Research Article

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Chemical compositions of *Litsea umbellata* and inhibition activities

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Abstract: *Litsea umbellata* (Lour.) Merr. is a plant commonly grown in Vietnam and some Asian countries. The plant is used in traditional medicines and exhibits significant biological activities. However, the sesquiterpenes' extraction in the essential oil (EO) of *L. umbellata* harvested from the northern region of Vietnam has been limitedly known. Therefore, in the present study, the *L. umbellata* leaves and stem EOs were obtained by hydrodistillation method, then determined for the phytochemical profile by using gas chromatography–mass spectrometry system, and investigated the cytotoxicity activities against five cancer cell lines. Results have identified a total of 21 and 26 compounds in the EOs of *L. umbellata* leaves and stem, respectively, with the main sesquiterpene compounds being β-caryophyllene (16.87–11.04%), (+)-spathulenol (9.74–6.57%), β-caryophyllene oxide (26.12–18.34%), and (−)-spatulenol (11.08–8.8%). *L. umbellata* leaves exhibit significant anticancer activity with IC_{50} values ranging from 29.58 to 62.96 μg/ml. Otherwise, *L. umbellata* EOs also exhibited the good inhibition activities against DPPH free radical and three bacteria strains. The chemical constituents and cytotoxicity activity of *L. umbellata* EO stems have been reported for the first time and provided the future applications of this plant.

Keywords: *Litsea umbellata*, Vietnam, cytotoxic activity, essential oil, sesquiterpene

1 Introduction

Litsea umbellata (Lour.) Merr. (*L. umbellata*) is a species of flowering plant in the Lauraceae family. *L. umbellata* is a plant located in China, Myanmar, Cambodia, Malaysia, Indonesia, Philippines, and Vietnam [1]. There are many chemical components in *Litsea* genus, such as monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, and oxygenated sesquiterpenes. Besides, oxygenated diterpenes, aldehydes, alcohols, ketones, and alkanes were identified [1]. *Litsea* genus is used in traditional medicine to treat various diseases, such as flu, stomach ache, diarrhea, and diabetes. *Litsea costalis* species in China and Malaysia is used in traditional medicine to treat flu, and stomachache [2]. *Litsea cubeba* has been used in the treatment of stomach cold hiccough, gastric cavity crymodynia, cold hernia celialgia, and stagnancy of cold-damp [3]. Recently, the bio-activities of *Litsea* genus have been reported, such as antimicrobial activity [4–7], antioxidant [8,9] and anti-inflammatory activities [10,11], and cytotoxic activity [9,12,13]. Particularly, caryophyllene oxide, which is one of the main components of *Litsea* genus, exhibited anti-cancer activities against various cancer cell lines, such as HeLa, A-2780, HepG2, AGS, SNU-1, and SNU-16 [21–23]. Caryophyllene oxide has been may reduce cancer cell invasion by inactivating the pathway of p-ERK and p-p38; otherwise, protein expression was also decreased by caryophyllene oxide on HT1080 cells [24]. In addition, caryophyllene oxide led to early and late apoptosis processes by the caspase-7 activation dependent [25] on PC-3 cells and it is an anti-proliferative agent against PC-3 cells, and inducing apoptosis with non-toxicity on normal cells [25]. However, up to the present, the knowledge about the phytochemical profile as well as the bioactivities of *L. umbellata* essential oils (Eos) has remained limited known. Herein, the present study has compared the chemical composition of the leaves and stem EO of *L. umbellata* cultivated in Thai Nguyen province, Vietnam by gas chromatography–mass spectrometry (GC/MS) method, followed by evaluation of the cytotoxic activities of the obtained *L. umbellata* EOs against MCF-7,

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MKN-7, SK-LU-1, A549, and HepG2 cell lines and inhibition activities against DPPH free radical and three bacterial strains.

2 Materials and Methods

2.1 Plant material

Fresh leaves and stem of *L. umbellata* (1.5 kg) were collected from a local farm in Thai Nguyen province (21°35′ 39.19″N, 105°50′53.41″E), Vietnam, in May 2021. The samples were authenticated by Dr Thuong, Faculty of Biology, Thai Nguyen University of Education. The plant samples were air dried at room temperature prior to the steam distillation process. Pure chemicals and reagents were obtained from Sigma-Aldrich (San Louis, MI, USA).

