Hindawi
Journal of Food Quality
Volume 2021, Article ID 9922283, 8 pages
https://doi.org/10.1155/2021/9922283

Research Article

The Chemical Composition and Biological Activities of Essential Oils from *Zanthoxylum rhetsa* Grown in Son La, Northwest Vietnam

Cao Bach Pham,[1] Thi Inh Cam,[1] Tuyen Tran Thi,[1] Pham Minh Quan,[1] Tran Quoc Toan,[1] Huu Nghi Do,[1] Dinh Thi Thu Thuy,[1] Thi Thuy Nguyen,[1] Anh Vien Trinh,[2] Thi Hong Minh Pham,[1] Quoc Long Pham,[1] Tran Thien Hien,[3,4] Dung Thuy Nguyen Pham,[4,5] and Hong Van Nguyen Thi[1,6]

[1]*Institute of Natural Products Chemistry (INPC), Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet Cau Giay, Ha Noi, Vietnam*
[2]*Hanoi Medical University, 01 Ton That Tung Đong Đa, Ha Noi, Vietnam*
[3]*Institute of Environmental Sciences, Nguyen Tat Thanh University, Ho Chi Minh City 70000, Vietnam*
[4]*Faculty of Environmental and Food Engineering, Nguyen Tat Thanh University, Ho Chi Minh City 70000, Vietnam*
[5]*NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City 70000, Vietnam*
[6]*Graduate University of Sciences and Technology, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet Cau Giay, Ha Noi, Vietnam*

Correspondence should be addressed to Dung Thuy Nguyen Pham; pntdung@ntt.edu.vn and Hong Van Nguyen Thi; nguyenthihongvan.inpc.vast@gmail.com

Received 8 March 2021; Accepted 29 June 2021; Published 12 July 2021

Academic Editor: Chunpeng Wan

Essential oils (EOs) from the stem barks, leaf petioles, fruit petioles, fresh leaves, and fresh and dried fruits of *Zanthoxylum rhetsa* were extracted by hydrodistillation. The volatile compounds of the products were analyzed by gas chromatography (GC-FID) and gas chromatography/mass spectrometry (GC/MSD). Monoterpene hydrocarbons formed the predominant fraction of all six EO samples, of which sabinene is one of the major components (from 12.37% to 41.13%). For the leaf petiole EO, limonene (25.01%), sabinene (14.56%), and linalool (12.63%) are the major constituents, while the main constituents of fruit petiole EO were terpinolene (19.66%), terpinen-4-ol (19.07%), and sabinene (17.83%). The major components of stem bark EO are terpinen-4-ol (18.23%), sabinene (12.37%), α-phellandrene (7.34%), β-phellandrene (6.32%), and γ-terpinene (6.12%), while sabinene (38.35%), terpinen-4-ol (13.71%), γ-terpinene (6.47%), and limonene (6.02%) are the major constituents of fresh leaf EO. For the EOs of dried fruits and fresh fruits, sabinene, terpinolene, limonene, and terpinen-4-ol are the major constituents. The essential oils were also tested for their cytotoxic and antimicrobial activities. The results revealed that six EOs at concentrations of 50 μg/mL exhibited inhibitory activity against at least one tested cancer cell line but were nontoxic on Vero normal cells. Most EOs showed moderate antimicrobial activity against *F. oxysporum*; however, there were no obvious activity against *B. subtilis* and *S. aureus*.

1. Introduction

Zanthoxylum rhetsa (Roxb.) DC (*Z. rhetsa*) is a flowering plant of the Rutaceae family found in India, Myanmar, Thailand, Lao, and Vietnam. The tree has a medium size (about 14–18 meter in height) with a straight body, thorny branches, and 10–15 cm lanceolate leaves. The *Z. rhetsa* flowering season is between June and July with clusters of gray-white flowers and fruiting in October and November [1, 2]. *Z. rhetsa* is an indigenous plant in the northwest of

Vietnam, where the Son La province accounted for 71% of the total production [3]. Fruit and seed powders of *Z. rhetsa* are used as spices for cooking or for meat preservation by ethnic minorities such as Thai and H'Mong. Moreover, the plant is also used as traditional treatment for toothache, abdominal and stomach pain, and improving digestion [3, 4].

The chemical composition of the essential oil of *Z. rhetsa* grown in India and Thailand has been reported. For instance, the seed EO of *Z. rhetsa* grown in Kerala (South India) contained mostly monoterpenes [5], while sesquiterpenes were predominant in the leaf EO, with the major components include caryophyllene oxide, β-caryophyllene, β-copaene, and spathulenol [6]. The phytochemical profile of the seed EO, i.e., the presence of sabinene, α-pinene, α-terpinene, β-pinene, γ-terpinene, myrcene, terpinolene, and limonene, was also varied according to the pH of environment [7]. Meanwhile, the seed coat of *Z. rhetsa* collected from Senapati (the northeast of India) mainly consisted of terpinen-4-ol (32.1%), α-terpineol (8.2%), sabinene (8.1%), along with β-phellandrene and 2-undecanone at 7.4% and 7.1%, respectively [8]. However, in some areas of Thailand such as Nan and Chiang Rai, the dried and the fresh fruits of *Z. rhetsa* contained different levels of limonene (27.10%–59.68%), β-phellandrene (10.88%–19.40%), and sabinene (25.03%–31.21%) [9]. On the other hand, sabinene (22.51%) and terpinene-4-ol (32.33%) were the major components of the EO extracted from fresh fruits of *Z. rhetsa* collected from Phayao (Thailand) [10].

