

E-ISSN: 2278-4136

P-ISSN: 2349-8234

<https://www.phytojournal.com>

JPP 2023; 12(5): 309-319

Received: 10-07-2023

Accepted: 15-08-2023

Md. Abdul Matin Sarker
Department of Pharmacy,
Jahangirnagar University,
Savar, Dhaka-1342, Bangladesh

AY Sk Feroz Uddin Ahmed Chowdhury
Professor, Department of
Pharmacy, Jahangirnagar
University, Savar, Dhaka-1342,
Bangladesh

Md. Ekramul Haque
Department of Pharmacy,
University of Rajshahi,
Rajshahi-6205, Bangladesh

Md. Aziz Abdur Rahman
Department of Pharmacy,
University of Rajshahi,
Rajshahi-6205, Bangladesh

Md. Zakir Sultan
Centre for Advanced Research in
Sciences (CARS), University of
Dhaka, Dhaka-1000, Bangladesh

Md. Emdad Hossain
Wazed Miah Science Research
Centre (WMSRC), Jahangirnagar
University, Savar, Dhaka-1342,
Bangladesh

Corresponding Author:
AY Sk Feroz Uddin Ahmed Chowdhury
Professor, Department of
Pharmacy, Jahangirnagar
University, Savar, Dhaka-1342,
Bangladesh

Phytochemical Investigation of the Bark of *Averrhoa bilimbi* Linn

Md. Abdul Matin Sarker, AY Sk Feroz Uddin Ahmed Chowdhury, Md. Ekramul Haque, Md. Aziz Abdur Rahman, Md. Zakir Sultan and Md. Emdad Hossain

DOI: <https://doi.org/10.22271/phyto.2023.v12.i5d.14742>

Abstract

The present study reports the phytochemical investigation of the bark of *Averrhoa bilimbi* Linn. Four phytoconstituents have been reported namely Bis-(2-ethylhexyl) phthalate; Myricetin; Quercetin; β -Sitosterol; and these four compounds have been reported for the first time from the bark of *A. bilimbi* Linn. The structures of these compounds were elucidated from their 1D NMR (^1H , ^{13}C , DEPT ^{13}C -NMR) and where required, from their 2D NMR (COSY, HSQC, HMBC) data and comparing the ^1H - and ^{13}C -NMR data where required with that of the published data in the literatures.

Keywords: *Averrhoa bilimbi*, Phytoconstituents, Bis-(2-ethylhexyl) phthalate, Myricetin, Quercetin, β -sitosterol

1. Introduction

Averrhoa bilimbi Linn. (Family: Oxalidaceae), locally known as Bilimbi, is a medicinal plant and is native to Moluccas (Indonesia) and Malaysia. The plant is also cultivated in all over the country but mainly found in Brahmanbaria, Cumilla, Chattogram and Dhaka in Bangladesh. *A. bilimbi* is 3-10 m in height evergreen and perennial small tree with small red flowers and sour oblong-angular fruits. It is basically a tree of tropical climate, lesser resistant to cold, grows excellent in wealthy and well-drained soil [1]. Bilimbi is medicinally used as a folk remedy for the treatment of many diseases such as its leaves is used for the treatment of itching, pimples, swellings of mumps, rheumatism, skin eruptions, bites of toxic organism, syphilis, cough, rectal inflammations; fruit is used in cough, beriberi, biliousness, pyrexia, inflammation, rectal bleeding, hemorrhoids, scurvy, whitlows, control obesity, hepatitis, diarrhea; flower infusions are effective against cough and thrush [1-5].

The stem bark of *A. bilimbi* indicated the presence of alkaloids, saponins and flavonoids; and possesses thrombolytic and antimicrobial activity [6]. Literature survey revealed that, leaf and fruit of *Averrhoa bilimbi* possess antioxidant, antimicrobial, anticoagulant, anti-fertility, antimalarial, anthelmintics, cytotoxic, hepatoprotective, hypoglycaemic, hypolipidemic, hypotensive, nephrotoxic, pediculicidal, thrombolytic and wound healing properties [7-9]. *A. bilimbi* leaf, bark and fruit possess significant analgesic activity [10]. A literature survey of this plant did not retrieve any information regarding the pure metabolites of bark of the Bilimbi plant. This research aimed to get pure metabolites of the bark of this plant and elucidate their structure by spectroscopic methods of analysis and evaluate the pharmacological actions of the plant.

2. Materials and Methods

2.1 Collection and Identification of Sample

The plant sample was collected from Institute of Health Technology (IHT) campus, Mohakhali, Dhaka, Bangladesh in March 2017. The medicinal plant sample was identified as part of *A. bilimbi* Linn. By a taxonomist of the Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh. The dried plant specimen was deposited in the Bangladesh National Herbarium for further reference (voucher specimen No. DACB 45297 dated 05-6-2017).

2.2 Preparation of Plant Material

Collected stem bark was dried and pulverized into coarse powder by grinder in Pharmacy Department, Jahangirnagar University, Savar, Dhaka, Bangladesh.

The powdered plant material was kept in an airtight container with proper labeling for further study.

2.3 Extraction of Plant material

The pulverized stem barks (500 gm) of *A. bilimbi* Linn was soaked in 3L of aqueous methanol (95%) for 7 days with occasional shaking and then filtered. The filtrate was evaporated off under reduced pressure at low temperature (40 ± 2 °C) with a Buchii Rotavapour to obtain the crude extract. The concentrated extract was preserved for the further work.

2.4 Isolation of the Chemical Constituents from the bark of *A. bilimbi* Linn.

Crude extract of the bark (5 gm) was made slurry with 90% aqueous methanol (100 ml) in a separating funnel, and chloroform (100 ml) was added and shaking well. Chloroform layer was then separated from the aqueous layer and the process was repeated three times. After drying, the solvent of the combined fraction was evaporated off under reduced pressure to obtain a syrup (1.2 gm) of chloroform extract from the bark.

On the aqueous methanol in the separating funnel ethyl acetate (100 ml) was added and shaken well. After separating the two layers, ethyl acetate layer was separated and the process was repeated for three times. After drying the solvent of the combined fraction solvent was evaporated off under reduced pressure to afford a semisolid mass (1.8 gm) ethyl acetate extract from the bark.

2.5 Work up with Chloroform soluble fraction of the stem bark of *A. bilimbi* Linn.

This fraction showed several prominent spots on TLC when eluted with *n*-hexane: ethyl acetate (1:1) and heating after spraying the chromatogram with vanillin- H_2SO_4 solution.

Similar types of spots were visualized under UV and iodine vapor also.

A portion (0.5 gm) of the chloroform fraction was subjected to a dry column of silica gel. The column was then eluted with *n*-hexane, *n*-hexane and chloroform with increasing portions of chloroform and then with chloroform only. The column was then further eluted with chloroform and ethyl acetate mixture with increasing portion of ethyl acetate and finally with ethyl acetate only.

The fraction eluted with *n*-hexane: chloroform (9:1) showed a single spot on TLC. The solvent of this fraction was dried and evaporated off under reduced pressure to afford a compound ABC-1 as off-white solid mass (7.25 mg) gave needles from *n*-hexane - ethyl acetate, mp. 134 °C

The fraction eluted with chloroform: ethyl acetate (7:3) showed a single spot on TLC. After drying, the solvent of this fraction was evaporated off under reduced pressure to afford a compound ABC-2 as a yellowish amorphous powder (5.50 mg).

The fraction eluted with chloroform: ethyl acetate (1:1) also showed a single spot on TLC. After drying, the solvent of this fraction was evaporated off under reduced pressure to afford a compound ABC-3 as a yellowish amorphous powder (6.10 mg).

