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RESEARCH ARTICLE

Spectrophotometric Determination and Biological Activity Study of Zn(II) Using a Newly Synthesized Azo Reagent Derivative from 4,5-Bis(4-methoxyphenyl) Imidazole

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Abstract A rapid and simple spectrophotometric method was submitted for Zn(II) determination using (E)-2-(4,5-bis(4-methoxyphenyl)-1H-imidazole-4-yl) diazenyl) benzoic acid as an analytical reagent. The suggested method is based on the chelation reaction between Zn(II) and the bidentate chelation ligand with a mole ratio of 1:2 (metal:ligand) with octahedral geometry. The maximum absorption of the formed violet complex was 560 nm, the linearity was in the concentration range of 0.5-15.0 µg mL^{-1}. The relative standard deviation (n =10) was 0.149%. The interfering effect of diverse cations and anions was tested. To clarify the biological efficacy of the synthesized Zn(II) complex, an in vitro biological activity study was carried out against four kinds of bacterial strains, and the results showed promising inhibition activity.

Keywords: Spectrophotometric determination, Imidazole derivative, Synthesized azo reagent, Zn(II)-azo complex, Biological activity.

Introduction

Azo compounds can be considered an important group of organic compounds that contain one or more azo chromophores (-N=N- bridge), which gives different characteristic colours to this well-known type of chemicals [1-4]. Heterocyclic azo compounds are very active toward the transition metal ions because they contain active donor groups and possess atoms such as nitrogen, oxygen, and sulfur, enabling them to form chelating coordination complexes, which are important in the biological field [5]. Azo compounds demonstrate high pharmacological activity, whereas administering these drugs as metal complexes forms makes them very active [6, 7]. Azo compounds are involved in some biological reactions, such as RNA and DNA inhibition, protein synthesis, and nitrogen fixation [8], they have many advantages, such as ease of preparation, abundant product, high selectivity and sensitivity, and desirable stability. These advantages make this class of chemicals highly used in different applications [9, 10], and utilized successfully as anticancer-treating chemicals [11, 12].

Zinc metal is an important component of the human body and a required cofactor for some intracellular proteins [13, 14]. More than 300 enzymes need zinc as a cofactor in their work and plays a structural role in the transcription factors [15]. It has a substantial role in the cellular metabolism processes of protein, lipids, and carbohydrates, its depletion may enhance the DNA damage through impairments of the DNA repair mechanisms [16]. Zn can be considered the only metal that is found in all classes of enzymes. The human body contains about 2-4 g of Zn distributing in different body organs such as the muscles, brain, kidneys, bones, and liver [17]. Its deficiency causes loss of appetite, immune problems, increase the susceptibility to different infections, and mental illness [18, 19]. Because the increase or decrease of the zinc concentration in foods, drinks, and medicines consumed by humans has a significant effect, different and efficient techniques for its estimation have been implemented, like spectrophotometric [13, 20], atomic absorption spectrometry [21], voltammetry [22], HPLC technique [23, 24], and flow injection method [25, 26]. Most of these methods are consuming for the time [5, 15, 19] and required additional solutions for complexation process such as buffer solutions, acids or bases, and surfactants [13, 15, 19, 26], while many of the other spectrophotometric methods required

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additional treatments like the sonication in the ultrasonic bath at high temperatures, and centrifugation [15, 19]. In this work, a sensitive and rapid spectrophotometric method was submitted for Zn(II) determination using (E)-2-(4,5-bis(4-methoxyphenyl)-1H-imidazole-4-yl) diazenyl) benzoic acid ligand as an analytical reagent at 560 nm, then studying the biological activity of the prepared Zn(II) complex against four kinds of bacterial strains.

