Nat. Volatiles & Essent. Oils, 2020; 7(3): 12-17
DOI: 10.37929/nveo.746965

NVEO
Natural Volatiles & Essential Oils

RESEARCH ARTICLE

Chemical composition of the essential oil of *Diospyros wallichii* King & Gamble (Ebenaceae)

Wan Mohd Nuzul Hakimi Wan Salleh[1,*] and Shamsul Khamis[2]

[1]Department of Chemistry, Faculty of Science and Mathematics, University Pendidikan Sultan Idris (UPSI), 35900 Tanjung Malim, Perak, MALAYSIA

[2]School of Environmental and Natural Sciences, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, MALAYSIA

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

Submitted: 02.06.2020; Accepted: 18.08.2020

Abstract

The chemical composition of the essential oil from the leaves of *Diospyros wallichii* (Ebenaceae) growing in Malaysia was investigated for the first time. The essential oil was obtained by hydrodistillation and fully characterized by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). A total of 34 components (95.8%) were successfully identified in the essential oil which were characterized by high proportions of β-eudesmol (28.5%), caryophyllene oxide (9.5%), β-caryophyllene (7.2%), α-eudesmol (6.5%) and germacrene D (6.2%).

Keywords: Ebenaceae, *Diospyros wallichii*, essential oil, hydrodistillation, β-eudesmol, GC-MS

Introduction

Essential oils are complex mixtures of volatile compounds, mainly terpenes and oxygenated aromatic and aliphatic compounds, such as phenols, alcohols, aldehydes, ketones, esters, ethers, and oxides, biosynthesized and accumulated in many plants (Dhifi et al., 2016). These naturally occurring mixtures of volatile compounds have been gaining increasing interest because of their wide range of applications in pharmaceutical, sanitary, cosmetics, perfume, food, and agricultural industries (Jugreet et al., 2020).

The Ebenaceae family contains approximately 5 genera and 500 species. The genus *Diospyros* contains about 200 species of trees, which are mainly distributed in tropical and subtropical regions (Yang & Liu, 1999). The genus has versatile uses including edible fruits, valuable timber, and ornamental uses. The leaves of *D. lotus* are approved as Chinese Medicine for the treatment of stroke and apoplexy syndrome in China or used as a hypotensive drug in Japan (Yang et al., 2020). The dry leaves of *D. kaki* are used in the treatment of hypertension, angina and internal haemorrhage in China, and have been used traditionally in Korea to promote maternal health (Han et al., 2002). In Thailand, the roots of *D. filipendula* are used as treatment for croup in children and fever (Wisetsai et al., 2019). The biological activities from the leaves of these plants such as antioxidant, anti-inflammatory, analgesic, antidiabetic, antibacterial, and antihypertensive have been validated by means of an *in vitro*, *in vivo*, and clinical tests (Tameye et al., 2020; Byun et al., 2020; Sulub-Tun et al., 2020). The chemical analyses of *Diospyros* species revealed the presence of bioactive molecules such as benzoic acid derivatives, coumarins (Wisetsai et al., 2019), ent-kaurane diterpene (Feusso et al., 2017), and triterpenes (Rauf et al., 2016). *Diospyros wallichii* commonly known as *tuba buah* in Malaysia, is grows in lowland mixed dipterocarp forests and limestone hills from sea-level to 2,300 ft altitude. It is distributed mainly in India, Thailand, Peninsular Malaysia, Sumatra, and Borneo (Utsunomiya et al., 1998). This plant grows to about 10-20 m in height, whose round fruits are poisonous and used for fishing. The twigs are rusty-hairy when young. The inflorescences bear up to nine flowers. The fruits are round, up to 2.5 cm in diameter. The leaves are alternate, simple, penni-veined, and usually hairy below. The flowers ca. 2.7 mm diameter,

