Review Article

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Synthesis, characterization, and anti-tumor application of a novel Zinc(II)-L-ascorbic acid derivative

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ABSTRACT

In this research, a derivative of L-ascorbic acid (L) as a ligand was synthesized from the reaction of this acid with acetone compound in an acidic medium, and then, a complex reaction was carried out between the synthesized derivatives with zinc chloride salt using ethanol as a solvent. This is a novel attempt to use 5,6-O-isopropylidene-L-ascorbic acid as a derivative of L-ascorbic acid in the synthesis of zinc complex [Zn(L)Cl_2]. Thin-layer chromatography was used to determine the time required for the completion of the reactions. The formation of the derivative (L) was confirmed by m.p, Rf, Fourier transform infrared (FT-IR), ^{1}H-nuclear magnetic resonance, and mass spectra, and the synthesis of [Zn(L)Cl_2] was proved by m.p, Rf, FT-IR, and mass spectra. The biological activity of the fabricated complex was evaluated against three human cancer cell lines, including prostate cancer cell line (PC-3), colon cancer cell line (Caco-2), and breast cancer cell line (MCF-7). The [Zn(L)Cl_2] exhibited a good reduction in cell viability in a dose-dependent system, where cell viability is reduced by increasing the concentration of the complex. In addition, its antioxidant activity was investigated and demonstrated significant activity at concentrations of 100 and 200 g/mL. Hence, this complex has the potential to be a promising drug for the treatment of PC-3, Caco-2, and MCF-7.

Keywords: Antioxidant, Ascorbic acid, Breast cancer, Zn(II) complex

INTRODUCTION

The Vitamin C discovery and its medical application go back to the 16th and 17th centuries. It was used to prevent the "scurvy" disease among sailors in Europe. Recently, the variety of Vitamin C is used in many fields such as pharmaceuticals, industry, food, and agriculture.[1] For many physiological functions, Vitamin C is required by the human body, and the functions of this vitamin are most important and represented by enhancement of response in body immune system, iron absorption, and pulmonary function.[2] Extensive clinical, animal, and epidemiological studies were conducted to examine the role of ascorbic acid for the prevention of different types of cancers. Except for gastric cancer, the results were found to be incomplete.[3]

Despite the fact of the simple structure of this molecule, the biochemistry of Vitamin C is not fully understood because of its complicated redox chemistry. For this reason, this molecule mostly is used in inorganic chemistry as a reducing agent. Udenfried *et al*.[4] have studied the interaction between metal ions and this vitamin almost 50 years ago. This work was about Vitamin C oxidation by oxygen gas (O_2). Other studies have been carried out on the interactive nature of ascorbic acid and metal ions. Martell *et al*.[5] examined the catalytic role of metals in the oxidation of L-ascorbic. The zinc-Vitamin C interaction is also very important, as Zn(II) is necessary for the activity of over 200 metallic enzymes in the human body.[6]

Zinc is one of the outstanding trace elements that have a crucial role in different cellular processes including cell proliferation, free radicals defense, and differentiation.[7,8] Zinc is an essential element in the structure of many enzymes and proteins, including cellular signaling proteins, transcription factors, and repair enzymes of DNA.[9,10] According to the

literature, zinc ion has been shown to have an important role in the regulation of growth in mammalian cells.[11-13] Constantly, the lowered Zn levels are associated with systemic abnormalities including cancer development.[14] Therefore, zinc complexes have been appeared with their distinctive properties as potential anticancer agents.[15]

However, this work aims to use one of the derivatives of L-ascorbic acid (L) as a vital ligand for the fabrication of [Zn(L)Cl_2]. The theoretical and spectroscopic analyses indicate the formation of the target complex. The antioxidant activity of the synthesized complex was evaluated, as well as its anti-tumor activity against prostate cancer cell line (PC-3), colon cancer cell line (Caco-2), and breast cancer cell line (MCF-7).

EXPERIMENTAL

Synthesis of 5,6-O-isopropylidene-L-ascorbic Acid

A saturated solution of L-ascorbic acid derivative (10.00 g, 57.00 mmol) in 100 ml of a mixture of absolute acetone and hydrochloric acid (V/V) was stirred at room temperature for 20 min. After that, 80 ml of n-hexane was added to the solution. The residue was washed 4 times with acetone-hexane (4:7 V/V); then, the solvent was removed under reduced pressure to yield a white crystallized L-ascorbic acid derivative.[16]

Preparation of Complex [Zn(L)Cl_2]

A 15 mL of L-ascorbic acid (0.2 g, 0.92 mmol) dissolved in ethanol were added to 15 mL of [$ZnCl_2$] (0.125 g, 0.92 mmol) suspended in ethanol. The mixture was refluxed for 2 h, and the formed brown precipitate was filtered and dried at room temperature.[17,18]