Materials and Methods

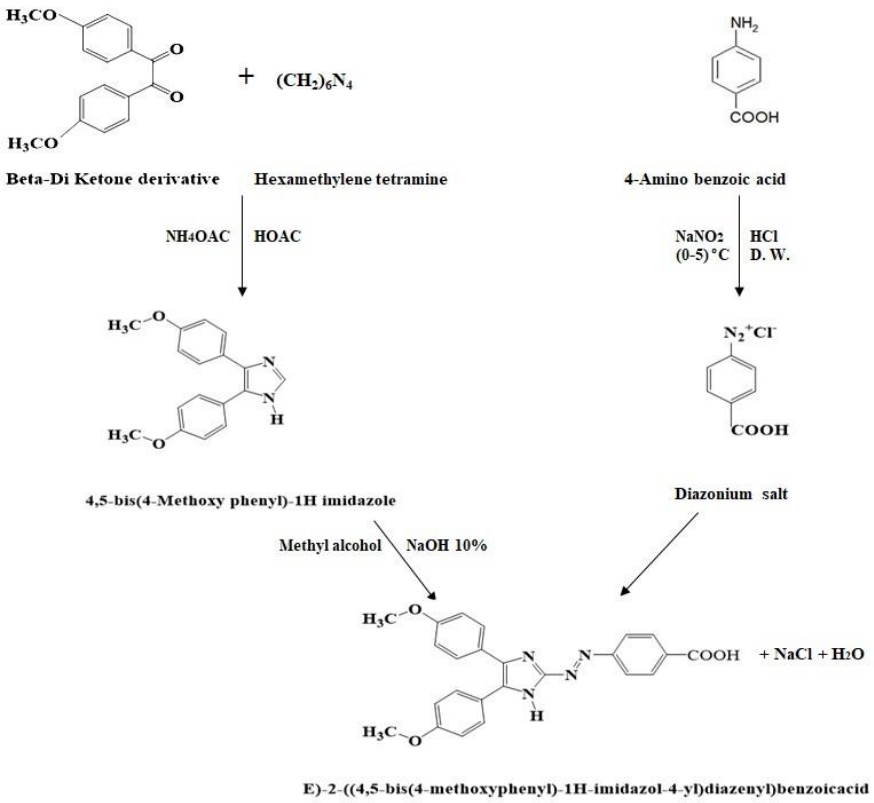
Chemicals and Instrumentation

All the chemicals used were purchased from Fluka, Merck, and Sigma-Aldrich and were of the highest purity without further purification. UV–Vis spectra were recorded by a Shimadzu (UV-1700) spectrophotometer. pH measurements were carried out using an Oakton 2100 Series pH meter.

Synthesis of (E)-2-(4,5-bis(4-methoxyphenyl)-1H-imidazole-4-yl) diazenyl) benzoic acid

To prepare the imidazole derivative 4,5-bis(4-methoxy phenyl) imidazole, as mentioned in a previous study [27], (2.70 g, 0.01 mol) 4,4-dimethoxy benzyl, (0.28 g, 0.002 mol) hexamethylene tetramine, (6.0 g, 0.077 mol) ammonium acetate, and 150 mL acetic acid were mixed, and then the final mixture was escalated for 90 minutes and cooled in an ice bath. The resulting precipitate was filtered, washed with distilled water to minimize impurities, and then dried. Recrystallization from ethanol was performed to obtain the pure crystalline precipitate.

To prepare the azo ligand, as mentioned in a previous study [28], dissolve (0.428 g, 0.001 mol) 4-amino benzoic acid salt in a mixture of (3.0 mL concentrated HCl and 20.0 mL distilled water). The final solution was kept in an ice bath, and then 10.0 mL of (0.70 g, 0.01 mol) $NaNO_2$ aqueous solution was added dropwise with stirring. Let the solution settle for 30 minutes, then mix this diazonium solution with (2.80 g, 0.01 mol) of 5,4-di (4-methoxyphenyl) imidazole compound dissolved in (150.0 mL methyl alcohol and 5.0 mL NaOH (10%)) solution (Scheme 1). After the colour of the solution changed to dark orange, the solution was allowed to settle for at least 24 hours, and the solution's pH value was adjusted to 7.0 using drops of 0.1 N HCl. The resulting solution was filtered and washed with distilled water to minimize any inorganic salts, dried, and recrystallized from ethanol.



