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Synthesis Characterization and *In Vitro* Pharmacological Evaluation of Oxalyldihydrazone and its Metal(II) Complexes

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Abstract

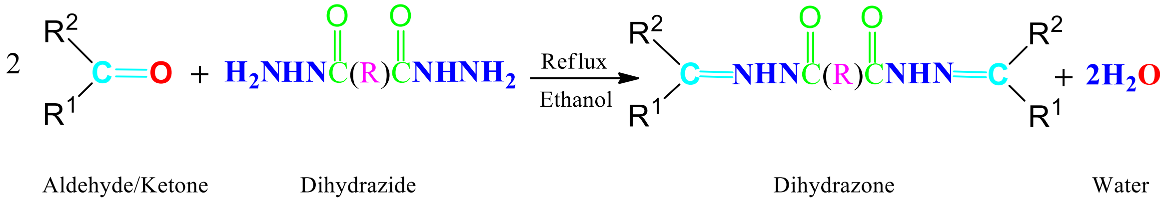
Drug resistance is a renowned condition that results when diseases become tolerant to chemotherapeutic agents. Even though, several classes of antibacterial and antifungal compounds are presently accessible, the resistance of microorganisms to these drugs has been continuously emerging. In order to address this problematic condition, it is crucial to discover new drugs with novel mechanisms of action, higher activity and improved selectivity. Complexes are gaining more attention in the design of drugs. This had led to lots of studies on metal-based drugs. Ni(II), Cu(II) and Zn(II) complexes of dihydrazone derived from oxalyldihydrazide and terephthalaldehyde were synthesized by salvo-thermal method and characterized by spectral and physicochemical techniques. The free ligand has a melting point of 176 °C and the complexes decomposed between 211 - 289 °C, which indicate their good thermal stability. The molar conductance values of the complexes were in the range of 9.8 - 20.3 $\Omega^{-1}cm^{-2}mol^{-1}$, signifying their non-electrolytic nature. Ni(II) and Cu(II) complexes were paramagnetic, whereas Zn(II) complex was diamagnetic. Electronic spectra show π - π^* and n - π^* transitions. The IR spectra of the compounds shows all the desired functional groups at the expected wavenumber. Elemental analyses agreed with the proposed formula of the compounds. The *in-vitro* antimicrobial, antioxidant and anti-inflammatory potency of the compounds were evaluated, the results revealed that the complexes displayed higher activities than the free dihydrazone. All the compounds exhibited appreciable activities against pathogenic gram positive and gram negative bacteria, harmful fungal species, remarkable radical scavenging activities and significant inflammatory inhibition.

Keywords: Anti-inflammatory, Antimicrobial, Antioxidant, Dihydrazone, Oxalohydrazide and Terephthalaldehyde

Introduction

Hydrazones are class of organic compounds that contain the group of atoms; RCH=NNHCO, they are generally derived from acid hydrazides [1]. Hydrazones are promising ligands due to their ability towards complexation and wide range of biological and non-biological applications [2-4]. As such they are used as important compounds in drug design [5], as bioactive ligands for metal complexes and for synthesis of many heterocyclic compounds [6]. Hydrazone ligands are of great importance due to their synthetic flexibility, chelate structure, electron-donating ability and structural resemblance with natural bioactive molecules. The bioactivity of hydrazones have encouraged synthetic researchers in the field of pharmacological chemistry to explore their potential applications [7-8], such as antibacterial [7], antifungal [9], anti-inflammatory [10], antiviral [11], antioxidant [12], anti-proliferative [13] and anticancer [14].

Dihydrazones are significantly important class of organic compounds, which are formed by condensation reaction of dihydrazides and carbonyl compounds [15-16]. The common structural features of dihydrazones are C=N, N–H, C=O and conjugated system [17-18]. These attractive and desirable features of dihydrazones can be effectively employed to synthesize many heterocyclic and biologically active compounds. In addition, they successfully provide various active and potential donor sites which can easily participate in complexation, hydrogen bonding and may also act as a site of protonation or deprotonation [19].



Scheme 1: General equation for the preparation of dihydrazones

Drug resistance is a renowned condition that results when diseases become tolerant to chemotherapeutic agents. Even though, several classes of antibacterial and antifungal compounds are presently accessible, the resistance of microorganisms to these drugs has been continuously emerging [20]. In order to address this problematic condition, it is crucial to discover new drugs with novel mechanisms of action, higher activity and improved selectivity. To combat these multidimensional challenges, there is increasing need for multifaceted bioactive agents [21,19]. This research is aimed at synthesis, characterization, *in-vitro* antibacterial, antifungal, antioxidant and anti-inflammatory evaluation of Ni(II), Cu(II) and Zn(II) complexes with dihydrazone derived from oxalyldihydrazide and terephthalaldehyde.