Numerous studies have reported the interesting biological activities of *Z. rhetsa* EOs. The fresh fruit EO of *Z. rhetsa* grown in Phayao, Thailand, has showed antiproliferative activity against breast cancer cells, and thus it was proposed as a potential food preservative and anticancer drug [10]. Meanwhile, terpinen-4-ol, which is the main constituent of pericarp EO, has the ability to inhibit the stress and diseases related to stomach and intestines [11].

In this paper, the EOs obtained from the different parts of *Z. rhetsa* (e.g., stem bark, leaf petiole, fruit petiole, fresh leaves, and fresh and dried fruit) grown in Son La, Northwest Vietnam, were extracted by hydrodistillation and its chemical composition was analyzed by GS/MS. In addition, these EOs have been evaluated for their biological activities, which included antibacterial and antiproliferative activities.

2. Materials and Methods

2.1. Materials. The stem bark, leaf petiole, fruit petiole, leaves, and fruits of *Z. rhetsa* were collected from the Thuan Chau district, Son La province, Vietnam. Plant identification was performed by Dr. Nguyen Quoc Binh, the Vietnam Museum of Nature (VMN), Vietnam Academy of Science and Technology (VAST). All the plant parts were washed with tap water three times, air-dried at room temperature, and then stored in a refrigerator. 500 g of each fresh sample of stem bark, leaf petiole, fruit petiole, and leaves was chopped into pieces, and 200 g of fresh fruits was crushed as samples for EO isolation. 500 g of fresh fruits was dried at room temperature and then were ground as samples for EO isolation.

2.2. Isolation of Essential Oils. The oil extraction was performed by hydrodistillation in the Clevenger-type apparatus for 3 h at normal pressure. The collected EOs were dehydrated with anhydrous sodium sulfate, weighted, and refrigerated until analysis. The samples were labeled as SB: stem bark; LP: leaf petiole; FP: fruit petiole; FL: fresh leaves; DF: dried fruit; and FF: fresh fruit.

2.3. GC-MSD and GC-FID Analysis. The chemical compositions of EOs were analyzed by Agilent 7890A gas chromatography (GC) equipped with an MSD Agilent 5975C detector and a HP-5MS column (60 m × 0.25 mm, 0.25 μm film thickness) (Agilent Technologies, CA, USA). Other conditions were set as follows: 250°C as injector temperature, helium as the carrier gas, 1 mL·min^{-1} as flow rate, and temperature program from 60°C to 240°C (4°C/min). The split ratio was 100 : 1, and the injection volume of EO was 1 μL. The MSD full-scan mode was applied under 70 eV of ionization voltage, 40 mA of emission current, and 35–450 amu of acquisition scan mass range.

The constituents were identified by comparing their mass spectrum with the W09N08 libraries and NIST Chemistry WebBook (http://webbook.nist.gov/chemistry/) database. The retention indices (RIs) of EO components were calculated by MassFinder 4.0 software base on homologous n-alkanes with same conditions. The relative content of each phytochemical component was estimated based on the GC-FID peak area with same conditions.

2.4. Antimicrobial Assays. The antimicrobial assays were performed by using four bacterial and two fungal strains purchased from American Type Culture Collection (ATCC, Manassas, VA, USA), including *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 27212, *Pseudomonas aeruginosa* ATCC 25923, *Staphylococcus aureus* ATCC 12222, *Aspergillus niger* ATCC 9763, and *Fusarium oxysporum* ATCC 48112. The culture of the microorganisms with an inoculum size of about 10^5 colony-forming units (CFU) per mL was prepared and loaded into 96-well microplates. Samples at different concentrations (50–200 μg/mL) were prepared by dissolving in 5% DMSO, then loaded into the plates, and incubated at 37°C for 24 h. Gentamycin (16 IU/mg, 8 IU/mg, and 4 IU/mg), doxycycline (0.4 IU/mg, 0.2 IU/mg, and 0.1 IU/mg), and nystatin (12 IU/mg, 6 IU/mg, and 3 IU/mg) (Merck KGaA, Darmstadt, Germany) were used as positive references. 5% DMSO was used as the negative control [12].

2.5. Cell Proliferation Assays. Five human cancer cell lines (e.g., HeLa, Hep-G2, A-549, MCF-7, and HGC-27) and a normal cell line Vero were obtained from ATCC and maintained in suitable media (RPMI 1640, MEM, DMEM; Sigma Aldrich Inc., Saint. Louis, MO, USA) at 37°C in 5% CO_2. MTT assay was performed to investigate the viability of cancer cells [13, 14]. Dilution was performed in a 96-well microplate to obtain a density of 5×10^4 cells per well. The samples (0.63–50 μg/mL), DMSO as the negative control (Merck KGaA) and ellipticine as the positive control (Merck

KGaA), were added to the wells and incubated at 37°C for 48 h. A total of 20 μL of MTT (Sigma-Aldrich, Saint. Louis, MO, USA) was then added, and incubation was continued for another 4 h at 37°C. Absorbance was measured at 540/720 nm using a Spark multimode reader (Tecan, Männedorf, Switzerland). All experiments were performed in triplicate. The growth inhibition (%) was calculated by using the formula: Inhibition rate (%) = $(1 - OD_{sampl}/OD_{con}) \times 100\%$, where OD_{sampl} and OD_{con} are the optical densities of the sample groups and control, respectively.

