Supporting Information

Spontaneous self-assembly of mangiferin in an aqueous medium into vesicles for anticancer drug encapsulation and antibacterial applications

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1. **Structure of Mangiferin (MGF) 1**

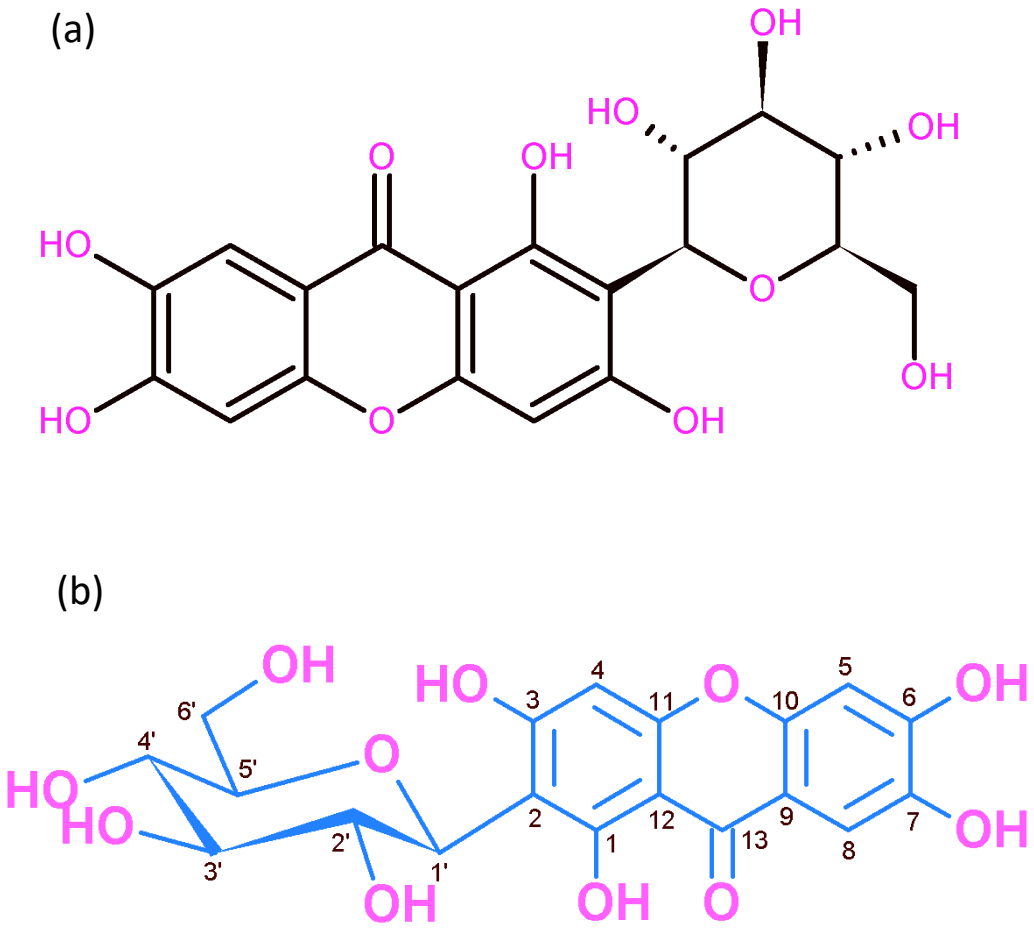

Figure S1: (a, b) are the structure of Mangiferin (MGF).

2. Energy minimized structure of Mangiferin 1, obtained by DFT calculation:

The energy minimized structure and the molecular length of Mangiferin **1** using Gaussian 09 software obtained by DFT calculation was 1.37 nm.

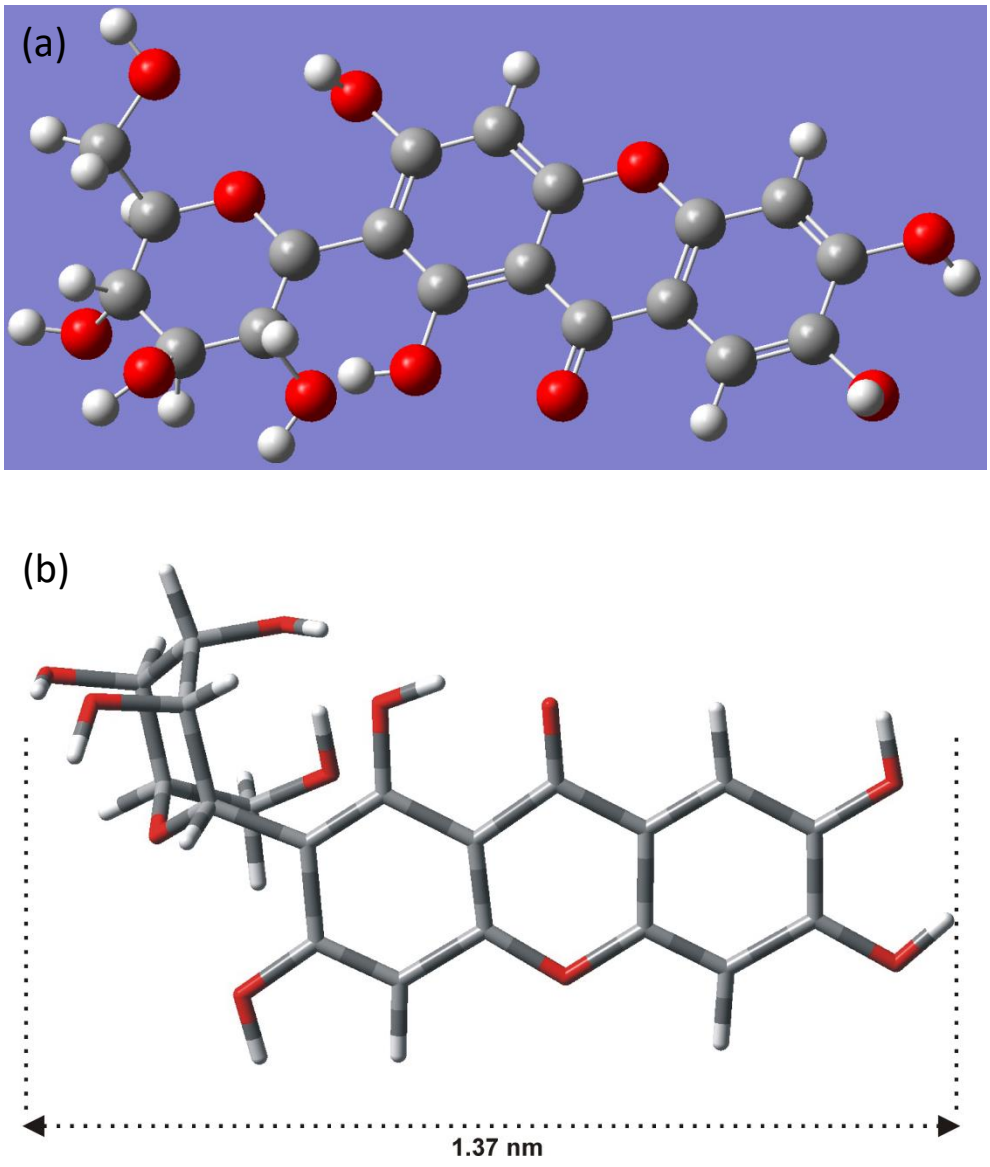

Figure S2: (a, b) The energy minimized structure of Mangiferin **1** using Gaussian 09 software on the basis of 6-31G, +, d, p, doublet spin. The molecular length of Mangiferin obtained by DFT calculation was 1.37 nm.

3. Energy minimized structure of mangiferin 1, obtained by PC model:

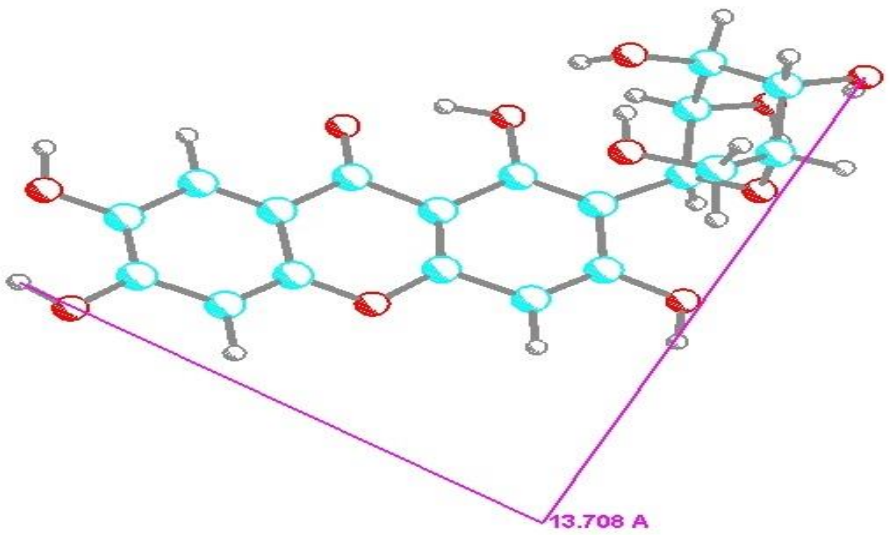

Figure S3: Energy minimized Structure of mangiferin **1** using MMX force field in PC MODEL version 9.2 (Serena Software), the molecular length is 1.37 nm.

4. Determination of Hydrophilic–Lipophilic Balance (HLB) value of Mangiferin

Griffin's method:

$$HLB = 20 * (M_h / M)$$

Where M_h is the molecular mass of the Hydrophilic portion of the molecule and M is the molecular mass of the whole Molecule, giving a result on a scale of 0 to 20.

$$\text{HLB of Mangiferin} = 20 * (M_h / M) = 9.28$$

Table TS1. Hydrophilic–Lipophilic Balance (HLB) value of Mangiferin 1.
Griffin's method
9.28

5. Morphology of the self-assemblies

5.1. Optical microscopic study with histograms:

The solution of MGF in different neat solvents and organic-aqueous solvent mixtures were taken for optical microscopy. Each sample (5 μL) was taken on a glass slide and covered with a cover slip and observed in a Carl Zeiss Microscope under normal light.

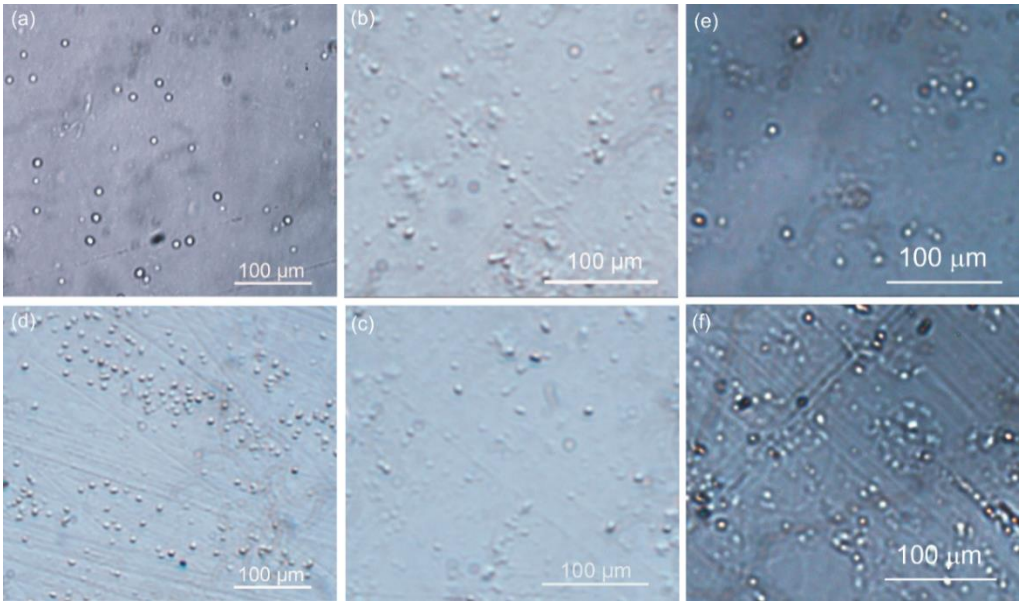

Figure S4: Optical microscopy images of self-assemblies of MGF in (a) DMSO (63.14 mM), (b, c) DMSO-H_2O (1:1 v/v, 2 mM), (d) DMSO-H_2O (1:2 v/v, 2 mM) and (e, f) in water (1 mM, 2 mM), showing spherical objects at lowers concentrations.

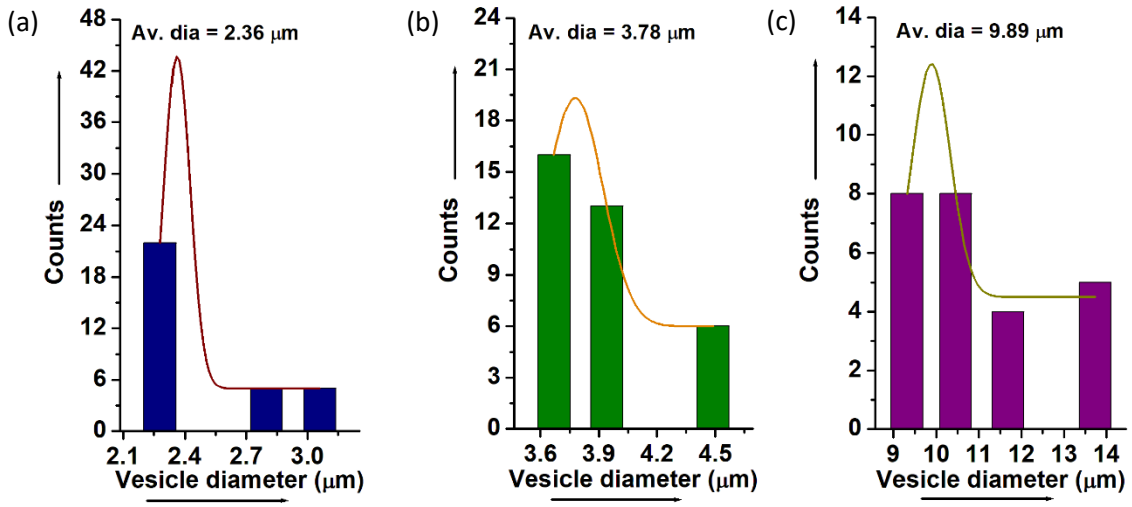

Figure S5: The average diameter of the spherical objects obtained from the histograms of the optical microscopic images of the self-assemblies of MGF in (a) DMF-H_2O (2:1 v/v, 31.57 mM), (b) DMSO-H_2O (1:1 v/v, 17.36 mM) and (d) Water (1 mM) are 2.36 μm, 3.78 μm and 9.89 μm respectively.

