Supplementary Materials

Bio-guided Isolation of SARS-COV-2 Main Protease Inhibitors from Medicinal Plants: *In vitro* Assay and Molecular Dynamics

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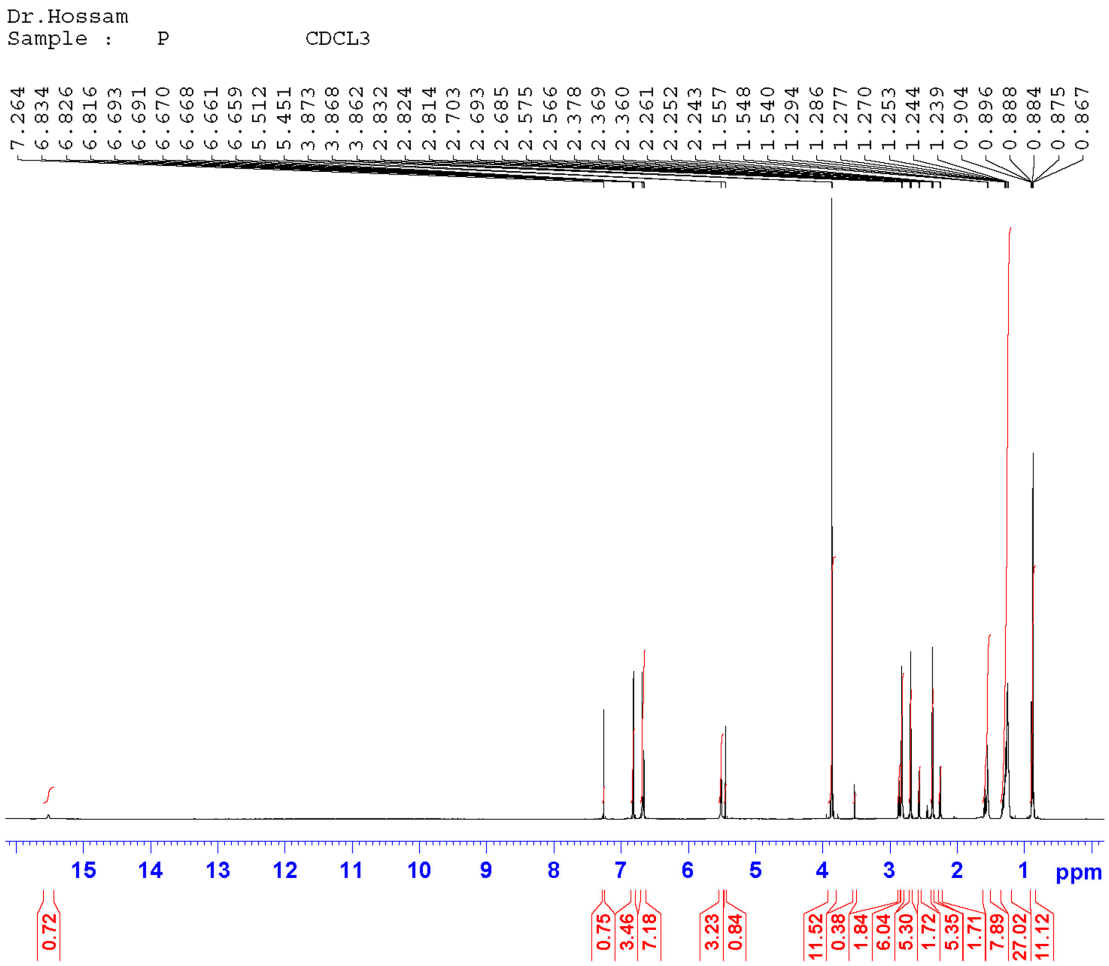

Figure S1. [1]HNMR spectra of 6-paradol.

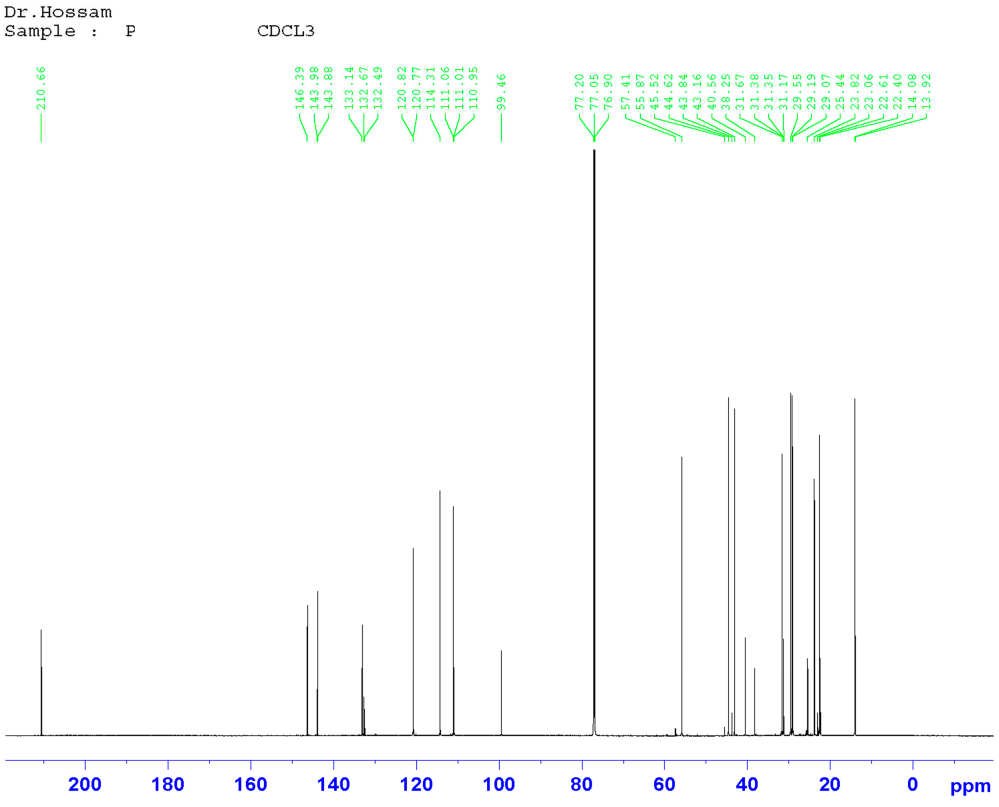

Figure S2. [13]CNMR spectra of 6-paradol.