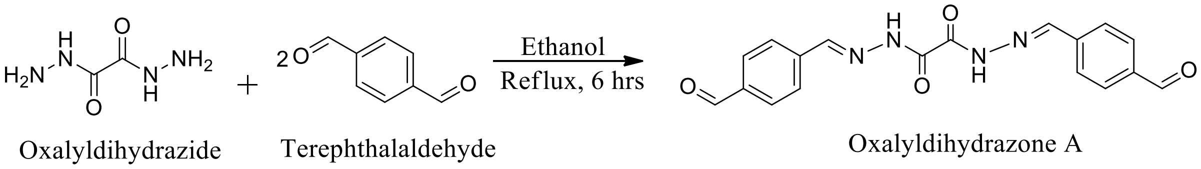
Materials and Methods

All the chemicals were of analytical grade and were used without further purification. The transition metal salts and other chemicals were obtained from either Merck or Sigma Aldrich. All glass wares were washed with detergent, rinsed with distilled water and dried in the oven at 110 °C. All weighing was carried out on electrical meter balance Toledo BI54. The *in vitro* antibacterial and antifungal screening were performed by well diffusion method. The antioxidant potency of the compounds was evaluated by DPPH radical scavenging activities.

The *in-vitro* anti-inflammatory evaluation was conducted by denaturation technique.

Synthesis of Oxalyldihydrazone A

Bis(terephthalaldehyde)oxalyldihydrazone was synthesized by refluxing ethanolic solution of oxalyldihydrazide (0.00l mol, 0.118 g) and terephthalaldehyde (0.002 mol, 0.268 g) for 6 hrs, after which the reaction mixture was concentrated to one-third of the original volume by slow evaporation on water bath, on cooling, the yellow coloured solid compound formed was filtered and washed several times with cold ethanol, followed by ether, then dried in desiccator over P_2O_5 and finally the yield was calculated [22]. The scheme for the synthesis was shown underneath (scheme 2).



Scheme 2: Synthesis of Dihydrazone A

Synthesis of Dihydrazone A Complexes

The complexes were synthesized by conventional refluxing method. The dihydrazone A (0.002 mol, 0.700 g) was added to 50 cm^3 ethanol. The resulting solution was mixed with ethanolic solution of metal(II) chloride (0.001 mol) and heated under reflux for 8 hrs, after which, the volume was reduced to 20 cm^3 by evaporation on water bath. The resulting reaction mixture was cooled to room temperature, the coloured solid complexes were filtered off, washed several times with cold ethanol and acetone to remove any excess of unreacted component and finally dried in a desiccator over P_2O_5. The dried compound was weighed, similarly, the yield was computed [22].

Antimicrobial Assay

The antibacterial and antifungal activity test of the synthesized dihydrazone and its respective complexes were carried out against three pathogenic bacterial isolates *Staphylococcus aureus, Escherichia coli and Salmonella typhi* and three deleterious fungal isolates, *Aspergillus flavus, Aspergillus fumigitus* and *Aspergillus niger* using well diffusion method by adopting the method reported by Laxman *et al.*, [23]. The result was shown in figure 2 and 3 respectively.

DPPH Radical Scavenging Assay

The stock solution of each standard control (Ascorbic acid), dihydrazone and the complexes were prepared (2 mg/mL each) and diluted to final concentration of 1000, 500, 250, 125, 62.5, 31.25, 15.63 and 7.81 µg/mL by serial dilution. 100 µL of 0.1mM 2,2-Diphenyl-1-picrylhydrazyl radical (DPPH) was used to evaluate the antioxidant potentials of the compounds. The absorbance was recorded at 517nm using a JASCO model V-550 UV-Vis spectrophotometer in triplicate [24]. The percentage radical scavenging activities (% RSA) were calculated by using the relation shown in equation beneath;

$$\% \text{ RSA } = \frac{\text{Absorbance of Control} - \text{Absorbace of Sample}}{\text{Absorbance of Control}} \times 100$$

In-vitro Anti-inflammatory Evaluation

The dihydrazone and its complexes were subjected to *in-vitro* anti-inflammatory test by protein denaturation technique at different concentrations; 62.5, 125, 250, 500, 1000 and 2000 µg/mL. The standard drug (diclofenac sodium) and the synthesized compounds were dissolved in a minimum amount of dimethylformamide (DMF) and diluted with phosphate buffer (0.2 M, pH 7.4). Final concentration of DMF in all the solution was less than 2%. 1 mL of the test solution containing different concentrations of the compounds were mixed with 1 mL of 1 mM egg albumin solution in phosphate buffer and incubated at $37^o \pm 2^o$ C for 15 min.