2.6 Work up with the Ethyl acetate soluble portion of bark of *A. bilimbi* Linn.

The fraction partitioned with ethyl acetate from the crude methanolic extract of *A. bilimbi* stem bark (0.5 gm) was subjected to a semi preparative C-18 HPLC column. The chromatographic condition was developed as 100% water to 50% aqueous acetonitrile for 10 min, then 50% aqueous acetonitrile to 100% acetonitrile for 5 min. Flow rate was at 4 ml/min and elution monitored at 210 nm. The HPLC afforded compound ABE-1 (4.1 mg, at R_t 4.0 min), compound ABE-2 (3.1 mg, at R_t 27.0 min) (Figure 1)

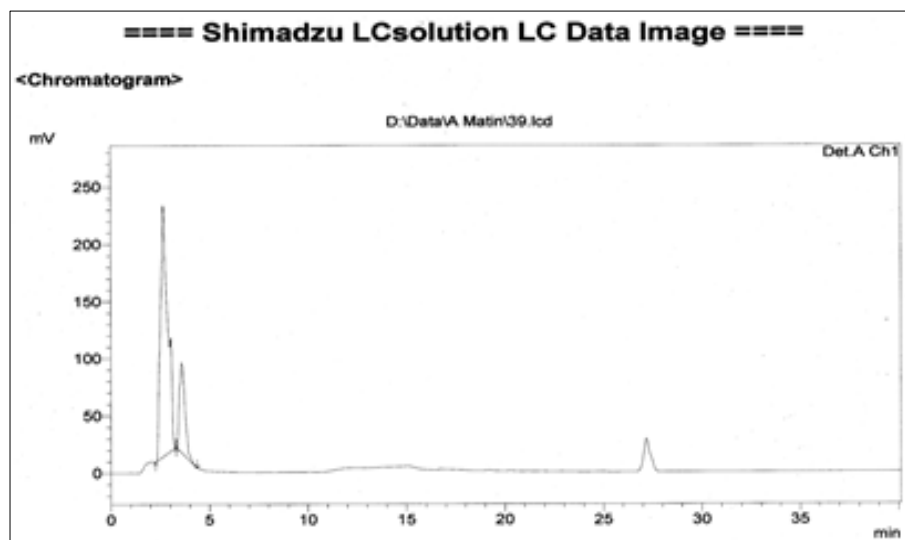


Fig 1: Semi preparative HPLC chromatogram of the EtOAc soluble fraction of stem bark of *A. bilimbi* Linn.

3. Results and Discussion

3.1 Characterization of Compound ABC-1 as β -sitosterol

The crystalline compound ABC-1 obtained from the chloroform fraction eluted with *n*-hexane: chloroform (9: 1) and after usual workup afforded needles from *n*-hexane-ethyl acetate, mp 134 °C and was found to be homogenous on TLC. The compound (ABC-1) gave positive test for steroid with Salkowski's and Liebermann-Burchard test and also gave a

positive test for unsaturation with Bromine water. From the above positive test, the compound ABC-1 was presumed to be a sterol and its melting point is almost same as β -Sitosterol (135 °C). Final identity of the compound was confirmed by analysis of its 1H and ^{13}C NMR spectral data. 1H -NMR spectrum of H-3 proton normally appeared as a multiplet, but unfortunately in this compound this peak coincided with a broad peak of water and appeared as broad singlet at δ 3.51. The olefinic H-6 showed a triplet in general but due to the low

resolution it appeared as a broad singlet at δ 5.31. Corresponding literature values of these two peaks are at δ 3.53 (1H, t, tdd, $J = 3.8, 4.2$ and 4.5 Hz) and at δ 5.31 (1H, t, $J = 6.1$ Hz), respectively. Six methyl protons appeared at δ 0.96, 0.81, 0.82, 0.84, 0.93 and 1.03 are almost coincided with the literature value (Figure 2; Table 1a).

In its ^{13}C NMR spectrum 6 methyl carbons at 11.9, 12.0, 18.8, 19.0, 19.4 and 19.8 ppm are almost similar to that of the

literature value (Figure 2; Table 1b). The olefinic carbons at C-5 and C-6 appeared at 140.8 and 121.7 ppm are almost identical with the literature value at 140.9 and 121.9 ppm, respectively. C-3 carbon appeared at 71.8 ppm deshielded due to the presence of an electro negative group OH. Other carbon chemical shifts are in accordance with the structure assigned and hence the structure of the compound ABC-1 has been assigned and confirmed as β -Sitosterol.

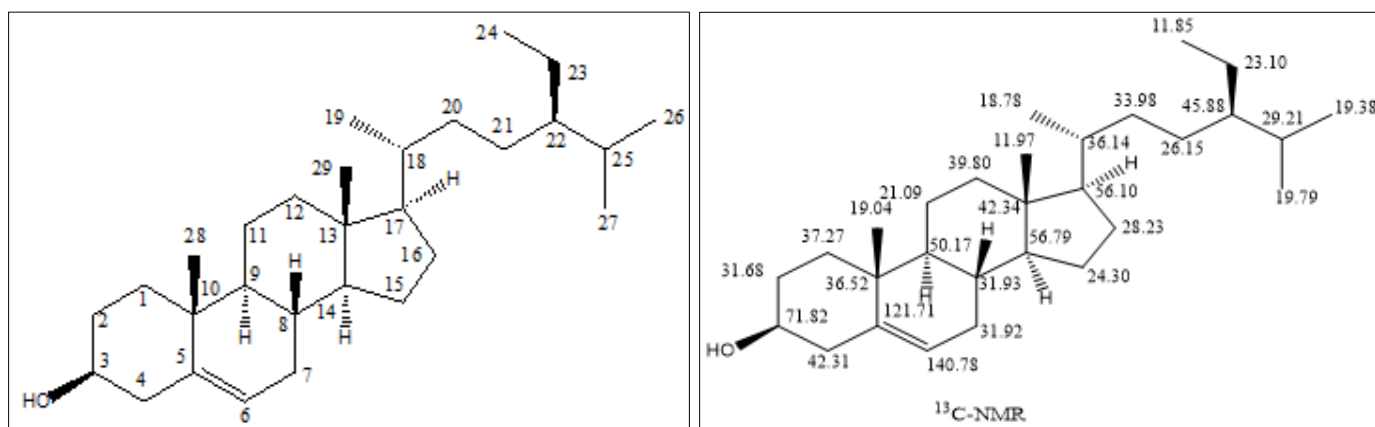


Fig 2: Structure of ABC-1 (β -sitosterol)

Table 1a: ^1H -NMR spectral data of Compound ABC-1 and Literature value of ^1H -NMR of β -Sitosterol ^[11] in CDCl_3 .

Position of protons	H-chemical shift (in δ values) of Compound ABC-1	H-chemical shift of β -Sitosterol (Lit. value) ^[11]
H-3	3.51(1H, bs)	3.53 (1H, tdd, $J = 4.5, 4.2$ and 3.8 Hz)
H-6	5.37 (1H, bs)	5.36 (1H, t, $J = 6.1$ Hz)
CH_3 -19	0.93 (3H, d)	0.93 (3H d, $J = 6.2$ Hz)
CH_3 -24	0.84 (3H, t)	0.84 (3H t, $J = 7.2$ Hz)
CH_3 -26	0.82 (3H, d)	0.83 (3H, d, $J = 6.4$ Hz)
CH_3 -27	0.81 (3H, d)	0.81 (3H, d, $J = 6.4$ Hz)
CH_3 -28	0.69 (3H,s)	0.68 (3H, s)
CH_3 -29	1.03 (3H,s)	1.01 (3H, s)

Table 1 b: ^{13}C NMR spectral data of Compound ABC-1 and Literature value of ^{13}C NMR of β -Sitosterol ^[11] in CDCl_3 .

Carbon No.	^{13}C NMR chemical shift of compound ABC-1	^{13}C NMR chemical shift of β -Sitosterol (Literature value) ^[11]
1	37.27	37.5
2	31.68	31.9
3	71.82	72.0
4	42.31	42.5
5	121.71	121.9
6	140.78	140.9
7	31.92	32.1
8	31.93	32.1
9	50.17	50.3
10	36.52	36.7
11	21.09	21.3
12	39.80	39.9
13	42.34	42.6
14	56.79	56.9
15	24.30	26.3
16	28.23	28.5
17	56.10	56.3
18	36.14	36.3
19	18.78	19.2
20	33.98	34.2
21	26.15	26.3
22	45.88	46.1
23	23.10	23.3
24	11.85	12.2
25	29.21	29.4
26	19.38	20.1
27	19.79	19.6
28	19.04	19.0
29	11.97	12.0

3.2 Characterization of Compound ABC-2 as Quercetin

The compound ABC-2 isolated from chloroform fraction of *A. bilimbi* Linn. bark as yellowish amorphous powder with n-hexane chloroform (Figure 3; Table 2) which gave positive test for flavonoids with Shinoda test and gave positive test for carbonyl function with 2,4-dinitrophenyl hydrazine reagent.

