

## Bioactive Compounds from Endophytic Bacteria *Bacillus subtilis* strain EP1 with their Antibacterial Activities

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**Abstract:** Endophytic bacteria boost host plant defense and growth by producing vital compounds. In the current study, a bacterial strain was isolated from the *Boswellia sacra* plant and identified as *Bacillus subtilis* strain EP1 (accession number: MT256301) through 16S RNA gene sequencing. From the identified bacteria, four compounds; **1** (4-(4-cinnamoyloxy)phenyl)butanoic acid), **2** (cyclo-(L-Pro-D-Tyr)), **3** (cyclo-(L-Val-L-Phe)), and **4** (cyclo-(L-Pro-L-Val)) were isolated and characterized by 1D and 2D NMR and mass spectroscopy. Moreover, antibacterial activity and beta-lactam-producing gene inhibition [(δ-(l-α-amino adipyl)-l-cysteinyl-d-valine synthetase (ACVS) and amino adipate aminotransferase (AADAT)] assays were performed. Significant antibacterial activity was observed against the human pathogenic bacterial strains (*E. coli*) by compound **4** with 13 ± 0.7 mm zone of inhibition (ZOI) followed by compound **1** having 11 ± 0.7 mm ZOI. In contrast, the least antibacterial activity among the tested samples was offered by compound **2** with 10 ± 0.9 mm ZOI compared to the standard (26 ± 1.2 mm). Similarly, the molecular analysis of beta-lactam inhibition determined that compounds **3** and **4** have inhibited the two genes (2 to 4-fold) in the beta-lactam