SUPPLEMENTARY MATERIAL

Phytochemical components and biological activities of *Silene arenarioides* Desf.

Lynda Golea[a,b], Mohammed Benkhaled[a], Catherine Lavaud[c], Christophe Long[d] and Hamada Haba[a]*

[a] *Laboratoire de Chimie et Chimie de l'Environnement (L.C.C.E), Département de Chimie, Faculté des Sciences de la Matière, Université de Batna-1, Batna, Algérie;* [b]*Département des Sciences de la Matière, Université de Khenchela, Khenchela 04000, Algérie ;* [c] *UMR CNRS 7312, Institut de Chimie Moléculaire de Reims, BP 1039, 51687 Reims Cedex 2, France;* [d] *Pôle Actifs Végétaux- Recherche Substances Naturelles Centre de R & D Pierre Fabre, 3 Avenue Hubert Curien BP 13562, 31035 Toulouse, Cedex 1, France.*

* Corresponding author: Hamada Haba Tel: +213 33 31 90 15; Fax: +213 33 31 90 15; E-mail address: haba.hamada@yahoo.fr

Abstract: In this study, six known compounds **1**–**6** were isolated from the aerial parts of *Silene arenarioides* Desf. by using different chromatographic methods. The structures of these compounds were identified as maltol glycoside (**1**), soyacerebroside I (**2**), chrysin (**3**), apigenin (**4**), quercetin (**5**) and stigmasterol glucoside (**6**). The compounds (**1**) and (**2**) are reported for the first time from this genus. The isolated compounds were determined by using NMR techniques (^{1}H-NMR, ^{13}C-NMR, COSY, HSQC, and HMBC) and mass spectroscopy (ESI-MS). The antibacterial and antioxidant activities of extracts and of compound (**1**) have been evaluated. The antioxidant activity was performed by DPPH radical scavenging method, which showed that methanol extract possesses a good antioxidant activity with value of IC_{50} = 8.064 ± 0.005 µg/mL.

Keywords: *Silene arenarioides* Desf., soyacerebroside, maltol, antibacterial activity, antioxidant activity

Experimental

General experimental procedures

The separation and purification of methanol extract were realized using column chromatography (SiO_2: 320–400 mesh, Merck, Polyamide: SC-6 and Sephadex LH-20 (25–100) μM). The TLC and preparative TLC were performed with silica gel (Kieselgel 60 F_{254}, Merck) and detection at 254 and 366 nm. The pure compounds were identified by UV spectra (Shimadzu UV-3101 spectrophotometer), IR (KBr, Shimadzu model IR-470 spectrometer), positive and negative ESIMS (ion trap Bruker Esquire), and extensive 1D and 2D NMR analysis (COSY, HSQC, HMBC, Bruker Avance spectrometer, ^{1}H 500 MHz, ^{13}C 125 MHz).

Preparation of plant extracts

The plant material of *Silene arenarioides* was collected in April 2008 in the vicinity of Biskra Algeria (GPS: 34.6543-6.6926) and was identified by Prof. Bachir Oudjehih, of the agronomic department of the University of Batna-1 (Number 685/LCCE). The dried aerial parts of *S. arenarioides* (700 g) were macerated twice with 5 L of petroleum ether at room temperature during 4 days. The residue was extracted successively with 2 × 5 L of ethyl acetate and methanol in the same conditions.

Study of methanol extract

The methanol extract (5 g) was chromatographed on a polyamide column eluted with H_2O/MeOH (100-0 → 0-100) to give seven main fractions (F1 → F7). Fraction F6 (200 mg) was separated on SiO_2 column chromatography ($CHCl_3$/MeOH: 100-0 → 0-100) and finally, purified by gel filtration on Sephadex LH-20 with MeOH as eluent to allow the isolation of two compounds **1** (18 mg) and **2** (13 mg). The mixed fractions F4 and F5 (350 mg) were fractionated on SiO_2 CC ($CHCl_3$/MeOH: 97-3, 95-5, 90-10) to afford two compounds **3** (3 mg) and **4** (4.5 mg) and several subfractions. Subfraction SF2 (18 mg) eluted with $CHCl_3$/MeOH (95-5), was further purified by crystallization using (CH_2Cl_2/Acetone) to give compound **5** (4 mg). The fraction F7 (82 mg) was subjected

to silica gel CC eluting with $CHCl_3$/MeOH (95:5, 90:10, 80:20) to yield compound **6** (11 mg). The fraction F3 contained a mixture of inseparable saponins.

Evaluation of antioxidant activity

The antioxidant activity of the methanol extract of *S. arenarioides* was evaluated with the 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) by the method described by Leitao (Leitao et al. 2002). Ascorbic acid (0 – 30 µg/mL) was used as reference and showed an antiradical activity value with IC_{50} = 7.645 µg/mL. The solution of DPPH (4 mg) was dissolved in 100 mL of methanol and samples were prepared by dissolving in methanol 50 µg/mL. Then, these solutions, known as stock solutions, were diluted for the following concentrations: 5, 10, 15, 25, 30, 40 and 50 µg/mL. The test works by mixing 900 µL of the above solution of DPPH with 100 µL of sample solution at different concentrations. The mixtures were kept in dark for 30 min and optical density was measured at 517 nm. The % inhibition was calculated using the formula given below:

$$\% \text{ inhibition} = [1 - (\text{Abs sample}/\text{Abs control})] \times 100$$

The IC_{50} value was calculated by linear regression of plots where the abscissa represented the concentration of the methanol extract and compared with the ascorbic acid. The test was realized in triplicate.

Evaluation of antibacterial activity

The petroleum ether, ethyl acetate and methanol extracts, and maltol benzoylglucoside (**1)** were tested against standard bacterial strains such as *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonas aeruginosa* ATCC 27853 for the determination of their antibacterial activity. The disc diffusion method was used to establish the inhibition zones of the tested samples against the standard bacterial strains (Celikel & Kavas 2008). Petri dishes with 60 mL of sterile Mueller-Hinton agar were seeded with the appropriate bacterial suspension. Sterile, 6 mm diameter filter paper discs were impregnated with 10 µL of each dilution of *S. arenarioides* extracts and placed on the inoculated agar gently tapped to remove excess liquid, and positioned on seeded plates. One other sterile blank disc impregnated with DMSO, was used as negative control. After incubation for 24 h at 37 °C, all plates were observed for zones

of growth inhibition, and the diameters were measured in millimeters. The test was realized in triplicate.

Statistical analysis

The statistical analyses were performed by a Microsoft Excel 2007 and the numerical results were expressed graphically. The analyses were made using GraphPad Prism (Version 5.0).

References

Celikel N, Kavas G. 2008. Antimicrobial properties of some essential oils against some pathogenic microorganisms. Czech J Food Sci. 26:174–181.

Leitao GG, Leitao SG, Vilagag W. 2002. Quick preparative separation of natural naphthopyranones with antioxidant activity by high-speed counter-current chromatography. Z Naturforsch. 57:1051–1055.

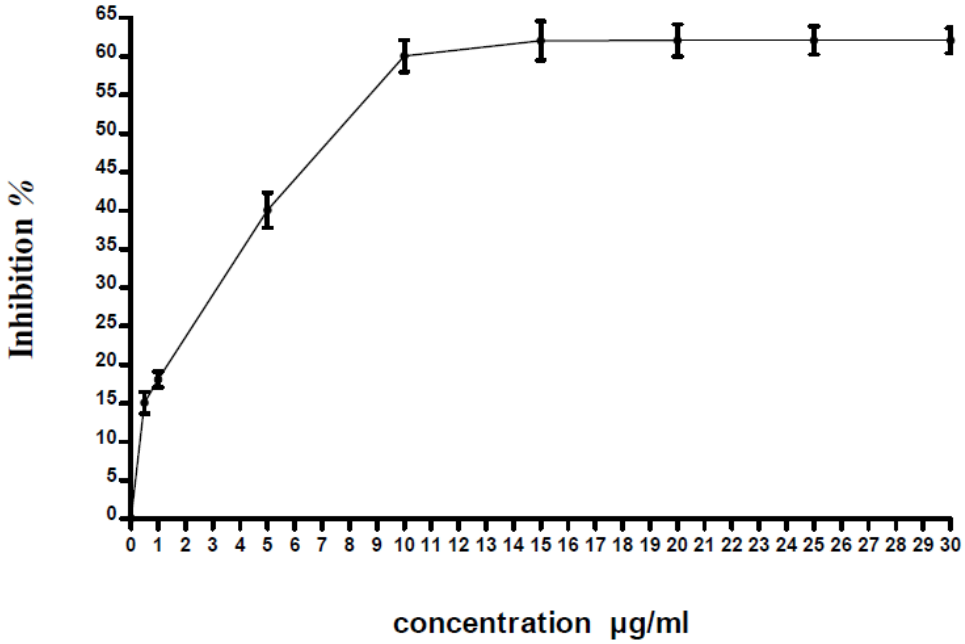

Figure S1. DPPH free radical scavenging activity of standard ascorbic acid

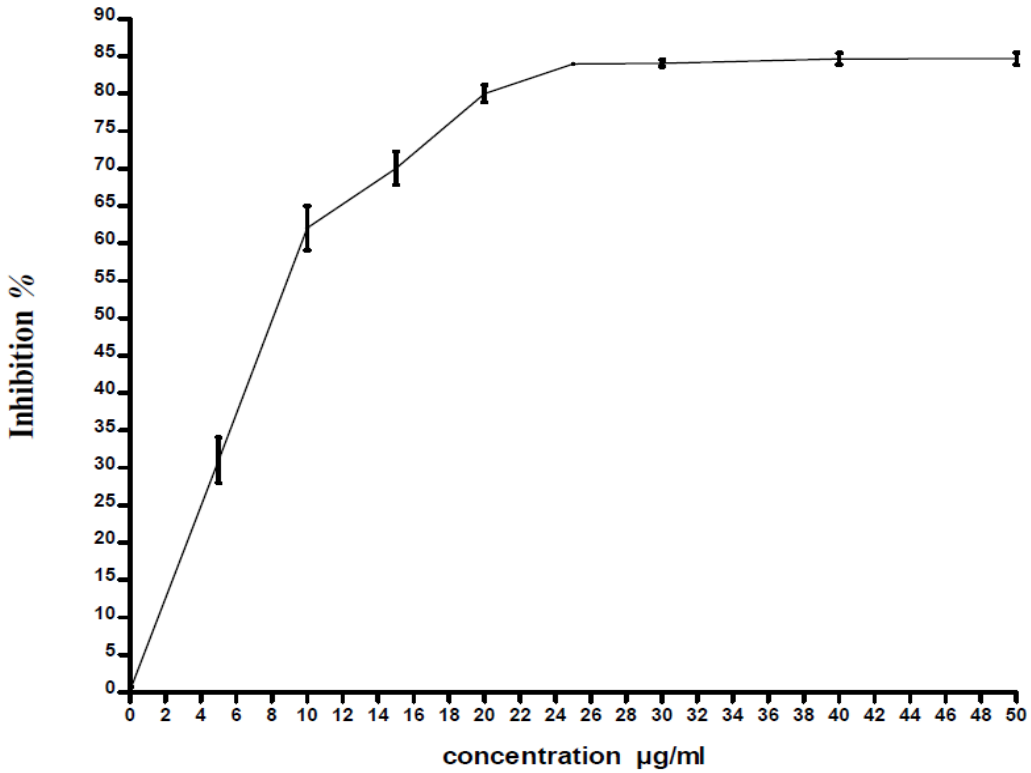

Figure S2. DPPH free radical scavenging activity of methanol plant extract

Table S1. Antibacterial activity of different extracts expressed in an agar diffusion test

Extracts and products	**Concentration**	***S. aureus***	***E. coli***	***P. aeruginosa***
Petroleum ether	100 µg/mL	-	-	-
Ethyl acetate	100 µg/mL	9.00 ± 1.50	13.00 ± 0.173	-
Methanol	100 µg/mL	21.00 ± 0.30	18.00 ± 0.265	23.00 ± 0.529
Compound **1**	0.4 µg/mL	0	9.00 ± 0.458	11.00 ± 1.082
AMP	10 µg	32	15	20
GEN	10 µg	28	14	19

(-): No inhibition. AMP: ampicillin; GEN: gentamicin.
Values are the average of three replicates.

Compound 1: maltol 3-*O*-[6-*O*-benzoyl]-β-D-glucopyranoside (Nakato et al. 2011)

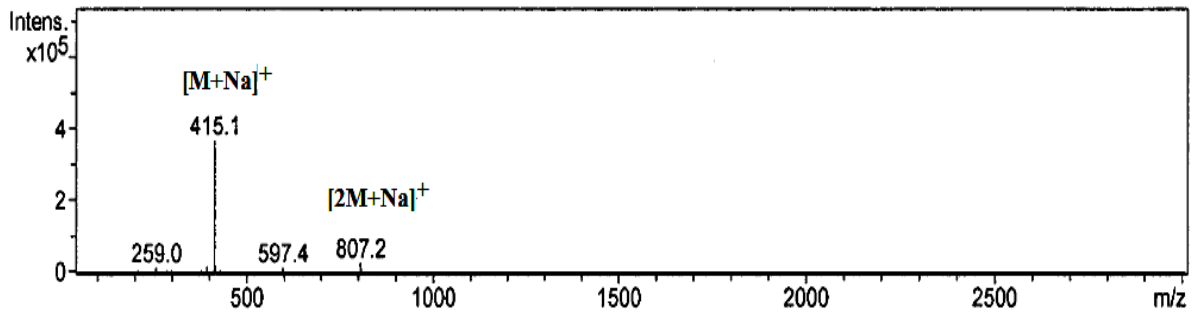

Figure S3. Mass spectrum ESI-MS of compound 1

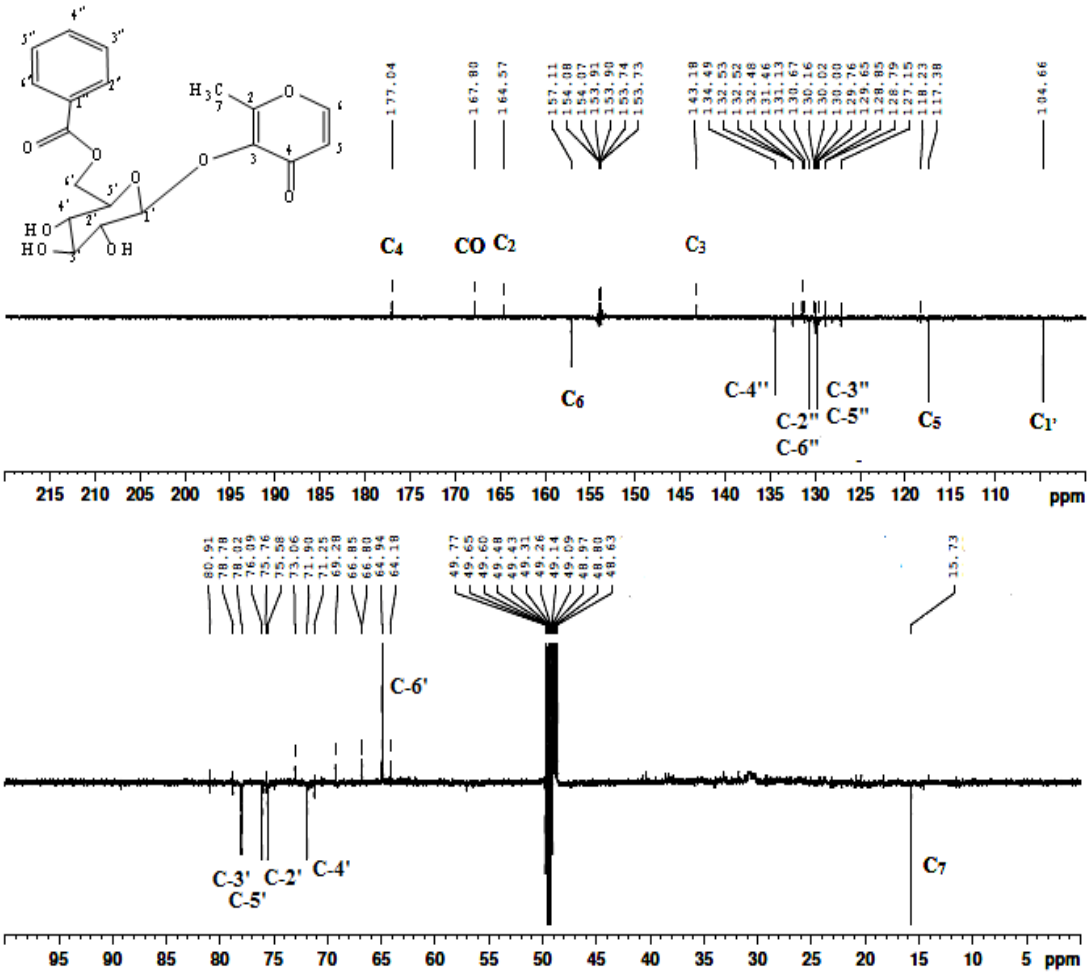

Figure S4. ^{13}C-NMR spectrum of compound 1 (125 MHz, CD_3OD)

