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SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF COBALT METAL COMPLEX AGAINST MULTI DRUG RESISTANT BACTERIAL AND FUNGAL PATHOGENS

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Subhasish Saha[1], Dharumadurai Dhanasekaran[1], Sarvanan Chandraleka[2], Annamalai Panneerselvam[2]

[1]Department of Microbiology, Bharathidasan University, Trichirapalli.620 024.
[2]P.G. & Research Department of Botany & Microbiology, A.V.V.M. Sri Pushpam College, (Autonomous), Poondi, 613 503 Thanjavur District, Tamil Nadu. India

Abstract. *Cobalt complex with histidine ligand was synthesized and physico-chemically characterized by solubility testing, melting point, UV-spectra and FTIR. The synthesized metal complex was evaluated for in-vitro antibacterial and antifungal activity against the multidrug resistant pathogens, such as Pseudomonas aeruginosa, Vibrio cholerae, Salmonella typhi, E. coli, Klebsiella pneumonia, Staphylococcus aureus, Aspergillus niger, Aspergillus flavus and Candida albicans. The metal complex showed the significant antibacterial and antifungal activity comparison with commercial antibiotics. Further work can be extended through In-silico docking of this metal complex and bacterial, fungal genome which can be given a better idea about genome metal interaction in molecular level.*

INTRODUCTION

Antimicrobial resistance is fast becoming a global concern with rapid increases in multidrug-resistant bacteria. Some previously treatable pathogens are now becoming untreatable, for example methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococcus (VRE) [1]. MRSA (resistant to methicillin, cephalosporins, all beta lactams, and occasionally gentamicin, erythromycin and trimethoprim/sulfamethoxazole) VRE (resistant to vancomycin, ampicillin, and gentamicin). This also includes *Enterococcus faecalis* and *Enterococcus faecium*. Other than these organism, some other organisms are also showing drug resistance like *M. tuberculosis* (Strain no: H37Rv ATCC 27294), susceptible to rifampicin, isoniazid, streptomycin, and ethambutol and two other clinical strains of multidrug-resistant *M. tuberculosis* (MDRTB), not susceptible to isoniazid and rifampicin and *Klebsiella pneumonia* is another drug resistance bacteria. The resistant microorganisms list also extends to other gram negative organisms like *Escherichia coli, Shigella flexeneri, Pseudomonas aeruginosa,* and *Salmonella typhi* and Gram-positive *Bacillus*

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subtilis bacterial strains. Even some fungal pathogens are also showing the resistance feature against drugs like *Candida albicans, Aspergillus flavus, Fusarium solani,* and *Candida glaberata*.

Throughout history, there has been a continual battle between humans and the multitude of microorganisms that cause infection and disease. Bubonic plague, tuberculosis, malaria, and more recently, the human immunodeficiency virus, acquired immunodeficiency syndrome pandemic, have affected substantial portions of the human population, causing significant morbidity and mortality. Beginning around the middle of the 20th century, major advances in antibacterial drug development and other means of infection control helped turn the tide in favor of humans [2].

To overcome the alarming problem of microbial resistance to antibiotics, the discovery of novel active compounds against new targets is a matter of urgency. Many of the crude drugs, which are sources of medicinal preparations, still originate from wild growing material. However, plant based drugs have shortened the life span of the source of material. There is a continuous search for more potent and cheaper raw materials to feed the industry. So now day's pharmaceutical industries are looking for synthesizing the alternative compounds which act as drug.

During the past decades, much attention has been given to the synthesis of new metal complexes and the evaluation of these agents for antibacterial activity. This revival interest was generated by the discovery of the antibacterial, antifungal and anticancer activity of several metal complexes. In the last few years so many studies has been done on the structure and chemical behavior of several metal complexes to find out an alternative against the drugs. The antimicrobial activity of Cobalt complex against the multi drug resistant organism is very scanty; the present study communicates the synthesis, characterization and antimicrobial activity of Cobalt complex with histidine ligand.

