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Research article

Extraction, isolation and characterization of chemical constituents of the leaves of *Magnolia lamdongensis*

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Abstract

Recently, a number of new species of the Magnoliaceae family have been discovered and published such as *Magnolia tiepii*, *M. lamdongensis*, *M. bidoupensis*. Phytochemical study of the leaves of *M. lamdongensis*, an endemic species of Lam Dong province, Vietnam, resulted in the isolation of four compounds, which were named stigmast-5-en-3*β*-ol-3-*O*-*β*-D-glucopyranoside (**1**), stigmast-5-en-3*β*-ol (**2**), quercetin (**3**), and palmitic acid (**4**). Their structural elucidations were confirmed by 1 D and 2 D NMR experiments and a comparison with those reported in the literature. These metabolites isolated for the first time from this species.

Introduction

Magnolia (Magnoliaceae), a genus of trees or shrubs, has about 250 species that grow in temperate and tropical climates, primarily in India, Malaysia, Japan, China, Vietnam and America [1]. Many species in this genus are of great value and have been used in traditional medicines to treat a wide range of diseases. Because of its anti-inflammatory activity, *M. fargesii* has been widely used in traditional medicine to treat empyema, nasal congestion, sinusitis, and allergic rhinitis [2]. *M. grandiflora* flowers and leaves have long been used to treat headaches, hypertension, fever, diarrhoea, and rheumatism [3]. The trunk bark of *M. ovata* has been used to treat fever, and the leaves are thought to be useful in the treatment of diabetes [4]. Previous chemical studies on *Magnolia* species revealed the presence of lignans [5, 6], alkaloids [7], and sesquiterpenoids [8]. The biological activities of compounds isolated from *Magnolia* genus have also been thoroughly investigated. Some of the most notable biological activities in this genus are cytotoxic activity [9], antioxidant activity [10], and anti-inflammatory activity [6]. Among them, sesquiterpenoids have been shown to have coronary vasodilator activity [11] as well as cytotoxicity [12], anti-inflammatory, and antihyperalgesic properties [13].

Magnolia lamdongensis belonging to the genus *Magnolia* were collected in Lam Dong province. In the previous report, three flavonoids (astragalin, quercetin 3-neohesperidoside, and quercetin 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranoside) and a sterol (stigmasterol) were isolated from the leaves of this species [14]. Continuing our research, four compounds **1-4** have been isolated for the first time from the leaves of *M. lamdongensis*. This is the first report about isolated compounds from this plant.

Material and methods

General experimental procedures

Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70–230 mesh and 230–400 mesh, Merck, Darmstadt, Germany) and reversed-phase silica gel (ODS-A, 12 nm S-150 mm, YMC Co., Ltd., Japan) resins. TLC used pre-coated silica gel 60 F_{254} (1.05554.0001, Merck) and RP-18 F254S plates (1.15685.0001, Merck), and compounds were visualized by spraying with aqueous 10% H_2SO_4 and heating for 3–5 min. The ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on an AVANCE III HD 500 (Bruker, Germany) FT-NMR spectrometer with tetramethylsilane (TMS) was used as an internal standard. ESI mass spectra were collected on Agilent 1100 LC/MS systems. The IR spectra were recorded on a JASCO FT/IR 4100 FT-IR in KBr.

Plant material

Magnolia lamdongensis samples were collected in September 2020 at the Phu Son slope in Lamdong, Vietnam, and identified by Dr. Nong Van Duy of the Tay Nguyen Institute for Scientific Research, VAST. A voucher specimen (TN3/163) was deposited at the Tay Nguyen Institute for Scientific Research, VAST.

Extraction and isolation

The air-dried and powdered leaves of *M. lamdongensis* (2.0 kg) were extracted three times with methanol (10L/time) at room temperature. The methanol solutions were filtered, combined, and concentrated under reduced pressure to obtain methanol residue (267 g). This was suspended in water (2L) and partitioned in turn with *n*-hexane, chloroform, and ethyl acetate to give the corresponding extracts: *n*-hexane (H, 15.0 g), $CHCl_3$ (C, 20.8 g), EtOAc (E, 19.9 g), and water layer (W, 2L).

Column chromatography

The extract C (20.8 g) was separated on silica gel CC with stepwise gradient elution of $CHCl_3$/MeOH (1:0-0:1, v/v) to yield thirteen fractions, C1-C11. Fraction C11 (1.94 g) was fractionated by Sephadex LH-20 CC with stepwise gradient elution MeOH/H_2O (9:1-1:0, v/v) to yield five subfractions, C11A-C11E. Subfraction C11C (234 mg) was subjected to a silica gel CC eluted with $CHCl_3$/MeOH/H_2O (5:1:0.1, v/v/v) to yield five subfractions, C11C1-C11C5. Subfraction C11C2 (104 mg) was purified by the RP-18 column eluted with MeOH/H_2O (4:1, v/v) to yield compound **1** (15 mg). Fraction C10 (1.4 g) was further separated by sephadex LH-20 CC with MeOH/H_2O (1:1-1:0, v/v) to give four subfractions C10A-C10D. Subfraction C10D (72 mg) was separated by silica gel CC eluting with $CHCl_3$/MeOH/H_2O (3:1:0.1, v/v/v) and purified by RP-18 column using MeOH/H_2O (1:1-1:0, v/v) as elution to yield compound **3** (10 mg).

The extract H (15.0 g) was separated on silica gel CC with stepwise gradient elution of hexane/EtOAc (1:0-0:1, v/v) to yield seven fractions, H1-H7. Fraction H4 (1.8 g) was fractionated by Sephadex LH-20 CC with stepwise gradient elution MeOH/H_2O (9:1-1:0, v/v) to yield six subfractions, H4A-H4F. Subfraction H4F (89 mg) was purified by the silica gel column eluted with *n*-hexane:CH_2Cl_2 (10:1, v/v) to yield compound **2** (12 mg). Fraction H3 (4.8 g) was further separated by column chromatography on silica gel CC using a mixture of *n*-hexane:CH_2Cl_2 (20:1, v/v) to afford seven subfractions, H3A-H3G. Subfraction H3B (1.1 g) was fractionated by RP-18 CC with MeOH/H_2O (9:1, v/v) to yield three subfractions, H3B1-H3B3. Subfraction H3B3 (82 mg) was subjected to chromatography on the silica gel column eluted with CH_2Cl_2:acetone (30:1, v/v) to yield compound **4** (22 mg).

The physical constants and NMR data of compounds 1-4

Stigmast-5-en-3β-ol-3-*O*-β-D-glucopyranoside (1): White needle; molecular formula $C_{35}H_{60}O_6$; ESI-MS *m/z* 577.43 $[M+H]^+$; IR (KBr): 3433 cm^{-1} (OH), 1639 cm^{-1} (C=C), 1461 cm^{-1} and 1380 cm^{-1} (*gem*-dimethyl group), 1053 cm^{-1} (C-O). ^1H NMR (500 MHz, DMSO) and ^{13}C NMR (125 MHz, DMSO):see table 1.

