SUPPORTING INFORMATION

Crude to Leads: A triple-pronged direct NMR approach in coordination with docking simulation

Sheraz A.K. Tanoli[a], Nazish U. Tanoli[a], Tatiani M. Bondancia[b], Saman Usmani[d] Rainer Kerssebaum[c], Antonio G. Ferreira[a*], Joao B. Fernandes[b], Zaheer-Ul-Haq[d]

[a]: Laboratory of nuclear Magnetic Resonance Federal University of Sao Carlos, Rodovia Washington Luiz, Km 235, Brazil

[b]: Department of Chemistry, Federal University of Sao Carlos, Rodovia Washington Luiz, Km 235, Brazil.

[c]: Bruker Bio Spin GmbH Silberstreifen, 76287 Rheinstetten, Germany

[d]:Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemicals and Biological Sciences, University of Karachi, Karachi-75210, Pakistan

Correspondence to:

Fax: +55 16 3351-8350; Tel: +55 (16) 3351-8068

Prof.drgiba@gmail.com

Table of contents

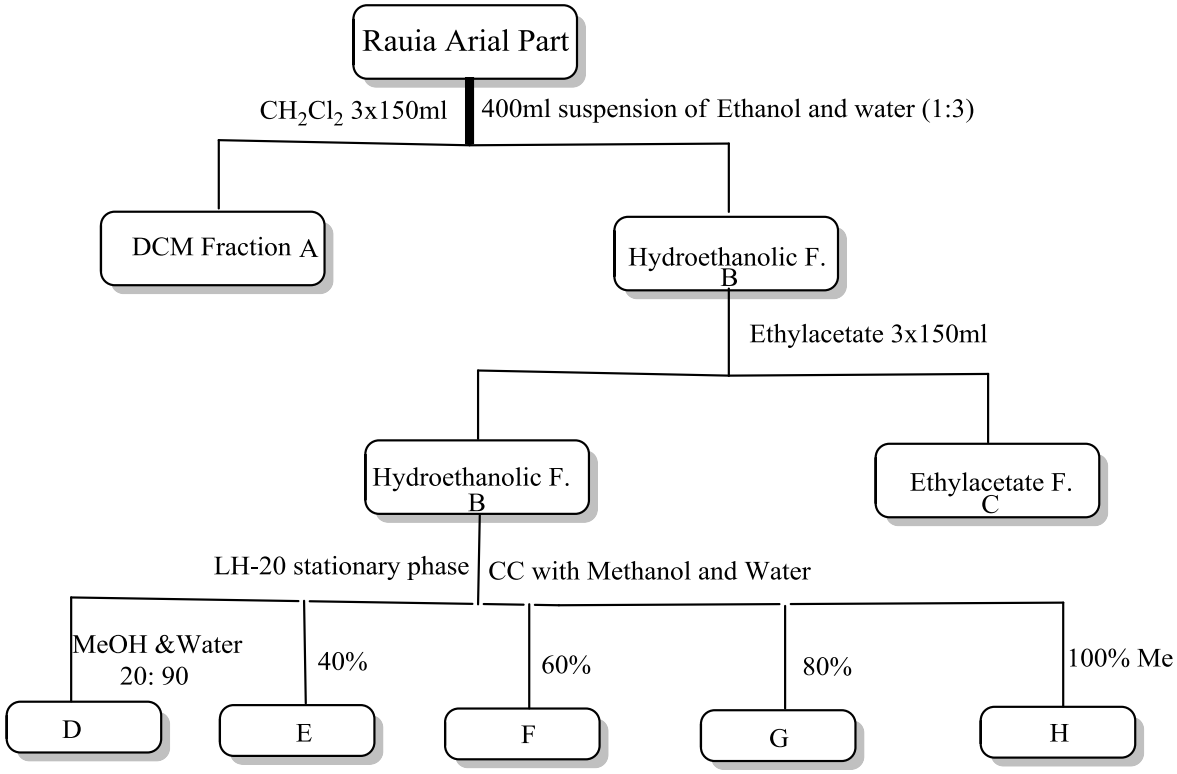

Figure S-1. Isolation Scheme for arial parts of Rauia plant

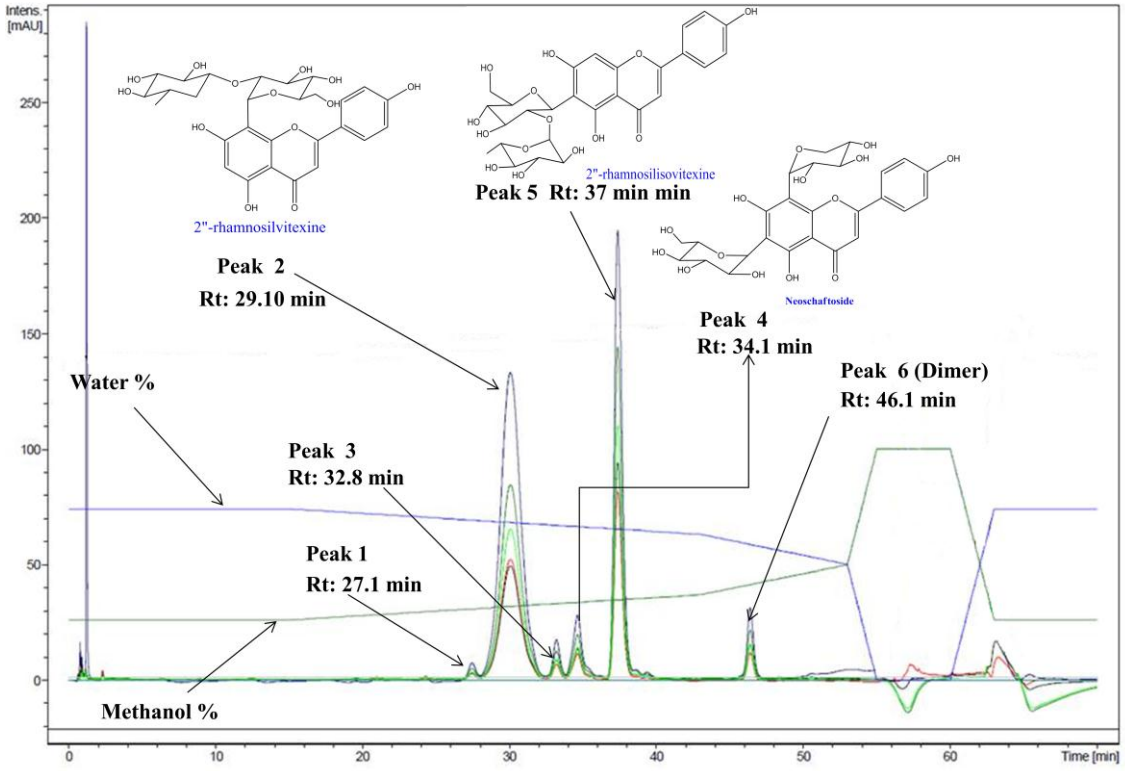

Figure S-2. Chromatogram obtained as result of separation with LC-Method by using water and methanol as solvent at different gradients.

