Egypt. J. Chem. **Vol. 65,** No. 12 pp. 39 - 47 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Synthesis and Antimicrobial Activity of Copper and Zinc Complexes based on Benzimidazole scaffold

Nagwa M. Abdelazeem,[a,*] Farid M. Sroor,[a] Mohamed S. Abdel-Aziz[b] and Wahid M. Basyouni[a]

[a]*Organometallic and Organometalloid Chemistry Dept., National Research Centre, Dokki, Giza, Egypt*

[b]*Microbial Chemistry Dept., National Research Centre, Dokki, Giza, Egypt*

Abstract

The fundamental goal of the present study was carried out to generate novel metal complexes based on benzimidazole (BI) as a precursor with Cu and Zn cations. Herein, Cu-BI and Zn-BI complexes were prepared and characterized *via* Fourier transform infrared spectroscopy (FT-IR), UV–visible, ^{1}H NMR, and mass spectra. Moreover, antimicrobial activity of the benzimidazole, zinc, and copper complexes was evaluated using different methods as disc agar and colony forming techniques. The obtained results revealed that the Cu-BI complex could offer the best antimicrobial activity.

Keywords: Benzimidazole; Metal complexes; Antimicrobial Agents.

1. Introduction

There have been various studies were directed for the preparation of different metal complexes with the evaluation of their biological activity [1–6]. The use of biologically active ligands with transition metals yields new metal complexes with higher biological activity when compared with the coordinated ligands or the metal ion [7–10]. Benzimidazole scaffold is a good structure that displays great therapeutic and physiological activity, and is being to a monodentate ligand that coordinates with the heterocyclic nitrogen atom [11]. Upon a comprehensive survey, benzimidazole moiety exhibited an increase and incorporated activities such as antimicrobial [12, 13], antithrombotic, anticoagulant and antiplatelet [14], anthelminthic [15], anti-inflammatory [16, 17], acetylcholinesterase and antifungal [18], antiulcer [19], antitubercular and antiprotozoal [20], antimycobacterial [21], antileishmanial [22], antiviral [23], antitumor, [24] and anti-HIV agents [25]. In addition, benzimidazole was described as indoleamine 2,3-dioxygenase-1 (IDO1) [26], hepatitis C virus [27] inhibitors, anti-hypertensive agent [28], *in vitro* alpha-glycosidase inhibitory [29], Zika Virus inhibitors [30], antiglycation and antioxidant [31], NOD2 antagonists [32], and antileukemic agents [33]. Moreover, benzimidazole core was reported to used with high efficiency for the treatment of tuberculosis [34].

Recently, benzimidazole scaffolds were obtained as anti-cancer agents in many reviews [35, 36]. As a consequence, various drugs originating from benzimidazole have been founded on the market as omeprazole (Anti-ulcer), albendazole (antimicrobial), Bendamustine (Anti-tumor), Candesartan (Antihypertensive), Enviradine (Anti-viral), and Benoxaprofen Analog (Anti-inflammatory) [37] Figure 1. Also, the activities of benzimidazole with transition metal complexes have been studied, where many benzimidazole-metal complexes showed effectiveness greater than free ligand [38-40]. In recent years various metal complexes have been attracted much attention due to their contribution to cancer therapy [41, 42].

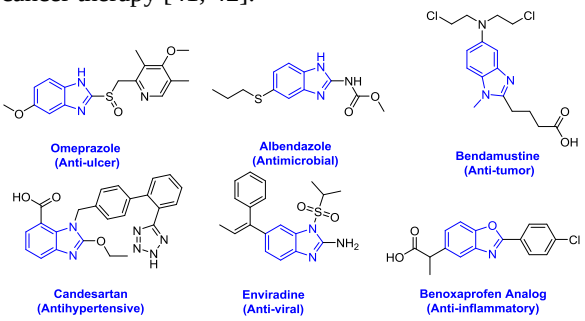


Figure 1: Chemical structure of biologically active Benzimidazole.

The main purpose of the present work was generating for synthesizing metal complexes that can offer antimicrobial activity. In this context, two complexes were prepared by the reaction between BI as an organic ligand and both Zn and Cu cations as inorganic species. FT-IR, ^{1}HNMR, UV-visible, and mass spectral determinations were used to characterize the structure of the synthesized complexes. Also,

*Corresponding author e-mail: nagwamorad@yahoo.com.; (Nagwa M. Abdelazeem).

Receive Date: 24 July 2022, Revise Date: 14 August 2022, Accept Date: 15 August 2022

DOI: 10.21608/EJCHEM.2022.152140.6592

antimicrobial activity of the complexes was studied by using both disc agar and colony forming techniques.

