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Essential Oil Composition and Acetylcholinesterase Inhibitory Activity of *Magnolia alba* (Magnoliaceae) Leaves from Malaysia

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Abstract: This study aims to investigate the chemical composition of the essential oil of *Magnolia alba* from Malaysia as well as acetylcholinesterase inhibitory activity. The hydrodistillation process was used to produce the essential oil, and gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC-MS) were used to analyse it. In total, 20 chemical components were identified in the leaf oil, accounting for 99.4%. The major components of the essential oil were linalool (65.4%), β-caryophyllene (6.7%), (*E*)-nerolidol (5.2%), and β-elemene (4.4%). Acetylcholinesterase inhibitory activity was evaluated using the Ellman method, respectively, in which the essential oil showed moderate inhibitory activity against acetylcholinesterase (I%: 73.5%). Thus, the findings may be helpful for identifying the medicinal and therapeutic uses of the essential oil from the *Magnolia* genus.

Keywords: essential oil, *Magnolia alba*, Magnoliaceae, linalool, acetylcholinesterase

1. Introduction

Essential oils from aromatic and medicinal plants have been known since antiquity to possess biological activity, most notably antibacterial, antifungal and antioxidant properties. Essential oils are important natural sources and are used as raw materials for the production of 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 (Salleh et al., 2016).

Acetylcholinesterase (AChE) is a cholinergic enzyme primarily found at postsynaptic neuromuscular junctions, especially in muscles and nerves. It immediately breaks down or hydrolyzes acetylcholine (ACh), a naturally occurring neurotransmitter, into acetic acid and choline. The primary role of AChE is to terminate neuronal transmission and signaling between synapses to prevent ACh dispersal and activation of nearby receptors. Hydrolysis of acetylcholine is required to allow a cholinergic neuron to return to its resting state after activation (Colovic et al., 2013). Essential oils and their chemical constituents have been shown to have effects on the central nervous system, including in the treatment of Alzheimer's disease and Parkinson's disease (Lahlou, 2004). The components of essential oils are characterized by their small size and lipophilicity, thus facilitating movement across the blood–brain barrier. Their characteristic volatility may facilitate their use in inhalation, avoiding the metabolic channel, with its facilitator denaturing the active components (Lomarat et al., 2015).

Magnoliaceae, a family of *Magnolia* and the order of Magnoliales consists of about 17 genera and 300 species. It is mainly dispersed in mild and tropical Asian and American continents. Several genera have been recognized such as *Alcimandra*, *Lirianthe*, *Manglietia*, *Michelia*, *Pachylarnax*, *Parakmeria*, *Talauma*, and *Yulania*, whereas the genus *Magnolia* and *Liriodendron* are restricted to China (Xia et al., 2008). The genus *Magnolia* comprises about 219 species and is widely distributed in Asian and American regions. These species are geologically located in the Southeastern United States, Mexico, Central America, the Caribbean, and Southeast Asia. The most common *Magnolia* species such as *M. salicifolia*, *M. kobus*, *M. macrophylla*, *M. ashei*, *M. acuminate*, *M. grandiflora*, *M. virginiana*, and *M. liliiflora* which are native to Japan, Korea, Southeastern US, Mexico, and China (Myers, 2020).

The genus embraces both deciduous and evergreen trees and shrubs with a height of 9 to 31 meters tall, with most species are thin, smooth bark and large leaves and flowers. The woods are soft and light in color and are used in making crates, boxes, and furniture. *Magnolia* species have unique ornamental values, strong anti-pollution ability, and wide adaptability, especially in China, Japan, Thailand, and India (Shen et al., 2008). The species is easily recognizable as they

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are valued for their large and fragrant white, yellow, pink, or purple flowers, frequently smooth and shining leaves, and cone-like fruits. The flowers, usually cuplike and fragrant, are located at the branch tips and have three sepals, 6 to 12 petals arranged in 2 to 4 series, and many spirally arranged stamens. The seeds, usually reddish, often hang pendulously by slender threads (Britannica, 2019).

Herbal medicines continue to be rapidly used by many people across the world for the treatment of various health and to cure illness. In this regard, the use of *Magnolia* species as an herbal medicine has also been widely embraced in many developed countries with complementary and alternative medicines (Anquez-Traxler, 2011). For example, in ancient Chinese and Japanese medications, *Magnolia* bark is an ingredient in *Hange-koboku-to*, which consists of five plant extracts, and in *Saiboku-to*, which consists of ten plant extracts. These extracts are used to decrease anxiety and nervous tension and boost sleep. Besides, some researchers reported that the bark and flower buds of *Magnolia* are employed for weight loss, digestion, constipation, inflammation, anxiety, stress, depression, fever, headache, stroke, and asthma (Kuribara et al., 2000).

