Vietnam Journal of Science and Technology ***60*** *(1) (2022) 43-48*

doi:10.15625/2525-2518/15867

ESSENTIAL OIL FROM THE AERIAL PART OF *ORCHIDANTHA VIETNAMICA* K. LARSEN AND ITS ANTIMICROBIAL ACTIVITY

Ninh The Son[1, *], Nguyen Duc Danh[2], Le Tuan Anh[3], Dinh Thi Thu Thuy[4], Nguyen Dinh Luyen[4], Tran Thi Tuyen[4, 5]

[1]*Institute of Chemistry, Vietnam Academy Science and Technology (VAST),18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam*

[2]*Institute of Applied Technology, Thu Dau Mot University, 06 Tran Van On, Phu Hoa, Binh Duong, Viet Nam*

[3]*Mien Trung Institute for Scientific Research, VAST, 321 Huynh Thuc Khang, Thua Thien Hue, Viet Nam*

[4]*Institute of Natural Products Chemistry, VAST, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam*

[5]*Graduate University of Science and Technology, VAST, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam*

[*]Email: *yamantson@gmail.com*

Received: 1 February 2021; Accepted for publication: 26 August 2021

Abstract. The hydro-distilled essential oil from the fresh aerial part of *Orchidantha vietnamica* K. Larsen collected from Lamdong-Vietnam was analyzed by GC-FID (Gas Chromatography-Flame Ionization Detection) and GC-MS (Gas Chromatography-Mass Spectroscopy) for the first time. The oil was characterized by the presence of volatile compounds type terpenoids, in which oxygenated monoterpenes (30.04 %) and sesquiterpene hydrocarbons (37.32 %) are the major components. The oxygenated monoterpene linalool reached the highest percentage of 16.66 %, followed by the sesquiterpene hydrocarbons (*E*)-caryophyllene (11.4 %), α-humulene (8.00 %), and bicyclogermacrene (6.93 %). Monoterpene hydrocarbons, oxygenated sesquiterpenoids, oxygenated diterpenes, carotenoids, and others are also detected in this oil (< 3.00 %). The essential oil of *O. vietnamica* aerial part has been subjected to antimicrobial assay against eight microorganisms, including two Gram (+) bacteria *Bacillus subtilis* ATCC 27212 and *Staphylococcus aureus* ATCC 12222, two Gram (-) bacteria *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 25923, two filamentous fungi *Aspergillus niger* ATCC 9763 and *Fusarium oxysporum* ATCC 48112, and two yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC9763. Thereby, it showed antimicrobial activity against two pathogenic bacterial strains *E. coli* ATCC 8739 and *C. albicans* ATCC 10231 with the same MIC (Minimum Inhibitory Concentration) value of 200 µg/mL.

Keywords: Orchidantha vietnamica, aerial part, essential oil, antimicrobial.

Classification numbers: 1.1.3, 1.2.1, 1.4.6

1. INTRODUCTION

Orchidantha is the only genus in the Lowiaceae family. This genus is mainly distributed in South-East Asia and South China with twenty species [1 - 4]. In Viet Nam, *Orchidantha* has four species, including *O. vietnamica* and three newly recorded species *O. stercorea, O. virosa*, and *O. anthracina* [4]. Phytochemical investigations on *Orchidantha* plants are not available. To date, there has been only one publication of GC-MS identification using *Orchidantha* materials, in which three essential oil compositions dimethyl disulfide, *p*-cresol, and indole were responsible for the fetid scent of *O. fimbriata* flower and separate organ [5]. In another result, (2*S*,5*R*)-2-ethyl-5-methylhexanedioic acid, thymine, and cerevisterol were isolated from endophytic fungus *Penicillium* sp. OC-4 parasitizing in the leaf of *O. chinensis* [6]. In traditional uses, *O. chinensis* whole plant was applied to treat fever and cough [6]. Leaf of *O. fimbriatum* was used to relieve chest pain and back pain, sometimes used for wrapping food while cooking [3].

O. vietnamica, locally named Hung lan Viet, is a perennial herb growing as bushes, up to 40 cm tall [1,2,7]. It was found in Lamdong, Viet Nam [2]. In the current study, we briefly report on the chemical composition of the essential oil obtained from the fresh aerial part of this species. The oil was further subjected to antimicrobial assay.

2. MATERIALS AND METHODS

2.1. Materials

The fresh aerial part of *Orchidantha vietnamica* K. Larsen species was collected from Myduc, Dateh, Lamdong, Viet Nam in December 2020, at around 11°35'06.4"N and 107°31'24.6"E. The plant material was identified by associate professor Vu Tien Chinh, Vietnam National Museum of Nature, VAST. The voucher specimen OV-1188 was deposited at Institute of Natural Products Chemistry, VAST.

