JSR Publications

Available Online

J. Sci. Res. **6** (3), 509-519 (2014)

JOURNAL OF SCIENTIFIC RESEARCH

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Synthesis and Characterization of some Ternary Metal Complexes of Curcumin with 1,10-phenanthroline and their Anticancer Applications

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Received 30 April 2014, accepted in final revised form 2 August 2014

Abstract

Some ternary complexes of curcumin have been synthesized by the reaction of M(II) nitrate with curcumin as primary ligand and 1,10-phenanthroline (phen) as supporting ligand in water/ethanol solution under a nitrogen atmosphere. The composition of the complexes has been characterized by elemental analysis, molar conductivity, thermogravimetric analysis, IR, UV–vis spectroscopy. The results reveal that curcumin ligand coordinates with M(II) in bidentate mode after deprotonation. The supporting ligand (phen) uses its two N atoms in coordination with metal ions in bidentate mode. The general formula of the complexes is $[M(Cur)(phen)]NO_3$ (M = Ni(II), Co(II), Cu(II) and Z(II)). The results of antibacterial activity indicated that the complexes have good antibacterial ability for the testing bacterium than that of curcumin. Furthermore, the $[M(Cur)(phen)]NO_3$ complexes were evaluated for its *in vitro* anticancer activity against hepatocellular carcinoma.

Keywords: Curcumin; Ternary complexes; Biological activity**;** Hepatocellular carcinom.

doi: http://dx.doi.org/10.3329/jsr.v6i3.18750 J. Sci. Res. 6 (3), 509-519 (2014)

1. Introduction

Curcumin, (bis[4-hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione) a yellow spice and pigment from *Curcumalonga* L. (Zingiberaceae), is by far known for its antioxidant [1-3], anti-inflammatory [4, 5] and anticancer activities [5, 6]. Some studies on curcumin are based on the ionic structure where the keto-enol equilibrium (Fig. 1) is present or when it is fully in keto form [7-9] with the resulting properties depending on the latter. The strong chelating ability of diketones has been widely investigated towards a great

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number of metal ions; therefore, curcumin could be of great importance in the chelating treatment of metal intoxication and overload [10].

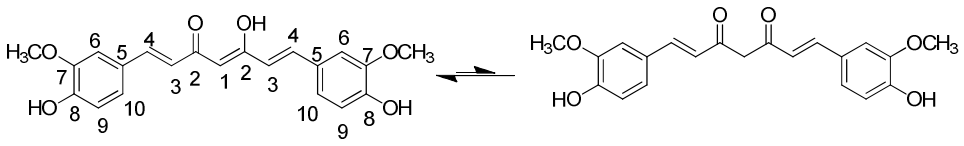


Fig 1. Keto-enol form of curcumin.

The formation of various metal complexes with curcumin has been investigated [10-12]. The stoichiometries of curcumin with some metal ions were also reported [10, 12]. Several metal complexes of curcumin have been synthesized, characterized and evaluated for various biological activities. The [Au(curcumin)$_2$Cl] was a five coordinate gold complex [13], which had anti-arthritic properties and assessed in an adjuvant-induced rat poly arthritis model. The [Cu(curcumin)$_2$] complexes [14] were most cytotoxic in cultured L929 cells and showed significant reduction in solid tumor volume in as cites tumor bearing mice. In addition, vanadyl, gallium and indium complexes of curcumin [15] have reported for medicinal applications. Curcumin metal complexes (M = Eu, Ce, La, Y, Cr, Pd) showed that curcumin coordinates with metal ions in bidentate mode in deprotonated form. Anti-bacterial study of the synthesized complexes indicated that these complexes had anti-bacterial activity against Klebsiella pneumonia and Escherichia coli. [16, 17]. Also, curcumin with other transition metals ions Ni(II), Zn(II), Pd(II), Fe(III), Cr(III), Mn(II), can form strong chelates [10, 18-20]. Curcumin complexes with Zn(II), Cu(II), Mg(II) and Se(II) are synthesized by mechanical mixture, without any conventional organic solvents and found to form complexes with 1:1 for Zn(II), Cu(II) and Mg(II) complexes and 1:2 for Se(II) complex [21].

