JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Schiff's base hetero-binuclear complexes of Ni(II) and Organic Salts of Sr(ll) metal derived from *N,N'*-bis (salicylidene)-o phenylenediamine as potent antimicrobial agent.

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Abstract:

Several stable hetero-binuclear Schiff base metal complexes of nickel and strontium, (**L**) = *N,N*-bis (salicylidene)-o-phenylenediamine; **Ni(II)L** = Nickle schiff base complex; **Ni(II)LSr(II)-S**$_1$ [where, S$_1$=ONP (o-nitrophenol)]; **Ni(II)L-Sr(II)-S**$_2$ [where, S$_2$=DNP (2,4- dinitrophenol)]; **Ni(II)L-Sr(II)-S**$_3$ [where, S$_3$=TNP (2,4,6-trinitrophenol)] have been synthesized using Ni(II) metal chelates of Schiff base and alkaline earth metal [Sr(II)] salts of ONP, DNP and TNP. The IR spectra indicate a coordination bond between the Sr(II) and the Ni(II) metal chelate, which is revealed by the oxygen atoms of the phenolic C-O. It is discovered that the nitrogen atoms of the imine and the oxygen atoms of the hydroxyl groups form an interaction between the ligands, the Nickle atom, and the strontium atom.These complexes are biologically active against bacteria viz. Gram-positive bacteria, Bacillus cereus and Staphyloccocus aureus, Gram-negative bacteria, Salmonella typhimurium and Escherichia coli, Yeast, Candida albicans, Mold, Aspergillus niger and Fungus, Aspergillus carbonarius, showing good antimicrobial and antifungal activity against several bacteria and fungi

Keywords : *hetero-binuclear metal complexes; Antimicrobial Studies; Schiff Base; organic salts of strontium*

Introduction :

Imine-containing (C=N) compounds known as azomethine Schiff's bases are typically synthesized through the Primary amines undergo condensation reactions with aldehydes or ketones, which are significant in elucidating the preferred coordination geometries of metal complexes [**1-4**]. Schiff bases possess the ability to form complexes with metals and are of significant interest due to their biological properties. In the field of inorganic chemistry, transition metal complexes of Schiff bases have garnered considerable attention and have been extensively studied [**5-6**]. The extensive study of Schiff-base metal complexes is attributed to their wide-ranging applications in industry, analytical processes, biology, medicine, and pharmacology [**7-8**]. Additionally, because of their intriguing structural characteristics, metal chelates, and antimicrobial properties [**9-10**], the condensation products have drawn more and more attention [**11**].

Schiff base ligands, particularly salens, are considered essential in biological applications. Over the past twenty years, various transition metal-salen complexes have been extensively employed for a wide range of biological applications [**12**]. Efforts to enhance the capabilities of Schiff base ligands have led multiple research teams to explore their use in heterobimetallic systems combining transition metals with alkali metals or group 13 metals or with Lanthanides [**13-16**]. Despite these advancements, there is limited research on heterobimetallic Schiff base catalysts that combine transition metals with alkaline earth metals.

The heterobinuclear metal complexes of Ni (II) Schiff base demonstrate biological activity, exhibiting enhanced effects compared to their parent ligand [**17**]. The development of novel heterobinuclear complexes from Ni (II) Schiff base complexes as ligands and its alkaline earth metal derivatives of organic acids has been the focus of our work. Gram-positive bacteria, Bacillus cereus (ATCC 1966) and Staphyloccocus aureus (ATCC 25923), Gram-negative bacteria, Salmonella typhimurium (ATCC 14028) and Escherichia coli (ATCC 25922), Yeast, Candida albicans (ATCC 10231), Mold, Aspergillus niger (NRRL 612) and Fungus, Aspergillus carbonarius (NRRL 368) were all susceptible to the antimicrobial activity of the ligand and its metal complexes.

