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# Synthesis and Anti-Microbial Activities Investigation of New Mixed Ligand Complexes

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**Abstract**

Transition ion complexes have various commercial and technical uses, including antibacterial, antifungal, and anticancer medications and catalysts; their characteristics are critical regarding their kinetic impact. This study aims to synthesize new complexes, copper and nickel complexes, characterize them using spectral techniques, determine their chloride content, evaluate their anti-oxidant effect, and examine the antibacterial activity of the ligands and their complexes against gram-positive bacteria (*Staphylococcus*), gram-negative bacteria (*Escherichia coli*), and *Candida albicans*. All chemicals were used strictly as provided, with no additional purification; $CuCl_2.2H_2O$ and $Ni(CH_3CO_2)_2$ salts were used 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 mixed ligand metal complexes. The results showed that the two metal complexes were more active in ($10^{-3}$ M) and had higher activity than the two ligands (melatonin and cysteine). According to the findings, the complexes have suggested structures: octahedral geometry for the Cu(II) complex and tetrahedral geometry for the Ni(II) complex. The scientific results demonstrated that the synthesized complexes have high antibacterial action against [*Escherchia coli (G-) (E. coli), Staphylococcus aureus (G+)*], and *antifungal action* against [*Candida (C. albicaus*)].

**Keywords:** Antimicrobial, copper complex, cysteine, nickel complex, melatonin, mixed ligand

## 1. Introduction

Recent research has shown that mixed ligand complexes are essential in biological systems (1) and highly active against pathogenic microorganisms (2). Depending on the number of ligands

linked to the metal ion, mixed ligand complexes can be di-ligand, tri-ligand, tetraligand, or multi-ligand complexes. Developing mixed ligand complexes and their characteristics is critical regarding their kinetic impact (3). Transition ion complexes have various commercial and technical uses, including antibacterial, antifungal, and anticancer medications and 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 complex increases the chances of variation in the expected properties of the complex (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 and tumor size reduction (1).

N-acetyl-5-methoxytryptamine, often known as melatonin (MLT), is a hormone 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 and other possible applications. Although applied topically in cosmetic goods, it also acts as a skin protectant against UV rays (8, 9). Melatonin has pharmacological effects on Alzheimer's disease therapy (10), Parkinson's illness (11), breast cancer, depression, and glaucoma, breast and prostate cancer (5). One of the non-essential amino acids is cysteine (Cys). Approximately 2% of proteins contain cysteine, which has a sulfur group in its structure. The sulfhydryl group of (CySH) plays an essential function in the biological activity of enzymes and proteins (12). Even though it is described as a"non-essential" amino acid, it becomes necessary in conditions of high nutrient demands. In the liver, a metabolic pathway called transsulfuration permits the supply of cysteine by converting an essential amino acid: methionine (13). It has many roles and applications, such as being necessary for tissue and cell growth in organisms (14), acting as an antibiotic to repair injured skin (15), acting as a radio-protective agent, and treating Parkinson's disease (16, 17). The metal complex was discovered to have higher antimicrobial activity, antifungal, and anticancer drugs than their parent ligands in all these cases.

This study aims to synthesize new complexes, copper and nickel complexes, characterize them using spectral techniques, determine their chloride content, evaluate their anti-oxidant effect, and examine the antibacterial activity of the ligands and their complexes against gram-positive bacteria (*Staphylococcus*), gram-negative bacteria (*Escherichia coli*), and *Candida albicans*.

## 2. Materials and Methods

### 2.1. 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. 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 $^1$H-NMR spectroscopy in $d^6$-DMSO.

**2.2. Synthesis of complexes**

Mixed ligand complexes were prepared from copper salts as chloride ($CuCl_2.2H_2O$), and nickel salts as acetate $Ni(CH_3CO_2)_2$, melatonin (L1), as a first ligand and amino acid (cysteine) (L2), as a second ligand. To an aqueous solution (10 mL) of (1 mmol) Cu(II), Ni(II), (0.134 g, 0.176 g respectively), an aqueous solution (10 mL) of melatonin (0.232.2 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. An aqueous solution (5 mL) of cysteine (0.121 g, 1 mmol) was added to this hot solution with constant stirring. The PH of the resulting mixture was adjusted to 7.5-8 with NaOH (NaOH as a regulator for the PH of the medium), and the mixture was heated again in a water bath (reflux 3.5 h). during this period, a light gray color appeared on the precipitated Cu-complex and a light green color on the precipitated Ni-complex. The precipitate was collected by filtration, washed with deionized water, then with absolute ethanol, dried with ether, and then put in the oven.

**2.3.Biomedical evaluation**

The antibacterial and antifungal activity of the synthesized complexes 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* (*C. albicans*). The inhibition diameters were measured to evaluate antimicrobial activity (18).

**3. Results and Discussion**

**Table 1** shows the data on metal content (atomic absorption), CHNS, physical properties, and the names of the ligands (L1, L2) and their metal complexes. The molecular formulae of studied compounds were suggested based on CHNS, atomic absorption analysis, chloride content, and spectral data.

**3.1. The FTIR spectroscopy of the complexes**

In characteristics of L-cysteine chelated, a band at (3307, 3282) $cm^{-1}$ was noticed due to stretching vibration of ($NH_2$) in the complexes of Cu(II), Ni(II), which differed slightly from the band seen in free cysteine indicating coordination through the nitrogen atom of the amino group of Cysteine (19). Strong band assigned to NH (indole) of melatonin at 3473 $cm^{-1}$ shifted to (3244, 3272) $cm^{-1}$ in Cu(II), Ni(II) complexes respectively, 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 metal (20). The Cysteine ligand spectrum showed 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 mixed ligand complex was also shifted to a lower frequency, indicating coordination of L-cysteine via the sulphur atom (20). New bands formed at (449-424) $cm^{-1}$ Cu(II) and Ni(II) complexes, respectively, indicating the appearance of metal ions coordinated via the N atom of the ligand (21).

