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Antibacterial and Antioxidant Properties of Mn (II), Co (II), Ni (II) and Zn (II) Complex of Schiff base derived from Cephalexin.

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ABSTRACT

In the present study, Schiff bases have been synthesized by the condensation of cephalexin with p-chlorobenzldehyde and cinnamaldehyde respectively in methanol. Further their metal complexes have been synthesized by metal salts of Mn (II), Co (II), Ni (II) and Zn (II). Structural assignment of these compounds has been made on the basis of TLC, molecular weight, molar conductivities, elemental analysis, UV, IR and ^{1}HNMR spectral data. Synthesized compounds were screened for their *in vitro* growth inhibiting activity against different strains of bacteria viz., gram positive *Staphylococcus aureus*, *Bacillus licheniformis*, *Micrococcus luteus* and gram negative Escherichia *coli* and were compared with the standard antibiotic oflaxocin. *In-vitro* antioxidant activities of all compounds were determined by nitric acid free radical scavenging assay.

Keywords: - antibacterial activities and antioxidant activity of cephalexin.

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INTRODUCTION

Compound containing imines bases have not only extensive application in organic synthesis, but several of these molecules display significant biological activity. Schiff bases form a significant class of compounds in medicinal and pharmaceutical chemistry with several biological applications that include antibacterial, antifungal and antitumor activity. They have been studied extensively as a class of ligand and are known to coordinate with metal ions through the azomethine nitrogen atom. The present study deals initially with the synthesis and characterization of Schiff base of *p*-chlorobenzaldehyde and cinnamaldehyde with cephalexin which showed moderate biological activities. Cephalexin is a very well known drug. It was found that the activity of this drug increases more when attached with cinnamaldehyde and *p*-chlorobenzaldehyde and further binded with metals such as Co (II), Zn (II), Ni (II) and Mn (II).

MATERIALS AND METHOD

All chemicals and solvent used were of analytical grade. All metal (II) salts were used as chloride. UV-VIS spectra were obtained on a Perkins Elmer spectrophotometer in the 250-900nm range in DMF.

IR spectra were recorded using KBR disc on a FT-IR spectrophotometer, shimadzu 8201PC in the range of 4000-400cm^{-1}. ^{1}HNMR spectra were recorded in MeOD at room temperature. Elemental analysis was carried out on a vario EL III Elementar Carlo- Erba 1108. Conductance measurement of 10^{-3} M solution of the complexes in DMF was carried out on an Equiptronic model no. Eq-660A. Melting point of the ligands and their metal complexes were determined by open capillary method using sunsim electric melting point apparatus and are uncorrected. Molecular weight of ligands and their metal complexes were determined by Rast camphor method.

EXPERIMENTAL

Synthesis of the organic ligand (PbC, CmC)

Cephalexin (1.827 gm, 1 mol.) was dissolved in methanol (10 ml) and added to the *p*-chlorobenzaldehyde (0.702gm, 1 mol.) and cinnamaldehyde (0.737 gm, 1 mol) respectively dissolved in methanol (10 ml). Few drops of KOH (0.1 % in methanol) were added to adjust the pH 7-8 and the mixture was refluxed for 4 hrs. After complete refluxation orange and brown coloured precipitates were separated after removal of the solvent at room temperature. After the ppt was recrystallized at room temperature with some solvent like petroleum ether and was dried under vacuum and kept in desiccator.