biosynthesis (ACVS and AADAT) pathway. From these results, it can be concluded that future research on these compounds could lead to the inhibition of antibiotic-resistant pathogenic bacterial strains.

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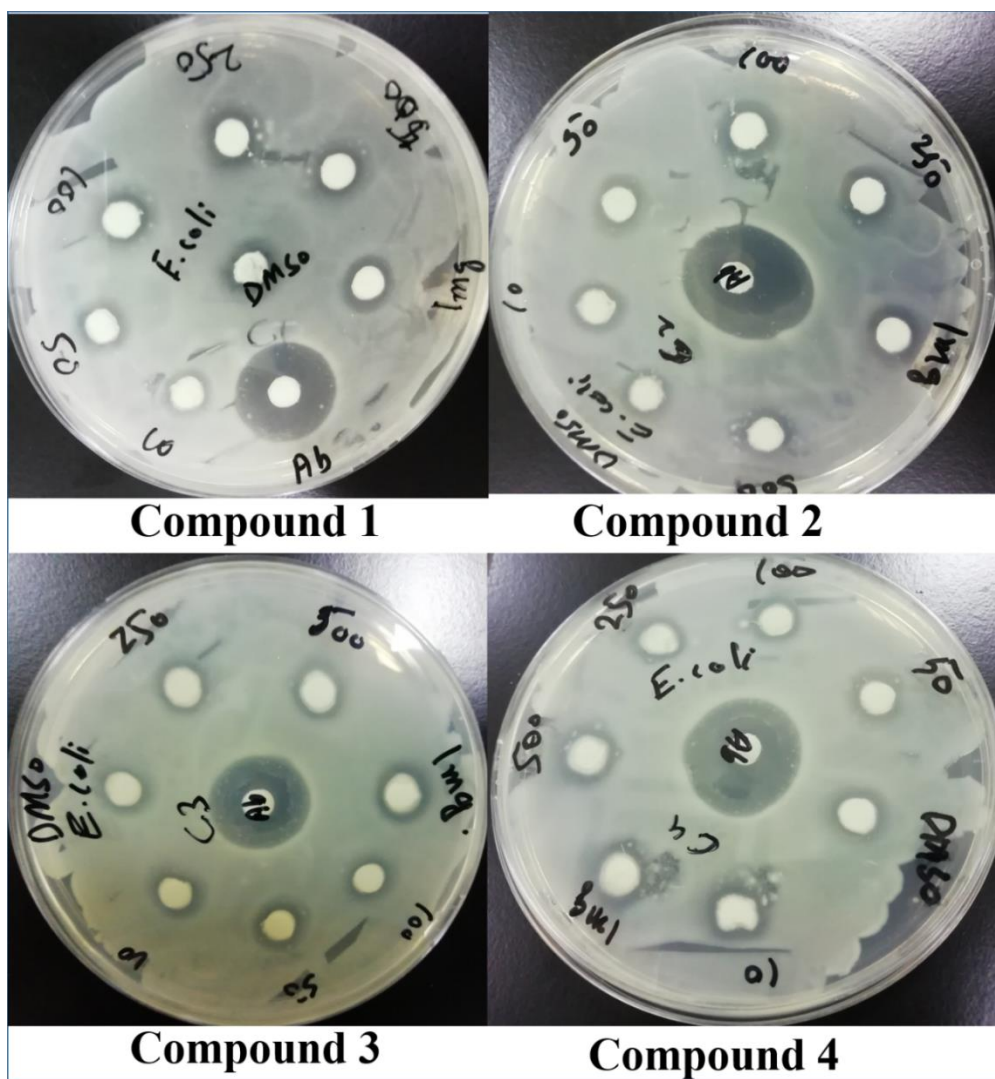
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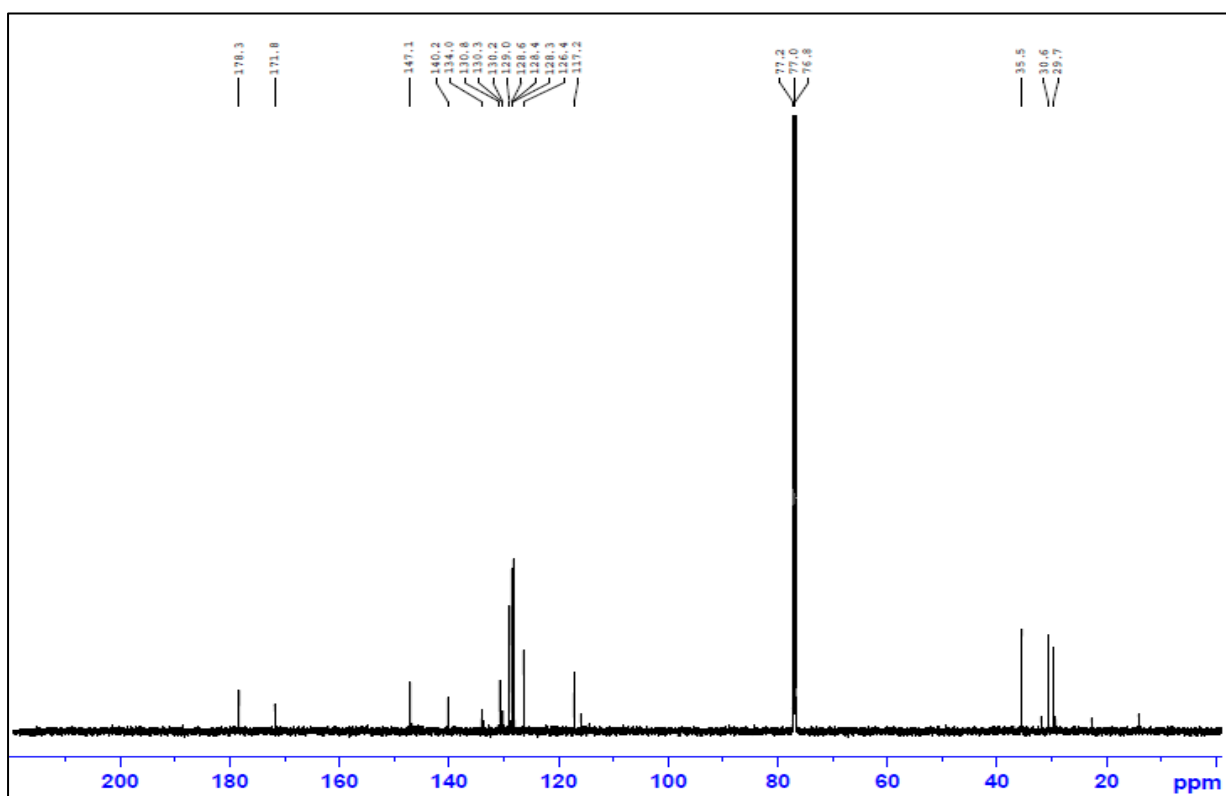
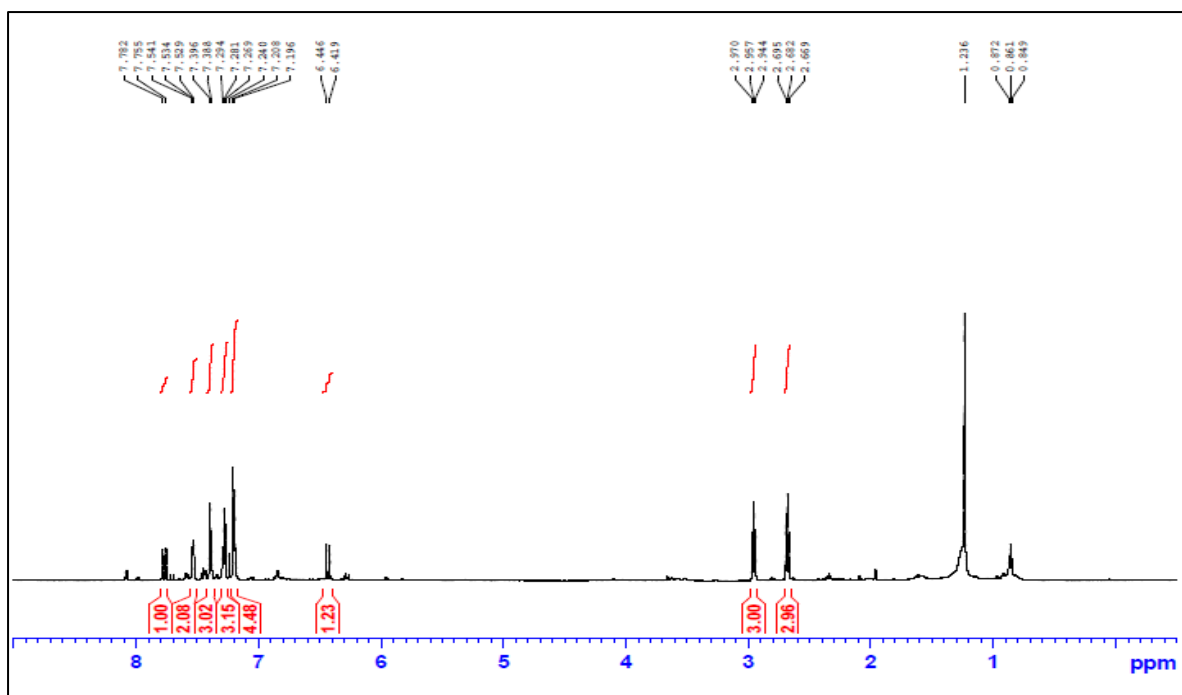
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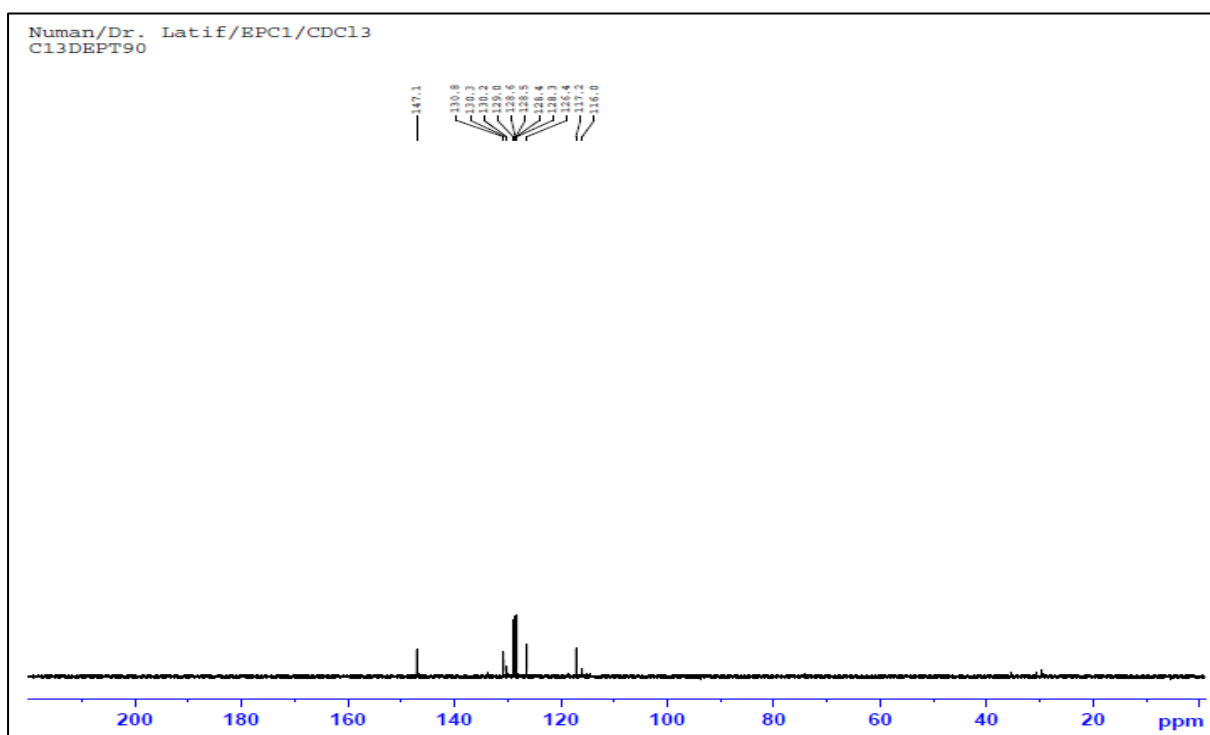
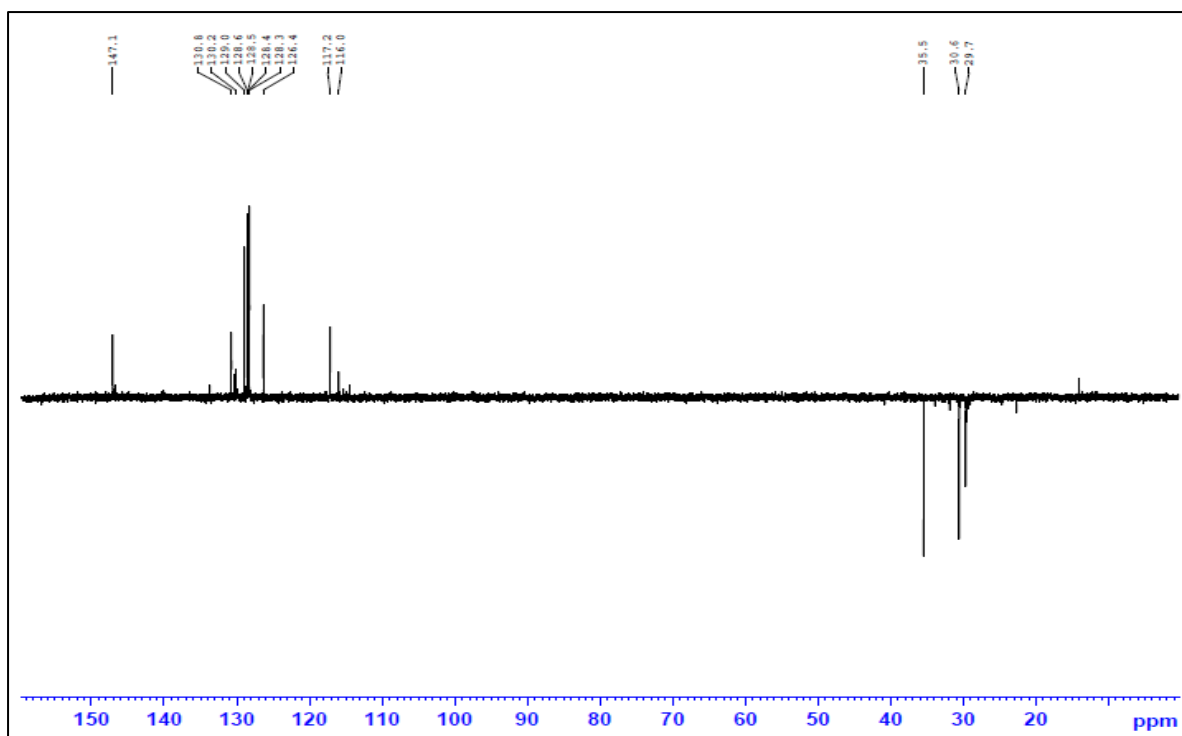
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**Keywords:** Secondary metabolites; *Bacillus subtilis* strain EP1; antibacterial activity; NMR spectroscopy; HR-ESI-MS.