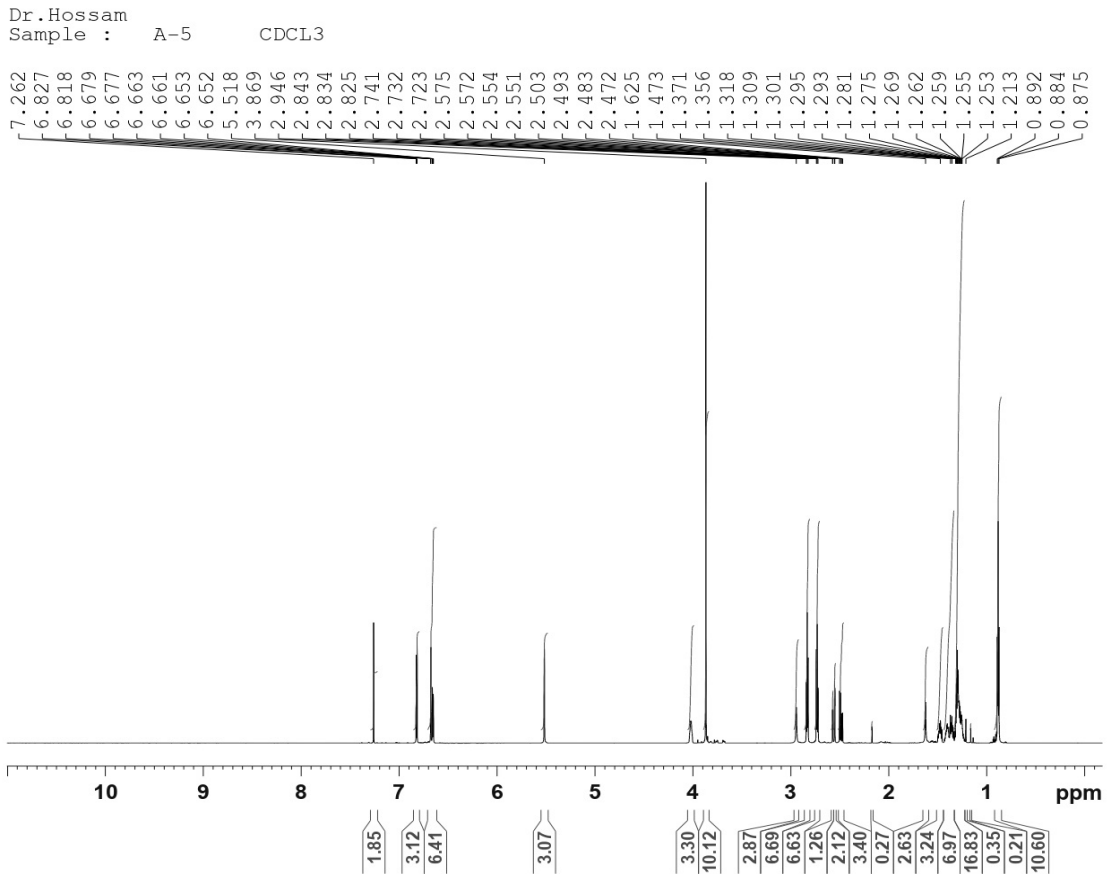

Figure S3. [1]HNMR spectra of 6-gingerol.

