



CONSTITUENTS OF ESSENTIAL OILS FROM THE LEAVES AND TWIGS OF *ZANTHOXYLUM AVICENNAE* (Lamk.) DC. GROWING IN LIMESTONE FOREST, NORTHERN VIET NAM

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Abstract. *Zanthoxylum avicennae* (Lam.) DC. is a medicinal and aromatic plant, belong to the *Zanthoxylum* genus. The plant is found in some Asian countries, including Viet Nam. The various parts of this plant has been used to medicine and contain essential oils. In this study, the essential oil of *Z. avicennae* was isolated. Two essential oils from the leaves and twigs of *Zanthoxylum avicennae* (Lamk.) DC. collected from a limestone forest in Northern part of Vietnam were separately obtained by hydrodistillation and analyzed by GC-MS. A total of 42 and 53 compounds were identified in the leaf and twig oil, respectively. Mono- and sesquiterpenes were found to be the main chemical classes in both oils. Major constituents of the leaf oil were β -caryophyllene (18.71 %), α -humulene (14.03 %), α -terpinene (10.65 %), γ -terpinene (10.64 %), and linalool (9.42 %). Major constituents of the twig oil were linalool (18.3 %), β -phellandrene (8.82 %), α -terpinene (7.85 %), o-cymene (7.79 %), γ -terpinene (7.99 %), and (*E*)- β -ocimene (6.62 %). In the leaf oil the total amounts of mono- and sesquiterpenes were nearly equal (49.15 % and 44.27 %, respectively), while the twig oil was dominated by monoterpenes (76.66 % and 2.65 %, respectively). Previous literature data on essential oils obtained from this plant species were reviewed and discussed.

Keywords: *Z. avicennae*, essential oil, monoterpenes, sesquiterpenes, limestone forest

Classification numbers: 1.1.1, 1.1.3, 1.4.6.

1. INTRODUCTION

Zanthoxylum avicennae (Lamk.) DC. belonging to the *Zanthoxylum* genus are small trees or shrubs found in some Asian countries [1-6]. In Vietnam, *Z. avicennae* is distributed mostly from Northern to Central provinces [7]. In Vietnamese folk medicine, it is used to rheumatism, hepatitis (roots), scabies, wound (leaf), abdominal pain, and stomachache (fruits) [8].

A wide range of natural products, such as alkaloids, coumarins, terpenoids, flavonoids, and neolignans have been found in *Z. avicennae*, some of which were shown to have anti-inflammatory, anti-platelet aggregation, or anti-hepatitis B virus activities [4,5,6,9,10-13]. Various parts of *Z. avicennae* growing in Vietnam, China, and Indonesia were reported to contain essential oils, many of which were found to show interesting bioactivities, such as anti-inflammatory, anti-microbial, or cytotoxic properties [3,5,6,11,14-18]. A literature survey on the analysis of these oils showed that their chemical compositions varied greatly, depending not only on the tree parts used for oil extraction, but also on the time of harvest and the location where the plant materials had been collected. Recently, we were able to collect leaves and twigs of *Z. avicennae* plants growing wild in a limestone forest in Northern Vietnam. Due to the extreme conditions, along with low humidity and poor humus, the limestone flora is found distinctive and different from other ecosystems. The aim of this study was to explore the essential oils from *Z. avicennae* plants growing under these special conditions.

2. MATERIALS AND METHODS

2.1. Plant material

Leaves and twigs of *Zanthoxylum avicennae* were collected in May 2019 from Huong Son limestone forest, My Duc District, Ha Noi, Viet Nam (50 m above sea level). The species was identified by Dr. N. Q. Binh from Department of Biology, Vietnam National Museum of Nature. A voucher specimen (HS300) was deposited at the Herbarium of Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology.

2.2. Extraction of essential oils

2 kg of the fresh leaves and 1.6 kg of the fresh twigs of *Z. avicennae* were used for extraction. Hydrodistillation was carried out in a 5 L glass Clevenger-type apparatus for 3-4 hours. After distillation, the essential oils were collected, dried over anhydrous sodium sulfate, and kept in dark glass bottles at 4 °C before analysis. The oil yields were calculated on a dried weight basis.

