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ESSENTIAL OIL COMPOSITION AND CYTOTOXICITY OF *Decaspermum parviflorum* (Lam.) A.J.Scott FROM MALAYSIA

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The present study analyzed the chemical composition and evaluated the cytotoxicity of essential oil extracted from Decaspermum parviflorum (Lam.) A.J.Scott (Myrtaceae). The chemical composition of the essential oil was analyzed using gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Thirty-five components were identified, accounting for 97.3% and comprising mainly of monoterpene hydrocarbons, 52.5%. α-Pinene (40.5%), β-pinene (9.2%), β-eudesmol (8.0%), globulol (7.5%), and β-caryophyllene (5.1%) were identified as its major components. The oil exhibits potent cytotoxicity (IC_{50} values of 68.5-70.2 μg/mL) against three human cancer cell lines; $HepG_2$ (liver), MCF7 (breast), and A549 (lung). This is the first report on the chemical composition and cytotoxicity of D. parviflorum essential oil from the context of Malaysia in this study. The present study highlights the potential of using essential oil as an excellent alternative for the development of anticancer chemotherapeutic agents for the pharmaceutical industry.

Keywords: *Myrtaceae, Decaspermum parviflorum, essential oil, α-pinene, cytotoxicity.*

INTRODUCTION

Essential oils are natural cocktails of components exhibiting various biological activities, including antioxidant, antimicrobial, analgesic, anti-inflammatory, and anticancer activities. A recent interest is growing in the potential of various essential oils as natural source of anticancer therapeutics which may minimize the development of multidrug resistance and serious side effects associated with currently used drugs[1]. It has also been found that some essential oils potentiate the chemotherapeutic effects of docetaxel, paclitaxel, and 5-fluorouracil which opens the possibility of their use as adjuvant therapeutics in cancer treatment[2].

Decaspermum, a genus including thirty species as small trees and shrubs, belongs to Myrtaceae family and is widely located in Asia, and Eastern Australia[3]. *Decaspermum parviflorum* is a shrub or treelet located at forest edges and clearings throughout Malaysia where it is locally known as "*baduk-baduk* or *mempoyang padang"*. It also grows largely in Thailand, Peninsular Vietnam, Indonesia, Myanmar, and the Philippines[3]. It produces numerous white flowers and small blue-black fruits. The leaf blade elliptic, ovate, oblong, or lanceolate. The 'hermaphrodite' flowers were shown to have sterile pollen so the species is functionally dioecious. The fruits were eaten by birds. The species displays many features typical of dioecious tropical plants with the unusual feature of pollen being the main food reward for pollinators[3]. The plant leaves are

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chewed with betel (*Areca catechu*) as astringent treatment for dysentery. Its roots are consumed as decoction or infusion with *Ixora elliptica* leaves and roots to treat fever and fatigue while fruits are used as remedy for stomach pains[4]. The plant is classified as 'Least Concern' in the IUCN Red List of Threatened Species[5]. Our research group has a continuous interest in systematic studies regarding volatile oils of Malaysian flora[6-9].

In this regard, the composition of *Decaspermum* genus essential oil is poorly explored. Herein, we report the chemical composition and cytotoxicity of *D. parviflorum* essential oil as obtained from the Malaysian flora.

MATERIALS AND METHODS

Plant material

D. parviflorum leaves were collected in October 2020 from Behrang Perak (N 3°44'43.8756", E 101°26'59.1864"), identified Dr Shamsul Khamis at the Universiti Kebangsaan Malaysia Bangi (UKMB), and deposited at UKMB Herbarium (voucher specimen NH-22/02).

Extraction of essential oil

The *D. parviflorum* crude essential oil (0.18% w/w based on fresh leaves weight) was obtained by hydro-distilling collected fresh leaves (300 g) for 4 hrs followed by drying over anhydrous $MgSO_4$ and storage at 4-6°C.

Analysis of essential oil

Gas chromatography (GC) was performed on an Agilent Technologies apparatus (7890B). The used column was DB-5 (30 m, 0.25 µm, 0.25 mm) with helium as a carrier gas (0.7 mL/min) and the temperature of the injector and detector set at 250 and 280°C, respectively. The oven was gradually heated (5°C/min) after 15 min holding at 50°C and isothermally kept at 280°C. Diluted samples in diethyl ether (1.0 µL, 1/100v/v) were manually injected (50:1 split) as triplicates. The peak area percentages (means ±SD) were calculated using the GC HP Chemstation software (Agilent Technologies). GC-MS chromatogram was recorded using an Agilent Technologies apparatus (7890A/5975C MSD). HP-5MS fused silica capillary column (30 m, 0.25 µm, 0.25 mm) was used and helium (carrier gas) was set at 1 mL/min. The injector was adjusted at 250°C and the oven was heated from 50°C to 250°C at 10°C/min after 5 min holding and finally held isothermally for 15 min. GC-MS detection was recorded at 0.5 s (cycle time: 0.2 s) for a mass range 50-400 amu at 70 eV. Essential oil components were identified by co-injection with standards (α/β-pinene, β-eudesmol, globulol, β-caryophyllene) and comparison with FFNSC2 and NIST08 libraries for their retention indices and mass spectra[10] and were semi-quantified using peak area normalization.

