World Journal of Pharmaceutical ReseaRch
SJIF Impact Factor 5.045
Volume 3, Issue 6, 2030-2039. Research Article ISSN 2277 – 7105

PREPARATION AND BIO-POTENTIAL CHARACTERIZATION OF Zn(II), Cd(II) AND Hg(II) COMPLEXES WITH PHENYLACETYLUREA AND BENZOATE ION LIGANDS

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Article Received on
28 June 2014,

Revised on 23 July 2014,
Accepted on 18 August 2014

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ABSTRACT

Phenylacetylurea (PAU) is a biologically active, neutral bidendate ligand. The present study deals with the microwave assisted preparation, physico-chemical, spectral and biological characterization of Zn(II), Cd(II) and Hg(II) complexes containing PAU and benzoate ion (ben) ligands. The complexes were prepared by microwave irradiation technique. The molecular formulae of the complexes were arrived at from the estimation of metals and electrical conductivity values. The electronic spectral data confirm the entry of ligands into the coordination sphere of the metals. The antibacterial and anti fungal screening of the three complexes against the bacteria, viz., *Raoultella planticola, Vibro cholera, Lactobacillus brevis, Pseudomonas aeruginosa, Micrococus luteus* and fungi, viz., *Candida albicans, Aspergillus oryzae, Aspergillus niger, Aspergillus flavus and Aspergillus sojae* were carried out. The results indicate that Hg(II) complex exhibits higher antibacterial activity against *Raoultella planticola, Vibro cholera, Lactobacillus brevis, Pseudomonas aeruginosa, Micrococus luteus* than Zn(II) complex, Cd(II) complex and pure ligands. All the complexes show high antifungal activity against *Candida albicans, Aspergillus oryzae, Aspergillus niger, Aspergillus flavus and Aspergillus sojae.* The diphenylpicrylhydrazyl free radical scavenging activity of the above three complexes are lower than the standard compound, vitamin-C.

KEY WORDS: Phenylacetylurea, benzoate ion, antibacterial, anti fungal, antioxidantal.

INTRODUCTION

The inorganic elements, especially the metals are vital for the functioning of biological systems. Metal ions and their complexes are essential components in all living organisms[1]. Alkali and alkaline earth metals tend to be present in fairly large quantities, whereas transition metals tend to be present in relatively small quantities, and are sometimes called as trace metals. Transition metals are extremely good catalytic active sites in enzymes. They are stable in a variety of geometries, have multiple coordination sites, stable in a variety of oxidation states, able to change the reactivity of ligands and are capable of stabilizing intermediates. They serve to enhance[2] the stability of various bio-molecules, active sites and cofactor of many enzymes. Metal ions and their complexes find their use in drugs for many diseases. These metal ions are not usually present as free metal ions, but instead as complexes. Also the ligands involved in the complexes, in which the donor atoms are usually oxygen, nitrogen, sulphur and occasionally, carbon.

Phenylacetylurea[3] is a urea based anticonvulsant, administered for controlling severe epilepsy, particularly, mixed forms of complex partial (psychomotor or temporal lobe) seizures, refractory to other anticonvulsants. Phenylacetylurea accelerates the threshold for minimal electroshock convulsions and abolishes the tonic phase of maximal electroshock seizures. It also prevents/modifies seizures induced by pentylenetetrazol or other convulsants.

Microwave assisted[4] preparation has become popular and gained greater attention in this decade. The microwave irradiation technique is used for carrying out chemical transformations. It is almost pollution free, eco-friendly, cheap, less time consuming, effluent free and offers high yield[5-6] when compared to conventional methods. The present work aims at the preparation of Zn(II), Cd(II) and Hg(II) complexes with phenylacetylurea and benzoate ion as ligands by microwave irradiation and characterization by physico-chemical, spectral and biological methods.