3. Results and Discussion

3.1. Chemical Composition of Z. rhetsa Essential Oils. Essential oils from the different parts of *Z. rhetsa* were obtained by hydrodistillation and analyzed for phytochemical profile by GC-MSD/GC-FID. The results are shown in Table 1 and Figure 1.

3.1.1. Stem Bark EO. Fifty-one constituents were detected, which comprised 98.85% of the total oil (Figure 1(a)). The major components were terpinen-4-ol (18.23%), sabinene (12.37%), α-phellandrene (7.34%), β-phellandrene (6.32%), and γ-terpinene (6.12%). The EO was rich in monoterpene hydrocarbons (52.17%) followed by oxygenated monoterpenes (24.41%), sesquiterpene hydrocarbons (12.61%), oxygenated sesquiterpenes (6.93%), and aliphatic ketones (2.77%).

3.1.2. Leaf Petiole EO. The chemical constituents accounted for 99.69% of the total oil (Figure 1(b)). Monoterpene hydrocarbons were the most abundant in leaf EOs, with limonene being the major compound (25.01%). Oxygenated monoterpenes represented 24.07% of the EO, with linalool (12.63%) as the major compound. A minor quantity (2.7%) of sesquiterpene hydrocarbons was represented with β-caryophyllene (1.23%) as the major compound. Oxygenated sesquiterpenes represented 1.11% of the oil.

3.1.3. Fruit Petiole EO. The chemical constituents accounted for 99.69% of the total oil (Figure 1(c)). Monoterpene hydrocarbons, which are mainly terpinolene and sabinene, formed the predominant fraction (54.66%) followed by oxygenated monoterpene (41.64%), sesquiterpene hydrocarbons (2.71%), and oxygenated sesquiterpenes (0.29%). The others represented 0.18% of the total oil.

3.1.4. Fresh Leaf EO. The chemical constituents accounted for 99.57% of the total oil (Figure 1(d)). Monoterpene hydrocarbons were the most abundant (74.64%) with sabinene, γ-terpinene, limonene, and α-pinene. Terpinen-4-ol (13.71%) was the major compound of the oxygenated monoterpene fraction (19.01%). A minor quantity of sesquiterpene hydrocarbons was found (3.72%) with β-caryophyllene (1.53%) as the major constituent. Oxygenated sesquiterpenes and the others represented 0.29% and 1.92% of the EO, respectively. This result was different from the study of Jirovetz et al., where sesquiterpenes and monoterpenes were presented with the quantities of 38.6% and 2.2%, respectively [6].

3.1.5. Fresh Fruit and Dried Fruit EOs. A total of 23 components from fresh fruit EO (FF) and 26 components from dried fruit EO (DF) were identified, accounting for 99.99% (Figures 1(e) and 1(f)). The monoterpene hydrocarbon fractions were enriched in the two EOs (83.71% and 84.28%, respectively), with sabinene (41.13% and 32.88%, respectively), terpinolene (27.05% and 30.37%, respectively), and limonene (7.84% and 8.29%, respectively). Next, the oxygenated monoterpene fractions were 15.04% and 14.74% for FF and DF, respectively. The major components of this fraction were α-terpineol (6.08%, 3.27%) and terpinen-4-ol (5.35%, 7.73%), respectively. In comparison with another result of chemical constituents of fresh fruit EO collected from the Mai Chau district of the Hoa Binh province in Vietnam, there were 24 components found, in which benzene, benzaldehyde-4-methoxy, 1-methoxy-4 (1-propenyl), 1-butanon, 1-(4-hydroxyphenyl), benzene-methanol, and alpha-ethyl-4-methoxy were the main components [18].

There are nineteen common compounds present in all six essential oil samples, including sabinene, limonene, terpinolene, terpinen-4-ol, α-terpineol, γ-terpinene, α-terpinene, linalool, trans-β-ocimene, myrcene, α-pinene, β-phellandrene, α-thujene, trans-sabinene hydrate, cis-sabinene hydrate, trans-p-menth-2-en-1-ol, cis-p-menth-2-en-1-ol, geranyl acetate, and germacrene D. In these common compounds, sabinene is present in high content in all six EOs (from 12.37% to 41.13%) followed by terpinen-4-ol (from 5.35% to 19.07%) and limonene (from 4.18% to 25.01%). Meanwhile, there are some compounds present in all six EO samples, but all in low content (less than 5%), such as α-terpineol, α-thujene, myrcene, α-terpinene, trans-β-ocimene, cis-sabinene hydrate, trans-sabinene hydrate, cis-p-menth-2-en-1-ol, trans-p-menth-2-en-1-ol, geranyl acetate, and germacrene D. However, there is a large difference in the content of some compounds in six EO samples, such as terpinolene is present in high content in dried fruits, fresh fruits, fruit petioles, and leaf petioles (30.37%, 27.05%, 19.66%, and 6.86%, respectively) but with low content in fresh leaves and stem barks (1.91% and 1.57%, respectively); linalool is present in relatively high content in leaf petioles and fruit petioles (12.63% and 11.64%, respectively), but it is only present in low content in fresh leaves, fresh fruits, stem barks, and dried fruits (1.80%, 1.71%, 1.61%, and 0.84%, respectively); α-pinene presents with 7.00% in leaf petioles and 5.62% in fresh leaves, but it has only trace content in the samples of dried fruits (0.67%) and fresh fruits (0.54%). These differences are displayed in Figure 2.

