Supplementary Information to the manuscript having the title

Multitargeting Antibacterial Activity of a Synthesized Mn$^{2+}$ Complex of Curcumin on Gram-Positive and Gram-Negative bacterial strains

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The supporting information for this manuscript includes

1) A figure (S1) showing the absorption spectra of Curcumin and the Mn$^{II}$ complex in DMSO.
2) A figure (S2) showing absorption spectra of Mn$^{II}$ interacting with Curcumin in ethanol-water as solvent during determination of stoichiometry of complex formation.
3) A figure (S3) showing the infra-red spectrum of Curcumin.
4) A figure (S4) showing the infra-red spectrum of [Mn$^{II}$(Cur)$_2$(HCur)].
5) A figure (S5) showing TGA of [Mn$^{II}$(Cur)$_2$(HCur)].
6) A schematic diagram (S6) showing the keto-enol tautomerism of Curcumin.
7) A Table (S1) showing the optimized bond lengths for the carbon-oxygen bonds of Curcumin involved in coordination of Mn$^{II}$.
8) A figure (S7) showing degradation of Curcumin and no degradation of the complex as realized from a change in absorbance in the UV-visible region after being taken in PBS buffer or in PBS buffer with 10 μM DTT.
9) A figure (S8) showing degradation of Curcumin and no degradation of the complex realized from a change in absorbance in the UV-visible region after being taken in different bacterial growth medium.
10) Double reciprocal plot for interaction of [Mn$^{II}$(Cur)$_2$(HCur)] with calf thymus DNA. using UV-Vis spectroscopy (Fig. S9).
11) Double reciprocal plot with y intercept = 1 for interaction of [Mn$^{II}$(Cur)$_2$(HCur)] with calf thymus DNA using UV-Vis spectroscopy (Fig. S10).
12) A figure (S11) showing bacterial survival in logarithmic scale to indicate antibacterial efficacy of [Mn$^{II}$(Cur)$_2$(HCur)] and Curcumin on S. aureus and E. coli cells.
13) A figure (S12) showing scanning electron microscope images of S. aureus ATCC 29213 treated with either no compound or with Curcumin, [Mn$^{II}$(Cur)$_2$(HCur)] and gramicidin D to realize membrane permeabilization of S. aureus by the calcein leakage assay.
14) Equations related to the dissociation of the three protons on Curcumin.
15) A figure (S13) showing the spectrophotometric titration of Curcumin followed at 467 nm.
16) Figures showing mole ratio plots (S14A & S14B) and Job’s plots of continuous variation (S14C) for Curcumin with Mn$^{II}$ that were followed at 430 nm.
17) A figure (S15) showing the spectrophotometric titration of Curcumin in the presence of Mn$^{II}$ followed at 430 nm.

18) Equations for the evaluation of stability constant of the complex formed in solution based on the interaction of HCur with Mn$^{II}$ by evaluation of pK$_a$ values of HCur in the absence and presence of Mn$^{II}$.

**Figure S1**

![Absorption spectra for HCur and [Mn$^{II}$(Cur)$_2$(HCur)] in DMSO.](image)

Figure S1: Absorption spectra for HCur and [Mn$^{II}$(Cur)$_2$(HCur)] in DMSO.
Figure S2: Absorption spectra for Mn$^{II}$ interacting with Curcumin in ethanol-water during a stoichiometry determination experiment.

Figure S3: FTIR spectrum of HCur
Figure S4: FTIR spectrum for [Mn^{II}(Cur)_{2}(HCur)]

Figure S5: TGA of [Mn^{II}(Cur)_{2}(HCur)]
Figure S6

Table S1: Optimized bond lengths for carbon-oxygen bonds of Curcumin involved in coordination of Mn$^{II}$.