Scheme 1. Azo ligand preparation steps

Preparation of Standard and Work Solutions

In a 100.0 mL volumetric flask, a 1000.0 µg mL^{-1} azo ligand stock solution was prepared by dissolving 0.1 g of the ligand in 96% ethanol and then diluting it with the same solvent to the flask mark. Additional dilutions were implemented with ethanol to prepare the working solutions. A 100.0 µg mL^{-1} Zn(II) stock solution was prepared by dissolving 0.01 g of $ZnCl_2$ salt in distilled water and then completing the volume with distilled water to 100.0 mL. Additional dilutions were implemented with distilled water to obtain the working solutions.

General Determination Procedure

Appropriate volumes of Zn(II) solution containing (5.0-150.0 µg) were transferred into a series of 10.0 mL volumetric flasks, and 0.25 mL of 20.0 µg mL^{-1} azo ligand solution was added to each flask. Then, the final solutions were diluted with distilled water to the desired level. The mixture absorbance was measured at 560 nm against the blank sample absorbance; the blank sample solution was 9.75 mL of distilled water and 0.25 mL of 20.0 µg mL^{-1} azo ligand solution in 10.0 mL volumetric flask. The calibration curve was drawn between the absorbance and the complex concentration values.

Results and Discussion

UV–Vis Spectra of the Synthesized Ligand (L) and the $[Zn(L)_2Cl_2)].H_2O$ Complex

The UV–Vis spectrum of the ethanolic solution of the synthesized ligand exhibits a maximum absorption peak at 470 nm (Figure 1), whereas the absorption spectrum of the Zn(II) complex exhibits a bathochromic shift in the ligand spectrum. This shift places the complex maximum absorption at 560 nm.

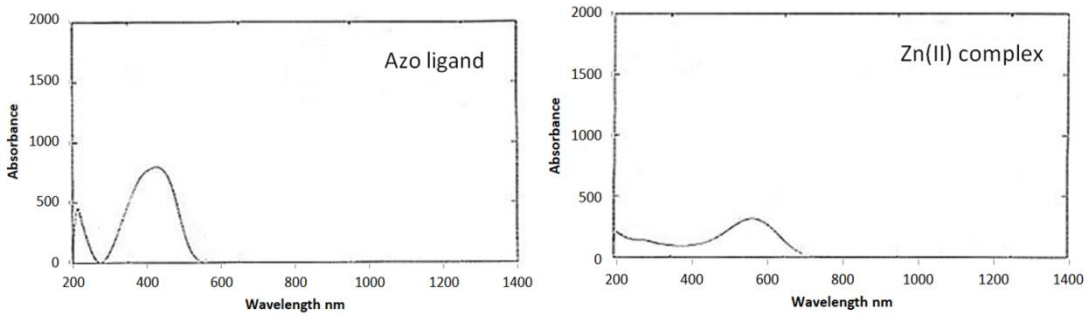


Figure 1. UV–Vis spectra of 100.0 µg mL^{-1} ethanolic solution of the azo ligand (L), and the $[Zn(L)_2Cl_2)].H_2O$ complex of 100.0 µg mL^{-1} Zn(II) aqueous solution at pH = 6.8

Reaction Conditions' Optimization

Solution pH Value Effect

Using (2.0 mL, 10.0 µg mL^{-1}) Zn(II) aqueous solution and (1.0 mL, 20.0 µg mL^{-1}) azo ligand solution, the effect of the pH function value of the Zn(II) solution on the complex formation was tested in the pH range of 3.0-9.0 by measuring the final solution absorbance value at graduated pH values at 560 nm against the blank absorbance. The study results, showed that the complex absorbance increased with the pH value increasing up to 6.8 (Figure 2a), and then decreased at higher values.

