



Research Article

Synthesis, Characterization and Antioxidant Activity of Mixed–Ligand Complexes of Mn(II), Co(II), Ni(II), Cu(II) And Zn(II) Ions with Nicotinic Acid and Phenylalanine

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ARTICLE INFO:

Keyword:

Nicotinic Acid (NA),
Phenylalanine (Phe),
Ascorbic Acid
DPPH, Mixed-Ligand

ABSTRACT

Mixed ligand metal complexes of nicotinic acid and phenylalanine with Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) have been synthesized and characterized using infrared spectroscopy, electronic spectra, conductivity, melting point, and solubility. Nicotinic acid and phenylalanine were reacted with some metal (II) chloride salts in the molar ratio of 1:2:1 (M: NA: Phe) which produced the complexes with various colours and a percentage yield range of 58-97%. The melting points of the compounds ranged from 320 to 348 °C, with conductivities ranging from 9.01-26.4 S cm⁻² mol⁻¹. The infrared spectra of the ligands and metal complexes revealed that nicotinic acid is a monodentate ligand that coordinates to the metal via the nitrogen of the pyridine ring, whereas phenylalanine is a bidentate ligand that coordinates to the metal via the carbonyl oxygen $\nu(\text{C}=\text{O})$ and nitrogen of the amine $\nu(\text{N}-\text{H})$ groups. The electronic spectra of the compounds are compatible with an octahedral geometry, except for the zinc complex, which probably has a tetrahedral structure. All the complexes were slightly soluble in some of the organic solvents used, methanol, ethanol, dimethylformide (DMF), acetone, chloroform, ethyl acetate, and benzene. The ligands and complexes were evaluated for antioxidant activity in vitro using the DPPH and hydrogen peroxide methods. According to the findings, all the complexes have higher activity than the ligand (nicotinic acid) but were less active than the standard (ascorbic acid). This indicates, the complexes have strong antioxidant properties.

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INTRODUCTION

Mixed ligand complexes contain at least two different types of ligands bound to the same metal ion, and the presence of multiple types of ligands in a complex increases the chances of the expected properties (Mahanada & Archama, 2019). Mixed ligand complexes are used in a variety of chemical and biological systems, including water softening, ion exchange resin, electroplating, dyeing, antioxidants, photosynthesis in plants, removal of Metals that are unwanted or dangerous to living organisms, and biological activity of many of these metal complexes against harmful bacteria has been observed (Maher *et al.*, 2018). Due to the extensive use of coordination molecules in different applications in recent decades, the chemistry of mixed ligand complexes has gotten a lot of attention. Mixed ligand complexes have been discovered to be extremely important (Abdullahi *et al.*, 2018). The importance of mixed ligand complexes in biological processes is widely understood. A review of the literature found that the synthesis, characterization, biological importance, and metabolic enzymatic activity of mixed ligand complexes of various transition metals with amino acids have been investigated (Shatha *et al.*, 2018). As models for metallo-enzymes, mixed ligand complexes containing metal ions bound to two distinct and biochemically significant ligands have attracted interest. These complexes have a wide range of biological actions, including activating, fungicidal, anti-inflammatory, bactericidal, and antiviral effects. Mixed ligand complexes of transition metals with amino acids are physiologically fascinating. They play a vital function in biological systems and have aroused researchers' curiosity (Oladunni *et al.*, 2021).

Nicotinic acid is an organic compound that is commonly referred to as vitamin B₃. It is a colourless, water-soluble solid with the

chemical formula C₆H₅NO₂ and a melting point of 2370° C. They are pyridine derivatives with a carboxyl group at position 3 (Shakeel *et al.*, 2017).

Nicotinic acid is required for a variety of biological functions, including energy production, signal transmission, gene control, fatty acid and lipoprotein reduction, and involvement in the lipid synthesis pathway (Safaa *et al.*, 2017). Anemia, headaches, exhaustion, nausea, and skin infections are all symptoms of a lack of nicotinic acid. Pellagra is caused by chronic deficiency (Hemat *et al.*, 2020). There have been prior reports of nicotinic acid complexes with various metals, nicotinic acid-copper complexes have been shown to have a variety of bioactivities (Safaa *et al.*, 2017). Cu nicotinate complexes' antioxidant activity was attributed to their superoxide dismutase mimicking activity, which scavenges free radicals, control nitric oxide level, accelerate the excessive production of antioxidant enzymes like glutathione peroxidase, glutathione-s-transferase, and catalase, and protect blood capillary damage by stimulating blood flow (Muhammed *et al.*, 2020). Phenylalanine is an aromatic amino acid with the formula C₈H₉NO₂ and has a non-polar phenyl group and is found in many living species. It is required for crossing the blood-brain barrier, where it converts to tyrosine and produces important hormones like norepinephrine and epinephrine (Ufuk *et al.*, 2020). Because of their diversity in terms of coordination mode and binding capabilities towards metals, amino acids are particularly important family of organic ligands that have been explored for the production of metallic complexes ions (Canaj *et al.*, 2018). Amino acids bind to transition metal ions via the carboxylate oxygen and the amine nitrogen donor atoms, forming a thermodynamically stable chelate ring under biological conditions (Alvarez, 2020; Ufuk *et al.*, 2020). Based on

the facts, the study's objectives are to synthesize the mixed ligand of nicotinic acid and phenylalanine with metal (II) complexes of manganese, cobalt, nickel, copper, and zinc, and characterize them using various physico-chemical techniques such as melting point, molar conductivity, atomic absorption spectroscopy, UV-visible, and FT-infrared, as well as determine the antioxidant activity of the complexes by DPPH and hydrogen peroxide assays.

MATERIALS AND METHODS

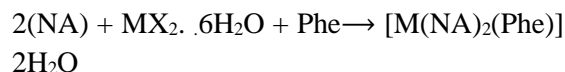
All chemicals and reagents were analytical grade and used as received without further purification, which included $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{ZnCl}_2 \cdot 4\text{H}_2\text{O}$, (CH_3OH) , ethanol ($\text{C}_2\text{H}_5\text{OH}$), *N*, *N*-dimethylformamide ($\text{C}_3\text{H}_7\text{NO}$), acetone ($\text{C}_3\text{H}_6\text{O}$), benzene (C_6H_6), ethyl acetate ($\text{C}_4\text{H}_8\text{O}_2$), and chloroform (CHCl_3). The drugs used were purchased from Rovert Scientific Nigeria Limited, Benin, Nigeria. They were utilized as obtained. They included ascorbic acid ($\text{C}_6\text{H}_8\text{O}_8$), nicotinic acid ($\text{C}_6\text{H}_5\text{NO}_2$) and phenylalanine ($\text{C}_9\text{H}_{11}\text{NO}_2$).

