SUPPLYMENTARY MATERIAL

Chemical composition and antioxidant activity of the essential oil of *Alseodaphne velutina* Chev. from Viet Nam

Nguyen Tien Cuong[a], Pham Hong Ban[a] and Mai Van Chung[a*] iD

[a] *Department of Biology and Application, Vinh University, Le Duan 182, 43108 Vinh, Nghe An, Viet Nam*

[*]Email: chungmv@vinhuni.edu.vn;

Abstract

Chemical composition and antioxidant activity of the essential oil of *Alseodaphne velutina* Chev. (Lauraceae) were investigated for the first time from Viet Nam. Leaf essential oil was hydrodistilled and analysed by GC and GC-MS that totally identified 32 terpenoid compounds (accouting for 89.18% of the total oil) with β-patchoulene (25.74%) and β-caryophyllene (12.81%) as two major sesquiterpene hydrocarbons compounds. The antioxidant potential of leaf essential oil was evaluated using DPPH, ABTS and FRAP assays, and revealed a moderate-to-high activity comparable with already known antioxidant standard Trolox.

Keywords: Essential oil; *Alseodaphne velutina*; Lauraceae; DPPH, ABTS; FRAP.

Experimental

Plant material: Leaves of *Alseodaphne velutina* plants were collected at an altitude of 790-800 meters in Pu Hoat Nature Reserve (Nghe An Province, Vietnam) in the 2019 dry season. The plant was identified by the botanically taxonomical specialist (Prof. Pham Hong Ban from Vinh University). A voucher specimen (NTC.NN11) was deposited in the Herbarium of Vinh University.

Extraction of essential oil: The essential oil was extracted by hydrodistillation of 4.0 kg of fresh leaves for 6 hours using the Clevenger-type apparatus, and then was stored in a sealed amber glass at -18°C for analyses. The yield calculation was performed in g of essential oil per 100 g of material (%, w/w).

Analysis of essential oil

Gas Chromatography (GC) analysis: The essential oil was analysed by using an Agilent Technologies HP 6890 Plus Gas chromatograph which was equipped with a flame ionization detector (FID). The analysis was carried out on an HP-5MS column (Agilent Technologies,

Santa Clara, CA 95051, USA) measuring 30 m x 0.25 mm i.d., film thickness 0.25 μm. The carrier gas as hydrogen was at a flow rate of 1 mL/min. The injector and detector temperatures were maintained at 250°C and 260°C, respectively. The column temperature was programmed from 40°C with a 2 min hold to 220°C with 10 min at 4°C/min. A volume of 1.0 mL of each oil sample was injected into the GC by splitting method with the split ratio of 10:1. The inlet pressure was maintained at 6.1 kPa. The relative amounts of each component were calculated based on the GC peak area percentage that was performed on basis of the FID signal using the GC HP-Chemstation software (Agilent Technologies, Santa Clara, CA, USA).

Gas Chromatography-Mass Spectrometry (GC-MS) analysis: GC-MS analysis of the essential oil was performed on an HP 6890N Plus Chromatograph (Agilent Technologies) which was fitted with a HP-5MS column (30 m × 0.25 mm i.d., film thickness 0.25 μm) and interfaced with a mass spectrometer HP 5973 MSD. The analytical conditions were the same as used for GC analysis, except that helium (1 mL/min) was used as the carrier gas. The ionization voltage was 70 eV with the emission current of 40 mA. The acquisitions scan mass range of MS was 35-350 amu with the sampling rate of 1.0 scan/s.

Identification of components: Components of essential oil was identified by comparison of their retention indices and mass spectra with data generated under identical experimental conditions. The retention time (t_R) was in relation to a homologous series of *n*-alkanes (C_4-C_{32}) and authentic compounds under the same chromatographic conditions. Further identification was used for processing and interpretation of mass spectra with several commercially available libraries included: NIST/EPA/NIH Mass Spectral Library (NIST 08) and Wiley Registry of Mass Spectral Data, 9th (ed.) (McLafferty, 2009), as well as by comparing with literature data (Adams, 2007; Babushok et al., 2011).

Data expressed in Table S1 are mean of triple analyses.

