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RESEARCH ARTICLE

GC-MS profiling and biological activities of *Conamomum vietnamense* leaf extracts from Vietnam

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Abstract

Conamomum vietnamense is a native species in Vietnam with significant potential pharmacological properties. This study evaluated the total phenolic (TPC) and total flavonoid (TFC) contents, antioxidant properties and *in vitro* cytotoxicity of *C. vietnamense* leaves. The volatile components of the leaf fractionated extract were analyzed using the gas chromatography-mass spectrometry (GC-MS) method. The TPC and TFC were evaluated with Folin-Ciocalteu and aluminum chloride in colorimetric methods, respectively. Antioxidant activity was evaluated through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, while cytotoxicity was assessed using the sulforhodamine B (SRB) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) methods. The main compounds identified in the leaf fractionated extract included 1,8-cineole (37.16%), α-terpineol (7.77%), limonene (4.96%), α-eudesmol (4.66%), β-eudesmol (2.95%) and α-thujene (2.25%). TPC and TFC values were determined to be 119.66 ± 1.03 mg gallic acid equivalent (GAE)/g E and 205.94 ± 1.97 mg RE/g E, respectively. The extract demonstrated antioxidant activity with an IC_{50} value of 122.46 ± 2.82 µg/mL. It also exhibited strong cytotoxic effects against MCF-7, SK-LU-1, HeLa, MKN-7 and HL-60 cell lines with IC_{50} values of 35.90 ± 1.24, 68.89 ± 3.76, 45.49 ± 1.64, 59.18 ± 1.77 and 51.41 ± 1.70 µg/mL, respectively, which may be attributed to the presence of diverse secondary metabolites with cytotoxic properties. This study provides a foundation for further research on isolating bioactive compoundswith pharmacological effects from *C. vietnamense* and elucidating the molecular mechanisms underlying its cytotoxic activity.

Keywords: anticancer; antioxidant; *Conamomum vietnamense* leaf; cytotoxicity; essential oil

Introduction

Free radicals are atom or molecule that contains one or more unpaired electrons in its outer shell, rendering them highly unstable and reactive. To achieve stability, they readily interact with nearby molecules abstrating electrons, thus returning to a ground state. Free radicals and other reactive molecules are mainly derived from oxygen and nitrogen, collectively called as reactive oxygen species (ROS) and reactive nitrogen species (RNS). Among them, ROS are naturally formed as the result of electron transfer reaction, by losing or accepting electron. ROS participate in many cell signaling pathways, including cellular proliferation, migration, apoptosis and necrosis (1, 2). Common ROS include superoxide, hydroxy radical and hydrogen peroxide, which play a pivotal role in the maintenance of redox homeostasis and regulation of transcription factors. Imbalance occurs when ROS levels are in excess and the antioxidant defense system loses its control. An excessive amount of ROS can lead to oxidative stress and damage biomolecules by oxidizing DNA, proteins, cellular sturdiness and cell membranes and cell proliferation. Therefore, uncontrolled ROS levels are also associated with the development of aging as well as various physiological disorders including cancer, respiratory, cardiovascular, obesity, diabetes and many digestive diseases (1-3). On the other hand, cancer in particular, is the most common and lethal disease, causing millions of deaths every year worldwide (4). Conventional treatment strategies include chemotherapy include chemotherapy, surgery and radiotherapy (5, 6). Among these, chemotherapy is considered the first-line intervention. However, regardless of their intial positive responses, conventional therapies have shown limited long term efficacy due to several drawbacks, including side effects, incomplete tumor eradication and the emergence of drug resistance in the treatment of some cancer diseases, these therapeutics have not fully achieved the desired (7). Furthermore, surgery methods might lead to mortality and morbidity, resulting in low quality of life (6).

