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SHORT COMMUNICATION

Antioxidant and Anticholinesterase Activities of Essential Oil of *Alseodaphne peduncularis* Meisn

Alseodaphne peduncularis Meisn. Uçucu Yağının Antioksidan ve Antikolinesteraz Aktivitesi

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ABSTRACT

This study was designed to investigate the antioxidant and anticholinesterase activities of the essential oils from *Alseodaphne peduncularis* Meisn. GC and GC/MS analysis of the leaves oil showed thirty one components representing 72.6% of the oil. The most abundant components were β-caryophyllene (24.0%), δ-cadinene (15.9%) and germacrene B (12.2%). The antioxidant activity was determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging and total phenolic content assays, while anticholinesterase activity assessed by measuring inhibition of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes. The essential oil showed weak activity in the DPPH radical scavenging (IC_{50} of 253.2 µg/mL) and phenolic content (32.5 mg GA/g), while moderate inhibition activity against AChE (I: 45.2%) and BChE (I: 48.6%).
Key words: Essential oil, *Alseodaphne peduncularis*, Antioxidant activity, Anticholinesterase activity, Lauraceae

ÖZ

Bu çalışma *Alseodaphne peduncularis* Meisn. uçucu yağının antioksidan ve antikolinesteraz aktivitelerini araştırmak için tasarlanmıştır. GC ve GC/MS analizleri yaprak yağında %72,6 oranında otuz bir bileşen bulunduğunu göstermiştir. En çok bulunan bileşenler β-karyofilen (%24,0), δ-kadinen (15,9) ve germakren B (%12,2) dir. Antioksidan aktivite DPPH (1,1- difenil-2-pikrilhidrazil) radikal süpürme ve toplam fenolik içerik yöntemleriyle değerlendirilirken, antikolinesteraz aktivite asetilkolinesteraz (AChE) ve bütirilkolinesteraz (BChE) enzim inhibisyonu ölçülerek değerlendirilmiştir. Uçucu yağ, zayıf DPPH radikal süpürme aktivitesi (IC_{50} değeri 253,2 mg/mL) ve fenolik içerik (32,5 mg GA/g) gösterirken, AChE (I: %45,2) ve BChE (I: %48,6) inhibisyon aktivitesi orta düzeydedir.
Anahtar kelimeler: Uçucu yağ, *Alseodaphne peduncularis*, Antioksidan aktivite, Antikolinesteraz aktivite, Lauraceae

INTRODUCTION

Alseodaphne is a genus of small to medium sized trees of the wet evergreen tropical forests. The genus is having more than fifty species and distributed in tropical belt of Cambodia, China, Indonesia, Laos, Malaysia, Myanmar, Philippines, Sri Lanka, Thailand, Vietnam and India. There is no information in the literature regarding medicinal uses of genus Alseodpahne. Most of the species of genus are unexplored, both, pharmacologically and phytochemically (1). Previous phytochemical investigations of Alseodaphne have resulted in the isolation of alkaloids (aporphines, bisbenzylisoquinoline, morphinandienones) (2), lactones (3), neolignans (4) and phenanthrenes (5). The isolated constituents of genus *Alseodaphne* might become useful as a source of pharmacologically interesting molecules.

A. peduncularis is a shrub or sometimes a small tree of up to 6-12 m height. The twigs colour is whitish. The leaves/stalk is slender and around 0.51 cm long in size. The blade is membranous and lanceolate with drying greenish brown colour. The midrib rise above and secondary nerves is about 610 pairs, curving and joining near margin and rise on both surfaces. The tertiary nerves are reticulate and visible on both the surfaces. The colour of the flowers is greenish. The shape of fruits is ellipsoid or globose with a dark purple colour and on enlarged red perianth tube (1).

To the best of our knowledge there is no report on the chemical compositions of the essential oil of *A. peduncularis*, therefore we would like to report their chemical composition and their antioxidant and anticholinesterase activities.