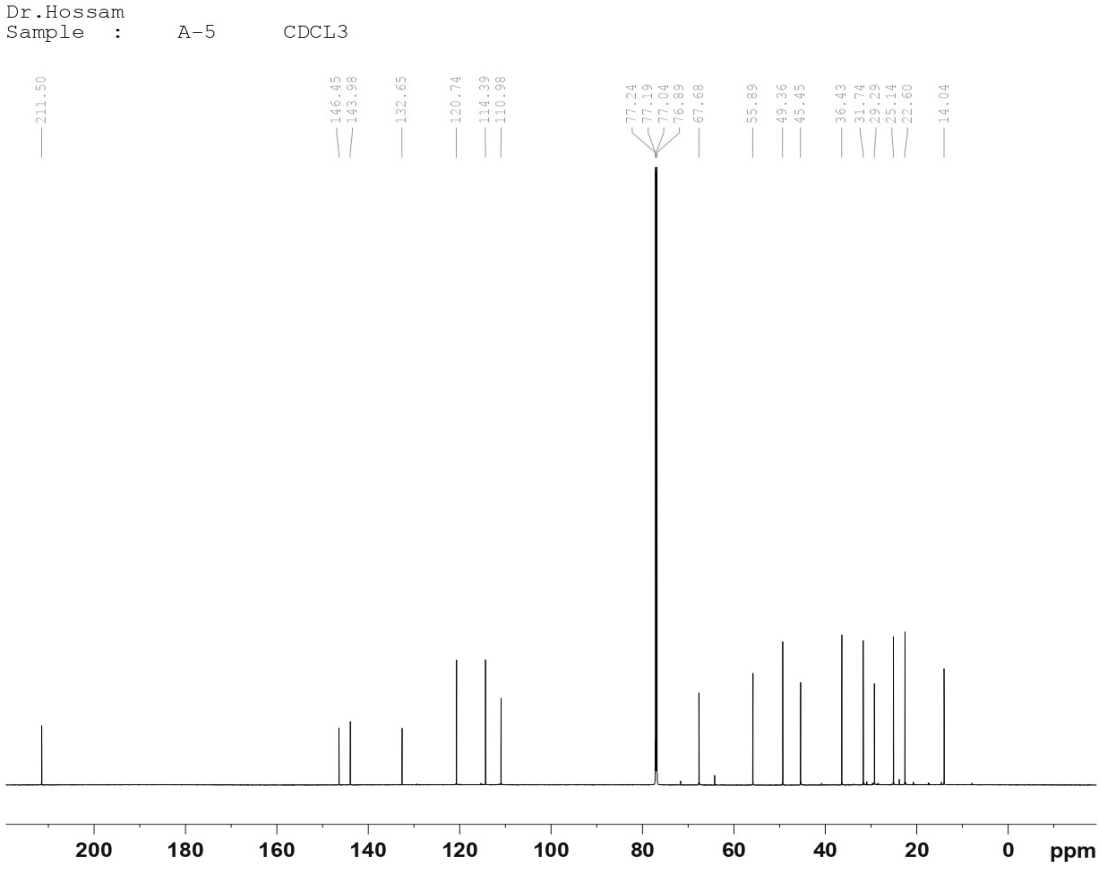

Figure S4. [13]CNMR spectra 6-gingerol.

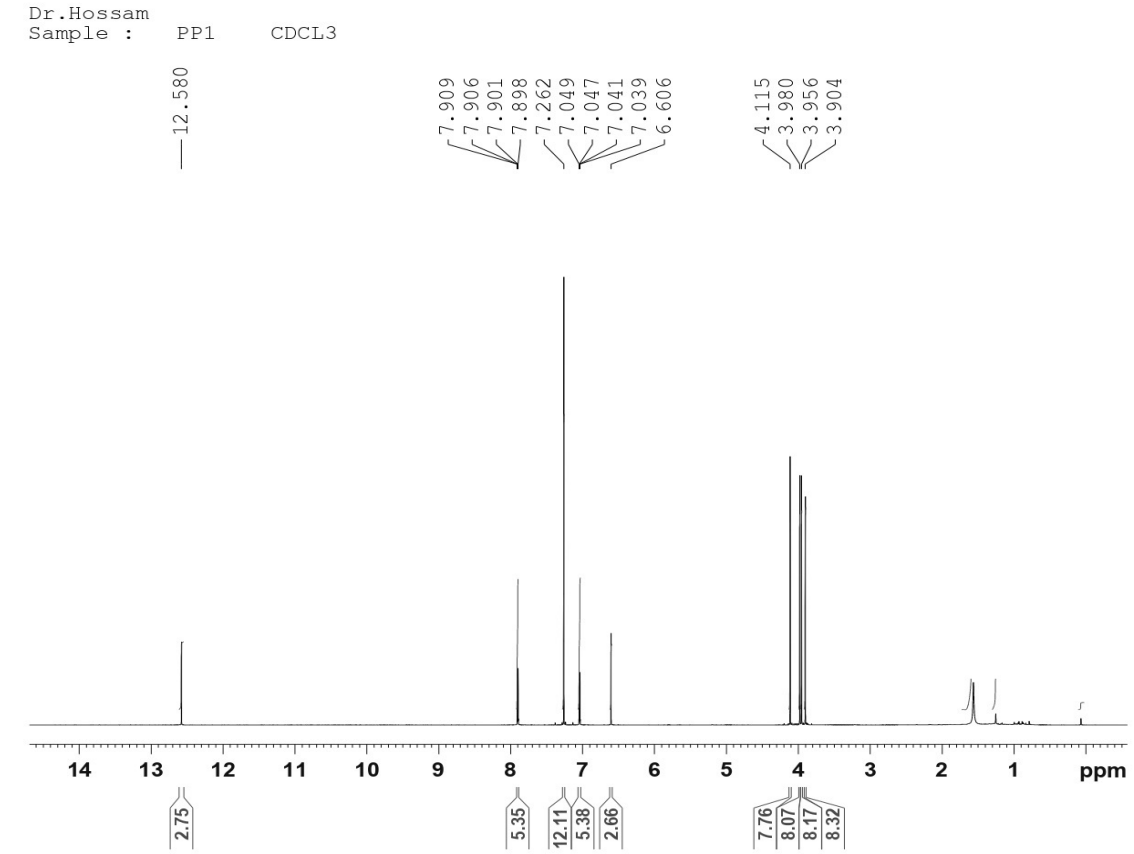

Figure S5. [1]HNMR spectra of gardenin B.