Magnolia alba, locally known as Cempaka Putih in Malaysia. It is distributed mainly in Java, Indonesia, Peninsular Malaysia, Singapore, and Borneo. In earlier reports, the traditional medicine of *M. alba* was used to treat headache, sinusitis, cough, inflammation, flatulence, nausea, and vaginal discharge (Khairan et al. 2021). Given the relevance of this genus' therapeutic benefits in the treatment of various ailments, it's evident that more research is needed. In fact, investigations on the essential oil composition and acetylcholinesterase inhibitory of *M. alba* have been conducted. Thus, this study reports the chemical composition and acetylcholinesterase inhibitory activity of the essential oil from the leaves of *M. alba*.

2. Material and Methods

2.1. Plant material

The leaves of *M. alba* was collected from Behrang, Perak (3° 45' 24.29"N 101° 29' 48.84"E), in September 2021 and identified by a botanist Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). The voucher specimen (BB-05/21) was deposited at the UKMB Herbarium, Faculty of Science and Technology UKM.

2.2. Isolation of essential oil

The fresh leaf (200 g) was subjected to hydrodistillation in a Clevenger-type apparatus for 6 h. The essential oil obtained was dried over anhydrous magnesium sulphate and stored at 4–6°C. On a fresh weight basis, the oil yield (w/w) was 0.12%, and the average moisture content was 86–89%.

2.3. Analysis of essential oil

2.3.1. Gas chromatography (GC)

The analysis was performed using a Hewlett Packard 6890 series II instrument. A gas chromatograph equipped with an HP-5 column (30 m × 0.25 mm × 0.25 m film thickness). 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 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 L were injected manually (split ratio 50:1). The injection was repeated three times, and peak area percentages were reported as means standard deviations of triplicates. The calculation of the peak area percentage was carried out by using the GC HP Chemstation software (Agilent Technologies).

2.3.2. Gas chromatography-mass spectrometry (GC-MS)

GC-MS chromatograms were recorded using a Hewlett Packard Model 5890A gas chromatograph and a Hewlett Packard Model 5989A mass spectrometer. The GC was equipped with an HP-5 column. Helium was used as a carrier gas at a flow rate of 1 mL/min. The injector temperature was 250 °C. The oven temperature was programmed to rise from 50°C (5 min hold) to 250°C at 10 °C/min before remaining isothermal for 15 min. For GC-MS detection, and electron an ionisation system with 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 of 50–400 amu.

2.4. Identification of components

For the identification of essential oil components, co-injection with the standards (linalool, β-caryophyllene, (E)-nerolidol, and β-elemene) were used, together with the correspondence of retention indices and mass spectra with respect to those reported in Adams, NIST 08, and FFNSC2 libraries. Semi-quantification of essential oil components was made by peak area normalization, considering the same response factor for all volatile components.

2.5. Acetylcholinesterase (AChE) inhibitory activity

The AChE inhibitory activity of the essential oil was initially measured by slightly modifying the spectrophotometric method (Salleh et al., 2015). Electric eel AChE was used, while acetylthiocholine iodide was employed as a substrate of the reaction, and DTNB acid was used for the measurement of the anticholinesterase activity. In brief, 140 µL of sodium phosphate buffer (pH 8.0), 20 µL of DTNB, 20 µL of essential oil, and 20 µL of AChE (0.22 U/mL) solution were added into a 96-well microplate and incubated for 15 min at 25°C. The reaction was then initiated by adding 10 µL of acetylthiocholine iodide. The hydrolysis of acetylthiocholine iodide was monitored by the formation of the yellow 5-thio2-nitrobenzoate anion as a result of the reaction of DTNB with thiocholines at 412 nm using a 96-well microplate reader (Epoch Micro-Volume Spectrophotometer, USA). The percentage of inhibition (I%) of AChE was determined by comparing the reaction rates of the relative to the blank sample (EtOH in phosphate buffer, pH 8). Analyses were expressed as means ± SD of triplicates, and galantamine at the same concentration as essential oil was used as a positive control.

3. Results and Discussion

The GC-FID and GC-MS (Fig. 1) analysis of the leaf oil of *M. alba* led to the identification of 20 components, representing 99.4% of the total oil.

