

FUDMA Journal of Sciences (FJS) ISSN online: 2616-1370 ISSN print: 2645 - 2944

Vol. 9 No. 12, December, 2025, pp 269 – 275



DOI: https://doi.org/10.33003/fjs-2025-0912-4274

SYNTHESIS, CHARACTERIZATION AND BIOACTIVITY EVALUATION OF MN(II), CO(II), NI(II) AND CU(II)-QUERCETIN COMPLEXES: ANTIMICROBIAL, ANTIOXIDANT AND CYTOTOXICITY PROPERTIES

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ABSTRACT

In this study, metal (II)- quercetin complexes are synthesized and characterized by using FT-IR, water of hydration, conductivity, magnetic susceptibility and elemental analysis. The metal(II) complexes of manganese, cobalt, nickel, and copper with the ligand were synthesized by refluxing respective metal ions with the corresponding ligand in a 1:2 mole ratio. The FT-IR spectra of the quercetin showed a broad band at 3248 cm ¹, assignable to phenolic (OH) stretching vibrations. Another sharp absorbance band seen at 1666 cm⁻¹ assigned to aromatic C=O stretch in the ligands were shifted to lower frequency range of 1596 – 1633 cm⁻¹, suggesting coordination through oxygen of the C=O of the ligands respectively. New bands at the lower region in the range of 530 – 616 cm⁻¹ which were not observed in the ligand, further confirmed M–O chelation in the complexes, which confirmed the deprotonation of phenolic (OH) in the ligands. Molar conductivity values obtained for the complexes were found to be low $(5.18-11.00~\Omega^{-1}~cm^2mol^{-1})$ indicating non-electrolytic nature. Magnetic moments determined were in the range 1.69 - 5.92 BM, indicating that all the complexes are paramagnetic suggesting a tetrahedral geometry for the complexes. The relative percentages of the constituent elements from CHN analysis showed strong agreement with theoretical percentages for all the respective complexes. The antimicrobial activity of quercetin and its complexes was evaluated using the well diffusion method against three bacterial and fungal isolates each. The result of the antioxidant assay obeys the Beer-Lambert law over the useful range, with both the ligand and its complexes showing great antioxidant potency in all fractions tested. The result of cytotoxicity showed that the Mn-Qur is more cytotoxic with an LC50 of 177.916 µg/mL. This study reveals metal-specific enhancements in bioactivity, with Mn(II)-quercetin showing superior cytotoxicity (LC~50~ 177.9 µg/mL), while Co(II) and Ni(II) complexes exhibited notable antioxidant effects $(IC\sim50\sim < 0.5 \mu g/mL)$.

Keywords: Cytotoxicity, Quercetin, Bacterial, Antifungal, Microorganisms

INTRODUCTION

Plant natural products have been used by humans as herbal medicine since ancient times. Among numerous plant natural products, flavonoids are phytochemicals used in medicines. They play a significant role in minimizing the effects of various diseases and there been reported to be beneficial for human health (Karak, 2019).

Flavonoids are known to be potent metal chelators and because of their polyphenolic nature, flavonoids can behave as antioxidants. These properties depend on the number of hydroxyl groups present on the flavonoid structure. It is said that if the number of hydroxyl groups in flavonoids is higher, the antioxidant capacity is also better (Anuj *et al.*, 2016). This makes them beneficial chemical compounds that impart various health benefits.

To further improve flavonoids in terms of their bioactivities, metal complexes with flavonoids as ligands to chelate transition metal ions have gained significant scientific interest (Thangavel *et al.*, 2018; Gençkal *et al.*, 2020; Silva *et al.*, 2020; Wu *et al.*, 2020). Therefore, approaches to synthesizing flavonoid—metal complexes with novel structures are highly desirable

The general procedure for complex formation involves the deprotonation of the phenolic hydroxyl group(s) of the flavonoid into the corresponding phenolate, which then chelates the metal ion in an air-saturated alkaline solution (Thangavel *et al.*, 2018; Gençkal *et al.*, 2020; Silva *et al.*, 2020; Wu *et al.*, 2020).