2.3. GC-MS Analysis

GC-MS analysis of the essential oils was carried out using an Agilent Technologies HP7890A GC equipped with a mass spectrum detector (MSD) Agilent Technologies HP5975C and a HP5-MS column (60 m × 0.25 mm, film thickness 0.25 µm, Agilent Technologies). The injector and detector temperatures were set at 250 and 280 °C, respectively. The temperature program started at 60 °C, followed by an increase to 240 °C at 4 °C/min. The carrier gas was helium at a flow rate of 1 mL/min. Samples were injected by splitting. The split ratio was 100:1. The volume injected was 1 µL of essential oils. The MSD conditions were as follows: ionization voltage 70 eV, emission current 40 mA, acquisitions scan mass range 35 - 450 amu under full

scan. A homologous n-alkane series was used as the standard to calculate the retention indices (RI) of each component. Relative amounts of individual components were calculated based on the GC peak areas (MSD response) without correction. MassFinder 4.0 software connected to the HPCH1607, W09N08 libraries, and the NIST Chemistry WebBook was used to match mass spectra and retention indices.

3. RESULTS AND DISCUSSION

Essential oils from the fresh leaves and twigs of *Z. avicennae* were obtained by hydrodistillation with yields of 0.10 % and 0.06 % (v/w, calculated on a dry weight basis), respectively. Both essential oils were yellow liquids with a lower density than water.

The chemical constituents of the essential oils were identified by GC/MS, the results are summarized in Table 1.

3.1. Chemical constituents of the leaf oil

A total of forty-two components were identified in the leaf oil, accounting for 99.09 % of the composition.

- Of the total oil, 49.15 % were monoterpenes (11.12 % were oxygenated), 44.27 % were sesquiterpenes (3.73 % were oxygenated).
- The main monoterpenes were α -terpinene (10.65 %), γ -terpinene (10.64 %), and linalool (9.42 %).
- The main sesquiterpenes were β -caryophyllene (18.71 %) and α -humulene (14.03 %).
- Other important compounds were phytol (1.47 %) and myristicine (0.21 %).

Thus, this oil is characterized by the dominance of mono- and sesquiterpene compounds (93.42 % of total oil). The accumulation of monoterpenes are comparable to that of sesquiterpenes (49.15 % and 44.27 %, respectively). Another characteristic of this oil is the high percentage of oxygenated monoterpenes (11.12 %) with a considerable amount of linalool (9.42 %).

3.2. Chemical constituents of the twig oil

Fifty-three components were identified in the twig oil, comprising 96.14 % of the total oil.

- Of the total oil, 76.66 % were monoterpenes (20.87 % were oxygenated), 18.92 % were sesquiterpenes (13.86 % were oxygenated).
- The main monoterpenes were linalool (18.3 %), β -phellandrene (8.82 %), α -terpinene (7.85 %), o-cymene (7.79 %), γ -terpinene (7.99 %), (E)- β -ocimene (6.62 %), sabinene (4.00 %), and α -pinene (3.51 %).
- The main sesquiterpenes were β -caryophyllene (1.84 %) and α -humulene (2.65 %).
- Other important compounds were methyl salicylate (0.30 %).

Thus, this oil is also characterized by the dominance of mono- and sesquiterpene compounds (95.58 % of total oil). But the accumulation of monoterpenes (76.66 %) is much higher than that of sesquiterpenes (18.92 %). Another characteristic of this oil is the high percentages of both oxygenated mono- and sesquiterpenes (20.87 % and 13.86 %, respectively). The content of linalool (18.3 %) is also higher than that of the leaf oil.

In general, the chemical compositions of the leaf and twig oils from this plant are qualitatively very similar. Both oils are characterized by the dominance of mono- and sesquiterpene compounds and the presence of considerable amount of the oxygenated monoterpene linalool. They are mainly different in the ratio of total monoterpenes to total sesquiterpenes and the percentage of their oxygenated compounds. The accumulation of monoterpenes and oxygenated compounds in the twig oil is higher than that in the leaf oil.

Table 1. Chemical constituents found in the leaf and twig oils of *Z. avicennae* (Lamk.) DC.

