VHNSN COLLEGE (AUTONOMOUS), VIRUDHUNAGAR– 626 001

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PG & RESEARCH DEPARTMENT OF CHEMISTRY

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NOTIFICATION OF ONLINE Ph.D PUBLIC VIVA- VOCE EXAMINATION

As per the regulations of Madurai Kamaraj University, Madurai, **Mrs. J. PORKODI (P4630),** Assistant Professor of Chemistry, S. F. R. College for Women, Sivakasi will defend her thesis at a Public Viva-Voce Examination through **Video Conference mode** using **Google Meet Platform**.

Title of the Thesis

CURCUMIN BASED MIXED-LIGAND COMPLEXES: SYNTHESIS, PHYSICO-CHEMICAL CHARACTERIZATION AND BIOLOGICAL STUDIES

Date & Time

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Department of Chemistry (MBA LAB), VHNSN College (Autonomous) Virudhunagar-626001

Video Conference Platform

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The Synopsis of the thesis is available in the College Website and a copy of the thesis is available in the Department Library, for reference. Faculty members, Scholars and Students are most welcome to attend the Viva-Voce Examination and take part in the discussion.

ALL ARE CORDIALLY INVITED

Place: Virudhunagar
Date: 27.10.2020

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CURCUMIN BASED MIXED-LIGAND COMPLEXES: SYNTHESIS, PHYSICO-CHEMICAL CHARACTERIZATION AND BIOLOGICAL STUDIES

SYNOPSIS submitted to Madurai Kamaraj University in partial fulfillment for the requirements of the Degree of

DOCTOR OF PHILOSOPHY IN CHEMISTRY

By

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DEPARTMENT OF CHEMISTRY
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CURCUMIN BASED MIXED-LIGAND COMPLEXES: SYNTHESIS, PHYSICO-CHEMICAL CHARACTERIZATION AND BIOLOGICAL STUDIES

Ancient human civilisation and culture uses food as medicine and not medicine as food. They depend on mineral, plant and animal products from the nature and utilize them as food and drugs. The compounds which are obtained from the plant origin have more potential and also they are promising alternatives for synthetic chemicals for the discovery of novel scaffolds with various biological activities. One such easily available and naturally available compound is curcumin. From the Vedic period itself it is widely used as curry species, dye, cosmetic and medical applications. It is also used in the treatment of flatulence, dyspepsia, liver disorders (jaundice), common colds, eye and ear infections, small-pox, chicken pox and above all a variety of skin diseases and inflammatory conditions. It is safer upto a higher dose level also. But the insolubility of curcumin in water makes it absorption poorly inside the gastrointestinal tract. Also its rapid metabolism and systemic elimination limits its biological action. Consequently abundant research has been done to improve its pharmacokinetic properties. Bioinorganic chemists utilise it as a chelating ligand because of the presence of β-diketo group in it so that it can easily forms Schiff bases with active amine moieties. These curcumin Schiff base and its metal chelates approach eradicates the bioavailability problem and to attain yet new potential health benefits.

From the prehistoric period, gold, silver and copper metals are used in medicine as antimicrobial agents. These metal ions easily involve in coordination of various organic moieties and they are essential for the existence and maintenance of plants and animals (chlorophyll, haemoglobin cytochrome *etc*). This kind of transition metal complexes with heterocyclic ligands are developed as chemotherapeutic agents because of their ability to interact with DNA molecules. Some metal chelates possess remarkable antitumour, antifungal, anti-inflammatory, antiviral and antidiabetic activities. This chelation therapy has paved way for chemotherapy by adopting different tactics and pharmacological strategy which reveal important prospects for the exploitation of metal complexes as drugs and presenting a budding field for medical bioinorganic chemistry.

At present, in numerous inorganic pharmaceuticals they are used as drugs against an assortment of diseases, ranging from antibacterial and antifungal to anticancer applications. The recent epoch of metal-based complexes like cisplatin, carboplatin, oxaliplatin, satraplatin, nedaplatin, lobaplatin *etc.,* is successful against various types of cancer (ovarian, head and neck, lung, testicular and bladder cancers) but with lot of side effects. Therefore, these compounds are needed to be modified to circumvent the toxicity and to enhance their efficiency in biological systems.