5.2. Atomic force microscopy study with histogram:

For AFM studies, 10 µL of freshly prepared colloidal solution of self-assembled Mangiferin (**1**) was drop-casted on a cover slip. The sample was initially dried in air (24 h) and then under reduced pressure (48 h). Then AFM study was performed by using VEECO, dicp-II, Model No-AP0100.

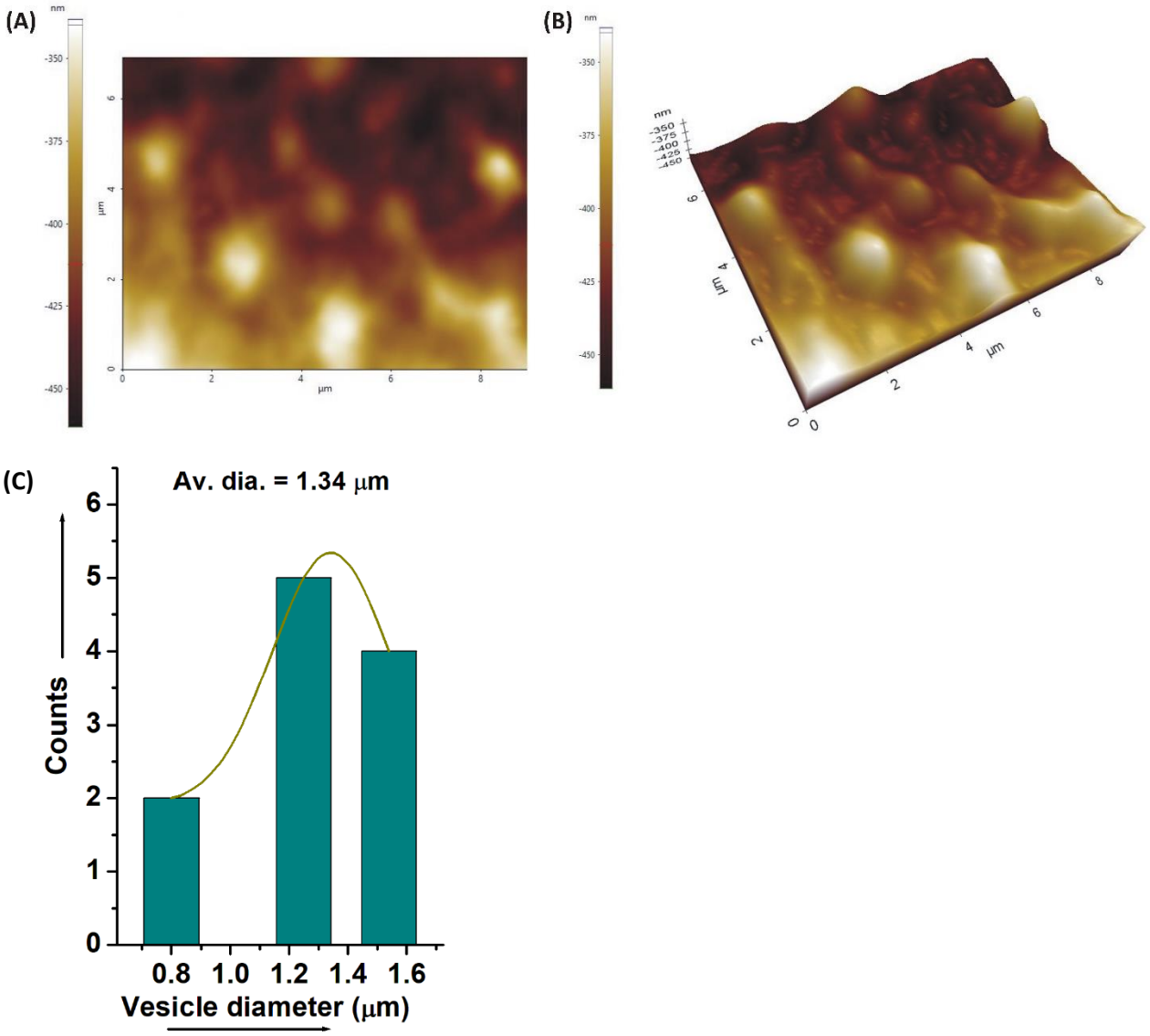

Figure S6: AFM images of the dried self-assemblies of MGF (**1**) in DMSO– H_2O (1 : 1 v/v, 2 mM) (a) 2D image of vesicular self-assemblies, (b) 3D image of vesicular self-assemblies and (c) histogram, from which the average diameter of the spherical object obtained was 1.34 µm.

5.3. Scanning electron microscopic (SEM) study with histogram:

The scanning electron microscope (SEM) is an electron microscope. It produces images of a sample by raster scanning the surface with a focused beam of electrons. These electrons interact with the atoms of the sample to produce various signals, which gives the structure information about the sample. It is used to know the topography and composition of samples. SEM was performed on a JOEL SEM with tungsten filament as electron source. Freshly prepared 10µL each of the colloidal solutions of self-assembled Mangiferin **1** were drop casted on aluminium surface and allowed to dry in air (2 days) and then under reduced pressure (5 days). Before recording the morphology, gold coating on the sample was carried out by sputtering method in a sputter coater. The self-assemblies of MGF in water (1 mM) forms vesicular self-assemblies at lower concentrations and we obtained the average diameter of the vesicles as 300.5 nm (Figure 4 and Figure S11). The shape of the vesicular self-assemblies are different from each other probably due to the different type of interactions between solvent and solute at different concentrations.

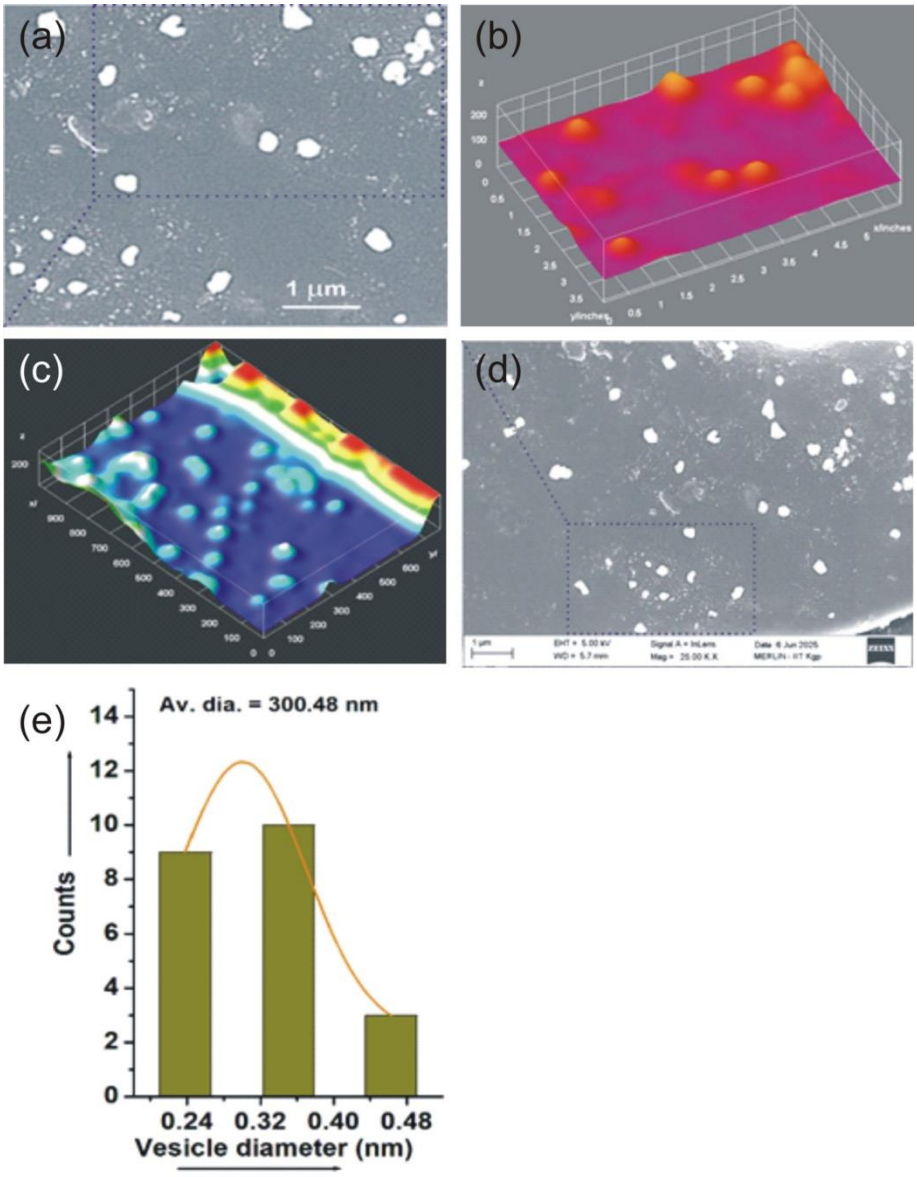

Figure S7: SEM images of the dried self-assemblies of MGF (**1**) in DMSO- H_2O (2:1v/v, 1 mM): (a, d) 2D images, (b, c) 3D images and (e) histogram. Average diameter of the vesicles was 300.5 nm.

5.4. Transmission Electron Microscopy (HRTEM) study with histograms:

High Resolution Transmission Electron Microscopy (HRTEM) study was performed using JEOL instrument. For HRTEM analysis, 10μL of a dilute colloidal sample was placed on carbon coated copper TEM grids (300 mesh). Then it was allowed to dry in air (24 h) and then under reduced pressure (24 h). Vesicular self-assemblies were observed from the HRTEM images of MGF (**1**) in DMF-water (2:1 v/v, 3 mM) and DMSO-water (1:1 v/v, 2 mM) solvent systems. The darkness of some of the vesicles indicated the soft nature of the vesicles.

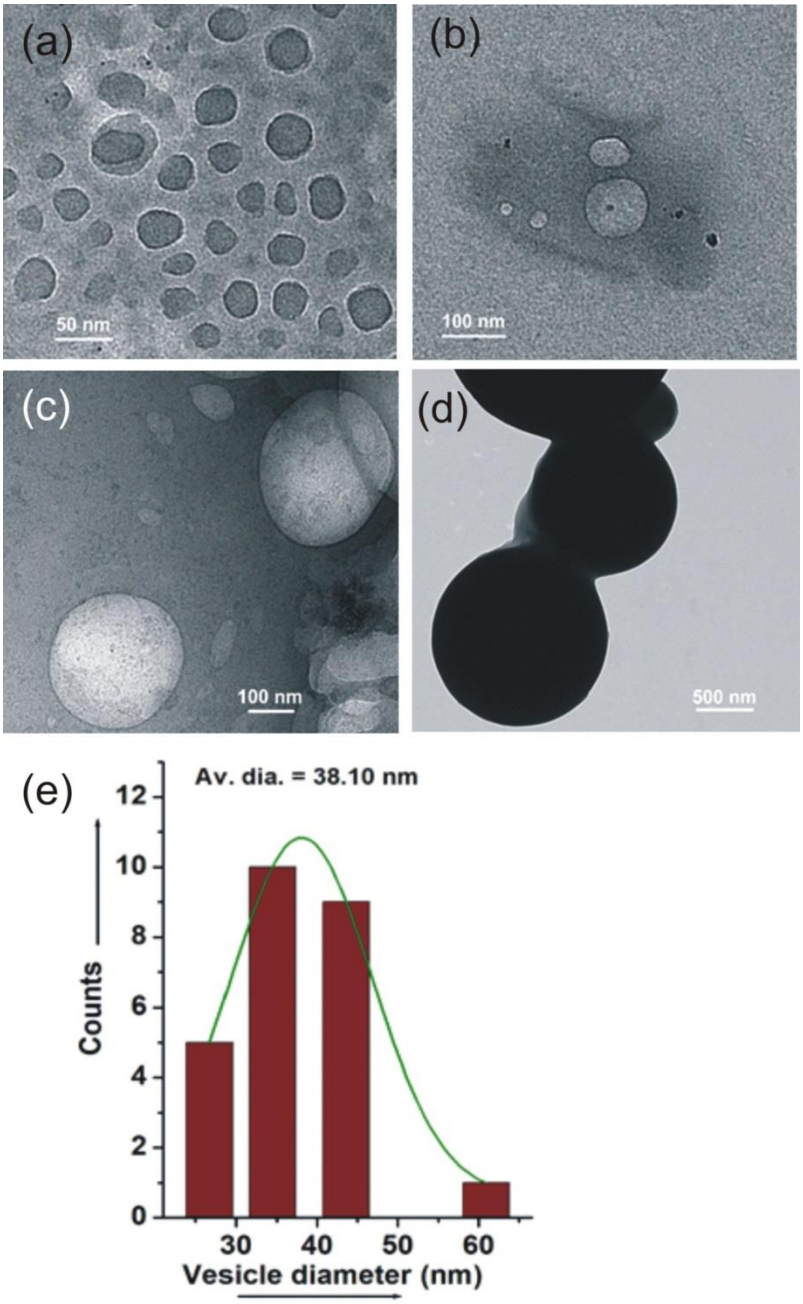

Figure S8: The HRTEM images of the dried self-assembled MGF (**1)** in: (a, b) DMSO-water (1:1 v/v, 2 mM); (c, d) DMF-water (2:1 v/v, 3 mM) and (e) histogram, the average diameter of spherical objects was 38.10 nm.

