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Original Article (Special Issue)

Synthesis, Spectral Analysis, Stability, Antibacterial, and Antioxidant of Fe(II) Mixed Ligands Complex of Imidazole and 1,10-Phenanthroline

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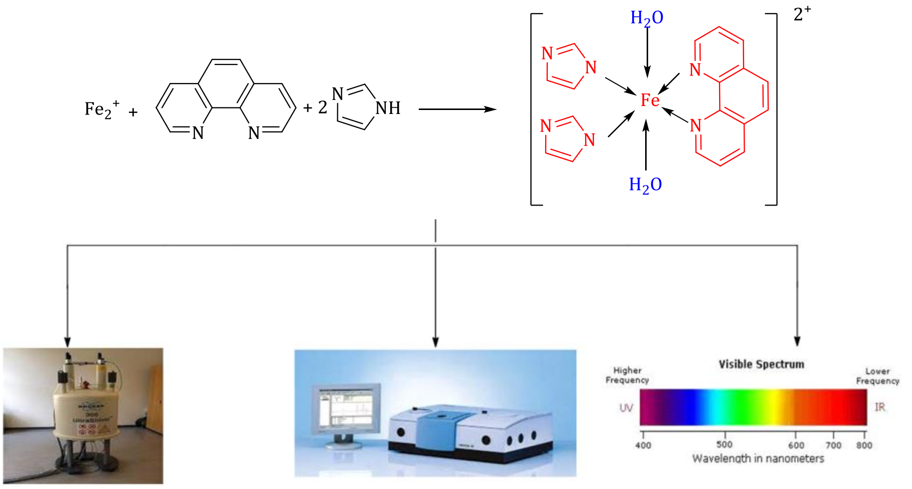
ABSTRACT

In this study, a new complex consisting of iron metal coordination in a binary oxidation state, synthesized with mixed ligands, which are imidazole and 1,10-phenanthroline. The absorbance measurement at the maximum wavelength of the complex with a concentration of 0.001M was run at 504 nm. For the new complex, the optimum conditions were studied and it was found that it is stable over 3 days at pH 6 at temperature range of (20-80°C). The complex conductivity was 99.7µs/L and it was found that the complex decomposed after 208°C). The formation ratio was 1:2:1 iron: imidazole: 1,10-phenanthroline, respectively. A calibration curve was constructed to determine the limit of detection which was 0.20 mg/L. This study was applied to measure the number of iron ions in the ferrous sulfate tablet and the result was very close to the standard amount of ferrous in tablet sample. The complex formation was detected by using different spectroscopic techniques such as FT-IR and ^{1}HNMR. On the other hand, the antibacterial activity and the antioxidant activity of the complex were studied.

GRAPHICAL ABSTRACT



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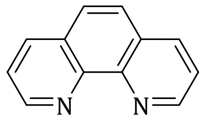
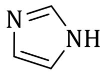
Introduction

Interest in the chemistry of complexes containing mixed ligands has caught more attention because of their apparent effectiveness in biological and physiological processes, as these ligands chelate in many vital systems, making them have a structure capable of storing and transporting active substances across membranes [1-4]. All mixed ligands, except mercury complex, have a negative effect against *Providencia* growth, which indicates a neutral effect [5]. 1,10-phenanthroline is one of the mixed ligands that form very stable chelates with most of the metals of the first class of transition elements, as it is considered as a good nitrogen donor which makes it contributes into many applications such as antibiotics, antimicrobial, anticancer, and antifungal agents [6, 7]. Phenanthrolines are diazaphenanthrene analogs - polycyclic aromatic hydrocarbons present in sex hormones, sterols, cardiac glycosides, alkaloids of morphine, and bile acids [8]. The aromatic ring of 1,10-phenanthroline (Scheme 1, 1-a) is a major driving force for metal bonding, depending on the electron density and its size [9]. The hardness of 1,10-phenanthroline, its hydrophobicity, the planarity, heterogeneity of its ring, and its inhibition of enzymes made it possess unique physical and chemical properties [10].