General Procedure for Synthesis of Mixed-Ligand Metal Complexes of Nicotinic acid and Phenylalanine

The technique developed by (Suksrichavalit *et al.*, 2008) was used to synthesize the complexes. Nicotinic acid dissolved in methanol (0.246g, 2mmol) was heated to 70 °C and stirred under reflux until a clear solution was formed. After that, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (0.198g, 1mmol) dissolved in methanol was added dropwise to the nicotinic acid solution and heated for about 1 hour, after which the reaction mixture was added drop wise into the phenylalanine (0.165g, 1mmol) solution in a mole ratio of 2:1:1 (metal–ligands). The colour of the solution changed from white to light brown after 30 minutes of heating under the same conditions. Filtration was used to recover

the precipitated solid, which was then washed with cold methanol/water and dried under vacuum on calcium chloride. The following complexes were synthesized in the same manner.

Equation for the Reaction



Where M = Mn (II), Co (II), Ni (II), Cu (II) or Zn (II)

$\text{X}_2 = \text{Cl}$, NA = nicotinic acid, Phe = phenylalanine

Physical Measurement

Melting points of the ligands and complexes were determined using the Gallenkamp melting point apparatus model of MF370. DDS-307 conductivity meter was used to measure the molar conductivity of the complexes at 35°C, in either methanol or DMF. The metal concentration (%M) was done on Atomic Absorption spectrophotometer of MODEL AA 6800 SHIMADZU. The infrared spectra were recorded using a KBr disc on SHIMADZU FTIR – 8400S FOURIER TRANSFORM INFRARED SPECTROPHOTOMETER in the range of 4000-400 cm^{-1} . The UV-Visible spectra of the ligands and the complexes in DMF solution at 10^{-3} M concentration at 25°C were run on the 270P MODEL in the wavelength range of 200- 800 nm. Antioxidant activity of the compounds and their ligands was determined spectrophotometrically with 270P MODEL in the wavelength range of 517nm of the DPPH method and 230nm of hydrogen peroxide method.

In vitro Antioxidant Determination of the Complexes

DPPH Method: DPPH (0.1mM) was prepared in 95% methanol which served as the blank. A stock concentration of the standard/sample was prepared in methanol with subsequent serial dilution at concentrations (10 – 100 $\mu\text{g/ml}$). The samples were mixed with DPPH solution

in the ratio 1:1 and incubated for 30 mins in the dark. Its absorbance was read at 517nm (Yesufu *et al.*, 2018). The percent antioxidant was calculated as follows;

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorbance of the control solution, and A_1 is the absorbance in the presence of sample solutions or standards for positive control.

Hydrogen Peroxide Method: Sroka and Gsowski, (2003) method was used with minor modifications (Yesufu *et al.*, 2017). A solution of hydrogen peroxide (2mM) was prepared in phosphate buffered saline (PBS), at pH 7.4. A stock concentration (1mg/ml) was prepared accompanied with serial dilutions (10 – 100 $\mu\text{g}/\text{mg}$). To the compound, were added 2 ml of

hydrogen peroxide solution in PBS. The absorbance was measured at 230nm after incubation for 10 mins. The level of inhibition was calculated as follows;

$$\% \text{ Scavenged } [\text{H}_2\text{O}_2] = [(AC - AS)/AC] \times 100$$

Where AC is the absorbance of the control and AS is the absorbance in the presence of the sample solutions or standards for positive control.

Statistical Analysis

The data obtained were expressed as mean \pm standard deviation and the statistical investigation was performed using MICROSOFT EXCEL. Inhibitory concentration IC_{50} is the concentration of a given compound that is required for 50% inhibition in vitro. The comparison was conducted using simple charts.

RESULTS

The Physical properties of the metal (II) complexes are reported in Table 1. The complexes were coloured except for the Zinc complex.

Table 1: Physical Characteristic of the Ligands and the Metal (II) Complexes

Compound	Molecular Mass	Colour	Melting point ($^{\circ}\text{C}$)	Yield (%)	Molar Conductivity ($\text{Scm}^2\text{mol}^{-1}$)	Metal Percentage(%) Found (cal)
NA	123.11	White	242	-	-	-
Phe	165.19	White	280	-	-	-
$[\text{Mn}(\text{NA})_2(\text{Phe})(\text{H}_2\text{O})_2]$	501.94	Brown	320	97	19.7	10.55(10.92)
$[\text{Co}(\text{NA})_2(\text{Phe})(\text{H}_2\text{O})_2]$	505.93	Pink	339	58	10.2	11.75(11.62)
$[\text{Ni}(\text{NA})_2(\text{Phe})(\text{H}_2\text{O})_2]$	505.69	Green	340 ^d	97	9.01	11.37(11.67)
$[\text{Cu}(\text{NA})_2(\text{Phe})(\text{H}_2\text{O})_2]$	510.54	Blue	343	90	26.4	12.77(12.42)
$[\text{Zn}(\text{NA})_2(\text{Phe})]$	512.37	Colourless	348	81	12.6	13.30(13.69)

Key: NA = Nicotinic Acid Phe = Phenylalanine Ligand and d= decomposition temperature

In **Table 2**, the Infrared spectra showed clear broad bands of vibrational frequency for nicotinic acid and phenylalanine at 3402cm^{-1} which was absent in the complexes. A metal to

oxygen band absent in the nicotinic and phenylalanine spectra was found on the complexes.

