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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL STUDY OF RARE EARTH COMPLEXES OF SCHIFF BASE DERIVED FROM 4-AMINO-3-HYDROXY BENZOIC ACID

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ABSTRACT: Schiff base was prepared by using 4-amino-3-hydroxy benzoic acid and 4-nitrobenzaldehyde, namely (E)-3-hydroxy-4-((4-nitrobenzylidene)amino)benzoic acid. In the preparation of rare earth complexes, nitrates of lanthanum, Cerium, and Praseodymium were used with Schiff base. Rare earth Schiff base complexes were prepared by using NaOH to maintain the pH of solvent. Synthesized Schiff base ligand and its complexes were structurally characterized by UV, IR, NMR, HRMS, TGA, and elemental analysis. Structural characterization shows 1:2 metal to ligand ratio in the complexes. The electronic absorption spectra of the ligands and their metal complexes are recorded after preparing the solution and after standing the solution for 3 weeks in DMF. No appreciable change was observed in the spectrum with time. Free ligands and their metal complexes have different electronic spectra's and blue shift observed in complexes which support the complex formation. HRMS are in good agreement with calculated values, which confirms the proposed structure of ligand. Carboxylate group of Schiff base ligand shows bidentate the coordination to central metal and azomethine group is not involved in coordination. TGA shows the presence of lattice and coordinated water molecules in complexes. The present study deals with antibacterial and antifungal activity of Schiff base ligand and their Ln(III) complexes by disc diffusion method. In the study of antibacterial activity of the free Schiff base ligand and their Ln(III) complexes were screened *in-vitro* against gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* as well as with gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* with Chloramphenicol as standard drug. In the study of antifungal activity of the free Schiff base ligand and their Ln(III) complexes were screened *in-vitro* against *Aspergillus niger* and *Saccharomyces cerevisiae* with Amphotericin B as a standard drug. Schiff base ligand and complexes showed good antimicrobial activity against *Saccharomyces cerevisiae*.

INTRODUCTION: The growth of bacteria, fungi, and soil nematodes is inhibited by rare-earth ions. Except for radioactive promethium, most of the rare earth elements had antibacterial and antifungal properties which are comparable to copper ions.

These elements also showed nematicidal properties because they disrupt the nematode's embryonic growth¹.

Due to the unique properties of rare-earth ions, for example, radiation emission or magnetism, approve to rare-earth ions to be applied in many various therapeutic and diagnostic uses in current medicine², Because of their high medical importance, the rare earth cerium element and its Ce(III) complexes have been attracted worldwide attention in recent years.

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Rare earth and their compounds playing important role as antimicrobial, anti-inflammatory, anti-coagulant, in cancer prevention and treatment, and in arterial hardening prevention and treatment³. Rare-earth doped with ZnO nanoparticles were studied for their photocatalytic and antimicrobial activities and found that the anti-microbial activities of these compounds are increased than the standard control⁴. Metal ion interactions with medicinal drugs are topic of significant attention because rare-earth ions with drugs can produce unique antibacterial and anticancer properties. Some medications are known to operate by chelating metals or inhibiting the development of metalloenzymes. As a result, metals can be essential in the biological course of drug utilization in the body⁵. Carboxylic acid ligand having O-atom and neutral ligand having N-atom played a role as antenna to sensitize the rare-earth ions. Furthermore, the rigid aromatic carboxylic acid can form a bond between central metal ion and ligand by several coordination modes, and these types of rare earth complexes displayed good antibacterial activity against the *Candida albicans*⁶.

4-Amino-3-hydroxybenzoic acid Schiff base is used in the preparation of various pharmaceutical compounds such as sphingosine kinase inhibitors⁷. Bidentate or multidentate Schiff base ligands with flexible donor atoms are more suitable ligand systems for stabilizing metal complexes, due to donor atom actively supports for the coordination to one another, and such complexes have good biological activity⁸. Schiff bases of amino acids have shown excellent antiaflatoxic activity⁹. Literature survey reveals that rare earth complexes have attracted attention in the medicinal field in recent years because the rare-earth ions are potential local luminescent probes for diagnostic application in biological systems and potential applications in areas as diverse as catalysis optics, magnetic resonance imaging (MRI)¹⁰. Schiff bases and their derivatives are competent to form stable complexes with rare earth and found that rare earth complexes exhibit the anticancer, antibacterial, antifungal activity, and the complexes of Ce, Gd, and Nd show extensive antifungal activity. Coumarin and its related compounds in complexes with rare earth show the photo biological properties, antitumor, anti-leukemia, and anti-HIV activities¹¹.