Characteristics of the L-ascorbic Acid Derivative

L-ascorbic acid derivative ($C_9H_{12}O_6$): Color (White). Yield (10.96 g, 91.33 %). m. p. (218–221°C). R_f (0.61). Fourier transform infrared (FTIR) (cm^{-1}): Broad band at 2400–3600 for carboxylic acid hydroxyl (O-H); 2995 v(C-H); 1756 v(C=O); 1664 v(C=C); 1141 w(C-O). ^{1}H-nuclear magnetic resonance (NMR) (400 MHz, d_6-DMSO): (ppm): 11.27 (s, for the proton of carboxylic acid hydroxyl OH [G group]), 8.46 (s, for alcohol hydroxyl OH [F group]), 4.71 (s, for the proton of OH [E group]), 4.25 and 4.26 (d, for the proton of CH [D group]), 4.07, 4.09, 4.11, and 4.12 (q, for the proton of the CH [C group]), 3.86 and 3.88 (d, for the 2 proton of the CH_2 [B group]), 1.26 (s, for 6 protons of $2CH_3$ [A groups]. Mass (m/z): 216.0 ($C_9H_{12}O_6$), 159.0 ($C_7H_{12}O_4^{+\bullet}$), 130.0 ($C_6H_{10}O_3^{+\bullet}$), 88 ($C_4H_8O_2^{+\bullet}$), 57.1 ($C_2H_2O_2^{+\bullet}$), 42.0 ($C_3H_6^{+\bullet}$), 30.1 ($CH_2O^{+\bullet}$). Microanalysis (The microanalysis of elements for prepared complex was estimated by Shimadzu-AA-670-Flame spectrophotometer in the laboratories of Gazi University in Turkey): 50.0 (C Theoretical), 50.6 (C Practically), 5.60 (H Theoretical), and 5.88 (H Practically).

Characteristics of the Prepared Complex

Perpetrated complex ([Zn(L)$(Cl)_2$]): Color (Brown). Yield (0.28 g, 86.41%). m. p. (288–289°C), R_f (0.56). FTIR (cm^{-1}): 2981, 2894 (C-H); 1743 (C=O); 1612 (C=C); 1139 (C-O); 2400–3610 (O-H). Mass (m/z): 350.2 ($C_9H_{11}Cl_2O_6Zn^{+\cdot}$), 289.8 ($C_4H_5Cl_3O_4Zn^{+\bullet}$), 156.0 ($C_7H_{10}ClO_2^{+\bullet}$), 133.8 ($C_4H_3ClO_3^{+\bullet}$), 84.0 ($C_4H_6O2^{+\bullet}$), 71.9 ($C_3H_4O_2^{+\bullet}$), 58.1 ($C_3H_6O^{+\bullet}$). Microanalysis: 30.58 (C Theoretical), 29.9 (C Practically), 2.88 (H Theoretical), 2.11 (H Practically), 20.23 (Cl Theoretical), 19.52 (Cl Practically), 18.66 (Zn Theoretical), and 17.92 (Zn Practically).

Biological Activity

Antioxidant activity of the prepared complex was detected using 2,2-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay according to Rajesh and Natvar.[19]

The cytotoxic effect of the complex was conducted using ready-to-use MTT kit.[20] The kit contents are: (1 mL × 10 vials MTT solution) and (50 mL × 2 bottle solubilizing solution).

Statistical Analysis

Statistical Analysis System (2012) program was employed for examining the different factors in the study parameters. To compare the means and Chi-square, test least significant difference–LSD test analysis of variance was used to compare between percentages. The estimation of correlation coefficient between variables has also been studied.[21]

RESULTS AND DISCUSSION

L-ascorbic Acid Derivative

L-ascorbic acid molecule has four (-O-H) groups, all these groups are active for known esterification reactions and other reactions. L-ascorbic acid derivatives synthesis at 2- and 3-position requires the conversion of L-ascorbic acid into its 5,6-isopropylidene derivative because the carbon-6 hydroxyl group (a primary hydroxyl group) is the most reactive group.[22] The acetal group is readily hydrolyzed by a dilute acid.[23,24] L-ascorbic derivative was synthesized from the reaction of acetone and L-ascorbic acid in acidic medium, according to the literature,[16] Scheme 1.

In the first step, the suggested mechanism of the synthesized derivative involved protonation, the second step was to attack the electron pair of the hydroxyl oxygen in the terminal continuous chain on the carbonyl carbon of the ketone, and in the third step, the transfer of a proton from the terminal hydroxyl group to the nearest hydroxyl group, followed by a fourth step by losing a water molecule and cyclic closure and in the fifth step occurred opening of the ring containing lactone group (O-C=O), Scheme 2:

The FTIR spectrum of L-ascorbic acid derivative [Figure 1] showed a broad band at 2400–3600 cm^{-1} for (O-H) of carboxylic acid and other bands were observed at 2995 cm^{-1} for (C-H aliphatic), 1756 cm^{-1} for (C=O) of carboxylic acid, 1664 cm^{-1} for aromatic (C=C), and 1141 cm^{-1} for (C-O).[25,26]

The ^{1}H-NMR spectrum of an L-ascorbic acid derivative [Figure 2] reveals a singlet signal at 11.27 ppm that corresponds to the proton of the hydroxyl group OH of carboxylic acid (g group), a singlet signal at 8.46 ppm for the proton of OH (f group), a singlet signal at 4.71 ppm for the proton of OH

