Short note

Chemical Composition and Cytotoxicity of the Essential Oil of *Rothmannia schoemannii* Tirveng

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The chemical composition of the essential oil of *Rothmannia schoemannii* Tirveng. (Rubiaceae) was investigated for the first time. The essential oil was obtained by hydrodistillation and fully characterised by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). In total, 28 components were identified in the essential oil, which made up 99.6% of the total oil. The essential oil is composed mainly of β-eudesmol (30.8%), α-cadinol (18.2%), caryophyllene oxide (6.5%), δ-cadinene (5.5%), and γ-eudesmol (5.4%). The essential oil exhibited cytotoxic activity against J5 (human hepatocellular carcinoma) and A549 (human lung adenocarcinoma) cells with IC_{50} values of 112.5 and 98.5 ug/mL, respectively.

Keywords: Essential oil, *Rothmannia schoemannii,* Rubiaceae, hydrodistillation, cytotoxicity.

1. INTRODUCTION

Rothmannia Thunb. is a genus of trees and shrubs belonging to the tribe Gardenieae, subfamily Ixoroideae of the family Rubiaceae. There are about 40 species of *Rothmannia*, occurring in tropical and subtropical Africa, Myanmar, the Seychelles, Indochina, the Andaman Islands, South China, the Malay Peninsula, Java, Sumatra, Borneo and Papua New Guinea [1]. Previous phytochemical investigations reported the abundance of iridoids, triterpenoids, and flavonoids [2-4]. *Rothmannia schoemannii* Tirveng. is locally known as *bengkil* in Borneo. It is native to Borneo, Peninsular Malaysia, Thailand, Sumatra, and Java. It can be found in undisturbed mixed dipterocarp and keranga forests up to 700 m altitude. The leaf has been used for kidney pain and diarrhoea with blood, drinking the leaf juice is said to help during childbirth [5]. Essential oils as secondary metabolites involve complex mixtures of natural compounds with versatile organic structures representing useful medicinal properties [6]. Essential oils are important natural sources and are used as raw materials to produce fragrance compounds in cosmetics, as flavouring additives for food and beverages, as scenting agents in a variety of household products, and as intermediates in the synthesis of other perfume chemicals [7]. Essential oils from aromatic and medicinal plants have been known since antiquity to possess biological activities, most notably antibacterial, antifungal, and antioxidant properties [8]. Regarding the essential oil composition of the genus *Rothmannia*, the literature search did not reveal any report on the essential oil composition of the genus except for *R. macrophylla*, which has been reported by us [9]. Hence, the aim of the study was to evaluate the chemical composition and cytotoxicity of the essential oils of *R. schoemannii*.

2. MATERIAL AND METHODS

2.1. PLANT MATERIAL

Sample of *R. schoemannii* was collected from Fraser Hill, Pahang (Latitude:

3°42'42.72"N Longitude: 101°44'11.6"E) in January 2023. The voucher specimens (SA-12-63) have been identified by Shamsul Khamis and deposited at UKMB Herbarium.

2.2. ISOLATION OF ESSENTIAL OILS

The fresh leaves of *R. schoemannii* (300 g) were weighed and then subjected to hydrodistillation using a Clevenger-type apparatus. A hydrodistillation run time of 4 hours was used to obtain the optimum yield without drastically affecting the oil components. The obtained oil was then dried using anhydrous magnesium sulphate, weighed, and stored in dry amber vials at 4°C until analysis. The average yield of oil was calculated as the percentage weight by weight (% w/w) of the plant material.