Table 2: Relevant Infrared Spectra of the Ligands and the Metal Complexes (cm^{-1})

Compound	$\nu(\text{OH})$	$\nu(\text{NH}_2)$	$\nu(\text{C}-\text{N})$	$\nu(\text{C}=\text{O})$	$\nu(\text{C}-\text{O})$	OH_{def}	$\nu(\text{M}-\text{N})$	$\nu(\text{M}-\text{O})$
NA	3402b	-	1504w	1651m	1026m	-	-	-
Phe	-	3402b	1504w	1635w	1018w	-	-	-
$[\text{Mn}(\text{NA})(\text{Phe})_2(\text{H}_2\text{O})_2]$	3842w	3410b	1558w	1627m,	1049m	1388s	532w	763m
$[\text{Co}(\text{NA})(\text{Phe})_2(\text{H}_2\text{O})_2]$	3842w	3417b	1388w	1627s	1126m	1327w	563w	748m
$[\text{Ni}(\text{NA})(\text{Phe})_2(\text{H}_2\text{O})_2]$	3880w	3394b	1388s	1620m	1049w	1249w	532w	763m
$[\text{Cu}(\text{NA})(\text{Phe})_2(\text{H}_2\text{O})]$	3842w	3410b	1566m	1627m	1049s	1396m	532w	756w
$[\text{Zn}(\text{NA})(\text{Phe})_2]$	-	3410b	1627m	1689m	1134m	-	555w	748w

Key: b = broad, s = strong, m = medium, w = weak

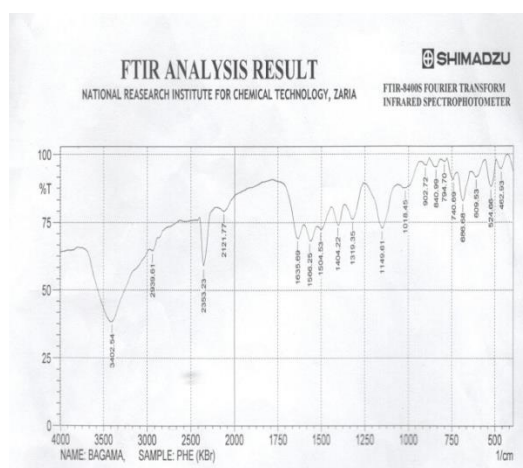


Figure 1: IR Spectrum of (Phe)

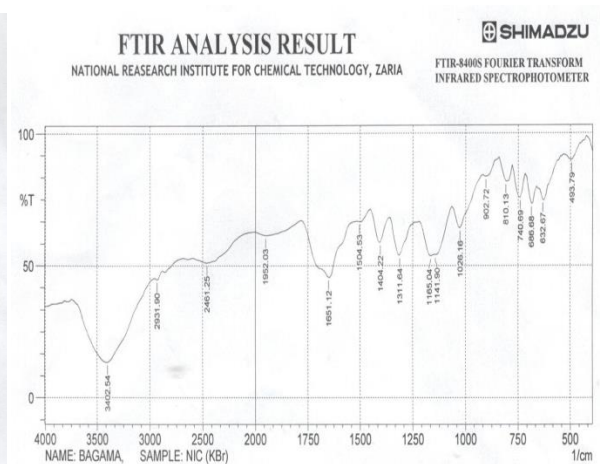
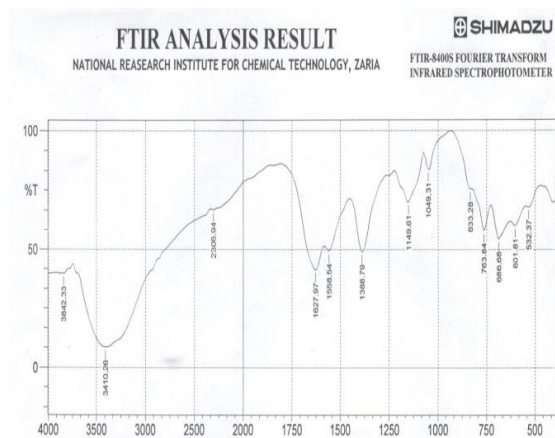
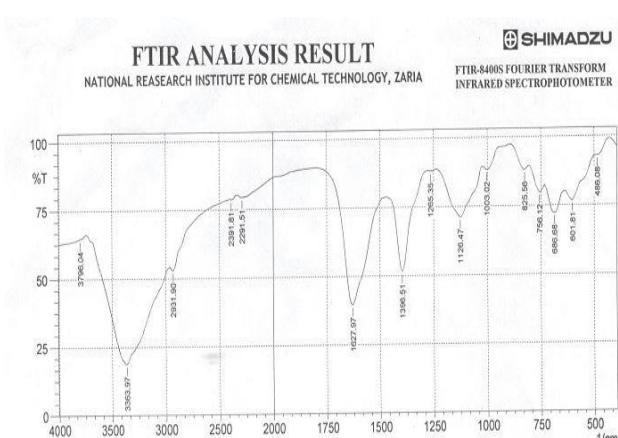
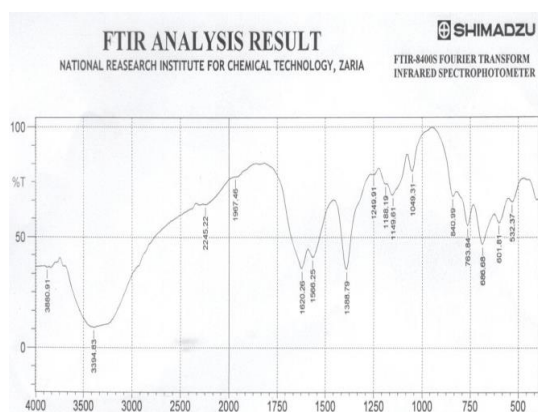
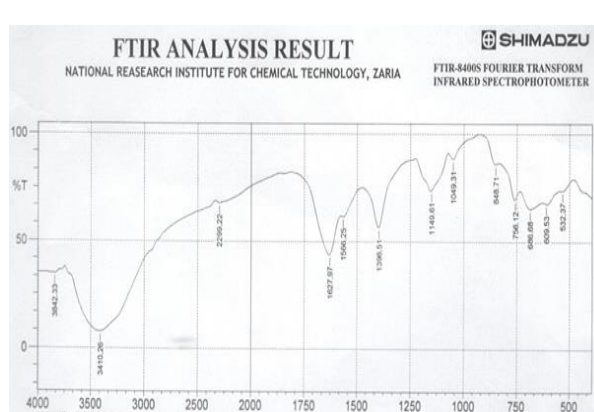
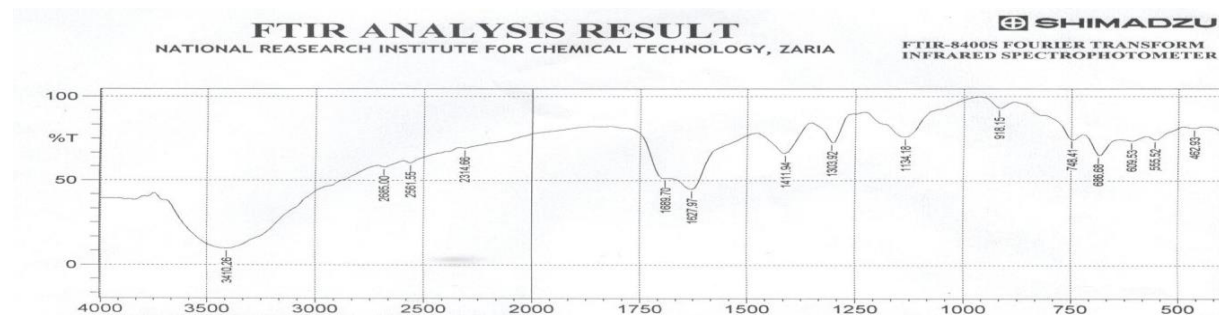