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MATERIALS AND METHODS

Plant materials

A sample of *A. peduncularis* was collected from Hutan Simpan Bangi, Selangor in September 2015, and identified by Dr. Shamsul Khamis from Institute of Biosience (IBS), Universiti Putra Malaysia (UPM). The voucher specimen (SK2955/16) were deposited at the Herbarium of IBS, UPM.

Solvents and chemicals

Antioxidant: 1,1-Diphenyl-2-picrylhydrazyl (DPPH), gallic acid and butylatedhydroxytoluene (BHT) were obtained from Sigma-Aldrich (Germany). Analytical grade methanol, ethanol and dimethylsulfoxide (DMSO), HPLC grade chloroform, Folin-Ciocalteu's reagent, anhydrous sodium sulphate, and sodium carbonate were purchased from Merck (Germany). Anticholinesterase: AChE enzyme (Type-VI-S, EC3.1.1.7), butyrylcholinesterase enzyme (BChE; EC3.1.1.8), acetylthiocholine iodide, butyrylthiocholine chloride, 5,5′-dithio-bis(2-nitrobenzoic)acid (DTNB), and galantamine were purchased from Sigma-Aldrich (Germany).

Extraction of essential oils

The fresh leaf (300 g) was subjected to hydrodistillation in an all glass Dean-stark apparatus for 6 hours. The oils obtained were dried over anhydrous magnesium sulfate and stored at 4-6˚C. The oil yield (w/w) was 1.55 g (0.52%) based on the fresh weight.

Gas chromatography (GC)

GC analysis were performed on a Hewlett Packard 6890 series II A gas chromatograph equipped with an Ultra-1 column (100% polymethylsiloxanes) (25 m long, 0.33 µm thickness and 0.20 mm inner diameter). Helium was used as a carrier gas at flow rate of 0.7 mL/min. Injector and detector temperature were set at 250 and 280˚C, respectively. Oven temperature was kept at 50˚C, then gradually raised to 280˚C at 5˚C/min and finally held isothermally for 15 min. Diluted samples (1/100 in diethyl ether, v/v) of 1.0 µL were injected manually (split ratio 50:1). The injection was repeated three times and the peak area percents were reported as means ± SD of triplicates. Calculation of peak area percentage was carried out by using the GC HP Chemstation software (Agilent Technologies).

Gas chromatography-mass spectrometry (GC-MS)

GC-MS chromatograms were recorded using a Hewlett Packard Model 5890A gas chromatography and a Hewlett Packard Model 5989A mass spectrometer. The GC was equipped with Ultra-1 column (25 m long, 0.33 µm thickness and 0.20 mm inner diameter). Helium was used as a carrier gas at a flow rate of 1 mL/min. Injector temperature was 250˚C. Oven temperature was programmed from 50˚C (5 min hold) at 10˚C/min to 250˚C and finally held isothermally for 15 min. For GC-MS detection, an electron ionization system, with ionization energy of 70 eV was used. A scan rate of 0.5 s (cycle time: 0.2 s) was applied, covering a mass range from 50-400 amu.

Identification of components

The constituents of the oil were identified by comparison of their mass spectra with reference spectra in the computer library (Wiley) and also by comparing their retention indices, with data in the literature (6). The quantitative data were obtained electronically from FID area percentage without the use of correction factor.

Antioxidant activity

DPPH radical scavenging

The free radical scavenging activity was measured by the DPPH method with minor modifications (7). Each sample of stock solution (1.0 mg/mL) was diluted to final concentration of 1000-7.8 µg/mL. Then, a total of 3.8 mL of 50 µM DPPH methanolic solution (1 mg/50 mL) was added to 0.2 mL of each sample solution and allowed to react at room temperature for 30 min. The absorbance of the mixtures was measured at 517 nm. A control was prepared without sample or standard and measured immediately at 0 min. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity, and vice versa. The percent inhibitions (I%) of DPPH radical were calculated as follow:

I% = [Ablank – Asample/ Ablank] ×100

where Ablank is the absorbance value of the control reaction and Asample is the absorbance values of the test samples. The sample concentration providing 50% inhibition (IC_{50}) was calculated by plotting inhibition percentages against concentrations of the sample. All tests were carried out in triplicate and IC_{50} values were reported as means ± SD of triplicates.