MATERIALS AND METHODS

Preparation of complexes

All the reagents viz., phenylacetylurea, nitrate zinc(II) nitrate, cadmium(II) nitrate and mercury(II) nitrate and sodium benzoate were of Analytical reagent grade. The solvents acetonitrile, dimethylsulphoxide (DMSO), dimethylformamide (DMF), ethanol and methanol were of AnalaR grade and used as such. All the three metal complexes were prepared by the addition of required mole ratios of phenylacetylurea (0.60 g, 3.53 mmol; 0.58 g, 3.41 mmol

and 0.65 g, 3.82 mmol) in methanol and sodium benzoate (0.97 g, 6.74 mmol; 0.93 g, 6.46 mmol and 1.05 g, 7.29 mmol) in ethanol to the methanolic solutions of Zn(II) nitrate, Cd(II) nitrate and Hg(II) nitrate (1.00g, 3.36 mmol; 1.00 g, 3.24 mmol and 1.00 g, 3.08 mmol) respectively followed by microwave irradiation for about 10 seconds after each addition of ligands. Microwave oven IFB 25PG1S model was used for the preparation of complexes. The precipitated complexes were filtered, washed with ethanol and dried.

Characterization

The estimation of the metals of the complexes was carried out by volumetric method by titration with EDTA. The molar conductance measurements of the 10^{-3} M complex solutions were carried out in acetonitrile using Systronic 304 Conductivity meter at 30 °C. Solid state UV-visible absorbance spectra of the complexes were carried out by using Varian Make, CARY-5000 Model UV-vis Spectrophotometer. The IR spectra of complexes and ligands were recorded in Shimadzu, FT-IR 8400 Spectrometer in 4000-400 cm^{-1} range using KBr pellet technique.

The antibacterial[7-9] activities of the individual ligands and complexes were carried out by agar well diffusion method. 20 ml of sterilized nutrient agar (NA) media was taken in a petri-dish and after solidification, 0.1 ml of test bacteria was spread over the medium. The test complex of concentrations viz., 25, 50, 75 and 100 μgml^{-1} in DMF were poured into the four numbers of 6 mm diameter holes already made. At the end of 24 hours incubation at 37 °C, the diameter of the inhibition zone detected around each hole was measured. DMF and streptomycin were used as control and standard drug respectively. By subtracting the diameter of the inhibition zone of control from the complexes, the antibacterial activities were calculated as a mean of three replicates. The anti fungal activities of the ligands and the complexes were studied by Agar plate technique. The complexes were directly mixed to the DMF in 100, 200 and 400 μgml^{-1} concentrations. Whatman No.1 filter paper of 5 mm diameter, sterilized at 140 °C was soaked with the fungus and was placed on the medium. The plate was inverted and kept in an incubator at 27 °C for 72 hours. The inhibition zone formed was measured after 72 hours. Ketoconazole was used as the standard and the DMF served as a control. The growth of fungus was measured by recording the diameter of fungal colony as a mean of three replicates. The fungal growth inhibition due to the presence of the complexes was calculated using the formula,

Percentage fungal growth inhibition = 100 * (C- T)/ C

Where, C is the diameter of fungal colony in control plate and T is the diameter of fungal colony in test plate. Antioxidantal activities of the complexes and ligands were carried out by DPPH free radical scavenging method[10]. The complexes were dissolved in a mixture of ethanol and 5% DMSO in various concentrations ranging from 1.95 to 1000 μgml^{-1}. Vitamin-C was used as a positive control. Assay mixture contained 500 µl of the complex, 125 µl DPPH (100µM) and 375 µl solvent (5% DMSO) were prepared. This mixture was incubated for 30 minutes at 25 °C in dark condition. The absorbance was measured at 517 nm spectrophotometrically. All determinations were carried out in triplicate. The free radical scavenging activity was calculated using the equation,

