

In silico and *in vitro* studies of (E)-4-((2-aminophenyl)imino)-4-phenylbutan-2-one based Transition Metal Complexes

Kalichamy Indra¹, Arulannandham Xavier²

Research Scholar, PG & Research Department of Chemistry, Madura College (Autonomous), Madurai.

Madurai Kamaraj University, Tamil Nadu, India¹

Head and Associate Professor, PG & Research Department of Chemistry, Madura College (Autonomous), Madurai

Madurai Kamaraj University, Tamil Nadu, India²

ABSTRACT: A series of three compounds with tridentate Schiff base derived from Benzyl acetone with O-phenylenediamine and their mononuclear Ni(II), Fe(III) and Zn(II) complexes in 2:1 molar ratio have been synthesized, characterized over spectral in addition to analytical techniques. The free (E)-4-((2-aminophenyl)imino)-4-phenylbutan-2-one (APIPB) as tridentate with amine, carbonyl and imine moiety were co-ordination sites. The FT-IR stretching frequency for the above co-ordination sites were varied after co-ordination with Ni(II), Fe(III) and Zn(II) metallic salts. The octahedral geometry of the metal complexes were confirmed by spectral techniques such as UV, fluorescence, ¹H-NMR, ¹³C-NMR, ESI-MS and EPR. The XRD studies provide additional information to confirm the nature of the ligand and its complex were as powder form. The SEM analysis predict the structure morphology of the APIPB was irregular ice pieces and the (APIPB)₂Zn had irregular ice pieces with sieves like structure. The anti-oxidant study revealed that the ligand and its metal complexes have well and good biological activity. The *in vitro* biological activities of the APIPB and its complexes have been tested against two bacterial strains such as *Staphylococcus aureus* and *Escherichia-coli* and two fungal strains such as *Candida albicans* (227), *Penicillium chrysogenum* or *P. notatum* (formerly) (160) in both the positive control and negative control. All the complexes have higher biological activity than the free APIPB. *In silico* biological activity score for the APIPB was derived by PASS online software.

KEYWORDS: O-phenylenediamine, Benzoyl acetone, biological activities.

I. INTRODUCTION

In this year itself, the corona virus make life threatening to all around the world. In this way, this and there, the world facing major therapeutic problem by means of microorganisms. This situation create the challenging to institutions, pharmaceutical companies and academics. This was because many of the microorganisms were naturally becoming resistant to previously existing drugs due to morphological variations, cell divisions and mutations of their genome or by tolerant anti-microbial genes from other microbes. Anti-microbial resistant occur when microorganisms change in certain way that reduces or eliminates the effectiveness of drugs designed to prevent alterations to cells. These natural microbial resistant properties shown by microbes have further complicated the world's challenge in health sector. Therefore to build a strong health sector in any nation, major advances towards the improvement of anti-microbial drugs have to be a nonstop process [1-4].

The rewards of metallodrug development for biological activity of the organic ligand through a new mechanism of metallotherapeutic action in comparison to organic ligands, reduced the toxic effects of drug or its metabolites produced in the cell. However, not all metal complexes have been emerged into successful drugs. Because of the biological activity of metallodrugs depends on numerous of their properties such as nature of the metal ion and its oxidation states, number of ligands and nature of ligands, stereochemistry of the metal complex, thermodynamics and kinetics of ligand exchange reactions in bio-environment, electrochemical and photo physical properties [1].

In this way, the platinum based metal complexes have been found to impending drugs for the treatment of life threatening diseases such as cancer, inflammatory, tuberculosis, viral, epilepsy, diabetes and cardiovascular. But because of platinum metal are costly, less abundant, more toxic due to severe side effects and most of the complexes were kinetically less labile, lack of activity for wide range on cancerous cells. In the same way the Ni(II), Fe(III) and Zn(II) complexes were more abundant, cost effective and less toxic, abundant trace essential elements, these metal ions exhibit excellent bio co-ordination chemistry by providing suitable co-ordination in physiological conditions [5-10].

Schiff base ligands exhibit rich co-ordination and structural chemistry with most of the transition metals, form constant and chelated complexes with additional donors. The azomethine nitrogen play a significant role in biological activity hence Schiff bases and their metal complexes have been mostly explored in medicine for the treatment of numerous diseases [11-12].