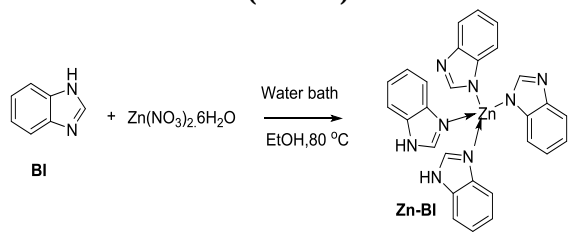
2. Materials and methods:

2.1. Materials:

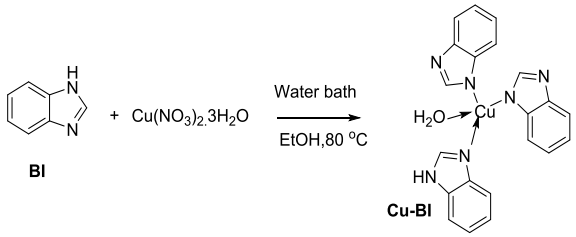
Benzimidazole was purchased from Merck Co., Copper nitrate $Cu(NO_3)_2 \cdot 3H_2O$ and zinc nitrate $Zn(NO_3)_2 \cdot 6H_2O$ were purchased from Sigma-Aldrich Co. Electrothermal 9300 melting point was used to determine melting points of the ligand and its complexes. The mass spectra were recorded with a GC/MS Finnigan SSQ 7000 spectrophotometer. Tensor 27 Brucker FT-IR spectrophotometer (4000-400cm^{-1}) with KBr disc was used to record IR spectra. The different NMR spectra were recorded with DMSO-d_6 as a solvent on a Bruker instrument at 500 MHz. UV-Vis. spectra (1100-400nm) were measured by the Labo. Med., inc. 1650Pc spectrophotometer and by using DMF as a solvent.

2.2. Preparation of the metal complexes:

Two complexes of Cu^{2+} and Zn^{2+} with BI were prepared by adding BI(2mmole in 25ml of ethanol) gradually to $Cu(NO_3)_2 \cdot 3H_2O$ *or* $Zn(NO_3)_2 \cdot 6H_2O$ (1mmole in 25 ml ethanol). The reaction mixture was placed on a water bath for 3hrs at 80°C. A green precipitate of the Cu-BI complex was observed after evaporation of ethanol solution, while a gray precipitate was obtained in the case of Zn-BI complex on hot. Schematic diagrams of the synthetic processes are shown in **Schemes (1 and 2)**.



Scheme 1: The suggested structure of Zn-BI complex



Scheme 2: The suggested structure of Cu - BI complex

BI: IR(ν/cm^{-1});3475 (NH),3104 (CH aromatic), 1604 (C=N), 1473 (C=C)$^{-1}$H NMR (400 MHz, DMSO): δ/ppm = 7.18-7.20 (m, 2H, Ar-H), 7.60-7.63 (m, 2H, Ar-H), 8.24 (s, 1H,Ar-H), 12.50 (*br*, 1H, NH).Anal. Calcd. for $C_7H_6N_2$ (118.14): C, 71.17; H 5.12; N, 23.71; Found: C, 71.16; H 5.10; N, 23.65%.

Zn-BI: Yield 93%; m.p. over 300°C; mass spectra of Zn(II) complex displayed peak concentrated on m/z=539(M^+) corresponding to the formula $C_{28}H_{22}ZnN_8$; IR (ν/cm^{-1}); 3409 (NH), 3073 (CH aromatic), 1677 (C=N), 1465 (C=C). Anal. Calcd. for $C_{28}H_{22}ZnN_8$ (538.12): C, 62.75; H 4.14; N, 20.91; Found: C, 62.73; H 4.12; N, 20.55%.

Cu-BI: Yield 95%; m.p. 230°C; IR (ν/cm^{-1}); 3297 (NH), 2900 (CH aromatic), 1639 (C=N), 1376 (C=C).Anal. Calcd. for $C_{21}H_{18}CuN_6O$ (433.95): C, 58.12; H 4.18; N, 19.37; Found: C, 58.09; H 4.11; N, 19.22%.

2.3. Antimicrobial Activity:

The four microorganisms, *Staphylococcus aureus* (Gram +ve bacteria), *Escherichia coli* (Gram -ve bacteria), *Candida albicans* (yeast), and *Aspergillus niger* (fungi), were selected for evaluating the antimicrobial activity of BI, Cu-BI complex and Zn-BI complex by using disc agar and colony forming techniques.

For disc agar diffusion; the bacterial and yeast test microbes were grown on a nutrient agar medium. While *Aspergillus niger* was cultivated on potato dextrose agar (PDA) medium. The culture of each test microbe was diluted by sterilized distilled water to 10^7 to 10^8 colony forming units (CFUs)/ml, and then 1 ml of each was used to inoculate 1L Erlenmeyer flask containing 250 ml of solidified agar media. These media were put onto previously sterilized Petri dishes (10 cm-diameter having 25 ml of solidified media). Prepared films were placed on agar plates seeded with test microbes and incubated for 24 hrs, at the appropriate temperature of each test organism. Antimicrobial activities were recorded as the diameter of inhibition zones (including the disc itself) that appeared around the discs [43, 44].

For the colony forming technique (CFU) against *Staphylococcus aureus* and *Escherichia coli*, the bacterial cells suspension (100 µL of stock of CFU value of about10^7) were inoculated into a 20 ml freshly prepared liquid nutrient broth in 100 ml-volume of Erlenmeyer flasks, and incubated overnight. Samples (250 mg) were added to the inoculated flasks (with 20 µL of inoculums). A control (inoculated flasks without samples) was also constructed. After the incubation period at 37°C, a serial dilution from each sample has been done (10^{-1}-10^{-4}). The microbial inhibition was determined *via* calculating the colony forming units (CFU) by inoculating petri-dishes containing solidified nutrient agar medium with 100 µL from each dilution [45].

