from the stem bark of *C. urucurana* exhibited an IC_{50} of 3.21 mg/mL (3,210 μg/mL) in the DPPH assay [36], a value much higher than *C. thorelii*'s IC_{50} (> 500 μg/mL), indicating significantly weaker antioxidant activity. The composition of *C. urucurana* essential oil was not specified, but its high IC_{50} suggests a limited presence of active antioxidant compounds, contrasting with *C. thorelii*'s more diverse terpenoid profile, which includes linalool and 1,8-cineole, both known for moderate radical scavenging properties.

C. thorelii essential oil's low monoterpene hydrocarbon concentration and absence of phenolic components, which are both necessary for potent radical scavenging, are the reasons for its weak antioxidant activity, especially in the DPPH experiment. However, its moderate ABTS activity may be driven by oxygenated monoterpenes like linalool and 1,8-cineole, which can stabilize radicals through hydrogen donation. The variability in antioxidant performance across *Croton* species highlights the influence of chemical composition, which is shaped by genetic, environmental, and geographical factors. While *C. thorelii*

essential oil does not exhibit the potency of phenolic-rich essential oils like *C. cajucara*, its moderate ABTS activity suggests potential for use in combination with other antioxidants, such as in natural preservative systems, warranting further exploration of its synergistic effects.

Anti-inflammatory properties

In LPS-stimulated RAW 264.7 macrophage cells, the essential oil from *C. thorelii* leaves showed considerable anti-inflammatory efficacy in the nitric oxide (NO) generation assay (Table 5). At a concentration of 100 μg/mL, the essential oil inhibited NO production by 61.46 ± 2.37%, though this was accompanied by a reduced cell viability of 57.91 ± 2.98%, suggesting potential cytotoxicity at higher concentrations. In comparison, the positive control, dexamethasone, achieved 86.59 ± 1.12% inhibition with a higher cell viability of 92.71 ± 2.45%. NO inhibition was dose-dependently reduced at lower doses by *C. thorelii* essential oil, with values of 10.57 ± 1.01% at 50 μg/mL, 5.85 ± 0.61% at 25 μg/mL, and 1.81 ± 0.12% at 12.5 μg/mL, while cell viability improved to 83.47 ± 2.20% at 50 μg/mL (viability data for 25 and 12.5 μg/mL were not available). The IC_{50} for *C. thorelii* essential oil could not be determined, whereas dexamethasone exhibited an IC_{50} of 13.24 ± 1.23 μg/mL. Although *C. thorelii* essential oil's potency is limited when compared to the standard, its chemical composition probably plays a role in its anti-inflammatory actions.

Table 5. In vitro anti-inflammatory activity of the leaf essential oil of *Croton thorelii*.

Concentration (μg/mL)	*Croton thorelii*		Concentration (μg/mL)	Dexamethasone[a]	
	NO inhibition rate (%)	Viability rate (%)		NO inhibition rate (%)	Viability rate (%)
100	61.46 ± 2.37	57.91 ± 2.98	100	86.59 ± 1.12	92.71 ± 2.45
50	10.57 ± 1.01	83.47 ± 2.20	20	53.83 ± 1.07	99.09 ± 1.54
25	5.85 ± 0.61	-	4	41.84 ± 0.98	-
12.5	1.81 ± 0.12	-	0.8	31.91 ± 0.92	-
IC_{50}[b]	NA (not available)	-	IC_{50}	13.24 ± 1.23	-

[a] Positive control; [b] concentration that inhibits 50% of NO production. -: not tested. Values are expressed as mean ± standard deviation (SD) (*n* = 3).

In LPS-stimulated RAW 264.7 macrophages, essential oils obtained from *C.* kongensis stems collected in Nhu Xuan and Thuong Xuan districts (Thanh Hoa province, Vietnam) inhibited NO production, yielding IC_{50} values of 105.71 ± 0.96 μg/mL and 94.93 ± 1.31 μg/mL, respectively [22]. The *C. kongensis* essential oil contained 1,8-cineole (6.53%), (*E*)-caryophyllene (7.91%), bornyl acetate (9.52%), and linalool (15.05%), sharing some compositional similarities with *C. thorelii*, particularly in linalool and 1,8-cineole content. Although an IC_{50} for *C. thorelii* was not determined, its inhibition of > 61% at 100 μg/mL suggests comparable or slightly better activity at this concentration than *C. kongensis*, which required a similar concentration range to achieve its IC_{50}. The presence of 1,8-cineole and linalool in both essential oils likely contributes to their anti-inflammatory effects, as these compounds are known to suppress pro-inflammatory pathways, including NO production.

