CHEMICAL COMPOSITION AND ACETYLCHOLINESTERASE ACTIVITY OF *Syzygium pyrifolium* (Blume) DC. ESSENTIAL OIL

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Abstract **– This study aims to investigate the chemical composition and acetylcholinesterase activity of essential oil from *Syzygium pyrifolium* leaves. The essential oil was obtained by hydrodistillation in a Clevenger-type apparatus and its chemical composition was analyzed by gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Thirty-two volatile components were identified in *S. pyrifolium* leaves oil, including geraniol (73.8%), β-pinene (7.0%), α-pinene (5.7%) and (*E*)-β-ocimene (4.5%) as the major components. Acetylcholinesterase activity was evaluated using Ellman method and the essential oil showed strong inhibitory activity (I%: 82.5%). Our findings demonstrate that *S. pyrifolium* essential oil could be useful for an alternative raw material of food and medicinal products for pharmaceutical applications.**

Keywords **– essential oil, Myrtaceae, *Syzygium pyrifolium*, GC-MS, geraniol, cholinesterase**

I. INTRODUCTION

Syzygium is the largest genus in the Myrtaceae family, with a wide range of species throughout Asia's tropical regions (Rahman *et al*., 2018). The genus consists of about 1800 species and mainly found in Southeast Asia, Southern China, Australia, New Caledonia, East Africa, Madagascar, the Mascarenhas Islands, the Southwest Pacific Islands, Taiwan, and Southern Japan (da Costa *et al*., 2020). The *Syzygium* species having a long history of use in traditional medicinal systems. For instance, *S. aromaticum* or known as clove have been used for centuries in the treatment of vomiting, flatulence, nausea, liver, bowel and stomach disorders, and as a stimulant for the nerves. In tropical Asia, cloves have been documented to relieve different microorganisms as scabies, cholera, malaria, and tuberculosis (Batiha *et al*., 2020).

Syzygium pyrifolium (Blume) DC. is locally known as *jambu hutan* or *kayu lelan* in Malaysia. The species can be found in primary rainforest and freshwater swamp forest in Malaysia, Thailand, Singapore, and Indonesia. It is a tree that can grow up to 18 m tall and 120 cm in trunk girth size (Kochummen, 1978; Parnell and Chantaranothai, 2022). There has been no information of this plant in traditional or folk medicine practice. Nevertheless, it is interesting to report the essential oil composition of *S. pyrifolium* for the first time.

Most of the species of genus are unexplored, both, pharmacologically and phytochemically. Bioactive compounds such as flavanone derivatives, ellagic acid derivatives and other polyphenolics, and terpenoids are reported from several species of the genus *Syzygium*. Meanwhile, antioxidant, antidiabetic, anticancer, toxicity, antimicrobial, anti-inflammatory and anthelmintic activities are reported in various extracts from different parts of *Syzygium* species (Aung *et al*., 2020). Although many members of the family Myrtaceae are renowned for their valuable essential oils, the genus *Syzygium* is still poorly explored as far as its essential oil composition is concerned.

Alzheimer disease (AD) is an irreversible neurodegenerative disorder which effects memory loss, learning disabilities, cognitive impairments, and diverse range of neuropsychiatric symptoms. Acetylcholinesterase (AChE) has proven to be the most viable therapeutic target for symptomatic improvement in AD. Besides AChE which is found primarily in the blood and neural synapscs is butyrylcholinesterase (BChE), an enzyme found in the liver. Many of drugs available for treatment of AD target both AChE and BChE but some are more selective than others (Salleh *et al*., 2016a; Marucci *et al*., 2021). Therefore, the search for other inhibitors from natural plants has become a necessity in the pharmaceutical field.

To the best of our knowledge, there is no report on the essential oil composition of *S. pyrifolium*.

II. METHODS

A. Plant material

Sample of *Syzygium pyrifolium* was collected from Fraser Hill, Pahang, Malaysia (3.7119°N, 101.7366°E) in September 2021 and identified by Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). The voucher specimen (SA01-12/2021) was deposited at UKMB Herbarium, Faculty of Science and Technology UKM.