DOI: 10.37929/nveo.746965

white-cream, with corolla tube, and placed in bundles in leaf axils (Smitinand, 2001). The Plant List includes two synonymous plant names of *D. wallichii* which are *Diospyros bakhuisii* Boerl. & Koord.-Schum. and *Diospyros pulchrinervia* Kosterm (The Plant List, 2012). There has been no information of this plant in traditional or folk medicine practice. Previous phytochemical investigations of *D. wallichii* have resulted in the isolation of naphthalene derivatives, naphthoquinones, coumarins, and triterpenes (Salae et al., 2010). In addition, the fruits extract of *D. wallichii* shown cytotoxicity effect against HTC116 colon carcinoma cell line (Nematollahi et al., 2012). Meanwhile, *D. wallichii* has not been previously studied for its essential oils. As a continuation part of our systematic evaluation of the aromatic flora of Malaysia (Salleh et al., 2014a, 2014b, 2014c, 2015, 2016a, 2016b, 2016c), we here report the volatile components of *D. wallichii* leaves.

Materials and Methods

Plant material

Sample of *Diospyros wallichii* was collected from Gambang, Pahang in September 2019, and identified by Dr. Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). The voucher specimen (SK358/19) was deposited at UKMB Herbarium, Faculty of Science and Technology UKM.

Isolation and analysis of essential oil

The fresh leaf (300 g) was subjected to hydrodistillation in Clevenger-type apparatus for 4 hours. The essential oil obtained was dried over anhydrous magnesium sulfate and stored at 4-6°C. Gas chromatography (GC-FID) analysis was performed on an Agilent Technologies 7890B equipped with HP-5MS capillary column (30 m long, 0.25 µm thickness and 0.25 mm inner diameter). Helium was used as a carrier gas at a flow rate of 0.7 mL/min. Injector and detector temperatures were set at 250 and 280°C, respectively. The 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 percent were reported as means ± SD of triplicates. Gas chromatography-mass spectrometry (GC-MS) analysis was recorded using a Hewlett Packard Model 5890A gas chromatography and a Hewlett Packard Model 5989A mass spectrometer. The GC was equipped with an HP-5 column. Helium was used as carrier gas at a flow rate of 1 mL/min. The injector temperature was 250°C. The oven temperature was programmed from 50°C (5 min hold) to 280°C at 10°C/min 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

For identification of essential oil components, co-injection with the standards (major components) were used, together with correspondence of retention indices and mass spectra with respect to those reported in Adams (2007). Semi-quantification of essential oil components was made by peak area normalization considering the same response factor for all volatile components. Percentage values were the mean of three chromatographic analyses.

Results and Discussion

The essential oil had a spicy odour and yielded 0.15% calculated from the fresh weight of the leaves. The GC-FID (Figure 1) and GC-MS analysis of the essential oil revealed the presence of 34 chemical components with the constitution of 95.8%.