Figure 2: IR Spectrum of (NIC)

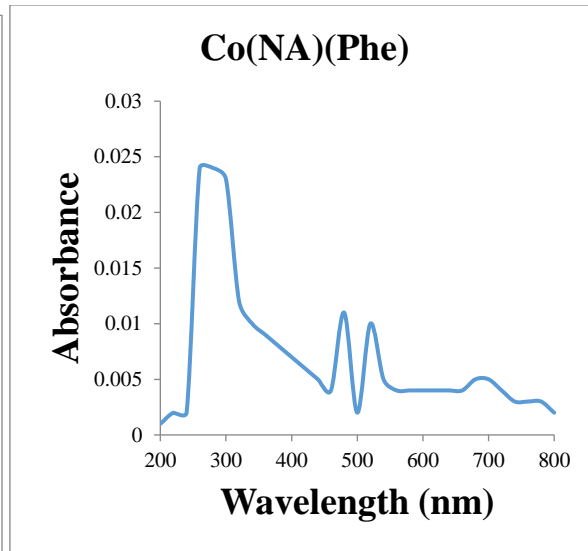
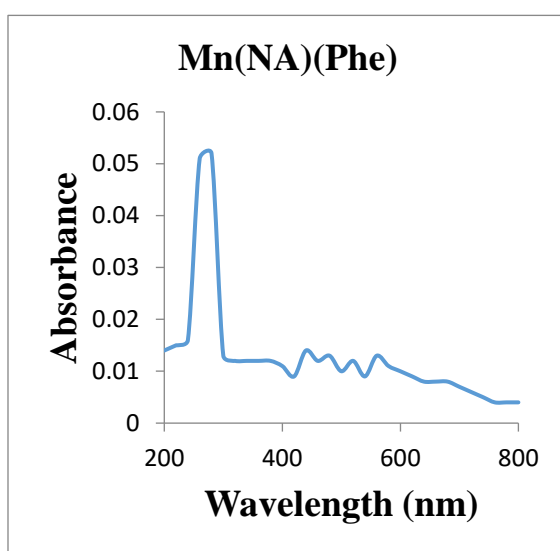
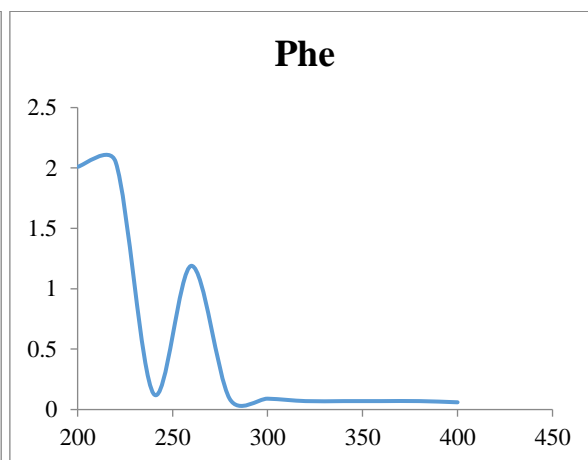
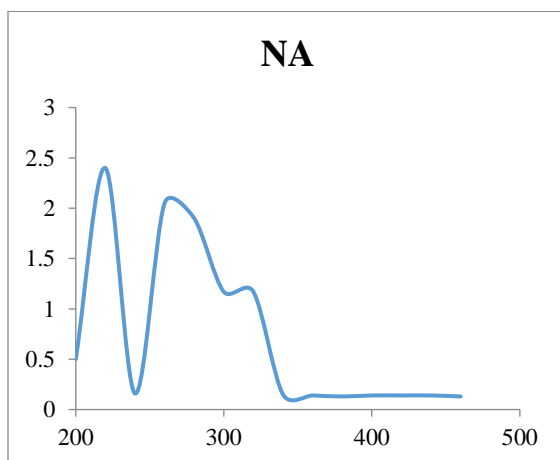
**Figure 3:** IR Spectrum of $\text{Mn(NA)(Phe)(H}_2\text{O)}_2$ **Figure 4:** IR Spectrum of $\text{Co(NA)(Phe)(H}_2\text{O)}_2$ **Figure 5:** IR Spectrum of $\text{Ni(NA)(Phe)(H}_2\text{O)}_2$ **Figure 6:** IR Spectrum of $\text{Cu(NA)(Phe)(H}_2\text{O)}_2$ **Figure 7:** IR Spectrum of Zn(NA)(Phe)

Electronic transitions arising from uv/visible spectrometry are shown in **Table 3**. The ligands (nicotinic acid and phenylalanine) showed mainly nonbonding

to π -anti-bonding transitions, while the metal complexes showed ligand to metal, and metal to ligand charge transfers.

Table 3: Data for the Electronic Absorption Spectra of the Ligands and the Complexes

Ligands/ compound	Absorption		Bands Assignment
	nm	cm ⁻¹	
NA	260,280	(38461,35715)	$n \rightarrow \pi^*$, $n \rightarrow \pi^*$
Phe	220,265	(45454,37735)	$n \rightarrow \pi^*$, $n \rightarrow \pi^*$
[Mn(NA)(Phe)(H ₂ O) ₂]	570,525,495,435	(17543,19047,20202,22988)	${}^6A_{1g} \rightarrow {}^4T_{1g}(G)$, ${}^6A_{1g} \rightarrow {}^4T_{2g}(G)$, ${}^6A_{1g} \rightarrow {}^4E_g(G)$, ${}^6A_{1g} \rightarrow {}^4A_{1g}(G)$
[Co(NA)(Phe)(H ₂ O) ₂]	530,490	(18867,20408)	${}^4T_{1g}(F) \rightarrow {}^4A_{2g}(F)$, ${}^4T_{1g}(F) \rightarrow {}^4T_{1g}(P)$
[Ni(NA)(Phe)(H ₂ O) ₂]	600,550,502	(16666,18181,19920)	${}^3A_{2g} \rightarrow {}^3T_{2g}$, ${}^3A_{2g}(F) \rightarrow {}^1E_g$, ${}^3A_{2g}(F) \rightarrow {}^3T_{1g}(F)$
[Cu(NA)(Phe)(H ₂ O) ₂]	640,450	(15625,22222)	${}^2E_g \rightarrow {}^2T_{2g}$, MLCT
[Zn(NA)(Phe)]	230,340	(43478,29411)	LMCT, MLCT