The essential oil from *C. rhamnifolioides* leaves, with 1,8-cineole as its major constituent (41.33%), exhibited significant anti-inflammatory activity in vivo [37]. The essential oil decreased *Croton* essential oil-induced edema by 42.1% at 20 mg/mL, but 1,8-cineole by itself (8.26 mg/mL) reduced it by 34.9%. Furthermore, the essential oil and 1,8-cineole (10.33–82.66 mg/kg) significantly decreased vascular permeability and paw edema brought on by carrageenan, dextran, histamine, and arachidonic acid at doses of 25–200 mg/kg. The high 1,8-cineole content in *C. rhamnifolioides* essential oil, compared to 20.7% in *C. thorelii*, likely enhances its anti-inflammatory efficacy, as 1,8-cineole is known to inhibit pro-inflammatory cytokines and mediators like NO. The in vitro NO inhibition by *C. thorelii* essential oil (> 61% at 100 μg/mL) aligns with the anti-inflammatory potential of 1,8-cineole, though its lower concentration and the presence of other terpenoids like β-selinene may dilute its overall potency compared to *C. rhamnifolioides*.

A study investigating the essential oil of *C. rhamnifolioides* complexed with β-cyclodextrin (COEFC) revealed anti-inflammatory effects in vivo, underscoring the critical role of 1,8-cineole [37]. In line with the results of the uncomplexed essential oil, all tested doses of COEFC decreased vascular permeability and acute paw edema brought on by carrageenan and dextran [37]. The complexation probably increased 1,8-cineole's bioavailability, which strengthened its anti-inflammatory properties. In contrast, *C. thorelii* essential oil, tested in vitro, showed a dose-dependent NO inhibition but with reduced cell viability at higher concentrations, suggesting that its anti-inflammatory potential may be limited by cytotoxicity, a factor not reported in the *C. rhamnifolioides* studies.

The moderate anti-inflammatory properties of *C. thorelii* leaf essential oil are due to the presence of 1,8-cineole and linalool, which are known to influence inflammatory pathways by lowering the production of NO and the expression of cytokines. The presence of (*E*)-caryophyllene (9.5%), a sesquiterpene with documented anti-inflammatory properties via cannabinoid receptor pathways, may also contribute to its activity. However, the high β-selinene content (22.0%), which lacks significant anti-inflammatory activity, may dilute the overall effect compared to essential oils with higher concentrations of active compounds like 1,8-cineole in *C. rhamnifolioides*. The variability in anti-inflammatory potency across *Croton* species highlights the influence of chemical composition, which is shaped by genetic, environmental, and geographical factors. The dose-dependent response of *C. thorelii* essential oil, coupled with its cytotoxicity at higher concentrations, suggests that further optimization, such as complexation or combination with other agents, could enhance its therapeutic potential. The observed > 61% NO inhibition at 100 µg/mL, while promising, coincides with a cell viability of only 57.91%, which may indicate nonspecific toxicity rather than a specific anti-inflammatory effect. This suggests that the anti-inflammatory activity at this concentration could be confounded by cytotoxic effects, particularly given the lower viability compared to dexamethasone (92.71%). At lower concentrations (e.g., 50 µg/mL), where viability improves to 83.47%, the inhibition drops significantly (10.57%), further supporting the possibility of toxicity-driven effects at higher doses. This limitation highlights the need for further investigation, such as in vivo studies or testing at a broader range of concentrations, to differentiate between specific anti-inflammatory activity and nonspecific cytotoxicity, and to optimize the therapeutic window of *C. thorelii* essential oil.

Conclusion

The essential oil from *C. thorelii* leaves, collected in Central Vietnam, exhibits a terpene-rich composition with potential cytotoxic and anti-inflammatory properties. While its antioxidant activity is limited, the essential oil shows promise for therapeutic applications, particularly in anticancer and anti-inflammatory contexts. Further optimization and in vivo studies are recommended to explore its full potential.

Abbreviations

ABTS: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

DMEM: Dulbecco's modified Eagle's medium

DMSO: dimethyl sulfoxide

DPPH: 2,2-diphenyl-1-picrylhydrazyl

FBS: fetal bovine serum

GC-MS: gas chromatography-mass spectrometry

LPS: lipopolysaccharide

OD: optical density

SD: standard deviation

SRB: sulforhodamine B

Supplementary materials

The supplementary figure for this article is available at: https://www.explorationpub.com/uploads/Article/file/1010121_sup_1.pdf.

Declarations

Author contributions

BCN: Conceptualization, Methodology, Investigation, Data curation, Writing—original draft. ATL, QTN, and TQL: Conceptualization, Data curation, Resources. TVP: Conceptualization, Data curation, Formal analysis, Visualization, Writing—original draft, Resources, Writing—review & editing, Supervision, Project administration. All authors read and approved the submitted version.

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