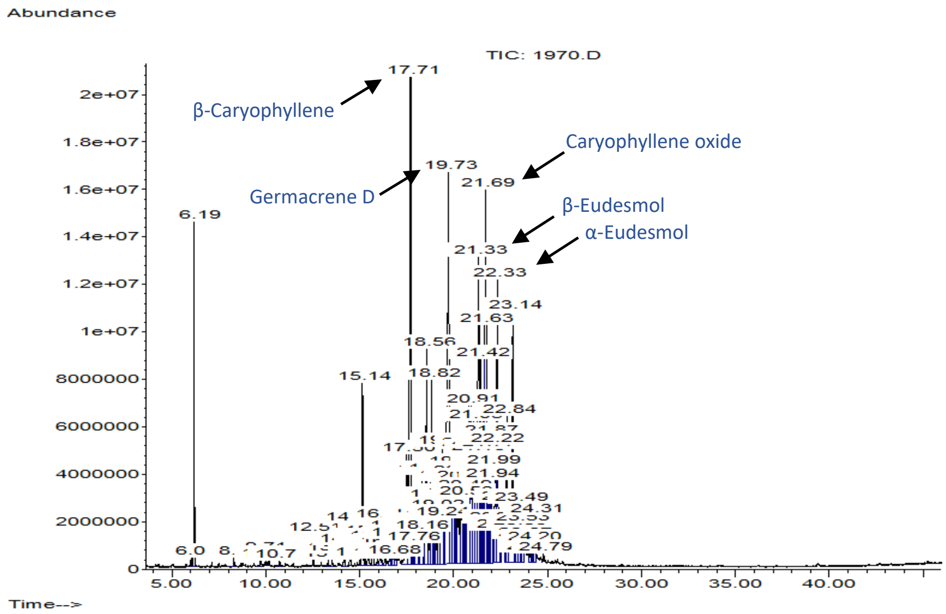
DOI: 10.37929/nveo.746965

Table 1. Chemical composition of the essential oil of *Diospyros wallichii*

RRI[a]	**RRI**[b]	**Components**	**Percentage**[c]	**Identifications**[d]
935	932	α-Pinene	2.5 ± 0.2	RI, MS
978	975	β-Pinene	2.2 ± 0.1	RI, MS
1014	1015	α-Terpinene	1.4 ± 0.2	RI, MS
1025	1029	Limonene	1.2 ± 0.2	RI, MS
1095	1097	Linalool	1.8 ± 0.2	RI, MS
1185	1189	α-Terpineol	2.5 ± 0.2	RI, MS
1351	1350	α-Cubebene	0.2 ± 0.1	RI, MS
1386	1385	δ-Elemene	2.0 ± 0.2	RI, MS
1425	1420	β-Caryophyllene	7.2 ± 0.2	RI, MS, Std
1434	1435	α-Bergamotene	0.2 ± 0.1	RI, MS
1455	1453	α-Humulene	0.2 ± 0.1	RI, MS
1458	1458	Aromandendrene	1.2 ± 0.2	RI, MS
1472	1470	β-Selinene	1.0 ± 0.2	RI, MS
1478	1480	Germacrene D	6.2 ± 0.1	RI, MS, Std
1500	1501	Bicyclogermacrene	2.4 ± 0.2	RI, MS
1502	1500	α-Muurolene	1.8 ± 0.1	RI, MS
1528	1528	α-Calacorene	0.4 ± 0.1	RI, MS
1529	1530	δ-Cadinene	4.2 ± 0.2	RI, MS
1563	1565	(*E*)-Nerolidol	3.2 ± 0.2	RI, MS
1580	1582	Caryophyllene oxide	9.5 ± 0.2	RI, MS, Std
1580	1581	Spathulenol	0.5 ± 0.1	RI, MS
1592	1595	Viridiflorol	0.8 ± 0.2	RI, MS
1600	1602	Guaiol	0.2 ± 0.2	RI, MS
1635	1639	α-Eudesmol	6.5 ± 0.1	RI, MS, Std
1640	1640	τ-Cadinol	1.2 ± 0.1	RI, MS
1650	1650	β-Eudesmol	28.5 ± 0.1	RI, MS, Std
1688	1685	α-Bisabolol	1.6 ± 0.2	RI, MS
1700	1700	Heptadecane	0.4 ± 0.1	RI, MS
1720	1722	Dodecanal	0.2 ± 0.1	RI, MS
1762	1765	(*E*)-2-Undecenal	0.8 ± 0.2	RI, MS
1945	1940	Phytol	1.5 ± 0.2	RI, MS
2400	2400	*n*-Tetracosane	0.2 ± 0.1	RI, MS
2500	2500	*n*-Pentacosane	0.2 ± 0.1	RI, MS
2931	2930	Hexadecanoic acid	1.9 ± 0.2	RI, MS
		Monoterpene hydrocarbons	7.3 ± 0.1	
		Oxygenated monoterpenes	4.3 ± 0.1	
		Sesquiterpene hydrocarbons	27.0 ± 0.2	
		Oxygenated sesquiterpenes	52.0 ± 0.2	
		Others	5.2 ± 0.1	
		Total identified	95.8 ± 0.2	

[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.