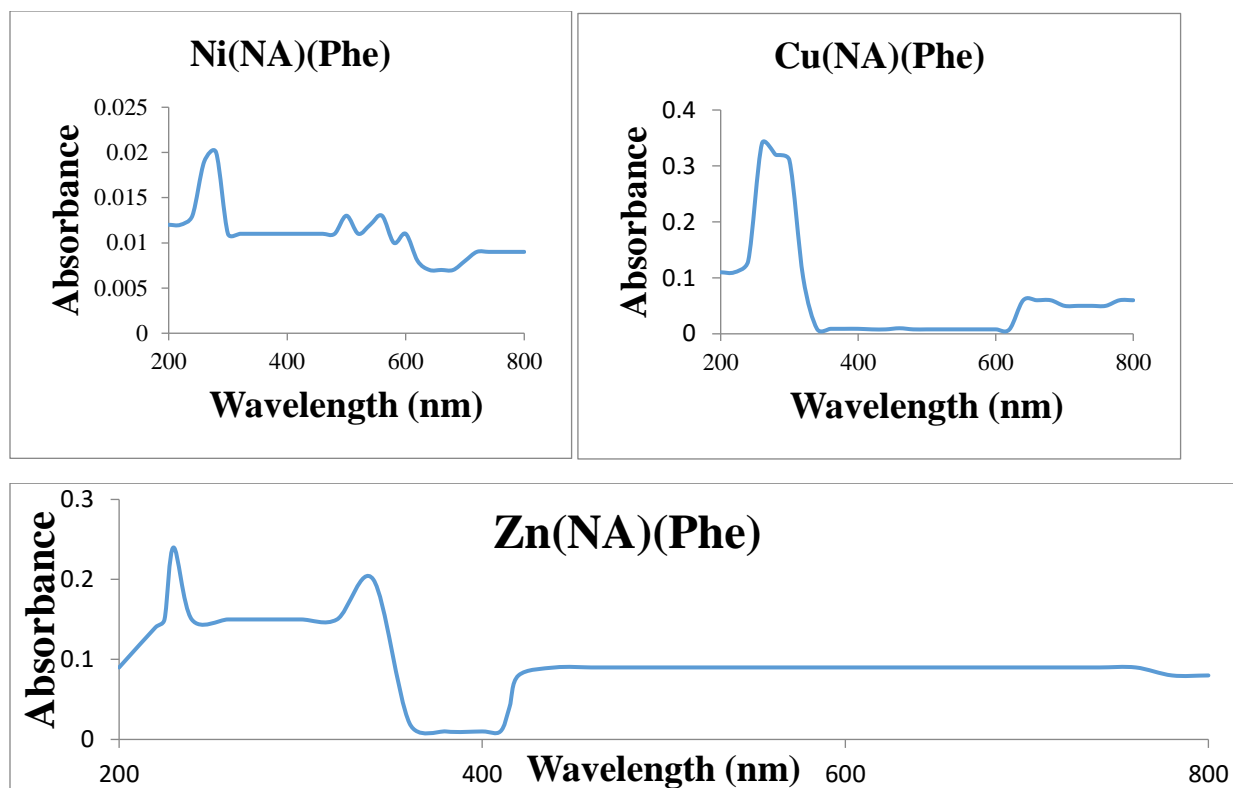


Figure 8: UV-Visible spectra of the ligands and its complexes

In **Table 4**, the free radical scavenging potential of the ligands and complexes is reported. The standard ascorbic acid (AA) gave the higher inhibition. However, significant inhibition was reported on the complexes.

Table 4: Antioxidant Activity of Ligands and the Metal Complexes by DPPH Assay

Compound	100($\mu\text{g/mL}$)	50($\mu\text{g/mL}$)	25($\mu\text{g/mL}$)	12.5($\mu\text{g/mL}$)	6.25($\mu\text{g/mL}$)	IC ₅₀
AA	93.56 \pm 0.04	82.65 \pm 0.02	72.42 \pm 0.02	59.26 \pm 0.10	30.29 \pm 0.20	5.9
NA	78.4 \pm 0.09	68.2 \pm 0.10	60.75 \pm 0.11	48.75 \pm 0.11	29.15 \pm 0.05	22.4
[Mn(NA)(Phe)(OH ₂) ₂]	83.98 \pm 0.01	70.68 \pm 0.15	67.15 \pm 0.16	55.29 \pm 0.20	29.79 \pm 0.05	13.2
[Co(NA)(Phe)(OH ₂) ₂]	84.72 \pm 0.08	73.54 \pm 0.06	68.54 \pm 0.11	58.45 \pm 0.02	30.01 \pm 0.01	9.1
[Ni(NA)(Phe)(OH ₂) ₂]	83.52 \pm 0.23	70.47 \pm 0.15	65.81 \pm 0.06	54.26 \pm 0.16	29.63 \pm 0.10	14.8
[Cu(NA)(Phe)(OH ₂) ₂]	83.99 \pm 0.00	71.6 \pm 0.33	67.47 \pm 0.35	56.29 \pm 0.08	29.9 \pm 0.03	12.1
[Zn(NA)(Phe)]	85.55 \pm 0.04	73.65 \pm 0.07	68.85 \pm 0.10	58.54 \pm 0.10	30.01 \pm 0.19	9.08

Values are expressed as Mean \pm SD (n = 3), IC₅₀ = concentration resulting in 50% inhibition of DPPH, derived from the graph of inhibition percentage *versus* concentration in $\mu\text{g/mL}$.