In this work (E)-4-((2-aminophenyl)imino)-4-phenylbutan-2-one (APIPB) and its Ni(II), Fe(III) and Zn(II) complexes have been synthesized and their structural characterization were done using spectrometric methods like UV-Vis, fluorescence, FT-IR, ¹H-NMR, ¹³C-NMR, ESI-MS, EPR, XRD and SEM spectroscopy methods. The anti-bacterial activities of compounds were studied against the species like *Staphylococcus aureus* and *Escherichia coli* in both the positive control and negative control by using well diffusion method. The anti-fungal studies against *Candida albicans* (227), *Penicillium chrysogenum* or *P. notatum* (formerly) (160) with Clotrimazole maintained as a standard.

II. EXPERIMENTAL

1. Materials and Physical Measurements

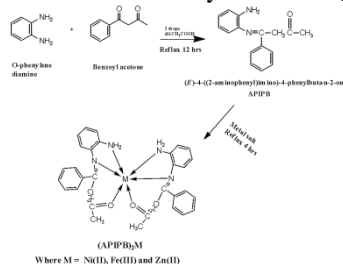
Benzoyl acetone (Sigma-Aldrich, 97%), O-phenylenediamine (Sigma-Aldrich, 95%), CH₃COOH, (Merck, 99%), NiSO₄, FeCl₃ and ZnSO₄·7H₂O were purchased. All the chemicals and the solvents were of analytical grade, used without any further purification.

2. Synthesis of (E)-4-((2-aminophenyl)imino)-4-phenylbutan-2-one (APIPB)

The azo (E)-4-((2-aminophenyl)imino)-4-phenylbutan-2-one (APIPB) was prepared by dissolving 1.63 gm (0.01 mol) of Benzoyl acetone in 25 ml of ethanol and 1.08gm (0.01mol) of O-phenylenediamine in 25 ml of ethanol and 2 drops of dil. acetic acid was added on the way to the overhead solution. The resulting solution was then refluxed for about 12 hrs. at 80⁰C. The precipitate was filtered off, then washed by distilled water and ethanol and finally dried in air (yield: 75%). Scheme 1 represents the synthetic procedure of the azodye APIPB.

3. Synthesis of Metal Complexes

Metal complexes have been prepared by mixing appropriate amounts of the APIPB (0.505 gm, 2 mmol) in ethanol (20% V/V) with (1 mmol) of the hydrated metal salts or unhydrated metal salts in absolute ethanol. The resulting solutions were then refluxed with stirring for 4 hrs., the solid complexes which separated out on hot have been collected by filtration and dried in vacuum desiccator over anhydrous CaCl₂.



Scheme 1: Synthesis of APIPB and its Metal Complexes

III. RESULT AND DISCUSSION

1. Physical analysis

All the metal complexes were intense colored and insoluble in water, but soluble only in DMSO and DMF. The elemental analysis (C, H, N, M) of the metal chelates showed that they may be represented by the formula [M (APIPB)₂], where (M = Ni(II), Fe(III) and Zn(II)) of complexes. The analytical data of the APIPB and its corresponding metal complexes were shown in (Table 1).

Table 1. Physical Data of APIPB and its Metal Complexes

Compound	Molecular formula	M. Wt. (calc.)	C % (Found)	H % (Found)	N % (Found)	O % (Found)	Colour	Melting Point(°C)	Yield (%)
APIPB	C ₁₆ H ₁₆ N ₂ O	252.31	76.16 (74.32)	6.39 (3.75)	11.10 (9.84)	6.34 (5.12)	Brown	101	64
(APIPB) ₂ Ni	C ₃₂ H ₃₂ NiN ₄ O ₂	563.32	68.23 (67.62)	5.73 (4.93)	9.95 (8.20)	5.68 (4.73)	Brown	192	39
(APIPB) ₂ Fe	C ₃₂ H ₃₂ FeN ₄ O ₂	560.47	68.58 (67.26)	5.75 (4.96)	10.00 (9.82)	5.71 (4.73)	Brown	207	56
(APIPB) ₂ Zn	C ₃₂ H ₃₂ ZnN ₄ O ₂	570.00	67.43 (66.19)	5.66 (4.34)	9.83 (8.64)	5.61 (4.13)	Light Yellow	226	48

2. Experimental FT-IR Spectroscopy

Fig. 1 exhibited the FT-IR bands for free APIPB as well as its complexes [13, 14]. O-phenylenediamine had strong bands at 3376 cm⁻¹ owing to the -NH₂ stretching frequency. Vaguish the APIPB these crests were absent minded, the imine >C=N band seems in place of a solid band at 1622 cm⁻¹ (>C=N) was get rid of to lesser frequency vogueish the metal complexes [13]. In APIPB the frequency through the side of 1666 cm⁻¹ was in lieu of -C=O group was shifted to higher frequency of around 5 - 45 cm⁻¹ in the case of metal complexes imitating the contribution of oxygen in the C=O-M bond [15 and 16].