**Figure S1.** Antibacterial activity of the isolated compounds measured as disc diffusion.





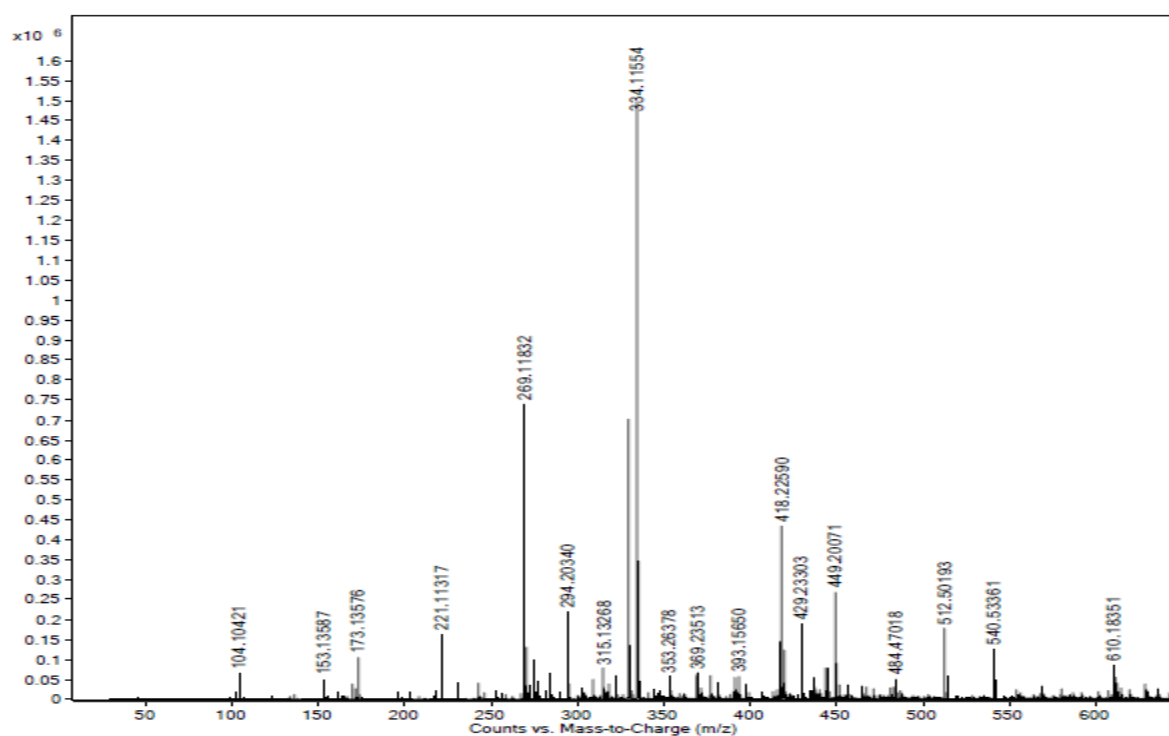
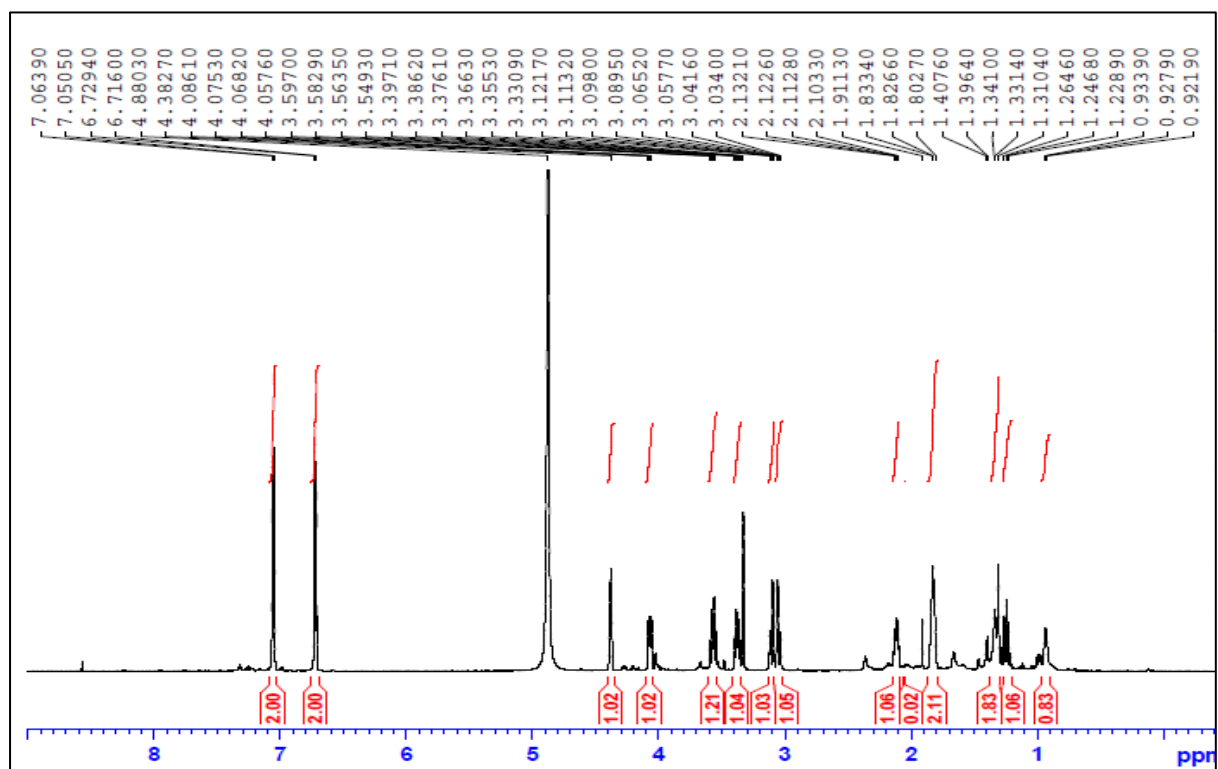
