

Mixed ligand complex: Synthesis, characterization, and investigation of biomedical activity

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Abstract

A new complex, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (M), was synthesized to react with melatonin (L1) and one of the amino acids (L-cysteine, L2) in a mole ratio of (1:1:1) (M: L1:L2) to produce the metal complex. The synthesized complex was then characterized using spectral techniques including infrared (FT-IR), thermal analysis (TG), flame atomic absorption (AAS), micro elemental analysis (CHNS), melting point (m.p.) measurement, and determination of chloride. Octahedral geometry was proposed for the complex. The copper complex was paramagnetic and non-electrolyte. The synthesized complex was evaluated as an antibacterial and antifungal agent against *Escherichia coli* (G-) and *Staphylococcus aureus* (G+). fungus *Candida*. The results showed that the copper complex was more active in 10–3 M than the two ligands; the complex's anti-cancer properties were also evaluated and gave a good result in the test.

Keywords: Biomedical activity, Cu complex, Cysteine, Melatonin, Mixed ligands.

Introduction

According to recent research, mixed ligand complexes play an important function in biological systems ¹, the main method for adjusting the properties of transition metal ions to achieve the intended applications involves the use of various ligands with different structures and properties ². The mixed ligand complexes can be di ligand, tri ligand, tetra ligand, or multi ligand complexes depending on the number of ligands linked to the metal ion. The development of mixed ligand complexes and their characteristics can be critical in terms of the kinetic impact ³.

Transition ion complexes have a wide range of commercial and technical uses, including antibacterial, antifungal, and anticancer medications, as well as catalysts. The metal atom itself may play a variety of functions in these complexes, depending

on its oxidation state, coordination geometry, and magnetic, electronic, and photochemical properties ⁴. It has been proved clearly that new chemical ligands show improvement as physiologically active ⁵. One type of ligand in the compound increases the chances of variation in the expected properties of the compound ⁶.

Many biological processes require organic molecules with pyridine rings. So complexes containing the pyridine ring (cyclic nitrogen) have been shown to have high anticancer efficacy as well as tumor size reduction¹.

N-acetyl-5-methoxytryptamine, often known as melatonin, is a hormone that can be found in all living organisms, including humans and algae. It is referred to as vertebrate pineal secretory product and was identified in 1958 ⁷. Melatonin can be used

orally as a supplement or medication for common diseases, including sleep problems, depression, Parkinsonism, Alzheimer's disease, and cancer. Melatonin also plays several physiological roles in humans. Exogenous MLT may also decrease age-related oxidative processes and act as a skin UV radiation protector in addition to its other possible applications. Although being applied topically in cosmetic goods, it also acts as a skin protectant against UV rays^{8,9}. Melatonin is a circadian rhythm-regulated and multifunctional molecule that plays a neuroprotective role against the pathogenesis of AD¹⁰, Parkinson's illness¹¹ Breast cancer, depression, and glaucoma, breast and prostate cancer⁵.

Cysteine (Cys), the main sulfur-containing amino acid that contains sulfur, is a semi-essential amino acid since it may be taken from food or synthesized through the transsulfuration path during the hydrolysis of methionine. It is believed to act as a biomarker of sulfur-containing amino acids in the mammalian diet¹². It becomes essential. Transsulfuration is a metabolic mechanism in the

Materials and Methods

The chemical and apparatus

All chemicals were used exactly as provided, with no additional purification. CHNS Elemental Analyzer Euro EA 3000/Italy was used to record micro elemental analyses (CHNS). The melting points of all compounds were determined using the Gallenkamp melting point instrument. The FTIR (Fourier Transform Infra-Red) spectra were obtained using a SHIMADZU 8400 s spectrophotometer for ligands in the (4000–400) cm^{-1} range with KBr and complexes in the range 4000-250 cm^{-1} with CsI. The electronic spectra (Uv-Vis). Thermal analysis (TG) was recorded by METTLER TA 4000 SYSTEM). The metal content was determined using a Nova350 spectrophotometer and flame atomic absorption spectroscopy. The Mohr technique was used to determine the chloride concentration in the complexes. Bruker 400 MHz NMR spectrometer was used to measure ¹H-NMR spectroscopy in d-DMSO⁶.

Synthesis Cu (II) complex

A mixed ligand complex was prepared from copper salts as chloride ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$), melatonin (L1) as a

liver that allows cysteine to be supplied by converting a necessary amino acid: methionine¹³.

Cysteine is considered a proteinogenic amino acid since it serves as a building component for about 2% of proteins and plays an essential role in biological processes in our bodies. Several key metabolic processes are catalyzed by it¹². Cysteine residues provide a variety of roles in proteins. In eukaryotes, the sulfur atom in cysteine, which is oxidatively sensitive and highly ionizable, responds to redox conditions and the pH of subcellular organelles¹⁴. Cysteine is oxidized at the thiol group (-SH), which can form a covalent bond by reacting with free radicals and other groups¹⁵. Cysteine acts as an antimicrobial to heal damaged skin¹⁶. It involves lipid biosynthesis¹⁵, Iron-sulfur biosynthesis¹⁴ is an essential component of skeletal muscles and a source of taurine, glutathione, and coenzyme A¹⁷.

In this research, a mixed ligand copper complex was prepared and its biological and toxic activity was studied.

primary ligand, and cysteine (an amino acid) (L2) as a secondary ligand.