Stigmast-5-en-3β-ol (2): White needle; molecular formula $C_{29}H_{50}O$; ESI-MS *m/z* 415.12 $[M+H]^+$; IR (KBr): 3424 cm^{-1} (OH), 2937 and 2870 cm^{-1} (C-H sp^3), 1641 cm^{-1} (C=C), 1379 cm^{-1} (*gem*-dimethyl groups), 1056 cm^{-1} (C-O). ^1H NMR (500 MHz, $CDCl_3$) and ^{13}C NMR (125 MHz, $CDCl_3$): see table 1.

Quercetin (3): Yellow powder; molecular formula $C_{15}H_{10}O_7$; ESI-MS: *m/z* 303.23 $[M+H]^+$. IR (KBr): 3406 cm^{-1} (OH), 1666 cm^{-1} (C=O), 1610 cm^{-1} (C=C, aren). ^1H NMR (500 MHz, CD_3OD)δ_H: 7.75 (1H, br s, H-2′), 7.65 (1H, d, *J* = 8.5 Hz, H-6′), 6.91 (1H, d, *J* = 8.5 Hz, H-5′), 6.41 (1H, br s, H-8), 6.21 (1H, br s, H-6);^{13}CNMR (125 MHz, CD_3OD) δ_C: 148.03 (C-2), 137.20 (C-3), 177.53 (C-4), 162.51 (C-5), 99.25 (C-6), 165.57 (C-7), 94.42 (C-8), 158.25 (C-9), 104.53 (C-10).

Palmitic acid (4): White amorphous powder; molecular formula $C_{16}H_{32}O_2$; ESI-MS *m/z* 257.35 $[M+H]^+$; IR (KBr): 1702 cm^{-1} (C=O), 3321 cm^{-1} (OH). ^1H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR (150 MHz, $CDCl_3$):see table 1.

Result and Discussion

Compound **1** was isolated as a white needle with the molecular formula $C_{35}H_{60}O_6$ determined by ESI-MS *m/z* 577.43 $[M+H]^+$. The IR spectrum suggested the presence of hydroxyl groups (3433 cm^{-1}), *gem*-dimethyl group (1461 and 1380 cm^{-1}), olefinic carbons (1639 cm^{-1}), and C-O bond

(1053 cm-1). The 1H-NMR spectra revealed the signals of six methyl groups including two *tert*-methyl groups at δ_H0.95 (s) and 0.65 (s), three secondary methyl groups at δ_H0.90 (d, *J* = 6.5 Hz), 0.80 (d, *J* = 5.5 Hz), and 0.78 (d, *J* = 5.5 Hz), and one primary methyl group at δ_H 0.82 (t, *J* = 7.5 Hz).

Table 1. The NMR data of 1, 2, and 4

	1		2		4	
Position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	36.85	1.79 m/1.00 m	37.30	1.68 m	179.83	7.26 s (OH)
2	29.28	1.80 m/1.46 m	31.71	1.50 m	34.02	2.34 t (7.2)
3	76.75	3.07 brd (5.0)	71.83	3.52 m	24.70	1.63 m
4	38.33	2.36 brd (9.5)/ 2.12 t (12.0)	42.35	2.23 m	29.08	1.26-1.34 m
5	140.48		140.81		29.37	1.26-1.34 m
6	121.23	5.33 brs	121.71	5.35 t(5.2)	29.45	1.26-1.34 m
7	31.39	1.92 m/1.90 m	31.95	1.99 m	29.61	1.26-1.34 m
8	31.45	1.39 m	32.93	1.43 m	29.70	1.26-1.34 m
9	49.63	0.89 m	50.21	0.82 m	29.70	1.26-1.34 m
10	36.24		36.54		29.70	1.26-1.34 m
11	20.61	1.47 m/1.39 m	21.12	1.46 m	29.70	1.26-1.34 m
12	39.1	1.94 m/1.11 m	39.83	1.23 m	29.70	1.26-1.34 m
13	41.88		42.37		29.70	1.26-1.34 m
14	56.2	0.98 m	56.82	0.95 m	31.94	1.26-1.34 m
15	23.88	1.52 m/1.04 m	24.33	1.58 m	22.70	1.26-1.34 m
16	27.81	1.78 m/1.23 m	28.26	1.16 m	14.11	0.88 t(7.2)
17	55.45	1.08 m	56.13	1.10 m		
18	11.69	0.65 s	12.00	1.00 s		
19	18.96	0.95 s	19.41	0.68 s		
20	35.49	1.33 m	36.17	1.86 m		
21	18.64	0.90 d (6.5)	18.81	0.92 d (6.2)		
22	33.37	1.30 m/1.00 m	34.01	1.05 m		
23	25.48	1.15 m	26.20	1.07 m		
24	45.17	0.91 m	45.91	1.52 m		
25	28.75	1.62 m	29.25	1.60 m		
26	19.12	0.78 d (5.5)	19.82	0.83 d (6.5)		
27	19.73	0.80 d (5.5)	19.08	0.79 d (5.2)		
28	22.64	1.23 m/1.19 m	23.13	1.27 m		
29	11.81	0.82 t (7.5)	12.01	0.84 t (5.2)		
1'	100.79	4.22 d (8.0)				
2'	73.5	2.90 m				
3'	76.97	3.48 m				
4'	70.15	3.01 m				
5'	76.78	3.11 m				
6'	61.13	3.63 dd (5.0, 11.0)/3.40 m				