Keeping this in view, Schiff base and its rare earth complexes were prepared and characterized by UV, IR, NMR, HRMS, TGA, and elemental analysis. Schiff base ligand and their Ln(III) complexes were screened for their antibacterial and antifungal activity.

MATERIALS AND METHODS: Chemicals used in the present investigation were purchased from S.D. Fine and Alfa aesar. ¹H NMR spectra were recorded on a Bruker-AV 400 MHz spectrometer using chloroform-d as solvent and TMS as an internal standard. The infrared spectra (4000–400 cm⁻¹) were recorded by using KBr pellet on Shimadzu IR Affinity⁻¹. UV measurements were taken on Shimadzu UV-1800 spectrophotometer. Elemental analyses were performed with Flash EA 1112 elemental analyzer. TGA was performed at a heating rate of 20 °C/min under nitrogen atmosphere in the range of ambient temperature to 850 °C. In the antimicrobial activity microorganism used, its strain name and reference are as [Gram+ve bacteria *Staphylococcus aureus* (NCIM 2079) and *Bacillus subtilis* NCIM 2250], [Gram-ve bacteria *Escherichia coli* (NCIM 2109) and *Pseudomonas aeruginosa* (NCIM 2036)] and [Fungi (yeast) *Aspergillus niger* (NCIM 545) and *Saccharomyces cerevisiae* (NCIM 3050)]. [NCIM: National Collection of Industrial Microorganisms, National Chemical Laboratory (NCL), Pune 411008 (India)].

Synthesis:

Synthesis of Schiff Base: (E)-3-hydroxy-4-((4-nitrobenzylidene)amino)benzoic acid Schiff base [L] was prepared by using 4-amino-3-hydroxy benzoic acid (0.002 m) was dissolved in ethanol and mixed with 4-nitrobenzaldehyde (0.002 m) dissolved in ethanol and stirred for 15 min at 60 °C. A yellow solid product was obtained and washed with methanol. Obtained product was filtered, dried and melting point was taken¹².

Yield-80%, M.P. 295-297, ¹H NMR: 8.88δ(1H, S, HC=N), 12.87(1H, S, OH-COOH), 9.70(1H, S, Ar-OH), 8.25-8.40(4H, M, Ar-H), 7.26-7.49(3H, Ar-H), IR(cm⁻¹): 1626(-HC=N-), 1692(-C=O, COOH), 3433(Ar-OH). Elemental Analysis calcd: C, 58.74; H, 3.52; N, 9.79; O, 27.95, Found: C, 58.44; H, 3.82; N, 9.75; O, 27.99, HRMS for C₁₄H₁₀N₂O₅ [M+H] calcd: 287.0668, found: 287.0664.

Synthesis of Rare Earth Complexes: The complexes were prepared by using nitrate of

Lanthanum, Cerium and Praseodymium. To an ethanolic solution of Schiff base ligand (0.004 mol) containing NaOH (0.004 mol), lanthanide nitrate (0.002 mol) in 10 mL ethanol was added drop wise

with constant stirring for half an hour on hot plate at 60 °C, in the solution metal to ligand ratio is 1:2. A solid product was obtained filtered off, washed with ethanol ¹³.