5.5. FTIR (ν, cm^{-1}) Study:

The FTIR spectrum study of neat solid powder and liquid self-assemblies were carried out by using a Perkin Elmer Spectrum Two model with KBr pellet. The FTIR data are 3358 cm^{-1} for O-H stretching (for solid neat powder of mangiferin) and 3363 cm^{-1} for O-H stretching, 3183 cm^{-1} O-H bending, 2942 cm^{-1} C-H symmetric stretching, 1655 cm^{-1} C=O stretching, 1621 cm^{-1} C=C stretching, 1255 cm^{-1} C-O stretching, 1092 cm^{-1} C-C stretching (for self-assembled of mangiferin in DMF−H_2O (2:1v/v, 31.57 mM)) (Figure S13).

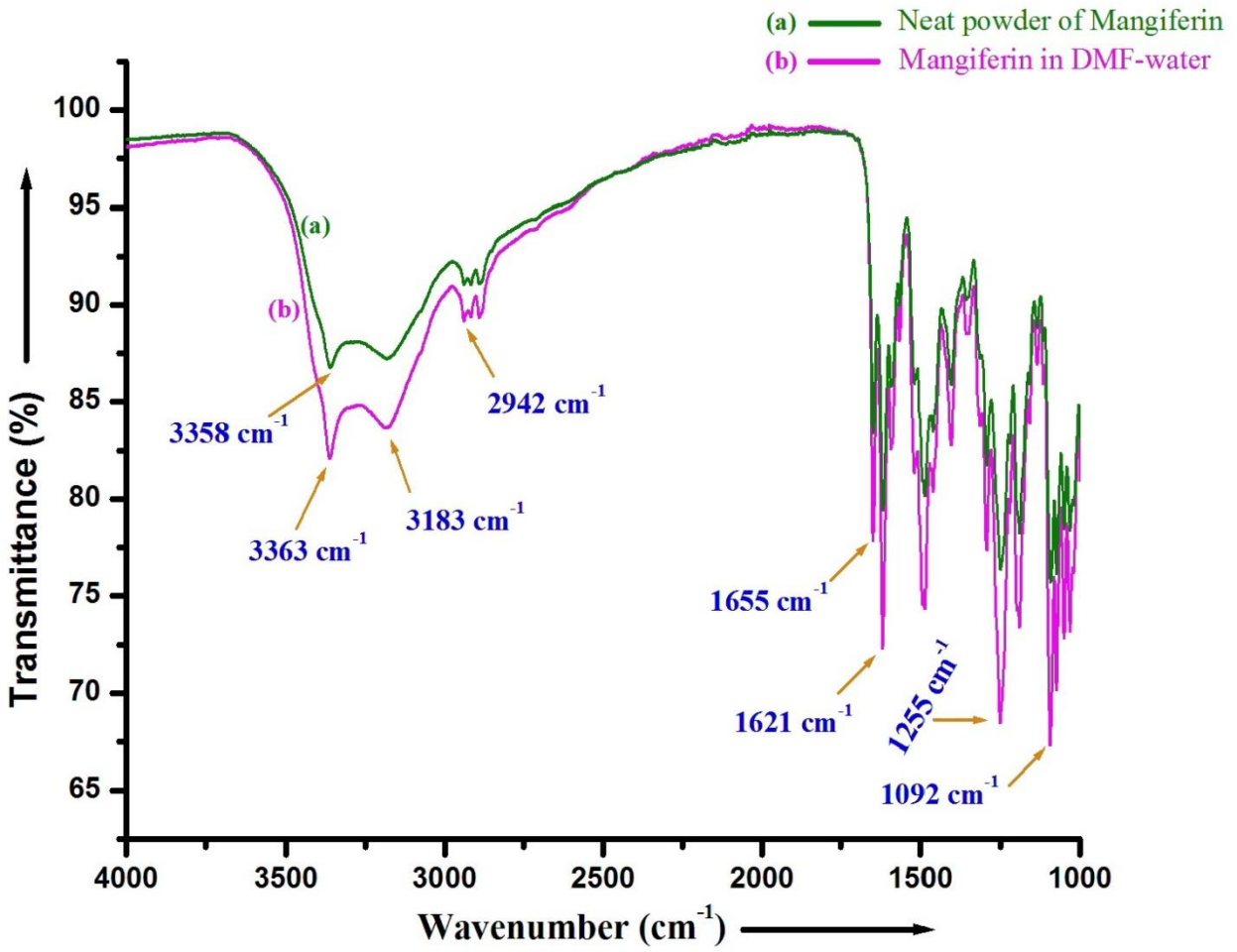

Figure S9: FTIR spectrum of (a) neat powder of the Mangiferin **1** (olive colour line) and (b) the vesicular self-assemblies of **1** in DMF-H_2O (2:1, 31.57 mM) (magenta colour line).

5.6. X-ray Diffraction study and crystal structure of Mangiferin:

The X-Ray diffraction (XRD) is a technique that gives the information about the crystallographic structure, chemical composition and physical properties of a material. It is based on Bragg's law ($n\lambda = 2d\sin\theta$), where 'n' is the diffraction order and λ is the wavelength,'d' is the interplaner spacing in the crystal lattice and θ is the diffraction angle. The powder X-ray diffraction (PXRD) was conducted at room temperature using a Bruker D6 PHASER X-ray diffractometer over a 2θ range of 5–50°. The simulated powder patterns were generated from single-crystal X-ray diffraction data and processed using the Mercury software, which was provided by the Cambridge Crystallographic Data Centre (CCDC). The PXRD patterns of commercial mangiferin and mangiferin from *Davallia solida* were compared with the simulated pattern, demonstrating a good match in their phase purity. Experimental PXRD patterns obtained for the dried self-assemblies of MGF obtained from DMSO (green), DMSO-H2O (2:1) (deep blue), DMSO-H2O (1:1) (violet) were also compared. Most of the peaks obtained from the self-assemblies were in close proximate indicating that these samples have identical ordered structures.

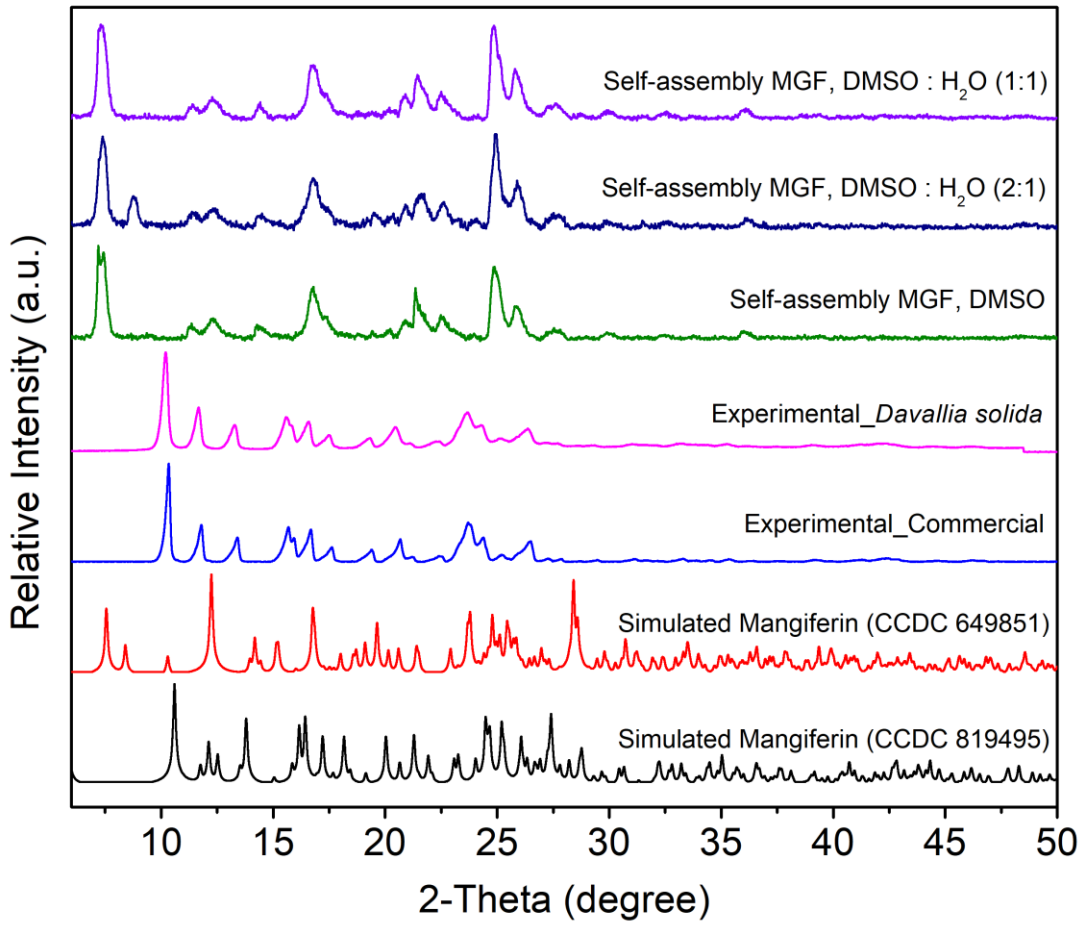

Figure S10: Overlay of PXRD spectra for mangiferin studies. Simulated pattern for mangiferin (CCDC No: 819495) (black), Simulated pattern for mangiferin (CCDC No: 649851) (red), experimental pattern for mangiferin from commercial 98% (blue), experimental pattern for mangiferin from *Davallia solida* (magenta), Self-assembly MGF in DMSO (green),Self-assembly MGF in DMSO-H2O (2:1) (deep blue), Self-assembly MGF in DMSO-H2O (1:1) (violet).

5.7. Crystal structure of mangiferin:

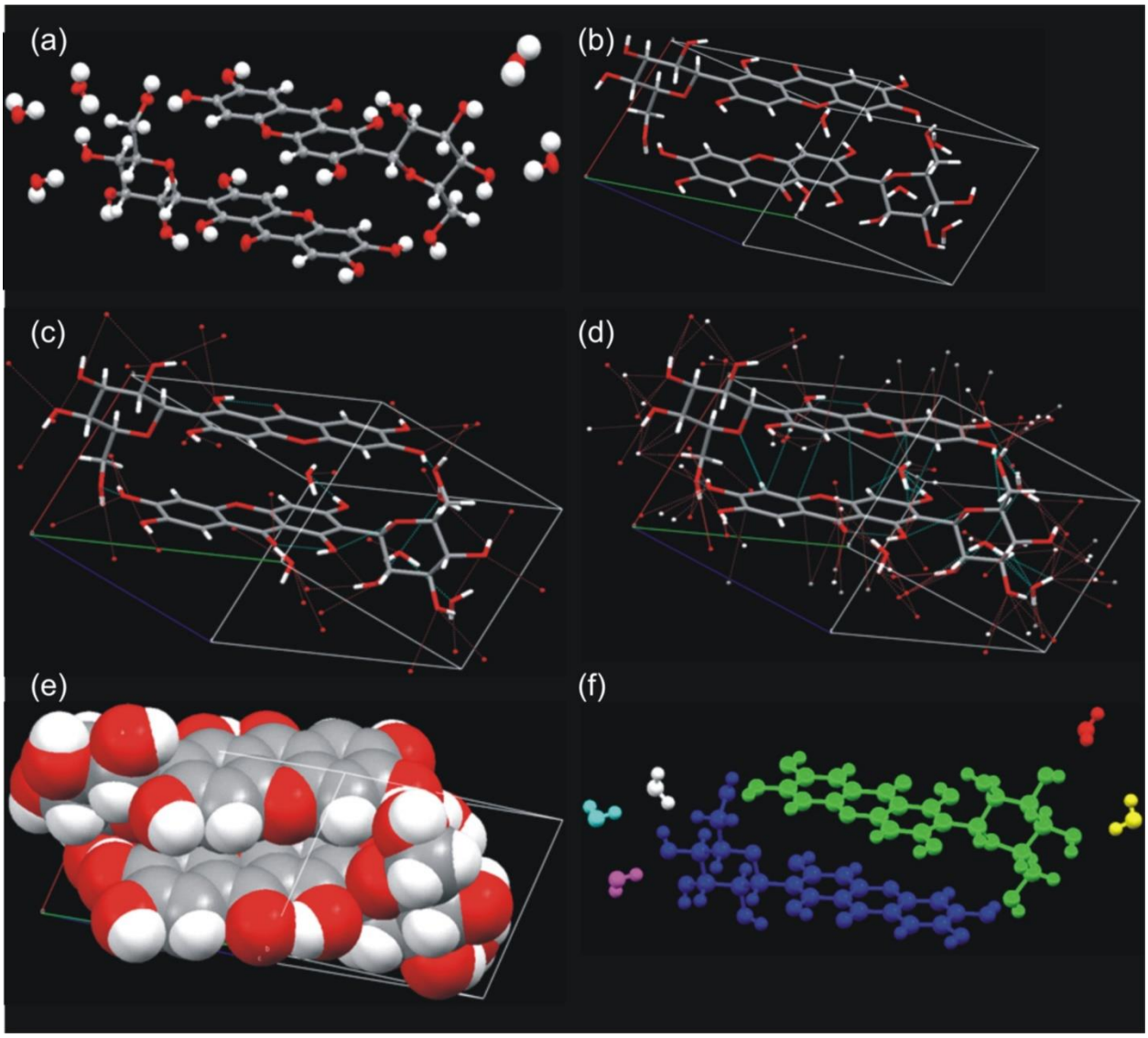

Figure S11: The crystal structure and crystal packing of mangiferin in H_2O solvent with (a) ellipsoid form, (b) capped stick form, (c) capped stick form with H-bondings, (d) capped stick form with H-bondings and Van der Waals interactions, (e) space fill structure and (f) ball & stick structure with color symmetry equivalence, generated using the 3D viewer of Mercury software, which was provided by the Cambridge Crystallographic Data Centre (CCDC), (ref. - Jr. J. W. da C., Moraes L. R. de, Santos M.H. dos, Silva G. A. da, Brigagao M. R. P. L., Ellena J., and Doriguetto A. C., *Helv. Chim.Act*, **2008** (91) :144-154, DOI: 10.1002/hlca.200890005).

