**SUPPLEMENTARY DATA**

**Enzyme inhibitory assessment of isolated constituents from *Plantago holosteum* Scop.**

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**Abstract**

Plants of the *Plantago* genus are widely used in Turkish folk medicine especially for the treatment of wound, abscess and inflammation. The aqueous extract and five phenylethanoid glycosidesacteoside (**1**), arenarioside (**2**), echinacoside (**3**), isoacteoside (**4**) and leucosceptoside A (**5**) isolated from the aerial parts and roots of *Plantago holosteum* Scop. (Plantaginaceae) were tested for their possible inhibitory activity against hyaluronidase, elastase and collagenase, related to wound pathogenesis. Even though, the aqueous extract prepared from aerial parts (36.26 %) and roots (47.01 %), as well among the isolated compounds, acteoside (29.13 %), echinacoside (28.73 %) and isoacteoside (31.69 %) exerted a notable inhibition, arenarioside and leucosceptoside A were found inactive in the hyaluronidase enzyme inhibition test. Similar results were obtained from collagenase enzyme inhibition test. The aqueous extract prepared from aerial parts (31.09 %) and roots (35.17 %), echinacoside (25.13 %) and isoacteoside (23.85 %) exerted a notable inhibition in this test. However, none of the extracts and isolated compounds displayed elastase enzyme inhibitory activity. The experimental data demonstrated that *P. holosteum* displayed remarkable enzyme inhibitory activity against hyaluronidase and collagenase. This paper is the first report regarding to *in vitro* enzyme inhibitory activity of *P. holosteum*.

**Keywords:** Collagenase; Elastase; Hyaluronidase; Phenylethanoid glycosides; Plantaginaceae; *Plantago holosteum*.

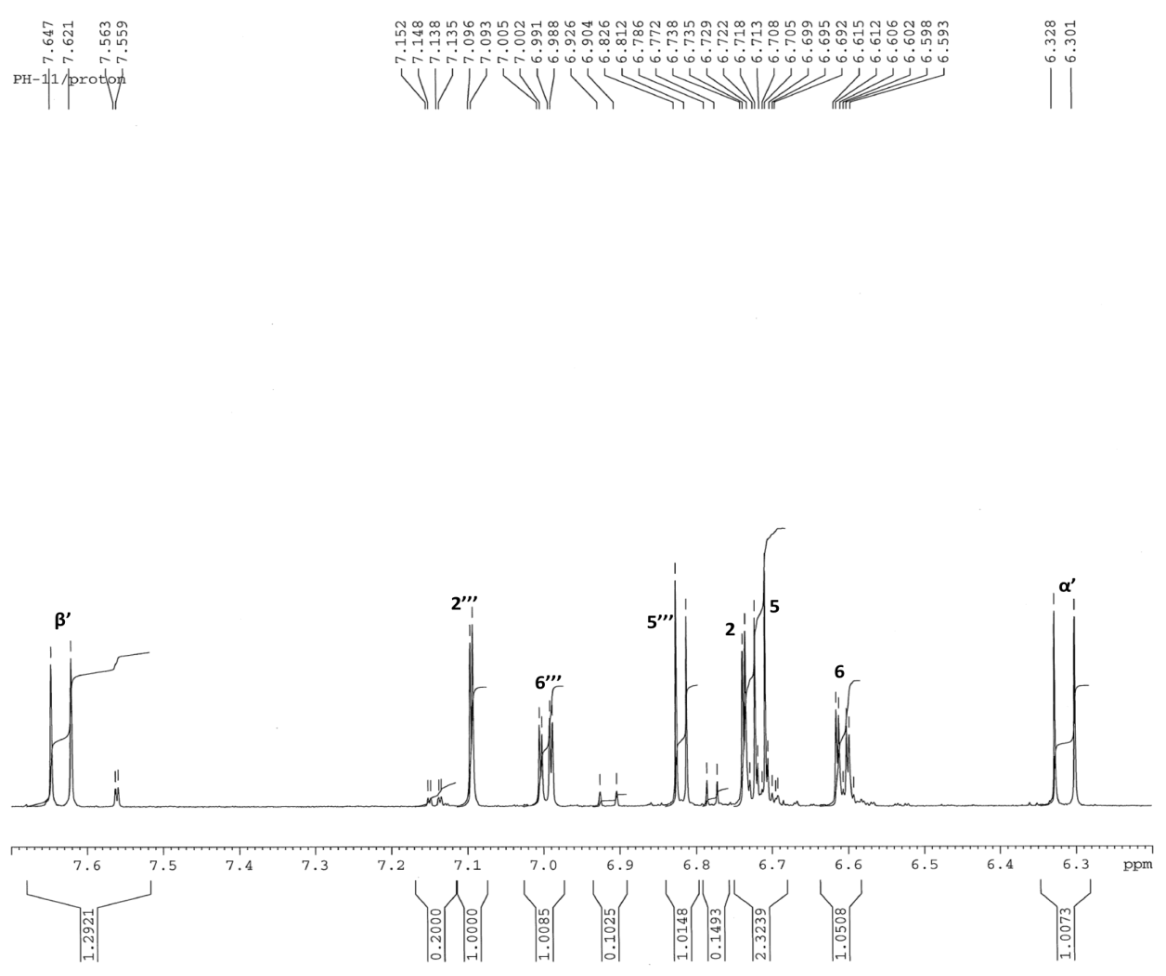
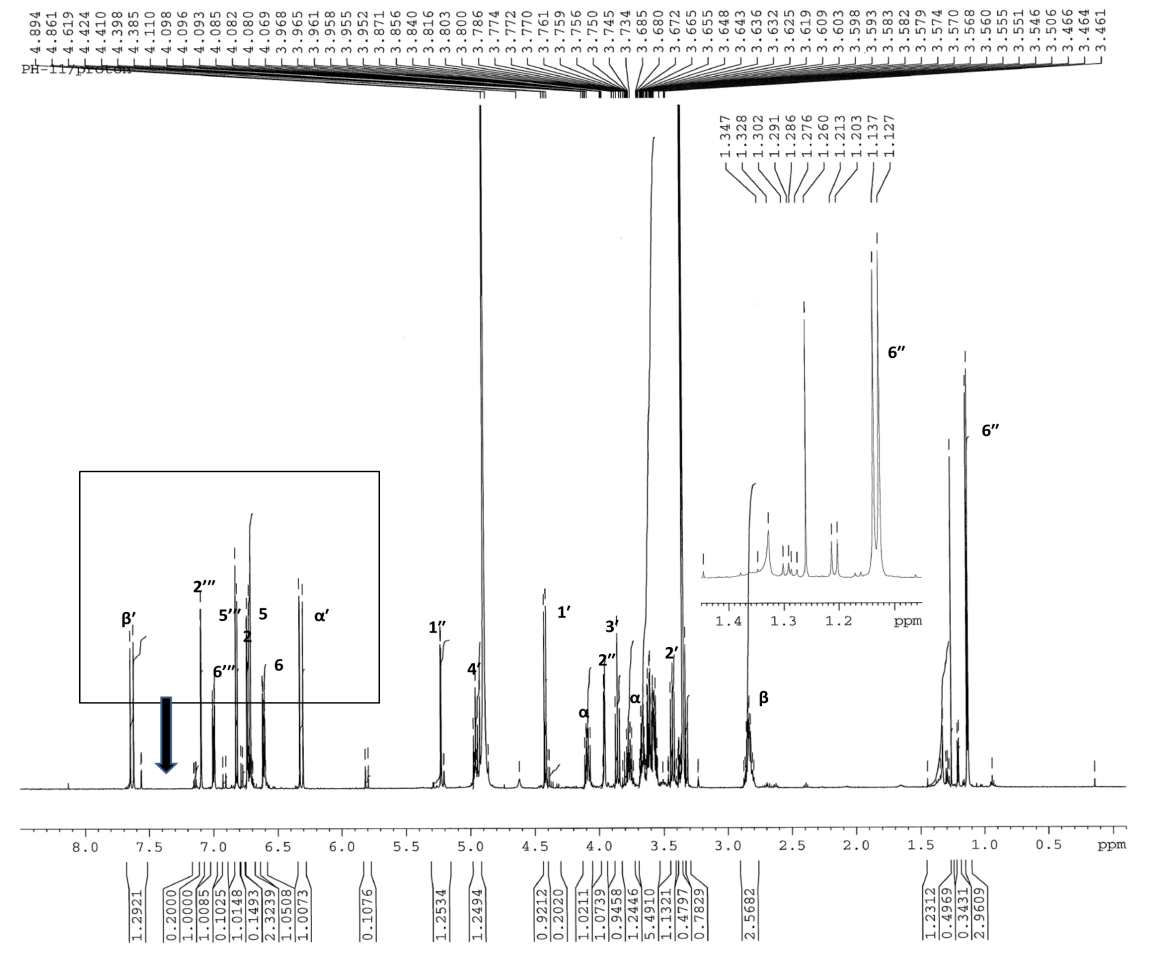
**SUPPLEMENTARY DATA**

**CONTENT**

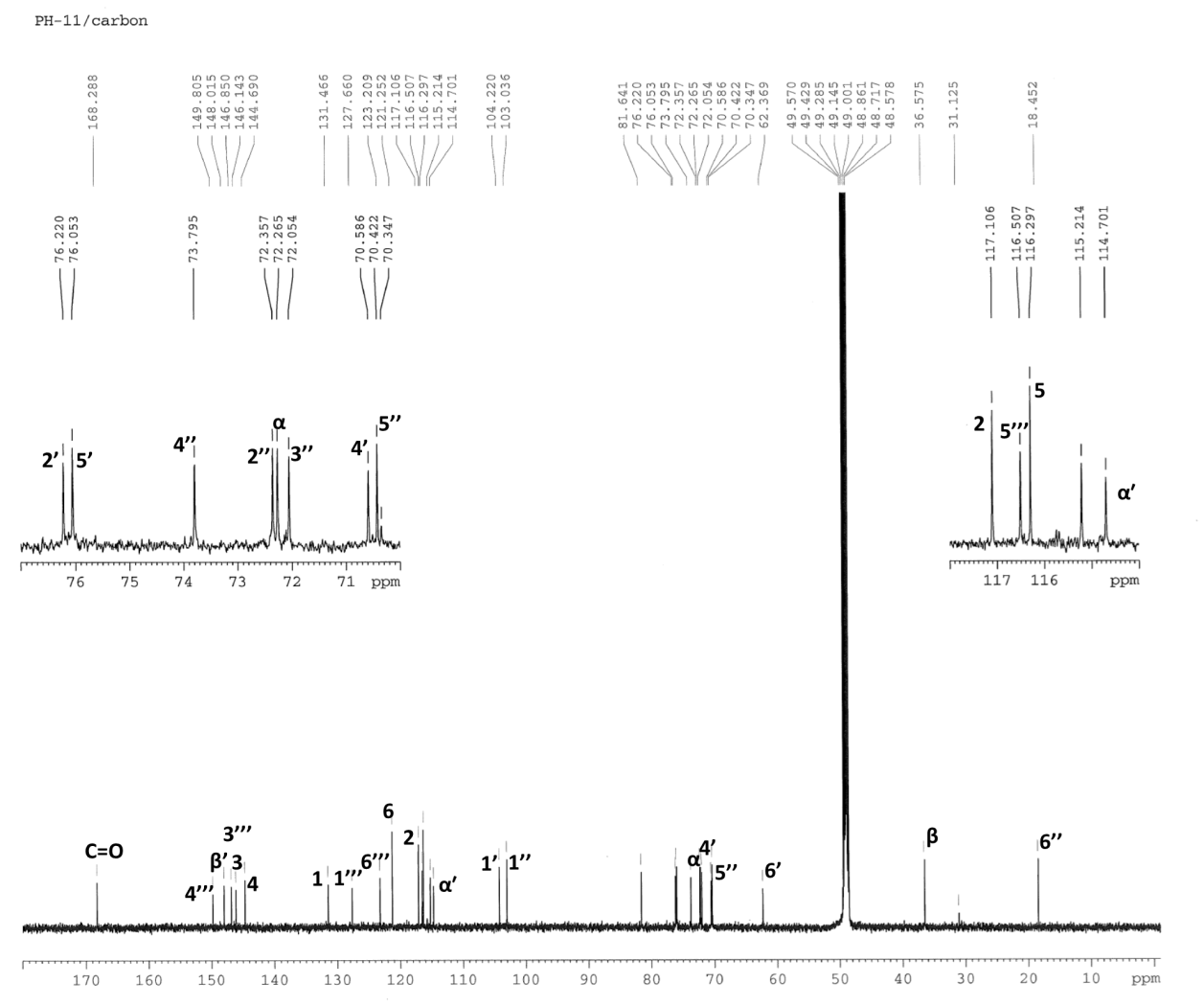
* 1H, 13C NMR, and DEPT spectra of **Acteoside (1)**
* 1H, 13C NMR and DEPT spectra of **Arenarioside (2)**
* 1H, and 13C NMR spectra of **Echinacoside (3)**
* 1H, 13C NMR, and DEPT spectra of **Isoacteoside (4)**
* 1H, 13C NMR, and DEPT spectra of **Leucosceptoside A (5)**
* NMR tables of Isolated Compounds **(Acteoside, Arenarioside, Echinacoside, Isoacteoside, Leucosceptoside A)**

