SYNTHESIS, CHARACTRIZATION AND ANTI-MICROBIAL ACTIVITY OF BIS(4-HYDROXY 3-METHOXY BEBZAYLIDENE)ETHYLENE DIAMINE

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ABSTRACT:-The various transition metal complexes of iorn(II),copper(II), and Nickel (II) with bis(4-hydroxy 3-methoxy bebzaylidene) ethylenediamine were prepared in methanol solution with nitrates. Separately in stichometric proportion (1;2) .The mixture was refluxed for 5 to 7 hours with adjusting the PH between 7 to 8 by using alcoholic ammonia solution. Metal complexes have been characterized on the bases of spectral, elemental analysis. The metal complexes were screened for their antimicrobial activities against various pathogens. The prepared metal complexes have 1:2 metal ligand ratios.

KEYWORDS:- Bis(4-hydroxy 3-methoxy benzaylidine)ethylene diamine spectroscopic and microbial study of some transition metal ion complex

INTIODUCATION:-

Ethylenediamine:

Ethylenediamine[19,20] is the organic compound with the formula $C_2H_8N_2$. It is a colorless to yellowish liquid, possesses ammonia-like odor. It is basic in nature and miscible in polar solvents such as water and ethanol. It is widely used as a building block for polymers and as a ligand for coordination compounds.**4-Hydroxy,3- methoxy benzaldehyde:-** The 4-Hydroxy,3- methoxy benzaldehyde with molecular formula $C_8H_8O_3$ **,**commonly known as vanillin, having methoxy(-OCH_3),Hydroxyl (-OH) and aldehyde (-OCH) functional groups. It is used as flavoring agent in confectionary, beverages and foods.

Preparation of Schiff base ligand:-

Bis-(4-hydroxy-3-methoxy benzylidene)- ethylenediimine :

To a mixture of vanillin **1** (608 mg, 4 mmol) dissolved in methanol (25 ml), ethylenediamine (120 mg, 2 mmol) dissolved in methanol (25 ml) was added 2-3 drops of glacial acetic acid and the mixture was refluxed for 5 to 7 hours. On cooling the reaction mixture was poured on to 250 ml cold water. The separated solid was **filtered** of, washed with methanol and dried under vacuum.

Prepration of Metal complexes:-

1. bis-[(4-hydroxy-3-methoxy benzylidene ethylenediimine)-Fe (II)] complex:

Bis-(4-hydroxy-3-methoxy benzylidene)-ethylenediimine (500 mg, 2 mmol) and nitrates of iron (250 mg, 1 mmol) were dissolved in methanol (25 ml) separately. The mixture was refluxed for 5 to 7 hours, PH of the solution is adjusted to 7-8 using alcoholic ammonia.A dark red product was isolated after reduction of volume by evaporation.The solid was filtered of, washed with methanol and dried under vacuum.

2. Bis-[(4-hydroxy-3-methoxy benzylidene ethylenediimine)] Cu (II) complex:

Bis-(4-hydroxy-3-methoxy benzylidene)-ethylenediimine (500 mg, 2 mmol) and nitrates of

Cupper (250 mg, 1 mmol) were dissolved in methanol (25 ml) separately. The mixture was refluxed for 5 to 7 hours pH of the solution is adjusted to 7-8 using alcoholic ammonia. A dark gray product was isolated after reduction of volume by evaporation. The solid was filtered of, washed with methanol and dried under vacuum.

3. Bis-[(4-hydroxy-3-methoxy benzylidene ethylenediimine)] Ni (II) complex: Bis-(4-hydroxy-3-methoxy benzylidene)-ethylenediimine (500 mg, 2 mmol) and nitrates of Nickel (250 mg, 1 mmol) were dissolved in methanol (25 ml) separately. The mixture was refluxed for 5 to 7 hours pH of the solution is adjusted to 7-8 using alcoholic ammonia.A dark brown product was isolated after reduction of volume by evaporation. The solid was filtered of, washed with methanol and dried under vacuum.

Analytical data of Fe(II), Cu(II), Ni(II) metal complexes with Bis (4-Hydroxy 3-methoxy benzlidene) ethylenediamine

Sr. No.	Complexes	Molecular weight	Empirical formula	Color	Decom-position point	Molar conductivity $\Lambda m\Omega^{-1}cm^2$	yield %	M:L ratio	Elemental analysis				
									C	H	N	O	M
1.	Bis(4-hydroxy 3-methoxy benzylidene ehtylenediamine)Fe	855.84	$FeC_{36}H_{40}N_4O_8$	Dark red	178	11.50	71.08 (73.40)	1:2	67.30 (68.50)	4.67 (5.01)	6.54 (7.75)	14.95 (15.50)	6.54 (07.60)
2.	Bis(4-hydroxy 3-methoxy benzylidene ehtylenediamine)Cu	863.54	$CuC_{36}H_{40}N_4O_8$	Gray	192	12.23	66.70 (70.00)	1:2	66.70 (71.20)	4.63 (5.20)	6.48 (7.70)	14.82 (16.20)	7.35 (8.50)
3.	Bis(4-hydroxy 3-methoxy benzylidene ehtylenediamine)Ni	858.69	$NiC_{36}H_{40}N_4O_{89}$	Brown	187	11.10	72.29 (76.06)	1:2	67.07 (68.50)	4.65 (5.25)	6.52 (7.60)	14.90(15.50)	6..83 (7.60)