No.	Compound	RI ^L	Content of leaf oil (%)	RI ^T	Content of twig oil (%)
1.	α -Thujene	930	1.65	930	2.08
2.	α -Pinene	939	1.38	939	3.51
3.	Sabinene	978	2.86	979	4.00
4.	β -Pinene	-	-	984	0.14
5.	Myrcene	992	1.09	992	1.65
6.	α -Phellandrene	1010	0.56	1010	0.81
7.	δ -3-Carene	1016	0.19	1016	0.20
8.	α -Terpinene	1022	10.65	1022	7.85
9.	o-Cymene	1030	2.78	1030	7.79
10.	Limonene	1034	1.22	1035	2.07
11.	β -Phellandrene	1036	3.66	1036	8.82
12.	(Z)- β -Ocimene	-	-	1038	0.52
13.	(E)- β -Ocimene	1049	2.66	1049	6.62
14.	Dihydrotagetone	-	-	1054	0.31
15.	γ -Terpinene	1064	10.64	1064	7.99
16.	Terpinolene	1094	1.47	1094	1.43
17.	Linalool	1104	9.42	1104	18.30
18.	(E)-Tagetone	-	-	1148	0.21
19.	Terpinen-4-ol	1187	1.18	1187	1.44
20.	Naphthalene	1197	0.39	1197	0.26
21.	α -Terpineol	1200	0.33	1200	0.30
22.	Methyl salicylate	-	-	1204	0.30
23.	(Z)-Ocimenone	-	-	1237	0.33
24.	(E)-Ocimenone	-	-	1246	0.16
25.	Geraniol	1257	0.19	1258	0.13
26.	α -Cubebene	-	-	1360	0.54
27.	α -Ylangene	1385	0.24	1384	0.17
28.	α -Copaene	1389	0.76	1389	0.51
29.	cis- β -Elemene	1403	0.58	1403	0.53
30.	β -Caryophyllene	1438	18.71	1437	1.84
31.	γ -Elemene	1445	0.18	1445	0.63
32.	α -Humulene	1473	14.03	1471	2.65
33.	γ -Muurolene	-	-	1490	0.13

34.	Germacrene D	1498	0.49	1498	0.92
35.	β -Selinene	1504	0.85	1505	0.37
36.	γ -Amorphene	1510	0.17	1511	0.30
37.	(<i>E,E</i>)- α -Farnesene	1512	1.72	1512	0.40
38.	Cadina-1,4-diene	1519	0.53	-	-
39.	Myristicine	1533	0.21	-	-
40.	γ -Cadinene	-	-	1533	0.16
41.	δ -Cadinene	1536	0.52	1537	1.04
42.	cis-Calamenene	1539	1.50	1539	1.62
43.	α -Calacorene	1560	0.25	1560	0.28
44.	Elemol	1565	0.22	1564	0.28
45.	(<i>E</i>)-Nerolidol	-	-	1570	0.31
46.	Germacrene B	1577	0.26	1577	1.77
47.	Spathulenol	-	-	1598	0.27
48.	Caryophyllene oxide	1605	0.57	1605	0.31
49.	Cedrol	1627	0.65	1627	0.67
50.	Humulene epoxide II	1632	0.31	1632	0.20
51.	1-epi-Cubenol	-	-	1647	0.28
52.	γ -Eudesmol	-	-	1653	0.30
53.	epi- α -Muurolol	-	-	1661	0.41
54.	α -Cadinol	1674	0.62	1674	0.82
55.	neo-Intermedeol	1677	1.11	1677	1.21
56.	n-Eicosane	2016	0.82	-	-
57.	Phytol	2118	1.47	-	-
	Total		99.05		96.14
	<i>Monoterpenes</i>		49.15		76.66
	<i>Oxygenated monoterpenes</i>		11.12		20.87
	<i>Sesquiterpenes</i>		44.27		18.92
	<i>Oxygenated sesquiterpenes</i>		3.73		13.86
	<i>Other constituents</i>		5.63		0.56

RI: Retention indices; ^L: leaf oil; ^T: twig oil.

