SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF CU (II) AND NI (II) MIXED COMPLEXES WITH PFA AND OTHER N-O$^-$, N-N, O$^-$-O$^-$ DONOR LIGANDS

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ABSTRACT

The binary and ternary complexes of the type ML and MLA were synthesized where M= Cu (II), Ni (II), L = Phosphonoformic acid and A = Alanine, phenyl alanine, ethylene diamine, 1, 10-phenanthroline, oxalic acid and Malonic acid. The complexes were non-electrolytic. The elemental analysis, magnetic measurements, conductivity measurements and spectral studies of these complexes were carried out. The complexes exhibited octahedral geometry. The antimicrobial activity of the ligand and its complexes were tested on staphylococcus aureus and Esherichia Coli. Metal chelates showed greater antimicrobial activity than the control and the ligand. The antioxidant activity of the metal, binary and mixed ligand complexes were studied on ragi seedlings and it was observed that the antioxidant levels increased in the case of binary and ternary complexes. PM3(Henre et al.,1970 & Stewart ,1989) a semi empirical method was employed to obtain a 3D- Geometry and relative energies of the possible isomers of Ni(II) complexes. The molecular modelling studies showed an octahedral geometry for these complexes.

Keywords:*PFA, Ni (II), Cu (II) complexes, antimicrobial activity, antioxidant levels,* ***N****-O$^-$,N-N, and O$^-$-O$^-$donor ligand, Molecular modelling studies.*

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INTRODUCTION

The recognition of the potential employment of metal complexes and chelates in therapeutic application provides useful outlets for basic research in transition metal chemistry(Obaleye et al.,2009) studies on metal complexes involving PFA moiety are receiving wider attention owing to its applications in various fields like medicine, industry and analytical chemistry. Therefore the study of the interaction of PFA with metal ions may throw some light on the nature of the metal ligand interactions that occur in biological systems. Phosphonoformic acid (PFA) which is in the form of its trisodium salt is known as Fascarnet acts as an antiviral agent and in particular against Herpes virus group (HSV-1, HSV-2, VZV, EBV and CMV). It was found to be an inhibitor of the deoxyribose acid polymerase induced by the herpes virus of Turkeys. It was able to block the replication in cell culture of Marcks disease herpes virus(Reno et al.,1978 &2001). Since PFA possess antiviral activity and it is a potential chelating agent it can be considered for its ability to form binary and mixed ligand complexes with metal ions in biological systems. Literature survey also reveals that PFA and Metal ions like Ni (II), Cu (II) have been extensively used as biologically active complexing agents. Considering these facts, the binary and mixed ligand complexes of transition metals Ni(II) and Cu(II) with primary ligand PFA and secondary ligands ethylene diamine, 1,10-phenanthroline (N-N donors), Alanine, phenyl alanine (N-O-donors), oxalic acid and malonic acid (O-,O-donors) were synthesized, analyzed and characterized by I.R Spectra. These complexes were also tested for biological activity on Eleucine coracona and antimicrobial activity against staphylococcus aureus (gram positive bacteria) and Esherichia coli (Gram negative bacteria). The molecular modelling studies showed an octahedral geometry for these complexes.Experimental Methods and Materials All the chemicals are of analar grade and were used as supplied without further purification.**Synthesis of binary complex of Ni (II) and Cu (II) with primary ligand phosphonoformic acid(PFA):**0.01M Concentration of metallic salt solution is mixed with 0.01M Concentration of ligand phosphonoformic acid in 1:1 ratio in 10 ml of distilled water and stirred for half an hour. To this mixture 0.01M concentration of NaOH is added to maintain the pH where by solid binary complex separates out after stirring for more than one hour. The solid product is filtered, washed with ethanol, distilled water and acetone followed by drying at 110^0C for one hour.

Synthesis of binary complexes of Ni (II), Cu (II) with secondary ligands (en, phen, a-ala, phen-ala, ox and mal):0.01M Concentration of Metallic salt solution is mixed with 0.01M Concentration of secondary ligands like ethylenediamines (en), 1,10-phenanthroline (phen), a-alanine(ala), phenyl alanine (phen-ala), oxalic acid(Ox) and Malonic acid(mal) in the ratio of 1:1 in 10ml of distilled water. The same procedure is followed as synthesis of binary complexes of metallic complexes with primary ligand.

