Synthesis, Characterization and Antioxidant Properties of Metal (II) Complexes of Gallic Acid and Quercetin

Hassan B. Yesufu[1], Salihu Alhaji Musa[1], Garba T. Mohammed[1], Mohammed B. Fugu[2]
[1]*Department of Pharmaceutical Chemistry, University of Maiduguri, P.M.B 1069 Maiduguri, Nigeria*
[2]*Department of Chemistry, University of Maiduguri, P.M.B 1069 Maiduguri, Nigeria*

Abstract :- **The purpose of this study is to synthesized complexes of Mn and Zn with Quercetin and Gallic acid as ligands using reported procedure and characterized using IR, UV, AAS and Micro Analysis techniques. The purity of the complexes was monitored and analyze using TLC, Melting point, Conductivity measurement and Solubility test and the complexes were evaluated for antioxidant activity against 1,1-diphenylhydrazyl (DPPH) free radical. All the complexes are of good yield, colored, air stable, non hygroscopic, non electrolyte and partially soluble in organic solvent but soluble in DMF. The statistical analysis using one-way ANOVA show that all the complexes are strong free radical scavengers compares to the parent ligands at all the concentration tested but Zn complex at 6.25 µg/ml and 12.5 µg/ml showed better scavenging activity than the standard Ascorbic Acid (VIT. C). The EC_{50} regressional output shows that complex of Zn is found to be more portent than the other investigated compounds. On the basis of the above described studies an octahedral geometry has been proposed for the complexes.**

Keywords: **Quercetin, Gallic acid, Ascorbic Acid, DMF, Antioxidant, and Octahedral geometry.**

I. INTRODUCTION

Among the natural sciences, medicinal inorganic chemistry is still considered a rather young discipline by many, but this is contrary to the historically proven use of metals in pharmaceutical potions, which traces back to the ancient civilizations of Mesopotamia, Egypt, India, and China (Magner, 2005; Thompson and Orvig, 2006). For thousands of years, from the antiseptic properties of copper complexes, to the long standing application of gold complexes in Chinese and Arabic medicine; the unique and useful therapeutic benefits of metals have long been recognized and harnessed (Annapurna *et al.,* 2006). A number of new bio active ligands involving natural product are reported to function properly upon co-ordination with metal ions (Aliaga and Lissi, 2004). In recent years, metal-organic complexes have drawn much attention because many organic molecules can coordinate with various metal ions to form metal complexes with diverse geometries, providing infinite possibilities for coordination numbers and applications (Korkina, 2001). Carboxylates are among the most ubiquitous compounds in nature and the increasing interest in their complexes can be attributed to significantly important biological properties. The antioxidant activity of flavonoids is related to the chelation of metal ions, which catalyse the production of hydroxyl and lipid radicals through the decomposition of preformed lipid hydroperoxides (Halliwell, 1978; McCord and Day, 1978). It has been reported that, the interaction of flavonoids with metal ions may change the antioxidant properties and enhance the pharmacological properties of the flavonoids (Boots *et al.,* 2008). Many transition metal complexes bearing flavonoid ligands have been investigated, synthesized and characterized in the past years (Afanas'ev *et al.,* 1989). Experimental data have shown metal-flavonoid complexes are considerably more effective free radical scavengers than the free flavonoids and moreover they play an important role in protection against oxidative stress (Hajji *et al.,* 2006; Van Acker *et al.,* 1998). It is generally accepted that the ability of flavonoids to chelate metals is very important for their antioxidant activity. Complexes of flavonoids have the impact on the reduction of toxic metals bioavailability and much stronger free radical scavenging properties than the free flavonoids (Bushra and Farooq, 2008). Starting in the early 1980's until now, approximately 40 complexes of flavonoids from different flavonoids subclasses (rutin, quercetin, 3-hydroxyflavone, morin and hesperidin) with a number of metal ions or metal groups have been investigated (Malešev and Kuntic, 2007). To the best of our knowledge and available literature on the subject no work appears to be done in the above field of study.

II. MATERIALS AND METHODS

Metal (II) salts; Zinc (II) shulphate heptahydrate ($ZnSO_47H_2O$), Manganese (II) chloride tetrahydrate ($MnCl_24H_2O$), Sodium hydroxide (NaOH). Ligand molecule; Gallic Acid ($C_7H_7O_5$) and Quercetin ($C_{15}H_{10}O_7$).