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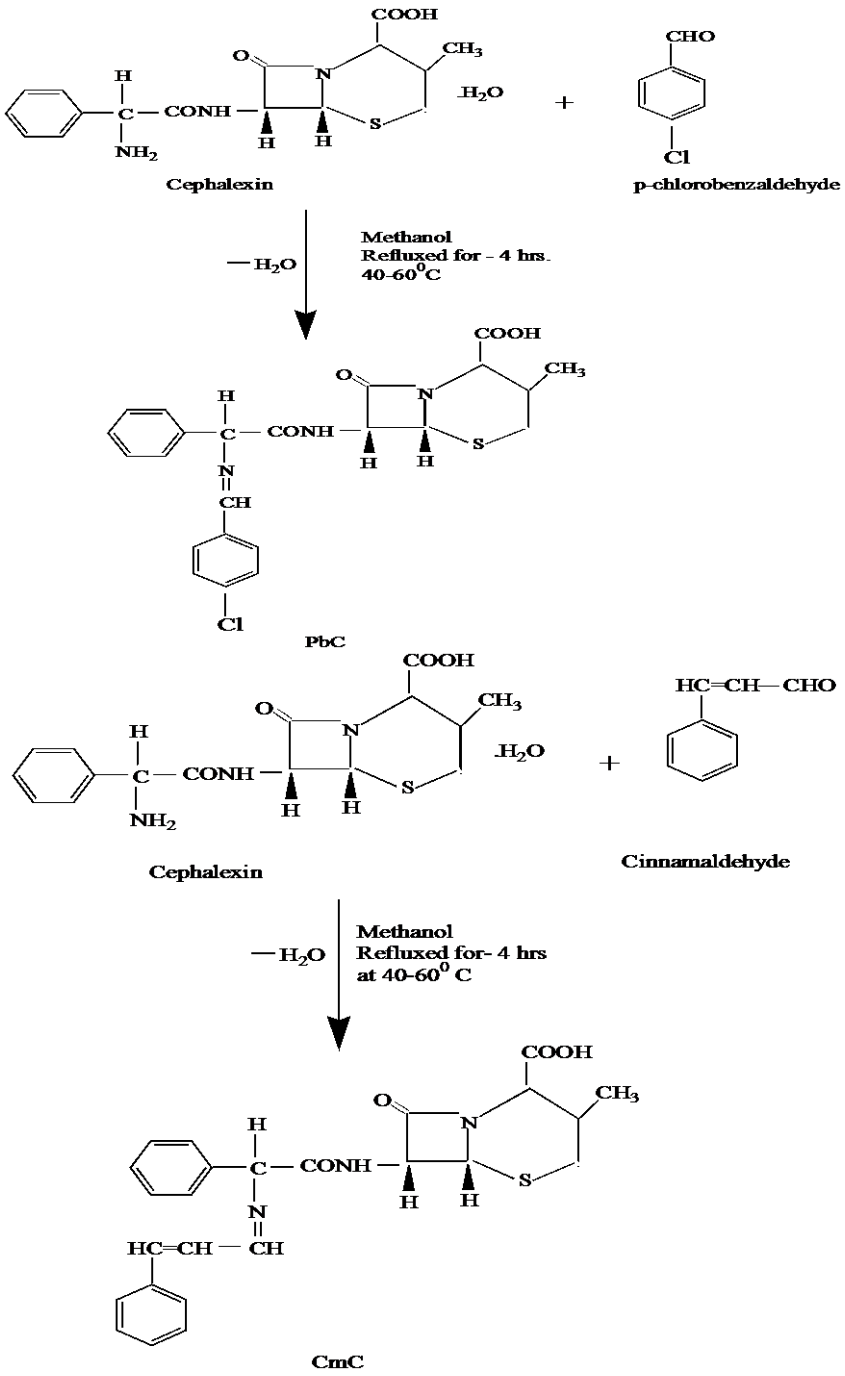

Synthesis of metal complex: - (PbCM, CmCM)

Cephalexin (0.2 mol.), *p*-chlorobenzaldehyde (0.2 mol.) Cinnamaldehyde (0.2 mol) and (0.1 mol.) metal chlorides of Ni (II), Zn (II), Mn (II), and Co (II), were dissolved in methanol (10 ml) separately. To this few drops of KOH (0.1 % in methanol) was added to adjust the pH of the solution between 7-8 and the mixture was refluxed for 4-5 hrs. A dark brown colored product was isolated after reduction of solvent volume by evaporation, which was filtered, washed with methanol and then recrystallised with methanol and dried over vacuum.

