Nat. Volatiles & Essent. Oils, 2021; 8(3): 29-33
DOI: 10.37929/nveo.878488

RESEARCH ARTICLE

Chemical composition and lipoxygenase inhibitory activity of *Alseodaphne peduncularis* Meisn. essential oil

Wan Mohd Nuzul Hakimi Wan Salleh[1,*] Mohd Azlan Nafiah[1,*], Shamsul Khamis[2], Salam Ahmed Abed[3]

[1]Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris, 35900 Tanjung Malim, Perak, MALAYSIA
[2]Department of Biological Sciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, MALAYSIA
[3]Department of Pharmacognosy, College of Pharmacy, Kerbala University, IRAQ

*Corresponding author. Email: wmnhakimi@fsmt.upsi.edu.my; azlan@fsmt.upsi.edu.my

Submitted: 11.02.2021; Accepted: 20.04.2021

Abstract

This study examined the chemical composition of the essential oil extracted from the bark of *Alseodaphne peduncularis* Meisn. (Lauraceae) by hydrodistillation with 0.2% yield. The essential oil was chemically characterised by gas chromatography-flame ionization detector and gas chromatography-mass spectrometry, and 31 constituents comprising 94.3% of the total oil content were identified. These constituents included β-caryophyllene (24.5%), viridiflorol (12.3%), δ-cadinene (9.8%), and bicyclogermacrene (4.2%), respectively. The tested essential oil showed moderate *in vitro* lipoxygenase activity with an IC_{50} = 60.2 µg/mL. To the best of our knowledge, there is no report and study on the bark part of this species.

Keywords: Lauraceae, *Alseodaphne peduncularis*, hydrodistillation, GC-MS, lipoxygenase

Introduction

The tree species *Alseodaphne peduncularis* Meisn. (Lauraceae), locally known as *medang*, occurs widely in mixed dipterocarp and sub-montane forests up to 1000 m above sea level in the Peninsular Malaysia, Sumatra, and Borneo (Turner, 1995; Thakur et al., 2012). Although the tree is a common wood source for fuel, furniture, house, and boat-building, little is known about its medicinal values. Recently, the chemical composition and biological activity of the essential oil extracted from the leaves of *A. peduncularis* were reported in our study (Salleh et al., 2016a). The leaf oil was weak in the DPPH radical scavenging (IC_{50}: 253.2 µg/mL) with relatively low phenolic content (32.5 mg GA/g) and modest inhibitory effect against acetylcholinesterase (I: 45.2%) and butyrylcholinesterase (IC_{50}: 48.6%). In the continuation of our studies on essential oils from this species *A. peduncularis*, herein we report the chemical constitution of essential oil extracted from its bark and the lipoxygenase inhibitory effect of the essential oil.

Materials and Methods

Plant material and isolation of essential oil

In this study, the essential oil was extracted from the fresh bark of *A. peduncularis*, which was sampled from the secondary forest at Behrang, Perak in September 2019. The tree was taxonomically identified by Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). Meanwhile, voucher specimens of the plant (SK128/19) were stowed at UKMB Herbarium. A Clevenger apparatus was used to extract the essential oil from the fresh bark (200 g) mixed with water via hydrodistillation for 4 h. The essential oil was desiccated using the dry magnesium sulfate and kept at 4 – 6 °C until analysis.

DOI: 10.37929/nveo.878488

Analysis of essential oil

Hydrocarbons were detected via a gas chromatography-flame ionization detector (Agilent Technologies 7890B, USA) equipped with an HP-5MS capillary column of 30 m in length, 0.25 µm thick, and an inner diameter of 0.25 mm. Analytes were moved through the column by helium gas at a flow rate of 0.7 mL/min. Temperatures were adjusted to 250 °C and 280 °C for the injector and detector, respectively, while the oven was set at 50 °C, but slowly increased to 280 °C at 5 °C/min and eventually kept isothermally for 15 min. Samples were diluted with diethyl ether at 1: 100 (v/v) and analyzed in triplicates, in which 1.0 µL of the diluted sample was manually interpolated at a split ratio of 50: 1. The average of triplicates was calculated to generate the percentage of peak area. Meanwhile, a gas chromatography-mass spectrometer (GC/MS; 5890A for GC, 5898A for MS, Hewlett Packard, USA) was used to detect various substances in the test sample. Analytes were moved by helium through the HP-5 column fixed to the GC at 1 mL/min. The injector was kept at 250 °C, and the oven set to gradually increase from 50 to 280 °C (5 min hold) at 10 °C/min and eventually kept isothermally for 15 min. An electron ionization energy of 70 eV was employed in the GC/MS detection at a scan rate of 0.5 s (cycle time: 0.2 s), encompassing a mass range of 50 - 400 atomic mass unit (Salleh et al., 2012; Salleh et al., 2016b).