Denaturation was induced by keeping the reaction mixture at $70^o \pm 2°$ C in water bath for 10 min. After cooling, the turbidity was measured at 660 nm. Each experiment was done in triplicate and average is taken. Diclofenac sodium was used as standard drug (positive control) to compare its inhibition with the inhibition of test samples. The absorbance of 1 mM egg albumin solution in phosphate buffer without the test samples was taken as the absorbance of control which was used in the computation of % inhibition [25]. The percentage of inhibition of denaturation was calculated using the equation below:

$$\% \text{ Inhibition } = \frac{\text{Ac}-\text{At}}{\text{Ac}} \times 100$$

Where, A_c = Absorbance of control, A_t = Absorbance of test sample

Statistical Analysis

The IC50 values of the standard drugs (ascorbic acid and diclofenac sodium), the dihydrazone and its complexes at different concentrations were statistically analysed by probit analysis using IBM SPSS software version 20 [28].

Results and Discussion

The results obtained from the physico-chemical analyses, spectral characterizations, *in-vitro* antibacterial, antifungal, antioxidant and anti-inflammatory evaluation of the dihydrazones A and its respective metal(II) complexes are presented in the tables below;

Table 1, show the percentage yield and some properties of the dihydrazone A and its corresponding metal(II) complexes.

Table 1: Percentage Yield and Some Properties of the Dihydrazone A and its Corresponding Metal(II) Complexes

Compounds	Colour	Melting Point (°C)	Decomposition Temperature (°C)	Percentage Yield (%)
A	Yellow	176	-	77
$[NiA_2Cl_2]$	Greenish-yellow	-	289	71
$[CuA_2Cl_2].2H_2O$	Greenish-blue	-	211	69
$[ZnA_2Cl_2].3H_2O$	Light yellow	-	232	55

Where A = $C_{18}H_{14}N_4O_4$

Table 2: Result for Solubility Test of the Dihydrazone A and its Metal(II) Complexes

Compounds	Solvents									
	Methanol	**Ethanol**	**Acetone**	**Acetonitrile**	**Chloroform**	**DMSO**	**DMF**	**Pet. ether**	**n-Hexane**	**Water**
Ligand A	SS	SS	SS	SS	IS	S	S	IS	IS	IS
[NiA_2Cl_2]	IS	IS	IS	IS	IS	S	S	IS	IS	IS
[CuA_2Cl_2].$2H_2O$	SS	SS	SS	IS	IS	S	S	IS	IS	IS
[ZnA_2Cl_2].$3H_2O$	SS	SS	SS	IS	IS	S	S	IS	IS	IS

Key: S = Soluble; SS = Slightly Soluble and IS = Insoluble

Table 3: Results for Molar Conductivity Measurement of the Dihydrazone A Complexes in 10^{-3} M DMF

Compounds	Specific Conductance ($\Omega^{-1}cm^{-1}$)	Molar Conductance ($\Omega^{-1}cm^2mol^{-1}$)
[NiA_2Cl_2]	15.6×10^{-6}	15.6
[CuA_2Cl_2].$2H_2O$	9.8×10^{-6}	9.8
[ZnA_2Cl_2].$3H_2O$	20.3×10^{-6}	20.3

Where A = $C_{18}H_{14}N_4O_4$

Table 4: Magnetic Susceptibility Data of Dihydrazone A Complexes.

Compounds	Xg ($erg.G^{-2}g^{-1}$)	Xm ($erg.G^{-2}mol^{-1}$)	μ_{eff} (B.M)	Magnetic property
[NiA_2Cl_2]	4.3390×10^{-6}	3.6021×10^{-3}	2.93	Paramagnetic
[CuA_2Cl_2].$2H_2O$	1.6813×10^{-6}	1.4640×10^{-3}	1.87	Paramagnetic
[ZnA_2Cl_2].$3H_2O$	-3.4251×10^{-7}	-3.1134×10^{-4}	-ve	Diamagnetic

