

Volatile Constituents and *In vitro* Antimicrobial Activities of Essential Oils from Leaves of *Siliquamomum oreodoxa* N.S. Lý & Škorničk and *Curcuma thorelii* Gagnep. (Zingiberaceae) Growing in Vietnam

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Original Article**Volatile Constituents and *In vitro* Antimicrobial Activities of Essential Oils from Leaves of *Siliquamomum oreodoxa* N.S. Lý & Škorničk and *Curcuma thorelii* Gagnep. (Zingiberaceae) Growing in Vietnam****Hieu Tran-Trung¹, Le Duc Giang¹, Dau Xuan Duc¹, Nguyen Thi Giang An², Dang Van Son³, Danh C. Vu⁴, Nguyen Thi Kim Anh⁵, Hien Nguyen-Thi-Thu⁶, Khieu Thi Tam⁷, Trang H.D. Nguyen^{5*}**¹ Department of Chemistry, Vinh University, 182 Le Duan, Vinh City, Nghean 43000, Vietnam² Faculty of Biology, Vinh University, 182 Le Duan, Vinh City, Nghe An 43000, Vietnam³ Institute of Tropical Biology, Vietnam Academy of Science and Technology, 85 Tran Quoc Toan, District 3, Ho Chi Minh City, Vietnam⁴ Institute of Applied Technology, Thu Dau Mot University, 06 Tran Van On, Phu Hoa Ward, Thu Dau Mot City, Binh Duong, Vietnam⁵ Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, 12 Nguyen Van Bao, Ward 4, Go Vap, Ho Chi Minh City 70000, Vietnam⁶ Department of Pharmacy, Nguyen Tat Thanh University, Ward 13, District 7, Ho Chi Minh City 70000, Vietnam⁷ Faculty of Chemistry, TNU-University of Sciences, Tan Thinh Ward, Thai Nguyen City, Vietnam

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Abstract: *Siliquamomum oreodoxa* and *Curcuma thorelii* in the family Zingiberaceae are two new species native to Vietnam. In the present work, volatile composition of essential oils (EOs) from leaves of the two plant species and their antimicrobial activities were reported for the first time. The gas chromatography-mass spectrometry analytical results revealed that the major constituents of the *S. oreodoxa* leaf EO were α -pinene (39.23%), β -pinene (26.34%), myrtenyl acetate (8.24%), and limonene (7.85%). The results also showed the *C. thorelii* EO was predominantly composed of β -pinene (21.58%) and caryophyllene (14.26%). In the antimicrobial assay, the two EOs were found to possess strong antimicrobial activities against multiple pathogenic bacterial and fungal strains, with minimum inhibitory concentrations ranging between 32 and 128 $\mu\text{g/mL}$.

Keywords: *Siliquamomum oreodoxa*, *Curcuma thorelii*, essential oil, antimicrobial activity.

Introduction

Siliquamomum, a genus of plant in the family Zingiberaceae, was first characterized by Baillon (1895) during an investigation in the Ba Vi mountainous region of Northern Vietnam. This genus contains four known species which have been so far found growing in China and Vietnam, including *S. tonkinense*, *S. phamhoangii*, *S. alcornae*, and *S. oreodoxa*¹⁻⁴. In general, these species show some morphological similarities, such as sizes of leafy shoots, leaf laminae, and petiolate leaves. Reportedly, they are often used locally for medicinal purposes. For example, *S. tonkinense* is traditionally used for relieving stomachache, treating gastrointestinal bleeding, or as herbal remedies for postpartum healing^{5,6}. Plant species belonging to the family Zingiberaceae have aroused much interest due to their fragrant leaves and rhizomes⁷⁻⁹. Nevertheless, very limited information about volatile profile of the *Siliquamomum* species has been reported. Recently, one study has revealed that essential oil (EO) from leaves of *S. tonkinense* mainly contain pinenes (45%) and sabinene (14.6%) while that from rhizomes was composed of high percentage of pinenes (21.3%), 1,8-cineole (19.1%) and γ -terpinene (14.9%)¹⁰. Among the *Siliquamomum* species, *S. oreodoxa* was identified and reported for the first time in 2010 in Southern Vietnam⁴. To the best of our knowledge, no data about chemical composition and bioactivities of *S. oreodoxa* are available in the literature.