At low frequencies appeared bands in the spectra of complexes attributed to υM-O, υM-Cl, and υM-S (18), as shown in **Table 2** and **Figures 1-4**.

**Table 1.** Analysis of data and physical properties of the two ligands and its metal complexes.

| Compound | The molecular formula | Color | Melting point (°C) | Yield % | Elemental Micro Analysis (Found) Calc. | | | | Metal found % Calc. (Found) | Chloride content % Calc. (Found) |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | C% | H% | N% | S% | | |
| MLT(L1) | $C_{13}H_{16}N_2O_2$ | White | 117 | - | 67.16<br>66.23 | 6.88<br>6.12 | 12.05<br>11.97 | - | - | - |
| L-CYS(L2) | $C_3H_7NO_2S$ | White | 240 decomposition | - | 29.71<br>29.01 | 5.77<br>4.92 | 0.8<br>0.8 | 26.41<br>26.33 | - | - |
| Cu(II)complex | $CuC_{16}H_{27}N_3O_6SCl_2$ | Light Gray | 198-200 | 98 | 36.64<br>35.98 | 5.15<br>5.94 | 8.01<br>8.30 | 6.10<br>7.06 | 12.12<br>12.60 | 13.55<br>12.73 |
| Ni(II)complex | $NiC_{20}H_{29}N_3O_8S$ | Light green | 242 | 98 | 45.27<br>45.33 | 5.47<br>5.65 | 7.92<br>8.18 | 6.03<br>6.87 | 11.07<br>10.11 | - |

**Table 2**. The spectral infrared data of two ligands and their metal complexes in ($cm^{-1}$)

| Compound | $H_2O$ Lattice (coordinate) | υNH | υNH indole | υOH | υSH | υC=O Carboxylic | υC-N | υC-S | S M- | υM-N | υMO | υM-Cl |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MLT | - | 3371 | 3473 | - | - | 1797 | 1157 | - | - | | - | - |
| CYS | - | 3176 | - | 3433 | 2551 | 1735 | 1197 | 692 | - | | - | - |
| Cu(ll) complex | 3436 (918) | 3307 | 3244 | 3467 | 2360 | 1712 | 1137 | 671 | 341 | 449 | - | 293 |
| Ni(ll) Complex | - | 3282 | 3272 | 3475 | 2375 | 1701 | 1188 | 678 | 347 | 424 | 457 | - |