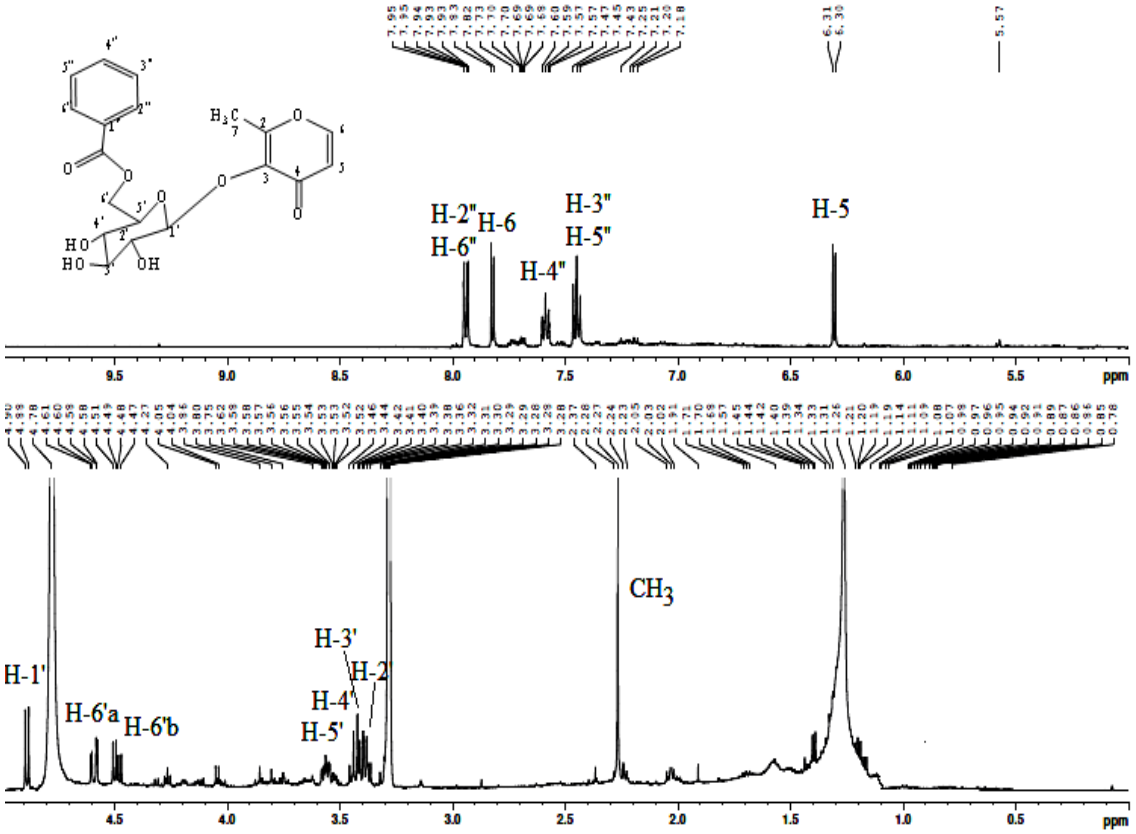

Figure S5. ^{1}H-NMR spectrum of compound 1 (500 MHz, CD_3OD)

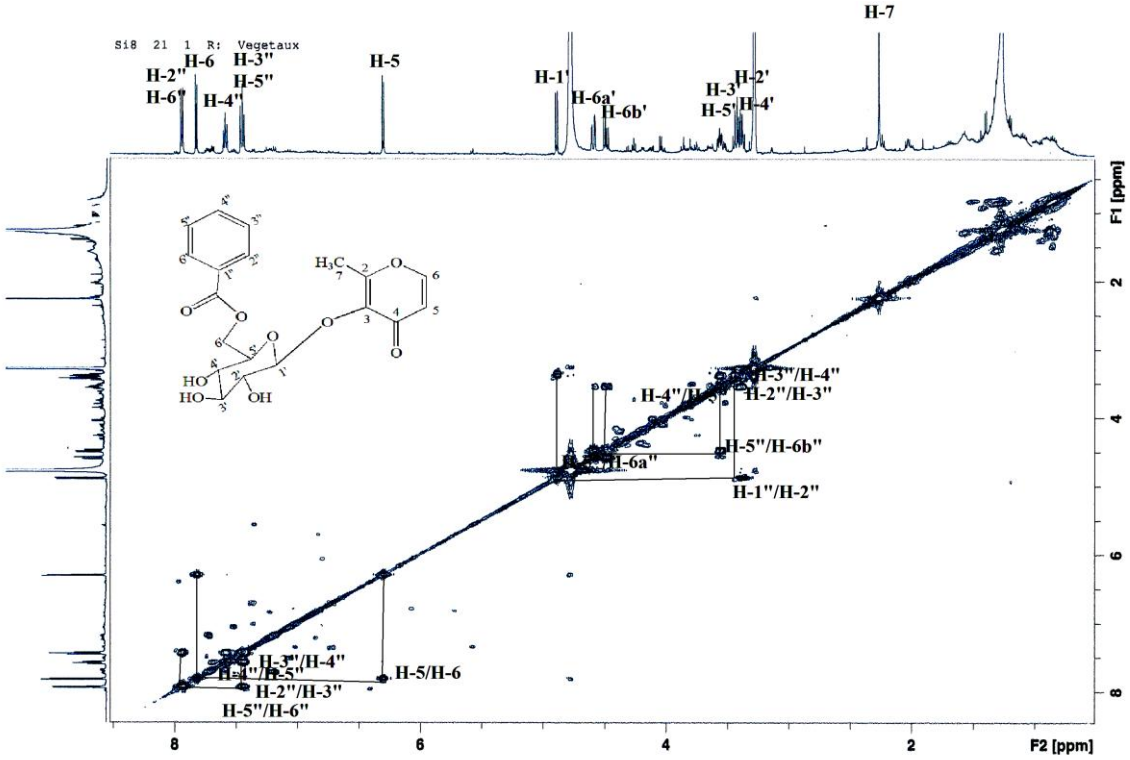

Figure S6. COSY spectrum of compound 1 (500 MHz, CD_3OD)

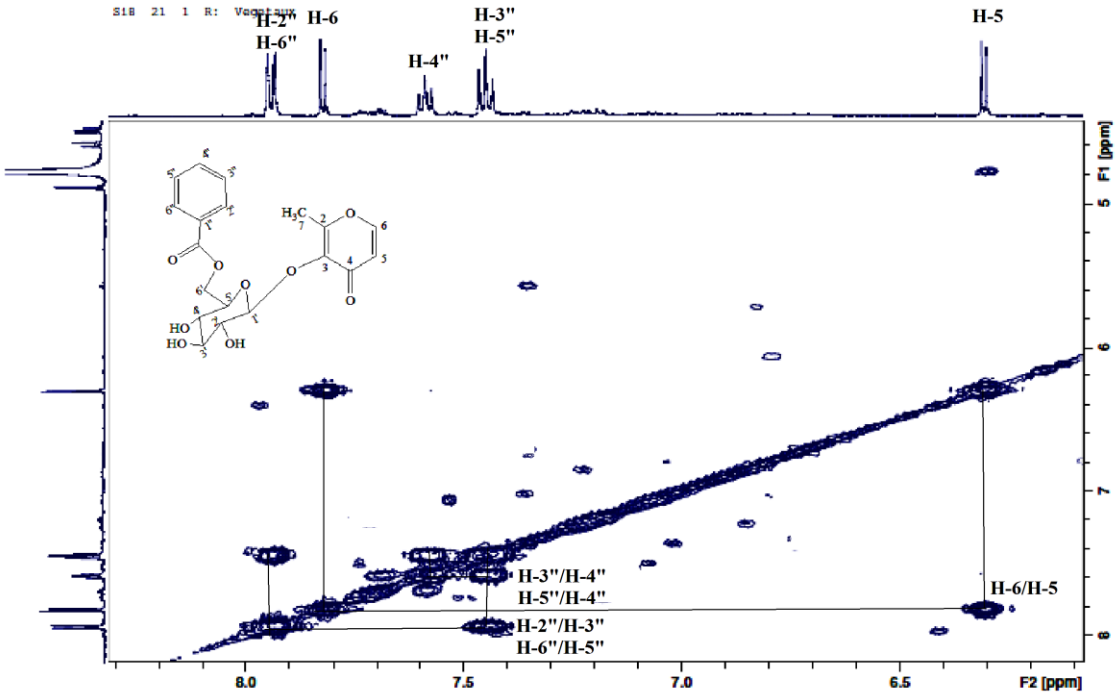

Figure S7. COSY spectrum (aromatic part) of compound 1 (500 MHz, CD_3OD)

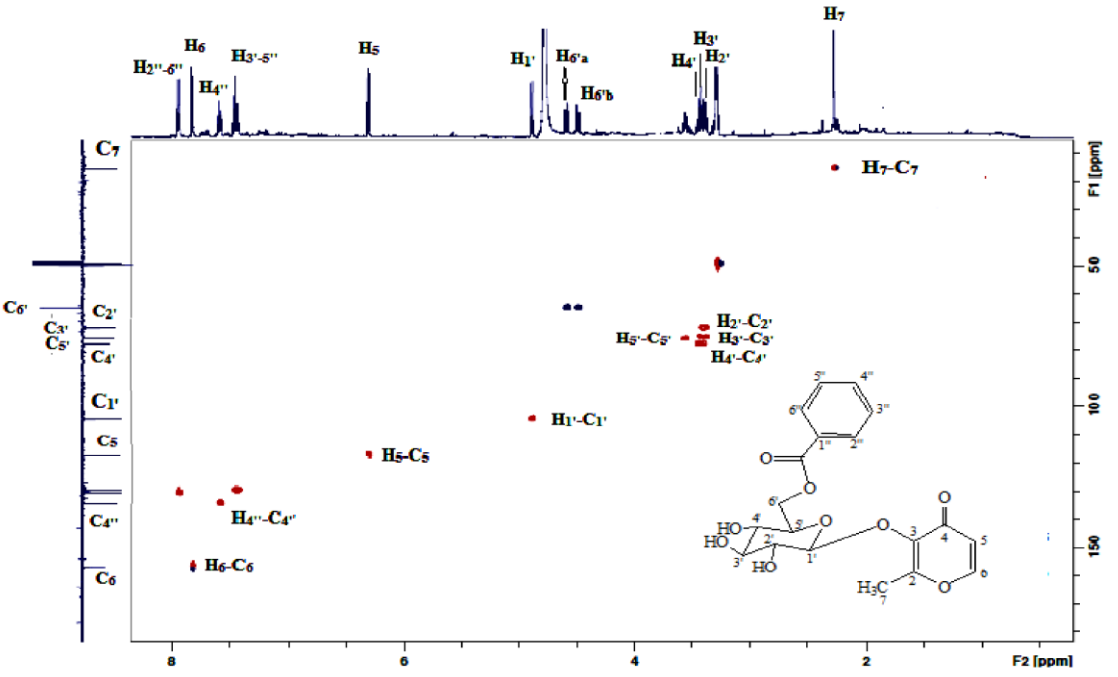

Figure S8. HSQC spectrum of compound 1 (500 MHz, CD_3OD)

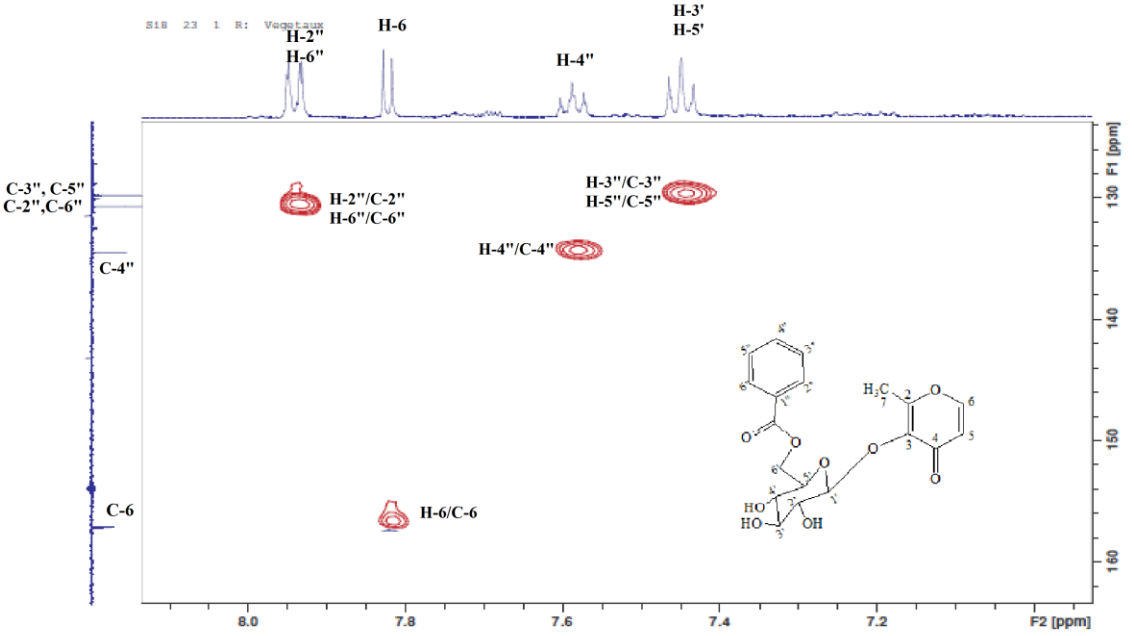

Figure S9. HSQC spectrum (aromatic part) of compound 1 (500 MHz, CD_3OD)