Quercetin, a yellow, crystalline solid with a molecular weight of 302.236 g/mol and a density of 1.799 g/cm³ is the major representative of the flavonol subclass which as powerful antioxidant prevents from oxidation of low density lipoproteins in vitro. It is a water-soluble plant pigment commonly found in green tea, red wine, apples, onions, leafy vegetables. It protects cellular structures and blood vessels from the damaging effects of free radicals (antioxidant and anti-inflammatory activity). Quercetin improves blood vessel strength and stems the activity catechol-O-methyltransferase that suppresses neurotransmitter norepinephrine. This action may lead to elevated levels of norepinephrine, thermogenesis, and fat oxidation. Furthermore, quercetin acts as antihistamine agent preventing from allergies or asthma. Antioxidant properties of quercetin have evinced cholesterol reduction and heart disease protection. It can also block an enzyme resulting in sorbitol accumulation which has been associated with nerve, kidney or eye damage in diabetics. Quercetin may protect against cataract formation. It could be also examined as phytoestrogen (Erlund, 2004).

Metal complex formation with quercetin plays an important role in biological systems and also provides sensitive color stabilization in higher plants. The flavonol's interaction with metal ions could improve the antioxidant properties and the stability of flavonoids. The presence of metal ions in the quercetin molecule can influence its biological activities. Generally, quercetin chelates with metal ions through its structural hydroxy and oxo-groups. Due to its biotic and medical activities, it exists in several foods such as apple, tea, onion, nuts, berries, cauliflower, and cabbage (Siva *et al.*, 2021).

Structure of Qur-M(II) Complex Figure 1: Chemical structures of quercetin and qurcetin-M(II) Complexes

MATERIALS AND METHODS

The glasswares used was washed with detergent, rinsed with distilled water and dried in an ovum at 110°C before used. All the reagents used are analar grade and flavonoids used are obtained from Sigma-Aldrich. All the solvents were used without further purification. Electric metler balance model H30AR was used for weighing. Melting/decomposition temperature are determined using Gallenkamp melting point apparatus. Molar conductance measurements were carried out in DMSO using a Denver Instrument Model 20. IR spectra of the flavonoids and metal(II) complexes were recorded using a Scimadzu FT-IR Fourier transform spectrophotometer in the range 4000 - 400 cm⁻¹ while Elemental analysis was carried out using a Series II CHNS/O Analyzer 2400 Perkin Elmer and electronic adsorption spectroscopy were performed at Musa Yar'adua University Katsina, Nigeria. Antimicrobial studies conducted at the Department of Microbiology, Bayero University Kano, Nigeria. Antioxidant and Cytotoxicity Assay carried out at the Department of Chemistry, Bayero University Kano, Nigeria.

Synthesis of Quercetin Metal (Mn(II). Co(II), Ni(II) and Cu(II)) Complex.

The quercetin (0.02mol in 20cm³ methanol) was stirred with a magnetic stirrer until the quercetin completely dissolved in a 100 mL beaker. About 20 cm³ methanolic NaOH was added to the solution for the deprotonation of the ligand. The solution pH was adjusted to 6 by adding 1.0 M H₂SO₄. Then, metal(II) chloride salts (0.01 mol) dissolved in 20cm³ methanol were added to the flask until the color of the solution transformed to dark yellow, and further, the solution was stirred for 2 hrs more at room temperature and filtered. The filtrate was slowly evaporated to a dark yellow product at room temperature and finally washed with diethyl ether followed by vacuum drying, which yielded the product of different colours (Siva and Badal. 2021).

Molar Conductivity Measurement of the Complex

Solution of each metal (II) complex (0.001 mol/dm³) was prepared in DMSO and the molar conductance was measured.

Magnetic Susceptibility Measurement

The prepared metal complex was introduced into a capillary tube up to a given mark and the reading was recorded using the magnetic susceptibility balance.