Synthesis of Mixed ligand complexes of Ni (II), Cu (II) with phosphonoformic acid and secondary ligands (en, phen, a-Ala, phen-Ala, ox and Mal in the ratio of 1: 1: 1

0.01M Concentration of metallic salt solution is mixed with 0.01M Concentration of PFA in the ratio of 1:1 in distilled water and stirred for half an hour. Then 0.01M concentration of secondary ligands (like en) was added, and 0.01M concentration of NaOH is added slowly to this mixture to maintain the pH and stirred for half an hour, slowly the solid mixed ligand complex separates out. This product is filtered, washed with ethanol, distilled water and acetone, later dried at 110°C for 1 hr. The synthesis is repeated with metallic salt solution, PFA and other secondary ligands in the similar manner.

Physical measurements

The elemental analysis was carried out using Perkin Elmer elemental analyzer. The percentage of metal contents was determined by Atomic absorption spectroscopy on Perkin Elmer Model 2380. Conductivity measurements of the synthesized complexes were determined using digital conductivity meter model D1 9009. IR Spectra for the primary ligand, secondary ligands, binary and mixed ligand complexes were determined in the region 400-4000cm^{-1}. I.R. Spectra were obtained by KBR Pellet technique using Perkin Elmer model 621 spectrophotometer at Vimta, laboratory Hyderabad. The magnetic data of the metal complexes were obtained at room temperature by the Gouy technique using Hg [Co (NCS)] as calibrant. The molecular weight of the samples was determined by Rast Method.

Biological Activity

Four day old Eleusine coracana (Ragi seedlings) were treated with Ni (II), Cu (II) and their binary and mixed ligand complexes separately at 50mm concentration. The solvent used for extract is ethanol. The effect of the metal ions and their complexes on the accumulation of antioxidants like Glutathione, Ascorbate and Tocopherol were studied by analyzing the samples for 24hrs exposure with control seedlings using reversed phase HPLC (Methanol : Water in 1:1 ratio) and the analyte volume 25µℓ spectrophotometer used is C_{18} column and the model schimadzus.

Antibacterial assay

Primary ligand and the synthesized complexes were tested for antimicrobial activity against Test cultures Escherichia coli and staphylococcus aureus, a gram positive and gram negative bacteria using Kirby Bauer method(James et al.,2004 & Wistreich et al.,1998) which is a disc diffusion method. Materials used are PFA, DMF as solvent and control copper sulphate solution, binary and mixed ligand complex solution (100µg/disc). The antimicrobial agent is loaded on the discs by soaking them in the test solutions and drying them overnight broth of test cultures were used to get carpeted growth on nutrient agar medium. The test culture was spread plated and discs were placed on the surface. The plates were incubated for 24 hrs at 37°C. The incubation zones were measured for the antibacterial activity of the test compounds in diameters.

Molecular Modelling studies

A semi empirical self consistent field method was employed to the 3D geometry and the relative energies of the possible isomers of the Ni (II) complexes.

RESULTS AND DISCUSSIONS

Physical and analytical data for the complexes are shown in Table – 1. Ni (II) Complexes were green in colour and Cu (II) complexes were light blue to bluish green in colour. All the complexes were stable at room temperature. The molar conductance values of binary and ternary complexes suggest that they are non-electrolytic in nature (Raman et al., 2001& Geary, 1971). The relevant I.R bands of the ligand and their binary and mixed ligand complexes are presented in Table – 2.Free ligand PFA shows absorption band at v694cm^{-1} due to its v(C=O) stretching frequency, the band at 1435 cm^{-1} is due to v(P-C) bond, the peaks at 1281cm^{-1} and 1313cm^{-1} shows due its v(P=O), v(P-O) bonds. The absorption peaks for the binary complexes of PFA with Ni(II) shows broad bands in the range 3644-3465 cm^{-1} due to v(OH) frequency and a peak at 588cm^{-1} due to v(M-O) and at 846cm^{-1} due to v(O-O) stretching frequency. All the ternary complexes of PFA with Ni (II) and other secondary ligands en, phen, phe ala shows bonds in the range 3434 to 3422 cm^{-1} due to v (N-H) stretching frequency, in the region 656 to 750cm^{-1} due to v(M-N) bond, at 518 to 526cm^{-1}due to v(M-O) bond, at 845cm^{-1} due to v(O-O) bond. Ox and Phen as secondary ligands shows bands in the range 520cm^{-1} due to v (M-O) bond, the bonds in the range of 520 cm^{-1} and 447 cm^{-1} show due to v(M-O) bond which confirms the formation of complex(Nakamoto *et al.* 1970 and 1978, Coates 1996, Bellamy 1975 and 1980, Steele 1971, Colthrup *et al.* 1990, Socrates 1994, Lin-Vien *et al.* 1991, Smith 1999, Nakamoto 1997, Nyquist *et al.* 1997, John Coates, B.D.Mistry).

