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To cite this article: Hieu Tran Trung, Tran Van Chen, Nguyen Ngoc Hieu, Van Son Dang, Nguyen Thi Giang An, Tran Dinh Thang, Le Thi Hong Minh, Hoang Van Trung, Dau Xuan Duc & Le Duc Giang (2023) Chemical components and antimicrobial properties of essential oil distilled from *Siliqueamomum oreodoxa* N.S. Lý & Škornick (Zingiberaceae) rhizomes, Journal of Essential Oil Bearing Plants, 26:3, 547-555, DOI: [10.1080/0972060X.2023.2226681](https://doi.org/10.1080/0972060X.2023.2226681)

To link to this article: <https://doi.org/10.1080/0972060X.2023.2226681>



Published online: 28 Jun 2023.



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Research Article

Chemical components and antimicrobial properties of essential oil distilled from *Siliquamomum oreodoxa* N.S. Lý & Škornick (Zingiberaceae) rhizomes

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Received 21 September 2022

Revised 25 May 2023

Accepted 29 May 2023

Abstract

Siliquamomum oreodoxa N.S. Lý & Škornick is a species of the family Zingiberaceae and is currently only found in Vietnam. Information on the chemical components and antimicrobial activity of *S. oreodoxa* essential oil has not been reported in the literature. In this work, the potentiality of the essential oil of *S. oreodoxa* rhizomes against bacteria and fungi were investigated using antimicrobial bioassays. The chemical components of the plant essential oil were also determined by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. A total of forty bioactive compounds represented 89.5% of the oil, the major components of which were β -pinene (31.25%), α -pinene (26.01%), 1,8-cineol (21.35%), α -limonene (4.66%), and γ -terpinene (4.39%). In the antibacterial test, the essential oil showed inhibitory activity against *Enterococcus faecalis* (MIC = 16 μ g/mL, IC₅₀ = 5.34 μ g/mL), *Pseudomonas aeruginosa* (MIC = 64 μ g/mL, IC₅₀ = 20.23 μ g/mL), and *Salmonella enterica* (IC₅₀ = 9.24 μ g/mL), *Candida albicans* (IC₅₀ = 9.27 μ g/mL), *Bacillus cereus* (IC₅₀ = 9.45 μ g/mL), *E. coli* (IC₅₀ = 9.76 μ g/mL), *Staphylococcus aureus* (IC₅₀ = 12.45 μ g/mL) at similar MIC value \approx 32 μ g/mL. These results encourage further experiments on biological effects and validation for the extract composition of other parts of *S. oreodoxa*, especially the essential oil compound, for functional food and drug development efforts.

Keywords

Siliquamomum oreodoxa, essential oil, GC-MS, antimicrobial activity

Introduction

The genus *Siliquamomum* Baill. was first described by Baillon in 1895 by a survey conducted in Ba Vi mountainous area, Northern Vietnam. For a very long period of time, the genus *Siliquamomum* was considered monotypic as only one species was discovered in Northern Vietnam and Southeastern China, which is *S. tonkinense*. In 2010, N.S. Lý & Škornick reported a new species of the genus, *S. oreodoxa* (Fig. 1) from Southern Vietnam with the most important differences in the leafy shoot, shorter petiole, smaller lamina, shorter and denser inflorescence¹. In 2014, the third member of the

