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Phytochemical Studies from *Magnolia alba* and Their Antioxidant Activity

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Abstract

The purposes of this study were to investigate the phytochemicals and antioxidant activity from *Magnolia alba* (Magnoliaceae). The antioxidant activity was investigated using total phenolic content and DPPH free radical scavenging assay. Isolation and purification of the leaf extracts of *M. alba* yielded six phytochemicals identified as squalene (**1**), parthenolide (**2**), vanillin (**3**), stigmasterol acetate (**4**), friedelin (**5**), and β-sitosterol (**6**). In antioxidant activity, the hexane extract of *M. alba* showed the highest value of phenolic content with 206.7 mg GAE/g, whereas methanol extract gave the highest inhibition in DPPH assay with 36.0%. However, all compounds were found weak activity in DPPH assay. The implication of this study demonstrates that *M. alba* could represent a source of molecules with antioxidant potential in the prevention of free radical-related diseases such as atherosclerosis and diabetes.

Keywords: Magnoliaceae; *Magnolia alba*; phytochemicals; parthenolide; antioxidant; DPPH

INTRODUCTION

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* [1]. Magnoliaceous plants are of extraordinary value in botanical research. Magnoliaceae trees and shrubs have two ranked stipulated leaves that enclose young buds, while the flowers are hermaphrodite and actinomorphic. Magnoliaceae is closely related to the Annonaceae in terms of floral structure and organization. Essentially, they are evergreen broad-leaved woodlands and deciduous broad-leaved timberlands from tropical to mild zones as well as celebrated trees for decorative, timber, therapeutic, and fragrance [2].

Most of the genus of the Magnoliaceae family have a woody plant with primitive flowering. Several plants in this family are valuable for traditional uses. For instance, the bark of *Magnolia officinalis* is very popular in China as a remedy for flatulent dyspepsia, cough, and asthma [3]. *Michelia champaca* has been used in traditional medicine to treat postpartum depression, fever, diabetes, and hypertension [4], whereas the root bark of *Liriodendron tulipifera* is used as a febrifuge in paroxysmal fever [5]. This family, also economically used for timber such as *Michelia excelsa* wood, is a valuable commercial timber known as whitewood [6]. Magnoliaceae plants appear to be of major importance due to their wide range of phytochemicals and biological characteristics that have been reported. Several Malaysian Magnoliaceae

species have not been thoroughly explored chemically nor biologically. Given the relevance of this genus' therapeutic benefits in the treatment of a variety of ailments (asthma, constipation, edema, abdominal distension, and malaria), it's evident that more research is needed [7].

Magnolia alba is locally known as *Cempaka Putih* in Peninsular Malaysia. It is mainly distributed in Indonesia, Peninsular Malaysia, Singapore, and Borneo. It has been traditionally used to treat headache, sinusitis, cough, inflammation, flatulence, nausea, and vaginal discharge [8]. In fact, investigations on the isolation of phytochemicals and the antioxidant activity of *M. alba* have been conducted. Thus, the outcomes of the study may contribute to the pharmaceutical industry in the future.

MATERIALS AND METHODS

Plant material

The leaves of *M. alba* were collected from Fraser's Hill (3.7119° N, 101.7366° E) on September 2020, and identified by Shamsul Khamis from Universiti Kebangsaan Malaysia (UKM). The voucher specimen (SB42-73) was deposited at UKMB Herbarium, Faculty of Science and Technology, UKM.

General Experimental Procedure

Cold extraction method was used to acquire the crude extract from the dried samples using *n*-hexane, ethyl acetate, dichloromethane, and methanol. Gravity column chromatography (CC) was carried out using Merck SiO_2 (70-230 Mesh). Thin layer chromatography (TLC) analysis was performed on 0.20 mm precoated silica gel aluminium sheets (Merck Kieselgel 60 F254). Preparative thin layer chromatography (PTLC) was conducted using a 1 mm thin glass plate of Merck SiO_2 $60F_{254}$. *n*-Hexane, EtOAc, DCM, $CHCl_3$, Et_2O, and MeOH were used as solvent systems in chromatographic method. The spot on TLC plate was detected by ultraviolet (UV) illumination at 254 nm and 365 nm, before being sprayed with vanillin sulphuric acid reagent. All reagents used in this study were analytical grade and purchased from Sigma Aldrich. For structural elucidation, Infrared Spectroscopy (IR), Nuclear Magnetic Resonance (NMR), and Mass spectrometry (MS) were used in this study. The IR spectrum was recorded on Perkin Elmer series 1600 spectrophotometer (KBr pellet for solid and NaCl discs for liquid sample) and Perkin Elmer Spectrum 100 FT-IR Spectrometer. 1D and 2D NMR spectra were recorded on a JEOL Spectrometer (JNM-ECX-500). Chemical shifts were reported in ppm. Residual solvents ($CDCl_3$) were used as an internal standard. Mass spectral data were obtained from Liquid Chromatography-Mass Spectrometry (Thermo Fisher Scientific).