Solvent; Dimethylformamide (DMF), Methanol, Ethanol, Chloroform and Acetone used in this research were of analytical grade, purchased from commercial sources and they were used without further purification.

Physical measurements and analysis of purity: The melting points of the synthesized complexes was determined using Gallenkemp melting point apparatus fitted with a thermometer at Pharmaceutical Chemistry Laboratory, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Maiduguri along Bama Road. Maiduguri, Borno state. Nigeria.

Thin layer chromatography: The reaction was monitored by TLC on silica gel G plates using acetone: toluene: acetic acid as developing solvent system in the ratio of 10:10:1 and iodine vapors was used as spray reagent. At the end of the reaction starting material disappeared and all the complexes appeared as a single spot.

Solubility: The solubility of the complexes was determined in some polar, mid polar and non polar solvents such as water, methanol, ethanol, acetone, chloroform and Dimethylformamide (DMF).

Conductivity: The conductivity of the complexes was measured in DMF solutions (10^{-3} M) at room temperature using DDS-307 conductivity meter at Soil Science Laboratory, Department of Soil Science, University of Maiduguri along Bama Road. Maiduguri, Borno state. Nigeria.

Infrared Spectra (IR): IR spectra of the ligands and complexes were recorded using KBr pellets in the range (4000-400 cm^{-1}) on SHIMADZU FT-IR-8400S FOURIER TRANSFORM INFRARED SPECTRPHOTOMETER at National Research Institute for Chemical Technology, Zaria (NARICT). Kaduna state. Nigeria.

Electronic spectra (UV): Electronic spectra of metal complexes in DMF were recorded on Perkin-Elmer. Lamda 35 UV/VIS Spectrometer. National Food Drugs and Control (NAFDAC), Maiduguri, Borno State. Nigeria.

Elemental microanalysis: The percentage composition of C and H of the complexes and ligands was determined by using micro analytical techniques at University of Johannesburg, South Africa.

Percentage of metal: The percentage of metal in the complexes was determined using Atomic Absorption Spectroscopy (AAS) at National Research Institute for Chemical Technology, Zaria (NARICT). Kaduna state. Nigeria.

Method: All glass wares used in this work were washed with detergent after soaking in conc. HNO_3 rinsed with distilled water and dried in an oven.

Synthesis of Mn (II) complex: The complex was prepared following reported procedure (Nadira *et al.*, 2010; Ogunniran *et al.,* 2008; Ibrahim *et al.,* 1995). A 1:1 ligand solution of quercetin (0.3022g, 1.0mmol) and gallic acid (0.1701g; 1.0mmol) dissolved in methanol (20ml) was prepared in100ml three-necked round-bottomed flask provides with electromagnetic stirrer and stirred until homogeneous solution is obtained. To this miscible solution, a $MnCl_2.4H_2O$ (0.1979g, 1.0mmol) dissolved in methanol (20ml) was added dropped wise with constant steering and the progress of the reaction was monitored by TLC. The resulting reaction mixture was stirred for 3hrs using magnetic stirrer at room temperature. The complexes were precipitated by adjusting the pH to 6-8 using buffer 7 by adding very dilute NaOH solution (1.0ml, 0.001M) drop wise and stirred using magnetic stirrer for 5-10 minutes, after which the solid complex formed were poured into a beaker and left to stand for 24 hours. The precipitate was filtered and washed with equal volume of water and methanol and dried over $CaCl_2$ in dessicator.

Equation for the reaction is presented as: $C_7H_6O_5 + C_{15}H_{10}O_7 + MnCl_2.4H_2O + NaOH \rightarrow [Mn(C_{22}H_{13}O_{12}).2H_2O] + NaHCl_2 + 3H_2O$.

Synthesis of Zn (II) complex: The complex was prepared following reported procedure (Nadira *et al.*, 2010; Ogunniran *et al.,* 2008; Ibrahim *et al.,* 1995). A 1:1 ligand solution of quercetin (0.3022g, 1.0mmol) and gallic acid (0.1701g; 1.0mmol) dissolved in methanol (20ml) was prepared in100ml three-necked round-bottomed flask provides with electromagnetic stirrer and stirred until homogeneous solution is obtained. To this miscible solution, a $ZnSO_4.7H_2O$ (0.2875g, 1.0mmol) dissolved in methanol (20ml) was added dropped wise with constant steering and the progress of the reaction was monitored by TLC. The resulting reaction mixture was stirred for 3hrs using magnetic stirrer at room temperature. The complexes were precipitated by adjusting the pH to 6-8 using buffer 7 by adding very dilute NaOH solution (1.0ml, 0.001M) drop wise and stirred using magnetic stirrer for 5-10 minutes, after which the solid complex formed were poured into a beaker and left to stand for 24 hours. The precipitate was filtered and washed with equal volume of water and methanol and dried over $CaCl_2$ in dessicator.