In the present study, we also focused on another species in the family Zingiberaceae, namely *Curcuma thorelli* Gagnep., which was previously described by Gagnepain in 1907¹¹. There is much evidence to show that species of *Curcuma* genus have been extensively employed for therapeutic purposes in traditional or cultural medicine across the globe¹². Many *Curcuma* plants are known to possess a significant degree of therapeutic potential that can help manage a range of health conditions, including stomach ulcers, spleen and liver enlargement, hepatic disorders, skin ailments, cough, diabetes, and the purification of blood. Previously, the chemical composition of EO from *Curcuma thorelli* rhizomes was reported¹³. It showed the

preponderance of sesquiterpene hydrocarbons and oxygenated sesquiterpenes in this EO.

To date, there have been no data about the volatile profiles of EOs extracted from leaves of the aforementioned plant species. Thus, the aims of the present study were to identify volatile organic compounds and antimicrobial properties of EOs from *S. oreodoxa* and *C. thorelli* leaves for the first time. The findings of the study will provide the first evidence of the bioactive chemicals present in leaves of the species, as well as contribute to the understanding of their potential health endorsing properties.

Materials and methods

Sample collection

The *S. oreodoxa* fresh leaves were collected from Bidoup Nui Ba National Park, located in Lac Duong District, Lam Dong Province, Vietnam in July 2021. The *C. thorelli* fresh leaves were collected from Tanh Linh District, Binh Thuan Province, Vietnam in August 2022. Both species were identified by Dr. Dang Van Son (Institute of Tropical Biology, Vietnam Academy of Science and Technology) and Dr. Nguyen Danh-Duc (Institute of Applied Technology, Thu Dau Mot University). The voucher specimen of *S. oreodoxa* (no. HC-006) was deposited at the Laboratory of the Department of Chemistry, Vinh University while the specimen of *C. thorelli* was deposited at the Institute of Applied Technology Herbarium, Thu Dau Mot University, under the no. NDD-236.

Preparation of the EOs

Five hundred grams of each leaf sample were subjected to washing, pulverization, and distillation utilizing a Clevenger apparatus until the amount of EO obtained achieved a state of constancy (about 4 h). Anhydrous sodium sulphate was then employed to fully eliminate any residual water content. The experimental procedure was replicated three times. Subsequently, the EOs were kept in a refrigerator (4°C) for further analyses.

Gas chromatography-Mass spectrometry (GC-MS)

Volatile organic compounds of the EOs were analyzed on an Agilent 7890B gas chromatograph

interfacing with an Agilent 5977B quadrupole mass spectrometer (GC-MS) ⁹. The constituents were separated by using an HP-5MS Ultra Inert capillary column (30 m × 0.25 mm I.D.). The oven temperature was initially set at 50°C for 2 min, and then raised to 150°C (held for 10 min) at 5°C/min. Finally, the temperature was increased to 280°C at 10°C/min (held for 10 min). A volume (1.0 µL) of EO solution (diluted with n-hexane, 1:30 v/v) was injected and the split injection with a ratio of 50:1 was set. Helium was used as a carrier gas with a constant flow of 1.0 mL/min. The injector temperature was held at 300°C, the MS Quad temperature was at 150°C, the transfer line temperature was at 300°C, and the MS source was held at 230°C. Mass spectral data were collected from 50 – 550 m/z. The tentative identification of the volatile constituents was conducted by comparing their mass spectra and retention indices (RI) with those compiled in the NIST publicly available database (National Institute for Standard and Technology) ¹⁴. The calculation of RI was carried out as follows:

$$RI = 100 \times \left[n + (N - n) \times \frac{(\log RT_{\text{unknown}} - \log RT_n)}{N/(\log RT_N - \log RT_n)} \right]$$

Where, RI is the retention index calculated for each tentatively identified compound; RT stands for retention time of the respective constituent; n and N denote the numbers of carbon atoms of the heading and trailing alkanes.

The relative percentage of volatile organic compounds was determined by calculating the peak areas of the chromatographic peaks.

Antimicrobial activity

The analysis of antimicrobial activity was performed on the leaf EOs of *S. oreodoxa* and *C. thorelii* against Gram-positive bacteria (*E. faecalis* ATCC 29212, *S. aureus* ATCC 2592, and *B. cereus* ATCC 14579), Gram-negative bacteria (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, and *S. enterica* ATCC 13076; and a yeast strain (*C. albicans* ATCC 10231). The aforementioned microbial strains were obtained from the

