JBEP

Journal of Biosciences and Experimental Pharmacology
ISSN-2960-284X

Short Communication

Isolation of Anthraquinone Derivatives Along with Other Constituents from *Polygonum flaccidum* Extract

Pritesh Ranjan Dash [1,2], Md. Sohel Rana[1], Choudhury Mahmood Hasan[3], Md. Abdul Muhit[4*]

[1]Department of Pharmacy, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka, Bangladesh

[2]Department of Pharmacy, Primeasia University, Banani Dhaka

[3]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Dhaka, Bangladesh

[4]Department of Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, University of Dhaka, Bangladesh

[*]***Correspondence E-mail***: *muhit@du.ac.bd*

Citation: Dash, P. R.; Rana M. S.; Hasan, C. M.; Muhit, M. A. Isolation of anthraquinone derivatives along with other constituents from *Polygonum flaccidum*. *J. Bio, Sci. Exp, Pharm.* 2024***, 2(1),* 94–102.**

https://doi.org/10.62624/JBEP00.0013

Academic Editor: Dr. Sheikh Jamal Uddin

Received date: May 22, 2024

Accepted date: June 28, 2024

Published date: July 15, 2024

Publisher's Note: JBEP stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: Total six compounds were isolated from the methanolic extract of *Polygonum flaccidum* and structural elucidation of the yielded compounds using intensive studies of their NMR spectrometry confirmed them as 1, 7, 8-trimethoxy-2, 3, 5-trihydroxy anthraquinone (1), 1, 2, 3, 6, 7, 8-hexamethoxy anthraquinone (2) 1, 7, 8-trimethoxy-2, 3, 5, 6-tetrahydroxy anthraquinone (3), β-sitosterol (4), decanoic acid (5) and 1-tetradecanol (6). Literature survey revealed that compound (1), (2), (3) is the new compound from natural products. The isolated compounds 5, 6 are the first time report from this plant. Therefore, the plant is a good candidate for further studies to isolate the bioactive principles to identify leads for drug development.

Keywords: *Polygonum flaccidum*, anthraquinone, NMR spectroscopy

1. Introduction

A considerable work has already been done to identify and isolate the chemical constituents from different extracts of *Polygonum flaccidum*. Numerous studies have exposed that different extracts of *Polygonum flaccidum* contains several bioactive compounds including acylflavone, α-santalene, caryophyllenepoxide, borneol, sitosterin and stigmasterol [1]. The potent bioactive compound named α-santalone has been identified by the aerial parts of *P. flaccidum* when extracted with methanol [2]. This investigation was undertaken to isolate and identify the potential bioactive compounds from *Polygonum flaccidum* extract.

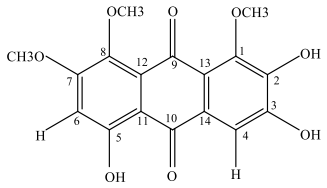
2. Materials and Methods

2.1 Plant Sources

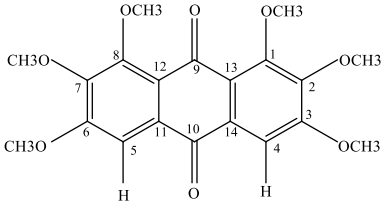
The plant of *Polygonum flaccidum* Meisn. was collected from the local area of Savar, Dhaka during January 2014. Dust, dirt and the undesirable materials were then separated manually. The collected plant was then identified by the taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka and a voucher specimen (DACB: 39,317) has been deposited for future reference.