Plant-based medicines have been known as an alternative source for the treatment of various diseases. Natural products isolated from different parts of medicinal plants have been used since ancient times. Back in 2600 BC, oils from *Cupressus sempervirens* and *Commiphora* species were used by Mesopotamian civilizations to treat coughs, colds and inflammatory diseases. In

China and India, the usage of herbs as medicine has been recorded since the 1st millennium BC (6, 8). There is growing evidence that natural products are favorable sources for curing various diseases and are becoming an interesting topic concerning research and drug discovery. Bioactive compounds are naturally derived from plants, fungi, bacteria and mammals (9). Among them, plant-derived compounds are not only food-derived substances, but also have significant therapeutic potential. Plants contain a variety of phytocompounds like alkaloids, flavonoids, terpenoids, phenolic derivatives and to name a few (10), which exhibit a wide range of activities and are considered as a major source for drug development with high efficacy and low side effects (6).

The Zingiberaceae family comprises medicinal plants that have long been utilized as cosmetics, foods, spices and for health protection for a long time. This family contains about 50 genera with more than 1600 species distributed across the world, mainly found in tropical regions of Asia, Africa and America. Various extracts and isolated compounds from this family exhibited many biological activities, like anticancer, hepatoprotective, antibacterial, antioxidant, anti-inflammatory and antidiabetic (11). Amongst the medicinal plants of this family, *Conamomum Vietnamese* N.S. Lý & T.S. Hoang is a native herb of Vietnam's flora. Previous studies about the essential components from non-polar to polar extracts of this species have been reported for mosquito larvicidal, antioxidant and antimicrobial activities (12-15). However, to the best of our knowledge, the cytotoxic activities of *C. vietnamense* leaf extract still remains unknown. Herein, the chemical compositions, total flavonoid and total phenolic contents, antioxidants and cytotoxicity of *C. vietnamense* leaf have been investigated.

Materials and Methods

Chemicals, reagents and cell lines

Chemicals and reagents: n-Hexane (Chemsol, Vietnam), ethanol (OPC, Vietnam), sodium bicarbonate and acetic acid (China), MEME (Minimum Essential Medium with Eagle salts), DMEM (Dulbecco's Modified Eagle Medium), L-glutamine, penicillin G, streptomycin, TCA (trichloroacetic acid), SRB (Sulforhodamine B), Tris-base buffer, PBS (phosphate-buffered saline), MTT (3-(4,5 -dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), FBS (10% Fetal Bovine Serum), sodium pyruvate ($C_3H_3NaO_3$), Trypsin-EDTA (0.05%), ellipticine (Sigma, USA) and DMSO (dimethyl sulfoxide) (Merck, Germany). Other analytical-grade chemical reagents were used in this work.

Cell lines

The cell lines were provided by Prof. JM Pezzuto (Long Island University, USA) and Prof. Jeanette Maier (University of Milan, Italy) and stored at the Institute of Biotechnology, Vietnam Academy of Science and Technology.

Plant material

In a continuation of the previous study, *C. vietnamense* leaves were selected due to the limitations in the chemical investigations and biological activities of this species as compared with other species in the genus *Conamomum* (15). The scientific name of the plant was elucidated in previous research (16). The morphology of *C. vietnamense* is presented in Fig. 1.

C. vietnamense leaves were cleaned and dried (< 45 °C), then ground into coarse powder and prepared to get the extract for the present experiment.

Preparation of plant extract

Using the percolation method, the powdered leaf was immersed in 96% ethanol (EtOH) and the mixture solution was filtered by using filter paper, followed by drying under low pressure using a vacuum evaporator (17, 18). The leaf *n*-hexane fractionated extract of *C. vietnamense* (LCV) was obtained after a series of extraction processes, which then was used for analyzing volatile components and screening the cytotoxic activity.

Preliminary phytochemical screening evaluation

Qualitative tests for secondary metabolites, including lipids, carbohydrates, carotenoids, essential oils (EOs), triterpenoids, alkaloids, amino acids, steroids/cardiac glycosides, saponins, coumarins, polyphenols, flavonoids and tannins, were conducted