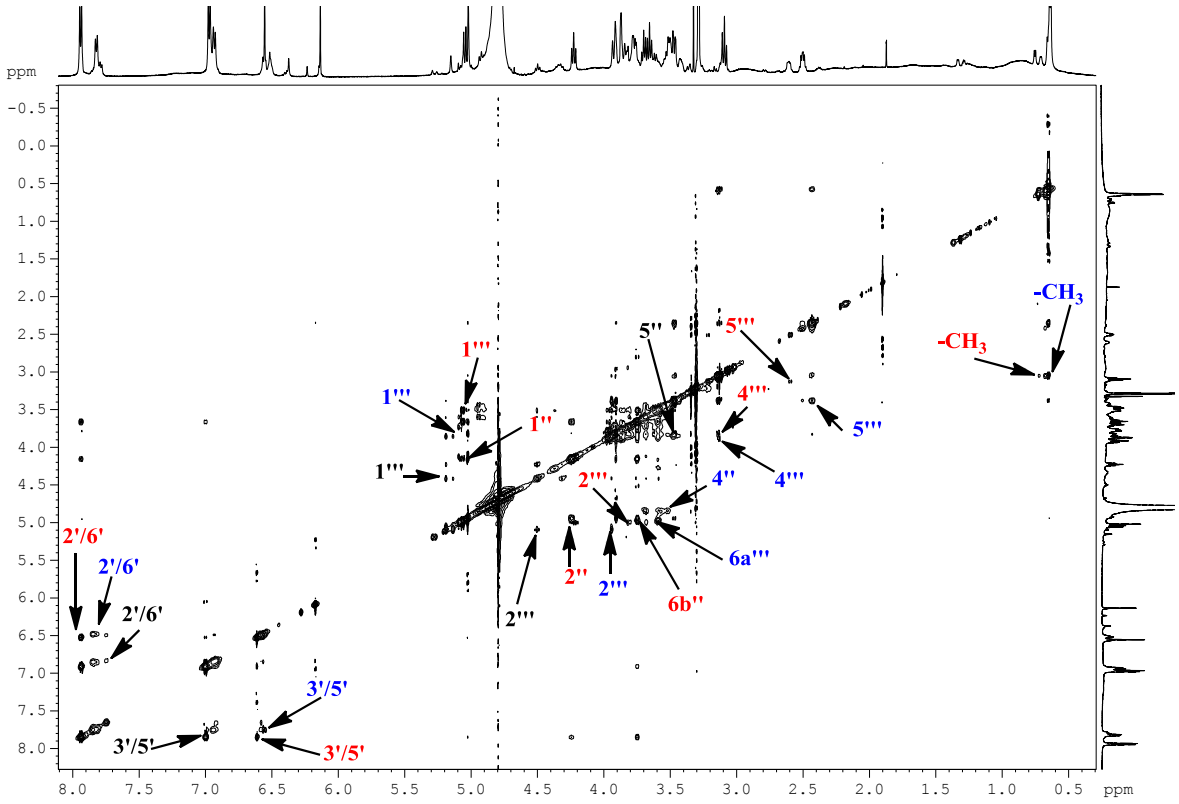

Figure S-3. Two-dimensional transfer NOESY with Bruker standard phase sensitive pulse sequence for the extract in D_2O and methanol (95:5% v/v) and BSA in buffer solution . In transfer NOESY pulse sequence is modified my putting the 3500Hz spin lock for the removal of unwanted protein signal before the first $\pi/2$ pulse. This spectrum is produced by the use of 400ms mixing time with at 600 MHz Bruker NMR spectrometer with a cryoprobe TCI at 298 K. NOE cross-peaks shown are of the same sign as of the diagonal signals (Negative NOEs).