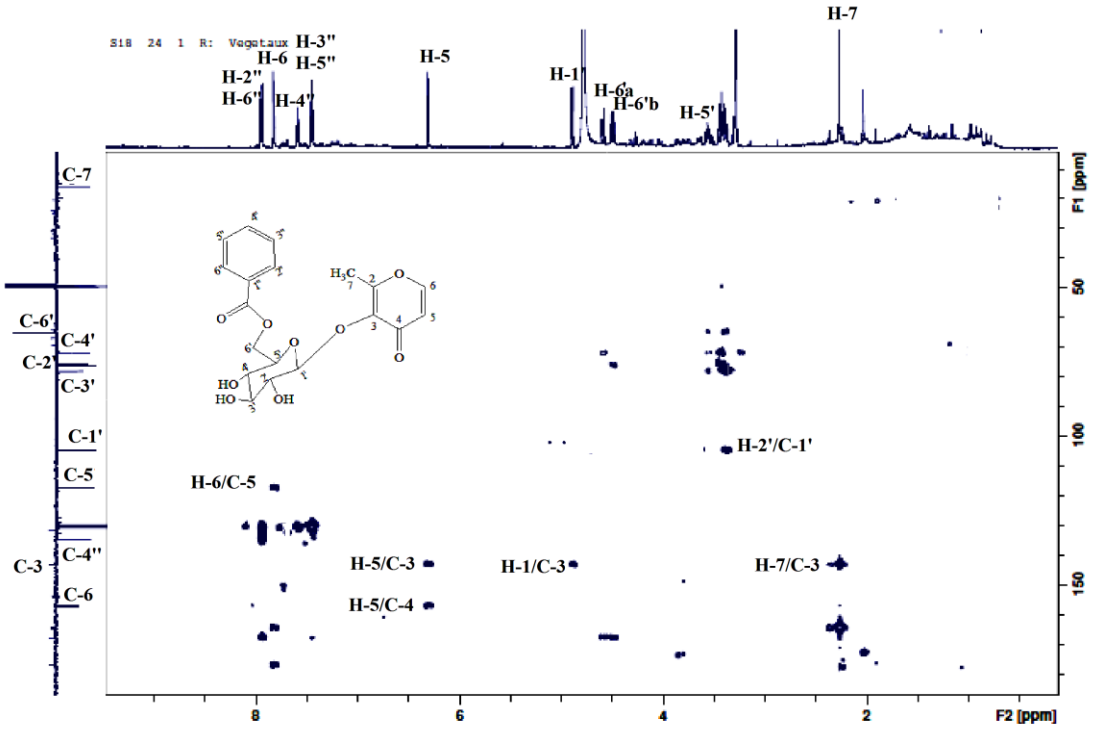

Figure S10. HMBC spectrum of compound 1 (500 MHz, CD_3OD)

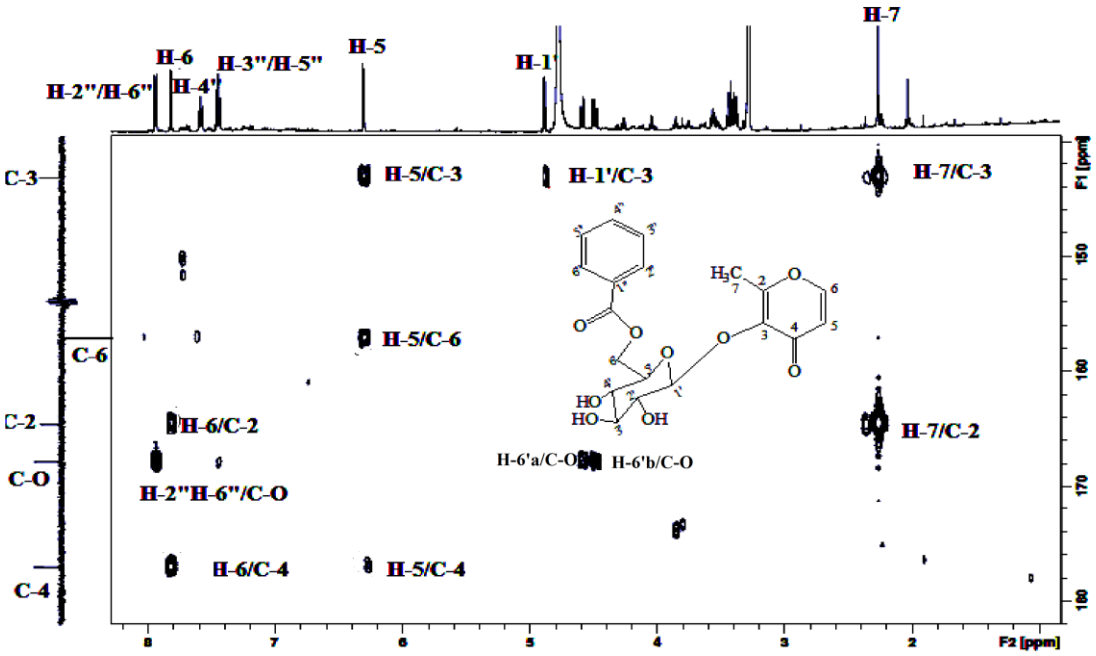

Figure S11. HMBC spectrum of compound 1 (500 MHz, CD_3OD)

Compound 2: Soyacerebroside I (Voutquenne et al. 1999)

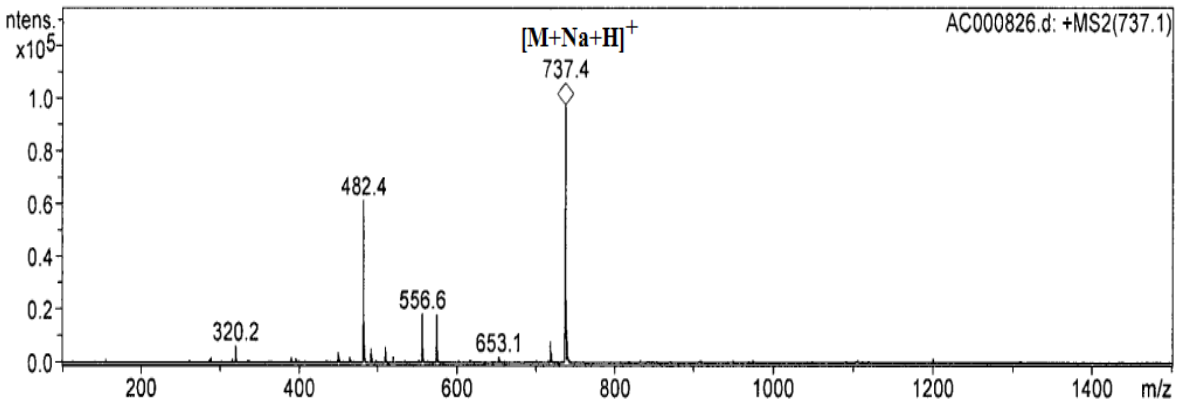

Figure S12. Mass spectrum ESI-MS of compound 2

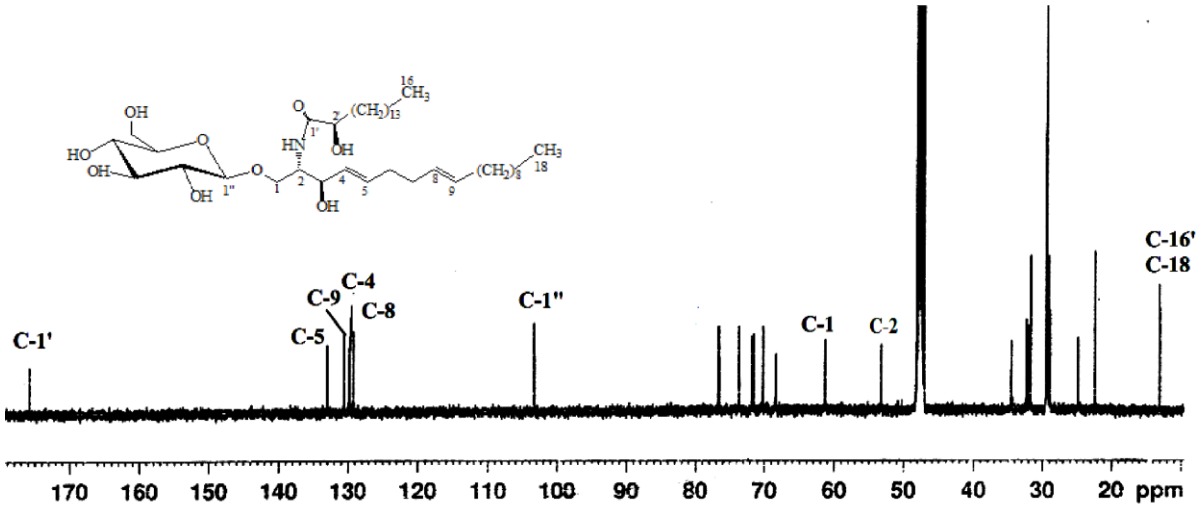

Figure S13 ^{13}C-NMR spectrum of compound 2 (125 MHz, CD_3OD**)**

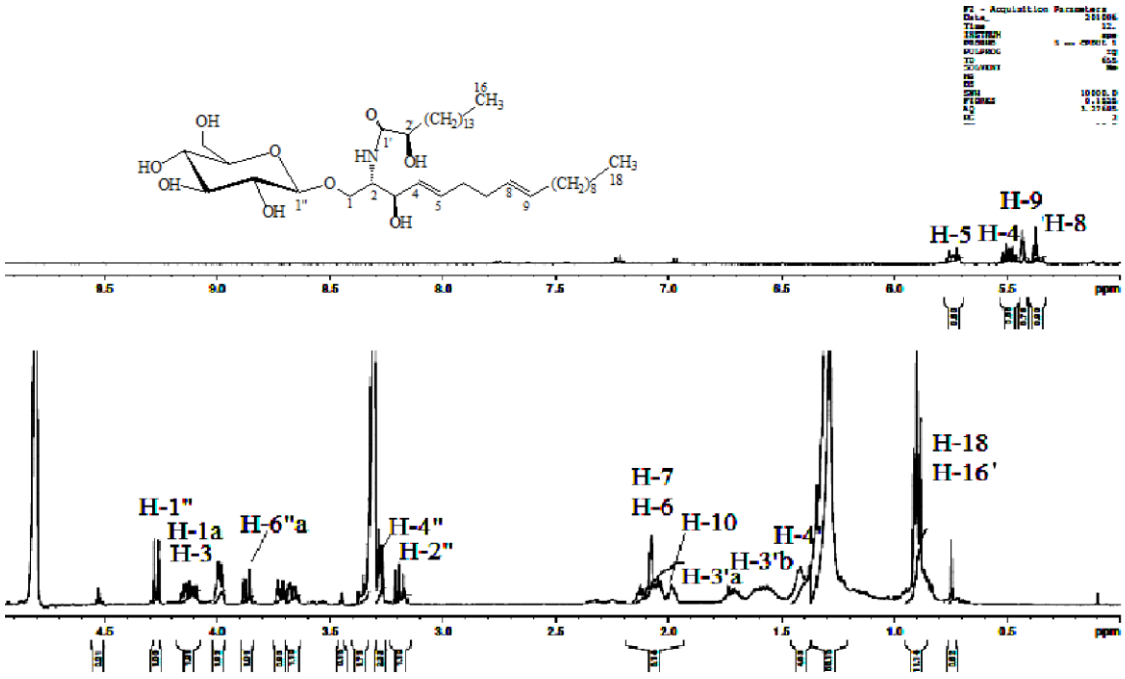

Figure S14. ^{1}H-NMR spectrum of compound 2 (500 MHz, CD_3OD)

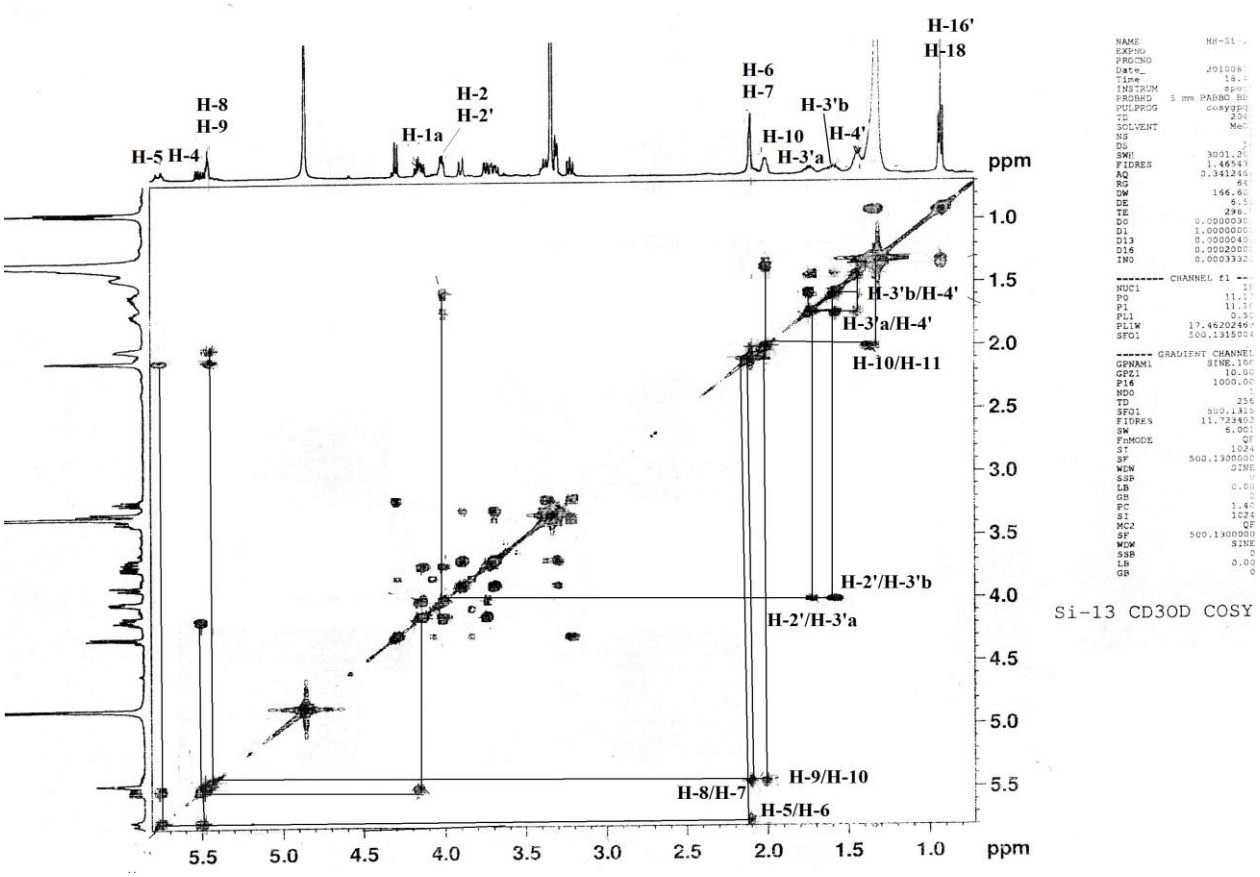

Figure S15. COSY spectrum of compound 2 (500 MHz, CD_3OD)