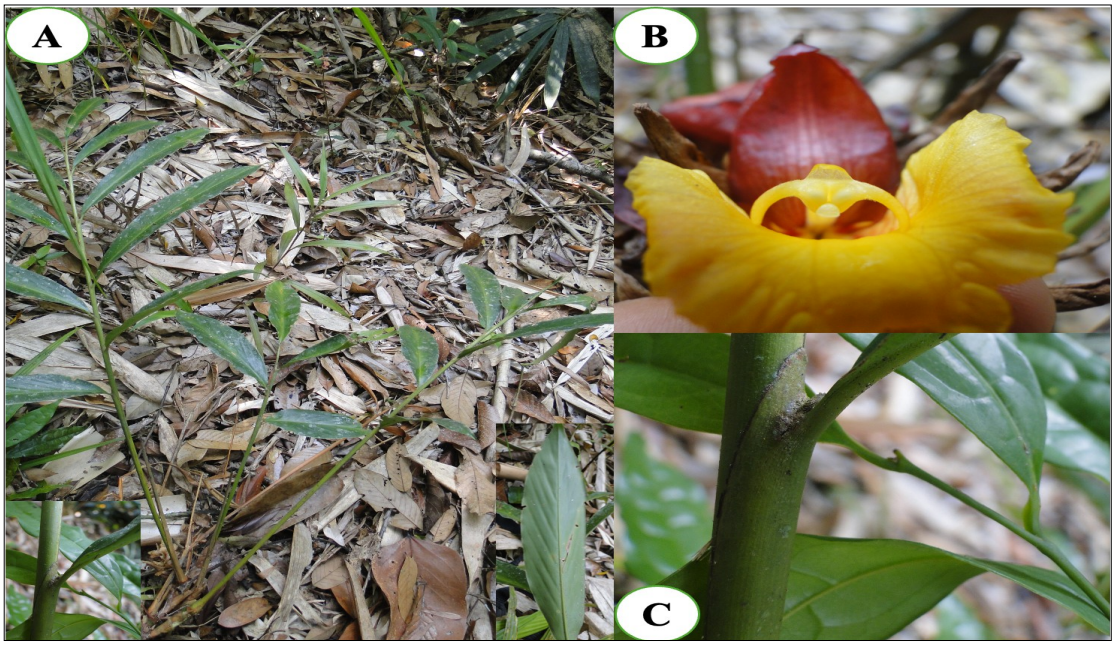

Fig. 1. The morphological features of *C. vietnamense* (A. Whole plant; B. Flowers; C. Pseudo-stems and leaves)

following the protocol of (19) and (20). Briefly, 50 g of *C. vietnamense* leaf powder was dissolved in 100 mL of 96% EtOH/30 minutes and the resulting extract was conducted to specific chemical reactions to qualitatively identify the presence of the above compounds (19).

Analysis of the volatile compounds

Sample preparation

The LCV was diluted with *n*-hexane solvent to obtain a final concentration of 20 mg/mL.

Gas chromatography-mass spectrometry (GC-MS) analysis: The volatile components of *C. vietnamese* leaves were analyzed using the GC-7980 gas chromatography system (Agilent, USA) with an HP-5MS UI column (30 m × 0.25 mm × 0.25 mm). Helium was used as a carrier gas (1.0 mL/min) and sample injection was set up at 1.0 mL. The column temperature was programmed: initial temperature 80 °C, then increase 20 °C/min to 300 °C and hold for 15 min. The injector, MS Quad and transfer line temperatures were set up at 300, 150 and 300 °C, respectively. Detector MS-5977C (Agilent, USA) was installed to scan ionic fragments in the range 50 -500 amu (2.0 scans/s) with an ionization energy of 70 eV. The retention indices (RI) of the chemical constituents were determined by co-injecting the samples with a homologous series of *n*-alkanes (C_7-C_{40}) and comparing the results. Identification of the compounds was achieved by matching their RI values with those reported in the literature (21). The MS fragmentation patterns were analyzed and compared with those of known compounds in the NIST17 library for identification (18). Each volatile compound was quantified based on the relative area of its peak in the total ion chromatogram (TIC).

Total phenolic content (TPC), total flavonoid content (TFC) and antioxidant activity

The TPC in the leaf extract were evaluated with Folin-Ciocalteu reagent according to previously reported in which gallic acid was used as a standard compound (19, 22). The data are expressed as mg gallic acid equivalent/g crude extract (mg GAE/g E). Meanwhile, the TFC was determined based on previous publish with some modifications (19, 22). The TFC was calculated as the rutin equivalent in mg/g crude extract (mg RE/g E).

The DPPH free radical scavenging activity of *C. vietnamense* extract was determined as previously published (19, 23). Ascorbic acid was used as a positive control.