In this study, the benzene rings present in 1,10-phenanthroline were linked to the aromatic imidazole ring via the iron(II) ion to extend the aromatic system [11]. The imidazole (1-b) is a stable, heterogeneous, and water-soluble nitrogen ring. It has an active acid π system with a single coordination center, so it is considered a weak canine ligand, but it has broad biological activity and acts as an antibacterial, antiviral, and anticancer agent [12-16].



1-a



1-b

Scheme 1: 1-a- 1,10-phenanthroline, 1-b- imidazole

The metal ion (Fe^{++}) is Lewis acid, while the ligands are Lewis base due to the presence of nitrogen atoms in the ring that contains electron pairs. These atoms are responsible for the appearance of the physical and chemical properties of the resulting complex [17-19].

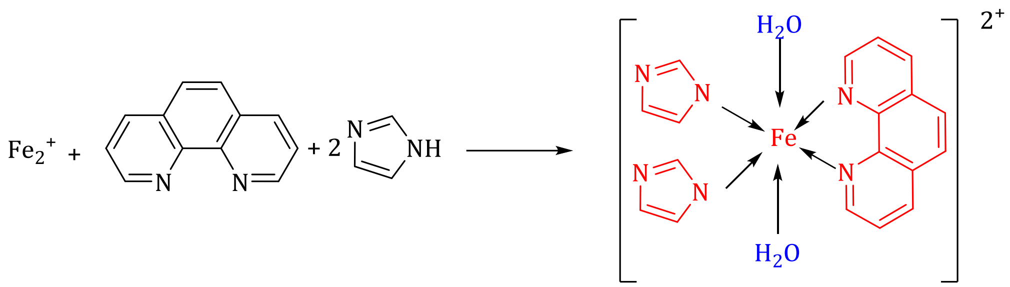
The presence of heterogeneous aromatic ligands in the donor structure gives the bonds additional properties, such as the poly-nitrogen donor, which increases the binding capacity of the ion with the physical properties of the ligand. Therefore, such ligands can be used as chemical sensors for the metal ion present in the solution [20, 21]. When forming a complex for a specific ion with mixed ligands, an increase in selectivity results in a decrease in the possibility of interaction with other ions [22]. The scientific importance of complexes with mixed ligands or mixed ions has prompted further studies of mechanistic, kinetic, structural, and other interesting properties [23, 24].

Material and Methods

The chemicals used in accomplishing the research, including 1,10-phenanthroline, ethanol, ferrous sulfate, sodium hydroxide, hydrochloric acid, and imidazole were utilized from FLUKA, BDH, and SDI Companies at a high purity state, and the spectra of the compounds were recorded by ultraviolet radiation within the range of 200-700 nm using T80 UV/Vis spectrometer PG Instruments Ltd., APEL PD-303 UV apparatus, while the infrared spectra of the ligands and the complex were recorded within the range 200 to 4000 cm^{-1} using the fourier transformed-infra red spectroscopy (FT-IR) Shimadzu, Japan apparatus, as well as the diagnosis of the complex. The melting points of each of the ligands and complexes were measured using the PHS-3CW, Melting point SMP30, and molar conductivity was measured at a concentration of 0.001 M in distilled water as solvent using Heidolph MR Hei- Standard m conductivity Lab Tech. ^{1}HNMR Inova, spectrometer frequency 499.42 Technologies Agilent Technologies, pH meter Radiometer, Denmark were used to detect the complex formation.

Synthesis of complexes

An aqueous solution of the metal salt $FeSO_4.7H_2O$ (1.39 g, 5 mmol) and an aqueous solution of the imidazole (0.68 g, 10 mmol) dissolved in water. 1,10-phenanthroline (0.99 g, 5 mmol) was dissolved in water. The imidazole and 1,10-phenanthroline solutions were mixed and added to the metal solution (Scheme 2) using stoichiometric amounts (1:2:1) (metal: ligand 1: ligand 2) [25].



Scheme 2: General reaction for the synthesis of prepared complex

Antibacterial activity [26]

A sensitivity test for the prepared complex against microbes was conducted according to the well diffusion method. The activity of the prepared complex was evaluated by two strains of bacteria, one Gram-negative G^- (*Klebsiella pneumonia*) and the other Gram-positive G^+ (*Staphylococcus aureus*). Samples were cultured for 24 hours on Mueller-Hinton agar medium at a temperature of 37°C and the results of the complex were good.