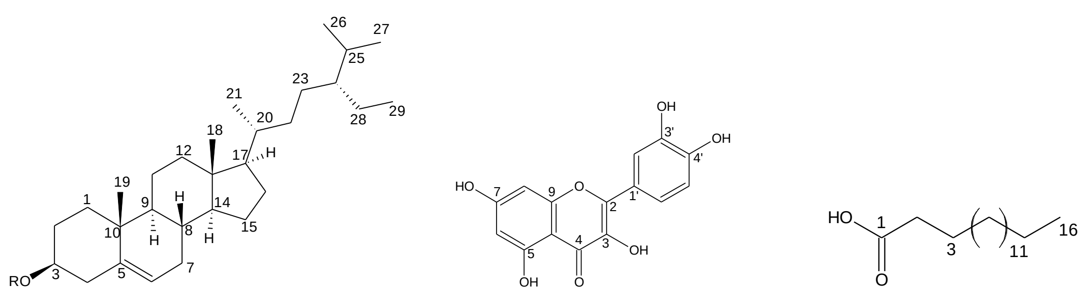
Besides, one olefinic methine group at δ_H 5.33 (brs), an oxymethine group at δ_H 3.52 (brd, *J* = 5.0 Hz), and one sugar anomeric proton at δ_H 4.22 (d, *J* = 8.0 Hz, H-1') were also observed in the 1H NMR spectra. The 13CNMR and DEPT spectra showed the presence of 35 carbon signals, including six methyls, twelve methylenes, thirteen sp3 methines, one oxygenated methine (δ_C 76.75, C-3), and three quaternary sp3 carbons. These signal were indicative of the presence of stigmastane-type steroid skeleton (aglycone) and one sugar moiety. The chemical shifts of sugar moiety is revealed by HSQC (Heteronuclear Single Quantum Coherence) spectra analysis [δ_C100.79 (CH, C-1'), 73.5 (CH, C-2'), 76.97 (CH, C-3'), 70.15 (CH, C-4'), 76.78 (CH, C-5'), 61.13 (CH_2, C-6')/δ_H 4.22 (1H, d, *J* = 8.0 Hz, H-1'), 2.90 (1H, q, *J* = 8.0 Hz, H-2'), 3.48 (1H, m, H-3'), 3.01 (1H, m, H-4'), 3.11 (1H, m, H-5'), 3.63 (1H, dd, *J* = 5.0, 11.0 Hz, H-6_a') and 3.40 (1H, m, H-6_b')]. Furthermore, the HMBC (Heteronuclear Multiple Bond Correlation) correlations observed from proton olefinic H-6 (δ_H 5.33) to C-4 (δ_C 38.33), C-8 (δ_C31.45), and C-10 (δ_C36.24) allowed to confirm a double bond (C5=C6). Also, the correlation between anomeric proton H-1' and carbon C-3 in the HMBC spectrum confirmed that the sugar moiety was attached to the *O*-atom of the aglycone. Compound **1** was identified as stigmast-5-en-3*β*-ol-3-*O*-*β*-D-glucopyranoside (daucosterol) (Figure 1) based on spectroscopic evidence and comparison with reported values in the literature [15].

Compound **2** was obtained as a white needle. The molecular formula $C_{29}H_{50}O$ was deduced from ESI-MS *m/z* 415.12 $[M+H]^+$. The IR spectra showed absorption peaks at 3424 cm^{-1} (OH), 2937 and 2870 cm^{-1} (CH sp^3), 1641 cm^{-1} (C=C), 1379 cm^{-1} (*gem*-dimethyl groups), and 1056 cm^{-1} (C-O). Comparison of the ^1H and ^{13}C NMR data of **2** with those of **1** indicated that the structures of both compounds were similar, except for the replacement of the *O*-*β*-D-glucopyranoside in **1** with a hydroxylgroup (OH) in **2**. Detailed analysis of the ^{13}CNMR and DEPT spectra revealed the presence of 29 carbon signals, including six methyls, eleven methylenes, eight sp^3 methines, one oxygenated methine, and three quaternary sp^3 carbons. The ^1H-NMR spectra of **2** showed signals for six methyl groups at δ_H 1.00 (s, H-18), 0.68 (s, H-19), 0.92 (d, *J* = 6.2 Hz, H-21), 0.83 (d, *J* = 6.5 Hz, H-26), 0.79 (d, *J* = 5.2 Hz, H-27), 0.84 (t, *J* = 5.2 Hz, H-29). The presence of a signal at δ_H 3.52 indicated an oxymethine proton and one trisubstituted olefin at δ_H 5.35 (t, *J* = 5.2 Hz, H-6). By comparison of the NMR data of **2** with those of the published data [16], **2** was identified as stigmast-5-en-3*β*-ol (*β*-sitosterol).

Compound **3** was isolated as a yellow powder with molecular formula $C_{15}H_{10}O_7$, which was deduced from the ESI-MS *m/z*: 303.23 $[M+H]^+$. The IR spectrum suggested the presence of hydroxyl groups 3406 cm^{-1}, carbonyl group 1666 cm^{-1}, doupble bonds 1610 cm^{-1} (aren). The ^1H NMR spectrashowedan ABX system at δ_H 7.75 (brs, H-2′), 7.65 (d, *J* = 8.5, H-6′), and 6.91 (d, *J* = 8.5 Hz, H-5′) of B ring and a meta-coupled pattern for H-6 and H-8 protons (δ_H6.21 and 6.41, br s). The ^{13}C NMR spectra showed the presence of 15 carbon signals in the flavonoid skeleton. Based on these data,compound **3** was identified as quercetin [17].

Compound **4** was isolated as a white amorphous powder. The IR spectrum suggested the presence of carbonyl group at 1701 cm^{-1} (C=O), hydroxyl group at 3321 cm^{-1} (OH). Based on the ESI-MS molecular ion peak at *m/z* 257.35 $[M+H]^+$, the spectral data of compound **4** indicated that it is palmitic acid, confirming the molecular formula of $C_{16}H_{32}O_2$. The ^{13}C NMR spectrum revealed the presence of one methyl signal at δ_C 14.11 was assigned to a terminal methyl (C-16), one quaternary carbon signal at δ_C 179.83 was assigned to the carboxylic acid (C-1). The signals at δ_C 22.70, 24.70, 31.94, and 34.02 were assigned to methylene carbons C-15, C-3, C-14, and C-2, respectively. The remaining methylene carbon signals from δ_C 29.08 to 29.25were assigned to carbons from C-4 to C-13. In the ^1H NMR spectra of compound **4** showed the following signals, one methyl triplet at δ_H 0.88 (t, *J* = 7.2 Hz, H-16), a multiplet at δ_H 1.63 (m, H-3), a triplet at δ_H 2.34 (t, *J* = 7.2 Hz, H-2), and a singlet at δ_H 7.26 (OH) which could be assigned to carbocylic group (COOH). In addition, the proton signals from δ_H1.26 to 1.34 (24H, m) were determined to be protons from H_2-4 to H_2-14. Based on the spectroscopic evidences and comparison with the reported values in the literature [18], compound **4** was identified as palmitic acid (*n*-hexadecanoic acid).

These results contribute to additional data on the chemical composition of a new species of the genus *Magnolia*.In addition to the previously reported flavonoid glycosides, terpenoids, flavonoid aglycone, and fatty compound were isolated from the leaves of *M. lamdongensis*.



(**1**) R = *β*-D-glucopyranoside (**3**) (**4**)

(**2**) R = H

Figure 1. Structure of compounds 1-4.