To an aqueous solution (10 ml) of (0.134g, 1 mmol) Cu(II), an aqueous solution (10 ml) of melatonin (0.232 g, 1 mmol) containing NaOH (0.04 g, 1 mmol) was added. The reaction mixture was stirred and kept in a boiling water bath for 10 minutes. To this hot solution, an aqueous solution (5 ml) of cysteine (0.121g, 1 mmol) was added with constant stirring. The PH of the resultant mixture was adjusted to 7.5–8.0 using NaOH. The mixture was heated again in a water bath (reflux 3.5 h), and during this period, a gray color appeared for the precipitated complex. The precipitate was collected by filtration, washed with deionized water, then with absolute ethanol, and finally dried with ether in the oven.

Biomedical evaluation (antimicrobial and anticancer activity).

The antibacterial and antifungal activity of the synthesized complex was accomplished using the agar diffusion technique with 10^{-3} M in deionized water solutions. Escherichia Coli (G-) (E. coli), Staphylococcus aureus (G+) (S. aureus), and Candida albicaus.

The preparation of metal-based anticancer drugs is considered one of the most important areas in this field. A lot of drugs that are used for cancer treatment

are cytotoxic. As a result, the most important challenge these days is to develop novel anticancer drugs with high efficacy and low toxicity.

Results and Discussion

The physical properties, elemental analysis data illustrated in Table 1.

Table 1. Analysis of data and physical properties of the two ligands and their metal complex.

Compound	The molecular formula	Colour	Molecular weight g/mol	Melting point °C	Yield %	Elemental Micro Analysis (Found) Calc.				Metal found % Calc. (Found)	Chloride content % Calc. (Found)
						C%	H%	N%	S%		
L1	C ₁₃ H ₁₆ N ₂ O ₂	White	121.16	117	-	-	-	-	-	-	-
L2	C ₃ H ₇ NO ₂ S	White	232.28	240	-	-	-	-	-	-	-
Cu(II) complex	CuC ₁₆ H ₂₇ N ₃ O ₆ SCl ₂	Light gray	523.92	198-200	98	36.64 (35.98)	5.15 (5.94)	8.01 (8.30)	6.10 (7.16)	12.12 (12.60)	13.55 (12.73)

* Dec=decomposition

FT-IR

In the characteristics of L-cysteine chelated, a band at 3307 cm⁻¹ was noticed due to stretching vibration of (NH₂) in the complex of Cu(II), which differed slightly from the band seen in free cysteine, indicating coordination through the nitrogen atom of the amino group of Cysteine¹⁸. The strong band

assigned to NH (indole) of melatonin at 3473 cm⁻¹ shifted to 3244 cm⁻¹ in complex, which may be linked to the formation of a nitrogen-metal bond. The complex's band of (C=O) carboxylic acid didn't change in comparison to the two ligands (melatonin and cysteine), indicating that the -(C=O) group of cysteine and melatonin was not involved in the coordination with metal¹⁹.

The Cysteine ligand spectrum indicated a band at 2551 cm⁻¹ that refers to (SH), which shifted to a lower frequency in the complex spectrum due to coordination with metal ions via the SH group, and the corresponding vibration (C-S) in the mixed ligand complex was also shifted to a lower frequency, indicating coordination of L-cysteine via the sulfur atom¹⁹. New bands formed at 424–474 cm⁻¹, indicating the appearance of metal ions coordinated via the N atom of the ligand²⁰.

At low frequencies, new bands also appeared in the spectrum of the complex, attributed to M-Cl and M-S²¹ (293, 341), respectively, see Table 2 and Fig. 1-3.

Table 2. The spectral infrared data of the two ligands and their metal complex in (cm⁻¹).

COMP.	H ₂ O Lattice (coordinate)	νNH indole	νNH indole	νOH	νSH	νC=O Carboxylic	νC-N	νC-S	νM-S	νM-N	νM-Cl
MLT	-	3371	3473	-	-	1797	1157	-	-	-	-
Cys	-	3176	-	3433	2551	1735	1197	692	-	-	-
Cu(II) complex	3436 (918)	3307	3244	3467	2360	1712	1137	671	341	449	293