Inhibition percentage = $100 * (Abs_{control} - Abs_{sample})/Abs_{control}$

RESULTS AND DISCUSSION

Physico-chemical properties

The complexes of the three metals were obtained as colourless precipitate. The yield of Zn(II), Cd(II) and Hg(II) complexes were 72.9%, 64.1% and 79.8% respectively. The zinc, cadmium and mercury metal contents[11] in the respective complexes were 13.53%, 21.12% and 32.18% as against the theoretical values of 13.70%, 21.44% and 32.74% respectively. The electrical conductivity of Zn(II), Cd(II) and Hg(II) complexes were 80.92, 63.86 and 58.84 $ohm^{-1}cm^2mol^{-1}$ respectively. The physico-chemical properties of Zn(II), Cd(II) and Hg(II) complexes are given in table-1.

Table-1 Physico-chemical properties of Zn(II), Cd(II) and Hg(II) complexes

S.No.	Complex	Yield, %	Metal, %		Electrical conductivity, $ohm^{-1}cm^2mol^{-1}$
			Theoretical	Experimental	
1	$[Zn(PAU)(ben)_2]$	72.9	13.70	13.53	80.92
2	$[Cd(PAU)(ben)_2]$	64.1	21.44	21.12	63.86
3	$[Hg(PAU)(ben)_2]$	79.8	32.74	32.18	58.84

From the metal content percentage, the molecular formulae of the three complexes were arrived as $[Zn(PAU)(ben)_2]$, $[Cd(PAU)(ben)_2]$ and $[Hg(PAU)(ben)_2]$. The electrical conductance measured was low, which indicate that the complexes are non electrolyte in nature and of 1:0 type.

IR Spectra

In IR spectrum, the PAU ligand exhibited frequencies at 1180 cm^{-1}, 1672 $cm^{-1}$and 3389 cm^{-1} and they are assignable to the **ν**(C-N) stretching, **ν**(N-H) (secondary amine) bending and **ν**(N-H) (primary amine) stretching vibrations respectively[12]. These vibrations were found at the frequencies of 1178 cm^{-1}, 1666 cm^{-1} and 3389 cm^{-1} in Zn(II) complex, 1178 cm^{-1},

1668cm^{-1} and 3389 cm^{-1} in Cd(II) complex and 1178 cm^{-1}, 1668 cm^{-1}and 3391 cm^{-1} in Hg(II) complexes and this ensures the entry of PAU into the coordination sphere of the metal.

In the IR spectrum of benzoate ligand, frequencies at 3067 cm^{-1} and 1307cm^{-1} correspond to **ν**(C-H) and **ν**(C-O) stretching vibrations. These vibrations were found at the frequencies of 3059 cm^{-1} and 1387 cm^{-1} in Zn(II) complex, 3065 cm^{-1} and 1387 cm^{-1} in Cd(II) complex and 3065 cm^{-1}and 1347 cm^{-1} in Hg(II) complexes and this ensures the entry of benzoate ligand into the coordination sphere. The IR spectra of the complexes and ligands are given in fig.1.