Cytotoxicity assay

Cytotoxic of the essential oil was evaluated using MTT assay[11] at Laboratory of Natural Products, UPSI. Briefly, tested samples (1-100 µg/mL) were added to cancer cell lines in a 96-well microplate (200 µL, 5×10^4 cells) in triplicates and incubated at 37°C for 48 h with 5% CO_2. Doxorubicin (0.05–1.56 µg/mL), a reference anticancer drug, was used as a positive control. MTT (20 µL) was used to determine cell viability after incubation at 37°C for 4 hrs and absorbance was recorded at 540/720 nm using a Spark multimode reader (Tecan). Inhibition (%) = $[1 - OD_{sample}/OD_{conc}] \times 100$; where OD_{sample} and OD_{conc} are the samples and control optical densities.

RESULT AND DISCUSSION

Result

Components of the *D. parviflorum* essential oil are listed with their percentages in Table 1 based on their elution from the HP-5 column. Thirty-five chemical components are identified and grouped into four classes which represents 97.3% of the total oil composition. It includes seven monoterpene hydrocarbons accounting for 52.5% of the total oil composition. The second major class is oxygenated sesquiterpenes with twelve components representing 25.6% of the oil. Both sesquiterpene hydrocarbons and oxygenated monoterpenes exist in substantial amounts as 14.0% and 5.2%, respectively. Identified major components are α-pinene (40.5%), β-pinene (9.2%), β-eudesmol (8.0%), globulol (7.5%), and β-caryophyllene (5.1%) as shown in Figure 1. Minor components exceeding 2% of the oil are δ-cadinene (3.5%), 1,8-cineole (2.5%), germacrene D (2.5%), limonene (2.2%), and spathulenol (2.2%).

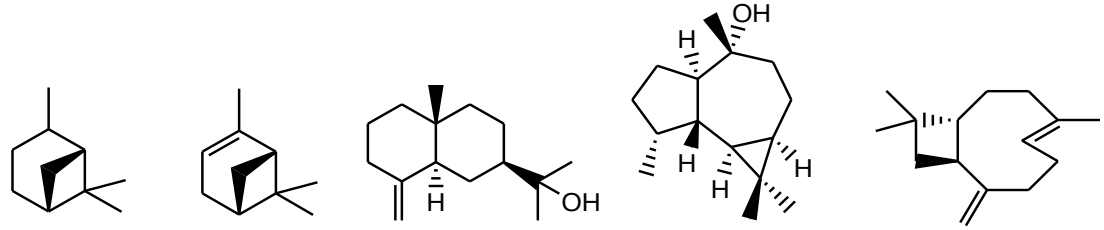
Previously, Khanh et al. (2020)[12] reported the chemical composition of essential oils

obtained from the leaves, fruits and flowers of *D. parviflorum* grown in Vietnam. The leaves oil contains mainly caryophyllene (43.9%) with other major components including humulene (10.7%), copaene (8.2%), α-selinene (6.4%), and eudesma-4(14)(11)-diene (6.1%). The fruit oil also consists of caryophyllene (23.4%) as the major component with eudesma-4(14,11)-diene (17.4%), α-selinene (13.6%), humulene (7.1%), and guaia-3,9-diene (4.5%). Meanwhile, the flower oil consists mainly of γ-elemene (37.0%), caryophyllene (14.5%), and ocimene (11.8%). In contrast, our results indicate that caryophyllene exists in low percentage (5.1%) in the oil obtained from plant leaves grown in Malaysia. Existing literature on essential oils of the genus *Decaspermum* reveals that α-pinene is the principal monoterpene component of the leaves oil of *D. struckoilicum* (37.5%) and *D. humile* (20.4%) collected from Australia[13] and the dominant component in *D. vitiense* from Fiji[14]. However, other major components (β-pinene, β-eudesmol, globulol, and β-caryophyllene) were found in minute quantities in these oils. Natural products obtained from plants growing in different geographical origins show diversity in their chemical and biological properties due to multiple factors including differences in their climatic conditions, cultivation area, vegetation phase, genetic modifications and harvesting season. This influence plant biosynthesis leading to a diversity in the type and percentage of natural products components[15].

Table 1: Chemical components identified from *D. parviflorum* essential oil.