Some compounds are only present in a certain EO and therefore are assumed to have properties specific to a certain EO: δ-3-carene, 2-undecanone, α-cubebene, β-cubebene, α-copaene, cis-β-elemene, β-selinene, elemol, spathulenol, 1-epi-cubenol, epi-α-cadinol, α-muurolol, and neo-

Table 1: Phytochemical profile of EOs from the different parts of *Z. rhetsa*.

Compound name	RI[a/b]	RI	Percentage					
			SB	LP	FP	FL	DF	FF
(*Z*)-Hex-3-en-1-ol	854	851	—	—	—	0.45	—	—
(*Z*)-Hex-2-en-1-ol	855	860	—	—	—	1.06	—	—
n-Hexanol	871	862	—	—	—	0.41	—	—
α-Thujene	930	930	0.75	0.42	0.27	1.14	0.61	0.35
α-Pinene	939	939	2.09	7.00	1.07	5.62	0.67	0.54
Sabinene	975	978	12.37	14.56	17.83	38.35	33.71	41.13
β-Pinene	979	984	0.14	0.26	—	1.12	—	—
Myrcene	991	991	1.89	1.87	1.17	2.00	2.02	1.76
n-Octanal	999	1003	—	—	—	—	—	0.26
α-Phellandrene	1003	1010	7.34	2.74	0.10	2.11	0.11	—
δ-3-Carene	1011	1016	0.15	—	—	—	—	—
α-Terpinene	1017	1021	3.63	1.90	2.23	3.68	2.03	1.12
o-Cymene	1026	1029	0.71	0.42	0.39	0.66	0.23	—
Limonene	1029	1034	4.18	25.01	4.44	6.02	8.29	7.30
β-Phellandrene	1030	1035	6.32	2.08	0.26	2.53	0.35	0.26
cis-*β*-Ocimene	1037	1037	0.89	0.51	0.12	0.20	—	—
trans-*β*-Ocimene	1050	1048	4.02	5.05	2.69	2.83	2.55	2.37
γ-Terpinene	1060	1063	6.12	3.16	4.43	6.47	3.34	1.83
n-Octanol	1068	1068	—	—	—	—	—	0.13
cis-Sabinene hydrate	1070	1072	0.38	0.24	0.79	0.49	0.65	0.54
Terpinolene	1089	1094	1.57	6.86	19.66	1.91	30.37	27.05
Linalool	1097	1101	1.61	12.63	11.64	1.80	0.84	1.71
trans-Sabinene hydrate	1098	1104	0.35	0.25	0.74	0.43	0.44	0.30
trans-4,8-Dimethylnona-1,3,7-triene	1103	1117	—	—	0.18	—	—	—
cis-p-Menth-2-en-1-ol	1122	1128	1.08	0.49	1.14	0.75	0.42	0.33
trans-*p*-Menth-2-en-1-ol	1141	1145	0.73	0.34	0.83	0.53	0.31	0.22
Terpinen-4-ol	1177	1186	18.23	7.78	19.07	13.71	7.73	5.35
p-Cymen-8-ol	1183	1190	—	—	0.17	—	0.32	—
α-Terpineol	1189	1197	0.89	1.55	5.35	0.70	3.27	6.08
cis-Piperitol	1196	1203	0.26	0.13	0.28	0.18	—	—
Decanal	1202	1206	—	—	—	—	0.34	0.40
Octyl acetate	1214	1210	—	—	—	—	0.23	0.22
trans-Piperitol	1208	1214	0.41	0.17	0.46	0.28	0.13	—
Nerol	1230	1231	0.12	—	0.25	—	—	—
Geraniol	1253	1255	0.19	0.22	0.58	—	—	—
2-Undecanone	1294	1294	2.77	—	—	—	—	—
α-Cubebene	1351	1360	0.16	—	—	—	—	—
Geranyl acetate	1381	1383	0.16	0.27	0.34	0.14	0.63	0.51
α-Copaene	1377	1389	0.36	—	—	—	—	—
β-Cubebene	1388	1401	0.21	—	—	—	—	—
cis-*β*-Elemene	1391	1403	0.21	—	—	—	—	—
(E)-Caryophyllene (*β*-Caryophyllene)	1419	1437	3.42	1.23	1.01	1.53	0.11	—
α-Humulene	1455	1471	0.67	0.21	0.19	0.25	—	—
β-Chamigrene	1478	1489	0.38	—	—	—	—	—
Germacrene D	1485	1498	3.46	0.65	0.76	0.88	0.27	0.24
β-Selinene	1490	1503	0.38	—	—	—	—	—
(*E*,*E*)-*α*-Farnesene	1506	1512	—	—	—	0.51	—	—
Bicyclogermacrene	1500	1513	2.28	0.40	0.32	0.36	—	—
γ-Cadinene	1514	1530	0.11	—	—	—	—	—
δ-Cadinene	1523	1536	0.97	0.21	0.12	0.19	—	—
Elemol	1550	1562	0.58	—	—	—	—	—
Spathulenol	1578	1595	0.38	—	—	—	—	—
Viridiflorol	1593	1603	0.45	0.15	—	—	—	—
Guaiol (=champacol)	1601	1613	0.55	0.23	0.31	—	—	—
1-epi-Cubenol	1629	1645	0.17	—	—	—	—	—
epi-*α*-Cadinol (=tau-cadinol)	1640	1657	0.26	—	—	—	—	—
epi-*α*-Muurolol (=tau-muurolol)	1642	1658	0.96	0.22	—	0.11	—	—
α-Muurolol (=*δ*-cadinol)	1646	1661	0.29	—	—	—	—	—
α-Cadinol	1654	1671	1.12	0.40	0.28	0.18	—	—