3. Results and discussion:

3.1. Chemistry:

Infrared and ^{1}H NMR spectra of the benzimidazole (BI) were carried out for comparing with the spectra of the synthesized metal complexes. Where, its IR spectrum showed two bands at 1604 $cm^{-1}$and 3475 cm^{-1} for the azomethine and (NH) groups, respectively.^{1}H NMR spectrum of BI exhibited a broad signal at δ: 12.5 ppm (D_2O-exchangeable) indicating the presence of NH proton. The spectrum showed also a signal at δ: 8.24ppm (singlet) in addition to δ: 7.20 and 7.63 ppm (multiplet) corresponding to 4-H (aromatic protons). The synthesized Zn^{2+} complex was found to be stable at room temperature (~ 25ºC) and poor solubility in most organic solvents (chloroform, ether, ethanol, DMF and DMSO) (**Scheme 1**). Structure of the Zn-BI complex was elucidated by mass spectroscopy which it displayed a peak concentrated on *m/z*= 539 as well as correct elemental analysis. Also, the Zn-BI complex was well indicated by the FT-IR spectrum, which representing two bands at 1677 cm^{-1} and 3409 cm^{-1} attributed to the azomethine and NH groups, respectively. On the other hand, when $Zn(NO_3)_2{\cdot}6H_2O$ was replaced by $Cu(NO_3)_2{\cdot}3H_2O$, no solid precipitate was separated out on hot or on cold, but a clear solution was obtained. The Cu-BI complex was obtained as a green precipitate upon concentrating the reaction mixture (**Scheme 2**). Structure of the Cu-BI complex was established by studying of its FT-IR and UV-visible spectra. In the FT-IR spectrum, the peaks of the azomethine and NH groups were appeared at 1639 cm^{-1} and 3297 cm^{-1}, respectively.

The electronic spectra of BI and Cu-BI complex in DMF solution at room temperature were measured. The UV-visible spectrum showed two absorption peaks for ligand in the wavelength region of 200-400 nm. The first absorption and the most intensive peak of ligand was obtained in the region of 255-270 nm, which is a result of aromatic benzene rings assigned to the π-π* bond and the second absorption peak was observed in the wavelength range of 290-331 nm, which is assigned to the n-π*bond. While the UV-visible spectrum of the Cu-BI complex includes four absorption peaks. The peak observed in the wavelength region of 255-270 nm for benzene aromatic ring assigned to the π-π* bond and 290-331 nm related to the n-π* bond. However, in the wavelength range of 275-290 nm, a new absorption peak was obtained related to the π-π* transition resulting from the Cu-BI complex. The chelation between the inorganic and organic parts of the Cu-BI can be obtained from the higher absorption of the Cu-BI complex in the wavelength range of 250-290 nm.The results indicated a strong chelation between the inorganic and organic parts of the Cu-BI complex.

3.2. Antimicrobial activity:

Results in **Table 1** and **Figure 2** demonstrated the antimicrobial activities of the prepared compounds against the previously mentioned test microbes using the cup plate diffusion technique. The obtained results are compared with the reference antibiotics namely, Cyclohexamide (fungicide) and Neomycin (bactericide). The obtained results showed that, the Cu-BI complex exhibited the highest antimicrobial activity against all the tested microorganisms with inhibition zones of 22, 32, 34, and 25 mm against *S. aureus, E. coli, C. albicans,* and *A. niger*, respectively. Moderate antimicrobial activities were noticed for the BI with inhibition zone values of 18, 14, 15, and 13 mm for *S. aureus, E. coli, C. albicans,* and *A. niger*, respectively. While the lowest antimicrobial activities were found with Zn-BI complex with inhibition values of 14, 12, 12, and 12 mm for *S. aureus, E. coli, C. albicans*, and *A. niger*, respectively. The antimicrobial activities of the prepared complexes were also assured using the counting technique (Colony forming units, CFU). Results in **Table 2** and **Figures 3 and 4** demonstrated that all the compounds at a concentration of 2.5mg/ml exhibited the highest reduction in growth against *S. aureus* and *E. coli*.

Table (1): Antimicrobial activity (inhibition zone, mm) of BI, Zn-BI complex, and Cu-BI complex against *S. aureus* (G+ve bacteria), *E. coli* (G-ve bacterium), *C. albicans* (yeast) and *A. niger* (fungi) using cup agar diffusion technique.

Samples	Clear zone(ϕ mm)			
	Staphylococcus aureus	***Escherichia coli***	***Candida albicans***	***Aspergillus niger***
BI	**18**	**14**	**15**	**13**
Zn-BI	**14**	**12**	**12**	**12**
Cu-BI	**22**	**32**	**34**	**25**
Neomycin	**27**	**31**	**33**	**0**
Cyclohexamide	**0**	**0**	**0**	**35**

Table 2: The colony forming units(CFU) values at different dilutions of BI, Zn-BI complex, and Cu-BI complex against *S. aureus* and *E. coli*.

