Research Article

Antimicrobial and Antioxidant Behaviour of Eu(III)Complex with 1-(2-hydroxy-4,6-dimethoxyphenyl)ethanone and Nitrogen Containing Heterocyclic Ancillary Ligand

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ABSTRACT

The ternary Eu(III) ion complex, $Eu(HDMPE)_3$.nphen was synthesized by using precipitation method. The synthesized complex was characterized on the basis of various techniques like elemental analysis, ^{1}H-NMR and FT-IR, We investigated the antimicrobial and antioxidant properties of the ligand and complex Eu $(HDMPE)_3$.nphen. The *in vitro* antibacterial activities were investigated by using Gram-positive bacteria: *B.subtilis, S.aureus* and gram-negative bacterium: *Escherichia coli.* The antifungal activities were investigated by using fungi *C. albicans* and *A.niger.* The antibacterial profile of ligand is poor but better of Eu(III) ion complex $Eu(HDMPE)_3$.nphen than standard drugs ciprofloxacin and fluconazole. The antioxidant activities of the synthesized complex were determined by using DPPH method. The Eu(III) ion complex $Eu(HDMPE)_3$.nphen have poor antioxidant activities.

Keywords: Eu(III) complex, elemental analysis, ^{1}H-NMR, FT-IR, Antimicrobial and antioxidant activities.

INTRODUCTION

According to laws of Darwinian development, antimicrobial utilize makes a determination weight on microorganisms: frail ones are executed, however more grounded ones may adjust and survive[1-2]. At the point when pathogenic microorganisms can increase past some minimum amount even with attacking antimicrobials, treatment result is traded off; this wonder is eluded as antimicrobial resistance. Antimicrobial resistance is a genuine and developing issue in every one of the three nation's. To date, there is not solid global union in the nation's resistance designs. This finding may change with the more prominent universal travel that will go with globalization. Future research on the determinants of medication resistance designs, and their worldwide meeting or difference, ought to be a need [3-6].

Several reactive oxygen species (ROS) are persistently created in plants as byproducts of aerobic metabolism. Based upon the nature the ROS species, some are very dangerous and quickly detoxified by different cell enzymatic and nonenzymatic systems. While plants are surfeited with components to battle expanded ROS levels amid abiotic resistance conditions[7-8]. Fat accumulation has been found to be correlated with systemic oxidative stress in humans and mice. Production of ROS increased selectively in adipose tissue of obese mice, accompanied by augmented expression of NADPH oxidase and decreased expression of antioxidative enzymes[9]. There is much evidence suggesting role of mitochondria in ageing-related neurodegenerative diseases. Mitochondria are critical regulators of cell death, a key feature of neurodegeneration. Mutations in mitochondrial DNA and oxidative stress both contribute to ageing, which is the greatest risk factor for neurodegenerative diseases[10-12]. To prevent oxidation, several kinds of antioxidants are in use for the last few decades and mainly include organic compounds and other metal complexes. In our earlier papers Eu(III), Tb(III) and Sm(III) complexes with β-Hydroxy ketones proved excellent antimicrobial agent[13-17]. Keeping this observation in mind and in continuation of our study on exploring the biological profile of complex compounds, we hereby report the synthesis, charatrization, antimicrobial and antioxidant investigation of novel ternary Eu(III) ion complex "$Eu(HDMPE)_3$.nphen" by using 1-(2-hydroxy-4,6-dimethoxyphenyl)ethanone (HDMPE) as main ligand and 5-Nitro-1, 10-phenanthroline (nphen) as ancillary ligand.