Where A = $C_{18}H_{14}N_4O_4$

Table 5: Percentage Water of Crystallizations for Metal(II) Complexes

Compounds	Initial mass (g)	Final mass (g)	Lost in mass (g)	% water of crystallization
[NiA_2Cl_2]	0.2	0.2000	-	-
[CuA_2Cl_2].$2H_2O$	0.2	0.1917	0.0083	4.15
[ZnA_2Cl_2].$3H_2O$	0.2	0.1881	0.0119	5.95

Where A = $C_{18}H_{14}N_4O_4$

Table 6: IR Spectral Data of the Dihydrazone A and its Metal(II) Complexes

Compounds	**υ(O-H) cm^{-1}**	**υ(N-H) cm^{-1}**	**υ(C=O) cm^{-1}**	**υ(C=N) cm^{-1}**	**υ(M-N) cm^{-1}**	**υ(M-Cl) cm^{1}**
Dihydrazone A	-	3223	1666	1603	-	-
$[NiA_2Cl_2]$	-	3272	1665	1618	615	485
$[CuA_2Cl_2].2H_2O$	3346	3253	1666	1629	622	460
$[ZnA_2Cl_2].3H_2O$	3368	3249	1663	1596	607	490

Where A = $C_{18}H_{14}N_4O_4$

Table 7: UV-Visible Spectral Data of the Dihydrazone A and its Metal(II) Complexes

Compounds	**π - π* (nm) Aromatic**	**π - π* (nm) C=O**	**π - π* (nm) C=N**	**n - π* (nm) C=O**	**n - π* (nm) C=N**
A	223	251	265	356	418
$[NiA_2Cl_2]$	211	254	281	359	400
$[CuA_2Cl_2].2H_2O$	217	248	275	397	411
$[ZnA_2Cl_2].3H_2O$	210	248	271	355	407

Where A = $C_{18}H_{14}N_4O_4$

Table 8: Micro-analytical Results of the Dihydrazone A and its Metal(II) Complexes.

	Percentage of Elements Calculated (Found)		
Compounds	**C**	**H**	**N**
Dihydrazone A	61.71 (59.94)	4.03 (3.86)	15.99 (14.52)
$[NiA_2Cl_2]$	52.08 (49.97)	3.40 (2.99)	13.50 (12.83)
$[CuA_2Cl_2].2H_2O$	49.63 (47.95)	3.70 (3.51)	12.86 (12.32)
$[ZnA_2Cl_2].3H_2O$	47.57 (45.44)	3.99 (3.67)	12.33 (10.99)

Where A = $C_{18}H_{14}N_4O_4$

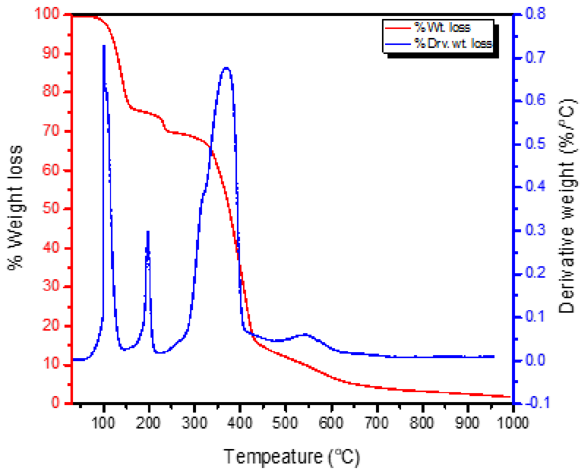


Fig.1: Thermogram of CuF$_2$Cl$_2$].2H$_2$O

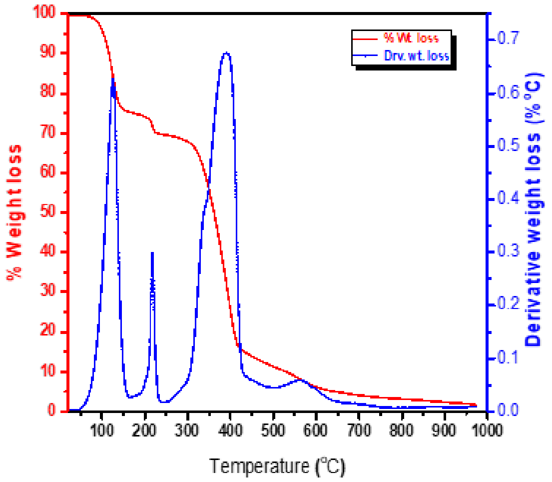


Fig. 2: Thermogram of [ZnA$_2$Cl$_2$].3H$_2$O

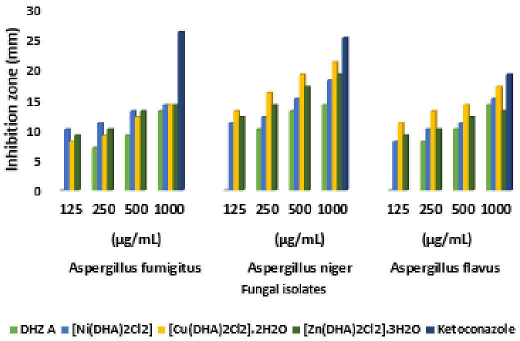


Fig. 3: Bar chart showing the antibacterial activity of dihydrazone B and its complexes