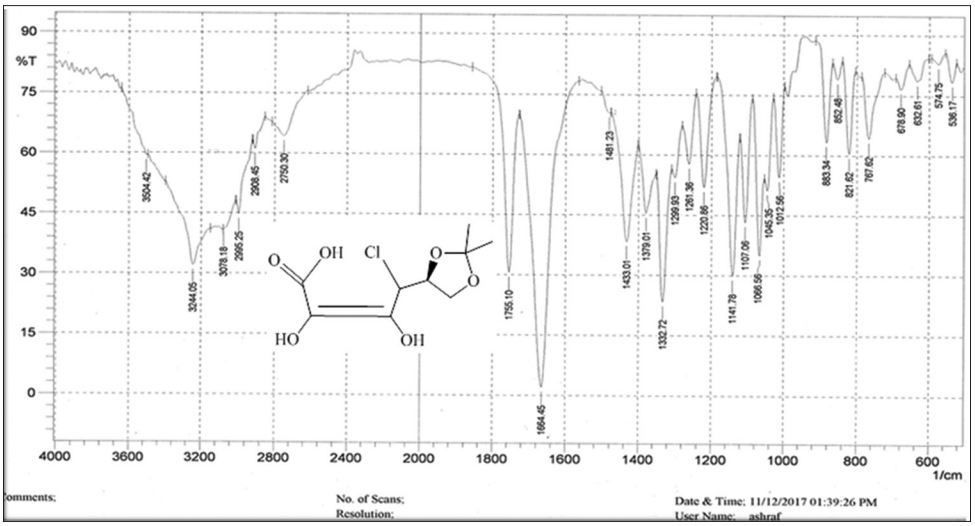


Figure 1: Fourier transform infrared of L-ascorbic acid derivative

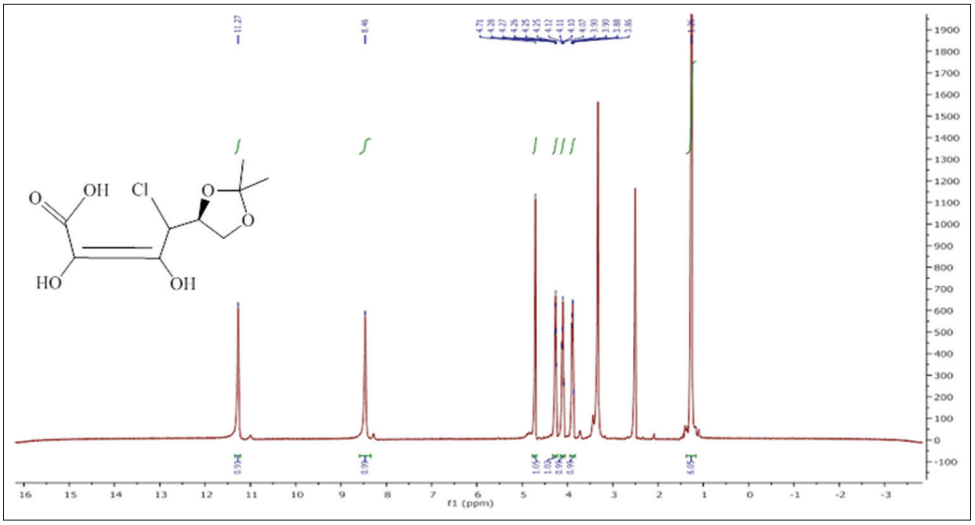


Figure 2: [1]H-nuclear magnetic resonance of L-ascorbic acid derivative

(e group), a doublet signal at 4.25 and 4.26 ppm for the proton of CH (d group), a quartet signal at 4.07, 4.09, 4.11, and 4.12 ppm for the proton of the CH (c group), a doublet signal at 3.86 and 3.88 ppm for the 2 proton of the CH_2 (b group), and a singlet signal at 1.26 ppm for 6 protons of $2CH_3$ (a groups).[27,28]

The signals of the mass spectrum of L-ascorbic acid derivative [Figure 3] are described in Table 1. These signals are representing the molecular weight of the derivative in addition to the emergence of multiple fissures (structure of L-ascorbic acid derivative and its fragmentation), which confirm the formation of the derivative with the proposed formulas. The suggested molecular weights were consistent with the other results.

The Synthesis of Complex $[Zn(L)(Cl)_2]$

This complex was prepared, as shown in Scheme 3. The reaction of Ligand (L-ascorbic acid derivative) with $ZnCl_2$, in the ethanol medium, completed within 2 h. A high yield (86.41%) of $[Zn(Cl)_2]$ was obtained with a brown color and a melting point of 288–289°C, Scheme 3.

The FTIR spectrum of $[Zn(L)(Cl)_2]$ complex shows stretching band at 2981 cm^{-1} asymmetric, 2894 cm^{-1} symmetric for (C-H aliphatic), 1743 cm^{-1} for (C=O) of carboxylic acid, 1612 cm^{-1} for (C=C), 1139 cm^{-1} for (C-O), and broad band at 2400–3610 cm^{-1} for (O-H) of carboxylic acid,[27,28] Figure 4 of FTIR spectra of $[Zn(Asc)(Cl)_2]$ complex.

The mass spectrum of the complex [Figure 5] shows the following signals in Table 2. These signals are representing the molecular weight of the complex in addition to the emergence of multiple fissures (structure of $[Zn(L)Cl_2]$ complex and it's fragmentation), which confirm the formation of this complex with the proposed formulas and the suggested molecular weights that are consistent with the other results.

A microanalysis of the synthesized L-ascorbic acid derivative and $[Zn(L)Cl_2]$ complex elements was measured and a match was found between theoretical percentages and the calculated practical percentages.

