Synthesis, Characterization and Antimicrobial studies of some transition metal complexes derived from Curcumin and 2-Aminopyrimidine

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Abstract

Schiff bases are an important class of compounds in medicinal and biological field. However various studies have shown that schiff bases derived from biologically active ingredients present in medicinal plants are more effective in biological and medicinal activity than in its pure form. In this paper Zn(II), Cu(II), Ni(II), Co(II) and Mn(II) complexes were synthesized from schiff bases derived from curcumin (a yellow bioactive component extracted from curcuma longa) and 2-Aminopyrimidine.The ligand and their complexes were characterized by powder XRD,SEM-EDAX, and Antioxidant studies. Also the synthesized ligand and metal complexes have been screened for their antimicrobial activity against E.coli, S.aureus, B.subtilis, P.aeruginosa, K.pneumoniae and against various fungi like A.flavus and P.notatum. Results reveals that the complexes showed enhanced activity than their corresponding ligand.

Keywords : Curcumin, 2-Aminopyrimidine, Transition metal complexes, Antioxidant activity, SOD etc.

1. INTRODUCTION

During the past three decades considerable attention has been paid to the chemistry of complexes of the schiff base containing nitrogen and other donors [1]. Schiff bases play an important role in coordination chemistry as they easily form stable complexes with most transition metal complexes. Schiff bases derived from an amino and carbonyl groups are an important class of compounds that contains azo-methine nitrogen ie; > C = N linkage which is essential for biological activity[2]. Curcumin or 1, 7-bis (4-hydroxy-3 methoxyphenyl)-1, 6-heptadiene-3, 5-dione or Feruloyl methane, the main bioactive component of turmeric (Curcuma longa) has been known to have the medicinal activity, since ancient times and this molecule has been the object of several investigations in the field of biological medicine such as antioxygenation, antibiosis, anti-tumour, herbicidal activities and Alzhimer's prevention[3]. It exhibits a wide spectrum of pharmacological effects including anticarcinogenic, antiinflammatory, antimicrobial activities [4-6], most of which are attributed to its antioxidant and free radical scavenging properties[7-8]. It is known that, pyrimidine moiety is present in nucleic acids, several vitamins, coenzymes and antibiotics which act as valuable substrates in the synthesis of antitumour agents, many of which exhibit useful biological activities and clinical applications[9-10]. The present investigation deals with the synthesis of metal complexes of Zn(II), Cu(II), Ni(II), Co(II), and Mn(II) with the schiff base derived from Curcumin and 2-Aminopyrimidine. The synthesized ligand and their metal complexes were characterized by SEM-EDAX and powder XRD. The synthesized compounds were studied for their antioxidant activity by DPPH method. Also the ligand and their metal complexes have been screened for their antimicrobial activities against the selected bacteria and fungi using the disc diffusion method.

2. EXPERIMENTAL

2.1 Materials and Methods

All the chemicals and solvents used in the preparation of ligands and their metal complexes were of A.R grade. Curcumin and 2-Aminopyrimidine were purchased from Sigma-Aldrich. Metal salts like Zn(II), Cu(II), Ni(II), Co(II) and Mn(II) chlorides and the solvents were purchased from Merck.

2.2 Synthesis of Schiff base ligand [4,4'-(1E,3Z,5Z,6E) -3-hydroxy -5- (pyrimidin-2-ylimino) hepta-1,3,6-triene-1,7-diyl) bis (2-methoxy phenol]

Curcumin (0.005 mol, 1.8445g) was dissolved in 20 ml methanol and stirred well at room temperature. Then methanolic solution of 2-Aminopyrimidine (0.005 mol, 0.4755g) was added to the prepared curcumin solution. The obtained orange coloured mixture was stirred and refluxed at 60^0C in presence of catalytic amount of glacial acetic acid (1-2 drops) for about 6 hrs. After cooling, the resulting orange fine precipitate was filtered and washed well with distilled ethanol repeatedly to remove any unreacted chemicals. The obtained orange crystals were then dried at room temperature.