Antioxidant activity [27]

Test tubes were covered with aluminum foil to protect the solution from light. 4 mg of DPPH was dissolved in 100 ml of ethanol. Different concentrations (25,50, and 100) ppm were made using the resulting compound, which was manufactured by dissolving 1 mg of the chemical in 10 ml of methanol to produce 100 ppm, and then it was diluted to lower concentrations of 50 and 25 ppm in the same way. 1 ml of the dilute solution at different concentrations of (25, 50, and 100) ppm was added to 1 ml of DPPH solution in a test tube in an incubator at a temperature of 37°C and after 30 minutes the absorbance measurement of each solution was performed at a wavelength of 517 nm by a spectrophotometer, as indicated in Table 2. The equation below was used to find out the possibility of searching for the DPPH roots.

I % = (Absorption control – Absorption sample) /Absorption blank x 10, Absorption blank = 0.003

Results and Discussions

The maximum wavelength between the UV-Visible spectrum and the complex composition agrees well. The molar conductivity values for the prepared complex were obtained in 1:1 ratio. These coordination units were provided with a concentration of 0.001 molar 99.7 $ohm^{-1}\ mol^{-1}cm^2$. The value explained any dissociation of the complex in the water solvent. Hence, this complex can be considered non-electrolytic [28]. This means that the complex is a solid that is insoluble in water but dissolves in some organic solvents such as DMSO, DMF, and THF. The melting point is 208°C for a complex. The infrared spectrum of the complex is given in Figure 1. It was indicated that there is a coordinated interconnection between the mixed ligands and the metal, as well as the appearance of a water molecule, which confirmed this bonding. Also, the ^{1}HNMR spectrum confirmed the appearance of the number and type of protons present in the complex explicitly.

UV-visible spectra [29]

The UV spectrum of 1,10-phenanthroline was measured in the ethanol solvent, as well as for the iron complex with mixed ligands (imidazole and 1,10-phenanthroline) using a cell with a diameter of 1 cm and at room temperature and pH= 6. It was noted that the 1,10-phenanthroline ligand showed the maximum wavelength at 340 nm [30]. As for the iron complex with mixed ligands, it recorded the maximum absorption at wavelength 504 nm.

FT-IR spectra [31]

Infrared spectral studies (Figure 1) of the mixed ligand complexes reveals the main vibrational bands of the FTIR spectrum appeared as sharp band at 3453 cm^{-1} which is characteristic for ν(OH) stretching in the free 1,10-phenanthroline monohydrate, while appeared at 3423 cm^{-1} (m) in $[Fe(imida.)_2(phen.)(H_2O)_2]SO_4$. This change in the absorption frequency of water explains the change like its interaction, consequently confirming the transformation of crystalline H_2O to coordinate H_2O. The band arising due to vibrational ν(C=N) mode at 1643 cm^{-1}(m) in the free 1,10-phenanthroline monohydrate was shifted to lower frequencies, at 1573.79 cm^{-1}(m) in $[Fe(imida.)_2(phen.)(H_2O)_2]SO_4$. This shift suggested that 1,10-phenanthroline binds with the metal center via ring nitrogen appeared at 646.37cm^{-1} due to ν (Fe…N) [32, 33].