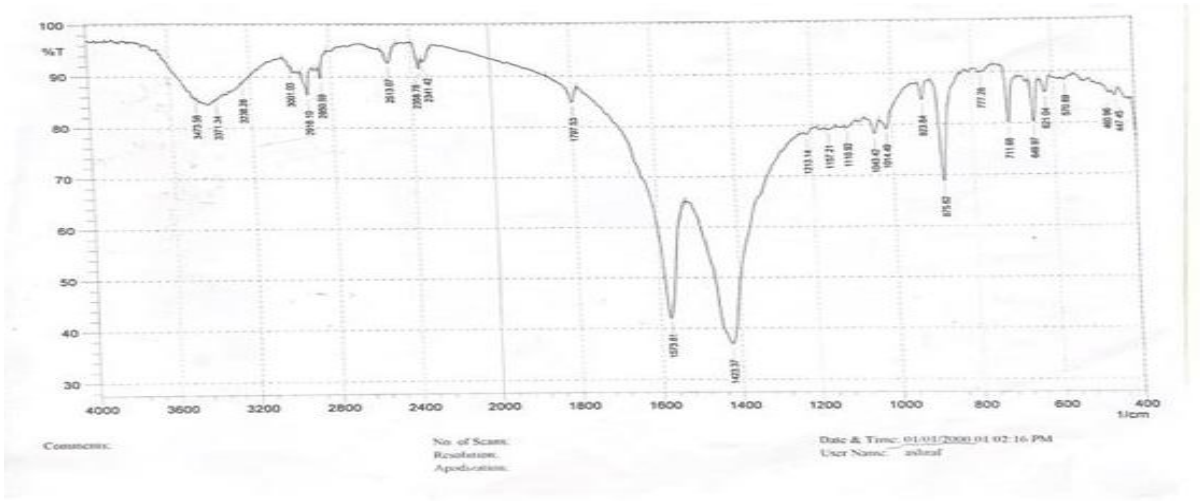


**Figure 1.** The FT-IR Spectrum of Melatonin(L1).

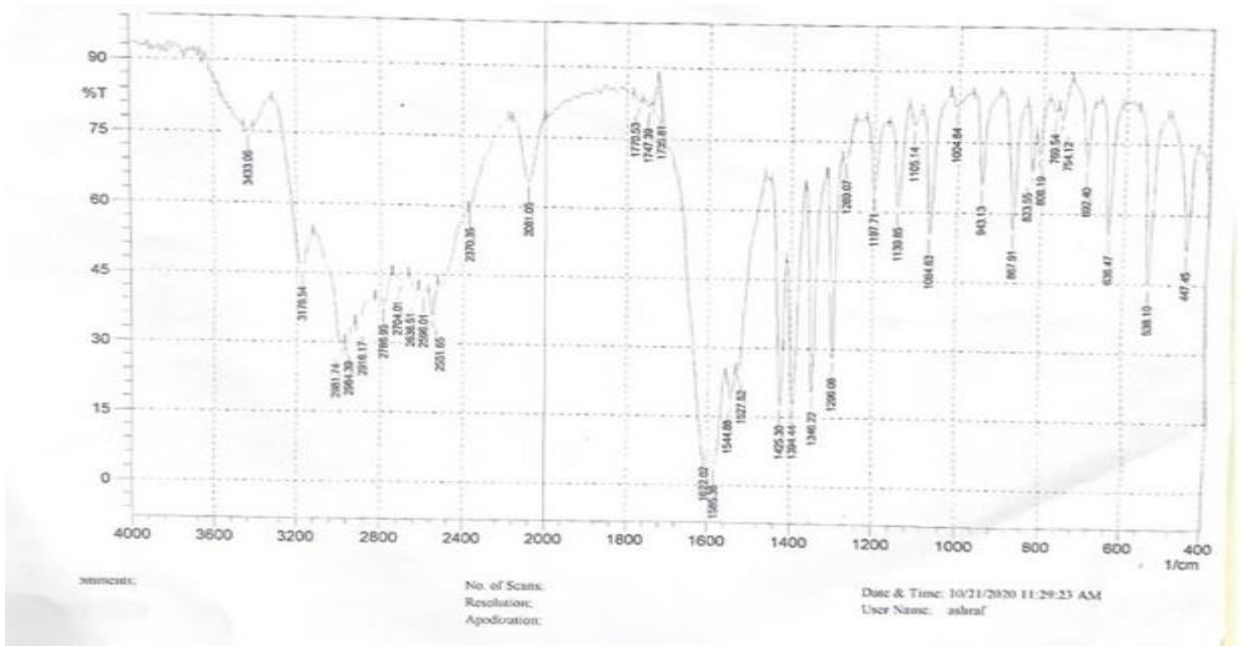


**Figure 2.** The FT-IR spectrum of cysteine (L2).

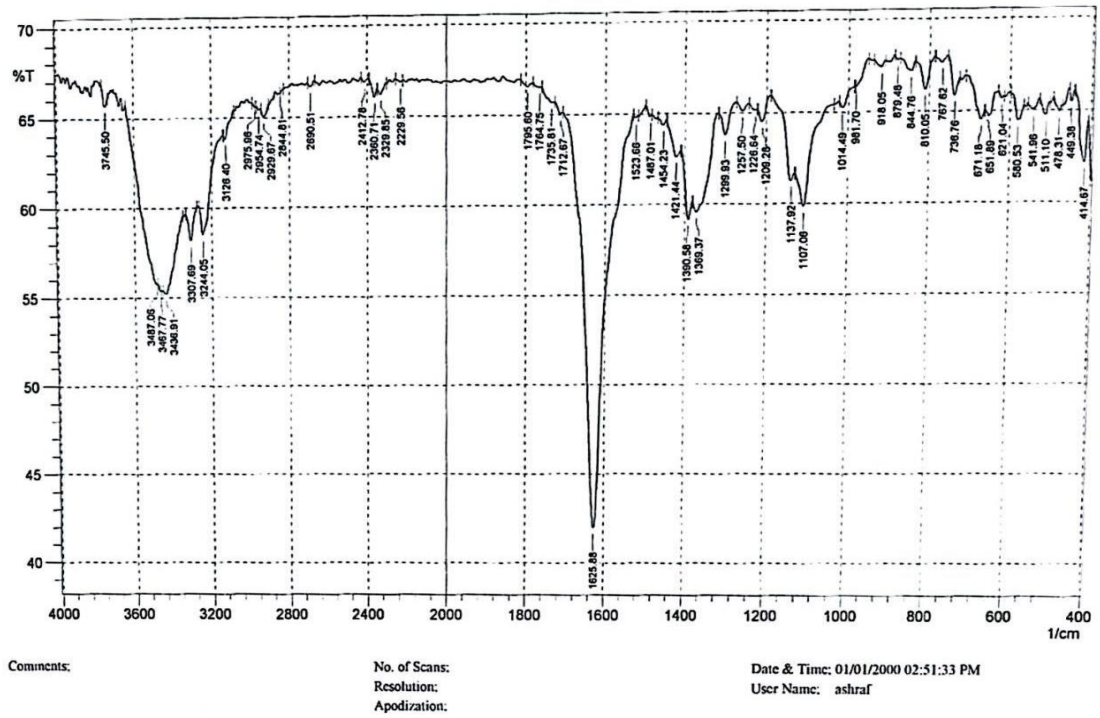


**Figure 3.** The FT-IR spectrum of Cu-complex.

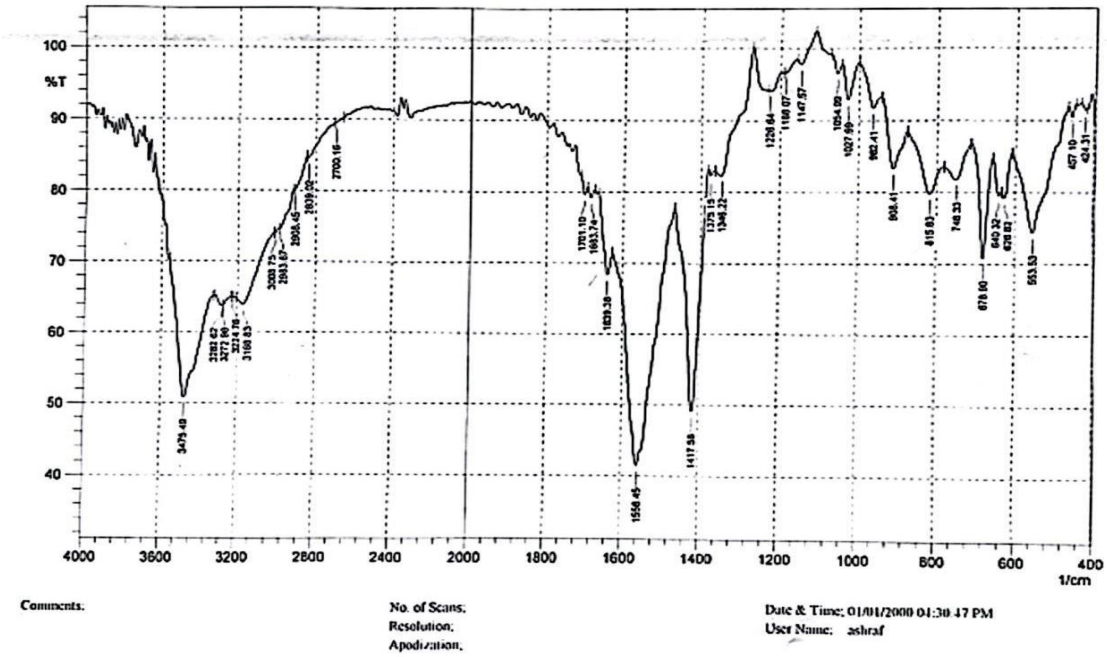


**Figure 4.** The FT-IR spectrum of Ni-complex.

### 3.2. The $^{1}$H-NMR

The $^{1}$H-NMR spectra of the two ligands showed characteristic peaks of (SH, CH2, CH, NH2) for cysteine and aromatic protons, cyclic protons ($CH_3$, $CH_2$, $CH_2$, $CH_3O$) for melatonin. All the chemical shifts δ (ppm) of the peaks mentioned above agreed with the literature (20, 22). **Figures 5-8** and **Tables 3, 4** show this.

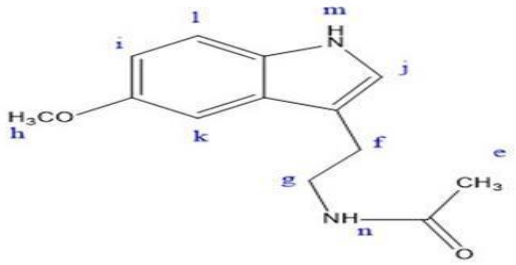


**Figure 5.** Structure formula of melatonin (L1).

**Table 3.** The $^{1}$H-NMR data of the melatonin (L1).

| Assignments in d$^{6}$ -DMSO | Mark | Chemical shifts δ (ppm) |
|---|---|---|
| $CH_3$ | e | 1.80 |
| $CH_2$ | f | 2.79 |
| $CH_2$ | j | 2.84 |
| $CH_3O$ | 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 |