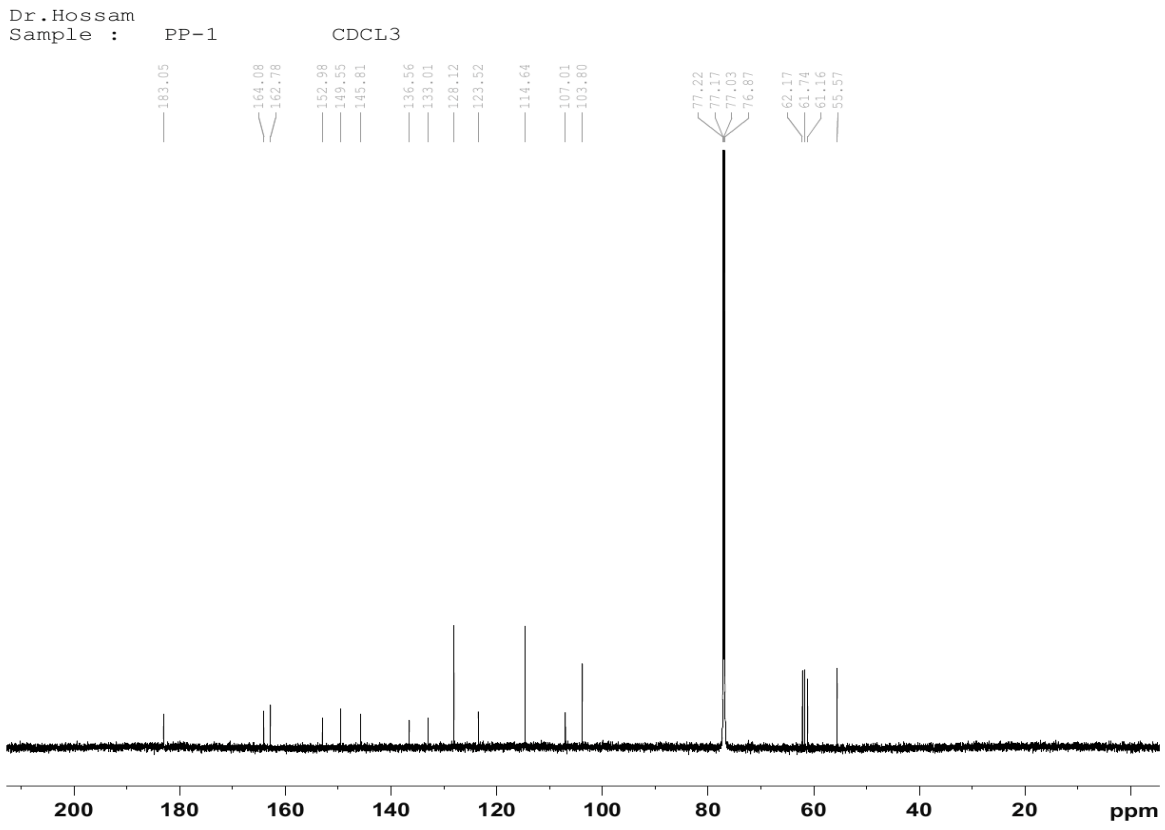

Figure S6. [13]CNMR spectra gardenin B.

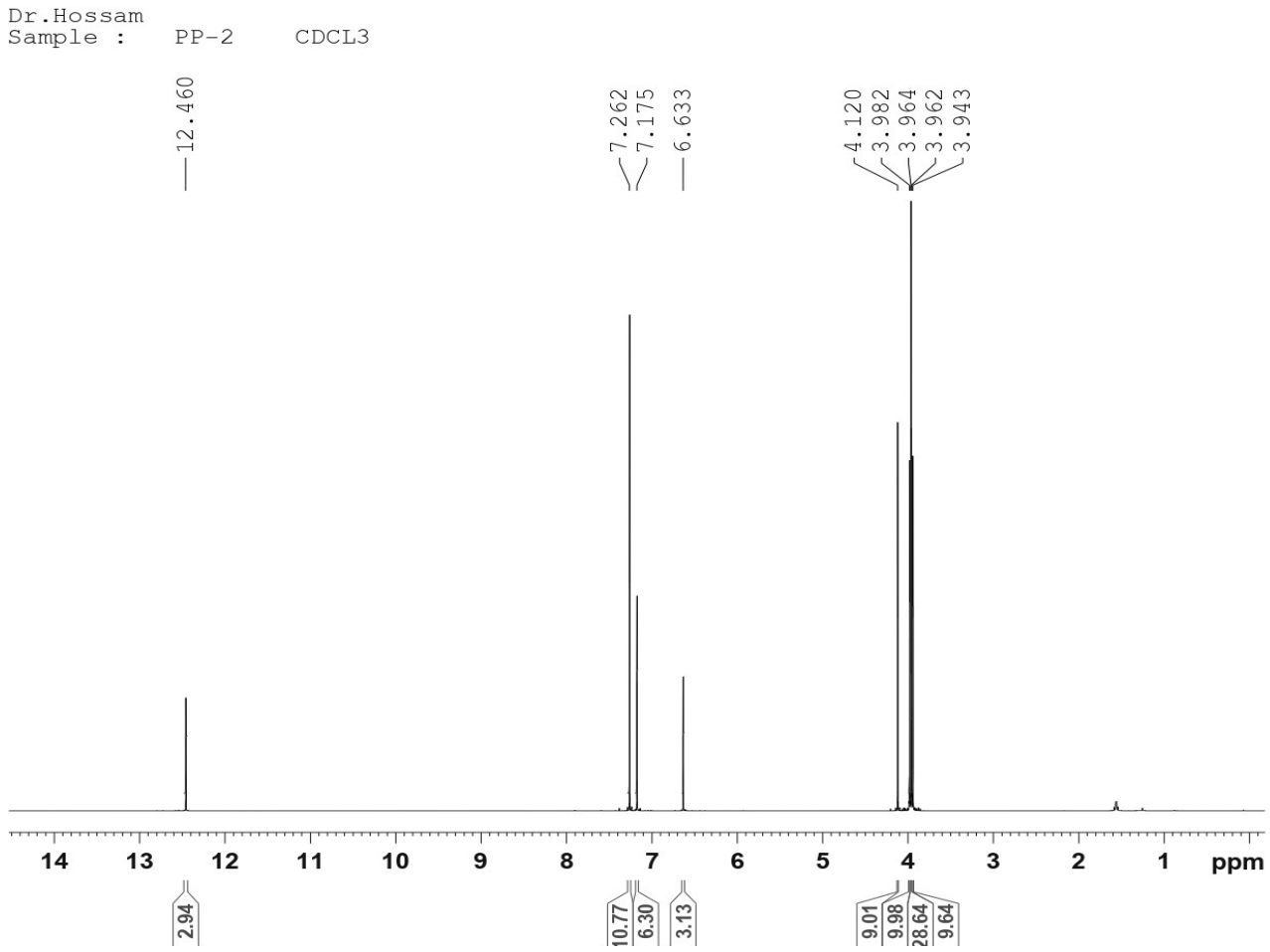

Figure S7. [1]HNMR spectra of gardenin A.