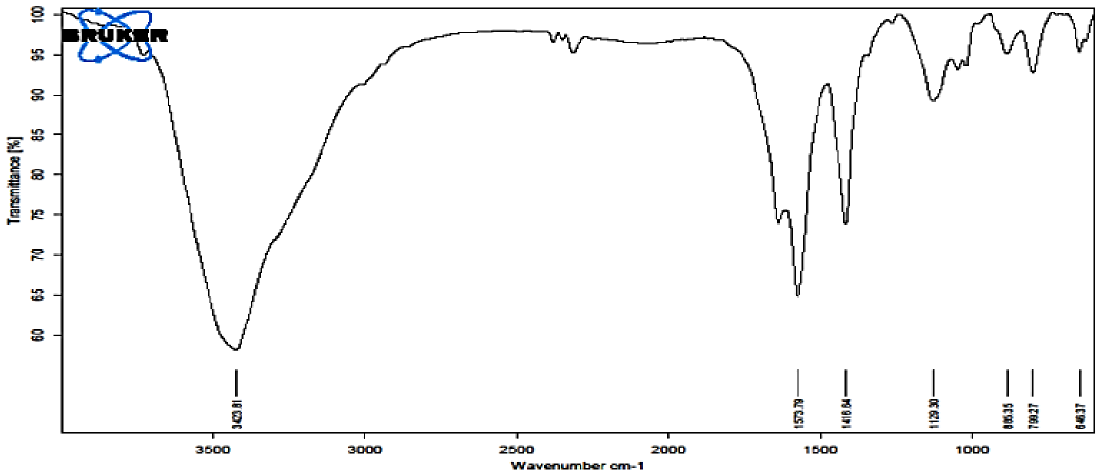


Figure 1: Infrared spectrum of the iron complex

^{1}HNMR spectrum [34]

The ^{1}HNMR spectra (Figure 2) were recorded for the mixed ligands and its metal complex in DMSO as the solvent and showed the following chemical shifts: multiple and signal chemical shifts between 7.13–8.8 ppm belong to the protons in aromatic rings, the singlet chemical shift at 8.39,7.89 ppm is attributed to the proton attached to C=N group. The ^{1}HNMR spectrum of the ligands showed the hydroxyl protons appeared at δ = 3.23 ppm for water. The chemical shifts of the DMSO appeared in 2.45-2.53 ppm [35, 36].

