Short note

Chemical components and cytotoxicity of *Syzygium filiforme* var. *filiforme* (Myrtaceae)

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Syzygium filiforme var. *filiforme* Chantar. & J.Parn. is a plant variety from the dicotyledonous plant family (Myrtaceae). This work is designed to examine the chemical composition and cytotoxicity of *Syzygium filiforme* essential oil. The essential oil was obtained through hydrodistillation and the volatile components were analysed using gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS) techniques. The cytotoxicity of essential oil was evaluated using an MTT assay. A total of 34 chemical components, which constitute 96.1% of oil content, were successfully identified. The most abundant components were α-cadinol (23.3%), t-muurolol (9.1%), geraniol (6.6%), germacrene D (6.3%), and γ-gurjunene (5.0%). The essential oil exhibited cytotoxicity against three cancer cell lines which are HepG2, MCF7, and A549 with the IC_{50} values ranging from 70.2-88.1 µg/mL. The current study highlights the potential of the use of essential oils as an alternative to the development of pharmaceutical anti-chemopreventive or cosmetics.

Keywords: *Syzygium filiforme*, Myrtaceae, essential oil, GC-MS, cytotoxicity, α-cadinol

1. INTRODUCTION

Essential oils can be extracted from a variety of plant parts, including leaves, flowers, roots, seeds, fruits, and wood. They are made up of volatile aromatic compounds that come from the secondary metabolism of plants. Mono- and sesquiterpenes, alcohols, aldehydes, ketones, and ethers are only a few of the compounds that make up essential oils. In plants, essential oils play a protective role against herbivores and solar radiation and in attracting pollinating insects. Essential oils have been the focus of several studies because they have several bioactive chemical substances with a great potential for use, and several biological activities have been demonstrated, such as antioxidant, antimicrobial, anti-inflammatory and antifungal activities. Their uses range from agriculture to therapies to treat diseases. Many plants produce essential oils, including species of the Myrtaceae family [1-5].

The Myrtaceae family has nearly about 55,000 species classified into two subfamilies, 17 tribes, and 142 genera. The species are evergreen shrubs or woody trees and are mainly found in North Africa and South America along the Mediterranean [6]. *Syzygium* is the largest genus in the Myrtaceae family, with a wide range of species throughout Asia's tropical regions. The genus consists of about 1800 species and can be found mainly in Southeast Asia, Southern China, Australia, New Caledonia, East Africa, Madagascar, the Mascarenhas Islands, the Southwest Pacific Islands, Taiwan, and Southern Japan [7].

Syzygium species have a long history of use in traditional medicinal systems. For instance, *S. aromaticum* has promising inhibitory activities on fatty acid synthase, which in turn helps reduce food intake and subsequently induces weight loss [8]. It is also well-known for its anticancer effects and has been

the focus of much research [9]. Besides, *Syzygium* species is also renowned for having a high concentration of volatile oils, especially in the parts of the fruit [10]. Meanwhile, the species have been reported previously to treat diabetes, diarrhoea, stomach-aches, colds, and ulcers [11].

Syzygium filiforme var. *filiforme*, locally known as *kelat merah* or *kelat manik* in Malaysia, is mainly distributed in Southwest Thailand, Peninsular Malaysia, and Singapore. It is a tree that can grow up to 42 m tall, and attain 210 cm in trunk girth size, with a dense crown, drooping branches, and slender twigs. It is mainly found in terrestrial (primary rainforest, secondary rainforest, freshwater swamp forest) and tropical forests. It is harvested for timber and used for house posts [12]. According to the literature study, phytochemical studies of *S. filiforme* extracts produced triterpenoid compounds [13], which showed antioxidant, antimicrobial, and antiglucoside activity [14].

In continuation of our search for bioactive components from Malaysian species [15-20], we have investigated the chemical composition present in the leaves oil of *S. filiforme.* To the best of our knowledge, this is the first report on the essential oil of this species and its cytotoxicity.