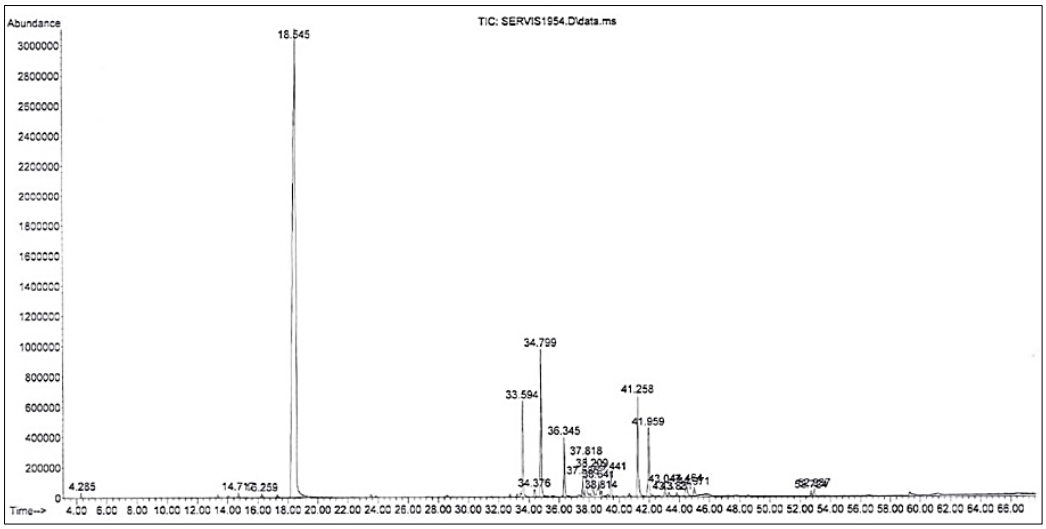

Fig. 1: Chromatogram of *M. alba* essential oil

In the order of elution on the HP-5 column, the identified components are listed in Table 1. Analysis of chemical components identified in *M. alba* leaf oil consists of several groups of components such as monoterpene hydrocarbon (0.2%), sesquiterpene hydrocarbons (21.7%), oxygenated monoterpenes (65.6%), oxygenated sesquiterpenes (11.5%), and phenylpropanoid (0.4%). The most contributed components identified in the leaf oil were linalool (65.4%), β-caryophyllene (6.7%), (*E*)-nerolidol (5.2%), and β-elemene (4.4%).

The monoterpenes were comprised of one monoterpene hydrocarbon (0.2%) and two oxygenated monoterpenes (65.6%). The oxygenated monoterpenes in *M. alba* leaf oil revealed a high abundance of linalool (65.4%) as well as (*Z*)-linalool oxide (0.2%). Previously, linalool was found to be dominant in the leaf and flower oil of the same species collected from Malaysia with a percentage of 76.6%, 67.1%, and 85.8%, respectively (Abu Shah, 2009; Nasution et al., 2019). Moreover, linalool was also reported previously from various *Magnolia* species such as *M. hookeri* (Vietnam: leaf oil, 21.3%; twig oil 17.1%) (Ha et al., 2021), *M. insignis* (Vietnam: leaf oil 24.1%; twig oil 26.9%) (Ha et al., 2021), *M. sirindhorniae* (Thailand: bud oil 58.9%; flower oil 51.0%) (Ghosh et al., 2021), *M. denudata* (Japan: branch oil 6.7%) (Fujita et al., 1977), *M. kobus* (Japan: bud oil 5.9%) (Nagasawa et al., 1969), and *M. salicifolia* (Japan: flower bud oil 28.0%) (Nagasawa et al., 1969). Furthermore, ten components of sesquiterpene hydrocarbons (21.7%) and six components of oxygenated sesquiterpenes (11.5%) were also recognized. β-Caryophyllene (6.7%) had the greatest percentage, followed by β-elemene (4.4%), and α-humulene (2.8%). In prior studies, β-caryophyllene has been reported in diverse *Magnolia* species specifically from *M. sirindhorniae* (Thailand: flower oil 6.4%) (Ghosh et al., 2021), *M. grandiflora* (USA: fruit oil 7.9%, seed oil 19.3%; flower oil 34.8%) (Ali et al., 2020; Schuhly et al., 2008; Luo et al., 2012; Garg and Kumat, 1999), *M. denudata* (Japan: leaf oil 18.4%; flower bud oil 5.0%) (Fujita et al., 1977), *M. kobus* (Korea: fruit oil 8.1%) (Sowndhararajan et al., 2016), and *M. tripetala* (USA: fruit oil 21.0%) (Schuhly et al., 2008).