Equation for the reaction is presented as: $C_7H_6O_5 + C_{15}H_{10}O_7 + ZnSO_4.7H_2O + 2NaOH \rightarrow [Zn(C_{22}H_{13}O_{12}).2H_2O] + Na_2SO_4 + 7H_2O$.

1,1-Diphenyl-2-picrylhydrazyl (DPPH): The DPPH free radical scavenging potential of the ligands and their complexes relative to the standard i.e. Ascorbic Acid (VIT.C) were determined using reported procedure as described by Khan *et al* (2013). In the assay, ligands and their complexes at different concentrations (6.25 µg/ml, 12.5 µg/ml, 25 µg/ml, 50 µg/ml, 100 µg/ml) dissolved in ethanol were prepared in a test tube using serial dilution method and 2ml of freshly prepared 0.004mg DPPH in ethanol is added to each concentration, the resulting mixture was allowed to stay in a dark for 30min at room temperature. After which, absorbance of the mixture was taken at 517nm using UV spectrophotometer. All measurements were taken in triplicates. DPPH radical scavenging activity was calculated using the formula:

$$\text{Scavenging Activity (\%)} = \frac{Ac - As}{Ac} \times 100$$

Where;-

Ac - Absorbance of control

As - Absorbance of sample

Determination of EC_{50}: The EC_{50} value required for 50% of the DPPH free radicals scavenging by the complexes were determined from a series of dose-response data (sample concentration and DPPH free radical scavenging (%)). Using an X-Y plot fitted with a linear regression line and the EC_{50} was estimated using the following relationship;

$$EC_{50}\ (X) = 50 - C/M$$

Where, C represents the intercept

M represent the gradient of the line

Statistical analysis: Statistical analysis was performed using SPSS software version 20 and one-way Analysis of variance (ANOVA). Data were presented as Mean ± S.E.M. from at least three independent experiments (n = 3). Where $P < 0.05$ is considered statistical significant.

III. RESULTS

Table 1: Analytical data and other physical properties

Empirical formula	Molecular weight(g)	Color	Mp ^0C	Actual Yield (cal)	% yield	Am $Scm^{-2}mol^{-1}$
$[Mn(C_{22}H_{13}O_{12}).2H_2O]$	559.938	Dark Brown	>300	0.3512 (0.5597)	62.7	14.4
$[Zn(C_{22}H_{13}O_{12}).2H_2O]$	570.38	Milky	292-294	0.3891 (0.5703)	68.2	15.7

Table 2: Solubility

Complexes	Water	Ethanol	Methanol	Acetone	Chloroform	DMF
$C_7H_6O_5$	S	S	S	S	I	S
$C_{15}H_{10}O_7$	I	S	S	S	I	S
$[Mn(C_{22}H_{13}O_{12}).2H_2O]$	I	SS	SS	SS	SS	S
$[Zn(C_{22}H_{13}O_{12}).2H_2O]$	I	SS	SS	SS	SS	S

Where: Soluble (S), Slightly (SS), Insoluble (I), Dimethylformamide (DMF)

Table 3: Elemental analysis

Complexes	Empirical Formula	%Carbon Found (Cal.)	%Hydrogen Found (Cal)	%Metal
$C_7H_6O_5$	-	49.03 (49.4)	3.43 (3.5)	-
$C_{15}H_{10}O_7$	-	58.86 (59.6)	3.18 (3.3)	-
$[Mn(C_{22}H_{13}O_{12}).2H_2O]$	$C_{22}H_{17}O_{14}Mn$	46.96 (47.2)	2.94 (3.0)	9.8
$[Zn(C_{22}H_{13}O_{12}).2H_2O]$	$C_{22}H_{17}O_{14}Zn$	46.21 (46.3)	2.87 (2.9)	11.1