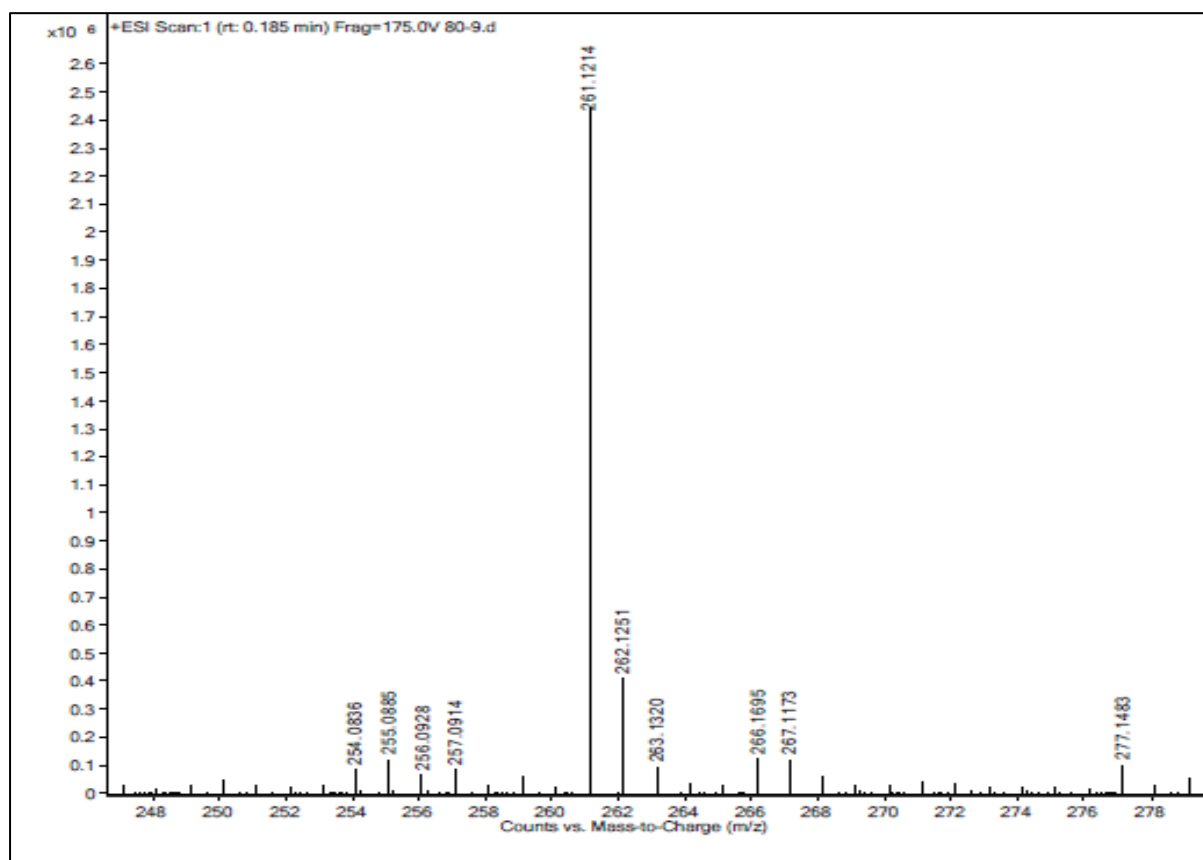
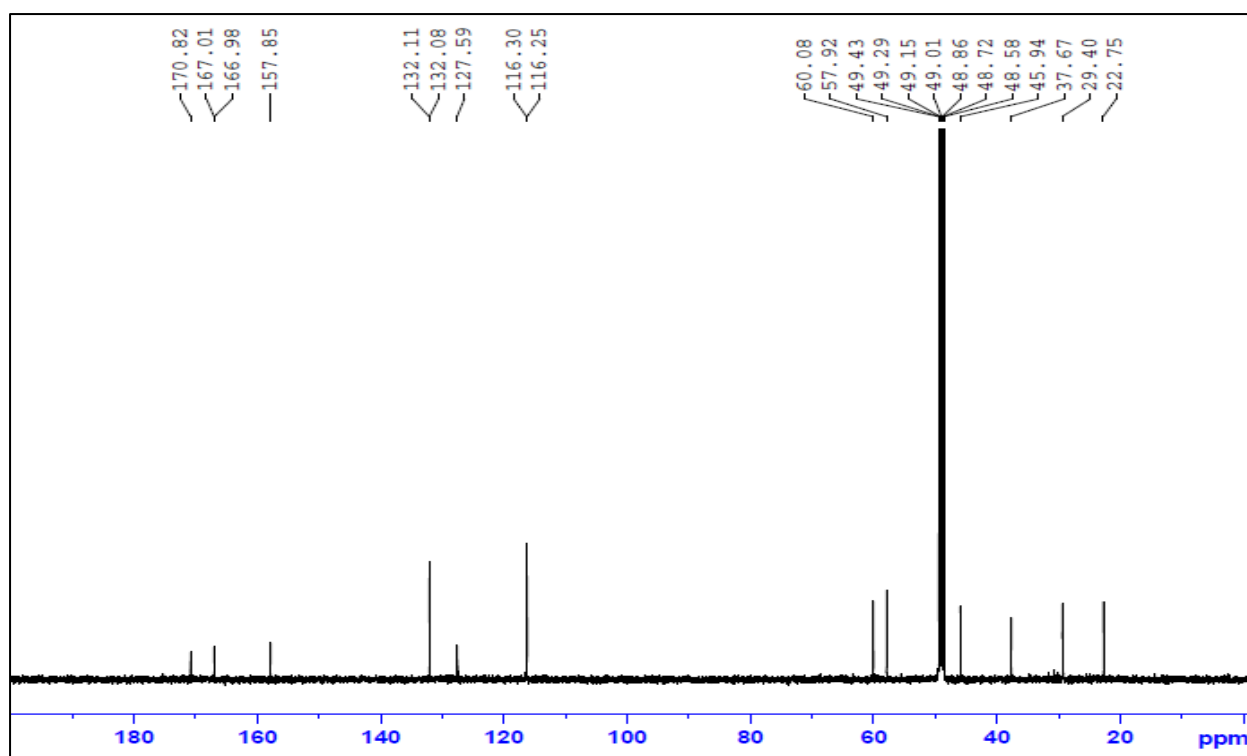
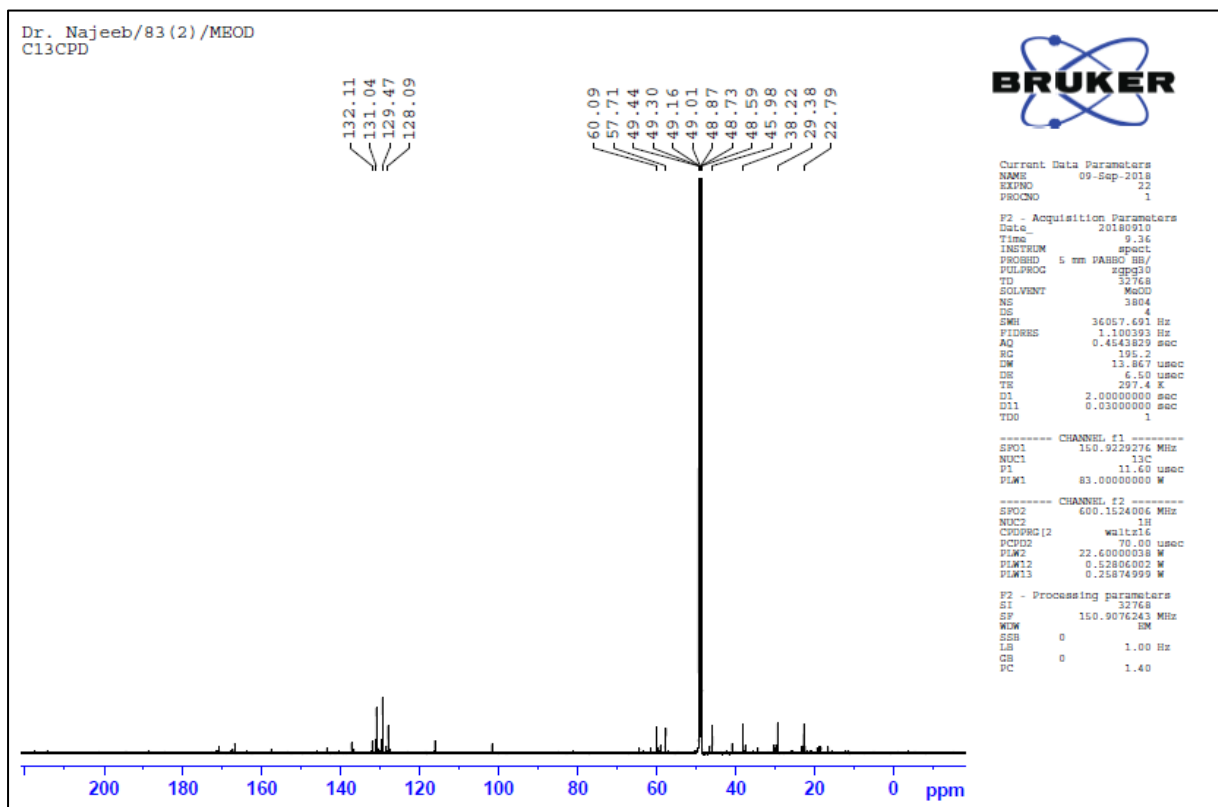
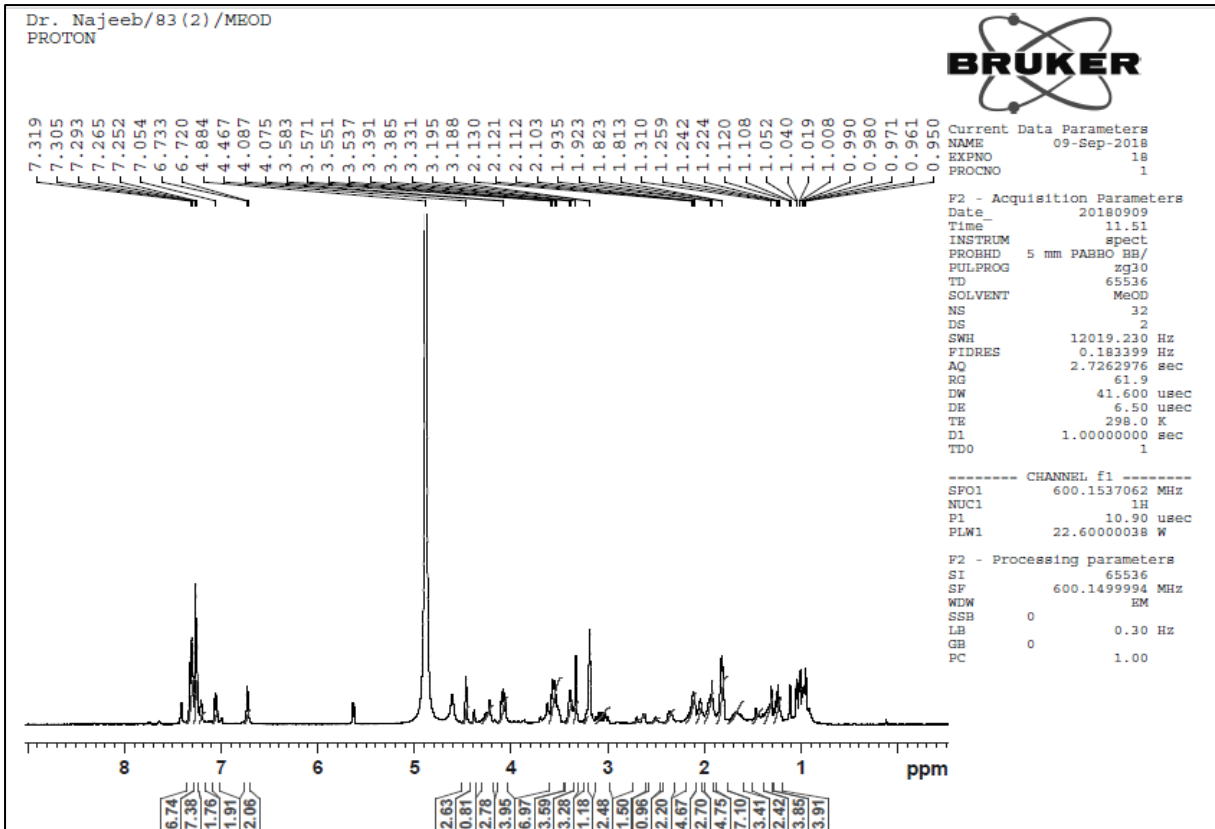