Eight microorganisms, including two Gram (+) bacteria *Bacillus subtilis* ATCC 27212 and *Staphylococcus aureus* ATCC 12222, two Gram (-) bacteria *Escherichia coli* ATCC 8739 and *Pseudomonas aeruginosa* ATCC 25923, two filamentous fungi *Aspergillus niger* ATCC 9763 and *Fusarium oxysporum* ATCC 48112, and two yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* ATCC9763, were used for anti-microbial assay. They were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA).

2.2. Extraction of essential oils

The fresh aerial part (1.5 kg) was cut into pieces, and was intermediately distilled in a Clevenger-type-apparatus for 4 h to produce a yellow colored essential oil (0.01 % yield, w/w). The obtained essential oil was dried over anhydrous Na_2SO_4 before analysis.

2.3. GC-MS procedure

Essential oil of *O. vietnamica* aerial part was analyzed by GC-MS (Gas chromatography-Mass spectrometry) and GC-FID (Gas Chromatography-Flame Ionization Detection). GC-MS analysis was carried out by a GC Agilent Technologies 7890A coupled with a mass spectrum detector (MSD) Agilent Technologies 5975C and the HP-5 MS column. The column dimensions

were 60 m × 0.25 mm, film thickness of 0.25 µm. The injector was established at 250 ^{o}C. The temperature program was 60 ^{o}C ramp of 4 ^{o}C/min up to 240 ^{o}C. Helium was used as the carrier gas at a flow rate of 1 mL/min. The split ratio was 100:1 and 1 mL of essential oil was injected. The GC/MSD data acquisition was based on full scan modes under an electron impact ionization voltage of 70 eV and an emission current of 40 mA in the acquisitions scan mass range of 35 - 450 amu. The GC-FID analytical procedure was performed under the same condition as the GC-MS method. The identification of the essential oil constituents was performed by comparing their RI and MS data with those from HPCH1607, W09N08 libraries, NIST standard database, and Adam book [8, 9]. The relative percentage amount of each volatile individual was calculated based on the GC-FID peak area without any correction.

2.4. Anti-microbial assay

The assay for anti-microbial activity has been described carefully in previous reports [10]. Briefly, the positive and negative Gram bacteria were cultured in tryptic soy environment (Merck, Germany), whereas fungi were grown in Sabouraud-2 % dextrose environment (SDB) (Merck, Germany) to a final inoculum size of about 150 × 10^6 colony-forming units (CFU) per mL. The sample (12.5 - 200 µg/mL) was loaded into 96-well microplate containing fresh culture and maintained at 37 ^{o}C for 24 h. The MIC (Minimum inhibitory concentration) value is the lowest concentration, which inhibited the visible growth of a bacterium or bacteria. Streptomycin and tetracyclin were used as positive controls for respective Gram (+) and Gram (-) bacteria, whereas nystatin was used for fungi and yeasts. Negative control was DMSO (5 %). Each experiment was run in triplicate.

Table 1. Essential oil of *O. vietnamica* aerial part.

No	[a]Rt	[b]RI_E	[c]RI_L	Constituents	Content [%]	Identification
1	10.00	938	939	α-Pinene	0.29	RI, MS
2	11.35	983	979	β-Pinene	0.39	RI, MS
3	12.81	1028	1026	*O*-cymene	0.19	RI, MS
4	13.10	1037	1037	(*Z*)-β-Ocimene	0.56	RI, MS
5	13.47	1048	1050	(*E*)-β-Ocimene	0.32	RI, MS
6	13.96	1062	1060	γ-Terpinene	0.27	RI, MS
7	15.02	1093	1091	*p*-Cymene	0.77	RI, MS
8	15.30	1101	1097	Linalool	**16.66**	RI, MS
9	15.40	1104	1101	Nonanal	0.88	RI, MS
10	17.82	1173	1169	Borneol	0.19	RI, MS
11	18.37	1189	1183	*p*-Cymen-8-ol	0.46	RI, MS
12	18.62	1196	1189	α-Terpineol	2.16	RI, MS
13	19.78	1230	1230	Nerol	2.41	RI, MS
14	20.26	1244	1238	Neral	1.43	RI, MS
15	20.63	1255	1253	Geraniol	3.73	RI, MS
16	21.24	1273	1267	Geranial	1.52	RI, MS
17	22.14	1299	1306	Dihydroedulan	0.56	RI, MS