3.3. Discussion

In 2012, Dai *et al.* published a study on an essential oil obtained with 0.11 % (w/w, calculated on a dry weight basis) yield from fresh leaves of *Z. avicennae* collected in December 2006 in Nghe An Province of Viet Nam [16]. GC-MS analysis revealed that:

- More than 80 compounds were detected, 53 components (87 % of the total oil) could be identified.
- Of the total oil, 44.30 % were monoterpenes (1.97 % were oxygenated), 40.75 % were sesquiterpene compounds (1.32 % were oxygenated).

- The main monoterpenes were α -pinene (10.07 %), β -phellandrene (9.42 %), γ -terpinene (4.53 %), (E)- β -ocimene (3.87 %) and α -terpinene (3.09 %).
- The main sesquiterpenes were β -caryophyllene (17.01 %) and α -humulene (10.38 %).
- Other important compounds were the phenylpropanoids: estragol (0.57 %), safrole (0.47 %), and asarone (0.3 %). Aliphatic compounds, such as 2-nonanone, nonanal, decanal, and benzyl hexanoate, together with hexahydrofarnesyl acetone were minor components (less than 0.3 %).

Thus, this oil is characterized by the dominance of mono- and sesquiterpene compounds (85.05 % of total oil), most of which are hydrocarbons. The accumulation of monoterpenes are comparable to that of sesquiterpenes (44.30 % and 40.75 %, respectively).

In 2016, Trung *et al.* published a study on essential oils obtained from mature leaves, flowers, and fruits of *Z. avicennae* collected in September 2011 from Pu Mat National Park, Nghe An Province of Viet Nam [18]. The essential oil yields from the leaves, flowers, and fruits were 0.20 %, 0.15 %, and 0.20 % (v/w, calculated on a dry weight basis), respectively.

The GC-MS results for the leaf oil were summarized as follows:

- Total monoterpenes: 10.40 % (1.2 % oxygenated); Total sesquiterpenes: 71.09 % (0.9 % oxygenated).
- Main monoterpenes: limonene (3.4 %), α -pinene (2.9 %), β -ocimene (2.5 %).
- Main sesquiterpenes: (E, E)- β -farnesene (19.6 %), β -elemene (17.3 %), β -caryophyllene (15.3 %) and α -humulene (12.3 %).
- Others: hexahydrofarnesyl acetone (11.4 %).

Thus, this oil is characterized by the dominance of mono- and sesquiterpene compounds (81.49 % of total oil), most of which are hydrocarbons. The accumulation of sesquiterpenes is much more than that of monoterpenes (71.09 % and 10.40 %, respectively). Another characteristic of this oil is the high percentage of hexahydrofarnesyl acetone (11.4 %).

The GC-MS results for the flower oil were summarized as follows:

- Total monoterpenes: 12.0 % (0.5 % oxygenated); Total sesquiterpenes: 77.4 % (2.7 % oxygenated).
- Main monoterpenes: limonene (7.6 %), α -pinene (1.6 %), β -terpinene (1.5 %).
- Main sesquiterpenes: β -elemene (23.7 %), β -caryophyllene (22.8 %), β -selinene (18.2 %).
- Others: phytol (9.4 %).

This oil is characterized by the dominance of mono- and sesquiterpene compounds (89.4 % of total oil), most of which are hydrocarbons (3.2 % oxygenated). The accumulation of sesquiterpenes is much more than that of monoterpenes (77.4 % and 12.0 %, respectively). Another characteristic of this oil is the high percentage of phytol (9.4 %).

The GC-MS results for the fruit oil were summarized as follows:

- Total monoterpenes: 89.3 % (10.1 % oxygenated); Total sesquiterpenes: 0.2 %.
- Main monoterpenes: limonene (41.2 %), sabinene (18.0 %), terpinen-4-ol (6.6 %), α -phellandrene (4.7 %), γ -terpinene (3.9 %), (E)- β -ocimene (3.6 %), α -terpinene (2.6 %).
- Others: n-decanal (2.5 %).

Thus, this oil is characterized by the dominance of monoterpene compounds (89.3 %). Another characteristic of this oil is the presence of notable percentage of n-decanal (2.5 %).