Extraction and Isolation of Phytochemicals

Cold extraction of the dried powdered leaves of *M. alba* was performed *via* a polarity gradient of *n*-hexane (4 days), EtOAc (4 days), and MeOH (4 days). The *n*-hexane leaf extract (MALH, 8.2 g) was subjected to CC and eluted with *n*-hexane:$CHCl_3$ to afford 4 fractions (MALH A-D). The combined fraction MALH A was purified and identified by PTLC to yield compound (**1**) as colourless oil. The fraction MALH B was purified followed by PTLC to afford compound (**4**) as a white solid. The fraction MALH D was purified and washed with diethyl ether to afford compound (**2**) as a white crystalline solid. Purification of the EtOAc extract (MALEA, 10.3 g) by CC eluted with *n*-hexane:EtOAc afforded 6 fractions (MALEA A-F). Fraction MALEA B was purified and followed by PTLC to attain compound (**3**), later washed with *n*-hexane and then formed a colorless solid. The fraction MALEA C was also purified and followed by PTLC to obtained compound (**5**) as white needle solid and compound (**6**) as white crystalline needles. Purification of the MeOH extract (MALM, 15.8 g) by CC eluted with *n*-hexane:EtOAc afforded 4 fractions (MALM A-D). Fraction MALM B was purified and identified as a compound similar to MALH extract, compound (**1**). The combined fraction MALM D was purified and washed with Et_2O and then produced a similar compound to MALH extract of compound (**4**). The chemical structures of isolated compounds (**1-6**) are shown in Figure 1.

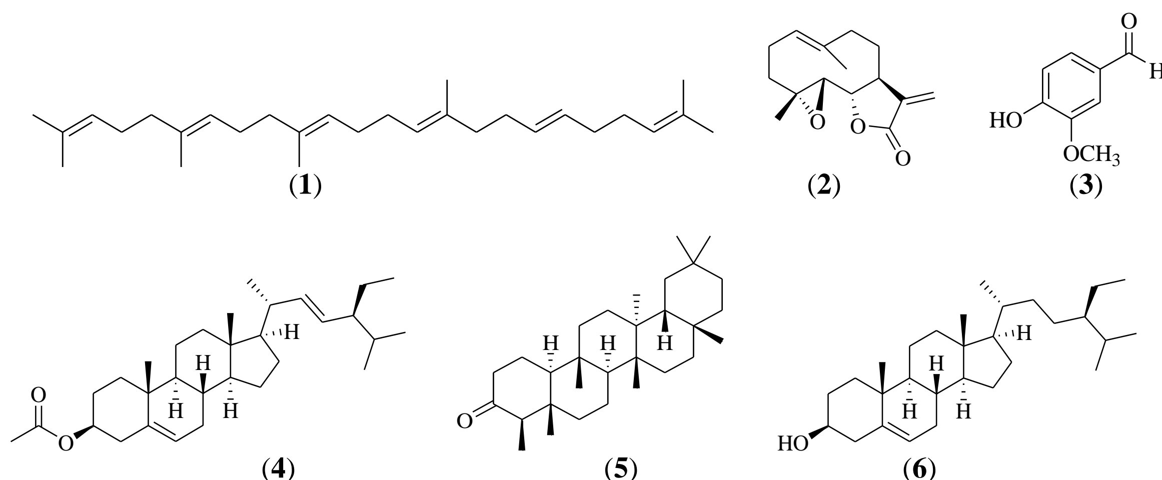

Figure 1. Chemical structures of isolated compounds (**1-6**).