The compound ABC-2 was made sufficiently pure until gave a single and homogeneous spot on TLC and was subjected to ^1H and ^{13}C NMR spectral analysis for structure elucidation. Compound ABC-2 showed two aromatic proton signals at δ 6.40 and 6.19 with a J value of 1.2 Hz indicating the meta coupling between each other and were designated as H-6 and H-8 protons of the aromatic ring A and this indicates that there is no proton on C-7 carbon instead an -OH group is present. The benzene ring (A) is fused with a cyclohexene ring (B) (a criterion of flavonol) and the ring is a heterocyclic ring having no proton is present, instead there is a carbonyl function present at C-4 position which showed a peak at 175.92 ppm in its ^{13}C -NMR spectrum. Moreover, on the neighboring C-3 carbon is attached with an -OH group.

Ring C is also a benzene ring and showed 3 aromatic protons at δ 6.90 as a doublet with a J value of 8.4 Hz indicating the presence of a single proton *vicinal* to it and is assigned as Ar-3' and the neighboring Ar-2' proton appeared as a dd with a J value of 8.4 and 1.6 Hz at δ 7.64 ppm due to the meta coupling with Ar-6' proton which gave its peak at δ 7.75 as a doublet with a J value of 1.6 Hz. Hence the benzene ring has three aromatic protons and two OH groups are disposed on Ar-4' and Ar-5' and Ar-1' is linked with the heterocyclic ring-B. Hence the structure of this compound is similar to the structure of Myricetin except there not an OH group at Ar-3' position and the structure is confirmed as Quercetin by comparing its ^1H and ^{13}C -NMR data that cited in the literature [12] a well-known flavonoids reported first time from this plant.

All the ^{13}C - chemical shifts of compounds ABC-2 were found to be identical with that of the authentic Quercetin cited in the literature of Ahmadu [12] isolated from leaf of the plant *Physalis angulata* Linn. Hence by analysis of its ^1H and ^{13}C NMR data, the structure of the compound is confirmed as Quercetin.

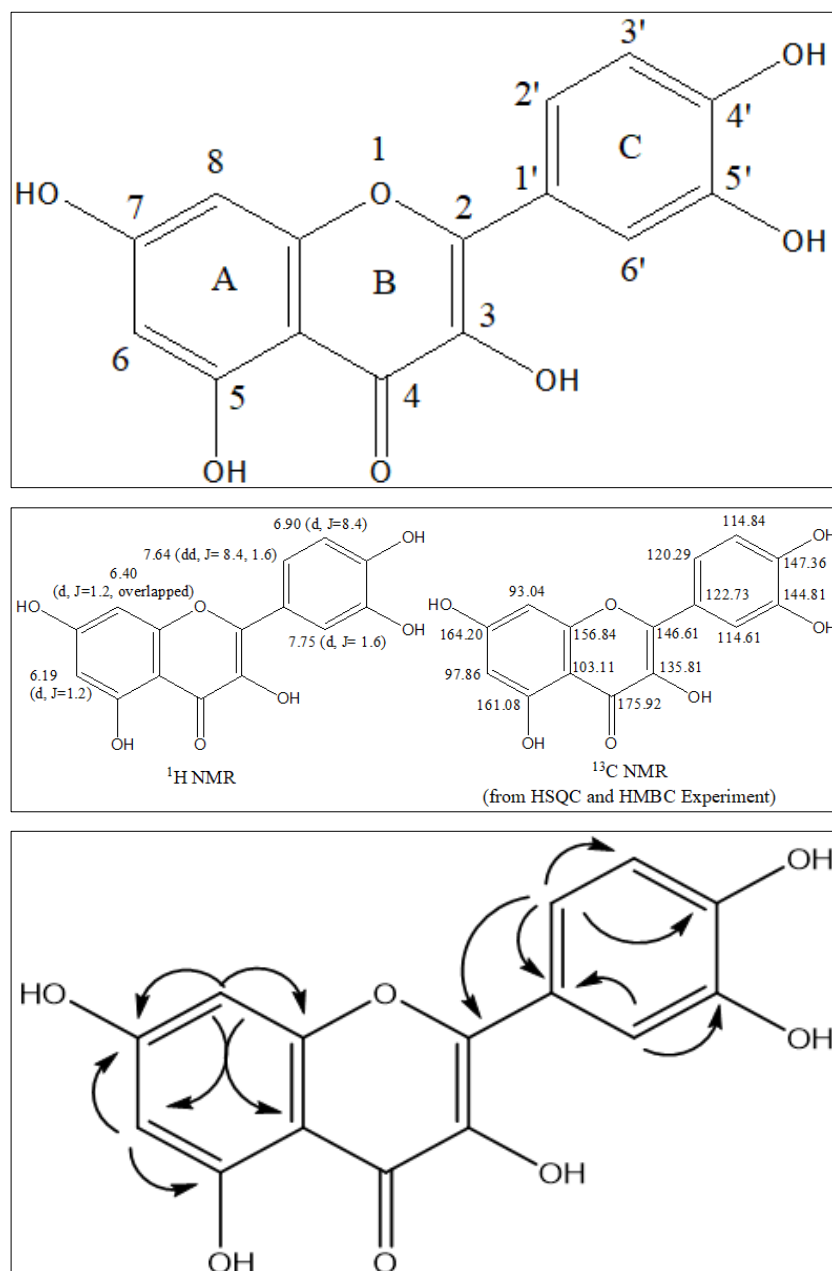


Fig 3: Structure of ABC-2 (Quercetin)

Table 2: $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ Spectral data of ABC-2 and Literature value of Quercetin ^[12] in CD_3OD

Position	$^1\text{H-NMR}$ (δ ppm, J in Hz)		$^{13}\text{C-NMR}$ (δ ppm)	
	Compound ABC-2 Value	Literature Value of Quercetin ^[12]	Compound ABC-2 Value	Literature Value of Quercetin ^[12]
1				
2			146.61	148.9
3			135.81	135.8
4			175.92	175.9
5			161.08	160.8
6	6.19 (1H, d, $J = 1.2$ Hz)	6.13 (1H, d, $J = 2$ Hz)	97.86	98.3
7			164.20	164.0
8	6.40 (1H, d, $J = 1.2$ Hz)	6.34 (1H, d, $J = 2$ Hz)	93.04	93.5
9			156.84	156.2
10			103.11	103.1
1'			122.73	122.1
2'	7.64 (1H, dd, $J = 8.4, 1.6$ Hz)	7.58 (1H, dd, $J = 8, 2$ Hz)	120.29	120.1
3'	6.90 (1H, d, $J = 8.4$)	6.83 (1H, d, $J = 8$ Hz)	114.84	115.5
4'			147.36	147.7
5'			144.81	145.1
6'	7.75 (1H, d, $J = 1.6$ Hz)	7.69 (1H, d, $J = 2$ Hz)	114.61	115.2

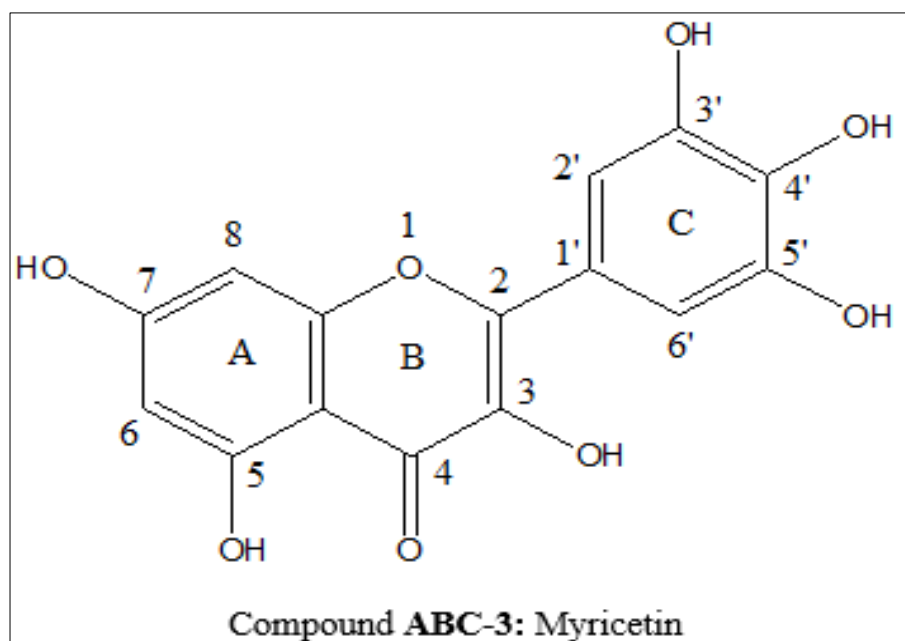
3.3 Characterization of Compound ABC-3 as Myricetin

The compound **ABC-3** isolated from the chloroform fraction of the bark of *A. bilimbi* Linn. As yellowish amorphous powder (Figure 4; Table 3) which gave positive test for flavonoid and carbonyl function with Shinoda test and 2, 4-dinitrophenyl hydrazine reagent, respectively.