Identification of constituents

For the identification of chemical constituents in GC/MS, samples were co-injected with the standards (major components) with the corresponding retention indices and mass spectra following the study of Adams (2007). Constituents of essential oil were semi-quantified through the normalization of peak areas by taking into account the same reactionary component for all the detected volatile constituents. The average of three GC analyses yielded the relative percentage (%) value.

Lipoxygenase inhibitory activity

The LOX inhibition was largely based on the technique of Ellman (Salleh et al., 2016c), in which 5 µL of essential oil was buffered with 1.74 mL borate (0.2 M, pH 9.2) and added with 5 µL (50,000 U/mL) of 5-LOX enzyme. The reaction began upon adding 250 µL linoleic acid (5 mg linoleic acid mixed with 15 µL ethanol and 15 mL borate in brisk shaking). The absorbance at 234 nm was assayed for 5 min in a UV-visible spectrophotometer (Genesys 10Se, Thermo Scientific, USA). The dimethyl sulfoxide (5 µL) served as a negative control, while the quercetin (Sigma-Aldrich, St. Louis, MO, USA), a positive control, was prepared in the same strength as the essential oil. The percentage inhibition (I%), which is equivalent to the concentration of drug required for 50% inhibition (IC_{50}) in µg/mL , was computed by the equation below:

$$I\% = [A_{initial\ activity} - (A_{inhibitor} / A_{initial\ activity})] \times 100$$

where $A_{initial\ activity}$ is the absorbance of the control, and $A_{inhibitor}$ is the absorbance of the test sample. Averaging the absorbance values of the triplicates and quercetin yielded the LOX inhibitory activity.

Results and Discussion

The bark of *A. peduncularis* yielded 0.2% yellow oil based on the fresh weight. Table 1 shows, in order of elution, 31 constituents identified in the essential oil, comprising 94.3% of the total chemical content. Sesquiterpenes were the main hydrocarbon groups found in the 16 oil components identified, constituting 59.2% of the total oil content. Besides, oxygenated sesquiterpenes, oxygenated monoterpenes, and monoterpene hydrocarbons contributed 25.7%, 5.7%, and 3.7%, respectively to the overall oil content.