2.3 Synthesis of Schiff base metal complexes.

To the hot solution of schiff base ligand (0.005 mol) in methanol (20ml) was added a hot methanolic solution (10ml) of respective metal chlorides (0.0025 mol) drop by drop in 2:1 (ligand: metal) molar ratio. P^H of the solution was maintained just below the value of hydrolysis of the metal ion using alcoholic ammonia. The reaction mixture was magnetically stirred and refluxed for 4 hrs at 60^0C. The coloured precipitate was filtered and washed by cold ethanol to remove the residue reactants. Finally the obtained powder was dried to get the complex.

2.4 Determination of antimicrobial Activity

2.4.1. Test organisms:

The in-vitro biological activity of the schiff base and its metal complexes in DMSO were tested against the bacterial species such as Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, Bacillus subtilis and fungal species like Aspergillus flavus and Pencillium notatum by Kirby Bayer disc diffusion method [11] using nutrient agar as medium.

2.4.2. Experimental methods:

The test organisms were grown on nutrient agar medium in petri plates. The compounds were dissolved in DMSO solution and soaked in filter paper disc of 6mm diameter and1mm thickness. The discs were placed on a previously seeded petri plates and incubated at 37^0C and the diameter of inhibition zone around each disc was measured after 24hr for bacterial and fungal species. The inhibition zone was developed at which the concentration was noted and the results were recorded. From the results, the activity index was calculated using the formulae.

Activity Index (AI) = Inhibition zone of the sample/ Inhibition zone of the standard.

2.5 Antioxidant assay (DPPH scavenging activity)

The antioxidant activity of the synthesized curcumin derivatives was evaluated using the DPPH (1,1-Diphenyl-2-picryl-hydrazyl) free radical scavenging assay[12]. DPPH scavenging is considered as a good in-vitro model and is widely used to assess antioxidant efficacy[13]. 100 g/ml of the test sample solution was added to 4ml of 100M methanolic DPPH at various concentrations (20, 40, 60, 80 g). After stirring, the mixture was incubated for 20 min at room temperature and the absorbance at 517 nm was measured. Ascorbic acid (100 g/ml) was used as the standard. A blank was prepared without adding standard or test compound (95% methanol). Lower the absorbance of the reaction indicates higher the free radical scavenging activity. The capability to scavenge the DPPH radical were calculated using the equation,

$$\% \text{ of inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of test compounds[14].

3. RESULTS AND DISCUSSIONS

The condensation of curcumin with 2-amino-pyrimidine give the schiff base 4,4'-(1E, 3Z, 5Z, 6E) -3-hydroxy-5-(pyrimidin-2-ylimino) hepta-1, 3, 6-triene-1,7-diyl) bis (2-methoxy phenol). The ligand L_1 which coordinated with Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+} and Mn^{2+} ions separately to give coloured complexes M_1, M_2, M_3, M_4 and M_5 respectively. The schiff base ligand L_1 and its metal complexes are stable at room temperature and soluble in almost all organic solvents like DMSO and DMF.

3.1. X-Ray Diffraction analysis

X-ray diffraction studies of curcumin were investigated from the angle of 10^0 to 80^0. The powder XRD patterns of L_1 and M_2 are recorded in the range 2θ = 0-80 A^0 were shown in fig: 1.

The average crystalline size d_{XRD} of the complexes was calculated using Scherrer's formula[15],

$$d = 0.89\lambda/\beta\cos\theta),$$

where'd' is the average crystalline size of the phase under investigation. 'λ' is the wavelength of X-ray beam used. 'β' is the full width at half maximum of diffraction and 'θ' is the Bragg's angle. From the observed XRD patterns, the average crystalline size for the ligand L_1 and M_2 are found to be 54.0 nm and 26.47 nm respectively. After complexetion, the particle size decreases. This suggests that the ligand and the complexes are nanocrystalline in nature.