National Institute for Food Control (Hanoi, Vietnam). Antibiotics (kanamycin, tetracycline, cycloheximide) were used as controls. The leaf EOs were diluted in 10% dimethyl sulfoxide. A volume (50 µL) of each EO and antibiotic solution was added into a 96-well plate in triplicate. Next, 50 µL of microbial suspension (2×10^5 CFU/mL) were added to have the final concentration of EOs and antibiotic as 256, 128, 64, 32, 16, 8, 4, and 2 µg/mL. The plate was left for 24 hours at 37°C in an incubator. After that, 30 µL of resazurin (0.015%) were added into each well and the plate was incubated for 2 hours for color change observation. The change of resazurin from blue to red or purple color was used as an indicator for bacterial growth. While the color change from blue to red was considered as negative results, the blue color indicated positive results ¹³. The lowest concentration of EOs to prevent (97% – 100%) the visible growth of the test bacteria and yeast was estimated. This value is known as minimum inhibitory concentration (MIC), which was measured using a BioTek Epoch spectrophotometer (USA). The experimental procedure was replicated three times.

Results and discussion

The yields and chemical constituents of the EOs

The hydrodistillation method was applied for the extraction of EOs from the leaves of *S. oreodoxa* and *C. thorelii*. After 4 h isolation, the yields of two EOs were 0.11 and 0.13 % (w/w, based on fresh plant sample), respectively.

Through the GC-MS analysis, the EO extracted from *S. oreodoxa* leaves was shown to comprise 44 volatile organic compounds (Table 1, Figure 1). The dominant component of the EO was monoterpenes, comprising 80.38% of its overall composition, while their oxygenated derivatives constituted the second largest proportion at 13.97%. Sesquiterpenes and their oxygenated derivatives were also present, making up 4.53% of the EO. Non-terpenoids, namely 3-phenyl-2-butanone and 4-phenyl-2-butyl acetate, accounted for a small fraction (0.44%). The major compounds detected in the EO were α-pinene (39.23%) and β-pinene (26.34%). Myrtenyl

Table 1. EO components of *Siliquamomum oreodoxa* leaves

No.	RT (min)	Components	RI (Exp.)	RI (Lit.)	(%)
1	6.984	α -Thujene	932	929	0.07
2	7.224	α -Pinene	942	937	39.23
3	7.522	α -Fenchene	953	950	0.37
4	7.567	Camphene	954	952	1.43
5	8.414	β -Pinene	983	979	26.34
6	8.763	β -Myrcene	993	991	0.82
7	9.141	α -Phellandrene	1006	1005	0.14
8	9.313	δ -Carene	1013	1011	0.06
9	9.496	α -Terpinene	1020	1017	0.15
10	9.730	p-Cymene	1028	1025	0.88
11	9.868	Limonene	1033	1030	7.85
12	9.942	1,8-Cineole (= Eucalyptol)	1040	1032	0.14
13	10.125	(Z)- β -Ocimene	1042	1038	0.50
14	10.743	γ -Terpinene	1063	1060	0.96
15	11.619	Terpinolene	1091	1088	1.36
16	11.945	Linalool	1100	1099	1.55
17	12.351	<i>exo</i> -Fenchol	1116	1115	0.49
18	12.803	<i>Neo-allo</i> -ocimene	1133	1131	0.22
19	13.101	<i>trans</i> -Pinocarveol	1143	1139	0.06
20	13.381	Camphene hydrate	1153	1148	0.13
21	13.885	<i>endo</i> -Borneol	1170	1167	0.39
22	14.222	Terpinen-4-ol	1181	1177	0.14
23	14.600	α -Terpineol	1193	1189	0.81
24	14.772	Myrtenol	1198	1195	0.56
25	16.093	3-Phenyl-2-butanone	1248	1244	0.33
26	17.284	Bornyl acetate	1289	1285	0.18
27	17.661	<i>trans</i> -Pinocarvyl acetate	1302	1297	0.49
28	18.371	Myrtenyl acetate	1331	1327	8.24
29	19.338	Nerol acetate	1368	1364	0.07
30	19.704	α -Copaene	1381	1376	0.39
31	19.836	Geranyl acetate	1386	1382	0.72
32	20.150	4-Phenyl-2-butyl acetate	1397	1399	0.11
33	20.848	Caryophyllene	1426	1419	0.40
34	21.180	γ -Elemene	1439	1433	0.12
35	21.701	Humulene	1460	1454	0.06
36	22.250	γ -Gurjunene	1481	1473	0.25
37	22.542	β -Eudesmene	1492	1486	1.25
38	22.771	α -Selinene	1500	1494	0.33
39	23.080	β -Bisabolene	1510	1509	0.60
40	23.171	β -Curcumene	1513	1514	0.06
41	23.543	δ -Cadinene	1525	1524	0.14
42	23.772	<i>trans</i> - γ -Bisabolene	1533	1533	0.66
43	25.540	Caryophyllene oxide	1587	1581	0.19
44	29.929	β -Bisabolol	1683	1684	0.08

table 1. (continued).

