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SHORT COMMUNICATION



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

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ABSTRACT

Chemical composition and antioxidant activity of the essential oil of *Aleodaphne 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 (accounting 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.

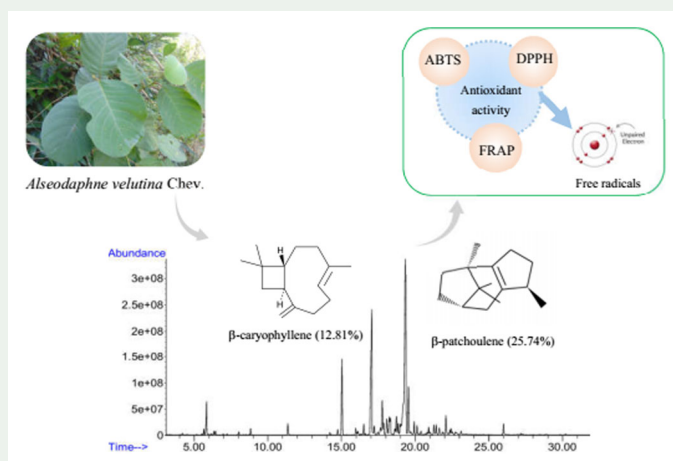
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Essential oil; *Aleodaphne velutina*; Lauraceae; DPPH; ABTS; FRAP



1. Introduction

Aleodaphne Nees, a genus of Lauraceae family, consists of approximately 60 species, of which about 90% are distributed in tropical Asia (Roskov et al. 2014). Although, many Lauraceae plants are renowned for their valuable essential oils (de Silva et al. 2009; Damasceno et al. 2019), the available information regarding the composition

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and bioactivity of the essential oil from *Alseodaphne* plants is still limited (Chang et al. 2000; Lee et al. 2001; Nafiah et al. 2010; Charles and Ramani 2011; Thakur et al. 2012). Recently, the essential oil of an Indian species *A. semecarpifolia* was introduced to be rich in sesquiterpenoids (Verma et al. 2016), and expressed antibacterial, antifungal, antioxidant activities (Charles et al. 2012). Leaf oil of Malaysian *A. peduncularis* plants also composed sesquiterpenoids as the most abundant components (Salleh and Ahmad 2016; Anuar et al. 2019), and showed weak activity towards DPPH radical scavenging (Salleh and Ahmad 2016).

A. velutina Chev. plants (Supporting Information, Figure S1) locally distributed at altitude of 750 m and higher in Central of Viet Nam (Nguyen 2010). This species was generally characterised as small trees of about 2–5 m in height; the broadly oval leaves with blackish hairs on surfaces are alternately arranged in the greyish white stem and twigs; the midrib raised, and secondary nerves is about 10–12 pairs, curving and joining near margin; the hermaphroditic flowers are in clustered arrangements, and the small fruits are in oval shape. To date, lack of documents on the essential oil of *A. velutina* plants was reported, therefore, it is worthwhile to study on its chemical composition and antioxidant activity for the first time from Viet Nam.

2. Results and discussion

2.1. Chemical composition

Hydrodistillation of *A. velutina* leaves gave pale yellow oil in 0.25% (w/w). Constituents of *A. velutina* essential oil are presented in Table S1 (Supporting Information). GC and GC-MS analyses had successfully identified 32 terpenoid compounds, which made up 89.18% of the chromatographical components. Sesquiterpene hydrocarbons (59.98%) were the most dominant components with β -patchoulene (25.74%) and β -caryophyllene (12.81%) as two substantial components, together with 12 other compounds. Oxygen-containing sesquiterpenes composed of 7 compounds (10.24%) with main component as isospathulenol (4.65%). Monoterpene hydrocarbons (18.96%) composed of 1-methyl-6-(1-methylethylidene)-bicyclo[3.1.0]hexane (7.82%) and α -phellandrene (4.43%) as major constituents, together with 9 other components; while oxygen-containing monoterpenes were not found in this essential oil.

A limited literature investigated the chemical composition of essential oils of *A. semecarpifolia* in India (Verma et al. 2016), *A. peduncularis* and *A. perakensis* in Malaysia (Salleh and Ahmad 2016; Anuar et al. 2019) introduced the major components as sesquiterpene hydrocarbons. Our results were in the agreement with previous studies to reveal that sesquiterpene hydrocarbons were the most dominant components of *A. velutina* essential oil. The most dominance of β -patchoulene, β -caryophyllene is probably associated with biological activities of their essential oil. β -Patchoulene expresses an anti-inflammatory activity (Yang et al. 2017), and a potential therapeutic efficacy for antiulcer treatment (Liu et al. 2017). The high quantity of β -caryophyllene obtained in the essential oil contributed to antioxidant, antibiotic, anticarcinogenic activities (Legault and Pichette 2007), and/or anti-inflammatory activity (Gertsch et al. 2008) ascribed to plants.

2.2. Antioxidant activity

Antioxidant activity of leaf oil of *A. velutina* was evaluated using different methods such as DPPH, ABTS and FRAP assays (Supporting Information, Table S2); and results are presented following Trolox equivalent antioxidant capacity (mg TEAC/g dw), meaning the level of antioxidant activity compared with standard Trolox. A moderate activity resulted from DPPH assay (1.08 mg TEAC/g dw) could be due to the low abundance of oxygen-containing sesquiterpenes in the essential oil (10.24%). The ABTS^{•+} scavenging ability was 2.53 mg TEAC/g dw, having by 2.34-fold higher than DPPH test. FRAP analysis recorded a strong ability to reduce Fe³⁺ into Fe²⁺ (2.79 mg TEAC/g dw), and expressed as the highest antioxidant activity in comparing with DPPH and ABTS. The above results revealed a moderate-to-high antioxidant potential of the essential oil from *A. velutina* leaves. It should be highlight that, antioxidant activity of *A. velutina* leaf oil is probably connected to a dominance of terpenoids (89.18%), which was previously identified as the main carriers of antioxidant activity in plants (Bakkali et al. 2008).

3. Experimental

See: Supporting Information.

4. Conclusion

Leaf essential oil of *A. velutina* plants from Viet Nam possesses a high presence of terpenoids (89.18%) with β -patchoulene and β -caryophyllene as two major components. Natural essential oil expressed a high scavenging free radical activity and a strong reducing antioxidant power, suggesting that it may be a promising source of natural antioxidant.

Disclosure statement

No potential conflict of interest was reported by the authors.

Authors' contribution

Nguyen Tien Cuong collected samples, extracted essential oils, operated GC/GC-MS systems and analyzed data. Pham Hong Ban collected samples and identified the botanical name of plants; Mai Van Chung analyzed antioxidant activity, prepared literatures and manuscript. No conflict of interest was made.

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SUPPLYMENTARY MATERIAL

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

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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 (accounting 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 x 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 (C₄-C₃₂) 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). $\text{ABTS}^{+\cdot}$ 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 $\text{ABTS}^{+\cdot}$ solution and mixed for 30 min, then the spectrophotometric measurement was done. Blank was the $\text{ABTS}^{+\cdot}$ solution without essential oil. All the determinations were performed in triplicate. Percent of $\text{ABTS}^{+\cdot}$ scavenging was calculated using the equation:

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

where, A_B is absorbance of $\text{ABTS}^{+\cdot}$ methanol solution; A_A is absorbance of $\text{ABTS}^{+\cdot}$ 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 $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ 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 $[\text{Fe}^{3+}\text{TPTZ}]$ (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.

DataTable 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.	Isolatedene	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° 31.900' N, 104° 42.285' E)



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