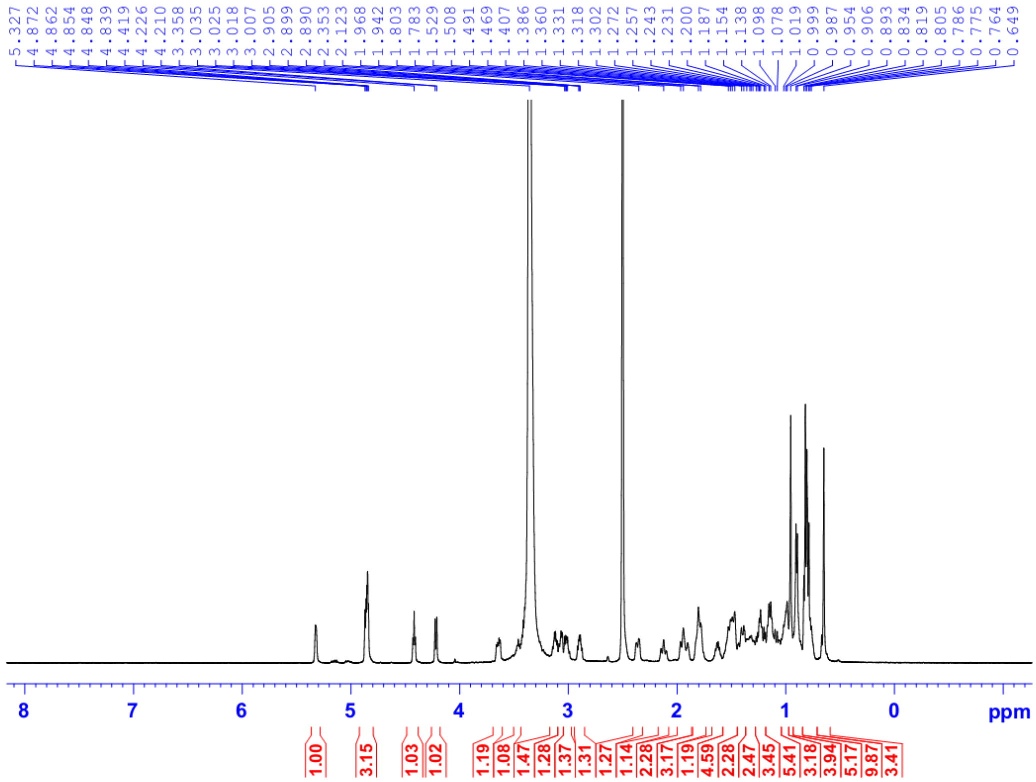


Figure 2.¹H NMR spectrum of compound 1.

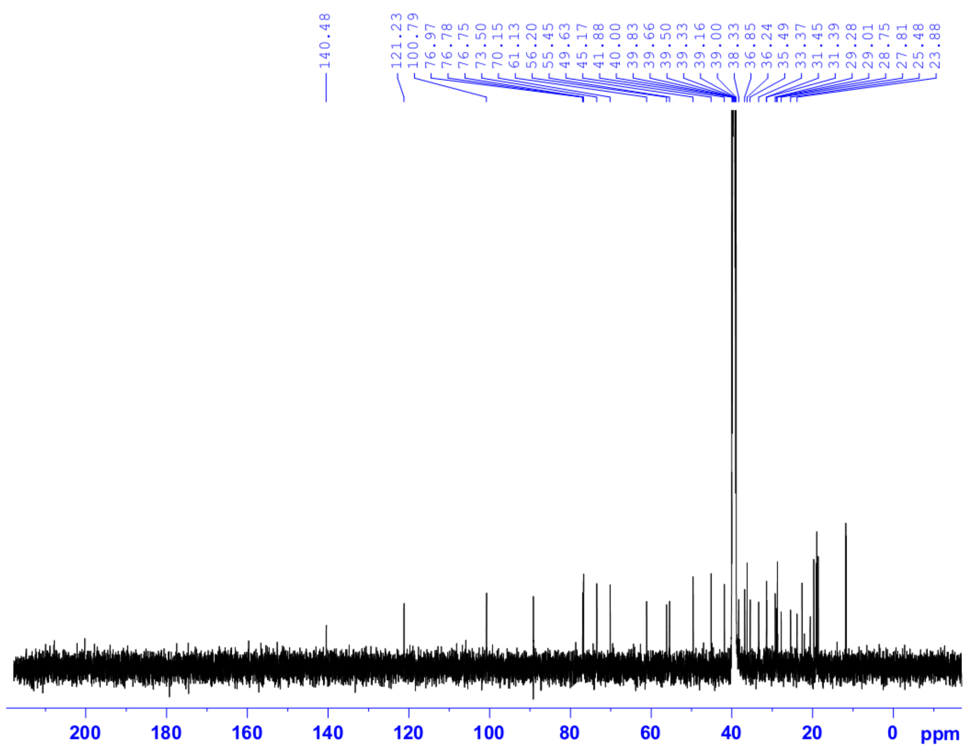


Figure 3.¹³C NMR spectrum of compound 1

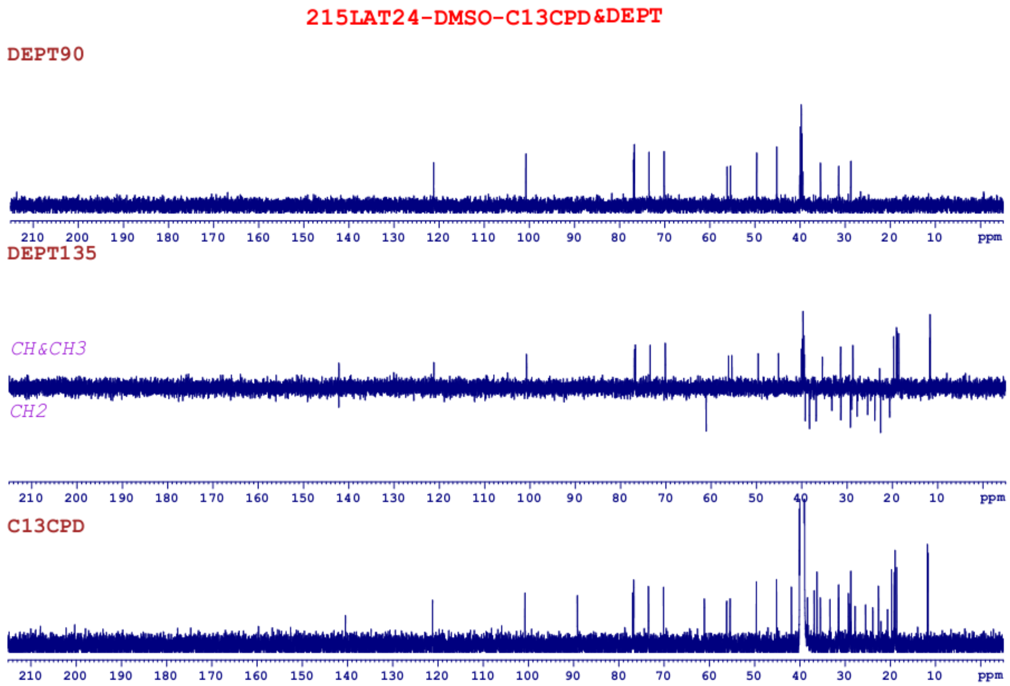


Figure 4. DEPT spectrum of compound 1.