The lanthanides complexes of Sm(III), Eu(III) and Dy(III) with curcumin and 1,10-phenanthroline-5,6-dione as mixed ligand complexes exhibited effective biological activities [22]. One of these activities were given as the results of agarose gel electrophoresis which suggested that the complex of SmL_3L' can cleave the plasmid DNA at physiological pH. The antibacterial activities tests showed that all these complexes had excellent antibacterial ability against *E. coli* and *H. bacillus*. Copper(II) mixed ligand complex of curcumin with diphenylphenanthroline, $Cu(Cur)(DIP)]^{2+}$ was synthesized and the binding mode of the complex with CT-DNA was investigated [23]. The aim of this work is synthesis, characterization and biological activities of the new Ni(II), Co(II), Cu(II) and Zn(II) ternary complexes with curcumin as primary ligand and 1,10-phenanthroline (phen) as supporting ligand. The supporting ligand, 1,10-phenanthroline (phen) is strong field bidentate ligand that form very stable chelates with many first row transition metals.

2. Experimental

2.1. *Chemicals and instruments*

Curcumin, nickel nitrate hexahydrate, cobalt nitrate hexahydrate, copper nitrate trihydrate, zinc nitrate hexahydarate and phen were purchased from Sigma-Aldrich and used as received. All solvents were of reagent grade and used without further purification.

IR spectra were recorded as KBr pellets on a Unicam-Mattson 1000 FTIR spectrometer. Electronic absorption spectra were measured on a Unicam UV2-300 UV-Vis spectrophotometer. NMR measurements were performed on a Spectrospin-Bruker AC 200 MHz spectrometer. Samples were dissolved in d_6-DMSO with TMS as internal reference. Magnetic susceptibility (Gouy method) measurements of the paramagnetic complexes were carried out on a Sherwood Scientific magnetic susceptibility balance. The complexes were also characterized by elemental analysis (Perkin-Elmer 2400 CHN elemental analyzer). The mass spectra were recorded by the EI technique at 70 eV using Finnigan MAT SSQ 7000 instrument. Thermogravimetric analysis was carried out with a heating rate of 10°C using a Perkin-Elmer 7 series thermal analyzer thermal analyzer. ESR spectrum of the Cu complex was recorded using a Bruker ESR-spectrometer model EMX.

2.2. *Synthesis of the ternary complexes of curcumin with phen*

The Ni(II)–curcumin complex was synthesized by mixing equi-molar amounts of Ni(II) nitrate (0.29 g, 1.0 mmol) and curcumin (0.37 g, 1.0 mmol) in ethanol and heated the mixture at 60°C for 1 h under a nitrogen atmosphere. After 1 h, 0.18 g (1.0 mmole) 1,10-phenanthroline was added and the reaction continued for 2h under reflux. Then the complex solution was concentrated and the solid residue was separated by filtration and washed several times by water/ethanol to remove unreacted curcumin, phen and metal salt. Similar methods were used for the synthesis of Co (II), Cu(II) and Zn (II) ternary complexes of curcumin with phen.

2.3. *Antimicrobial studies*

The curcumin, phen ligands and their ternary complexes were screened for their antibacterial activity using the agar diffusion technique [24**]**. A 5 mg/mL solution in DMF was used and 100 μL was tested. The tested organisms were two gram positive bacteria *viz., Bacillus subtilis, Staphylococcus aureus, E-Coli* and two gram negative bacteria *viz., Escherichia coli, Pseudomonas aerogenasa* and three fungi as *Aspergillus fumigates, Saccharomyces cerevisiae and Candida albicans.* The bacteria and fungi were maintained on nutrient agar medium and Czapeks Dox agar medium, respectively. The agar media were inoculated with different test microorganisms. After 24h of incubation at 30°C for bacteria and 48 h of incubation at 28°C for fungi, the diameter of inhibition zone (mm)

was measured. A reference standard for both gram positive and gram negative bacteria was made by dissolving accurately weighed quantity of streptomycin in sterile distilled water, separately. Clotremazole was used as reference standard for fungi. The assay was repeated thrice and results recorded as mean ± standard deviation of triplicated experiments. Compounds were considered as active when the inhibition zone was greater than 6 mm.