METHODOLOGY

Preparation of amino acid Ligands

All the required chemicals were collected for the preparation of amino acid ligands. 20 mmol acetylacetone was added in 10 ml of ethanol, mixed well and this mixture was added into a stirred solution of the 20 mmol amino acid histidine in 30 ml of water. Then the mixture was refluxed for 6-8 hours at 55 to 60^0C. During refluxing the colour of the solution turned to yellow initially and later turned to orange. (Chohan *et al*., 2006)

Recovery of the amino acid Ligands

The reaction mixture was transferred to a beaker and cooled in freezer for 2 to 3 hours to afford a solid product. After cooling the mixture, solid product (amino acid ligand) was settled down at the bottom of the beaker. Then solid product was recovered by three steps, evaporation of excess solution from the mixture by heating the solution mixture at 30^0C temperature for 20 to 30 minutes and after heating the solution mixture was kept in freezer for few minutes to make it cool and finally solid residue was filtered through 3mm Watman filter paper.

Purification of amino acid Ligands

Solid product was washed with ethanol twice, again washed with ether and then dried at room temperature. Now dried amino acid (histidine) ligand was collected and stored in clean, sterile reagent bottle.

Preparation of Metal complex

20 ml of hot methanol was taken and 500 mg of histide ligand was added into that hot methanol and mixed well.

Simultaneously 500 mg of metal chloride (cobalt chloride) was taken and mixed with 18 ml of ethanol with a magnetic stirrer. Then both the solution mixed properly. In this preparation of metal complex 1:1 molar ratio of metal and ligand (Metal : Ligand) was used. Finally the whole mixture was refluxed for 4 to 5 hours in refluxer unit at 40 to 45^0C temperature. After completion of refluxing the solution mixture was transferred into a beaker and then cooled to room temperature. Once the solution cooled the purple colour solid product (metal complex) was settled down at the bottom of the beaker.

Recovery of Metal complex

The solid product was obtained through filtering by 3 mm Watman filter paper. Then obtained solid product was washed with ethanol twice, washed with ether and finally once again washed with ethanol. Then filtrate product was dried in air to afford the desired product. In this last step of recovery, the product was handled very carefully as the product was highly hygroscopic in nature, long expos to air can damaged the product badly.

Characterization of Metal complex

The metal complex was characterized by physico-chemical characterization test.

Solubility test

The solubility of the metal complex was tested using various polar solvents like water, methnol, ethanol, butanol, acetone, ethyl acetate and non polar solvents like benzene and di ethyl ether.10 mg of metal complex was taken and dissolved into 2 ml of corresponding solvent and checked the solubility.

Melting point and Colour determination

Melting point was determined using an apparatus consisting round bottom flask filled with conc. H_2SO_4. It was fitted with thermometer. One side opened capillary tube with powdered metal complex along with thermometer in a test tube was introduced in to the flask. The flask was heated and the temperature was noted when the metal complex was

first get melted to a clear liquid. Colour of the metal complex was determind by the visual observation.

Ultra Violet spectrum

The ultra violet spectral measurement of the metal complex obtained from solution of metal complex was made 200-400 nm by using U2800 Spectrophotometer, Hitachi. Sterile distilled water was used as a solvent.

Infra Red spectrum

The metal complexes were subjected to IR spectral analysis. IR spectrum was recorded on a AVATAR 330 FT-IR, Thermo Nicolet instrument.

BIOLOGICAL ACTIVITY

Source of microorganism

The organisms used were Gram-negative *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Klebsiella pneumonia, Vibrio cholera* and Gram-positive *Staphylococcus aureus* as bacteria for testing antibacterial activity, were collected from several clinical laboratories.

Preparation of Bacterial Pathogens

The overnight cultures (0.2ml) of each bacterium was dispensed into 20ml of sterile nutrient broth and incubated for about 3-5h to standardize the culture. A loopful of the standard cultures was used for the antibacterial assay.

Antibacterial assay

Muller Hilton Agar was prepared and sterilized. 20 ml of media was poured in petriplates and allowed for solidification. The bacterial lawn culture was made using sterile cotton swab and labelled. The wells were made in the media with the help of a metallic borer with centers at least 24 mm. Recommended concentration 50 μl of the test sample 100 mg/ml in water was introduced in the respective wells. Other wells supplemented with and reference antibacterial drug [4]. The plates were incubated immediately at 37◦C for 24 hours. Activity was determined by measuring the diameter of zones showing complete inhibition (mm). Growth inhibition was compared with the drug.