Determination of Water of Hydration

Water of hydration of the prepared complex was determined as recorded in (Table 2) by weighing 0.2 g each of the compounds in a watch glass and putting it in an oven maintained at a temperature of 110^{0} C until constant weight was obtained. The average loss of weight was recorded after cooling as the weight of water of hydration (Vogel, 1978). The percentage composition of the water of hydration in each of the complexes was calculated as follows;

 $\frac{Loss\,in\,weight}{Weight\,of\,complex\,taken}~x~100$

Determination of Metalions in the Metal Complexes

0.01g of the powdered sample was added into $10cm^3$ digestion flask containing $1cm^3$ concentrated H_2SO_4 and about $0.7\ ml$ 60-62% of nitric acid. The flask was then heated in a fume cupboard until dense white fume appeared. The solution will then be allowed to cool and then filtered into $10\ cm^3$ volumetric flasks. Deionized water will then be added up to the mark.

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Antibacterial Activity

Antimicrobial activity of quercetin and its metal(II) complexes was determined using the well diffusion method. The suspension (4000, 2000, 1000 and 500 μL) of the test organism were spread on the agar (NA) and the well (6 mm diameter) impregnated with 10 μL of the target compounds and DMSO (negative control) was placed on the inoculated agar, which was incubated for 24 h and 48 h at 37°C respectively for bacterial and fungal isolate. Ciprofloxin and griseofulvin were used as the positive controls for the tested organism. Clear inhibition zones around the wells indicated the presence of antimicrobial activity and the activity was determined by measuring the zone of inhibition that appeared after the incubation period. DMSO was used as a negative control and all tests and analyses were carried out in triplicate (Pooja *et al.*, 2020).

Determination of Antioxidant Activity.

The assay was performed in 96-well plates. The reaction mixture, containing $200 \,\mu\text{L}$ of $100\mu\text{M}$ DPPH solution and $100 \,\mu\text{L}$ of the diluted test sample, was incubated in the dark at room temperature for 30min. The absorbance was measured at 517 nm. Ascorbic acid (AA) was used as a positive control (Nithianantham *et al.*, 2011).

Percent DPPH radical scavenging activity was calculated as follows:

Percent radical scavenging activity (AA%) $\{ [\frac{(Abssample-Absblank)}{Abscontrol}] x 100 \}$

The radical scavenger activity was expressed in terms of the amount of antioxidants necessary to decrease the initial DPPH absorbance by 50% (IC50). The IC50 value for each sample was determined graphically by plotting the percentage disappearance of DPPH as a function of the sample concentration as recorded in the Table 1.

Cytotoxicity Assay Hatching Shrimp

Brine shrimp eggs, *Artemia salina* were hatched in seawater. After 48 hrs incubation at room temperature, the larvae were attracted to one side of the vessel with a light source and collected by pipette. Larvae were separated from eggs by aliquoting them three times in small beakers containing seawater (Ibrahim and Abdullahi, 2015).

Brine Shrimp Lethality Assay

Toxicity of the compounds was monitored by the brine shrimp lethality test according to the method described by Lilybeth and his co-workers (2013), with slight modification. Each of the compounds/fractions (1 mg/mL) was dissolved in DMSO,

from which 5000, 500 and 50 μ L of each solution was transferred into vials corresponding to 1000, 100 and 10 μ g/mL respectively.

This was allowed to evaporate to dryness in about 24 hrs at room temperature. Each dosage was tested in triplicate (9 per test sample). Sea water (4 mL) and 10 larvae were introduced into each vial. The final volume of solution in each vial was adjusted to 5 mL with sea water immediately after adding the shrimps, and DMSO as a negative control was prepared as a drug-free.

Survivors were counted after 24 h, and LC_{50} was determined by probit analysis using SPSS version 20 to establish the therapeutic index. Brine shrimp assays followed institutional ethical guidelines for invertebrate studies.