No.	RT (min)	Components	RI (Exp.)	RI (Lit.)	(%)
		Monoterpene hydrocarbons (Sr. No. 1-11, 13-15, 18)			80.38
		Oxygenated monoterpenes (Sr. No. 12, 16, 17, 19-24, 26-29, 31)			13.97
		Sesquiterpene hydrocarbons (Sr. No. 30, 33-42)			4.26
		Oxygenated sesquiterpenes (Sr. No. 43, 44)			0.27
		Others (Sr. No. 25, 32)			0.44
		Total identified			99.32

RT (min): Retention time

RI (Exp.): Retention indices on HP-5MS UI column

RI (Lit.): Retention indices in literature

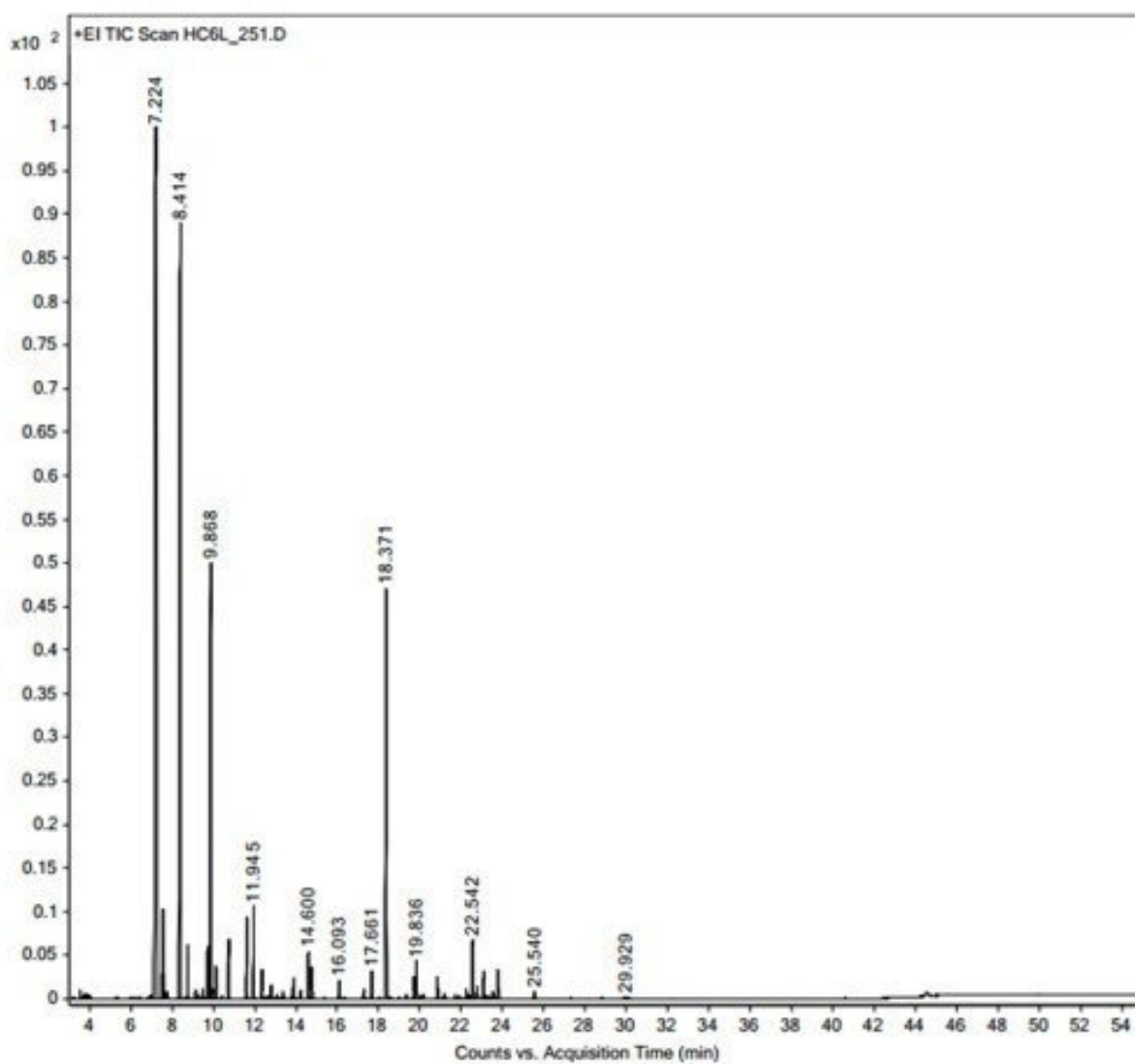


Figure 1. GC-MS chromatogram of *S. oreodoxa* leaf EO on HP-5MS Ultra-Inert column (30 m × 0.25 mm)