Table 1: Continued.

Compound name	RI[a/b]	RI	Percentage					
			SB	LP	FP	FL	DF	FF
Neointermedeol	1660	1674	1.93	—	—	—	—	—
Bulnesol	1672	1685	0.24	0.11	0.23	—	—	—
Total			98.85	99.69	99.69	99.57	99.99	99.99
Monoterpene hydrocarbons			52.17	71.84	54.66	74.64	84.28	83.71
Oxygenated monoterpenes			24.41	24.07	41.64	19.01	14.74	15.04
Sesquiterpene hydrocarbons			12.61	2.7	2.71	3.72	0.38	0.24
Oxygenated sesquiterpenes			6.93	1.11	0.51	0.29	—	—
Aliphatic ketones			2.77	—	—	—	—	—
Others			—	—	0.18	1.92	0.57	1.01

RI[a/b]: retention index compared between software predictions [15–17]; SB: stem bark; LP: leaf petiole; FP: fruit petiole; FL: fresh leave; DF: dried fruit; FF: fresh fruit.

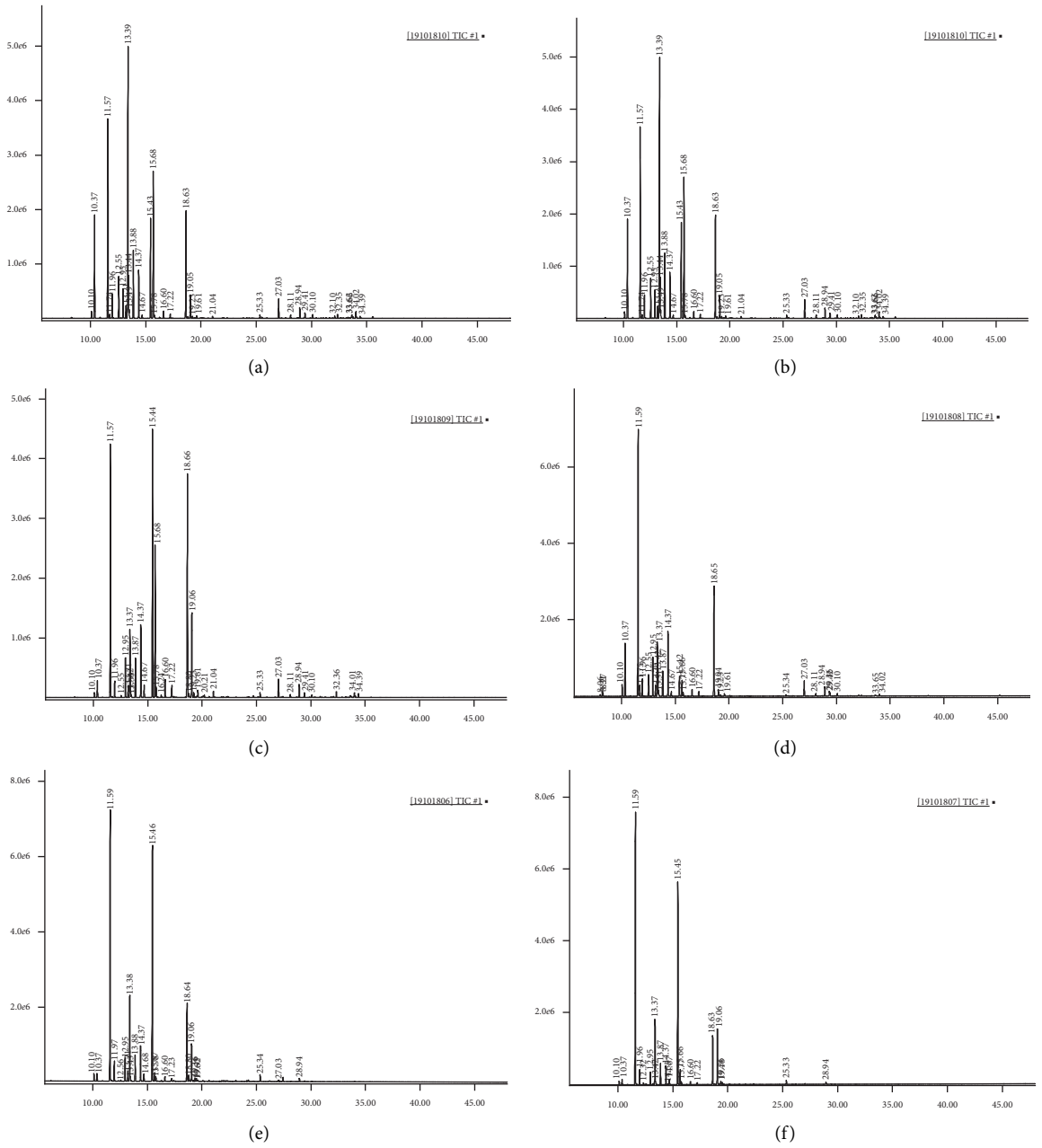

Figure 1: Chromatography of EOs from (a) SB, (b) LP, (c) FP, (d) FL, (e) DF, and (f) FF.

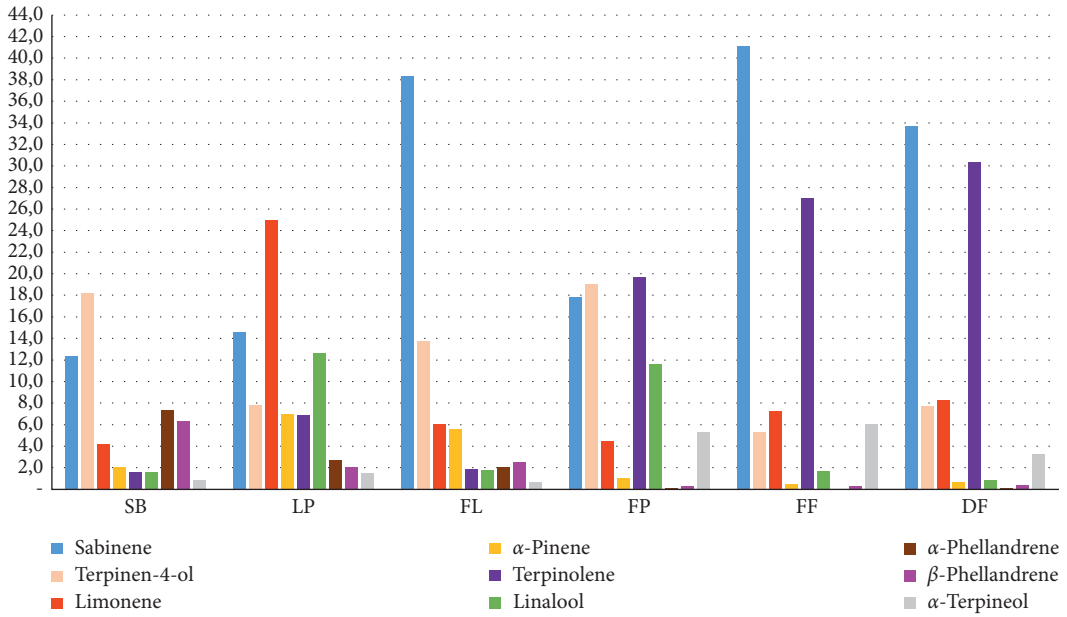

Figure 2: The main constituents in six essential oil samples.

intermedeol are present only in stem bark EO; trans-4,8-dimethylnona-1,3,7-triene is present only in fruit petiole EO; (Z)-hex-3-en-1-ol, (Z)-hex-2-en-1-ol, *n*-hexanol, and (E,E)-*α*-farnesene are present only in fresh leaf EO; and the two compounds decanal and octyl acetate are specific to fresh and dried fruit EOs.

3.2. Biological Activity of *Z. rhetsa* EOs