I.R. Spectra of PFA, its binary and Mixed ligand complexes of Ni (II) and Cu (II)

PFA shows a band in the region of 1694cm^{-1}, 1435 cm^{-1}, 1313 cm^{-1}, 1281 cm^{-1} due to v(C=O), v(P-C), v(P=O) and v(P-O) bonds. The binary complex of PFA with Cu(II) shows bands in the region 1678 to 1578 cm^{-1} due to v(C=O)) bond, a band in the region 1436 to 1324 cm^{-1} due to v(P-C) bond, band in the region 1360cm^{-1}-1280 cm^{-1} due to v(P=O) bond. Its complexes with Cu (II) and mal absorption at 937 to 918 cm^{-1} due to v(P-O) bond, a band in the region 3854 to 3500cm^{-1} due to v(OH) group. A band in the region 3466 to 3332 cm^{-1} due to v(N-H) bond, a band at 755 to 500 cm^{-1} due to v(M-N) band, at 557 to 422 cm^{-1} due to v(M-O) band, a band at 745cm^{-1} to 810 due to v(O-O) bond. These bands indicate the formation of complex.

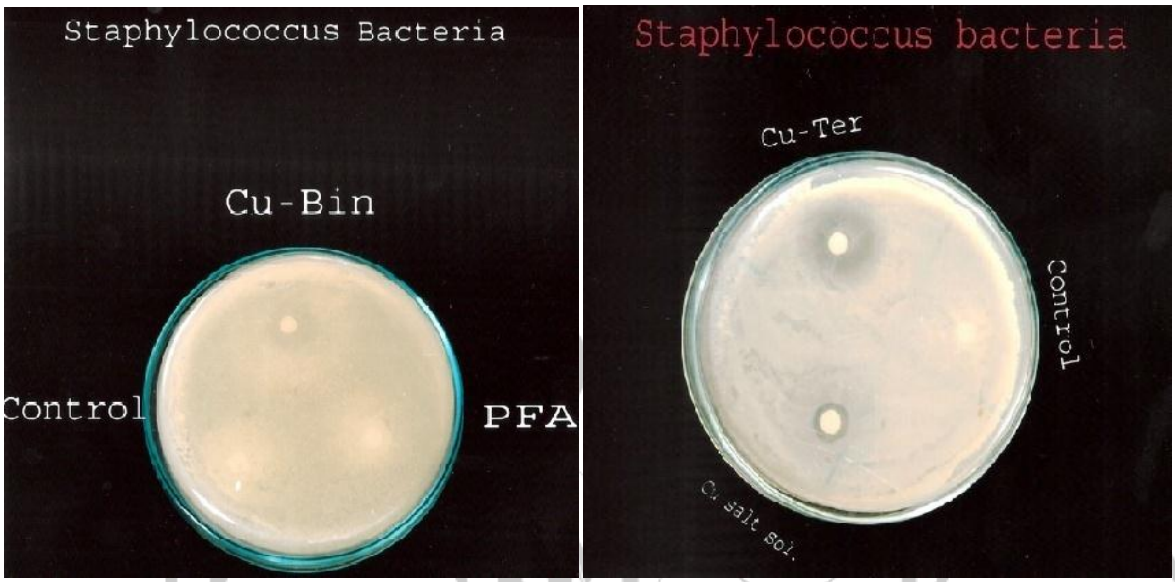

Figure 1(a) **(b)**

Figure 1 (a) and (b) showing antimicrobial activity of staphylococcus bacteria of bBinary and mixed ligand complex of Cu (II)

Table – 1. Physical characteristics and elemental analysis of PFA, binary and mixed ligand complexes of Ni (II) and Cu (II)