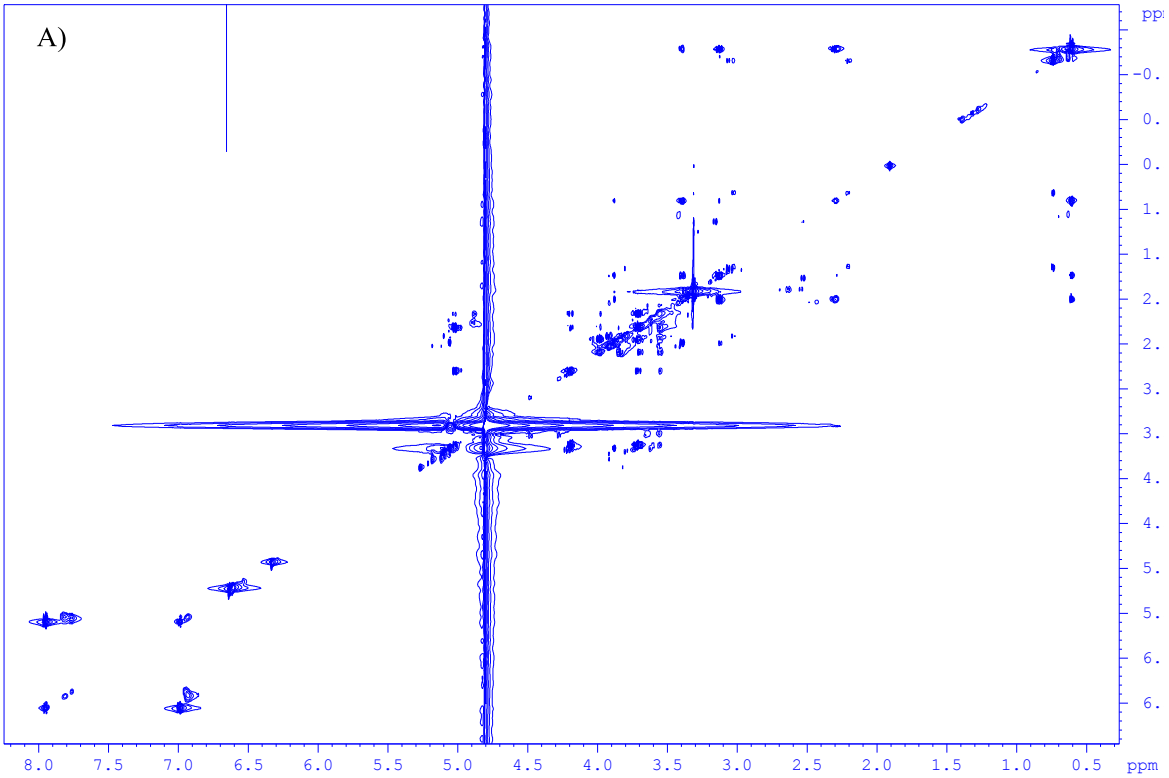

Figure S-4a. A standard 2D TOCSY NMR spectrum of crude extracts with 50ul BSA is shown. Experiment was done by using a Malcolm Levitt's composite-pulse decoupling sequence (MLEV-17) with mixing time of 60 ms at 298K with 600MHz Spectrometer. For standard TOCSY MLEV-17 sequence the experimented was recorded with 7211.539Hz spectral width in both dimensions (F_1 and F_2) with a total number of 56 scans,with 256 increments in t_1 were collected with 2s relaxation delay for each scan; with the experimental time less than 10hrs..Other conditions are according to the experimental section. TOCSY spectrum was acquired in 1mg of the extract in D_2O: methanol (95:5% v/v) and BSA in buffer (7.4pH) solution respectively.