5.8. Dynamic Light Scattering (DLS) studies:

DLS analysis of the self-assembled MGF samples were carried out with the DLS instrument (***Malvern Zetasizer Nano-ZS90*)*.***

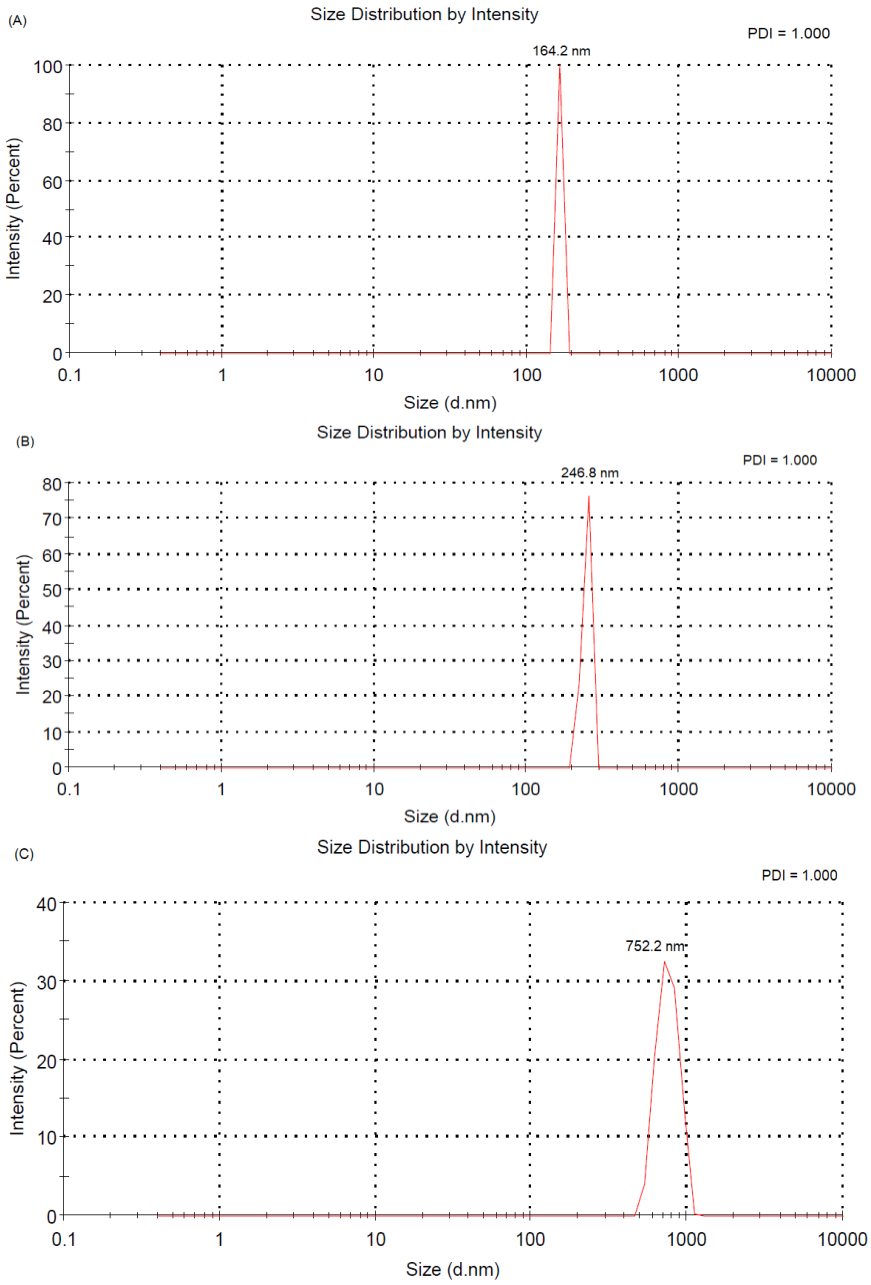

Figure S12: The size distribution curves with PDI values of mangiferin in only water medium with concentrations (a) 0.1 mM, (b) 1 mM and (c) 2 mM.

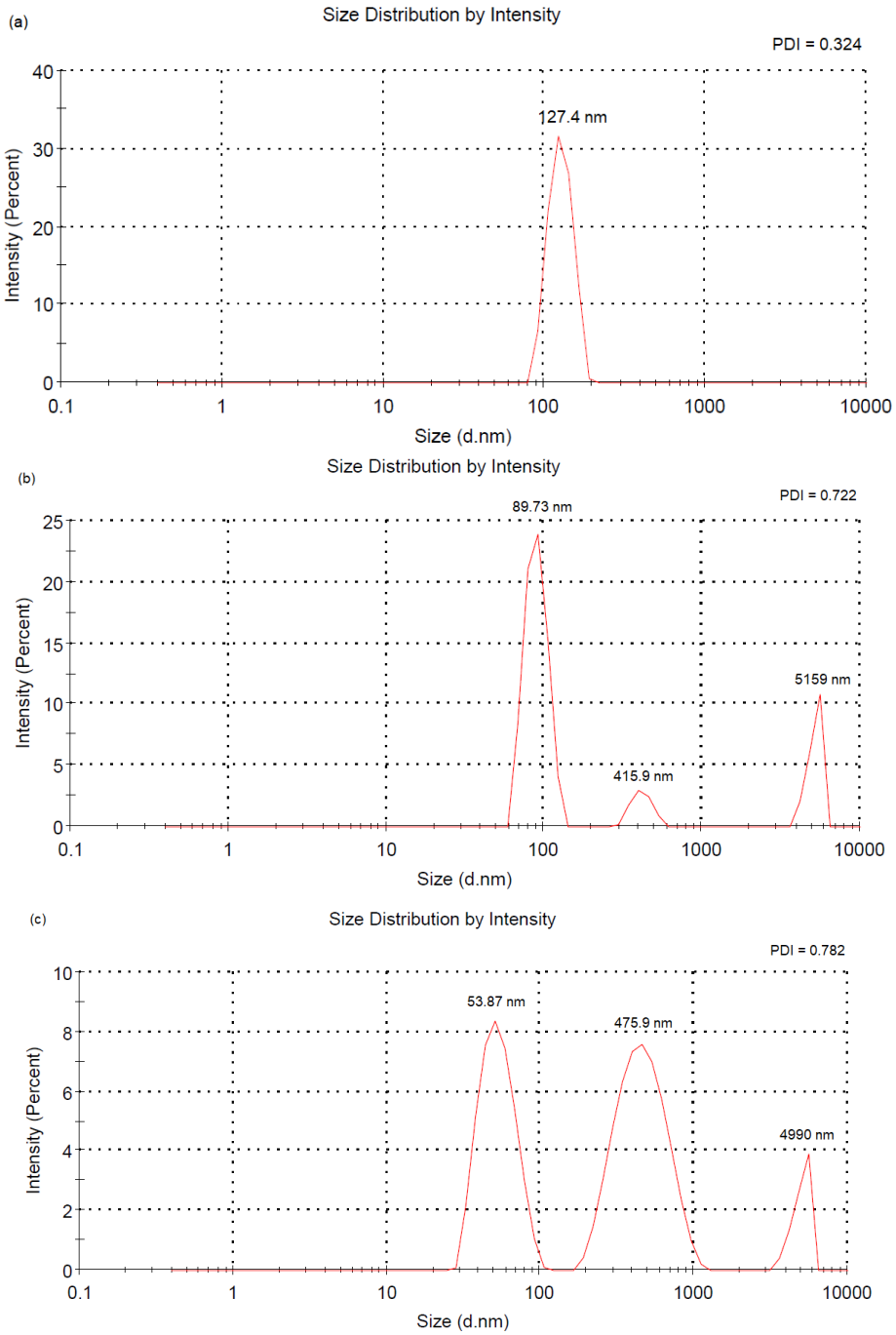

Figure S13: The size distribution curves with PDI values of mangiferin in DMSO-water (1:1 v/v) medium with concentrations (a) 0.1 mM, (b) 1 mM, and (c) 2 mM.

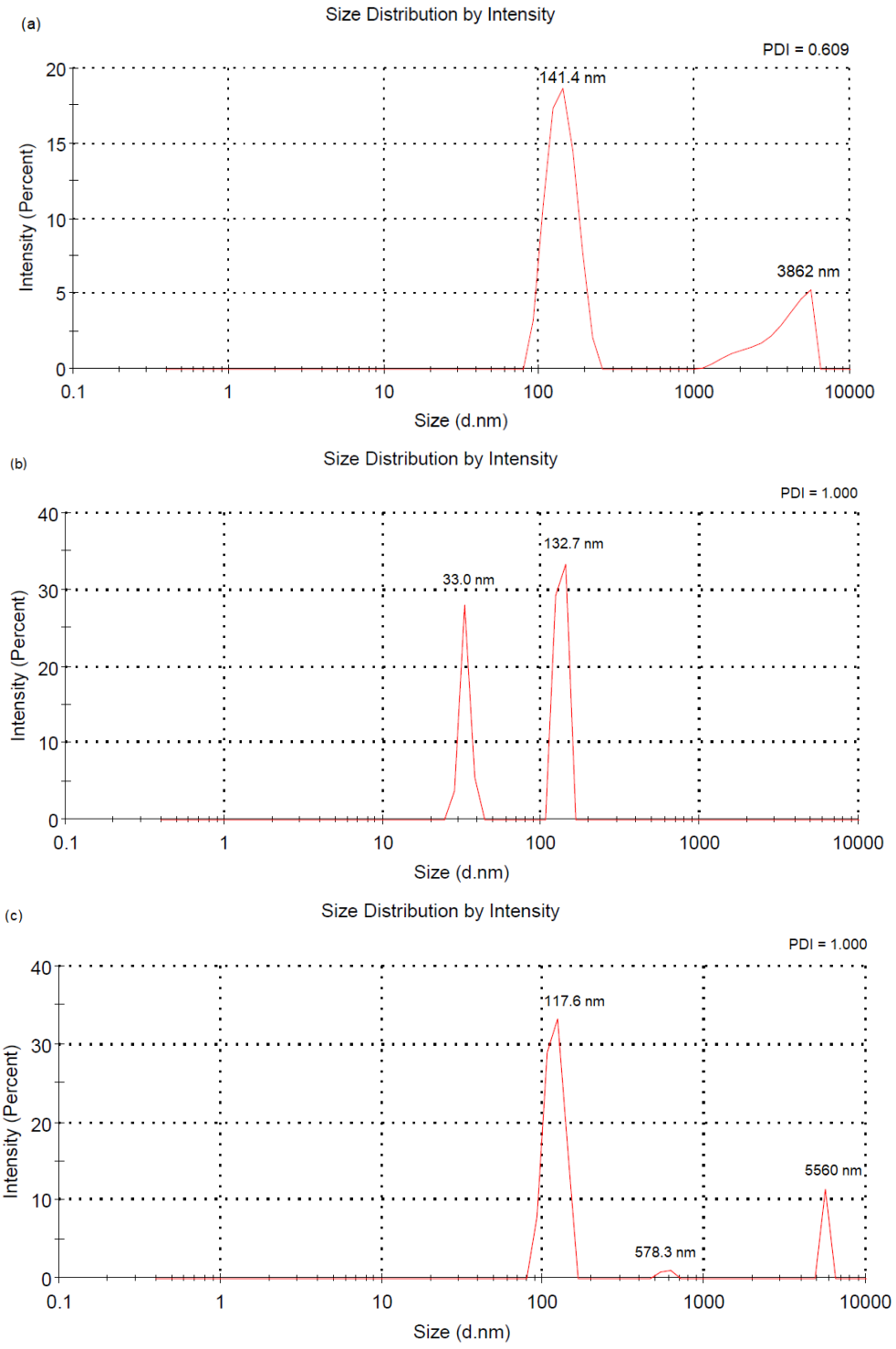

Figure S14: The size distribution curves with PDI values of mangiferin in DMSO-water (1:1 v/v) medium after (a) 10 min, (b) 20 min and (c) 30 min.