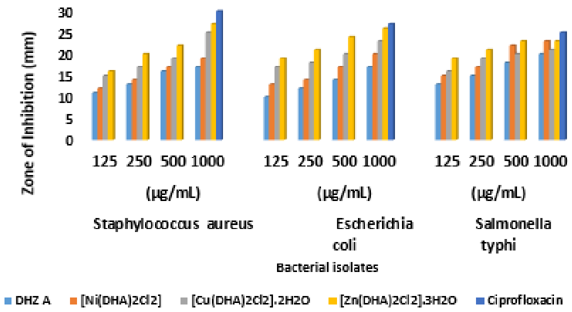


Fig. 4: Bar chart showing the antifungal activity of dihydrazone B and its complexes

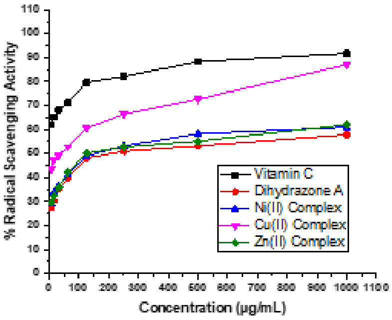


Fig. 5: Percentage RSA of ascorbic acid, dihydrazone A and its metal(II) complexes

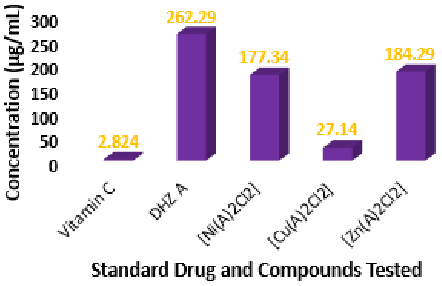


Fig. 6: Antioxidant IC50 Bar chat of vitamin C, dihydrazone A and its metal(II) complexes

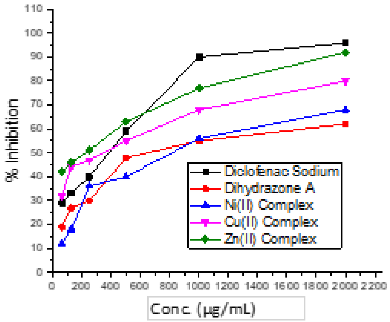


Fig. 7: Anti-inflammatory percentage inhibition of dihydrazone A and its metal(II) complexes

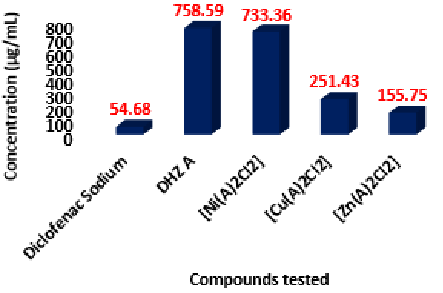


Fig. 8: Anti-inflammatory IC50 Bar-chat of diclofenac sodium, dihydrazone A and its metal(II) complexes

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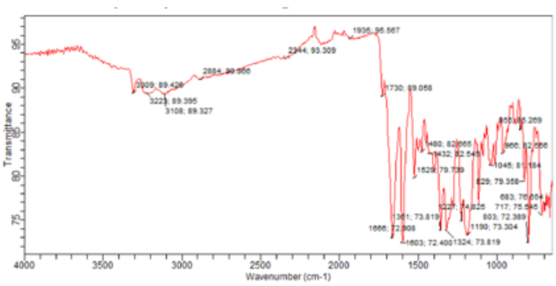