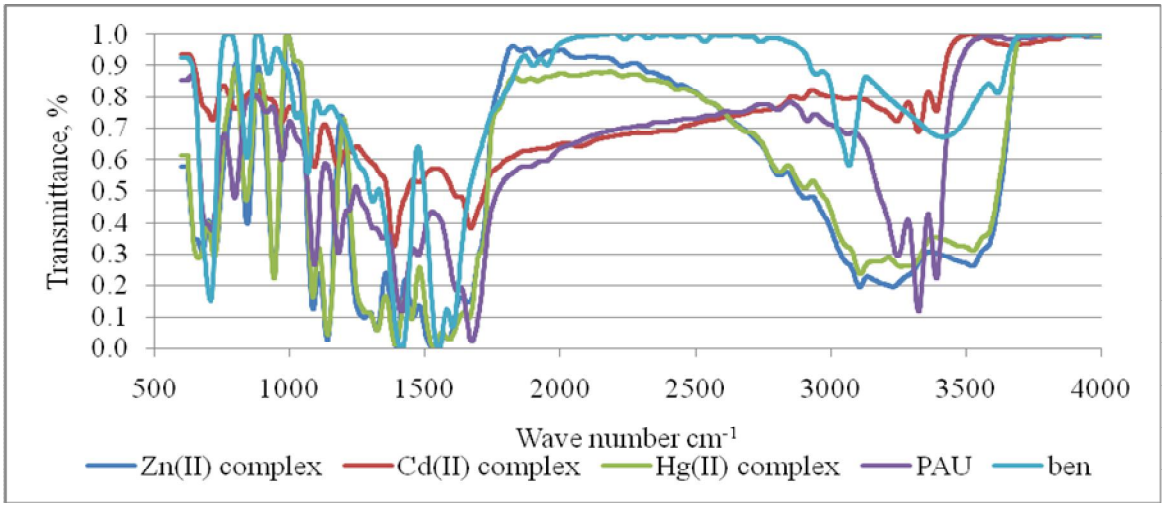

Fig.1. IR spectra of Zn(II), Cd(II) and Hg(II) complexes, PAU and ben ligands.

UV spectra

UV absorption frequencies obtained at 260nm, 262 nm and 262 nm for Zn(II), Cd(II) and Hg(II) complexes respectively, indicate the charge transfer transition. The UV spectra of the complexes are given in fig.2.

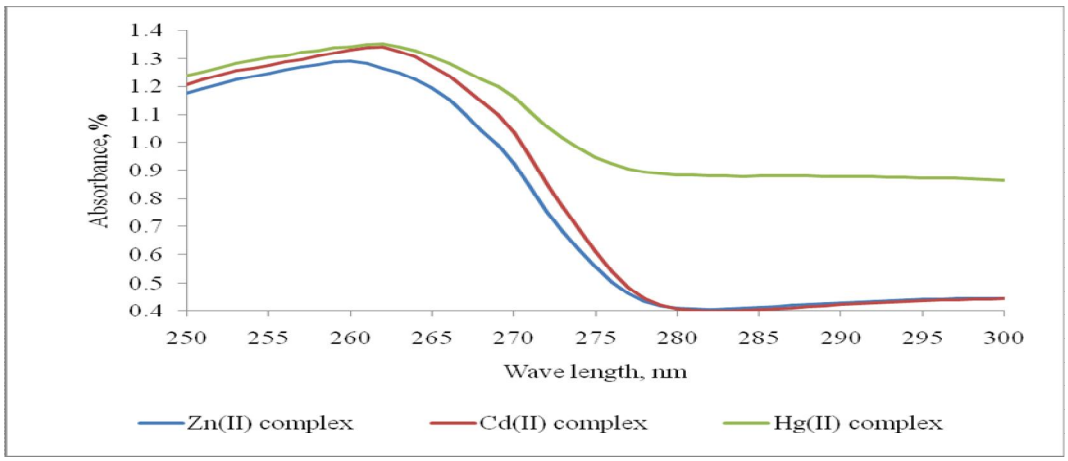

Fig.2 UV-Visible spectra of Zn(II), Cd(II) and Hg(II) complexes

Biological activities

Antibacterial activity

All the three complexes exhibit enhanced antibacterial activity than the pure ligands. Streptomycin was used as reference standard. $[Hg(PAU)ben)_2]$ exhibited highest activity than **[**$Zn(PAU)(ben)_2]$ and $[Cd(PAU)(ben)_2]$. $[Hg(PAU)ben)_2]$ showed higher activity against *Lactobacillus brevis* than streptomycin. **[**$Zn(PAU)(ben)_2]$ showed high activity against *Raoultella planticola, Vibro cholera, Lactobacillus brevis* and *Micrococus luteus.* $[Cd(PAU)(ben)_2]$ showed high activity against *Raoultella planticola* and *Pseudomonas aeruginosa.* The high antibacterial activity of Hg(II) complex may be due to the toxic nature of metal itself[13-14]. The results of antibacterial activities of the complexes, PAU and ben are given in table-2 and graphically in fig.3.