**1H-NMR spectra of Acteoside**

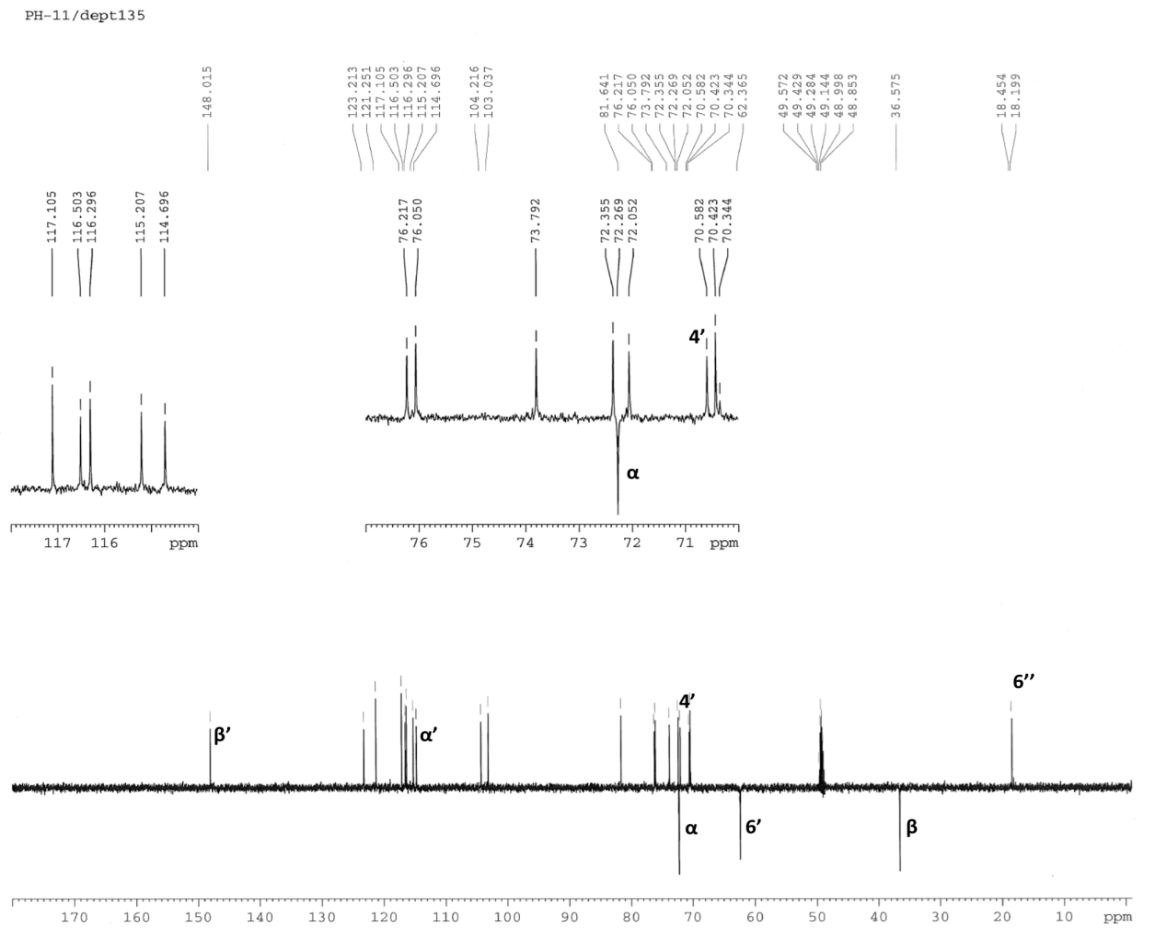
**(A)**



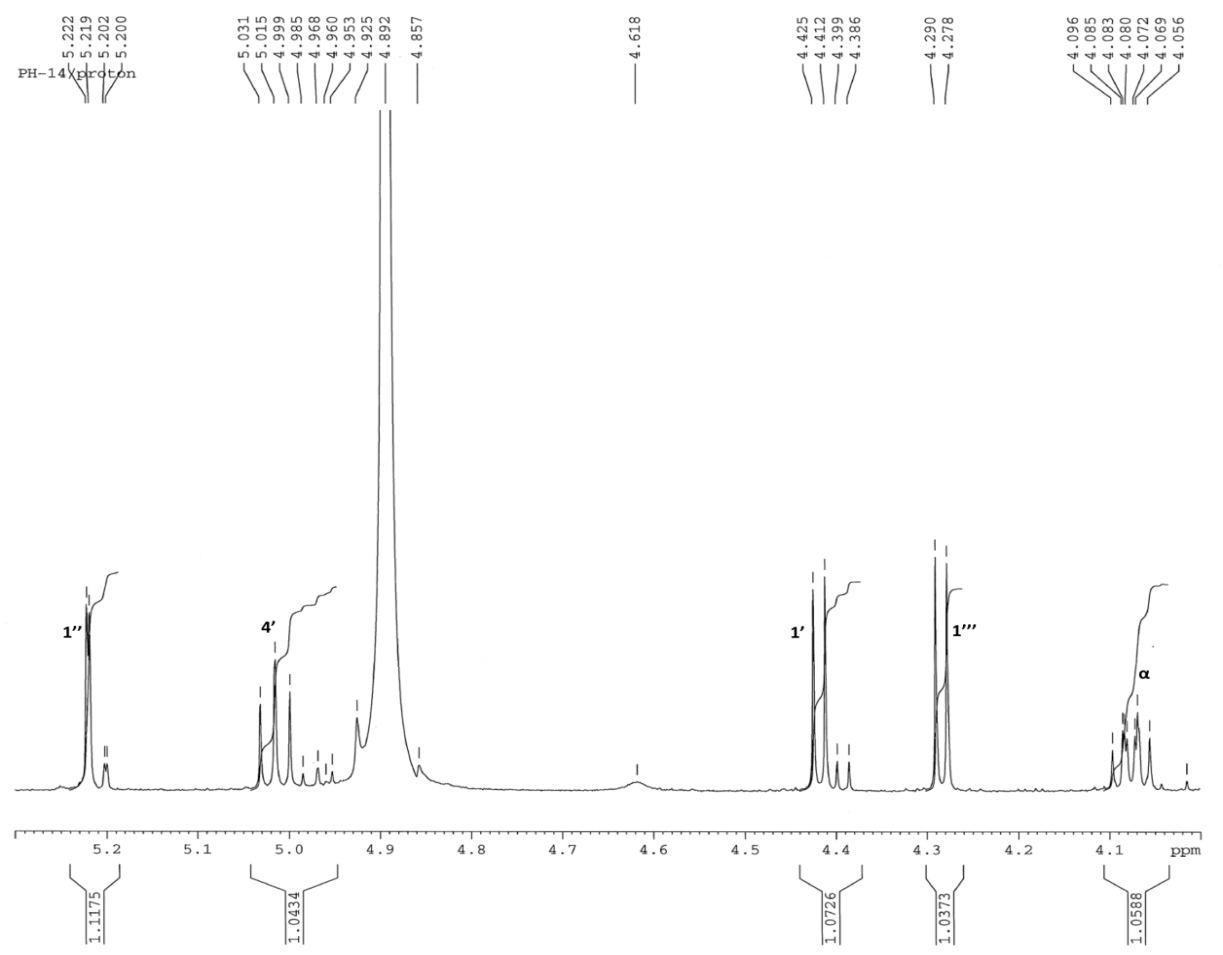
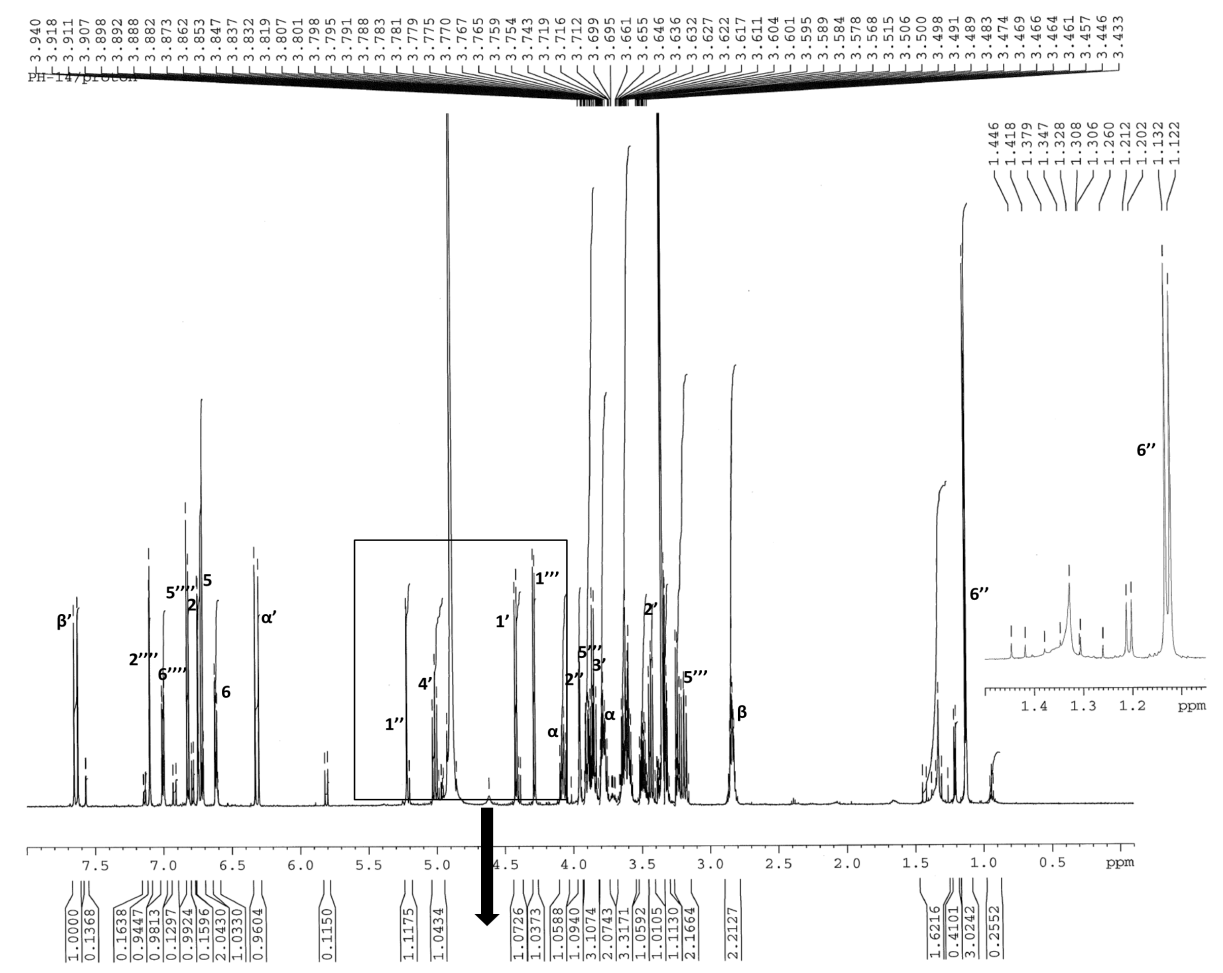
**13C-NMR spectrum of Acteoside**