The testing procedure is detailed in the supplementary file.

In vitro cytotoxic activity assay

The cytotoxic activities of LCV (0.8-500 µg/mL) on human breast carcinoma (MCF-7), human lung carcinoma (SK-LU-1), human cervical carcinoma (HeLa) and human gastric carcinoma (MKN-7) cell lines were evaluated following the protocol of (18) and (24). The cytotoxic effects of LCV (0.8-500 µg/mL) on human acute leukemia (HL-60) cell lines were assessed using a modified version of the procedure established by (18) and (25). Ellipticine, used as a positive control, was prepared at concentrations of 10, 2.0, 0.4 and 0.08 µg/mL. The testing procedure is detailed in the supplementary file.

Data analysis

Using the Minitab software (Version 20), the experimental results of phytochemical screening, TPC, TFC, antioxidant and cytotoxic activity were conducted in triplicate. Microsoft Excel 2023 software was used to calculate the data as mean ± SD, n = 3. The IC_{50} value (µg/mL) was determined using TableCurve 2Dv4 software. The outcomes were then subjected to a one-way ANOVA and Tukey test to assess the significance of the differences between samples.

Results

Phytochemical evaluation

The preliminary phytochemical screening of LCV displayed the presence of carbohydrates, carotenoids, EOs, triterpenoids, alkaloids, amino acids, polyphenols, flavonoids and tannins (Table S1). In contrast, lipids, steroid/cardiac glycosides and saponins were absent in this extract under the same analytical conditions.

The chemical composition of LCV was determined using GC-MS analysis. The relative amount of each compound was calculated based on the percentage of peak area in the total ion chromatogram. As shown in Table 1 and Fig. 1, a total of 22 components were found in this extract, accounting for 79.07%. The main components were *α*-thujene (2.25%), *β*-eudesmol (2.95%), *α*-eudesmol (4.66%), limonene (4.96%), *α*-terpineol (7.77%) and 1,8-cineole (37.16%). Meanwhile, camphor (0.57%), linalool (0.66%), *α*-cubebene (0.67%), caryophyllene oxide (0.81%), neophytadiene (0.87%), *α*-santalene (0.87%), terpinen-4-ol (0.87%), *γ*-cadinene (0.98%), *endo*-fenchol (1.23%), pinocarvone (1.29%), nerolidol (1.46%), *cis*-calamenene (1.69%), benzylacetone (1.80%), cryptomeridiol (1.80%), 10-*epi*-*γ*-eudesmol (1.81%) and *endo*-borneol (1.94%) were also determined with an area lower than 2.0% (Table 1 and Fig 2).

TPC, TFC and antioxidant activity

The TPC was measured using the Folin-Ciocalteu reagent and expressed in gallic acid equivalents per gram of the crude extract (mg GAE/g E). The TPC in *C. vietnamense* leaf extract was 119.66 ± 1.03 mg GAE/g E. Furthermore, the TFC was determined using aluminum chloride in a colorimetric method and expressed in rutin equivalents per gram of the crude extract (mg RE/g E). The TFC in the leaf extract was 205.94 ± 1.97 mg RE/g E. The DPPH free radical scavenging activity of leaf extract was displayed in Table 2, in which its IC_{50} value was 122.46 ± 2.82 µg/mL, which was higher than that of the positive control, ascorbic acid (IC_{50} = 25.65 ± 0.12 µg/mL).

Cytotoxic effect evaluation

The results from *in vitro* cytotoxicity tests show that LCV exhibits significant anticancer cytotoxicity. As shown in Table 3, the extract demonstrates strong anticancer activity with low IC_{50} values. Specifically, among the tested cell lines, the extract exhibited the strongest inhibitory effect on the growth of the MCF-7 cell line (IC_{50} = 35.90 ± 1.24 µg/mL), followed by HeLa (IC_{50} = 45.49 ± 1.64 µg/mL, HL-60 (IC_{50} = 51.41 ± 1.70 µg/mL), MKN-7 (IC_{50} = 59.18 ± 1.77 µg/mL) and SK-LU-1 (IC_{50} = 68.89 ± 3.76 µg/mL). Meanwhile, the positive control compound exhibited cytotoxic activity with IC_{50} values ranging from 0.33 to 0.52 µg/mL. These findings highlight the diverse molecular characteristics of cancer cell lines and their varying sensitivities to phytochemicals present in the leaves of *C. vietnamense*.