Squalene (**1**). Colourless oil (10.5 mg); Melting point: -75°C; IR (KBr) υ_{max}: 2924, 1651 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.56 (18H, s, H-25, H-26, H-27, H-28, H-29, H-30), 1.65 (6H, s, H-1, H-24), 2.01 (8H, m, H-4, H-12, H-13, H-21), 2.05 (12H, m, H-5, H-8, H-9, H-16, H-17, H-20), 5.09 (4H, m, H-3, H-7, H-18, H-22), 5.15 (2H, m, H-11, H-14); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 15.7 (C-27, C-28), 16.0 (C-26, C-29), 17.6 (C-25, C-30), 25.5 (C-1, C-24), 26.6 (C-8, C-17), 26.7 (C-4, C-21), 28.2 (C-12, C-13), 39.7 (C-9, C-16), 39.7 (C-5, C-20), 124.0 (C-11, C-14), 135.0 (C-10, C-15); MS *m/z* 409.3433 $[M+H]^+$, $C_{30}H_{50}$ [13].

Parthenolide (**2**). White crystalline solid (11.9 mg); Melting point: 115-117°C; IR (KBr) υ_{max}: 2924, 1764, 1664, 1246, 943 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.20-1.28 (2H, m, H-3a), 1.27 (3H, s, H-15), 1.68 (3H, s, H-14), 1.70-1.72 (1H, m, H-8b), 2.11-2.21 (1H, s, H-2a, H-3b, H-8a, H-9a, overlapping peak), 2.32-2.38 (1H, s, H-2b, H-9b, overlapping peak), 2.74-2.77 (1H, s, H-7, overlapping peak), 2.76 (1H, d, J = 8.82 Hz, H-5), 3.83 (1H, t, J =8.58 Hz, H-6), 5.18 (1H, dd, J = 2.52 and 12.00 Hz, H-1), 5.59 (1H, d, J = 3.3 Hz, H-13b), 6.30 (1H, d, J = 3.72 Hz, H-13a); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 17.1 (C-14), 17.4 (C-15), 24.3 (C-2), 30.8 (C-8), 36.5 (C-3), 41.3 (C-9), 47.8 (C-7), 61.7 (C-4), 66.5 (C-5), 82.6 (C-6), 121.3 (C-13), 125.4 (C-1), 134.7 (C-10), 139.4 (C-11), 169.4 (C-12); MS *m/z* 249.1490 $[M+H]^+$, $C_{15}H_{20}O_3$ [14].

Vanilin (**3**). Colourless solid (10.1 mg); Melting point: 80-81°C; IR (KBr) υ_{max}: 3189, 2976, 1663, 1586, 1508, 1198 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 3.96 (3H, s, OCH_3), 6.50 (1H, s, OH), 7.04 (1H, d, J = 8.4 Hz, H-5), 7.42 (1H, d, J = 2.0 Hz, H-2), 7.44 (1H, dd, J = 8.4 and 2.0 Hz, H-6), 9.83 (1H, s, CHO); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 56.1 (3-OCH_3), 108.7 (C-5), 114.4 (C-2), 127.6 (C-1), 129.7 (C-6), 147.2 (C-4), 151.7 (C-3), 191.1 (CHO); MS *m/z* 153.0545 $[M+H]^+$, $C_8H_8O_3$ [15].

Stigmasterol acetate (**4**). White solid (10.1 mg); Melting point: 140-144°C; IR (KBr) υ_{max}: 2920, 1738, 1463, 1177 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.67 (3H, s, H-18), 0.78 (3H, d, J =7.0 Hz, H-29), 0.80 (3H, d, J = 6.8 Hz, H-26), 0.81 (3H, d, J = 6.8 Hz, H-27), 0.88 (3H, d, J = 6.6 Hz, H-21), 1.15 (3H, s, H-19), 1.22-2.33 (25H, m, overlap CH and CH2), 2.14 (3H, s, H-31), 3.60-3.63 (1H, m, H-3), 4.97-5.13 (1H, m, H-23), 5.30-5.34 (1H, m, H-22), 5.69 (1H, s, H-6); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 12.0 (C-18), 12.1 (C-29), 19.0 (C-26), 19.2 (C-19), 19.6 (C-11), 20.0 (C-27), 20.4 (C-31), 21.3 (C-21), 23.3 (C-15), 24.5 (C-28), 26.0 (C-2), 28.8 (C-25), 29.1 (C-16), 31.9 (C-8), 32.1 (C-7), 36.3 (C-10), 36.7 (C-1), 38.1 (C-4), 39.7 (C-12), 40.4 (C-20), 42.5 (C-13), 45.6 (C-24), 50.1 (C-9), 56.0 (C-17), 56.8 (C-14), 72.0 (C-3), 121.9 (C-6), 129.3 (C-23), 138.2 (C-22), 140.9 (C-5), 170.5 (C-30); MS *m/z* 454.3724 $[M+H]^+$, $C_{31}H_{50}O_2$ [16].