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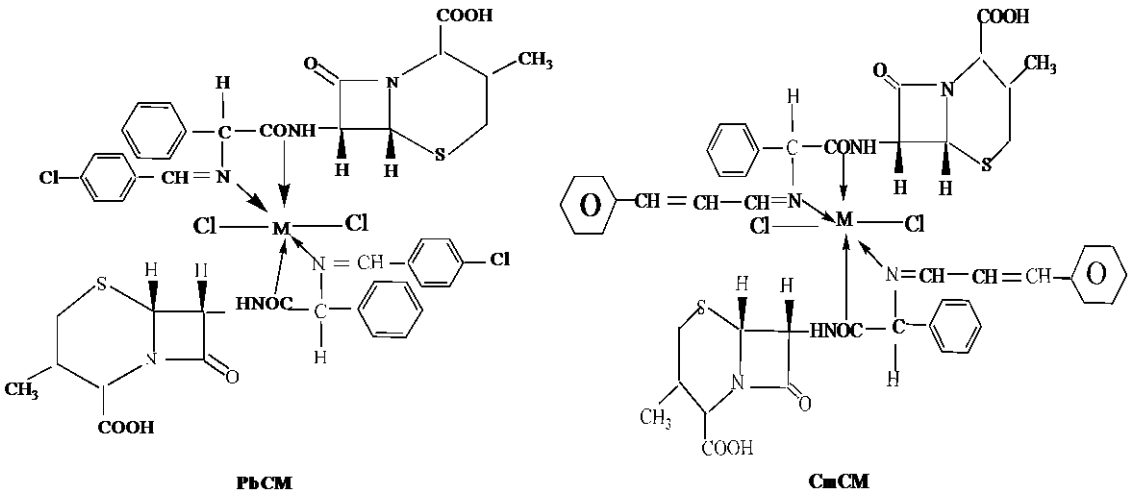

PbCM CuCM

ANTIBACTERIAL STUDIES

The synthesised metal complexes and Schiff base ligands were screened for their antibacterial activity against pathogenic bacterial species like gram (-) *E. coli* and gram (+) *S. aureus, M. luteus and B. lichenformis* (ATCC), were grow in nutrient agar medium at 37 ^{0} C for 24 hrs. The paper disc diffusion method was adopted for the determination of antibacterial activity. Antibiotics ofloxacin was used as positive control.

Antibacterial activities of the compounds were tested against *using* Muller Hinton agar medium. The sterilized (autoclaved at 121°C for 15 min) medium (40-50°C) was poured into the Petri dishes to give a depth of 3-4 mm and allowed to solidify. The suspension of the microorganism then streaked on plates. The paper discs impregnated with the test compounds were placed on the solidified medium. The plates were pre-incubated for 1 h at room temperature and incubated at 37°C for 24 h. Ofloxacin was used as standard. The observed zone of inhibition is presented as mean ±SEM in table 4 and also the MIC values are shown in table 3.

Scavenging of nitric oxide

Sodium nitroprusside (5mM) in standard phosphate buffer solution was incubated with different concentration of (125,100,75,50 µg/ml) the test extracts dissolved in standard phosphate buffer (0.025M, pH 7.4) and the tubes were incubated at 25 °C for 5 hr. After 5 h, 2 ml of incubation solution was removed and diluted with 2 ml Griess reagent (prepared by mixing equal volume of 1% sulphanilamide in 2% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in water). The absorbance of solution formed was read at 546 nm. The experiment was performed in triplicate and % scavenging activity was calculated using the formula (%) = Ao – A_1 / Ao x100

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Where Ao is control absorbance and A_1 is the absorbance of the sample. The activity was compared with ascorbic acid, which was used as a standard antioxidant. Then % inhibitions were plotted against respective concentrations used and from the graph IC50 values were calculated and show in table 5.