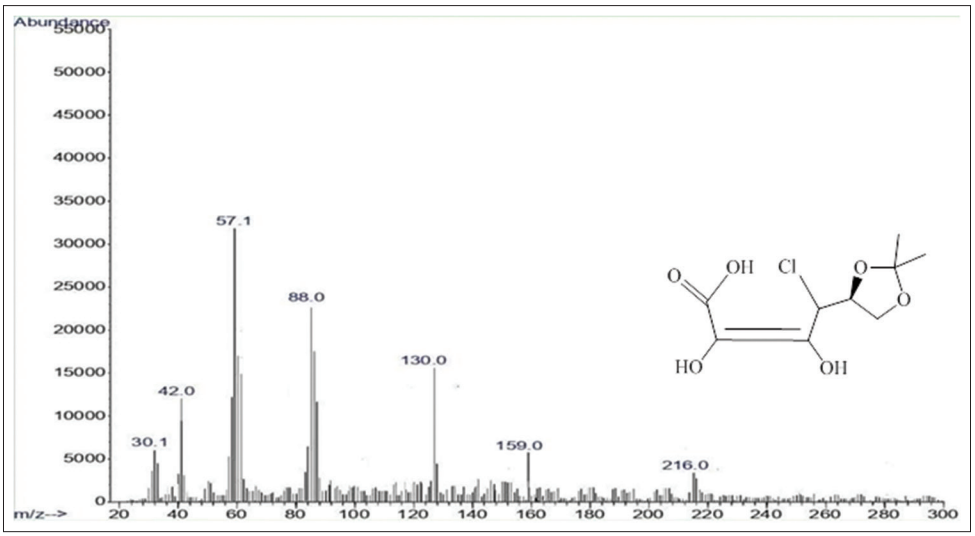
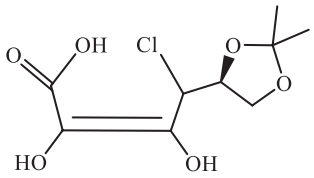
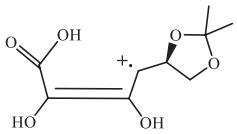
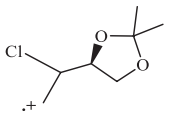
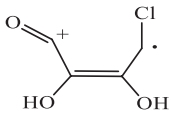
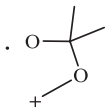
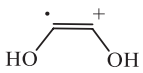


Figure 3: The mass spectrum of L-ascorbic acid derivative

Table 1: Mass spectra and fragmentation of L-ascorbic acid derivative

Molecule	Structure of fragments	Fragmentation chemical formula	Mass/charge: m/z
		$C_9H_{13}O_6^{+\cdot}$	216.0
		$C_7H_{10}ClO_2^{+\bullet}$	159.0
		$C_4H_3ClO_3^{+\bullet}$	130.0
		$C_4H_8O_2^{+\bullet}$	88.0
		$C_2H_2O_2^{+\bullet}$	57.1
		$C_3H_6^{+\bullet}$	42.0
		$CH_2O^{+\bullet}$	30.1

Quantum Chemical Properties

The global chemical reactivity indices of $[Zn(L)Cl_2]$ were revealed by molecular orbital calculations depending on lowest unoccupied molecular orbital (LUMO) and highest occupied molecular orbital (HOMO) calculations [Table 3]. These agents are useful for predicting the interaction of $[Zn(L)Cl_2]$ with biological molecules in a human body and their diffusion across cell membranes and the brain–blood barrier.[29,30] Figure 6 shows that the energy difference between HOMO and LUMO of $[Zn(L)Cl_2]$ is 4.400 eV, so there is a contribution of the prepared complex on the ability to accept electrons. Related parameters such as electronic chemical potential (μ), chemical hardness (η), and electrophilicity index (ω) were estimated using Equations 1, 2, and 3, which found to be 2200 eV, 3930 eV, and 1786 eV, respectively. These values reveal the biologically pharmacodynamic activity of $[Zn(L)Cl_2]$. Furthermore, the negative value (–3.459) of the solubility class (Log S) means having the ability to dissolve rabidly with serum and proteins. Finally, using Swiss ADME

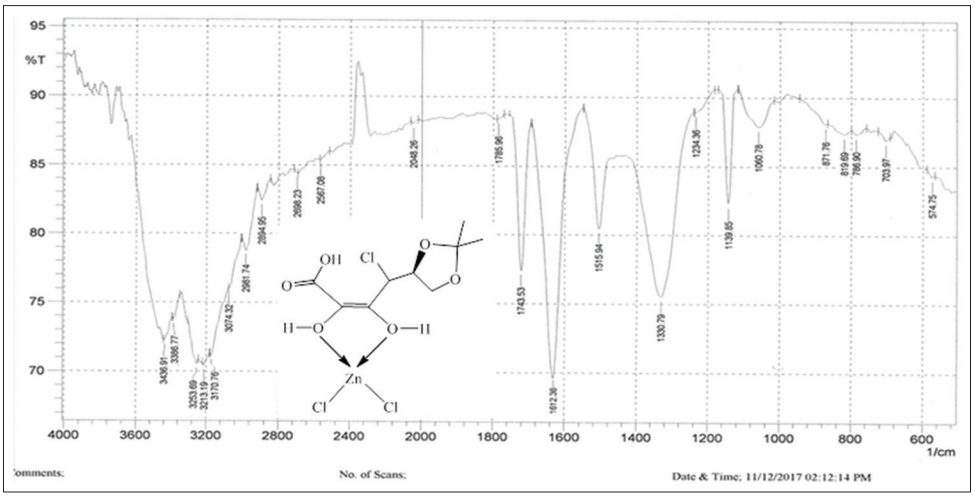
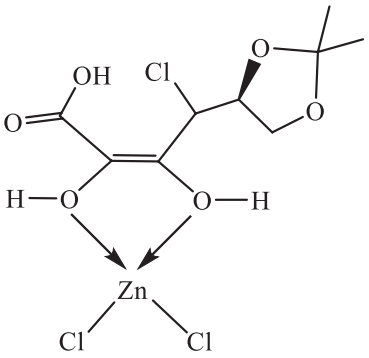
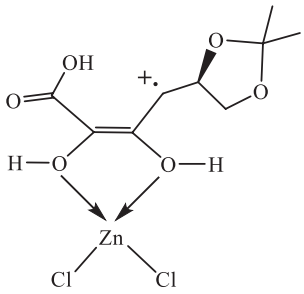
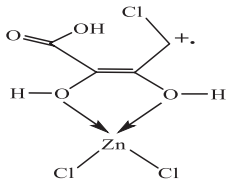
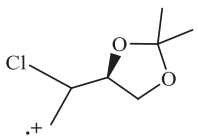
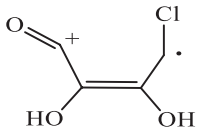
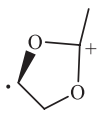
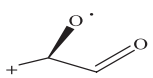
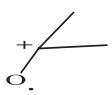