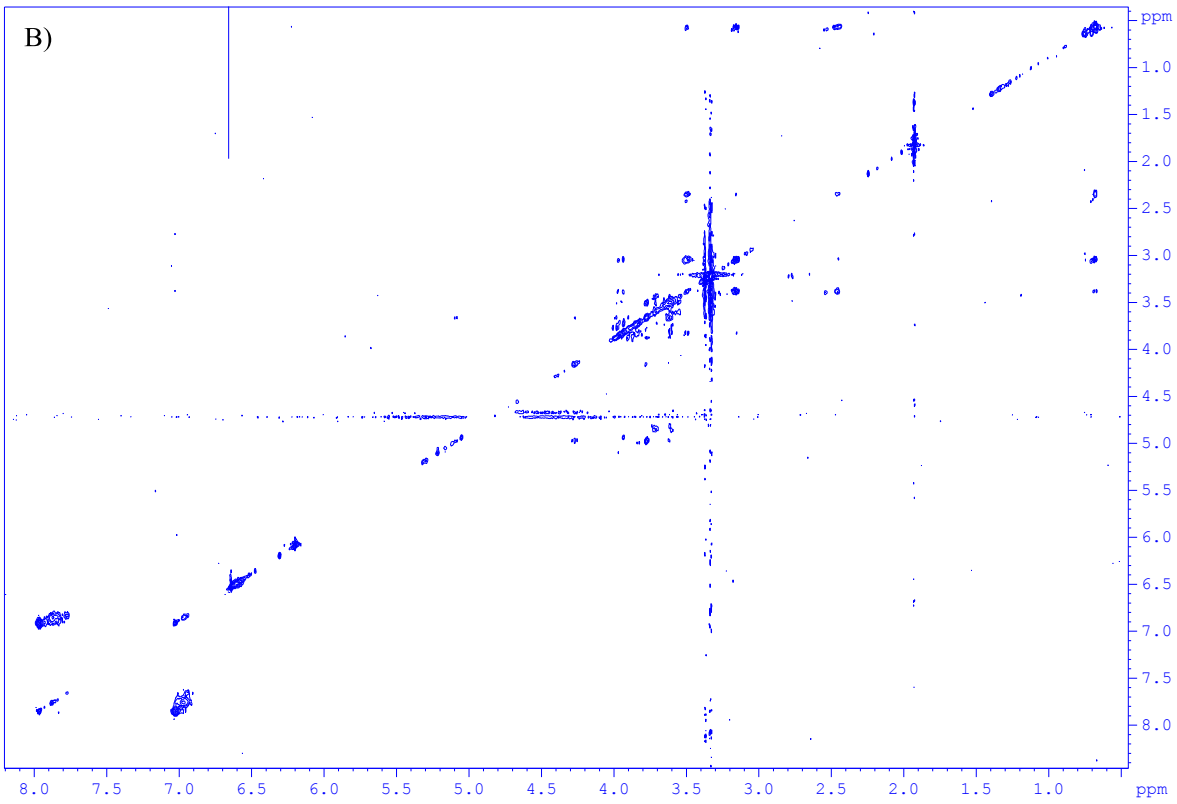

Figure S-4b. Two-dimensional STD-TOCSY NMR spectra of crude extracts with 50uM BSA by using a Malcolm Levitt's composite-pulse decoupling sequence (MLEV-17) with mixing time of 60 ms at 298K with 600MHz Spectrometer. In STD-TOCSY saturation of the protein was achieved with a cascade of 50Gaussian pulse (50 ms duration each) with a delay of 200 ms in between each pulse, with a total saturation time of 2.5 s. The on-resonance saturation frequency was set to -0.5 ppm and off-resonance to 30 ppm. 2D STD-TOCSY Spectrum was recorded with 7211.539Hz spectral width in both dimensions (F_1 and F_2) with 64 transients in t_2 and with 384 increments for the *on* and *off* resonance in t_1 with the total experimental time of more than 16hrs. Both spectra (2D standard and STD TOCSY) were acquired in 1mg of the extract in D_2O: methanol (95:5% v/v) and BSA in buffer(7.4pH) solution respectively.