Table-2 Antibacterial activity of Zn(II), Cd(II) and Hg(II) complexes, PAU, ben and Streptomycin (Diameter inhibition in mm at 100µgml^{-1} concentration)

S. No.	Complex	*Raoultella planticola*	*Vibro cholera*	*Lactobacillus brevis*	*Pseudomonas aeruginosa*	*Micrococus luteus*
1	$[Zn(PAU)(ben)_2]$	22	22	21	10	28
2	$[Cd(PAU)(ben)_2]$	31	18	18	28	10
3	$[Hg(PAU)(ben)_2]$	36	40	39	38	29
4	PAU	6	13	15	12	10
5	ben	23	17	9	13	16
6	Streptomycin (standard)	48	52	28	38	36

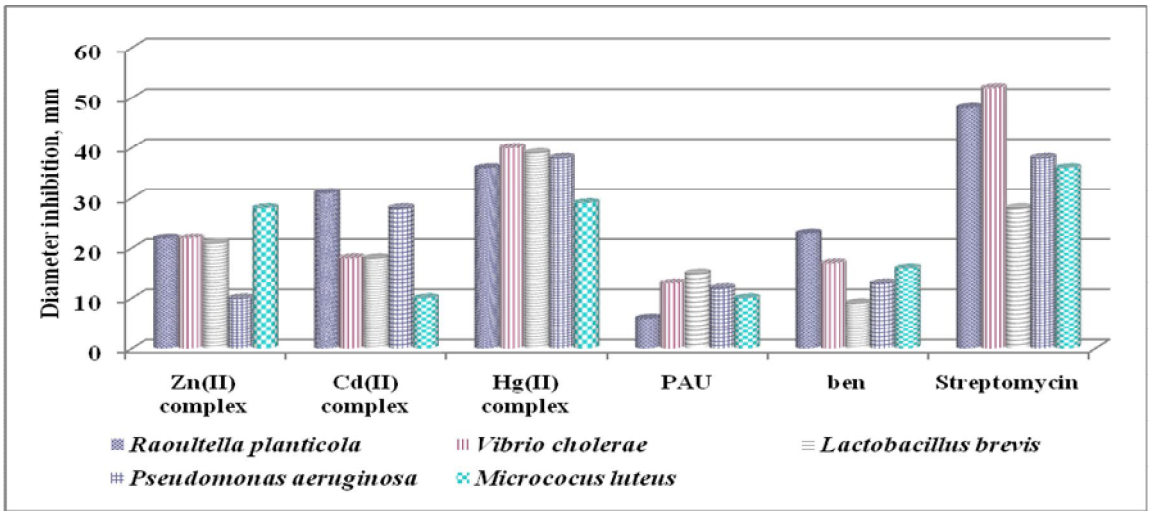

Fig.3 Comparison of antibacterial activities of Zn(II), Cd(II) and Hg(II) complexes against pure PAU, ben and streptomycin (standard) (Diameter inhibition at 100µgml^{-1} concentration)

Anti fungal activity

All the complexes showed lower anti fungal activity than the standard, ketoconazole. $[Cd(PAU)(ben)_2]$ and $[Hg(PAU)(ben)_2]$ showed higher anti fungal activity than $[Zn(PAU)(ben)_2]$. $[Cd(PAU)(ben)_2]$ showed very low anti fungal activity against *Candida albicans* and *Aspergillus sojae.* The results of anti fungal activities of the complexes, PAU and ben are given in table-3 and graphically in fig.4.