Figure 4: Fourier transform infrared of [Zn(l)$(Cl)_2$] complex

Table 2: Mass/Charge (m/z) of the fragmentation of [Zn (L) Cl_2] complex

[Zn (Asc)$(Cl)_2$] complex	Structure of fragments	Chemical formula of complex fragments	Mass/charge: m/z
		$C_9H_{11}Cl_2O_6Zn^{+\cdot}$	350.2
		$C_4H_5Cl_3O_4Zn^{+\bullet}$	289.8
		$C_7H_{10}ClO_2{}^{+\bullet}$	156.0
		$C_4H_3ClO_3{}^{+\bullet}$	133.8
		$C_4H_6O2^{+\bullet}$	84.0
		$C_3H_4O_2{}^{+\bullet}$	71.9
		$C_3H_6O^{+\bullet}$	58.1

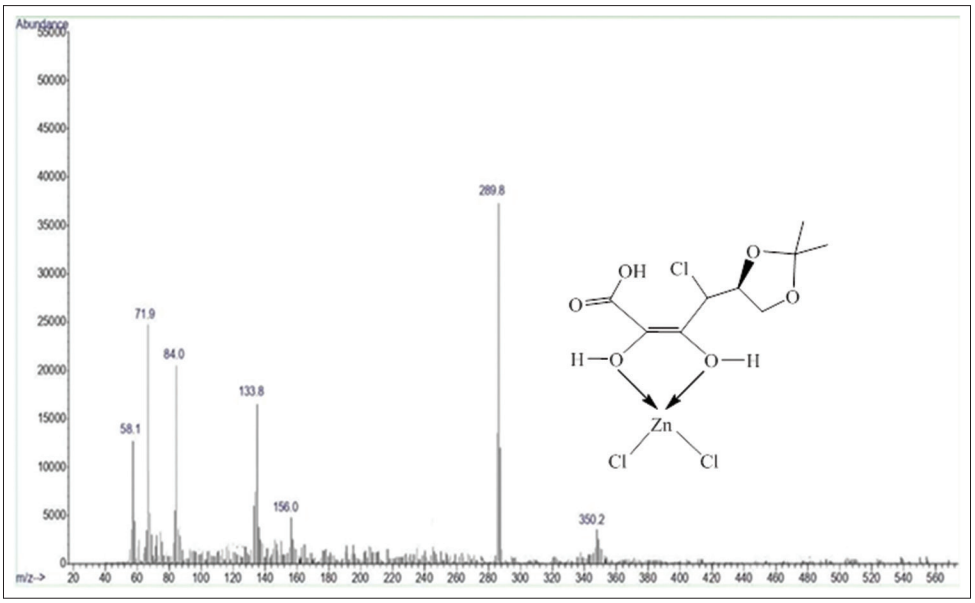


Figure 5: Mass spectrum of $[Zn(l)(Cl)_2]$ complex

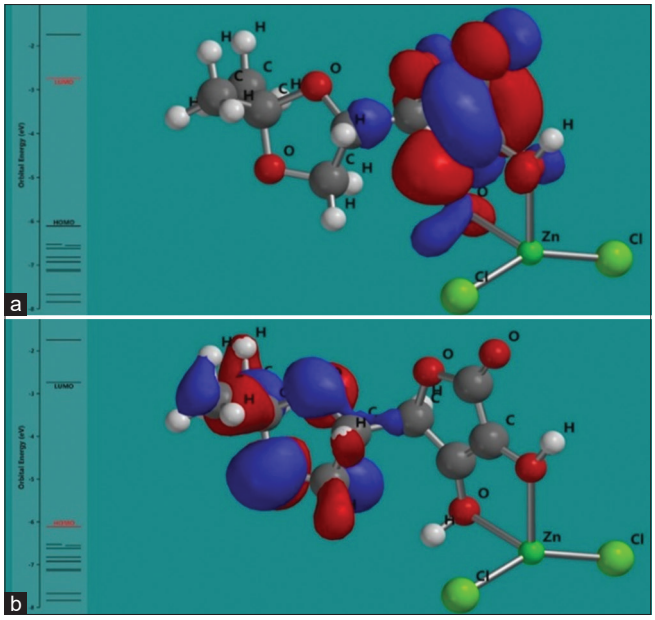


Figure 6: (a and b) Frontier molecular orbitals of $[Zn(l)Cl_2]$

online, the prepared complex was effective on drug-likeness criteria derived from the rules of Lipinski, Ghosh, and Veber.[31]