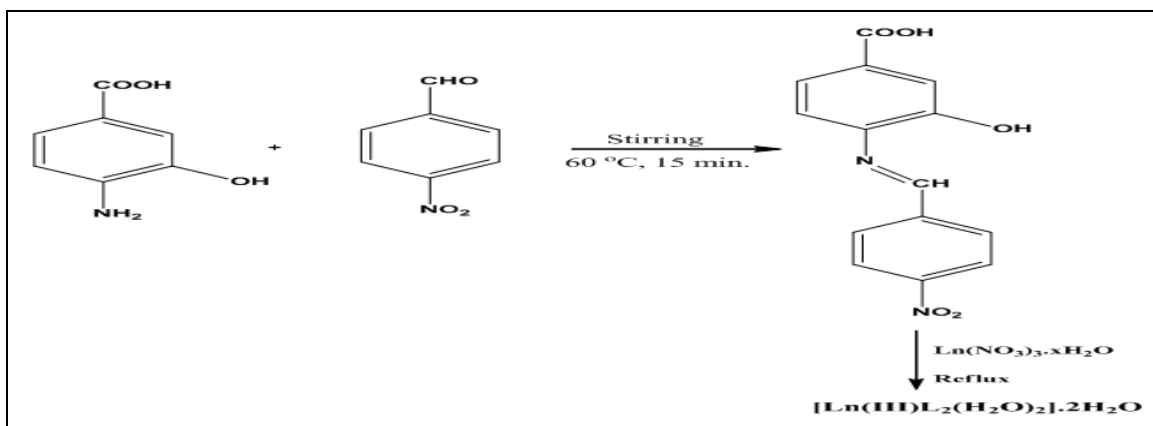


FIG. 1: SCHEME OF REACTION

TABLE 1: ANALYTICAL AND PHYSICAL DATA OF THE SCHIFF BASE LIGAND (L) AND ITS COMPLEXES

Molecular Formula	Mol. Wt.	M.P. °C	Colour	Calculated/Found %			
				C	N	H	M
Ligand	286.2396	295-297	Yellow	58.74/ 58.44	9.79/ 9.75	3.52/ 3.82	-
[La(III)(L) ₂ (H ₂ O) ₂].2H ₂ O	781.4299	>300	Lime	43.04/ 42.98	7.17/ 7.10	3.35/ 3.47	17.78/ 17.92
[Ce(III)(L) ₂ (H ₂ O) ₂].2H ₂ O	782.6404	>300	Lime	42.97/ 43.08	7.16/ 7.34	3.35/ 3.04	17.90/18 .05
[Pr(III)(L) ₂ (H ₂ O) ₂].2H ₂ O	783.4321	>300	Lime	42.93/ 43.02	7.15/ 7.09	3.35/ 3.27	17.99/ 18.08

Antimicrobial Activity: The present study deals with antibacterial and antifungal activity of Schiff base ligand and their Ln(III) complexes by disc diffusion method. In the study of antibacterial activity of the free Schiff base ligand and their Ln(III) complexes were screened *in-vitro* against gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* as well as with gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* with Chloramphenicol as standard drug. In the study of antifungal activity of the free Schiff base ligand and their Ln(III) complexes were screened *in-vitro* against *Aspergillus niger* and *Saccharomyces cerevisiae* with Amphotericin B as standard drug. Antibacterial activities were carried out by the paper disc plate method ^{14, 15}. The nutrient agar medium and 6 mm diameter paper discs were used. The compounds were dissolved in DMSO, making known stock solution. The Whatmann no. 42 paper discs of 6 mm in diameter were soaked; after that, they were cut and sterilized in an autoclave. Paper discs were soaked in desired concentration of the compounds were placed

aseptically in the plates containing nutrient agar media seeded with *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa* strains separately. The plates were incubated at 37°C for 24 h and inhibition zones were recorded in terms of diameter, which is zone of inhibition of the growth of bacteria. The antibacterial activity of a standard drug Chloramphenicol was also recorded by maintaining the same protocol at the same concentrations.

Antifungal activities were carried out by the paper disc plate method ^{14, 15}. The potato dextrose agar medium and 6 mm diameter paper discs were used. The compounds were dissolved in DMSO making known stock solution. The Whatmann No. 42 paper discs of 6 mm in diameter were soaked, after that they were cut and sterilized in an autoclave. Paper discs were soaked in desired concentration of the compounds were placed aseptically in the plates containing potato dextrose agar media seeded *Aspergillus niger* and *Saccharomyces cerevisiae* on MGY medium separately. The plates were

incubated at 37°C for 24 h and inhibition zones were recorded in terms of diameter, which is zone of inhibition of growth of fungi.

The Antifungal activity of a standard drug Amphotericin B was also recorded.

TABLE 2: ANTIMICROBIAL ACTIVITY OF LIGAND AND ITS COMPLEXES (DISC DIFFUSION ASSAY)

Test Compound	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. niger</i>	<i>S. cerevisiae</i>
Ligand [L]	14.40	10.02	14.56	10.09	00.00	27.12
[La(III)(L) ₂ (H ₂ O) ₂] ₂ H ₂ O	00.00	11.21	00.00	11.32	00.00	21.14
[Ce(III)(L) ₂ (H ₂ O) ₂] ₂ H ₂ O	9.34	7.01	10.11	00.00	00.00	12.48
[Pr(III)(L) ₂ (H ₂ O) ₂] ₂ H ₂ O	00.00	9.12	00.00	8.0	00.00	17.33
Chloramphenicol	25.11	26.12	25.44	24.53	NA	NA
Amphotericin B	NA	NA	NA	NA	12.58	28.19