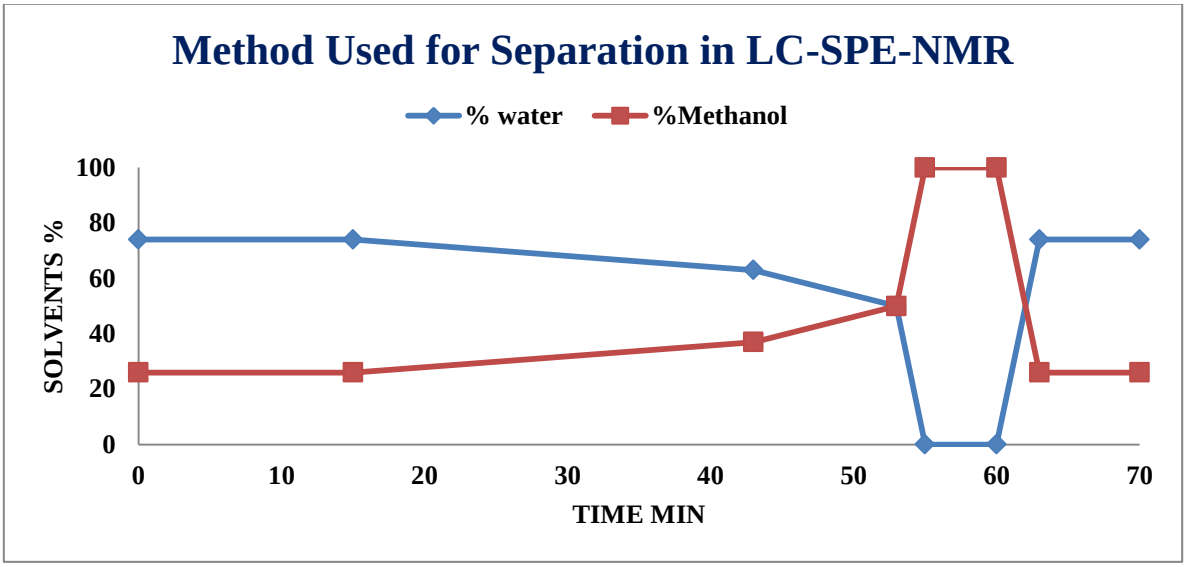

Figure S-5. Separation method and the solvent gradient used in LC-Method

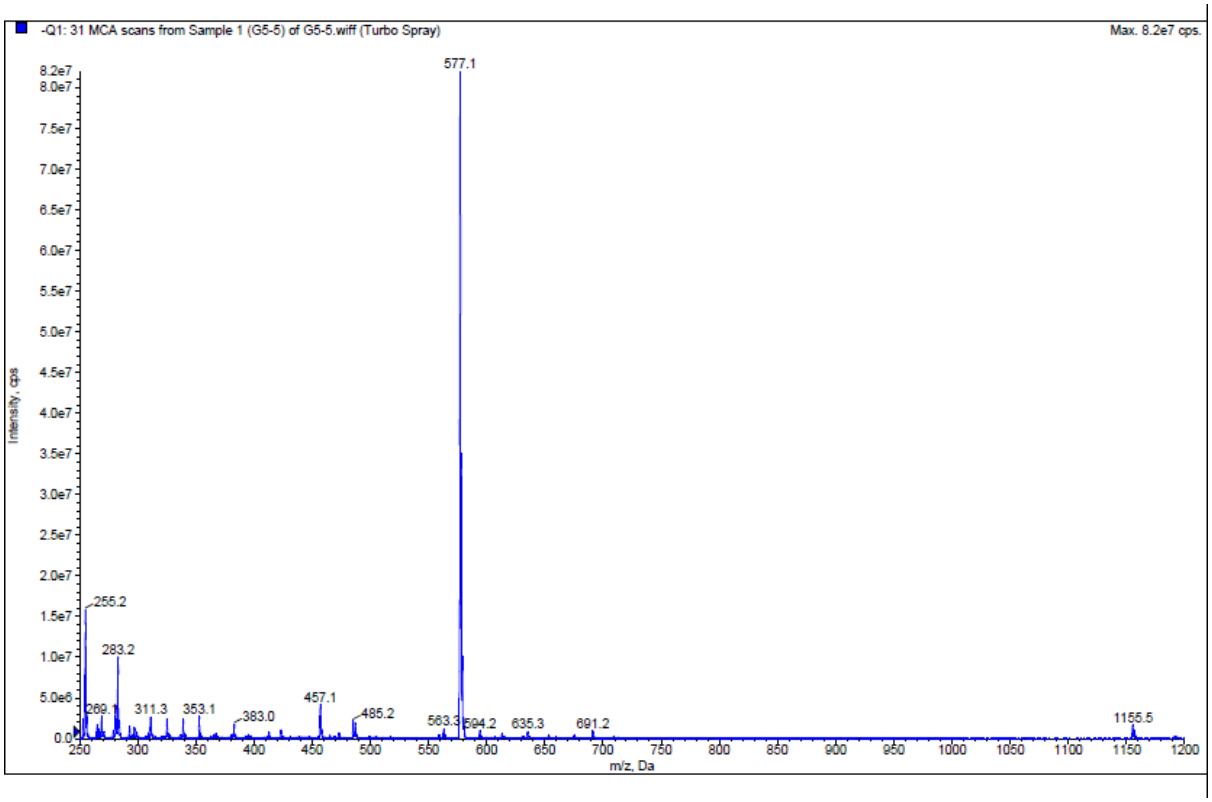

Figure S-6. ESI spectrum of peak 6 of LC-chromatogram, showing the molecular weight 1155.5 a.m.u.