2.3. ANALYSIS OF ESSENTIAL OIL

Gas Chromatography with Flame Ionisation Detection (GC-FID) analysis was performed on an Agilent Technologies 7890B and an Agilent 7890B FID equipped with HP-5 column (30 m long, 0.25 µm film thickness and 0.25 mm inner diameter). At a flow rate of 0.7 mL/min, helium was used as a carrier gas. Injector and detector temperatures were set at 250 and 280°C, respectively. The oven temperature was maintained at 50°C, then slowly increased to 280°C at 5°C/min and lastly detained 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 percents were reported as means ±SD of triplicate [10]. Gas chromatography-mass spectrometry (GC-MS) chromatograms were recorded using Agilent Technologies 7890A and Agilent 5975 GC MSD equipped with HP-5MS column (30 m long, 0.25 µm thickness and 0.25 mm inner diameter). Helium was used as the 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 [11]. 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 m/z 50-400 amu. To determine the chemical components of essential oil, standards (major components) need to be injected, together with the correspondence of retention indices. The data of mass spectra were compared with those occurring in Wiley, NIST08, and FFNSC2 libraries [12]. Each peak was considered the same response factor for all components for semi-quantification of essential oil components. Quantification was done by the external standard method using calibration curves generated by running GC analysis of representative authentic compounds.

2.4. CYTOTOXICITY

Human hepatocellular carcinoma J5 and human lung adenocarcinoma A549 were obtained from ATCC (Rockville, MD, USA) and propagated in RPMI-1640 medium supplemented with 10% heat-inactivated FCS and 2 mM L-glutamine (Life Technologies, Inc., MD), and cultured in a 37°C, 5% CO_2 incubator. The cytotoxicity of the essential oil was assessed using the alamarBlue® proliferation assay according to a protocol from AbD Serotec. Cells (3000 cells/well) were incubated with either essential oils (dissolved in DMSO, final 0.1% DMSO in medium) or vehicle control (0.1% DMSO) for 24 h and 48 h, followed by replacing with fresh medium containing 10% alamarBlue® reagent for an additional 6 h. The absorbances at 570 nm and 600 nm were measured by a microplate reader. All values are given as means ± SD of 3 independent experiments [13].

2.5. STATISTICAL ANALYSIS

Data obtained from essential oil analysis and cytotoxicity were expressed as mean values. The 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 was obtained at a 0.2% (w/w) yield. The identified components in the essential oil are listed in Table 1, according to their Kovats indices (KI) on an HP-5 column. The essential oil of *R. schoemannii* revealed the presence of 28 components with a percentage of 99.6%. The essential oil was characterised by a high concentration of oxygenated sesquiterpenes (69.9%) followed by sesquiterpene hydrocarbons (26.8%). The essential oil was demonstrated by its richness in β-eudesmol (30.8%), α-cadinol (18.2%), caryophyllene oxide (6.5%), δ-cadinene (5.5%), and γ-eudesmol (5.4%). Meanwhile, other minor components detected in the oil exceeding 2%, were β-caryophyllene (3.2%), α-gurjunene (2.9%), spathulenol (2.8%), α-copaene (2.5%), germacrene D (2.5%), elemol (2.5%), α-amorphene (2.4%), β-cubebene (2.2%), and globulol (2.1%). In comparison to the previous study, β-eudesmol has also been reported as the major component in *R. macrophylla* which constituted of 20.9% [9]. Meanwhile, the chemical differences in the essential oil composition of plant species concerning their geographical origins and harvesting season have been reported, showing that the chemical and biological diversity of aromatic and medicinal plants depend on factors such as cultivation area, climatic conditions, vegetation phase, and genetic modifications. In fact, these factors influence the plant's biosynthetic pathways and consequently, the relative proportion of the main characteristic components [14, 15].

For the evaluation of the cytotoxicity of *R. schoemannii* essential oil, we tested its effect on the viability of two human cancer cell lines: J5 (human hepatocellular