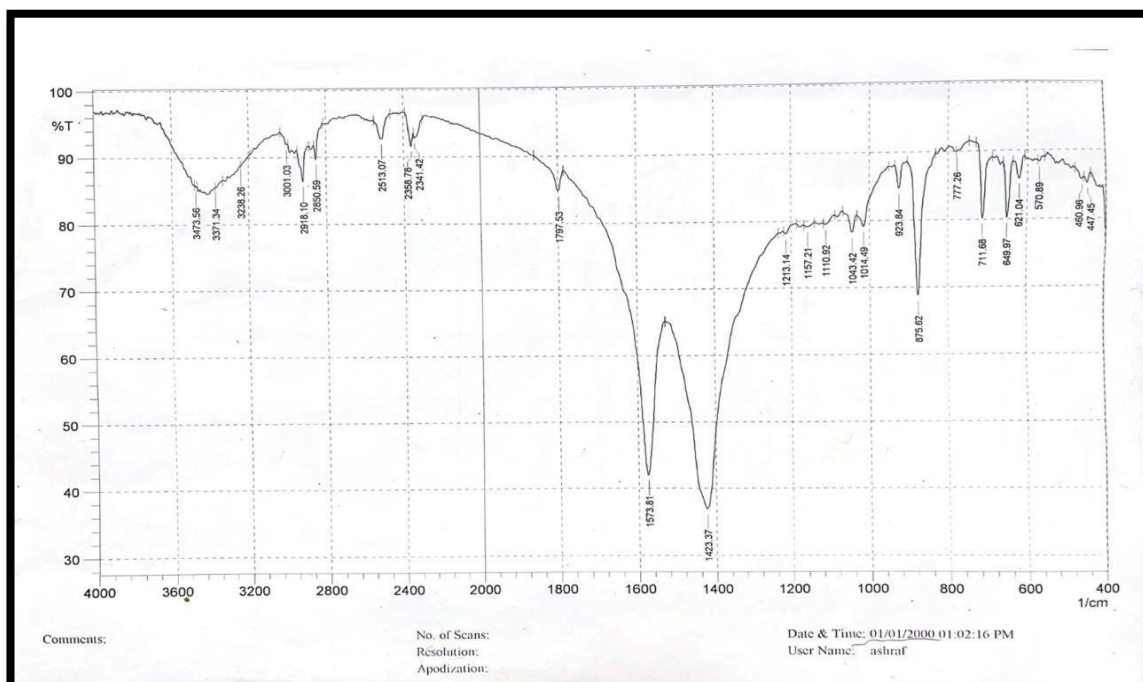


Figure 1. FT-IR Spectrum of Melatonin(L1).

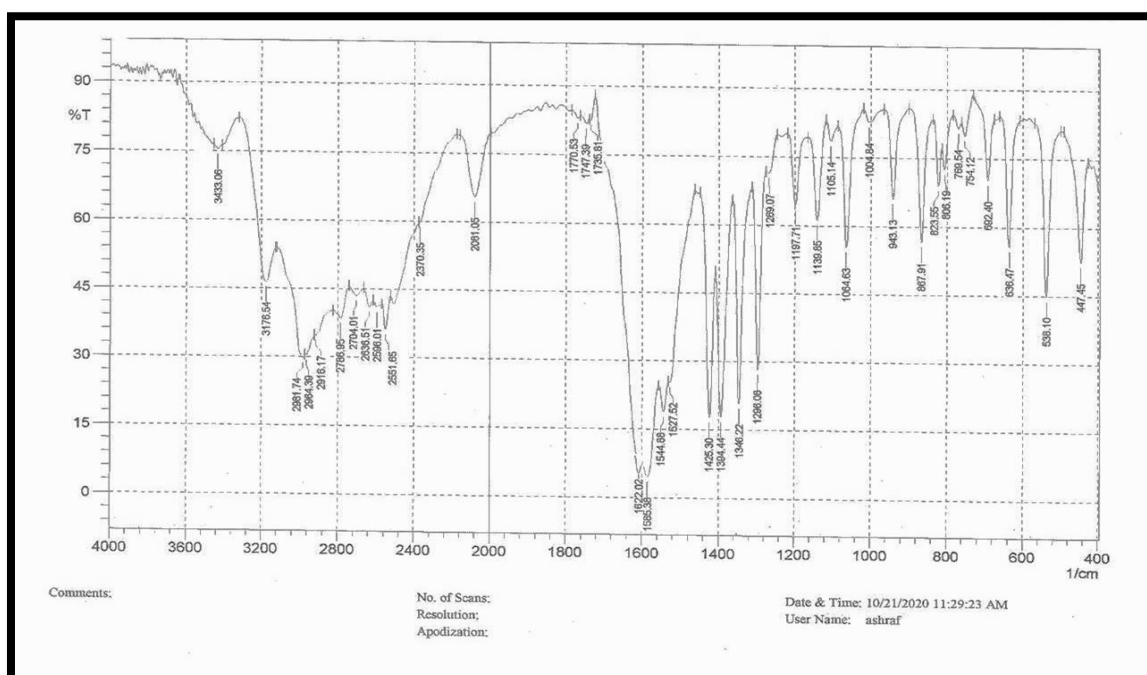


Figure 2. FT-IR Spectrum of Cysteine(L2).