Table 1. Volatile constituents of LCV

No.	RT (min)	Compounds	MF	MW (g/mol)	RI (E.)	RI (L.)	Content (%)
1	5.158	*α*-Thujene	$C_{10}H_{16}$	136.23	927	924	2.25
2	5.470	Limonene	$C_{10}H_{16}$	136.23	1090	1024	4.96
3	5.837	1,8-Cineole	$C_{10}H_{18}O$	154.25	1033	1026	37.16
4	7.541	Linalool	$C_{10}H_{18}O$	154.25	1101	1095	0.66
5	7.921	*endo*-Fenchol	$C_{10}H_{18}O$	154.25	1116	1114	1.23
6	8.701	Camphor	$C_{10}H_{16}O$	152.23	1144	1141	0.57
7	9.088	Pinocarvone	$C_{10}H_{14}O$	150.22	1166	1160	1.29
8	9.177	*endo*-Borneol	$C_{10}H_{18}O$	154.25	1165	1165	1.94
9	9.441	Terpinen-4-ol	$C_{10}H_{18}O$	154.25	1180	1174	0.87
10	9.727	*α*-Terpineol	$C_{10}H_{18}O$	154.25	1193	1186	7.77
11	10.867	Benzylacetone	$C_{10}H_{12}O$	148.20	1201	1199	1.80
12	13.440	*α*-Cubebene	$C_{15}H_{24}$	204.35	1354	1348	0.67
13	14.187	*α*-Santalene	$C_{15}H_{24}$	204.35	1420	1416	0.87
14	15.762	*γ*-Cadinene	$C_{15}H_{24}$	204.35	1516	1513	0.98
15	15.898	*trans*-Calamenene	$C_{15}H_{22}$	202.33	1524	1521	1.69
16	16.441	(*E*)-Nerolidol	$C_{15}H_{26}O$	222.37	1567	1561	1.46
17	16.868	Caryophyllene oxide	$C_{15}H_{24}O$	220.35	1594	1582	0.81
18	17.554	10-*epi*-*γ*-Eudesmol	$C_{15}H_{26}O$	222.37	1624	1622	1.81
19	17.853	*β*-Eudesmol	$C_{15}H_{26}O$	222.37	1651	1649	2.95
20	17.887	*α*-Eudesmol	$C_{15}H_{26}O$	222.37	1654	1652	4.66
21	20.073	Cryptomeridiol	$C_{15}H_{28}O_2$	240.38	1816	1813	1.80
22	20.256	Neophytadiene	$C_{20}H_{38}$	278.50	1836	1840	0.87
						Total (%)	79.07

Note: RT: retention time; RI (E.): Experimental retention indices; RI (L.): Retention Indices in literature; MF: Molecular Formula; MW: Molecular Weight; (%) in “Bold” indicates major compounds (> 2.0%).

Table 2. TPC, TFC and antioxidant properties of *C. vietnamense* leaf extract.

Samples	TPC (mg GAE/g E)	TFC (mg RE/g E)	DPPH scavenging activity (IC_{50}, [μg/mL])
Leaf extract	119.66 ± 1.03	205.94 ± 1.97	122.46 ± 2.82
Ascorbic acid	-	-	25.65 ± 0.12

Mean ± S.D, n = 3; (-) Not tested.