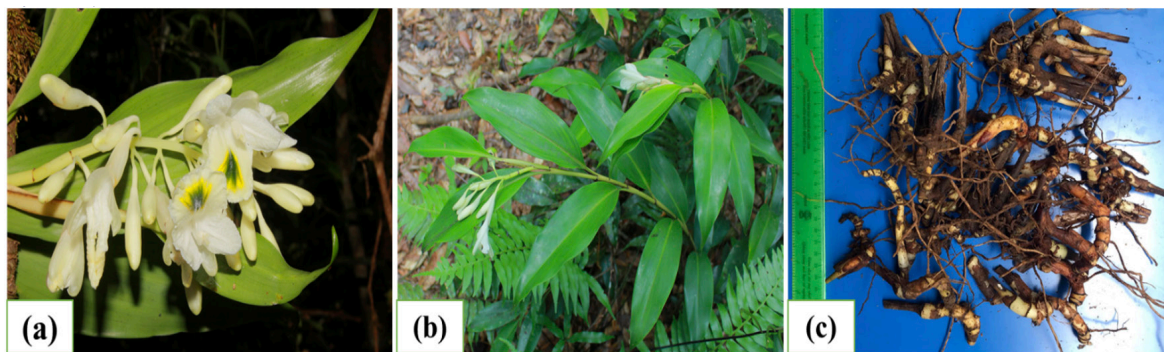


Figure 1. Flowers (a), the aerial parts (b), and rhizomes (c) of *S. oreodoxa*

genus *Siliquamomum* (*S. alcicorne*) was also introduced by J. Leong-Škorničková with sulfur-yellow labellum and green patches². Another species, *S. phamhoangii* was described as a new species of the genus in Kon Ka Kinh National Park (Central Highlands, Vietnam)³. Among discovered species, *S. tonkinense* is the most distributed and recorded in botanical books and herbarium records in six Vietnamese provinces, namely Ha Giang, Lao Cai, Tuyen Quang, Vinh Phuc, Phu Tho, and Hoa Binh, and in Southern Yunnan area². In Vietnamese folklore medicine, *S. tonkinense* stems and leaves have been used for the treatment of stomachache and bleeding stomach. Dao ethnic people in Da Bac (Hoa Binh) and Ba Vi (Ha Noi) have also used the stems and leaves parts for herbal bathing to recover women's health after childbirth.

There has been a very limited number of studies on the phytochemical and pharmacological aspects of *Siliquamomum* plants. To date, there have been only publications on the chemical composition of *S. tonkinense* essential oils prepared from different plant parts. The content of essential oils from *S. tonkinense* rhizomes and leaves collected in Vinh Phuc were 0.12 and 0.16% (w/w), respectively. While the major chemical compositions of leaf oil were monoterpene hydrocarbons (79.90%), the rhizomes mainly contained both monoterpene hydrocarbons (58.80%) and oxygenated derivatives (21.90%). The major compounds found in the leaf oil were β -pinene (29.30%), α -pinene (15.70%), and sabinene (14.60%). Meanwhile, the rhizome oil was shown to contain 1,8-cineol (19.10%), γ -terpinene (14.90%), *o*-cymene (14.00%), and

α -pinene (12.50%)⁴.

The present study is the first report, which described the chemical components and antimicrobial properties of essential oil distilled from *S. oreodoxa* rhizomes, in order to provide more insights into the characterization of volatile compounds and their bioactivity of new species from Zingiberaceae.

Materials and methods

Plant materials

The fresh rhizomes of *S. oreodoxa* were collected from Bidoup-Nui Ba National Park, Lac Duong district, Lam Dong province, Vietnam (12°09'42.7" N; 108°44'23.5" E) in June 2020. The collected sample was identified by Dr. Van Son Dang (Institute of Tropical Biology, Vietnam Academy of Science and Technology, Ho Chi Minh City). The voucher specimen (No. HC-006) was stored at the Laboratory of the Department of Chemistry, Vinh University, Vinh City, Vietnam.

Essential oil preparation

The fresh rhizomes (experiments were performed in triplicate, 800 g for each) of *S. oreodoxa* were cleaned, cut into small pieces and hydro-distilled separately using Clevenger-type apparatus for 4 h, according to the Vietnamese Pharmacopoeia⁵. The obtained volatile oil was isolated and dehydrated over anhydrous sodium sulfate to remove all water traces and preserved at 4°C for GC-MS analysis and antimicrobial test⁶⁻⁸.

Analysis of the essential oil

The chemical components of the rhizomes

essential oil of *S. oreodoxa* were analyzed on an Agilent Technologies 7890B GC System coupled to an Agilent 5977B MSD model, equipped with an Agilent HP-5MS Ultra Inert column (30 m × 0.25 mm × 0.25 μm). Helium was used as the carrier gas. In a typical procedure, the sample (1.0 μL was diluted with *n*-hexane with a 1:100 ratio) was injected by a split ratio of 50:1 and at a temperature of 230°C. The GC temperature program was initiated at 50°C (hold time 2 min), linearly increasing to 150°C (hold time 10 min) at 5°C/min, then raised at 10°C/min to the temperature of 180°C (hold time 10 min). The MS conditions were as follows: ionization voltage 70 eV, solvent delay 3 min, scanning set from 50 to 550 amu (2 scans per second).

Identification of chemical constituents

The volatile compounds in *S. oreodoxa* rhizomes essential oil were identified by comparing their retention index (RI) and their mass spectral fragmentation patterns with those in literature⁹. The formula used to calculate of RI was:

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

Where: $RI_{(calc.)}$ = retention index determined with reference to a homologous series of *n*-alkanes (Sigma-Aldrich); RT = retention time of the respective component; N= no. of carbon atoms in the larger alkane; n= no. of carbon atoms in the smaller alkane^{6,7,10,11}.

