Short Note

Chemical composition and acetylcholinesterase inhibitory activity of *Chassalia curviflora* (Wall.) Thwaites essential oil

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Chemical composition and acetylcholinesterase activity of the essential oil of *Chassalia curviflora* (Wall.) Thwaites (Rubiaceae) was examined for the first time. The essential oil was obtained by hydrodistillation and fully characterised by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS). A total of twenty-seven components were identified in the essential oil, which made up 91.2% of the total oil. The essential oil is composed mainly of caryophyllene oxide (22.4%), β-caryophyllene (16.5%), germacrene D (11.8%), (*E*)-nerolidol (9.2%), and elemol (5.5%). The essential oil showed significant activity against acetylcholinesterase with a percentage inhibition of 70.5%.

Keywords: Essential oil. *Chassalia curviflora*. Rubiaceae. Caryophyllene oxide. Acetylcholinesterase.

1.INTRODUCTION

The genus *Chassalia* is a shrub or small trees that belongs to the Rubiaceae family. It consists of more than 110 species and is distributed in tropical Africa, Madagascar, and tropical Asia [1]. Literature reviews indicate that some species of the *Chassalia* genus are known for their ability to synthesize cyclotides, macrocyclic and cysteine knotted peptides [2]. The genus *Chassalia* was also reported for its anti-hepatotoxic [3], anti-hypertensive [4], antibacterial [5], acaricidal [6], antimicrobial, insecticidal, and cytotoxic activities [7]. *Chassalia curviflora* locally known as '*jarum-jarum*' is a flowering and tropical woody plant. It is a shrub or small tree up to 1.5 metres of height and commonly found in South and East Asia mainly in India, Sri Lanka, China, Philippines, Indonesia, Borneo and Peninsular Malaysia [8]. The different parts of the plant are used to cure ear and eye disease, headache, skin diseases, ulcers, phlegm, rheumatism, jaundice, pneumonia, wounds and sour throat [9]. Previous phytochemical investigation on *C. curviflora* species led to the isolation of benzoic acid, benzoquinone derivatives, [10], flavonoids [11], and monoterpenoid indole alkaloids [12]. A literature survey revealed that no report on the essential oil composition of the genus *Chassalia* has been carried out. In continuation of the studies on Malaysian Flora [13-17], therefore this study reports the chemical composition and acetylcholinesterase inhibitory activity of the essential oil from the leaves of *C. curviflora*.

2. MATERIAL AND METHODS

2.1. PLANT MATERIAL

A sample of *Chassalia curviflora* was collected from Behrang, Perak in Septem-

ber 2019, and botanical identification was performed by Dr. Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). The voucher specimen (SK131/19) was deposited at UKMB Herbarium, Faculty of Science and Technology UKM.

2.2. ISOLATION OF ESSENTIAL OIL

The fresh leaf (350 g) was 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.

2.3. ANALYSIS OF ESSENTIAL OIL

Gas chromatography (GC) 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. The calculation of the peak area percentage was carried out by using the GC HP Chemstation software (Agilent Technologies). 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 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 COMPONENTS

For the identification of essential oil components, co-injection with the standards (major components) were used, together with the correspondence of retention indices and mass spectra with respect to those reported in Adams, NIST 08 and FFNSC2 libraries [18-20]. Semi-quantification of essential oil components was made by peak area normalisation considering the same response factor for all volatile components. Percentage values were the mean of three chromatographic analyses.

2.5. ACETYLCHOLINESTERASE INHIBITORY ACTIVITY

Acetylcholinesterase (AChE) inhibitory activity of the essential oil was measured by slightly modifying the reported spectrophotometric method [21, 22]. Electric eel AChE (0.22 U/mL) was used, while acetylthiocholine iodide was employed as substrates of the reaction. DTNB acid was used for the measurement of the activity. Briefly, 140 µL of sodium phosphate buffer (pH 8.0), 20 µL of DTNB, 20 µL of essential oil (conc. of 1,000 µg/mL) and 20 µL of AChE solution was added by multichannel automatic pipette in a 96-well microplate and incubated for 15 min at 25°C. The reaction was then initiated with the addition of 10 µL of acetylthiocholine iodide. Hydrolysis of acetylthiocholine iodide was monitored by the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines, catalysed by enzymes at 412 nm utilising a 96-well microplate reader (Epoch Micro-Volume Spectrophotometer, USA). The AChE inhibition percentage (I%) was determined by comparison of rates of reaction of samples relative to blank sample (EtOH in phosphate buffer pH 8) using the formula: $I\% = [E - S / E] \times 100$; where E is the enzyme activity without test sample and S is the enzyme activity with the test sample. Galantamine (conc. of 100 µg/mL) was used as a reference.

2.6. STATISTICAL ANALYSIS

Data obtained from essential oil analysis and bioactivity 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 had 0.15% calculated from the fresh weight of the leaves. The list of chemical components identified in the essential oil are shown in Table I. The GC and GC-MS analysis of the essential oil discovered the occurrence of twenty-seven components with the constitution of 91.2%. Oxygenated sesquiterpenes were the most dominant components in the essential oil accounting for 46.6%, followed by sesquiterpene hydrocarbons (34.9%). Low number of monoterpenes were also present ranging from 4.5-5.2% of the total composition. The major components of the essential oil were caryophyllene oxide (22.4%), β-caryophyllene (16.5%), germacrene D (11.8%), (E)-nerolidol (9.2%), and elemol (5.5%). The other minor components detected in the essential oil for more than 2% were δ-cadinene (3.2%), α-cadinol (2.8%), borneol (2.7%), sabinene (2.5%), t-muurolol (2.2%) and α-humulene (2.0%).