$$\mu = (\varepsilon HOMO + \varepsilon LUMO) / 2 \quad (1)$$

$$\eta = (\varepsilon LUMO - \varepsilon HOMO) / 2 \quad (2)$$

$$\omega = \mu^2 / 2\eta \quad (3)$$

Antioxidant Activity of $[Zn(L)Cl_2]$ Complex

The activity of free radical scavenging of the synthesized complex has been studied using the stable (DPPH) of free radical scavenging assay. DPPH is characterized as a stable radical due to the delocalization of the maximum absorption about 517 nm and the spare electron. The activity of DPPH in scavenging has been commonly used to estimate the antiradical activities of different compounds,[32-34] Table 4.

Table 3: Physicochemical properties of $[Zn (L) Cl_2]$

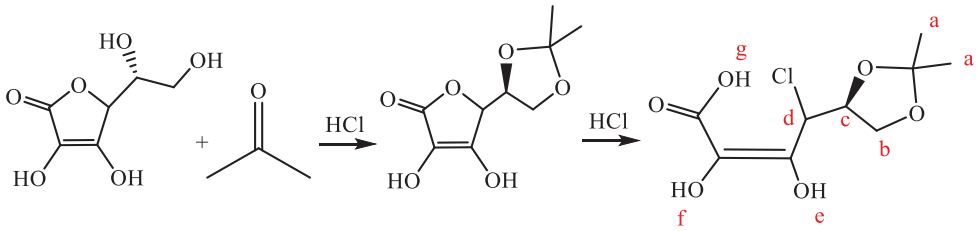
$[Zn (L)(Cl)_2]$	Physicochemical properties
−6.130	HOMO (eV)
−1.730	LUMO (eV)
	Dipole moment (debye)
–3.459	Log S
2.982	Log P
4.400	Energy gap (Δ) (eV)
3.930	μ (eV)
2.200	η (eV)
1.786	ω (eV)

Results in Table 4 revealed that 200 μg/mL of complex had the highest scavenging activity (86.03%) that is showing the more concentration of the complex, and the more free radicals scavenging percentage. When compared to free radical scavenging positive antioxidant ascorbic acid activity, the prepared complex exhibits nearly the same pattern of free radical scavenging activity, particularly at concentrations of 12.5–50 g/mL (Figure 7). While the concentrations of 100 and 200 μg/ml showed higher, activities compared with ascorbic acid (as a positive control). Free radicals play a critical role developing some health conditions, example cataracts, cancer, and cardiovascular disease. Free radicals have been represented in both acceleration and initiation of the aging process.[35] It is well-known that free radicals are the main cause of oxidation and that they can be prevented or controlled by the use of various antioxidants.[36] Many chemical compounds possess

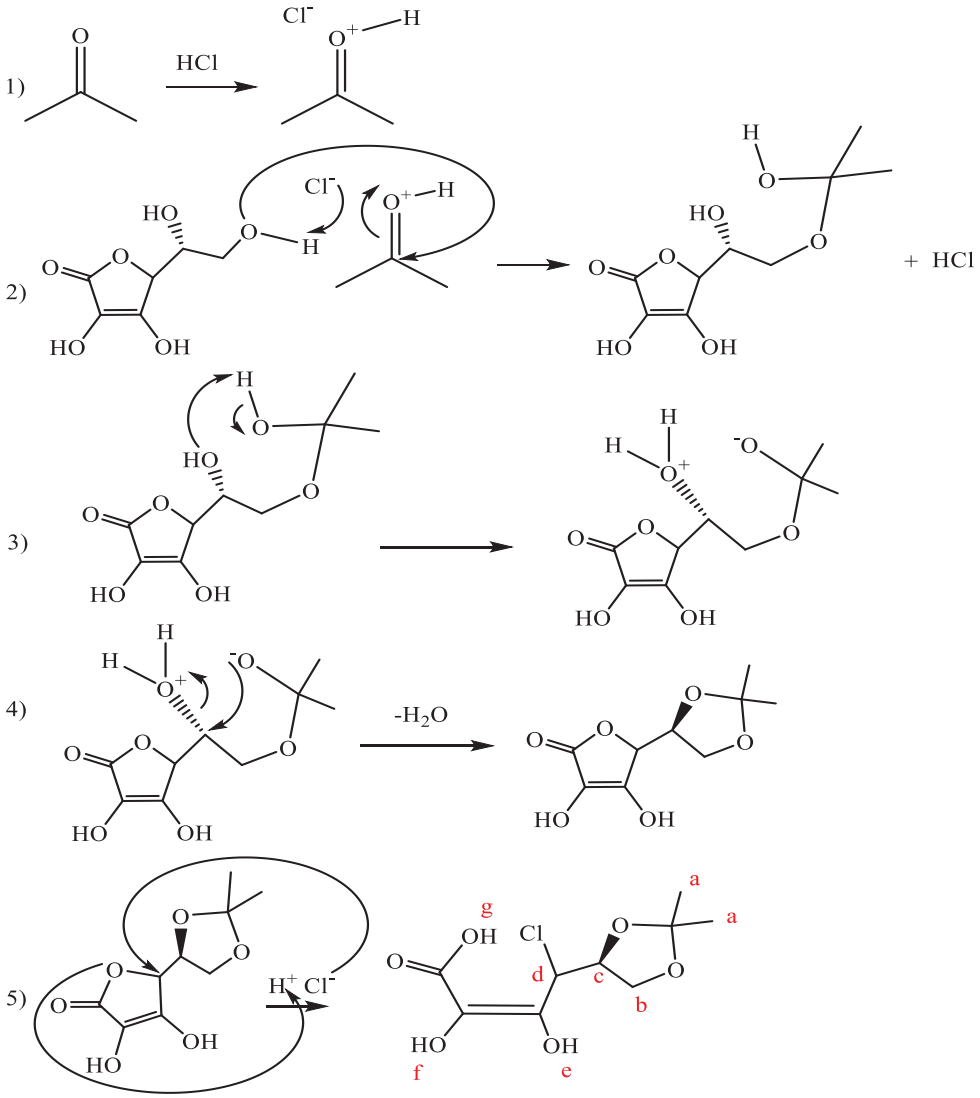
Table 4: Antioxidant activity of [Zn (L) Cl2] complex using 2,2-Diphenyl-2-Picrylhydrazyl method