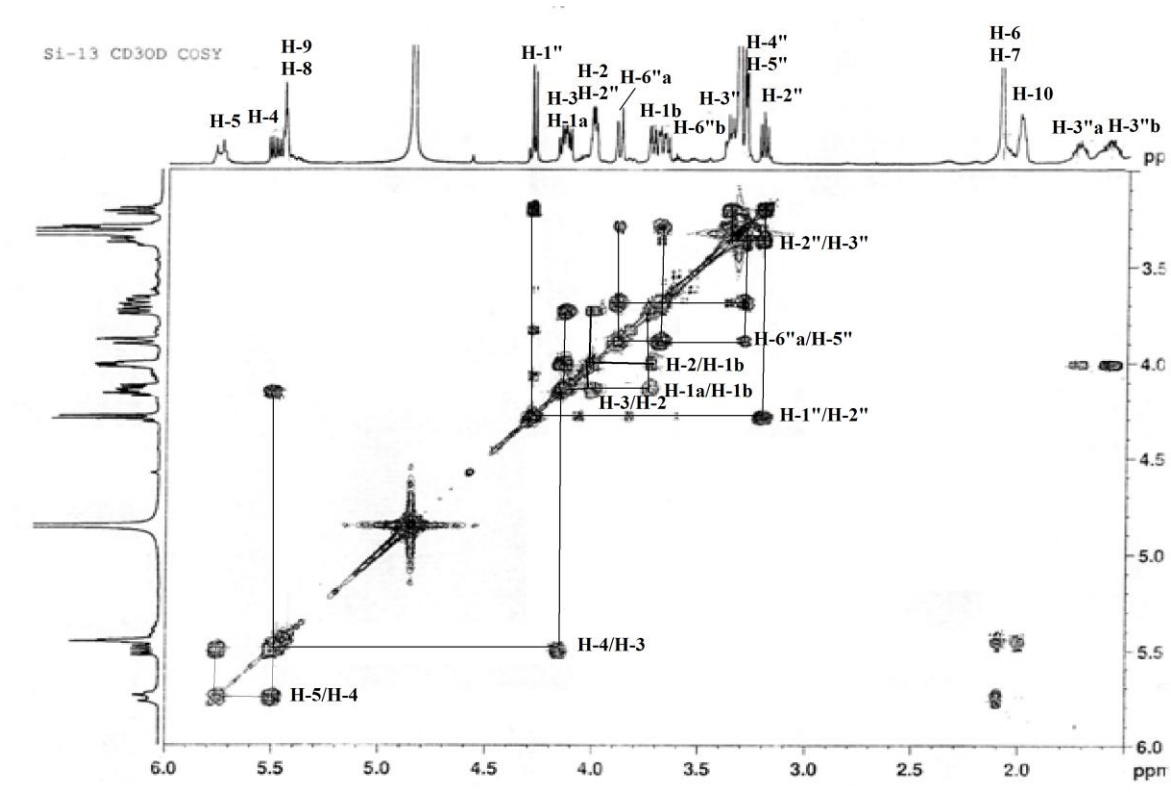

Figure S16. COSY spectrum of compound 2 (500 MHz, CD_3OD)

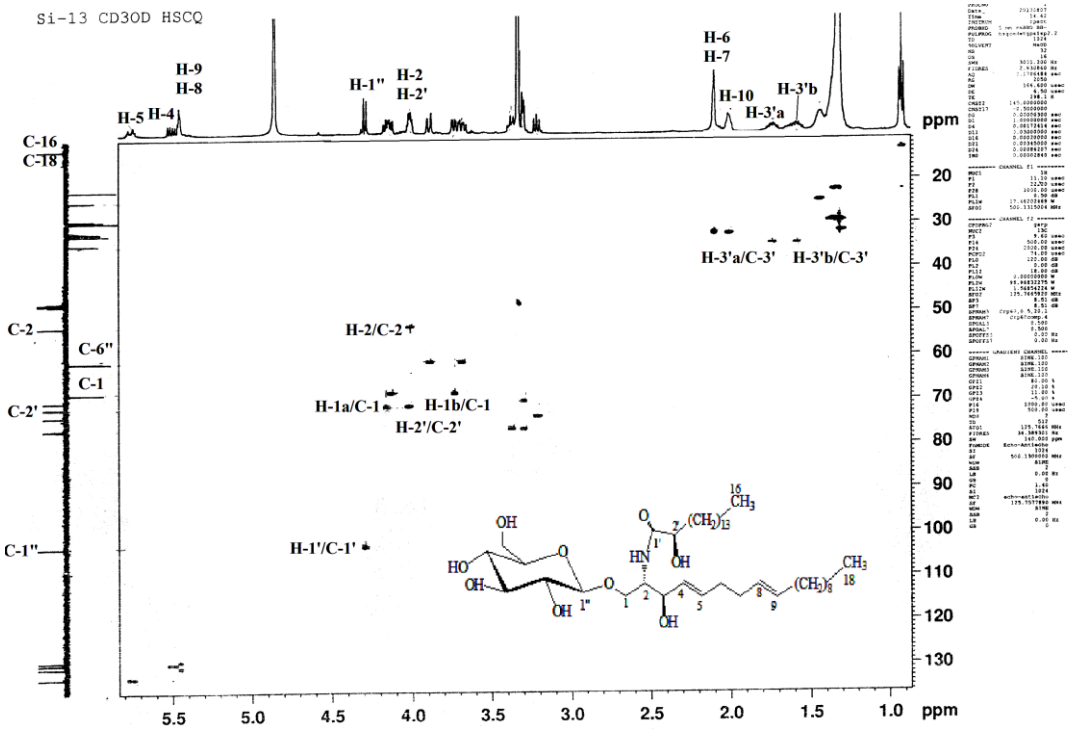

Figure S17. HSQC spectrum of compound 2 (500 MHz, CD_3OD)

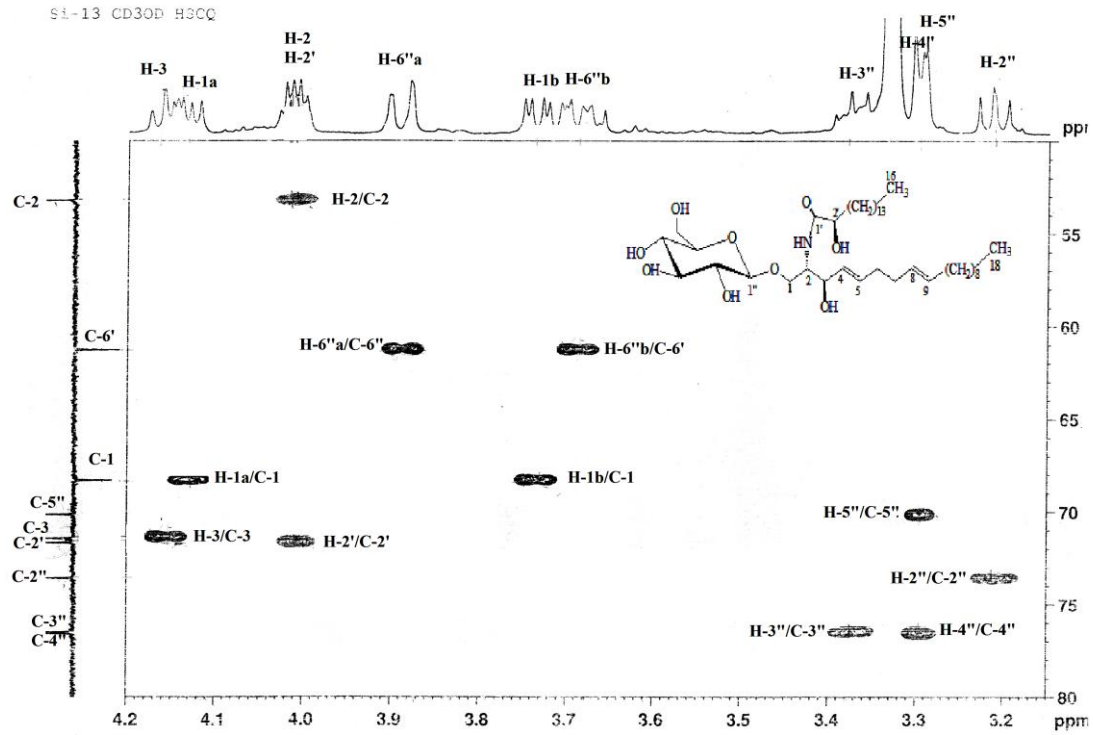

Figure S18. HSQC spectrum of compound 2 (500 MHz, CD_3OD)

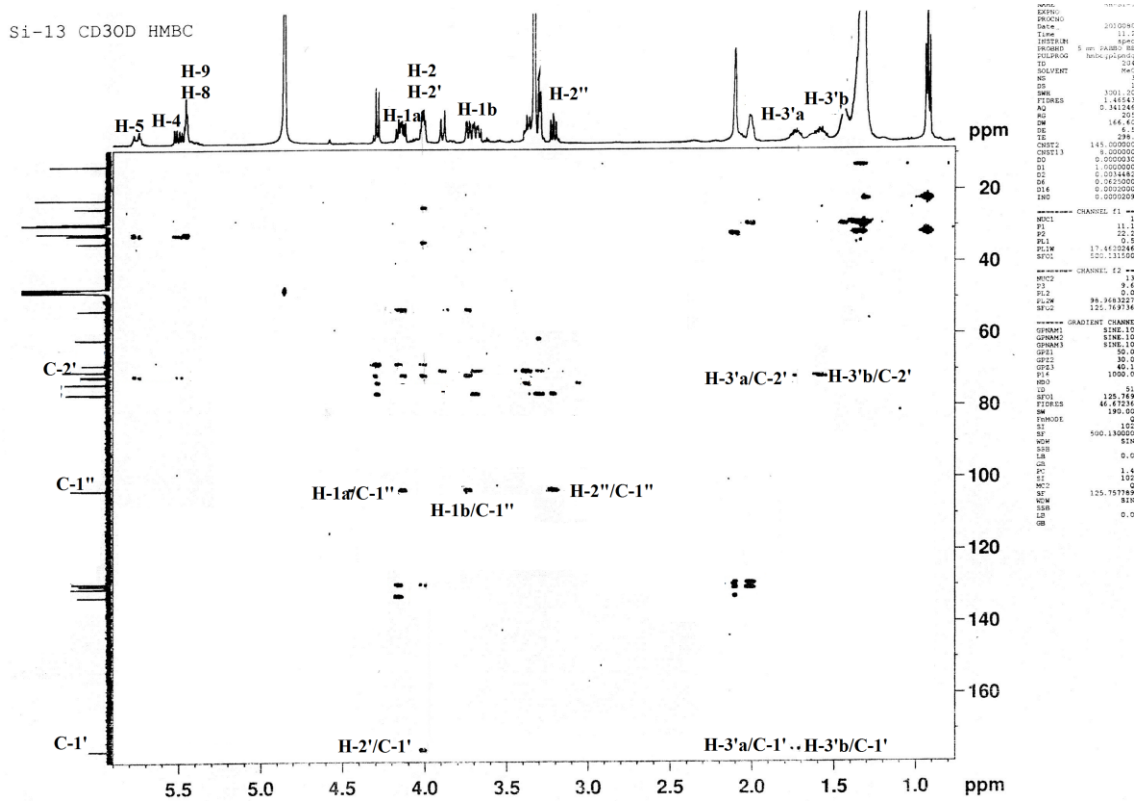

Figure S19. HMBC spectrum of compound 2 (500 MHz, CD_3OD)

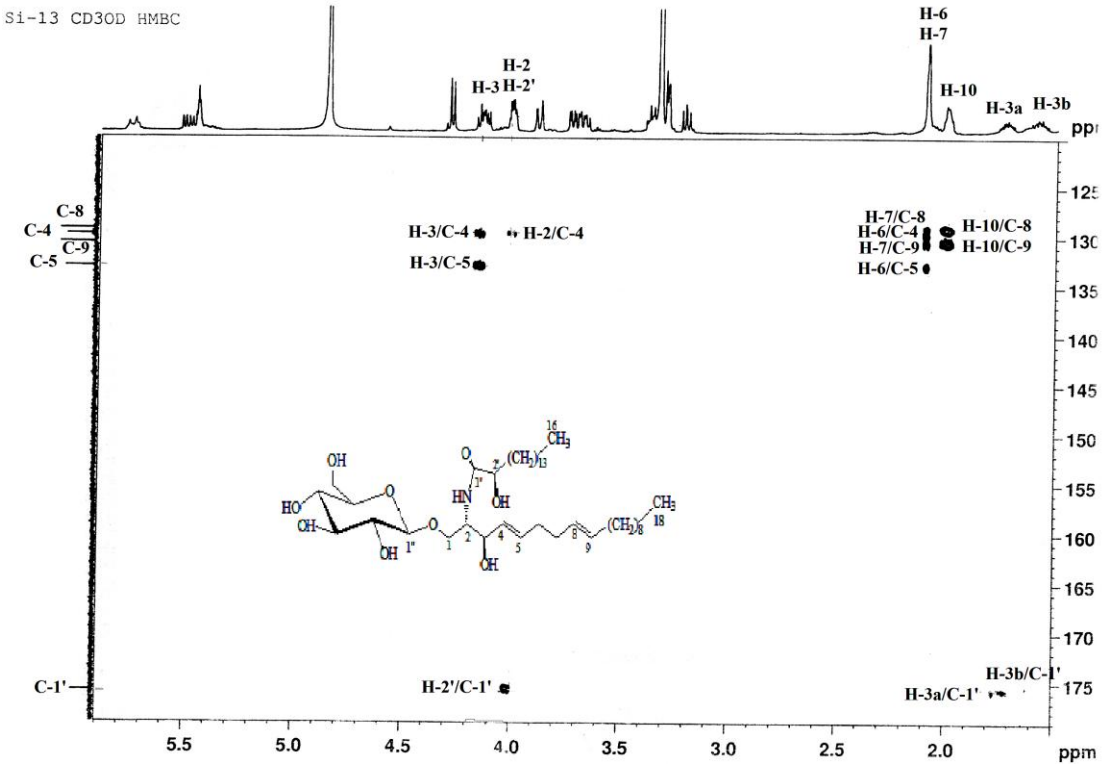

Figure S20. HMBC spectrum of compound 2 (500 MHz, CD_3OD)