Comparison of the results of the leaf oil obtained in this study with those of the above mentioned studies (Table 2) suggested that all of these leaf oils are characteristic in containing mainly mono- and sesquiterpene compounds. The oil obtained from the sample collected in September 2011 in Nghe An Province (Trung 2016) is different in the high percentages of total sesquiterpenes (71.09 %) and hexahydrofarnesyl acetone (11.4 %). This difference could be explained by the fact that this sample was collected when the plant carried flowers and fruits. The high percentages of oxygenated monoterpene compounds (11.12 %) and linalool (9.42 %) in the sample of this study, which was taken from the rocky mountains in Huong Son (Ha Noi) in May 2019, could be explained by the special geographical conditions where it came from.

Table 2. Chemical composition of leaf oils from Vietnamese samples of different origins.

Sample	Monoterpenes	Sesquiterpenes	Others
This study	- Total: 49.15 % (11.12 % oxyg.) - Main constituents: α -terpinene (10.65 %), γ -terpinene (10.64 %), linalool (9.42 %)	- Total: 44.27 % (3.73 % oxyg.) - Main constituents: β -caryophyllene (18.71 %), α -humulene (14.03 %)	Main constituents: phytol (1.47 %), myristicine (0.21 %)
Dai 2012 [16]	- Total: 44.30 % (1.97 % oxyg.) - Main constituents: α -pinene (10.07 %), β -phellandrene (9.42 %), γ -terpinene (4.53 %), (E)- β -ocimene (3.87 %), α -terpinene (3.09 %)	- Total: 40.75 % (1.32 % oxyg.) - Main constituents: β -caryophyllene (17.01 %), α -humulene (10.38 %)	Main constituents: estragol 0.57 %, safrole 0.47 %, asarone 0.3 %
Trung 2016 [18]	- Total: 10.40 % (1.2 % oxyg.) - Main constituents: limonene (3.4 %), α -pinene (2.9 %), β -ocimene (2.5 %)	- Total: 71.09 % (0.9 % oxyg.) - Main constituents: (E, E)- β -farnesene (19.6 %), β -elemene (17.3 %), β -caryophyllene (15.3 %), α -humulene (12.3 %)	Main constituents: hexahydrofarnesyl acetone (11.4 %)

In 2014, Liu *et al.* published a study on an essential oil obtained with 0.07 % (v/w) yield from dried aerial parts (leaves and stems) of *Z. avicennae* harvested in October 2012 from Qujing City, Yunnan Province, China [6]. The essential oil was found to exhibit larvicidal activity against the Asian tiger mosquito (*Aedes albopictus* Skuse) with a median lethal concentration (LC50) value of 48.79 μ g/mL. A total of 31 compounds (99.67 % of total oil) were identified by GC-MS analysis:

- Of the total oil, 65.70 % were monoterpenes (63.61 % oxygenated) and 33.45 % were sesquiterpenes (11.21 % oxygenated).
- The main monoterpenes were 1,8-cineol (53.05 %), linalool (3.28 %), α -terpineol (3.20 %), camphor (2.08 %), and α -pinene (1.56 %).
- The main sesquiterpenes were β -elemene (6.13 %), β -caryophyllene (5.09 %), α -humulene (5.96 %), caryophyllene oxide (4.59 %), and humulene oxide II (3.42 %).
- Other compounds were phytol (0.28 %) and isobutyl 2-methylbutyrate (0.24 %).

Thus, this oil is similar to the corresponding Vietnamese oils in consisting mainly of mono- and sesquiterpenes. It is further characterized by its large content of 1,8-cineol (53.05 %) and total oxygenated mono- and sesquiterpenes (74.82 %).

In 2012, Zhang *et al.* published a study on an essential oil obtained from leaves of *Z. avicennae* harvested from Hainan Island, China [14]. The article was written in Chinese, only abstract in English was available to us. According to the abstract, this oil exhibited strong antitumor activity against K-562 human tumor cell lines with IC₅₀ of 1.76 μ g/mL. It also

exhibited moderate antimicrobial activity against three bacteria. By GC-MS, 72 components were identified, accounting for 98.15 % of the total peak areas. According to Liu *et al.* (2014), the main constituents of this oil were linalool (24.36 %), β -elemene (12.03 %), (E)-2-hexen-1-ol (11.73 %), and caryophyllene oxide (10.84 %) [6].