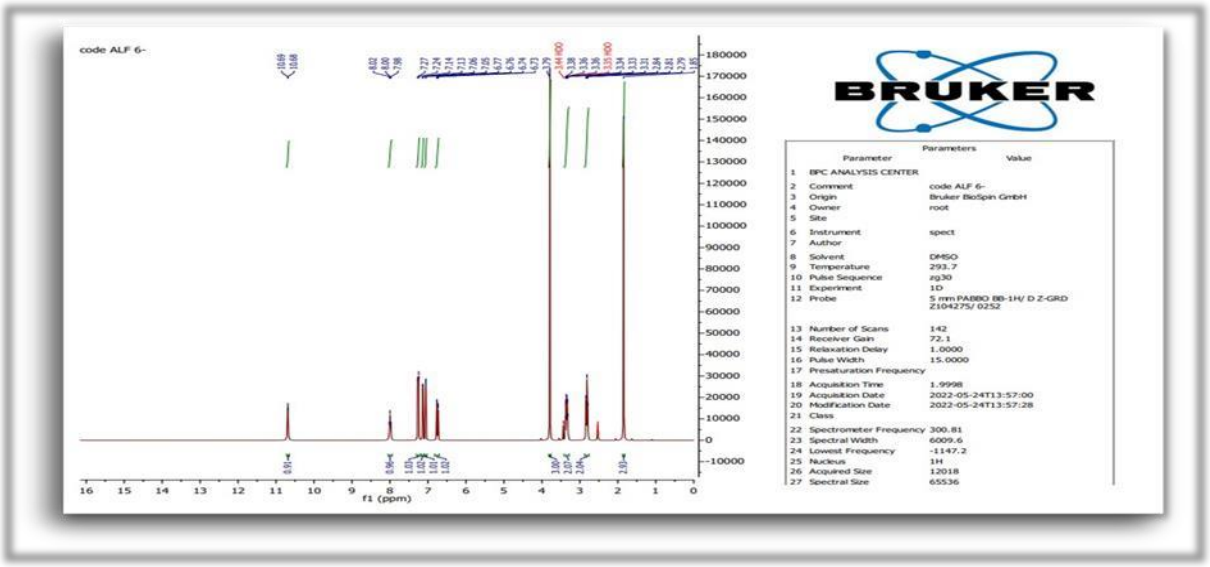


**Figure 6.** The $^{1}$H-NMR spectrum for the melatonin (L1).

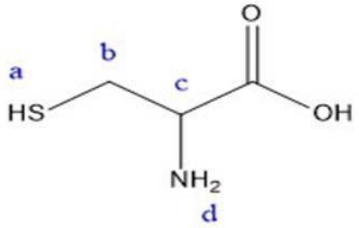


**Figure 7.** Structure formula of Cysteine (L2).

**Table 4.** The [1] H-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) |
| NH2 proton | d | 6.61 |

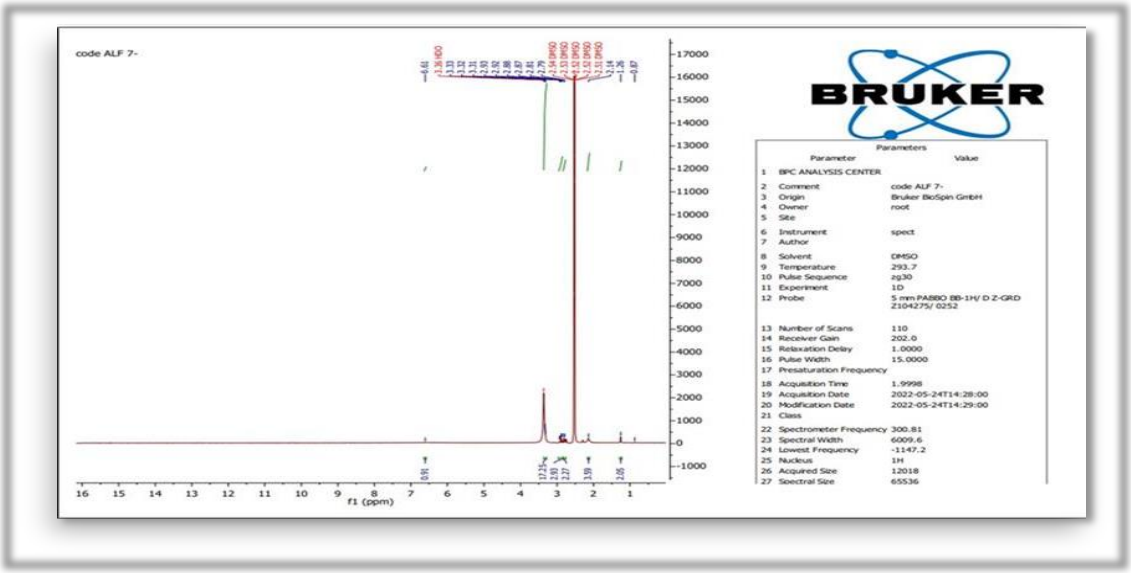


**Figure 8.** The [1]H-NMR spectrum for the cysteine (L2).

## 3.3. Electronic spectra (UV-Visible)

### 3.3.1. Electronic spectra of the ligands (L1, L2)

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 5** and **Figures 9-12**.

The electronic spectrum of the first ligand melatonin (L1) exhibits one band appeared at 218.2 nm (45829) $cm^{-1}$ due to ($\pi \rightarrow \pi^*$) transition (23), the electronic spectrum of the second ligand cysteine (L2) exhibits one band appeared at 277 nm (36101) $cm^{-1}$ due to ($\pi \rightarrow \pi^*$) transition (20).
The electronic spectrum of the Cu-complex was shown in the (d-d) transition. υ1 =[$^2B_1g \rightarrow {}^2A_1g$] 924 nm,10822 $cm^{-1}$.
υ2=($^2B_1g \rightarrow {}^2B_2g$) was obscured with CT at (673 nm, 14858 $cm^{-1}$) (25). Because this transition occurs within the Jahn-Teller deformation, the octahedral forms the $D_4h$ shape.
While The Ni(II) complex (**Figure 10**) exhibited a shift of ligand, two bands showed at [1034 nm (9671 $cm^{-1}$) and 717 nm (13947 $cm^{-1}$)], which were assigned to [ $^3T_1(F) \rightarrow {}^3A_2$ and $^3T_1(F) \rightarrow {}^3T_1(P)$] transitions of Tetrahedral Ni(II) complex (18).

**Table 5.** Electronic spectra and suggested geometry of metal complexes

| Compound | Positions of the bands nm ($cm^{-1}$) | Assignment | Geometry suggestions |
|---|---|---|---|
| L1 | 218.2(45829) | ($\pi \rightarrow \pi^*$) | - |
| L2 | 277(36101) | ($\pi \rightarrow \pi^*$) | - |
| Cu-complex | 267(37453) | ($\pi \rightarrow \pi^*$) | Distorted |
| | 673(14858) | $^2B_1g \rightarrow {}^2B_2g$(υ2) | octahedral |
| | 924(10822) | $^2B_1g \rightarrow {}^2A_1g$(υ1) | |
| Ni-complex | 717(13947) | $^3T1(F) \rightarrow {}^3T1(P)$(υ1) | Tetrahedral |
| | 1034(9671) | $^3T_1(F) \rightarrow {}^3A_2(F)$(υ1) | |