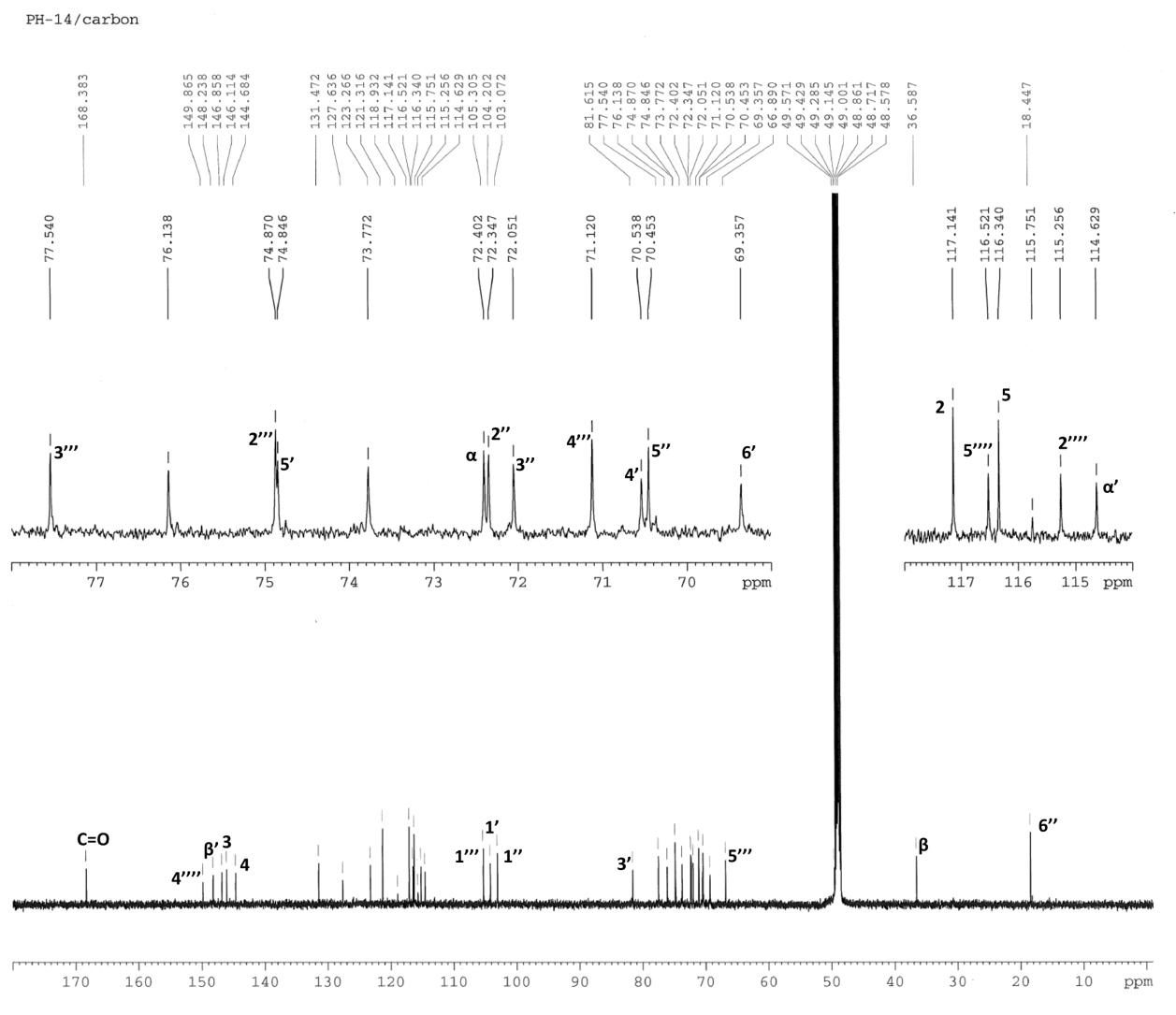
**DEPT-90 and DEPT-135 spectra of Acteoside**



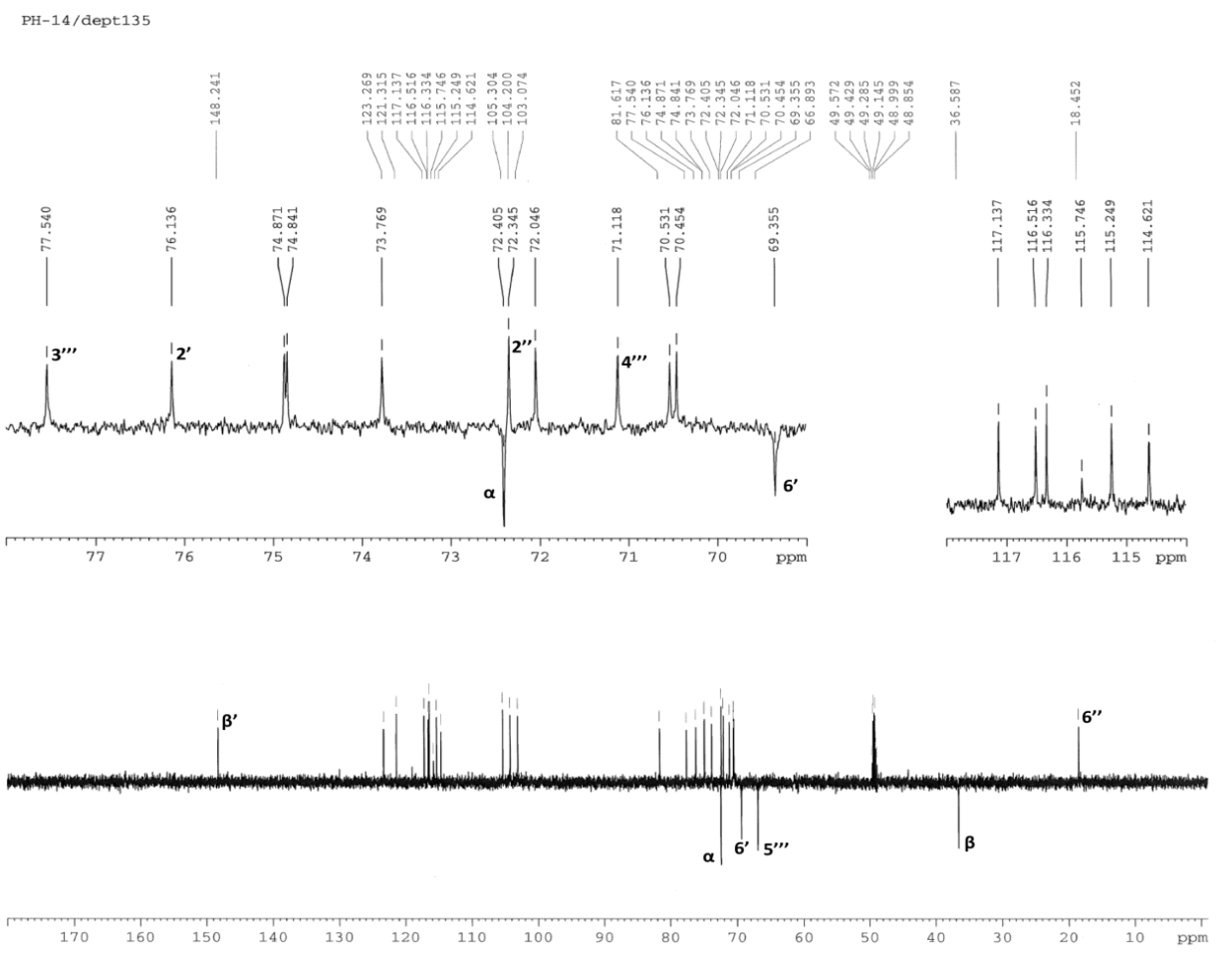
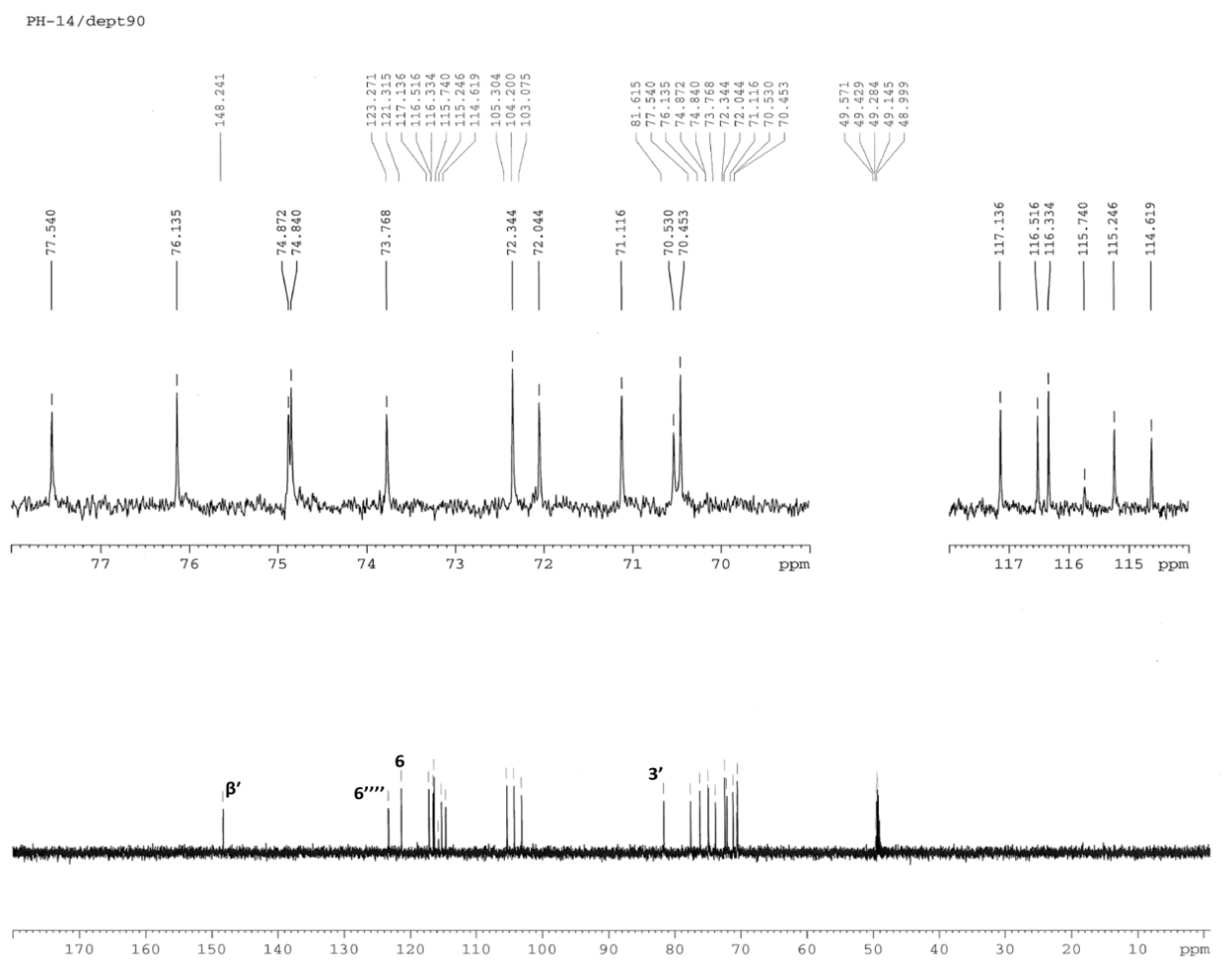
**1H-NMR spectra of Arenarioside**