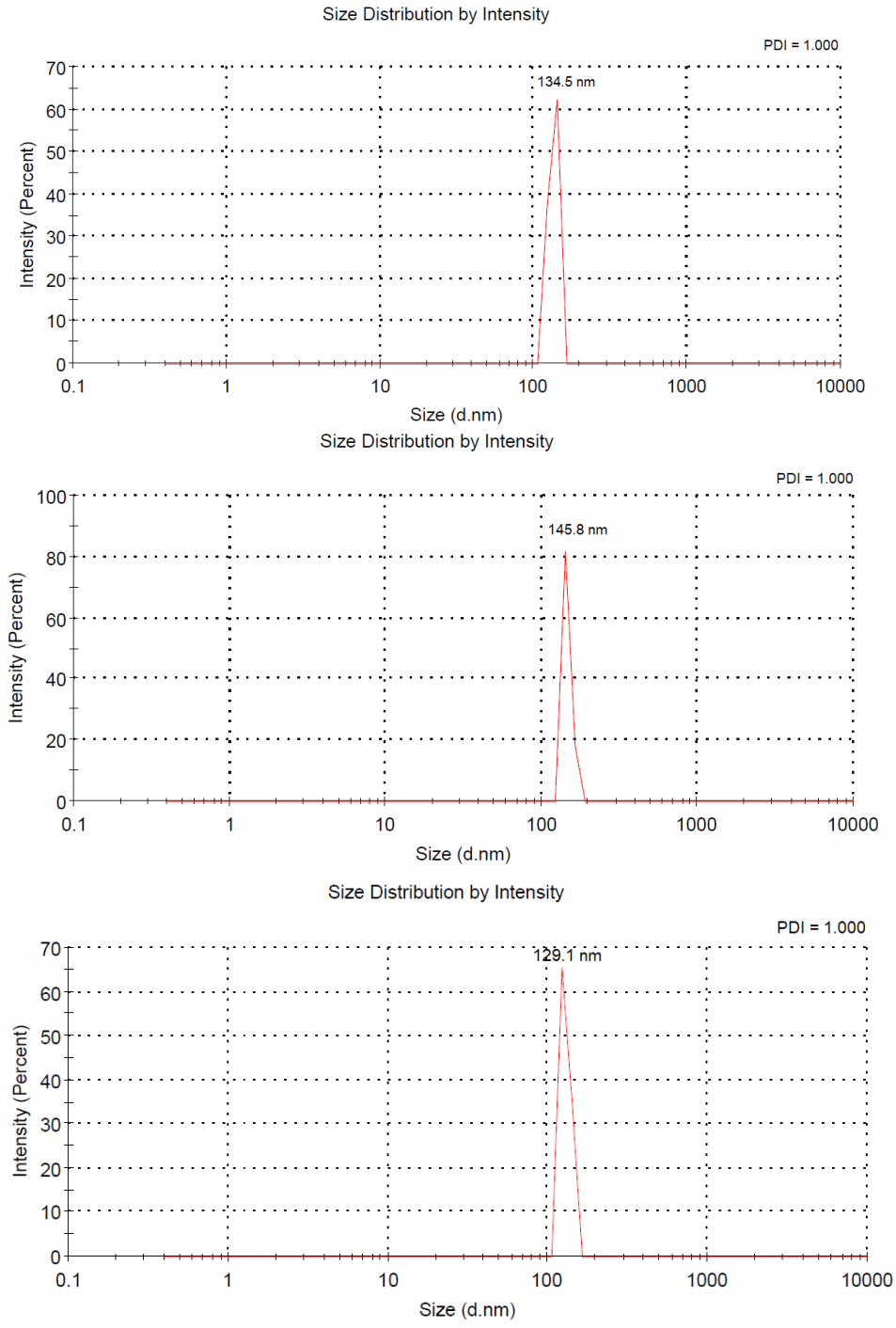

Figure S15: The size distribution curves with PDI values of mangiferin in water medium after (a) 10 min, (b) 20 min and (c) 30 min.

5.9. Critical Vesicular Concentration (CVC) study

The Critical Vesicular Concentration (CVC), is the minimum concentration which is necessary to form vesicles in a given liquid at room temperature. Formation of the vesicular self-assemblies and its utilization in drug delivery applications inspired us to study the cvc of mangiferin. CVC of mangiferin was determined in DMSO-water (1:1 v/v) binary liquid mixtures using pyrene as a fluorescence probe. Pyrene (1 mg) was weighed in a vial and dissolved in dichloromethane (1 mL) to obtain a clear solution (4.9 mM). An aliquot of 20.5 μL (4.9 mM) was taken in another vial and the volume of the solution made upto 1 mL to obtain a solution of concentration 0.1 mM. Aliquots of 20 μL each of of 0.1 mM pyrene solution were placed in twelve different clean and dry vials and the solvent was evaporated so that each vial contain fixed amount of pyrene (0.4 μg). Mangiferin (2 mg) was taken in a vial and dissolved in DMSO (2 mL) to obtain a clear solution (2.368 mM). For the determination of the cvc, a number of solutions were prepared at different concentration of mangiferin from 0.01mM to 0.20 mM and the concentration of probe was fixed at 1 μM for each case. For this purpose varied amount of previously prepared mangiferin solution in DMSO (2.368 mM) was added to each vial containing fixed amount of pyrene. Then distilled water was added maintaining the ratio of DMSO: H_2O at 1:1 in total volume of 2 mL. Thus, mangiferin solution in DMSO: H_2O of 0.01 mM to 0.2mM containing pyrene (1μM) was prepared. All of these samples were heated with stirring and kept at room temperature for 24 h before measuring the fluorescence. The excitation wavelength was 335 nm. When pyrene is excited at 335 nm, five predominant peaks are observed in fluorescence emission spectrum at 375 nm, 380 nm, 385 nm, 395 nm and 417 nm which are denoted as I_1 I_2, I_3, I_4 and I_5 respectively. The CVC value of mangiferin in DMSO-water (1:1 v/v) is 50 μM (Figure S16). Similarly, the CVC value of mangiferin obtained in DMSO-water (2:1 v/v) solvent system was 60 μM (Figure S17).

Table TS2. CVC values of Mangiferin carried out in DMSO-water

Entry	Binary liquid mixtures	CVC (μM)
1.	DMSO-water (2:1 v/v)	60
2.	DMSO-water (1:1 v/v)	50
3.	Water	30

5.9.1. CVC value of Mangiferin in DMSO-water (1:1 v/v)

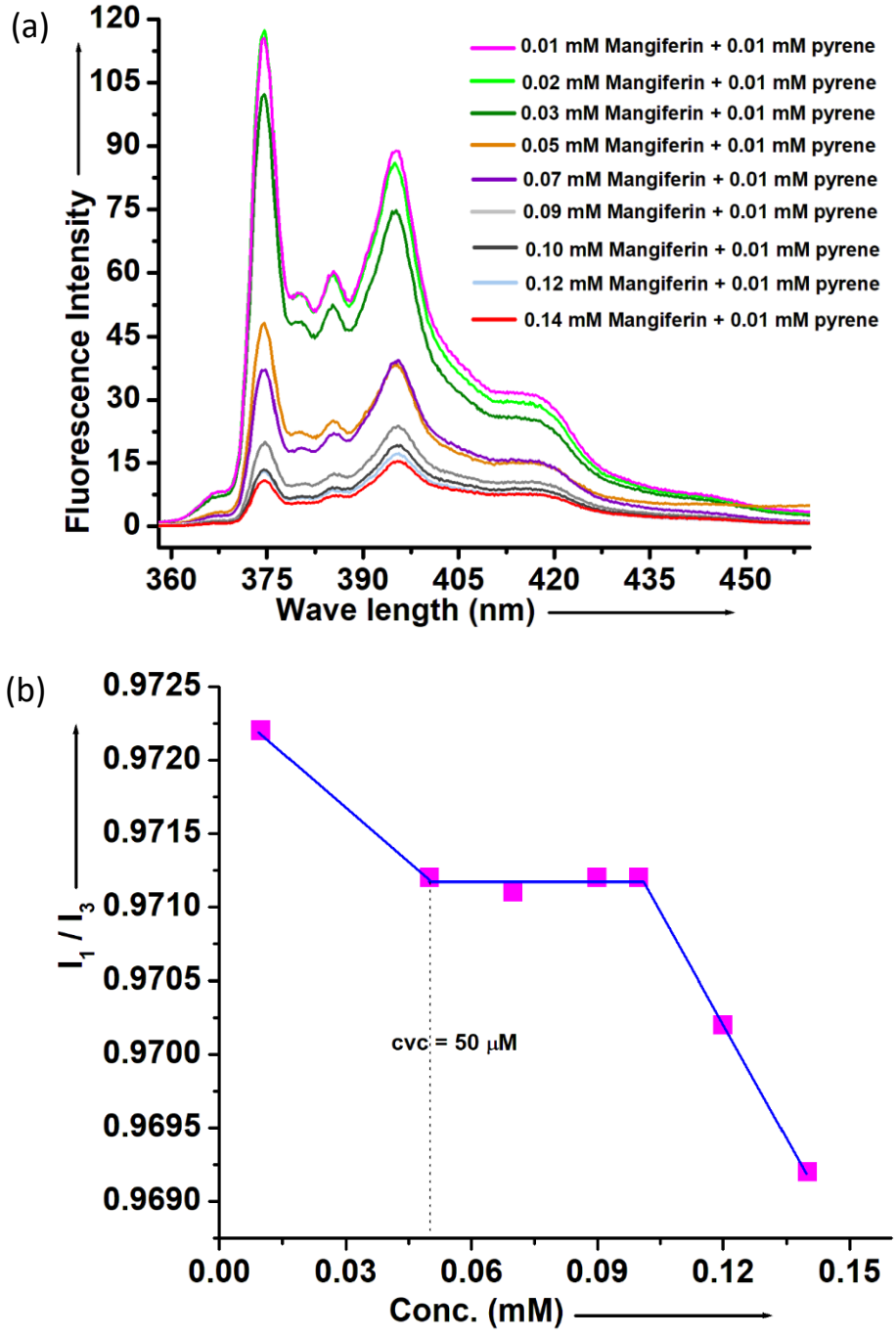

Figure S16: Fluorescence emission spectra of pyrene in DMSO-water (1:1 v/v) solvent system of mangiferin (a) Fluorescence intensity vs. wave length plot and (b) I_1/I_3 vs. concentration plot, from which the CVC value determined was 50 μM.

5.9.2. CVC value of Mangiferin in DMSO-water (2:1 v/v)

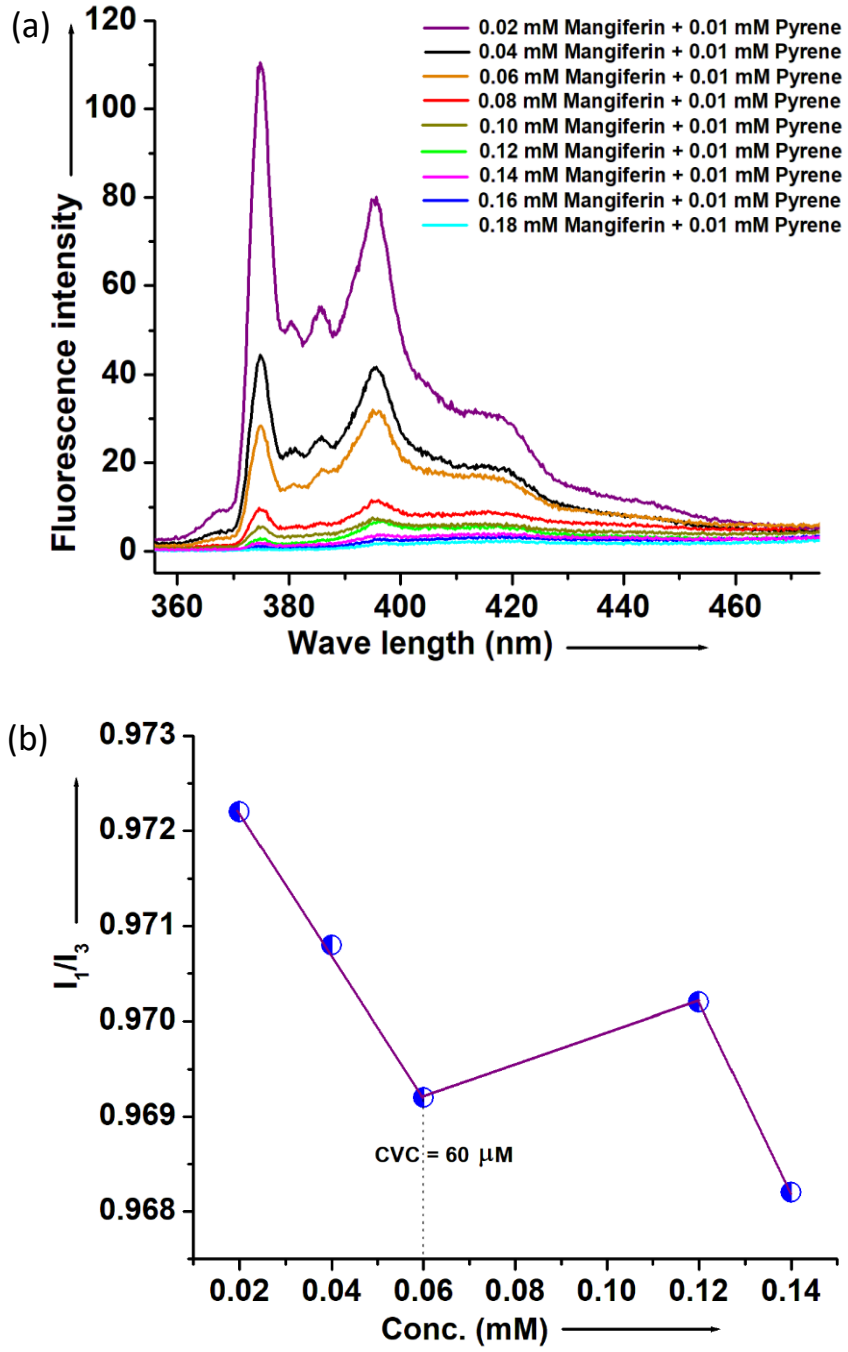

Figure S17: Fluorescence emission spectra of pyrene in DMSO-water (2:1 v/v) solvent system of mangiferin (a) Fluorescence intensity vs. wave length plot and (b) I_1/I_3 vs. concentration plot, from which the CVC value determined was 60 μM.