Fig. 9: FTIR Spectrum of dihydrazone A

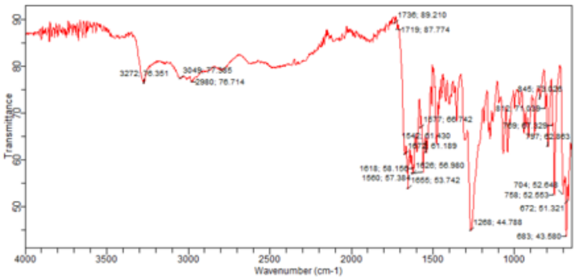

Fig. 10 FTIR Spectrum of $[NiA_2Cl_2]$ Complex

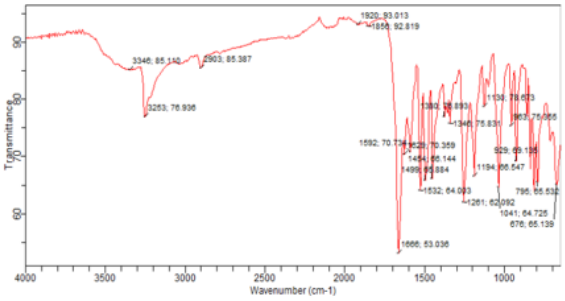

Fig. 11: FTIR Spectrum of $[CuA_2Cl_2].2H_2O$ Complex

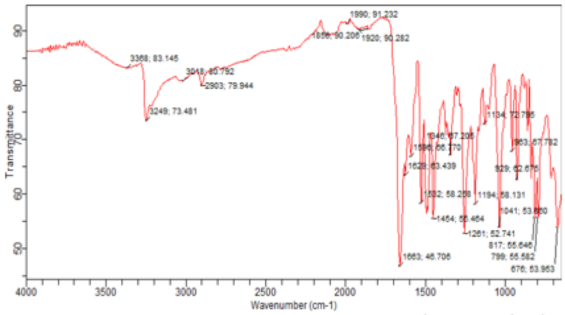

Fig. 12: FTIR Spectrum of $[ZnA_2Cl_2].3H_2O$ Complex

Discussion

The reaction between oxalyldihydrazide and terephthalaldehyde in a molar ratio of 1:2 respectively, produced yellow solid of dihydrazone A, the colour was due to transition of electrons between different energy levels of chromophores ($\pi - \pi^*$ electronic transitions), comparable with the colour of the compounds reported by Jadhav *et al.,* [27]. The percentage yield of the dihydrazone A was 77 %. The interaction of the ethanolic solution of dihydrazone A and that of Ni(II), Cu(II) and Zn(II) chlorides in a molar ratio of 2:1 respectively produced the desired complexes of the respective metal(II) ions, which are of different colours with a percentage yield between 55 – 71 % as displayed in Table1. Nickel(II) complex was obtained in exceptionally high yield, which was as good as the yield reported by Laylatul *et al.,* [28].

These indicated the practicability (feasibility) of the process for their synthesis. Likewise, the complexes have different colours due to d-d electron(s) transitions, the number of electrons in the d-orbitals of the metal ions differs, as such the wavelength at which they absorb light in the visible region varies leading to variation in colour of the complexes. All the complexes have relatively high decomposition temperature in the range of 211 – 289 ℃. The temperature at which all the complexes decomposed were by far above the melting point of the free dihydrazone which was 179 ℃, this indicated their good thermal stability. The decomposition temperature of these complexes were in good agreement with those of the compounds reported by Mouayed and Abduljleel, [29]. In the case of $[CuA_2Cl_2].2H_2O$ and $[ZnA_2Cl_2].3H_2O$ complexes, the temperature at which they decomposed coincide with their TGA decomposition temperature. The results for solubility tests of the dihydrazone A and its corresponding complexes were displayed in tables 2. The tests were carried out in strongly polar, slightly polar and non-polar organic solvents including water in order to identify the most suitable solvent for the purification and other spectroscopic and analytical measurements and also to know the nature of the compounds whether they are polar, partially polar or non-polar based on intermolecular attraction between the synthesized compounds and the solvents used. All the compounds were found to be insoluble in non-polar solvents but readily soluble in DMSO and DMF likely, due to the high dielectric constant of the solvents.

The molar conductance measurement of metal (II) complexes, were carried out in 10^{-3} M DMF and the values obtained were in the range of 9.8 – 20.3 $\Omega^{-1}cm^2mol^{-1}$. The values were presented in Table 3. The low values of molar conductance suggested the non-electrolytic behaviour of all the complexes. Since the dihydrazone is a neutral ligand and all the metal ions were in +2 state, this further confirmed that the two chloride ions were inside the coordination sphere [30].