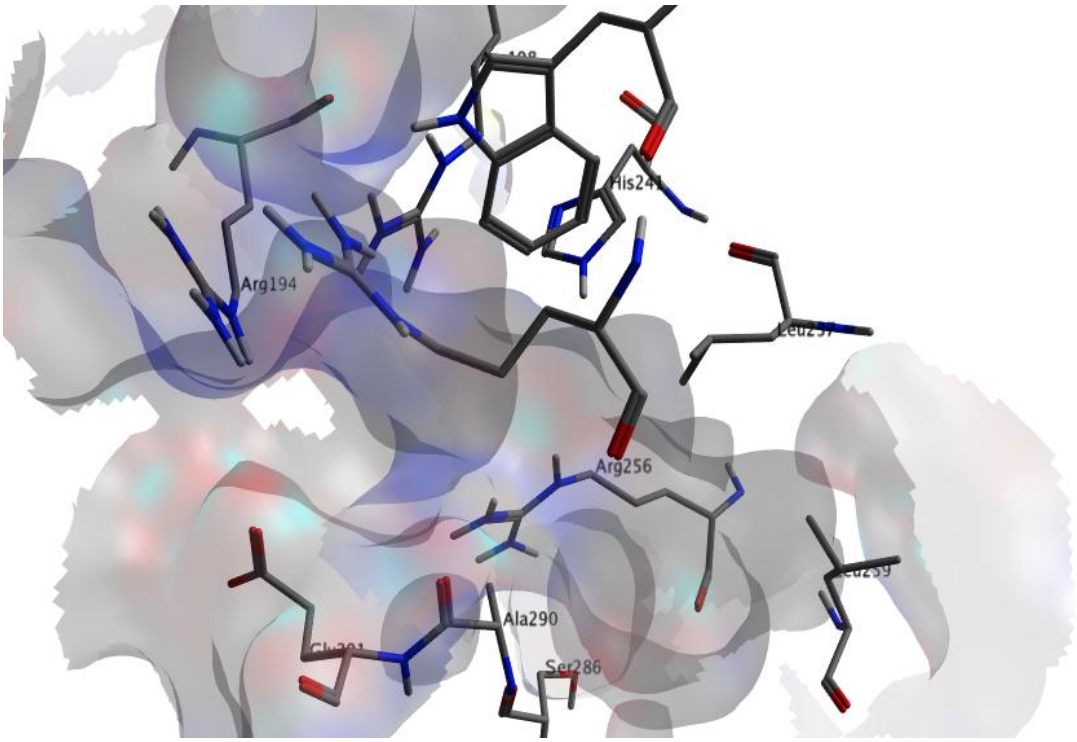

Figure S-7: Selected Active site of chain B of the Bovine Serum Albumin (BSA), generated by using Molecular Operating Environment (MOE) 2011.10. BSA PDB file was taken from protein data bank (http://www.rcsb.org/pdb/explore.do?structureId=4F5S) (4F5S). The classical binding sites were marked in corresponding subdomain cations.