Ligand Solution Volume Effect

Using (2.0 mL, 10.0 µg mL^{-1}) Zn(II) aqueous solution, the volume effect of a 20.0 µg mL^{-1} azo ligand solution on the complexation reaction was examined in the volume range of 0.10-1.25 mL by measuring the final solution absorbance value at different ligand solution volumes at 560 nm against the blank absorbance. The complex maximum absorbance was reached when adding 0.25 mL of the ligand (Figure 2b), and decreased when adding higher volumes.

Ligand Concentration Effect

Using (2.0 mL, 10.0 µg mL^{-1}) Zn(II) aqueous solution, the concentration effect of 0.25 mL azo ligand on the complexation reaction was investigated in the concentration range of 10.0-50.0 µg mL^{-1} by measuring the final solution absorbance value at different ligand solution concentrations at 560 nm against the blank absorbance. The complex absorbance increased as the reagent concentration increased to 20.0 µg mL^{-1} (Figure 2c), and then decreased at higher concentrations.

Time and Temperature Effect

The effect of the complex formation time and the reaction temperature on Zn(II)-complex formation was tested in the time and temperature ranges of 0.5-40 minutes and 25-40 ˚C, respectively. The study results showed that the absorbance value increased with the complex formation time up to 1 minute and stayed at the same absorbance value for at least 24 hours at room temperature until 30 ˚C and then decreased at higher temperature degrees.

Zn(II) Solution Volume Effect

Using (0.25 mL, 20.0 µg mL^{-1}) azo ligand aqueous solution, the volume effect of 10.0 µg mL^{-1} Zn(II) on the complex formation was examined in the volume range of 0.5-2.5 mL by measuring the final solution absorbance value at different Zn(II) aqueous solution volumes at 560 nm against the blank absorbance. The complex maximum absorbance was reached when using 2.0 mL of the Zn(II) solution (Figure 2d).

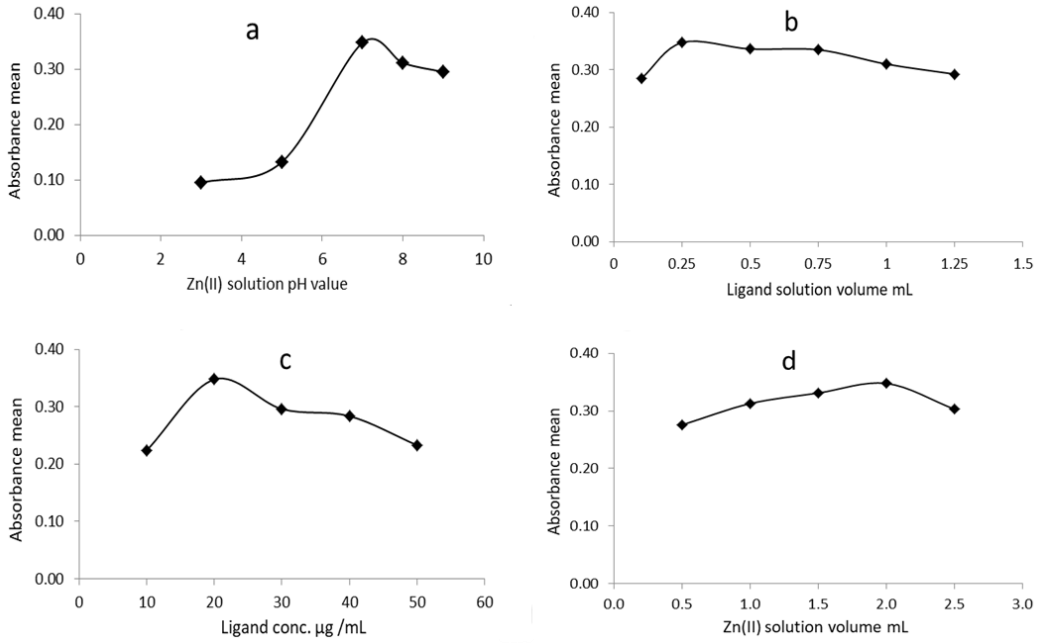


Figure 2. Effect of various variables on the absorbance value of the $[Zn(L)_2Cl_2)].H_2O$ complex.