Methodology :

AR-grade chemicals were used. Chemicals from Sigma Aldrich were utilized in the synthesis without further purification. All of the compound synthesis was performed in a solvent that had been dried as well as purified according to accepted literature methodologies. Melting points of the ligand and its metal complexes were determined using a Systronics melting point instrument. The Elemental analysis results were taken out through Thermo Fisher Scientific Flash Smart instrument. Infrared spectra were recorded in the region (4000 400 cm^{-1}) using KBr disks on a Shimadzu 4000 IR spectrophotometerHeterobinuclear complexes thus formed were investigated for their antimicrobial properties. Bacillus cereus (ATCC 1966), Staphyloccocus aureus (ATCC 25923), Salmonella typhimurium (ATCC 14028) and Escherichia coli (ATCC 25922) were tested for antibacterial activity, whereas Candida albicans (ATCC 10231), Aspergillus niger (NRRL 612) and Aspergillus carbonarius (NRRL 368) were tested for antifungal activity.

Synthesis :

Synthesis of Schiff base N,N'-bis(salicylidene)-o-phenylenediamine (L)

As per the literature method, the Schiff base ligand was prepared. Salicylaldehyde and orthophenylenediamine were used in 2:1 molar reactant ratios to create the Schiff base ligand following the literature method [**18**]. In a 100 mL beaker, 20 mL of ethanol and 20 mmol (2.08 mL) of salicylaldehyde were dissolved at room temperature. While stirring, a dropwise addition of orthophenylenediamine (10 mmol, 1.08 g) dissolved in 20 mL of ethanol was added to the salicylaldehyde solution. After three hours of reflux heating to 80°C in a water bath, the mixture was allowed to cool to room temperature. After filtering and drying, the resulting yellowish-orange crystalline powder was obtained.

Synthesis of Ni(II)L Complex

The synthesis of the nickel(II) complex serves as an example. To a 20 mL ethanol solution of nickel(II) chlorite hexahydrate (1.5 mmol, 0.255 g), a solution of N,N'-bis(salicylidene)-ophenylenediamine (1.5 mmol, 0.475 g) was added gradually while stirring. For almost two hours, the reaction mixture was allowed to reflux in the water bath. Following the separation of the colored complex, the product was filtered and allowed to dry at room temperature. The complex was found to be a brick-red solid [Ni(II)L].

Synthesis of Strontium salts of o-nitrophenol, 2, 4-dinitrophenol and 2,4,6- trinitrophenol

Following the literature method [**19**] organic salts of strontium metal was prepared. Strontium carbonate (5g) was gradually added to 1500ml o-nitrophenol/dinitrophenol/ trinitrophenol solution (1%) and the mixture was agitated for two hours at room temperature. As a result of the carbon dioxide byproduct, bubbles were produced from the reacting mixture. Following the reaction, the unreacted strontium carbonated was isolated and dried, and the solution was filtered. Impurities were thus removed by dissolving the crystal in 500 ml of acetone. In order to remove excess organic acids, the filtrate was concentrated to 100 ml, and the concentrated solution was then added to 2000 ml of dichloromethane for purification.

Synthesis of binuclear complexes containing Ni(II) metal and Strontium metal salts

Strontium metal salts of o-nitrophenol, dinitrophenol, and trinitrophenol were added to Ni(II)L complex in a conical flask containing ethanol in a 1:1 molar ratio. On a hot plate between 80 °C and 85 °C, the mixture was refluxed for one hour while being stirred. After precipitating in heated conditions, the distinctive colour adducts were filtered, carefully washed with pure ethanol, dried in an electric oven, and then stored in a desiccator over anhydrous calcium chloride.