Diameter in mm calculated by Vernier Caliper, '-' means no zone of inhibition, NA- Not applicable

RESULTS AND DISCUSSION: In the investigation, the Schiff base ligand [L] and its rare earth complexes were prepared and characterized by using sophisticated analytical instrument techniques. The ligand [L] was synthesized by the condensation of 4-amino-3-hydroxy benzoic acid with 4-nitrobenzaldehyde and characterized by UV-Vis, FT-IR, ¹HNMR, HRMS and elemental analysis. All the Ln(III) complexes were synthesized as per the scheme given in **Fig. 1**, in which Ln(NO₃)₃.xH₂O was used as the source of metal and they correspond to the formula [Ln(III)(L)₂(H₂O)₂].2H₂O. All the complexes were stable in air and non-hygroscopic, some of them completely and some of the sparingly soluble in DMSO and DMF. Their characterization analysis as follows

NMR: Schiff base ligand [L] formation was confirmed by NMR, a strong singlet pick observed at 8.88 δ assigned to azomethine proton ¹⁶ and a singlet observed for aromatic hydroxyl (OH) proton at 9.70 δ. The acidic proton of Carboxylic group

observed singlet at 12.87 δ ¹⁷. Four protons of aromatic ring attached to nitro group observed at 8.25 to 8.40 δ and second aromatic ring protons observed at 7.26 to 7.49 δ ¹⁸.

IR: The Schiff base ligand [L] infrared spectrum displayed a strong band at 1626 cm⁻¹ assigned to [HC=N] the azomethine group ¹⁹, and carbonyl [C=O] of COOH group observed at 1692 cm⁻¹ ²⁰. Strong band found at 3433 cm⁻¹ ^{21, 22} which may be assigned to the stretching frequencies of aromatic hydroxyl group. Aromatic ring C=C stretching frequency found at 1588 cm⁻¹ ²³. In the complexes, azomethine group does not participate in coordination, it does not show considerable change in their frequencies, and carboxylate group shows bidentate coordination. Asymmetric and symmetric stretching frequencies between the range 1527 cm⁻¹ to 1546 cm⁻¹ and 1394 cm⁻¹ to 1431 cm⁻¹ respectively, have Δ difference between 106 cm⁻¹ to 133 cm⁻¹ which confirms bidentate coordination ²⁴. The metal-oxygen band found between the range 447 cm⁻¹ and 449 cm⁻¹ ²⁵.

TABLE 3: IR STRETCHING FREQUENCIES (CM⁻¹) OF LIGAND AND COMPLEXES

Compd.	C=N	OH	COOH	asym(COO ⁻)	sym(COO ⁻)	Ln-O
Ligand [L]	1626	3433	1692	-	-	-
[La(III)(L) ₂ (H ₂ O) ₂] ₂ H ₂ O	1624	3127	-	1527	1394	447
[Ce(III)(L) ₂ (H ₂ O) ₂] ₂ H ₂ O	1624	3365	-	1539	1431	447
[Pr(III)(L) ₂ (H ₂ O) ₂] ₂ H ₂ O	1629	3385	-	1546	1440	449

UV: UV-Vis spectra of ligand and complexes were measured at 1×10⁻⁴ molL⁻¹ using DMF as solvent. The electronic absorption spectra of complexes are different from that of the free ligands, which indicates the formation of new complexes. The electronic absorption spectra of Schiff base ligand and complexes are compared. The electronic absorption peak positions obtained are used to assign the observed λ_{max} values to prominent chromophores, such as >C = N, -OH group and

benzene ring present in the ligands. The electronic absorption spectra of the ligands are recorded after preparing the solution and later on, allowing it to stand in DMF for three weeks. It was found that no appreciable change in the spectrum with time. The ligand's absorption is characterized by key absorption bands in the 200-600 nm range. The benzene ring exhibits three characteristic absorptions at 257, 263 and 287 nm; all assigned to π→π* type transitions. Bands observed due to the

$\eta \rightarrow \pi^*$ transition of the C=N chromophores found at 385 nm which changes and shows a blue shift, which may occur because of coordination of ligand to central metal ion. The complexes of lanthanum, Cerium, and Praseodymium displayed the blue shift in the electronic absorption spectrum²⁶.