Friedelin (**5**). White needle solid (11.5 mg); Melting point: 261-264°C; IR (KBr) υ_{max}: 2859, 1714, 1444, 1381 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.69 (3H, s, H-24), 0.83 (3H, s, H-23), 0.85 (3H, s, H-25), 0.92 (3H, s, H-29), 0.96 (3H, s, H-27), 0.97 (3H, s, H-30), 1.01 (3H, s, H-26), 1.14 (3H, s, H-28), 1.93 (1H, m, H-1), 2.22 (1H, m, H-4), 2.34 (1H, dd, J = 2.2 and 6.0 Hz, H-2a), 2.37 (1H, dd, J = 2.2 and 6.0 Hz, H-2b); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 6.8 (C-23), 14.7 (C-24), 17.9 (C-25), 18.3 (C-7), 18.7 (C-26), 20.3

(C-27), 22.3 (C-1), 28.2 (C-20), 30.0 (C-17), 30.5 (C-12), 31.8 (C-30), 32.1 (C-28), 32.4 (C-21), 32.8 (C-15), 35.0 (C-29), 35.4 (C-22), 35.6 (C-16), 36.0 (C-11), 37.5 (C-9), 38.3 (C-13), 39.3 (C-19), 39.7 (C-14), 41.3 (C-6), 41.5 (C-2), 42.2 (C-5), 42.8 (C-18), 53.1 (C-8), 58.3 (C-4), 59.5 (C-10), 213.2 (C-3); MS *m/z* 426 $[M+H]^+$, $C_{30}H_{50}O$ [17].

β-Sitosterol (**6**). White crystalline needles (12.3 mg); Melting point: 133-134°C; IR (KBr) υ_{max}; 3427, 2934, 1666, 1055 cm^{-1}; 1H NMR ($CDCl_3$, 500 MHz): δ 0.65 (3H, s, H-18), 0.78 (3H, d, J = 6.8 Hz, H-27), 0.79 (3H, d, J = 6.8 Hz, H-26), 0.81 (3H, d, J = 7.8 Hz, H-29), 0.89 (3H, d, J = 6.5 Hz, H-21), 0.97 (3H, s, H-19), 1.02-2.36 (29H, m, overlapping CH and CH2), 3.49 (1H, m, H-3), 5.32 (1H, d, J = 5.2 Hz, H-6); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 12.0 (C-29), 12.0 (C-18), 18.7 (C-21), 19.0 (C-27), 19.8 (C-26), 20.2 (C-19), 21.0 (C-11), 23.1 (C-28), 24.2 (C-15), 26.1 (C-23), 28.2 (C-16), 29.4 (C-25), 31.5 (C-2), 32.0 (C-8), 31.9 (C-7), 33.9 (C-22), 36.1 (C-20), 36.6 (C-10), 37.5 (C-1), 39.6 (C-12), 42.3 (C-4), 42.4 (C-13), 45.8 (C-24), 53.8 (C-9), 55.9 (C-17), 56.0 (C-14), 71.9 (C-3), 123.7 (C-6), 140.5 (C-5); MS *m/z* 414 $[M+H]^+$, $C_{29}H_{50}O$ [18].