The ν(N-H) stretching frequency of -NH₂ group was being to band at 3191 cm⁻¹ in the free APIPB [14]. This band was get rid of to higher frequency in circumstance of metal complexes. This output endorse that the nitrogen atom of the amino group co-ordinated to metal ions [14]. The bending vibration motion δ(NH₂) of -NH₂ group at 1576 cm⁻¹ in APIPB was get rid of to 1538-1573 cm⁻¹ vogueish the circumstance of its metal complexes. This outcome was owing to the partaking of NH₂ group in complexation [14]. In all the metal complexes a novel band seemed in the array 540 cm⁻¹ to 622 cm⁻¹ in addition en route for 460 cm⁻¹ to 487 cm⁻¹ as regards M-O and M-N bond [14, 17].

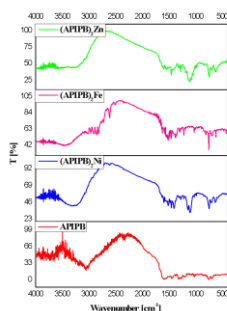


Fig.1 - FT-IR Spectrum of APIPB and its metal complexes

3. Electronic Spectral Studies

Electronic spectral revision report was exposed in Fig. 2. The APIPB reveals binary bands by the side of (223) 44,843 cm⁻¹ and (349) 28,653 cm⁻¹ could remain assigned towards intra π→π* as well as n → π* transition. In complexes these frequencies endure a bathochromic shift owing en route on behalf of co-ordination of imino nitrogen as well as carbonyl group of O-phenylenediamine and Benzoyl acetone moiety [18]. The Ni (II) (716) 13,966 cm⁻¹, (605) 16, 529 cm⁻¹ and (504) 19,841 cm⁻¹ regarding ³A_{2g}(F)→³T_{2g}(F), ³A_{2g}(F)→³T_{1g}(F) and ³A_{2g}(F)→³T_{1g}(P) transition relays to octahedral geometry [19]. Fe(III) exhibited bands at (723) 13,831 cm⁻¹ and (516) 19,380 cm⁻¹

apropos ${}^6A_{1g}(F) \rightarrow {}^4T_{2g}(G)$ and ${}^6A_{1g} \rightarrow {}^4A_{1g} + {}^4E_g$ of octahedral geometry [17]. Zn(II) complex was a diamagnetic, it had a frequency at $(477) 20,964 \text{ cm}^{-1}$ was for LMCT of octahedral geometry [18 and 19].

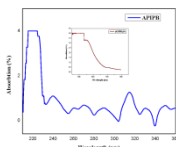


Fig. 2 – UV-Visible Spectrum of the APIPB and (APIPB)₂Fe

4. Fluorescence Spectra

The fluorescence spectrum was dignified in DMSO solution to decide the fluorescence behaviour for APIPB and (APIPB)₂Fe and displayed in Fig. 3(a) and Fig. 3(b). The extreme emission band of APIPB – 347 nm and (APIPB)₂Fe – 365 nm were pragmatic vognuish the spectrum, in lieu of that the compounds have impending fluorescence property. The fluorescence emission intensity of the complexes were higher than the APIPB. This increase in intensity was owing to the chelation of APIPB to metal ion center [20].

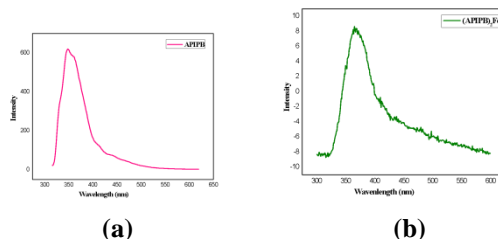


Fig. 3 – Fluorescence Spectrum of the APIPB (a) and (APIPB)₂Fe (b)

5. ¹H-NMR spectroscopy

The ¹H-NMR spectroscopy of APIPB exhibited in Fig. 4(a) and Fig. 4(b), the result crowning at 8.05 ppm was in authority intended for NH₂ functional group. The multiplet peaks at 7.20-7.98 ppm were owed to the aromatic protons. The peaks at 1.22-2.81 ppm was meant for aliphatic (CH₂) and (CH₃) protons. In the (APIPB)₂Zn complex shifting take place owing to the co-ordination of the (NH₂) functional groups. NH₂ group had a lower shift from 8.05 to 6.84 ppm [14].