18	23.71	1346	1338	*δ*-Elemene	0.51	RI, MS
19	24.93	1383	1381	Geranyl acetate	0.33	RI, MS
20	25.07	1388	1377	α-Copaene	1.31	RI, MS
21	25.21	1392	1385	(*E*)-β-Damascenone	1.05	RI, MS
22	25.52	1402	1391	*Cis*-β-Elemene	2.68	RI, MS
23	26.59	1435	1419	(*E*)-Caryophyllene	**11.14**	RI, MS
24	26.86	1444	1435	*Trans*-α-bergamotene	0.64	RI, MS
25	27.19	1455	1455	Geranylacetone	1.15	RI, MS
26	27.29	1458	1446	Selina-5,11-diene	0.58	RI, MS
27	27.66	1470	1455	α-Humulene	**8.00**	RI, MS
28	28.24	1488	1480	**γ**-Muurolene	0.70	RI, MS
29	28.43	1494	1489	(*E*)-β-Ionone	0.85	RI, MS
30	28.48	1496	1485	Germacrene D	2.56	RI, MS
31	28.67	1502	1494	α-Zingiberene	0.28	RI, MS
32	28.91	1510	1497	Viridiflorene	0.43	RI, MS
33	28.97	1512	1500	Bicyclogermacrene	**6.93**	RI, MS
34	29.43	1527	1514	**γ**-Cadinene	0.43	RI, MS
35	29.55	1532	1523	β-Sesquiphellandrene	0.36	RI, MS
36	29.64	1535	1523	*δ*-Cadinene	0.77	RI, MS
37	30.62	1567	1563	(*E*)-Nerolidol	0.28	RI, MS
38	31.04	1582	1572	Dendrolasin	0.77	RI, MS
39	31.41	1594	1578	Spathulenol	0.45	RI, MS
40	33.15	1655	1640	*Epi*-α-cadinol	0.39	RI, MS
41	33.19	1657	1642	*Epi*-α-Muurolol	0.30	RI, MS
42	33.55	1670	1654	α-Cadinol	0.94	RI, MS
43	34.82	1715	1713	Pentadecanal	1.37	RI, MS
44	36.50	1777	1760	Benzyl benzoate	0.33	RI, MS
45	38.27	1846	1846	6,10,14-Trimethylpentadecan-2-one	0.32	RI, MS
46	44.77	2115	1943	Phytol	2.92	RI, MS
				Total	81.24	
				Monoterpene hydrocarbons	2.79	
				Oxygenated monoterpenes	30.04	
				Sesquiterpene hydrocarbons	37.32	
				Oxygenated sesquiterpenoids	3.13	
				Oxygenated diterpenes	2.92	
				Carotenoids	2.46	
				Others	2.58	

[a]Retention time, [b]Retention indices relative to *n*-alkanes (C_7-C_{30}) on HP-5 MS column, [c]Retention indices from NIST standard database

3. RESULTS AND DISCUSSION

The chemical composition of essential oil extracted from *O. vietnamica* fresh aerial part was provided in Table 1. A total of forty-six constituents were identified, which accounted for 81.24 % of the total oil. The essential oil was almost characterized by the presence of sesquiterpene hydrocarbons (37.32 %) and oxygenated monoterpenes (30.04 %). This oil also contained oxygentated sesquiterpenes (3.13 %), oxygenated diterpenes (2.92 %), monoterpene hydrocarbons (2.79 %), carotenoids (2.46 %), and other classes (2.58 %). In detail analysis, fifteen compounds were assigned to the group of sesquiterpene hydrocarbons, of which six compounds reached more than 1.00 %, comprising of (*E*)-caryophyllene (11.14 %), α-humulene (8.00 %), *cis*-β-elemene (2.68 %), bicyclogermacrene (6.93 %), germacrene D (2.56 %), and α-copaene (1.31 %). Among the ten oxygenated monoterpenes, linalool (16.66 %) was the main compound in *O. vietnamica* oil. In addition, the group of oxygenated monoterpenes was also characterized by geraniol (3.73 %), nerol (2.41 %), α-terpineol (2.16 %), geranial (1.52 %), neral (1.43 %), and geranylacetone (1.15 %).

The essential oil of *O. vietnamica* aerial part was also found to contain minor compounds. Seven compounds, including α-pinene, β-pinene, *o*-cymene, (*Z*)-β-ocimene, (*E*)-β-ocimene, γ-terpinene, and *p*-cymene, ranging in content from 0.19 to 0.77 %, are assigned to the group of monoterpene hydrocarbons, while six consecutive compounds (*E*)-nerolidol, dendrolasin, spathulenol, *epi*-α-cadinol, *epi*-α-muurolol, and α-cadinol, and 6,10,14-trimethylpentadecan-2-one belong to the group of oxygenated sesquiterpenes. Phytol was the only oxygenated diterpene, reaching 2.92 %. Taking non-terpenoid compounds into account, carotenoid derivatives (*E*)-β-damascenone (1.05 %), dihydroedulan (0.56 %), and (*E*)-β-ionone (0.85 %) were detected, while other types such as an aliphatic compound (pentadecanal at 1.37 %) and an ester (benzyl benzoate at 0.33 %) were also observed.

