Index No : 234/18/Chem./26

Curcumin and its transition metal complexes: Synthesis, characterization and investigation of their antibacterial activity through model DNA binding experiments coupled with an *in silico* molecular modeling approach

Abstract

Curcumin is a polyphenolic compound found in the rhizomes of Curcuma longa, recognized as a yellow species, turmeric. It has attracted a substantial amount of interest owing to several pharmacological qualities it possesses, like that of anti-inflammatory, antioxidant and anticancer activities. However, the potential of this substance to operate as an antibacterial agent, particularly as metal complexes is not yet fully explored. It was demonstrated through this study that metal complexes of Curcumin have improved stability, solubility and bio-availability, all of which have the potential to boost antibacterial activity. As a part of this study, some metal complexes of Curcumin were synthesized, characterized and antibacterial activity was evaluated on gram positive and gram negative bacteria. In addition, *in silico* modeling studies were conducted to compare results with *in vitro* investigations.

Over the last four decades, researchers have been involved in carrying out studies to learn about effects of Curcumin in pharmacology. Their focus on this pigment went beyond its function as a food colorant to be used against multiple chronic diseases and also as a chemo-preventive agent. Although identified for the first time in powdered rhizome of *Curcuma longa* Linn in 1815, until the 1970s, there was little progress related to research with it pertaining to its structure and activities as an antioxidant. However, as time passed, the science associated with Curcumin became popular and it is now a topic of discussion in different branches of chemistry (analytical, inorganic, organic and physical).

Owing to its hydrolytic instability and aqueous insolubility, Curcumin is poorly bio-available; a major obstacle for achieving its full therapeutic potential. Researchers have shown that under physiological conditions it breaks down in solution into several smaller fragments. Hence, from the pharmacological reports available on Curcumin, it becomes extremely difficult to realize whether these are due to Curcumin present as a single unit or a collective effect of all fragments formed from it in solution. Most researchers concentrating on the pharmacological or medicinal aspects of the molecule have remained largely silent on this important issue.

In an attempt to overcome its inherent limitations, three metal complexes of Curcumin, one with Mn(II), another with Cu(II) and a third with Zn(II) having formulae $[Mn^{II}(Cur)_2(HCur)]$, $[Cu^{II}(Cur)(OCOCH_3)(OH_2)]$ and $[Zn^{II}(Cur)(OCOCH_3)(OH_2)]$ respectively were synthesized. Physico-chemical studies performed in solution using manganese(II) chloride, copper(II) acetate and zinc(II) acetate with Curcumin indicate formation of a 1:3 Mn^{II}:Curcumin, a 1:2 Cu^{II}:Curcumin and 1:2 Zn^{II}:Curcumin species. However, in case of Cu^{II} and Zn^{II}, attempts to synthesize complexes based on physicochemical studies always led to formation of 1:1 Cu^{II}: Curcumin and 1:1 Zn^{II}:Curcumin species, when Cu^{II}-acetate and Zn^{II}-acetate were used. UV spectra of prepared complexes were recorded in DMSO. λ_{max} was obtained at 400 nm for Mn^{II}, at

428 nm for Cu^{II} and at 426 nm for Zn^{II} complexes respectively. Curcumin itself has a λ_{max} at 432 nm. Mass spectrum of the complexes were recorded. These indicate formation of a 1:1 species for Cu^{II} and Zn^{II} and a 1:3 species for Mn^{II}. Thermo-gravimetric analysis also support the results of mass spectra. Since single crystals were not obtained, structure of all the said complexes was obtained through quantum chemical calculations. TDDFT was also done to forecast the electronic structure of the complexes using Gaussian software.

Before proceeding with *in vitro* studies, it was important to check whether the complexes were stable in various biological milieu and/or buffer medium. The synthesized Mn(Cur), Cu(Cur) and Zn(Cur) were found to be stable in different buffer systems and in different biological milieu than when Curcumin is present alone. Curcumin degrades in 20 minutes.

Hence, with such improved stability in biological milieu, obtained as a part of this study, all the complexes were tried for their DNA binding ability showing an effective binding with calf thymus DNA. It was found that Cu(Cur) had a reasonably strong affinity for DNA, that was realized from its binding constant value recorded at different temperatures.

Metal complexes exhibit potent and significant enhancement in activity over that of Curcumin when tried on *E. coli* and *S. aureus. These were carried out in the laboratory of* Prof. Kasturi Mukhopadhyay at Jawaharlal Nehru University (JNU), New Delhi. *Bacterial killing assay showed that when targets were exposed to 25 μM metal complexes for 60 minutes around 97-98% killing was achieved while for the same concentration and exposure time, Curcumin could kill only 36-37%. On increasing concentration to 50 μM, complexes were able to kill approximately 99% cells in 60 minutes while a similar concentration of Curcumin could only kill 66-67% in that time. All complexes showed significant improvement in antibacterial activity in comparison to Curcumin.*

Calcein leakage assay also performed through a collaboration in the laboratory of Prof. Kasturi Mukhopadhyay at JNU New Delhi revealed complexes triggered immediate membrane permeabilization in *S. aureus*. Excellent potency of complexes was supported by their safe toxicological profile, being non-hemolytic and non-cytotoxic towards mammalian cells.

In silico molecular docking and molecular dynamics simulation support the interaction of said complexes with DNA, *E. coli* and *S. aureus*. With that aim in mind, the investigation of FTSz inhibiting activity was checked. Protein of *E. coli* and *S. aureus* were taken from the RCSB protein data bank and confirmed by docking studies. Calculations were performed using Autodock 4.2 and Autodockvina algorithm and it was confirmed that in all cases, the prepared complexes showed more negative binding energy than Curcumin. To check the stability of docked complexes over time molecular dynamics was performed with the help of Desmond - a Linux based software. Results were analysed through RMSD, RMSF, ROG, SASA, H-Bond and MMGBSA.

Therefore, this study provides a scientific basis for the development of Curcumin based metal complexes as effective antibacterial agents. Given the rise of antibiotic-resistant bacteria, novel antibacterial agents are a need of the hour. The findings with regard to metal complexes of Curcumin presented in this dissertation might contribute to new therapeutic strategies against bacterial infections, that might help to advance the field of medicinal chemistry.

Tanmoy Saha
(Signature of the Candidate)
27/12/2024

Saurabh Das 27.12.2024
(Signature with Seal of the Supervisor)

Dr. Saurabh Das
Professor
Department of Chemistry