Currently, the development of new drugs depends mainly on the modern technology like high-throughput selection, combinatorial chemistry, QSAR, docking, *etc*., by using computers and software (*in silico)* to reduce the time and cost. This also provides valuable information about target molecules, lead compounds, screening and optimization of the drug like molecule. This type of screening technique is very useful before the clinical trials in drug discovery process. Due to the ample array of biological applications of curcumin derivatives, this thesis mainly concentrate on the curcumin Schiff base transition metal complexes of Co(II), Ni(II), Cu(II), Zn(II) and VO(IV) with bioactive co-ligands from plant origin. These metal complexes have been synthesized and characterised by various spectral analyses. *In vivo* and *in vitro* biological screening studies have been carried out based on the *in silico* results. Also from the DNA interaction studies, these metal complexes can be developed as lead compounds for clinical trials.

The current thesis has been split into seven chapters. The essences of these chapters are portrayed hereunder.

Chapter I

General introduction

This chapter emphasizes a general introduction of Schiff bases and their metal complexes and their roles in medicinal field. A brief introduction about the importance of drugs derived from the biological origin is also discussed. Moreover, it also encompasses the role of metal ions and metal complexes derived from curcumin in biological systems. The survey of earlier work in this field covers the period from sixties to the beginning of 2019. Furthermore, fundamental structure of DNA and its different binding approaches like

intercalation and groove binding are also discussed. It highlights the significance of cheminformatics in the process of drug discovery. This chapter mainly concerned with the metal complexes of curcumin Schiff bases containing 4-aminoantipyrine with VO(IV), Co(II), Ni(II), Cu(II) and Zn(II) metal ions and their DNA interaction, antimicrobial, antioxidant, anti-inflammatory and anticancer studies. The justification of the choice, scope and objective of the present work are given at the last part of the chapter.

Chapter II

Materials and methods

This chapter deals with the general experimental techniques, analytical procedures, spectroscopic methods such as IR, NMR, EPR, Mass, UV-Vis., and electrochemical methods used in this research work. It also displays the experimental procedures for DNA binding and cleavage, molecular docking, *in vitro* antimicrobial, cytotoxocity, antidiabetic and antioxidant activities and *in vivo* anti-inflammatory and antidiabetic activities. Moreover, it deals with the steps to predict the biological activity and pharmacokinetic properties of the Schiff base and its metal complexes using PASS online, VLS3D and SWISS ADME online softwares.

Chapter III

Synthesis, characterization and biological screening studies of mixed ligand complexes using the flavonoid moieties

In the present chapter, a new series of transition mixed ligand complexes of Co(II), Ni(II), Cu(II) and Zn(II) were synthesized by incorporating curcumin Schiff base formed by the condensation reaction of curcumin with 4-aminoantipyrine (L_1) and chrysin flavonoid as co-ligand (L_2). The structural features of the synthesized complexes had been explored by elemental analyses, UV-Vis, IR, NMR, Mass, TGA, EPR spectral analyses and conductivity measurements. The low molar conductance values (10-16 $\Omega^{-1}cm^{-2}$ mol^{-1}) indicate the non-electrolytic nature of the synthesized complexes. UV-Vis spectroscopy and magnetic susceptibility values reveal the octahedral nature of the synthesized complexes. From the elemental analyses and mass spectra of the ligands and their complexes, the stoichiometry of the complexes is found to be $[ML_1L_2H_2O]$. The

monomeric nature of the complexes is confirmed by their magnetic susceptibility and EPR spectral data. The presence of water in the coordination site is confirmed by endothermic peak at 176-300.18°C in differential scanning calorimetry.