Antioxidant: Total Phenolic Content

The method was carried out as described by Salleh et al. [9] with slight modifications. Sample of stock solution (1.0 mg/mL) was diluted in MeOH to a final concentration of 1000 µg/mL. A 100 µL aliquot of sample was pipetted into a test tube containing 900 µL of MeOH, then 50 µL Folin-Ciocalteau reagent was added, mixed well, and left for about 2 min. After 2 min, 500 µL of 5% Na_2CO_3 solution was added and the contents were vortexed for 15 sec. Then, 2.5 mL of MeOH was added and left to stand in the dark for 1 h. The absorbance measurements were recorded using UV spectra (Shimadzu UV 1601PC spectrophotometer) at 765 nm. The same procedure was repeated for the standard gallic acid solutions and a standard curve was obtained with the following equation; $y = (3\times10^{-7})x - 0.001$, $r^2 = 0.7252$. The total phenolic contents in all samples were calculated using the formula: C = c V/m; where, C = total phenolic content, mg gallic acid (mg GA/g) equivalents per gram dry extract, c = concentration of gallic acid obtained from the calibration curve in µg/mL, V = volume of extract in mL, m = mass of extract in mg. Test was carried out in triplicate and gallic acid equivalent value was reported as mean ± SD of triplicate.

DPPH Free Radical Scavenging

The DPPH free radical scavenging assays of extracts and selected phytochemicals were investigated as a previous method [10] with slight modifications. The DPPH solution was freshly prepared in MeOH. The samples were in MeOH (200 µL) with a 1000 µg/mL concentration and mixed with the DPPH solution (3.8 mL). The mixture was allowed to stand for 30 min at room temperature in the dark, and then the absorbance was recorded at 517 nm. The percentage inhibition of DPPH (%) was calculated using the following formula; $I\% = [A_{blank} - A_{sample} / A_{blank}] \times 100$; where A_{blank} is the absorbance value of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance value of the test compounds. Ascorbic acid was used as a standard and diluted to the same concentration as the samples.

RESULTS AND DISCUSSION

Six compounds were isolated from the leaf extract of *M. alba* comprising of sesquiterpene lactone; parthenolide (**2**), phenolic; vanillin (**3**), and triterpenes; squalene (**1**), stigmasterol acetate (**4**), friedelin (**5**), and β-sitosterol (**6**). These compounds were identified by modern techniques and comparison with those reported in the literatures [13-18].

The IR spectrum of compound (**1**) showed a strong band at 2924 cm^{-1} from sp^3 C-H stretching and a medium band at 1651 cm^{-1}, indicating the presence of a C=C olefinic. The ^1H NMR spectrum displayed a multiplet at δ 5.09 attributed to an olefinic group. Another multiplet signals at δ 1.96 and 2.01 were assigned to methylene groups, and the singlet signals at δ 1.56 and 1.65 integrated for 24 protons were attributed to 8 methyl groups. The COSY spectrum showed strong correlations between the olefinic and methylene groups, also correlations between the methylene groups itself. The ^{13}C NMR and DEPT spectra showed 15

peaks from 15 carbons, which were classified as four methyl, five methylene, three methine, and three quaternary carbons. Meanwhile, the MS spectrum showed a molecular ion peak at *m/z* 410, consistent with the molecular formula $C_{30}H_{50}$. Based on these physical properties and by comparison with the spectroscopic data, compound (**1**) was identified as 2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene namely as squalene [13].

The IR spectrum of compound (**2**) displayed an aliphatic sp^3 C-H stretching at 2924 cm^{-1}, the carbonyl group (C=O) at 1764 cm^{-1}, C=C at 1664 cm^{-1} and the C-O at 1246 cm^{-1}. In addition, the band at 943 cm^{-1} suggested the presence of epoxide. The ^1H NMR spectrum showed the presence of two singlets at δ 1.68 (H-14) and 1.27 (H-15), both integrating for two methyl protons. The methylene protons at δ 1.20-1.28 (H-3a), 1.70-1.72 (H-8b), 2.11-2.21 (H-2b, H-9b), and 2.32-2.38 (H-2a, H-3b, H-8a, H-9a) were observed as multiplet as overlapping signals. In addition, the olefinic protons H-13a and H-13b appeared as doublet signals at δ 6.30 (J = 3.7 Hz) and 5.59 (J = 3.3 Hz), respectively. The methine groups were observed as one doublet of doublet at δ 5.18 (J = 2.5 and 12.0 Hz, H-1), one triplet at δ 3.83 (J = 3.58 Hz, H-6), one doublet at δ 2.76 (1H, d, J = 8.82 Hz, H-5), and one multiplet at δ 2.74-2.77 (1H, s, H-7, overlapping peak). The ^{13}C NMR and DEPT spectra showed the presence of fifteen carbon atoms, comprising of four quaternary at δ 61.7 (C-4), 134.7 (C-10), 139.4 (C-11), 169.4 (C-12); two methyls at δ 17.1 (C-14), 17.4 (C-15); five methylenes at δ 24.3 (C-2), 30.8 (C-8), 36.5 (C-3), 41.3 (C-9), 121.3 (C-13); and four methines at δ 47.8 (C-7), 66.5 (C-5), 82.6 (C-6), 125.4 (C-1). The MS spectrum showed a molecular ion peak at *m/z* 249.1490, which was compatible with a molecular formula of $C_{15}H_{20}O_3$. Based on its spectroscopic data, compound (**2**) was identified as 4α,5β-epoxy-germacra-1-(10),11(13)-dien-12,6α-olide, or also known as parthenolide [14].