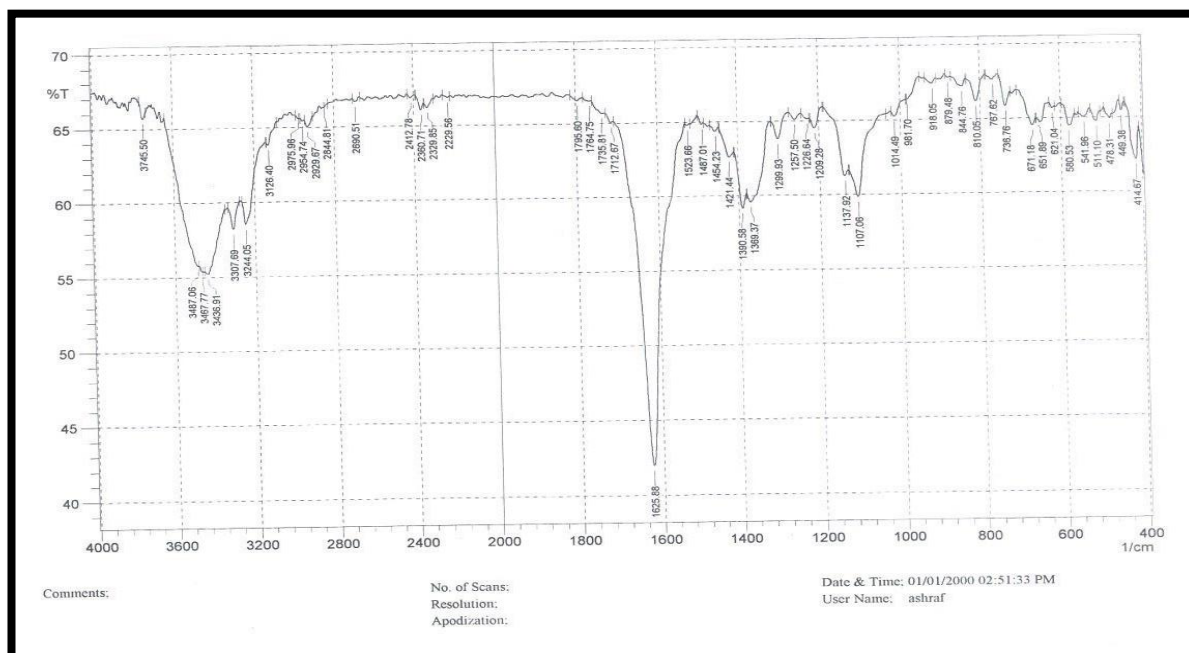


Figure 3. FT-IR Spectrum of Cu-complex.

¹H-NMR Spectroscopy

The ¹H-NMR spectra of the two ligands showed characteristic peaks of (SH, CH₂, CH, NH₂) for cysteine and aromatic protons and cyclic protons (CH₃, CH₂, CH₂, CH₃O) for melatonin. The ¹H-NMR spectra of cysteine showed peaks ranging from (δ 0.87) ppm that were attributed to SH proton and peaks ranging from (δ 2.79–2.93) and (3.31–3.33) ppm that refer to CH₂ and CH protons, respectively, as well as peaks at (δ 6.61) that were assigned to NH₂ proton. Later, the donor atoms SH and OH showed a shift to a lower frequency because of the coordination of metal ions.

As opposed to melatonin, which has distinct peaks denoted by letters "e-n" that stand for the -CH₃-, two -CH₂-, CH₃O-, three phenyl -CH-, cyclic -CH-, and the two -NH- (indole and amide), respectively. These peaks were measured at various points, including (1.8, 2.79, 2.84, 3.79, 6.73, 7.24, 7.27, 7.98, and 10.69 and 10.68) ppm, respectively.

All the chemical shifts δ (ppm) of the peaks mentioned above were in agreement with literature^{19, 22}. As shown in Figs. 4–7 and Tables 3 and 4.

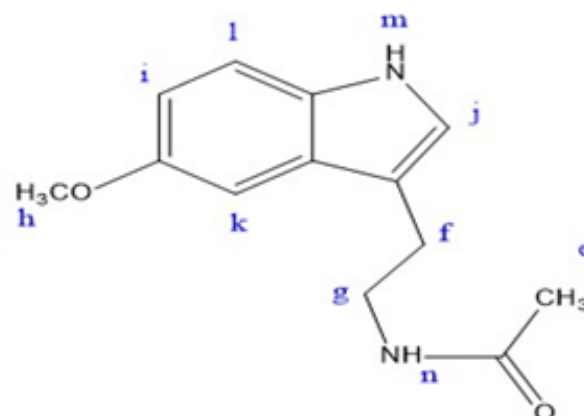


Figure 4. The structural formula of the melatonin (L1).

Table 3. ¹H-NMR data of the Melatonin(L1).

Assignments in d-DMSO ⁶	Mark	Chemical shifts δ (ppm)
CH ₃	E	1.80
CH ₂	F	2.79
CH ₂	J	2.84
CH ₃ O	H	3.79
phenyl -CH-	I	6.73
phenyl -CH-	J	7.24
phenyl -CH-	K	7.27
cyclic -CH-	L	7.98
Indole-NH-	M	10.69
amide-NH-	N	10.68