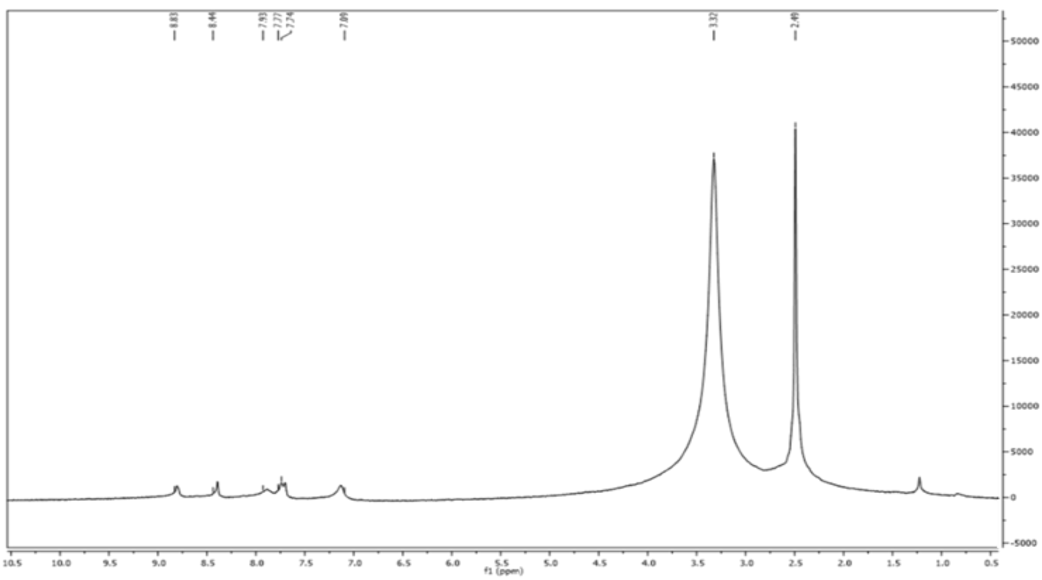


Figure 2: ^{1}HNMR spectrum of Fe(II) complex

Stability studies of the complex

According to the selected maximum wavelength and the preferred molar ratio in this research, measurements were made for the prepared complex after making some necessary adjustments in the absorbance values according to the preferred and standardized concentration of 0.001 M in 1:2:1 ratio meaning that 1 metal: 2 imidazole: and 1 of 1,10-phenanthroline. This was confirmed by Job's method [37], which showed the best absorbance given by the complex at wavelength 504 nm when the number of moles of imidazole is twice the number of moles of each of 1,10-phenanthroline and the metal, as displayed in (Figure 3). To find out the extent of the stability of the complex over time, the complex was measured at different times and proved its stability for more than three days (Figure 4). The stability of the complex when exposed to temperatures was also known, and it was found that it was possible to work with it within wide temperatures, from 20 to 80 °C as in (Figure 5). Likewise, assessments were made to show the best acidic function in which the complex works, and it was found that it gives the best results at function 6, as depicted in (Figure 6). When making measurements of the complex and the ability of the ligands to sense the least amount of ferrous ion, it was found that it is possible to sense very small amounts of ferrous ion, as the detection limit was 0.25 ppm, as illustrated in (Figure 7). Thus, the ferrous ion presents in a ferrous sulfate pill (Ferrous Sulfate Tablets accord, UK) was used as a real sample to investigate the precision of the current method and it was find the practical value was very close to the actual value that is available and proven with the drug package information (the theoretical value was 0.7 ppm while the practical value was 0.643 ppm). Table 1 represents the obtained data in this research compared with pervious data.

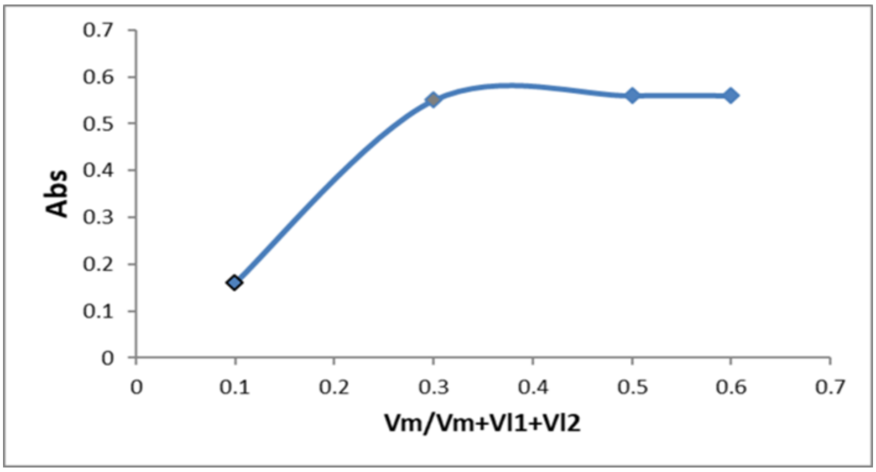


Figure 3: The absorbance values relative to (Job's method)