RESULTS AND DISCUSSION

Characterization of the compound

All the synthesis compounds are air and moisture stable, intensely colored amorphous solid which decomposes of ligands range between 180-190^0 C and metal complexes range between 260-340^0 C. The ligands are soluble in methanol, DMF and DMSO and metal complexes are soluble in DMF and DMSO and they are insoluble in common organic solvent like chloroform, acetone, ether, ethanol and carbon tetra chloride.

Table 1:- Micro analytical data of their ligand and metal complexes

S. No.	Name of compound	Carbon Found (calc.)	Hydrogen Found (calc.)	Oxygen Found (calc.)	Nitrogen Found (calc.)	Conductivity $ohm^{-1}cm^2 mol^{-1}$	M.P. (^{o}C)	M.W. Found (calc.)	Colour	Yield in %
1.	PbC	54.32 (54.27)	3.73 (3.61)	12.75 (12.65)	11.15 (11.08)	0.49	184	484 (487)	Orange	75
2.	PbC -Ni^{+2}	50.05 (49.82)	3.44 (3.24)	11.77 (11.68)	10.31 (10.26)	0.32	320	1059 (1068)	Brown	72
3.	PbC -Co^{+2}	50.37 (50.22)	3.45 (3.25)	11.84 (11.55)	10.36 (10.28)	0.38	298	1062 (1068)	Dark brown	81
4.	PbC-Mn^{+2}	50.66 (50.41)	3.46 (3.26)	11.91 (11.66)	10.43 (10.35)	0.24	310	1058 (1063)	Yellow	76
5.	PbC -Zn^{+2}	49.98 (49.88)	3.41 (3.33)	11.77 (11.65)	10.28 (10.17)	0.26	308	1072 (1075)	Brown	72
6.	CmC	52.64 (52.55)	4.38 (4.25)	20.66 (20.46)	10.84 (10.65)	0.47	190	494 (499)	brown	79
7.	CmC-Co^{+2}	48.97 (48.94)	3.82 (3.74)	11.51 (11.49)	10.08 (10.05)	0.28	348	1084 (1092)	brown	78
8.	CmC-Ni^{+2}	48.68 (48.49)	3.80 (3.74)	11.40 (11.31)	10.02 (9.76)	0.35	338	1088 (1091)	black	75
9.	CmC-Mn^{+2}	49.25 (49.22)	3.85 (3.79)	19.33 (19.23)	10.14 (9.86)	0.40	318	1082 (1087)	yellow	71
10.	CmC-Zn^{+2}	48.55 (48.45)	3.81 (3.78)	11.45 (11.38)	10.00 (9.76)	0.38	308	1092 (1098)	brown	74

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The molar conductance of the complexes value range between (0.25-0.50 ohm^{-1} cm^2 mol^{-1}) of the complexes which was carried out in DMF solvent indicates that the complexes under study are non-electrolytic in nature. Insolubility of these complexes in water and there non-electrolytic nature provide sufficient evidence for covalence of the ligands. Purity of compounds was confirmed as both ligands and complexes moves as a single spot indicating the presence of only one component. Molecular weight determined by Rast Camphor method and were found in accordance with calculated value the range of ligands (484-494) and metal complexes (1060-1092), confirming the monomeric nature of the compounds. The yield of compounds found in the range of (70-82 %) show in table 1

In the IR spectrum, the (C=O) observed at 1662-1659 cm^{-1} in the ligands spectra shifted to 1632-1620 cm^{-1} in the spectra of the complexes showing the participation of the C=O group in coordination. The ligand shows strong band in the region 1604-1606 cm-1 due to -C=N- which is assignable to the Schiff bases, which appeared in both synthesized ligands. This band gets shifted to lower frequency in the complexes, indicating the coordination through azomethine nitrogen to metal atom. It is found from the IR spectra of the complexes that there are wide and strong band at 540 –560 cm^{-1} for (M-N) bonding and 432-455 cm^{-1} for (M-O) which are assigned to metal stretching vibration. The ^{1}HNMR spectral data of ligands (PbC) and **(**CmC) shows signal between δ7.55-7.59 and δ7.50-7.68 respectively due to aromatic ring which gets shifted downfield in their metal complexes. The VU-VIS spectra of ligands (PbC and CmC) showed two bands between 270-280 nm and 290-330 nm. The first band may be due to Π – Π* transition with in the aromatic ring. The second band would be due to n- Π * transition which is shifted in metal complexes. Show in table 2