Figure 1. GC chromatogram of essential oil of *Diospyros wallichii* King & Gamble



Oxygenated sesquiterpene hydrocarbons were the most dominant components in the essential oil accounting for 52.0%, followed by sesquiterpene hydrocarbons indicated as 27.0%. Besides, monoterpene hydrocarbons were present in appreciable amounts which accounted for 1.3-7.3% of the total composition. The most abundant components of the essential oil were β-eudesmol (28.5%), caryophyllene oxide (9.5%), β-caryophyllene (7.2%), α-eudesmol (6.5%), and germacrene D (6.2%). The other minor components detected in the essential oil in more than 2% were δ-cadinene (4.2%), (*E*)-nerolidol (3.2%), α-pinene (2.5%), α-terpineol (2.5%), bicyclogermacrene (2.4%), β-pinene (2.2%), and δ-elemene (2.0%). The genus *Diospyros* is still poorly explored as far as its essential oil composition is concerned. A review of the existing literature on essential oils of the genus *Diospyros* revealed the presence of a few studies. The essential oils of *D. blancoi* (Pino et al., 2008) and *D. malabarica* (Viswanathan et al., 2002) shown the foremost components were benzyl butyrate (33.9%) and *trans*-α-methyl isoeugenol (31.5%), respectively. In addition, two studies have been reported from *D. discolor* essential oils, which discovered benzyl salicylate (26.9%) and (2Z,6E)-farnesol (35.0%) as the major components (Su et al., 2015; Smith & Belardo, 1992).

The most abundant component *D. wallichii* essential oil are endowed with important biological activities. For instance, β-eudesmol, which is one of the main volatile components of the Chinese traditional herb *Atractylodis lancea* (Asteraceae). It has shown to induce neurite outgrowth in rat pheochromocytoma cells, thus being a promising lead compound for potential neuronal function (Obara et al., 2002). Besides, it shows antiepileptic action in electroshock seizure mice (Chiou et al., 1997) and ameliorated conditions in mice after intoxication from organophosphorus anticholinesterase agents (Chiou et al., 1995). The oral administration of β-eudesmol ameliorated gastrointestinal motility in mice (Kimura & Sumiyoshi, 2012). In another study, β-eudesmol showed anticancer (Plengsuriyakarn et al., 2015) and antiangiogenic (Tsuneki et al., 2005) activities, whereas the intravenous administration of β-eudesmol caused a dose-dependent decreasing of blood pressure in rats (Lim & Kee, 2005). In conclusion, this is the first report of the chemical composition of the essential oil from *Diospyros wallichii* and the results of this study could contribute to the valorisation of this Malaysian aromatic and medicinal plant.

ACKNOWLEDGMENT

The authors would like to thank the Department of Chemistry, Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris for research facilities.

REFERENCES

Adams, R. P. (2007). *Identification of essential oil components by gas chromatography/mass spectroscopy*. Carol Stream (IL): Allured.

Byun, E. B., Kim, M. J., Kim, S. J., Oh, N. S., Park, S. H., Kim, W. S., Song, H. Y., Han, J., Kim, K., & Byun, E. H. (2020). Antioxidant activity and neuroprotective effects of ethanol extracts from the core of *Diospyros kaki. Korean Journal of Food Science and Technology*, *52*, 60-66.

Chiou, L. C., Ling, J. Y., & Chang, C. C. (1995). β-Eudesmol as an antidote for intoxication from organophosphorus anticholinesterase agents. *European Journal of Pharmacology*, *292*, 151-156.

Chiou, L. C., Ling, J. Y., & Chang, C. C. (1997). Chinese herb constituent β-eudesmol alleviated the electroshock seizures in mice and electrographic seizures in rat hippocampal slices. *Neuroscience Letters*, *231*, 171-174.

Dhifi, W., Bellili, S., Jazi, S., Bahloul, N., & Mnif, W. (2016). Essential oils' chemical characterization and investigation of some biological activities: A critical review. *Medicines*, *3*, 25.