The IR spectrum of compound (**3**) displayed the characteristic absorption bands at 3189 cm^{-1} (O-H), 1663 cm^{-1} (C=O), 1586 and 1508 cm^{-1} (C=C aromatic) stretching. The ^1H NMR spectrum showed two singlet signals corresponding to hydroxyl and methoxyl groups at δ 6.10 and 3.98, respectively. It also contained three aromatic protons of a 1,3,4-trisubtituted aromatic ring appearing as doublets at δ 7.60 (J = 1.6 Hz, H-2) and 6.98 (J = 8.4 Hz, H-5). In addition, the doublet of doublets was observed at δ 7.72 (1H, dd, J = 8.4 and 1.6 Hz) corresponding to the proton of H-6. A singlet signal which was observed at δ 9.83 was confirmed the presence of aldehyde protons. The ^{13}C NMR and DEPT spectra showed the presence of eight carbon atoms attributed to three methine at δ 108.8 (C-2), 114.5 (C-5), 129.8 (C-6), three quaternary at δ 127.6 (C-1), 147.2 (C-3), 151.8 (C-4), one methoxyl at δ 56.1 (3-OCH_3), and one carbonyl at δ 191.1 (CHO). The presence of an aldehyde moiety was proven by a downfield signal at δ 191.1 in the ^{13}C NMR spectrum. The molecular formula was determined as $C_8H_8O_3$ based on the molecular ion peak at *m/z* 153.0545 in the MS spectrum. Based on the analysis of its physical properties and comparison with spectroscopic data with literatures, compound (**3**) was determined to be 4-hydroxy-3-methoxybenzaldehyde or known as vanillin [15].

The IR spectrum of compound (**4**) indicated an absorption band for 2920 cm^{-1} (sp^3 C-H), 1738 cm^{-1} (C=O), 1463 cm^{-1} (C=C), and 1177 cm^{-1} (C-O). The ^1H NMR spectrum displayed three singlet signals at δ 0.67, 1.15, and 5.69 attributed to methyl groups, 18-CH_3, 19-CH_3, and olefinic proton, H-6, respectively. Two doublets resonated at δ 0.78 and 0.79 were assigned to H-27 and H-26, each with J = 6.8 Hz. Another doublet signal resonated at δ 0.78 (J = 7.0 Hz) and 0.88 (J = 6.6 Hz) were attributed to two methyl groups at H-29 and H-21, respectively. The ^{13}C NMR and DEPT spectra indicated the presence to 31 carbons attributed to four quaternary carbons, seven methyl carbons, nine methylenes carbons, and eleven methines carbons. The ^1H and ^{13}C NMR parameters and data comparison from the literature are tabulated in Table 4.6. Furthermore, the MS spectrum showed a molecular ion peak at *m/z* 454.3724 which attributed to a molecular formula $C_{31}H_{50}O_2$. Based on the basis of the studied spectral data and a literature comparison, the compound (**4**) was identified as stigmast-5-en-3β-ol acetate or namely as stigmasterol acetate [16].