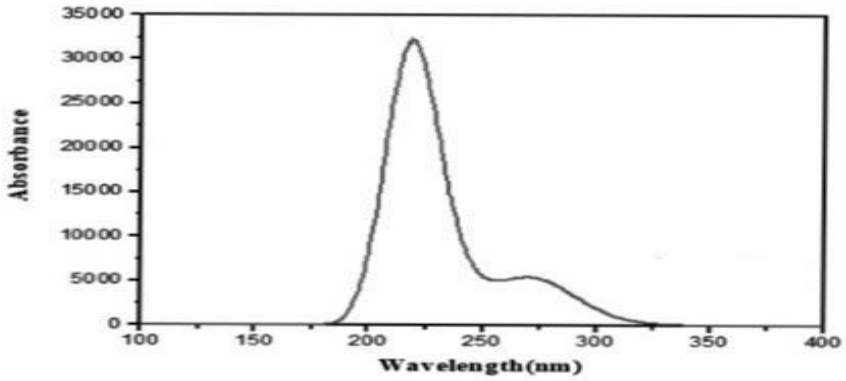


**Figure 9.** Electronic spectrum of melatonin(L1) in DMSO.

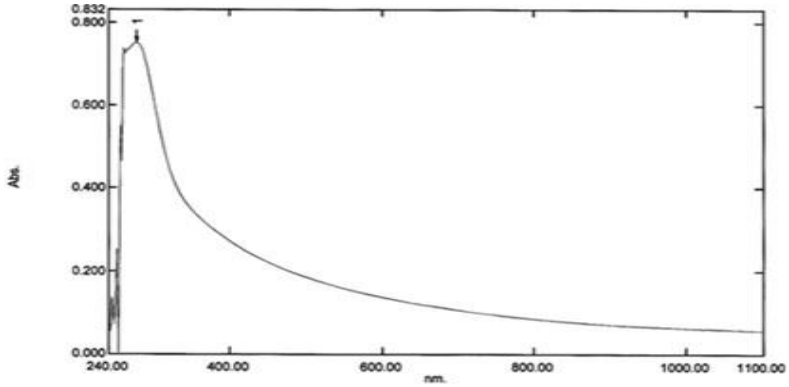


**Figure 10.** Electronic spectrum of cysteine(L2) in DMSO.

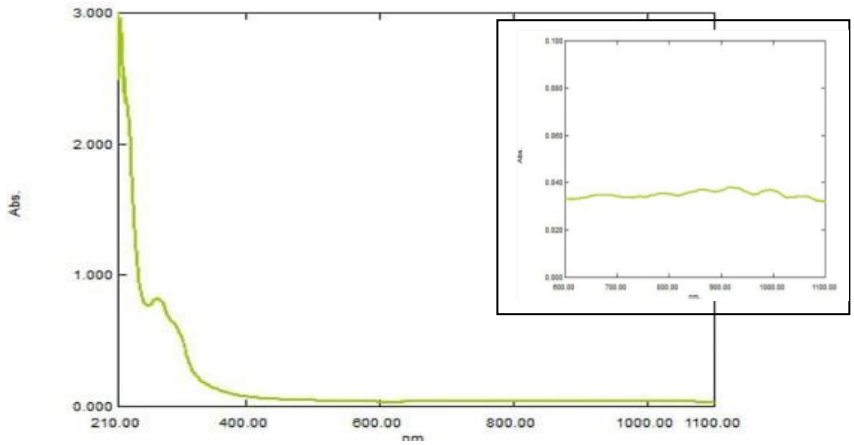


**Figure 11.** Electronic spectrum of Cu-complex in DMSO.

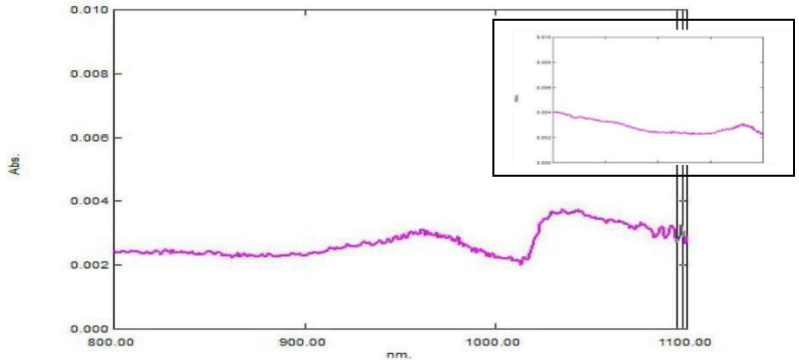


**Figure 12.** Electronic spectrum of Ni-complex in DMSO.

### 3.4. Thermal analysis of synthesized complexes

The complex TG analysis was performed under nitrogen gas at temperatures ranging from (01000) °C and (10 °C/min). This technique (heat dissociation) was used to evaluate the thermal stability of the synthesized complexes and to describe their proposed structures (24), **Figures 13-16** and **Table 6.** The thermal stability of metal complexes increases in the following orders: (Ni-complex < Cu-complex). Since the results showed good agreement in practical and theoretical percentage of mass loss.