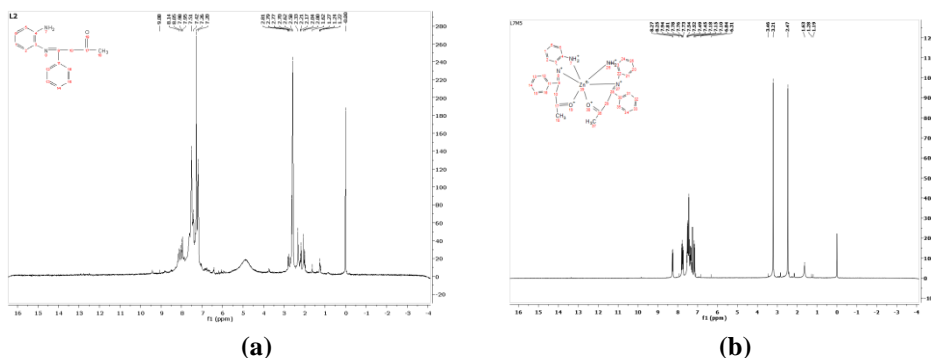


Fig. 4 - ¹H-NMR Spectrum of APIPB (a) and (APIPB)₂Zn (b)

6. ¹³C-NMR Spectral Studies

The ¹³C-NMR record of APIPB exhibited in Fig. 5 and it revealed signals at 31.41, 41.61, 77.34, 77.65, 117.20, 123.17, 126.06, 128.02, 131.16, 137.23, 141.56, 143.36, 164.58 and 202.94 ppm due to the functional groups C=C, C=N, C-N, C=O in addition C-O respectively [14 and 21].

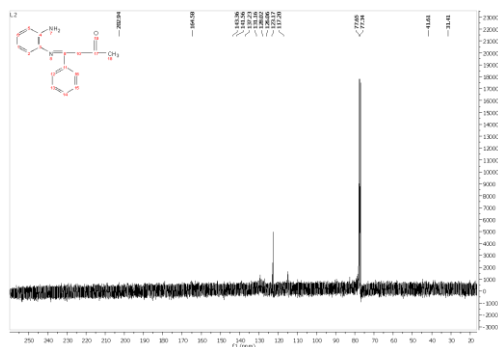
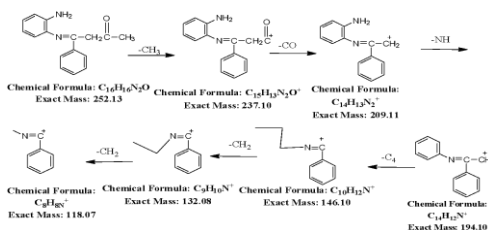


Fig. 5 - ¹³C-NMR Spectrum of APIPB

7. ESI-MS spectral studies

The ESI-MS spectrum of the APIPB displays a representative quasi molecular ion peak at m/z 252 which might accord the mass of the APIPB [C₁₆H₁₆N₂O]. The mass spectrum of the (APIPB)₂Fe exhibited a molecular ion peak at m/z 560 that could be rendering the composition (APIPB)₂Fe partaking the molecular formula as [C₃₂H₃₂FeN₄O₂]. The APIPB fragmentation peaks seemed at 252, 235, 213, 195, 149, 133 and 117 were intended in lieu of [C₁₆H₁₆N₂O], [C₁₅H₁₃N₂O⁺], [C₁₄H₁₃N₂⁺], [C₁₄H₁₂N⁺], [C₁₀H₁₂N⁺], [C₉H₁₀N⁺] as well as [C₈H₈N⁺] [14]. The fragmentation pattern and spectral results were reported in the Scheme 2 and in Fig. 6(a) as well as in Fig. 6(b).