Then, using integral spectrogram peak areas, the proportional fraction of volatile contents was calculated.

Antimicrobial assay

The antimicrobial effect of the essential oil of *S. oreodoxa* rhizomes was performed using the broth micro-dilution method as reported by Nguyen DD *et al.*¹². Several bacterial and fungal strains with specific ATCC (American Type Culture Collection) code were used for the anti-microbial activity evaluation, including gram-positive pathogenic bacteria (*Bacillus cereus* ATCC 14579, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 299212), Gram-negative pathogenic bacteria (*Salmonella enterica* ATCC 13076, *Escherichia*

coli ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853), and *Candida albicans* ATCC 10231 (a yeast responsible for thrush and vaginal yeast infection). The antimicrobial activity assay was carried out on a 96-well plate. The essential oil of *S. oreodoxa* rhizomes was first diluted in 10% DMSO to prepare a diluted concentration range (2, 4, 8, 16, 32, 64, 128, 256 μg/mL). The microbial samples were standardized to 2×10^5 CFU/mL. All experiments were conducted three times for replication. The plates were incubated in different conditions for bacteria (37°C in 18-24h) and for yeast (35-37°C in 36-48h). After 24 and 48-h treatments, the MIC values were determined from the lowest concentration of the sample, at which the growth of bacteria or yeast were shown to be completely inhibited. On the other hand, the IC_{50} values were measured accurately based on the turbidity measurement data by Biotek microplate spectrophotometer (USA) and RawData software (Brussels, Belgium), according to the following equations^{13,14}:

$$\% \text{ inhibition} = \frac{A_o - A_t}{A_o - A_{oc}} \times 100\%$$

$$IC_{50} = H_c - \frac{(H_i - 50\%) \times (H_c - L_c)}{(H_i - L_i)}$$

Where: A_o = the absorbance of the cells with medium without essential oil sample. A_{oc} = the absorbance of the culture medium without cells. A_t = the absorbance of the test sample (i.e., the cells with medium and test essential oil). H_c and L_c = the concentration (%) of the test agent at high/low concentrations, respectively. H_i and L_i = the % inhibition at high/low concentrations, respectively.

Results and discussion

Chemical composition of isolated essential oil

By Clevenger hydrodistillation, the average obtained yield of essential oil from *S. oreodoxa* rhizomes was 0.15% (w/w), calculated on a fresh weight basis. Essential oil were a light-yellow liquid having lower densities than water. The total ion chromatogram (TIC) obtained through GC-MS is shown in fig. 2. The chemical analysis identified forty different compounds accounting for 99.48% of the oil contents in the rhizomes of *S. oreodoxa* (Table 1). Based

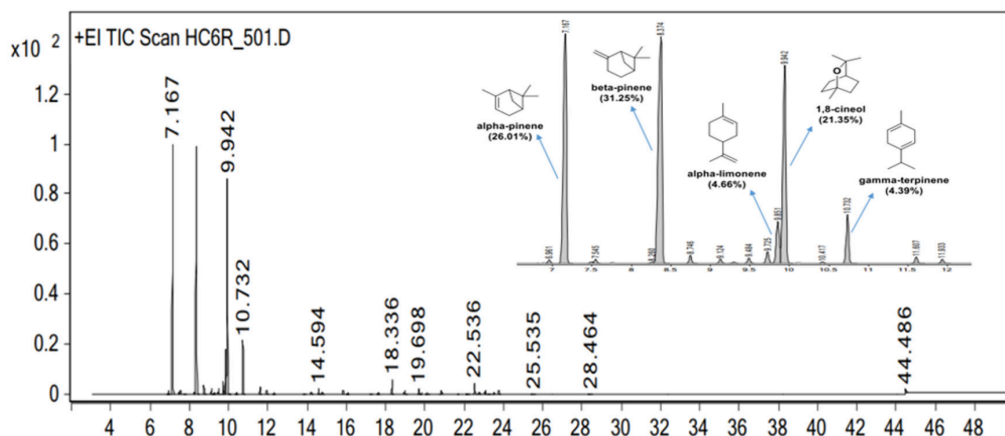


Figure 2. GC-MS chromatogram of volatile oil isolated from the rhizomes of *S. oreodoxa*

Table 1. Chemical components of essential oil distilled from *S. oreodoxa* rhizomes