Minimum Inhibitory Concentration

The minimum inhibitory concentration was determined using the tube dilution method by preparing different concentration of metal complex solution (300 µg/ml, 500 µg/ml, 600 µg/ml, 800 µg/ml, 1 mg/ml) [5]. Cleaned test tubes were taken and different concentration of metal complex such as 300 µl/ml, 500 µl/ml, 600 µl/ml, 800 µl/ml and 1 mg/ml was made and made the volume of medium upto 2 ml with nutrient broth and prepared the control with 2 ml of nutrient broth without any metal complex and sterilized the

medium at 121^0C temperature at 15 lbs pressure for 15 minutes in autoclave. After sterilization, the medium was allowed to cool and 0.2ml of overnight cultures of each organism was dispensed into sterile medium and incubated for 24 hours. The activity was measured by turbidity in the broth.

Antifungal assay

The antifungal activities of all metal complexes were studied against three fungal cultures, *Candida albicans, Aspergillus flavus,* and *Aspergillus niger*. Sabouraud dextrose agar was prepared, sterilized and prepared the culture plates same like Muller Hilton Agar. After solidification of media, respective fungal spore suspensions were transferred to petri plates. The wells were made in the media with the help of a sterile metallic borer with centers at least 24 mm. Recommended concentration 50 μl of the test sample 100 mg/ml in water was introduced in the wells. The plates were incubated at 30^0C for 72 hours. The results were recorded as zones of inhibition in mm [6].

RESULT AND DISCUSSION

Physico-chemical properties of Metal complex

The metal complex was prepared by refluxing an appropriate amount of amino acid histidine with acetylacetone in ethanol. Metal complex was prepared by using the metal salts as chloride (cobalt chloride) with the amino acid histidine in molar ratios of metal: ligand as 1: 1.

Metal complex was dissolved in all polar solvent such as water, methnol, ethanol, butanol, acetone, ethyl acetate and non polar solvents like benzene and di ethyl ether (Table 1). Cobalt metal complex was melted at 220^0C (Table 1). The UV visible spectroscopic data of cobalt metal complex is 2.292. The result has supported the presence of Cobalt molecule in the ligand metal complex, as Cobalt molecule shows the highest absorption in the range between 200 to 300 nm (Table 1) wavelengths [7].

The infrared spectra of the cobalt metal complex was exhibited a band at 3448.99 cm-1. The lowering of this band frequency was due to the hydroxyl (OH) group. Another band was appeared at about 1638.31 cm-1 and this band frequency was due to the carboxylic group (Table 1). The other bands in the spectrum of cobalt complex were due to the carbon and hydrogen bonding [8].

Antibacterial activity of Cobalt complex with histidine ligand and histidine ligand

Antibacterial activity of cobalt complex with histidine ligand and histidine ligand was analysed with several bacterial pathogens such as *Pseudomonas aeruginosa, Escherichia coli* and *Salmponella typhi.* Cobalt complex with histidine ligand was shown the zone of inhibition in culture plates but histidine ligand was not shown any zone of inhibition in culture plates (Table 2). The present finding evidence that antibacterial activity is due to the cobalt metal in histidine ligand, where as histidine ligand alone fails to show the antibacterial activity.

Antibacterial activity of Metal complexes and Commercial drugs

Antibacterial activity of metal complex and commercial drugs (Ampicillin and Streptomycin) were checked against six bacterial pathogens such as *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Vibrio cholerae* and *Salmponella typhi.* Cobalt complex was shown better result in the form of zone of inhibition in culture plates rather than commercial drugs (Table 3) against three pathogens (*Pseudomonas aeruginosa, Vibrio cholerae* and *Salmponella typhi*).

The zone of inhibition of cobalt metal complex in the culture plates are Pseudomonas *aeruginosa* (23 mm), *Vibrio cholera* (24 mm), *Salmponella typhi* (22 mm).

Minimum Inhibitory Concentration Assay

Pseudomonas aeruginosa, Vibrio cholerae and *Salmponella typhi* were checked for the Minimum inhibitory concentration. Minimum inhibitory concentrations of bacterial pathogens were observed such as *Pseudomonas aeruginosa* (500 µg/ml), *Vibrio cholera* (600 µg/ml*)* and *Salmponella typhi* (500 µg/ml) (Table 8). This result is similar to the finding of Chohan *et al.* [3] (Table 4).