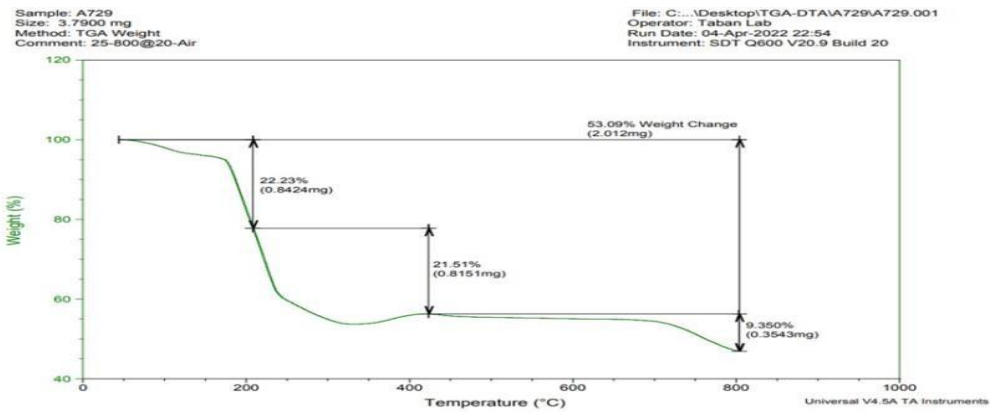


**Figure 13.** Thermogram of Cu-complex.

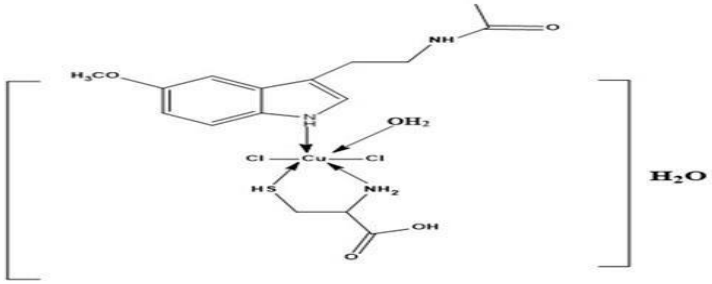


**Figure 14.** The structure formula of Cu-complex.

**Table 6.** Thermal decomposition data of the complexes.

| Comp. | Molecular formula and Molecular weight g/mole | Steps | Temp. rang of Decomposition °C | Suggested the Formula of loss | Mass loss% Cal. | Mass loss % Found |
|---|---|---|---|---|---|---|
| [Cu(MLT)(Cys)$Cl_2$.2$H_2O$] | CuC16H27N3O6SCl2 | 1 | 25-210 | C1H6O2Cl2 | 23.09 | 22.23 |
| | 523.92 | 2 | 210-425 | C7H14N | 21.37 | 21.51 |
| | | 3 | 425-800 | $C_3H_4N$ | 10.30 | 9.35 |
| | | residue | 1000 | $C_5H_3NO_4SCu$ | 45.15 | 46.91 |
| [Ni(MLT)(Cys)] | NiC20H29N3O8S | 1 | 25-100 | C1H6 | 3.41 | 3.41 |
| | 530.22 | 2 | 100-275 | C7H10N3 | 25.64 | 25.68 |
| | | 3 | 275-340 | C7H10S | 24.30 | 23.76 |
| | | 4 | 340-800 | C5H3O3 | 20.93 | 21.29 |
| | | residue | 1000 | $NiO_5$ | 26.15 | 25.31 |

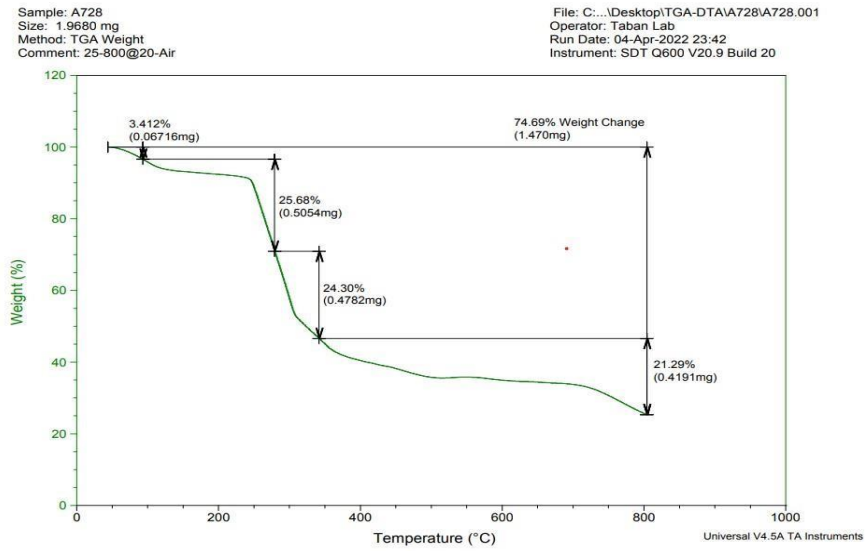


**Figure 15.** Thermogram of Ni-complex.

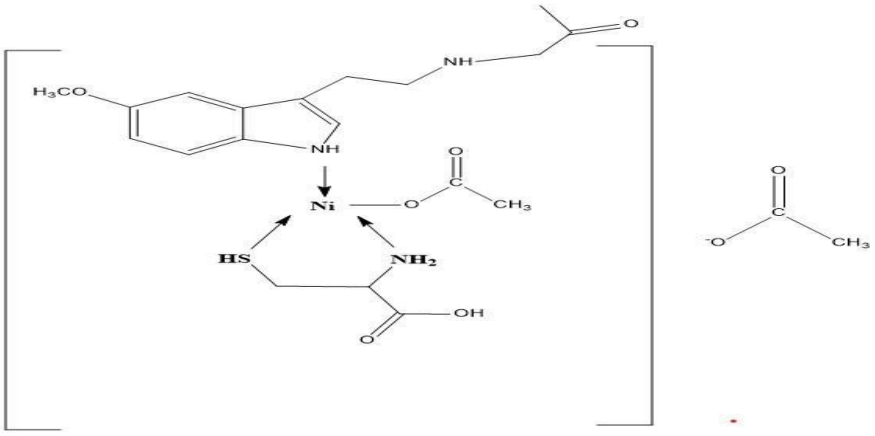

**Figure 16.** The structure formula of Ni-complex.

### 3.5. Antimicrobial activity

The antibacterial and antifungal activity of ligands and the synthesized complexes were tested against [ *Escherichia coli* (*G-*) (*E. coli*), *Staphylococcus aureus* (*S. aureus*) (*G+*), and fungi (*Candida*) (*C. albicaus)*], it took (0.04, 0.05) g from the Cu-complex, Ni-complex respectively then, dissolved in 5 mL of deionized water and the direct inhibitory effect of two complexes against pathogenic microorganisms was determined by well diffusion method under aerobic condition (24). The results indicated that the synthesized complexes [Cu-complex, Ni-complex] possess activity approximately equal to each other and have higher activity than the ligands. As shown in **Table 7** and **Figures 17**, **18**.

**Table 7.** The biological activity for studied compounds in ($10^{-3}$M).

| Compound | Inhibition zone Escherichia coli (-) | Inhibition zone Staphylococcus aureus (+) | Candida |
|---|---|---|---|
| **MLT** | 7 | 8 | - |
| **CYS** | 6 | 5 | - |
| **Cu(ll)-complex** | 12 | 14 | 14 |
| **Ni(ll)-complex** | 12 | 14 | 13 |
| **Amoxicillin** | 15 | 14 | --- |