Antimicrobial activity

The activity of the synthesized compounds was assessed using the conventional disk-agar diffusion method [**20**]. The examined bacteria were transferred into Petri dishes after being homogenized in agar suspensions and seeded in test tubes with nutrient broth (NB) 10^8 cells/m. After 16 h of incubation at 30°C in a thermostat, the inhibition (sterile) zone diameters (including disk) were measured and reported in millimeters. The holes, which had a diameter of 4 mm, were made in the cold medium. Following their dissolution in dimethylformamide (DMF) solvent, the tested compounds were prepared in three concentrations: 20, 30 and 40 mg/mL. 10 microliters of each preparation were then dropped onto disk-agar with a diameter of 6 mm using a micropipette.

A binuclear metal complexes ability to inhibit the growth of sensitive organisms, such as Gram-positive bacteria, Bacillus cereus (ATCC 1966) and Staphyloccocus aureus (ATCC 25923), Gram-negative bacteria, Salmonella typhimurium (ATCC 14028) and Escherichia coli (ATCC 25922), Yeast, Candida albicans (ATCC 10231), Mold, Aspergillus niger (NRRL 612) and Fungus, Aspergillus carbonarius (NRRL 368), is demonstrated by an inhibition zone diameter exceeding 6 mm. For every extract, three duplicates were tested against every test organism. For Gram-positive bacteria, the standard reference was cephalothin, for Gram-negative bacteria, it was chloramphenicol and for yeasts and fungi, it was cycloheximide.

Results and Discussion :

Both nickel (II) metal complexes and their alkaline earth metal (strontium) adducts are stable and have characteristic solid colours. After melting, most of the complexes were decomposed. The compounds' elemental analysis results showed a good agreement with their computed values. Table 1, displays the complexes and its molecular formula along with molecular weight, colour, melting point, yield, and elemental analytical data. These adducts are insoluble in water but soluble in organic solvents like acetone, methanol, benzene and DMF.

Table 1.

Physical and analytical data of the metal complex of ligand.

Sl. No.	Complexes [Molecular Formula] {Molecular Weight (g/mol)}	Colour	Melting point (°C)	Yield (%)	Elemental analysis (%) Found (Calculated)				
					C	H	N	Ni	Sr
1.	Schiff Complex (L) [$C_{20}H_{16}N_2O_2$] {316.360}	Yellowish Orange	164.8	84.6	75.82 (75.93)	4.93 (5.10)	8.71 (8.86)	-	-
2.	Ni(II)-L [$C_{20}H_{14}N_2O_2Ni$] {373.037}	Brick Red	>300	93.2	64.31 (64.40)	3.69 (3.78)	7.38 (7.51)	15.68 (15.73)	-
3.	Sr-S_1 [$C_{12}H_8N_2O_6Sr$] {363.824}	Yellowish Orange	>300	42.5	39.50 (39.62)	2.10 (2.22)	7.62 (7.70)	-	23.97 (24.08)
4.	Sr-S_2 [$C_{12}H_6N_4O_{10}Sr$] {453.818}	Deep Orange	>300	37.4	31.68 (31.76)	1.24 (1.33)	12.17 (12.35)	-	19.28 (19.31)
5.	Sr-S_3 [$C_{12}H_4N_6O_{14}Sr$] {543.812}	Orange Red	>300	32.7	26.41 (26.50)	0.61 (0.74)	15.39 (15.45)	-	16.05 (16.11)
6.	Ni(II)-L-Sr-S_1 [$C_{32}H_{22}N_4O_8NiSr$] {736.861}	Deep Orange	>300	78.6	52.03 (52.16)	2.94 (3.01)	7.53 (7.60)	7.89 (7.97)	11.81 (11.89)
7.	Ni(II)-L-Sr-S_2 [$C_{32}H_{20}N_6O_{12}NiSr$] {826.855}	Reddish Orange	>300	72.8	46.41 (46.48)	2.36 (2.44)	10.07 (10.16)	7.02 (7.10)	10.52 (10.60)
8.	Ni(II)-L-Sr-S_3 [$C_{32}H_{18}N_8O_{16}NiSr$] {916.849}	Yellowish Orange	>300	75.4	41.84 (41.92)	1.90 (1.98)	12.15 (12.22)	6.33 (6.40)	9.48 (9.56)