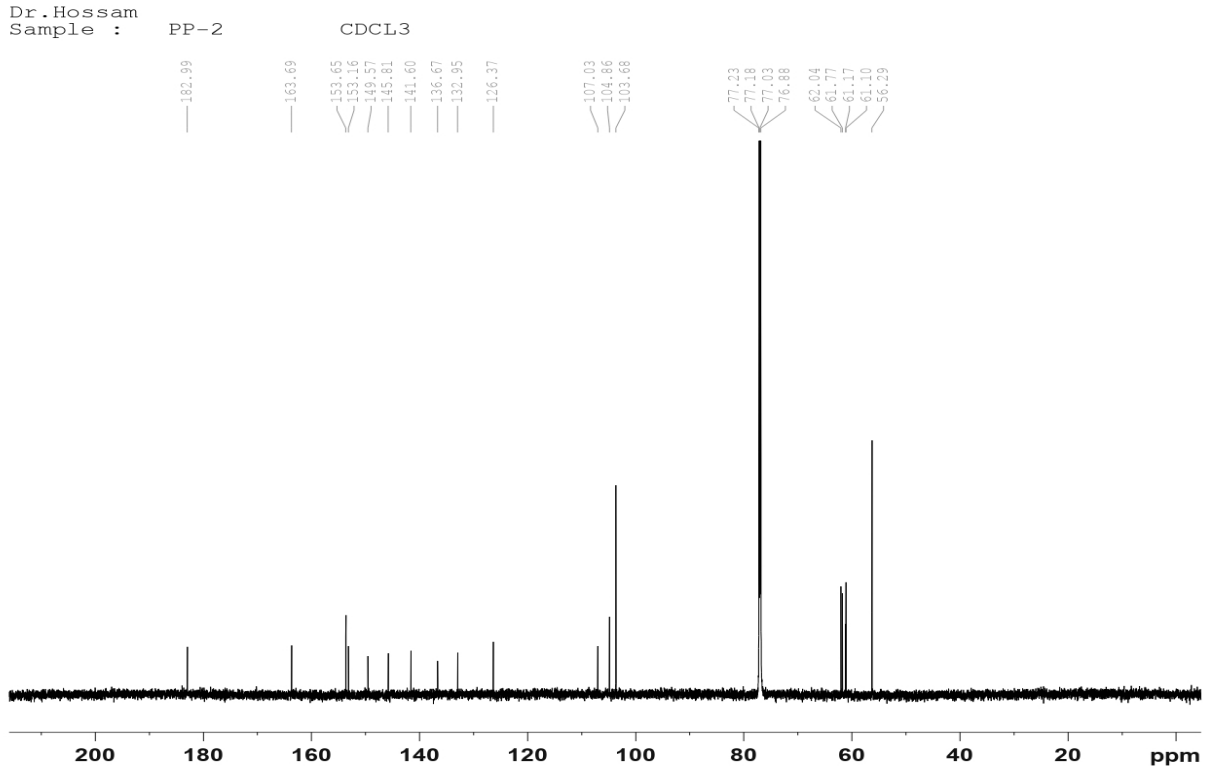

Figure S8. [13]CNMR spectra gardenin A.

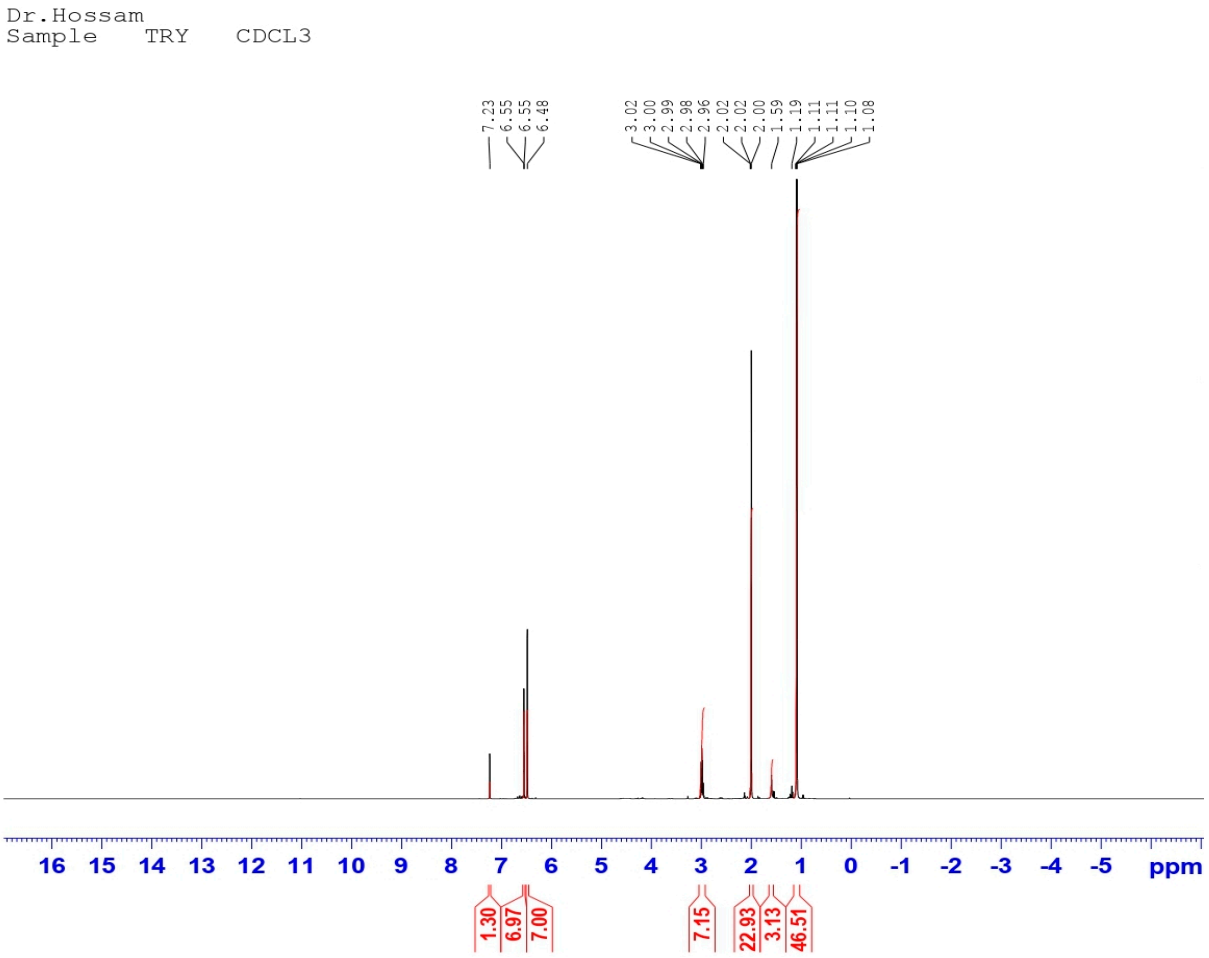

Figure S9. [1]HNMR spectra of thymoquinone.

