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SHORT COMMUNICATION

VOLATILE COMPONENTS OF *Dillenia reticulata* King (DILLENIACEAE) ESSENTIAL OIL AND THEIR CYTOTOXICITY

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ABSTRACT. This work aimed to investigate, for the first time, the chemical composition and cytotoxicity of *Dillenia reticulata* King's essential oil. The essential oil was obtained through hydrodistillation, and its volatile components were analyzed through gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS) techniques. Twelve components, which constitutes more than 99.49% of oil content, were successfully identified. The most prominent components were bicyclogermacrene (44.18%), germacrene D (14.49%), β-gurjunene (12.59%), and elemol (12.25%). The cytotoxicity of essential oil was evaluated using an MTT assay. The essential oil exhibited cytotoxicity against three cancer cell lines which are HepG2, MCF7 and A549 with the IC_{50} values ranging from 61.5-68.5 µg/mL. The present study highlights the potential of using essential oil as an alternative for the development of chemopreventive or cosmetic agents for the pharmaceutical industry.

KEY WORDS: *Dillenia reticulata*, Dilleniaceae, Essential oil, Cytotoxicity, Bicyclogermacrene

INTRODUCTION

Dilleniaceae is a family of evergreen shrubs, sub-shrubs, or climbers comprising about 12 genera. It is native to tropical and warm-temperate regions such as Australia and Asia. Most of the members of Dilleniaceae consist of woody plants including *Dillenia* [1]. *Dillenia* is a genus of about 100 species of flowering plants in tropical and subtropical trees of Southern Asia, Australasia, and the Indian Ocean Islands. Several species have been reported to be used traditionally in different countries for various medicinal folklore to treat cancers, wounds, jaundice, fever, cough, diabetes mellitus, and diarrhea as well as hair tonics [2]. Phytochemical investigation of *Dillenia* species resulted in the isolation of flavonoids and triterpenoids [2]. Their extracts and phytochemicals have been reported for antimicrobial, anti-inflammatory, cytotoxic, antidiabetes, antioxidant, antidiarrheal, and antiprotozoal activities [3]. *Dillenia reticulata* King is a perennial tree with a maximum height 30 to 40 m. It is mainly distributed in Sumatra, Peninsular Malaysia, Singapore, and Borneo. In Peninsular Malaysia, it is locally known as *simpoh jangkang* or *simpoh gajah* and mainly found in terrestrial (primary rainforest, freshwater

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swamp forest) and tropical forests [4]. In continuation of our search for bioactive components from Malaysian species [5-9], we have investigated the chemical composition present in the leaves oil of *D. reticulata.* To the best of our knowledge, this is the first report on the essential oil of this species and its cytotoxicity.

RESULTS AND DISCUSSION

The identified essential oil components with their percentages are listed in order of their elution from the HP-5 column in Table 1. The GC-FID and GC-MS analysis (Figure 1) of the essential oil revealed the presence of 12 volatile chemical components with the constitution of 99.49%.

Table 1. Chemical components identified from *D. reticulata* essential oil.

No	Components	KI[a]	KI[b]	Percentage (%)	Identification[c]
1	Hedycaryol	1546	1545	1.67	RI, MS
2	β-Elemene	1389	1389	1.78	RI, MS
3	Germacrene D	1484	1484	14.49	RI, MS, Std
4	Bicyclogermacrene	1500	1500	44.18	RI, MS, Std
5	γ-Elemene	1434	1432	1.92	RI, MS
6	Valencene	1495	1496	5.58	RI, MS
7	δ-Cadinene	1524	1522	1.87	RI, MS
8	Elemol	1548	1548	12.25	RI, MS, Std
9	β-Gurjunene	1431	1430	12.59	RI, MS, Std
10	Totarene	1922	1920	0.87	RI, MS
11	Phytol	1942	1942	1.36	RI, MS
12	Manool oxide	1987	1985	0.93	RI, MS
	Sesquiterpene hydrocarbons			83.28	
	Oxygenated sesquiterpenes			13.92	
	Diterpene			2.29	
	Total identified			99.49	

[a]Linear retention index experimentally determined using homologous series of C_6-C_{30} alkanes. [b]Linear retention index taken from Adams, Wiley or NIST08 and literature. [c]Quantification was done by the external standard method using calibration curves generated by running GC analysis of representative authentic compounds.

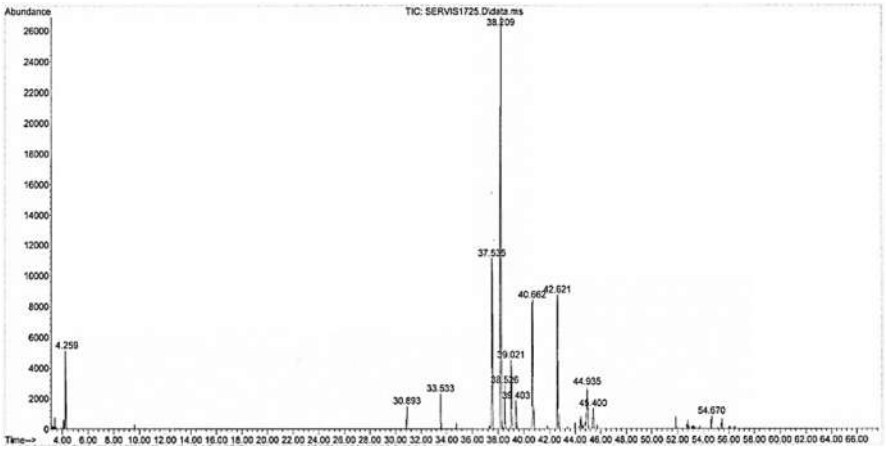

Figure 1. Chromatogram of *D. reticulata* essential oil.