DOI: 10.37929/nveo.878488

Table 1. Chemical composition of *Alseodaphne peduncularis* essential oil

No	RRI[a]	RRI[b]	Components	Percentage[c]	Identifications[d]
1	935	935	α-Pinene	2.0 ± 0.1	RI, MS
2	945	946	Camphene	1.2 ± 0.1	RI, MS
3	967	965	Sabinene	0.5 ± 0.2	RI, MS
4	1082	1082	Linalool	1.2 ± 0.1	RI, MS
5	1175	1175	Terpinen-4-ol	2.3 ± 0.2	RI, MS
6	1185	1189	α-Terpineol	2.2 ± 0.1	RI, MS
7	1352	1350	α-Cubebene	1.2 ± 0.2	RI, MS
8	1374	1374	α-Copaene	0.2 ± 0.2	RI, MS
9	1385	1386	β-Cubebene	0.5 ± 0.1	RI, MS
10	1409	1405	α-Cedrene	0.2 ± 0.2	RI, MS
11	1425	1420	β-Caryophyllene	24.5 ± 0.2	RI, MS, Std
12	1455	1453	α-Humulene	0.5 ± 0.2	RI, MS
13	1458	1458	Aromadendrene	2.5 ± 0.1	RI, MS
14	1478	1480	Germacrene D	5.2 ± 0.2	RI, MS, Std
15	1485	1482	α-Amorphene	3.5 ± 0.2	RI, MS
16	1495	1495	Cadina-1,4-diene	1.2 ± 0.1	RI, MS
17	1500	1501	Bicyclogermacrene	4.2 ± 0.2	RI, MS, Std
18	1502	1500	α-Muurolene	2.0 ± 0.1	RI, MS
19	1529	1530	δ-Cadinene	9.8 ± 0.2	RI, MS, Std
20	1535	1537	α-Cadinene	1.4 ± 0.1	RI, MS
21	1542	1545	Germacrene B	2.1 ± 0.2	RI, MS
22	1545	1543	γ-Cadinene	0.2 ± 0.2	RI, MS
23	1546	1545	Elemol	1.8 ± 0.2	RI, MS
24	1570	1570	Globulol	2.0 ± 0.1	RI, MS
25	1575	1575	Spathulenol	2.2 ± 0.2	RI, MS
26	1592	1595	Viridiflorol	12.3 ± 0.2	RI, MS, Std
27	1602	1602	Guaiol	1.0 ± 0.1	RI, MS
28	1635	1635	t-Muurolol	0.2 ± 0.2	RI, MS
29	1652	1650	α-Cadinol	2.5 ± 0.1	RI, MS
30	1654	1652	α-Eudesmol	3.2 ± 0.2	RI, MS
31	1682	1685	α-Bisabolol	0.5 ± 0.1	RI, MS
			Monoterpene hydrocarbons	3.7	
			Oxygenated monoterpenes	5.7	
			Sesquiterpene hydrocarbons	59.2	
			Oxygenated sesquiterpenes	25.7	
			Total identified	**94.3**	

[a]Linear retention index, experimentally determined using homologous series of C_6-C_{30} alkanes. [b]Linear retention index taken from Adams (2007). [c]Relative percentage values are means of three determinations ±SD. [d]Identification methods: Std, based on comparison with authentic compounds; MS, based on comparison with Wiley, Adams, FFNSC2, and NIST08 MS databases; RI, based on comparison of calculated RI with those reported in Adams, FFNSC2 and NIST08.

The major constituents of the essential oil consisted of β-caryophyllene (24.5%), viridiflorol (12.3%), δ-cadinene (9.8%), and bicyclogermacrene (4.2%). Other notable constituents included α-amorphene (3.5%), α-eudesmol (3.2%), aromadendrene (2.5%), α-cadinol (2.5%), terpinen-4-ol (2.3%), α-terpineol (2.2%), spathulenol (2.2%), germacrene B (2.1%), α-pinene (2.0%), α-muurolene (2.0%), and globulol (2.0%). Both β-

DOI: 10.37929/nveo.878488

caryophyllene and δ-cadinene occurred in a substantial amount in the bark oil when compared to the leaf oil of the same plant species (Salleh et al., 2016a), and the majority of the bark oil constituents also occurred in the leaf oil. However, 12 constituents were not detected in the bark oil; they were α-cedrene, α-humulene, aromadendrene, germacrene D, α-amorphene, cadina-1,4-diene, bicyclogermacrene, α-muurolene, elemol, viridiflorol, guaiol, and α-cadinol. Such differences were probably due to different genotypes and chemotypes of the plant, which may affect the content of essential oil (Salleh et al., 2014; Marjo et al., 2001).

Arachidonate 5-lipoxygenase is the key enzyme in leukotriene biosynthesis and catalyzes the initial steps in the conversion of arachidonic acid to biologically active leukotrienes. Leukotrienes are considered potent mediators of inflammatory and allergic reactions and regarding their pro-inflammatory properties, the inhibition of 5-lipoxygenase pathway is considered to be interesting in the treatment of a variety of inflammatory diseases. Besides 5-lipoxygenase inhibitors, drugs able to block the 5-lipoxygenase as well as the cyclooxygenase metabolic pathway are also of therapeutic value. The LOX inhibitory effect of essential oil extracted from the bark was modest at inhibition of 60.2%, compared to that of quercetin with inhibition of 92.5%. Since sesquiterpene hydrocarbons of essential oils extracted from various plant species (e.g., *Syzygium aromaticum*, *Cannabis sativa*, *Rosmarinus officinalis,* and *Tagetes minuta*) were generally reported to show anti-inflammatory effect (Gertsch et al., 2008; Ghiasvand et al., 2011), thus, the high amount of β-caryophyllene and δ-cadinene in the essential oil of *A. peduncularis* might at least partially contribute to the anti-inflammatory potential.