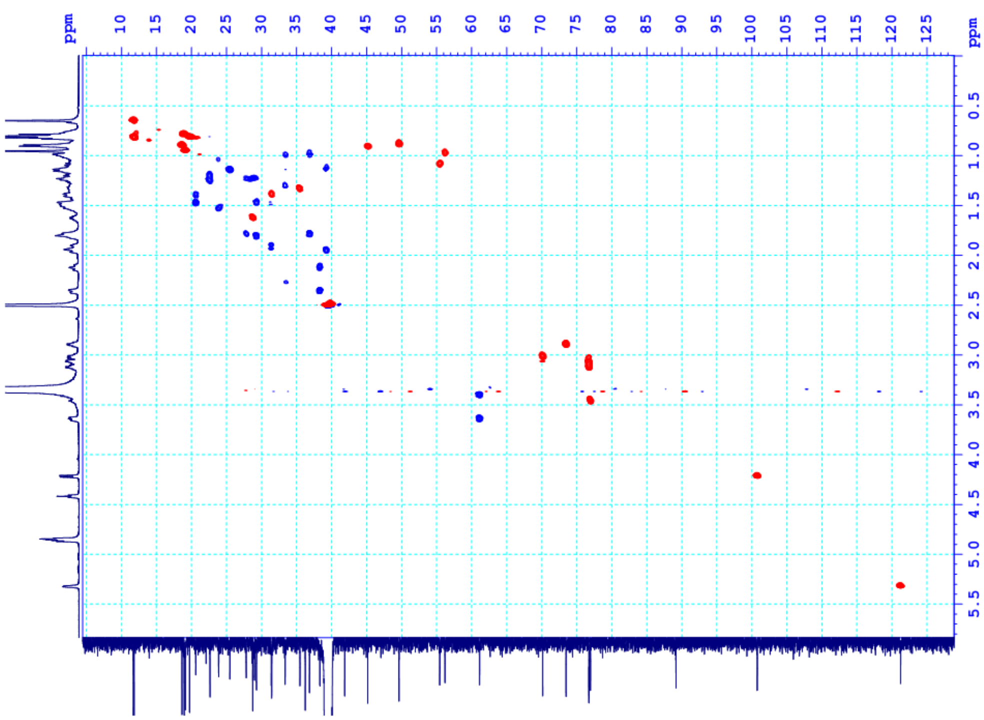


Figure 5. HSQC spectrum of compound 1.

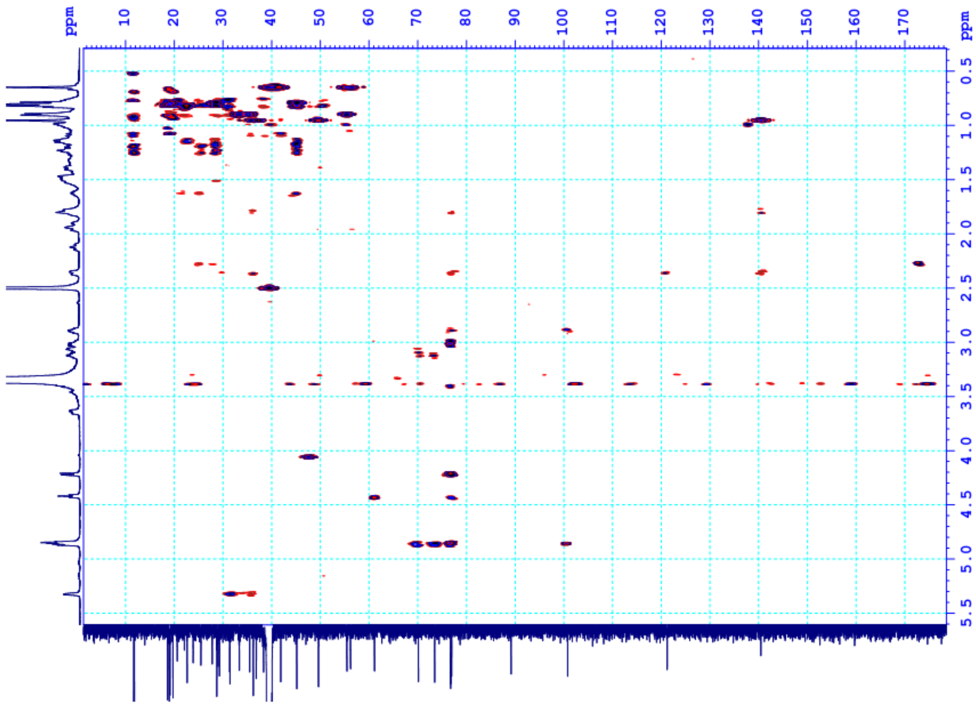


Figure 6. HMBC spectrum of compound 1.

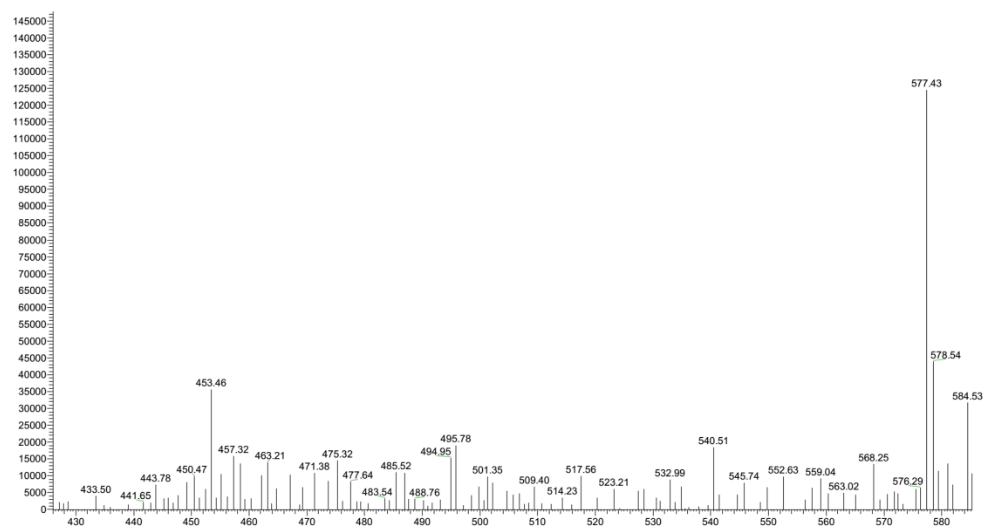


Figure 7. Mass spectrum (positive) of compound 1.