| Bond Type | Bond Length (Å) |
|-----------|-----------------|
| C16-O2    | 1.3051          |
| C17-O4    | 1.3001          |
| C15-O6    | 1.3193          |
| C12-O5    | 1.3099          |
| C11-O3    | 1.3101          |
| C8-O7     | 1.3096          |
Figure S7: UV-visible spectra of (A) HCur and (B) [Mn^{II}(Cur)_{2}(HCur)] in presence of (i) only PBS buffer (pH 7.4) and (ii) PBS buffer with 10 μM DTT.
Figure S8: UV-visible absorption spectra of (A) HCur and (B) [MnII(Cur)2(HCur)] in different bacterial growth medium (i) BHI, (ii) MHB, and (iii) DMEM.
Figure S9: A double reciprocal plot for the interaction of [Mn\textsuperscript{II}(Cur)\textsubscript{2}(HCur)] with calf thymus DNA leading to the determination of apparent binding constant (K\textsubscript{app}) at pH 7.4 (30 mM phosphate buffer) and ionic strength 0.15 M; [Mn\textsuperscript{II}(Cur)\textsubscript{2}(HCur)] = 40 µM, pH = 7.4; Temperature = 298 K.
Figure S10: Double reciprocal plot for a UV-Vis titration of 40 µM [Mn(II)(Cur)$_2$(HCur)] by calf thymus DNA using phosphate buffer (~pH 7.4) at 298 K.
Figure S11: Antibacterial efficacy of 25 and 50 μM of [Mn$^{II}$\((\text{Cur})_2\)(HCur)]] and HCur in PBS buffer against (A) \textit{S. aureus} and (B) \textit{E. coli} cells (10$^6$ CFU/mL). Grey columns, columns with stripes, and white columns denote the time of exposure (2 min, 60 min, and 120 min, respectively) to the compounds. The data represent mean (± SD) of three independent experiments (*** p ≤ 0.001).
Figure S12: Scanning electron microscope images of *S. aureus* ATCC 29213 treated with 50 μM of HCur, [Mn\textsuperscript{II}(Cur)\textsubscript{2}(HCur)], and 20 μg/mL gramicidin D for 2 hours. (A) Untreated control cells, (B) HCur, (C) [Mn\textsuperscript{II}(Cur)\textsubscript{2}(HCur)] and (D) gramicidin D treated cells.
Equations with regard to the dissociation of the three protons on HCur.

\[
\text{LH}_2H^+ \rightleftharpoons \text{LH}_2^- + H^+ \quad K_1 = \frac{[H^+][\text{LH}_2^-]}{[\text{LH}_2H^+]} \quad (S1)
\]

\[
\text{LH}_2^- \rightleftharpoons \text{L}^{2-} + 2H^+ \quad K_2 = \frac{[\text{L}^{2-}][H^+]^2}{[\text{LH}_2^-]} \quad (S2)
\]

Figure S13

Figure S13: Spectrophotometric titration of HCur as shown by a variation in absorbance at 467 nm; [HCur] = 5 µM, [NaNO\(_3\)] = 0.1 M, Temperature = 305K.

The change in absorbance of HCur at 467 nm was fitted to Eq. S3

\[
A_{\text{obs}} = \frac{A_1}{(1 + 10^{pH-pK_{a1}} + 10^{pH-pK_{a2}} + 10^{pH-pK_{a3}})} + \frac{A_2}{(1 + 10^{pK_{a1} - pH + 10^{pH - pK_{a2}} + 10^{pH - pK_{a3}}})} + \frac{A_3}{(1 + 10^{pK_{a1} - pH + 10^{pK_{a2}} - pH + 10^{pH - pK_{a3}}})} + \frac{A_4}{(1 + 10^{pK_{a1} - pH + 10^{pK_{a2} - pH} + 10^{pK_{a3} - pH}})} \quad (S3)
\]

A\(_1\), A\(_2\), A\(_3\) and A\(_4\) refer to absorbance due to LH\(_2\)\(^+\), LH\(_2\)\(^-\), LH\(_2\)\(^{2-}\) and L\(^{3-}\) respectively while pK\(_{a1}\), pK\(_{a2}\), pK\(_{a3}\) are pK\(_a\) values for the dissociation of three protons on Curcumin (Eqs. S1 and S2).
Experiments to determine stoichiometry of complex formation:

In experiments for mole-ratio and Job’s method of continuous variation appropriate amounts of HCur was mixed with Mn(II) in 10 mL volumetric flasks and after shaking the solution for a constant time of 3 minutes, absorbance was recorded. This was then plotted for all the three types of experiments.

If we consider the metal ion to be M and ligand L, then for our case since there is the formation of a 1:3 metal to ligand complex, sequence of reactions would be

\[
\begin{align*}
M & \quad + \quad L \quad \xrightarrow{\text{e}} \quad ML \quad \text{(1)} \\
ML & \quad + \quad L \quad \xrightarrow{\text{e}} \quad ML_2 \quad \text{(2)} \\
ML_2 & \quad + \quad L \quad \xrightarrow{\text{e}} \quad ML_3 \quad \text{(3)}
\end{align*}
\]

Since each step is an equilibrium step and we are allowing only 3 minutes of shaking time before recording the absorbance using a spectrophotometer it is only likely that for each solution having a certain composition, all species (ML, ML_2 and ML_3) would be present simultaneously. If attainment of equilibrium 1 is fast and other two relatively slow, we should see responses for ML_2 and ML_3. However, if equilibrium 1 is slow we would see responses for ML and ML_2. Sometimes in such cases, we may not see an exclusive response for ML_3 in the time-frame of our analysis but rather the existence of two species say ML_2 and ML_3. However, if one refluxes M and L, taking L in excess, for say 4 to 5 hours, which we did in order to prepare the complex one may get ML_3 exclusively.