This study provides valuable and useful information and indications for further exploring the potential nutraceutical and pharmaceutical applications of the genus Lauraceae. The next step will be to evaluate the *in vivo* of the essential oil in order to valorize this species with a special ecological character.

ACKNOWLEDGMENT

The authors would like to thank the Fundamental University Research Grant (GPUF) for financial support under vote 2019-0225-103-01 and the Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris for research facilities.

CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

REFERENCES

Adams, R. P. (2007). *Identification of Essential oil Components by Gas chromatography/Mass spectroscopy*. Carol Stream (IL): Allured.

Gertsch, J., Leonti, M., Raduner, S., Racz, I., Chen, J. Z., Xie, X. Q., Altmann, K. H., Karsak, M., Zimmer, A. (2008). Beta-caryophyllene is a dietary cannabinoid. *Proceedings of the National Academy of Sciences of the United States of America*, 105, 9099-9104.

Ghiasvand, A. R., Nasseri, M., Farsizaeh, S., Meshkatalsadat, M. H., Sadeghi-Sarabi, R., Shadabi, S., Borzoei, M. (2011). Chemical characterization of cultivated *Tagetes minuta* L. by use of ultrasound-assisted headspace SPME and GC-MS. *Chromatographia*, 73, 1031-1035.

Marjo, K., Eija, P., James, E. S. (2001). Variation in volatile compounds from tansy (*Tanacetum vulgare* L.) related to genetic and morphological differences of genotypes. *Biochemical Systematics and Ecology*, 29, 267-85.

Ng, F. S. P. (1989). *Malayan forest records, tree flora of Malaya*. Kuala Lumpur, Malaysia: FRIM, 4:98.

Salleh, W. M. N. H. W., Ahmad, F., Sirat, H. M., Khong, H. Y. (2012). Chemical compositions and antibacterial activity of the leaf and stem oils of *Piper porphyrophyllum* (LINDL.) N.E.Br. *EXCLI Journal*, 11, 399-406.

DOI: 10.37929/nveo.878488

Salleh, W. M. N. H. W., Ahmad, F., Khong, H. Y. (2014). Chemical composition of *Piper stylosum* Miq. and *Piper ribesioides* Wall. essential oils and their antioxidant, antimicrobial and tyrosinase inhibition activities. *Latin American and Caribbean Bulletin of Medicinal and Aromatic Plants*, 13(5), 488-497.

Salleh, W. M. N. H. W., Ahmad, F. (2016a). Antioxidant and anticholinesterase activities of essential oil of *Alseodaphne peduncularis* Meisn. *Turkish Journal of Pharmaceutical Sciences*, 13(3), 347-350.

Salleh, W. M. N. H. W., Ahmad, F. (2016b). Antioxidant and anti-inflammatory activities of essential oils of *Actinodaphne macrophylla* and *A. pruinosa* (Lauraceae). *Natural Product Communications*, 11(6), 853-855.

Salleh, W. M. N. H. W., Ahmad, F., Khong, H. Y., Zulkifli, R. M. (2016c). Anticholinesterase and anti-inflammatory constituents from *Beilschmiedia pulverulenta* Kosterm. *Natural Product Sciences*, 22, 225-230.

Thakur, B. K., Anthwal, A., Rawat, D. S., Rawat, B., Rashmi, Rawat, M. S. M. (2012). A review on genus *Alseodaphne*: phytochemistry and pharmacology. *Mini-Reviews in Organic Chemistry*, 9, 433-445.

Turner, I. M. (1995). A catalogue of the Vascular Plants of Malaya. *Gardens' Bulletin Singapore*, 47(1), 1-346.