B. Extraction of essential oil

The fresh leaf (300 g) was subjected to hydrodistillation in Clevenger-type apparatus for 5 hours. The essential oil

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obtained was dried over anhydrous magnesium sulphate and stored at 4-6°C. The oil yield (w/w) was 0.90% based on fresh weight basis.

C. Analysis of essential oil

Gas chromatography (GC-FID): GC-FID analysis was performed on an Agilent Technologies apparatus (7890B) equipped with a DB-5 capillary column (30 m, 0.25 µm, 0.25 mm) and helium as a carrier gas (0.7 mL/min). The injector and detector temperatures were set at 250 and 280°C, respectively. The oven temperature was gradually increased (5°C/min) and isothermally kept at 280°C after 15 min holding at 50°C. Diluted samples in diethyl ether (1.0 µL, 1/100 v/v) were manually injected (split ratio 50:1) as triplicates. The peak area percentages were calculated using the GC HP Chemstation software (Agilent Technologies) and reported as means ± SD. Gas chromatography-mass spectrometry (GC-MS) chromatogram was recorded using an Agilent Technologies apparatus (7890A/5975C MSD) equipped with HP-5MS fused silica capillary column (30 m, 0.25 µm, 0.25 mm) and helium as a carrier gas (1 mL/min). The injector temperature was adjusted at 250°C and the oven temperature was increased from 50°C (after 5 min hold) to 250°C at 10°C/min and finally held isothermally for 15 min. GC-MS detection was recorded at a scan rate of 0.5 s (cycle time: 0.2 s) covering a mass range from 50-400 amu with an ionization energy of 70 eV.

Essential oil components were identified by co-injection with standards of the major components (α-pinene, β-pinene, β-eudesmol, globulol, β-caryophyllene) and comparison of their retention indices and mass spectra with NIST08 and FFNSC2 libraries. Semi-quantification of the essential oil components was obtained by peak area normalization (FID) considering the same response factor for all volatile components.

D. Anticholinesterase inhibitory activity

AChE inhibitory activity of the essential oil was measured by slightly modifying the spectrophotometric method developed by Salleh *et al*. (2016b). Electric eel AChE was used, while acetylthiocholine iodide was employed as substrates of the reaction. DTNB acid was used for the measurement of the anticholinesterase activity. Briefly, in this method, 140 µL of sodium phosphate buffer (pH 8.0), 20 µL of DTNB, 20 µL of essential oil 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, catalyzed by enzymes at 412 nm utilizing a 96-well microplate reader (Epoch Micro-Volume Spectrophotometer, USA). Percentage of inhibition (I%) of AChE 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 activity of enzyme without test sample and S is the activity of enzyme with test sample. The experiments were done in triplicate. Galantamine was used as a reference and the preparation was compared with previous study (Cheraif *et al*., 2020).

III. RESULTS

The essential oil of *S. pyrifolium* was a yellow color with a strong odor. The yield of oil, obtained by hydrodistillation of the leaves, 0.90%. The characterization of the essential oil by GC-FID and GC-MS analyses resulted in the identification of thirty-two volatile components, accounting for 99.5% of the total oil composition (Table 1).

The essential oil was characterized by the high concentrations of oxygenated monoterpenes (75.5%) with four components. In addition, there were eighteen sesquiterpenes (4.9%), thirteen monoterpenes (94.5%), and