Table 3. Cytotoxic potential of LCV and Ellipticine

	% inhibition of cell growth of LCV				
Concentration [μg/mL]	**MCF-7**	**SK-LU-1**	**Hela**	**MKN-7**	**HL-60**
500	100 ± 1.29	96.44 ± 2.30	99.05 ± 2.46	91.63 ± 3.26	100 ± 2.67
100	97.43 ± 1.62	71.06 ± 1.36	92.86 ± 1.90	79.85 ± 1.68	89.32 ± 1.83
20.0	19.68 ± 1.06	10.57 ± 1.06	10.69 ± 0.83	12.43 ± 0.29	5.44 ± 0.52
4.0	8.59 ± 0.77	5.80 ± 0.42	4.65 ± 0.33	1.62 ± 0.17	2.01 ± 0.22
0.8	4.60 ± 0.28	2.19 ± 0.30	2.67 ± 0.22	0.81 ± 0.08	0.86 ± 0.09
IC_{50} [μg/mL]	35.90 ± 1.24^{d}	68.89 ± 3.76^{a}	45.49 ± 1.64^{c}	59.18 ± 1.77^{b}	51.41 ± 1.70^{bc}
Ellipticine (IC_{50}, [μg/mL])	0.49 ± 0.04	0.52 ± 0.05	0.42 ± 0.03	0.44 ± 0.05	0.33 ± 0.05

Note: Mean ± S.D, n = 3; Values with the same superscripts in the same row are not significantly different at the 99% level of confidence based on the Tukey test ($p<0.01$).

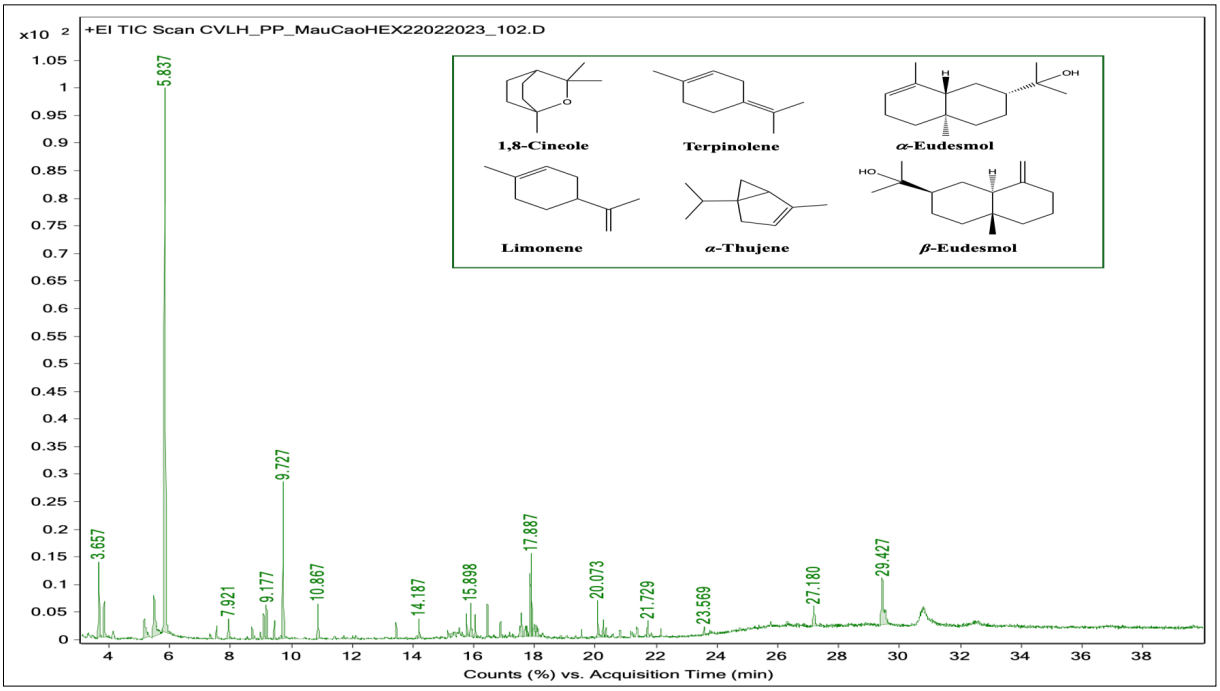

Fig. 2. GC-MS chromatogram and the identified main components in LCV

Discussion

This is the first study to identify the volatile components in LCV, wherein 22 compounds were identified. Among them, 1,8-cineole was found as the major component (37.16%). This result was similar to the previous reports that 1,8-cineole presents with a high amount in the EOs of *C. vietnamense* leaves, varying from 21.09% to 49.49% in relation to the extraction solvents (12, 13, 26). Meanwhile, α-terpineol (7.77%) and terpinolene (4.96%) were found to be rich in the EOs in this study, but their contents were lower than 2% in the previous study (12, 27). In the same pattern, the LCV extract was also found to be rich in *α*-thujene (2.25%), *α*-eudesmol (4.66%) and *β*-eudesmol (2.95%); however, these compounds were not detected in both acetone and aqueous extracts in the earlier researches (12, 13). These significant differences in the components and contents of the *C. vietnamense* leaves could be explained by differences in the extraction solvents, geographical locations and harvesting conditions.