Table 4 displays the magnetic susceptibility measurement results of the synthesized complexes. Gram magnetic susceptibility and molar magnetic moment values were calculated and used to compute the effective magnetic moments of all the complexes. Generally, square planer Ni(II) complexes are diamagnetic, while octahedral and tetrahedral complexes are paramagnetic with magnetic moment of 2.8 – 3.4 and 3.4 – 4.0 B.M respectively. In this study Ni(II) complex has a magnetic moment of 2.93 B.M which indicate two unpaired electrons in octahedral environment [30]. A magnetic moment of 1.7 – 2.2 B.M is usually observed for mononuclear Cu(II) complexes regardless of their geometries. i.e. (t_2g^6, eg^3) sometimes the values are higher than the spin only moment, likely due to orbitals contribution and spin orbit coupling [31]. In this study the Cu(II) complex has a total effective magnetic moment of 1.87 B.M indicative of one unpaired electron observable for six coordinate octahedral geometry [29]. Zn(II) complex is diamagnetic with negative molar susceptibility value. The complex assumed six coordinate geometry [32].

Tables 5 shows the percentage water of crystallization of the hydrated complexes. Nickel complex contain no water of crystallization as there was no decrease in mass of the compound after been placed in ovum at 110 °C overnight, likewise no broad band for O-H stretching frequency was observed in their respective FTIR spectrum, as such it was anhydrous. $[CuA_2Cl_2].2H_2O$ and $[ZnA_2Cl_2].3H_2O$ complexes were obtained in hydrated form, containing 2 and 3 molecules of water of crystallisation respectively and their respective percentage of water found were 4.15 and 5.95 %.

Table 6 show the infrared spectral data of the dihydrazone A and its complexes. The υ(O-H) absorption band for water of crystallization were observed at 3346 cm^{-1} and 3368 cm^{-1} in the spectra of Cu(II) and Zn(II) complexes respectively. The υ(N-H) absorption band in the spectrum of dihydrazone was observed at 3223 cm^{-1}, which were observed in the range of 3249 – 3272 cm^{-1} in the spectra of the complexes [31]. So also, υ(C=O) stretching frequency for the dihydrazone was seen at 1666 cm^{-1} and for the complexes between 1663 – 1666 cm^{-1}. The strong absorption band at 1603 cm^{-1} in the spectrum of the dihydrazone is due to υ(C=N) which was shifted to 1596 – 1629 cm^{-1} in the spectra of the complexes due to coordination through nitrogen atom of azomethine group [31]. New absorption bands at 607 - 622 cm^{-1} and 460 - 490 cm^{-1} in the spectra of the complexes were due to υ(M-N) and υ(M-Cl) respectively. This confirm the coordination of the dihydrazone to the metal ions. The result is similar with the results reported by Karthika *et al.,* [25] and Racheal *et al.,* [33].

UV-visible spectra of the dihydrazone and that of its complexes were recorded and presented in Table 7. The dihydrazone A spectrum displayed high-pitched absorption bands, λmax (nm) attributed to the following electronic transitions, 223 (π - π* aromatic ring), 251 (π - π* C=O), 265 (π - π* C=N), 356 (n - π* C=O) and 418 nm (n - π* C=N). These are consistent with the results stated by Ayman *et al.,* [22]. The complexes showed high intensities electronic transitions at 211 - 217 (π - π* aromatic ring), 248 - 254 (π - π* C=O), 271 - 281 (π - π* C=N), 355 - 397 (n - π* C=O) and 400 – 411 nm (n - π* C=N). The shift in absorption bands to lower λ_{max} (blue shift) or higher λ_{max} (red shift) in the spectra of the complexes are due to coordination of the dihydrazone to the metal ions. These are comparable to the results reported by Idris *et al.,* [30]. The results for elemental analysis of the dihydrazone and that of the metal(II) complexes were presented in Table 8. The experimental values obtained were in good agreement with the calculated percentages of C, H and N in the compounds. The variation was insignificant. This agrees with the proposed structures of the complexes, that is, 1:2 metal to ligand ratio. These were in good agreement with the results reported by Uba [35].