S.No	Complex(s)	Formula	Color	M.W grams	Elemental analysis (cal, %)					
					Ni/Cu	C	H	N	P	O
1	L(PFA)	$C_1H_2PO_3$	white	300.00	-	4.0	0.6	-	10.33	26.66
2	ML	CH_8P_9O	green	253.69	22.91 (22.92)	4.73 (4..74)	3.15 (3.16)	-	12.24 (12.25)	56.93 (56.91)
3	Ni-L-A(en)	$C_3H_8N_2PO_7$	green	273.69	21.23 (21.24)	13.15 (13.18)	2.92 (2.93)	10.24 (10.25)	11.34 (11.35)	41.04 (41.02)
4	Ni-L-A(phen)	$C_{12}HN_2PO_7$	green	381.69	14.72 (14.75)	39.20 (39.22)	3.28 (3.01)	7.01 (7.04)	7.71 (7.79)	28.11 (28.16)
5	Ni-L-A(ala)	$C_4H_{10}NPO_9$	green	305.69	19.02 (19.01)	15.72 (15.73)	3.26 (3.27)	4.57 (4.59)	10.15 (10.16)	7.24 (47.21)
6	Ni-L-A(phen-ala)	$C_9H_{14}PO_9$	green	305.69	15.21 (15.22)	31.48 (31.49)	3.66 (3.67)	3.66 (3.67)	8.12 (8.13)	37.80 (37.79)
7	Ni-L-A(Ox)	$C_3H_4PO_{11}$	green	305.69	19.02 (19.01)	11.81 (11.80)	1.30 (1.31)	-	10.14 (10.16)	57.52 (57.70)
8	Ni-L-A(mal)	$C_4H_6PO_{11}$	green	319.69	18.17 (18.18)	15.02 (15.04)	1.86 (1.880	-	9.70 (9.71)	55.19 (55.17)
9	Cu-(L)PFA	CH_8P_9O	light blue	258.546	24.39 (24.37)	4.63 (4.64)	3.11 (3.09)	-	11.97 (11.99)	55.72 (55.70)
10	Cu-L-A(en)	$C_3H_8N_2PO_7$	bluish green	278.546	22.78 (22.80)	12.92 (12.92)	2.88 (2.87)	10.06 10.05	11.14 (11.13)	40.18 (40.20)
11	Cu-L-A(phen)	$C_{12}HN_2PO_7$	bluish green	402.50	15.71 (15.78)	38.79 (38.80)	2.95 (2.98)	6.90 6.95	7.68 (7.70)	27.79 (27.82)
12	Cu-L-A(ala)	$C_4H_{10}NPO_9$	bluish green	310.50	20.44 (20.45)	15.43 (15.45)	3.21 (3.22)	4.51 4.50	9.97 (9.98)	46.38 (46.37)
13	Cu-L-A(phen-ala)	$C_9H_{14}PO_9$	bluish green	386.50	16.42 (16.40)	31.02 (31.02)	3.63 (3.62)	3.63 3.62	8.01 (8.02)	37.27 (37.25)
14	Cu-L –(Ox)	$C_3H_4PO_{11}$	bluish green	310.546	20.43 (20.45)	11.60 (11.59)	1.27 (1.28)	1027 1.28	9.97 (9.98)	56.69 (56.68)
15	Cu-L-A(mal)	$C_4H_6PO_{11}$	bluish green	324.546	19.55 (19.56)	14.77 (14.79)	1.83 (1.84)	1.83 1.84	9.56 (9.55)	54.21 (54.23)

L= ligand Phosphonoformic acid (PFA); M= Cu (II), Ni (II); A = Alanine (ala); phenyl alanine (phen-ala); ethylene diamine (en); 1, 10-phenanthroline (phen); oxalic acid (Ox) and Malonic acid (mal).

Table–2. IR spectral data of PFA, binary and mixed ligand complexes of Ni (II) and Cu (II) (cm^{-1})

S.No	Complex(s)	ν(C=O)	ν(P-C)	ν(P=O)	ν(P-O)	ν(OH)	ν(N-H)	ν(M-N)	ν(M-O)	ν(O-O)
1	L(PFA)	1694	1435	1313	1281	0-	-	-	-	-
2	Ni-L(PFA)	1694	1426	1259	-	3644-3465	-	-	588	846
3	Ni-L-A(en)	1649	1364	-	-	3854	3434	656	-	-
4	Ni-L-A(phen)	1586	1427	-	-	3854-3649(BB)	3447(str) 1516(def)	725	-	845
5	Ni-L-A(ala)	1578	1384	-	-	3642	3447	800-700	518	-
6	Ni-L-A(phen-ala)	1617	1384	-	1037	3642	3422	750	526	-
7	Ni-L-A(Ox)	1636	1384	-	-	3649	-	-	519	-
8	Ni-L-A(mal)	1582	1384	-	1021	3647	-	-	520,447	-
9	Cu-(L)PFA	1579	1362	1280	918	3468	-	-	527,424	-
10	Cu-L-A(en)	1578	1436	1360	-	3600	3466	600-500	422	-
11	Cu-L-A(phen)	1585	1432	-	-	3854-3649	3412	721	458	-
12	Cu-L-A(ala)	1617	1384	-	-	3568	3422	698	463	-
13	Cu-L-A(phen-ala)	1620	1324	-	-	3500	3332	755	557	-
14	Cu-L –(Ox)	1678	1414	1278	-	3535	-	-	492	810
15	Cu-L-A(mal)	1578	1363	1279	937	3649	-	-	492	745