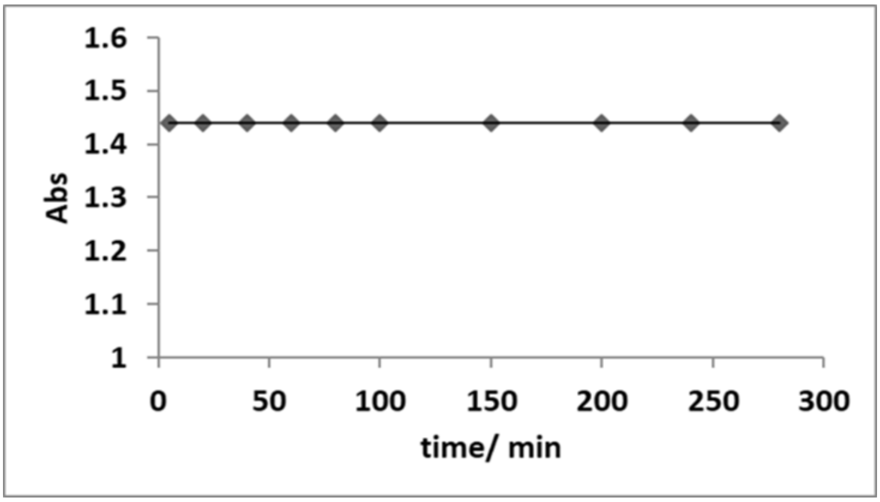


Figure 4: Absorbance values over time

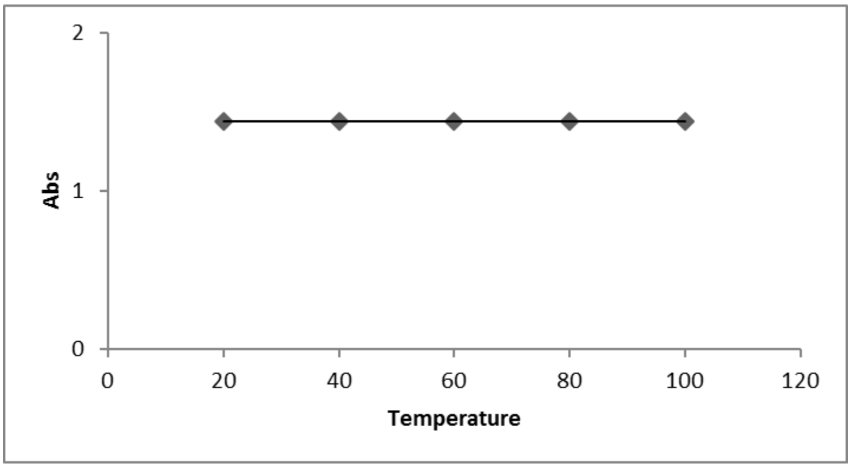


Figure 5: Absorption values with changing temperatures

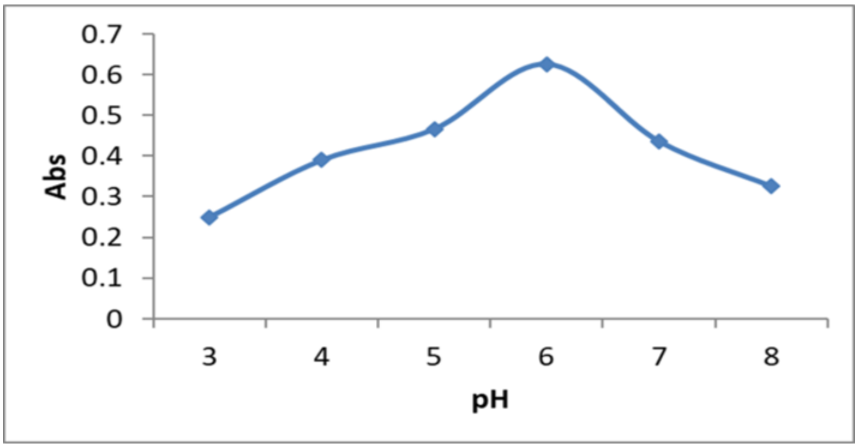


Figure 6: Absorbance values as the acidity function changes

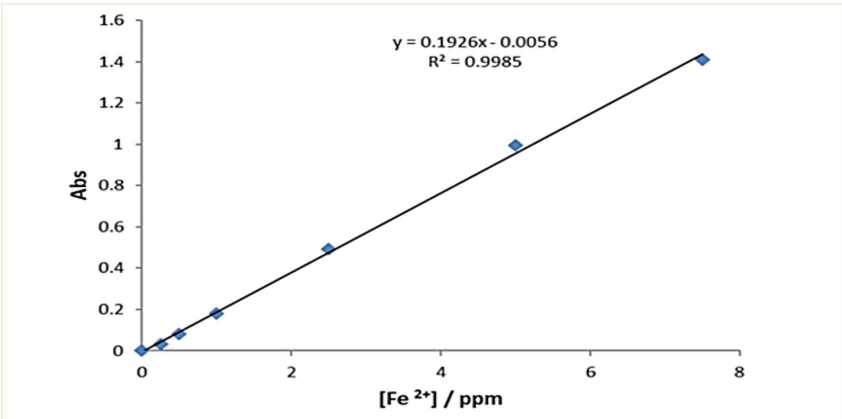


Figure 7: The calibration curve of Fe^{2+} at optimum conditions

Table 1: Differences between Fe^{2+}:1,10-phenanthroline and Fe^{2+}:imidazole:1,10-phenanthroline

Differences	ferrous:1,10-phenanthroline	ferrous:imidazole:1,10-phenanthroline
Complex absorbance and maximum wavelength	A=1.19, λ_{max} = 510-512nm	A=1.445, λ_{max} = 504 nm
pH	3-4	6
Color complex	Orange-red	Dark red
Mole ratio	1:3	1:2:1
ppm	0.5	0.20
standard deviation	2.8	0.054
Relative standard deviation	17.14%	11.8%

Antibacterial activity

Antibacterial activity increases when mixed ligands are coordinated (Figure 8 A and B, Table 2). It has been suggested that bonds containing nitrogen donor systems inhibit enzyme activity. In addition, the coordination reduces the polarity of the metal ion mainly due to the partial sharing of its positive charge with the donor groups within the chelating ring system, and then the production of metal chelates can act as an antibacterial [38, 39].