Scheme 2: Fragmentation pattern of the novel APIPB

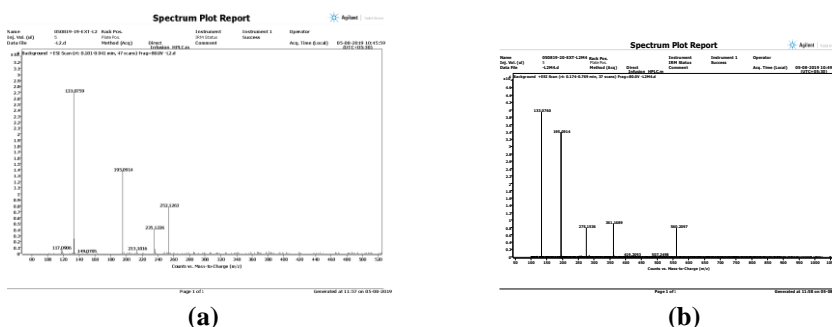


Fig. 6 - ESI-MS spectrum of the APIPB (a) and (APIPB)₂Fe complex (b)

8. EPR Spectroscopy

The EPR spectrum of (APIPB)₂Fe was displayed in Fig. 7 and Table 2. The report exhibited the g_{||} = 3.0676, g_⊥ = 1.628 and g_{av} = 2.1079 correspondingly. The g_{||} > g_⊥, so the unpaired electrons were confined in the d_{x²-y²} orbital. These data allocated en route for octahedral geometry of the Fe (III) complex. The G = 2.8461, this value was less than 4 which specifies that there was an exchange dealings between the neighboring Fe (III) cores [22].

Table 2. The spin Hamiltonian parameters of (APIPB)₂Fe complex in DMSO solution at LNT

Complex	g-tensor				A × 10 ⁻⁴ (cm ⁻¹)			g / A	G
	g	g _⊥	g _{iso}	g _{av}	A	A _⊥	A _{iso}		
(APIPB) ₂ Fe	3.0676	1.628	3.1618	2.1079	172	20	16	178	2.8461

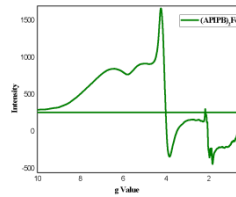


Fig. 7 - EPR spectrum of (APIPB)₂Fe complex

9. XRD studies

The primed complexes weren't single crystals, subsequently it was challenging to acquire the single crystal XRD structure. In order to regulate the degree of crystallinity of the APIPB as well as its metal complexes, the powder XRD were executed and the results were displayed in Fig. 8(a) and in Fig. 8(b). The XRD pattern of altogether compounds were scanned in the goni axis, through range ($2\theta=10-80^\circ$), goniometer ratio = 240mm, specimen length = 10mm, $K-\alpha_1 = 1.54060^\circ$, $K-\alpha_2 = 1.54443^\circ$, $K-\beta = 1.392250$, $K-A2/K-A1$ Ratio = 0.50000. The XRD of APIPB and (APIPB)₂Ni complex were liable for amorphous phase [23 and 24]. The (APIPB)₂Ni complex the $\theta = 25^\circ 7'$ designated the Ni-N bond vouguish the complex.



Fig. 8 - XRD spectrum of the APIPB (a) and (APIPB)₂Ni (b) complex

10. SEM analysis

SEM report used to enquire the surface morphology of the APIPB and its complex reports were given away in Fig. 9(a) and Fig. 9(b). The SEM images of the APIPB presented crooked ice pieces like structure with particle size 6.6° to $4.5 \mu\text{m}$. The (APIPB)₂Zn complex revealed that the particles were agglomerated by means of the controlled morphology and the irregular ice pieces with sieves like structure with particle size 7.1° to 472.4 nm [25]. This report deep-rooted that depending on the metal and its co-ordination the structure morphology of the compounds were wide-ranging.



Fig. 9 - SEM spectrum of APIPB (a) and (APIPB)₂Zn (b) complex

IV. BIOLOGICAL STUDIES

1. Anti-oxidant activity

The free APIPB was found to be located 65.30%, however upon complexation, it increased expressively. It was pragmatic that the metal complexes have higher doings than the free APIPB [12]. The DPPH radical scavenging ability of the verified samples could thus be displayed in Table 3 and graded in the order: Ascorbic acid > (APIPB)₂Zn > (APIPB)₂Ni > (APIPB)₂Fe > APIPB



Table 3. DPPH scavenging activity of AIPB and its metal complexes

Compound	DPPH scavenging (%)
Ascorbic acid	84.93
AIPB	65.30
(AIPB) ₂ Ni	70.68
(AIPB) ₂ Fe	67.33
(AIPB) ₂ Zn	76.32

2. Anti-bacterial studies

The Schiff base AIPB had more anti-bacterial doings than other AIPBs charity as well as this activity was owing to the existence of electron emancipating groups such as -Ph, -OH, and >N=C< [26]. The anti-bacterial revision consequences were presented in the Plate 1. Grounded on the revision report in lieu of the positive strain *Staphylococcus aureus* as well as the negative strain *Escherichia coli*, the metal complexes have improved doings than the AIPB [27- 31]. The anti-bacterial doings of the compounds increases with increased in concentration [32]. The (AIPB)₂Ni had better activity than other test samples for both postive and negative strains.