2.2 Extraction and Isolation

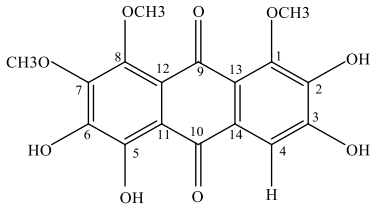
The powder plant (1 kg) of *P. flaccidum* was extracted by cold extraction method with 6 L of methanol for 7 days at room temperature with occasional stirring. The extract was then filtered and evaporated on rotary evaporator under reduced pressure to obtain 49 gm extract. The crude extract was subjected to VLC using solvent system of n-hexane, n-hexane-chloroform, chloroform and finally with the mixture of methanol-chloroform with increasing polarities to obtain 9 (Fraction 1-fraction 9) major fractions. The crude extract of *P. flaccidum* (PF), as well as fraction-1 (F-1, 0.15 g), fraction-2 (F-2, 0.15 g), fraction-3 (F-3, 0.17 g), fraction-4 (F-4, 0.33 g), fraction-5 (F-5, 0.28 g), fraction-6 (F-6, 2.84 g), fraction-7 (F-7, 0.54 g), fraction-8 (F-8, 3.2 g) and fraction-9 (F-9, 2.9 g) were subjected to chemical investigations. Upon the TLC behavior, the fraction-6 (0.54gm), fraction-7 (3.20gm) and fraction-8 (2.90gm) were combined to form a mixture. The mixture of 6.13 gm was then fractionated using column chromatography with silica gel (Kieselgel 60 and mesh 70-230). Elution of the column were done using petroleum ether, followed by mixtures of petroleum ether-ethyl acetate, ethyl acetate and finally with the mixtures of ethyl acetate-methanol, along with increasing degree of polarities for providing a total of 96 fractions each with 100 ml. After TLC screening, similar fractions were mixed together and purified the compounds by washing with different solvent treatments to give 1, 7, 8-trimethoxy-2, 3, 5-trihydroxy anthraquinone (1), 1, 2, 3, 6, 7, 8-hexamethoxy anthraquinone (2) 1, 7, 8-trimethoxy-2, 3, 5, 6-tetrahydroxy anthraquinone (3), β-sitosterol (4), Decanoic Acid (5) and 1-tetradecanol (6). In the current investigation, a total of six compounds have been isolated from the plant of *Polygonum flaccidum*. Literature survey revealed that compound (1), (2), (3) is the new compound from natural products. The isolated compounds 5, 6 are the first time report from this plant.



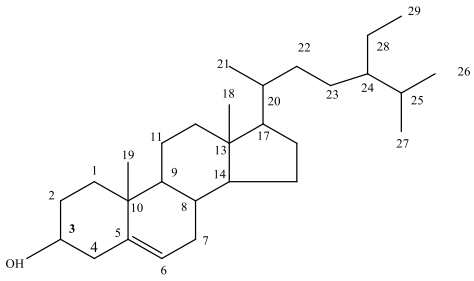
(1)



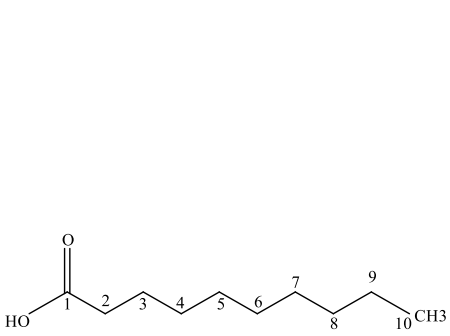
(2)



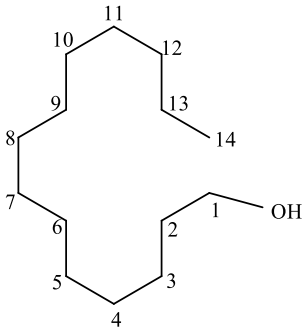
(3)



(4)



(5)



(6)

Figure 1. Compounds **1**-**6** isolated from *Polygonum flaccidum*

3. Results

1, 7, 8-trimethoxy-2, 3, 5-trihydroxy-anthraquinone (1): Yellow power; 1H-NMR (400 MHz, CDCl3): δ4.24 (s, OCH3-1), 7.76 (s, H-4) 10.29 (s, OH-5), 7.68 (s, H-6) 4.05 (OCH3-7), 4.40 (OCH3-7).