Table 4: IR Spectral Data

Complexes	v(OH)	v(C=O)	γ(C-O)	γ(C-OH)	H_2O	M-O
$C_7H_6O_5$	3492.79	1737.28	1283.27	1350.07	-	-
$C_{15}H_{10}O_7$	3400.63	1668.12	1257.23	1385.79	-	-
$[Mn(C_{22}H_{13}O_{12}).2H_2O]$	3429.22	1625.14	1235.71	1357.40	845.35	601.82
$[Zn(C_{22}H_{13}O_{12})._2H_2O]$	3373.32	1643.12	1250.67	1378.40	870.23	609.02

Table 5: Electronic Absorption Spectra (UV)

Complexes	Absorption (nm)	Transitions		Proposed geometry
		CT	d-d	
$C_7H_6O_5$	210 270	$\Pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	- -	- -
$C_{15}H_{10}O_7$	250 370	$\pi \rightarrow \pi^*$ $n \rightarrow \pi^*$	- -	- -
$[Mn(C_{22}H_{13}O_{12}).2H_2O]$	380 470 600	CT - -	- $^6A_{1g} \rightarrow {}^4T_{1g}$ (4P) $^6A_{1g} \rightarrow {}^4T_{2g}$(4G)	Octahedral
$[Zn(C_{22}H_{13}O_{12}).2H_2O]$	320 430	LMCT	- -	Octahedral

LMCT= ligand metal charge transfer, CT= charge transfer

Table 6: Antioxidant studies

Conc. µg/ml	DPPH Radical Scavenging Activity (% Scavenging)				
	Zn ± SEM	Mn ± SEM	Gallic Acid ± SEM	Quercertin ± SEM	Ascorbic A. ± SEM
6.25	82.74±0.02	76.16±0.03	70.020.03	68.9±20.02	80.07±0.03
12.5	86.52±0.04	80.52±0.04	76.18±0.02	70.28±0.01	85.85±0.02
25	88.88±0.10	85.43±0.02	78.23±0.03	74.88±0.03	92.47±0.02
50	92.70±0.03	88.03±0.03	83.11±0.04	80.46±0.06	95.30±0.05
100	95.08±0.02	92.95±0.02	86.62±0.03	82.55±0.03	97.47±0.02
EC_{50}	0.0036	0.070	0.166	0.219	0.0043

Anova: p-value = 0.000; p-value < 0.05 compared to standard AA was considered statistically significant (By one way ANOVA, Turkey HSD).

IV. DISCUSSIONS

All the complexes are colored, air stable, non-hygroscopic and possess high melting points, which indicate good thermal stability of the complexes and strong metal-ligand bond (Table 1). They showed yields that ranged from good to excellent (62.7%, 68.2%). The complexes are insoluble in water and partially soluble in common organic solvents such as ethanol, methanol acetone and chloroform but are soluble in Dimethyl Formamide (DMF) suggested polymeric behavior of the complexes probably due to large number of OH group present on the structure (Table 2). The elemental analysis data (Table 3) of metal complexes is consistent with their general formulation as 1:1:1, Ligand: metal: ligand ratio of the type $[M(G)(Q)\cdot 2H_2O]$, this is quite in agreement with the proposed geometry of the complexes which also indicate the presence of the two ligands in the complexes. The molar conductance values (Table 1) which helps in ascertaining its ionic and covalent nature of the complexes (14.4, 15.7 Scm^{-2} mol^{-1}) are too low to account for any dissociation of the complexes in DMF, which indicate non electrolytic nature of the complexes.