The TGA curve of $[Cu(A)_2Cl_2].2H_2O$ complex show four sequential steps of weight loss. The first step occurred within the temperature range of 100 - 130 ℃, causing a weight loss of 4.11 % (calculated 4.14 %) which matches to the loss of two uncoordinated water molecules, i.e. water of crystallization ($2H_2O$), the DTG curve gave maximum temperature peak at exactly 100 ℃. This confirmed the existence of uncoordinated water molecules. The second step was detected between 190 - 220 ℃ resulting in weight loss of 8.10 % (calculated 8.14 %), attributed to the loss of Cl_2 molecule, and the DTG curve gave a maximum peak temperature at 210 ℃. The third step was detected between 280 - 420 ℃, with a huge weight loss of 80.39 % (calculated 80.41 %), which is due to the breakdown of the ligand moiety at maximum peak temperature of 370 ℃ designated by DTG curve. At temperatures above 520 ℃, the remaining percentage indicate the complete decomposition of the residual complex, with the formation of copper oxide. $[Zn(A)_2Cl_2].3H_2O$ complex compound bearing the same ligand displayed a similar decomposition pattern, further emphasizing the consistent behaviour of the studied compounds during thermal degradation. These were comparable with the results reported by Fayed *et al.,* [36] and Bassam *et al.,* [37].

Fig. 3 and 4 show the *in-vitro* antibacterial and antifungal activities of the dihydrazone and its metal(II) complexes. The compounds were tested against three clinically isolated pathogenic bacteria (*Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*), and three fungal strains (*Aspergillus fumigitus*, *Aspergillus niger* and *Aspergillus flavus*). The results indicated that, the metal complexes exhibit higher antibacterial and antifungal activities than the free dihydrazone A. The presence of metal ions increases the activity of the compound. Incorporation of metal ions into the ligands increases its liposolubility (lipid soluble). According to Overtone’s concept of cell permeability, the lipid membrane that surrounds the microbial cells favours the passage of only lipid soluble materials, due to which, liposolubility is an important factor, that determine the antibacterial and antifungal activities of the tested compounds [33]. The variation in the effectiveness of different compounds against different organisms depends either on the permeability of the microbial cells or on differences in ribosome of microbial cells. That is the reason why some compounds are more potent against microbes than others [25]. Moreover, the activity is concentration dependent, it increases generally, with increase in concentrations.

The dihydrazone A ligand its respective metal(II) complexes were screened for their antioxidant potential using DPPH assay, in which the result is presented in Figure 5. The antioxidant activity results exhibit that the % radical scavenging power increases with increase in concentration of the tested compounds. The percentage radical scavenging potential of the ligand (dihydrazone

A) was 57.88 % at 1000 µg/mL which got enhanced on complexation with metal ions to 87.10 % in the case of copper(II) complex [34]. Among the screened compounds, the ligand possesses very low radical scavenging activity with IC50 = 262.29 ± 0.02 µg/mL, as compared to the standard ascorbic acid, IC50 = 2.82 ± 0.01 µg/mL. The results were shown in Figure 6. The complexes generally showed enhanced antioxidant activity with IC50 = 177.34 ± 0.04, 27.14 ± 0.02 and 184.29 ± 0.03 µg/mL) for Ni(II), Cu(II) and Zn(II) complexes respectively. Cu(II) complex has the least IC50 value, indicating its highest antioxidant potency compared to the other complexes.

These were in agreement to the results reported by Nighat *et al.,* [26]. The synthesized compounds were subjected to *in-vitro a*nti-inflammatory activity using albumin inhibition denaturation technique according to the method reported by Karthik *et al.,* [25]. All the complexes exhibited significant anti-inflammatory activity. Zn(II) complex was found to have comparable anti-inflammatory activity with the standard diclofenac sodium as shown in Figure 7. The Zn(II) complex has the highest percent inhibition of 92 % at 2000 µg/mL and was found to be most significant with IC50 155.75 µg/mL. From the overall percentage inhibition, all the complexes have displayed enhanced and significant anti-inflammatory activity, that are comparable or equipotent with the standard drug, diclofenac sodium.

Conclusion

The dihydrazone and its corresponding bio-active complexes were successfully synthesized and characterized by different physicochemical techniques and spectral analysis. All the synthesized compounds were obtained in appreciable yield with significant thermal stability. The complexes are non-electrolytic due to low values of molar conductance. The values of effective magnetic moment indicated the paramagnetic nature of the complexes with the exception of zinc(II) complex which was found to be diamagnetic. Infrared spectra show all the prominent absorption bands at the desired wave number. Electronic spectra show π - π^* and n - π^* transitions at expected wavelength values. Additionally, the results for elemental analyses show 1:2 metal to ligand ratio which are in good agreement with the proposed structures. The dihydrazone and all the complexes display appreciable activities against pathogenic bacterial and fungal isolates, as such the compounds are potent antimicrobial agents. Moreover, they have displayed significant antioxidant and appreciable anti-inflammatory activities, indicating their potentials as good antioxidant and anti-inflammatory agents.

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