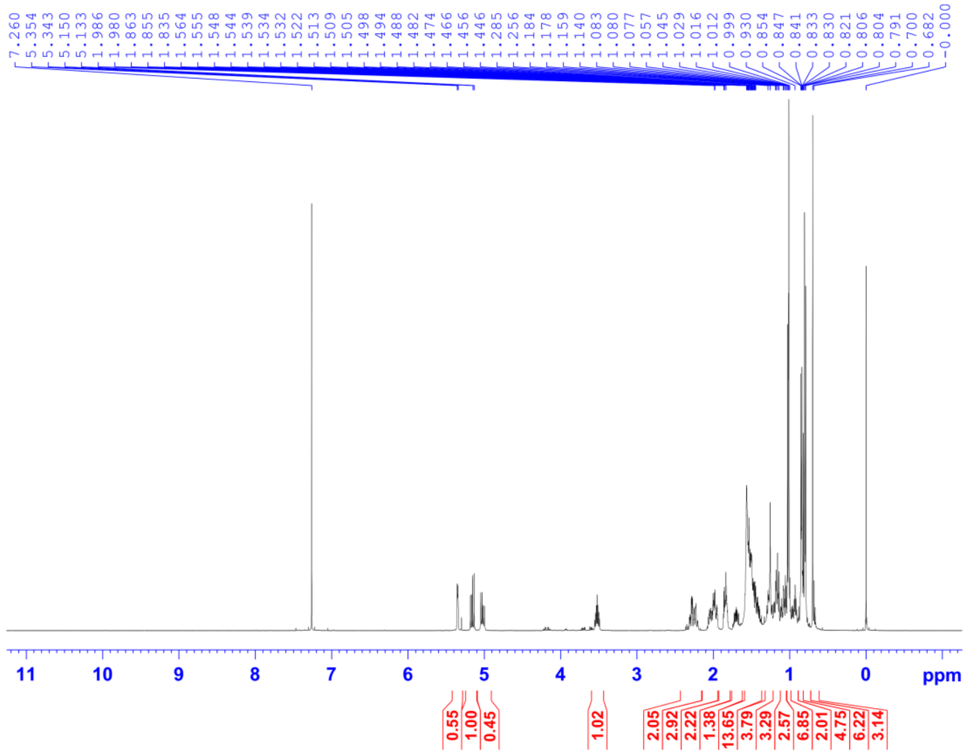


Figure 8.¹H NMR spectrum of compound 2.

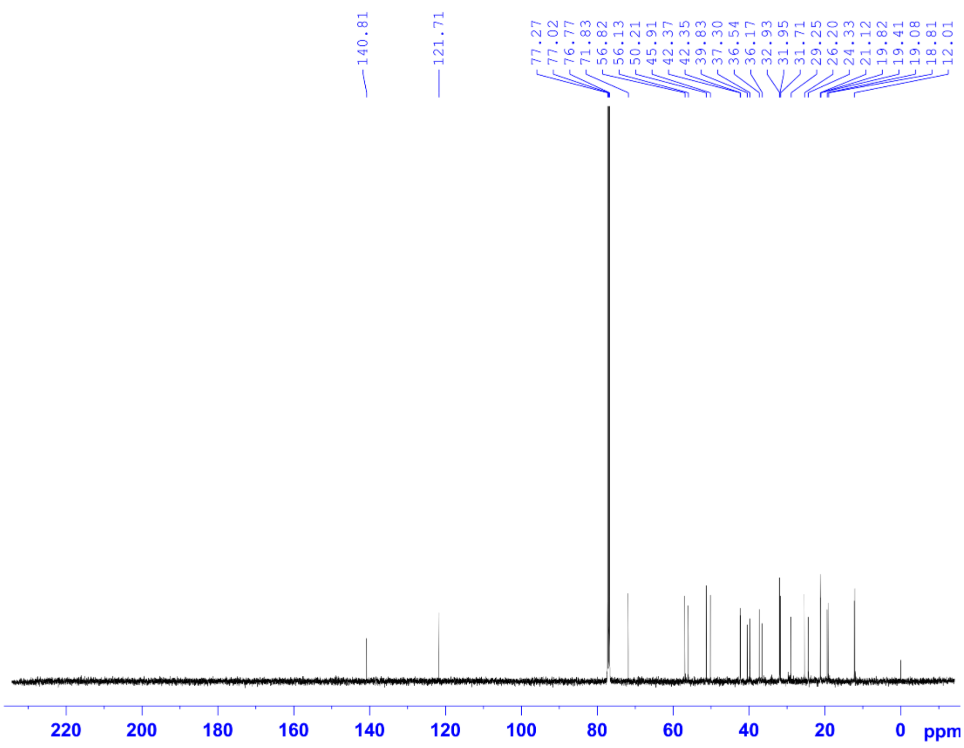


Figure 9. ¹³C NMR spectrum of compound 2.

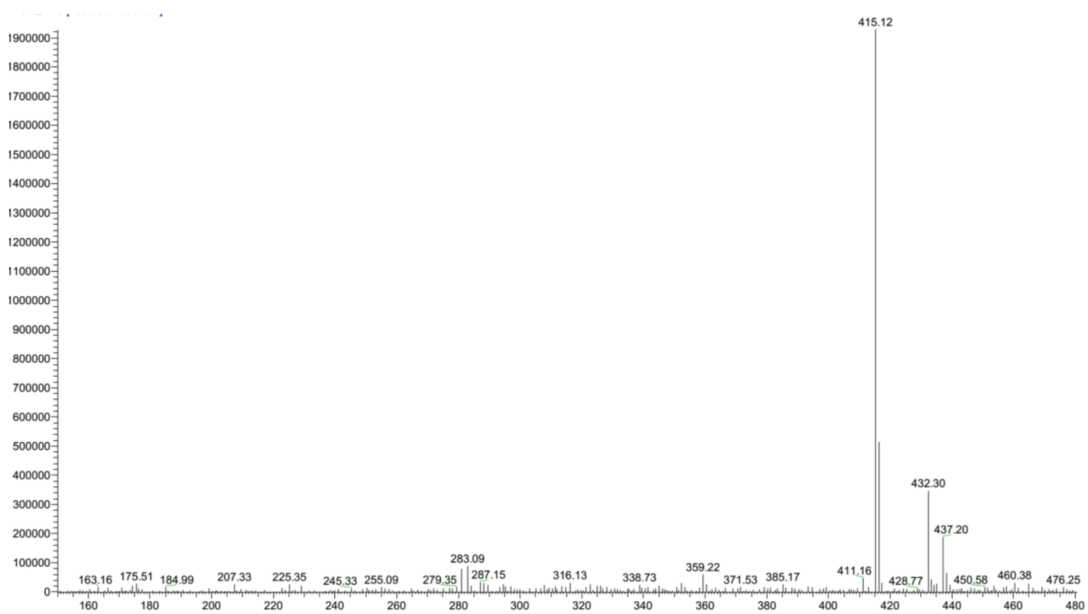


Figure 10. Mass spectrum (positive) of compound 2.