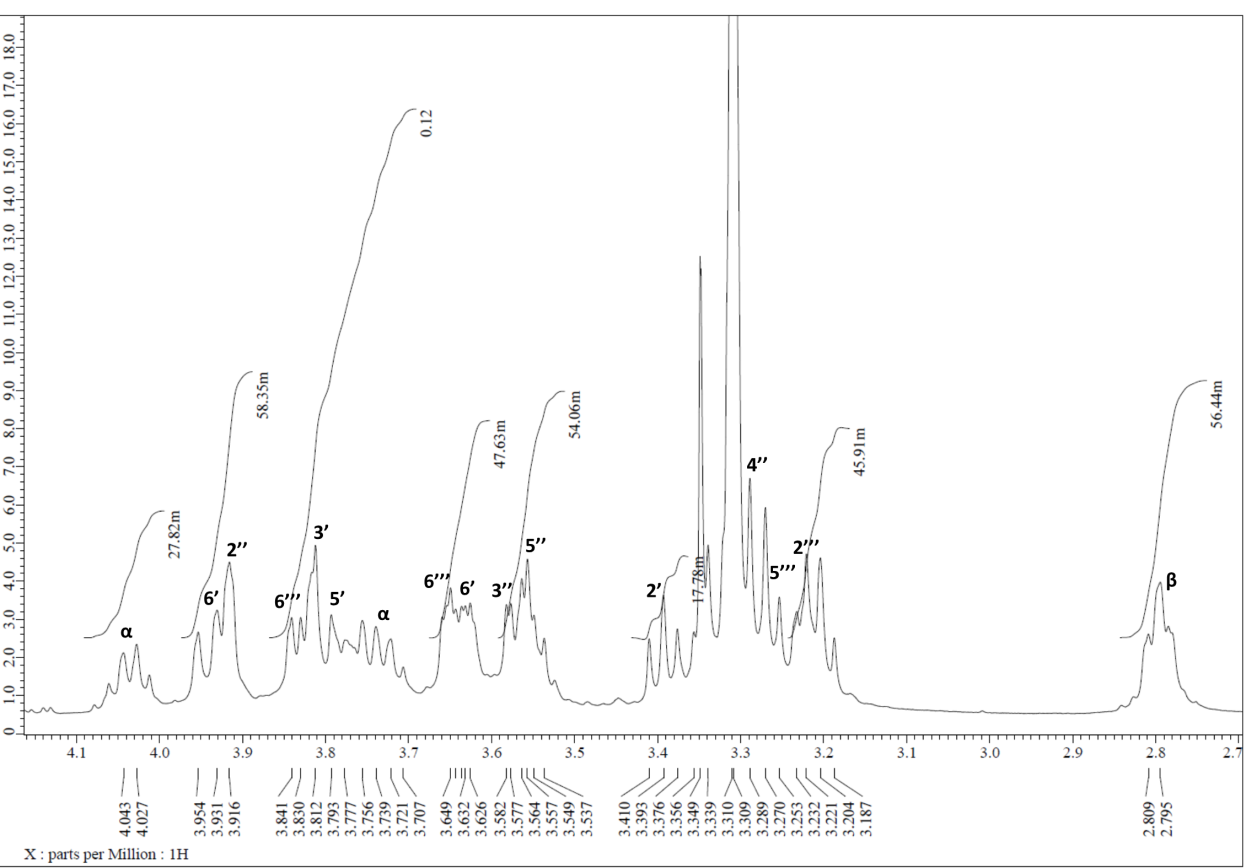
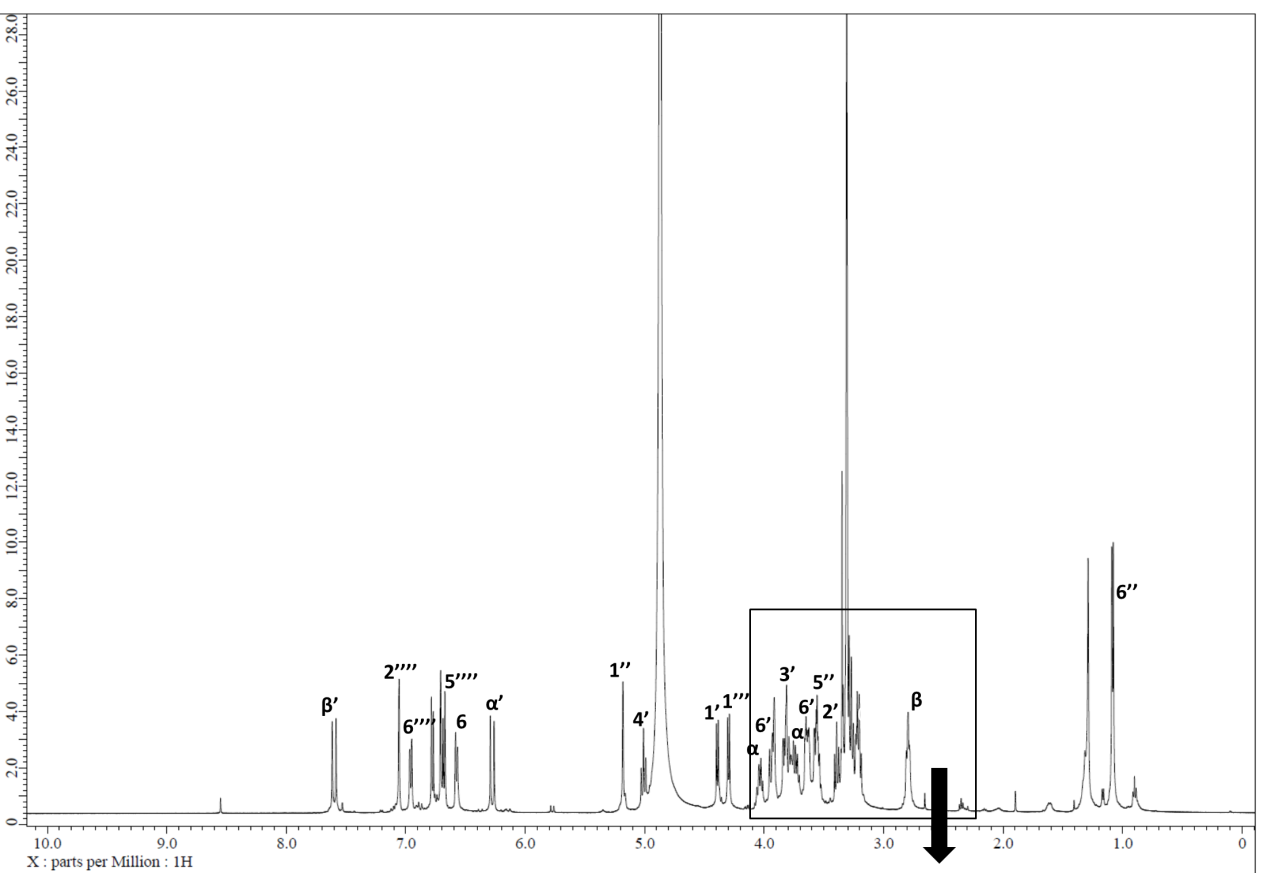
**13C-NMR spectrum of Arenarioside**



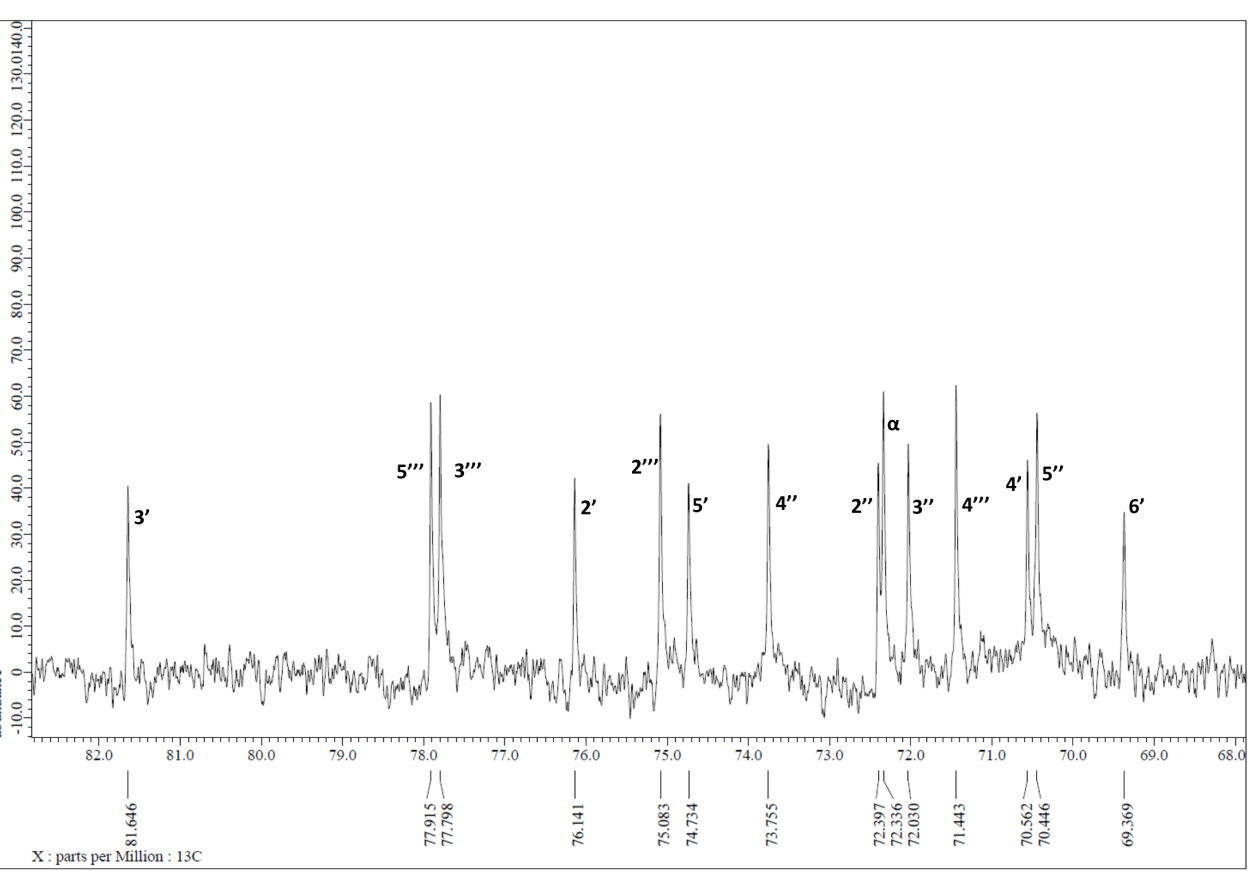
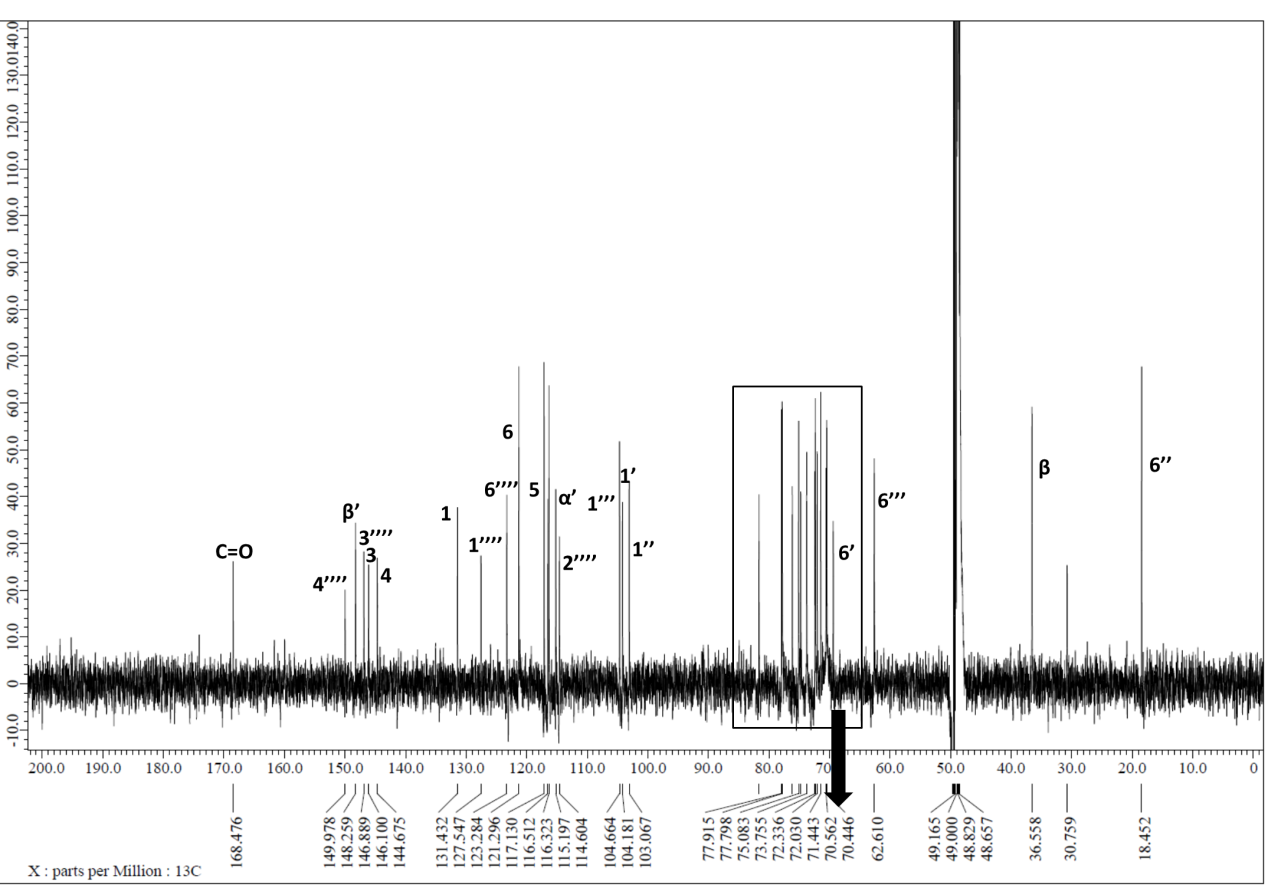
**DEPT-90 and DEPT-135 spectra of Arenarioside**