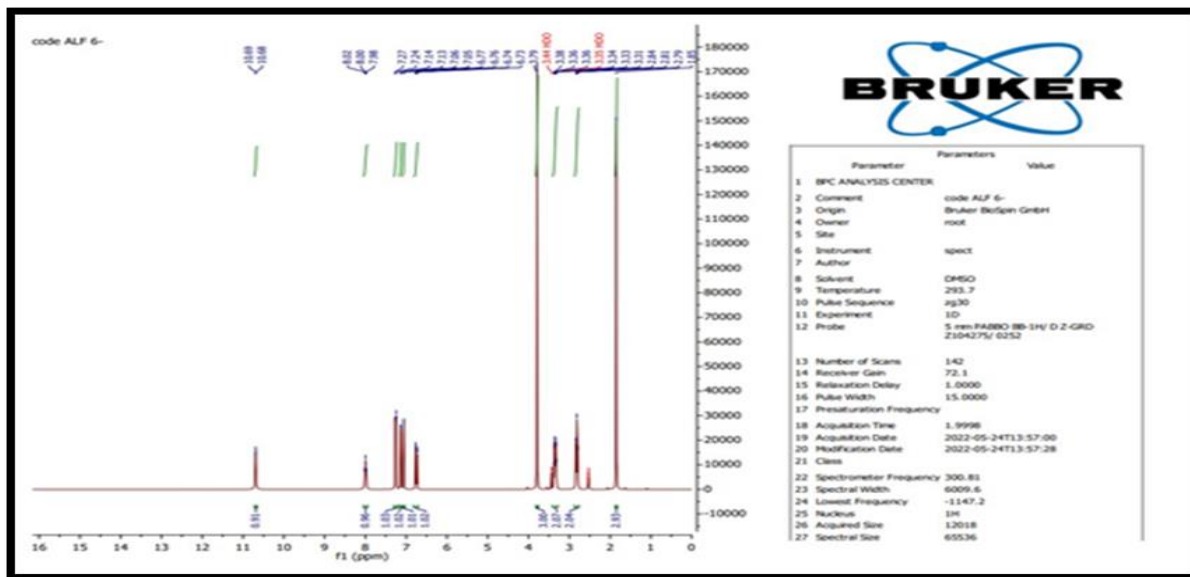


Figure 5. ¹H-NMR spectrum for the melatonin (L1).

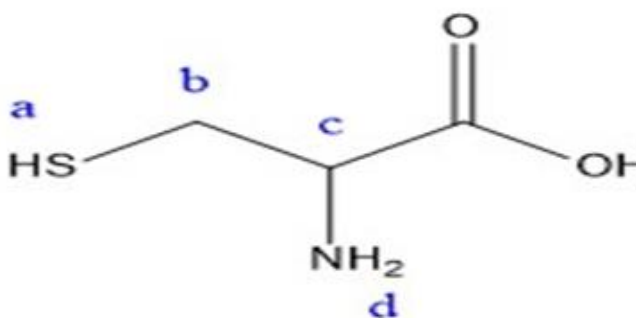


Table 4. ¹H-NMR data of the Cysteine (L2).

Assignments in d ⁶ -DMSO	Mark	Chemical shifts δ (ppm)
SH proton	A	1.26
CH ₂ proton	B	(2.79_2.93)
CH proton	C	(3.31_3.33)
NH ₂ proton	D	6.61

Figure 6. The structural formula of the cysteine (L2).

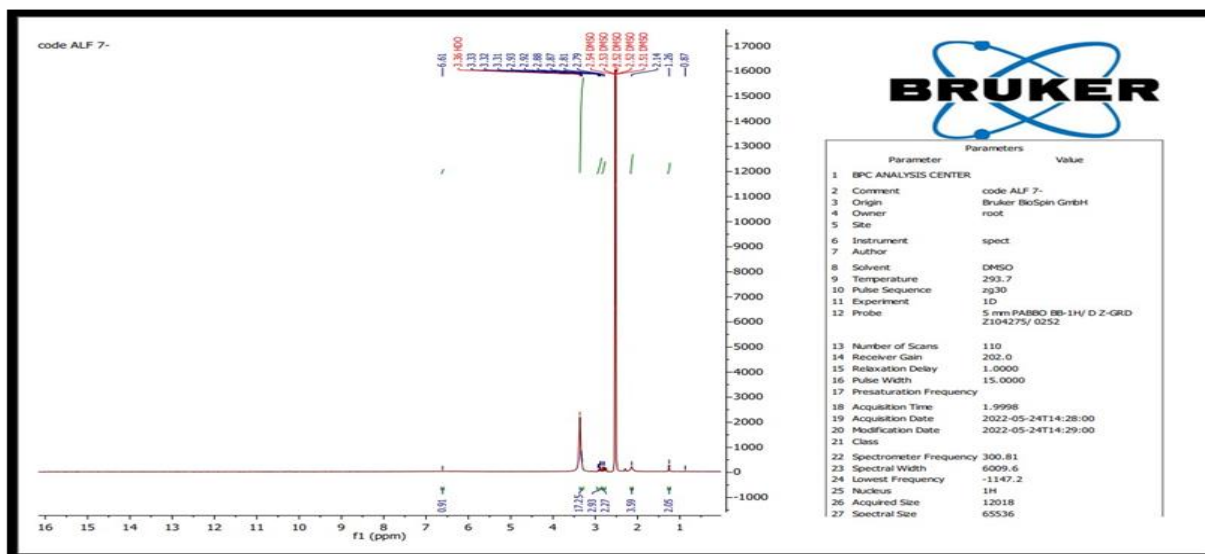


Figure 7. ¹H-NMR spectrum of the cysteine (L2).

Thermal analysis of the synthesized complex

The TG analysis of the complex was performed under nitrogen gas at temperatures ranging from 25 to 800) °C and a heating rate of (10 °C/min)²³. Fig .8. This technique (heat dissociation) was used to evaluate the thermal stability of the synthesized compound and to describe its proposed structure. The weight losses for the compound obtained from the thermal graph were utilized to calculate the

decomposed species. The water molecule outside the coordination sphere (lattice water) was lost in the first step, and at higher temperatures, the water molecule within the coordination sphere was lost as well, followed by other lightweight components of the complex.

Fig. 9. Since the results showed good agreement in the real and theoretical percentages of mass loss, Table 5

Table 5. Thermal decomposition data of the Cu-complex.