The compound ABC-3 was made sufficiently pure and subjected to ^1H and ^{13}C NMR spectra for structure determination. Compound ABC-3 indicated two aromatic proton signals at δ 6.37 and 6.18 as doublets with a J value of 1.6 Hz showing the meta coupling between each other and were designated as H-6 and H-8 of the aromatic ring A and this indicates that there is no proton on C-7 carbon instead an -OH group is present. The benzene ring (A) is fused with a cyclohexene ring (B) (a criteria of flavonol) and the ring is a heterocyclic having no proton is present, instead there is a carbonyl function present at C-4 position which showed a peak at 176.23 ppm in its $^{13}\text{C-NMR}$ spectrum. Moreover, on the neighboring C-3 carbon a -OH group is present. Ring C is also a benzene ring and showed a broad singlet equivalent to two protons at meta positions assigned as H-2' and H-6' protons should chemically shifted to the aromatic region at δ

7.24. The other 4 positions of the ring C must be occupied with other functional groups.

From the analysis of the flavonoid chemistry, it is seen that C-1' is linked with a bond to the heterocyclic ring and the rest 3 positions (C-3', C-4' and C-5') are occupied with -OH groups. Molecular formula of the compound ABC-1 showed 15 carbons and its $^{13}\text{C-NMR}$ also showed the presence of 12 carbons peaks including carbonyl carbon at 176.23 ppm and the rest 3 carbon signals are coincided with other peaks and two rings olefinic carbons at C-2 and C-3 positions of the heterocyclic ring appeared at 147.33 and 136.32, respectively. From the analysis of above spectral data the compound ABC-3 is presumed to be identical with a known flavonoidal compound Myricetin. The structure was thus confirmed by comparing the ^1H - and ^{13}C - NMR data with that of the data of authentic myricetin cited in literature ^[13] shown in the table 4.3. All the data were identical with the literature data published by He ^[13] and they isolated myricetin from *Ribes nigrum* Linn. Thus, the compound ABC-3 isolated from the chloroform fraction of the bark of *A. bilimbi* Linn. was confirmed as Myricetin.



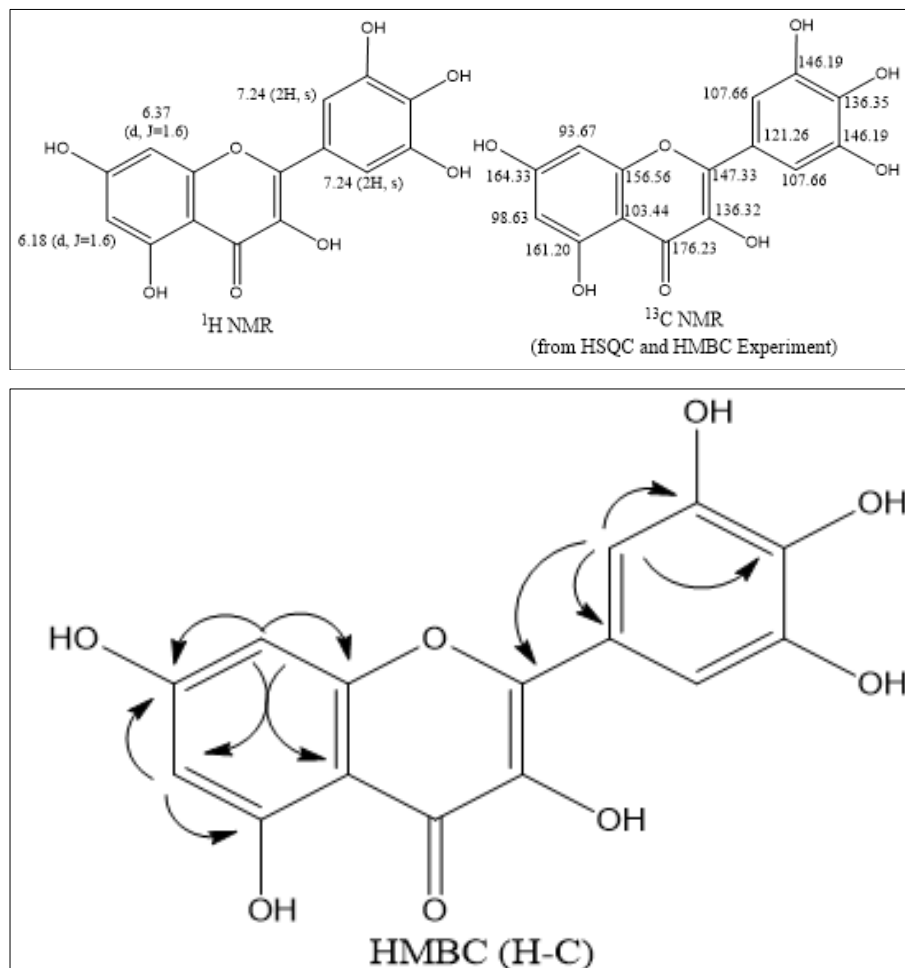


Fig 4: Structure of ABC-3 (Myricetin)

Table 3: ^1H -NMR and ^{13}C -NMR Spectral data of ABC-3 and Literature value of Myricetin ^[13] in DMSO.

Position	^1H -NMR (δ ppm, J in Hz)		^{13}C -NMR (δ ppm)	
	Compound ABC-3	Literature value of Myricetin ^[13]	Compound ABC-3	Literature value of Myricetin ^[13]
1			147.33	146.75
2			136.32	135.88
3			176.23	175.71
4			161.20	160.69
5	6.18 (1H, d, $J = 1.6$)	6.18 (1H, d, $J = 2.4$)	98.63	98.20
6			164.33	164.08
7	6.37 (1H, d, $J=1.6$)	6.37 (1H, d, $J = 1.8$)	93.67	93.21
8			156.56	156.07
9			103.44	102.86
10			121.26	120.74
11	7.24 (2H, s)	7.24 (2H, s)	107.66	107.11
12			146.19	145.73
13			136.35	135.84
14			146.19	145.73
15	7.24 (2H, s)	7.24 (2H, s)	107.66	107.11

3.4 Characterization of Compound ABE-1 as Bis-(2-ethylhexyl) phthalate

The compound ABE-1 isolated from the bark *A. bilimbi* Linn. Using HPLC as color less viscous liquid (Figure 5; Table 4). Compound ABE-1 was found to be identical with Bis-(2-ethylhexyl) phthalate by analysis of its ^1H , ^{13}C , HSQC and HMBC ^{13}C -NMR data and in comparison, with the data cited in the literature ^[14].

The four aromatic protons on C-3, 4 and 5, 6 appeared in the aromatic region as two sets of doublets of a doublet at δ 7.72 and δ 7.55 with a coupling constant 3.2 and 4.8 Hz, respectively. From this evidence it can be concluded that the other two aromatic carbons having no proton could be

engaged in bonding. Four methylene protons on C-1' and C-1'' appeared in the downfield region at δ 4.24 as a multiplet equivalent indicated their presence as *vicinal* to an electron withdrawing group and two methylene protons on C-2' and C-2'' appeared at δ 1.70 as multiplet as it coupled with their neighboring protons at C-3' and C-7', and C-3'' and C-7'' protons. Rest 16 methylene protons appeared between δ 1.20 - 1.50 as multiplets. Moreover, 12-methyl protons appeared as multiplets between δ 0.90 - 0.96.

^{13}C -NMR peaks appeared in pairs demonstrated the appearance of double and similar long aliphatic chain present in the molecule. In ^{13}C -NMR spectrum of the compound two carbonyl carbon ($>\text{C}=\text{O}$) peak appeared a single peak at 167.7

ppm and six aromatic carbons appeared in 3 peaks. Two aromatic carbons bearing no protons appeared as a singlet at 132.5 and proton bearing 4 carbons appeared as doublets at 130.8 and 128.8 ppm. C-1' and C-1'' methylene carbons also suffered a downfield shift due to the presence of a neighboring ether function and appeared as a single peak at 68.2 ppm again demonstrate the appearance of a methylene group *vicinal* to an ether function. C-2' and 2'' carbon signal appeared at 38.8 ppm and accordingly total 8 methylene

carbons appeared as 4 peaks in a set and similarly 4-methyl carbons appeared as a pairs of signals at 14.4 and 10.9 ppm.