Assay of antioxidant activity

DPPH radical scavenging activity: Free radical scavenging activity of essential oil were investigated using the DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) method (Brand-Williams et al., 1995) with minor modification. The radical solution is prepared by dissolving 2.4 mg DPPH in 100 mL methanol 99.9%. The studied solution was made by adding 10 μL solution of 50 mg essential oil/mL to 3.990 mL of the radical solution. The mixture was vigorously shaken and then kept in the dark at 25°C for 30 min. Absorbance of the reaction mixture was measured at 515 nm using the UV-Vis CARY 60 spectrophotometer (Agilent, USA); data were analysed by Cary WinUV Software. Blank was the DPPH radical solution without essential oil. Methanol

was used to zero spectrophotometer. All the measurements were carried out at least three times. The DPPH radical scavenging capability was calculated using the following equation as:

$$\text{DPPH scavenged (\%)} = (A_B - A_A / A_B) \times 100 \text{ (Yen and Duh, 1995)}$$

where A_B is the absorbance of blank, and A_A is the absorbance of the investigated solution. The final results were expressed as mg of Trolox equivalent per gram of dried weight sample (mg TEAC/g dw).

ABTS radical scavenging activity: Free radical scavenging activity of essential oil was determined using ABTS (2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid) radical cation decolorization assay (Re et al., 1999). ABTS$^{.+}$ cation radical solution was formed by the reaction between 7 mM ABTS and 2.45 mM potassium persulfate (1:1; v:v), kept in the dark at 25°C for 15h, then diluted with methanol to obtain an optical density value of 0.700 at 734 nm wavelength in the UV-Vis CARY system. 10 μL of essential oil solution (concentration of 50 mg essential oil/mL) was added to 3.990 mL of ABTS$^{.+}$ solution and mixed for 30 min, then the spectrophotometric measurement was done. Blank was the ABTS$^{.+}$ solution without essential oil. All the determinations were performed in triplicate. Percent of ABTS$^{.+}$ scavenging was calculated using the equation:

$$\text{ABTS}^{.+} \text{ scavenging (\%)} = [(A_B - A_A)/ A_B] \times 100,$$

where, A_B is absorbance of ABTS$^{.+}$ methanol solution; A_A is absorbance of ABTS$^{.+}$ essential oil /standard solution. Trolox was used as a positive control. The final results were expressed as mg TEAC/g dw.

Ferric reducing antioxidant power: The ferric reducing antioxidant power (FRAP) activity of essential oil was investigated using spectrophotometric method (Benzie and Strain, 1996). The FRAP solution was prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mL tripyridyl triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in the proportion of 10:1:1 at 37°C. The freshly prepared working FRAP solution (3.990 mL) was mixed with 10 μL of the essential oil solution (concentration of 50 mg essential oil/mL). A blue color complex was formed when Fe^{3+} in [Fe^{3+}TPTZ] (a colorless complex) was reduced to Fe^{2+} form. After incubation for 30 min at 37°C, the absorbance at 593 nm was recorded against a blank (3.990 mL FRAP solution + 10 μL distilled water) by using the UV-Vis CARY system. All the determinations were performed in triplicates. Trolox was used as antioxidant standard. The FRAP values were expressed as mg TEAC/g dw.

Data

Table S1. Chemical composition of the essential oil from *Alseodaphne velutina* leaves