acetate and limonene were found at moderate proportions (8.24% and 7.85%, respectively). The other compounds, such as β -eudesmene, terpinolene, camphene and linalool, were also detected at levels exceeding 1.0% of the EO. Previous research into the chemical composition of *S. tonkinense* from Vietnam reported that β -pinene (29.3%) was the most abundant volatile constituent of the leaf EO, followed by α -pinene (15.7%) and sabinene (14.6%)¹⁰. This indicates the leaf EOs from the two *Silicium* species contained comparable amounts of β -pinene. However, the *S. oreodoxa* EO was composed of more than twice as much of α -pinene compared to the *S. tonkinense* counterpart. Besides, a smaller percentage of limonene was found and no presence of myrtenyl acetate was detected in the leaf EO of the latter species. Overall, the percentages of monoterpenes constituted roughly equal proportions of the two leaf EOs.

As seen in Table 2 and Figure 2, a total of 49 compounds were identified in the *C. thorelii* leaf EO, representing 90.72% of its composition. Unlike the composition of *S. oreodoxa* EO, monoterpenes and their oxygenated derivatives amounted to 45.34% and 0.73% of the EO from *C. thorelii* leaves. Sesquiterpenes combined with

their oxygenated forms accounted for 41.42% of the EO. The EO was mainly composed of β -pinene (21.58%), caryophyllene (14.26%), caryophyllene oxide (8.84%), camphene (8.65%), and α -pinene (7.42%). The other significant terpenoids detected in the EO included trans- β -elemenone (3.12%), limonene (2.64%), and humulene (2.57%). The other class of compounds (i.e., non-terpenoids) was detected at 3.23%. Previously, we reported the chemical composition of EO extracted from *C. thorelii* rhizomes¹³. Interestingly, pinenes showed up as minor volatile constituents in the rhizome EO, with levels of 1.0 – 1.2%. It is noted that the rhizome EO contained xanthorrhizol (40.7%), which was not detected in the leaf EO in the present study. Additionally, β -curcumene (20.7%), which was identified as a major compound in the former EO, was recorded in a trace amount in the latter. Together, sesquiterpenes and their oxygenated derivatives made up 81.8% of the total EO. These point out considerable differences in volatile profiles between the *C. thorelii* leaf and rhizome EOs.

According to the results, the EOs from leaves of the two examined species in the present study possessed 27 volatile organic compounds in common. Notably, pinenes were among these

Table 2. EO components of *Curcuma thorelii* leaves

No.	RT (min)	Components	RI (Exp.)	RI (Lit.)	(%)
1	6.829	Tricyclene	926	925	0.33
2	6.972	α -Thujene	932	929	0.10
3	7.184	α -Pinene	940	937	7.42
4	7.596	Camphene	955	952	8.65
5	8.437	β -Pinene	983	979	21.58
6	8.775	β -Myrcene	994	991	1.43
7	9.313	δ -Carene	1013	1011	0.61
8	9.496	α -Terpinene	1020	1017	0.05
9	9.730	p-Cymene	1028	1025	0.10
10	9.862	Limonene	1033	1030	2.64
11	9.936	1,8-Cineole (= Eucalyptol)	1036	1032	0.22
12	10.125	(Z)- β -Ocimene	1042	1038	0.50
13	10.434	(E)- β -Ocimene	1053	1049	1.35
14	10.738	γ -Terpinene	1063	1060	0.15
15	11.613	Terpinolene	1091	1088	0.24
16	11.939	Linalool	1100	1099	0.06

table 2. (continued).