¹H-¹H COSY and carbon-proton correlation spectra also showed the identical results. Finally, the structure of the compound was confirmed as octadecyl phthalate by comparing its ¹H and ¹³C-NMR data with the data of Ragasa [14] cited in the literature. They isolated this compound from *Cymodocea serrulata* R. Brown.

The structure of compound ABE-2 could not be determined due to the small quantity of isolated compound.

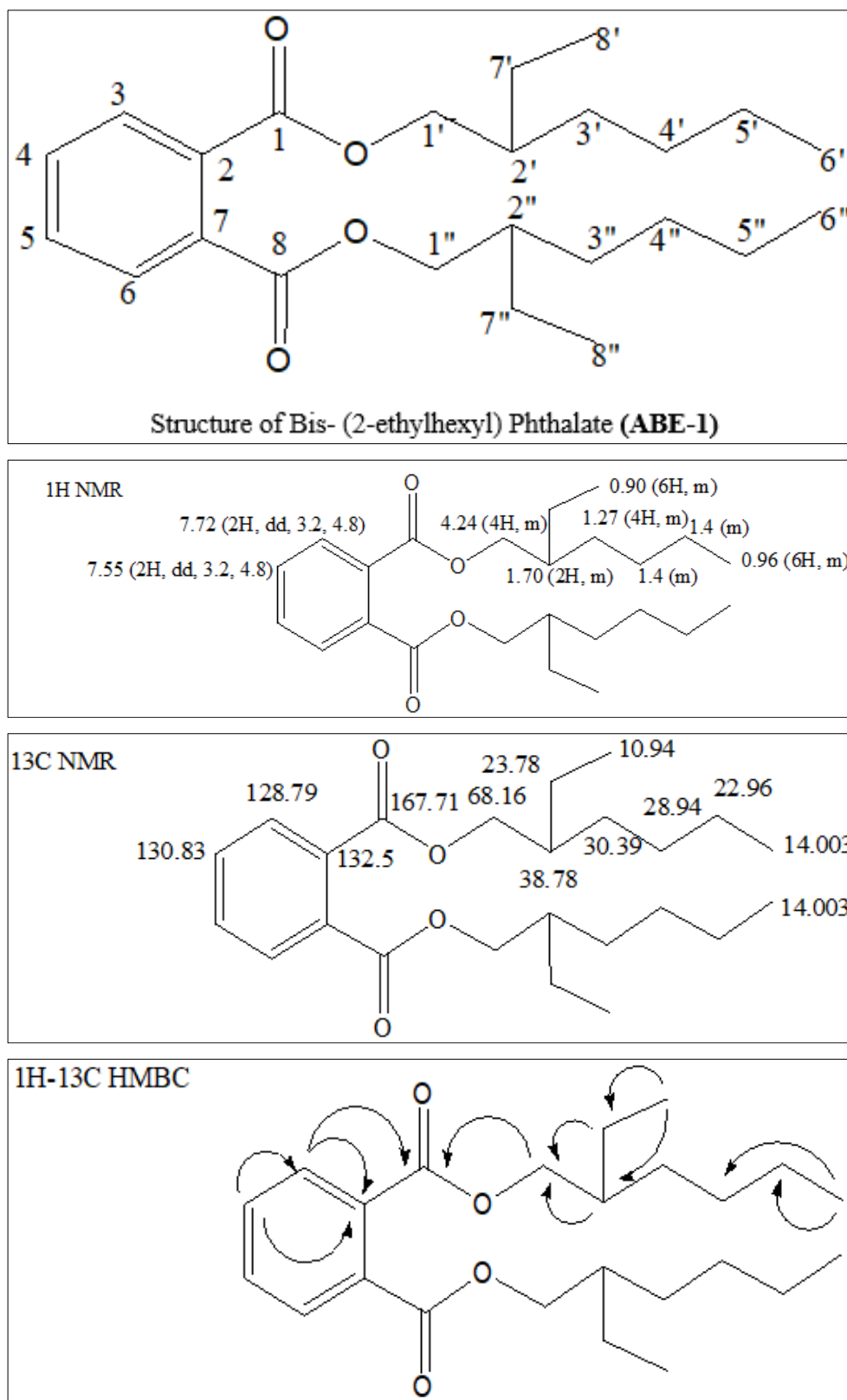


Fig 5: Structure of ABE-1, Bis- (2-ethylhexyl) Phthalate

Table 4: ¹H-NMR and ¹³C-NMR Spectral data of Compound ABE-1 and in comparison with the Literature value of Bis-(2-ethylhexyl) phthalate ^[14] in CDCl₃.

Position	¹ H-NMR (δ ppm, J in Hz)		¹³ C-NMR (δ ppm)	
	Compound ABE-1	Literature value	Compound ABE-1	Literature value
1			167.71	167.74
2			132.5	132.45
3	7.72 (2H, dd, J = 3.2, 4.8)	7.68 (2H, dd, J = 3.6, 6.0)	128.79	128.78
4	7.55 (2H, dd, J = 3.2, 4.8)	7.51 (2H, dd, J = 3.6, 6.0)	130.83	130.87
5	7.55 (2H, dd, J=3.2, 4.8)	7.51 (2H, dd, J = 3.6, 6.0)	130.83	130.87
6	7.72 (1H, dd, J=3.2, 4.8)	7.68 (2H, dd, J = 3.6, 6.0)	128.79	128.78
7			132.5	132.45
8			167.71	167.74
1', 1"	4.24 (4H,m)	4.20 (4H, m)	68.16	68.14
2', 2"	1.70 (2H, m)	1.65 (2H, m)	38.78	38.72
3', 3"	1.27 (4H, m)	1.34 (4H, m)	30.39	30.34
4', 4"	1.4 (m)	1.30 (8H, m)	28.94	28.91
5', 5"	1.4 (m)	1.30 (8H, m)	22.96	22.97
6', 6"	0.96 (6H, m)	0.88 (6H, t, J = 7.2 Hz)	14.00	14.04
7', 7"	1.41 (4H,m)	1.40 (4H)	23.78	23.73
8', 8"	0.90 (6H, m)	0.90 (6H, t, J = 7.2)	10.94	10.94

4. Conclusion

Four compounds were isolated from the bark extracts of *A. bilimbi* and their structures were elucidated and were found to be the compounds not previously reported to isolate from the bark of this plant. These compounds showed significant pharmacological actions.

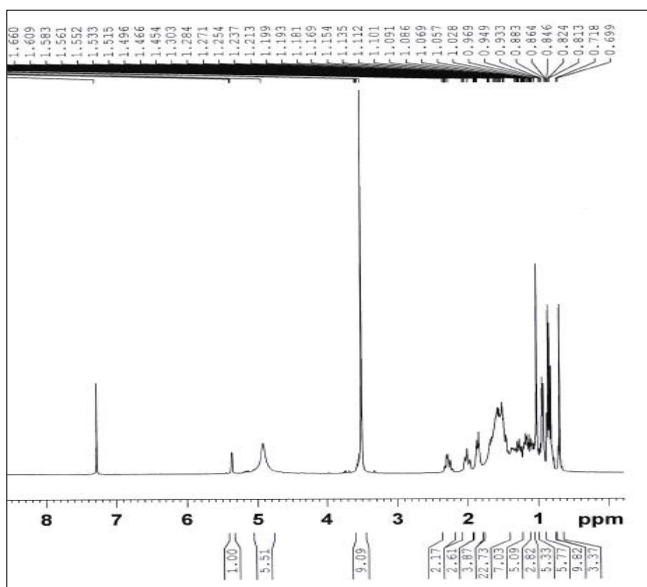
5. Acknowledgements

The authors would like to acknowledge the University Grants Commission of Bangladesh; the Centre for Advanced Research in Sciences (CARS), University of Dhaka for providing all possible supports in compound isolation; Wazed Miah Science Research Centre (WMSRC), Jahangirnagar University for help in NMR experiments and Department of Pharmacy, Jahangirnagar University for the use of their research facilities.