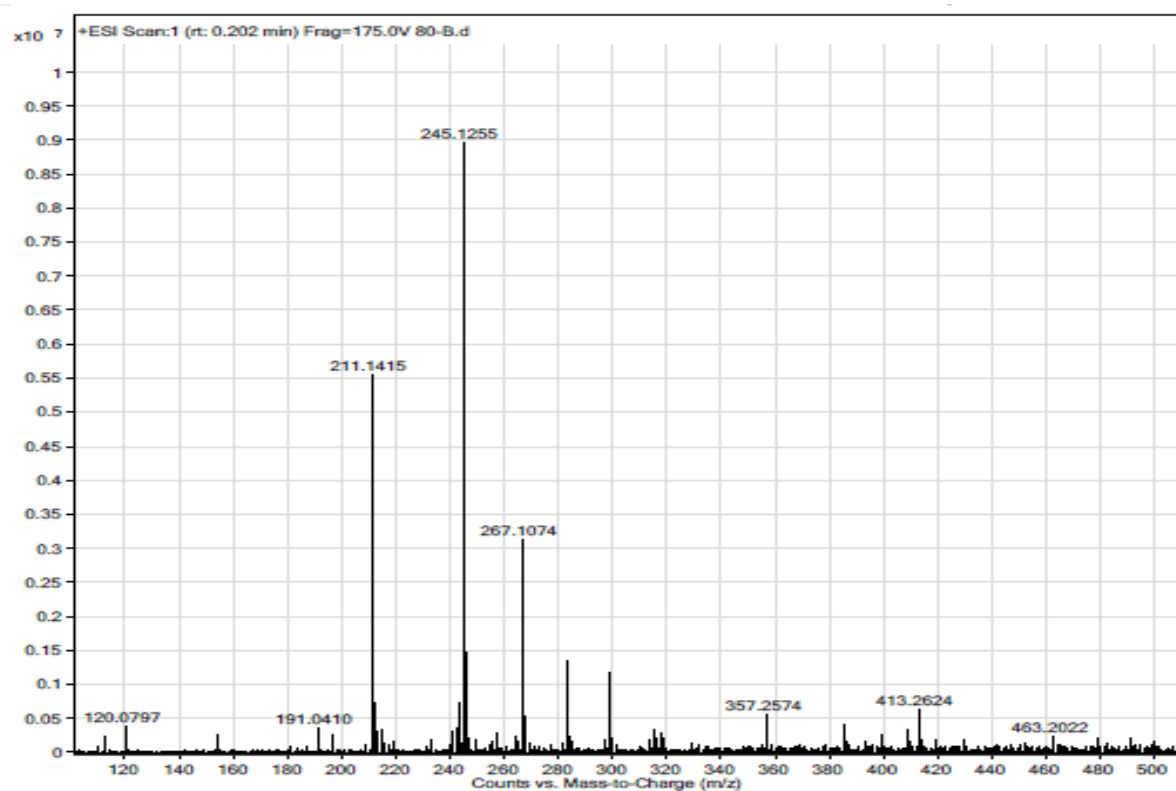
Figure S2:  $^1\text{H}$ -,  $^{13}\text{C}$ -NMR and HR-ESI-MS spectrum of 1



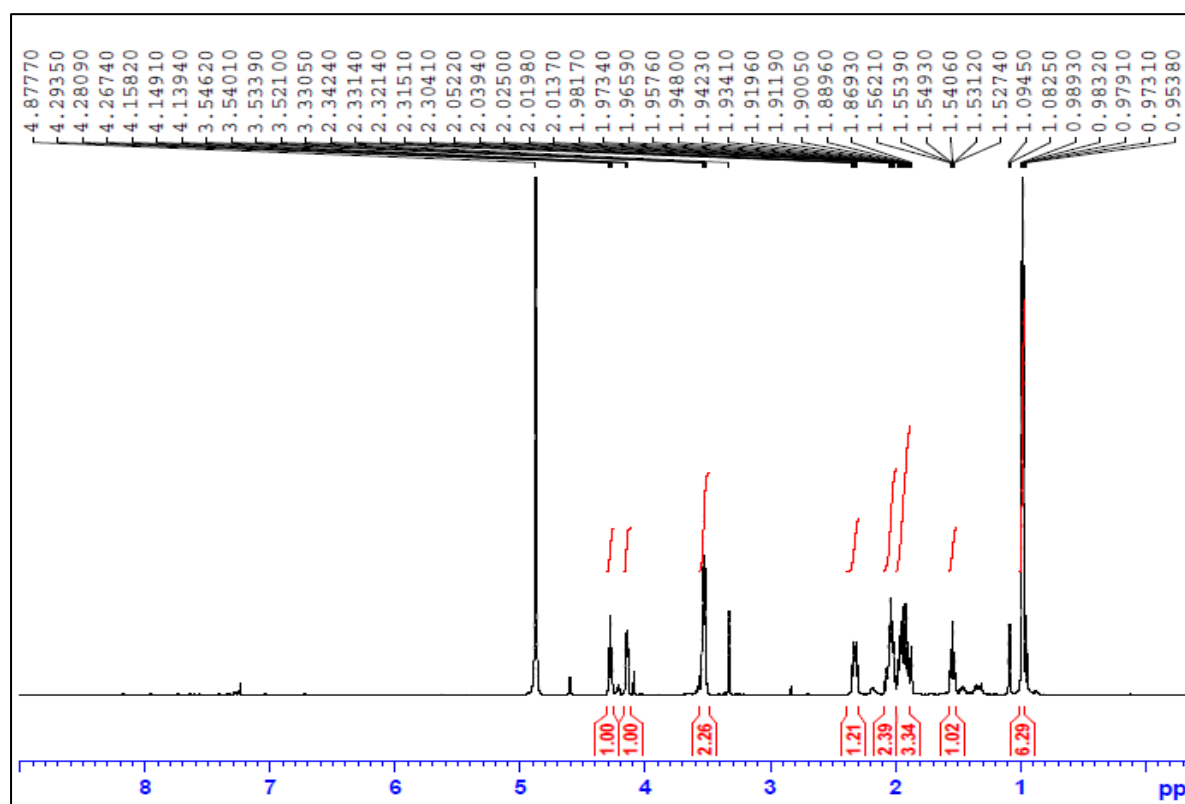