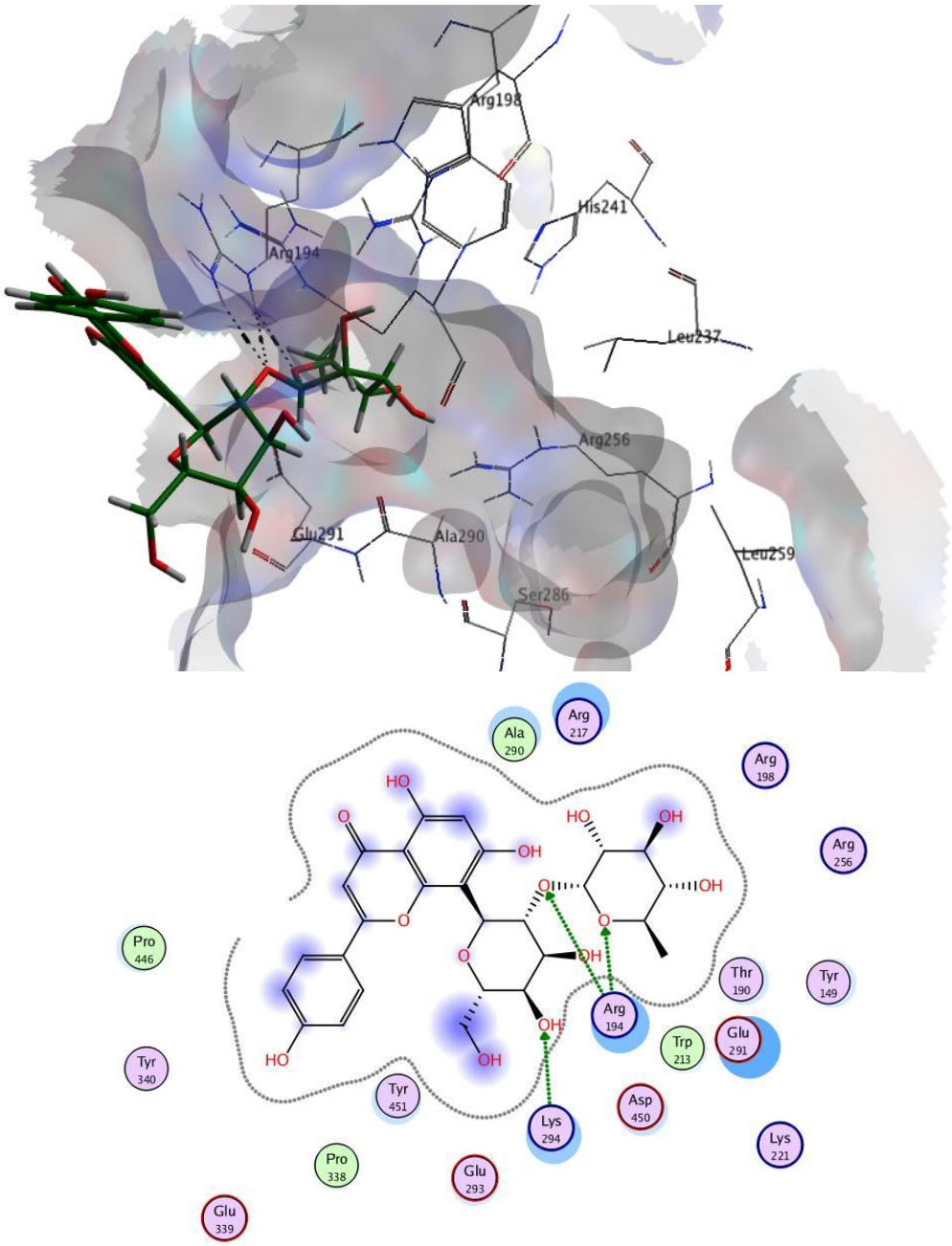

Figure S-8a-b: Molecular Docking model of interactions between the Bovine Serum Albumin (BSA) and Vetexine-2-rhamnosoide, **1,** generated by using Molecular Operating Environment (MOE) 2011.10. BSA PDB file was taken from protein data bank (http://www.rcsb.org/pdb/explore.do?structureId=4F5S) (4F5S). The classical binding sites were marked in corresponding subdomain cations. See more details in experimental section.