2.4. *In vitro anticancer screening*

The synthesized compounds were supplied to the Regional Center of Microbiology and Biotechnology Cell Al Azhar University, Cairo, Egypt, for *in vitro* antitumor screening on hepatocellular carcinoma (HePG2). Cell viability was assessed by the mitochondrial-dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan.

3. Results and Discussions

Characterization of the metal-curcumin-phen mixed ligand complexes

Reaction of curcumin with $M(NO_3)_2$ where M= Ni(II), Co(II), Cu(II) or Zn(II) in the molar ratio of 1:1, under nitrogen atmosphere gave complexes with 1:1 ratio of M:curcumin as found by many authors before. With the addition of 1 mole equivalent of phen, complexes with the molecular formula $[M(Cur)(phen)]NO_3$ were obtained. All complexes were stable at room temperature and were soluble in DMF and DMSO and partly soluble in methanol and ethanol. The formation of the ternary complexes of curcumin with phen was confirmed on the bases of elemental analysis, FTIR, and thermogravimetric analysis. Elemental analyses of the mixed-ligand complexes indicate that one mole of metal nitrate reacted with one mole of phen and one mole curcumin giving the complexes $[M(cur)(phen)]NO_3$; M= Ni(II), Co(II), Cu(II) and Zn(II). The elemental analysis of the complexes is depicted in Table 1. The conductivity, Λ_M value of the complex in water is 53-85 Ohm-1 cm^2 mol^{-1}, which indicated that the complex is a 1:1 electrolyte [25].

The IR spectra of curcumin, Fig. 2, shows stretching vibrations at 1628 cm^{-1} attributed predominantly to the overlapping stretching vibrations of alkenes (C=C) and carbonyl (C=O) character. Infrared of curcumin ligand show stretching vibration at 3200–3500 cm^{-1} due to O-H groups, C=C aromatic stretching vibration at 1427 cm^{-1} and high intensity band at 1512 cm^{-1} attributed to the mixed vibrations including stretching carbonyl bond vibrations ν(C=O), in plane bending vibrations around aliphatic δ CC-C, δ CC=O and in plane bending vibrations around aromatic δ CC-H of keto and enol configurations and stretching vibrations around aromatic νCC bonds of keto and enolic form of curcumin [26]. Furthermore, significant intense band at 1277 cm^{-1} attributed to the bending vibration of the ν(C-O) phenolic band.

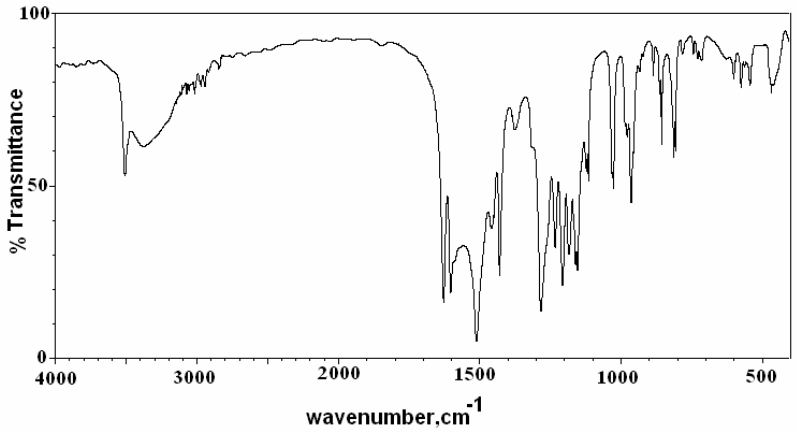


Fig. 2. FTIR spectrum of curcumin.

Table 1. Elemental analysis and mass spectrometry of the curcumin complexes.