No.	Components	RT (min)	RI (calc.)	RI (Lit.)	Concentration (%)
1	α -thujene	6.961	931	930	0.29
2	α-pinene	7.167	939	939	26.01
3	camphene	7.545	953	946	0.34
4	sabinene	8.260	978	975	0.11
5	β-pinene	8.374	981	979	31.25
6	β -myrcene	8.746	993	990	0.66
7	α -phellandrene	9.124	1005	1002	0.37
8	3-carene	9.296	1012	1011	0.11
9	α -terpinene	9.484	1019	1017	0.48
10	p-cymene	9.725	1028	1024	1.07
11	α-limonene	9.851	1033	1029	4.66
12	1,8-cineol	9.942	1036	1031	21.35
13	(Z)- β -ocimene	10.114	1042	1037	0.07
14	(E)- β -ocimene	10.417	1053	1050	0.11
15	γ-terpinene	10.732	1063	1059	4.39
16	terpinolene	11.607	1090	1088	0.56
17	β -linalool	11.933	1100	1096	0.35
18	β -fenchol	12.345	1116	1121	0.09
19	borneol	13.879	1170	1169	0.12
20	terpinen-4-ol	14.217	1181	1177	0.16
21	α -terpineol	14.594	1193	1188	0.53
22	myrtenol	14.766	1198	1195	0.26
23	thymol methyl ether	15.830	1238	1235	0.32
24	3-phenyl-2-butanone	16.093	1248	1244	0.10
25	bornyl acetate	17.278	1289	1288	0.09
26	<i>trans</i> -pinocarvyl acetate	17.656	1302	1298	0.09
27	myrtenyl acetate	18.336	1329	1326	1.26
28	α -terpinyl acetate	18.966	1354	1349	0.29
29	α -copaene	19.698	1381	1376	0.49
30	geranyl acetate	19.824	1385	1381	0.09

table 1. (continued)

No.	Components	RT (min)	RI (calc.)	RI (Lit.)	Concentration (%)
31	β -elemene	20.110	1396	1390	0.18
32	β -caryophyllene	20.843	1425	1419	0.33
33	α -humulene	21.695	1459	1454	0.05
34	β -selinene	22.536	1491	1490	1.07
35	α -selinene	22.765	1500	1498	0.35
36	β -bisabolene	23.080	1510	1505	0.30
37	δ -cadinene	23.532	1525	1523	0.20
38	(E)- γ -bisabolene	23.767	1532	1529	0.41
39	caryophyllene oxide	25.535	1586	1583	0.08
40	diisooctyl phthalate	44.486	2547	2543	0.44
Total identified					99.48
Monoterpene hydrocarbons (Sr. No. 1-11, 13-16)					70.48
Oxygenated monoterpenes (Sr. No. 12, 17-22, 25-28, 30)					24.68
Sesquiterpene hydrocarbons (Sr. No. 29, 31-38)					3.38
Oxygenated sesquiterpene (Sr. No. 39)					0.08
Phenyls (Sr. No. 23, 24, 40)					0.86
Sr. No.: Compound serial number; RT (min): Retention time (minutes); RI (calc.): Retention index determined with reference to a homologous series of <i>n</i> -alkanes on HP-5MS Ultral Inert column; RI (Lit.): Retention index from the databases (NIST17 and Adams book)					

on the component analysis, the main classes of compounds were monoterpene hydrocarbons (accounted for the highest fraction at 70.48% of the total), oxygenated monoterpenes (accounted for 24.68%), meanwhile, the minor classes of components were sesquiterpene hydrocarbons (3.38%), phenyls (0.86%), and oxygenated sesquiterpene (0.08%). Among which, the most abundant constituents present in this oil were β -pinene (31.25%), α -pinene (26.01%), 1,8-cineol (21.35%), α -limonene (4.66%), and γ -terpinene (4.39%).