Compound	Compound				
	12.5 (µg/mL)	25 (µg/mL)	50 (µg/mL)	100 (µg/mL)	200 (µg/mL)
Ascorbic acid	17.63±7.196	40.43±7.081	54.48±2.412	61.77±1.351	72.11±3.002
Complex	22.90±1.836 (NS)	39.00±1.732 (NS)	57.60±2.207 (NS)	74.07±1.007**	86.03±4.028**

**Significant at level ($P<0.001$). NS: Non-significant



Scheme 1: Synthesis of L-ascorbic acid derivative



Scheme 2: Suggested mechanism of L-ascorbic acid derivative preparation

great antioxidant activities with capabilities of reducing the oxidative reactions in cells due to their ability to donate hydrogen atoms, therefore, they are considered useful in the treatment of many diseases including inflammatory, cancer,

Table 5: Anticancer activity of [Zn (L) Cl2] complex on MCF-7, prostate cancer-3, and Caco-2 cell lines after 24 h of incubation at 37°C using the MTT method

Concentration (µg/mL)	400	200	100	50	25	12.5	6.2	IC50	*P*
MCF7	44.445±1.208	47.801±1.947	51.235±2.032	61.458±1.365	75.502±2.453	85.764±1.445	94.522±0.571	72.46	0.0001>
WRL68	78.665±2.549	81.636±1.438	86.150±1.275	90.394±1.029	93.904±1.112	94.985±0.678	94.753±0.964	248.5	
PC3	61.574±3.478	65.548±1.237	70.447±1.993	79.012±2.166	86.883±1.563	94.252±1.142	94.830±0.241	128.2	<0.0001
WRL68	73.349±1.474	81.991±5.005	92.477±1.505	93.866±0.613	95.062±0.177	94.753±0.571	95.216±0.241	470.1	
Caco-2	32.793±7.442	37.847±1.736	63.812±2.744	90.046±2.425	96.219±0.678	96.644±1.365	97.26±0.3	98.70	<0.0001
WRL68	70.409±1.414	84.028±1.002	91.667±1.621	95.332±1.183	95.216±0.821	95.949±1.028	95.45±0.3	333.7	

PC: Prostate cancer, Caco: Colon cancer cell line (Caco-2), MCF: Breast cancer cell line

and cardiovascular diseases.[37] Zn complexes are more active DPPH scavengers than the corresponding free compounds.[38] A zinc complex of 2-hydroxy-salicylhydrazide-isatin hydrazone has two free hydroxyl groups in its [ZnL_2] formula which exhibited good radical scavenging activity (SC50 = 31.13 µM). Özdemir[39] suggested that this higher activity might be dependent on the stabilization of radical with the phenolic moieties of zinc complex. In addition, Andelescu *et al*. reported that Zn(II) complexes containing four free hydroxyl groups in their formulae which showed remarkable scavenging activity (SC50 = 8.10–12.17 µM).[40]

Effect of [Zn(L)Cl_2] Complex on Different Cancer Cell Lines Using the MTT Method

3-(Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was achieved to evaluate the cytotoxic effect of the complex against three different types of cancer cell lines; Caco-2, PC-3, and MCF-7. This assay was conducted to determine the cell viability and the rate of inhibition by the implementation of different concentrations of the complex on the targeted cancer cell lines. For the most significant IC_{50}