Table 2: The anti-bacterial activity of Fe(II) complex

No. of Components	Antibacterial activity test	
	Staphylococcus aureus (Gram-positive bacteria)	*Klebsiella pneumonia* (Gram-negative bacteria)
Control	-	-
complex	14	28

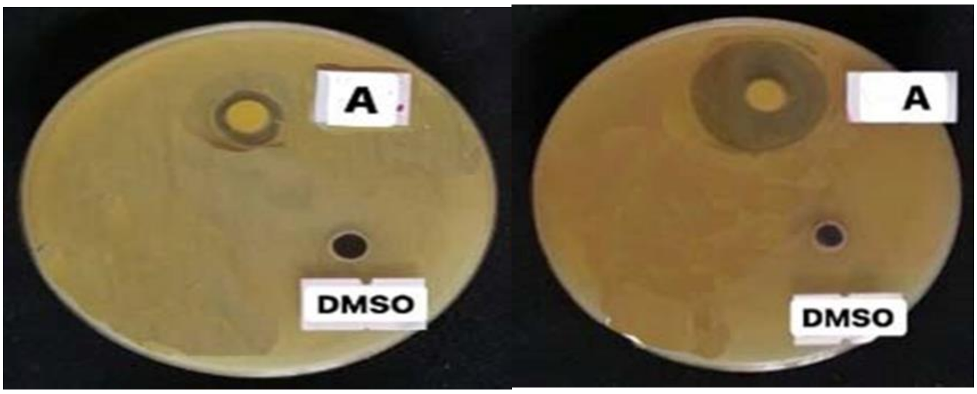


Figure 8: (A) *Staphylococcus aureus* activity test, (B) *Klebsiella pneumonia* activity test

Antioxidant

The free radicle is an atom or molecule that contains unpaired electrons. It causes damage when another molecule is encountered on another electron for a purpose of duplication. The interaction with adjacent particles leads to a sequential chemical reaction to radical production. Such an event causes molecule damage, and cell contents. The role of antioxidants is to reduce and/or prevent damage caused by free radical reactions because of their ability to donate the electrons that extend the radical without another configuration. One of these radicals is DPPH, which contains a non-paired electron in its structure and is usually used to detect the radical scavenging activity in chemical analysis. The stable DPPH radical scavenging model is a widely used method for assessing antioxidant activities in a relatively short time compared with other methods. The maximum absorbance of the DPPH radical in DMSO was at 517 nm. When there is an interaction between the antioxidant molecules and the DPPH radical, it results in the removal of the radicals by donating hydrogen or an electron, resulting in a lower uptake of the DPPH molecule. Hence, DPPH is usually used as a substrate to assess the antioxidant activity (Figures 9 and 10) of antioxidants. The effect of antioxidants on the radical scavenging of DPPH is thought to be due to its ability to donate hydrogen or electron. The scavenging ability of the mineral complex was compared with ascorbic acid as a standard, as presented in Table 3. The complex showed good activity compared to ascorbic acid [29, 40].

Table 3: Complex inhibition compared with STD

Comp. No.	Inhibition %			IC_{50} mg/mL
	25 mg/mL	50 mg/mL	100 mg/mL	
A	48.22	55.44	56.55	33.87
Ascorbic acid(STD)	46.12	60.14	65.01	28.72