The binding mode between the synthesized complexes and CT DNA is examined using electronic absorption titration, viscosity measurements, cyclic voltammetry and molecular docking. Due to the damage of the double helical structure of CT DNA by the metal complex, hyperchromic bands are appeared during the successive addition of 30 μL of CT DNA with the metal complexes. This confirms the groove mode of binding between the CT DNA and metal complexes. Blue shifts are shown by all the metal complexes in the range of 3-7 nm. Among all the complexes, $[CuL_1L_2H_2O]$ complex has higher K_b value ($2.2 \times 10^{-4}\ M^{-1}$) which reveals that it has a strong binding efficiency towards the CT DNA. The binding mode is also examined by the CV behaviour of the metal complexes in DMSO solution which is encountered in the presence and absence of the CT DNA with all the synthesized metal complexes. This study also confirms the groove mode of binding between the metal complexes and CT DNA by the decrease in anodic and cathodic peak current in CV analysis as well as the E_{pc} and E_{pa} values which are moved towards the negative side. This is again verified with viscosity measurements and molecular docking data.

Moreover, all the synthesized complexes have good potential to cleave the circular plasmid pUC19 supercoiled DNA efficiently in presence of H_2O_2. *In silico* biological activity score for the ligand was predicted using PASS online software. The data imply that the anti-inflammatory activity of the curcumin derived Schiff base L_1 (0.86 pa) is higher when compared to that of curcumin (0.45 pa). ADMET properties were studied by VLS3D online software. It is predicted that curcumin derived Schiff base is readily absorbed in Human intestine (HIA-0.9890 pa value) and moderate Caco-2 permeability (0.5857 pa value). It also has the capacity to penetrate the BBB (0.5519 pa value). It is distributed in mitochondria. It is metabolized by CYP4503A4 enzyme. It has low AMES toxic value and it is non–carcinogen.

In vivo anti-inflammatory activity of the ligand L_1 and metal complexes was examined by carragenan induced mice paw edema inhibition method and antioxidant

activities have been screened by DPPH assay. These examinations prove that the theoretical predictions are agreed with the experimental results. MIC values of the synthesized complexes reveal that the complexes have better antimicrobial efficacy than the ligands. The outcomes of all the studies suggest that the copper complex possesses potent activity than the free ligands. This study aids to assess the potentiality and effectiveness of newer mixed ligand complexes from biological origin to use as effective DNA probes and drug like molecule. In conclusion, the significant results achieved from all the experiments demonstrate that the existence of metal ion along with the bioactive ligands plays a very important role in the pharmaceutical behaviour of the complexes, thereby addressing their application as effective biomarkers.

Chapter IV

Synthesis, characterization and biological screening studies of mixed ligand complexes using curcumin Schiff base and quercetin

This chapter deals with the potential of curcumin Schiff base ligand (L_1) and its complexes and accentuates higher biological activity by introducing quercetin as a co-ligand (L_2) in the metal complexes. The octahedral geometry of the synthesized complexes had been confirmed by elemental analyses, UV-Vis, IR, NMR, Mass, TGA, EPR spectral analyses and conductivity measurements. Molar conductance values (10-18 $\Omega^{-1}cm^{-2}$ mol^{-1}) indicate the non-electrolytic nature of the synthesized complexes. The UV–Vis spectral transitions and the magnetic moment values corroborate the octahedral environment around the metal ion. From the IR and ^{13}C NMR data, the involvement of C=O functional group from ligand L_1 and L_2 and C=N from L_1 in the coordination with the metal ion is confirmed. ^{1}H NMR confirms the coordination of enolic OH group from ligand L_1 and 5^{1} phenolic OH from ligand L_2 to the central metal ion. The presence of water in the coordination site is confirmed by thermogravimetric analysis and differential scanning calorimetry. From the EPR data, it is calculated that $A_{\parallel}$ (180) > $A_{\perp}$ (113.5). The axially symmetric g-tensor with $g_{\parallel}$ (2.182) > $g_{\perp}$ (2.026) proves the existence of $d_{x^2-y^2}$ as ground state in the copper complex. This is due to the octahedral geometrical characteristic of $[CuL_1L_2H_2O]$ complex.

The interaction between the synthesized complexes and DNA is examined using complementary techniques like electronic absorption titration, viscosity measurements, cyclic voltammetry and molecular docking. The synthesized complexes interact with CT DNA through groove binding mode with the binding constant values of 2.75×10^{-4}, 2.09×10^{-4}, 1.75×10^{-4}, 1.2×10^{-4} M^{-1} for Cu(II), Zn(II), Ni(II) and Co(II) complexes respectively. This result is also confirmed by viscosity measurements, docking analyses and CV studies also.