Evaluation of *in vitro* antimicrobial activity

The *in vitro* antimicrobial activity of essential oil of *S. oreodoxa* rhizomes was determined based on the ability against the Gram-positive and -negative bacteria, and yeast screening. The MIC and IC₅₀ values of the microbial inhibitory activity of essential oil were presented in table 2. The results showed good antimicrobial activity against tested microorganisms. However, essential oil exhibited varying degree of antimicrobial activity with MIC below 64 μ g/mL and IC₅₀ below 20.23 μ g/mL. In this study, essential oil showed the highest *E. faecalis*

ATCC 299212 inhibitory activity with MIC and IC₅₀ values of 16 μ g/mL and 5.34 μ g/mL, respectively. Generally, there was no significant difference in the *in vitro* antimicrobial ability of essential oil against *S. aureus* ATCC 25923, *B. cereus* ATCC 14579, *E. coli* ATCC 25922, *S. enterica* ATCC 13076, and *C. albicans* ATCC 10231 strains at the mentioned concentrations with MIC value of 32 μ g/mL. Meanwhile, the essential oil exhibited lower activity against *P. aeruginosa* ATCC 27853 with MIC value of 64 μ g/mL. In brief, the antimicrobial activity of the essential oil against tested microorganisms followed the order: *E. faecalis* ATCC 299212 (MIC = 16 μ g/mL, IC₅₀ = 5.34 μ g/mL) > *S. enterica* ATCC 13076 (MIC = 32 μ g/mL, IC₅₀ = 9.24 μ g/mL) \approx *C. albicans* ATCC 10231 (MIC = 32 μ g/mL, IC₅₀ = 9.27 μ g/mL) \approx *B. cereus* ATCC 14579 (MIC = 32 μ g/mL, IC₅₀ = 9.45 μ g/mL) \approx *E. coli* ATCC 25922 (MIC = 32 μ g/mL, IC₅₀ = 9.76 μ g/mL) > *S. aureus* ATCC 25923 (MIC = 32 μ g/mL, IC₅₀ = 12.45 μ g/mL) > *P. aeruginosa* ATCC 27853 (MIC = 64 μ g/mL, IC₅₀ = 20.23 μ g/mL). It can be concluded that the antimicrobial activity of essential oil is dependent upon concentration.

According to the results of table 2, streptomycin

Table 2. The *in vitro* antimicrobial activity of essential oil (EO) of *S. oreodoxa* rhizomes

Microorganisms	MIC ($\mu\text{g/mL}$)			IC ₅₀ ($\mu\text{g/mL}$)		
	EO*	ST	CY	EO*	ST	CY
<i>E. faecalis</i> ATCC 299212	16 \pm 2.57	256	NT	5.34 \pm 1.32	50.34	NT
<i>S. aureus</i> ATCC 25923	32 \pm 2.99	256	NT	12.45 \pm 0.05	45.24	NT
<i>B. cereus</i> ATCC 14579	32 \pm 1.69	128	NT	9.45 \pm 0.17	20.45	NT
<i>E. coli</i> ATCC 25922	32 \pm 3.45	32	NT	9.76 \pm 0.01	9.45	NT
<i>P. aeruginosa</i> ATCC 27853	64 \pm 2.59	256	NT	20.23 \pm 2.12	68.67	NT
<i>S. enterica</i> ATCC 13076	32 \pm 1.59	128	NT	9.24 \pm 0.74	45.67	NT
<i>C. albicans</i> ATCC 10231	32 \pm 2.52	-	32 \pm 0.07	9.27 \pm 0.96	-	10.46

ST: Streptomycin; CY: Cycloheximide; NT: Not tested; -: No activity; *: Mean \pm SD, n = 3

was used as a positive control for bacteria and showed antimicrobial activity with MIC values in the range 32 $\mu\text{g/mL}$ to 256 $\mu\text{g/mL}$ as well as IC₅₀ values in the range of 9.45 $\mu\text{g/mL}$ to 68.67 $\mu\text{g/mL}$. This finding was similar to previously reported studies^{13,14}. Additionally, cycloheximide was also used as a positive control (i.e., anti-candidal agent) with MIC and IC₅₀ values of 32 $\mu\text{g/mL}$ and 9.27 $\mu\text{g/mL}$, respectively. Interestingly, the MIC and IC₅₀ values for essential oil were lower than positive controls, suggesting that the essential oil from *S. oreodoxa* rhizomes had the ability to be antimicrobial which was better than positive controls.

In recent studies, researchers have reported that bioactive substances were considered to have good antibacterial activity when the MIC value was less than 100 $\mu\text{g/mL}$ ¹³⁻¹⁶. Additionally, with higher concentration MIC value, tested samples (e.g., essential oil, extract sample) with MIC value \leq 200 $\mu\text{g/mL}$ exhibited significant antibacterial activity as reported by Tuan *et al.* (2021)¹⁷. Thus, the results of this study have shown that the essential oil of *S. oreodoxa* rhizome had strong inhibitory ability against the tested bacterial strains (with MIC value $<$ 64 $\mu\text{g/mL}$ and IC₅₀ value $<$ 20.23 $\mu\text{g/mL}$).