1, 2, 3, 6, 7, 8-hexamethoxy anthraquinone (2): Yellow power; 1H-NMR (400 MHz, DMSO): δ 4.04 (s, OCH3-1), 3.89 (s, OCH3-1), 4.15(s, OCH3-1) 7.53, 7.53, δ4.04 (s, OCH3-1), 3.89 (s, OCH3-1), 4.15 (s, OCH3-1).

1, 7, 8-trimethoxy-2, 3, 5, 6-tetrahydroxy anthraquinone (3): Yellow power; 1H-NMR (400 MHz, CDCl3): δ4.31 (s, OCH3-1) 7.80 (s, H-4), 10.31 (s, OH-5), 4.04 (s, OCH3-7), 4.40 (s, OCH3-8).

β -sitosterol (4): White powder; ^{1}H-NMR (400 MHz, CDCl3): δ3.55 (1H, s, H-3), 5.36 (1H, m, H-6), 1.02 (3H, s, CH3-10), 0.69 (3H, s, CH3-13), 0.94 (3H, d, J=6.4 Hz, CH3-20), 1.27 (H-21); 1.3 (dd, H-22), 1.13 (m, H-23); 0.94(m, H-24) 1.58(m, H-25) 0.87(d, H-26) 0.84 (d, H-27) 1.23(m, H-28) 0.71(t, H-29).). 13C-NMR (100 MHz, CDCl3): δ37.27(C-1), 31.66 (C-2), 71.79 (C-3), 42.23 (C-4), 140.77(C-5), 121.70(C-6), 31.88(C-7), 31.92(C-8), 50.16(C-9), 36.51(C-10), 23.09 (C-11), 39.80 (C-12), 42.30 (C-13), 56.78 (C-14), 24.37(C-15), 28.25(C-16), 56.09(C-17), 11.86 (C-18), 19.40 (C-19), 36.15 (C-20), 18.79 (C-21), 33.97 (C-22), 26.13 (C-23), 45.86 (C-24), 29.19 (C-25), 19.82 (C-26), 19.06 (C-27), 21.23 (C-28), 11.99 (C-29).

Decanoic Acid (5): Brown powder; ^{1}H-NMR (400 MHz, CDCl3): δ 2.36 (2H, m, H-2), 2.35 (1H, m, H-3), 1.63 (2H, m, H-4), 1.84 (2H, m, H-5), 1.67 (1H, m, H-6), 1.65 (2H, m, H-7), 2.15 (2H, m, H-8), 1.27 (2H, m, H-9), 0.90 (H-10). 13C-NMR (100 MHz, CDCl3): δ39.43(C-2), 24.7(C-3), 29.0(C-4), 29.35(C-5), 25.69(C-6), 29.3(C-7), 31.9(C-8), 22.7(C-9), 14.07(C-10).

1-Tetradecanol (6): White powder; ^{1}H-NMR (400 MHz, CDCl3): δ4.06 (1H, m, J= 6.4Hz, 6.8Hz, H-1), 1.27 (1H, s), H-3), 0.88 (J=7.2 Hz, H-14). 13C-NMR (100 MHz, CDCl3): δ63.10 (C-1) 31.92(C-2), 22.67(C-3), 29.68(C-4), 25.74(C-5), 29.52(C-6), 29.42(C-7), 29.34(C-8), 29.64(C-9), 29.06(C-10), 32.82(C-11), 29.59(C-12), 33.50(C-13), 14.07(C-14).

3.1 Chemotaxonomic Significance

1, 7, 8-trimethoxy-2, 3, 5-trihydroxy anthraquinone (1), 1, 2, 3, 6, 7, 8-hexamethoxy anthraquinone (2) and 1, 7, 8-trimethoxy-2, 3, 5, 6-tetrahydroxy anthraquinone (3) were isolated and identified as anthraquinone derivatives. Literature survey revealed that compound (1), (2) and (3) appears to be new compound, we could not find any compound like this. β-sitosterol (4) has been reported from *Polygonum amplexicaule* [3], *Polygonum*

aviculare [4] and *Polygonum bistorta* [5, 6] respectively. Decanoic acid (5) and 1-tetradecanol (6) were isolated and identified as known fatty acid derivatives compounds, although had not previously been isolated from this plant.