The compound, 1,8-cineole is an oxidized terpenoid that predominates in the EO composition of plants in the genus *Eucalyptus*. Previous studies reported that this compound has exhibited several significant pharmacological effects, including antimicrobial, anti-inflammatory, antioxidant, analgesic and spasmolytic properties (28, 29). It has shown efficacy in managing various chronic conditions like respiratory diseases, pancreatitis, colon damage and cardiovascular and neurodegenerative diseases (29). The cytotoxic effects of 1,8-cineole have also been documented, involving diverse mechanisms across various cancer cell lines, primarily through the regulation of p53 expression and the expression of apoptotic proteins (Bax/Bcl-2, cytochrome c (Cyt -c) and caspase-9/3), leading to apoptosis and G2/M phase cell cycle arrest (30).

The presence of α-terpineol in the EO of *C. vietnamense* leaves may also help explain the cytotoxic potential of this plant extract. Particularly, *α*-terpineol is a naturally occurring monoterpene widely distributed in nature, known for its pharmacological effects, including antioxidant, anticancer, anticonvulsant, antiulcer, antihypertensive and anti-nociceptive properties (31). Notably, *α*-terpineol exhibits cytotoxicity against various cancer cell lines through multiple mechanisms. In the murine sarcoma 180 cell line, *α*-terpineol induces DNA fragmentation, chromosomal breaks and loss of cell membrane integrity, leading to early apoptosis, late apoptosis and necrosis (32). In the breast cancer cell line (MCF-7), this compound inhibits the cell cycle by causing cell accumulation in the Sub-G1 phase and promoted apoptosis through the downregulation of Bcl-2 and upregulation of Bax expressions (33). Interestingly, a recent study has shown that *α*-terpineol exerts selective cytotoxicity on cancer cells without affecting healthy cells (32).

Limonene is also a promising compound for cancer treatment, exhibiting cytotoxic activity against various cancer cell lines, such as K562 and HL-60 leukemia cancer cell lines, LS174T colon cancer cell line, HepG2 hepatocellular carcinoma cancer cell line, A549 and H1299 lung cancer cell lines, MCF-7 breast cancer cell line, etc. (34-36). A distinctive feature of limonene's cytotoxic mechanism is its ability to increase the production of ROS in cancer cells, leading to elevated expression of apoptosis-related proteins, including BAX, Cyt-c, cleaved-PARP and pro-caspase-9, without causing genotoxicity, along with inhibition of the activity of the Ras/Raf/MEK/ERK and PI3K/Akt pathways (34). In addition, limonene has also been reported to have many pharmacological effects, such as anti-inflammatory, anti-diabetic, anti-stress, hepatoprotective, cardioprotective, gastroprotective, immunomodulatory, antiatherogenic, hypolipidemic effects, etc. (35).

Eudesmol isomers are bicyclic sesquiterpenoid alkene alcohols (including three isomers: *α*-, *β*- and *γ*-eudesmols) found in various plant species, known for their cytotoxic effects on multiple cancer cell lines (37). Among them, the cytotoxic mechanism of *β*-eudesmol has been extensively studied. In cholangiocarcinoma cell lines (CCA: HuCCT1 and CL-6), *β*-eudesmol inhibits STAT1/3 phosphorylation, activates heme oxygenase (HO)-1, reduced NF-*κ*B expression and activates caspase-3/7, thereby promoting apoptosis. It also inhibits the enzyme NAD(P)H-quinone oxidoreductase 1 (NQO-1), reversing CCA resistance to 5-fluorouracil and doxorubicin, highlighting its potential as a chemosensitizing agent. In human leukemia HL-60 cells, *β*-eudesmol induces caspase-3 and caspase-9 cleavage, downregulates Bcl-2 expression, reduces mitochondrial membrane potential and promotes Cyt-c release from mitochondria, thereby triggering apoptosis (38). In HepG2 liver cancer cells, *β*-eudesmol increases caspase-3 activity and reduces mitochondrial membrane potential, leading to cell death. Similarly, *α* -eudesmol exhibits the same cytotoxic mechanism as *β*-eudesmol in HepG2 cells (39).