Statistical Analysis Computer software

All the tested parameters were subjected to statistical analysis using SPSS version 20. Means were compared by analysis of variance (ANOVA) followed by Tukey's multiple range test procedures of SAS software version 9.1. All the results obtained were expressed as \pm Standard error of the Mean (SEM) of triplicates of each sample and the differences between means were regarded as significant at P < 0.05.

RESULTS AND DISCUSSION

Table 1: Physical Properties of Ligand Quercetin and its Metal(II) Complexes

Compound	Colour	Melting Point	Decomposition Temperature	Percentage Yield
La	Yellow	316	-	-
$[Mn(L^a)_2].5H_2O$	Brown	-	338	86.67
$[Co(L^{a})_{2}].5H_{2}O$	Brown	-	356	78.48
$[Ni(L^{a})_{2}].9H_{2}O$	Green	-	355	77.38
$[Cu(L^a)_2].4H_2O$	Black	-	342	84.00

 $L^a = Quercetin \; (C_{15}H_{10}O_7)$

The quercetin-metal(II) complexes were synthesized by the reaction of quercetin with the corresponding manganese(II), cobalt(II), nickel(II) and copper(II) salts. The complexes were produced in high yield (77.38 – 86.67%) with different colours as shown in Table 1. The colour change can be attributed to the d-d electronic transition of the metal ions, charge transfer from metal to ligand and vice versa or the nature of the ligand. The melting point of quercetin

determined was 316°C. The complexes were found to be amorphous and were stable in air and moisture at room temperature; they decomposed without melting at higher temperatures (338-356°C). Table 1 indicating good stability of the complexes due to chelation between the ligand and the respective metal ions. The values were in close agreement with work reported by Stepan *et al.*, (2023).

Table 2: Solubility of the Quercetin and its Metal Complexes

Solvent	$\mathbf{L}^{\mathbf{a}}$	$[Mn(Qur)_2].5H_2O$	$[Co(Qur))_2].5H_2O$	$[Ni(Qur))_2].9H_2O$	$[Cu(Qur))_2].4H_2O$
Water	SS	SS	IS	SS	IS
Methanol	S	SS	SS	SS	SS
Ethanol	S	SS	SS	IS	SS
Acetone	S	IS	SS	SS	SS
Chloroform	IS	IS	IS	IS	IS
DMSO	S	S	S	S	S
DMF	S	S	SS	S	SS
Diethylether	S	IS	IS	IS	IS
N-Hexane	IS	IS	IS	SS	IS

Key: S=soluble SS= slightly soluble IS= Insoluble

Qur = Quercetin

The results of the solubility test revealed that the ligand was soluble in most common solvents, such as DMSO, DMF methanol, ethanol and diethylether while completely insoluble in n-hexane. However, the solubility result of the

complexes indicated that they were insoluble in common organic solvents such as chloroform, acetone n-hexane, but slightly soluble in diethyl-ether, methanol, ethanol and water, but completely soluble in DMSO and DMF (Table 2).

Table 3: FTIR Spectral Data Quercetin and its Metal Complexes

Compound	υ(OH) cm ⁻¹	υ(C=O) cm ⁻¹	υ(M-O) cm ⁻¹	
La	3248	1666	-	
$[Mn(L^{a})_{2}].5H_{2}O$	3217	1618	589	
$[Co(L^{a})_{2}].5H_{2}O$	3212	1596	616	
$[Ni(L^{a})_{2}].9H_{2}O$	3167	1618	613	
$[Cu(L^a)_2].4H_2O$	3085	1633	530	