4. Discussion

Compound 1 as 1, 7, 8-trimethoxy-2, 3, 5-trihydroxy-anthraquinone

Compound 1 was isolated from column fraction by elution with PE/50-50% EtOAc. It was obtained as a yellow crystal (3 mg). It appeared as a white yellowish spot on the TLC plate under UV light at 254 nm and also exhibited blue fluorescence at 365 nm. Compound 1 is soluble in $CDCl_3$.

The NMR (400 MHz, $CDCl_3$) data of compound 1 showed unexpectedly three methoxy signals, one hydroxyl signal and two aromatic proton signals only. Revealing the characteristic of the compound. It was appeared as anthraquinone derivatives.

The ^{1}H NMR showed a clear spectrum of three proton singlets at δ 4.24, δ 4.05 and δ 4.40 due to three methoxyl groups. The spectrum also showed only two proton signals at δ 7.76 and δ 7.68 in the aromatic region and one chelated hydroxy signal at δ 10.22. Due to low resolution and low amount of yield ^{13}C NMR data could not be revealed. Nevertheless, the above signals directed it as an anthraquinone derivative. Anthraquinones have 14 carbon skeletons, among which eight positions can be substituted either with hydroxyl or methoxy group. Based on the above evidence compound 1 is 1, 7, 8-trimethoxy-2, 3, 5-trihydroxy-anthraquinone. Meticulous observation at the spectrum and extensive literature survey analysis of anthraquinone structural properties, we could not find any compound like this. It appears to be new compound but to confirm it will need more spectral data because due to paucity of compound we could not do any other spectral studies. So, we could not confirm whether this compound is new.

Compound 2 as 1, 2, 3, 6, 7, 8-hexamethoxy anthraquinone

Compound 2 was isolated from column fraction by elution with MeOH/1-99% DCM. It was obtained as a yellow crystal (3 mg). It appeared as a white yellowish spot on the TLC plate under UV light at 254 nm and also exhibited blue fluorescence at 365 nm. Compound 2 is soluble in DMSO.

A clear ^{1}H NMR (400 MHz, DMSO) spectrum of the compound showed 4 signals only. Among the signals, 3 signals appeared at δ 3.89, δ 4.15 and δ 4.04 revealed integration of three protons each. These signals can be attributed to methoxy protons. The spectrum also showed only one proton signal at δ 7.53 in the aromatic region. Due to low resolution and low amount of yield, ^{13}C NMR data could not be revealed. Nevertheless, the above signals directed it as an anthraquinone derivative. Anthraquinones have 14 carbon skeletons, among which eight positions can be substituted either with hydroxyl or methoxy group. A thorough observation at the spectrum and extensive literature survey analysis of anthraquinone structural properties, the compound seems to be asymmetrical in nature. Due to low resolution the position of the single aromatic proton could not be confirmed. Most of the anthraquinones H-4 position is not substituted. That's why here, H-1, 2, 3 position can be substituted with methoxy protons. Therefore, the structure of compound 2 is 1, 2, 3, 6, 7, 8- hexamethoxy anthraquinone. Based on the above observations and literature survey, we could not find any compound like this reported previously. It appears to be new compound but to confirm it will need more spectral data. However, due to paucity of compound we could not do any other spectral studies.

Compound 3 as 1, 7, 8-trimethoxy-2, 3, 5, 6-tetrahydroxy anthraquinone

Compound 3 was isolated from column fraction by elution with MeOH/2-98% DCM. It was obtained as a yellow crystal (3 mg). It appeared as a white yellowish spot on the TLC plate under UV light at 254 nm and also exhibited blue fluorescence at 365 nm. Compound 3 is soluble in $CDCl_3$.