**Figure S3:** <sup>1</sup>H-, <sup>13</sup>C-NMR and HR-ESI-MS spectrum of **2**

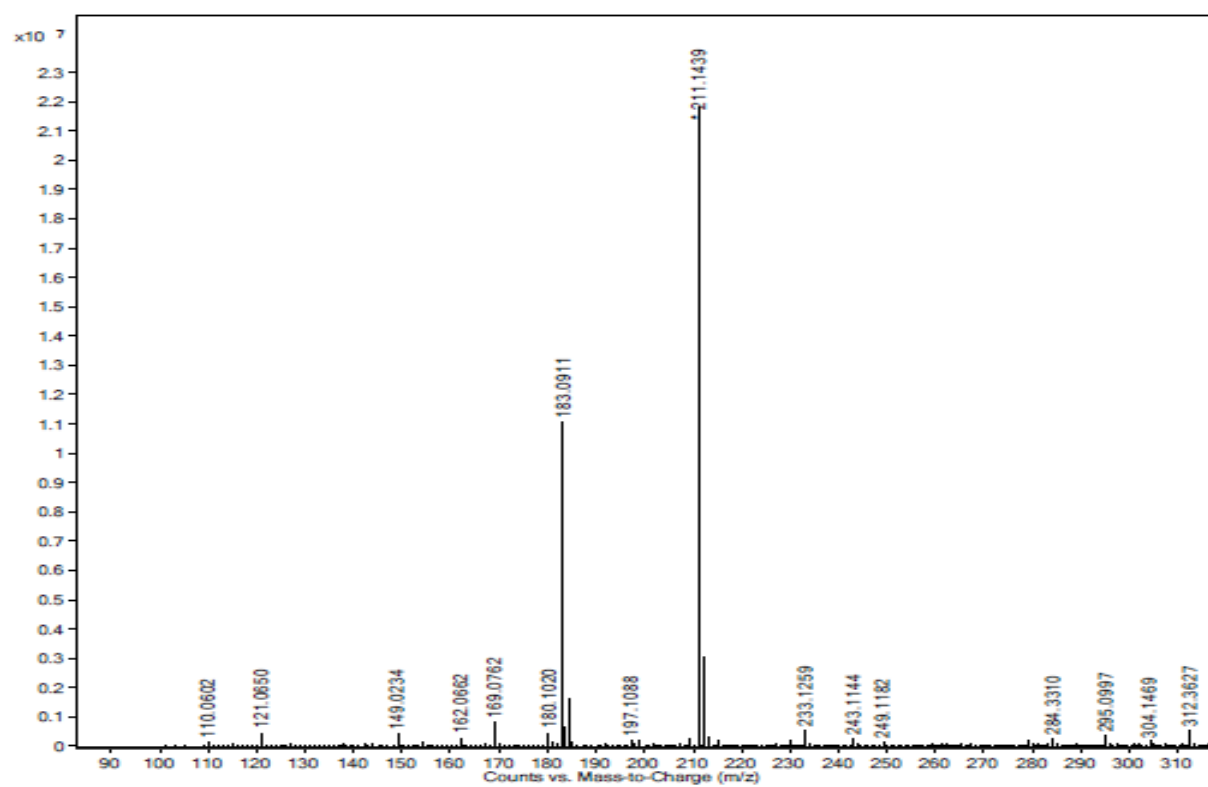
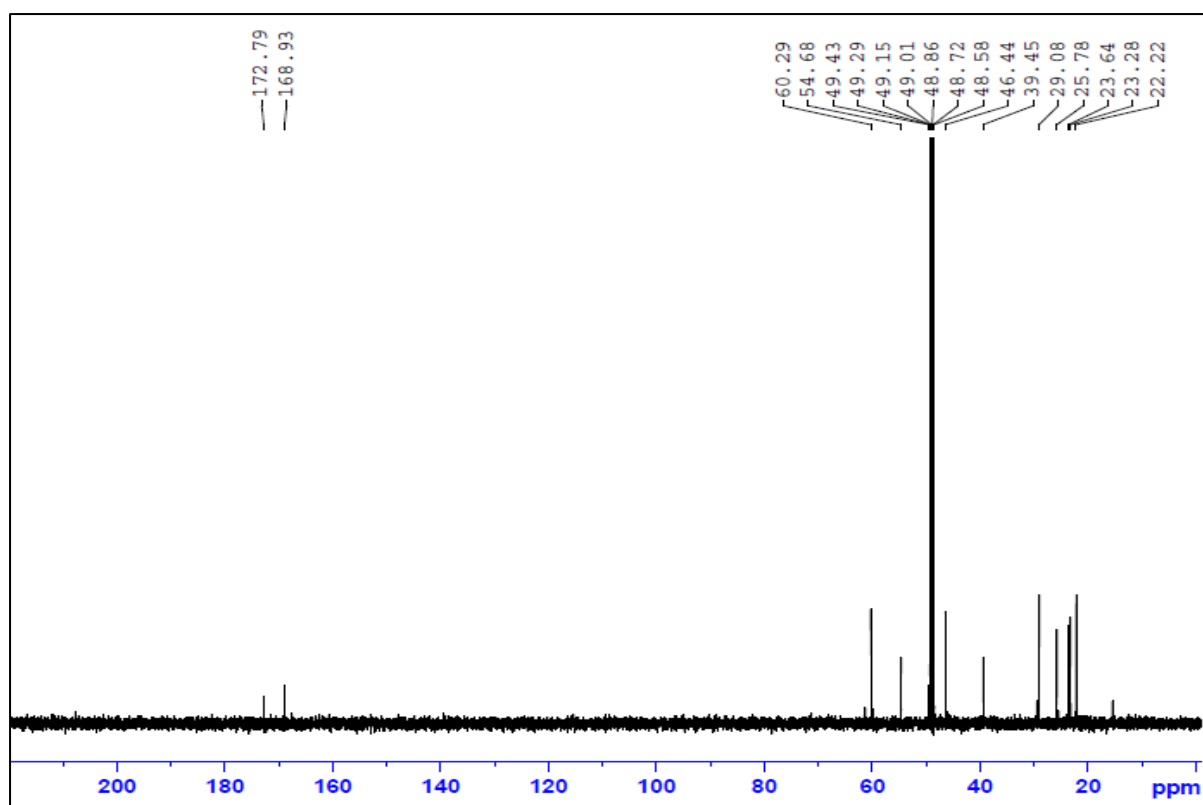




**Figure S4:**  $^1\text{H}$ -,  $^{13}\text{C}$ -NMR and HR-ESI-MS spectrum of **3**







**Figure S5:** <sup>1</sup>H-, <sup>13</sup>C-NMR and HR-ESI-MS spectrum of **4**