SPECTRAL STUDY:-

The U.V. spectrum consist of transition between L and M charged transfer and also transition within the ligand it self which are usually $\prod \rightarrow \prod^*$ and $\sigma \rightarrow \sigma^*$ transition .The ligand transition in all cases are charactistic of the coordinated ligand and not free ligand.

However the spectrum of free ligand AIDS in classifying the transition of coordinated ligand. The intensities of crystal field transition of coordinated ligand. The electronic transition that are involved in U.V. and near visible region are of type $\sigma \rightarrow \sigma^*$, $\prod \rightarrow \sigma^*$, $n \rightarrow \prod^*$,$\prod \rightarrow \prod^*$

The compound that contain non bonding electron on nitrogen atom are capable for showing absorption n to $n \rightarrow \sigma^*$, the transition which are of lower energy then $\sigma \rightarrow \sigma^*$. $\prod \rightarrow \prod^*$ are intermediate energy absorption to those transition which are usually between the *, $n \rightarrow \prod^*$,

and $n \rightarrow \sigma^*$,[12-13]

In the present investigation following absorption are found U.V.vissible spectra of ligand and complexes were recorded on U.V. = SHIMDZU U.V. 3600 Spectrometer at range of 200-700 by using DMSO solvent at shivaji university Kolhapur .

I .R. spectra of ligand and complexes are recorded at yeshwant mahavidyalaya nanded. I.R.Spectra of bis-[(4-hydroxy-3-methoxy benzylidene ethylenediimine Complexes A strong band absoeved at 1668 cm-1 in ligand bis-[(4-hydroxy-3-methoxy benzylidene ethylenediimine spectra is assigned –C=O (aldehyde group in Fe(bis-[(4-hydroxy-3-methoxy benzylidene ethylenediimine) H S shift ed 1563 cm-1nthis shifting of bond indicate that oxgen of aldehyde is involved in formation of shiff base[14-15].

One bond is observed at 3430 cm-1. In I R sectra of complex it is due to present of –OH group.These are also found in ligand it means that –OH group not involved in complex formation. Another bond is formed in complex 537cm^{-1} which is not found in spectra of ligand it indicate that M-L bond is formed[16-18].

IIn I.R.spectra of ligand another bondis observed at 3135 cm^{-1}. It may be presence of NH-group.in I.R. spectra of complex it is shifted at 2985 cm^{-1}. It indicate that nitrogen of shiff base ligand involved in the complex formation[19-20]. One stretching bond in ligand at 1590 cm $^{-1}$.it is due to -C=C- stretching.

AGAR DISC DIFFUSION METHOD FOR BACTERIA:

All the bacteria were cultured for 24h on various specialized agar plates specific for fungi and bacteria. The agar medium was prepared as per standard microbiological protocols and the sterile medium was cooled to 40°C, poured in sterile glass Petri plates and the medium was allowed to solidify. Sterile Whatman No.1 paper discs (4 mm diameter) were dipped in the filtered lanthanide chelate solutions.

The extracts were dissolved in 10% aqueous dimethyl sulfoxide (DMSO) to a final concentration of 50µg/all. Pure DMSO was taken as the control. 100µL of inoculums was aseptically introduced on to the surface of sterile agar plates after dipping them in the lanthanide chelate. Filter paper discs dipped in sterile distilled water and DMF solvent separately were used as controls. Replicates of two plates were maintained for each of the concentrations tested. The plates were incubated at 37°C for 24h and observed for inhibition zones. A compound is regarded as active at a particular concentration if a minimum of 1 mm circle around a disc showed no growth of the bacteria.

Petriplates with 0.5mL of 24hour old grown bacterial culture[3]. Chloramphenicol which is a bacteriostatic antimicrobial is considered a prototypical broad-spectrum antibiotic, alongside the tetracycline's, and as it is effective against a wide variety of Gram-positive and Gram-negative bacteria, including most anaerobic organisms it was employed as a control to measure comparable efficacy.