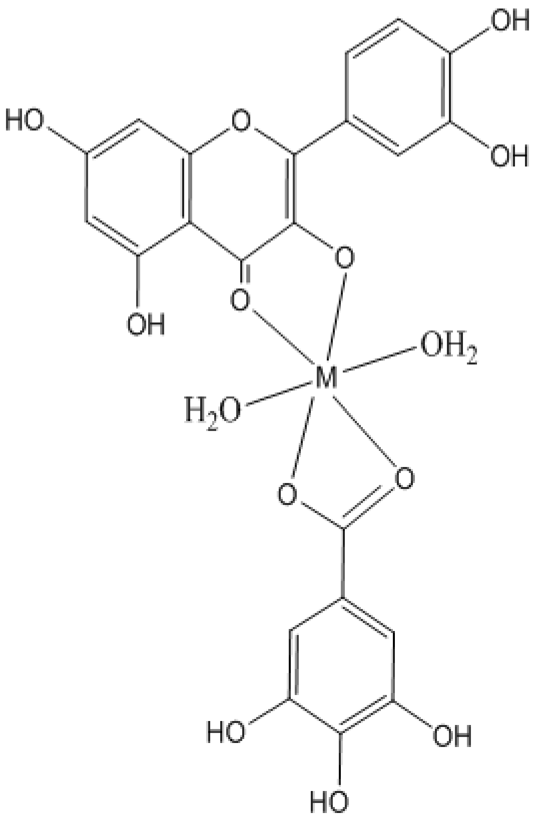
Infrared spectra of ligands and their metal complexes: The FTIR spectra of compounds were quite complex due to presence of numerous bands with varying intensities making the task quite difficult. However, an attempt has been made to assign some important bands on the basis of reported spectra of several metal ligand complexes. In this work, fundamental bands of Gallic acid and Quercetin compounds and their complexes are found and presented in table 4. Comparing the infrared spectra of the complexes with that of the free parent ligands, it should be possible to determine the possible coordination sites of the complexes. The broad band observed at 3492.79cm^{-1} and 3400.63cm^{-1} in the spectra of the parent ligands; Gallic acid and Quercetin is due to stretching vibrations of –OH group. These vibrations are reported to occur around 3610-3300 cm^{-1} for the compounds containing free –OH functional group (Pavia *et al.*, 2001). The appearance of broad band in the spectrum of the complexes at 3429.22 cm^{-1} and 3373.32 cm^{-1} region suggests deprotonation of C–OH of the phenolic O-H and subsequent coordination of phenolic oxygen atom to the metal ion to form bond. The appearance of non-ligand bands at 845.35 cm^{-1} and 870.23 cm^{-1} region is assign to the rocking mode of water which suggests the presence of coordinated water in the complexes （Nakamoto, 2009). The γ(C-O-H) deformation mode observed at 1350.07 cm^{-1} and 1385.79 cm^{-1} in the parent ligands is shifted in all the complex 1357.40 cm^{-1} and 1378.40 cm^{-1}, indicating an increase in bond order, which is normally observed when metal coordination involves with the O-H group of quercetin. The band observed at 1235.71 cm^{-1} and 1250.67 cm^{-1} region in the spectra of the complexes is due to (C-O) phenolic while usual absorbance of this group is around 1200 cm^{-1} as reported by pavia *et al* (2001) so the shifting towards higher frequency is a consequence of the weaken O-H bond which leads to increase in electron flow toward benzene ring causing shift in the C-O bond. A medium intensity band exhibited in the spectrum of free quercetin at 1668.12 cm^{-1} is due to stretching mode of carbonyl v(C=O) group, this band has downshifted which indicate bond formation through the carbonyl (C=O) group and the hydroxyl (OH) of the ligands this agree with reported findings by Nilanjan, *et al.*, (2015). While the stretching band due to carbonyl v(C=O) group observed at 1737.28 cm^{-1} in the free gallic acid remains unaffected after complex formation which also indicate C=O of the gallic acid is involved to formed bond. The low intense bands appearing in the spectra of the complexes and not observed in the spectra of the parent ligands at 601.82 cm^{-1} and 609.02 cm^{-1} is assigned to M-O bond, which suggest that the heteroatoms oxygen is also involved in the co-ordination (Pavia *et al.*, 2001). From the infrared spectral data obtained, one comes to the following conclusion; Gallic acid and Quercetin behave as mono basic bidentate chelating agents and coordinate to the metal ion through the oxygen atom of the hydroxyl (OH) group and carbonyl (C=O) group in the binuclear complex with a proposed geometrical octahedral structure.