All the synthesized complexes are effective in the cleavage of the circular plasmid pUC19 supercoiled DNA efficiently in presence of H_2O_2. In accordance with the theoretical data from the PASS online biological activity prediction software, anti-inflammatory and antioxidant activities have been experimentally validated which prove that these complexes possess higher activity when compared to the mixed ligand complexes synthesized in chapter III. This is due to the presence of more number of OH groups compared to chrysin. MIC values of the synthesized complexes reveal that the complexes have better antimicrobial efficacy than the ligands. The outcomes of all the binding and cleavage studies suggest that the copper complex possesses good interaction with DNA than the free ligands and the other complexes. These complexes may have further extent in developing them as DNA probes. In this chapter, the effect of increasing hydroxyl substituents in the side chain of flavonoids is noted.

Chapter V

Synthesis, characterization and biological screening studies of mixed ligand complexes incorporating lawsone as co-ligand

The chapter-V discusses the synthesis, characterization, DNA interaction, *in vivo* and *in vitro* studies of a new series of transition mixed ligand complexes of Co(II), Ni(II), Cu(II) and Zn(II) which were synthesized by using curcumin Schiff base (L_1) and lawsone as co-ligand (L_2). The design and synthesis of these complexes have been investigated with the aim of increasing stability and planarity of the co-ligand, thereby exploring their DNA binding affinity and other biological applications. The physicochemical studies and the spectral data reveal the octahedral geometry of the synthesized complexes. The lower molar conductance values of the synthesized complexes

indicate their non-electrolytic nature. The monomeric nature of the complexes is revealed by their magnetic susceptibility values and EPR spectral data. The presence of water in the sixth coordination site is corroborated by 30% weight loss in the range of 176-300^{0}C which is attributable to the loss of coordinated water in thermogravimetric analysis and an endothermic band observed in respective DTA curve in the temperature region 176- 300^{0}C.

The interaction between the synthesized complexes and CT DNA is scrutinized by adopting different techniques *viz.,* electronic absorption titration, viscosity measurements, cyclic voltammetry and molecular modelling studies. Interestingly all the synthesized complexes interact with CT DNA through groove binding mode like distamycin, mitomycin, netripsin and spermine. Among all the complexes, copper complex has effective binding with CT DNA (binding constant of 3.5×10^{-4} M^{-1}). All the complexes display minor increase in the relative viscosity of CT DNA compared to ethidium bromide, also suggesting primarily groove binding nature of the complexes. This binding nature is also confirmed by CV studies and molecular docking studies.

Moreover, all the synthesized complexes have good potential to cleave the circular plasmid pUC19 supercoiled DNA efficiently in presence of H_2O_2. Zinc complex disturbed the pUC19 DNA into open circular form, whereas Cu(II), Co(II) and Ni(II) metal complexes damaged the pUC19 DNA into nicked and linear forms. Cytotoxicity examination is carried out by using *Artemia salina* and it is found that all the complexes possess higher cytotoxic effect than the ligand. The copper and zinc complexes have significant cytotoxicity activity with LC_{75} values 1.135×10^{-4} and 1.167×10^{-4} M^{-1}/mL respectively. Anti-inflammatory activities have been studied to validate the theoretical prediction. Among all the tested compounds, the copper(II) complex showed the highest anti-inflammatory activity with 80.1% inhibition and is noted to be significantly more potent than felbinac (62.44%) but quit comparable to clinically used drug ibuprofen (81.5 %). Antimicrobial and antioxidant activity studies also confirm the higher biological character of the synthesized metal complexes. The outcomes of all the studies suggest that the copper complex possesses potent anti-inflammatory activity than other complexes. This is due to the effect of co-ligand with more planarity that has higher biological impact on the metal complexes which is also examined.