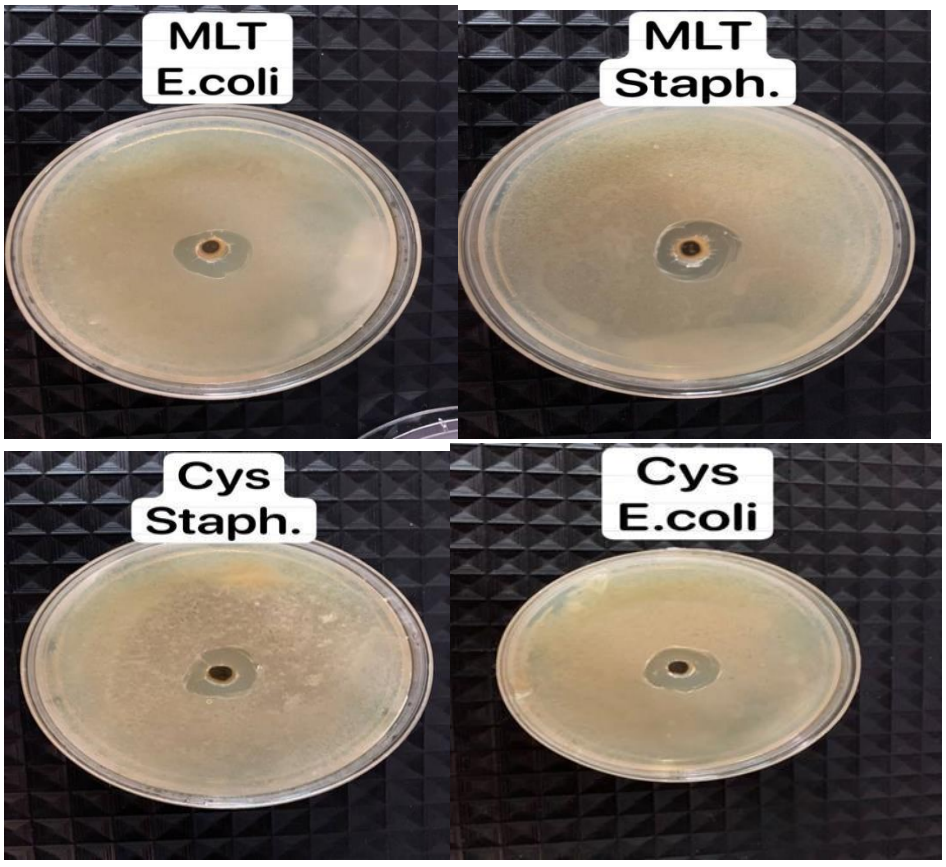


**Figure 17.** The inhibition zones versus bacterial gram positive and gram negative of two ligands.

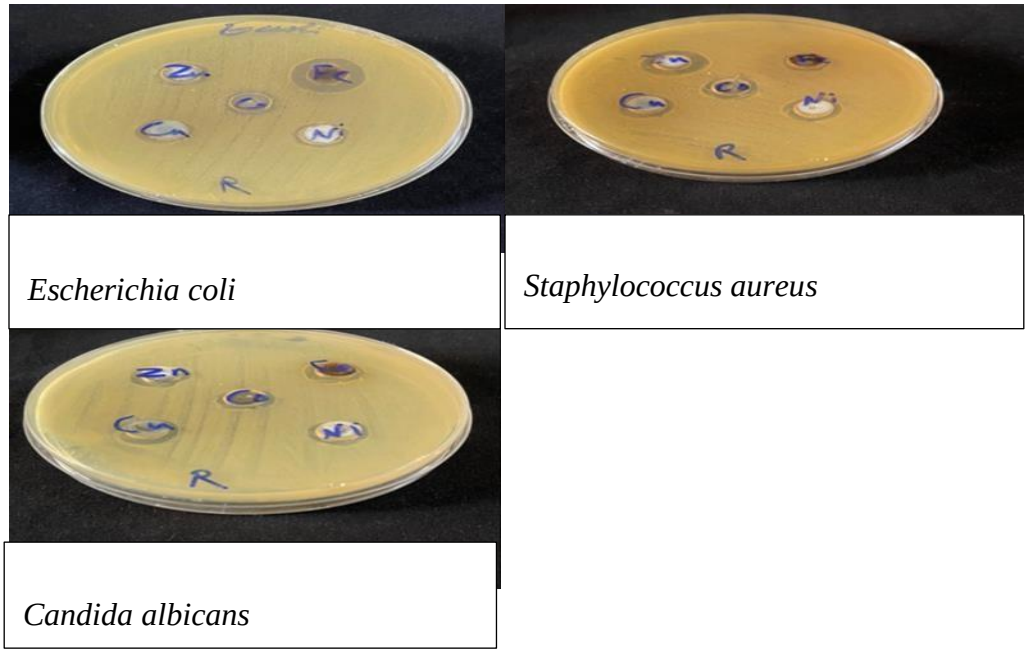


**Figure 18.** The inhibition zones versus bacterial gram positive, gram negative and Candida of Cu-complex, Ni-complex.

## 4. Conclusion

Melatonin and cysteine were reacted with $CuCl_2.2H_2O$, $Ni(CH_3CO_2)_2$ in a mole ratio 1:1:1 (L1:M:L2), yielding new metal complexes. The produced compounds were analyzed, and the suggested structures were supported using spectral and physicochemical approaches. According to the findings, the complexes have suggested structures: octahedral geometry for the Cu(II) complex, and tetrahedral geometry for the Ni(II) complex. The scientific results demonstrated that the synthesized complexes have high antibacterial action against *[Escherchiacoli (G-) (E. coli), Staphylococcus aureus (G+)]*, and antifungal action against *[Candida (C. albicaus)]*.

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## Conflict of Interest

The authors declare that they have no conflicts of interest.

## Funding

There is no funding for the article.

## Ethical Clearance

The study was conducted with ethical principles. The study protocol was reviewed and approved by Baghdad University, College of Science, a local ethics committee, according to document number CSEC/044/0071, on 26 September 2021 to get this approval.

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