5.9.3. CVC value of Mangiferin in water medium

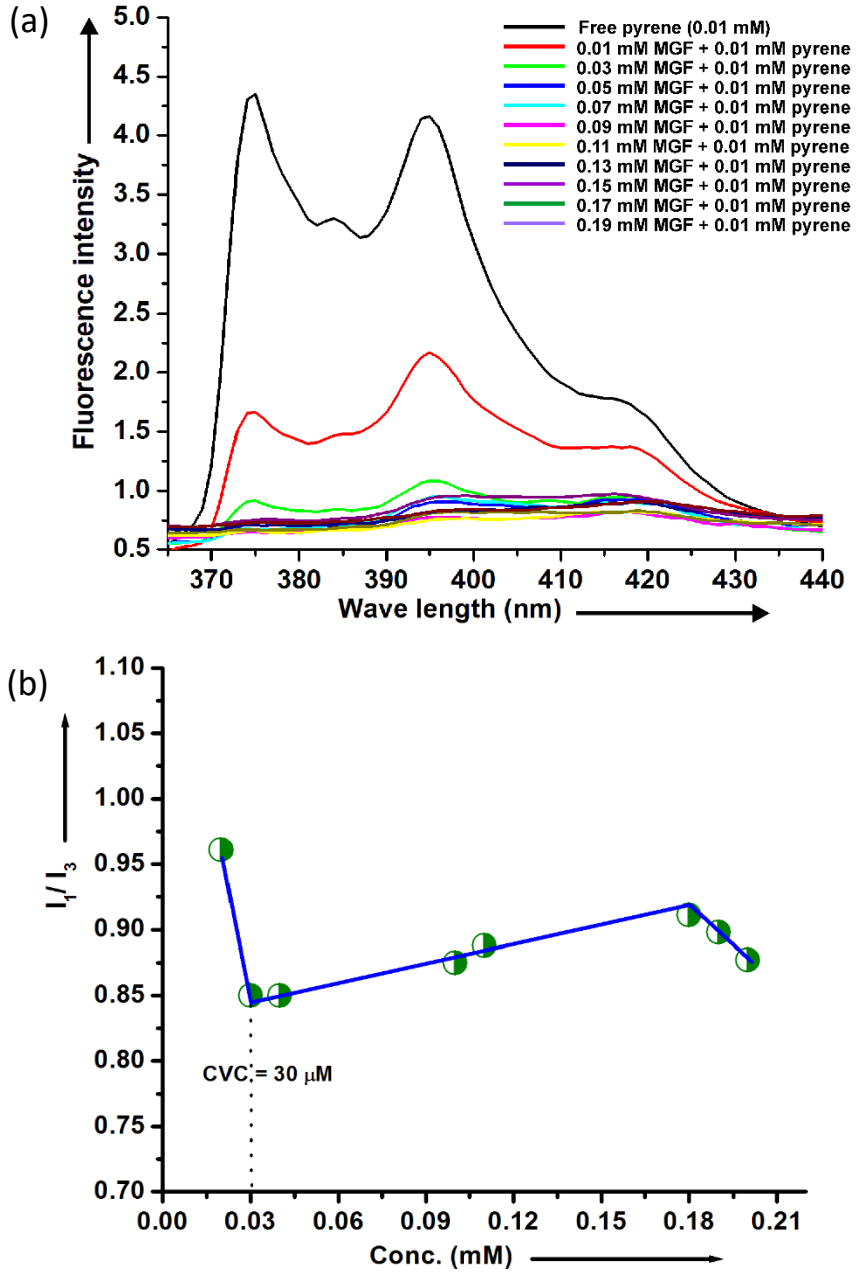

Figure S18: Fluorescence emission spectra of pyrene in water solvent system of mangiferin (a) Fluorescence intensity vs. wave length plot and (b) I_1/I_3 vs. concentration plot, from which the CVC value determined was 30 μM.

6. Utilization of vesicular self-assemblies

6.1. Efficiency (%) of entrapment and release of the dye Rhodamine-B (Rho-B)

Entrapment of the Rho-B was studied by fluorescence emission experiments by a fluorimeter. Gradual decrease in intensities of fluorescence emission ($\lambda_{ex.}$ = 510 nm, λ_{em} = nm) were observed and we measure % of entrapment of Rho-B after 40, 70, 110, and 160 min are 2.9, 19.03, 20.5, 28.8 % respectively. Similarly, a gradual increase in fluorescence emission intensity was observed by treating with Triton-X 100 of the entrapped Rho-B solution and we also measure % of release of Rho-B after 5, 40, 130 and 210 min are 10, 19.3, 23.5, 33.5 % respectively.

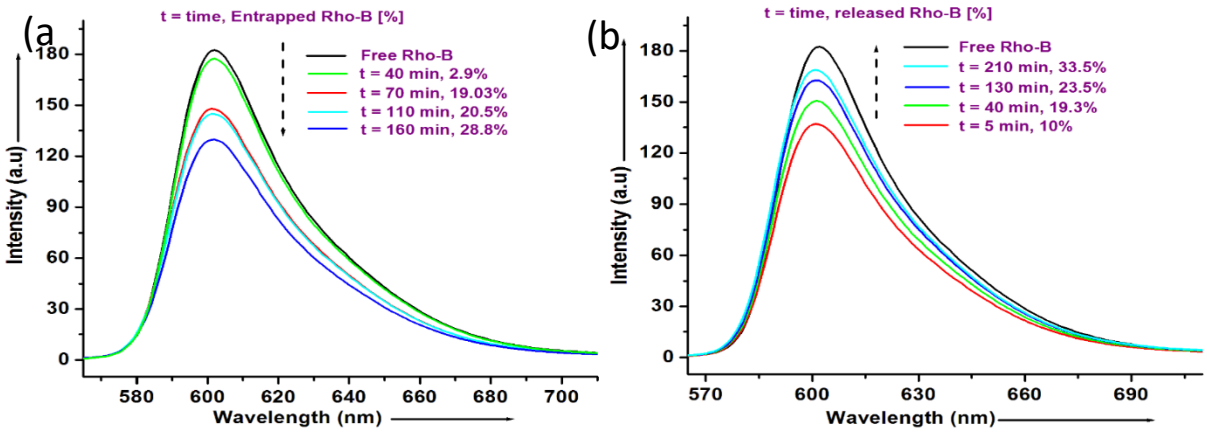

Figure S19: Fluorescence emission spectra (a) Rho-B entrapment efficiency (%) of mangiferin after time intervals 40-160 min is 2.9-28.8%, (b) Rho-B release efficiency (%) of mangiferin after time intervals 5-210 min is 10-33.5%.

6.2. Efficiency (%) of entrapped and released of the anticancer drug doxorubicin

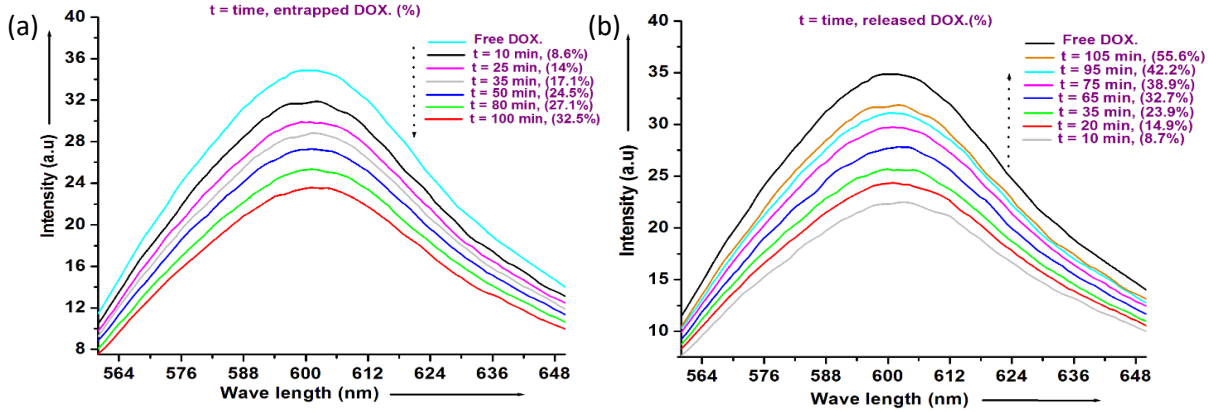

Figure S20: Fluorescence emission spectra ($\lambda_{ex.}$ = 470 nm, λ_{em} = 600 nm): (a) doxorubicin (DOX) entrapment efficiency (%) of mangiferin after time intervals 10-100 min is 8.6-32.5%, (b) Rho-B release efficiency (%) of mangiferin after time intervals 10-105 min is 8.7-55.6%.

6.3. Entrapment study of bioactive compound Curcumin from the vesicular self-assemblies of 1 in DMSO-water (1:1 v/v)

Curcumin has several medicinal activities such as anti-bacterial, anti-viral, anti-cancer, anti-inflammatory, anti-diabetic and antioxidant etc. It is poorly soluble in water and so it has low bioavailability for biological studies. To overcome this problems of low bioavailability and poor absorption of curcumin, we studied the entrapment of curcumin inside the vesicular self-assemblies of mangiferin. Interestingly, curcumin was entrapped inside the vesicular self-assemblies of mangiferin in DMSO-water (1:1 v/v, 17.76 mM) (Figure S18).

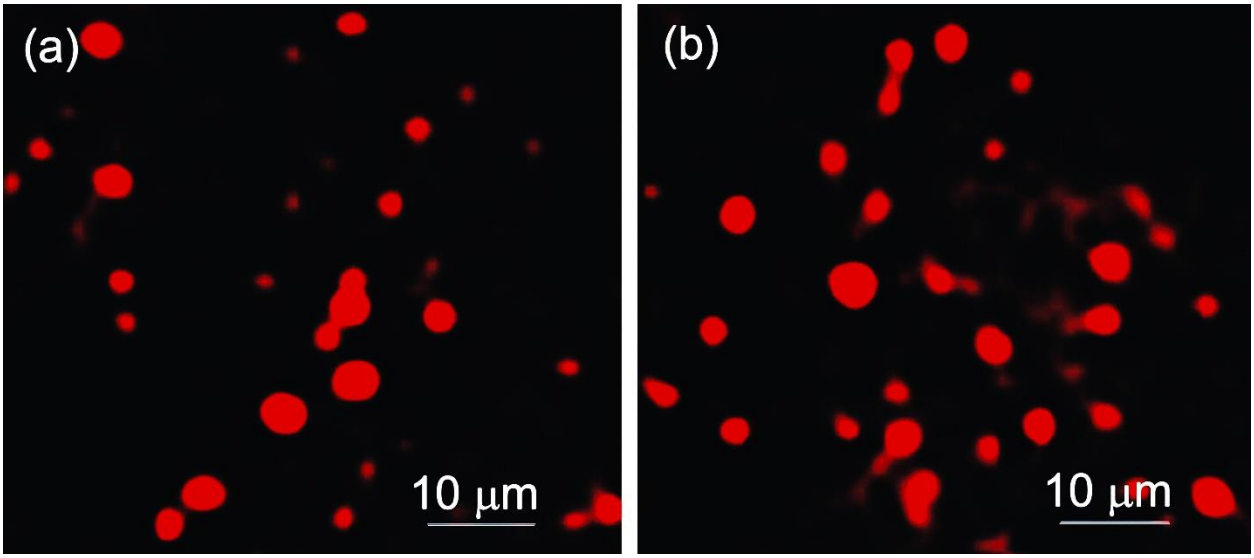

Figure S21: Entrapment of curcumin inside the vesicular self-assemblies of mangiferin (a,b) Fluorescence images of self-assembled mangiferin in DMSO-H_2O (1:1 v/v, 17.76 mM) containing curcumin (1.36 mM) when exposed under green emission light.

6.4. Antibacterial activity Study

We examined the antibacterial activities of curcumin loaded self-assembled mangiferin via zone-inhibition method. First we prepared agar gel on petri dishes. Then bacteria strain applied on it for bacterial growth and four spots are created on this gel plate. Then these spots are filled with 25 μL each of mangiferine (MGF) in water, self-assembled MGF in DMSO-water (1:1), Curcumin in DMSO and self-assembled MGF - Curcumin (1:1) in DMSO-water (1:1) solutions. No antibacterial activities against both for Gram positive bacteria (*Staphylococcus aureus*) as well as Gram negative bacteria (*Esherichia coli*) were observed after 18 hours with pure mangiferine in water medium and self-assembled mangiferine in DMSO-water (1:1) medium at the concentration (1500 μg/mL) but curcumin loaded self-assembled mangiferin showed antibacterial activities. Interestingly, curcumin loaded self-assembled mangiferin showed greater antibacterial activity than only curcumin in DMSO-water (1:1 v/v) solvent system against both for Gram positive bacteria (*Staphylococcus aureus*) as well as Gram negative bacteria (*Esherichia coli*) (Table T2 and Figure S19).

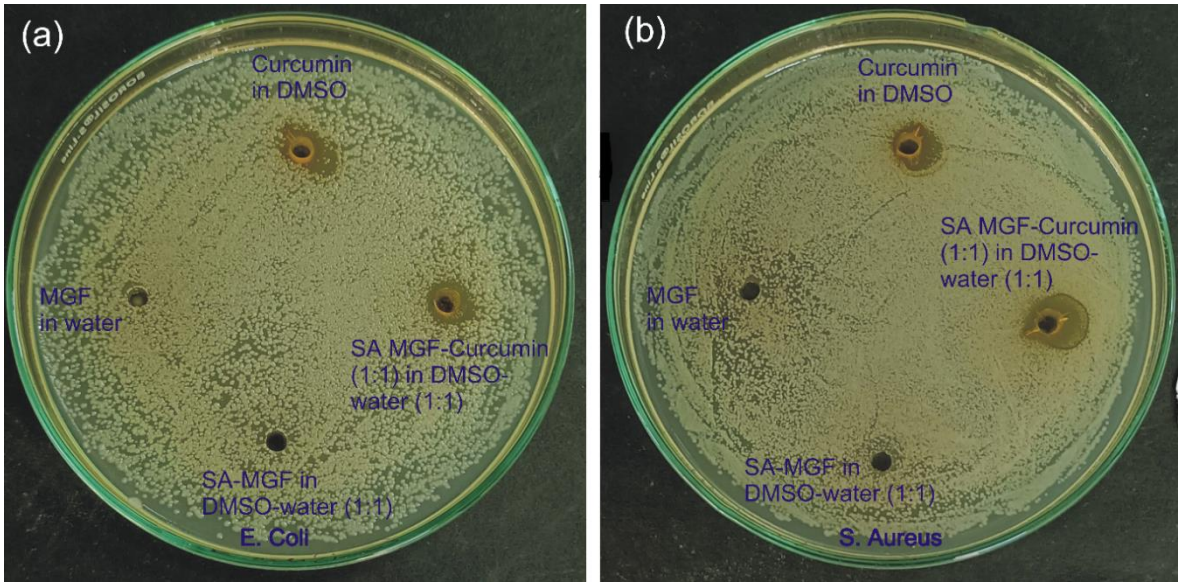

Figure S22: Antibacterial activity studies are carried out with the mangiferine (MGF) in water, self-assembled MGF in DMSO-water (1:1), Curcumin in DMSO and self-assembled MGF - Curcumin (1:1) in DMSO-water (1:1) solutions, both for Gram positive strain *S. aureus* as well as Gram negative strain *E. coli* and inhibition zone observed after 18 hours at molar concentration of 1500 μg/mL.