The ^1H NMR (400 MHz, $CDCl_3$) data of compound 3 showed unexpectedly three methoxy signals, one hydroxyl signal and an aromatic proton signal only. Revealing the characteristic of the compound. It was appeared as anthraquinone derivatives.

The ^1H NMR showed a clear spectrum of three proton singlets at δ 4.04, δ 4.40 and δ 4.31 due to three methoxy groups. The spectrum also showed only one proton signal at δ 7.80 in the aromatic region and one chelated hydroxy signal at δ 10.31. Due to low resolution and low amount of yield, ^{13}C NMR data could not be revealed for this compound. Nevertheless, the above signals directed it as an anthraquinone derivative. Anthraquinones have 14 carbon skeleton, among which eight positions can be substituted either with hydroxyl or methoxy group. Based on the above evidence compound 3 is 1, 7, 8-trimethoxy-2, 3, 5, 6-tetrahydroxy anthraquinone. A careful observation at the spectrum and extensive literature survey analysis of anthraquinone structural properties, we could not find any report suggesting that it appears to be new compound. However, to confirm it will need more spectral data. Due to the scarcity of the isolated compound, we could not do any other spectral studies.

5. Conclusions

Based on the above observations and literature survey, we could not find any compound like as 1, 7, 8-trimethoxy-2, 3, 5-trihydroxy anthraquinone (1), 1, 2, 3, 6, 7, 8-hexamethoxy anthraquinone (2) 1, 7, 8-trimethoxy-2, 3, 5, 6-tetrahydroxy anthraquinone (3). They appears to be new compound. However, it will need more spectral data. Further research is warranted to do more spectral analysis.

Author Contributions: The authors confirm contribution to the paper as follows: study conception and design: MSR. Methodology: PRD; data analysis and interpretation of results: PRD, CMH and MAM. Supervision: MSR Draft manuscript preparation: PRD, CMH and MAM. All authors reviewed the results and approved the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

Conflict of interest statement: Authors declare no conflict of interest.

Funding: This research did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Acknowledgment: The authors are thankful to the authority of BCSIR for providing laboratory facilities and also thankful to the authority of Wazed Miah Science Research Centre, Jahangirnagar University, Savar, Dhaka for the measurement of NMR spectra and logistic supports.

References:

1. Dash, P. R.; Sohrab, M. H.; Rana, M.S. *Polygonum Flaccidum* (*Polygonaceae*): A Review. *Int J Life Sci Rev* **2016**, *2*, 109-13.
2. Ahmed, M.; Datta, B. K.; Rouf, A. S. S.; Jakupovic, J. Flavone and α-santalene derivatives from *Polygonum flaccidum*. *Phytochem*, **1991**, *30*, 3155–3156.
3. Ren, H.; Wan, D.; Zou, Z. Studies on chemical constituents from roots of *Polygonum amplexicaule*. Zhongguo Zhong Yao Za Zhi **2009**, *34*, 183-5.
4. Al-Hazimi, H.; Haque, S. A new naphthoquinone from *Polygonum aviculare*. *Nat Prod Lett* **2002**, 16, 115-118.
5. Manoharan K.P.; Benny, T. K. H.; Yang, D. Cycloartane type triterpenoids from the rhizomes of *Polygonum bistorta*. *Phytochem* **2005**, 66, 2304–2308

6. Manoharan K. P.; Yang, D.; Hsu, A.; Benny, T. K. H. Evaluation of *Polygonum bistorta* for anticancer potential using selected cancer cell lines. *Med Chem* **2007**, 3, 121-126.

Supplementary Materials:

Table 1: ^{1}H NMR (400MHz) data for **Compound-1** in $CDCl_3$.