In 2014, Lin *et al.* published a study on an essential oil obtained with 2.10 % (v/w) yield from dried leaves of *Z. avicennae* collected in Sanya city, Hainan Island of China in August 2011 [3]. The oil was obtained by steam distillation followed by extraction with ethyl ether. This oil was shown to possess strong antibacterial activity against the fungi *Rhizoctonia solani* AG1-1A and strong cytotoxicity in the prawn larvae lethality bioassay method. GC-MS analysis showed that the main components of this oil were 2-methoxy-3-(2-propenyl)-phenol (42.94 %), caryophyllene (23.33 %), 1,1,4,8-tetramethyl-cis,cis,cis-4,7,10-cycloundecatriene (8.98 %), 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-[1S-(1 α ,2 α ,4 α)]-cyclohexane (6.20 %), 3,7-dimethyl-1,6-octadien-3-ol (linalool, 1.73 %), caryophyllene oxide (1.58 %), 1,2-dimethoxy-4-(2-propenyl)-benzene (methyl eugenol, 1.40 %), copaene (1.04 %), and α -farnesene (1.02 %). Thus, this oil is qualitatively different from other corresponding oils in containing the rare phenylpropanoid compound 2-methoxy-3-(2-propenyl)-phenol (42.94 %) as the main component. The compounds 1,1,4,8-tetramethyl-cis,cis,cis-4,7,10-cycloundecatriene (8.98 %) and 1-ethenyl-1-methyl-2,4-bis(1-methylethenyl)-[1S-(1 α ,2 α ,4 α)]-cyclohexane (6.20 %) were also rarely found in nature.

Prior to the above studies, in 1990 Cheng *et al.* published a study on an essential oil obtained by steam distillation of fruits from *Z. avicennae* collected in Guangdong Province of China. Separation of this essential oil yielded 27 compounds, of which 22 were identified by GC-MS and NMR data. In tests of 15 compounds using 8 moulds (*Aspergillus niger*, *A. sydowi*, *A. terreus*, *Penicillium chrysogenum*, *Paecilomyces varioti*, *Chaetomium globosum*, *Cladosporium herbarum* and *Trichoderma sp.*), citral, 1-octanol, 4-methyl-6-acetoxyhexanal and linalool showed strong anti-mildew activities. GC-MS analysis showed:

- Of the total oil, 80.04 % were monoterpenes (2.34 % oxygenated), 4.69 % were sesquiterpenes (0 % oxygenated), and 12.22 % were aliphatic oxygenated compounds.
- The main monoterpenes were sylvestrene (50 %), α -pinene (16 %), ocimene (3.2 %), and α -thujene (3.2 %).
- The main sesquiterpenes were β -elemene (6.13 %), β -caryophyllene (5.09 %), α -humulene (5.96 %), caryophyllene oxide (4.59 %), and humulene oxide II (3.42 %).
- The main aliphatic oxygenated compound was octanal (8.7%).

Thus, this fruit oil is similar to the corresponding Vietnamese oil in consisting mainly of monoterpenes and a notable amount of aliphatic aldehyde.

In 2018, Andila *et al.* published a study on essential oils obtained from fresh fruits and leaves of *Z. avicennae* harvested in March 2016 from the Bali Botanical Garden, Indonesia [15]. This plant was collected from natural forest, Batulanteh Mountain, Sumbawa Regency, West Nusa Tenggara, Indonesia and planted at Bali Botanical Garden on December 15, 1993. Hydrodistillation of the leaves and fruits produced essential oils with the yields of 1 % (v/w) and 2.7 % (v/w, according to fresh material), respectively. The major components found in the leaf oil were estragol (87.13 %), 1,8-cineol (4.28 %), trans-anethole (3.51 %), myrtenyl acetate (0.65 %), isopiperitenone (0.62 %), and eugenol (0.45 %). The main compounds of the fruit oil were 1,8-cineol (12.34 %), 1-p-menthen-8-yl acetate (6.24 %), 1-limonene (6.09 %), 3-methyl-2-(2-methyl-2-butenyl)-furan (rosefuran, 3.74 %). The dominance of phenylpropanoid compounds in the leaf oil, such as estragol (87.13 %), trans-anethole (3.51 %), and eugenol (0.45

%), suggested that this plant from Indonesia is more similar to the plant from Hainan Island of China, which was studied by Lin *et al.* [3].