Electronic Spectra of the ligands and their complexes: The complexes show intra-ligand charge transfer and d-d transitions in the range 200 – 800 nm (table 4.5). The UV absorption band observed between 200 - 300 nm is assigned to $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transitions in the aromatic rings of the free ligands (Shivakumar *et al.*, 2008). The charge transfer (CT) transition which mostly occur around 300 - 400 nm are classified as Ligand to Metal charge transfer (LMCT) or

Metal-to-Ligand charge transfer (MLCT). LMCT can occur in all complexes including ones with empty or completely filled d orbital while MLCT is seen in complexes where the ligands have low-lying empty orbital. The d-d transitions which were confined to the d orbital of the metal ion occur in the visible region of the electromagnetic spectrum between 400 – 800 nm. In the electronic spectra of the free ligands; Gallic acid and Quercetin, two prominent absorption bands were observed, at 210 nm (47619 cm^{-1}) and 270 nm (37037 cm^{-1}) for Gallic acid and at 250 nm (40000 cm^{-1}) and 370 nm (27027 cm^{-1}) for Quercetin which are due to π - π^* and n - π * intra ligand charge transfer transitions respectively. These bands undergo a hypochromic effect (a shift to longer wavelength) in the spectrum of the metal complexes due to complexation of the ligand to the metal ions. The electronic spectra of Mn (II) d^5 ion also exhibits three spin allowed electronic transition which correspond to; ${}^6A_{1g} \rightarrow {}^4T_{1g}$ (4G), ${}^6A_{1g} \rightarrow {}^4T_{2g}$ (4G) and ${}^6A_{1g} \rightarrow {}^4T_{1g}$ (4P) transitions in an octahedral field. The complexes are found to be diamagnetic as expected for six-coordinated d^5 octahedral complexes. The electronic spectrum of the Mn complex show a band at 600 nm (16666 cm^{-1}) and 470 nm (21276 cm^{-1}) which are attributed to d-d transitions and are assign to; ${}^6A_{1g} \rightarrow {}^4T_{1g}$ (4G) and ${}^6A_{1g} \rightarrow {}^4T_{2g}$ (4G) transition respectively and a band at 380 nm (26315 cm^{-1}) is assign to charge transfer transition. These transitions strongly favor octahedral geometry around the Mn (II) ion forming an octahedral complex.

Zn (II) complex is diamagnetic in nature and its electronic spectra do not furnish any characteristic d-d transitions except charge transfer bands as expected for d^{10} configuration. The two bands at 320 nm (31250 cm^{-1}) and 430 nm (23255 cm^{-1}) exhibited in the spectrum of Zn (II) complex are due to charge transfer (LMCT) transition.

Antioxidant studies: DPPH is a stable organic compound and it is extensively used for oxidative assay of various complexes and other materials. The scavenging ability of the complexes in comparison to the parent ligands and to the standard Ascorbic Acid is found to be statistically significant with p-value < 0.05. This implies that the complexes exhibit dose-dependent response against DPPH free radicals and showed significant differences in their scavenging capacity when compare to the parent ligands and the standard Ascorbic Acid. A comparative study of the ligands and their complexes indicates that all the complexes exhibited higher antioxidant scavenging activity than the free ligands at all the concentration tested. Complex of Mn, at 25 µg/ml, 50 µg/ml and 100 µg/ml show less scavenging activity than the reference standard Ascorbic Acid (VIT. C). At 6.25 µg/ml and 12.5 µg/ml complex of Zn show better scavenging activity than the reference standard (VIT. C). Patel, (2012) reported that, the lower the EC_{50} value the strong ability of the complex to act as DPPH scavenger while the higher the EC_{50} value the lower scavenging activity of the complex. On this note, the EC_{50} regression analysis obtained evidently show that complex of Zn (0.0036 µg/ml) is found to have stronger scavenging activity with lower EC_{50} when compare to all investigated compounds but a marked enhancement of activity was generally exhibited by complex of Mn against free radical DPPH when compare to the free Ligands. These strong scavenging activities could be due to coordination of metal ion to the ligands which increased its capacity to stabilize unpaired electrons and thereby increased its ability to scavenge free radicals. This findings indicated that the metal ions significantly change the biological chemical properties of the free ligands such that; the activity of the free ligands increases with coordination with the metal ion which agrees with the same finding by Svetlana and Dorina (2016) and also justified the statement by Foti *et al.,* (2011), Boots *et al.,* (2008), Bukhari *et al.*, (2009) and Delaney *et al.*, (2002), that metal complexes of flavonoid are much more effective free radical scavengers than the free flavonoid.. The order of activity is presented as Zn (0.0036 µg/ml) > AA > Mn (0.070 µg/ml) > G (0.166 µg/ml) > Q (0.219 µg/ml).