Table 1 - Components identified from *S. pyrifolium* oil

Components	KI[a]	KI[b]	(%)	Identification[c]
α-Pinene	932	932	**5.7**	RI, MS, Std
β-Pinene	978	974	**7.0**	RI, MS, Std
β-Myrcene	990	988	0.7	RI, MS
α-Phellandrene	1004	1001	0.1	RI, MS
Limonene	1020	1024	0.6	RI, MS
(*Z*)-Ocimene	1037	1032	0.2	RI, MS
(*E*)-β-Ocimene	1041	1044	**4.5**	RI, MS, Std
γ-Terpinene	1053	1054	0.1	RI, MS
α-Terpinolene	1086	1083	0.1	RI, MS
Linalool	1092	1095	1.0	RI, MS
4-Terpineol	1177	1174	0.1	RI, MS
α-Terpineol	1187	1186	0.6	RI, MS
Geraniol	1250	1249	**73.8**	RI, MS, Std
α-Cubenene	1347	1345	0.1	RI, MS
α-Copaene	1375	1374	0.3	RI, MS
Geranyl acetate	1380	1379	0.3	RI, MS
β-Cubebene	1390	1387	0.5	RI, MS
Longifolene	1406	1407	0.1	RI, MS
β-Caryophyllene	1417	1417	0.8	RI, MS
Aromadendrene	1440	1439	0.1	RI, MS
(*E*)-Isoeugenol	1444	1448	0.1	RI, MS
α-Amorphene	1080	1483	0.1	RI, MS
Germacrene D	1490	1484	0.1	RI, MS
β-Selinene	1487	1489	0.1	RI, MS
Cadina-1,4-diene	1450	1495	0.1	RI, MS
Ledene	1497	1496	0.1	RI, MS
Bicyclogermacrene	1499	1500	0.5	RI, MS
(*E*,*E*)-α-Farnesene	1502	1505	0.6	RI, MS
δ-Cadinene	1527	1522	0.3	RI, MS
Spathulenol	1575	1577	0.1	RI, MS
τ-Muurolol	1640	1644	0.4	RI, MS
α-Cadinol	1650	1652	0.3	RI, MS
Monoterpene hydrocarbons			19.0	
Sesquiterpene hydrocarbons			4.1	
Oxygenated monoterpenes			75.5	
Oxygenated sesquiterpenes			0.8	
Phenylpropanoid			0.1	
Total identified (%)			**99.5**	

RI: based on comparison of calculated RI with Adams; MS: based on comparison with Wiley; Std: based on comparison with standard; [a]Linear retention index experimentally determined using homologous series of C6-C30 alkanes; [b]Linear retention index taken from Adams (2007); [c]Quantification was done by the external standard method using calibration curves generated by running GC analysis of representative authentic compounds.

one phenylpropanoid (0.1%) were also detected. The major components identified in the leaf oil were geraniol (73.8%), β-pinene (7.0%), α-pinene (5.7%) and (*E*)-β-ocimene (4.5%). In comparison to prior investigations, geraniol was also presence in *S. jambos* (France: fruit: 7.3%, Malaysia: fruit: 2.3%) (Vernin *et al*., 1991) and *S. malaccense* (Malaysia: fruit: 1.0%) (Wong and Lai, 1996) essential oils, though it has been reported in a minute quantity.

Anticholinesterase inhibitory activity (I%) 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 strong AChE (I%: 82.5%) inhibitory activity at 1,000 mg/mL concentration, compared to galantamines which gave 95.9% inhibition. These results supported the hypothesis that insect AChE is a potential target for some monoterpenoids (Mukherjee *et al*., 2007). The capacity to inhibit AChE can be explained by monoterpenoids chemical structure. Although many authors showed that a bicyclic monoterpene hydrocarbon containing an allylic methyl group was a strong inhibitor of AChE activity, and they also reported the importance of the position of the double bond on the activity (Orhan *et al*., 2008). Geraniol is a monoterpenic alcohol with a pleasant rose-like aroma, occurring in the essential oils of several aromatic plants. It is known as an important ingredient in many essential oils, and is used commercially as a fragrance compound in cosmetic and household products (Chen *et al*., 2020). It was also noted that this compound has very interesting biological properties such as an antimicrobial agent, as a plant insect repellent, anti-inflammatory agent, anthelmintic, as well as a substrate in the synthesis of other compounds of health-promoting importance, such as vitamins A and E (Maczka *et al*., 2020).

V. CONCLUSIONS

The chemical composition of the volatile oil from *S. pyrifolium* collected from Malaysia was identified and described. The essential oil and its active constituents, such as geraniol, are promising sources of bioactivity substances, and the data presented in this study highlight the potential roles that monoterpenoids can contribute to this field. Hence, these findings demonstrate that *S. pyrifolium* essential oil may be an alternative natural product and a potential source of pharmaceutical agent.

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