Evaluating all the data above, especially the data for the leaf oils, we suggest that:

- The *Z. avicennae* species, unlike other economic essential oil plants, affords essential oils with very diverse chemical compositions. The chemical profiles of its essential oils can vary due to a wide range of factors, such as geographical location, environmental condition, harvest season, and techniques of extraction.
- The *Z. avicennae* plants which have been studied for their essential oils so far could be divided into two groups. The first group gives leaf oils with low yield (around 0.2 % v/w, calculated on dry weight basis) and evident dominance of mono- and sesquiterpen compounds in their chemical composition. The second group gives leaf oils with high yield (more than 1 % v/w, calculated on dry weight basis) and evident dominance of phenylpropanoid compounds class in their chemical composition.
- All the Vietnamese *Z. avicennae* plants which have been discussed above showed similar essential oil profiles and could be assigned to the first group. The difference in their essential oil profiles might rather be caused by environmental impacts or growth status, than by major genetic variations.
- The *Z. avicennae* plants which were the subjects of study by Lin (2014) [3] and Andila (2018) [15] could be assigned to the second group.

4. CONCLUSION

Leaf and twig oils of *Z. avicennae* collected from the Huong Son limestone forest in Northern Vietnam were obtained in low yield (0.10 % and 0.06 % v/w, respectively). The chemical compositions of the leaf and twig oils from this plant are qualitatively very similar. Both oils are characterized by the dominance of mono- and sesquiterpene compounds and the presence of a considerable amount of the oxygenated monoterpene linalool. They are mainly different in the ratio of total monoterpenes to total sesquiterpenes and the percentage of their oxygenated compounds. The accumulation of monoterpenes and oxygenated compounds in the twig oil is higher than that in the leaf oil. Comparison of the results for the leaf oil obtained in this study with those of the leaf oils obtained from *Z. avicennae* growing in Nghe An Province of Vietnam showed that the special geographical conditions of the limestone forest did not change the characteristic profile of the oil. Notable difference was only observed in the accumulation of a significant amount of the oxygenated monoterpene compound linalool.

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Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