Comp.	Molecular formula and Molecular weight g/mole	Steps	Temp. range of the Decomposition °C	Suggested Formula of mass loss	Mass loss%	
					Calc.	Found
[Cu(L1)(L2)Cl ₂ .H ₂ O]	CuC ₁₆ H ₂₇ N ₃ O ₆ SCl ₂ 523.92	1	25-210	C ₁ H ₆ O ₂ Cl ₂	23.09	22.23
		2	210-425	C ₇ H ₁₄ N	21.37	21.51
		3	425-800	C ₃ H ₄ N	10.30	9.35
		Residue	800<	C ₅ H ₃ NO ₄ SCu	45.15	46.91

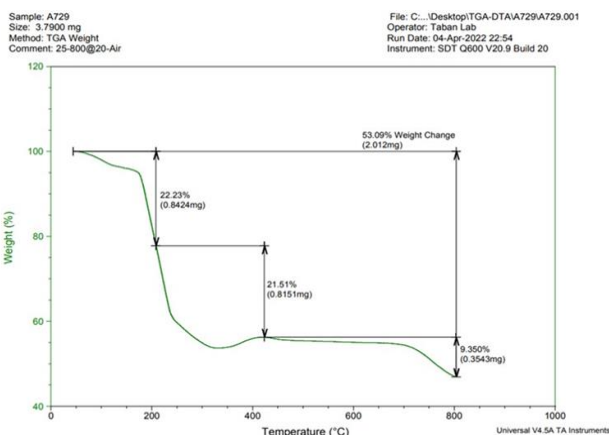


Figure 8. Thermogram of Cu-complex.

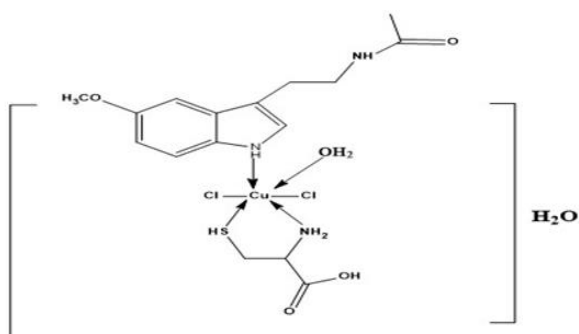


Figure 9. The suggested structural formula of the Cu-complex.

Electronic spectra (UV-Visible) of the ligands and their Cu-complex

The electronic absorption data of the ligands (L1, L2) and their metal ion complex in (1*10⁻⁴ M) were

recorded in DMSO at room temperature, and they are shown in Table 6 and Figs. 10, 11, 12. The electronic spectrum of the first ligand melatonin (L1) exhibits one band at 218.2 nm (45829) cm⁻¹ due to (π → π*) transition²⁴. The electronic spectrum of the second ligand cysteine (L2) exhibits one band at 277 nm (36101) cm⁻¹ due to (π → π*) transition¹⁹.

The electronic spectrum of Cu-complex was shown in the d-d transition:

$$\nu_1 = ({}^2B_{1g} \rightarrow {}^2A_{1g}) \text{ 924 nm, } 10822\text{cm}^{-1}.$$

$\nu_2 = ({}^2B_{1g} \rightarrow {}^2B_{2g})$ was obscured with CT at (673 nm, 14858 cm⁻¹). Because this transition takes place within the Jahn-Teller deformation, the octahedral forms the D_{4h} shape²⁵.

Table 6. Electronic spectra and suggested geometry of metal complexes

Comp.	Positions of the bands nm (cm ⁻¹)	Assignment	Geometry suggestion
L1	218.2(45829)	(π → π*)	-
L2	277(36101)	(π → π*)	-
Cu-complex	267(37453) 673(14858) 924(10822)	(π → π*) ${}^2B_{1g} \rightarrow {}^2B_{2g}$ ν_2 ${}^2B_{1g} \rightarrow {}^2A_{1g}(\nu_1)$	Distorted octahedral

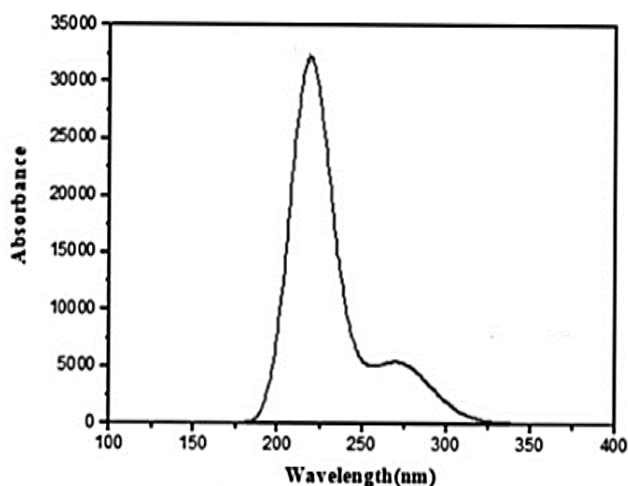


Figure 10. Electronic spectrum of Melatonin(L1) in DMSO.