The Evaluation of the Suggested Method

As stated in the specified experimental conditions, at 560 nm, the calibration curve of the suggested method was built by preparing a series of Zn(II) aqueous solutions containing increasing concentrations; each solution had five replicates. The obtained absorbance values were recorded against the blank absorbance. Then the curve between absorbance and concentration values was drawn. The Beer`s-Lambert law was followed in the concentration range of (0.5-15.0) µg mL^{-1}, the detection limit (LOD) and quantitation limit (LOQ) values were calculated to evaluate the sensitivity of the suggested method. At the optimum conditions, the method's precision was checked by calculating the relative standard deviation value (RSD%). 10 replicate samples containing 10.0 µg mL of Zn(II) were analysed by applying the suggested method, then calculating the RSD% value for them, which was equal to 0.149, indicating the high precision of the suggested method. The analytical data are listed in Table 1.

Table 1. The analytical data of the Zn(II) determination suggested method.

Analytical parameter	Zn(II) complex
λmax nm	560
Regression equation	0.0269x + 0.0519
Specific absorption coefficient L g-1 cm-1	26.9
Molar absorption coefficient L mol-1 cm-1	1.759×10^{3}
Sandell's sensitivity µg cm-2	3.717×10^{-5}
Correlation coefficient r2	0.9991
Detection limit D. L µg mL-1	0.170
Quantitation limit Q. L µg mL-1	0.568
Linear range µg mL-1	0.5-15.0
Standard deviation n = 10	0.001
Relative standard deviation% n = 10	0.149

The $[Zn(L)_2Cl_2)].H_2O$ Complex Composition Study

The $[Zn(L)_2Cl_2)].H_2O$ complex composition was investigated by the mole ratio method (Figure 3). The results indicated that the complex's molar ratio is 1:2 (metal: ligand) and the general formula is $[Zn(L)_2Cl_2)].H_2O$. From these results and based on the imidazole compound's properties, the hybridization of the $[Zn(L)_2Cl_2)].H_2O$ complex is sp^3d^2 and the geometry is octahedral as shown in Scheme 2.

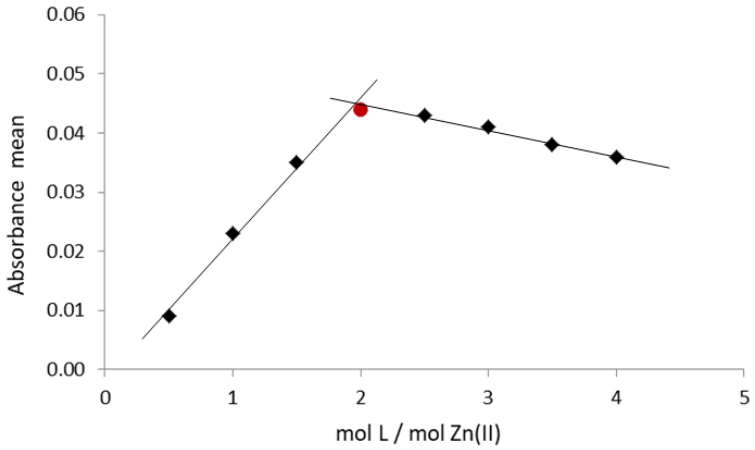
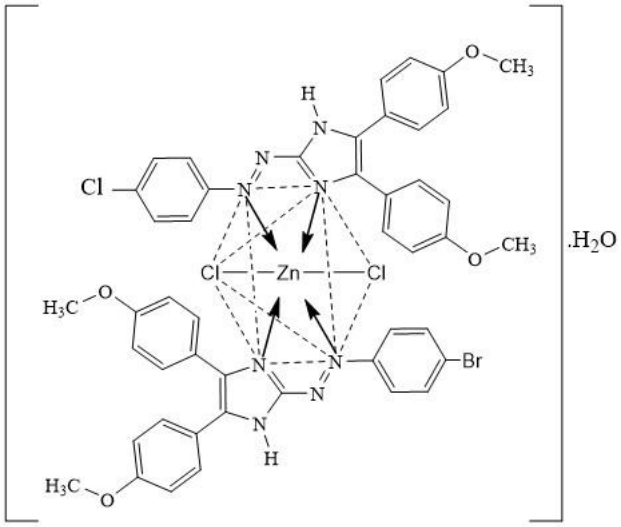