Complexes	Mol. Wt.	C %		H%		N%		% metal		Mass spectrometry m/z
		Cal.	Found	Cal.	Found	Cal.	Found	Cal.	Found	
[Ni(cur)(phen)]NO_3	668.28	59.29	58.63	4.07	4.12	6.29	6.80	8.77	8.63	603 (P-NO3)+
[Co(cur)(phen)]NO_3	668.52	59.31	57.87	4.07	4.08	6.29	6.43	8.78	8.63	667 (P-1)+
[Cu(cur)(phen)]NO_3	673.13	58.88	57.41	4.04	4.21	6.24	6.66	9.44	9.70	673 (P)+
[Zn(cur)(phen)]NO_3	674.69	58.72	57.41	4.03	4.21	6.23	6.36	9.69	10.33	672 (P-2)+

In the IR spectra of the [M(Cur)(phen)]NO_3 complexes as shown in Fig. 3, the νC=O band of the free curcumin is shifted from 1628 cm^{-1} to 1625-1598 cm^{-1} depending on the metal used [20, 22]. The presence of an intense band at 1277 cm^{-1} region which attributed to υ(CO) of phenolic group, confirmed the absence of -OH phenolic in the chelation process. The υ (OH) of the two phenolic groups in curcumin showed broad band in the 3200–3500 cm^{-1} region. The IR spectra of the complexes exhibited new bands at 460–479 and 551-555cm^{-1} are assigned to υ (M-O) and υ (M-N) stretching frequency, respectively [27]. The band due to ring vibrations of the uncoordinated phen observed at 1618 cm^{-1} was shifted to 1583-1599 cm^{-1} in the complexes indicating the participation of phen in the coordination. From the elemental analysis and IR spectra the proposed structure of the prepared complexes is as shown in Scheme 1.

The proton magnetic resonance spectrum of the Zn(II)-curcumin-phen complex was performed in d_6-DMSO. The previous results of the NMR studies showed that curcumin exists in solution as keto-enol tautomers [28-30]. According to these authors the chemical shifts data δ (ppm) of curcumin free ligand: 2.517(DMSO), 3.365 (H_2O; moisture), 3.855

(6H; -OCH_3), 6.073 (1H; C1), 6.737 (1H; C3), 6.825 (1H; C9), 7.173 (1H; C10), 7.338 (1H; C6), 7.594 (1H; C4), 9.82 (1H; -OH phenol) and 16.45 (1H; -OH enol). However, the signal of the enol -OH could not be detected [20] for curcumin in DMSO. The discrepancy between these measurements could be due to the sensitivity and resolution of the ^{1}H NMR instruments used for measurements. The ^{1}H NMR spectrum of [Zn(Cur)(phen)]NO_3 complex in Fig. 4 showed signals due to curcumin with respective shift and signals due to phen, δ (ppm): 2.506 (DMSO), 3.344 (H_2O; moisture), 3.840 (6H; -OCH3), 5.714 (1H; C1), 6.667 (1H; C3), 6.768 (1H; C9), 7.079 (1H; C10), 7.271 (1H; C6), 7.489 (1H; C4), 9.86 (1H; AOH phenol) besides multiplets at 8.89 and 8.19 due to protons of phen.

Table 2. Assignment of important infrared spectral bands (cm^{-1}) for curcumin and their metal complexes.

Complexes	νOH	ν(C=O) ketonic	ν(C=C) aliphatic	δCO enol	νC-O phenol	νC=N phen	νM-N	νM-O
Curcumin	3508m	1628m 1603s	1510s	1427s	1278m	------	-----	-----
[Ni(Cur)(phen)]NO3	3327m	1628m 1603s	1515s	1427s	1276m	1582s	551m	470w
[Co(Cur)(phen)]NO3	3348m	1627m	1513s	1427s	1277m	1599m	554m	468w
[Cu(Cur)(phen)]NO3	3422w,br	1617m 1599m	1510vs	1430m	1274s	1587m	555w	476w
[Zn(Cur)(phen)]NO3	3252m	1627m 1599m	1514s	1429s	1272m	1583m	551w	470w