Table-3 Anti fungal activity activities of Zn(II), Cd(II) and Hg(II) complexes, PAU, ben ligands and streptomycin (Diameter inhibition in mm at 400µgml^{-1} concentration)

S. No.	Complex	*Candida albicans*	*Aspergillus oryzae*	*Aspergillus niger*	*Aspergillus flavus*	*Aspergillus sojae*
1	$[Zn(PAU)(ben)_2]$	18	21	17	22	15
2	$[Cd(PAU)(ben)_2]$	9	29	30	28	15
3	$[Hg(PAU)(ben)_2]$	32	30	28	29	19
4	PAU	4	7	11	12	10
5	ben	22	22	24	16	13
6	Ketoconazole (standard)	86	91	94	89	92

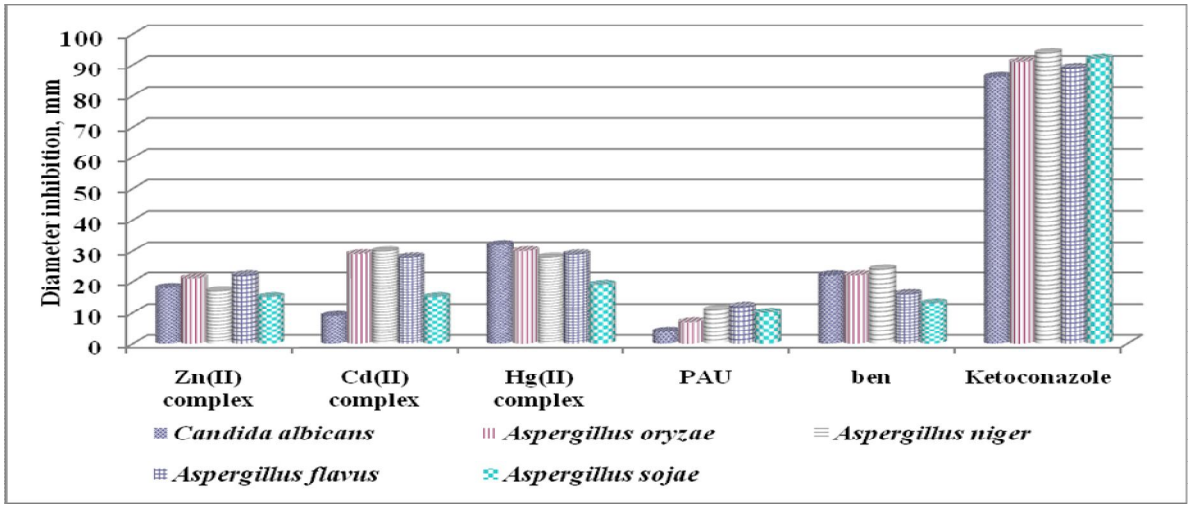

Fig.4 Comparison of anti fungal activity activities of Zn(II), Cd(II) and Hg(II) complexes, PAU, ben and streptomycin (Diameter inhibition in mm at 400 µgml^{-1} concentration)

Antioxidantal activity

All the three complexes showed lower inhibition of DPPH free radical scavenging activity at a concentration of 1000 µgml^{-1} than the standard, Vitamin-C. The inhibition percentage of Zn(II), Cd(II) and Hg(II) complexes were 22.35, 26.95 and 18.89 respectively.

Table-4 Antioxidantal activities of Zn(II), Cd(II) and Hg(II) complexes, PAU, ben and streptomycin - DPPH free radical scavenging activity (Percentage inhibition)

S. No.	Concentration, µg/ml	Zn(II) complex	Cd(II) complex	Hg(II) complex	PAU	Ben	Vitamin-C
1	1000	-	-	-	12.09	7.66	-
2	500	59.58	53.78	56.58	11.01	5.20	90.23
3	250	55.41	48.50	55.87	5.14	2.98	92.03
4	125	49.61	35.20	60.10	4.01	3.03	93.22
5	62.5	38.46	26.66	55.15	3.96	2.88	93.09
6	31.3	24.84	20.01	42.76	4.17	3.03	88.92
7	15.6	17.99	15.39	28.49	3.91	2.93	75.23
8	7.81	14.54	13.17	24.06	4.06	3.03	38.72
9	3.90	15.06	10.56	22.49	4.06	3.03	25.68
10	1.95	14.60	10.89	16.24	3.86	2.31	11.99