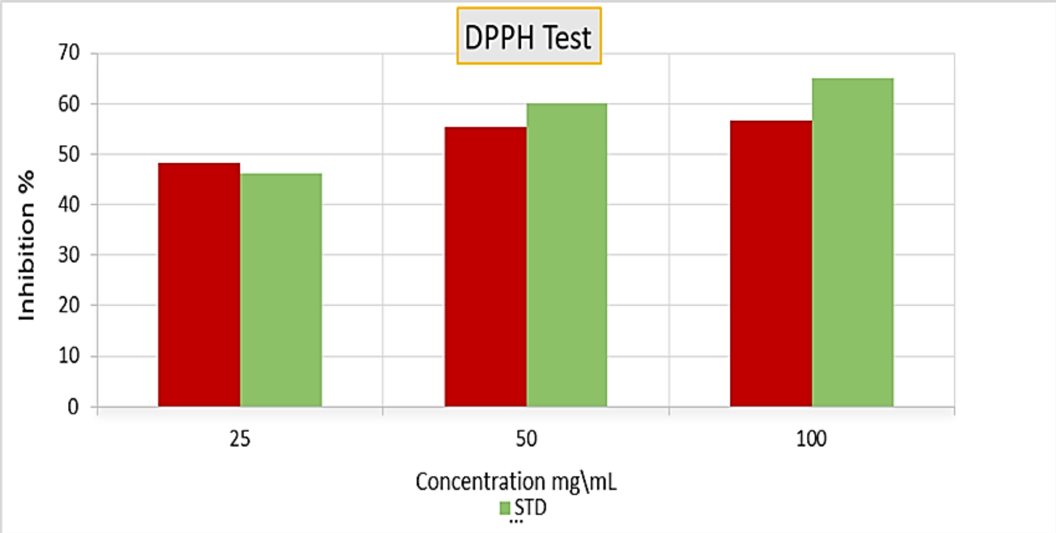


Figure 9: Concentration of Fe(II) complex with STD

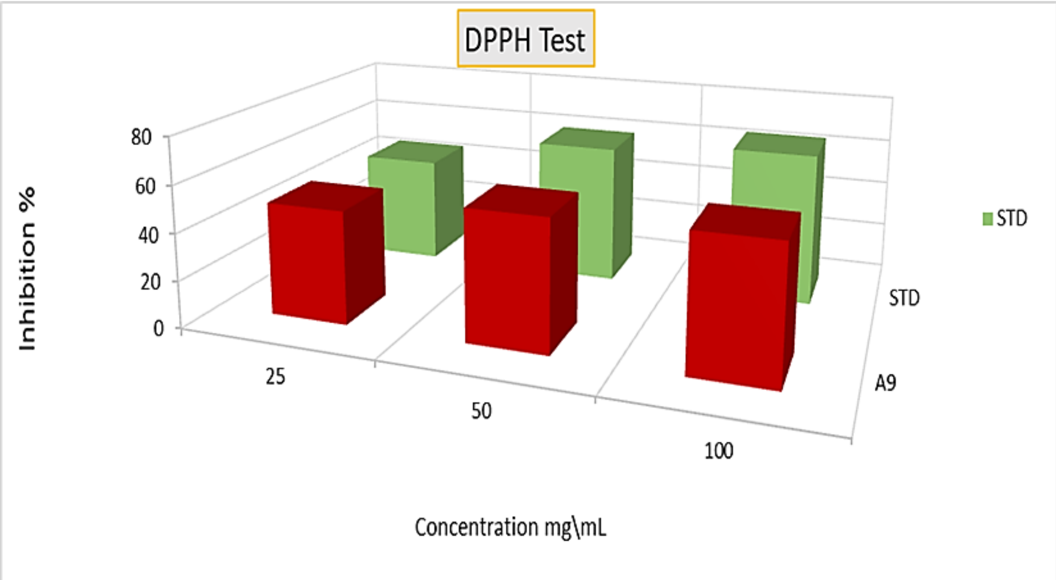


Figure 10: Concentration of Fe(II) complex with STD

Conclusion

In this the research, a ferrous complex was produced, which contains mixed ligand represented by imidazole and 1,10-phenanthroline, and it was perfectly distinguished by its solubility, where it was found that its solubility was good in ethanol and slight in water while clear in DMSO. The melting point of the complex was 204°C and the conductivity of the complex was 99.7 µS/L, and this indicates that the solution of the complex is neutral and does not have any charge. Similar to the complex formation was distinguished by infrared, ultraviolet, and proton nuclear magnetic resonance and by comparing it with the complex (ferrous: 1,10-phenanthroline), it was found that the prepared complex was more stable than Fe^{2+}-1,10-phenanthroline complex. The prepared complex can be used for iron determination as well as the antibacterial and antioxidant agents.

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Authors' contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

Conflict of Interest

There are no conflicts of interest in this study.

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