Samples	Serial dilution			
	10^{-1}	10^{-2}	10^{-3}	10^{-4}
S. aureus	∞	∞	∞	284
BI (1mg/ml)	86	16	0	0
BI (2.5mg/ml)	1	1	0	0
Zn-BI (1mg/ml)	∞	95	17	1
Zn-BI (2.5mg/ml)	62	1	0	0
Cu-BI (1mg/ml)	49	4	0	0
Cu-BI (2.5mg/ml)	19	5	0	0
E. coli	∞	∞	∞	66
BI (1mg/ml)	2	0	0	0
BI (2.5mg/ml)	1	0	0	0
Zn-BI (1mg/ml)	∞	∞	∞	192
Zn-BI (2.5mg/ml)	1	0	0	0
Cu-BI (1mg/ml)	15	5	2	1
Cu-BI (2.5mg/ml)	4	1	1	0

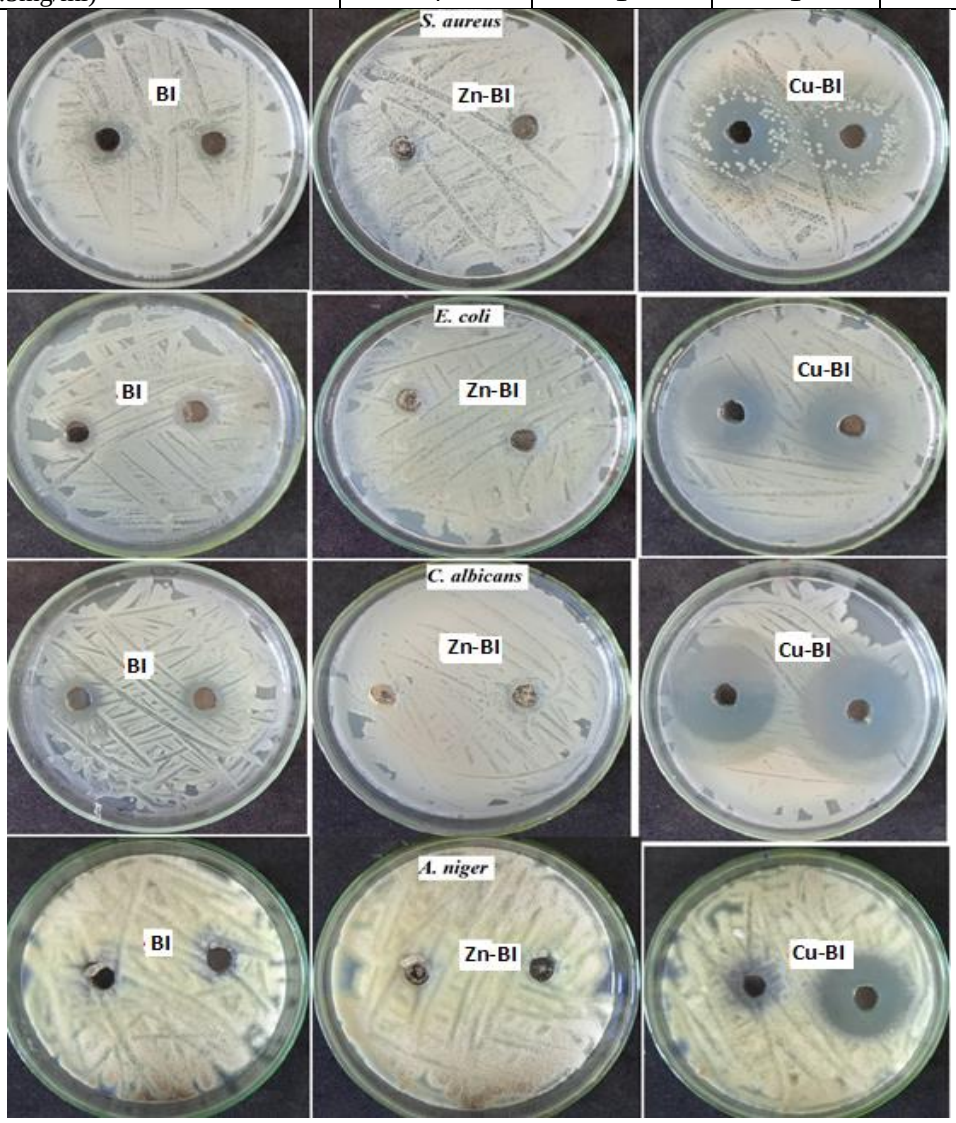


Figure 2: The antimicrobial activity of BI, Zn-BI complex, and Cu-BI complex against the microorganisms *S. aureus* (G+ve bacterium), *E. coli* (G-ve bacterium), *C. albicans* (yeast), and *A. niger* (fungi) using cup agar diffusion.