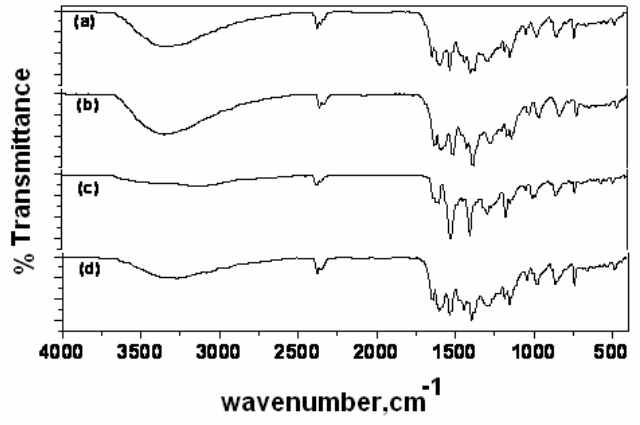
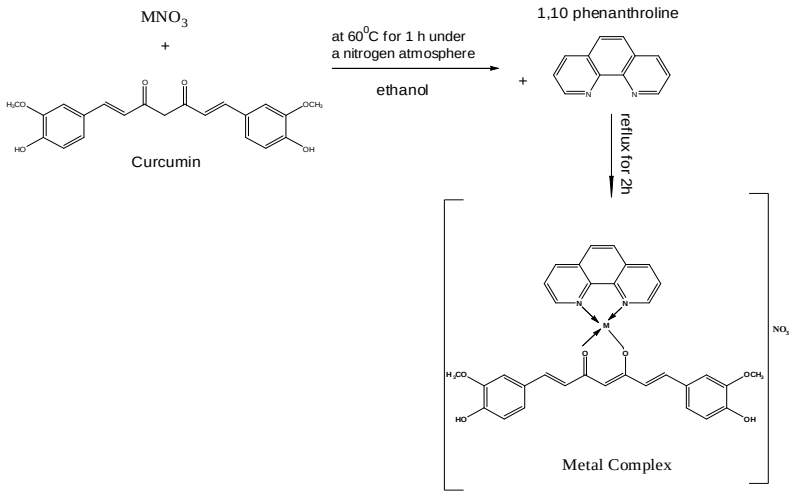


Fig. 3. FTIR spectra of a) [Ni(Cur)$(phen)_2$]NO_3; b) [Co(Cur)$(phen)_2$]NO_3; c) [Cu(Cur)$(phen)_2$]NO_3 and d) [Zn(Cur)$(phen)_2$]NO_3.



Scheme 1. Proposed structure of the prepared metal complexes.

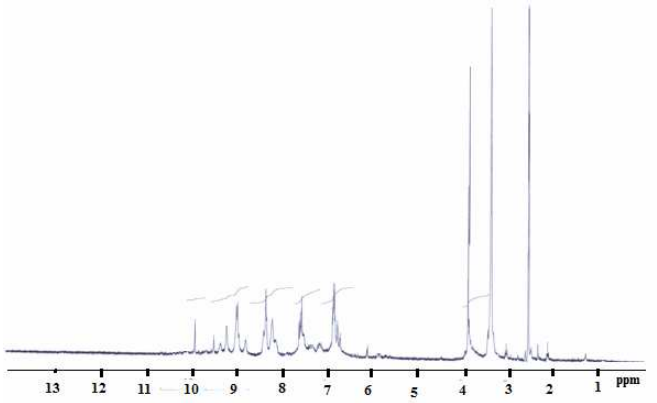


Fig. 4. ^{1}H NMR spectrum of [Zn(Cur)(phen)]NO_3 complex.