MATERIALS AND METHODS

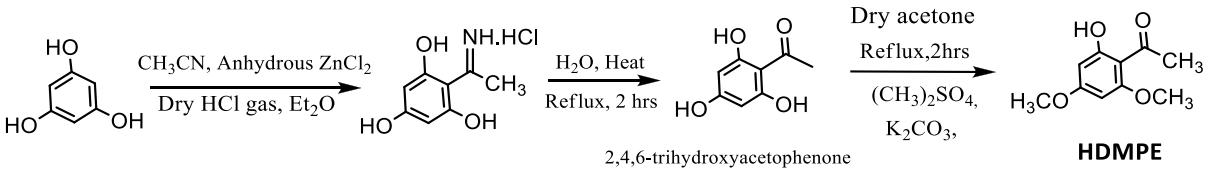
5-Nitro-1, 10-phenanthroline, $Eu(NO_3)_3.xH_2O$, benzene-1, 3, 5-triol, dimethyl sulphate, potassium carbonate, xylenol orange and 1,1-Diphenyl-2-picrylhydrazylradical (DPPH) were purchased from Sigma-Aldrich and used without additional purification. The microorganisms used in antimicrobial activities were purchased from Institute of Microbial Technology, Sector39-A, Chandigarh, India. Nutrient broth medium, nutrient agar medium, subourand dextrose agar medium and subourand dextrose broth medium were purchased from Hi-Media Pvt Ltd. The synthesized ligand HDMPE was recrystalized three times with methanol before synthesis of complexes. The elemental analysis was performed using thermoscientific flash 2000 elemental analyzer. The percentage of Eu(III) was estimated by complexometric titration with EDTA. The ^{1}H-NMR spectra were measured on Bruker Avance II 400 spectrometer using tetramethyl silane (TMS) as an internal reference (chemical shift in δ ppm). Infrared spectra were recorded (Perkin Elmer spectrum 400) from 4000–400 cm^{-1} in KBr pellets. Antimicrobial and Antioxidant investigation were made by tube dilution method and DPPH method respectively. All

measurements were made at room temperature unless otherwise stated.

Synthesis

Synthesis of ligand HDMPE

The ligand HDMPE was synthesized by adopting conventional method as per literature[18] and is given in Scheme 1 as follow:



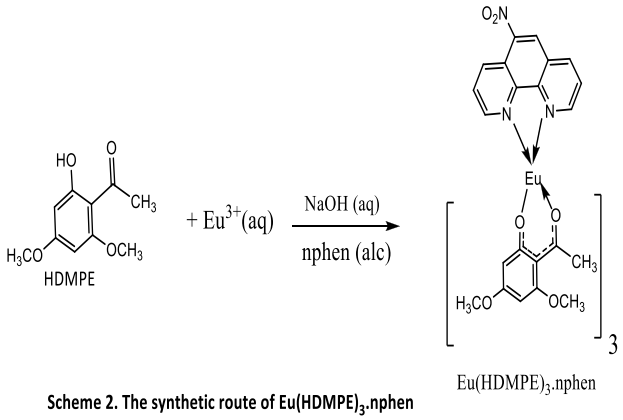
2,4,6-trihydroxyacetophenone

HDMPE

Scheme 1. The synthetic route of ligand HDMPE.

Synthesis of complex Eu(HDMPE)$_3$.nphen

The complex was synthesized by mixing ethanolic solution of 3 mmol HDMPE ligand, and 1 mmol nphen with ethanolic solution of 1 mmol $Eu(NO_3)_3.xH_2O$. Afterwards the pH of mixture was adjusted to 6.5 - 7, using NaOH (0.05 M) solution with constant stirring. This resulted into formation of white precipitates (Scheme 2). These precipitates were stirred for 3 h at about 40°C and then allowed to digest for 1 h. Finally, a suction filter was used to filter precipitates, washed with doubly distilled water and then with ethanol, dried in vacuum oven at 50 °C. The obtained complex was white powder with 79% yield. The powdered the complex was stored in sample tube in vacuum desiccators.



Scheme 2. The synthetic route of Eu(HDMPE)$_3$.nphen

Biological activity

Antimicrobial activity

The synthesized ligand HDMPE and their corresponding Eu(III) ion complex 'Eu(HDMPE)$_3$.nphen' was evaluated using tube dilution method[19]. The following bacteria were used for *in vitro* antibacterial activities, Gram-positive bacteria: *B.subtilis, S.aureus* and gram-negative bacterium: *Escherichia coli.* The following fungi were used for antifungal activity *C.albicans* and *A.niger.* The standard drugs ciprofloxacin and fluconazole[20] have also tested for their antibacterial and antifungal activity at the same concentration under the same condition as that of the tested HDMPE and Eu (HDMPE)$_3$.nphen. The dilutions of synthesized complex as well as standard drugs have been prepared in double strength nutrient broth I.P and sabouraud dextrose broth I.P media for bacteria and fungi respectively[21]. The standard, ligand and complex were dissolved in DMSO to give concentration of 100µg/mL. The incubation period for HDMPE and Eu(HDMPE)$_3$.nphen were 24 h at 37 °C for bacteria, 48 h at 37°C for *C.albicans* and 7 days at 25 °C for *A.niger* respectively. The zone of inhibitions of the antimicrobial activity has been recorded in terms of minimum inhibitory concentration (MIC).