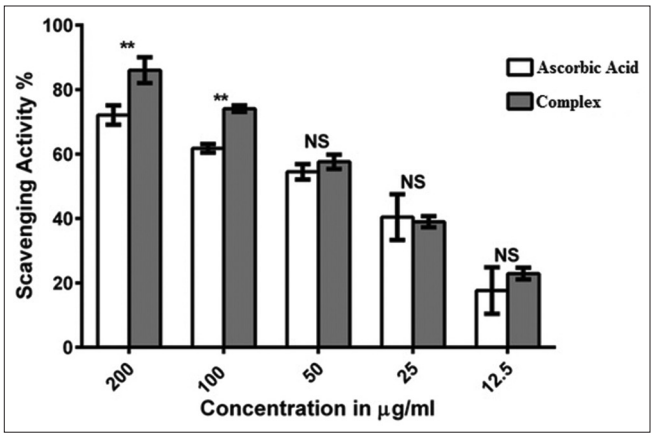


Figure 7: Antioxidant activity of [Zn(l)$(Cl)_2$] complex using DPPH method

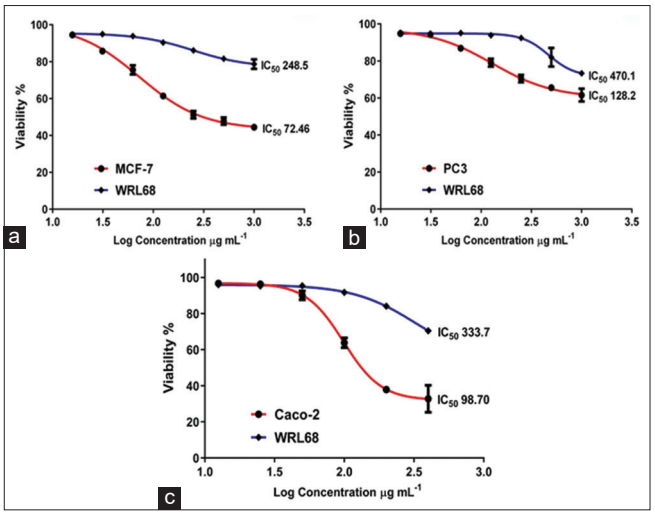
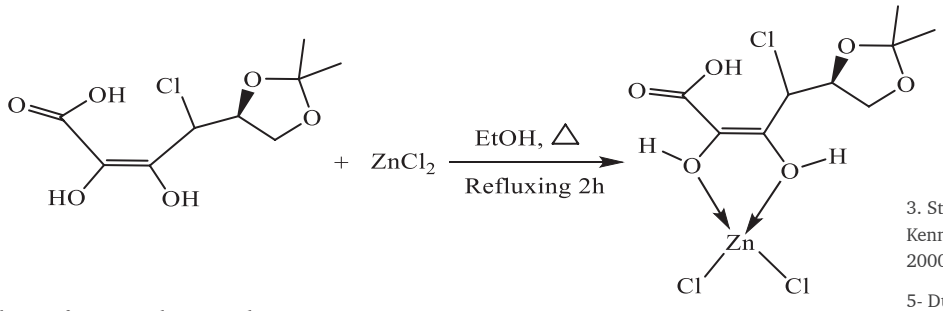


Figure 8: Cytotoxicity effect of [Zn(l)$(Cl)_2$] complex on breast cancer cell line-7 (a), prostate cancer-3 (b), and colon cancer cell line-2 (c) cell lines after 24 h of incubation at 37°C



Scheme 3: The synthesis of $[Zn(L)(Cl)_2]$ complex

values, the best values were chosen. MTT colorimetric assay was used to determine the cell viability at every time point. The cytotoxic effect of the complex toward three different types of cancer cell lines is reported in Table 5 and Figure 8.

Results indicated that the incubation of tumor cell lines with the complex at different concentrations ranged between 12.5 and 400 µg/mL for 24 h showed that viability of cell reduced in a dose-dependent pattern by the increase of the complex concentration. The cell viability percentage (%) of a lowest value was recorded at the concentration of 400 µg/mL. The explanation of this case is still unrevealed, but it is known that significant amounts of the complex are hydrolyzed outside the cell and excreted by renal on plasma proteins binding, with only few DNA binds. It may be attributed to the strong Zn-O bond, which may result in more cell penetration by the complex and becoming bound to DNA. Significant differences were observed in the complex inhibition rate among different cell lines by the calculating of the IC_{50}. The complex showed the most cytotoxic activity on the MCF-7 cells with an IC_{50} value of 72.46 µg/mL, whereas IC_{50} values of PC-3 and Caco-2 were 128.2 and 98.70 µg/ml, respectively.

Researchers have paid attention toward designing metal complexes as anticancer agents. The metal complexes designing process depends on the donation ability and the metal ion type along with its oxidation state.[41] Cancer cell toxicity varied from one cell line to another, due to different types of cancer cell lines in their receptors the presence of the complex affected certain receptors on the surfaces of these cells, and through these receptors, the cells are responsive to the action of many substances that stimulate apoptosis.[42] Particular zinc thiosemicarbazone complexes have been studied and confirmed to have anti-cancer activity as antioxidant agents. They have shown cytotoxicity similar to cisplatin and significantly effective against cancer cell lines. The zinc complexes seem to be concentrated in a relatively small zone of the cell with a low general uptake in the rest of the cytoplasm. However, the cell absorption of mineral complexes depends on the cell type.[43]

CONCLUSION

The formation of both the L-ascorbic acid derivative and $[Zn(L)(Cl)_2]$ was demonstrated by spectroscopic studies (FTIR, ^{1}H-NMR, and mass spectrum). This structure had a significant effect on the cancer cells under evaluation. DPPH is characterized as a stable radical due to the delocalization of the maximum absorption about 517 nm and the spare electron. Significant differences were observed in the complex inhibition rate among different cell lines by the calculating of the IC_{50}. The complex showed the most cytotoxic activity on the MCF-7 cells with a IC_{50} value of 72.46 µg/mL, whereas IC values of PC-3 and Caco-2 were 128.2 and 98.70 µg/ml, respectively.

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