Antifungal Activity of Cobalt Metal Complex

The inhibitory activity of Cobalt metal complex was observed in fungal pathogens. Among the three fungal pathogens *Candida albicans* was highly susceptible (Co complex 45 mm) than the *Aspergillus niger* (Co complex 34 mm) followed by *Aspergillus flavus* (Co complex 21 mm). (Table 5)

Table 1. Physico-chemical Characterization of Cobalt Metal Complex

Sl. No.	Character	Co complex
1.	Colour	Purple
2.	Nature	Powdery, Highly hygroscopic
3.	Solubility	Soluble in methnol, ethanol, butanol, acetone, ethyl acetate benzene and di ethyl ether
4.	Melting temperature	220^0C
5.	UV spectrum (200-400 nm)	2.276, 2.292, 1.971, 1.442, 0.807
6.	[UV λ Max X nm] IR_Y Max (KBr)cm^{-1}	3448.99, 2088.61, 1638.31, 1384.72, 593.08

Table 2. Antibacterial Activity of cobalt complex with histidine ligand and histidine ligand

Sl. No.	Name of Bacterial Pathogens	Zone of Inhibition (mm)	
		Metal Complex	Control
1.	*Pseudomonas aeruginosa*	32	No zone
2.	*Escherichia coli*	27	No zone
3.	*Salmponella typhi*	29	No zone

Note: Metal complex - Co complex with histidine ligand, Control - Histidine ligand

Table 3. Antibacterial activity of metal complexes and commercial drugs

Sl. No.	Name of Bacterial Pathogens	Zone of Inhibition (mm)	Zone of Inhibition (mm)	
		Co Metal	Ampicillin	Streptomycin
1.	*Escherichia coli*	28	37	36
2.	*Pseudomonas aeruginosa*	23	20	21
3.	*Salmonella typhi*	22	28	20
4.	*Klebsiella pneumonia*	31	42	40
5.	*Staphylococcus aureus*	33	44	38
6.	*Vibrio cholerae*	24	18	26

Table 4. Minimum Inhibitory Concentration assay of metal complex against bacterial pathogens

Sl. No.	Name of Bacterial Pathogens	Zone of Inhibition (mm)				
		300 µg/ml	500 µg/ml	600 µg/ml	800 µg/ml	1 mg/ml
1.	*Pseudomonas aeruginosa*	+	-	-	-	-
2.	*Vibrio cholera*	+	+	-	-	-
3.	*Salmonella typhi*	+	-	-	-	-

Note: + = Growth of bacteria, - = No growth of bacteria

Table 5. Antifungal Activity of Cobalt metal complex

Sl. No.	Name of Fungal Pathogens	Zone of Inhibition (mm)
		Cobalt Complex
1.	*Aspergillus niger*	34
2.	*Aspergillus flavus*	21
3.	*Candida albicans*	45

CONCLUSION

Cobalt metal complex were synthesized and physico-chemically characterized by solubility testing, melting point, UV-spectra and FTIR. Antimicrobial analysis of Cobalt metal complex was evaluated among the different bacterial strains such as *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi* and *Klebsiella pneumonia, Vibrio cholera* and *Staphylococcus aureus* and fungal strains such as *Aspergillus niger, Aspergillus flavus* and *Candida albicans.* Among the pathogens *Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholera* and *Candida albicans* were highly susceptible to the metal complex. The present study concluded that cobalt complex will be used as good drug of choice to manage the bacterial and fungal diseases after evaluating the in-vivo effect of metal complex on experimental animal and clinical trials.

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SINTEZA I KARAKTERIZACIJA KOMPLEKSA JONA KOBALTA I HISTIDINA, KAO I NJEGOVA MIKROBIOLOSKA AKTIVNOST PROTIV VISESTRUKO OTPORNIH PATOGENIH SOJEVA BAKTERIJA I GLJIVICA

Subhasish Saha, Dharumadurai Dhanasekaran, Sarvanan Chandraleka, Annamalai Panneerselvam

Ostvarena je sinteza kompleksa jona kobalta i histidina kao liganda koji je fiziko-hemijski okarakterisan (odredena je njegova rastvorljivost, tacka topljenja, a i snimljeni su njegovi UV i IC spektri). In vitro antibakterijska i antifungalna aktivnost sintetisanog kompleksa je testirana na visestruko otpornim patogenim sojevima, kao sto su: Pseudomonas aeruginosa, Vibrio cholerae, Salmonella typhi, Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus, Aspergillus niger, Aspergillus flavus i Candida albicans. Kompleks je pokazao znacajnu antibakterijsku i antifungalnu aktivnost u poredenju sa komercijalnim antibioticima koji su paralelno testirani sa njim. Dalja istrazivanja u smislu in silico ekperimenata bi pruzila informacije o interakciji ovog kompleksa kobalta sa genomom bakterija i gljivica na molekulskom nivou.