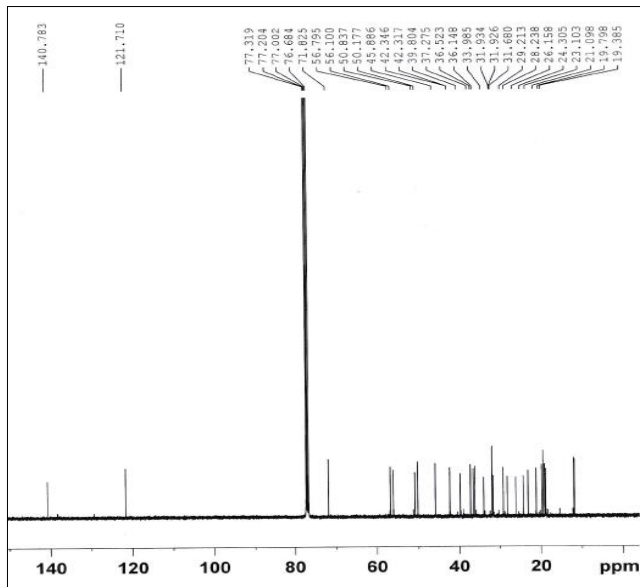
6. References

- Ghani A. Medicinal Plants of Bangladesh. 2nd Ed. The Asiatic Society of Bangladesh, Dhaka, Bangladesh; c2003. p. 115-116, 500-504, 540-541.
- Ali MR, Hossain M, Runa JF, Hasanuzzaman M. Preliminary cytotoxic activity of different extracts of *Averrhoa bilimbi* (fruits). Int. Curr. Pharm. J. 2013;2(3):83-84. doi: 10.3329/icpj.v2i3.13634
- Jayaweera DMA. Medicinal plants (Indigenous and exotic) used in Ceylon. Part IV. The National Science Council of Sri Lanka, Colombo, Sri Lanka; c1982. p. 166-167.
- Kirtikar KR, Basu BD. Indian medicinal plants. Volume 1. 2nd Ed. 44, International Book Distributors, Dehradun, India; c1935. p. 443.
- Yusuf M, Chowdhury JU, Wahab MA, Bejom J. Medicinal Plants of Bangladesh, 1st Ed. BCSIR Laboratories, Chittagong, Bangladesh; c1994. p. 31.
- Siddique KI, Uddin MMN, Islam MS, Parvin S, Shahriar M. Phytochemical screenings, thrombolytic activity and antimicrobial properties of the bark extracts of *Averrhoa bilimbi*. J App Pharm Sci. 2013;3(03):094-096. DOI: 10.7324/JAPS.2013.30318.
- Anitha Roy, Geetha RV, Lakshmi T. *Averrhoa bilimbi* Linn.-Nature's Drug Store-A Pharmacological Review. Int J Drug Dev & Res. 2011;3:101-106.
- Kumar KA, Gousia SK, Anupama M, Latha JNL. A review on phytochemical constituents and biological assays of *Averrhoa bilimbi*. Int J Pharm Pharm Sci Res. 2013;3:136-39.
- Alhassan AM, Ahmed QU. *Averrhoa bilimbi* Linn. A review of its ethnomedicinal uses, phytochemistry, and pharmacology. J Pharm Bioall Sci. 2016;8:265-71.
- Sarker MAM, Chowdhury AYSFUA. Analgesic effect of methanolic extract of *Averrhoa bilimbi* Linn. Bangladesh Medical Res Counc Bull. 2022;48:120-126. DOI: 10.3329/bmrcb.v48i2.62298
- Chaturvedula VSP, Prakash I. Isolation of Stigmasterol and β-Sitosterol from the dichloromethane extract of *Rubus suavisissimus*. Int. Curr. Pharm. J. 2012;1(9):239-242.
- Ahmadu AA, Omonigho U. Flavonoids from the leaves of *Physalis angulata* Linn. Afr. J. Pharm. Res. Dev. 2013;5(1):40-43.
- He D, Gu D, Huang Y, Ayupbek A, Yang Y, Aisa HA, Ito Y. Separation and Purification of Phenolic Acids and Myricetin from Black Currant by High Speed Countercurrent Chromatography. J. Liq. Chromatogr. Relat. Technol. 2009;32(20):3077-3088. doi:10.1080/10826070903320756.
- Ragasa CY, Perez JDV, Shen CC. Chemical constituents of *Cymodocea serrulata* R. Brown. Res. J. Pharm. Biol. Chem. Sci. 2016;7(6):1630-1633.

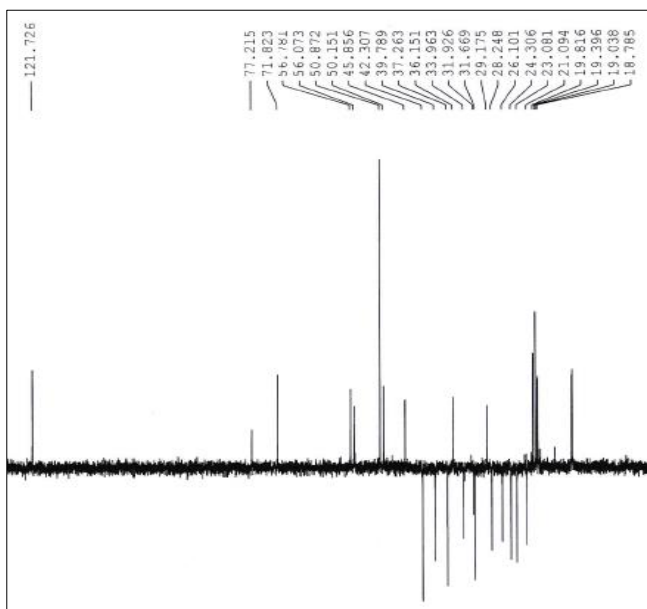
Appendix: Spectroscopic Data of Isolated Compounds Spectroscopic data of Compound ABC-1



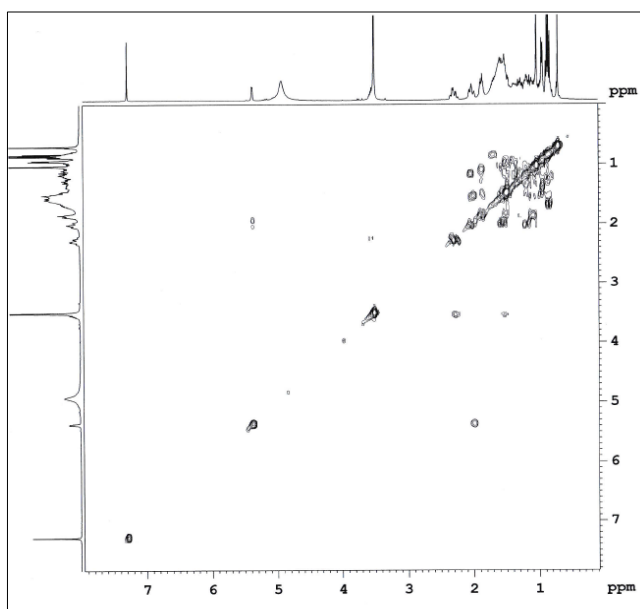
¹H-NMR Spectrum of ABC-1 (CDCl₃ at 400 MHz)



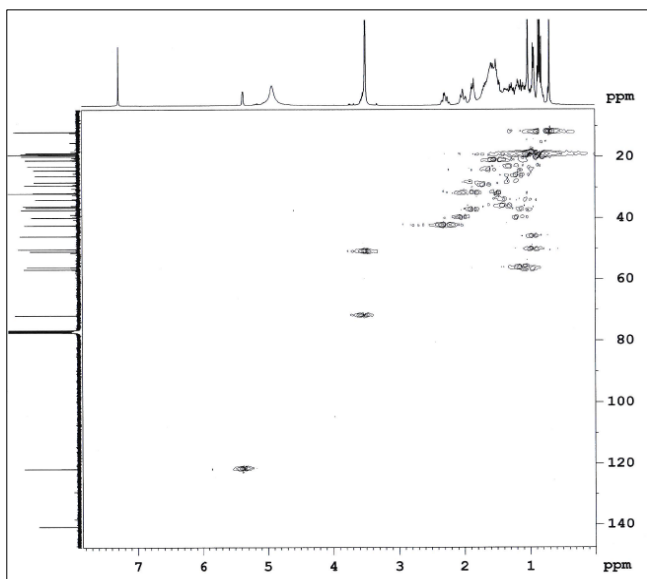
¹³C-NMR Spectrum of ABC-1 (CDCl₃ at 400 MHz)