 $L^a = Quercetin (C_{15}H_{10}O_7)$

The FT-IR spectral studies of the ligand (quercetin) and its complexes were determined. The FT-IR spectral data of the ligand (quercetin) showed a band in the region 3249 cm⁻¹, characteristic of $\upsilon(OH)$ stretching frequency. The complexes of quercetin also showed similar bands in the range 3085-3217 cm⁻¹, which were attributed to $\upsilon(OH)$ stretching vibration due to both water of crystallization and the phenolic group which were in agreement with the report by (Monika *et al.*, 2016). Other bands in the range 525–615cm⁻¹ were assigned to $\upsilon(M-O)$ stretching vibrations, confirming the ligand coordination to the respective metal ions after

deprotonation of the phenolic OH. The band due to $\upsilon(C=O)$ of the quercetin at 1663 cm⁻¹ was also shifted to lower frequencies in the range 1596-1618 cm⁻¹ for the complexes, suggesting the coordination of the oxygen of the C=O to the respective metal ions. The values were consistent with the result obtained by (Siva and Badal, 2021), at 1662cm^{-1} for the ligand and $1627\text{-}1653\text{cm}^{-1}$ for the respective complexes. Thus, the FT-IR results revealed that the ligand acted as bidentate ligand coordinating via the two O of phenolic and carbonyl as shown in Figure 1.

Table 4 Elemental and Metal Analysis of Quercetin Metal Complexes

		% Calculated(For	und)	
Compound	C	H	M	
[Mn(L ^a) ₂].5H ₂ O	48.21(48.58)	2.41(2.31)	7.34(7.36)	
$[Co(L^{a})_{2}].5H_{2}O$	47.95(47.94)	2.41(2.41)	7.84(7.86)	
[Ni(L ^a) ₂].9H ₂ O	43.77(43.38)	2.20(2.09)	7.13(7.64)	
$[Cu(L^{a})_{2}].4H_{2}O$	48.82(48.23)	2.46(2.26)	8.61(8.62)	

L^a=Quercetin

To establish the formation of the metal(II) complexes with the ligand, the elemental analysis of the metal(II) quercetin complexes was determined. The found and theoretical percentages for C and H in the complexes were in good agreement, supporting 1:2 metal to quercetin ratio (Table 4).

This was tallied with similar work reported by (Emad *et al.*, 2019 and Hassan *et al.*, 2013). As for the metals ions (Mn, Co, Ni and Cu) they were found by AAS but not elemental analysis.

Table 5 Molar Conductance of the Quercetin-Metal(II) Complexes in 10⁻³M DMSO

Complex	Specific Conductance (Ohm ⁻¹ cm ²)	Molar Conductance(Ohm ⁻¹ cm ² mol ⁻¹)
[Mn(L ^a) ₂].5H ₂ O	6.39x10 ⁻⁶	6.39
$[Co(L^{a})_{2}].5H_{2}O$	11.0×10^{-6}	11.0
$[Ni(L^{a})_{2}].9H_{2}O$	5.21×10^{-6}	5.21
$[Cu(L^{a})_{2}].4H_{2}O$	5.18x10 ⁻⁶	5.18

Qur = Quercetin

The molar conductance values of the synthesized metal(II) complexes were obtained in Ω^1 cm²mol⁻¹ at room temperature using DMSO as a solvent and their results are given in Table 5. Molar conductance values of the complexes in DMSO

indicated that all the complexes of quercetin were non-electrolyte in nature, having lower values of $5.18-11.00~\Omega^1$ cm²mol⁻¹. These readings were found to agree with work reported by Ezalden and Omar, 2023.

Table 6 Magnetic Moment Values of Quercetin Metal Complexes

Complex	Xg(erg.G ⁻² g ⁻¹)	Xm(erg.G ⁻ ² mol ⁻¹)	$\mu_{\text{eff}}(BM)$	No. of unpaired electrons	Property	Geometry
$[Mn(L^{a})_{2}],5H_{2}O$	19.68x10 ⁻⁶	14.70x10 ⁻³	5.92	5	Paramagnetic	Tetrahedral
$[Co(L^{a})_{2}].5H_{2}O$	0.90×10^{-6}	6.80×10^{-3}	4.01	3	Paramagnetic	Tetrahedral
$[Ni(L^a)_2].9H_2O$	0.45×10^{-6}	3.70x10 ⁻³	2.97	2	Paramagnetic	Tetrahedral
$[Cu(L^{a})_{2}].4H_{2}O$	0.16×10^{-6}	$0.71x10^{-3}$	1.68	1	Paramagnetic	Tetrahedral