Table 2:-Characteristic IR and ^{1}HNMR spectral data of the ligands and their metal complexes

S.No.	Compound.	IR spectra cm^{-1}					^{1}HNMR Spectra ppm			U.V. Visible	
		(C=O)	(O-M)	(N-M)	(C=N)	(Ar-CH)	δ(CH=CH)	δ(Ar-H)	(N=H)	(C=C)	(C=N)
1.	PbC	1662	-	-	1604	3022	4.82	6.78-7.4	7.58	280	320
2.	PbC -Ni^{+2}	1632	445	558	1580	3014	4.39	6.17-7.1	7.48	281	300
3.	PbC -Co^{+2}	1630	448	552	1582	3010	4.78	6.44-7.2	7.44	282	295
4.	PbC -Mn^{+2}	1633	432	560	1570	3018	4.57	6.12-6.26	7.45	280	300
5.	PbC -Zn^{+2}	1623	435	542	1574	3014	4.58	6.15-7.01	7.51	280	330
6.	CmC	1659	-	-	1606	3026	5.29	6.79-7.32	7.59	270	290
7.	CmC -Co^{+2}	1628	440	548	1584	3016	5.16	6.45-7.18	7.42	270	300
8.	CmC -Ni^{+2}	1632	455	552	1578	3012	4.84	6.23-7.16	7.48	272	310
9.	CmC -Mn^{+2}	1624	445	558	1588	3018	4.78	6.77-6.75	7.47	272	320
10.	CmC -Zn^{+2}	1620	448	540	1586	3019	4.86	6.42-7.18	7.45	270	300

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All the compounds were evaluated for their antibacterial activity *in vitro* by using zone inhibition technique against *E.coli* (-) *S.aureus* (+) *M.luteus* (+) and *B.licheniformis* (+) at different concentration (100, 500 and 1000ppm). Experiments were repeated three times and the results were expressed as (Mean±SEM) values in table 4. The results obtained were compared with the standard drug Ofloxacin. The ligand PbC showed better efficiency 0.40 mg/ml for *S.aureus* and the ligand CmC showed 0.42 mg/ml better efficiency for *B.licheniformis.* The metal complexes showed an increase in activity in comparison with ligands. The MIC values are also shown in table 3.

Table 3:- MIC of the ligand and their metal complexes in concentration mg/ml

Name of Compound	*E. Coli* (-)	*S.Aureusi*(+)	*M. Luteus*(+)	*B. Lichenformis*(+)
	mg/ml	mg/ml	mg/ml	mg/ml
PbC	0.42	0.40	0.42	0.44
PbC-Ni^{+2}	0.30	0.28	0.26	0.28
PbC-Co^{+2}	0.30	0.26	0.25	0.26
PbC-Zn^{+2}	0.29	0.25	0.22	0.24
PbC-Mn^{+2}	0.27	0.24	0.20	0.19
CmC	0.43	0.44	0.50	0.42
CmC -Ni^{+2}	0.30	0.26	0.29	0.24
CmC -Zn^{+2}	0.29	0.24	0.24	0.25
CmC –Co^{+2}	0.28	0.27	0.25	0.26
CmC -Mn^{+2}	0.20	0.21	0.22	0.19

All compounds showed significant free radical scavenging action against nitric oxide (NO) induced release of free radicals at different concentration 125, 100, 75, 50 µg / ml. Ascorbic acid was used as reference standard. The antioxidant activity are in shown as Table 5

Table 4:- Antimicrobial activity of ligands and their metal complexes.