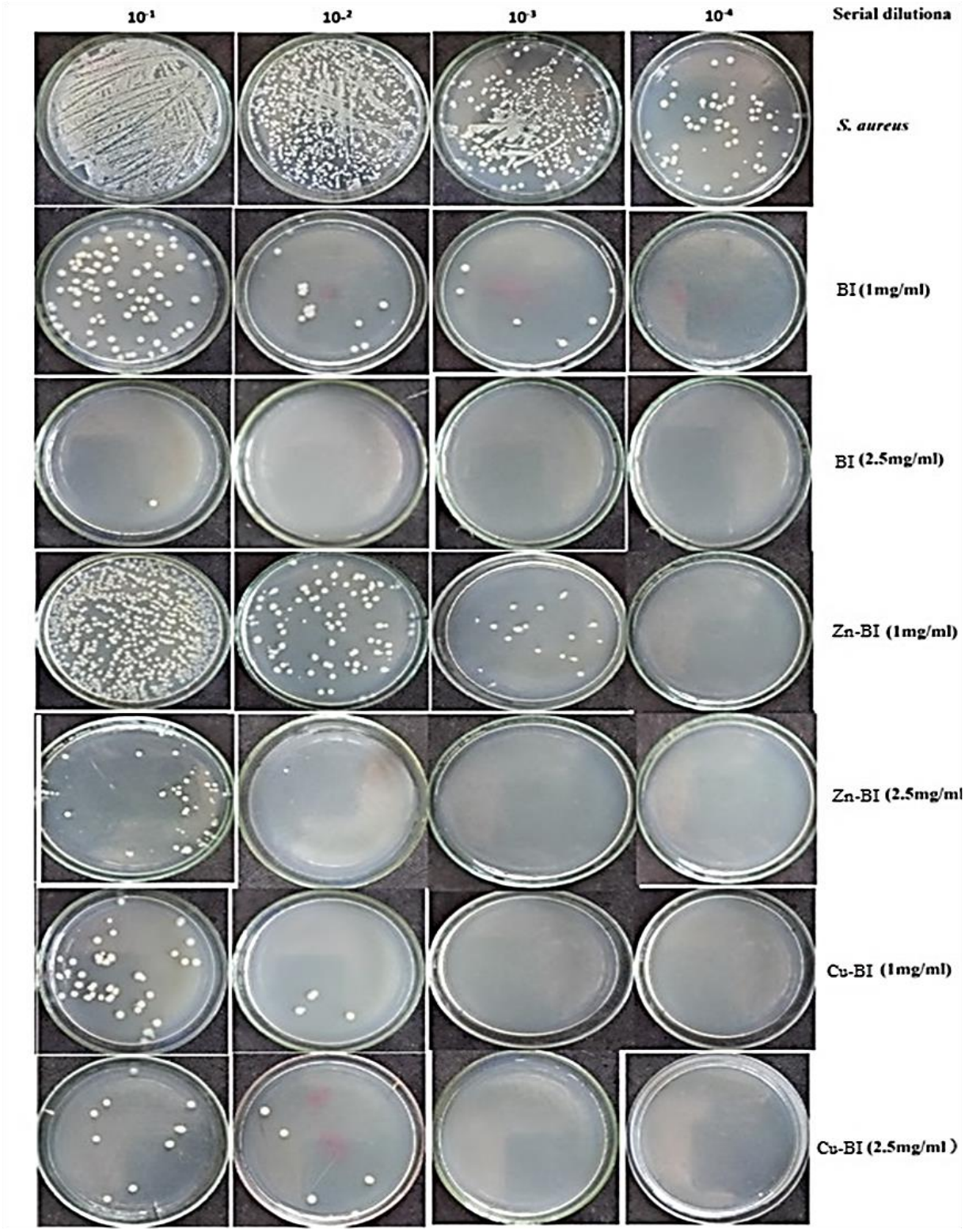


Figure 3: The CFU value at different dilutions of BI, Zn-BI complex, and Cu-BI complex against *S. aureus*.