3.2.1. Cytotoxicity. Six EO samples extracted from different parts of *Z. rhetsa* collected from the Son La province in Vietnam were tested for their cytotoxicity effect against five cancer cell lines (MCF-7, HeLa, HGC-27, Hep-G2, and A-549) and a normal cell line Vero. The cytotoxic activities were expressed by IC_{50} values, which revealed that all EOs at maximum concentration slightly inhibited at least one tested cell line (IC_{50} ranges from 46.21 to 89.39 μg/mL; Table 2).

Particularly, the EO of fresh leaves (FL) exhibited stronger cytotoxicity against four tested cancer cell lines, while the EO of stem bark (SB) and of fresh fruit (FF) exhibited cytotoxicity against HGC-27 and A-549, respectively. Significantly, these EOs demonstrated no cytotoxicity against the normal Vero cell line at the final concentration of samples up to 100 μg/mL. Naik et al. suggested that the EO from *Z. rhetsa* fruits could inhibit the cell viability and proliferation of breast cancer [10]. It was found the EO obtained from dried fruits collected from Nan of Thailand exhibited inhibitory effect on the growth of human lung cancer cell line (H460) with an EC_{50} value of 1.79 μL/mL. Meanwhile, the dried *Z. rhetsa* fruits collected from some districts of Thailand (Nan, Phayao, and Chiang Rai) revealed a wide range of EC_{50} values from 2.03 μg/mL to 7.07 μg/mL against human lung cancer cells (MRC-5) [9].