BB=broad band; str.= stretching frequency; def= deformation;

Table – 3. Biological activity of binary and mixed ligand complexes of Ni (II) and Cu (II) on Eleusine Coracana (Ragi) in µg/mℓ.

S.No.	Antioxidants	control	Ni metal	Ni-(L)PFA	Ni-L-A(en)	Cu metal	Cu-(L)PFA	Cu-L-A(en)
1.	Ascorbate	0.9	24	24.8	9.2	24.5	7.9	13
2.	Glutathione	0.9	3.6	14.6	2.4	0.9	2.9	1.8
3.	Tocopherol	1.1	7.8	3.5	15	1.1	1.1	1.1

Biological activity of Ni (II) and Cu (II) complexes

The activity of metal precursors, binary and mixed ligand complexes on the Ragi seedlings are discussed. Controlled seedlings exhibited very low levels of ascorbate (0.9µg/mℓ), Glutathione (0.9µg/mℓ) and tocopherol (1.1µg/mℓ). In seedlings with Ni (II) as precursor the ascorbate levels were elevated (24µg/mℓ) compared to those treated with Cu (II) (24.5µg/mℓ). However the seedlings treated with binary complexes of these metals showed increased levels of ascorbate with Ni (II) (24.8µg/mℓ) Cu (II)7.9µg/mℓ. The mixed ligand complexes caused an enhancement of ascorbate levels of Ni (II), Cu (II) in the seedlings to 9.2µg/mℓ, and 13 µg/mℓ.

Glutathione an ubiquitous antioxidant was found to be present in high levels in seedlings treated with Ni (II) (3.6µg/mℓ). However the levels of glutathione in plants treated with Cu (II) had the same levels as control (0.9µg/mℓ). The binary complexes of the Cu(II) had little effect on the accumulation of glutathione (2.9 µg/mℓ) as compared to the control and accumulation of glutathione of Ni (II) is 14.6 µg/mℓ and mixed ligand complexes of Cu(II) showed minimum increase (1.8 µg/mℓ).

Ragi seedlings responded differently to treatment with both metal and metal complexes with reference to tocopherol. The level of tocopherol in control is 1.1µg/mℓ which remained unmodified when treated with Cu precursor and their binary and mixed ligand complexes 1.1µg/mℓ where as Ni (II) metal treatment produced enhanced production of tocopherol to 7.8 µg/mℓ. The Ni (II) mixed ligand complexes exhibited maximum response of 15µg/mℓ whereas the binary complexes of Ni (II) showed the response to 3.5 µg/mℓ.

Antimicrobial activity

The antimicrobial activity of ligand PFA, metal precursors, synthesized binary and ternary complexes of Ni (II), Cu (II) and the solvent DMF were tested against E-coli and staphylococci aureus. Both the metal ions used have varying antibacterial activity on the bacterial species but the activity of the synthesized complexes has greater effect. E-coli cultures did not show any resistance but it was sensitive to all the compounds, while staphylococcus aureus was resistant to the mixed ligand, binary and copper solution in a descending order while it was resistant to the ligand. The staphylococcus aureus is inhibited by

ternary complex was the best followed by the binary complexes. The results are shown in figures 1(a) & (b). The results of antimicrobial testing reveal an increase of inhibition zone with the increase of the chelates weight placed on the bacterial culture.

Conclusion

The synthesized binary and mixed ligand complexes of PFA had shown distorted octahedral geometry. From the present study it is concluded that not only metal but also the binary and mixed ligand complexes induce biological activity by increase in the antioxidant levels of Glutathione, Ascorbate and Tocopherol in the plants. The synthesized complexes were also tested for antimicrobial activity on staphylococcus aureus and Escherichia coli. The results reveal that there is an increase of inhibition zone with the increase of the chelates weight placed on the bacterial culture. More detailed studies are needed which may lead to specific application of these metal complexes.

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