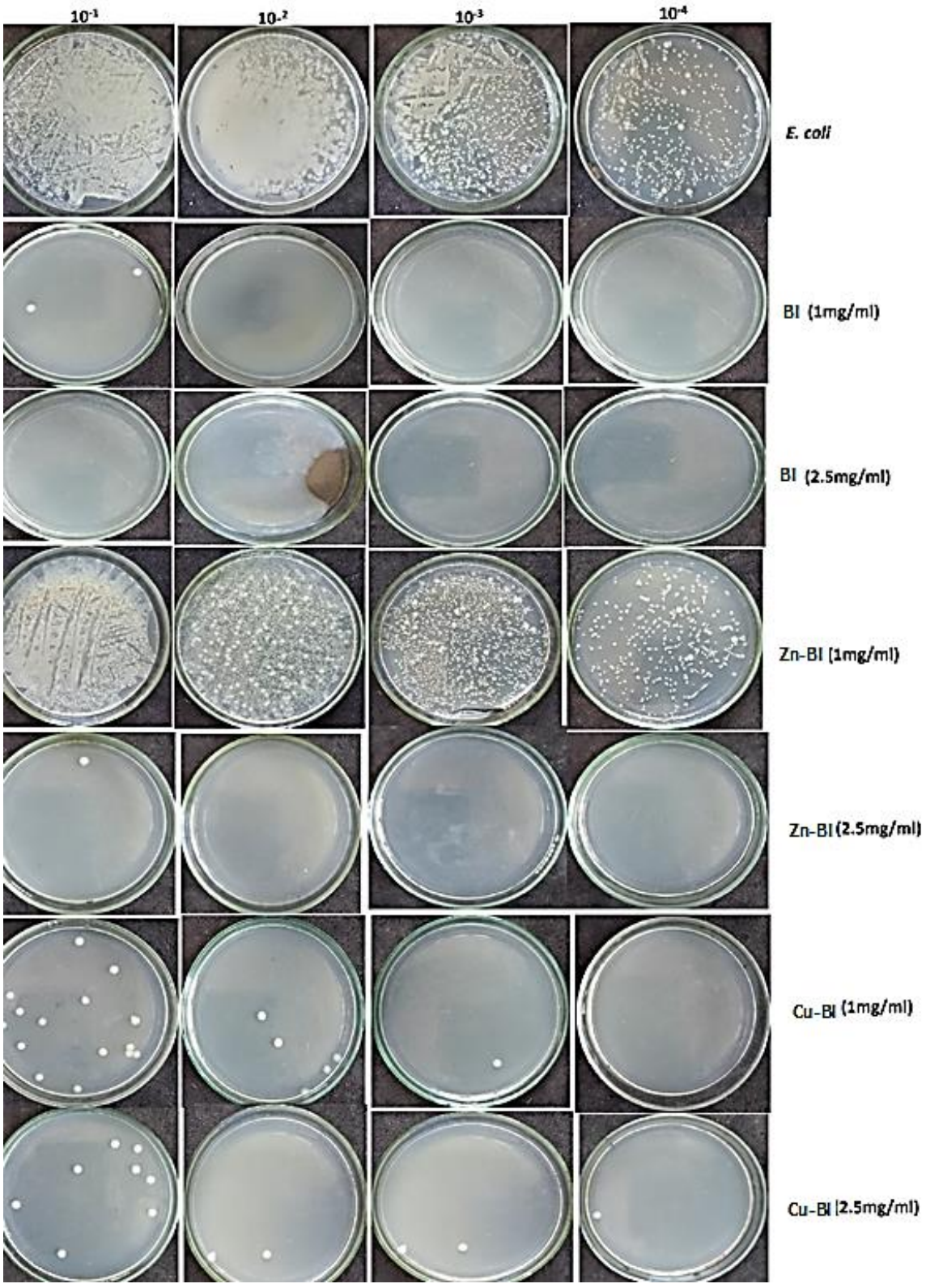


Figure 4: The CFU value at different dilutions of BI, Zn-BI complex, and Cu- BI complex against *E. coli*.

4. Conclusions

The prepared Zn-BI, and Cu-BI were characterized by using FT-IR, UV-visible, ^{1}H NMR, and mass spectra. The formation of the two complexes between the Zn and Cu cations with the functional group of BI (NH) was confirmed. The antimicrobial activity of BI, Zn-

BI complex and Cu-BI complex were investigated *via* disc agar and colony forming techniques. The results of the prepared complex revealed that the Cu-BI complex has the highest antimicrobial activity.

References

[1] Rafique S., IdreesM., Nasim A., Akbar A. and AtharA.;"Transition metal complexes as potential therapeutic agents". Biotechnol. *Mol. Biol. Rev.*, (**5)**, 38–45 (2016).

[2] SchatzschneiderU., "Photoactivated Biological Activity of Transition-Metal Complexes". *Eur. J. Inorg. Chem.*,(10),1451–1467 (2010).

[3] FreiA., "Metal Complexes, an Untapped Source of Antibiotic Potential?" *Antibiotics*, **9** (90), (2020).

[4] IakovidisI., Delimaris I. and Piperakis S.M.; "Copper and Its Complexes in Medicine: A Biochemical Approach". *Mol. Biol. Int.*, 1–13 (2011).

[5] Duncan C. and White A.R.; "Copper complexes as therapeutic agents". *Metallomics.*, (4), 127–138 (2012).

[6] Ashraf S. Hassan. Mixed isatin with 3-(2-(aryl)hydrazono)acetylacetoneMn(II), Co(II) and Ni(II) complexes: antibacterial evaluation and molecular properties prediction. *Bull. Chem. Soc. Ethiopia*, 34 (3), 533-541 (2020).

[7] Obaleye J.A., Ajibola A.A., Bernardus V.B. and Hosten E.C.;"Synthesis, X-ray crystallography, spectroscopic and in vitro antimicrobial studies of a new Cu(II) complex of trichloroacetic acid and imidazole". *J. Mol. Struct.*, (1203), 127435 (2020).

[8] Ashraf S. Hassan, Taghrid S. Hafez. Antimicrobial Activities of Ferrocenyl Complexes: A Review. *J. Appl. Pharm. Sci.*, 8 (5): 156-165 (2018).

[9] Efthimiadou E.K., Katsaros N., Karaliota A. and Psomas G.;"Mononuclear copper(II) complexes with quinolones and nitrogen-donor heterocyclic ligands: Synthesis, characterization, biological activity and interaction with DNA". *Inorg. Chim. Acta.*, (360), 4093–4102 (2007).

[10] Renfrew A.K.;"Transition metal complexes with bioactive ligands: Mechanisms for selective ligand release and applications for drug delivery". *Metallomics.*, (6), 1324–1335 (2014).

[11] Li H., Yin K.-L. and Xu D.-J.;"catena-Poly[[bis-(1H-benzimidazole-κN^3)(salicylato-κO)copper (II)]-μ-salicylato-O,O′:O″]". *ActaCryst.*,**61**(1), m19–m21 (2005).

[12] Dokla E. M., Abutaleb N.S., Milik S.N., Li D., El-Baz K., Shalaby M.A., Al-Karaki R., Nasr M., Klein C.D., Abouzid K.A. and Seleem M.N.;"Development of benzimidazole-based derivatives as antimicrobial agents and their synergistic effect with colistin against gramnegative bacteria". Eur. *J. med. Chem.*, (186), 111850 (2020).

[13] Tayade A. P., Pawar R. P.;"The Microwave Assisted and Efficient Synthesis of 2- Substituted Benzimidazole Mono-Condensation of O-Phenylenediamines and Aldehyde". *Polyc. Arom. Comp.*,**42**(4) 1474-1478 (2020).

[14] Kuo H. L., Lien J. C., Chung C. H., Chang C. H., Lo S. C., Tsai I. C., Peng H. C., Kuo S. C. and Huang T. F.;"NP-184 [2-(5-methyl-2-furyl) benzimidazole], a novel orally active antithrombotic agent with dual antiplatelet and anticoagulant activities". *Naunyn-Schmiede berg's., Arch. Pharmaco.*,**381**(6), 495-505 (2010).