Table 2. Anti-microbial activity of tested sample.

Microbial strains		**Minimum Inhibitory concentration (MIC: μg/mL)**			
		Essential oil	**Streptomycin**	**Tetracyclin**	**Nystatin**
Gram (+)	*B. subtilis*	(-)	7.20		
	S. aureus	(-)	14.38		
Gram (-)	*E. coli*	200		5.5	
	P. aeruginosa	(-)		11.0	
Fungi	*A. niger*	(-)			23.13
	F. oxysporum	(-)			11.57
Yeasts	*C. albicans*	200			11.56
	S. cerevisiae	(-)			5.78

The essential oil of *O. vietnamica* aerial part was subjected to anti-microbial assay (Table 2). This oil inhibited the growth of Gram (-) bacterium *E. coli* and yeast *C. albicans* with the same MIC value of 200 μg/mL, but it failed to control the remaining bacterial strains.

4. CONCLUSIONS

The yield of essential oil from fresh aerial part of *Orchidantha vietnamica* K. Larsen was 0.01 % (w/w). By using GC-FID and GC/MS analysis, forty-six constituents were identified, accounting for 81.24 % of the total oil. Oxygenated monoterpenes and sesquiterpene hydrocarbons were the main chemical classes with the highest amounts at 30.04 and 37.32 %, respectively. The main constituents of essential oil were linalool (16.66 %), (*E*)-caryophyllene (11.44 %), α-humulene (8.00 %), and bicyclogermacrene (6.93 %). The essential oil showed anti-microbial activity against Gram (-) bacterium *E. coli* and yeast *C. albicans*. This is the first report on the chemical composition of the essential oil from *O. vietnamica* fresh aerial part.

Acknowledgements. This work was funded by Vietnam Academy of Science and Technology under international cooperation project, code number QTRU 02.04/20-21.

CRediT authorship contribution statement. NTS: Methodology, formal analysis, supervision, writing-original draft, writing-review and editing, NDD and LTA: Investigation, DTTT, NDL and TTT: Methodology and formal analysis.

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. Ho P. H. - An illustrated flora of Viet Nam, Youth Publisher, Ho Chi Minh City, Viet Nam, **3** (1993).
2. Chi V. V. - Dictionary of common plants, Science and Technology Publisher, Ha Noi, Viet Nam **2** (2004) 1824.
3. Lemmens R. H. M. J., Bunyapraphatsara N. - Medicinal and poisonous plants. No 12(3). Plant resources of South-East Asia, Backhuys Publishers, Leiden **3** (2003) 320.
4. Tran H. D., Luu H. T., Leong-Škorničková J. - *Orchidantha anthracina* (Lowiaceae), a new species from Viet Nam, Blumea **20** (2020) 90-93. https://doi.org/10.3767/blumea.2020.65.01.12.
5. Feulner M., Lauerer M., Dotterl S. - Es stinkt! Komponenten im Blütenduft von *Orchidantha fimbriata*. Der Palmengarten **79** (2015) 52-58. https://doi.org/10.21248/palmengarten.265.
6. Yu L., Wuhai C., Lu W., Lin Z., Xi K., Gang C. - A new hexanedioic acid analogue from the endophytic fungus *Penicillium* sp. OC-4 of *Orchidantha chinensis*, Chemistry of Natural Compound **53** (2020) 834-838. https://doi.org/10.1007/s10600-017-2135-8.
7. Larsen K. - A new species of *Orchidantha* (Lowiaceae) from Viet Nam. Adansonia **2** (1973) 481-482.
8. http://webbook.nist.gov/chemistry (Accessed February 1, 2021).
9. Adams R. P. - Identification of essential oil components by gas chromatography/mass spectrometry, Allured Publishing Corporation, Carol Stream IL, 4th edn, (2007).
10. Son N. T., Oda M., Naoki H., Daiki Y., Yu K., Fumi T., Kenichi H., Cuong N. M., Fukuyama Y. - Antimicrobial activity of the constituents of *Dalbergia tonkinensis* and structural-bioactive highlights. Natural Product Communication **13** (2018) 157-161. https://doi.org/10.1177/1934578X1801300212.