IR Spectra :

Table 2. lists the significant infrared spectrum information of the ligand and its metal complexes along with their approximate assignments. Following the condensation reaction of N, N'-bis (salicylidene)-o-phenylenediamine, the ligand's infrared spectra did not show a distinctive band for a C=O group. This outcome shows that the Schiff metal complex was completely formed. The υ(C=N) stretching vibration of the ligand's azomethine group is responsible for the medium band at 1611 cm^{-1}. After complexation, this band shifts to a lower wavenumber by 8–15 cm^{-1}. These shifts to lower wavenumbers provide evidence that

the azomethine group of the ligand is involved in binding to the nickel (II) ion [**21**]. This can be explained by the donation of electrons from nitrogen to vacant d-orbitals of the metal atoms [**22**].

Table 2.
Characteristic IR spectral data of the Schiff complex (L) and its metal complexes.

Compound	**IR Spectra (cm^{-1})**			
	υC=N	**υC-O**	**υM-N**	**υM-O**
Schiff complex (L)	1611	-	-	-
Ni(II)L	1603	1516	464	583
Ni(II)L-Sr-S_1	1598	1560	461	591
Ni(II)L-Sr-S_2	1598	1543	472	598
Ni(II)L-Sr-S_3	1596	1550	458	595

The phenolic C-O stretching may be the reason of the bands in the 1516–1560 cm^{-1} range seen in the infrared spectral bands of Ni(II) transition metal complexes and their binuclear complexes with the alkaline earth metal strontium [**23**].When compared to their binuclear complexes, the C-O bands for Ni(II) transition metal complexes as ligands are slightly higher. By virtue of υC-O (phenolic), the nickel metal complexes as ligands exhibit absorption at 1516 cm^{-1}. This band shifts up to 44 cm^{-1} toward the lower wavenumber side on the formation of a Ni(II)L schiff base complex with different salts of Sr(II), this is because phenolic oxygen coordinates with the metals, signifying the existence of a phenoxobridge [**24-26**].

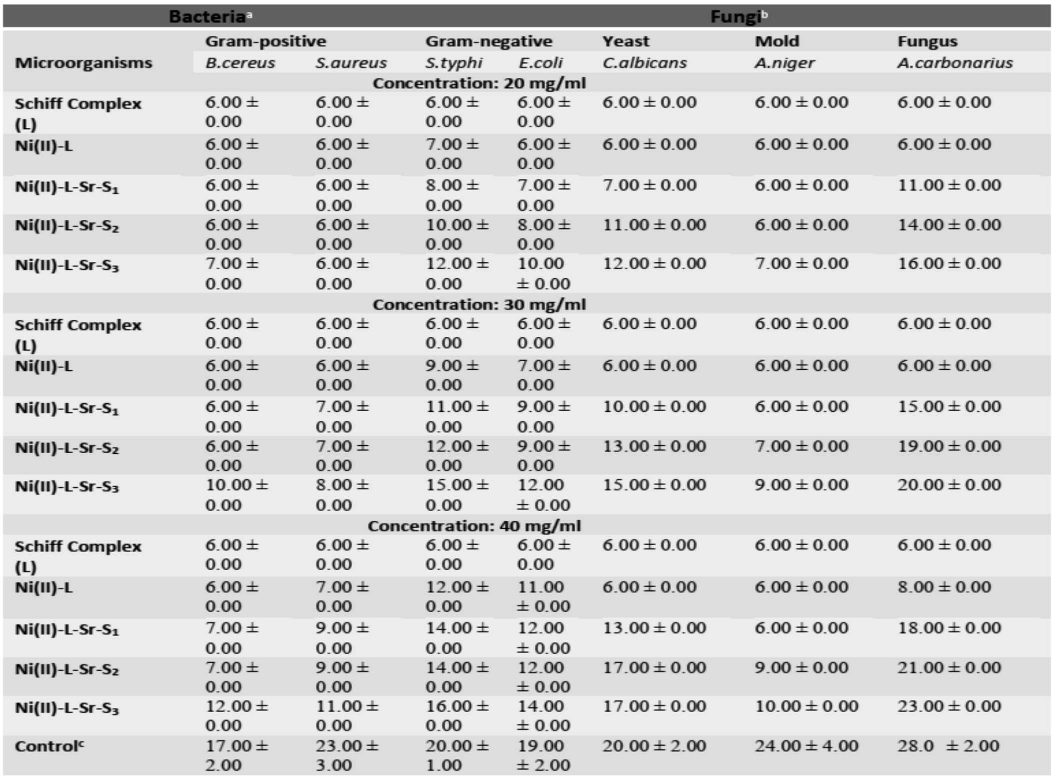
Shifts are anticipated due to the phenolic C-O bond and a ring current that results from the delocalization of electrons in the chelating ring. υM-O and υM-N bonds are represented by the far infrared bands at 583-598 cm^{-1} and 458-472 cm^{-1}, respectively [**27-29**]. Their coordination through the oxygen atom of the Schiff base is facilitated by these positive shifts in the absorption band of the binuclear complex compared to those of the transition metal complex assigned to the synthesis complex. Based on the electronegativity these are proposed. The M-O bond is significantly more ionic than the M-N bond because oxygen has a higher electronegativity than nitrogen. Thus, according to the IR spectra, the Schiff complex (L) ligand acts as a tetradentate ligand coupled to the metal ions via the azomethine.