Figure 3. Mole ratio method for the $[Zn(L)_2Cl_2)].H_2O$ complex



Scheme 2. The suggested structure of the $[Zn(L)_2Cl_2)].H_2O$ complex

Effect of the Interferences Study

The specificity of the suggested determination method was examined by studying the interfering effect of various cations and anions that may compete with Zn(II) or the synthesised ligand in the complex formation or affect the complex stability. By following the general determination procedure using synthetic solutions of 10.0 µg mL^{-1} Zn(II) containing the studied interfering ions in the proportions 1:1 and 1:5 (w/w) (ion:interference). To eliminate the interfering effect of the cations, diverse masking agents were examined, such as NaCl, NH_3, EDTA, trisodium citrate ($Na_3C_6H_5O_7$), and sodium acetate. 100.0 µg mL^{-1} of sodium chloride solution was used as a masking agent for the Hg(II), while the other cations were masked by 100.0 µg mL^{-1} of $Na_3C_6H_5O_7$ as shown in Table 2. However, anions such as Cl^-, $CH3COO^{2-}$, NO^{3-}, $SO4^{2-}$, and CrO_4^{2-} have no interfering effect on Zn(II) complex formation at the two tested concentrations.

Table 2. The interference effect on 10.0 µg mL^{-1} Zn(II) determination.

Interference type	Interference conc. µg mL^{-1}	Masking agent[1] volume mL	Recovery%
Hg(II)	10.0	0.5 NaCl	100.532
	50.0	2.0 NaCl	98.404
Cu(II)	10.0	0.5 $Na_3C_6H_5O_7$	97.701
	50.0	2.5 $Na_3C_6H_5O_7$	98.936
Mn(II)	10.0	1.0 $Na_3C_6H_5O_7$	96.552
	50.0	2.0 $Na_3C_6H_5O_7$	98.276
Ca(II)	10.0	1.0 $Na_3C_6H_5O_7$	99.734
	50.0	2.0 $Na_3C_6H_5O_7$	102.394
Pb(II)	10.0	1.0 $Na_3C_6H_5O_7$	97.872
	50.0	2.5 $Na_3C_6H_5O_7$	101.596

[1] masking agent concentration is 100.0 µg mL^{-1}

Biological Activity Study

The microbial efficacy of the $[Zn(L)_2Cl_2)].H_2O$ complex against four strains of bacteria, *Staphylococcus aureus* and *Enterococcus faecalis* (gram-positive bacteria) and *Klebsiella pneumoniae* and *Escherichia coli* (gram-negative bacteria), was checked and compared with the microbial efficacy of the synthesized ligand employing the agar-well diffusion method [29]. This method involves swabbing the selected bacteria on the solidified surface of the agar, then making wells with a 6.0 mm diameter in the plates and filling them with 0.1 mL of 25.0, 50.0, 100.0, 200.0, and 400.0 µg mL^{-1} of the ligand and the $[Zn(L)_2Cl_2)].H_2O$ complex. The plates were then incubated at 37 °C for one day, and the biological activity was recorded by calculating the inhibition zone diameter for each well in mm units.