Total phenolic content (TPC)

Total phenolic contents of the essential oils were determined as described previously (8). A sample of stock solution (1.0 mg/mL) was diluted in methanol to final concentrations of 1000 µg/mL. A 0.1 mL aliquot of samplewas pipetted into a test tube containing 0.9 mL of methanol, then 0.05 mL Folin-Ciocalteu's reagent was added, and the flask was thoroughly shaken. After 3 min, 0.5 mL of 5% Na_2CO_3 solution was added and the mixture was allowed to stand for 2 h with intermittent shaking. Then, 2.5 mL of methanol was added and left to stand in the dark for 1 h. The absorbance measurements were recorded at 765 nm. The same procedure was repeated for the standard gallic acid solutions. The concentration of total phenolic compounds in the oils was expressed as mg of gallic acid equivalent per gram of sample. Tests were carried out in triplicate and the gallic acid equivalent value was reported as mean ± SD of triplicate.

Anticholinesterase activity

AChE/BChE inhibitory activity of the essential oils was measured by slightly modifying the spectrophotometric

Table 1. Constituents identified from the essential oil of *A. peduncularis*

No	Components	KI[a]	Percentage (%)
1	δ-Elemene	1335	1.5
2	α-Cubebene	1345	0.5
3	α-Ylangene	1373	0.1
4	α-Copaene	1374	2.5
5	β-Copaene	1374	0.8
6	Isoledene	1375	0.3
7	β-Cubebene	1387	0.4
8	α-cis-Bergamotene	1411	0.4
9	**β-Caryophyllene**	1417	**24.0**
10	α-trans-Bergamotene	1432	0.1
11	γ-Elemene	1434	5.2
12	α-Guaiene	1437	0.0
13	6,9-Guaiadiene	1442	0.6
14	cis-Cadina-1(6)4-diene	1461	0.2
15	trans-Cadina-1(6)4-diene	1475	0.4
16	γ-Muurolene	1478	0.9
17	β-Selinene	1489	0.6
18	β-Guaiene	1492	0.1
19	γ-Amorphene	1495	0.3
20	Valencene	1496	0.3
21	Cubebol	1514	0.3
22	**δ-Cadinene**	1522	**15.9**
23	α-Cadinene	1537	0.1
24	γ-Cadinene	1543	0.2
25	Germacrene B	1559	**12.2**
26	(E)-Nerolidol	1561	0.1
27	Spathulenol	1577	0.2
28	Caryophyllene oxide	1582	0.8
29	Globulol	1590	0.2
30	Epicubenol	1627	1.0
31	trans-Longipinocarveol	1634	0.2
32	t-Muurolol	1644	1.9
33	β-Eudesmol	1649	0.1
34	α-Bisabolol	1685	0.1
35	Phytol	1942	0.1
Group components			
	Sesquiterpene hydrocarbons		67.6
	Oxygenated sesquiterpenes		5.0
Identified components (%)			**72.6**

[a]Kovat's indices (KI) experimental: n-alkanes (C_9-C_{30}) were used as reference points in the calculation of KI

method developed by Ellman et al. (9) and Orhan et al. (10). Electric eel (Electrophorus electricus) AChE and horse serum BChE were used, while acetylthiocholine iodide (AChI) and butyrylthiocholine chloride (BChI) were employed as substrates of the reaction. DTNB acid was used for the measurement of the anticholinesterase activity. Briefly, in this method, 140 µL of sodium phosphate buffer (pH 8.0), 20 µL of DTNB, 20 µL of essential oils and 20 µL of AChE/BChE solution were added by multichannel automatic pipette in a 96-well microplate and incubated for 15 min at 25˚C. The reaction was then initiated with the addition of 10 µL of AChI/BChI. Hydrolysis of AChI/BChI was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalyzed by enzymes at 412 nm utilizing a 96-well microplate reader (Epoch Micro-Volume Spectrophotometer). Percentage inhibition (I%) of AChE/BChE was determined by comparison of rates of reaction of samples relative to blank sample (EtOH in phosphate buffer pH 8) using the formula:

I%=[E-S/E]×100

where E is the activity of enzyme without test sample and Sis the activity of enzyme with test sample. The experiments were done in triplicate. Galantamine was used as reference.