[15] Mavrova A. T., Anichina K. K., Vuchev D. I., Tsenov J. A., Denkova P. S., Kondeva M. S. and Micheva M. K.;"Antihelminthic activity of some newly synthesized 5 (6)-(un)substituted-1H-benzimidazol-2-ylthioacetyl piperazine derivatives", *Eur. J. Med. Chem.*,**41**(12) 1412-20 (2006).

[16] Maghraby M.T., Abou-Ghadir O.M., Abdel-Moty S.G., Ali A.Y. and Salem O.I.;"Novel class of benzimidazole-thiazole hybrids: The privileged scaffolds of potent anti-inflammatory activity with dual inhibition of cyclooxygenase and 15-lipoxygenase enzymes". *Bioorg. Med. Chem.*, (28), 115403-22 (2020).

[17] Imran M., Al Kury L.T., Nadeem H., Shah F.A., Abbas M., Naz S., Khan A.U. and Li S.;"Benzimidazole Containing Acetamide Derivatives Attenuate Neuroinflammation and Oxidative Stress in Ethanol-Induced Neurodegeneration". *Biomolecules*, **10**(1), 108-24(2020).

[18] Khodja I.A., Boulebd H., Bensouici C. and Belfaitah A.;"Design, synthesis, biological evaluation, molecular docking, DFT calculations and in silico ADME analysis of benzimidazolehydrazone derivatives as promising antioxidant, antifungal, and anti-acetylcholinesterase agents". *J. Mol. Str.*, (1218), 128527 (2020).

[19] Noor A., Qazi N.G., Nadeem H., Khan A.U., Paracha R.Z., Ali F. and Saeed A.; "Synthesis, characterization, anti-ulcer action and molecular docking evaluation of novel benzimidazole-pyrazole hybrids". *Chem. Central J.*, **11**(1), 85-98 (2017).

[20] Patel V. M., Patel N. B., Chan-Bacab M. J. and Rivera G.;"N-Mannich bases of benzimidazoleas a potent antitubercular and antiprotozoal agents: Their synthesis and computational studies".*Synth. Commun.*,**50**(6), 858-878 (2020).

[21] Bakhotmah D. A., Al-Ahmadi A. A.;"Design and Synthesis of Some New 3-Oxo/thioxo-1, 2, 4-triazolo [4, 3-a] benzimidazole Derivatives Bearing a 4-Tollyl Sulfonyl Moiety as Antimycobacterial Agents". *Polycycl. Aromat. Compd.*, 1-13 (2019).

[22] Azadbakht M., Davoodi A., Hosseinimehr S.J., Keighobadi M., Fakhar M., Valadan R., Faridnia R., Emami S., Azadbakht M. and Bakhtiyari A.;"Tropolone alkaloids from Colchicum kurdicum (Bornm.) Stef.(Colchicaceae) as the potent novel antileishmanial compounds; purification, structure elucidation, antileishmanial activities and molecular docking studies". *Exper. Parasitology*, (213), 107902 (2020).

[23] Francesconi V., Cichero E., Schenone S., Naesens L. and Tonelli M.;"Synthesis and Biological Evaluation of Novel (thio) semicarbazone-Based Benzimidazoles as Antiviral Agents against Human Respiratory Viruses". *Molecules.*, **25** (7) 1487-1508 (2020).

[24] Hassan A.M. , Heakal B.H., Khamis H., Abd El-Naeem G., Marzouk E., Abdelmoaz M.A. and Younis A., "Design, Synthesis, DFT Studies and Anticancer Activity of Novel Metal Complexes Containing 1,3,5-triazino[1,2-a] benzimidazole Moiety Using Microwave as an Approach for Green Chemistry". *Egypt. J. Chem.*, 64, 323-340 (2021).

[25] Pan T., He X., Chen B., Chen H., Geng G., Luo H., Zhang H., and Bai C.; "Development of benzimidazole derivatives to inhibit HIV-1 replication through protecting APOBEC3G protein". *Eur. J. med. chem.*, (95), 500-13 (2015).

[26] Serafini M., Torre E., Aprile S., Grosso E.D., Gesù A., Griglio A., Colombo G., Travelli C., Paiella S., damo A A. and Orecchini E.;"Discovery of highly potent benzimidazole derivatives as indoleamine 2, 3-dioxygenase-1 (IDO1) inhibitors: from structure-based virtual screening to in vivo pharmacodynamic activity". *J. Med. Chem.*,**63**(6),3047-65 (2020).

[27] Schmit D., Milewicz U., Boerneke M.A., Burley S., Walsworth K., Um J., Hecht D., Hermann T. and Bergdahl B. M.;"Syntheses and Binding Testing of N1-Alkylamino-Substituted 2-Aminobenzimidazole Analogues Targeting the Hepatitis C Virus Internal Ribosome Entry Site". *Aust. J. Chem.*,**73**(3), 212-21(2020).

[28] Marcus A. J., Iezhitsa I., Agarwal R., Vassiliev P., Spasov A., Zhukovskaya O., Anisimova V. and Ismail N. M.;"Intraocular pressure-lowering effects of imidazo[1,2-*a*]-and pyrimido[1,2-*a*]benzimidazole compounds in rats with dexamethasone-induced ocular hypertension". *Eur. J. Pharm*., (850), 75-78 (2019).