6.5. Minimum Inhibitory Concentration (MIC) determination

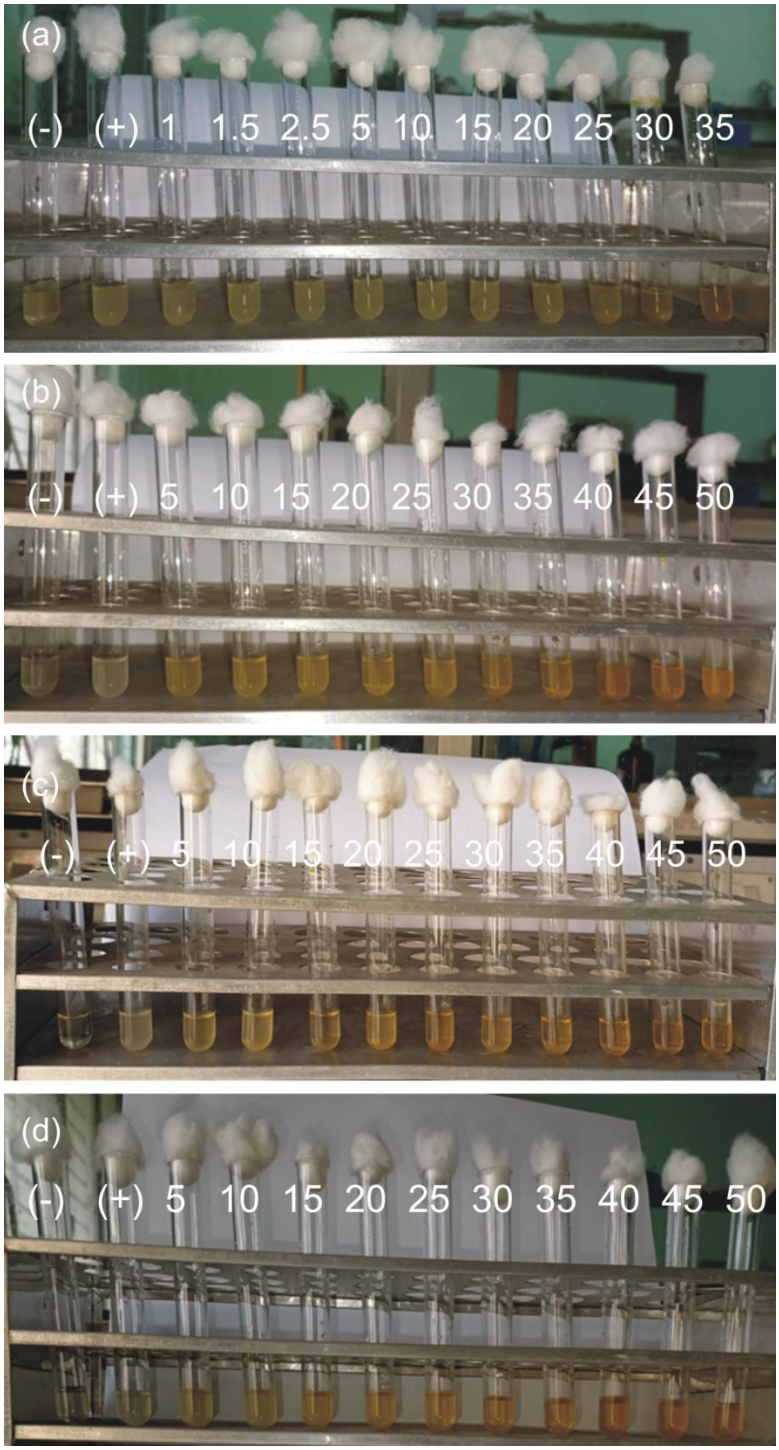

Figure S23: Minimum Inhibitory Concentration (MIC) value of curcumin are (a) 25 μg/mL for *E.Coli*, (b) 30 μg/mL for *S. aureus*. Minimum Inhibitory Concentration (MIC) value of MGF-curcumin (1:1 v/v) are (c) 15 μg/mL for S. *aureus*, (d) 15 μg/mL for *E. Coli*.

6.6. Minimum Bactericidal Concentration (MBC) determination

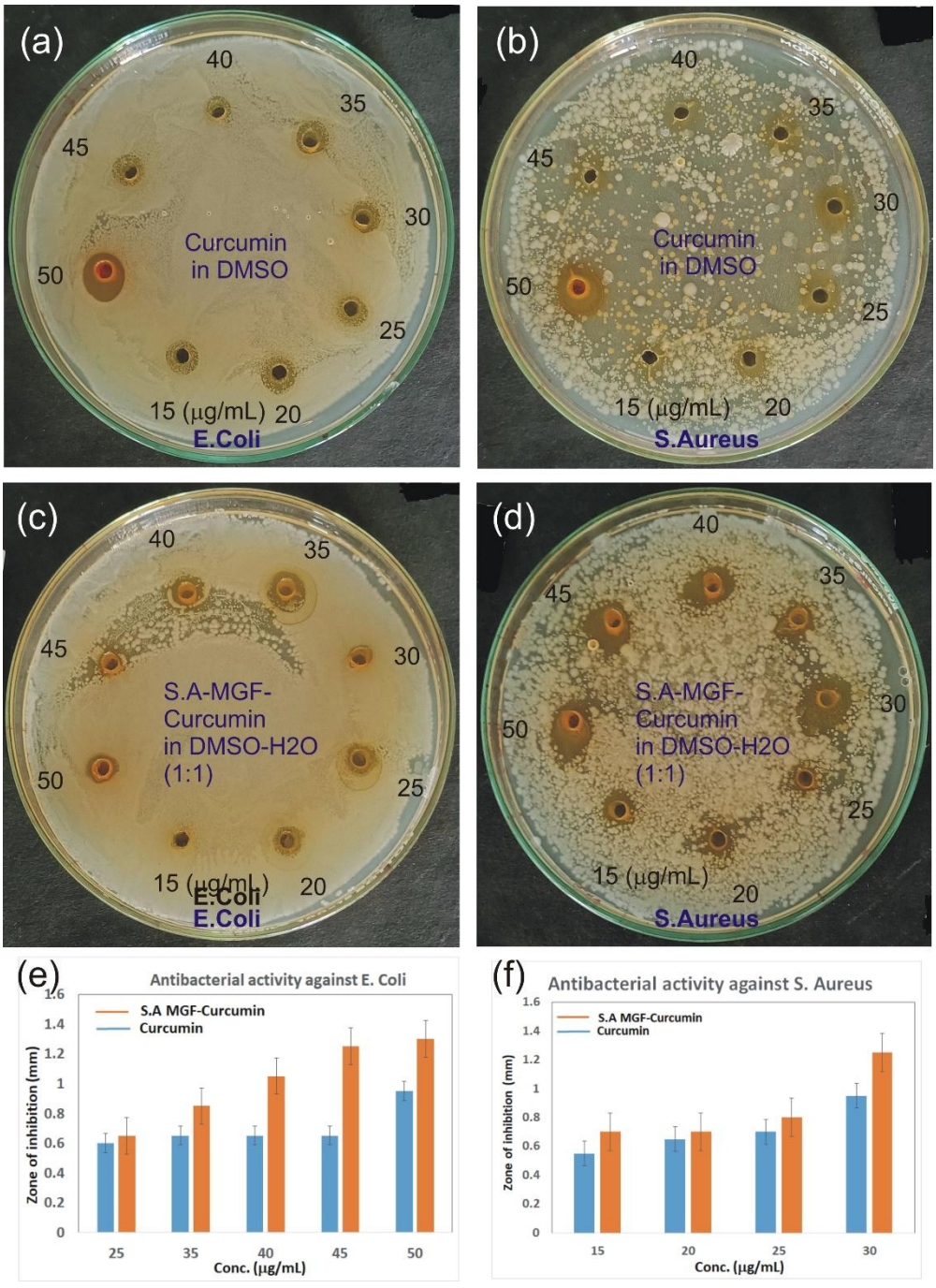

Figure S24: The Minimum Bactericidal Concentration (MBC) value of curcumin are (a) 50 µg/mL for E.Coli strain, (b) 50 µg/mL for S.Aureus strain. The Minimum Bactericidal Concentration (MBC) value of MGF-curcumin (1:1 v/v) are (c) 30 µg/mL for S.Aureus strain, (d) 35 µg/mL for E.Coli strain. (e,f) plots of conc. (µg/mL) vs zone of inhibition (mm) of self-assembled MGF – curcumin and curcumin alone for for *E.Coli* and *S. Aureus* bacteria respectively

7. Experimental Procedure:

7.1 Materials:

The liquid solvents DMSO, THF, DMF are purchased from Merk, EtOH is an analytical laboratory reagents and distilled water (H_2O) are used for the self-assembly studies. The dyes are analytical reagents used for entrapment. The anticancer drug doxorubicin hydrochloride (2% w/v) is used for drug delivery works, purchased from Fresenius Kabi Oncology in India.

7.2 Extraction and Isolation of Mangiferin:

The *Davallia solida* is a fern and it was collected from the Royal Project Foundation, Kasetsart University, Bangkok, Thailand. The dried rhizome of *Davallia solida* (1.19 kg) was soaked in MeOH (9 L x 3) at room temperature. Mangiferin (4.81 g, 3.12% yield) was precipitated during the evaporation of MeOH extracts. The compound was purified by repeated crystallizations from ethanol.

7.3 Identification of Mangiferin by ^{1}H-NMR, ^{13}C-NMR, DEPT-90, DEPT-135 NMR and FTIR spectra Data:

The structure of the compound **1** was identified by Bruker 400 MHz NMR including ^{1}H-NMR, ^{13}C-NMR, DEPT-90, DEPT-135 NMR and FTIR spectroscopy.

7.3.1 ^{1}H NMR (400 MHz, DMSO-d_6):

δ: 13.77 (1-OH, s), 10.45 (6'-OH, w), 7.36 (8-H, s), 6.85 (5-H, s), 6.36 (4-H, s), 4.88, 4.59, 4.56, 3.41, 3.40, 3.38, 3.37, 3.18, 3.16, 2.50, 1.22 ppm (Figure S4).

7.3.2 ^{13}C NMR (100 MHz, DMSO-d_6):

δ: 179.53 (C=O), 164.30, 162.31, 156.67, 154.50, 151.27, 144.24, 112.24, 108.49, 108.08, 103.05, 101.75, 93.75, 82.07, 79.46, 73.53, 71.11, 70.68, 61.79 ppm (nineteen C- atoms) (Figure S5).

7.3.3 DEPT-90 (^{13}C NMR, 100 MHz, DMSO-d_6):

δ: 108.41, 103.03, 93.73, 82.06, 79.44, 73.51, 71.09, 70.64 ppm (eight -CH groups) (Figure S6).

7.3.4 DEPT-135 (^{13}C NMR, 100 MHz, DMSO-d_6):

δ: 108.40, 103.03, 93.73, 82.04, 79.43, 73.50, 71.08, 70.63 ppm (eight -CH groups) (Figure S7). **Up (+):** -CH_3, -CH groups.

δ: 61.95 ppm (one -CH_2 groups) (Figure S7). **Down (-):** -CH_2 groups only.

7.4. ^{1}H NMR spectrum of Mangiferin 1 (400 MHz, DMSO-d_6):

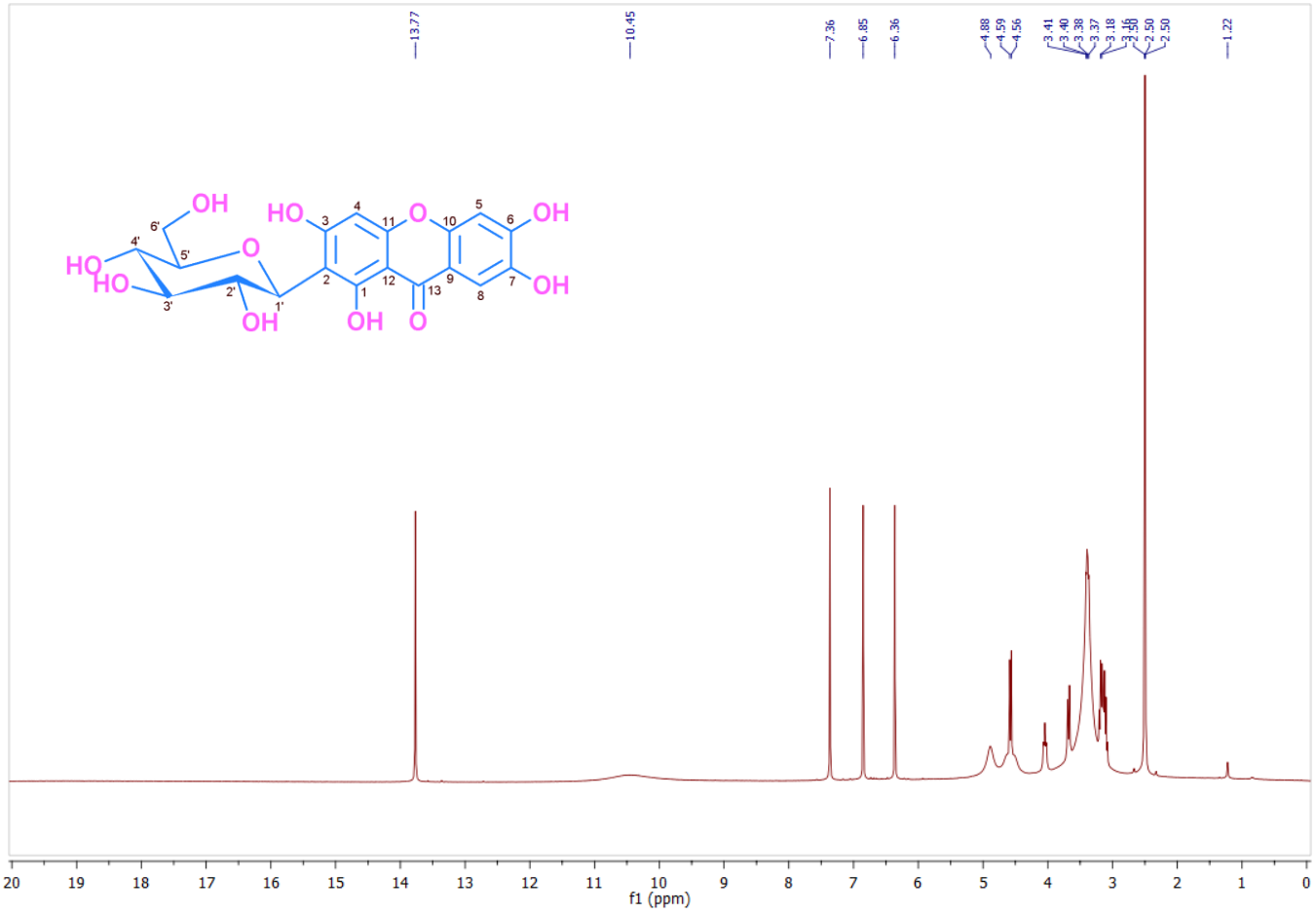

Figure S25: ^{1}H-NMR of Mangiferin (400 MHz, DMSO-d_6).