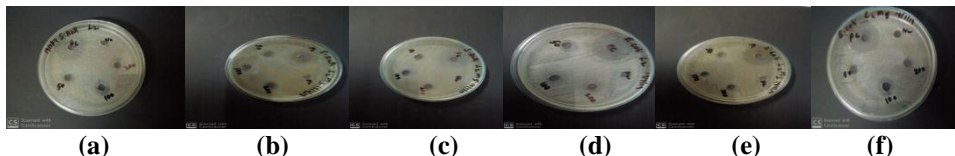


Plate 1- MIC of *Staphylococcus aureus* with AIPB (a), (AIPB)₂Ni (b) and (AIPB)₂Zn (c) and MIC of *Escherichia-coli* with AIPB (d), (AIPB)₂Ni (e) and (AIPB)₂Zn (f)

3. Anti-fungal study

The anti-fungal doings consequences were listed in Plate 2. Grounded on the report both the metal complexes and the AIPB have well and good activity, but the metal complexes have higher doings than the AIPB [33]. The Schiff base as well as its chelates could enrich the anti-microbial activity in both (*Candida albicans* and *Penicillium notatum*) the strains because of the azomethine nitrogen group. The activity of the metal complexes might be clarified on the basis of chelation theory in addition to overtone effect [27, 34-36]. The anti-fungal activity increased with increase in concentration of the compound [29, 32].

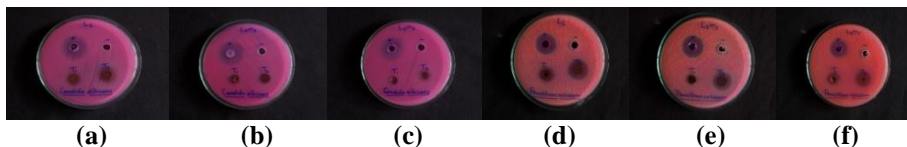


Plate 2 - MIC of AIPB (a), (AIPB)₂Ni (b) and (AIPB)₂Zn (c) against the tested organisms *Candida albicans* and MIC of AIPB (d), (AIPB)₂Ni (e) and (AIPB)₂Zn (f) against the tested organisms *Penicillium notatum*

V. COMPUTATIONAL STUDIES

1. Biological activity prediction

The biological activity of the AIPB was assessed by revenues of PASS online software and displayed in Table 4. The AIPB Schiff base had superior activity towards Heart failure treatment than its corresponding amine and aldehyde [37].

Table 4. Biological activity prediction of APIPB

Pa	Pi	Activity
0,925	0,003	Heart failure treatment
0,910	0,004	Gluconate 2-dehydrogenase (acceptor) inhibitor
0,740	0,005	Platelet derived growth factor receptor kinase inhibitor
0,764	0,048	Phobic disorders treatment
0,747	0,036	Mucomembranous protector
0,716	0,022	Glucose oxidase inhibitor
0,692	0,029	Glutamyl endopeptidase II inhibitor
0,668	0,005	Beta glucuronidase inhibitor
0,683	0,024	Omptin inhibitor

Pa- probability of active

Pi- probability of inactive

VI. CONCLUSION

Three metal complexes (Ni(II), Fe(III) and Zn(II)) and its Schiff base ((E)-4-((2-aminophenyl)imino)-4-phenylbutan-2-one) have been synthesized. These complexes were displayed octahedral environment. The FT-IR, UV, fluorescence, ¹H NMR, ¹³C NMR, ESI-MS and EPR spectroscopy consequences were inferred. The XRD studies inform APIPB and (APIPB)₂Ni as powder in nature. The SEM analysis predicted the structure morphology of the synthesized APIPB had as crooked ice pieces and (APIPB)₂Zn had irregular ice pieces with sieves like structure. The biological activities of the APIPB and its complexes have been tested alongside anti-microbial strains. All the complexes have higher biological activity than the free APIPB. The APIPB Schiff base had better activity towards Heart failure treatment than its corresponding amine and aldehyde.

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