Position	**Compound-1**
	δ_H (ppm) (mult., *J* in Hz)
1-OCH_3	**4.24 s**
2	
3	
4	**7.76 s**
5-OH	**10.29 s**
6	**7.68 s**
7-OCH_3	**4.05**
8-OCH_3	**4.40**

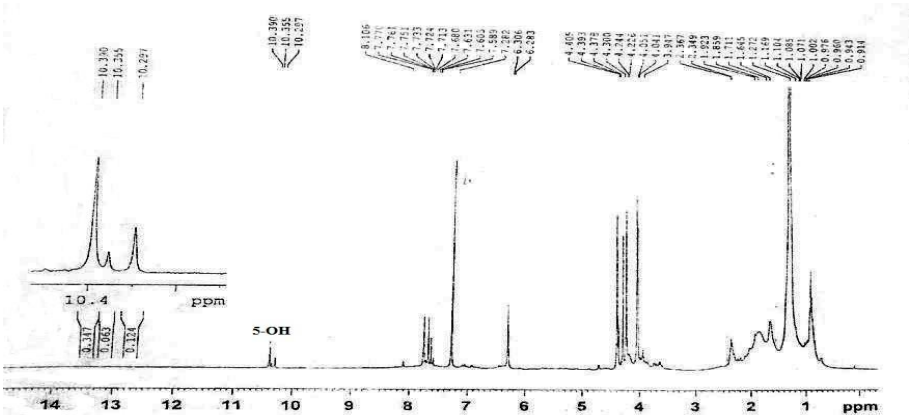


Figure 1: ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of **Compound-1**

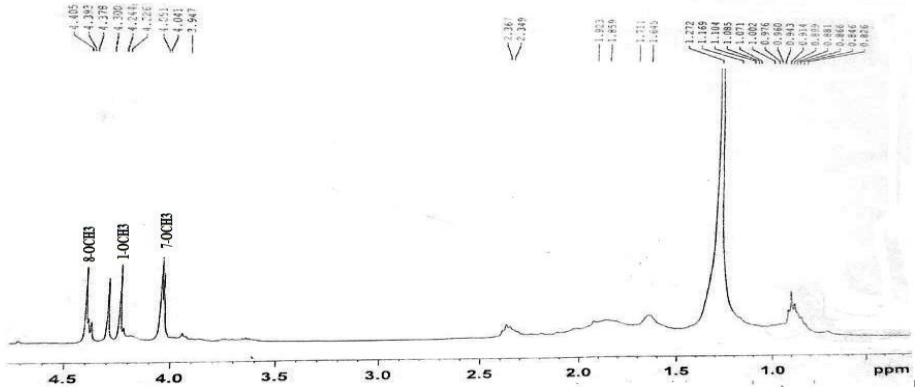


Figure 2: ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of **Compound-1 (expanded)**

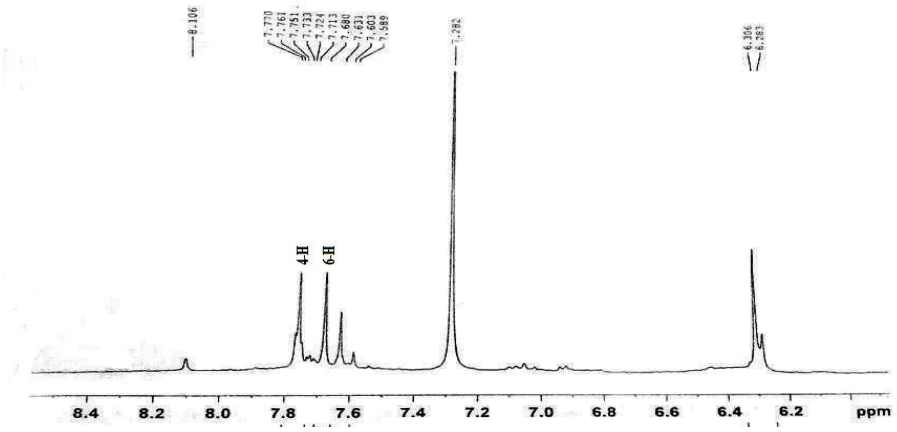


Figure 3: ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of **Compound-1 (expanded)**

Table 2: ^{1}H NMR (400MHz) data for **Compound-2** in DMSO.