2. MATERIAL AND METHODS

2.1. PLANT MATERIAL

The leaves of *Syzygium filiforme* were collected from Fraser Hill, Pahang (N 3°44'43.8756", E 101°26'59.1864") (January 2019) and identified by Shamsul Khamis. The voucher specimen (SK359/19) was deposited at UKMB Herbarium.

2.2. 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 0.12% based on a fresh weight basis.

2.3. ANALYSIS OF ESSENTIAL OIL

Gas chromatography (GC) analysis was performed on an Agilent Technologies 7890B equipped with a 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. The 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 percentage was 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) 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. The 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 ionisation system, with an ionisation 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.

2.4. IDENTIFICATION OF OIL COMPONENTS

For the identification of essential oil components, co-injection with the standards was used, together with correspondence of their retention indices and mass spectra as reported in Adams [21], NIST 08, and FFNSC2 libraries. Semi-quantification of essential oil components was made by peak area normalisation considering the same response factor for all volatile components.

2.5. CYTOTOXICITY ASSAY

Cytotoxic examination of the essential oil was carried out using the MTT assay [22]. 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 (I%) = $(1 - OD_{sample}/OD_{conc}) \times 100\%$; 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.

3. RESULTS AND DISCUSSION

The essential oil components identified with their percentages are listed in Table 1 in order of their elution from the HP-5 column. The GC-FID and GC-MS analysis of the essential oil revealed the presence of thirty-four chemical components with a constitution of 96.1%. The identified components were oxygenated sesquiterpenes (10 components) (48.2%), sesquiterpene hydrocarbons (23 components) (41.3%), and oxygenated monoterpene (1 component) (6.6%). The most abundant components were α-cadinol (23.3%), t-muurolol (9.1%), geraniol (6.6%), germacrene D (6.3%), and γ-gurjunene (5.0%). Meanwhile, other minor components which exceeded >2%, were

Table I - Chemical components identified in *Syzygium filiforme* essential oil

No	Components	KI[a]	KI[b]	Percentage (%)[c]	Identifications[d]
1	Geraniol	1250	1249	6.6 ± 0.2	RI, MS, Std
2	δ-Elemene	1335	1335	0.8 ± 0.1	RI, MS
3	α-Copaene	1375	1374	2.5 ± 0.1	RI, MS
4	β-Bourbonene	1385	1386	0.3 ± 0.2	RI, MS
5	β-Elemene	1390	1390	1.7 ± 0.1	RI, MS
6	α-Cedrene	1408	1410	0.3 ± 0.1	RI, MS
7	β-Caryophyllene	1415	1416	3.7 ± 0.2	RI, MS
8	γ-Elemene	1434	1435	3.3 ± 0.1	RI, MS
9	Aromadendrene	1440	1440	0.4 ± 0.2	RI, MS
10	α-Humulene	1450	1452	0.9 ± 0.2	RI, MS
11	Alloaromadendrene	1456	1458	0.2 ± 0.1	RI, MS
12	γ-Gurjunene	1470	1470	5.0 ± 0.2	RI, MS
13	γ-Muurolene	1476	1478	2.4 ± 0.2	RI, MS
14	Amorpha-4,7(11)-diene	1480	1479	0.6 ± 0.1	RI, MS
15	δ-Amorphene	1483	1485	0.5 ± 0.2	RI, MS
16	Germacrene D	1484	1485	6.3 ± 0.1	RI, MS, Std
17	β-Selinene	1490	1489	0.9 ± 0.2	RI, MS
18	γ-Selinene	1492	1492	0.5 ± 0.2	RI, MS
19	Bicyclogermacrene	1500	1500	2.5 ± 0.1	RI, MS
20	α-Muurolene	1501	1500	1.3 ± 0.1	RI, MS
21	γ-Cadinene	1513	1512	0.7 ± 0.1	RI, MS
22	δ-Cadinene	1522	1523	3.6 ± 0.2	RI, MS
23	Zonarene	1528	1530	0.5 ± 0.2	RI, MS
24	α-Cadinene	1537	1537	2.4 ± 0.1	RI, MS
25	Spathulenol	1577	1578	3.0 ± 0.2	RI, MS
26	Caryophyllene oxide	1582	1584	1.2 ± 0.1	RI, MS
27	Globulol	1590	1590	4.3 ± 0.2	RI, MS
28	Rosifoliol	1600	1600	1.2 ± 0.2	RI, MS
29	Ledol	1602	1602	0.7 ± 0.1	RI, MS
30	Junenol	1618	1618	2.7 ± 0.2	RI, MS
31	t-Muurolol	1645	1644	9.1 ± 0.1	RI, MS, Std
32	α-Cadinol	1653	1652	23.3 ± 0.1	RI, MS, Std
33	Selin-11-en-4α-ol	1685	1658	2.4 ± 0.2	RI, MS
34	β-Acoradienol	1762	1762	0.3 ± 0.1	RI, MS
Group components					
	Oxygenated monoterpenes			6.6	
	Sesquiterpene hydrocarbons			41.3	
	Oxygenated sesquiterpenes			48.2	
Identified components (%)				96.1	