Statistical analysis

Data obtained from essential oil analysis and their bioactivities were expressed as mean values. The statistical analyses were carried out by employing one way ANOVA ($p<0.05$). A statistical package (SPSS version 11.0) was used for the data analysis.

RESULTS AND DISCUSSION

Hydrodistillation of the fresh leaves of *A. peduncularis* gave pale yellow oil in 0.52% (w/w). The chemical compositions of the leaves oil of *A. peduncularis* are listed in Table 1. GC and GC-MS analysis of the essential oil had successfully found thirty five components, which accounted for 72.6% of the chromatographical components. Sesquiterpene hydrocarbons were the major components in the essential oil (67.6%) with β-caryophyllene (24.0%) being the most substantial component, followed by δ-cadinene (15.9%) and germacrene B (12.2%). Furthermore, oxygenated sesquiterpenes made

Table 2. Antioxidant and anticholinesterase activities of the essential oil of *A. peduncularis*

Samples	DPPH IC_{50} (µg/mL)[a]	TPC (mg GA/g)[b]	AChE (I%)c	BChE (I%)[c]
Essential oil	253.2	32.5 ± 0.1	45.2 ± 0.2	48.6 ± 0.2
BHT	18.5	-	-	-
Galantamine	-	-	95.9 ± 0.2	88.7 ± 0.2

[a]IC_{50} value at concentrations of 1000-7.8µg/mL; [b]TPC at a concentration of 1 mg/mL;[c]Percentage inhibition at a concentration of 1 mg/mL; ± represents SD of three independent experiments ($p < 0.05$)

up a minor fraction which gave 5.0%, while monoterpenoids were not found in this essential oil.

The antioxidant activities were investigated by DPPH free radical scavenging assay together with the Folin-Ciocalteu assay which evaluated the total phenolic content of the essential oil. The results are displayed in Table 2. The antioxidant activity on DPPH radical scavenging is due to their hydrogen donating ability. The capability of substances to donate hydrogen is able to convert DPPH into their non-radical form DPPH and the reaction can be followed spectrophotometrically (11). The essential oil exhibited weak DPPH radical scavenging activity (IC_{50} of 253.2 µg/mL) compared to standard antioxidant, BHT (IC_{50} of 18.5 µg/mL). The low activity was attributed to the low phenolic content of the essential oil which is responsible for antioxidant activity. This was supported by the results of the Folin-Ciocalteu assay on the essential oils, which showed low amount of phenolic (32.5%). AChE plays an important role in the central nervous system. It is one of the fastest known enzymes and catalyses the cleavage of acetylcholine in the synaptic cleft after depolarisation. Inhibitors of AChE, such as galanthamine, are used frequently in the pharmacotherapy of Alzheimer disease (12). The essential oil indicated moderate AChE and BChE activity at 1000 µg/mL concentration, which gave 45.2% and 48.6% for AChE and BChE activity, respectively. In previous reports, AChE inhibition can be explained by the high content of monoterpenes. It has been mentioned that 1,8-cineole, camphor, α-pinene, β-pinene, borneol, linalool, menthone, carvone, anetole, anisole, have anticholinesterase eactivity (13-15). This current oil lacked the presence of monoterpenes, hence contributed to the low AChE/BChE inhibition.

CONCLUSION

In conclusion, essential oil and their components generally displayed significant bioactivity properties, which are useful as preventive agents from various diseases. In the case of *A. peduncularis* oil, although there is no striking on their bioactivity in the oil, it is still worthwhile to investigate the other parts of the plant as a natural source for essential oil composition or their phytochemical studies.

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