The IR spectrum of compound (**5**) showed a strong absorption band at 1716 cm^{-1} which was associated with C=O of cyclic ketone. The ^1H NMR spectrum indicated the presence of eight methyl groups resonated as a singlet signal at δ 0.69 (H-24), 0.83 (H-23), 0.85 (H-25), 0.92 (H-29), 0.96 (H-27), 0.97 (H-30), 1.01 (H-26), and 1.14 (H-28). Two multiplet signals at δ 1.93 and 2.22 correspond to methylene protons of H-1 and methyl proton of H-4, respectively. Two set doublet of doublets signals, each resonated at δ 2.34 (J = 2.2 and 6.0 Hz) and 2.37 (J = 2.2 and 6.0 Hz) were assigned to H-2a and 2b, respectively. The ^{13}C NMR spectrum showed the presence of 30 carbon signals. The identification of these signals was confirmed by the DEPT spectra and deduced as seven quaternary carbons, four methine carbons,, eleven methylene carbons and eight methyl carbons. The MS spectrum displayed a molecular ion peak at *m/z* 426, which was

in agreement with the molecular formula $C_{30}H_{50}O$. Compound (**5**) was elucidated as friedooleanan-3-one also known as friedelin, based on the comparison of the physical properties and spectral data [17]. To the best of our knowledge, this is the first report of the presence of friedelin in *M. alba*.

Compound (**6**) displayed the presence of OH (3427 cm^{-1}), sp^3 C-H (2934 cm^{-1}), C=C (1666 cm^{-1}) and C-O stretching (1055 cm^{-1}) in the IR spectrum. The ^{1}H NMR displayed a similar profile to that of compound (**4**) except the replacement of the acetyl group with an addition of hydroxyl group. The ^{13}C NMR and DEPT spectra showed the presence of 29 carbon atoms consisting of six methyls, eleven methylenes, nine methines, and three quaternary carbons. The MS spectrum showed a molecular ion peak at *m/z* 414 which attributed to a molecular formula $C_{29}H_{50}O$. Compound (**6**) was assigned as β-sitosterol based on the comparison of its physical properties and spectroscopic data [18].

In this study, the crude extracts and selected phytochemicals were evaluated for their antioxidant activity using total phenolic content (TPC) and DPPH free radical scavenging assays. The total phenolic content of the extracts were evaluated based on Folin-Ciocalteu's method at the concentration of 1 mg/mL. The results were expressed as mg gallic acid equivalent (GAE) per gram of the extracts, as shown in Table 1. The number of phenolic compounds in the hexane extract was found to be the highest with 206.7 mg GAE/g. For the phenolic assay, the presence of hydroxyl groups in phenolic compounds was recognised to be responsible for the antioxidant activity in plant extracts. The higher the phenolic contents, the effective sample for antioxidant activity [11].

Table 1. TPC of the *M. alba* leaf extracts

Samples		Total Phenolic Content Gallic Acid Equivalent (mg GAE/g)
Extracts of *M. alba*	MALH	206.7
	MALEA	176.7
	MALM	93.3

MALH = *M. alba* leaf n-Hexane, MALEA = *M. alba* leaf EtOAc, MALM = *M. alba* leaf MeOH

In addition, the DPPH free radical scavenging activity of the extracts and selected phytochemicals were also evaluated. The results are shown in Table 2. The *M. alba* extracts displayed a moderate percentage inhibition with ranging 26.6-36.0%. These findings could be due to the presence of non-polar compounds in *M. alba* extracts which have minimal ability to act as free radical scavengers [12]. As for the isolated compounds, all compounds are shown weak activity ranging 13.3-28.0% inhibition.

Table 2. DPPH radical scavenging of the extracts and isolated phytochemicals

Samples		Percentage inhibition (I%) at 1000 µg/mL
Extracts of *M. alba*	MALH	34.2
	MALEA	26.6
	MALM	36.0
Compounds	Squalene (**1**)	28.0
	Parthenolide (**2**)	13.3
	Vanillin (**3**)	20.5
Standard	Ascorbic acid	91.7

MALH = *M. alba* leaf n-Hexane, MALEA = *M. alba* leaf EtOAc, MALM = *M. alba* leaf MeOH

CONCLUSION

Plant secondary metabolites provide a promising source for the discovery of antioxidant inhibitors which are important for the treatment of various diseases. In the current study, phytochemicals from *M. alba* leaves extracts had yielded sesquiterpene lactone, phenolic, and terpenes. Besides, further studies are needed to reveal the mode of action of these compounds to understand their possible roles in human physiology

DECLARATION OF INTEREST

The authors confirmthat there is no conflict of interest.

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