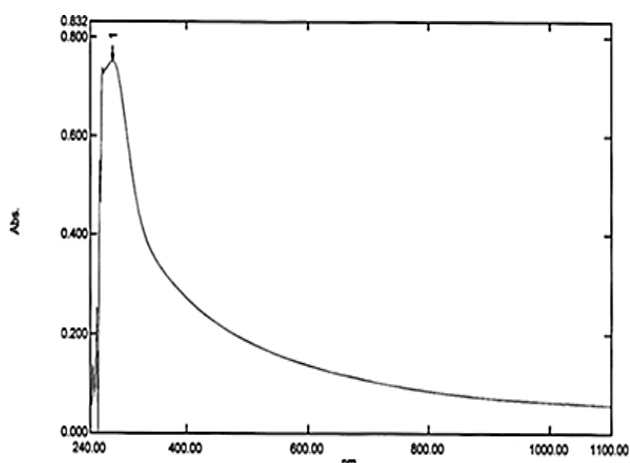


Figure 11. Electronic spectrum of Cysteine(L2) in DMSO.

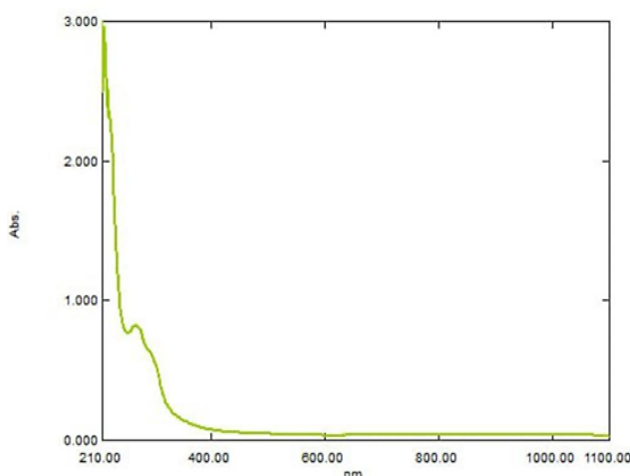


Figure 12. Electronic spectrum of Cu-complex in DMSO.

Antimicrobial activity

The antibacterial and antifungal activity of the synthesized complex was tested against *Escherichia coli* (G-) (*E. coli*), *Staphylococcus aureus* (*S. aureus*) (G+), and fungi (*Candida*) (*C. albicans*)]. The results showed that the synthesized complex (Cu(II) complex) and ligands have good activity against gram-negative *Escherichia coli* and very good activity against *Staphylococcus aureus*, and it is noted that the complex has more activity than the ligands. As shown in Table 7 and Figs. 13, 14.

The results indicated that L1 and L2 have activities approximately equal to each other in *Escherichia coli* (G-), while L1 was more active than L2 in *Staphylococcus aureus* (*S. aureus*) (G+), according to the following activity order: L1>L2, depending on inhibition zone (8>5) mm. The inhibition diameters were measured for the evaluation of antimicrobial activity.

Table 7. The biological activity for studied compounds in (10-3M).

Compound	Inhibition zone <i>Escherichia coli</i> (-)	Inhibition zone <i>Staphylococcus aureus</i> (+)	<i>Candida</i> a
MLT (L1)	7	8	-
CYS (L2)	6	5	-
Cu(II)-complex	12	14	14
Amoxicillin	15	14	---

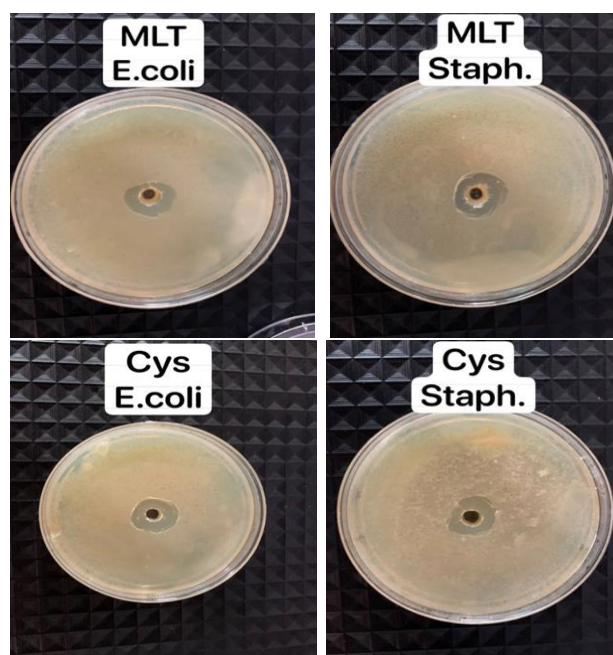


Figure 13. The inhibition zones versus bacterial gram positive and gram negative of two ligand.

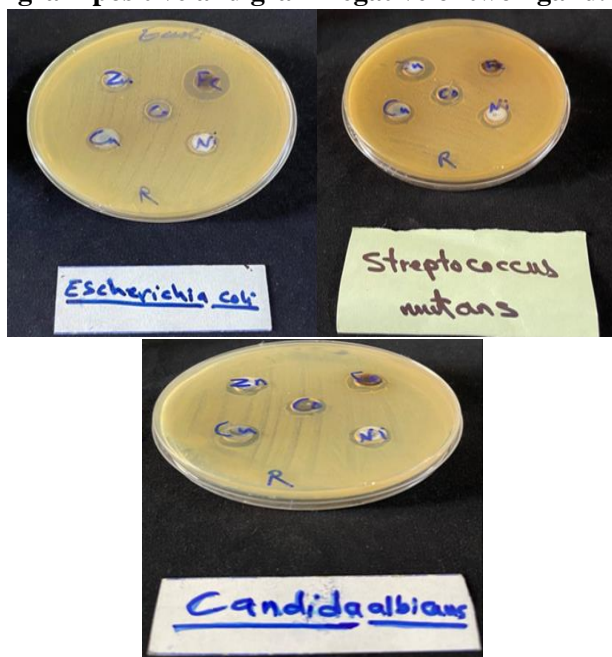


Figure 14. The inhibition zones versus bacterial gram positive, gram negative and Candida of Cu-complex.