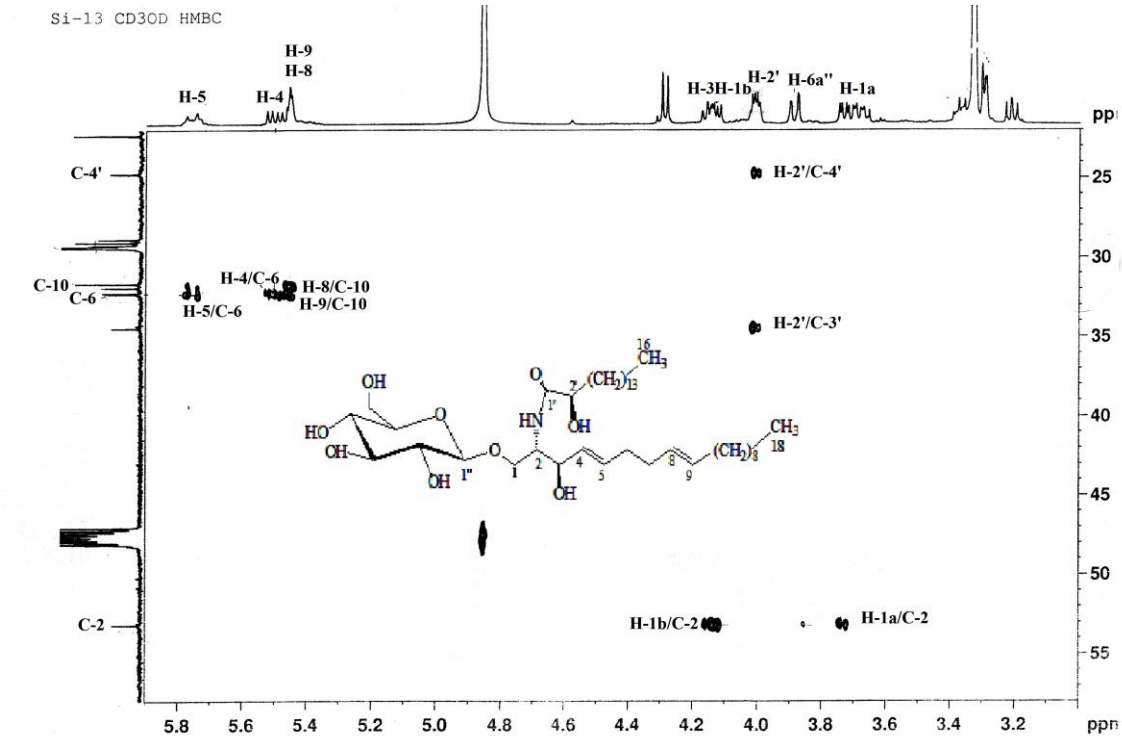

Figure S21. HMBC spectrum of compound 2 (500 MHz, CD_3OD)

Compound 3: Chrysin (Shen et al. 1993)

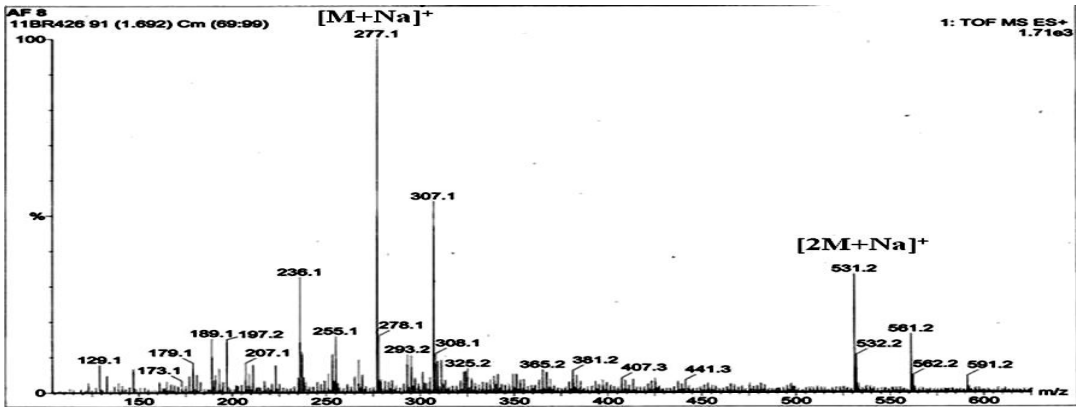

Figure S22. Mass spectrum ESI-MS of compound 3

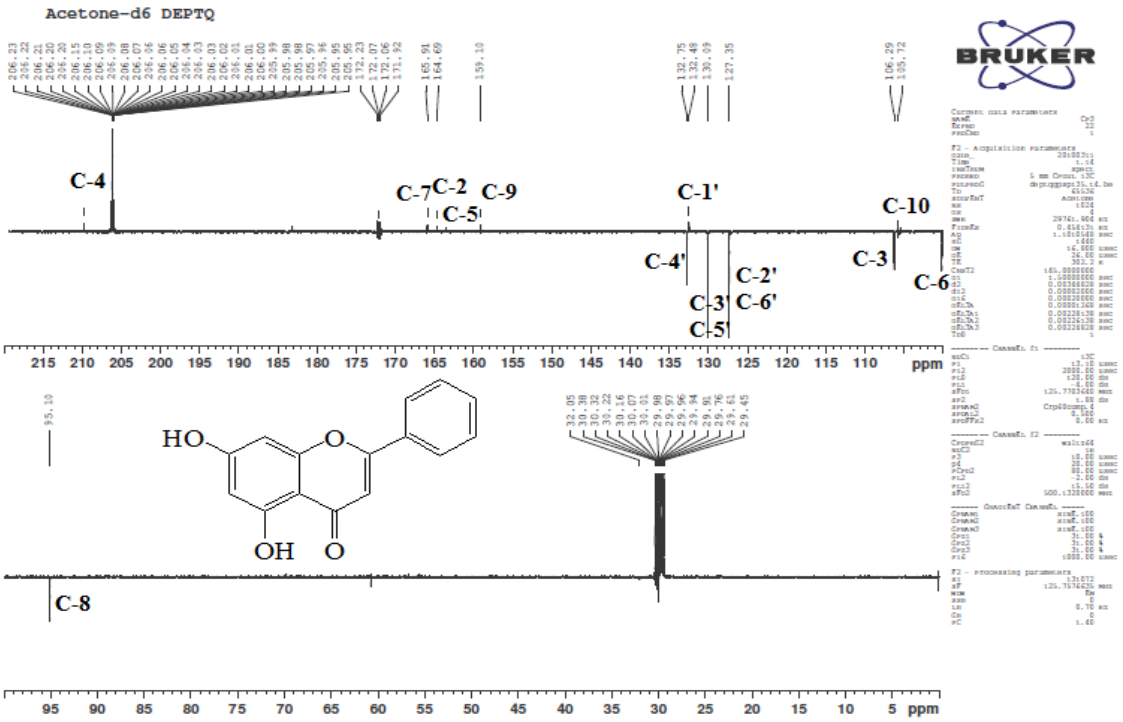

Figure S23. ^{13}C-NMR spectrum of compound 3 (125 MHz, CD_3COCD_3)

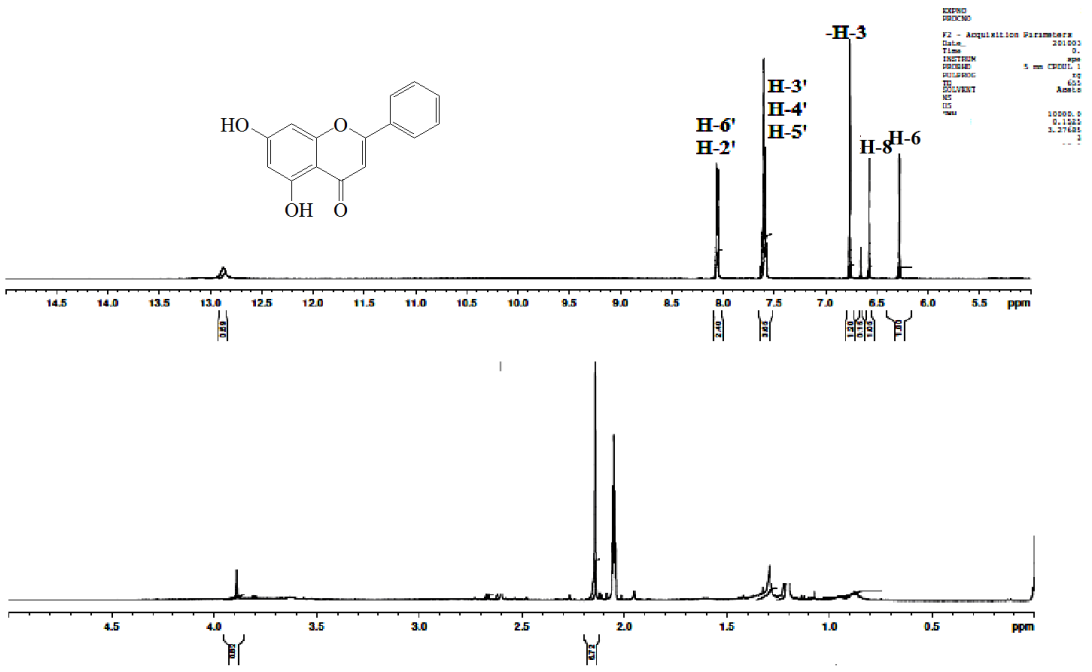

Figure S24. ^{1}H-NMR spectrum of compound 3 (500 MHz, CD_3COCD_3)

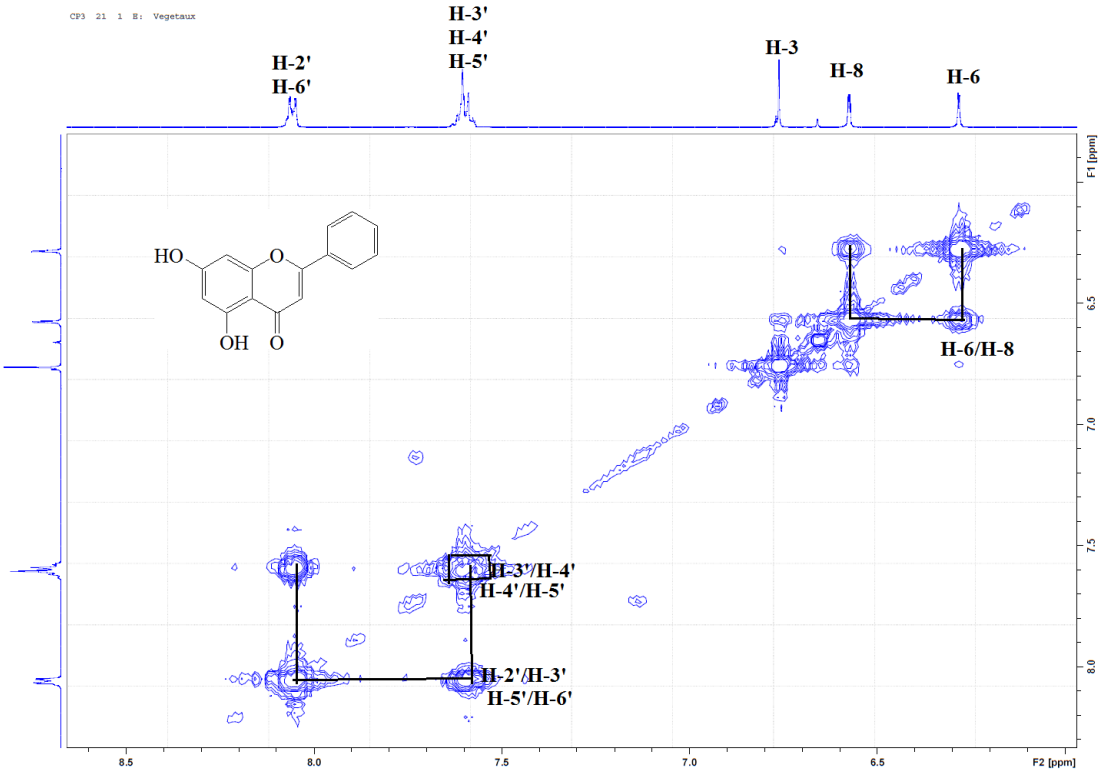

Figure S25. COSY spectrum of compound 3 (500 MHz, CD_3COCD_3)

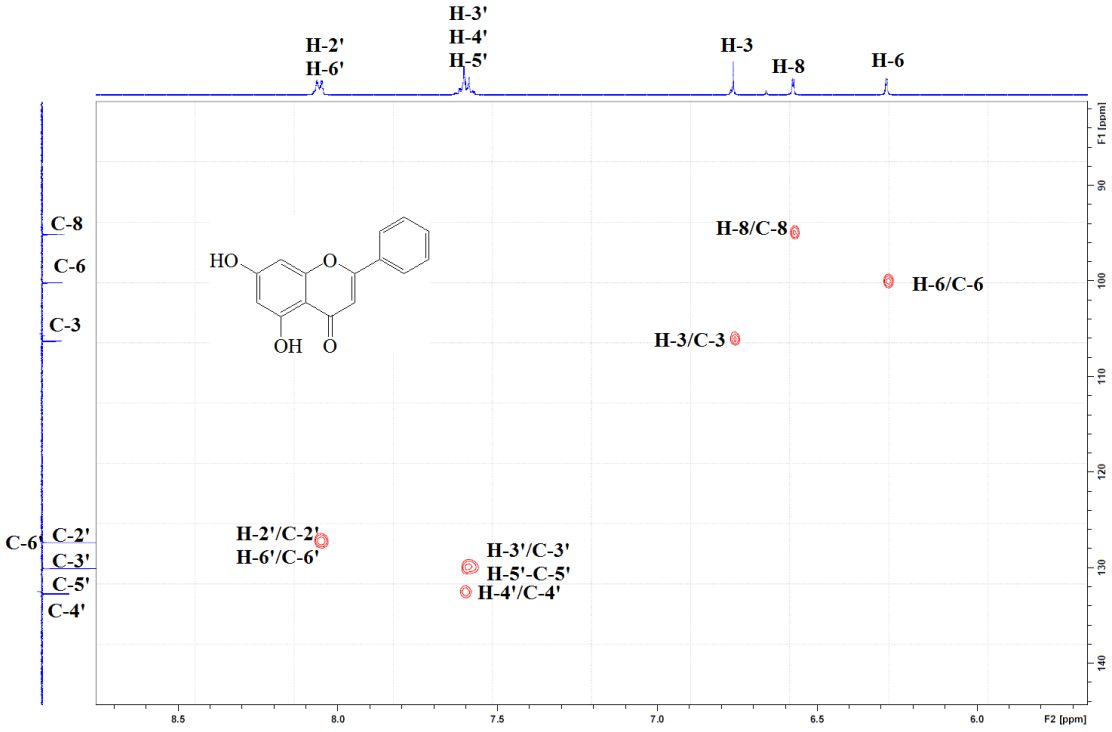

Figure S26. HSQC spectrum of compound 3 (500 MHz, CD_3COCD_3)

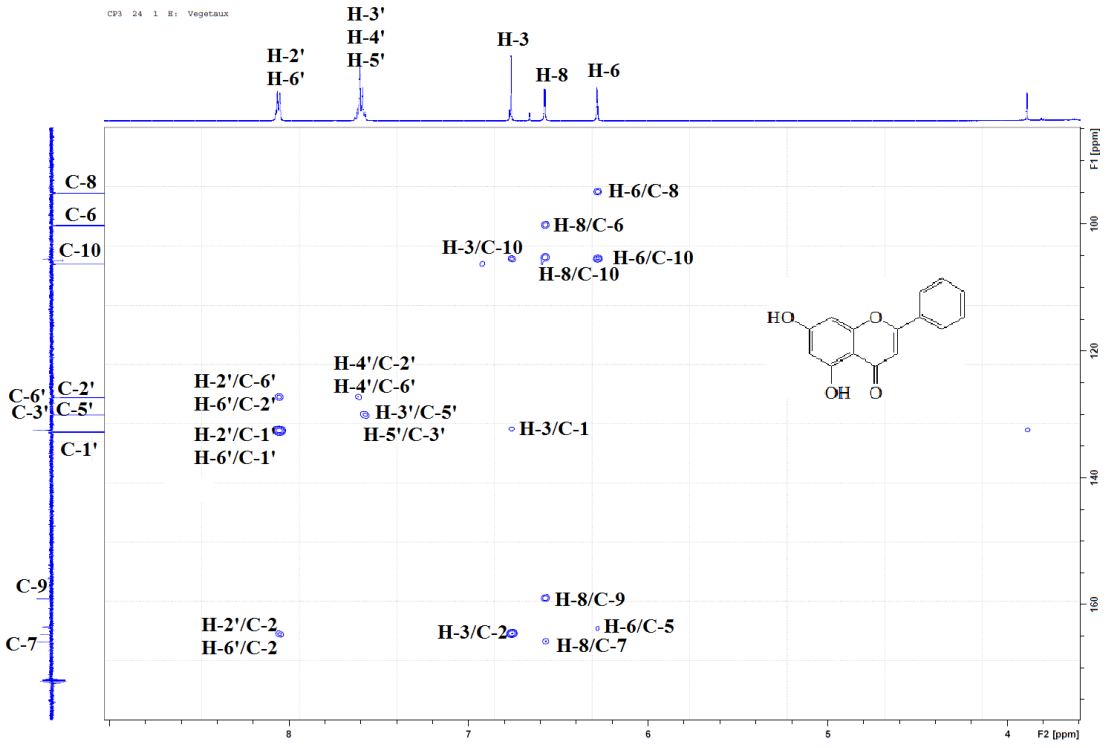

Figure S27. HMBC spectrum of compound 3 (500 MHz, CD_3COCD_3)