The essential oil was characterized by sesquiterpene hydrocarbons which constitute 83.28% of the total composition. Meanwhile, oxygenated sesquiterpenes and diterpene were present in substantial amounts accounting for 13.92% and 2.29% of the total composition, respectively. The most prominent components were bicyclogermacrene (44.18%), germacrene D (14.49%), β-gurjunene (12.59%), and elemol (12.25%). In a previous report, bicyclogermacrene has been isolated from the aerial parts of *Senecio oxyodontus* [10], and its fungitoxic activity was further reported [11], as along with its contribution to trypanocidal and antileishmanial activities of the *Annona coriacea* oil, in which it was found in high concentration [12]. A review of the existing literature on essential oils of the genus *Dillenia* revealed the presence of only one study [13]. *D. indica* fruit oil composed mainly of sesquiterpene hydrocarbons (71.3%). The most predominant components were germacrene D (26.1%), β-caryophyllene (15.5%), α-pinene (13.3%), bicyclogermacrene (7.3%), and α-copaene (6.8%). The chemical differences among *Dillenia* species may depend on the extraction procedure, the season, the stage of development and the distinct habitat in which the plant was collected [14].

The essential oil was subjected to cytotoxic examination using MTT assay. The essential oil showed activity against three cancer cell lines HepG2, MCF7, and A549 with the respective IC_{50} values of 68.5, 61.5, and 65.2 µg/mL, as compared with those of the positive control doxorubicin (IC_{50} 0.76 µg/mL for HepG2, IC_{50} 0.20 µg/mL for MCF7, and IC_{50} 0.95 µg/mL for A549). At the highest concentration of 100 µg/mL, the essential oil responses for inhibitory 80.5% at least. As previous studies showed that the bicyclogermacrene presents cytotoxic activity against B16F10-Nex2 and HCT cell lineages, this compound was also evaluated against human lineages U87 (glioblastome), HeLa (cervical carcinoma), Siha (cervical tumor), and HFF (non-tumorigenic) [15]. The present result suggesting that the occurrence of this compound as the major component (44.1%) could be associated to the cytotoxic activity detected in the essential oil.

EXPERIMENTAL

Plant material. The leaves of *D. reticulata* were collected from Behrang Perak (N 3°44'43.8756", E 101°26'59.1864") (January 2022) and identified by Shamsul Khamis. The voucher specimen (NH-22/01) was deposited at UKMB Herbarium.

Extraction of essential oil. The fresh leaves (300 g) were subjected to hydrodistillation in Clevenger-type apparatus for 4 hours. The essential oil obtained was dried over anhydrous magnesium sulphate and stored at 4-6 °C. The oil yield (w/w) was 0.15% based on a fresh weight basis.

Gas chromatography (GC) analysis of essential oil. GC analysis were performed on an Agilent Technologies 7890B equipped with DB-5 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 temperature 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 percentage were reported as means ± SD of triplicates. Calculation of peak area percentage was carried out by using the GC HP Chemstation software (Agilent Technologies) [10].

Gas chromatography-mass spectrometry (GC-MS) analysis of essential oil. GC-MS chromatograms were recorded using an Agilent Technologies 7890A/5975C MSD equipped with HP-5MS fused silica capillary column (30 m long, 0.25 µm thickness and 0.25 mm inner diameter). Helium was used as carrier gas at a flow rate of 1 mL/min. Injector temperature was

250 °C. The oven temperature was programmed from 50 °C (5 min hold) to 250 °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 [16].

Identification of oil components. For identification of essential oil components, co-injection with the standards (germacrene D, bicyclogermacrene, elemol, β-gurjunene) were used, together with correspondence of their retention indices and mass spectra as reported in Adams [17], NIST 08 and FFNSC2 libraries. Semi-quantification of essential oil components was made by peak area normalization considering the same response factor for all volatile components.

Cytotoxicity. Cytotoxic examination of the essential oil was carried out using the MTT assay [18]. Briefly, the cells were diluted in a 96-well microplate (5×10^4 cells per well of 200 µL mixture). The samples (1-100 µg/mL) and the positive control, doxorubicin (0.05–1.56 µg/mL), were added to the cells and incubated at 37°C for 48 h with 5% CO_2. MTT (20 µL) was added to the wells and incubation was continued at 37°C for 4 h. Absorbance was recorded at 540/720 nm using a Spark multimode reader (Tecan). Each experiment was repeated in triplicate. Inhibitory percentage (%) = ([1 − OD_{sample}/OD_{conc}]) × 100%; where OD_{sample} and OD_{conc} stand for the optical densities of the samples and the control, respectively. Data obtained from the cytotoxicity are expressed as mean values. 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.

CONCLUSION

This work is the first report on the essential oil composition of *D. reticulata* growing in Malaysia. In the present study, the GC-FID and GC-MS analysis of the essential oil allowed us to identify bicyclogermacrene, germacrene D, β-gurjunene, and elemol as the most abundant components, which revealed a potent cytotoxicity. Thus, the species might be a source of natural products for further investigation into the development of chemopreventive or cosmetic agents. Future studies are also required to evaluate the side effects, safety, and efficacy of the essential oil from *Dillenia* species in order to facilitate their clinical applications as modern medicines for human health.

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