Qur = Quercetin

The magnetic moment (B.M) values represented in Table 6 of the quercetin complexes were determined at room temperature. The magnetic moment values of the Mn(II), Co(II), Ni(II) and Cu(II) complexes were found to be 5.92,

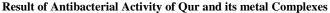
4.01, 2.97 and 1.68 B.M respectively, which were consistent with high spin tetrahedral geometry showing five, three, two and one unpaired electrons respectively. These values were in agreement with report by Safa *et al.*, (2014).

Table 7 Water of Hydration of Quercetin-Metal(II) Complexes

Compound	Weight Loss (g)	Percentage (%)	
[Mn (L ^a) ₂].5H ₂ O	0.024	12.05	
$[Co(L^a)_2].5H_2O$	0.034	11.97	
$[Ni(L^a)_2].9H_2O$	0.039	19.69	
$[Cu(L^a)_2].4H_2O$	0.019	12.09	

 $L^a = Quercetin (C_{15}H_{10}O_7)$

The water of hydration of the quercetin-complexes were determined. The metal complexes were found to contain water of crystallization, in the range 4-9 (Table 7)



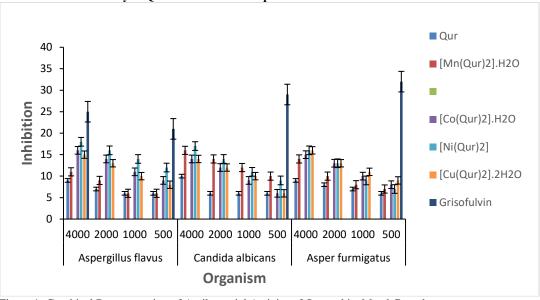


Figure 1: Graphical Representation of Antibacterial Activity of Qur and its Metal Complexes

The in-vitro antibacterial activity of Qur and its metal(II) complexes was tested against three bacterial isolates; *Escherichia coli, Staphylococcus aureus* and *Streptococcus pneumonia* using ciprofloxacin 500µg/ml as control during the experiment, agar well diffusion method was employed to evaluate the antibacterial activity of the synthesized compounds. The antibacterial activity results of Qur showed moderate activity against the tested organisms at higher

concentration, but no activity was observed at the lower concentration (Scheme 1). The results obtained indicated that the complexes were more active against the bacterial isolate used than the free ligand (Scheme 1). It is worth noting that, as expected the activity of the complexes also increases with an increase in concentration. Even though the activity was moderately high, it falls short of the activity of the standard drug ciprofloxacin.



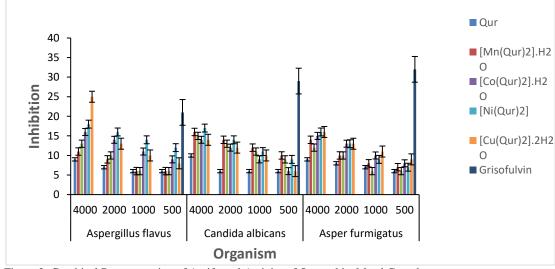
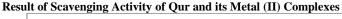


Figure 2: Graphical Representation of Antifungal Activity of Qur and its Metal Complexes

The in-vitro antifungal activity of ligand L^a and its metal(II) complexes was tested against three fungal isolates: *Aspergillus flavus, Candida albicans* and *Aspergillus furmigatus* and results were compared with those of Grisofulvin as a positive control. From the antifungal activity results (figure 2), Qur showed moderate activity against the tested organisms at 4000 µg/ml concentrations but no activity

at lower concentrations which is an indication that activity increases with an increase in concentration. However, all the complexes showed good to moderate activity against all the tested organisms and the complexes were more active against the fungal isolates used than the free ligand (Figure 2) at all concentrations, with the activity increasing with an increase in concentration.