Components	**KI[a]**	**KI[b]**	**(%)**	**Components**	**KI[a]**	**KI[b]**	**(%)**
α-Thujene	924	925	0.2	δ-Cadinene	1522	1520	3.5
α-Pinene	932	930	40.5	α-Calacorene	1544	1545	0.2
Sabinene	969	965	0.2	Germacrene B	1559	1560	0.5
β-Pinene	974	975	9.2	(*E*)-Nerolidol	1561	1560	1.9
Myrcene	988	986	0.1	Palustrol	1567	1565	0.2
α-Terpinene	1014	1012	0.1	Spathulenol	1577	1575	2.2
Limonene	1024	1024	2.2	Caryophyllene oxide	1582	1582	0.5
1,8-Cineole	1025	1026	2.5	Globulol	1590	1590	7.5
Linalool	1095	1095	1.8	Viridiflorol	1592	1590	1.7
Terpinen-4-ol	1174	1172	0.5	Guaiol	1600	1600	1.0
α-Terpineol	1186	1185	0.4	Ledol	1602	1603	0.2
δ-Elemene	1335	1335	0.2	γ-Eudesmol	1630	1630	0.2
α-Copaene	1374	1375	0.2	β-Eudesmol	1649	1650	8.0
α-Gurjunene	1408	1409	0.1	α-Eudesmol	1652	1652	1.0
Aromadendrene	1439	1438	0.6	Bulnesol	1670	1670	1.2
α-Humulene	1452	1452	0.8	***Monoterpene hydrocarbons***			**52.5**
Germacrene D	1484	1484	2.5	***Oxygenated monoterpenes***			**5.2**
β-Caryophyllene	1489	1488	5.1	***Sesquiterpene hydrocarbons***			**14.0**
α-Selinene	1498	1496	0.1	***Oxygenated sesquiterpenes***			**25.6**
Bicyclogermacrene	1500	1502	0.2	***Total identified***			**97.3**

[a]Linear retention index experimentally determined using homologous series of C_6-C_{30} alkanes; [b]Linear retention index taken from Adams.



α-Pinene β-Pinene β-Eudesmol Globulol β-Caryophyllene

Fig. 1: The major components identified in *D. parviflorum* essential oil.

The essential oil of *D. parviflorum* was evaluated for its cytotoxicity using MTT assay in the current study. A potent cytotoxicity was observed in three cancer cell lines HepG2 (liver), MCF7 (breast), and A549 (lung) with their respective IC_{50} values as 70.2, 68.5, and 69.2 µg/mL. At 100 µg/mL, the essential oil achieved inhibitory activity exceeding 82.5%, comparable to doxorubicin as a positive control with its respective IC_{50} at 0.76 µg/mL (HepG2), 0.20 µg/mL (MCF7), and 0.95 µg/mL (A549). Previous reports correlated cytotoxicity to high content of monoterpenes such as α- and β-pinene[16 & 17]. This may explain the potent cytotoxicity of the essential oil reported in the current study due to its high content of monoterpenes.

Conclusion

This study reports the composition and cytotoxicity of the essential oil obtained from *D. parviflorum* leaves growing in Malaysia. It consists of α-, β-pinene, β-eudesmol, globulol, and β-caryophyllene as major components and exhibits potent cytotoxicity. Our study highlights the potential of this species as a source of new natural anticancer agents.

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نشرة العلوم الصيدلية
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التركيب الكيميائي والسمية الخلوية للزيت المستخلص من نبات ديكاسبرموم بارفيلوروم (لام) إيه جي سكوت من ماليزيا

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تشير الدراسة الحالية إلى التركيب الكيميائي والسمية الخلوية للزيت العطري المستخلص من نبات ديكاسبرموم بارفيلوروم من ماليزيا. أظهر التحليل الطيفي لكروماتوجرافيا الغاز والكروماتوغرافيا الغازية ذات المطياف الكتلي وجود خمسة وثلاثين مكونًا تمثل ٩٧.٣ ٪ من إجمالي تركيبة الزيت. يحتوي الزيت على خمسة مكونات رئيسية: الفا بينن (٤٠.٥٪)، بيتا بينين (٩.٢٪)، إيوديسمول (٨.٠٪)، جلوبيولول (٧.٥٪)، بيتاكاريوفيللين (٥.١٪). أظهرالزيت العطري سمية خلوية ذات فعالية عالية للتركيزالنصفي المانع ضد ثلاثة خطوط خلايا سرطانية بشرية بقيم تركيزات ٦٨.٥–٧٠.٢ ميكروجرام / مل. يسلط البحث الضوء على إمكانات الزيوت الأساسية كبديل طبيعي لتطوير العلاجات الكيميائية المضادة للسرطان.