Antioxidant activity

The antioxidant activities of the synthesized ligand HDMPE and complex Eu(HDMPE)$_3$.nphen were determined by using DPPH method[22]. When DPPH reacts with antioxidant HDMPE and complex 'Eu(HDMPE)$_3$.nphen' it shows a significant absorption decrease at 517 nm. Methanol was used as a solvent for preparation of various concentrations (25, 50, 75, and 100) µg/ml of the materials. After a 30 minutes incubation period at room temperature, the absorbance was read against a blank at 517 nm. Tests were carried out in triplicate and ascorbic acid was used as a standard antioxidant. The DPPH scavenging activity is expressed as IC_{50}, whose concentration is sufficient to obtain 50% of maximum scavenging activity. Standard curve is plotted for different concentration of ascorbic acid, ligand and complex. Scavenging of DPPH free radical was calculated as:

DPPH scavenging activity (%) = [(Ac-At) / Ac] × 100

Where, Ac is the absorbance of the control reaction and At is the absorbance of the test sample.

RESULTS AND DISCUSSION

Solubility

The complex Eu(HDMPE)$_3$.nphen was stable under atmospheric condition. The complex Eu(HDMPE)$_3$.nphen was found to be soluble in dimethyl sulfoxide, dimethyl formamide, chloroform and acetone, sparingly soluble in methanol and ethanol but insoluble in benzene and hexane.

Elemental analysis, ^{1}H-NMR and IR Spectra

The elemental analysis data for HDMPE ($C_{10}H_{12}O_4$) were found (calculated) % C, 60.87 (61.22); H, 6.14 (6.16); O, 32.19 (32.61) IR (KBr) cm^{-1} 3430 (b), 3099 (m), 3005 (w), 2943 (w), 2847 (w), 1640 (s), 1538 (s), 1457 (m), 1366 (s), 1324 (m), 1270(s), 1221 (s), 1207 (s), 1112 (m), 1079 (m), 1045 (w), 896 (m), 835 (m), 658 (m), 594 (s). ^{1}HNMR (400

MHz, DMSO): d 2.52 (s, 3H, CH3), 3.83 (s, 6H, OCH3), 6.02 (s, 2H, Ar-H), 13.84 (s, 1H, OH).

The elemental analysis data for Eu(HDMPE)$_3$.nphen ($C_{42}H_{40}O_{14}N_3Eu$) was found (calculated) % C, 52.08 (52.40); H, 4.11 (4.19); N, 4.29 (4.37); O, 23.02 (23.27); Eu, 15.21 (15.78). IR(KBr):cm^{-1} 2930 (m), 2457 (m), 2332 (w), 1613 (m), 1588 (s), 1557 (s), 1488 (s), 1369 (s), 1324 (m), 1237 (s), 1212 (m), 1148 (m), 1126 (s), 1061 (m), 908 (m), 866 (s), 841 (m), 824 (s), 787 (m), 768 (s), 689 (s), 626 (m), 547 (m), 431 (m).^{1}HNMR (400 MHz, DMSO): d 2.49 (bs, 9H, CH3), 3.46 (bs, 18H, OCH3), 6.22 (bs, 6H, Ar-H),7.66 (d, H, nphen), 7.95 (d, H, nphen), 8.43 (d, H, nphen), 8.61 (s, H, nphen), 8.87 (d, H, nphen), 9.02 (d, H, nphen), 9.11 (d, H, nphen).