2.2 Extraction of EOs by steam distillation extraction

Extraction of *L. umbellata* leaves and stems was performed by using the steam distillation method, as previously described by Kong et al. [2]. The prepared samples were subjected to the steam distillation system for 7 h with 3,000 ml of distilled water. Then, the EOs were extracted with a separating funnel and dehydrated using anhydrous sodium sulfate. The EO with strong flavor was obtained and stored in a sealed glass vial in a refrigerator at 4–5°C prior to analysis. The oil yield (%) was calculated by dividing the volume of the obtained EO by the mass of the initial plant material of *L. umbellate*.

2.3 GC/MS and GC/flame ionization detector (FID) analysis

The GC–FID analysis was performed with a Hewlett Packard GC (HP5890 series II) equipped with HP-5 MS (30 m × 0.25 mm × 0.25 μm) with an FID. A microliter of EO in *n*-hexane samples (1/50, v/v) was initially injected (split mode, split ratio 1:15) 1/15. Helium (0.9 ml/min) was used as the carrier gas. The temperature of injector and detector was set at 210 and 295°C, respectively. The oven temperature was kept at 35°C, then gradually raised to 295°C at 3°C/min, and finally held isothermally for 23 min.

The GC/MS analysis was also performed with a Hewlett Packard GC (HP5890 series II) equipped with HP-5 MS (25 m × 0.25 mm × 0.4 μm) and MS (HP MSD5971 model). Helium (0.9 ml/min) was used as the carrier gas. The oven program started with an initial temperature of 70°C held for 3 min and then, the oven temperature was heated at 10°C/min to 270°C and finally held isothermally for 20 min. The electron impact spectra were recorded at an ion voltage of 70 eV over a scan range of 30–600 uma. The compounds were identified by comparison of their retention indices (RI) and their RI on HP-5MS column with those reported in NIST Chemistry WebBook (http://webbook.nist.gov/chemistry/). Further identification was made by comparison of their mass spectra with those stored in the Wiley NBS75K.L and NIST/EPA/NIH (2002 and 2014 version) mass spectral libraries. The experimental RI values were determined by a homologous series of *n*-alkanes (C6–C25) under similar conditions.

2.4 Cytotoxic assay

The cytotoxicity of *L. umbellata* leaves and stem EOs was investigated against four established cell lines using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay as described by Skehan et al. [14]. Briefly, MCF-7 cell (human breast carcinoma), MKN-7 cell (human gastric carcinoma), SK-LU-1 cell (human lung carcinoma), A549 cell (adenocarcinomic human alveolar basal epithelial cells), and HepG2 cell (human hepatocarcinoma) were maintained at the Bioassay Group Lab, Institute of Biotechnology, Vietnam Academy of Science and Technology. The culture medium included Dulbecco's modified Eagle's medium, Eagle's minimum essential medium, and 10% fetal bovine serum thermoactive activity purchased from Sigma-Aldrich (St. Louis, MO, USA). The conditions of cell line incubation include 5% CO_2, 95% air, 37°C in a CB 220 incubator (Thermo Scientific). The optical density (OD) measurement was performed at 540 nm on ELISA Plate Reader. The cytotoxicity of *L. umbellata* leaves and stem EOs was expressed as an IC_{50} value by using TableCurve 2Dv4 software.

2.5 DPPH free radical assay

The antioxidant capacity of EOs from leaf and trunk of *L. umbellata* by DPPH free radical neutralization was performed by Tabart et al. [27]. Conduct to aspirate 100 μl of

each type of EO at concentrations of 1, 2, 4, 8, 16, 32, 64, 128, and 256 µg/ml into test tubes, then add 2.9 ml of 0.1 mM DPPH mixed in methanol, shake well, and allow to stand for 30 min and using a UV–Vis 1800 Shimadzu manual, measured at a wavelength of 517 nm. The DPPH free radical scavenging capacity of the extract was determined according to the following formula: free radical scavenging ability DPPH (%) = $100 \times (A_c - A_s)/A_c$. In which, A_c is the optical absorbance of the control sample, and A_s is the optical absorbance of the sample that needs to be determined. The antioxidant capacity was determined based on the value of EC_{50} (is the concentration of sample with DPPH free radical scavenging capacity of 50%) [27]. After measuring the OD, we proceed to build the standard curve and the correlation equation for the antioxidant activity of the *L. umbellata* EOs.