No.	RT (min)	Components	RI (Exp.)	RI (Lit.)	(%)
17	12.437	(E)-4,8-Dimethylnona-1,3,7-triene	1119	1116	0.55
18	12.798	<i>Neo-allo</i> -ocimene	1132	1131	0.19
19	13.879	<i>endo</i> -Borneol	1170	1166	0.05
20	14.772	Myrtenol	1198	1195	0.05
21	15.670	Isobornyl formate	1232	1232	0.07
22	17.278	Bornyl acetate	1289	1285	0.28
23	17.450	2-Undecanone	1295	1294	0.11
24	19.704	α -Copaene	1381	1376	0.14
25	20.122	β -Elemene	1396	1391	0.40
26	20.351	Cyperene	1405	1399	1.04
27	20.940	Caryophyllene	1429	1419	14.26
28	21.169	γ -Elemene	1439	1433	0.09
29	21.712	Humulene	1460	1454	2.57
30	21.890	<i>epi</i> - β -Caryophyllene	1467	1466	0.15
31	22.393	Germacrene D	1486	1481	0.49
32	22.542	β -Eudesmene	1492	1486	0.33
33	22.771	α -Selinene	1500	1494	0.55
34	23.280	β -Curcumene	1516	1514	0.19
35	23.538	δ -Cadinene	1525	1524	0.43
36	24.802	<i>trans</i> -Nerolidol	1565	1564	1.75
37	25.300	(3E,7E)-4,8,12-Trimethyltrideca-1,3,7,11-tetraene	1579	1577	2.38
38	25.644	Caryophyllene oxide	1590	1581	8.84
39	26.164	<i>cis</i> - β -Elemenone	1604	1593	0.79
40	26.359	<i>trans</i> - β -Elemenone	1608	1597	3.12
41	26.565	Humulene epoxide II	1613	1606	0.86
42	27.669	Caryophylladienol II	1637	1637	1.52
43	28.476	α -Cadinol	1653	1653	0.24
44	29.288	β -Bisabolol	1670	1671	1.60
45	30.582	Germacrone	1695	1693	0.80
46	33.597	Neocurdione	1765	1762	0.30
47	35.056	Ambrial	1797	1809	0.71
48	36.155	Curcumenone	1845	1844	0.25
49	40.378	Phytol	2115	2114	0.19
Monoterpene hydrocarbons (Sr. No. 1-10, 12-15, 18)					45.34
Oxygenated monoterpenes (Sr. No. 11, 16, 19-22)					0.73
Sesquiterpene hydrocarbons (Sr. No. 24-35)					20.64
Oxygenated sesquiterpenes (Sr. No. 36, 38-48)					20.78
Others (Sr. No. 17, 23, 37, 49)					3.23
Total identified					90.72