Feusso, H. M. F., Mvot Akak, C., Feussi Tala, M., Azebaze, A. G. B., Vardamides, J. C., & Laatsch, H. (2017). Mannic acid, a new ent-kaurane dimer diterpenoid and other chemical constituents from different parts of *Diospyros mannii*. *Biochemical Systematics and Ecology*, *74*, 51-56.

Han, J., Kang, S., Choue, R., Kim, H., Leem, K., Chung, S., Kim, C., & Chung, J. (2002). Free radical scavenging effect of *Diospyros kaki*, *Laminaria japonica* and *Undaria pinnatifida*. *Fitoterapia*, *73*, 710-712.

Jugreet, B. S., Suroowan, S. Rengasamy, R. R. K., & Mahomoodally, M. W. (2020). Chemistry, bioactivities, mode of action and industrial applications of essential oils. *Trends in Food Science & Technology*, *101*, 89-105.

Kimura, Y., & Sumiyoshi, M. (2012). Effects of an *Atractylodes lancea* rhizome extract and a volatile component β-eudesmol on gastrointestinal motility in mice. *Journal of Ethnopharmacolpgy*, *141*, 530-536.

Lim, D. Y., & Kee, Y. W. (2005). Influence of β-eudesmol on blood pressure. *Natural Product Sciences*, *11*, 33-40.

Nematollahi, A., Aminimoghadamfarou, N., Bradshaw, T. D., & Wiart, C. (2012). In vitro cytotoxic activity of two malaysian rainforest plants on colon carcinoma cell-line. *Asian Journal of Pharmaceutical and Health Sciences*, *2*(1), 293-295.

Obara, Y., Aoki, T., Kusano, M., & Ohizumi, Y. (2002). β-Eudesmol induces neurite outgrowth in rat pheochromocytoma cells accompanied by an activation of mitogen-activated protein kinase. *Journal of Pharmacology and Experimental Therapeutics*, *301*, 803-811.

Pino, J., Glory, L. C., & Fuentes, V. (2008). Volatile components of Mabolo (*Diospyros blancoi* A.DC.) Grown in Cuba. *Journal of Essential Oil Research*, *20*(6), 506-508.

Plengsuriyakarn, T., Karbwang, J., & Na-Bangchang, K. (2015). Anticancer activity using positron emission tomography-computed tomography and pharmacokinetics of β-eudesmol in human cholangiocarcinoma xenografted nude mouse model. *Clinical and Experimental Pharmacology and Physiology*, *42*, 293-304.

Rauf, A., Uddin, G., Khan, H., Arfan, M., & Siddiqui, B. S. (2016). Bioassay-guided isolation of antibacterial constituents from *Diospyros lotus* roots. *Natural Product Research*, *30*, 426-428.

Salae, A. W., Karalai, C., Ponglimanont, C., Kanjana-Opas, A., & Yuenyongsawad, S. (2010). Naphthalene derivatives from *Diospyros wallichii*. *Canadian Journal of Chemistry*, *88*, 922-927.

Salleh, W. M. N. H. W., Ahmad, F., & Khong, H. Y. (2014a). Chemical compositions and antimicrobial activity of the essential oils of *Piper abbreviatum*, *P. erecticaule* and *P. lanatum* (Piperaceae). *Natural Product Communications*, *9*(12), 1795-1798.

Salleh, W. M. N. H. W., Ahmad, F., & Khong, H. Y. (2014b). Chemical composition of *Piper stylosum* Miq. and *Piper ribesioides* Wall. essential oils and their antioxidant, antimicrobial and tyrosinase inhibition activities. *Boletin Latinoamericano y del Caribe de Plantas Medicinales y Aromaticas*, *13*(5), 488-497.

Salleh, W. M. N. H. W., Ahmad, F., & Khong, H. Y. (2014c). Antioxidant and anti-tyrosinase activities from *Piper officinarum* C.DC (Piperaceae). *Journal of Applied Pharmaceutical Sciences*, *4*(5), 87-91.