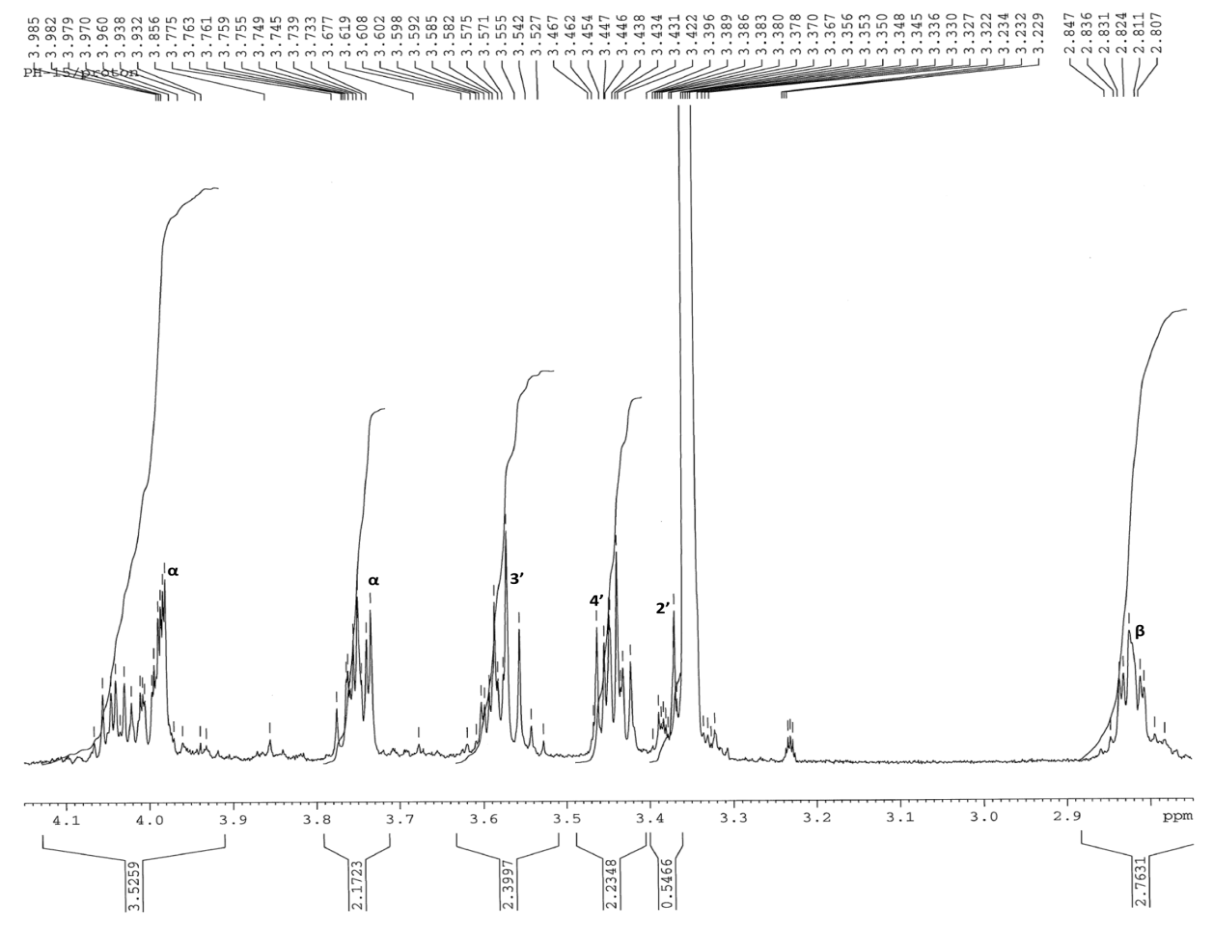
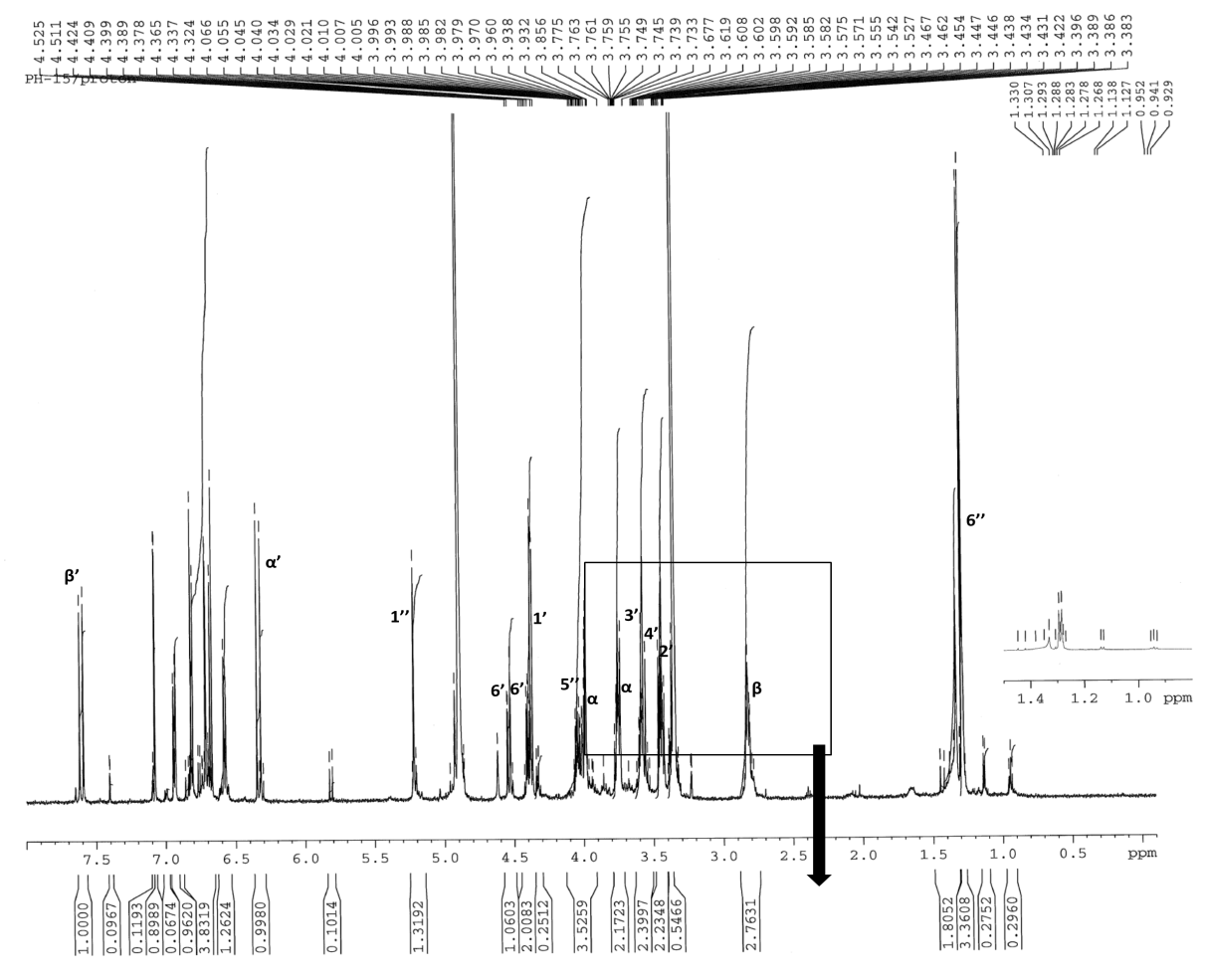
**1H-NMR spectra of Echinacoside**



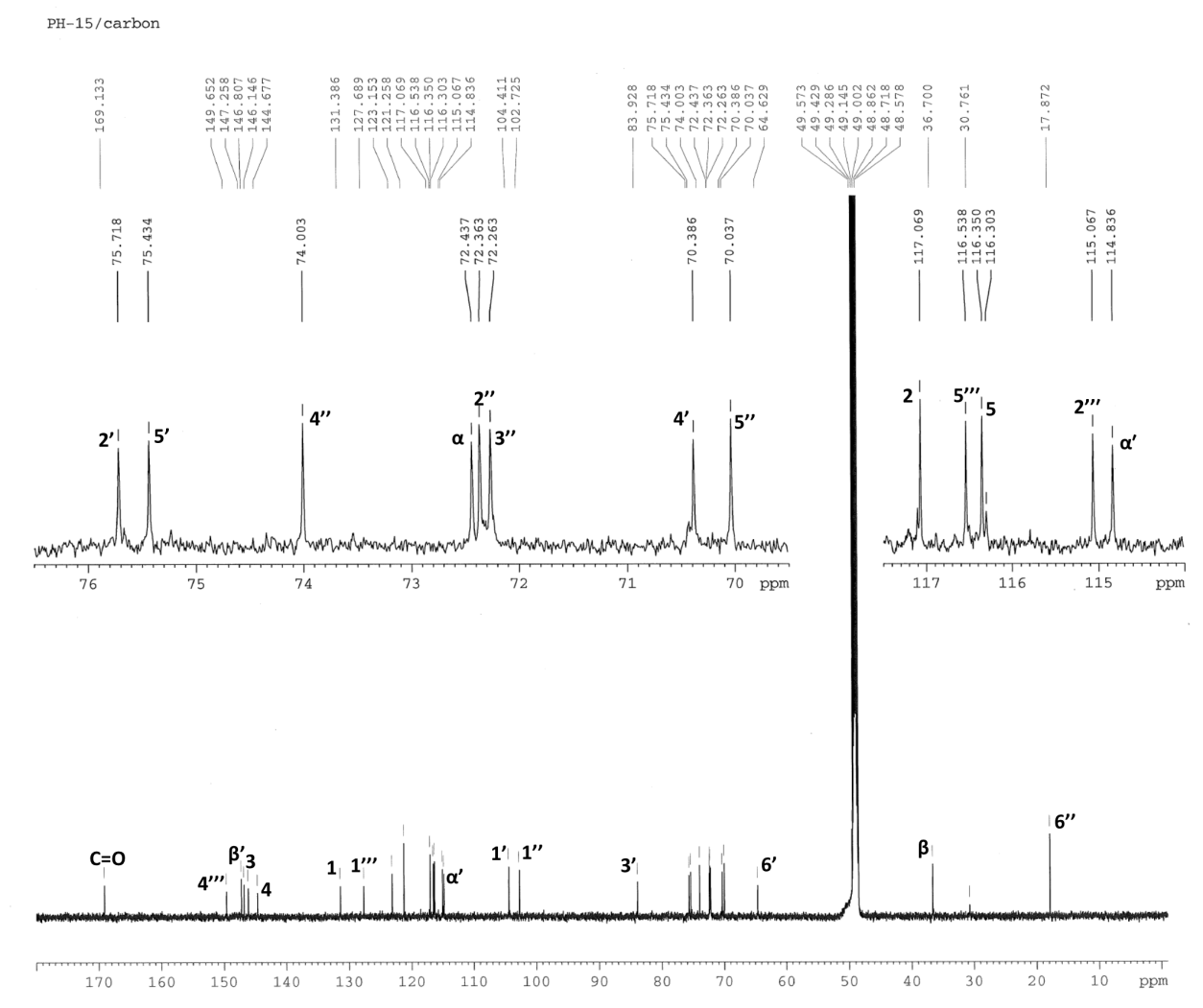
**13C-NMR spectra of Echinacoside**



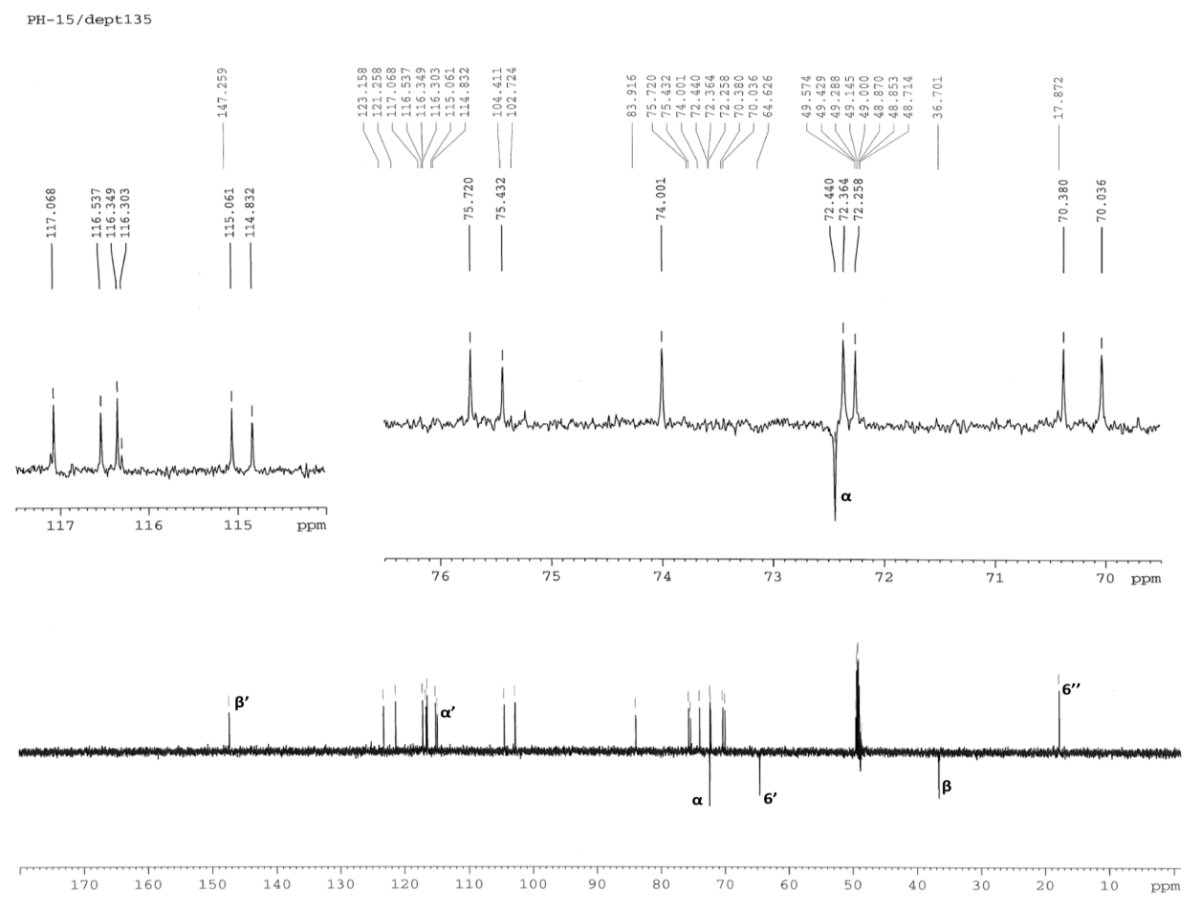
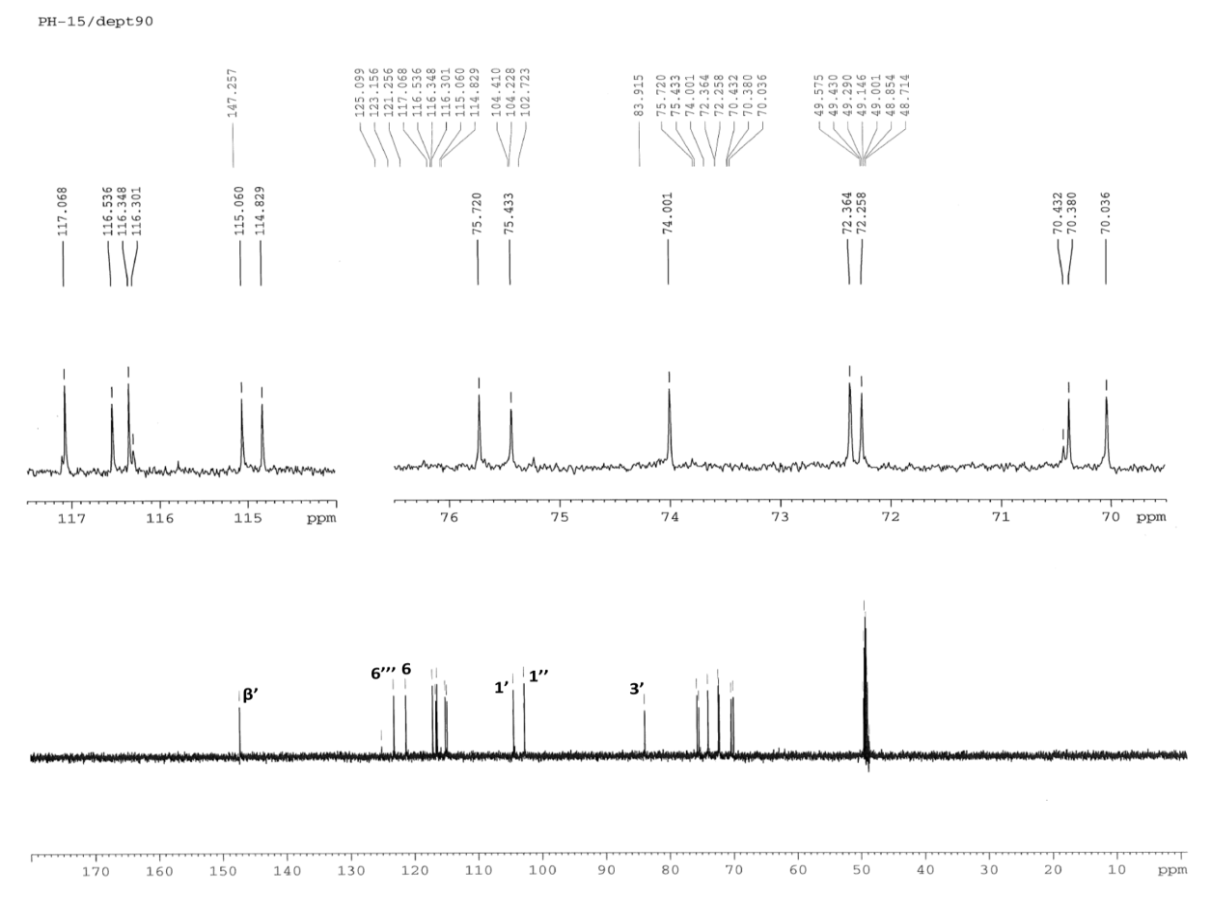
**1H-NMR spectra of Isoacteoside**