The above elemental analytical data indicate the stoichiometry of the ternary complex Eu(HDMPE)$_3$.nphen to be 3:1:1 (HDMPE: Eu: nphen). The ^{1}H-NMR spectrum of the ligand HDMPE showed singlet at δ 13.84 due to phenolic proton which disappeared in the complex Eu(HDMPE)$_3$.nphen was indicating that ligand is coordinated with Eu(III) ion through the oxygen atom of phenolic OH group of the ligand HDMPE. The FT-IR spectra of ligand HDMPE exhibits abroad absorption band at 3430 cm^{-1} assigned to ν(O-H) stretching vibration which disappeared in the IR spectra of complex the Eu(HDMPE)$_3$.nphen. The ligand also displays the intense C=O stretching vibration band at 1640 cm^{-1}, which was red shifted 27 cm^{-1} in complex the Eu(HDMPE)$_3$.nphen, indicating that phenolic and carbonyl group of HDMPE participated in coordination with Eu(III) ion[14-16,23]. The strong absorption band at 1588 cm^{-1} in complex the Eu(HDMPE)$_3$.nphen assigned to C=N stretching vibration, provided good evidence that the nitrogen atoms of nphen were coordinating with the Eu(III) ion[24]. The strong absorption band at 1557 cm^{-1} in complex the Eu(HDMPE)$_3$.nphen assigned to N=O stretching vibration. The peak for Ph-O vibration of the ligand HDMPE present at 1270 cm^{-1} showed a red shift of 37 cm^{-1} in the complex Eu(HDMPE)$_3$.nphen, indicating that the phenolic group is involved in coordination with the Eu(III) ion. The appearance of absorption bands at 547 cm^{-1} and at 431 cm^{-1} in the complex Eu(HDMPE)$_3$.nphen was assigned to ν(Eu-N) and ν(Eu-O)[14,25] respectively, which affirms that the nitrogen atoms of the nphen and oxygen atoms of the ligand HDMPE participated in coordination with the Eu(III) ion. Finally, it can be concluded from the FT-IR and ^{1}H-NMR spectra of the ligand HDMPE and complex Eu(HDMPE)$_3$.nphen, that the coordination of Eu(III) was through the oxygen atoms of phenolic and carbonyl group of ligand HDMPE and nitrogen atoms of the nphen.

Antimicrobial activity

The synthesized ligand HDMPE and Eu(HDMPE)$_3$.nphen were evaluated for their *in vitro* antimicrobial activity as tabulated in Table 1and presented as bar diagram Figure 1. The antimicrobial activity has been investigated by taking ciprofloxacin and fluconazole[20] as standard drugs for antibacterial and antifungal activity respectively. The results revealed that the ligand HDMPE was having insignificant antimicrobial activity against bacterial and fungal strains, while Eu(HDMPE)$_3$.nphen showed moderate to good activity compared to the standard antibiotics and showed excellent activity against *S.aurius.* Moreover, it was interesting to note that Eu(HDMPE)$_3$.nphen proved to be better than the standard ciprofloxacin against *S. aurius*. Further it was noticed that complex was excellently active in case of *C.albicans, while* moderately active in case of *A.niger.* Moreover, it was interesting to note that Eu(HDMPE)$_3$.nphen proved to be better than the standard fluconazole against *C. albicans.* The increase in antimicrobial activity of the complex may be due to the presence of Eu (III) ion coordinated with the donor atom of the ligand which leads to the π- electron delocalization over the chelate rings[26].

Table 2: Minimum inhibitory concentration of HDMPE and Eu(HDMPE)$_3$.nphen

Compound	Minimum Inhibitory Concentration (µM/mL)				
	B. subtillis	***S.aureus***	***E.coli***	***C.albicans***	***A.niger***
HDMPE	31.8	31.8	31.8	31.8	63.7
Eu(HDMPE)$_3$.nphen	13.51	**6.75**	13.51	**6.75**	13.51
Standard.	8.71[a]	8.71[a]	8.71[a]	10.09[b]	10.09[b]

[a]Ciprofloxacin [b] Fluconazole

Antioxidant activities

In DPPH free radical scavenging activity, antioxidant reacting with stable free radical 2,2-diphenyl-2-picrylhydrazyl (DPPH) produced colorless 2,2-diphenyl-2-picrylhydrazyl. The absorbance decreased, after receiving hydrogen radical. The DPPH scavenging activity is expressed as IC_{50}. The IC_{50} value of ligand and Eu(HDMPE)$_3$.nphen were calculated from the graph plotted as inhibition percentage against concentration of HDMPE and Eu(HDMPE)$_3$.nphen as shown in Table 2 and Figure 2. The results show that ligand HDMPE and complex showed poor antioxidant activity as compared to standard ascorbic acid (IC_{50}= 43.78µg/ml).