Compound 4: Apigenin (Chaturvedula & Prakash 2013)

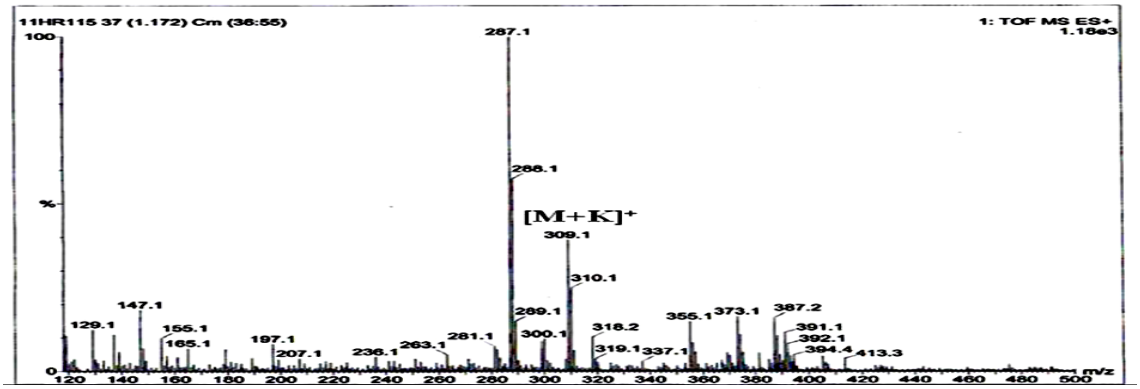

Figure S28. Mass spectrum ESI-MS of compound 4

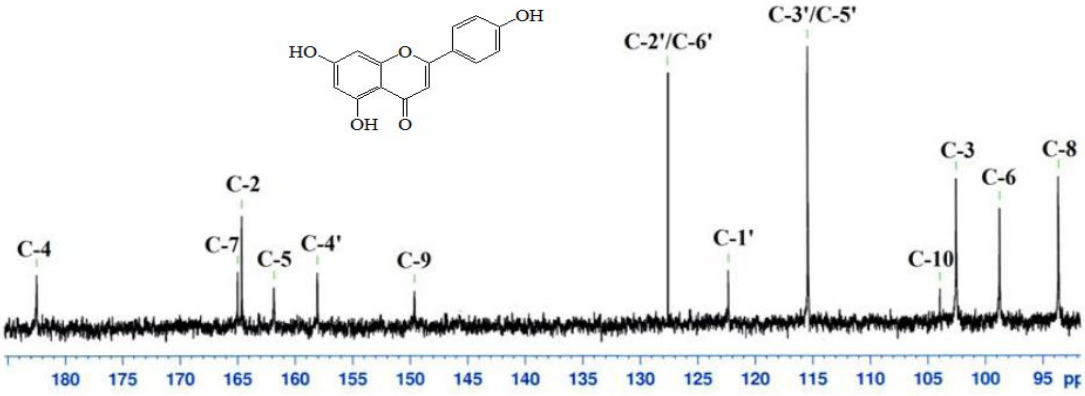

Figure S29. ^{13}C-NMR spectrum of compound 4 (125 MHz, CD_3OD)

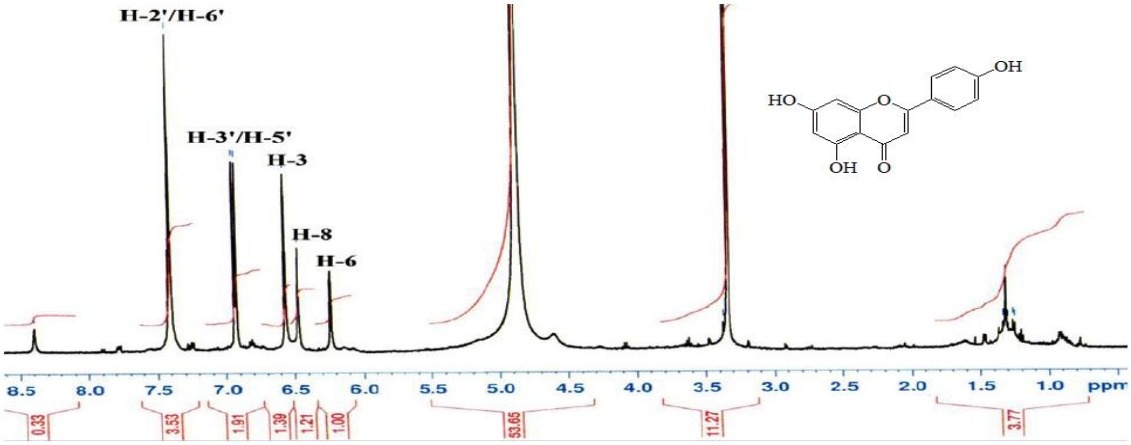

Figure S30. ^{1}H-NMR spectrum of compound 4 (500 MHz, CD_3OD)

Compound 5: Quercetin (Choi et al. 2006)

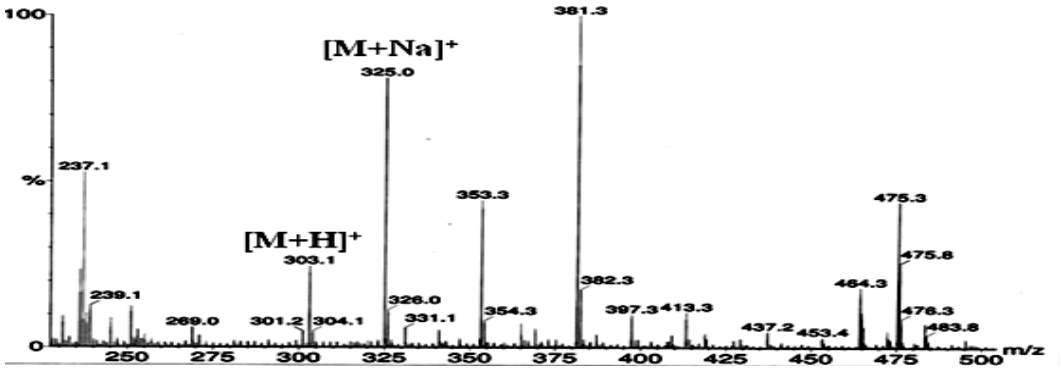

Figure S31. Mass spectrum ESI-MS of compound 5

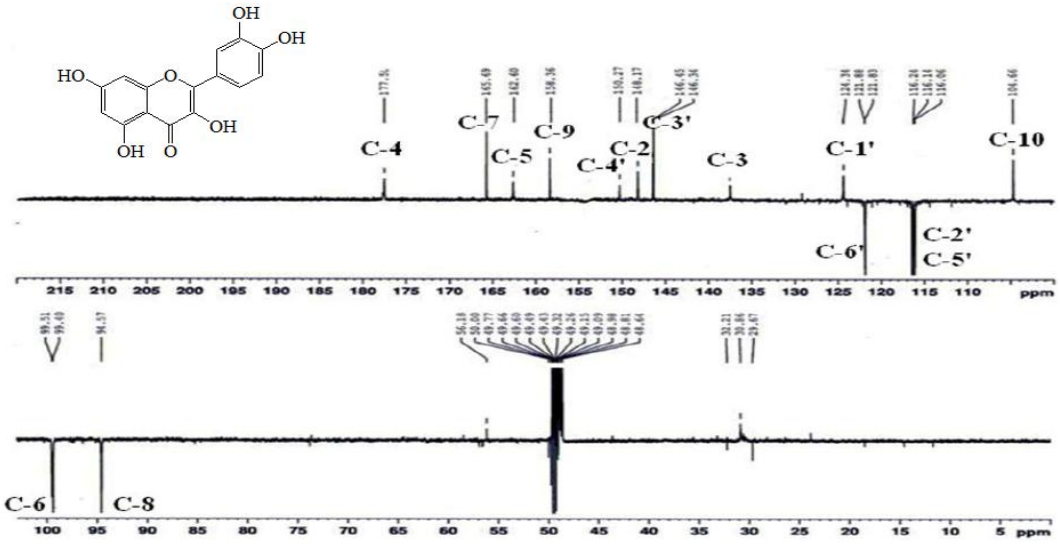

Figure S32. ^{13}C-NMR spectrum of compound 5 (125 MHz, CD_3OD)

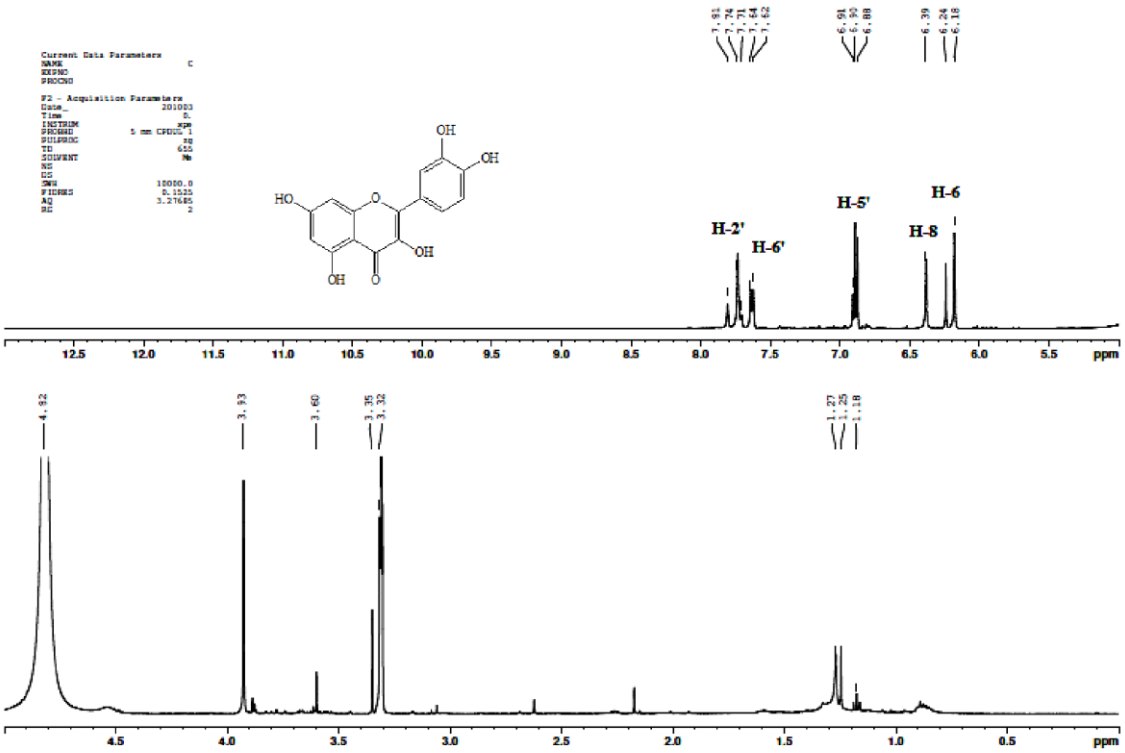

Figure S33. ^{1}H-NMR spectrum of compound 5 (500 MHz, CD_3OD)

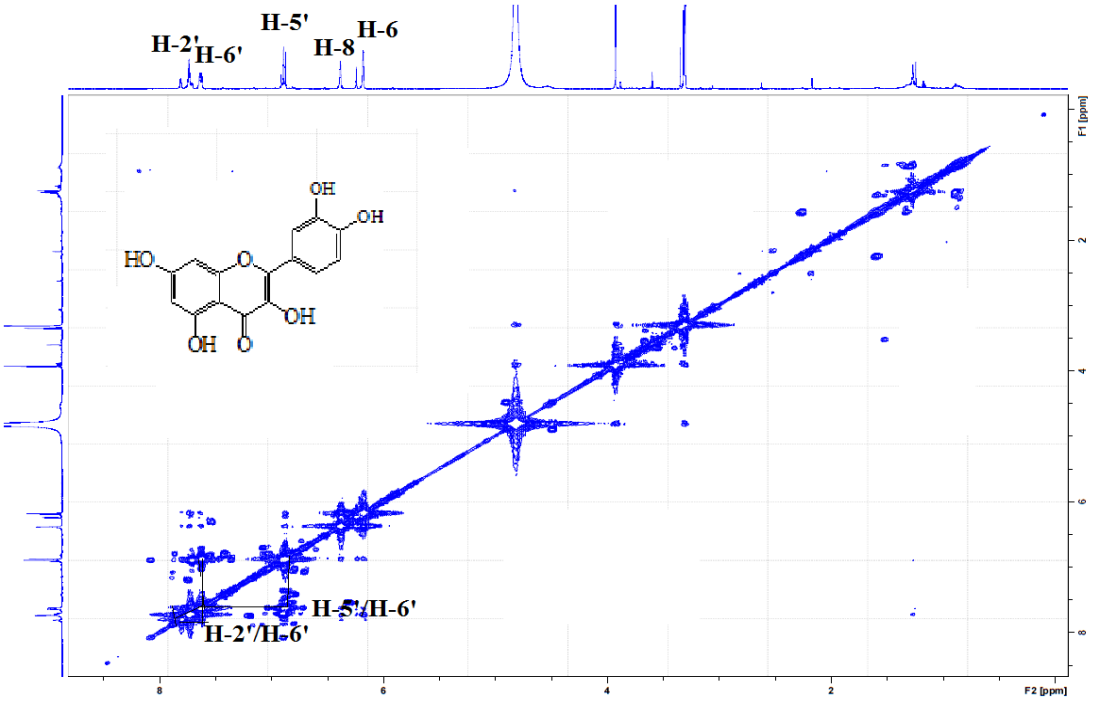

Figure S34. COSY spectrum of compound 5 (500 MHz, CD_3OD)