Position	**Compound-2**
	δ_H (ppm) (mult., *J* in Hz)
1-OCH3	4.04 s
2-OCH3	3.89s
3-OCH3	4.15s
4	7.53
5	7.53
6-OCH3	4.04s
7 -OCH3	3.89s
8-OCH3	4.15s

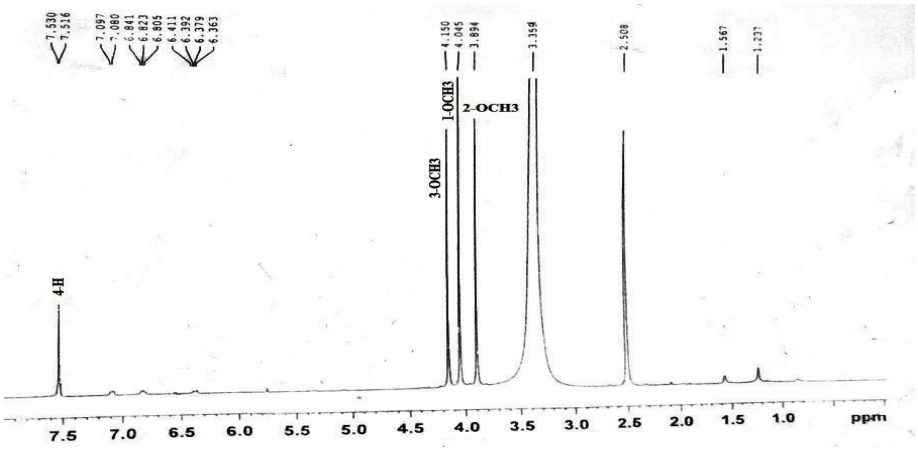


Figure 4: ^{1}H-NMR spectrum (400 MHz, DMSO) of **Compound-2**

Table 3: ^{1}H NMR (400 MHz) data for **Compound-3** in $CDCl_3$.

Position	**Compound-3**
	δ_H (ppm) (mult., *J* in Hz)
1-OCH3	4.31s
2	
3	
4	7.80s
5 -OH	10.31s
6	
7 –OCH3	4.04s
8 –OCH3	4.40s

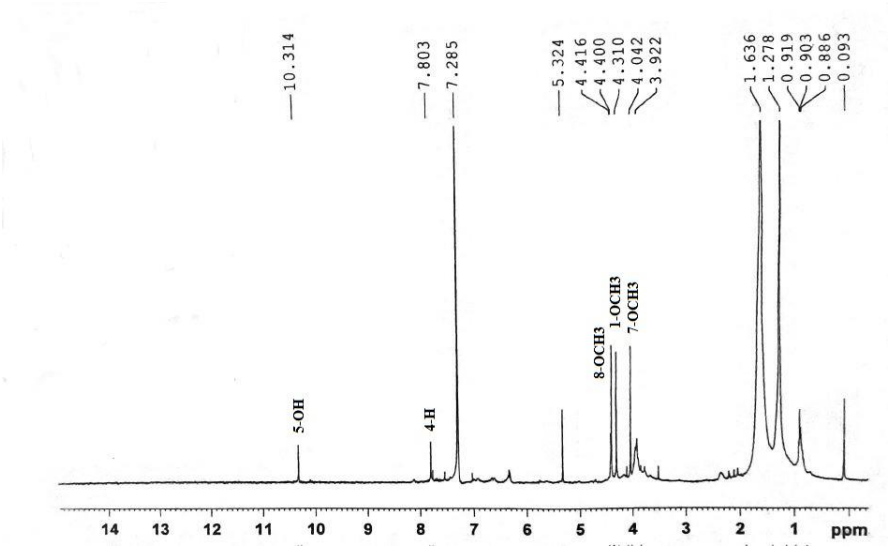


Figure 5: ^{1}H-NMR spectrum (400 MHz, $CDCl_3$) of **Compound-3**