The leaf oil also contains oxygenated sesquiterpenes, presence mainly of (*E*)-nerolidol (5.2%), accompanied by caryophyllene oxide (3.7%). Earlier, (*E*)-nerolidol was reported abundantly in *M. hookeri* (Vietnam: leaf oil, 12.2%) (Ha

et al., 2021), *M. insignis* (Vietnam: leaf oil 22.5%) (Ha et al., 2021), *M. denudata* (Japan: leaf oil 25.9%; branch oil 5.3%) (Fujita et al., 1977), and *M. acuminata* (USA: fruit oil 20.0%) (Schuhly et al., 2008) essential oils. Furthermore, there are seven components that were not identified in *Magnolia* species by comparing with the previous studies. They were (*Z*)-linalool oxide, methyl eugenol, viridiflorene, β-bisabolene, germacrene A, humulene epoxide II, and 1-epi-cubenol. The percentage of these components is displayed in the range of 0.2-1.3%. The chemical variations across *Magnolia* species could be attributable to developmental phases and the habitat whereby the plant was harvested. Aside from that, the chemical and biological variety of aromatic and medicinal plants is influenced by factors such as environmental situation, vegetation phase, and gene mutations. Such factors influence the plant's biosynthetic pathways and, as a result, the relative fraction of the primary distinctive chemicals (Salleh et al., 2015).

Table 1: Chemical components identified from the leaf oil of *M. alba*

No	Components	KI[a]	KI[b]	Percentage (%)
1	(*E*)-β-Ocimene	1044	1044	0.2
2	(*Z*)-Linalool oxide	1067	1067	0.2
3	Linalool	1095	1095	65.4
4	β-Elemene	1389	1389	4.4
5	Methyl eugenol	1403	1403	0.4
6	β-Caryophyllene	1417	1417	6.7
7	α-Humulene	1454	1452	2.8
8	Germacrene D	1483	1484	1.0
9	β-Selinene	1490	1489	2.1
10	Viridiflorene	1496	1496	0.3
11	α-Selinene	1497	1498	1.6
12	β-Bisabolene	1505	1505	0.3
13	Germacrene A	1508	1508	1.3
14	δ-Cadinene	1522	1522	1.2
15	(*E)*-Nerolidol (3)	1561	1561	5.2
16	Caryophyllene oxide	1583	1582	3.7
17	Viridiflorol	1592	1592	0.6
18	Humulene epoxide II	1610	1608	0.6
19	1-epi-Cubenol	1627	1627	0.3
20	ι-Muurolol	1640	1640	1.1
Group components				
	Monoterpene hydrocarbon			**0.2**
	Sesquiterpene hydrocarbons			**21.7**
	Oxygenated monoterpenes			**65.6**
	Oxygenated sesquiterpenes			**11.5**
	Phenylpropanoid			**0.4**
Total identified				**99.4**

KI[a] – Kovats index experimental; KI[b] – Kovats index of literature (Adams, 2001)

Regarding the biological activities, we investigated the acetylcholinesterase inhibitory properties of the isolated essential oil. AChE is the key enzyme that catalyses the breakdown of some choline ester compounds that function as neurotransmitters. The most important among them is acetylcholine, which is found in all autonomic ganglia and different synapses in the central nervous system. Reversible inhibitors of acetylcholine have been used for the treatment of some neurodegenerative disorders, especially Alzheimer's disease (Masondo et al., 2019). In this study, the acetylcholinesterase activity is based on the hydrolysis of acetylthiocholine iodide 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 a wavelength of 412 nm using a spectrophotometer or a microplate reader. Acetylthiocholine iodide work as substrates of the reaction, while the DTNB is utilized for the measurement of cholinesterase activity. The percent inhibition of enzymatic activity is calculated from the rate of change in the absorption of the reaction mixture. It was compared with that of galantamine, as a standard drug against Alzheimer's disease. In this study, the essential oil revealed moderate AChE (I%: 73.5%) inhibitory activity at 1000 mg/mL concentration, compared to galantamine, which gave 95.9% (AChE) inhibition, at the

same concentration (Table 2). In previous reports, AChE inhibition can be explained by the high content of monoterpenes. It has been mentioned that 1,8-cineole, camphor, a-pinene, b-pinene, borneol, linalool, menthone, carvone, anethole, anisole, have anticholinesterase activity (Savelev et al. 2004; Picollo et al. 2008). This study shows that the essential oils had low content of monoterpenes, hence contributed to the moderate AChE inhibition.

Table 2: Acetylcholinesterase inhibitory activity of the leaf oil of *M. alba*

Sample	Inhibition (%)
Essential oil	73.5
Galantamine	95.9

4. Conclusion

A study on the essential oil of *M. alba* the existence of oxygenated monoterpenes as the major group component, dominated by linalool. Based on the reported enzyme inhibition activities, the essential oil might be used for aromatherapy in patients with Alzheimer's dementia or inflammatory disease in order to improve the oxidative status or as an adjuvant to regular therapy. Nevertheless, it remains to identify the distribution of the aromatic compounds and their impact on certain types of receptors to better establish their mechanism of action.

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