7.5 ^{13} C-NMR spectrum of Mangiferin (100 MHz, DMSO-d_6):

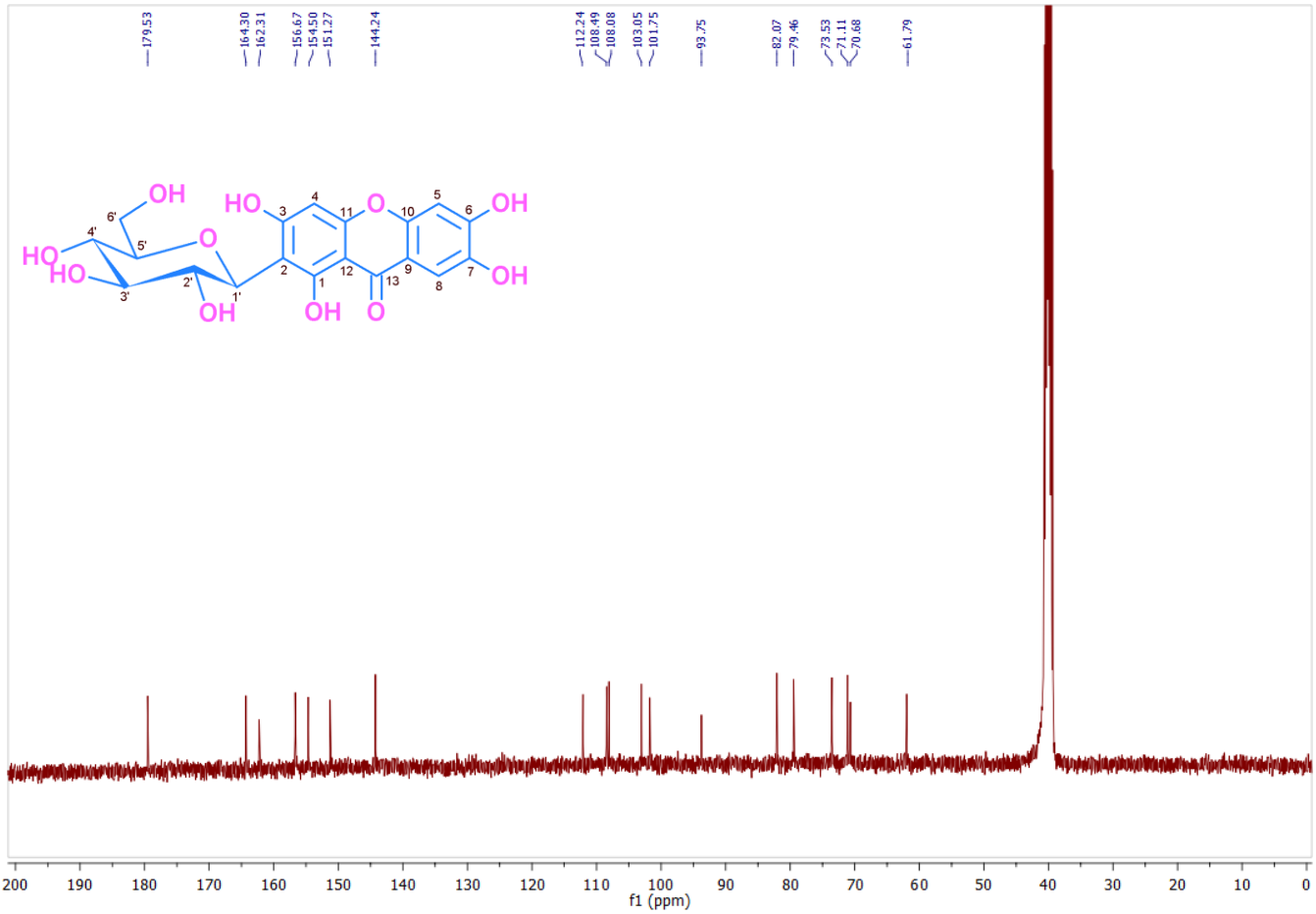

Figure S26: ^{13}C-NMR of Mangiferin (100 MHz, DMSO-d_6).

7.6 DEPT-90 (^{13} C-NMR) spectrum of Mangiferin (100 MHz, DMSO-d_6):

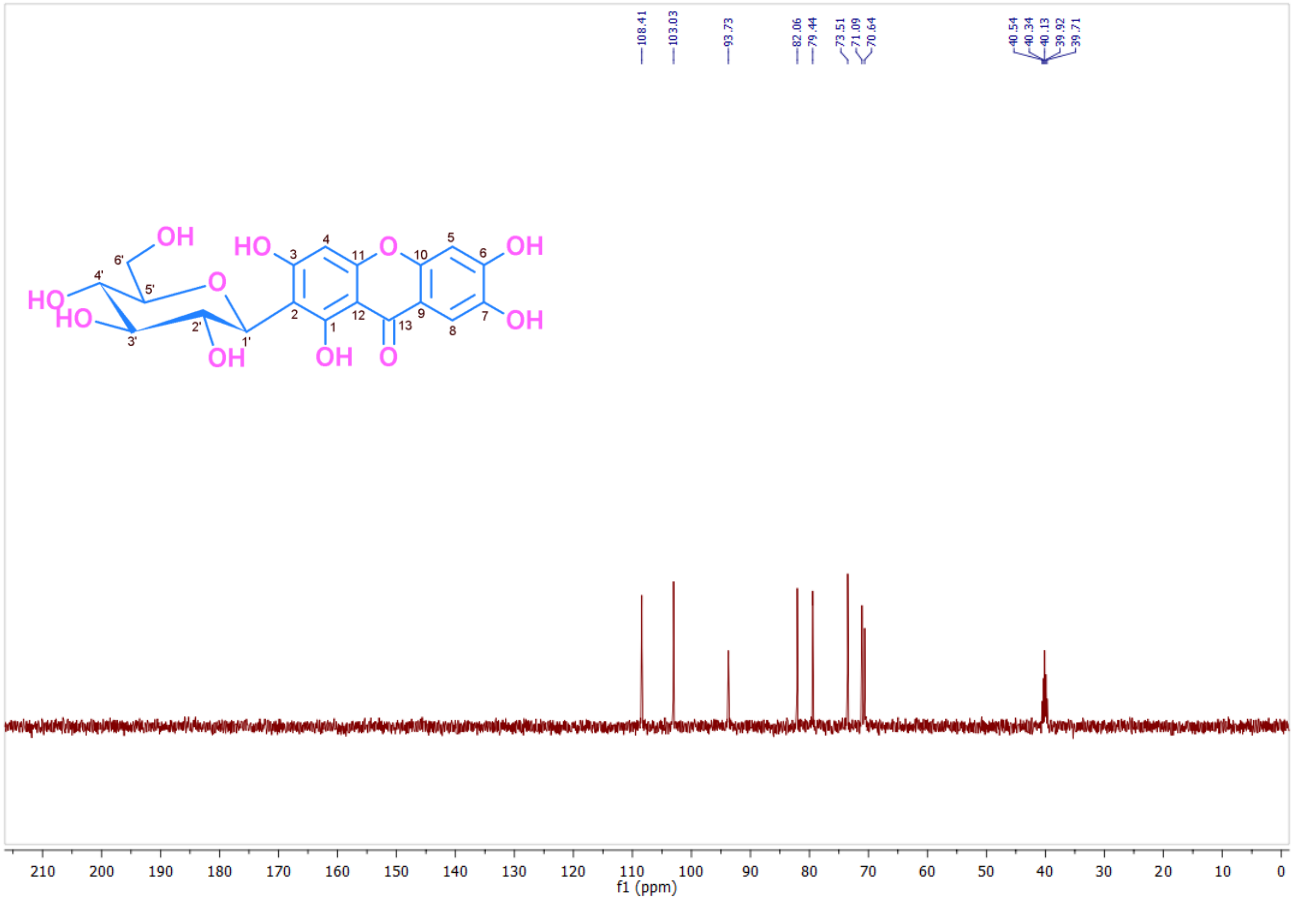

Figure S27: DEPT-90 (^{13}C-NMR) of Mangiferin (100 MHz, DMSO-d_6).

7.7 DEPT-135 (^{13} C-NMR) spectrum of Mangiferin (100 MHz, DMSO-d_6):

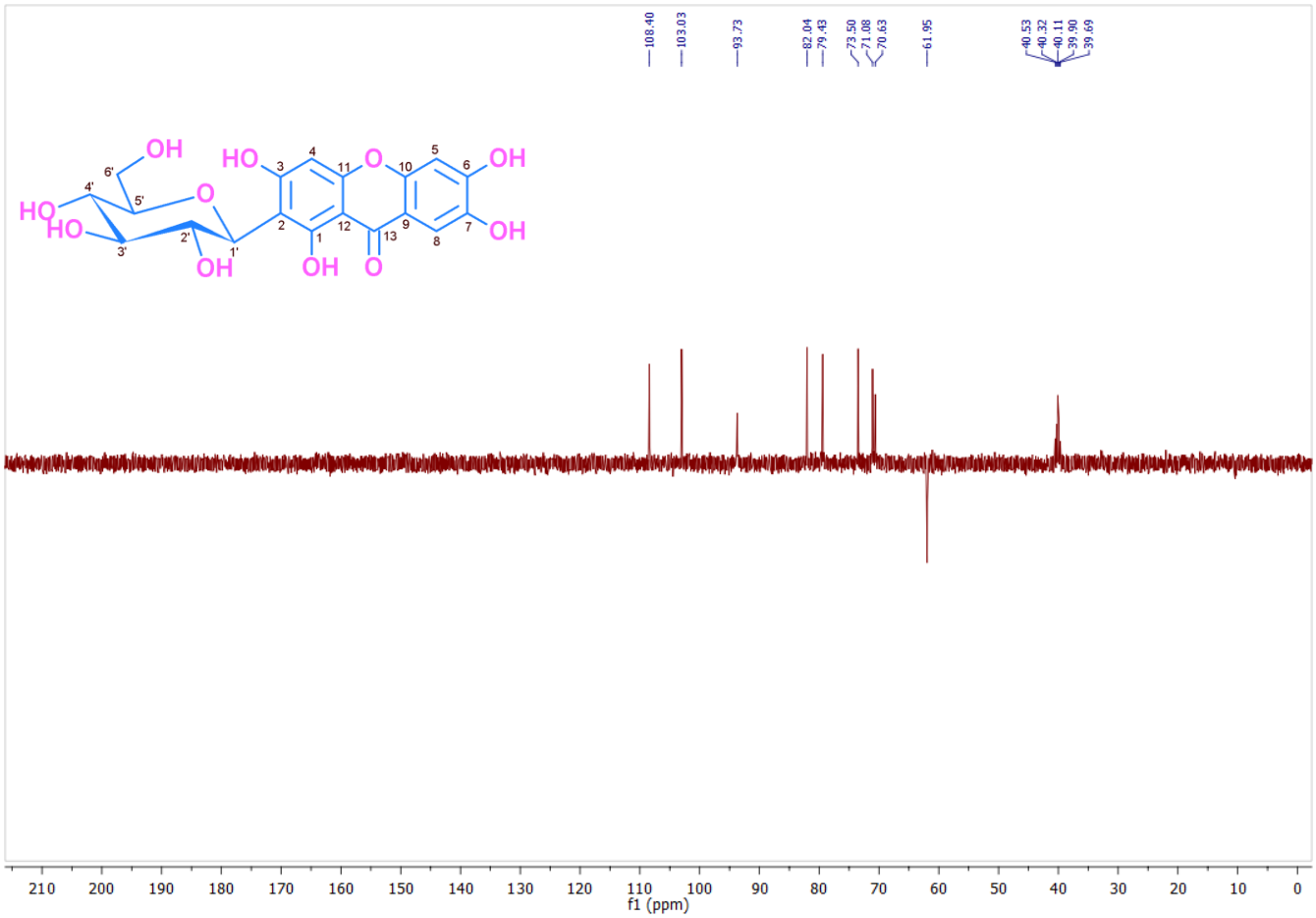

Figure S28: DEPT-135 (^{13}C-NMR) of Mangiferin (100 MHz, DMSO-d_6).