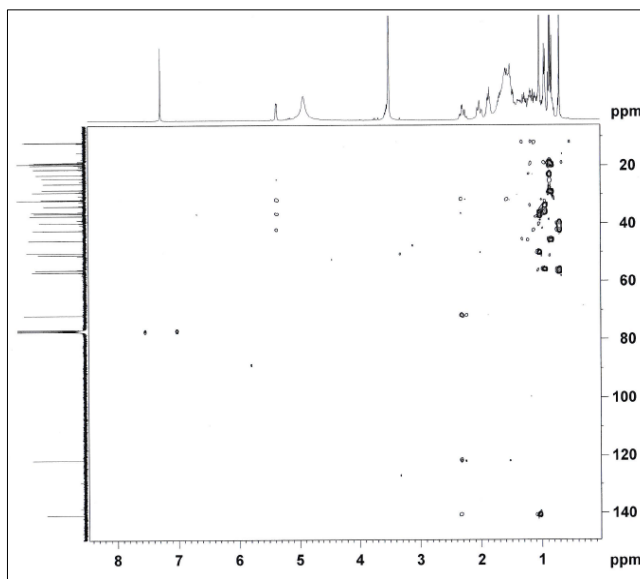
¹³C (DEPT) NMR Spectrum of ABC-1 (CDCl₃ at 400 MHz)



COSY Spectrum of ABC-1 (CDCl₃ at 400 MHz)

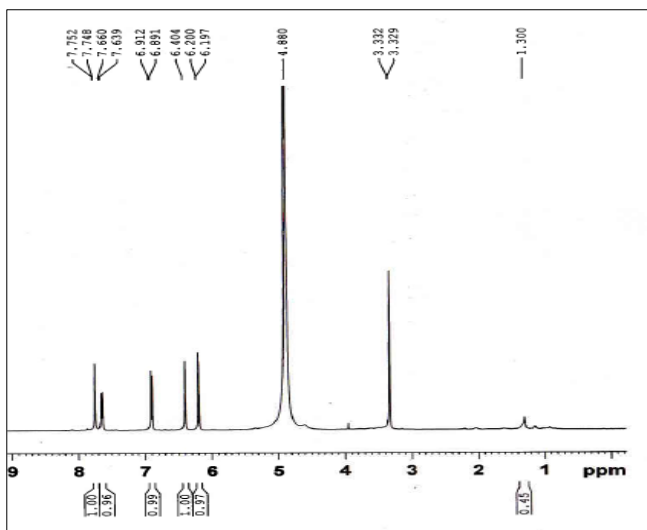


HSQC Spectrum of ABC-1 (CDCl₃ at 400 MHz)

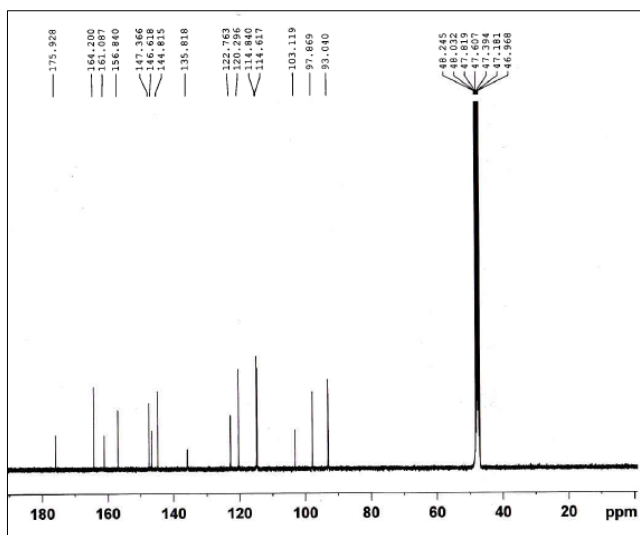


HMBC Spectrum of ABC-1 (CDCl₃ at 400 MHz)

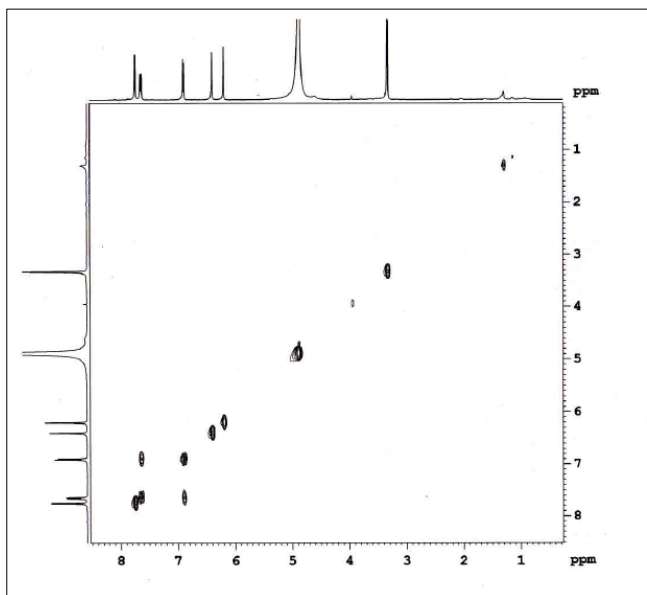
Spectroscopic data of Compound ABC-2



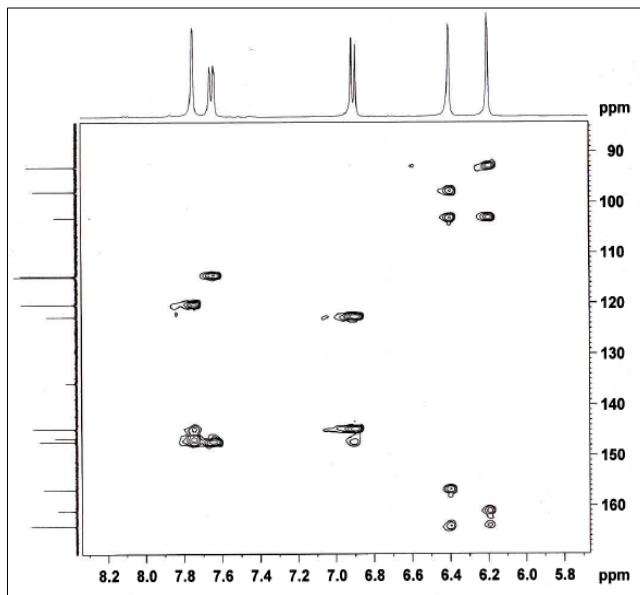
¹H-NMR Spectrum of ABC-2 (CD₃OD at 400 MHz)



¹³C-NMR Spectrum of ABC-2 (CD₃OD at 400 MHz)

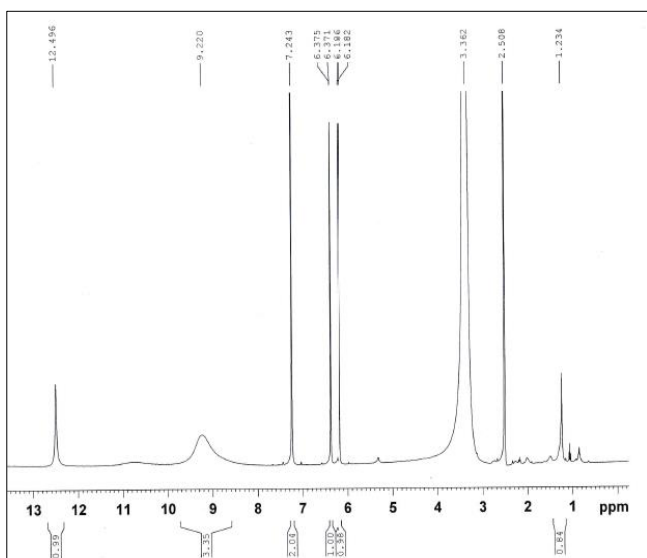


COSY Spectrum of ABC-2 (CD₃OD at 400 MHz)

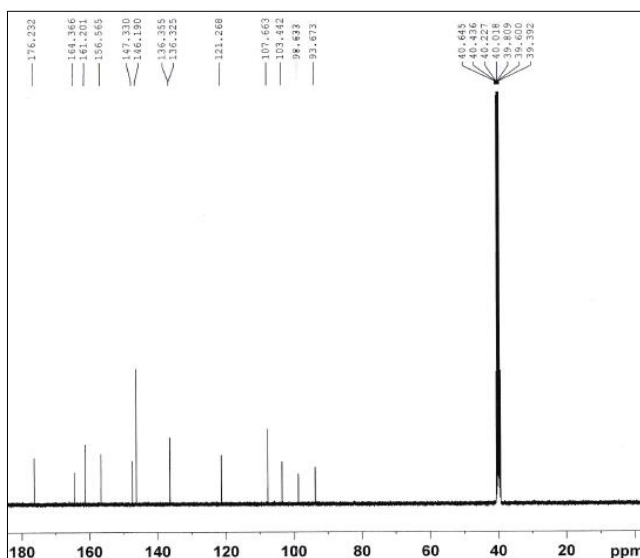


HMBC Spectrum of ABC-2 (CD₃OD at 400 MHz)