Antimicrobial Studies :

The Schiff ligand (L) and its metal complexes viz Ni(II)-L, Ni(II)L-Sr-S_1, Ni(II)L-Sr-S_2, Ni(II)L-Sr-S_3 was investigated for their antimicrobial activity against sensitive organisms. Gram-positive bacteria include Bacillus cereus (ATCC 1966) and Staphyloccocus aureus (ATCC 25923), Gram-negative bacteria include Salmonella typhimurium (ATCC 14028) and Escherichia coli (ATCC 25922), Yeast is Candida albicans (ATCC 10231), Mold is Aspergillus niger (NRRL 612) and Fungus is Aspergillus carbonarius (NRRL 368). The findings are presented on Table 3.

Table – 3

Diameter (in mm) of inhibition zones of Schiff complex L, Ni(II)L, Ni(II)-L-Sr-S_1, Ni(II)-L-S_2, and Ni(II)L-Sr-S_3 and of different drugs against bacteria and fungi at differnt concentrations.



	Bacteria[a]				Fungi[b]		
	Gram-positive		Gram-negative		Yeast	Mold	Fungus
Microorganisms	*B.cereus*	*S.aureus*	*S.typhi*	*E.coli*	*C.albicans*	*A.niger*	*A.carbonarius*
			Concentration: 20 mg/ml				
Schiff Complex (L)	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
Ni(II)-L	6.00 ± 0.00	6.00 ± 0.00	7.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
Ni(II)-L-Sr-S_1	6.00 ± 0.00	6.00 ± 0.00	8.00 ± 0.00	7.00 ± 0.00	7.00 ± 0.00	6.00 ± 0.00	11.00 ± 0.00
Ni(II)-L-Sr-S_2	6.00 ± 0.00	6.00 ± 0.00	10.00 ± 0.00	8.00 ± 0.00	11.00 ± 0.00	6.00 ± 0.00	14.00 ± 0.00
Ni(II)-L-Sr-S_3	7.00 ± 0.00	6.00 ± 0.00	12.00 ± 0.00	10.00 ± 0.00	12.00 ± 0.00	7.00 ± 0.00	16.00 ± 0.00
			Concentration: 30 mg/ml				
Schiff Complex (L)	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
Ni(II)-L	6.00 ± 0.00	6.00 ± 0.00	9.00 ± 0.00	7.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
Ni(II)-L-Sr-S_1	6.00 ± 0.00	7.00 ± 0.00	11.00 ± 0.00	9.00 ± 0.00	10.00 ± 0.00	6.00 ± 0.00	15.00 ± 0.00
Ni(II)-L-Sr-S_2	6.00 ± 0.00	7.00 ± 0.00	12.00 ± 0.00	9.00 ± 0.00	13.00 ± 0.00	7.00 ± 0.00	19.00 ± 0.00
Ni(II)-L-Sr-S_3	10.00 ± 0.00	8.00 ± 0.00	15.00 ± 0.00	12.00 ± 0.00	15.00 ± 0.00	9.00 ± 0.00	20.00 ± 0.00
			Concentration: 40 mg/ml				
Schiff Complex (L)	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00
Ni(II)-L	6.00 ± 0.00	7.00 ± 0.00	12.00 ± 0.00	11.00 ± 0.00	6.00 ± 0.00	6.00 ± 0.00	8.00 ± 0.00