Something like this happened for Mn(II)-Curcumin where we got responses both from mole-ratio and Job’s plots for species in between 1:2 and 1:3 (but tending to 1:3). Had equilibrium for
reaction 3 been fast we would have got a response for ML₃ only but probably that was not the case. In the course of our study, when we refluxed Mn(II) and Curcumin for 4 hours, to prepare the complex we obtained a 1:3 Mn(II)-Curcumin complex that provided a molecular ion peak in mass spectrometry corresponding to the molecular weight of a 1:3 species. Here we are providing all figures related to such experiments leading to determination of stoichiometry.

Mole ratio plots where concentration of Curcumin was constant, Mn(II) varied:
1: Absorbance recorded immediately i.e. after mixing for 3 minutes
2: Absorbance recorded after 6 hours from mixing.
3: Absorbance recorded after 24 hours from mixing.

Figure S14A

Figure S14A: Mole-ratio plots showing variation in absorbance at 430 nm for a change in concentration of Mn^{II} for a fixed concentration of HCur = 10 µM; (1) immediately after HCur and Mn(II) were mixed; (2) after 6 hours from the time HCur and Mn(II) were mixed; (3) after 24 hours from the time HCur and Mn(II) were mixed; pH of the medium: ~7.4, [NaNO₃] = 0.01 M, Temperature = 303 K.
Mole ratio plots where concentration of Mn(II) constant, Curcumin was varied:

1: Absorbance recorded immediately i.e. after mixing for 3 minutes
2: Absorbance recorded after 24 hours.

Figure S14B

Figure S14B: Mole-ratio plots showing variation in absorbance at 430 nm for a change in concentration of HCur for a fixed concentration of Mn$^{II} = 10$ µM; (1) immediately after HCur and Mn(II) were mixed; (2) after 24 hours from the time HCur and Mn(II) were mixed; pH of the medium: ~7.4, [NaNO$_3$] = 0.01 M, Temperature = 303 K.
Job’s plots from three separate experiments where both Mn(II) and Curcumin were varied continuously

Figure S14C

Figure. S14C: Plot showing a variation in absorbance at 430 nm for a continuous variation of HCur and Mn$^{II}$ for three different experimental sets, Set 1, Set 2 and Set 3 at pH (~7.4). Strength of stock solutions of Mn$^{II}$ and HCur were 100 µM; [NaNO$_3$] = 0.01 M, Temperature = 303 K.
Figure S15: Titration of HCur performed in the presence of Mn$^{II}$, as shown by a variation in absorbance at 430 nm; [HCur] = 30 µM, [Mn$^{II}$] = 10 µM, [NaNO$_3$] = 0.01 M, Temperature = 305 K.

$$A_{\text{obs}} = \frac{A_1}{(1 + 10^{pH - pK_{a1} + 10pH - pK_{a2}})} + \frac{A_2}{(1 + 10^{pK_{a1} - pH + 10pH - pK_{a2}})} + \frac{A_3}{(1 + 10^{pK_{a1} - pH + 10pK_{a2} - pH})}$$ (S4)

$A_1$, $A_2$ and $A_3$ are absorbances due to LH$_2$H*, LH$_2^-$ and L$^-$ respectively in the presence of Mn$^{II}$.

$$\text{Mn}^{2+} + 3\text{LH}_2\text{H}^* \rightleftharpoons [\text{Mn(LH}_2\text{)}_3]^− + 3\text{H}^{\text{I}+}$$ (S5)

$$\beta^* = \frac{[\text{Mn(LH}_2\text{)}_3][\text{H}^{\text{I}+}]^3}{[\text{Mn}^{2+}][\text{LH}_2\text{H}^*]^3}$$ (S6)

or, $$\text{Mn}^{2+} + 3\text{LH}_2^- \rightleftharpoons [\text{Mn(LH}_2\text{)}_3]^−$$ (S7)

$$\beta = \frac{[\text{Mn(LH}_2\text{)}_3]}{[\text{Mn}^{2+}][\text{LH}_2^-]^3}$$ (S8)

$$\beta = \frac{\beta^*}{K_1^{-3}}$$ (S9)

LH$_2$H* represents HCur; $K_1$ is the dissociation constant of the enolic-OH proton of HCur.