[29] Rahim F., Zaman K., Taha M., Ullah H., Ghufran M., Wadood A., Rehman W., Uddin N., Shah S.A., Sajid M. and Nawaz F.;"Synthesis, in vitro alpha-glucosidase inhibitory potential of benzimidazole bearing bis-Schiff bases and their molecular docking study". *Bioorg. Chem.*,(94), 103394 (2020).

[30] Hue B.T., Nguyen P.H., De T.Q., Van Hieu M., Jo E., Van Tuan N., Thoa T.T., Anh L.D., Son N.H. and Thanh D.D.;"Dupont-Rouzeyrol M. Benzimidazole Derivatives as Novel Zika Virus Inhibitors". *Chem. Med. Chem.*, (15),1-2 (2020).

[31] Taha M., Mosaddik A., Rahim F., Ali S., Ibrahim M. and Almandil N. B.;"Synthesis, antiglycation and antioxidant potentials of benzimidazole derivatives". *J. King Saud Univ-Sci*., **32**(1), 191-194 (2020).

[32] Guzelj S., Gobec M., Urbančič D., Mlinarič-Raščan I., Corsini E. and Jakopin Ž.;"Structural features and functional activities of benzimidazoles as NOD2 antagonists". *Eur. J. Med. Chem*,. (190), 112089 (2020).

[33] Sharma M. C.;"QSAR studies of novel 1-(4-methoxyphenethyl)-1H-benzimidazole-5-carboxylic acid derivatives and their precursors as antileukaemic agents". *J. Taibah Univ. Sci.*,**10**(1), 122-130 (2016).

[34] Surineni G., Gao Y., Hussain M., Liu Z., Lu Z., ChhotarayC., Islam M. M., Hameed H. M. A. and Zhang T.;"Design, synthesis, and in vitro biological evaluation of novel benzimidazole tethered allylidenehydrazinylmethylthiazole derivatives as potent inhibitors of Mycobacterium tuberculosis". *Med. Chem. Comm.*,**10**(1), 49-60 (2019).

[35] Sivaramakarthikeyan R., Iniyaval S., Saravanan V., Lim W. M., Mai C. W. and Ramalingan C.;"Molecular Hybrids Integrated with Benzimidazole and Pyrazole Structural Motifs: Design, Synthesis, Biological Evaluation, and Molecular Docking Studies".*ACS omega.*,**5**(17), 10089-10098 (2020).

[36] Scott F., Fala A. M., Pennicott L. E., Reuillon T. D., Massirer K. B., Elkins J. M. and Ward S. E.;"Development of 2-(4-pyridyl)-benzimidazoles as PKN2 chemical tools to probe cancer". *Bioorg. Med. Chem. Lett.*, **30**(8), 12704 (2020).

[37] Bansal Y., Silakari O.;"The therapeutic journey of benzimidazoles: a review". *Bioorg. Med. Chem.*,**20**(21), 6208-6236 (2012).

[38] Poyraz M., Sari M., Guney A., Demirci F., Demirayak S. and Sahin E.;"Synthesis, characterization and antimicrobial activity of a Zn(II) complex with 1-(1H-benzoimidazol-2-yl)-ethanonethiosemicarbazone". *J. Coord. Chem.*, (61), 3276-3283 (2008).

[39] Galal S.A., Hegab K.H., Kassab A.S., Rodriguez M.L., Kerwin S.M.A., El-Khamry AM. and El-Diwani H.I.;"New transition metal ion complexes with benzimidazole-5-carboxylic acid hydrazides with antitumor activity". *Eur. J. Med. Chem.*, (44), 1500-1508 (2009).

[40] Wu H.,Yuan J., Bal Y., Pan G., Wang H., Shao J., Gao J. and Wang Y.; "Synthesis, crystal structure, DNA-binding properties, and antioxidant activity of a V-shaped ligand bis(Nmethylbenzimidazol-2-ylmethyl) benzylamine and its zinc(II) complex". *J. Coord. Chem.*, (65), 4327-4341 (2012).

[41] Zhao J., Li S., Zhao D., Chen S. and Hu J.; "Metal and structure tuned in vitro antitumor activity of benzimidazole-based copper and zinc complexes". *J. Coord. Chem.*,(66), 1650-1660 (2013).

[42] Streciwilk W., Cassidy J., Hackenberg F., Muller-BunzH., Paradisi F., Tacke M.; "Synthesis, cytotoxic and antibacterial studies of p-benzyl-substituted NHC–silver(I) acetate compounds derived from 4,5-di-p-diisopropylphenyl- or 4,5-di-p-chlorophenyl-1Himidazole". *J. Organomet. Chem.*,(749), 88-99 (2014).

[43] Ghareeb M.A., Refahy L. A., Saad A. M., Osman N. S., Abdel-Aziz M. S., El-Shazly M. A. and Mohamed A. S.;"In vitro antimicrobial activity of five Egyptian plant species". *J. Appl. Pharm. Sci.*, (5), 045-049 (2015).

[44] Bauer A.W., Kirby W.M., Sherris J.C and Turck M.; "Antibiotic susceptibility testing by a standardized single disk method". *J. Am. Clin. Pathol.*, (45), 493-496 (1966).

[45] Gupta D., Khare S.K and Laha A.; "Antimicrobial properties of natural dyes against Gram-negative bacteria". *J. Color Technol.*, (120), 167-170 (2004).