Caryophyllene oxide which is a bicyclic sesquiterpene naturally occurring in essential oils from various medicinal and edible plants and used as a flavouring agent, showed a significant central as well as a peripheral analgesic, along with anti-inflammatory activity

Table I - Chemical composition of the essential oil of *Chassalia curviflora*

N.	Components	KI[a]	KI[b]	Percentage (%)	Identification[c]
1	α-Pinene	0935	0932	0.2 ± 0.1	RI, MS
2	Sabinene	0970	0969	2.5 ± 0.1	RI, MS
3	β-Pinene	0975	0974	0.2 ± 0.1	RI, MS
4	α-Terpinene	1015	1014	1.2 ± 0.1	RI, MS
5	Limonene	1025	1024	0.4 ± 0.1	RI, MS
6	Linalool	1095	1095	0.8 ± 0.2	RI, MS
7	Borneol	1165	1162	2.7 ± 0.2	RI, MS
8	Terpinen-4-ol	1174	1175	1.5 ± 0.1	RI, MS
9	Thymol	1289	1285	0.2 ± 0.1	RI, MS
10	α-Copaene	1376	1375	0.5 ± 0.1	RI, MS
11	α-Gurjunene	1410	1409	0.2 ± 0.1	RI, MS
12	β-Caryophyllene	1415	1417	16.5 ± 0.2	RI, MS, Std
13	α-Guaiene	1435	1437	0.2 ± 0.1	RI, MS
14	Aromadendrene	1440	1442	0.5 ± 0.2	RI, MS
15	α-Humulene	1450	1452	2.0 ± 0.1	RI, MS
16	Germacrene D	1485	1484	11.8 ± 0.2	RI, MS, Std
17	δ-Cadinene	1520	1522	3.2 ± 0.1	RI, MS
18	Elemol	1555	1555	5.5 ± 0.2	RI, MS
19	(*E*)-Nerolidol	1562	1562	9.2 ± 0.2	RI, MS, Std
20	Palustrol	1567	1565	0.2 ± 0.1	RI, MS
21	Caryophyllene oxide	1580	1582	22.4 ± 0.2	RI, MS, Std
22	Viridiflorol	1595	1596	1.2 ± 0.1	RI, MS
23	Guaiol	1602	1600	0.5 ± 0.2	RI, MS
24	Ledol	1602	1602	0.2 ± 0.1	RI, MS
25	β-Eudesmol	1650	1649	2.4 ± 0.2	RI, MS
26	t-Muurolol	1635	1635	2.2 ± 0.1	RI, MS
27	α-Cadinol	1655	1655	2.8 ± 0.1	RI, MS
	Monoterpene hydrocarbons			4.5 ± 0.1	
	Oxygenated monoterpenes			5.2 ± 0.1	
	Sesquiterpene hydrocarbons			34.9 ± 0.2	
	Oxygenated sesquiterpenes			46.6 ± 0.2	
	Total identified (%)			91.2 ± 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) or NIST 08 (2008) and literature
[c]Relative percentage values are means of three determinations ± SD
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, FFNSC 2 and NIST08

[23]. It acts as a potent anti-inflammatory agent and modulator of a newly established therapeutic target, such as 15-lipoxygenase [24]. Caryophyllene oxide was found previously to inhibit multiple myeloma, breast, and prostate cancer cell lines and also believed to be an apoptotic agent inhibiting NF-κB activation [25]. Caryophyllene oxide has been found as a major component in the essential oil of *Canthium dicoccum* (leaves 19.25%) [26].

Acetylcholinesterase (AChE) inhibitors have been known to be one of the promising drugs that can be used to treat diseases associated with nervous system such as Alzheimer disease, Parkinson disease and dementia. However, the efficacy of these drugs is limited as they may cause adverse side effects and are not able to completely arrest the progression of the disease. Thus, it is important to search the novel inhibitors with reduced toxicity and preserved pharmacological activity [27]. In this study, acetylcholinesterase inhibitory activity was tested against acetylcholinesterase (AChE) enzyme. It was compared with that of galantamine, as a standard drug against Alzheimer's disease. The essential oil indicated significant AChE (I%: 70.5%) inhibitory activity at 1,000 µg/mL concentration, compared to galantamine which gave 95.9% inhibition, at 100 µg/mL concentration. In previous reports, AChE inhibition can be explained by the presence of β-caryophyllene and caryophyllene oxide which have shown cholinesterase activity. This study shows that the high content of these components obtained in the essential oil may contribute, at least in part, to the activity ascribed to the plant [28].

This study rendered a preliminary overview of anti-cholinesterase activity from the genus *Chassalia* that provided the medical relevance toward these native plant species, especially those ones with limited ethnobotanical record or practice.

4. CONCLUSIONS

The chemical composition of the essential oil isolated from fresh leaves of *C. curviflora* growing in Malaysia was studied for the first time with gas chromatography combined with mass spectrometry (GC/MS). The essential oil of *C. curviflora* consisted mainly of caryophyllene oxide (22.4%), β-caryophyllene (16.5%), germacrene D (11.8%), (*E*)-nerolidol (9.2%), and elemol (5.5%). These results shed light on the phytochemistry of this unexplored flora species found in Malaysia. In addition, to validate the above-mentioned activity, further investigation should be carried out to study the safe use of the essential oil as therapeutic agents especially against its effect with neurodegenerative diseases.

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