RT (min): Retention time

RI(Exp.): Retention indices on HP-5MS UI column

RI(Lit.): Retention indices in literature

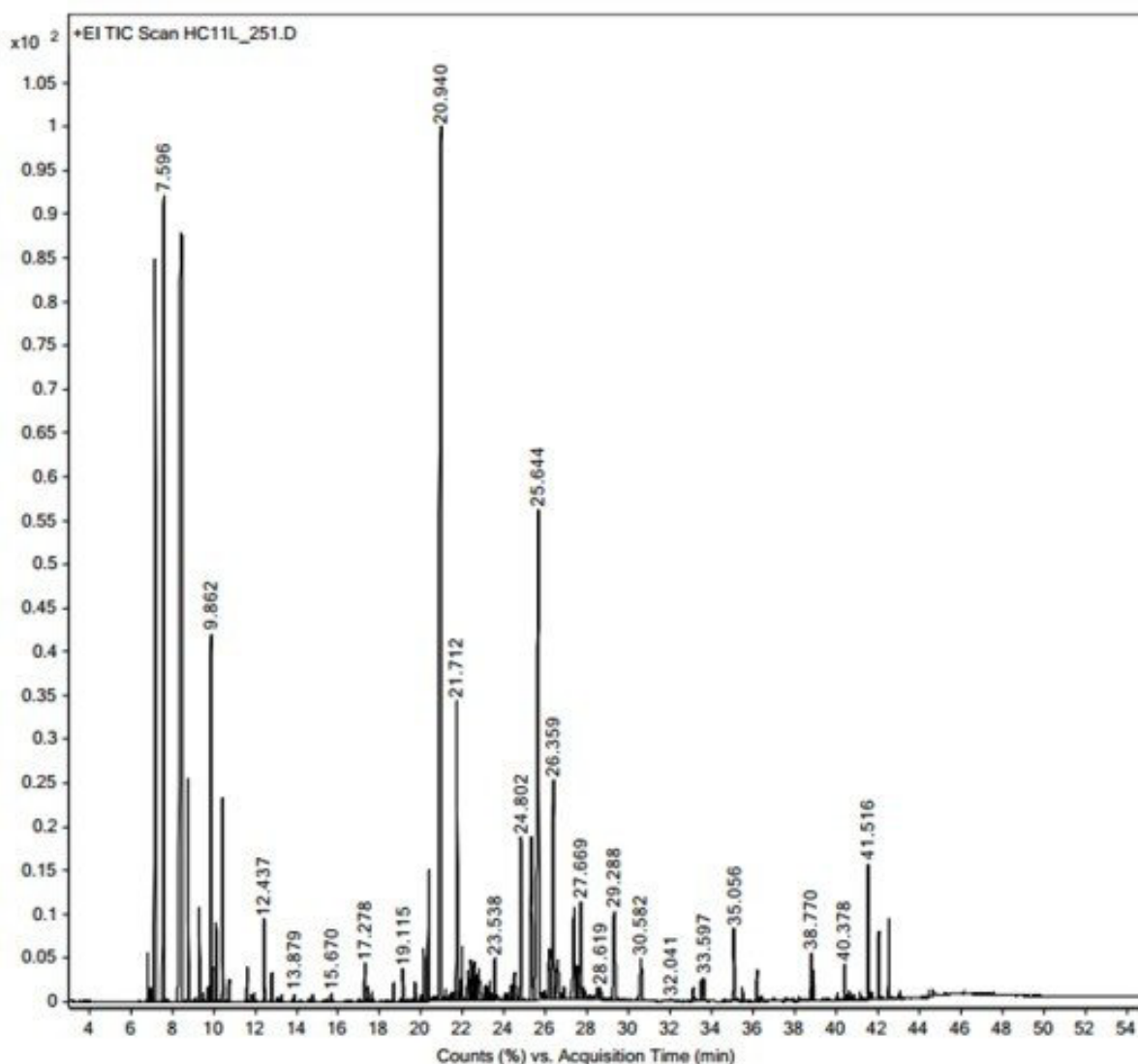


Figure 2. GC-MS chromatogram of *C. thorelii* leaf EO on HP-5MS Ultra-Inert column (30 m × 0.25 mm)

shared constituents. High levels of pinenes in EO were often shown to be linked with potent antibacterial properties against multiple pathogenic bacteria¹⁵. The 27 compounds that were found in both EOs may play a key role in contributing to their shared bioactivities. However, the unique compounds found in each EO may also have distinctive bioactivities that set them apart from each other. Understanding these similarities and differences in composition between different EOs can provide insights into their potential uses and help guide their application in different fields, such as aromatherapy, natural medicine, and cosmetic products.

The antimicrobial test on the EOs

The results of the antimicrobial assay of two EOs from *S. oreodoxa* and *C. thorelii* leaves were presented in Table 3.

The MIC of *S. oreodoxa* EO against Gram-positive bacteria was lower than the MIC for Gram-negative bacteria, indicating that Gram-positive bacteria were more sensitive to *S. oreodoxa* EO than Gram-negative bacteria in the present study. MICs ranged from 32 µg/mL (*E. faecalis*) to 64 µg/mL (*S. aureus*, *B. cereus*), while the MIC was 128 µg/mL for all three Gram-negative bacteria.

The chemical composition of EO from both

Table 3. Antimicrobial activity of two EOs from leaves of *S. oreodoxa* and *C. thorelii*

Microbial strains	Minimum inhibitory concentration (MIC: µg/mL)				
	<i>S. oreodoxa</i>	<i>C. thorelii</i>	Kanamycin	Tetracycline	Cycloheximide
<i>E. faecalis</i> ATCC 29212	32±1.56	16±0.54	128	4	-
<i>S. aureus</i> ATCC 25923	64±1.79	64±1.07	4	16	-
<i>B. cereus</i> ATCC 14579	64±2.09	32±0.44	8	64	-
<i>E. coli</i> ATCC 25922	128±2.12	64±1.06	128	8	-
<i>P. aeruginosa</i> ATCC 27853	128±1.78	64±1.40	64	256	-
<i>S. enterica</i> ATCC 13076	128±2.67	32±0.21	16	64	-
<i>C. albicans</i> ATCC 10231	64±1.52	32±1.13	-	-	32

“-”: Not tested; Mean ± SD; n = 3

plant species contained high levels of bactericidal substances, including pinenes. Studying the ability of pinenes to inhibit the growth of *S. aureus*, *S. choleraesuis*, *S. pneumoniae* and *S. pyogenes*, the MIC values were recorded from 2.5 to 40 µl/mL. In particular, *S. aureus* was completely destroyed within a maximum period of 24 hours, indicating that these active chemicals have potential applications in bactericidal therapy¹⁶. Another study showed that pinenes were able to inhibit *C. albicans*, methicillin-resistant *S. aureus*, and another Gram-positive strain *C. neoformans* with MIC values ranging from 117 – 4150 µg/mL. Among these, pinenes are toxic to *C. albicans*, killing 100% of yeast within 60 minutes¹⁵. Obviously, in this study, the EOs from both plants contained α-pinene and β-pinene and both have effective inhibitory results on *C. albicans* due to the presence of these compounds.