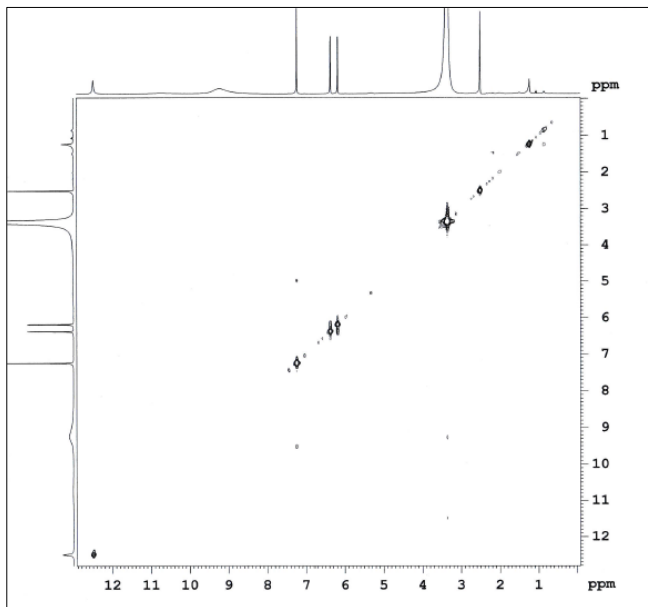
Spectroscopic data of Compound ABC-3



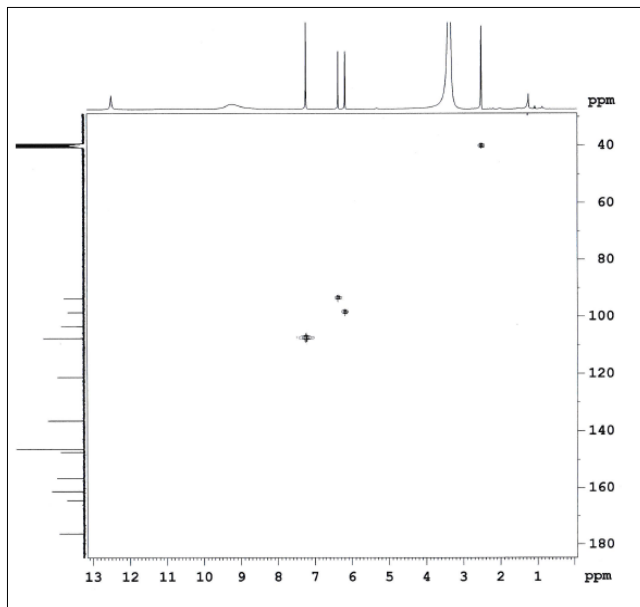
¹H-NMR Spectrum of ABC-3 (DMSO at 400 MHz)



¹³C-NMR Spectrum of ABC-3 (DMSO at 400 MHz)

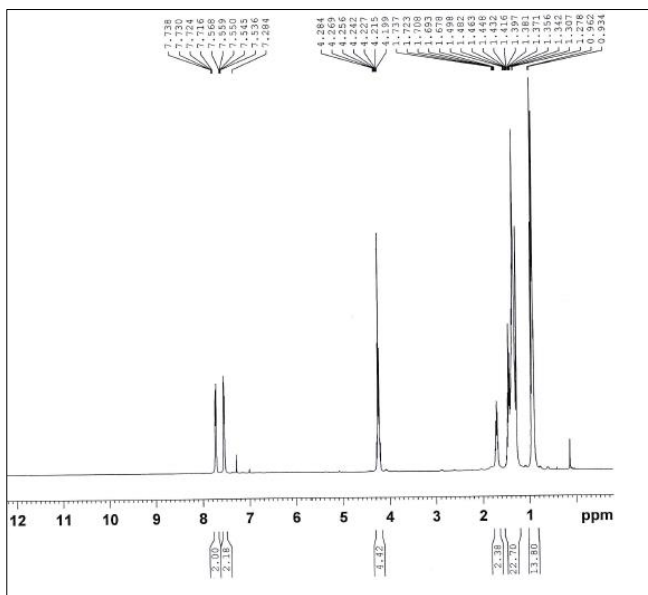


COSY Spectrum of ABC-3 (DMSO at 400 MHz)

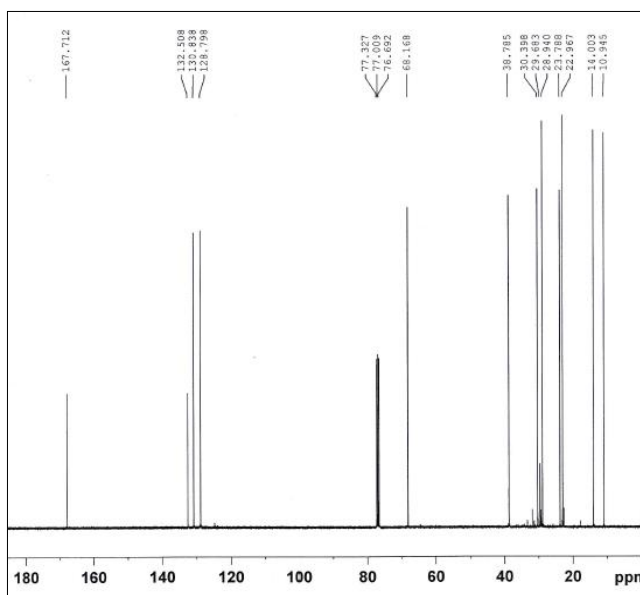


HMBC Spectrum of ABC-3 (DMSO at 400 MHz)

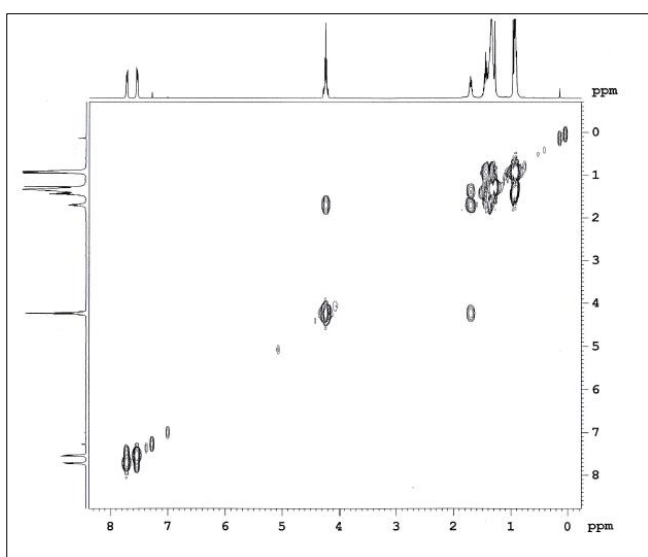
Spectroscopic data of Compound ABE-1



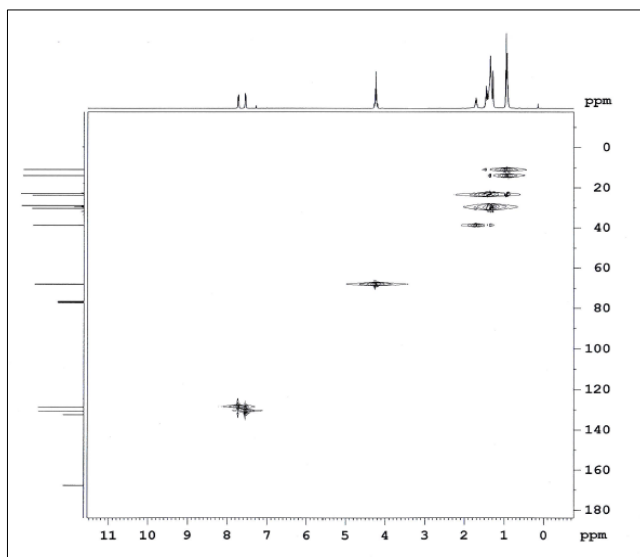
¹H-NMR Spectrum of ABE-1 (CDCl₃ at 400 MHz)



¹³C-NMR Spectrum of ABE-1 (CDCl₃ at 400 MHz)



COSY Spectrum of ABE-1 (CDCl₃ at 400 MHz)



HSQC Spectrum of ABE-1 (CDCl₃ at 400 MHz)