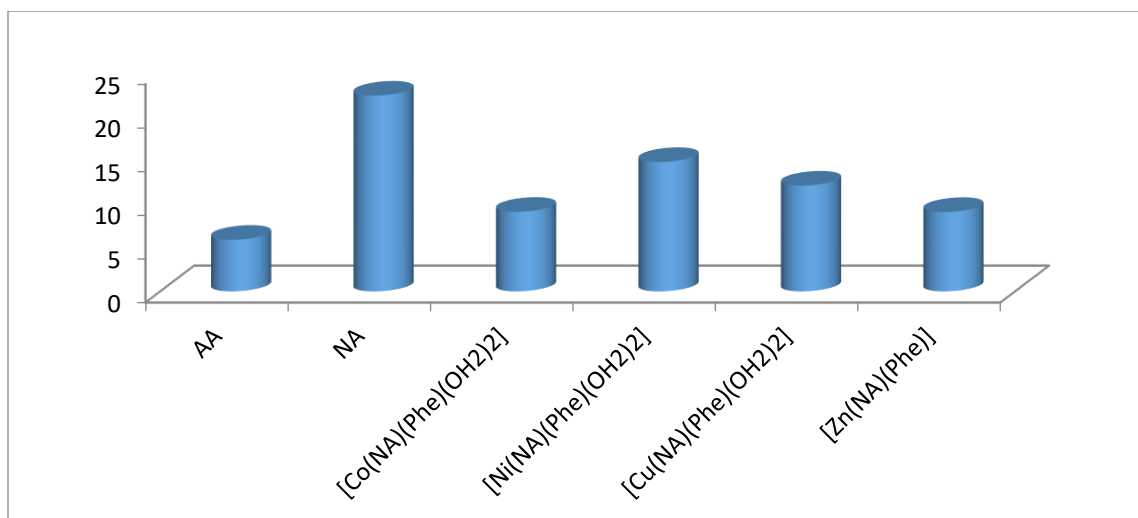


Figure 9: Simple chart showing the result of scavenging activities (IC_{50}) of (standard drug) Ascorbic acid (AA), ligand and their complexes by DPPH method

Table 5: Antioxidant Properties of Ligands and the Metal Complexes by Hydrogen Peroxide Method

Compound	100(μg/mL)	50(μg/mL)	25(μg/mL)	12.5(μg/mL)	6.25(μg/mL)	IC ₅₀
AA	93.56±0.04	82.65±0.02	72.42±0.02	59.26±0.10	30.29±0.20	5.9
NA	78.4±0.09	68.2±0.10	60.75±0.11	48.75±0.11	29.15±0.05	22.4
[Mn(NA)(Phe)(OH ₂) ₂]	84.01±0.00	71.87±0.10	65.84±0.06	53.29±0.07	29.2±0.00	15.4
[Co(NA)(Phe)(OH ₂) ₂]	87.2±0.01	73.7±0.05	66.58±0.10	53.58±0.08	29.45±0.10	14.4
[Ni(NA)(Phe)(OH ₂) ₂]	83.84±0.15	71.4±0.05	65.64±0.05	53.18±0.05	29.18±0.02	15.7
[Cu(NA)(Phe)(OH ₂) ₂]	84.07±0.04	72.54±0.11	66.25±0.05	53.38±0.07	29.28±0.03	14.8
[Zn(NA)(Phe)]	87.33±0.10	74.79±0.02	68.08±0.02	53.91±0.45	29.45±0.57	13.1

Values are expressed as Mean \pm SD (n = 3), IC_{50} = concentration resulting in 50% inhibition of hydrogen peroxide, derived from the graph of inhibition percentage *versus* concentration in μ g/mL

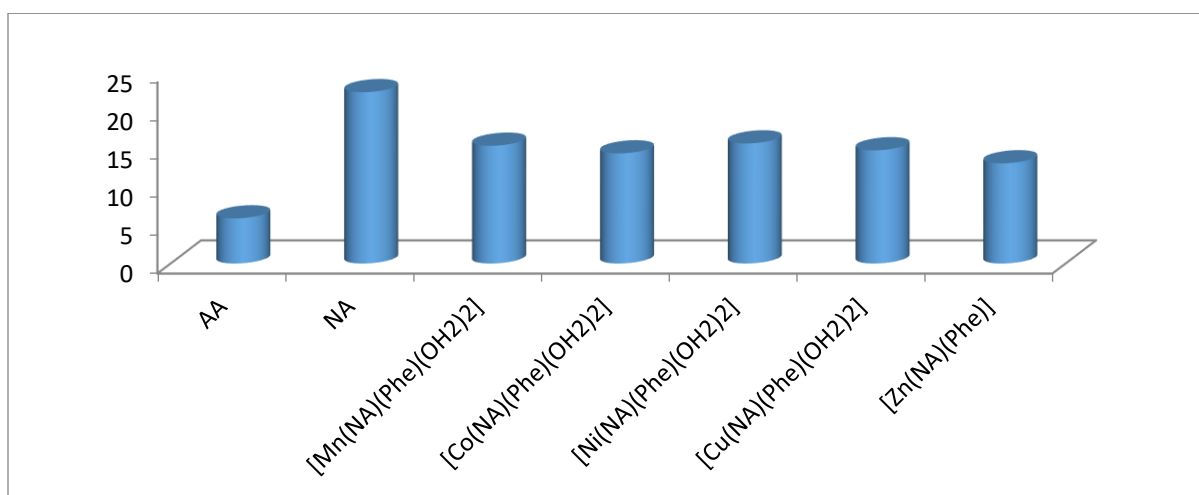


Figure 10: Simple chart showing the result of scavenging activities (IC_{50}) of (standard drug) Ascorbic acid (AA), ligand and their complexes by hydrogen peroxide method

DISCUSSION

Physical Properties of the Ligands and the Metal Complexes

The physical properties and percentage (%) yield of the complexes are shown in **Table 1**. nicotinic acid and phenylalanine ligands were reacted in the molar ratio of 1:2:1 (M:NA:Phe) with metal(II) salts to produce corresponding metal(II) complexes with diverse colors and percentage yields ranging from 58 to 97 %. The metal (II) complexes formed are powdery and non-hygroscopic. **Table 1** shows the melting points of the ligands and the decomposition temperatures of the metal (II) complexes, which range from 242 to 280 °C and 320 to 348 °C, respectively. The higher values for the complexes indicate their stability (Anthony *et al.*, 1969). The solubility determination was done using water and each of the following solvents: methanol, ethanol, dimethylformide (DMF), acetone, chloroform, ethyl acetate, and benzene. It was observed that both the nicotinic acid and the phenylalanine were slightly soluble in water, methanol, and ethanol. Metal (II) complexes were slightly soluble in some organic solvents used (acetone, chloroform, ethyl acetate, and benzene) but were soluble in hot water, methanol, ethanol, and dimethylformide. The results of molar conductance measurement in DMF solution at 10^{-3} M showed that all the complexes gave values in the range of 9.01–26.4 $Scm^{-2}mol^{-1}$ (**Table 1**). These values are relatively low, which indicates that the complexes are non-electrolytes. Based on the (AAS) results, the percentage (%) of metal obtained was found to be in close

agreement with the calculated percentages (%).