HRMS: HRMS were recorded on Bruker Compass Data Analysis 4.2 from Savitribai Phule Pune University, Pune. Results obtained in mass spectroscopy are compared with calculated mass and the ligand found mass is in good agreement with calculated. For Schiff base ligand [L] calculated mass [M+H] is 287.0668 and it is found at 287.0664 in high-resolution mass spectroscopy.

Thermo Gravimetric Analysis: Thermo gravimetric analysis was carried out for the Schiffbase Ce(III) complex within the temperature range from ambient temperature to 850 °C under N₂ flow. Corresponding weight losses are discussed in terms of proposed formula of the complex with the correlation between the different decomposition steps of Ce(III) complex. The water content was determined by the thermogravimetric analysis (TGA). In the thermogram of Ce(III) complex, the coordinated water molecules and lattice water molecules decomposes with a weight loss about 10.56%²⁷. The TGA results showed that the Schiff base ligand [L] is thermally stable at room temperature and complex decomposes with a weight loss of 48.87%. Finally, these processes were followed by the last step in which oxidation of Ce(III) occurs to give the corresponding oxide as residue²⁸.

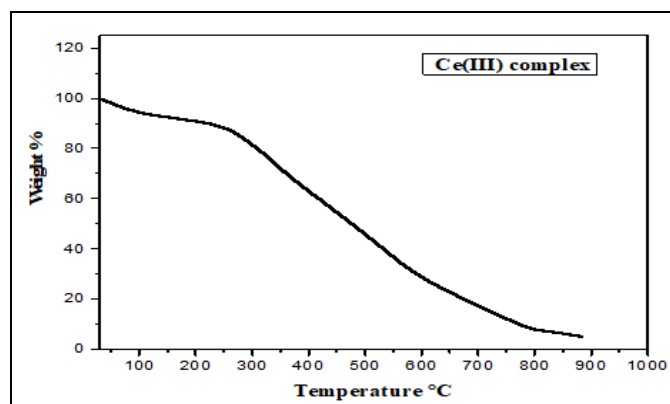


FIG. 2: TGA OF [Ce(III)L₂(H₂O)₂].2H₂O COMPLEX

Antimicrobial Activity: The Schiff base ligands and their metal complexes were tested for their antibacterial activity against gram-positive strains

Staphylococcus aureus and *Bacillus subtilis* and against gram-negative strains *Escherichia coli* and *Pseudomonas aeruginosa*. The inhibitory zone data reveals that both Schiff base ligand and their complexes show inhibition against these bacterial strains. Although with respect to standard, the majority of the tested compounds were found to be moderately active. The La(III) complex displayed increased activity than the free ligand against *Pseudomonas aeruginosa*. In the antifungal activity, inhibitory zone data reveals that both Schiff base ligand and their complexes showed a zone of inhibition against *Saccharomyces cerevisiae* and not against *Aspergillus niger*. With respect to the standard majority of the tested compounds were found to be moderately active against *Saccharomyces cerevisiae*. The metal complexes showed 7.01 to 21.14 mm zone of inhibition. The result of the investigation found that the ligand showed good activity with 27.12 mm zone of inhibition against strain *Saccharomyces cerevisiae* which is near to standard drug.

CONCLUSION: Schiff base ligand and its complexes were prepared by using 4-amino-3-hydroxybenzoic acid. Ligand shows the formation of imine bridge between aldehyde and amine group. Synthesized compounds were confirmed by UV, IR, NMR, HRMS, TGA, and elemental analysis, and all the characterization data were in good agreement with illustrated structures. In the complexes, azomethine group does not participate in the coordination, it does not show considerable change in their frequencies, and the carboxylate group shows bidentate coordination to central lanthanide metal ion. In the UV-Vis spectra, complexes displayed blue shifts, which support the formation of the complex. The present study deals with the antibacterial and antifungal activity of Schiff base ligand and their Ln(III) complexes by disc diffusion method. Schiff base ligand and complexes showed good antimicrobial activity against *Saccharomyces cerevisiae*. With respect to the standard majority of the tested compounds were found to be moderately active against *Saccharomyces cerevisiae*. The La(III) complex displayed increased activity than the free ligand against *Pseudomonas aeruginosa*. Ligand showed good activity in the zone of inhibition against strain *Saccharomyces cerevisiae* which is near to standard drug.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest.

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