Chapter VI

Biological screening studies of mixed ligand vanadyl complexes derived from co-ligands of plant origin

Over the past few decades, several biological effects of vanadium metal complexes have been studied for their insulin-mimetic action both amelioration of hyperlipidemia and hypertension. It also has the tendency to influence various enzymatic systems, like phosphatases, ATPases, peroxidases, ribonucleases, protein kinases and oxidoreductases respectively. This chapter deals with the synthesis of mixed ligand vandyl complexes from curcumin Schiff base and co-ligands *viz.,* quercetin/chrysin/lawsone which are of plant origin. The octahedral geometry of the synthesized complexes is confirmed by various analytical and spectral techniques like elemental analyses, magnetic susceptibility, molar conductivity measurements, UV-Vis, FT-IR, EPR and mass. The non-electrolytic nature of the synthesized complexes is confirmed by low molar conductance values and their magnetic susceptibility. From the EPR data, it is evaluated that $A_{\|}$ (150.5, 147.8 and 146.7) > $A_{\perp}$ (47, 49 and 44). The axially symmetric g- tensor with $g_{\|}$ (1.975, 1.983, 1.968) > $g_{\perp}$(1.946, 1.956 and 1.924) which proves the existence of unpaired electron in t_{2g} orbital (d_{xy}) as ground state in the oxovanadium complex. The g values of all the complexes are between 1.9 and 2.0. This is due to the octahedral geometrical characteristic of the synthesized oxovanadium complexes.

The binding efficiency of all the metal complexes has been prospected by electronic absorption titrations, electrochemical titrations, viscosity measurements and molecular docking studies. All the synthesized complexes interact with CT DNA through groove binding mode with binding constant values of 1.8×10^{-5}, 1.5×10^{-5} and 0.8×10^{-5} M^{-1}. Gel electrophoresis examination reveals that the synthesized complexes are efficient metallonucleases in the presence of H_2O_2. These vanadyl complexes are screened for cytotoxicity activity using *Artemia Salina* and it is found that all the complexes possess higher cytotoxic effect than the ligand. *In vivo* (strip method) and *in vitro (*α-amylase inhibition assay) diabetic analyses are examined for all three vanadyl complexes, which exhibit higher activity than the respective ligand and mimic the standard drug. SWISS ADME online software is used to predict the pharmacokinetic behaviour of the synthesized

molecules. Based on the PASS online results, *in vivo* anti-inflammatory activity studies are carried out in *albino* rats which reveal that the synthesized complexes are better candidates for lead discovery. Antimicrobial activity studies also reveal that the synthesized complexes have higher competency than the ligands. The outcomes of all the studies suggest that similar to copper complexes, vanadyl complexes too possess potent activity.

Chapter VII

Summary and future scope

This chapter portrays the summary of the thesis and pave a way for the future scope of the work. The present work mainly concentrated on the synthesis of mixed ligand transition metal complexes derived from the curcumin Schiff base and different bioactive co-ligands *viz*., chrysin, quercetin and lawsone. From the knowledge of the literature survey, it is found to be the first report on the *in vitro* and *in vivo* examination of the mixed ligand metal complexes from curcumin derivatives. All the synthesized compounds are characterised by elemental analyses, magnetic susceptibility, molar conductivity measurements, and UV- Vis, FT-IR, EPR, TGA and mass spectral data. The DNA binding efficiency of the metal complexes is studied by electronic titrations, viscosity measurements, CV studies and molecular docking studies. Moreover, the preliminary anticancer properties of the metal complexes are examined by the cleavage of pUC19 DNA.

Before starting to investigate the biological screening studies, *in silico* analysis is carried out on the curcumin Schiff base by PASS online and VLS3D software. Based on the results obtained from the *in silico* analysis, *in vivo* and *in vitro* examinations of the synthesized compounds are done. Amid all, copper complex derived from curcumin Schiff base and lawsone co-ligand has higher DNA binding ability and anti-inflammatory properties. Similarly, the vanadyl mixed ligand complex derived from the curcumin Schiff base and lawsone co-ligand has similar antidiabetic activity of the standard glibenclambide. This is due to the planarity and aromaticity of the lawsone moiety. Also the copper complex having quercetin co-ligand possesses higher antioxidant and antimicrobial property. This is due to the presence of more number of OH groups in its structure. This contemporary work clearly shows that the metal complexes from biological origin will be

used as lead molecules for antimicrobial, anti-inflammatory and antidiabetic activities after further evaluating them in experimental animals and clinical trials.