The magnetic moments are 1.78 and 2.83 BM for [Cu(Cur)(phen)]NO_3 and [Co(Cur)(phen)]NO_3, respectively, while [Ni(Cur)(phen)]NO_3 complex was diamagnetic indicating square planar geometry. Curcumin is soluble in most of the organic solvents, lipids, and micellar solutions, and is insoluble in neutral solutions. The curcumin complexes were insoluble in organic solvents like methanol and acetonitrile, and were soluble only in DMSO and DMF. The UV-vis spectrum of curcumin ligand showed main absorption bands in the UV–vis region at 265, 374 (shoulder) and 427 nm. The band at 265 and 374 nm corresponds to a $\pi \rightarrow \pi^*$ transition, whereas the band at 427 nm can be due either to an $n \rightarrow \pi^*$ transition or to a combination of $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions [31, 32]. The shifts of these bands in the complexes can be detected on the complexes, indicative of involvement of the carbonyl group of curcumin in metal complexation [22] as illustrated in Fig. 5. Bands at 291, 330 nm are due to $\pi \rightarrow \pi^*$ transitions of phen. The

variation of the absorption peak of curcumin in the different complexes depends on the nature of metal (M^{2+}) ion implication.

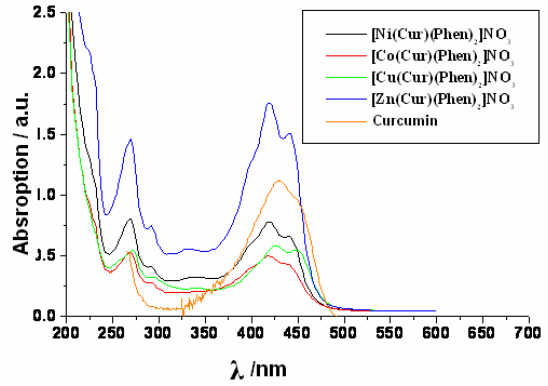


Fig. 5. Absorption spectra of curcumin and its complexes.

To obtain further information about the stereochemistry and the site of the metal ligand bonding and to determine the magnetic interaction in the metal complexes, ESR spectra of the Cu(II) complex was recorded in the solid state. Fig 6 shows EPR spectra of the copper(II) complex which exhibits well-defined single isotropic feature at $g_{//}$ (parallel) value of 2.11 and $g\perp$ (perpendicular) value of 2.06 which is associated with square planar coordination. In square planar complexes, the unpaired electron lies in the d_{x2-y2} orbital giving $g// > g\perp > 2$ while the unpaired electron lies in the d_{z2} orbital giving $g\perp > g// > 2$. This provides evidence that the unpaired electron is localized in the d_{x2-y2} orbital and the ground state is $^2B_{1g}$ [33]. The broadening of the lines is usually a result of intermolecular spin exchange. This intermolecular type of spin exchange is caused due to the spin coupling which occurred during a coupling of two paramagnetic species.

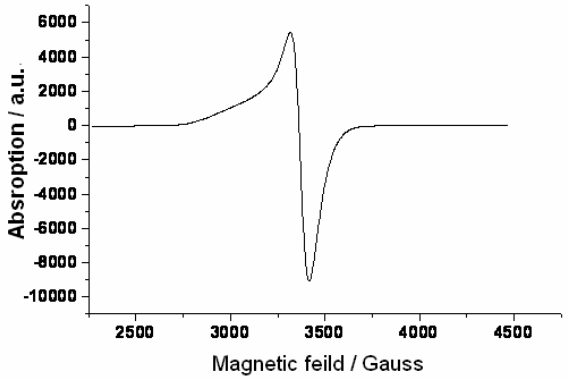


Fig. 6. ESR spectrum of $[Cu(Cur)(phen)_2]NO_3$.