Table 2: Cytotoxic activity of essential oils.

Samples	IC_{50}, μg/mL					
	MCF-7	Hela	HGC-27	HepG-2	A-549	Vero
SB	>100	>100	**83.48**	>100	>100	>100
FL	**75.19**	>100	**72.69**	**89.39**	**66.27**	>100
LP	**46.21**	>100	>100	**54.67**	**56.8**	>100
FP	**72.93**	>100	>100	>100	>100	>100
FF	>100	>100	>100	>100	**74.82**	>100
DF	>100	>100	>100	**48.45**	**62.57**	>100
Ellipticine	0.42	0.36	0.51	0.34	0.35	1.84

MCF-7: human breast adenocarcinoma cells; HeLa: cervical cancer cells; HGC-27: human stomach carcinoma cell; Hep-G2: hepatocellular carcinoma; A-549: human lung adenocarcinoma epithelial cells; Vero: kidney epithetical cells.

3.2.2. Antimicrobial Activity. Six EO samples from different parts of *Z. rhetsa* collected from the Son La province in Vietnam were also tested for their antimicrobial activities (Table 3). The results demonstrated that most of the EOs showed moderate antimicrobial activity against *F. oxysporum* yet did not inhibited bacteria *B. subtilis* and *S. aureus*.

Vanden Bergher and Vlietinck also observed various degrees of inhibition of the fresh leaf EO of *Z. rhetsa* at different concentrations against the test fungal isolates. The obtained results have shown that the concentration of 12.5% exhibited the highest activity against *A. niger*, *A. fumigatus*, *A. flavus*, and *Penicillium italicum* in agar dilution tests [19]. Pham et al. suggested that terpinen-4-ol that is the main active constituent in *Z. rhetsa* pericarp EOs had the ability to inhibit stomach and intestine diseases [11]. Some other studies have also shown that essential oils obtained from plants exhibited potential antibacterial and antifungal activities [20–22].

Table 3: Antimicrobial activities of essential oils.

Samples	Minimum inhibitory concentration (MIC, μg/mL)*					
	E. coli	*P. aeruginosa*	*B. subtilis*	*S. aureus*	*A. niger*	*F. oxysporum*
SB	>200	>200	>200	>200	>200	>200
FL	>200	>200	>200	>200	>200	100
LP	>200	100	>200	>200	>200	100
FP	50	>200	>200	>200	>200	200
FF	100	200	>200	>200	>200	100
DF	>200	>200	>200	>200	>200	200

*The highest test concentration 200 μg/mL.

4. Conclusions

Six EO samples were obtained by hydrodistillation from different parts of *Z. rhetsa* (e.g., stem barks, fresh leaves, leaf and fruit petioles, fresh and dried fruits) collected in the Son La province in Vietnam. Monoterpene hydrocarbons were found to be the predominant compound of all six EO samples, of which sabinene is one of the major components (from 12.37% to 41.13%) followed by limonene (from 4.18% to 25.01%). Oxygenated monoterpenes is present in quite high content in six EO samples, in which terpinen-4-ol was found to be the main compound of this fraction (from 5.35% to 19.07%). Sesquiterpene hydrocarbons and oxygenated sesquiterpenes were present at a relatively high concentration in stem bark EO (12.61% and 6.93%, respectively) but only in a trace amount in other samples. Especially, aliphatic ketones were found only in stem bark EO (2.77%) and completely absent in the remaining five EO samples. Some compounds were present in all six EO samples but at different concentrations, such as terpinolene is present in high content in dried fruits, fresh fruits, fruit petioles, and leaf petioles (30.37%, 27.05%, 19.66%, and 6.86%, respectively) but is in low content in fresh leaves and stem barks (1.91% and 1.57%, respectively); linalool is present in relatively high content in leaf petioles and fruit petioles (12.63% and 11.64%, respectively), but it is only present in trace amounts in fresh leaves, fresh fruits, stem barks, and dried fruits (1.80%, 1.71%, 1.61%, and 0.84%, respectively). The cytotoxicity results have shown that six EOs at a concentration of 50 μg/mL exhibited inhibitory activity against at least one tested cancer cell line but were nontoxic on Vero normal cells. For the antimicrobial activity, most EOs showed moderate inhibitory effect against *F. oxysporum*, yet no effects were observed against *B. subtilis* and *S. aureus*.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This research was funded by the Vietnam Academy of Science and Technology (VAST) under grant number VAST04.06/20-21.

References

[1] R. P. Adams, *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy*, Allured Publishing Corporation, Carol Stream, IL, USA, 3rd edition, 2004.

[2] D. S. Cao and T. M. C. Nguyen, "Features of periods of mac khen *Zanthoxylum rhetsa* (Roxb. DC) in Son La," *Journal of Science-Tay Bac University: Natural Science and Technology*, vol. 2, pp. 9–105, 2015.