AGAR WELL DIFFUSION METHOD FOR FUNGI :

Potato Dextrose Agar for fungal species[21] was used as the bacteriological medium. The four fungi employed in the study were *Aspergillums Niger, Pencillumnotatum, Fusariumoxysporum* and *Rhizoctoniasolani*; the extracts were dissolved in 10% aqueous dimethyl sulfoxide (DMSO) to a final concentration of 50µg/all. Pure DMSO was taken as the control. 100µL of inoculums was aseptically introduced on to the surface of sterile potato dextrose agar plates and sterilized cotton swabs were used for even distribution of the inoculums. Wells were prepared in the agar plates using a sterile cork borer of 6.0mm diameter. 50µL of test and control compound was introduced in the well. The same procedure was used for all the strains. The plates were incubated aerobically at 35°C and examined after 24 hours. The diameter of the zone of inhibition produced by each agent was measured with a ruler[22]. Nystatin (originally named Fungicidin) is a polygene antifungal medication to which many molds and yeast infections are sensitive and this was used as a standard to compare the efficacy of Lanthanides as an antifungal agent[23].

GROWTH AND MAINTENANCE OF THE TEST ORGANISMS:

Microorganisms need nutrients, a source of energy and certain environmental conditions in order to grow and reproduce. In the environment, microbes have adapted to the habitats most suitable for their needs, in the laboratory, however, these requirements must be met by a culture medium. This is basically an aqueous solution to which all the necessary nutrients have been added. Depending on the type and combination of nutrients, different categories of media can be made[24].

MEDIA FOR BACTERIA:

Antibiotic Assay Medium

Composition (Ingredients Gms / Litre)

Peptic digest of animal tissue	-	(Peptone) 6.000
Casein enzymichydrolysate	-	4.000
Yeast extract	-	3.000
Beef extract	-	1.500
Dextrose	-	1.000
Agar	-	15.000
Final pH	-	(at 25°C) 6.6±0.2

PREPARATION :

Agar was weighed to 30.5 grams and suspended in 1000 ml purified/distilled water. It was then heated to boiling to dissolve the medium completely and sterilize by autoclaving at 15 lbs pressure (121°C) for 15

minutes.

MEDIA FOR GROWTH AND MAINTENANCE OF FUNGI :

Potato Dextrose Agar (PDA)

Composition (g/liter)

Potato infusion	-	4.0
(Infusion from 200g potatoes)		
Dextrose	-	20.0
Agar	-	15
pH	-	5.1 ± 0.2 at 25°C

PREPARATION:

Potato infusion was made by boiling 200 grams of sliced (washed but unpeeled) potatoes in ~ 1 liter distilled water for 30 minutes and then decanting or straining the broth through cheesecloth. Distilled water was added such that the total volume of the suspension was 1 liter. 20 grams dextrose and 20 grams agar powder was then added and the medium was sterilized by autoclaving at 15 pounds per square inch (100 kPa) for 15 minutes.

Anti-Microbial Activity Of Ligands and its Metal Complexes.

The Anti -bacterial activity of bidetate Schiff base and their metal(II) complexes were Screened against micro-organism. The Microorganism in the present investigation included E.Coli, Stathylococcus Aureus and Salmonela TyphiMinimum inhabitary concentration(MICS) Method was used to determine antibacterial activity of synthesized complex. The diffusion method is very simple, it requires commercial disk, medium used is Mucller Hinton agar with 2% of Glucose and diameter of inhibition zone is usually read at 24 hours after incubation at 37^0C.The Antibacterial activity was estimated on the basis of size of inhibition zone around the paper disk on seeded agar plates. Streptomycin was used as Standard. The Results are prepared in table.

	Pathogen		
Compound	Escherichia Coli	Stathylococcus aureus	Salmonella Typhi
Bis (4-Hydroxy-3-Methoxy benzylidene) ethylenediamine	_ _ _ _	-- --	-- --
Bis (4-Hydroxy-3-Methoxy benzylidene) ethylenediamine) Fe (II) Complex	+++	++	++
Bis (4-Hydroxy-3-Methoxy benzylidene) ethylenediamine) Cu (II) Complex.:	+++	+++	++
Bis (4-Hydroxy-3-Methoxy benzylidene) ethylenediamine) Ni (II) Complex.	+++	++	+++

Results:

1. No inhibition Zone to the Schiff base.i.e. Bis (4-Hydroxy-3-Methoxy benzylidene) ethylenediamine
2. Bis (4-Hydroxy-3-Methoxy benzylidene) ethylenediamine) NI (II) Complex show highest antibacterial activity against E.Coli.
3. Bis (4-Hydroxy-3-Methoxy benzylidene) ethylenediamine) Cou (II) Complex.:show highest antibacterai activity against E.Coli and Stathylococcus aureus
4. Bis (4-Hydroxy-3-Methoxy benzylidene) ethylenediamine) Zn (II) Complex show highest antibacterial activity against E.coli and Salmonella Typhi.

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