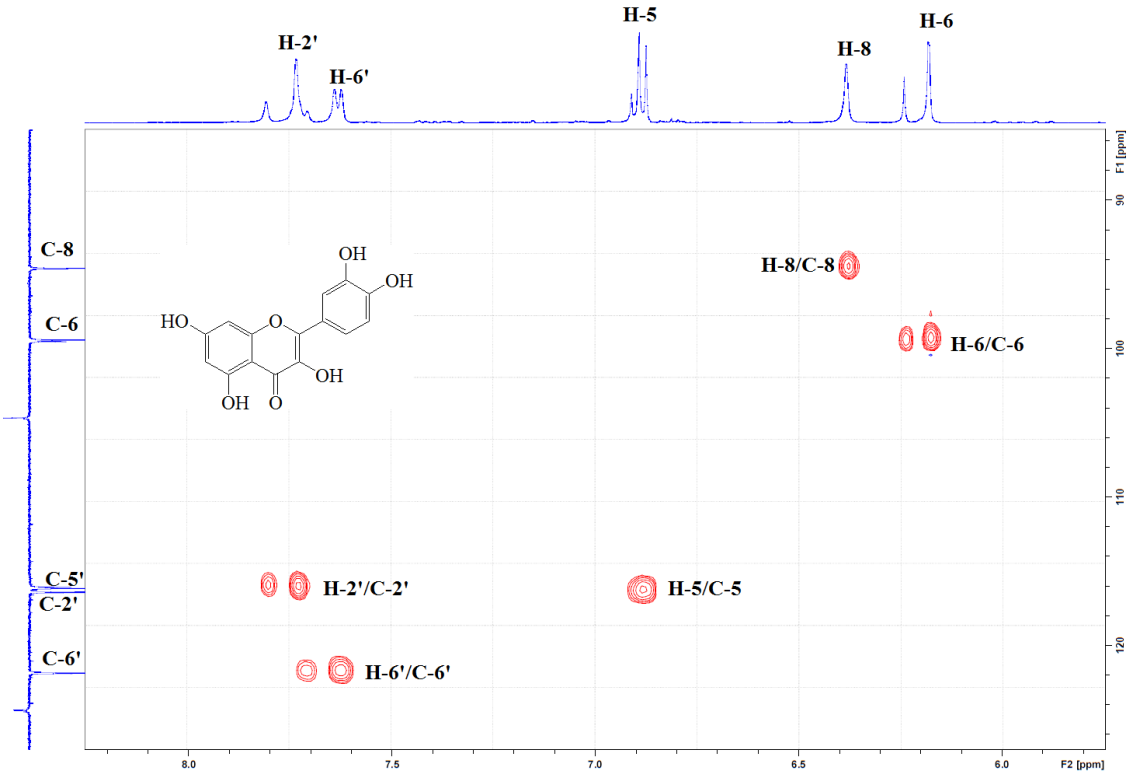

Figure S35. HSQC spectrum of compound 5 (500 MHz, CD_3OD)

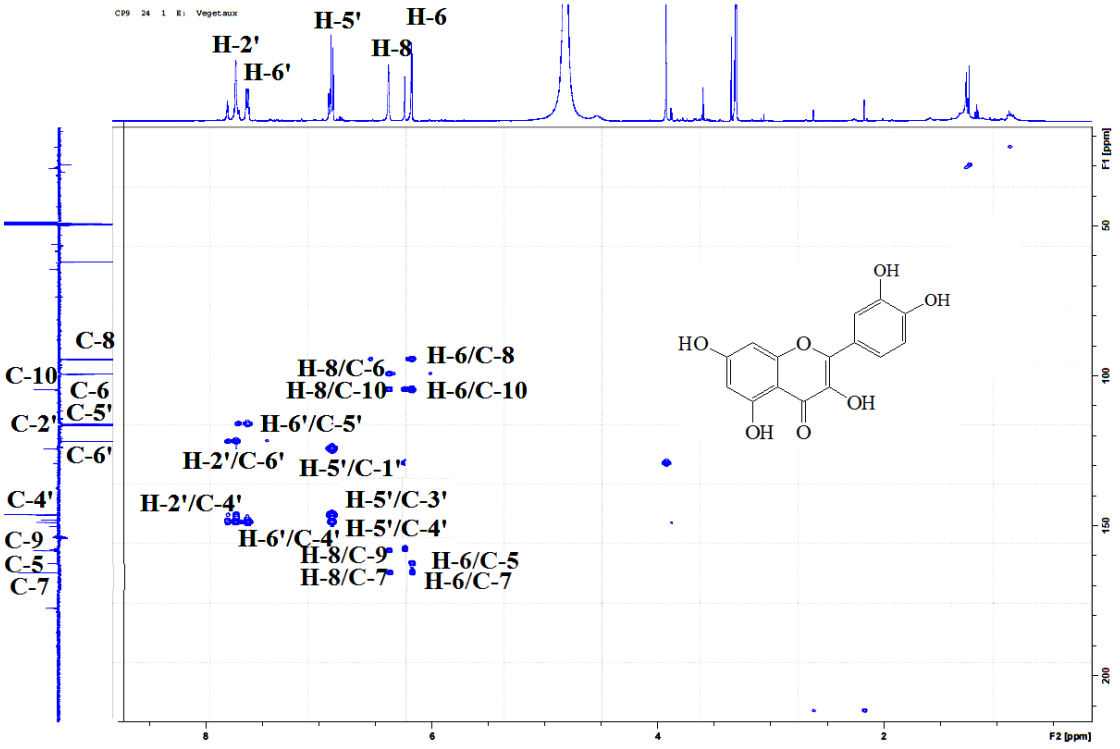

Figure S36. HMBC spectrum of compound 5 (500 MHz, CD_3OD)

Compound 6: stigmasterol glucoside (El-Askary 2005)

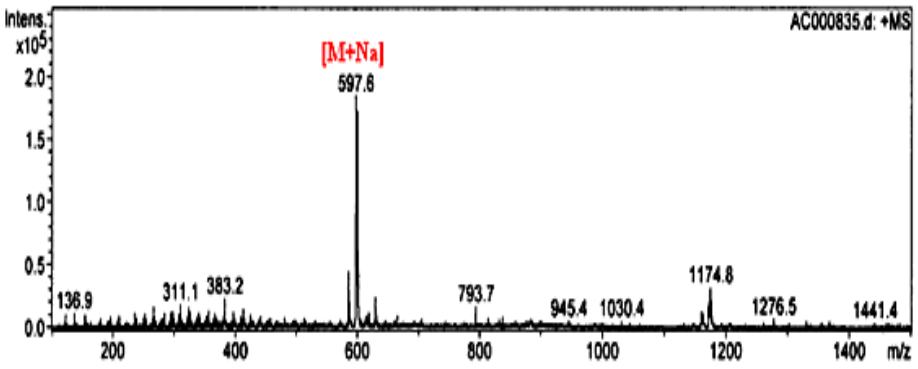

Figure S37. Mass spectrum ESI-MS of compound 6

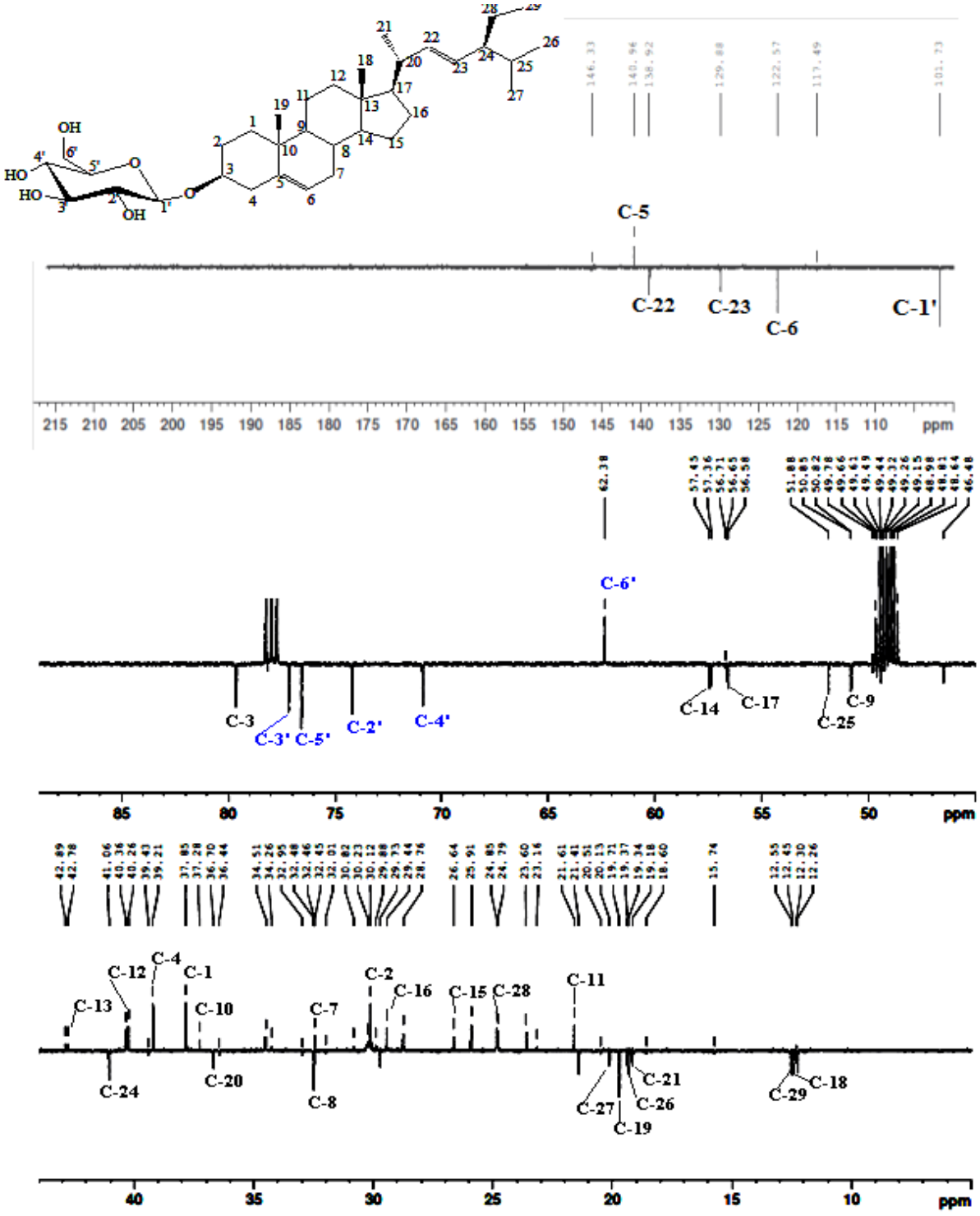

Figure S38. ^{13}C-NMR spectrum of compound 6 (125 MHz, $CDCl_3$ + CD_3OD)

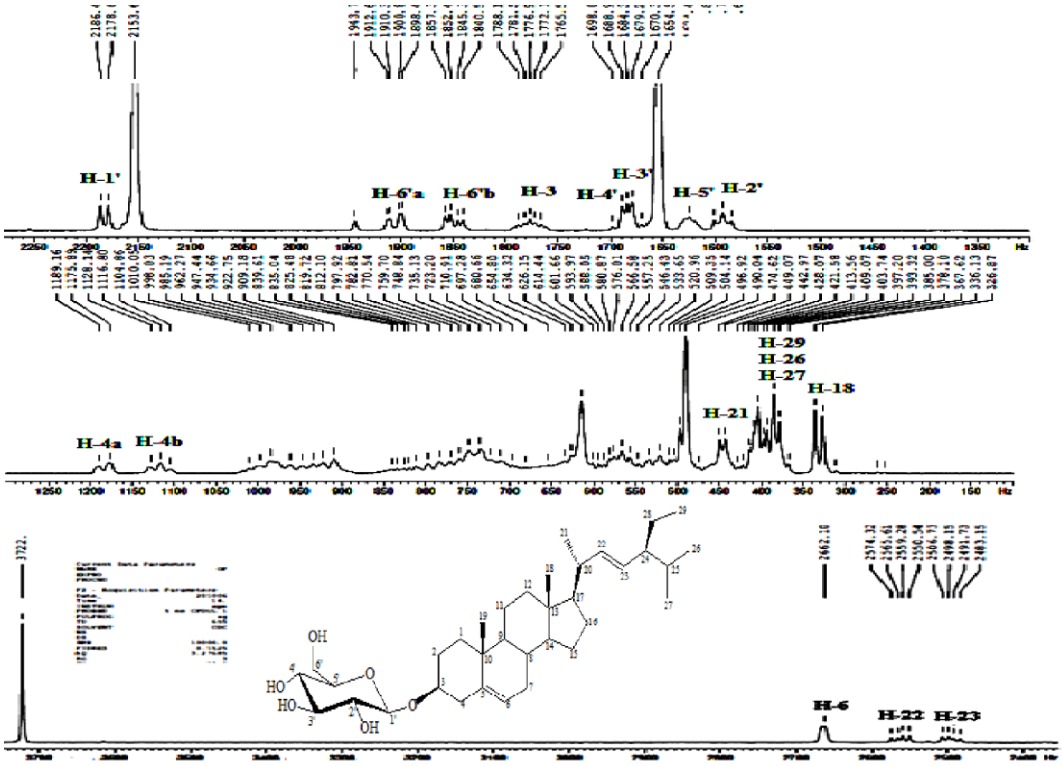

Figure S39. ^{1}H-NMR spectrum of compound 6 (500 MHz, $CDCl_3$ + CD_3OD)

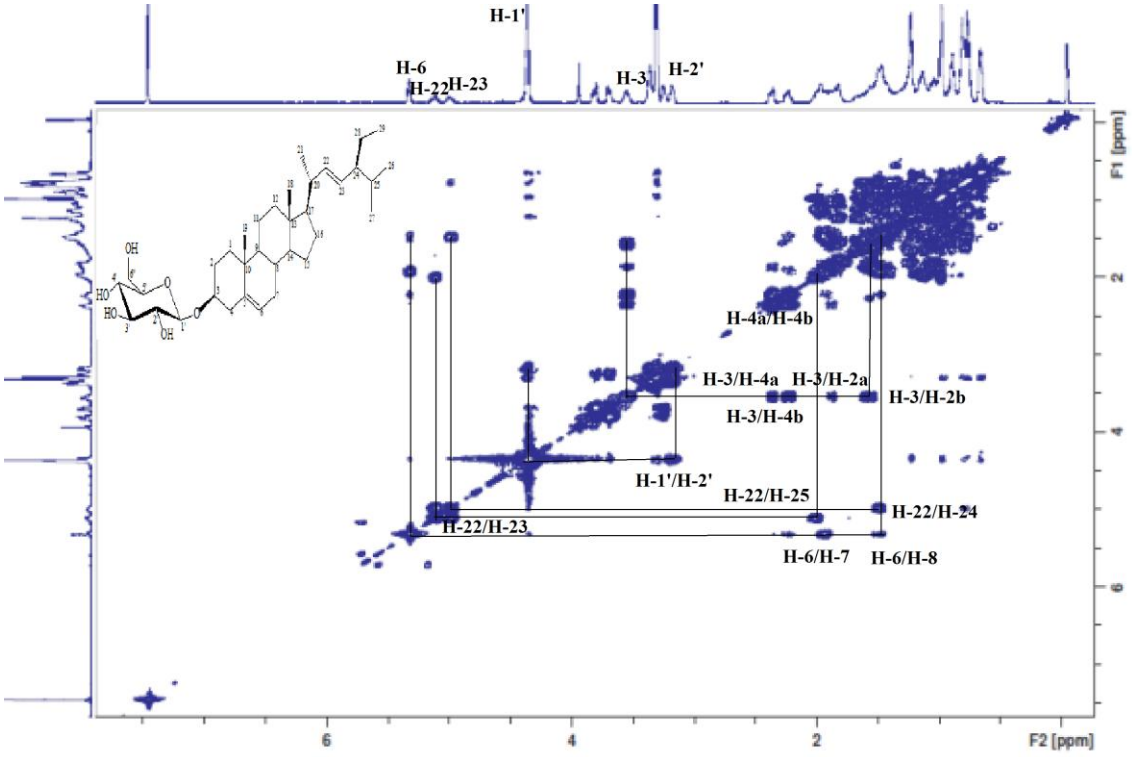

Figure S40. COSY spectrum of compound 6 (500 MHz, $CDCl_3$ + CD_3OD)