[3] H. N. Do, T. N. H. Tran, D. G. Vu et al., "Screening for antiproliferative and antimicrobial activity of total lipids of some marine invertebrates collected from Vietnam's north central coast," *Vietnam Journal of Chemistry*, vol. 55, no. 6E, pp. 124–130, 2017.

[4] T. L. Do, *Vietnamese Medicinal Plants and Ingredients*, Medical Publishing House, Hanoi, Vietnam, 2001.

[5] F. Hu, X.-F. Tu, K. Thakur et al., "Comparison of antifungal activity of essential oils from different plants against three fungi," *Food and Chemical Toxicology*, vol. 134, Article ID 110821, 2019.

[6] L. Jirovetz, G. Buchbauer, M. P. Shafi, and A. Saidutty, "Analysis of the aroma compounds of the essential oil of seeds of the spice plant *Zanthoxylum rhetsa* from southern India," *Zeitschrift for Lebensmitteluntersuchung und -Forschung A*, vol. 206, no. 3, pp. 228-229, 1998.

[7] W. A. König, D. Joulain, and D. H. Hochmuth, "Terpenoids library-terpenoids and related constituents of essential oils," 2018, https://massfinder.com/wiki/Terpenoids_Library.

[8] H. J. Kro, S. Das, and K. Tayung, "Fungi associated with contaminated stored grains and their biological control using *Zanthoxylum rhetsa* essential oil," *International Journal of Advances in Agricultural Science and Technology*, vol. 4, no. 11, pp. 10–26, 2017.

[9] T. Mosmann, "Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays," *Journal of Immunological Methods*, vol. 65, no. 1-2, pp. 55–63, 1983.

[10] R. R. Naik, A. K. Shakya, N. A. Khalaf, S. Abuhamdah, G. A. Oriquat, and A. Maraqa, "GC-MS analysis and biological evaluation of essential oil of *Zanthoxylum rhesta*

(Roxb.) DC pericarp," *Jordan Journal of Pharmaceutical Sciences*, vol. 8, no. 3, pp. 181–193, 2015.

[11] D. T. Pham, "Indigenous knowledge from the use of Mac Khen plant products (*Zanthoxylum rhetsa* (Roxb.) DC.) of Thai community in Son La," *Journal of Science-Tay Bac University: Natural Science and Technology*, vol. 14, pp. 90–96, 2018.

[12] R. P. Adams, *Identification of Essential Oils by Ion Trap Mass Spectroscopy*, Academic Press, San Diego, CA, USA, 1990.

[13] V. S. Rana and M. A. Blazquez, "Volatile constituents of the seed coat of *Zanthoxylum rhetsa* (Roxb.) DC," *Journal of Essential Oil Research*, vol. 22, no. 5, pp. 430–432, 2010.

[14] P. M. Shafi, B. Jose, K. T. Radhamani, and R. A. Clery, "Influence of pH on essential oil composition of *Zanthoxylum rhetsa* seeds obtained by steam distillation," *Flavour and Fragrance Journal*, vol. 21, no. 2, pp. 317-318, 2006.

[15] P. M. Shafi, A. Saidutty, and R. A. Clery, "Volatile constituents of *Zanthoxylum rhetsa* leaves and seeds," *Journal of Essential Oil Research*, vol. 12, no. 2, pp. 179–182, 2000.

[16] S. Theeramunkong and M. Utsintong, "Comparison between volatile oil from fresh and dried fruits of *Zanthoxylum rhetsa* (Roxb.) DC. and cytotoxicity activity evaluation," *Pharmacognosy Journal*, vol. 10, no. 5, pp. 827–832, 2018.

[17] H. T. Tran, M. H. Tran, and Q. H. Nguyen, "Chemical components of essential oil extracted from fruit of *Zanthoxylum rhetsa* (Roxb.) DC in Vietnam," *Journal of Pharmacology*, vol. 10, pp. 12-13, 2004.

[18] X.-F. Tu, F. Hu, K. Thakur, X.-L. Li, Y.-S. Zhang, and Z.-J. Wei, "Comparison of antibacterial effects and fumigant toxicity of essential oils extracted from different plants," *Industrial Crops and Products*, vol. 124, pp. 192–200, 2018.

[19] D. A. Vanden Bergher and A. J. Vlietinck, "Screening methods of antibacterial and antiviral agents from higher plants," in *Methods in Plant Biochemistry*, P. M. Dey and J. D. Harbone, Eds., pp. 47–69, Academic Press, London, UK, 1991.

[20] V. C. Vu, *Dictionary of Vietnamese Medicinal Plants*, pp. 622-623, Medical Publishing House, Hanoi, Vietnam, 1997.

[21] X. Wang, Y. Shen, K. Thakur et al., "Antibacterial activity and mechanism of ginger essential oil against *Escherichia coli* and *Staphylococcus aureus*," *Molecules*, vol. 25, no. 17, p. 3955, 2020.

[22] N. Wongkattiya, C. Akekawatchai, P. Sanguansermsri, I. H. Fraser, C. Pratoomsoot, and D. Sanguansermsr, "Chemical compositions and biological properties of essential oils from *Zanthoxylum rhetsa* (Roxb.) DC and *Zanthoxylum limonella* Alston," *African Journal of Traditional, Complementary and Alternative Medicines*, vol. 15, no. 2, pp. 12–18, 2018.