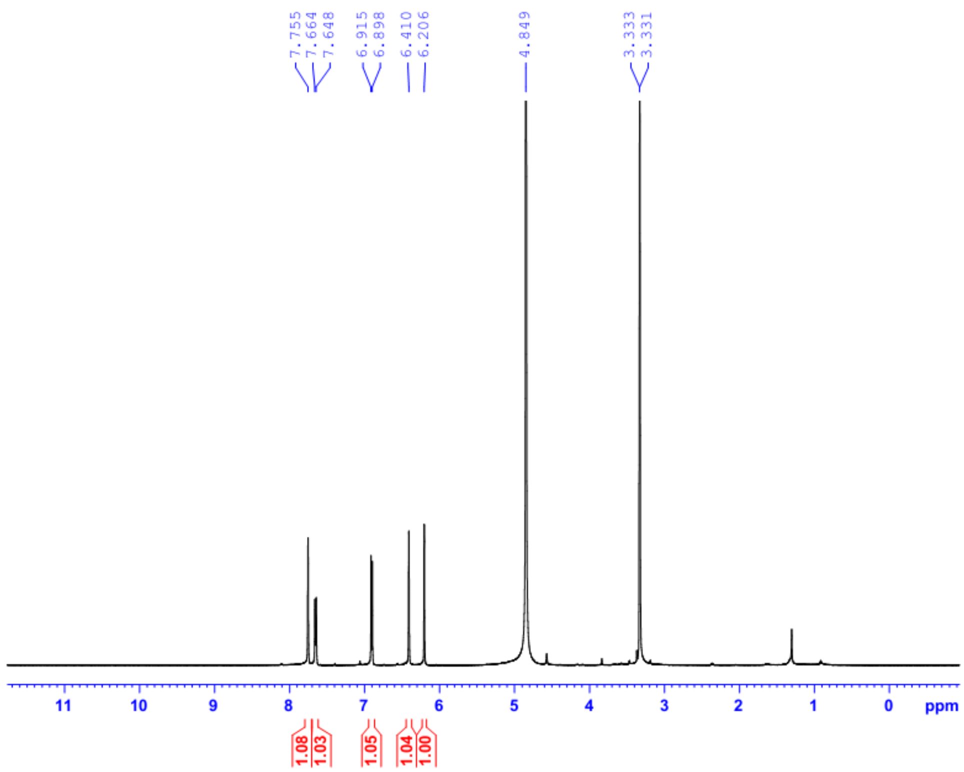


Figure 11. [1]H NMR spectrum of compound 3.

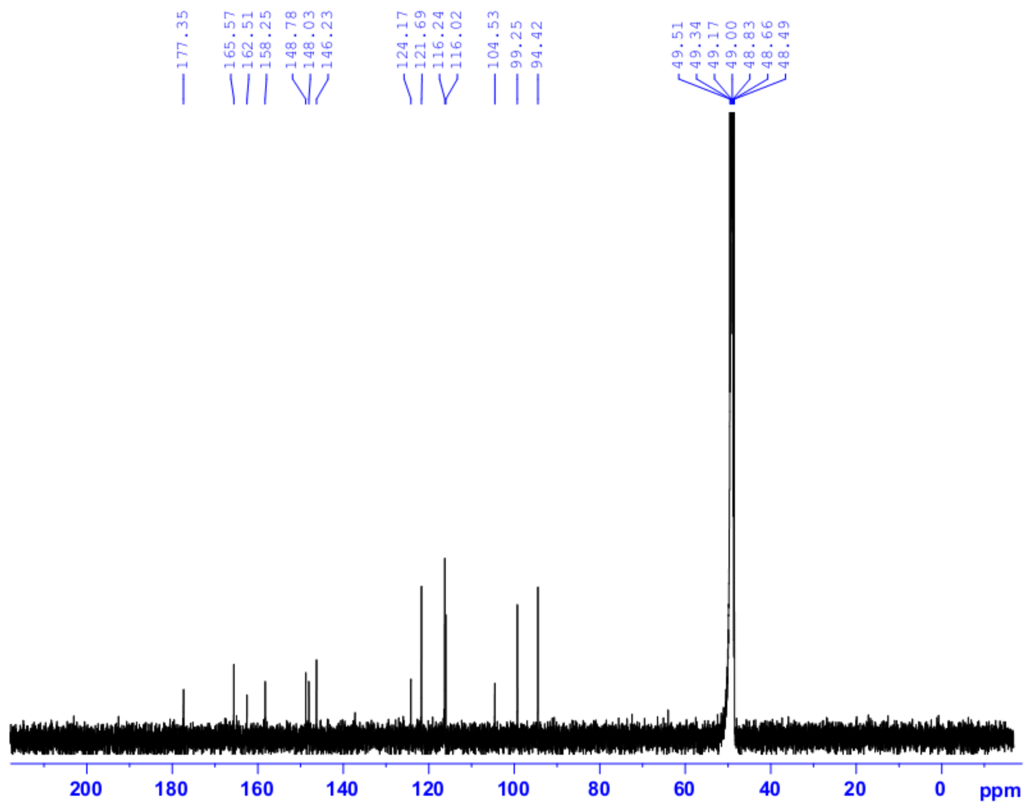


Figure 12. ^{13}C NMR spectrum of compound 3.

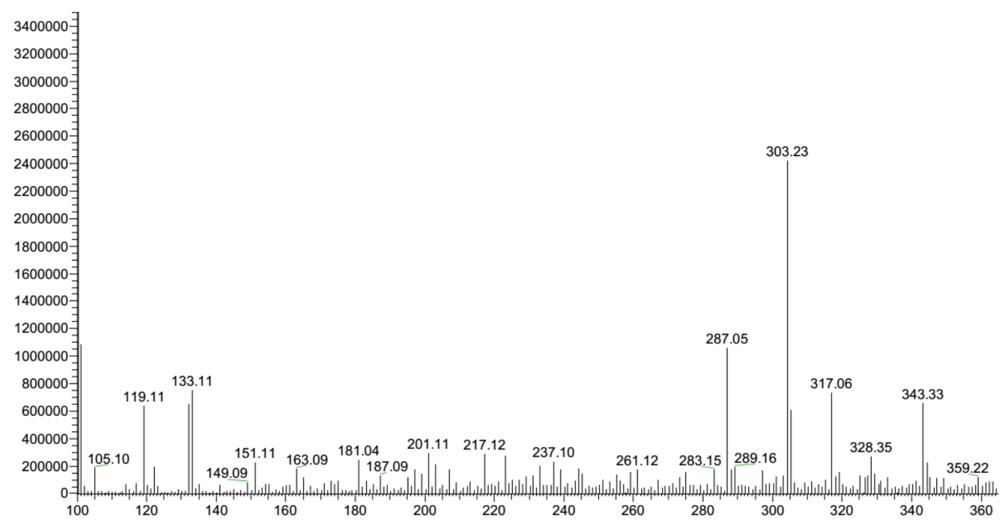


Figure 13. Mass spectrum (positive) of compound 3.