Anticancer activity

The cytotoxic effect of the Cu-complex on human colon cells (CACO⁻²) was tested using the 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (MTT) assay procedure in various concentrations²⁶, (HdFn: normal cell, CACO⁻²: cancer cell), the results exhibited that the Cu-complex had the ability to inhibition (CACO⁻²) cells viability in different concentration at range 400 – 6.25 µg/mL and slight inhibition for normal cells at the same concentrations As shown in Table 8 and Fig. 15.

Table 8. Cytotoxicity effects of Cu complex against CACO⁻² tumor cell line and normal cell line HdFn

Cell line	Conc. µg/ml	400	200	100	50	25	12.5	6.25	IC ₅₀	P value
CACO ⁻²	42.82±2.7	52.43±5.1	67.25±1.4	74.54±5.4	87.08±3.5	96.76±1.1	96.30±0.	101.	<0.000	
	7	2	5	2	1	4	81	6		
HdFn	71.95±0.8	80.03±3.8	84.34±2.6	86.07±1.9	95.22±0.8	95.95±1.0	95.95±0.	416.	8	
	1	8	6	2	2	3	20	8		

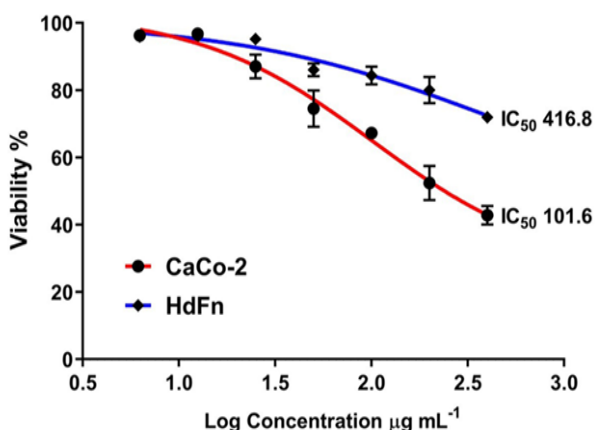


Figure 15. Cytotoxic effect of [Cu(MLT)(CYS)Cl₂]₂H₂O on CACO⁻² and HdFn cells after 24-hour incubation at 37 °C.

From the result above, it concluded that the Cu-complex needs a low concentration to kill the CACO⁻² cell line and at the same time its effect is little on normal cells (HdFn).

Conclusion

Melatonin and cysteine were reacted with $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ in a mole ratio of 1:1:1 (L1:M:L2), yielding a new metal complex. The produced compound was analyzed, and the suggested structure was supported using spectral and physicochemical approaches. According to the findings, the compound has octahedral geometry and a non-

electrolyte feature. The scientific results demonstrated that the synthesized complex has high antibacterial action against *Escherichia coli* (G-) (*E. coli*), *Staphylococcus aureus* (G+), and *Candida albicans*. The synthesized complex that was created has anticancer properties.

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Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Authors sign on ethical consideration's approval.
- Ethical Clearance: The project was approved by the local ethical committee at University of Baghdad.

Authors' Contribution Statement

E.A.M.: I did the conception of paper and acquisition and analysis of the data. B.I.A.: conceived the idea of the paper.

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معقد مختلط الليكاند: تحضير وتشخيص واستقصاء النشاط الحيوي الطبي

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الخلاصة

تحضير مركب جديد، تم تحضير معقد النحاس الجديد عن طريق تفاعل $CuCl_2 \cdot 2H_2O$ مع الميلاتونين وأحد الأحماض الأمينية (-L-cysteine) بنسبة مولية (1:1:1) (M:L1:L2)، وتم تشخيص المعقد بواسطة طرق طيفية تتضمن الأشعة تحت الحمراء (FT-IR) والرنين النووي المغناطيسي للبروتون (^1H-NMR) والتحليل الحراري (TG) والامتصاص الذري للهببي (AAS)، وتحليل العناصر الدقيقة (CHNS)، وكذلك قياس درجة الانصهار (m.p) وتقدير محتوى الكلوريد. من معطيات الطرق الطيفية المشار إليها أنفا تشير إلى أن الشكل الهندسي المقترح للمعقد هو ثماني السطوح. أظهرت قياسات التوصيل الكهربائي والمغناطيسية بأن معقد النحاس غير الكتروليتي وذي صفة بارامغناطيسية. تم فحص فعالية تركيز المعقد المحضر كعامل مضاد للبكتيريا من نوع *Escherichia coli* (G-) و *Staphylococcus aureus* (G+) ومضاد للفطريات (*fungus Candida*) حيث أظهر المعقد فعالية أفضل من الليكاندين وتم اختبار خاصيته كمضاد للسرطان.

الكلمات المفتاحية: النشاط الطبي الحيوي، معقد النحاس، سيسئين، ميلاتونين، مزيج الليكاندات.