S. No.		*E. Coli (-)*	
		100 ppm	500 ppm
1	PbC	17±.526	24±.574
2	PbC-Ni^{+2}	19±.306	26±.304
3	PbC-Co^{+2}	18±.418	28±.584
4	PbC-Zn^{+2}	19±.310	27±.294
5	PbC-Mn^{+2}	20±.209	28±.304
6	CmC	18±.208	24±.555
7	CmC-Ni^{+2}	20±.309	28±.260
8	CmC-Zn^{+2}	19±.251	27±.940*
9	CmC-Co^{+2}	19±.116	28±.232
10	CmC-Mn^{+2}	20±.419	29±.344
11	**Ofloxacin**	24±.236	29±.265

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	S. aureus (+)			M. luteus (+)			B. lichenformis (+)		
1000 ppm	100 ppm	500 ppm	1000 ppm	100 ppm	500 ppm	1000 ppm	100 ppm	500 ppm	1000 ppm
34±.303	18±.727*	21(±.424	35±.372	17±.554	23±.096	33±.304	18±.502	24±.305	33±.155
37±.502	19±.434	29±.26	36±.300	22±.304	26±.305	37±.094	20±.265	28±.557	34±.304
38±.491	20±.155	28±.67	36±.304	21±.740*	27±.712	38±.494	20±.301	27±.465	35±.306
39±.370	20±.495	29±.354	37±1.05	20±.206	27±.712	39±.059	21±.106	28±.156	36±.305
39±.759	21±.255	2±.582	39±.154	23±.405	27±.416	39±.205	21±.304	29±.304	34±.208
35±.328	17±.152	27±.980*	34±.261	18±.234	22±.251	34±.054	17±.105	25±.308	35±.269
37±.207	20±.402	29±.400	38±.153	22±.584	24±.416	37±.155	20±.302	29±.204	38±.308
38±.466	20±.090	28±.208	37±.175	22±.114	26±.416	36±.201	21±.232	28±.106	39±.374
38±.204	20±.058	28±.379	38±.205	21±.940*	26±.208	38±.502	20±.236	29±.498	38±.466
37±.394	22±.494	29±.208	38±.266	20±.559	27±.379	39±.262	21±.790*	30±.347	38±.615
33±.452	25±.254	30±.659	34±.564	24±.452	28±.635	32±.542	20±.125	25±.154	30±.562

P< .001, P<.01*

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Table 5 Antioxidant activity of the ligands and their metal complexes

Name of compounds	50 µg/ml	75 µg/ml	100 µg/ml	125 µg/ml
PbC	35.14(±.328)	31.941(±.500)	33.32(±.016)	34.80(±.018)
PbC-Ni	35.14(±.205)	36.90(±.144)	37.4(±.678)	39.30(±.104)
PbC-Mn	38.18(±.304)	39.26(±.200)	40.6(±.611)	43.41(±.872)
PbC-Zn	37.24(±.204)	38.41(±.201)	39.72(±.314)	41.6(±.095)
PbC-Co	37.16(±.180)	37.7(±1.98)*	38.4(±.201)	42.7(±.104)
CmC	28.14(±.018)	30.28(±.200)	31.04(±1.58)*	33.14(±.180)
CmC-Ni	34.28(±.017)	36.19(±.204)	37.17(±.500)	38.28(±.114)
CmC-Mn	32.19(±.200)	37.14(±1.96)*	38.28(±.728)	40.44(±.402)
CmC-Zn	31.78(±.104)	32.24(±.109)	35.42(±.180)	38.16(±1.87)*
CmC-Co	30.40(±.094)	34.36(±.210)	37.68(±.140)	39.74(±.304)

P< .001, P<.01*

CONCLUSION

The result of this investigation supports the suggested structure of the metal complexes. A square planner structure was suggested for all the complexes, the Schiff base ligands were found to be biologically active and their metal complexes show enhanced antimicrobial activity against one or more strains, chelation tends to make the ligands act as more powerful and potent bactericidal agent.

All compounds showed varying antioxidant (free radical scavenging) activities when compared to ascorbic acid. The results suggest that the antioxidant activity of these compounds may contribute to their claimed antioxidant property and may lead to chemical entities with potential for clinical use.

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