Ni(II)-L-Sr-S_1	7.00 ± 0.00	9.00 ± 0.00	14.00 ± 0.00	12.00 ± 0.00	13.00 ± 0.00	6.00 ± 0.00	18.00 ± 0.00
Ni(II)-L-Sr-S_2	7.00 ± 0.00	9.00 ± 0.00	14.00 ± 0.00	12.00 ± 0.00	17.00 ± 0.00	9.00 ± 0.00	21.00 ± 0.00
Ni(II)-L-Sr-S_3	12.00 ± 0.00	11.00 ± 0.00	16.00 ± 0.00	14.00 ± 0.00	17.00 ± 0.00	10.00 ± 0.00	23.00 ± 0.00
Control[c]	17.00 ± 2.00	23.00 ± 3.00	20.00 ± 1.00	19.00 ± 2.00	20.00 ± 2.00	24.00 ± 4.00	28.0 ± 2.00

a. Bacillus cereus (ATCC 1966); Staphyloccocus aureus (ATCC 25923); Salmonella typhimurium (ATCC 14028); Escherichia coli (ATCC 25922).
b. Candida albicans (ATCC 10231); Aspergillus niger (NRRL 612); Aspergillus carbonarius (NRRL 368).
c. For Gram-positive bacteria, the standard reference was cephalothin, for Gram-negative bacteria, it was chloramphenicol and for fungi, it was cycloheximide.

With an inhibitory zone diameter of around 6 mm, the Schiff base (L) has a weakly active effect on all microorganisms at concentration 20, 30, and 40mg/ml. Conversely, Ni(II)L-Sr- S3 showed greater activity on the bacteria (Gram-positive bacteria and Gram-negative bacteria), yeast, mold as well as fungus than L, Ni(II)L and other binuclear metal complexes. The ligand and its metal complexes demonstrated for antimicrobial behavior, according to an examination of the data in Table 3, the binuclear metal complexes are typically more active than Ni-L. The observed enhanced activity upon chelation may be explained by the fact that the donor atoms of the ligand share some of the metal's positive charge in the chelated complex.

The entire chelate ring exhibits electron delocalization. Consequently, this will enhance the metal's lipophilic properties [30-31]. The metal complexes' antimicrobial activity progressively increases with increasing concentration, and they perform remarkable better at high concentrations [32].

According to the Tweedy chelation theory [33], these findings have clarified how the compound's lipophilicity increases as the metal cloud's polarizability decreases, ultimately causing the cells' permeability to collapse [34]. In addition, the chelation decreased the polarity of metal ions because of its positive charge which is share by the π-electron delocalization and donor groups [35].