2.6 Antibacterial activity assay

The antibacterial activity of EOs was determined by the bacterial inhibitor activity test method, which is described by Hadacek and Harald [26]. Bacterial species tested from slanted agar tubes in a 4°C refrigerator were cultured on solid Luria-Bertani (LB) medium and then activated in liquid LB medium for 8–16 h at 28°C, shaking at 200 rpm. Aspirate 100 µl of activated bacterial solution on the plate of solid LB medium and spread evenly on the agar plate until dry. Punch five wells with a diameter of 1 cm on the agar plate and add 100 µl of EO extract from *L. umbellata* mixed in 2% DMSO solution (the negative control well was supplemented with DMSO, and the positive control well was supplemented with the antibiotic ampicillin 100 mg/ml). Place the diluted Petri dishes in the refrigerator at 4°C for 1–2 h and then in the incubator at 28°C for 18–24 h. Measure the diameter of the antibacterial ring, take a picture, and record the result. Each experiment was repeated three times. The diameter of the antibacterial ring was determined by the formula: $H = D - d$ (mm), where D is the diameter of the sterile ring from the center of the hole (mm) and d is the diameter of the agar perforation (mm).

Convention: $(D - d) \geq 25$ mm: has very strong antibacterial activity.

$(D - d) \geq 20$ mm: has strong antibacterial activity.

$(D - d) \geq 15$ mm: has medium antibacterial activity.

$(D - d) \leq 15$ mm: has weak antibacterial activity.

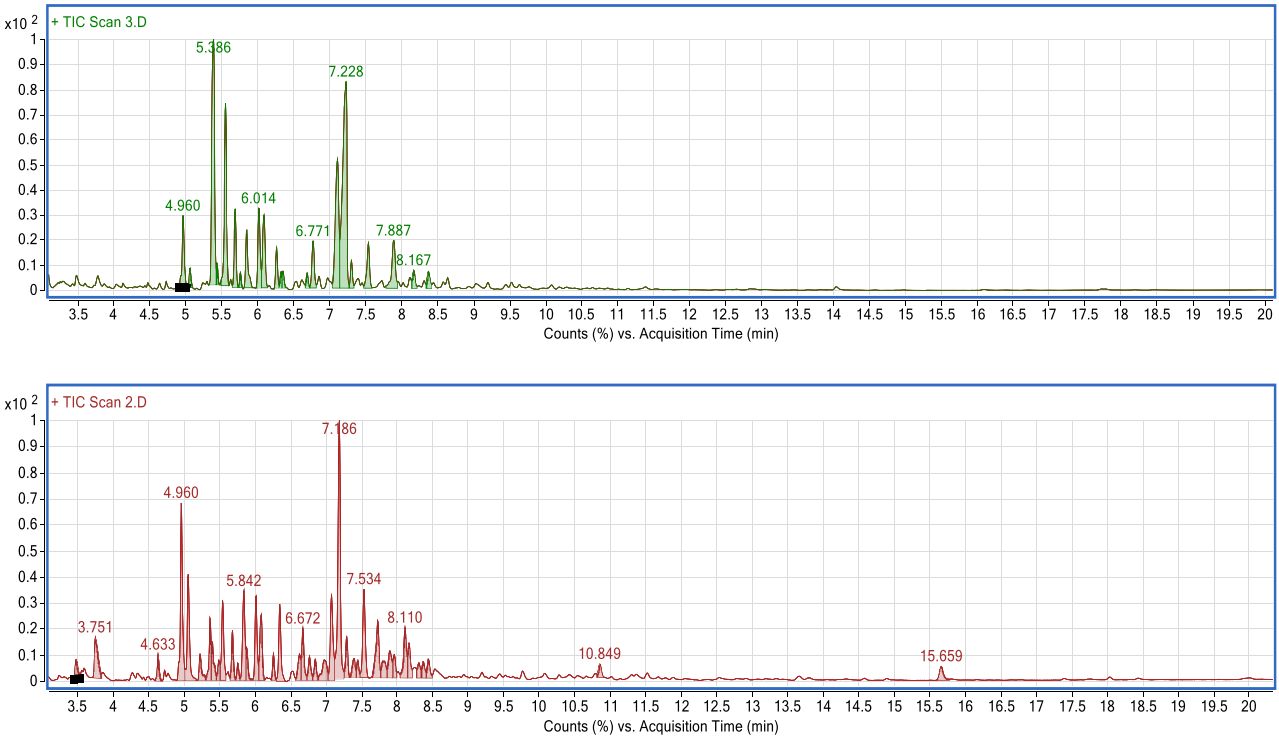

Figure 1: The gas chromatography spectrum of *L. umbellata* leaves (up) and stem (down) EOs.