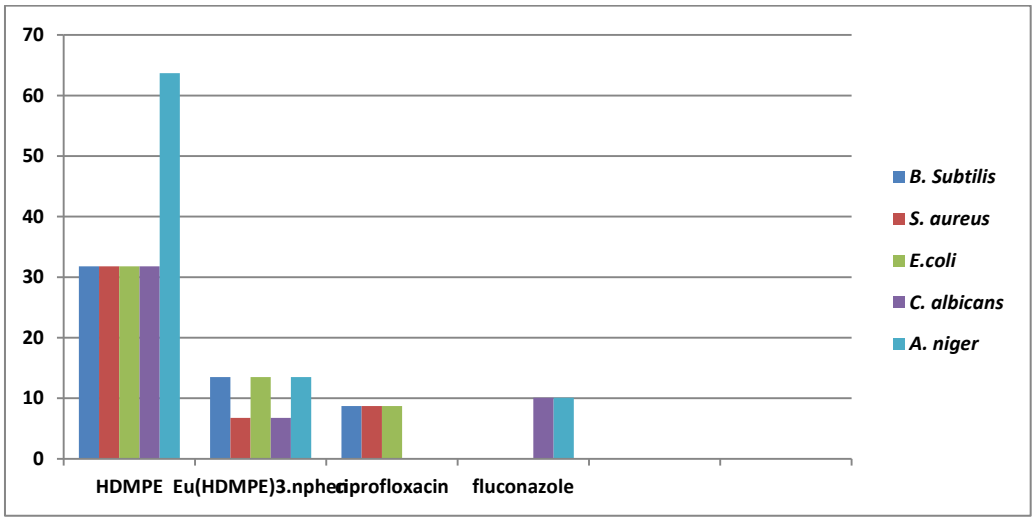


Figure 1: Bar diagram showing the antimicrobial activities of HDMPE and Eu(HDMPE)$_3$.nphen with respect to standard drugs.

Table 2: Percentage inhibition and IC_{50} values of DPPH radical scavenging activity of synthesized HDMPE and Eu(HDMPE)$_3$.nphen.

Compound	Concentration (µg/mL)				
	25	50	75	100	IC_{50}
HDMPE	23.12	43.02	60.08	80.83	60.42
Eu(HDMPE)$_3$.nphen	22.89	46.41	66.23	85.38	55.81
Ascorbic acid	34.02	56.22	76.12	92.01	43.78

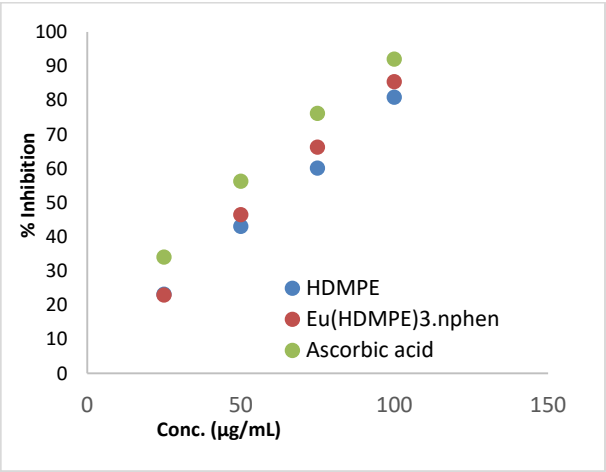


Figure 2: Percentage inhibition of HDMPE and Eu(HDMPE)$_3$.nphen with respect to standard ascorbic acid.

CONCLUSION

In this work, ternary Eu(III) complex, Eu(HDMPE)$_3$.nphen have been synthesized and characterized through various techniques like elemental analysis, FT-IR, ^{1}H-NMR spectroscopy. Variation in FT-IR and NMR spectra of free ligand (HDMPE) and complex have indicated that oxygen atoms of both phenolic as well as carbonyl group of ligand (HDMPE) and nitrogen atoms of ancillary ligand (nphen) were effectively coordinated to Eu(III)ion. This complex has exhibits excellent *in vitro* antimicrobial profile against *S.aureus* and *C.albicans* but poor antioxidant profile as compared to standard ascorbic acid (IC_{50}= 43.78µg/ml).

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