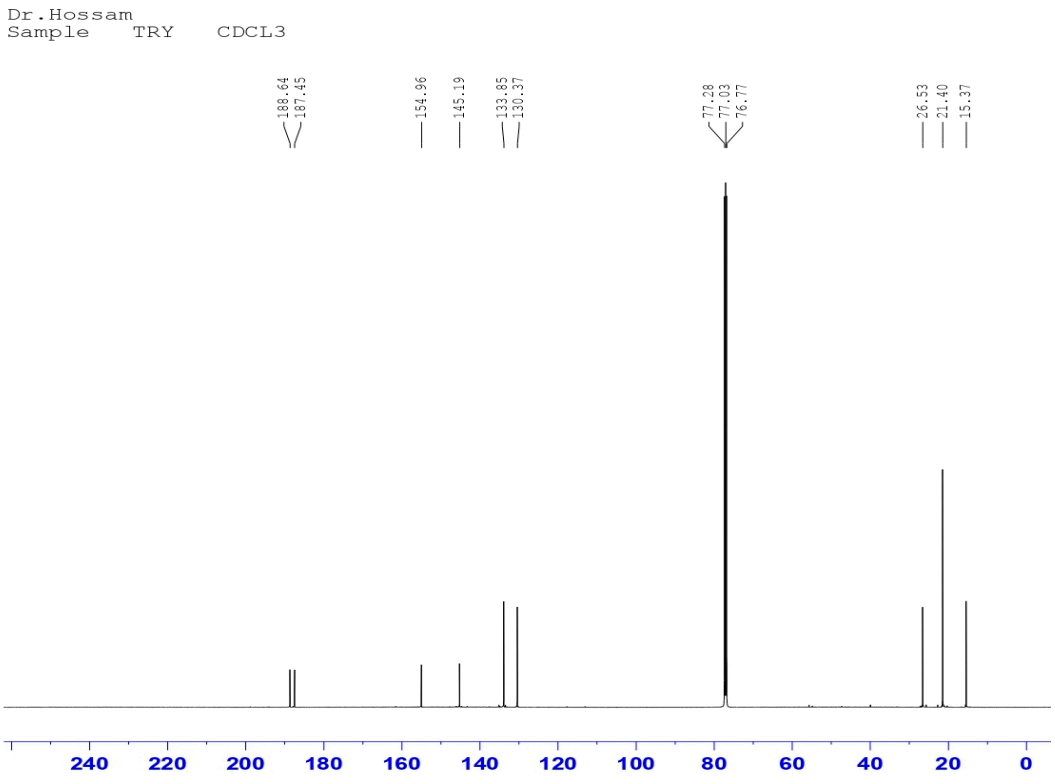

Figure S10. [13]CNMR spectra thymoquinone.

(**A**) (**B**)

Figure S11. Superimposing the co-crystallized (blue sticks) and redocked (green sticks) ligands. (**A**) non-covalent X77 (PDB: 6W63); (**B**) covalent GC376 (PDB: 7CBT) forming hemi-thioacetal adduct with Cys145 (lines) for validating the adopted docking protocols.

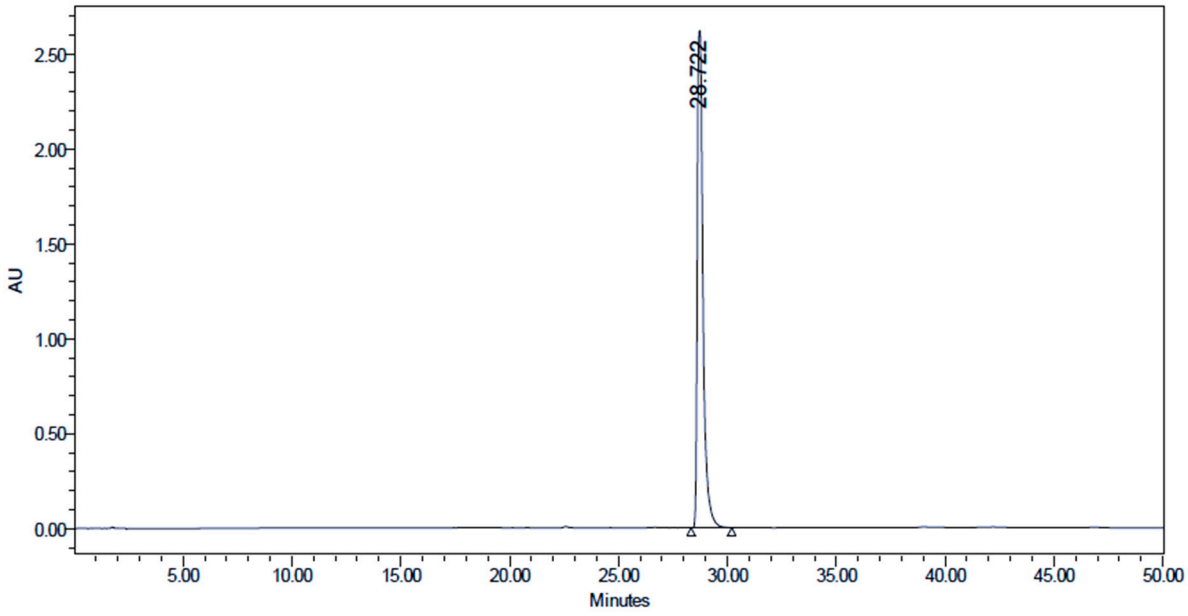

Figure S12. Thymoquinone HPLC chromatogram
HPLC was carried out on Waters W2690/5 system equipped with 996 PAD and Empower 3 Software Build 3471 SPs Installed: Feature Release 3. The column used was Kromasil (Sweden) 4.6X150mmX5um. Thymoquinone purity assessment was carried out using mobile phase A (phosphate buffer 20mM Ph 2.) and B; CH_3CN at 1ml/min flow rate according to the following gradient: 0 min. 95% A, 2 in 95% A, 40 min 20% A43min 20% A. Spectra were recorded at 253nm. Using the previous condition, the compound purity was found to be 99.5%