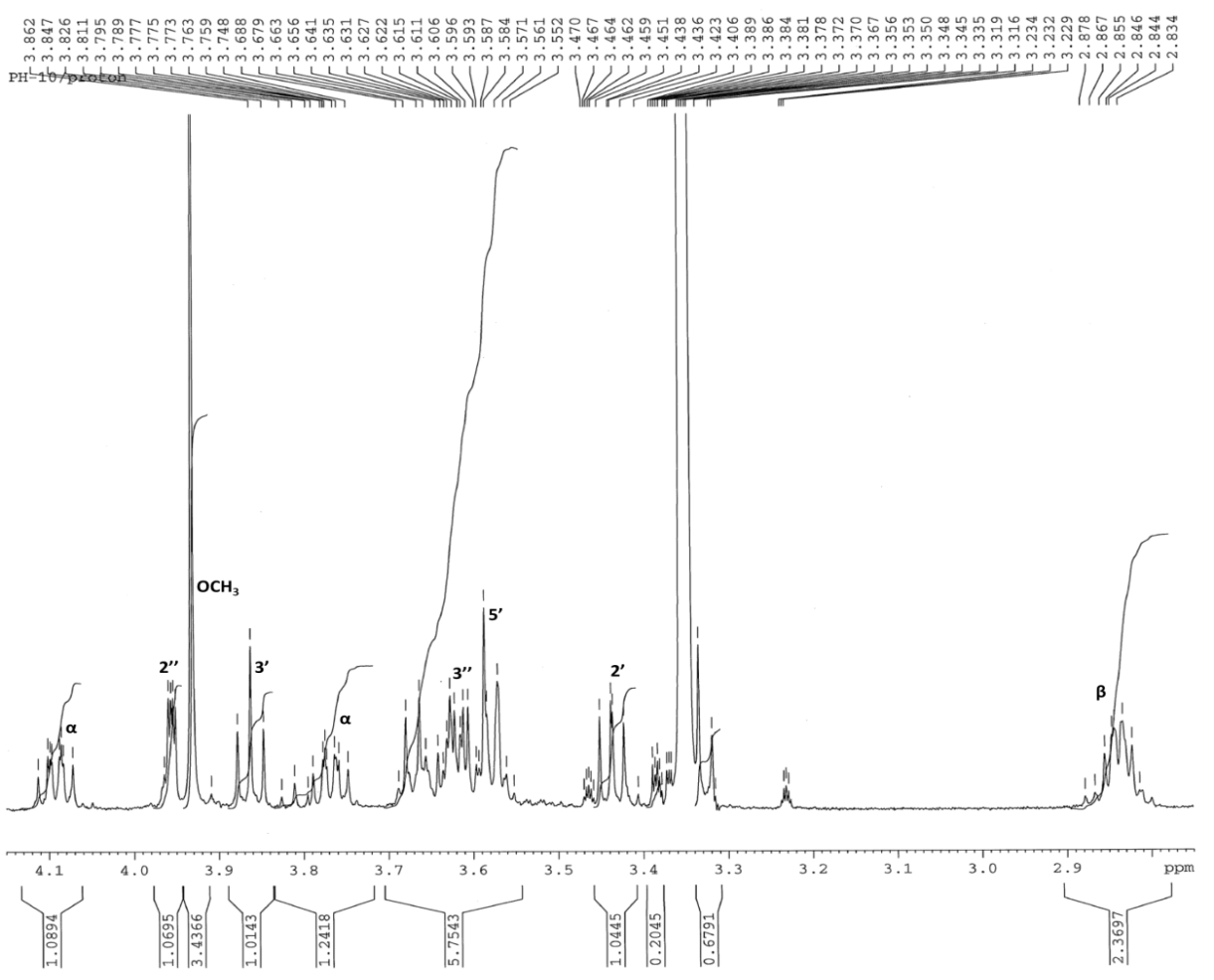
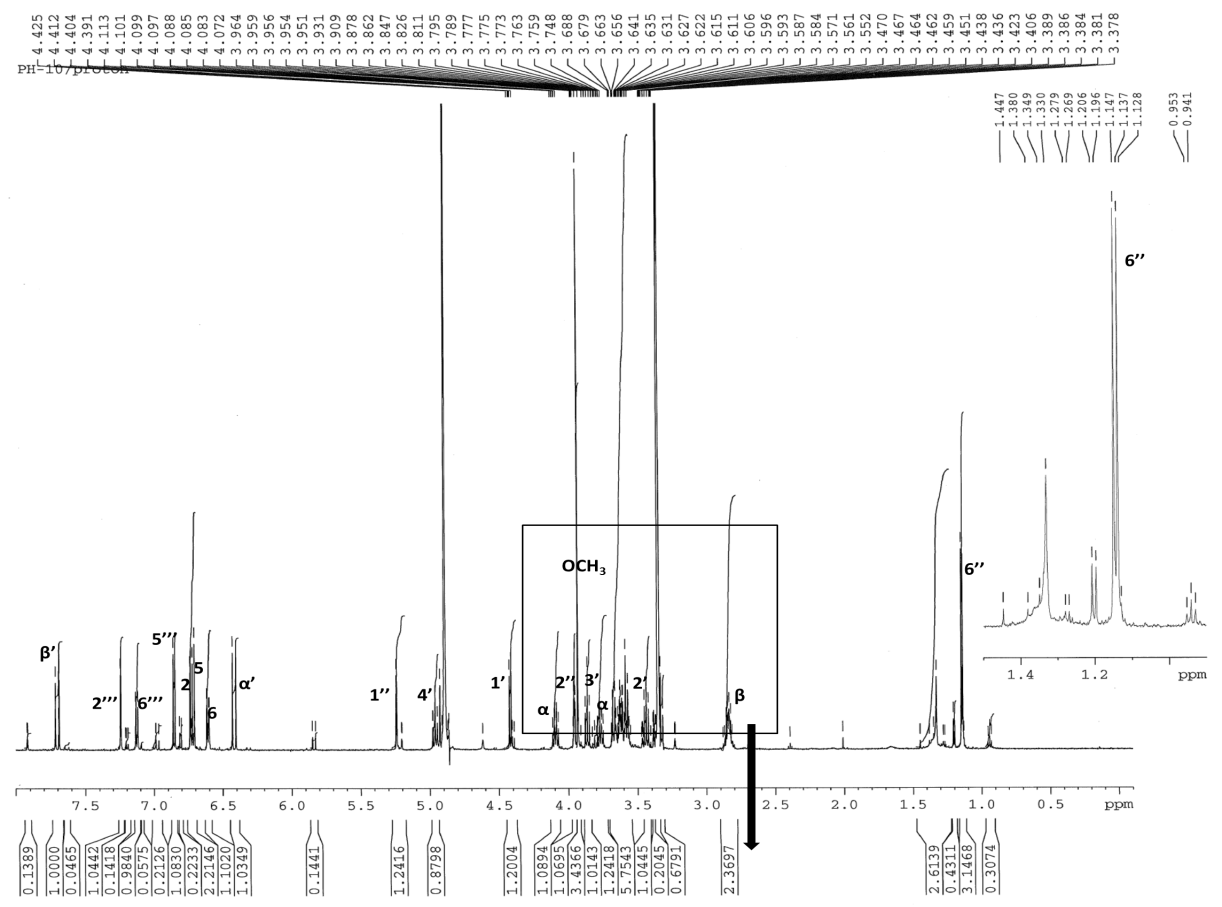
**13C-NMR spectrum of Isoacteoside**



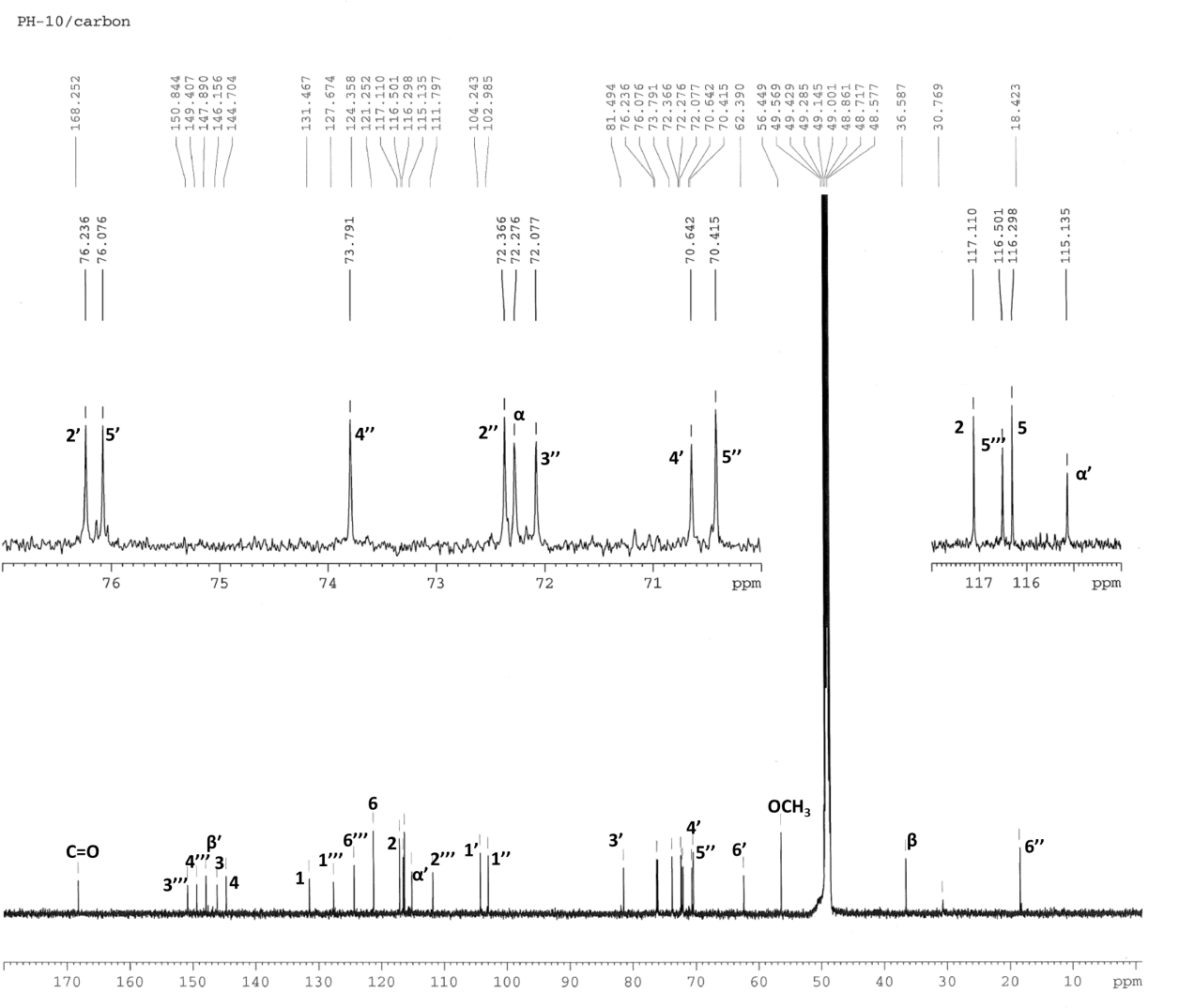
**DEPT-90 and DEPT-135 spectra of Isoacteoside**