Table 1: Chemical composition of the *L. umbellata* EOs

No.	Compositions	RI (lit.)	RI (exp.)	Relative content (%)	
				Leaves	Stem
1.	α-Copaene	1,376	1,375	3.85	2.11
2.	β-Elemene	1,394	1,392	0.88	1.12
3.	β-Caryophyllene	1,415	1,415	16.87	11.04
4.	α-Himachalene	1,428	1,426	—	0,87
5.	γ-Maaliene	1,435	1,434	0.8	—
6.	α-Caryophyllene	1,452	1,451	3.62	5.23
7.	β-Cedrene	1,424	1,464	0.59	0.43
8.	γ-Himachalene	1,481	1,485	4.31	3.13
9.	(−)-Alloaromadendrene	1,487	1,487	—	0.67
10.	β-Selinene	1,509	1,510	—	0.57
11.	γ-Cadinene	1,511	1,511	1.85	1.45
12.	*cis*-γ-Cadinene	1,513	1,512	3.34	2.11
13.	δ-Cadinene	1,519	1,517	0.69	1.23
14.	Cadina-1,3,5-triene	1,543	1,540	0.84	1.56
15.	(*R*)-(−)-*trans*-Nerolidol	1,551	1,552	0.8	1.54
16.	1,1,7-Trimethyl-4-methylenedecahydro-1*H*-cyclopropa[*e*]azulen-7-ol	1,567	1,564	—	0.56
17.	(+)-Spathulenol	1,571	1,570	9.74	6.57
18.	Isocaryophyllene oxide	1,572	1,571	2.78	1.23
19.	β-Caryophyllene oxide	1,578	1,577	26.12	18.34
20.	(−)-Spatulenol	1,582	1,584	11.08	8.8
21.	(+)-Viridiflorol	1,593	1,592	1.36	0.79
22.	Isoaromadendrene epoxide	1,594	1,591	1.25	0.57
23.	Cadinol	1,601	1,600	—	0.54
24.	β-Costol	1,611	1,614	—	0.78
25.	1-Heptatriacotanol	1,690	1,692	—	1.03

2.7 Statistical analysis

All data in the present study were obtained from one-way analysis of variance and are represented as mean ± standard deviation with $p < 0.05$ being considered as statistically different.

3 Results and discussion

The crude EOs were obtained from leaves and stem of *L. umbellata* with the yield of 0.08 and 0.04%, respectively. The chemical components of the obtained EOs were analyzed by GC/MS and GC–FID systems (Figure 1) and their relative percentages are reported in Table 1. It can be known that most chemical compositions were similar in the two EOs, except for some components. Specifically, 20 and 23 compounds have been identified in the EO from *L. umbellata* leaves and stem grown in the north mountain region of Vietnam. The main components were found as β-caryophyllene (16.87 and 11.04%), (+)-spathulenol (9.74 and 6.57%), β-caryophyllene oxide (26.12 and 18.34%), and (−)-spatulenol (11.08 and 8.8%) in the *L. umbellata* leaves and stem, respectively (Table 1).

The chemical composition of EO of *Litsea* plants was varied based on different extraction methods, cultivar, plant parts, time of sampling, and processing [15]. The sesquiterpene group accounted for 37% of the total EOs obtained from the leaves of this plant [16]. Meanwhile, our samples, which were collected in the northern mountainous area of Vietnam, contained a total of 70% of sesquiterpenes of the EO. Besides, the main β-caryophyllene

Table 2: Cytotoxic activities of *L. umbellata* leaves and stem EO

Cancer cell lines	Leaves	IC_{50} value (μg/ml)		
		SI	Stem	Ellipticine
HepG2	54.82 ± 3.52	3.45	>100	0.40 ± 0.04
MKN-7	33.51 ± 2.39	3.76	>100	0.27 ± 0.04
SK-LU-1	62.96 ± 1.19	3.15	>100	0.30 ± 0.02
A549	26.23 ± 1.40	3.50	>100	0.41 ± 0.07
MCF-7	43.01 ± 1.55	3.23	>100	0.41 ± 0.05

Data represented as mean ± SD of three independent replicates, $p < 0.05$ is considered significant. SI: selective index.