Conclusion :

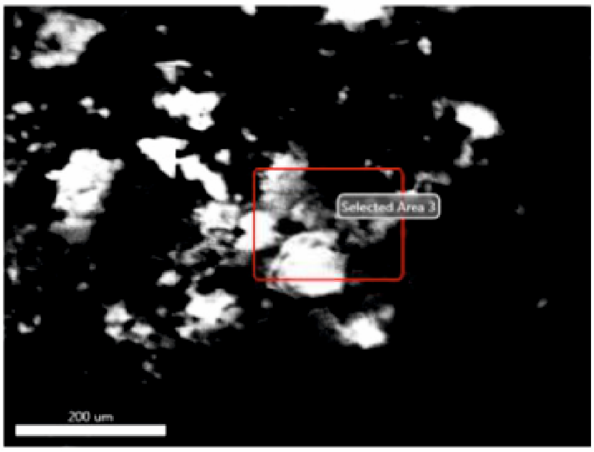
The synthesis and coordination chemistry of some novel binuclear complexes derived from the tetra-dentate Schiff-base ligand (L) are explored in this research paper. The bonding of [N,N'-bis (salicylidene)-o phenylenediamine]Ni(II) complex and its alkaline earth metal complex is probably by the coordinate bonding though the two phenolic oxygen atoms, which is supported by the infrared spectra. The metal complexes showed antibacterial activity towards selected kinds of Gram-positive bacteria, Gram-negative bacteria, yeast and fungus. However, the most promising heterobinuclear metal complex having remarkable antimicrobial potency was given by Ni(II)-L-Sr-S3.

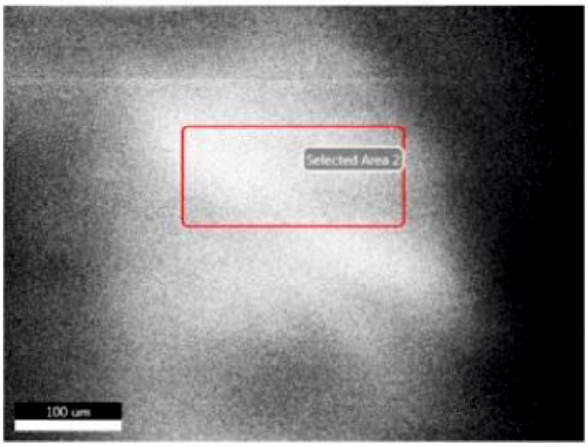
Acknowledgement :

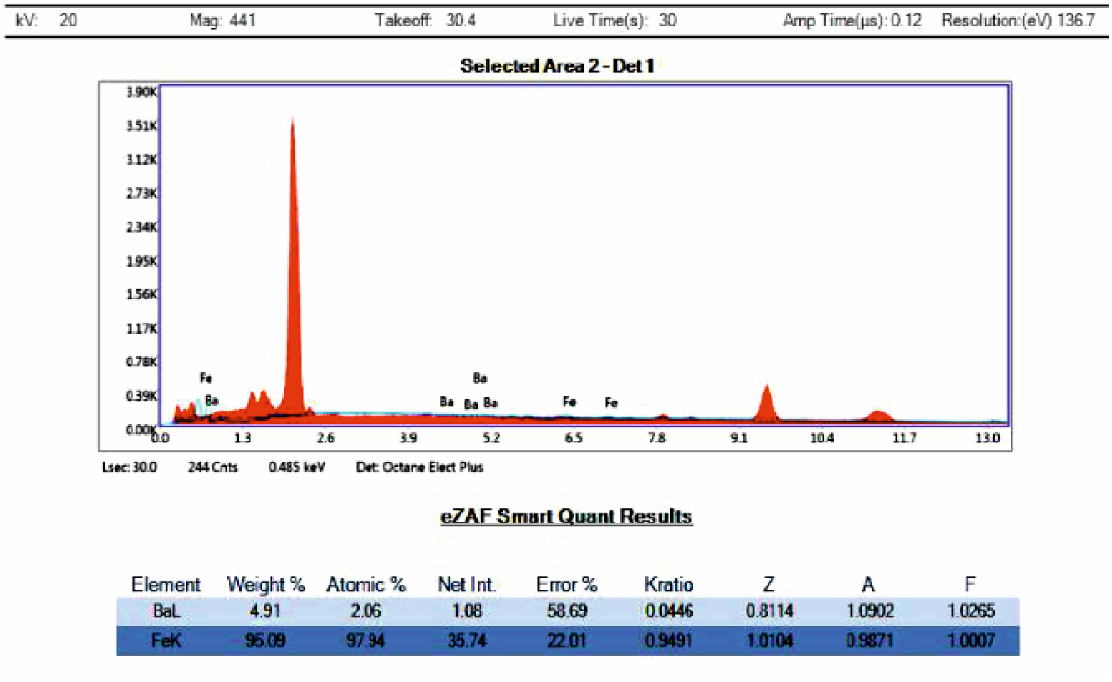
The authors express their sincere thanks to the Department of Chemistry, Patna University, Bihar, India.