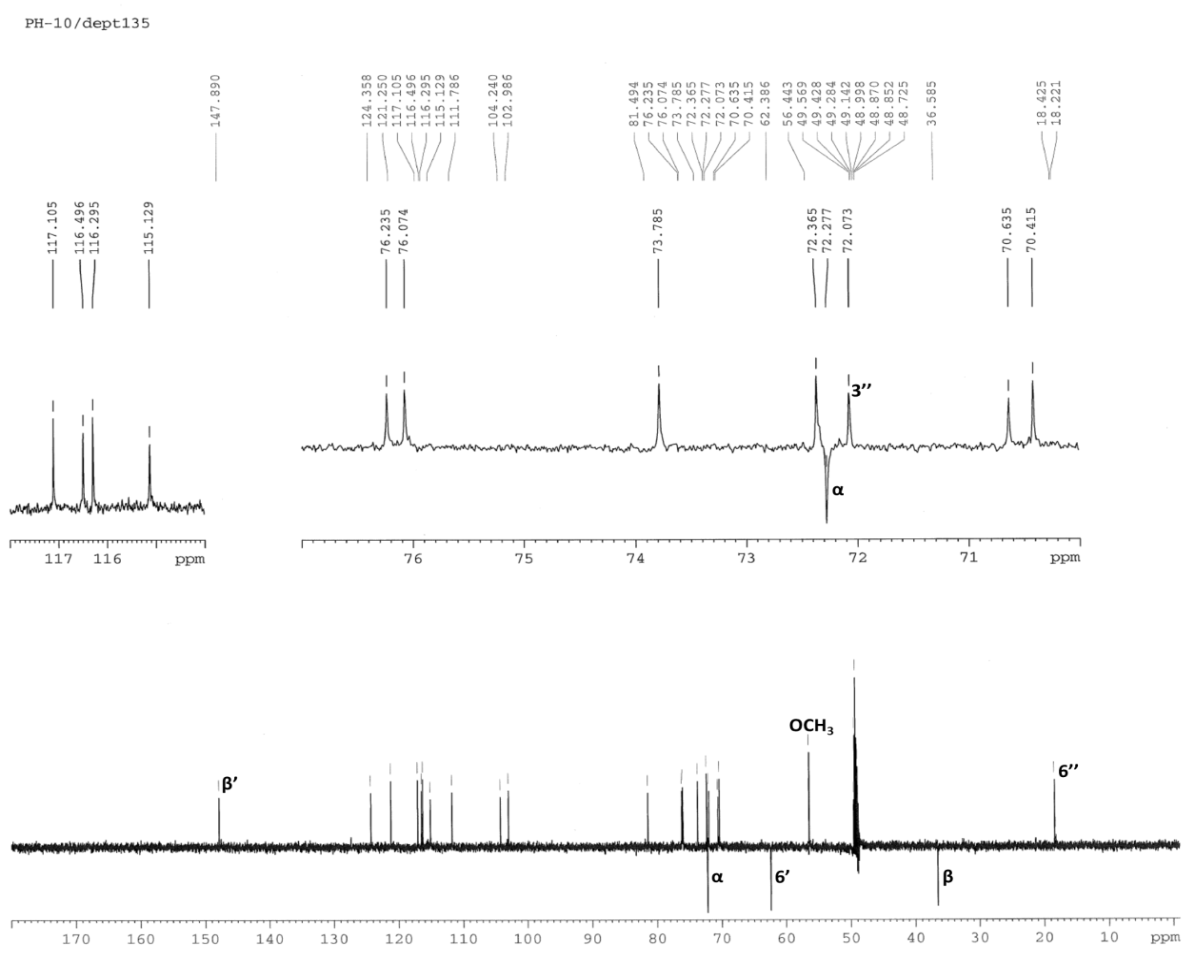
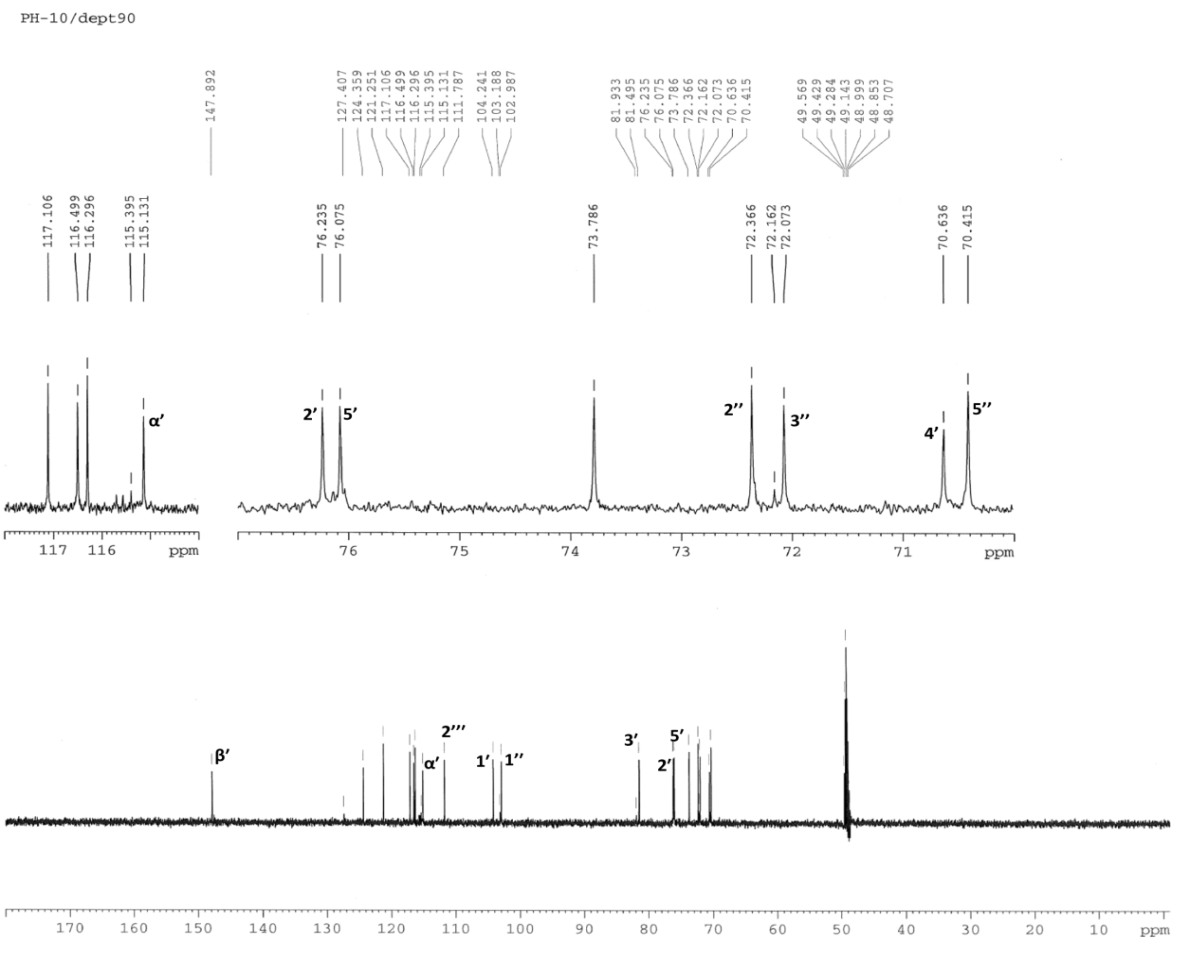
**1H-NMR spectra of Leucosceptoside A**



**13C-NMR spectrum of Leucosceptoside A**



**DEPT-90 and DEPT-135 spectra of Leucosceptoside A**



**Table 1**

**13C- and 1H- NMR data of acteoside** (CD3OD; 13C:150 MHz; 1H: 600 MHz)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| C/H Atom | DEPT | δC ppm | δH ppm | *J* (Hz) | HMBC (H→C) |
| Aglycone |  |  |  |  |  |
| 1 | C | 131.4 |  |  |  |
| 2 | CH | 117.1 | 6.73 d | (2.0) | C-3, C-4, C-6 |
| 3 | C | 146.1 |  |  |  |
| 4 | C | 144.6 |  |  |  |
| 5 | CH | 116.3 | 6.71 d | (8.0) | C-1, C-4, C-6 |
| 6 | CH | 121.2 | 6.61 dd | (8.0/2.0) | C-2, C-4 |
| *α* | CH2 | 72.2 | 4.09 m |  | C-1 |
|  |  |  | 3.77 m |  | C-1, C-1' |
| *β* | CH2 | 36.5 | 2.84 m |  | C-1, C-2, C-6 |
| Glucose |  |  |  |  |  |
| 1′ | CH | 104.2 | 4.41 d | (8.0) | C- *α* |
| 2′ | CH | 76.2 | 3.43 dd | (9.1/8.0) | C-3′ |
| 3′ | CH | 81.6 | 3.85 t | (9.2) | C-2', C-4′ |
| 4′ | CH | 70.6 | 4.96 t | (9.5) | C=O |
| 5′ | CH | 76.0 | 3.58 m |  |  |
| 6′ | CH2 | 62.3 | 3.66 gd | (13.1) |  |
|  |  |  | 3.56 dd | (12.0/6.5) |  |
| Rhamnose |  |  |  |  |  |
| 1'' | CH | 103.0 | 5.22 d | (1.7) | C-3′, C-4′ |
| 2'' | CH | 72.3 | 3.96 dd | (3.2/1.8) |  |
| 3'' | CH | 72.0 | 3.62 dd | (9.7/3.4) |  |
| 4'' | CH | 73.8 | 3.32 t | (9.5) |  |
| 5'' | CH | 70.4 | 3.59 m |  |  |
| 6'' | CH3 | 18.4 | 1.13 d | (6.2) | C-4′, C-4'' |
| Acyl |  |  |  |  |  |
| 1''' | C | 127.6 |  |  |  |
| 2''' | CH | 115.2 | 7.09 d | (2.0) | C-3''', C-4''', C-6''' |
| 3''' | C | 146.8 |  |  |  |
| 4''' | C | 149.8 |  |  |  |
| 5''' | CH | 116.5 | 6.82 d | (8.2) | C-3''', C-4''', C-6''' |
| 6''' | CH | 123.2 | 7.00 dd | (8.2/2.0) | C-3''', C-4''', C-5''' |
| *α*' | CH | 114.7 | 6.31 d | (16.0) | C-1''', C=O |
| *β'* | CH | 148.0 | 7.63 d | (16.0) | C-2''', C-6''', C=O |
| C=O | C | 168.3 |  |  |  |

The 13C and 1H assignments were based on HSQC, HMBC and COSY experiments

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The 13C and 1H assignments were based on HSQC, HMBC and COSY experiments