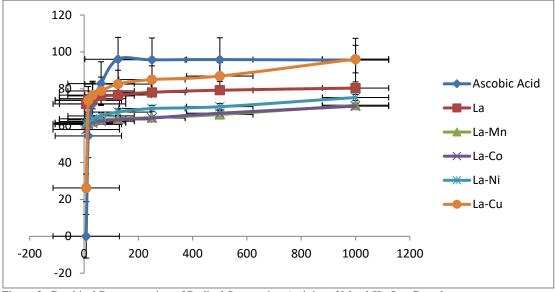
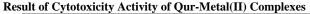


Figure 3: Graphical Representation of Radical Scavenging Activity of Metal(II)-Qur Complexes

The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen-donating ability. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Brighente *et al.*, 2007). Both the ligand (L^a) and its complexes displayed effective radical scavenging properties against DPPH. The assay was based on the measurement of the scavenging capacity of antioxidants towards it. The odd electron of the nitrogen atom in DPPH was usually reduced by receiving a hydrogen atom from antioxidants to the corresponding compounds. DPPH showed a strong absorption band at 517 nm due to its odd electron and the solution appeared deep

violet in colour, the absorption vanished as the electrons paired up. The resulting decolorization is dependent on the number of electrons donated by the active constituent. The result of the spectrophotometric absorption and concentration of the samples/standard obeys the Beer-Lambert law over the useful range.

The complexes with higher activities above the standard ascorbic acid were Mn(II), Co(II) and Ni(II) with IC50 of 0.037 $\mu g/mL$ 0.251 and 0.318, respectively. However, Cu(II) complex recorded a good but lower antioxidant activity with IC50 9.157 $\mu g/mL$ in comparison with standard ascorbic acid with IC50 9.073 $\mu g/mL$ (Table 4.1.1).



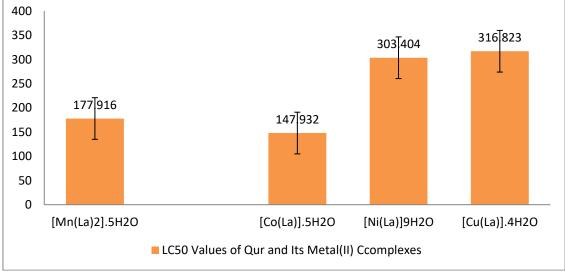


Figure 4: Graphical Representation of Cytotoxicity Study of Metal (II)-Qur Complexes

The degree of lethality was observed to be proportional to the concentration of the extracts/fractions, with the highest observed mortality at the highest concentrations (1000 $\mu g/mL$) in all the samples. In general, analysis of the result showed that Co(II) complex displayed moderate activity with LC50 147.9342 $\mu g/mL$ followed by Mn(II) complex with LC50 values 177.916 $\mu g/mL$. No mortality was observed in the negative control (Sea water), Scheme 4. Additionally, result from this assay can serve as a lead to the isolation of novel chemical compounds with medicinal properties Siva and Badal (2021).

CONCLUSION

The condensation reactions of some flavonoids (quercetin) with metal (II) ions produced the metal (II) complexes of manganese, cobalt, nickel and copper. In each case, the pure compounds obtained were characterized using different physical, spectroscopic and analytical techniques such as Infra-red spectroscopy (FTIR), CHN microanalysis, molar conductance measurement, magnetic susceptibility, Solubility test and melting point/decomposition temperature. The Mn(II) and Co(II) complexes show promise for further development as antimicrobial/anticancer agents, pending in vivo studies. Also, while magnetic data suggest tetrahedral geometry, XRD studies are needed to confirm structural details. Cu(II)'s lower antioxidant activity may arise from redox inactivity in DPPH assays (Wu et al., 2020)

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