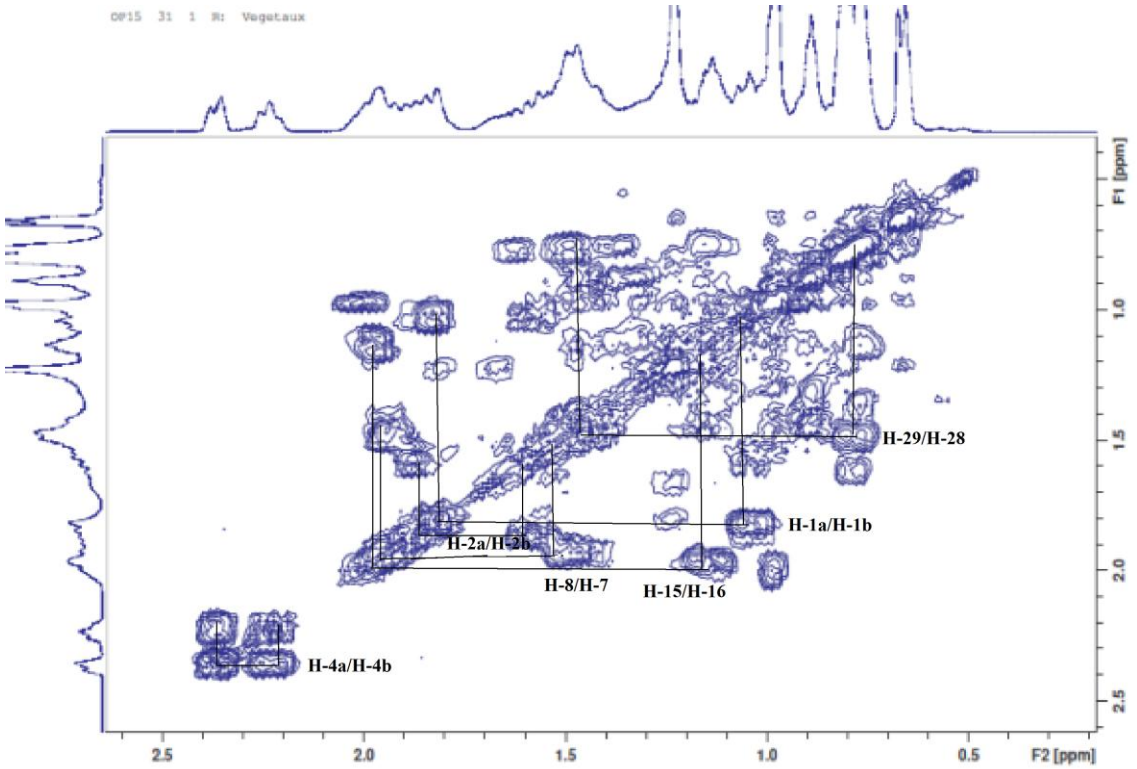

Figure S41. COSY spectrum of compound 6 (500 MHz, $CDCl_3$ + CD_3OD)

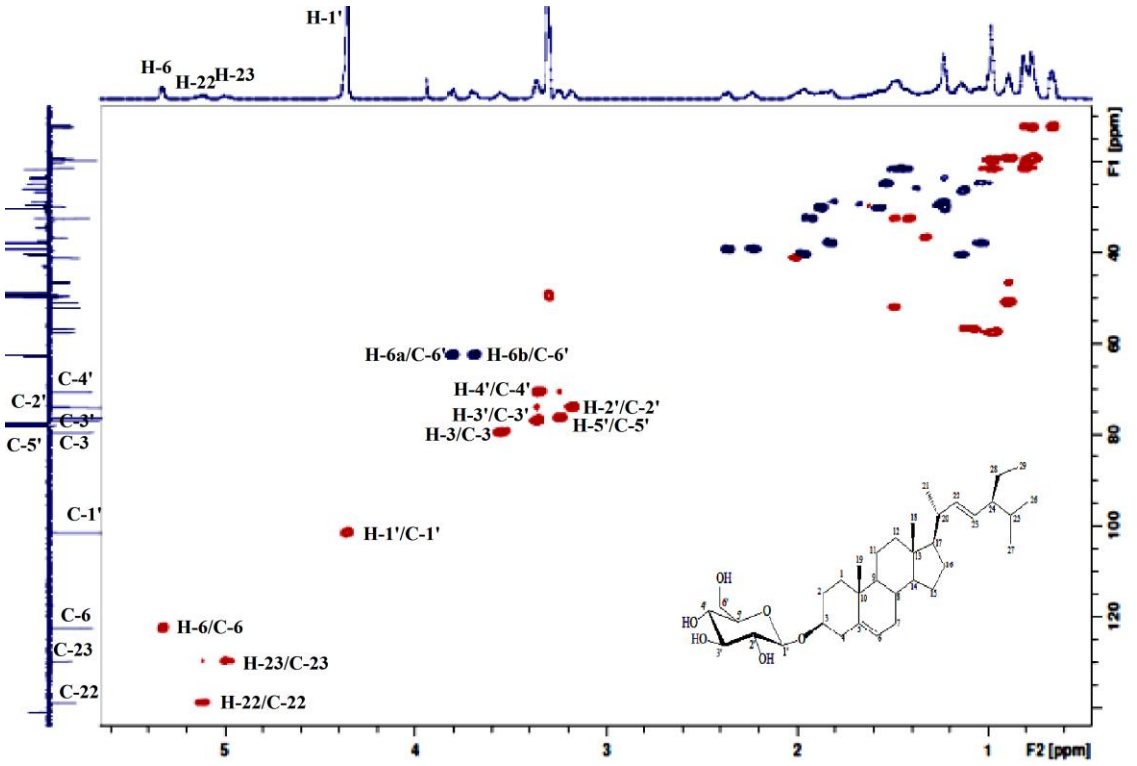

Figure S42. HSQC spectrum of compound 6 (500 MHz, $CDCl_3$ + CD_3OD)

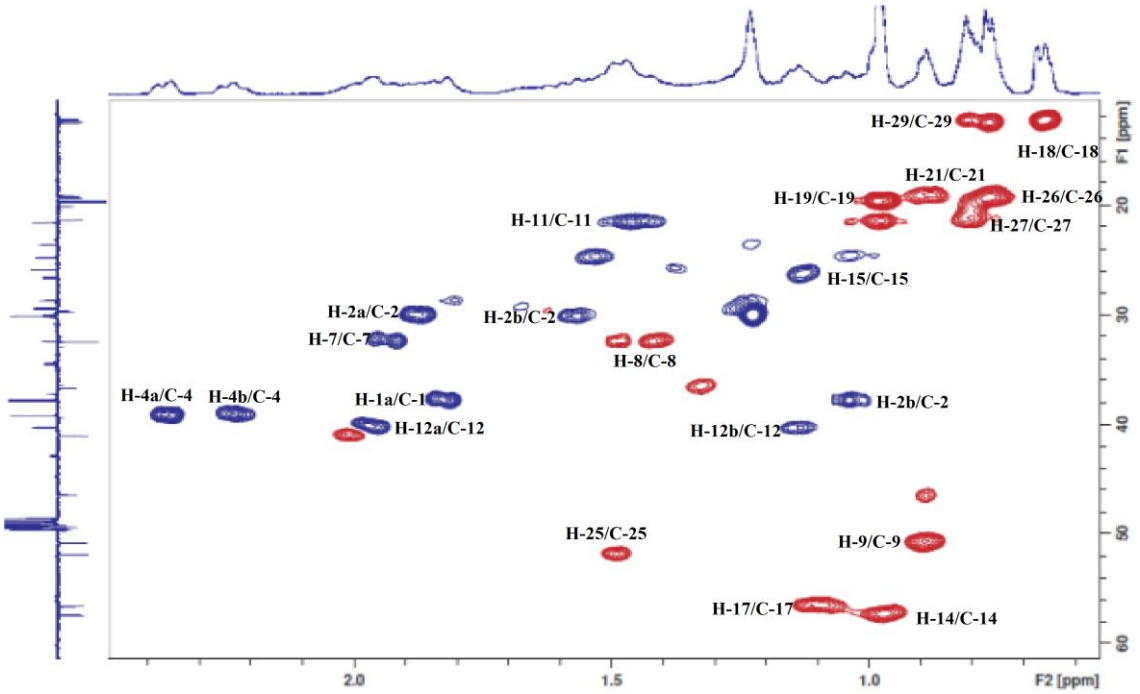

Figure S43. HSQC spectrum of compound 6 (500 MHz, $CDCl_3$ + CD_3OD)

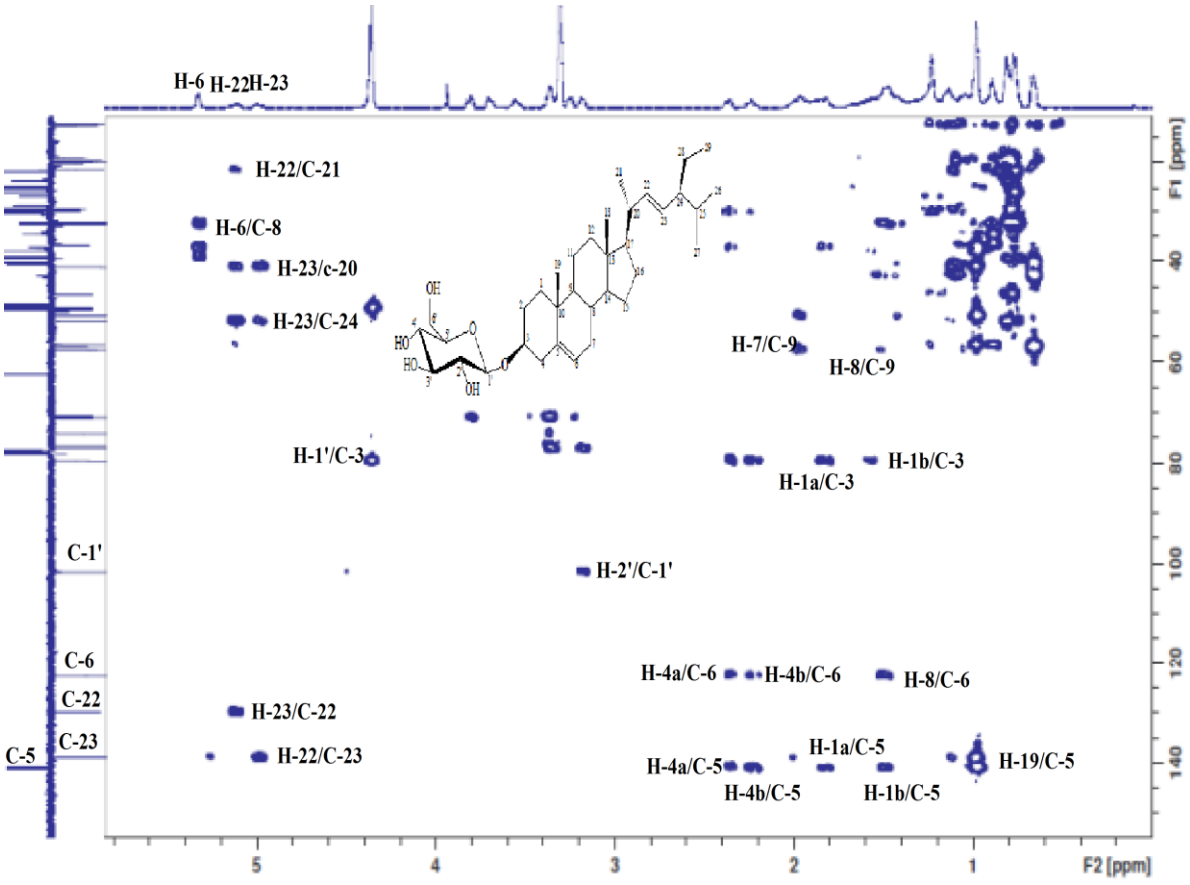

Figure S44. HMBC spectrum of compound 6 (500 MHz, $CDCl_3$ + CD_3OD)

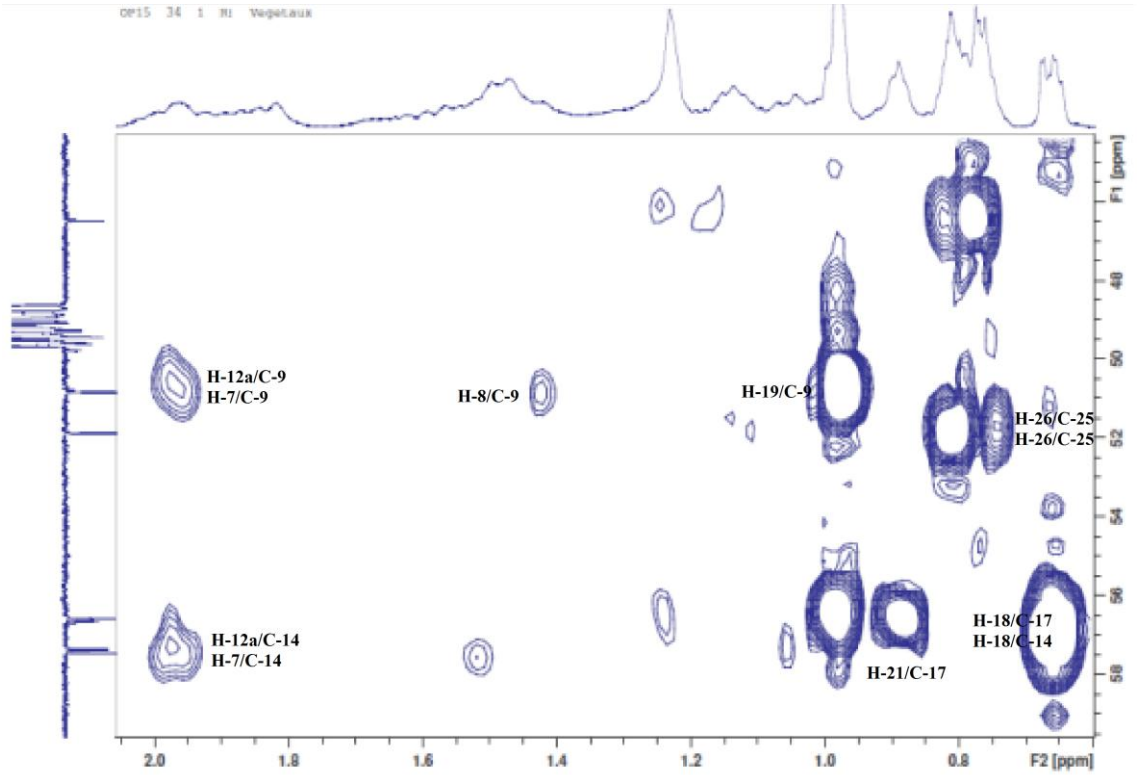

Figure S45. HMBC spectrum of compound 6 (500 MHz, $CDCl_3$ + CD_3OD)