In the composition of the *S. oreodoxa* EO, there were also limonene and myrtenyl acetate. Limonene is a monoterpene, which is found in a number of EOs with good antibacterial activity against food-borne pathogens. This compound inhibits Gram-positive bacteria more effectively than Gram-negative bacteria. Limonene exhibited a significant growth inhibitory effect against *S. aureus* at a minimum inhibitory concentration (MIC) of 20 µg/mL¹⁷. According to Gupta et al. (2021), limonene has no effect against *P. aeruginosa* and the MIC value against *E. coli* was 16 µg/mL¹⁸.

Myrtenyl acetate is known to be a plant-derived compound with antimicrobial properties. The

EO extracted from *Myrtus communis*, commonly used to fight microorganisms that contaminate food, mainly contains myrtenyl acetate, α-pinene, limonene and some other compounds. This EO shows the ability to inhibit many types of bacteria, but the effect is better against Gram-positive bacteria than Gram-negative bacteria, and is a potential source of ingredients for application in the food and pharmaceutical industries^{19, 20}. As stated earlier, *S. tonkinense* is often used in traditional medicine to treat abdominal pain and stomach bleeding⁵. The results of this study suggest that *S. oreodoxa* may have the potential for use in the food and pharmaceutical industries due to its antimicrobial activity.

In addition to α-pinene and β-pinene like the EO of *S. oreodoxa*, the EO of *C. thorelii* contain high concentrations of constituents including camphene, caryophyllene, and caryophyllene oxide. The EOs from the leaves of *C. thorelii* had MICs for all the bacteria and yeast examined, ranging from 32 – 64 µg/mL for pathogenic bacteria and yeast. In particular, the MIC value was only 16 µg/mL for *E. faecalis* - a probiotic. It is obvious that the EO extracted from *C. thorelii* in this study showed a stronger inhibition to the investigated bacteria and yeast than that from *S. oreodoxa*. Perhaps, camphene and caryophyllene is responsible for the results. Previously, camphene derivatives showed the ability to inhibit drug-resistant bacteria including MRSA and *Enterococcus* spp. resistant to vancomycin with MIC values of 1.9 and 31.2 µg/mL, respectively²¹. Research reported that the MIC

of β -caryophyllene for *B. cereus* was 2.5% (v/v)²². β -caryophyllene inhibits the growth of caries-forming biofilm-forming *S. mutans* and may be a candidate agent for the prevention of caries²³. Research also showed that β -caryophyllene exhibited antibacterial effects on *S. aureus*, as well as enhanced the effectiveness of norfloxacin against *S. aureus*, *P. aeruginosa* and *E. coli*²⁴.

The varying effectiveness of certain EOs against Gram-positive and Gram-negative bacteria can be attributed to several factors, including differences in the bacterial cell structure and the chemical composition of the EOs. Gram-positive bacteria have a thick peptidoglycan layer, while Gram-negative bacteria have a thinner peptidoglycan layer surrounded by an outer membrane. This structural difference affects the susceptibility of the bacteria to the EOs. The thicker peptidoglycan layer in Gram-positive bacteria may make it easier for EO compounds to penetrate and disrupt the cell wall, leading to more effective inhibition or killing of these bacteria. Some volatile constituents in the EOs, such as limonene and linalool have been reported to possess antimicrobial properties against Gram-positive bacteria, including *S. aureus* and *Streptococcus* species as discussed earlier.

Overall, the results of this study demonstrated that the EO extracted from the leaves of *C. thorelii* has good inhibitory ability against the investigated bacteria and yeast, with a low concentration of only 32 – 64 $\mu\text{g/mL}$. This is a potential plant species that can be considered in the application of the food and pharmaceutical industries.

Conclusion

This study presents the first report on the volatile profile and antimicrobial properties of the EOs obtained from *S. oreodoxa* and *C. thorelii* leaves. The findings indicate that *S. oreodoxa* EO is mainly composed of monoterpenes, while *C. thorelii* EO comprised approximately equal amounts of monoterpenes and sesquiterpenes. The EOs were found to exhibit strong antimicrobial activity against the tested microorganisms. These results suggest that these EOs could be potentially useful in various industrial applications, including food and pharmaceuticals. Further investigations should focus on isolating and characterizing

non-volatile compounds from these species and evaluating their biological activities.

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Conflict of interest

There are no conflicts of interest to declare.

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