The results revealed that at a concentration of 25.0 µg mL^{-1} of the free azo ligand and its Zn(II) complex there is no biological efficacy against all strains of bacteria as shown in Figure 4. The free ligand exhibits increasing inhibition activity against all bacteria strains with a concentration that increases from 50.0 µg mL^{-1} and reaches 400.0 µg mL^{-1}, except for the strain *K. pneumonia*, which was not affected by the ligand concentration of 50.0 µg mL^{-1}, while it started to be affected at the concentration of 100.0 µg mL^{-1} with an inhibition diameter of 14.0 mm increasing to 16.0 mm at 400.0 µg mL^{-1}. The highest biological activity of the azo ligand was against the *E. faecalis* strain, with an inhibition diameter in the range of (11.0-19.0) mm. The $[Zn(L)_2Cl_2)].H_2O$ complex shows a higher biological efficacy against all types of bacteria than the free ligand; the higher activity was against the *K. pneumoniae* strain with an inhibition diameter in the range of (16.0-22.0) mm, and the lower activity was against the *E. coli* strain with an inhibition diameter in the range of (11.0-18.0) mm.

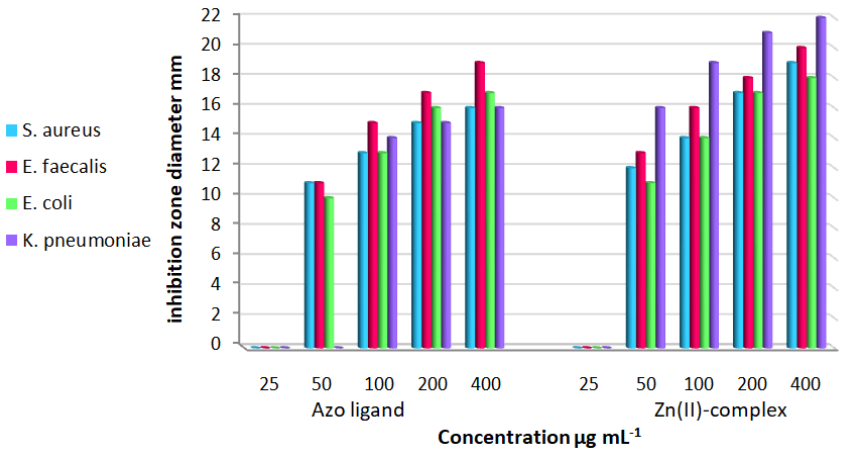


Figure 4. The biological efficacy of the ligand and the $[Zn(L)_2Cl_2)].H_2O$ complex.

Conclusions

The presented work describes the ability to use the synthesized azo ligand, (E)-2-(4,5-bis(4-methoxyphenyl)-1H-imidazole-4-yl)diazenyl) benzoic acid, as an analytical reagent for efficient and rapid spectrophotometric determination of Zn(II) based on the chelation reaction between Zn(II) and the bidentate azo ligand. The mole ratio method was applied to examine the Zn(II)-complex's composition, and the results revealed that the complex's molar ratio is 1:2 (metal:ligand) and that the complex has an octahedral geometrical shape with the chemical formula $[Zn(L)_2Cl_2)].H_2O$. The interfering effect for some cations and anions was studied, and the appropriate masking agents were used with specific concentration and volume to eliminate the interference effect if exists.

The biological efficacy of the $[Zn(L)_2Cl_2)].H_2O$ complex was tested and compared with the biological efficacy of the azo ligand (L). The study results revealed that the free azo ligand and its complex with Zn(II) display increasing biological activity against all the types of the studied bacteria, starting from 50.0 µg mL^{-1} of the ligand and its complex until 400.0 µg mL^{-1}. Except for the *K. pneumoniae* strain which is starting to be affected at 100.0 µg mL^{-1} of the free ligand. The $[Zn(L)_2Cl_2)].H_2O$ complex displays higher biological activity against all bacterial strains than the free ligand alone.

Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this paper.

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