IR Spectra

The nature of the bonding mode and the functional group linked to the metal ion can be determined using infrared spectra. A details comparison of the infrared spectra of the ligands and complexes was done in order to ascertain the ligand's bonding to the metal ion (**Table 2**). In the wavelength range of 400 – 4000 cm^{-1} , the important frequency bands of free ligands and complexes are displayed (Raman *et al.*, 2004). The IR bands of the ligands and the metal complexes were allocated based on data from the literature (Simo *et al.*, 2000) and all the spectral data were consistent with the proposed structure of the compounds. IR spectra of the ligands and the complexes were compared, and it was discovered that various peaks found in the ligands were shifted to lower or higher frequencies in the complex. In the free nicotinic acid spectrum, a band at 1504 cm^{-1} was ascribed to (C–N), which was shifted to the lower or higher of 1558 cm^{-1} , 1388 cm^{-1} , 1566 cm^{-1} , and 1627 cm^{-1} in the spectra of Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) complexes of nicotinic–phenylalanine, indicating that metal ion coordination occurred through the nitrogen atom of the pyridine ring in all complexes (David, 2000). In the spectrum of free phenylalanine, the band at 1638 cm^{-1} assigned to the (C–O) stretching vibration shifted to wavelengths of 1627 cm^{-1} , 1620 cm^{-1} , 1627 cm^{-1} , and 1687 cm^{-1} for the Mn(NA)(Phe), Co(NA)(Phe), Ni(NA)(Phe), Cu(NA)(Phe), and Zn(NA)(Phe) complexes of nicotinic–phenylalanine. This demonstrated that the carbonyl group of the ligand was involved in the coordination of phenylalanine to metal ions (Ufuk *et al.*, 2020; Bougherra *et*

al., 2018). In the spectrum of free phenylalanine, (N–H) stretching vibrations appeared at 3402 cm^{-1} , which shifted significantly to lower or higher frequencies in all the complexes at 3394 cm^{-1} for Mn (AA) (Phe), 3363 cm^{-1} for Co (AA) (Phe), 3410 cm^{-1} of Ni (AA) (Phe), 3394 cm^{-1} of Cu (AA) (Phe), and 3417 cm^{-1} of Zn (AA) (Phe). This could be due to coordination for metal ions with phenylalanine which occurs through the NH_2 group nitrogen atom (Taghreed *et al.*, 2019). New bands found in the nicotinic acid-phenylalanine complex in the ranges ($555\text{--}408\text{ cm}^{-1}$) and ($748\text{--}756\text{ cm}^{-1}$) were assigned to (M–N) and (M–O), respectively. These were not present in the spectra of free nicotinic acid and phenylalanine. These are indications of nitrogen and oxygen coordination with metal ions. The coordination of metal–nitrogen in the ligands was facilitated by the presence of a lone pair of electrons on the N atom of the amino group (Osunlaja *et al.*, 2009). This is also further evidence of coordination in nicotinic–phenylalanine complexes, which occurs through the nitrogen of the pyridine ring in nicotinic acid, as well as the nitrogen and oxygen of phenylalanine (Hadi, 2009).

Electronic Spectra

Free nicotinic acid (NA) and phenylalanine (Phe) exhibit two bands in the UV region in the range of $45454\text{--}34483\text{ cm}^{-1}$, which correspond to $\pi - \pi^*$ and $n - \pi^*$ transitions, respectively, according to their electronic spectra. Due to complexation, these bands are shifted to longer or shorter wavelengths (bathochromic and hypochromic shifts) in the metal ion complexes (Aderoju, & Sherif, 2015).

Electronic Spectra of Mixed Nicotinic – Phenylalanine Metal Complexes

The assignments of nicotinic acid and phenylalanine UV/Visible spectra to mixed-ligands metal complexes were done by comparing the observed values to earlier work on similar systems (Ndahi & Nasiru, 2012).

Both nicotinic acid and phenylalanine showed two absorption bands at 260 nm (38461 cm^{-1}) and 280 nm (35715 cm^{-1}) in nicotinic acid and 220 nm (45454 cm^{-1}) and 265 nm (37735 cm^{-1}) in phenylalanine, respectively. The visible spectrum of the Mn(II) nicotinic–phenylalanine complex revealed four bands at 570 nm (17543 cm^{-1}), 525 nm (19047 cm^{-1}), 495 nm (20202 cm^{-1}), and 435 nm (22988 cm^{-1}) assigned to ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{1g}(\text{G})$, ${}^6\text{A}_{1g} \rightarrow {}^4\text{T}_{2g}(\text{G})$, ${}^6\text{A}_{1g} \rightarrow {}^4\text{E}_g(\text{G})$ and ${}^6\text{A}_{1g} \rightarrow {}^4\text{A}_{1g}(\text{G})$. This suggests that the Mn (NA) (Phe) complex has an octahedral geometry (Temitayo *et al.*, 2012). The absorption bands in the electronic spectrum of Co(NA)(Phe) at 530 nm (18867 cm^{-1}) and 490 nm (20408 cm^{-1}) can be attributed to the ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{A}_{2g}(\text{F})$ and ${}^4\text{T}_{1g}(\text{F}) \rightarrow {}^4\text{T}_{1g}(\text{P})$ transitions, respectively. This could be related to the octahedral shape of the Co(II) complex (Siddappa *et al.*, 2008). In the electronic spectrum of the Ni(NA) (Phe) complex, three absorption bands at 600 nm (16666 cm^{-1}), 550 nm (1881 cm^{-1}), and 502 nm (1992 cm^{-1}) correspond to ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{2g}$, ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^1\text{E}_g$, and ${}^3\text{A}_{2g}(\text{F}) \rightarrow {}^3\text{T}_{1g}(\text{F})$, respectively. The octahedral geometry is also suggested by these transitions (Anaconda, & Rodriguez, 2004). Cu(II) complex of nicotinic – phenylalanine exhibits bands in the region of 640 nm (15625 cm^{-1}) and 450 nm (22222 cm^{-1}), attributed to ${}^2\text{E}_g \rightarrow {}^2\text{T}_{2g}$ transitions and MLCT, which correspond to octahedral geometry (Lawal *et al.*, 2017). Because the Zn(II) complex of nicotinic–phenylalanine did not show any visible peak due to its filled d-shell, the bands at 230 nm (43478 cm^{-1}) and 340 nm (29411 cm^{-1}) could be attributed to charge transfer transition. This result is in good agreement with previous works on its tetrahedral geometry (Lawal, 2010).