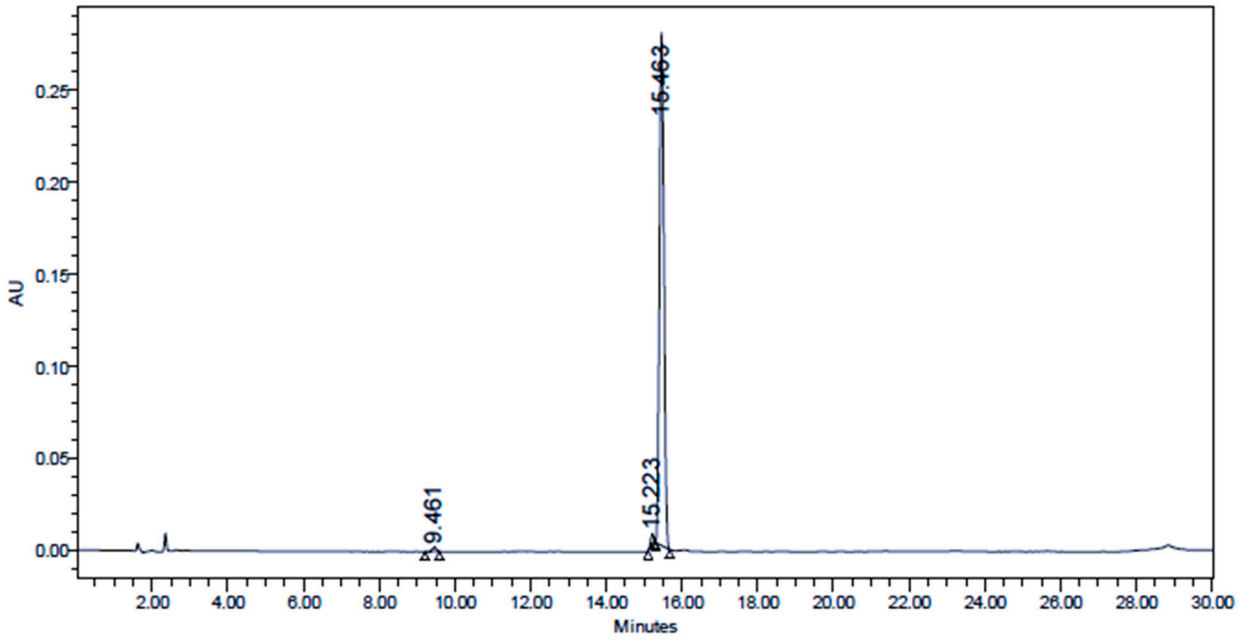

Figure S13. 6-Gingerol HPLC chromatogram
HPLC was carried out on Waters W2690/5 system equipped with 996 PAD and Empower 3 Software Build 3471 SPs Installed: Feature Release 3. The column used was Kromasil (Sweden) 4.6X150mmX5um. Gingerol purity was determined using Inertsil C18 4.6X150mmX5um and mobile phase consisting of 0.1% orthophosphoric acid (A) and CH_3CN (B) gradient elution was as follows; 0 min. 80%A, 2 min 80% A, 20 min 20% A25 min 20% A. elution was carried out at flow rate of 1 ml/ min and spectra were recorded at 280 nm