Table 3: Antibacterial activity of EOs of *L. umbellata* leaves and stem

Bacterial inhibitor	Concentration	Bacterial strains (zone of inhibition, mm)		
		E. coli	*P. aeruginosa*	*S. aureus*
Ampicillin	100 mg/ml	18.3 ± 0.1	19.0 ± 0.2	16.0 ± 0.1
Leaves	25 µg/ml	0	0	0
	50 µg/ml	19.1 ± 0.2	19.9 ± 0.3	21.9 ± 0.2
	100 µg/ml	29.0 ± 0.3	30.9 ± 0.2	31.1 ± 0.2
Stem	25 µg/ml	0	0	0
	50 µg/ml	19.9 ± 0.3	27.6 ± 0.2	20.1 ± 0.4
	100 µg/ml	29.9 ± 0.3	35.1 ± 0.4	32.1 ± 0.4

oxide (26.12%) to be different in comparison with major chemical compositions (β-pinene [18.8%], β-caryophyllene [16.2%]) in the sample collected in central Vietnam reported by Dai et al. [15]. Caryophyllene oxide is also found in other species of the genus *Litsea*, including *Litsea deccanensis* leaves (8.5%) [17], *Litsea glutinosa* fruit (5%) [18], and *Litsea monopetala* flowers (9.5%) in India [19] and *Litsea megacarpa* leaves in China (56.8%) [2]. Caryophyllene oxide is a sesquiterpenoid oxide in *Melissa officinalis* and *Melaleuca stypheloides*, whose content in EOs of 43.8% [20].

The cytotoxic activity of leaves and stem EO of *L. umbellata* was investigated against MCF-7, MKN-7, SK-LU-1, A549, and HepG2 cell lines. The results are presented in Table 2. Results have shown that no cytotoxicity effect was observed for the *L. umbellata* stem EO samples at the studied concentrations, as indicated by IC_{50} value >100 µg/ml. On the other hand, as compared to ellipticine (i.e., positive control), the EO of *L. umbellata* leaves exhibited high inhibitory activity against five tested cancer cell lines with IC_{50} values ranging from 26.23 to 62.96 µg/ml (Table 2). Previous studies have shown that caryophyllene oxide exhibited inhibitory activities against several cancer cell lines, such as HeLa, A-2780, HepG2, AGS, SNU-1, and SNU-16 [21–23]. Therefore, the higher content of caryophyllene oxide in *L. umbellata* leaves EO than its stem EO may have given rise to its high cytotoxicity effect. Previously, the anti-cancer activities of *L. cubeba* EO against OEC-M1, J5, and A549 cells were significant, as indicated by IC_{50} values of around 50, 50, and 100 ppm, respectively [12]. Thus, it can be concluded that *L. umbellata* leaves EOs exhibited higher cytotoxic activities than *L. cubeba* EOs against A549 cells [12].

In addition, *L. umbellata* EOs was experimented with the anti-oxidant activities by using DPPH free radical assay and anti-bacterial activities by using the bacterial inhibitor activity test method. The results show that *L. umbellata* EOs exhibited the anti-oxidant activities with IC_{50} = 3.24 µg/ml and the results of anti-bacterial activities are summarized in Table 3. In Table 3, EOs from leaves exhibited better inhibitory activities against *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* strains than EOs from the trunk.

4 Conclusions

The medicinal plant *L. umbellata* has exhibited many important biological and pharmacological activities. To date, many traditional medicines have used this medicinal ingredient. The sesquiterpenes have many biological activities, particularly inhibition activities against cancer cells, so in this study, the aim of this study was to identify the main components of the sesquiterpene group present in the medicinal plant *L, umbellata* and evaluate the inhibition activity against cancer cell lines MCF-7, MKN-7, SK-LU-1, A549, and HepG2 and against DPPH free radical and three bacterial strains are important in providing more scientific basis for the use of this plant in the medicinal industry.

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Conflict of interest: The authors declare no conflict of interest.

Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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