3.2 SEM – EDAX Analysis

Morphology of synthesized ligand and complexes were characterized by SEM analysis. SEM images of ligand L_1 and M_2 were shown in fig:2. SEM picture of the metal complexes shows that the particles are agglomerated with controlled morphological structure and the presence of small grains in non-uniform size. After agglomeration, SEM image of compounds shows irregular shaped grains with elongated morphology and increased particle size. The SEM-EDAX images of L_1 and M_2 were shown in Fig: 3. The results of Energy Dispersive X-ray analysis (EDAX) data reveals the purity of the complex which indicates that there is no elemental contamination present in the complex. The % content of elements in the complex is C (74.15), O (22.86), Cl(1.70) and Cu (1.29) respectively.

3.3 Antioxidant activity

Antioxidant activity evaluation of ligand and its complexes was measured in terms of decreases in absorbance at 517 nm of DPPH methanolic solution (0.1 mmol) produced by the effect of each compound as a result of their ability to donate a hydrogen giving to the reduced form of DPPH radical. The reducing abilities of the synthesized compounds were determined by their interaction with the free radical DPPH at 20 mg concentrations for 15 min. This investigation indicates that there is a greatest possibility of finding potent antioxidants. The antioxidant activity of ligand and metal complexes are given in table 1. The ligand (L_1) and its Cu complex (M_2) have exhibited very good free radical scavenging activity.M_3 and M_4 complexes were shown moderate activity. M_1 and M_5 showed less activity compared to standard. This study determined the antagonistic activity of the complexes when compared to ligand. The bar graph representation of percentage of free radical scavenging activity is shown in fig: 4.

3.4 Antimicrobial activity

The in-vitro biological screening effects of the investigated compounds were tested against various bacterial species like E.coli, S.aureus, K.pneumoniae, P. aeruginosa,

B.subtilis and fungal species like A.flavus and P.notatum. Streptomycin is used as +ve standard for antibacterial and Flucanazole for antifungal studies. The presence of clear zones noted that the compounds were active.

The antimicrobial activities of ligand and its metal complexes are shown in fig.5-6. Antimicrobial results showed that all the synthesized compounds possess biological activity. Comparative study of ligand and its metal complexes showed moderate to better antibacterial activity[16].The increased activity of metal complexes may be considered due to chelation of metal ions with schiff base which enhanced lipophilicity due to delocalization of pi-electrons over the whole chelate ring[17]. These increased lipophilicity enhances the penetration of complexes into the lipid membranes and blocks the meta binding sites in enzymes of microorganisms. These complexes also disturb the respiration process of the cell and thus block the synthesis of proteins, which restricts the growth of organisms. This data revealed that the activity of the ligand enhanced on complexetion but less than the standard used [18]. Overall comparison of observed data gives information that metal complexes are more active than free ligand against all bacteria. M_2 showed 15mm zone of inhibition against Streptococcus mutans. All other complexes show moderate activity against all other bacterial species. L_1 and M_4 show good activity against S.mutans but no activity against B.subtilis. Results reveals that Cu complex, M_2 showed good antibacterial activity against all bacterial species. The orders of activity of synthesized compounds are as follows.

Cu(II) > Zn(II) > Ni(II) > Co(II) > Mn(II). Also M_3, M_4 and M_5 complexes showed good antifungal activity against Pencillium notatum but moderate activity against Aspergillus flavus. M_1 and M_2 have no antifungal activity against A.flavus and P. notatum when compared with standard flucanazole. M_5 shows excellent activity against p.notatum and can be tested for further in-vivo studies and can be used as drug.