Together with the volatile components, polyphenols, often referred to as phenolic compounds, are also important components in the mechanism of signaling and defense of plants (40). Polyphenols can be grouped into four main classes: phenolic acids, flavonoids, stilbenes and lignans (41, 42). These compounds are also prevalent in a variety of foods integral to the human diet, such as fruits, vegetables, seeds, coffee and by-products from agricultural and industrial processes (43). Polyphenols and flavonoids exhibit numerous notable pharmacological effects, one of which is their antioxidant capacity by inhibiting free radicals. The reaction mechanism of a phenolic compound with a peroxyl radical includes a concerted transfer of the hydrogen cation from the phenol to the radical, forming a transition state of an H-O bond with one electron (44, 45). Since free radicals play a significant role in the pathogenesis of various conditions (e.g., cardiovascular diseases, inflammation and cancer), polyphenols and flavonoids are considered promising agents for research into treatments for these disorders (41). The antioxidant effect of acetone extract from *C. vietnamense* leaves, flowers and rhizomes had also been tested using the DPPH scavenging model (IC_{50} = 48.15 ± 9.32 µg/mL - 121.64 ± 6.18 µg/mL) (13). The results from this experiment, along with previous reports (12-15), suggest that the *C. vietnamense* leaves may serve as a potential resource for research and development of novel antioxidant agents.

C. vietnamense is an endemic plant species of Vietnam. The volatile compounds in LCV were identified using GC-MS analysis. Interestingly, *in vitro* results demonstrated that the extract exhibited strong cytotoxic effects against the MCF-7, SK-LU-1, HeLa, MKN-7 and HL-60 cancer cell lines. The high content of compounds such as *α*-thujene, *β*-eudesmol, *α*-eudesmol, limonene, *α*-terpineol and 1,8-

cineole, each with diverse cytotoxic mechanisms, may account for the potent anticancer activity of this extract. These findings suggest that *C. vietnamense* is a promising candidate for research into development of new, effective and safe anticancer drugs. However, further research is needed to evaluate its tumor-inhibiting potential and safety in animal models, as well as to elucidate the molecular mechanisms underlying this effect.

Conclusion

The chemical composition, total polyphenol and flavonoid contents, antioxidant potential and *in vitro* cytotoxic effects of the *n*-hexane extract from *C. vietnamense* (LCV) leaves were investigated. The results indicated that the LCV contained high amounts of *α*-thujene, *β*-eudesmol, *α*-eudesmol, limonene, *α*-terpineol and 1,8-cineole. The total polyphenol and flavonoid content of the extract were quantified as 119.66 ± 1.03 mg GAE/g E and 205.94 ± 1.97 mg RE/g E, respectively. The extract exhibited antioxidant activity in the DPPH scavenging capacity assay with an IC_{50} value of 122.46 ± 2.82 µg/mL. In the *in vitro* cytotoxicity assays, the LCV strongly inhibited the growth of human breast carcinoma, human lung carcinoma, human cervical carcinoma, human gastric carcinoma and human acute leukemia cell lines. Notably, to the best of our knowledge this is the first report on the chemical constituents, as well as the antioxidant and the cytotoxic properties of the *n*-hexane extract of *C. vietnamense* leaves. Further studies are needed to evaluate the safety, molecular mechanisms of cytotoxicity and tumor-inhibiting efficacy of this extract in animal models.

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Authors' contributions

TTTQ carried out the phytochemical evaluation and discussed the manuscript. DMK, DPND, VMT carried out the *in vitro* experiments and wrote the manuscript. VTT participated in the design of the study and performed the statistical analysis. TVP carried out the GC-MS analysis. NDD: collected samples and performed the extraction of plant material. TVC participated in its design and coordination and revised the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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