[a]Linear retention index experimentally determined using homologous series of C6-C30 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
[d]RI, based on comparison of calculated RI with those reported in Adams; MS, based on comparison with Wiley; Std, based on comparison with standard compounds

globulol (4.3%), β-caryophyllene (3.7%), δ-cadinene (3.6%), spathulenol (3.0%), γ-elemene (3.3%), junenol (2.7%), α-copaene (2.5%), bicyclogermacrene (2.5%), γ-muurolene (2.4%), α-cadinene (2.4%), and selin-11-en-4α-ol (2.4%).

There have been no reports on the leaves part in this study. These subtle differences in the chemical components may be attributed to the differences in environmental and genetic factors, chemotypes, and nutritional status of the plants, which may influence their oil composition [23]. α-Cadinol has been identified as the major and most common sesquiterpene present in some *Syzygium* species. Among them are *S. cumini* (fruit oil, 25.8%) [18], *S. caryophyllatum* (leaf oil, 18.3%) [24], *S. samarangense* (leaf oil, 12.7%) [25], and *S. zeylanicum* (leaf oil, 12.2%) [26]. In addition, α-cadinol is also reported as the major component in other plants of the genus *Horsfieldia* [27], *Goniothala-*

mus [28], and *Beilschmiedia* [29]. Recently, the in-silico drug-likeness analysis showed that α-cadinol is appropriate for the human system with no predicted hepatotoxicity or mutagenicity (AMES toxicity) [30].
The essential oil was subjected to cytotoxic examination using an MTT assay. The essential oil showed activity against three cancer cell lines HepG2, MCF7, and A549 with the respective IC_{50} values of 88.1, 70.2, and 75.5 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 69.5% at least. As the previous study showed that the α-cadinol was found to exhibit selective potent cytotoxicity in breast adenocarcinoma cells (MCF7) with IC_{50} value of 18.0 µg/mL [31]. The present result suggests that the occurrence of this compound as the major component (23.3%) could be associated to the cytotoxic activity detected in the essential oil. In addition, essential oils are complex mixtures of different volatile components, where their synergistic and antagonistic interactions could affect the effectiveness of oil samples as inhibitors [32]. Further studies must be carried out to understand better the basic mechanism involved in the activity of this essential oil.

4. CONCLUSION

This study is the first report on the composition of essential oil of *S. filiforme* growing in Malaysia. In the present study, the analysis of GC-FID and GC-MS of essential oil gave α-cadinol as the most abundant component and reveals significant cytotoxicity. This species may therefore become a source of natural products and is intended to further explore the development of chemotherapy or cosmetics. In addition, future studies are needed to assess the side effects, safety, and effectiveness of the *Syzygium* essential oil to facilitate its clinical application as modern medicine for human health.

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