Salleh, W. M. N. H. W., Kamil, F., Ahmad, F., & Sirat, H. M. (2015). Antioxidant and anti-inflammatory activities of essential oil and extracts of *Piper miniatum*. *Natural Product Communications*, *10*(11), 2005-2008.

Salleh, W. M. N. H. W., & Ahmad, F. (2016a). 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. (2016b). Essential oil composition of Malaysian Lauraceae: A mini review. *Pharmaceutical Sciences*, *22*(1), 60-67.

Salleh, W. M. N. H. W., Ahmad, F., Khong, H. Y., Zulkifli, R. M., Sarker, S. D. (2016c). Madangones A and B: Two new neolignans from the stem bark of *Beilschmiedia madang* and their bioactivities. *Phytochemistry Letters*, *15*, 168-173.

Smith, R. M., & Belardo, L. O. (1992). Volatile compounds from the fruit peelings of *Diospyros discolor* Willd. *Journal of Essential Oil Research*, *4*, 287-289.

Smitinand, T. (2001). *Thai Plant Names*. The Forest Herbarium, Royal Forest Dept., Bangkok.

Su, Y. C., Hsu, K. P., Wang, E. I. C., & Ho, C. L. (2015). Composition, in vitro cytotoxic, and antimicrobial activities of the flower essential oil of *Diospyros discolor* from Taiwan. *Natural Product Communications*, *10*, 1311-1314.

Sulub-Tun, R. A., Rodríguez-García, C. M., Peraza-Echeverría, L., Torres-Tapia, L. W., Peraza-Sánchez, S. R., Pérez-Brito, D., & Vera-Ku, B. M. (2020). Antifungal activity of wild and nursery *Diospyros cuneata*, a native species of dune scrub. *South African Journal of Botany*, *131*, 484-493.

Tameye, N. S. J., Akak, C. M., Happi, G. M., Frese, M., Stammler, H. G., Neumann, B., Lenta, B. N., Sewald, N., & Nkengfack, A. E. (2020). Antioxidant norbergenin derivatives from the leaves of *Diospyros gilletii* De Wild (Ebenaceae). *Phytochemistry Letters*, *36*, 63-67.

The Plant List (2012). Version 1. Retrieved from http://www.theplantlist.org/

Tsuneki, H., Ma, E. L., Kobayashi, S., Sekizaki, N., Maekawa, K., Sasaoka, T., Wang, M. W., & Kimura, I. (2005). Antiangiogenic activity of β-eudesmol in vitro and in vivo. *European Journal of Pharmacology*, *512*, 105-115.

Utsunomiya, N., Subhadrabandhu, S., Yonemori, K., Oshida, M., Kanzaki, S., Nakatsubo, F., & Sugiura, A. (1998). *Diospyros* species in Thailand: their distribution, fruit morphologhy and uses. *Economic Botany*, *52*, 343-351.

Viswanathan, M. B., Maridass, M., Thangadurai, & D., Ramesh, N. (2002). Chemical constituents of the fruit essential oil of *Diospyros malabarica* (Desr.) Kostel (Ebenaceae). *Acta Pharmaceutica*, *52*, 207-211.

Wisetsai, A., Schevenels, F. T., & Lekphrom, R. (2019). Chemical constituents and their biological activities from the roots of *Diospyros filipendula*. *Natural Product Research*, in press. https://doi.org/10.1080/14786419.2019.1656630

Yang, H. Q., Chen, G. H., Dong, S., Sun, Z., Wang, Y., Luo, X., Chen, B., Yao, G., Gao, Y., Chunxia, L. V., Zheng, D., Zhao, Y., Wang, T., Yan, S., & Yang, Y. (2020). Chemical constituents and medical function of leaves of *Diospyros lotus* L. *Thermal Science*, *24*, 1633-1639.

Yang, Y. P., & Liu, H. Y. (1999). *Manual of Taiwan vascular plants*. Council of Agriculture, Taipei, Taiwan.