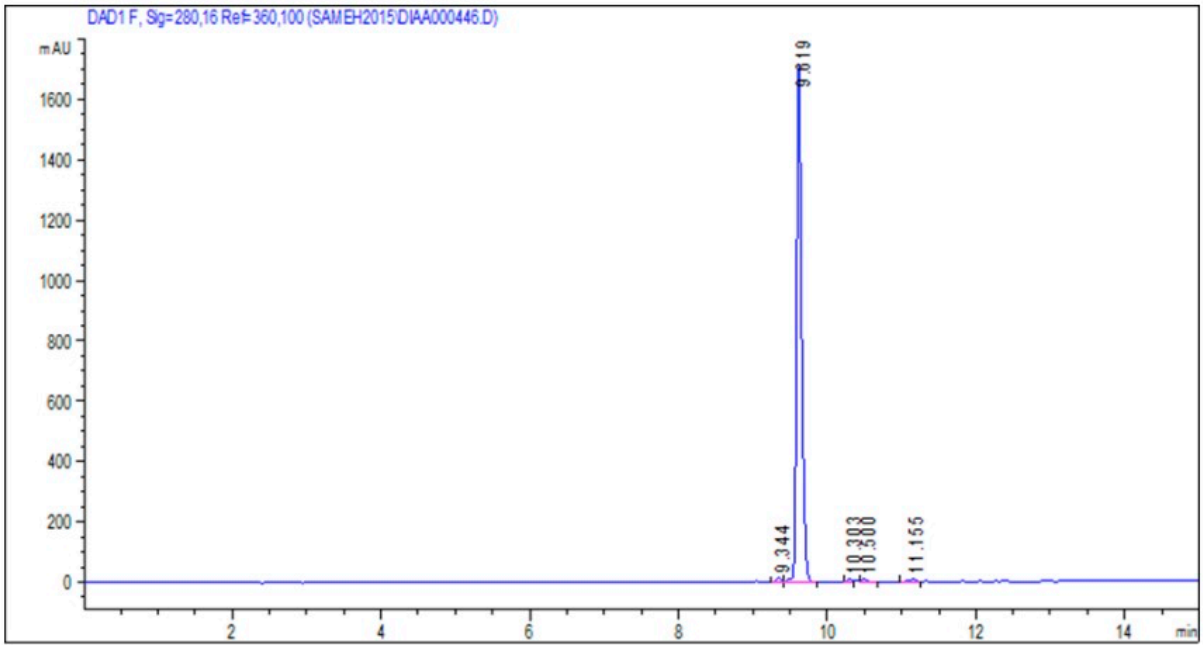

Figure S14. 6-Paradol HPLC chromatogram

HPLC was carried out on Agilent 1200 infinity instrument equipped with automatic injector and DAD detector. Paradol was chromatographed gradiently using a mobile phase A (acetonitrile) and B (0.1% TFA). The gradient elution program was: 35 % A (0-2 min), 35 - 60 % A (2-10 min), 60 - 100 % A (10-12 min) and 100 - 35 % A (12-16 min). The flow rate was 1.0 mL/min and the peaks were monitored 280 nm, respectively. Purity of paradol was 97% at these conditions

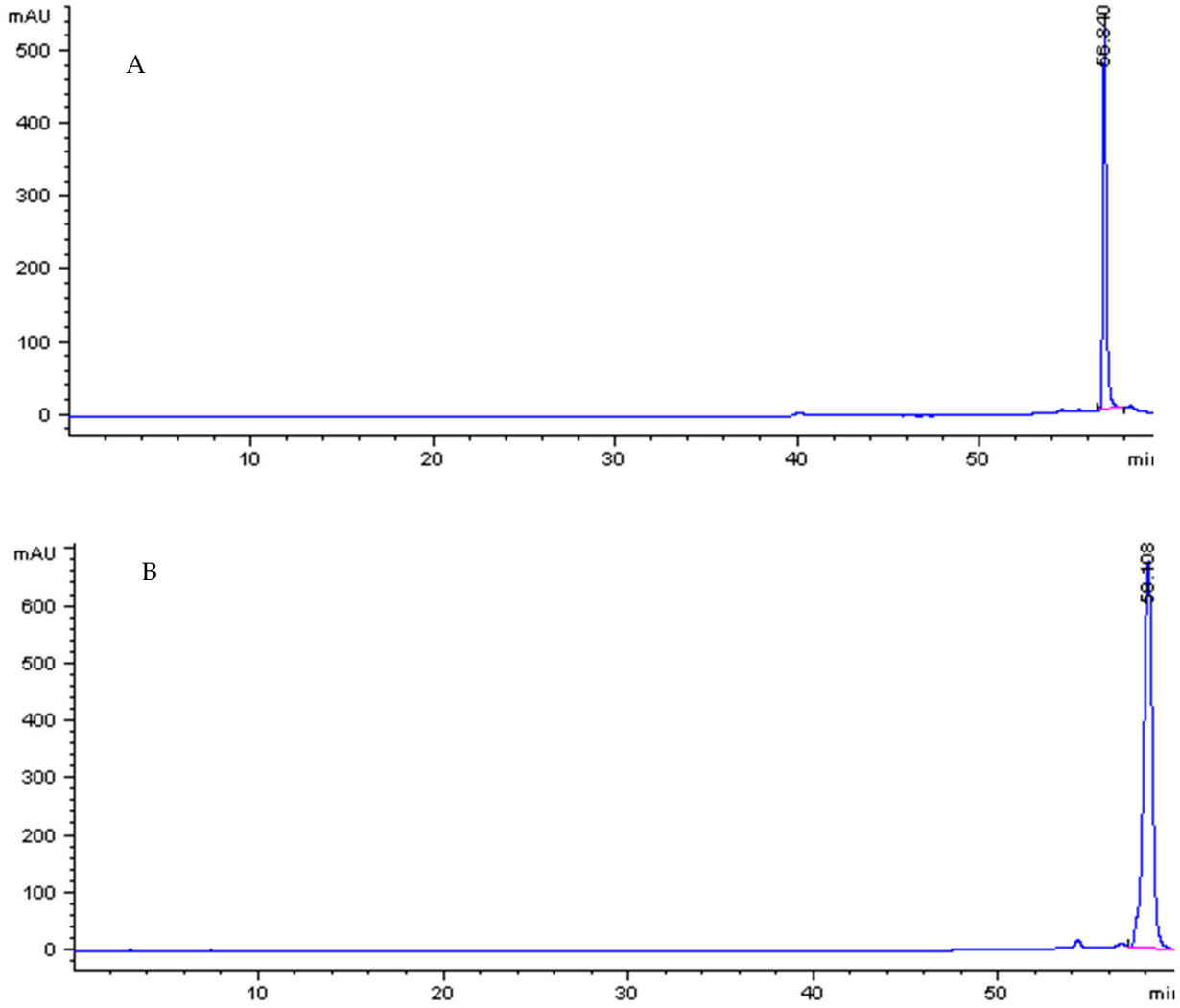

Figure S15. Gardenin A (A) and Gardenin B (B) HPLC chromatogram

HPLC system consisted of an Agilent 1200 system, a solvent delivery module, a quaternary pump, an autosampler, a diode-array detector (DAD), and a column compartment (Agilent Zorbax Extend-C18 column (250 mm length × 4.6 mm, i.d, µm) using Mobile phase: Acetonitril (solvent A) & 0.1% formic acid (solvent B), The gradient elution program was: 20-8% A (0-3 min), 8-42% A (3-45 min), 42-64% A (45-54 min), 64-20% A (54-60 min), 20% A (60-65 min). The flow rate was 1.0 mL/min and the peaks were monitored 280 nm, respectively. Purity of Gardenin A (A) and Gardenin B (B) were 97% at these conditions