No	Compound names	Molecular formula	RI[a]	RI[b]	Content (%)
1.	α-Pinene	$C_{10}H_{16}$	939	934.5	0.15
2.	Camphene	$C_{10}H_{16}$	953	947.4	0.07
3.	β-Pinene	$C_{10}H_{16}$	977	973.1	0.07
4.	β-Myrcene	$C_{10}H_{16}$	989	983.1	1.09
5.	2-Carene	$C_{10}H_{16}$	1002	997.7	1.09
6.	α-Phellandrene	$C_{10}H_{16}$	1006	999.1	**4.43**
7.	p-Cymene	$C_{10}H_{14}$	1016	1015.1	1.24
8.	D-Limonene	$C_{10}H_{16}$	1028	1023.7	0.94
9.	β-Ocimene	$C_{10}H_{16}$	1042	1038.4	0.86
10.	γ-Terpinene	$C_{10}H_{16}$	1054	1050.3	0.23
11.	α-Cubebene	$C_{15}H_{24}$	1357	1352.2	0.27
12.	β-Bourbonene	$C_{15}H_{24}$	1385	1381.7	0.76
13.	β-Elemene	$C_{15}H_{24}$	1391	1388.0	1.39
14.	1-Methyl-6-(1-methylethylidene)-bicyclo[3.1.0]hexane	$C_{10}H_{16}$	1395	-	**7.82**
15.	Isoledene	$C_{15}H_{24}$	1403	-	1.23
16.	α-Gurjunene;	$C_{15}H_{24}$	1409	1405.6	0.28
17.	α-Cedrene	$C_{15}H_{24}$	1413	1410.9	0.63
18.	β-Caryophyllene	$C_{15}H_{24}$	1422	1419.3	**12.81**
19.	trans-α-Bergamotene	$C_{15}H_{24}$	1434	1431.1	1.34
20.	Aromadendrene	$C_{15}H_{24}$	1441	1439.0	**5.21**
21.	β-Farnesene	$C_{15}H_{24}$	1451	1449.3	1.46
22.	β-Patchoulene	$C_{15}H_{24}$	1455	1451.3	**25.74**
23.	γ-Muurolene	$C_{15}H_{24}$	1478	1473.0	1.72
24.	Germacrene D	$C_{15}H_{24}$	1479	1475.9	1.17
25.	(Z)-γ-Bisabolene	$C_{15}H_{24}$	1514	1511.7	**6.99**
26.	Nerolidol	$C_{15}H_{26}O$	1553	1550.1	2.59
27.	Viridiflorol	$C_{15}H_{26}O$	1581	1579.9	0.21

28.	Globulol	$C_{15}H_{24}O$	1583	1578.9	1.73
29.	Ledol	$C_{15}H_{26}O$	1586	1582.5	0.27
30.	Isospathulenol	$C_{15}H_{24}O$	1628	1625.6	**4.65**
31.	tau-Cadinol	$C_{15}H_{26}O$	1631	1626.4	0.26
32.	α-Cadinol	$C_{15}H_{26}O$	1644	1640.2	0.51
Monoterpene hydrocarbons					18.96
Oxygen-containing monoterpenes					-
Sesquiterpene hydrocarbons					59.98
Oxygen-containing sesquiterpenes					10.24
Total identified					**89.18%**

RI[a] Calculated from retention indices on HP-5MS capillary column;

RI[b] Retention indices on literatures (Adam, 2007; NIST 08, 2008; McLafferty, 2009; Babushok et al., 2011);

(-) no information

Table S2. Antioxidant activity of the essential oil from *Alseodaphne velutina* leaves

Antioxidant index	**Antioxidant activity** *(mg TEAC/g dw) **
DPPH	1.08 ± 0.11
ABTS	2.53 ± 0.16
FRAP	2.79 ± 0.14

* TEAC, Trolox equivalent antioxidant capacity. Absorbance was converted to equivalent activity of Trolox per g of dry weight based on a standard curve. Unit of antioxidant activity was expressed as mg TEAC/g dw).

Figure S1. *Alseodaphne velutina* Chev.

(Leaves in young plant found in habitat of slope with shrubs in Pu Hoat Nature Reserve; at an altitude of 793m; coordinates: $19^o 31.900'$ N, $104^o 42.285'$ E)

References

Adams RP. 2007. Identification of essential oil components by gas chromatography/ mass spectrometry. 4th, Allured Publishing Corporation, Carol Stream. 804 pp.

Benzie IF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as measurement of "antioxidant power". The FRAP assay. Anal Biochem. 239:70-76.

Brand-Williams W, Cuvelier ME, Berset C. 1995. Use of free radical method to evaluate antioxidant activity. Lebens-Wiss Technol. 28:25-30.

Babushok VI, Linstrom PJ, Zenkevich IG. 2011. Retention indices for frequently reported compounds of plant essential oils. J Phys Chem Ref Data. 40(4): 043101, doi: 10.1063/1.3653552.

McLafferty FW. 2009. Wiley Registry of Mass Spectral Library (9th ed.). Wiley.

NIST 08. 2008. Mass spectral library (NIST/EPA/NIH). Gaitherburg, USA: National Institute of Standards and Technology.

Re R, Pellegrini N, Proteggente A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cationdecolorization assay. Free Radic Biol Med. 26:1231-1237.

Yen GC, Duh PD. 1994. Scavenging effect of methanolic extracts of peanut hulls on free radical and active oxygen species. J Agric Food Chem. 42:629-32.