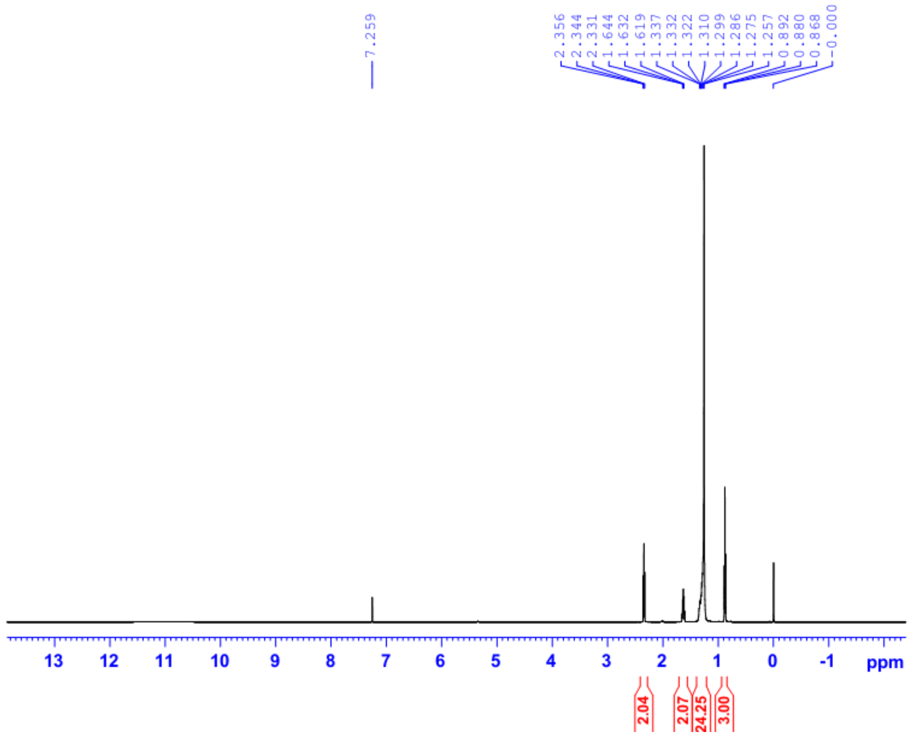


Figure 14. ^{1}H NMR spectrum of compound 4.

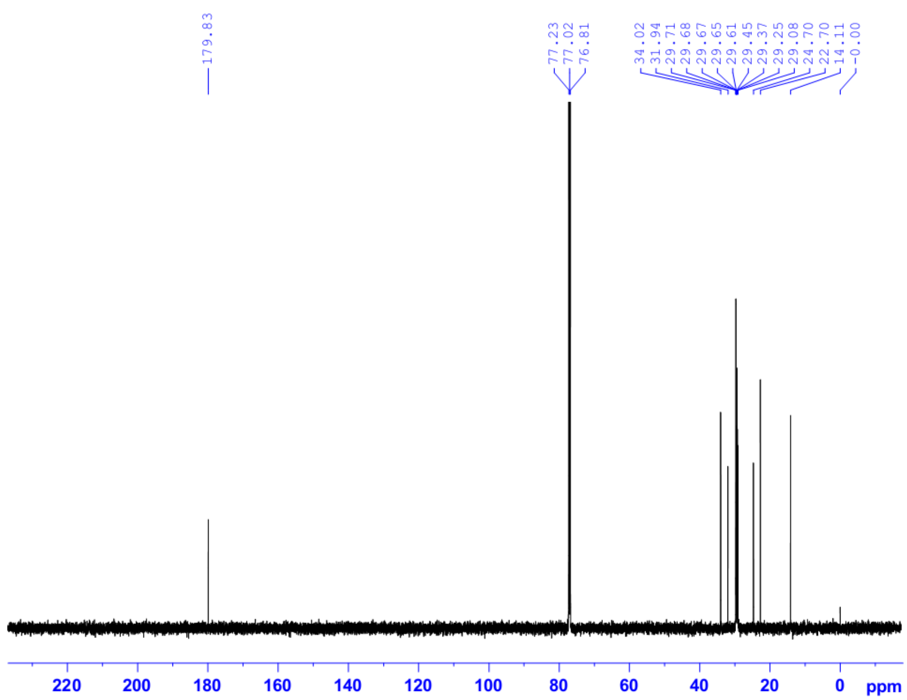


Figure 15. ^{13}C NMR spectrum of compound 4.

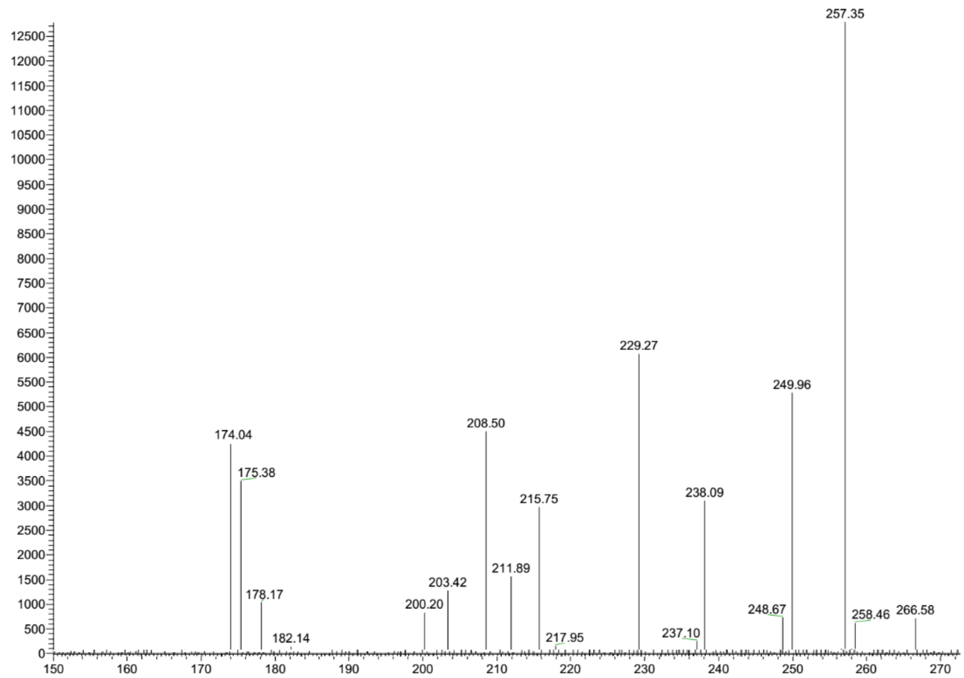


Figure 16. Mass spectrum (positive) of compound 4.

Conclusion

According to the results above, four compounds including stigmast-5-en-3β-ol-3-*O*-β-D-glucopyranoside, stigmast-5-en-3β-ol, kaempferol, and palmitic acid were isolated from the leaves extract of *Magnolia lamdongensis* for the first time. Their structures were identified by nuclear magnetic resonance (NMR), fourier transform-infrared spectroscopy (FT-IR), electrospay ionization mass spectrum (ESI-MS) as well as comparison with published data.

Acknowledgement

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Conflict of Interests

The authors declare that there is no conflict of interests.

Authors Contribution

All the authors have contributed equally in designing, drafting the manuscript as per the journal submission format. All authors read and approved the final manuscript.

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