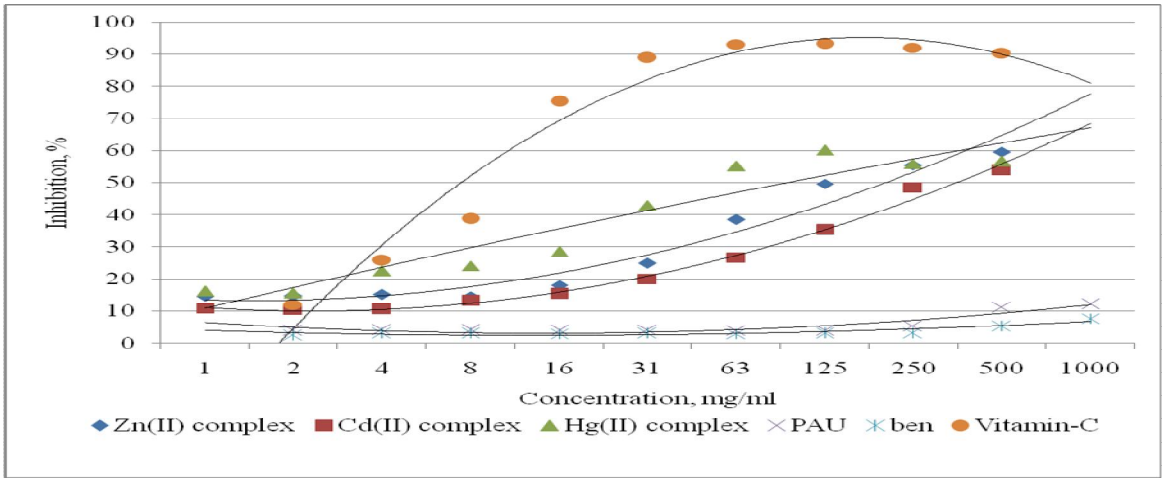

Fig.5 Comparison of antioxidantal activities Zn(II), Cd(II) and Hg(II) complexes, PAU, ben and Vitamin-C - DPPH free radical scavenging activity - Percentage inhibition at various concentration of metal complexes

CONCLUSION

From the analytical data, UV-visible and IR spectral studies, the molecular formulae were arrived at for the complexes. All the three complexes show high antibacterial activity than pure ligands. Hg(II) complex exhibits higher activity than Zn(II) and Cd(II) complexes. Hg(II) complex show higher activity against *Lactobacillus brevis* than streptomycin. Zn(II) complex show high activity against *Raoultella planticola, Vibro cholera, Lactobacillus brevis* and *Micrococus luteus.* Cd(II) complex show high activity against *Raoultella planticola* and *Pseudomonas aeruginosa.* The high antibacterial activity of Hg(II) complex is due to the toxic nature of metal itself.

All complexes show lower anti fungal activity than the standard, ketoconazole. Cd(II) and Hg(II) complexes show higher anti fungal activity than Zn(II) complex.

All the three complexes exhibit lower inhibition of DPPH free radical scavenging activity at a concentration of 1000 μgml^{-1} than the standard, Vitamin-C.

ACKNOWLEDGEMENTS

The authors express their thanks to the Principal, Management and the Head, Department of Chemistry, Bishop Heber College (Autonomous), Tiruchirappalli for providing the laboratory facilities. They also thank the Principal and Head, Department of Chemistry, Holy Cross College (Autonomous) for extending their support. The authors express their gratitude to the Management of Tamilnadu Newsprint and Papers Limited (TNPL), Kagithapuram, Karur–639 136 for encouragement and support.

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