Table I - Chemical components identified in from *R. schoemannii* essential oil

No	Components	KI[a]	KI[b]	Percentage (%)	Identifications[c]
1	Camphene	946	944	0.5	RI, MS
2	*α*-Terpinene	1014	1016	0.4	RI, MS
3	Limonene	1033	1032	0.2	RI, MS
4	Borneol	1165	1165	1.0	RI, MS
5	Terpinen-4-ol	1175	1172	0.8	RI, MS
6	*α*-Cubebene	1345	1345	0.5	RI, MS
7	Cyclosativene	1370	1369	0.2	RI, MS
8	*α*-Ylangene	1372	1373	0.4	RI, MS
9	*α*-Copaene	1375	1374	2.5	RI, MS
10	*β*-Cubebene	1388	1387	2.2	RI, MS
11	Longifolene	1407	1407	1.0	RI, MS
12	*α*-Gurjunene	1409	1410	2.9	RI, MS
13	*β*-Caryophyllene	1415	1417	3.2	RI, MS
14	*α*-Humulene	1435	1436	1.8	RI, MS
15	Aromadendrene	1439	1439	1.5	RI, MS
16	*α*-Amorphene	1480	1483	2.4	RI, MS
17	Germacrene D	1485	1484	2.5	RI, MS
18	**δ-Cadinene**	1520	1522	**5.5**	RI, MS, Std
19	*α*-Calacorene	1544	1545	0.2	RI, MS
20	Elemol	1548	1548	2.5	RI, MS
21	Palustrol	1565	1567	0.4	RI, MS
22	Spathulenol	1575	1577	2.8	RI, MS
23	**Caryophyllene oxide**	1582	1582	**6.5**	RI, MS, Std
24	Globulol	1590	1590	2.1	RI, MS
25	Ledol	1600	1602	1.2	RI, MS
26	**γ-Eudesmol**	1630	1630	**5.4**	RI, MS, Std
27	**β-Eudesmol**	1648	1649	**30.8**	RI, MS, Std
28	**α-Cadinol**	1650	1652	**18.2**	RI, MS, Std
	Monoterpene hydrocarbons			1.1	
	Oxygenated monoterpenes			1.8	
	Sesquiterpene hydrocarbons			26.8	
	Oxygenated sesquiterpenes			69.9	
	Total identified (%)			**99.6**	

RI: based on comparison of calculated RI with that reported in Adams; MS: based on comparison with Wiley, Adams, FFNSC2, and NIST08 MS databases; Std: based on comparison with authentic/standard compounds

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

[b]Linear retention index taken from Adams, Wiley, NIST08 and literature.

[c]Quantification was done by the external standard method using calibration curves generated by running GC analysis of representative authentic compounds.

carcinoma) and A549 (human lung adenocarcinoma) cells. Cells were incubated with various concentrations of essential oils for 48 h, and then the cell viabilities were measured by the alamarBlue® proliferation assay. The results showed that treatment for 48 h reduced the viability of J5 cells and A549 cells, with IC_{50} values around 112.5 and 98.5 µg/mL, respectively. This represents the first report of the cytotoxic activities of *R. schoemannii* essential oil against liver and lung cancer cells.

β-Eudesmol, a sesquiterpenoid alcohol, has been reported to exhibit diverse biological and therapeutic activities. It shows potent antiproliferative activity against cholangiocarcinoma (CCA), liver cancer, leukaemia, and melanoma cells [16]. Previous research on CCA cells in vitro and in animal models has shown promising anti-CCA activity of β-eudesmol. It induced apoptosis in CCA cell lines through the activation of caspase-3 and 7 [17]. The expression of the detoxifying enzymes heme oxygenase (HO)-1 and NAD(P)H quinone dehydrogenase (NOQ)-1 in CCA cells is also suppressed by β-eudesmol [18]. The antiproliferative activity of β-eudesmol against CCA cells is attributed to its inhibitory activity on STAT1/3 phosphorylation and NF-κB expression [19]. In a xenografted nude mouse model of CCA, a high dose of β-eudesmol (100 mg/kg body weight for 30 days) prevented tumour volume and lung metastasis [20]. Based on these previous reports on β-eudesmol, the cytotoxicity in this study might be explained by the presence of β-eudesmol. This study shows that the high content of this component obtained in the essential oil may contribute, at least in part, to the activity ascribed to the plant.

4. CONCLUSIONS

In this study, the GC-FID and GC-MS analysis of the essential oil allowed us to identify Oxygenated sesquiterpenes as the major group components with

the presence of β-eudesmol, α-cadinol, caryophyllene oxide, δ-cadinene, and γ-eudesmol. as the most abundant components. In addition, according to cytotoxicity, the essential oil revealed significant activity with IC_{50} value 98.5-112.5 µg/mL. The species might be a source of natural products for further investigation into the development of new antiproliferative agents, which could be used as natural additives in the food, cosmetic, and pharmaceutical industries. Thus, further phytochemical and biological studies should be carried out to identify their active constituents and toxicities.

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