To deal with antibiotic resistance, researchers have been looking for treatment alternatives, one of which includes the use of naturally derived products such as essential oil. Hitherto, essential oil obtained from plants of the Zingiberaceae family (e.g., *Alpinia kwangsiensis*, *Alpinia malaccensis*, *Amomum cinnamomeum*, *Boesenbergia pandurata*, *Boesenbergia*

quangngaiensis, *Zingiber zerumber*, etc)^{13,15,18-20} have been shown to present significant inhibitory effects on bacteria, fungi, and viruses. Therefore, many studies have been carried out to evaluate the effectiveness of essential oils on microorganisms inhibition.

In this work, β -pinene (31.25%), α -pinene (26.01%), 1,8-cineol (21.35%), α -limonene (4.66%), and γ -terpinene (4.39%) are the major constituents of *S. oreodoxa* rhizome essential oil. The recorded antibacterial activities may be related to the major compounds or synergistic effects of all the major and minor components identified in the rhizome essential oil of *S. oreodoxa*. α - and β -pinenes are important chemical components of the plant essential oil, typically pine. In previous studies, it was demonstrated that β - and α -pinene were effective against bacterial (e.g., *Pseudomonas putida*, *Staphylococcus epidermidis*, *S. aureus*, *Escherichia coli*, *Micrococcus luteus*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *Bacillus cereus*, *Streptococcus pneumoniae*, *S. pyogenes*, etc), fungal (e.g., *Candida albicans* or *Candida* spp.)^{21,22}. α -pinene was also found to be a potential antibiotic resistance modulator for *Campylobacter jejuni*, which is a multidrug-resistant strain causing gastroenteritis by significantly reducing the MIC values of well-known antibiotics, such as ciprofloxacin, erythromycin, and triclosan (512 times)²³. 1,8-cineol, commonly known as eucalyptol, is the main component of eucalyptus oil, which showed potential anti-microbial activities against both gram-positive and gram-negative bacteria²⁴,

and even antibiotic-resistant strains by inhibiting microbial biofilm formation and through quorum sensing²⁵. Eucalyptol also showed synergistic anti-bacterial effects when combined with antibiotics, such as amoxicillin/ clavulanic acid or gentamicin²⁶. Furthermore, α -limonene and γ -terpinene, which are common constituents of essential oils, were also reported to have antibacterial and antifungal activities^{27,28}. As a result, the antibacterial activity of the essential oil of *S. oreodoxa* rhizomes against the test microbial strains revealed by *in vitro* experiment would be the first proof of an alternative. Moreover, with its promising antimicrobial, further investigation on the *S. oreodoxa* chemical composition as well as other bioactivity is highly justified.

Conclusions

This study initially included both GC-MS chemical composition analysis and experiments *in vitro* antimicrobial to test the potential of *S. oreodoxa* essential oil against microorganisms. GC-MS characterizes forty bioactive compounds with five main compounds in essential oil: β -pinene (31.25%), α -pinene (26.01%), 1,8-cineol (21.35%), α -limonene (4.66%), and γ -terpinene (4.39%). Antimicrobial assay to determine the antibacterial and antifungal activities of essential oil of *S. oreodoxa* rhizome against *E. faecalis* (MIC = 16 μ g/mL, IC₅₀ = 5.34 μ g/mL), *P. aeruginosa* (MIC = 64 μ g/mL, IC₅₀ = 20.23 μ g/mL); meanwhile, the MIC value \approx 32 μ g/mL of the essential oil was similar for microorganisms such as *S. enterica* (IC₅₀ = 9.24 μ g/mL), *C. albicans* (IC₅₀ = 9.27 μ g/mL), *B. cereus* (IC₅₀ = 9.45 μ g/mL), *E. coli* (IC₅₀ = 9.76 μ g/mL), *S. aureus* (IC₅₀ = 12.45 μ g/mL). Thus, the essential oil of *S. oreodoxa* rhizome had strong inhibitory ability against the tested bacterial strains (i.e., MIC value < 64 μ g/mL and IC₅₀ value < 20.23 μ g/mL). Moreover, the results obtained in this work will encourage further experimental attempts to specify the biological activities of each component against bacteria and other their activities.

Acknowledgments

The authors would like to thank the National Institute for Food Control in Ha Noi and the

Institute of Drug Quality Control in Ho Chi Minh City (Vietnam) for providing microorganisms, streptomycin, and cycloheximide for analysis.

Competing interests

The authors declare no conflict of interest.

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