REFERENCES

1. Wu W. Z., Zhu Z. H. - Chemical constituents of Lie - Dang (*Zanthoxylum avicennae*), Chin Tradit Herb Drugs. **23** (1992) 115-116
2. Thuy T. T., Porzel A., Ripperger H., Sung T. V., Adam G. - Bishordeninyl terpene alkaloids from *Zanthoxylum avicennae*, Phytochemistry **50** (1999) 903-907
3. Lin Y., Han W., Ge W. C., Yuan K. - Chemical composition of the volatile oil from *Zanthoxylum avicennae* and antimicrobial activities and cytotoxicity. Pharmacogn. Mag. **10** (1) (2014) 164-170. <https://doi.org/10.4103/0973-1296.127369>.
4. Fish F., Gray A. I., Waterman P. G. - Coumarin, alkaloid and flavonoid constituents from the root and stem barks of *Zanthoxylum avicennae*, Phytochemistry **14** (1975) 841-842
5. Cho J. Y., T. L. Hwang T. H., Chang Y. P., Lim P. J., Sung T. H., Lee J. J. Chen - New coumarins and anti-inflammatory constituents from *Zanthoxylum avicennae*, Food Chemistry **135** (1) (2012) 17-23. <https://doi.org/10.1016/j.foodchem.2012.04.025>.
6. Liu X. C., Liu Q. Y., Zhou L., Liu Q. R., Liu Z. L. - Chemical composition of *Zanthoxylum avicennae* essential oil and its larvicidal activity on *Aedes albopictus* Skuse, Trop. J. Pharm. Res. **13** (3) (2014) 399-404. <http://dx.doi.org/10.4314/tjpr.v13i3.13>.
7. Nguyen Tien Ban - Checklist of plant species of Vietnam, Agricultural publisher, Ha Noi, 2003 (in Vietnamese).
8. Chi V. V. - Vietnamese Medical Plant Dictionary, Medical Publishing House, 2012, (in Vietnamese).
9. Chen J. J., Wang T. Y., Hwang T. L. - Neolignans, a coumarinolignan, lignan derivatives, and a chromene: anti-inflammatory constituents from *Zanthoxylum avicennae*, J. Nat. Prod. **71** (2008) 212-217. <https://doi.org/10.1021/np07059k>.
10. Chen J., Yang C., Kuo Y., Hwang T., Kuo W. L., Lim Y., and Cheng M. J. - New coumarin derivatives and other constituents from the stem bark of *Zanthoxylum avicennae*: effects on neutrophil pro-inflammatory responses, International Journal Molecules Sciences **16** (5) (2015) 9719-9731. <https://doi.org/10.3390/ijms16059719>.
11. Wu H. C., Lay I. S., Shibu M. A., Ho T. J., Cheng S. M., Lin C. H., Dung T. D., Jeng L. B., Viswanatha V. P., Huang C. Y. - *Zanthoxylum avicennae* extract enhances GSK-3 β to attenuate β -catenin via phosphatase 2A to block metastatic effects of HA22T cells and hepatocellular carcinoma xenografted nude mice, Environ Toxicol **32** (9) (2017) 133-2143. <https://doi.org/10.1002/tox.22426>.
12. Chen I. S., Lin Y. C., Tsai I. L., Teng C. M., Ko F. N., Ishikawa T., Ishii H. - Coumarins and anti-platelet aggregation constituents from *Zanthoxylum chinifolium*, Phytochemistry **39** (1995) 1091-1097.
13. Chang C. T., Doong S. L., Tsai I. L., Chen I. S. - Coumarins and anti-HBV constituents from *Zanthoxylum schinifolium*, Phytochemistry **45** (1997)1419-1422.
14. Zhang D. S., Zhong Q. X., Song X. M., Liu W. J., Wang J., Zhang Q. Y. - Study on chemical components, antimicrobial and antitumor activities of essential oil from leaves of *Zanthoxylum avicennae*, J. Chin. Med. Mater. **35** (8) (2012) 1263-1267.
15. Putri Sri Andila, I Putu Agus Hendra Wibawa, I Gede Turta, Deden Fardenan - First report from Indonesia, phytochemical composition of essential oils from leaves and fruits

- of *Zanthoxylum avicennae* (Lam.) DC, Journal of Biological Researches **23** (2) (2018) 101-106. <https://doi.org/10.23869/bphjbr.23.2.20189>
16. Dai D. N., Luong N. X., Thang T. D., Jirovetz L., Hoferl M., Schmidt E. - Chemical composition of the essential oil of *Zanthoxylum avicennae* (Lam.) DC leaves (Rutaceae) from Viet Nam, J. Essent. Oil-Bearing Plants **15** (1) (2012) 7-11. DOI: 10.1080/0972060X.2012.10644012.
 17. Dung T. D., Feng C. C., Kuo W. W., Pai P., Chung L. C., Chang S. H., Hsu H. H., Tsai F. J., Lin Y. M., Huang C. Y. - Suppression of plasminogen activators and the MMP-2/-9 pathway by a *Zanthoxylum avicennae* extract to inhibit the ha22t human hepatocellular carcinoma cell migration and invasion effects in vitro and in vivo via phosphatase 2a activation, Biosci Biotechnol Biochem **77** (9) (2013) 1814-1821.
<https://doi.org/10.1271/bbb.130060>.
 18. Hoang D. Trung, Tran D. Thang, Khoi N. K., Do N. Dai, and Isiaka A. Ogunwande - Chemical Constituents of Essential Oils from the Leaf, Flower and Fruit of *Zanthoxylum avicenna* (Lam.) DC. (Rutaceae) from Viet Nam, J. Essent Oil-Bearing Plants **19** (2016) 1019-1024. <https://doi.org/10.1080/0972060X.2016.1201433>