Conflict of interest :

The authors declare no conflict of interest.







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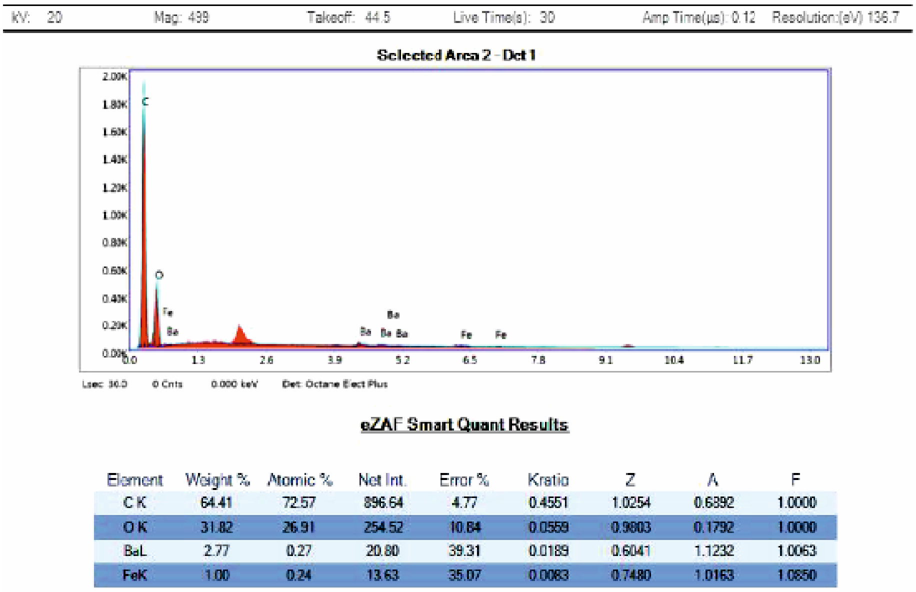
Selected Area 2 - Det 1

Lsec: 30.0 244 Cnts 0.485 keV Det: Octane Elect Plus

eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
BaL	4.91	2.06	1.08	58.69	0.0446	0.8114	1.0902	1.0265
FeK	95.09	97.94	35.74	22.01	0.9491	1.0104	0.9871	1.0007



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Selected Area 2 - Det 1

Lsec: 30.0 0 Cnts 0.000 keV Det: Octane Elect Plus

eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	64.41	72.57	896.64	4.77	0.4551	1.0254	0.6392	1.0000
O K	31.82	26.91	254.52	10.84	0.0559	0.9803	0.1792	1.0000
BaL	2.77	0.27	20.80	39.31	0.0189	0.6041	1.1232	1.0063
FeK	1.00	0.24	13.63	35.07	0.0083	0.7480	1.0163	1.0850

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