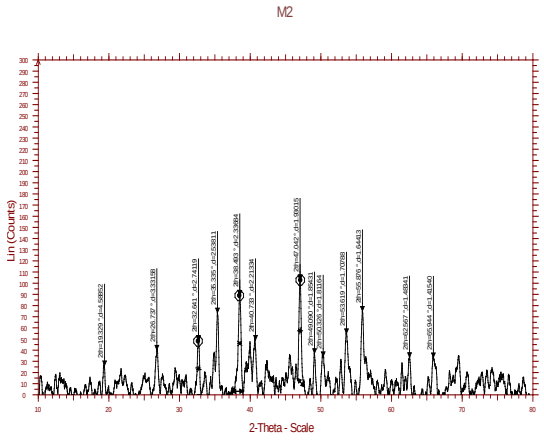


Fig.1 : Powder XRD pattern of ligand of L_1 and M_2 complex

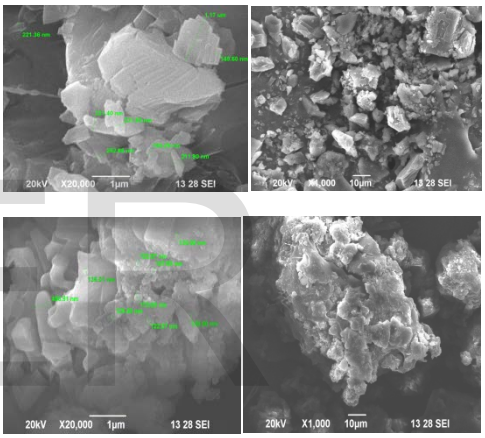
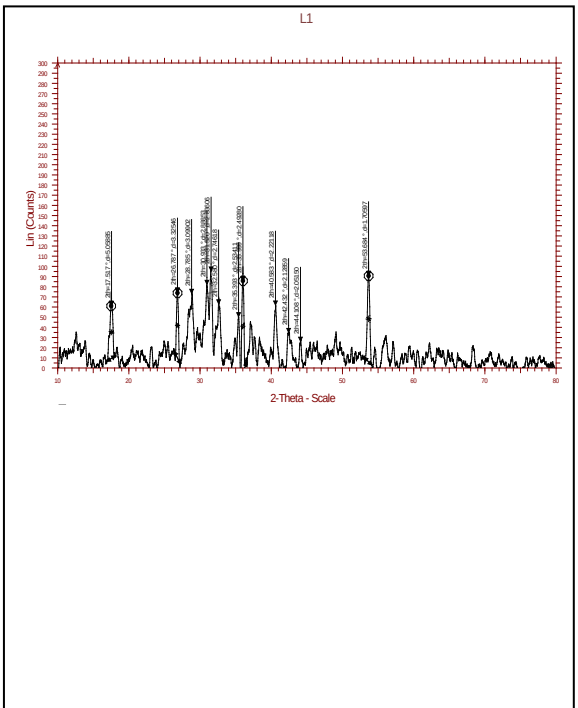
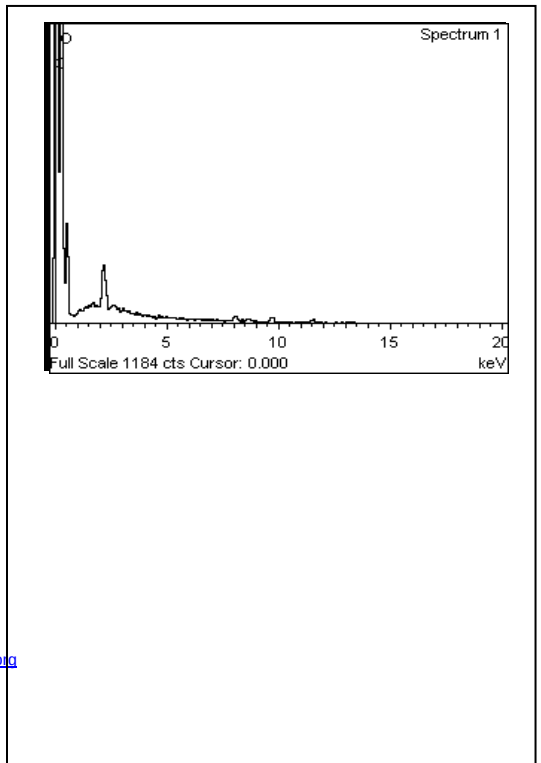