Antioxidant Activity

Due to the complexation of some metals with ligands, it is recommended that antioxidant activity be assessed using a variety of methods

(Anelise *et al.*, 2014). DPPH (1, 1- diphenyl – 2- picrylhydrazyl) and hydrogen peroxide tests are currently utilized (Brand-Williams *et al.*, 1995). In both assays, all the complexes had lower activity than the ascorbic acid (standard drug) but higher scavenging activity than the ligand (nicotinic acid), as shown in **Table 4**

and 5. The ability of metal complexes to scavenge decreases in the following order: Zn > Co > Cu > Mn > Ni. As a result, these complexes have been shown to have therapeutic potential in the treatment of oxidative stress-related diseases.

Proposed Structure based on the IR and UV-Visible spectra

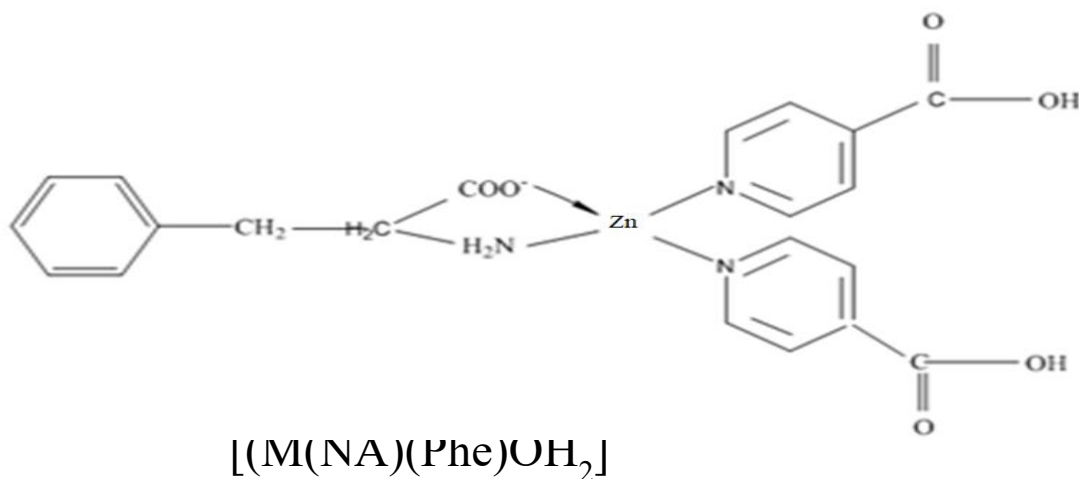


Figure 11: Proposed Structure of $[(M(NA)(Phe)]$ where M= Zn

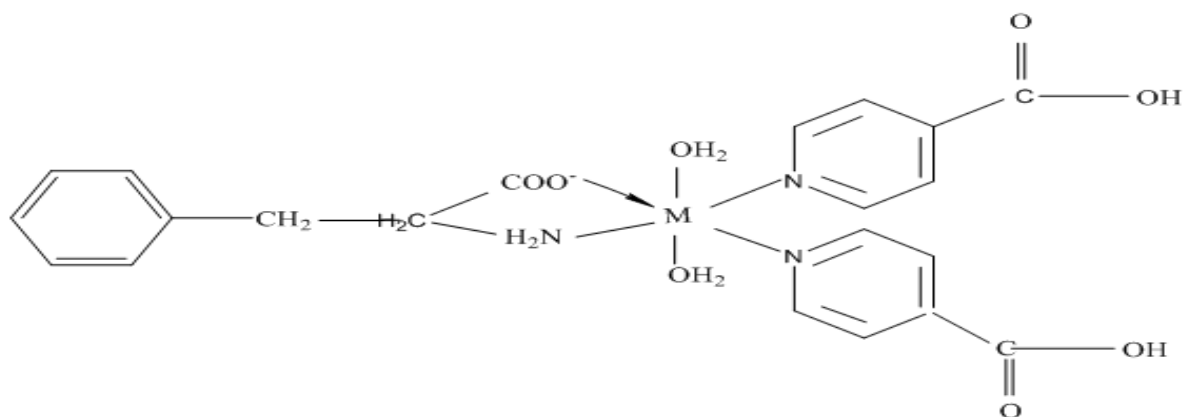


Figure 12: Proposed Structure of $[(M(NA)(Phe)OH_2)]$ where M= Mn, Co, Ni and Cu

CONCLUSION

The mixed ligands nicotinic – phenylalanine was synthesized and characterized using UV – Visible, FT-IR, conductivity measurements and antioxidant capacities were also determined.

All the complexes synthesized were slightly soluble in some of the organic solvents, but soluble in hot distilled water, methanol, ethanol and DMF. The results of the conductivity measurement suggested that the compounds are

non-electrolytes. Electronic spectra of all complexes observed were consistent with an octahedral geometry except that of zinc complex of nicotinic-phenylalanine which suggested a tetrahedral geometry. The infrared spectral results revealed that phenylalanine acts as a bidentate ligand, while nicotinic acid acts as a monodentate ligand in their mode of complexation. The phenylalanine coordinates through carbonyl oxygen and nitrogen of amine whereas the nicotinic acid coordinated through nitrogen of pyridine ring. *In vitro* antioxidant studies revealed that, all the complexes were found to be fewer effective antioxidants than the standard (ascorbic acid) but higher activities than the ligand (nicotinic acid). Therefore, the complexes demonstrated potential as therapeutic agents in the management of oxidative stress related diseases.

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ACKNOWLEDGEMENTS

The authors are grateful to the Departments of Pure and Applied Chemistry and Pharmaceutical Chemistry of the University of Maiduguri, Maiduguri, Nigeria, for providing chemicals (metal salts and some solvent) and research facilities (Laboratory) to carry out some work, as well as Prof. Noami P. Ndahi for her unwavering support in realization of this work.

STATEMENTS OF CONTRIBUTION

The undersigned authors contributed equally to the design and implementation of this work, as well as the analysis of the findings and the writing of the manuscript.

CONFLICT OF INTEREST

The authors declare that this study does not include any conflict of interest.

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