Proposed structure of the complex (M=Zn and Cu)

V. CONCLUSION

The synthesized Complexes of Mn and Zn with Gallic acid and Quercetin show that all the complexes are strong free radical scavengers' compares to the parent ligands at all the concentration tested. Zn complex showed better scavenging activity than the standard Ascorbic Acid (VIT. C). The EC_{50} regression shows that complex of Zn is found to be more portent than all the investigated compounds.

REFERENCES

[1] Annapurna M. M., Bhanoji Rao M.E. and Ravi Kumar B.V.V. (2006). Synthesis, Spectral Characterization and Evaluation of Pharmacodynamic Activity of Copper and Complexes of Ethambutol Dihydrochloride, *E-J Chem.* **3** (13): 274 - 277

[2] Afanas'ev, I. B., A. I. Dorozhko, A. V. Brodskii, V. A. Kostyuk, and A. I. Potapovitch (1989). Chelating and free radical scavenging mechanisms of inhibitory action of rutin and quercetin in lipid peroxidation. *Biochem. Pharmacol*. **38**: 1763 - 1769.

[3] Foti, M. C., Daquino, C., DiLabio, G. A. (2011) Kinetics of the oxidation of quercetin by 2,2-diphenyl-1-picrylhydrazyl (dpph•). *Org Lett.* **13**, 4826-4829. 541.

[4] Halliwell, B., (1978). Superoxide-dependent formation of hydroxyl radicals in the presence of iron chelates: Is it a mechanism for hydroxyl radical production in biochemical systems? *FEBS Lett*. **92**, 321-326.

[5] Hajji H. E., E. Nkhili, V. Tomao, O. Danglas, (2006). Interactions of quercetin with iron and copper ions: complexation and autoxidation. *Free. Rad. Res*. **40**, 303-310.

[6] Ibrahim, SA., Shaker, AM., Ismail, NM., Mahmoud, MR., (1995). Mixed ligands complexes of Ni(ii), Th(iv) and Vo(ii) with some benzoic acid derivatives and amino acids. *Chem. Abst.* 122: 1028.

[7] Korkina, (2001). Enhancement of antioxidant and anti-inflammtory activities of bioflavonoid rutin by complexation with transition metal, *Biochem. Pharmacol*. **61**, 677- 684.

[8] Khan SA, Rasool N, Riaz M, Nadeem R, Rashid U, Rizwan K, Zubair M, Bukhari IH, Gulzar T. (2013).Evaluation of Antioxidant and Cytotoxicity Studies of Clerodendrum inerme. Asian J Chem; **13**: 7457-7462.

[9] Malešev, D. and KunV. KuntIc,. (2007). Investigation of metal–flavonoid chelates and the determination of flavonoids via metal–flavonoid complexing reactions. *J. Serb. Chem. Soc*. **72** (10): 921 – 939

[10] Nilanjan, Ghosh,, Tania Chakraborty., Sougata, Mallick., (2015).Synthesis, characterization and study of antioxidant activity of quercetin–magnesium complex, Spectrochimica Acta Part A: *Molecular and Biomolecular Spectroscopy*, **151**, 807-813.

[11] Ogunniran K.O., Ajanaku K.O., James O.O., Ajani O.O., Nwinyi C.O., Allansela. (2008). Fe(iii) and Co(ii) complexes of mixed antibiotics: synthesis, characterization, antimicrobial potential and their effect on alkaline phosphatase activities of selected rat tissues. *Int. J. Phys. Sci*. **3**(8): 177-182.

[12] Patel K. B., Kharadi G. J., Vyas K. B. and Nimavat K. S. (2012). *Int. J. Pharma. Res. Scholars*, **1(2)**, 474

[13] Pavia; D. L. Lampman G. M. and Kriz, G. S., (2001) "Introduction to Spectroscopy" third edition; Thomson United States.

[14] Shivakumar K., Shashidhar, P. Vithal Reddy, M.B. Halli, (2008) J. *Coord. Chem.*, **61:** 2274.

[15] Thompson K.H. and Orvig C., (2006). Concepts and Models in Bioinorganic Chemistry. In: Kraatz HB, Metzler-Nolte N (eds.), Wiley-VCH: Weinheim, Germany, p: 25.

[16] Van Acker S., G. P. van Balen, B. van der Berg, W. J. F. van der Vijgh, (1998). Influence of iron chelation on the antioxidant activity of flavonoids. *Biochem. Pharmacol*. **56**, 935-943