The 13C and 1H assignments were based on HSQC, HMBC and COSY experiments

**Table 2**

**13C- and 1H- NMR data of arenarioside** (CD3OD; 13C:150 MHz; 1H: 600 MHz)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| C/H Atom | DEPT | δC ppm | δH ppm | *J* (Hz) | HMBC (H→C) |
| Aglycone |  |  |  |  |  |
| 1 | C | 131,4 |  |  |  |
| 2 | CH | 117,1 | 6,75 d | (2,0) | C-3, C-4, C-6 |
| 3 | C | 146,1 |  |  |  |
| 4 | C | 144,7 |  |  |  |
| 5 | CH | 116,3 | 6,72 d | (8,0) | C-1, C-4, C-6 |
| 6 | CH | 121,3 | 6,62 dd | (8,0/2,0) | C-2, C-4 |
| *α* | CH2 | 72,4 | 4,07 m |  | C-1 |
|  |  |  | 3,77 m |  | C-1, C-1’ |
| *β* | CH2 | 36,6 | 2,82 t | (7,6) | C-1, C-2, C-6 |
| Glucose |  |  |  |  |  |
| 1′ | CH | 104,2 | 4,42 d | (7,9) | C- *α* |
| 2′ | CH | 76,1 | 3,43 dd | (9,0/7,9) | C-3′ |
| 3′ | CH | 81,6 | 3,84 t | (9,2) | C-2’, C-4′ |
| 4′ | CH | 70,5 | 5,01 t | (9,8) | C=O |
| 5′ | CH | 74,8 | 3,78 m |  |  |
| 6′ | CH2 | 69,3 | 3,90 dd | (11,5/2,1) |  |
|  |  |  | 3,63 dd | (11,5/6,0) |  |
| Rhamnose |  |  |  |  |  |
| 1ʹʹ | CH | 103,1 | 5,21 d | (1,7) | C-3′, C-4′ |
| 2ʹʹ | CH | 72,3 | 3,95 dd | (3,2/1,8) |  |
| 3ʹʹ | CH | 72,1 | 3,61 dd | (9,5/3,2) |  |
| 4ʹʹ | CH | 73,7 | 3,33 t | (9,5) |  |
| 5ʹʹ | CH | 70,4 | 3,59 m |  |  |
| 6ʹʹ | CH3 | 18,4 | 1,13 d | (6,2) | C-4′, C-4’’ |
| Xylose |  |  |  |  |  |
| 1''' | CH | 105,3 | 4,28 d | (7,5) | C-6′, C-5''' |
| 2''' | CH | 74,9 | 3,23 dd | (9,0/7,5) |  |
| 3''' | CH | 77,5 | 3,34† |  |  |
| 4''' | CH | 71,1 | 3,49 m |  |  |
| 5''' | CH2 | 66,9 | 3,87 dd | (11,5/5,3) | C-1''', C-4''' |
|  |  |  | 3,19 dd | (11,5/10,2) |  |
| Acyl |  |  |  |  |  |
| 1ʹʹʹʹ | C | 127,6 |  |  |  |
| 2ʹʹʹʹ | CH | 115,2 | 7,10 d | (2,0) | C-3ʹʹʹʹ, C-4ʹʹʹʹ, C-6ʹʹʹʹ |
| 3ʹʹʹʹ | C | 146,8 |  |  |  |
| 4ʹʹʹʹ | C | 149,9 |  |  |  |
| 5ʹʹʹʹ | CH | 116,5 | 6,82 d | (8,1) | C-3ʹʹʹʹ, C-4ʹʹʹʹ, C-6ʹʹʹʹ |
| 6ʹʹʹʹ | CH | 123,3 | 7,00 dd | (8,3/2,1) | C-3ʹʹʹʹ, C-4ʹʹʹʹ, C-5ʹʹʹʹ |
| *α*' | CH | 114,6 | 6,31 d | (16,0) | C-1ʹʹʹʹ ,C=O |
| *β'* | CH | 148,2 | 7,64 d | (16,0) | C-2ʹʹʹʹ , C-6ʹʹʹʹ, C=O |
| C=O | C | 168,4 |  |  |  |

**Table 3**

**13C- and 1H- NMR data of echinacoside** (CD3OD; 13C:150 MHz; 1H: 600 MHz)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| C/H Atom | DEPT | δC ppm | δH ppm | *J* (Hz) | HMBC (H→C) |
| Aglycone |  |  |  |  |  |
| 1 | C | 131,4 |  |  |  |
| 2 | CH | 116,5 | 6,77 gs |  | C-3, C-4, C-6 |
| 3 | C | 146,1 |  |  |  |
| 4 | C | 144,6 |  |  |  |
| 5 | CH | 117,1 | 6,71 d | (8,0) | C-1, C-4, C-6 |
| 6 | CH | 121,3 | 6,57 gd | (8,0) | C-2, C-4 |
| *α* | CH2 | 72,4 | 4,03 m |  | C-1, C-*β* |
|  |  |  | 3,72 m |  | C-1, C-1’, C-*β* |
| *β* | CH2 | 36,5 | 2,80 t | (7,0) | C-1, C-2, C-6, C- *α* |
| Glucose |  |  |  |  |  |
| 1′ | CH | 104,1 | 4,39 d | (8,0) | C- *α* |
| 2′ | CH | 76,1 | 3,39 t | (8,5) | C-3′ |
| 3′ | CH | 81,6 | 3,81 t | (9,0) | C-2’, C-4′ |
| 4′ | CH | 70,5 | 5,01 t | (9,5) | C-1'', C-3′,C=O |
| 5′ | CH | 74,7 | 3,78 m |  |  |
| 6′ | CH2 | 69,4 | 3,93 gd | (11,5) |  |
|  |  |  | 3,62 dd | (11,5/6,0) |  |
| Rhamnose |  |  |  |  |  |
| 1ʹʹ | CH | 103,1 | 5,18 d | (1,7) | C-3′, C-4′ |
| 2ʹʹ | CH | 72,3 | 3,91† |  |  |
| 3ʹʹ | CH | 72,1 | 3,58 dd | (9,0/2,5) |  |
| 4ʹʹ | CH | 73,7 | 3,28 t | (9,5) |  |
| 5ʹʹ | CH | 70,4 | 3,56 m |  |  |
| 6ʹʹ | CH3 | 18,4 | 1,08 d | (6,0) | C-4′, C-4ʹʹ |
| Glucose |  |  |  |  |  |
| 1''' | CH | 104,6 | 4,30 d | (7,5) | C-6′ |
| 2''' | CH | 75,1 | 3,22 t | (8,5) |  |
| 3''' | CH | 77,8 | 3,35† |  |  |
| 4''' | CH | 71,4 | 3,27 dd | (9,5/8,5) |  |
| 5''' | CH | 77,9 | 3,24† |  | C-1''', C-4''' |
| 6''' | CH2 | 62,6 | 3,83 gd | (12,5) |  |
|  |  |  | 3,64 dd | (11,5/6,0) |  |
| Acyl |  |  |  |  |  |
| 1ʹʹʹʹ | C | 127,5 |  |  |  |
| 2ʹʹʹʹ | CH | 115,2 | 7,06 gs |  | C-3ʹʹʹʹ, C-4ʹʹʹʹ, C-6ʹʹʹʹ |
| 3ʹʹʹʹ | C | 146,9 |  |  |  |
| 4ʹʹʹʹ | C | 149,9 |  |  |  |
| 5ʹʹʹʹ | CH | 116,3 | 6,68 d | (8,0) | C-3ʹʹʹʹ, C-4ʹʹʹʹ, C-6ʹʹʹʹ |
| 6ʹʹʹʹ | CH | 123,3 | 6,95 gd | (8,5) | C-3ʹʹʹʹ, C-4ʹʹʹʹ, C-5ʹʹʹʹ |
| *α*' | CH | 114,6 | 6,28 d | (16,0) | C-1ʹʹʹʹ ,C=O |
| *β'* | CH | 148,2 | 7,60 d | (16,0) | C-2ʹʹʹʹ , C-6ʹʹʹʹ, C=O |
| C=O | C | 168,4 |  |  |  |

**Table 4**

**13C- and 1H- NMR data of isoacteoside** (CD3OD; 13C:150 MHz; 1H: 600 MHz)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| C/H Atom | DEPT | δC ppm | δH ppm | *J* (Hz) | HMBC (H→C) |
| Aglycone |  |  |  |  |  |
| 1 | C | 131.4 |  |  |  |
| 2 | CH | 117.1 | 6.71 d | (1.9) | C-3, C-4, C-6 |
| 3 | C | 146.1 |  |  |  |
| 4 | C | 144.7 |  |  |  |
| 5 | CH | 116.3 | 6.67 d | (8.0) | C-1, C-4, C-6 |
| 6 | CH | 121.2 | 6.58 dd | (8.0/1.9) | C-2, C-4 |
| *α* | CH2 | 72.4 | 3.98 m |  | C-1 |
|  |  |  | 3.74 m |  | C-1, C-1' |
| *β* | CH2 | 36.7 | 2.82 t | (7.6) | C-1, C-2, C-6 |
| Glucose |  |  |  |  |  |
| 1′ | CH | 104.4 | 4.37 d | (8.0) | C- *α* |
| 2′ | CH | 75.7 | 3.38 dd | (9.4/7.6) | C-3′ |
| 3′ | CH | 83.9 | 3.56 t | (8.9) | C-2', C-4′ |
| 4′ | CH | 70.4 | 3.45 dd | (10.0/8.0) |  |
| 5′ | CH | 75.4 | 3.58 m |  |  |
| 6′ | CH2 | 64.6 | 4.53 dd | (11.8/2.0) |  |
|  |  |  | 4.38 dd | (11.9/6.0) |  |
| Rhamnose |  |  |  |  |  |
| 1'' | CH | 102.7 | 5.21 d | (1.5) | C-3′, C-4′ |
| 2'' | CH | 72.4 | 3.98 dd | (3.4/1.8) |  |
| 3'' | CH | 72.3 | 3.74 dd | (9.5/3.3) |  |
| 4'' | CH | 74.0 | 3.44 t | (8.8) |  |
| 5'' | CH | 70.0 | 4.04 m |  |  |
| 6'' | CH3 | 17.9 | 1.28 d | (6.2) | C-4′, C-4'' |
| Acyl |  |  |  |  |  |
| 1''' | C | 127.7 |  |  |  |
| 2''' | CH | 115.1 | 7.07 d | (2.0) | C-3''', C-4''', C-6''' |
| 3''' | C | 146.8 |  |  |  |
| 4''' | C | 149.6 |  |  |  |
| 5''' | CH | 116.5 | 6.80 d | (8.1) | C-3''', C-4''', C-6''' |
| 6''' | CH | 123.1 | 6.93 dd | (8.1/2.0) | C-3''', C-4''', C-5''' |
| *α*' | CH | 114.8 | 6.33 d | (16.0) | C-1''', C=O |
| *β'* | CH | 147.2 | 7.60 d | (16.0) | C-2''', C-6''', C=O |
| C=O | C | 169.1 |  |  |  |

**Table 5**

**13C- and 1H- NMR data of leucosceptoside A** (CD3OD; 13C:150 MHz; 1H: 600 MHz)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| C/H Atom | DEPT | δC ppm | δH ppm | *J* (Hz) | HMBC (H→C) |
| Aglycone |  |  |  |  |  |
| 1 | C | 131,4 |  |  |  |
| 2 | CH | 117,1 | 6,73 d | (2,0) | C-3, C-4, C-6 |
| 3 | C | 146,1 |  |  |  |
| 4 | C | 144,7 |  |  |  |
| 5 | CH | 116,3 | 6,71 d | (8,0) | C-1, C-4, C-6 |
| 6 | CH | 121,2 | 6,61 dd | (8,0/2,0) | C-2, C-4 |
| *α* | CH2 | 72,2 | 4,09 m |  | C-1 |
|  |  |  | 3,77 m |  | C-1, C-1' |
| *β* | CH2 | 36,5 | 2,84 m |  | C-1, C-2, C-6 |
| Glucose |  |  |  |  |  |
| 1′ | CH | 104,2 | 4,42 d | (7,8) | C- *α* |
| 2′ | CH | 76,2 | 3,43 dd | (9,1/7,9) | C-3′ |
| 3′ | CH | 81,5 | 3,86 t | (9,2) | C-2', C-4′ |
| 4′ | CH | 70,6 | 4,96 t | (9,4) | C=O |
| 5′ | CH | 76,1 | 3,58 m |  |  |
| 6′ | CH2 | 62,4 | 3,67 gd | (10,0) |  |
|  |  |  | 3,58 dd | (12,0/5,5) |  |
| Rhamnose |  |  |  |  |  |
| 1'' | CH | 102,9 | 5,24 d | (1,6) | C-3′, C-4′ |
| 2'' | CH | 72,3 | 3,95 dd | (3,2/1,7) |  |
| 3'' | CH | 72,1 | 3,62 dd | (9,4/3,2) |  |
| 4'' | CH | 73,8 | 3,33 t | (9,5) |  |
| 5'' | CH | 70,4 | 3,63† |  |  |
| 6'' | CH3 | 18,4 | 1,13 d | (6,2) | C-4′, C-4'' |
| Acyl |  |  |  |  |  |
| 1''' | C | 127,6 |  |  |  |
| 2''' | CH | 111,8 | 7,24 d | (1,8) | C-3''', C-4''', C-6''' |
| 3''' | C | 149,4 |  |  |  |
| 4''' | C | 150,8 |  |  |  |
| 5''' | CH | 116,5 | 6,85 d | (8,1) | C-3''', C-4''', C-6''' |
| 6''' | CH | 124,3 | 7,13 dd | (8,3/1,8) | C-3''', C-4''', C-5''' |
| *α*' | CH | 115,1 | 6,41 d | (16,0) | C-1''', C=O |
| *β'* | CH | 147,9 | 7,70 d | (16,0) | C-2''', C-6''', C=O |
| C=O | C | 168,2 |  |  |  |
| OCH3 | CH3 | 56,4 | 3,93 s |  | C-3''' |

**(A)**