Fig.2 SEM images of L_1 and M_2 complex





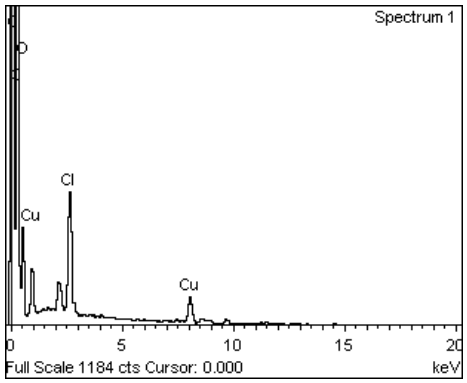


Fig.3 EDAX spectrum of L_1 and their metal complex

TABLE 1: DPPH ASSAY OF L_1 AND ITS COMPLEXES

S.No.	Compounds	% of Inhibition (mg/ml)
Control	-	100
1	L_1	94.2
2	M_1	42.2
3	M_2	87.7
4	M_3	80.1
5	M_4	71.9
6	M_5	36.5

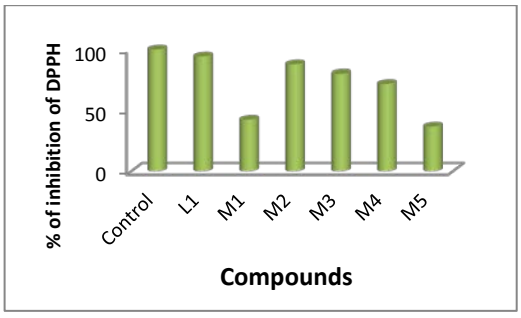


Fig.4 Bar diagram representation of antioxidant activity

Fig.5 Inhibition zone against screened bacteria and fungi by the ligand and complexes

TABLE 2: ANTIMICROBIAL ACTIVITY OF L_1 AND THEIR METAL COMPLEXES

Ligand/ Complexes	Antibacterial activity						Antifungal activity	
	E. coli	*S. aureus*	*P.aeruginosa*	*S.mutans*	*B.subtilis*	*K.pneumoniae*	A.flavus	*P.notatum*
L1	8	8	9	10	-	8	-	-
M1	9	8	9	9	10	-	-	-
M2	7	9	8	15	8	8	-	-
M3	9	8	8	9	8	11	9	11
M4	8	10	9	10	-	7	8	10
M5	7	8	11	9	10	8	9	18
Streptomycin	14	14	20	21	21	22		
Flucanazole							19	18

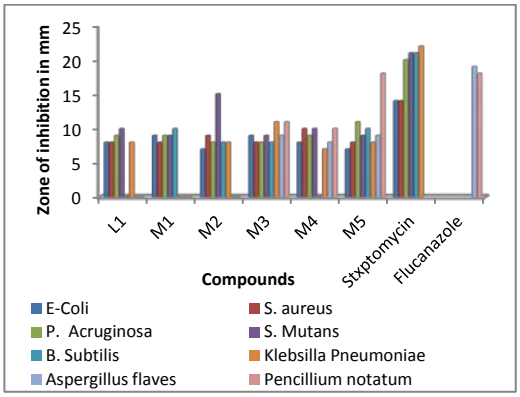


Fig.6 Antimicrobial activities of L_1 and their metal complexes

4. CONCLUSION

In this study, a schiff base ligand (Curcumin and 2-Aminopyrimidine) was synthesized. They formed stable complexes (2:1) with transition metal ions such as Zn(II), Cu(II), Ni(II), Co(II) and Mn(II).The ligand and its complexes were stable at room temperature and are completely soluble in almost all organic solvents. The synthesized compounds were characterized by XRD and SEM-EDAX analysis. They are also tested for antioxidant and antimicrobial activities. The XRD and SEM analysis explains the crystalline structure of the compounds. EDAX studies gives information about metal purity and elemental composition. Antioxidant studies reveal that most of the synthesized compounds have potential antioxidant activity. Antimicrobial study showed that all the complexes were moderately active against the tested organisms. Comparatively Cu complex, M_2 shows higher antibacterial activity than all other complex which is due to its higher lipid solubility. Also M_5 complex shows higher antifungal activity against P.notatum and can be used as drugs after invivo studies.

5. REFERENCES

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