3.2. *Biological activity*

From the data presented in Table 3 it may be seen that the curcumin was active against the gram-positive and gram-negative bacteria as well as the three fungi used. Upon complexation the activity against the tested micro-organisms generally decreased but the complexes showed higher activity against some gram-negative bacteria, for example, $[Cu(Cur)(phen)]NO_3$ against *E. coli*, $[Ni(Cur)(phen)]NO_3$ and $[Zn(Cur)(phen)]NO_3$ for P. aeruginosa. Also, the complexes $[Ni(Cur)(phen)]NO_3$, $[Cu(Cur)(phen)]NO_3$ and $[Zn(Cur)(phen)]NO_3$ showed higher activity against *Candida Albicans*. It should be mentioned that binary complexes of Ni, Cu with curcumin showed no activity against *E. Coli*, *P. aeruginosa*, *Bacillus subtili*s and *Staphylococcus aureus* while only Co-curcumin complex exhibit activity against these strains [20]. This result suggests the importance of the ternary complexes of curcumin with phen [34]. The phen ligand measured under the same conditions showed the greatest antimicrobial activity with very high zones of inhibition against the various bacterial species and *Candida Albicans* compared to the complexes.

Table 3. Biological activity of curcumin ternary complexes.

Complexes	Inhibition zone diameter (mm) Mean ± RSD*						
	S.Aureus	*B.Subtilis*	*E-Coli*	*Pp. aeruginosa*	*A.fumigate*	*S.cerevisiae*	*Candida Albicans*
[Ni(Cur)(phen)]NO3	10.4± 0.5	10.9± 0.3	9.8± 0.4	10.4± 0.5	10.3± 0.4	9.1± 0.4	8.7± 0.3
[Co(Cur)(phen)]NO3	8.3± 0.06	9.6± 0.03	9.8± 0.3	9.1± 0.3	11.4± 0.4	9.7± 0.4	8.1± 0.3
[Cu(Cur)(phen)]NO3	9.3± 0.06	8.8± 0.03	11.3± 0.4	9.6± 0.4	10.9± 0.3	10.2± 0.4	9.7± 0.3
[Zn(Cur)(phen)]NO3	10.7± 0.06	10.2± 0.3	10.1± 0.4	9.9± 0.4	8.8± 0.4	9.3± 0.3	9.7± 0.4
Curcumin	15.3± 0.9	16.1± 0.5	10.3± 0.7	9.7± 0. 4	11.4± 0.5	12.4± 0.5	8.2± 0.1
Phen	18.5+0.7	14.0+0.05	16.0+0.6	13.8+0.5			12.5+0.5
Clotrimazole	-	-	-	-	26.1± 0.5	23.1± 0.4	18.3± 0.4
Streptomycin	25.1± 0.5	30.1± 0.5	25.6±0.4	23.3± 0.5	-	-	-

Cytotoxicity evaluation of the curcumin mixed complexes

In vitro anticancer activity evaluation of the newly synthesized compounds was carried out against human cancer cell lines hepatocellular carcinoma (HePG2) using MTT method [35]. Doxorubicin hydrochloride is one of the most effective anticancer agents was used as a reference drug in this study. The relationship between drug concentrations and cell viability was plotted to calculate IC_{50} (μg) the value which corresponds to the concentration required for 50% inhibition of cell viability and the data are shown in Table

4. It can be seen that curcumin exhibited inhibitory activity against HePG2 with IC_{50} = 19.2 µg. All the complexes under investigation showed better inhibitory activity than curcumin. While the Ni and Co complexes showed inhibitory activity with IC_{50} of 16.1 and 12.6 µg, respectively, the inhibitory activity IC_{50} for Cu and Zn complexes were 5.6 and 7.5 µg, respectively.

Table 4. Cytotoxicity evaluation of the curcumin mixed complexes.

Complexes	IC50 / µg
Curcumin	19.2
[Ni(Cur)(phen)]NO_3	16.1
[Co(Cur)(phen)]NO_3	12.6
[Cu(Cur)(phen)]NO_3	5.6
[Zn(Cur)(phen)]NO_3	7.5
Doxorubicin HCl	1.2

4. Conclusion

New ternary complexes using curcumin as a primary ligand and 1,10-phenanthroline as supporting ligand have been synthesized and characterized. The complexes exhibited antibacterial activities even lower than the curcumin and/or 1,10-phenanthroline alone. The antitumor activity of complexes displayed good cytotoxic activities against the HePG2 cancer cell lines. The highest antitumor activity was found for the copper and zinc complexes.

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