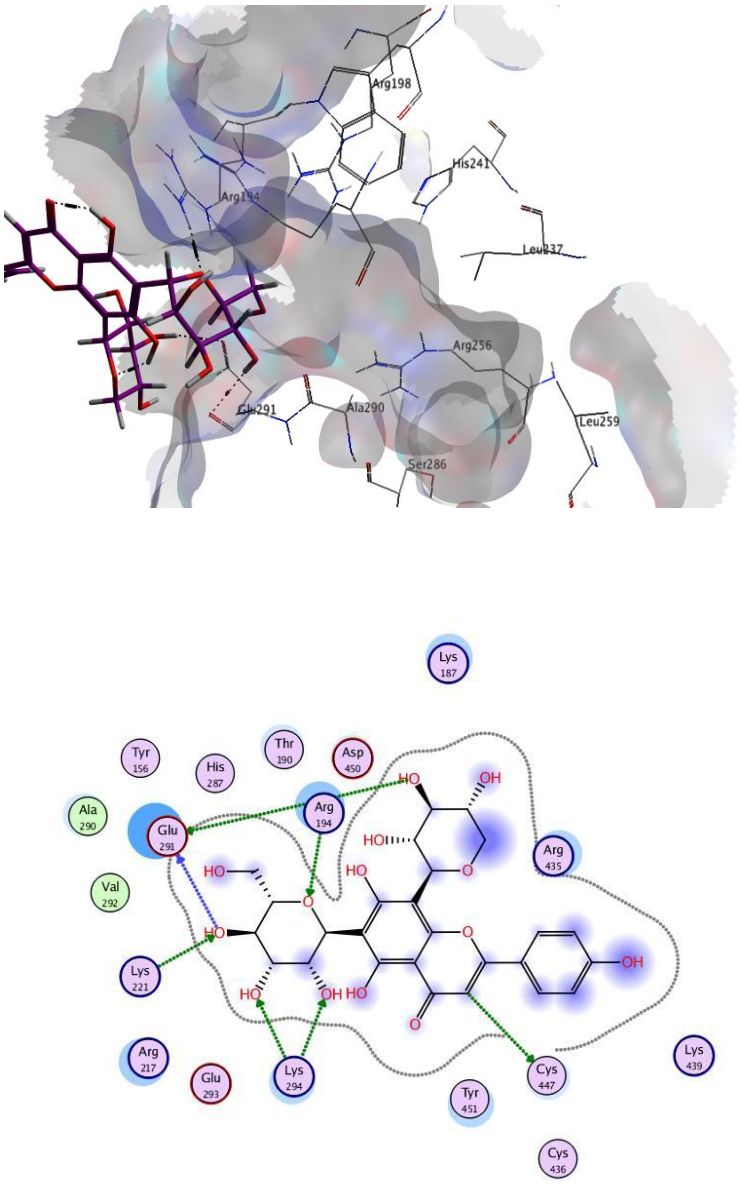

Figure S-9a-b: Molecular Docking model of interactions between the Bovine Serum Albumin (BSA) and Neoschiftosoide, **3,** generated by using Molecular Operating Environment (MOE) 2011.10. BSA PDB file was taken from protein data bank (http://www.rcsb.org/pdb/explore.do?structureId=4F5S) (4F5S). The classical binding sites were marked in corresponding subdomain cations. See more details in experimental section.