



STUDY ON THE INTERACTION OF COPPER (II) COMPLEX OF MORIN AND ITS ANTIMICROBIAL EFFECT

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ABSTRACT

Complex formation between copper (II) chloride dihydrate and morin (3,5,7,2',4'-pentahydroxyl flavone) have been studied. Structure of the complex was determined through various analytical techniques including UV-Vis, FT-IR, and elemental analysis. The results of these analyses indicate that the complex has a Cu (II) ion coordinated via positions 4 C=O and 5-OH of the morin and has the general structure $[CuL_2(H_2O)_2]$.

Antimicrobial (antibacterial and antifungal) activities of this complex was evaluated by using wells-diffusion method and compared with the activity of free morin, which showed that the antimicrobial activity of complexed morin has higher value as compared to the free morin.

Key words: Morin, Copper (II) complex, Antimicrobial.

INTRODUCTION

Flavonoids are a group of polyphenolic compounds found in fruits and vegetables, which display a variety of biological activities, such as antioxidant, anti-inflammatory, blood, lipid-lowering and anti-carcinogenic activities¹⁻³.

This class of compounds has received much attention because of their pharmacological activities in the treatment of diseases such as allergy, diabetes mellitus, cancer, viral infection inflammation and others⁴⁻⁸. Most of these effects are related to enzymatic inhibition, anti-cancer and antioxidant activity, and interference with reactions such as the formation of free radicals⁹.

Morin (Fig. 1) is a light yellowish natural plant dye. The bioactivity of morin is associated with its structural features which can be involved in complex formation with metals. Due to well-situated location of the 5 OH and 4 CO as well as 3 OH and 4 CO group

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in a molecule, morin forms chelate complexes with ion of p-, d- and f- electron metals. Morin has been used as a colorimetric reagent for spectrophotometric determination of metal ions¹⁰⁻¹².

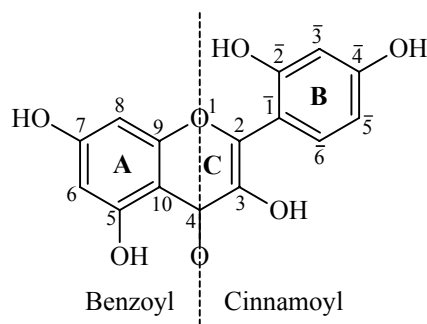


Fig. 1: Structure of morin (3,5,7,2',4'-pentahydroxy flavone)

Morin can frequently be used as a food preservative and has been shown to prevent lipid per oxidation. It is also known to protect against oxyl-radicals. The chelation of morin with metal cation is an important factor both in its bioactivity and its role as carrier and monitoring metal concentration¹³.

Copper have a major role in the production of the very reactive hydroxyl radical (OH) through the Fenton and Haber-Weiss reaction¹⁴. Copper (II) can bind and manifests large hyper. Chromic and bathochromic shifts in the molecular absorption spectra. Interests pressed in copper (II) complexes are increasing due to their possible medical uses as antitumor agents, and new bioactive ligands, involving natural product ligands^{15,16}, have been applied for the design of Cu-coordination novel drugs, for that naturally occurring compounds have served as a major source of drugs for centuries¹⁷.

The aim of these studies was to investigate the interaction between Cu (II) ion and the flavonoid. Herein, a modified method for the complexation of copper with morin has been adapted as well as the characterization and comparative study of ligand and its metal complex has been carried out. It is believed that it is the active form of compound, which is medicinally beneficial.

EXPERIMENTAL

Materials

All reagents and solvents were of analytical or chemically pure grade. Morin (3,5,7,2',4'-penthydroxyflavone) by gift supplied from Ass. Prof. AbdulKadir M. N. Jassim,

CuCl₂·2H₂O by Fluka, ammonia solution by Fluka. All reagents were weighted with an accuracy of ± 0.00010 g.

The biological activity determined by using wells-diffusion method. The isolates (*Staphylococcus aureus*, *pseudomonas aeruginosa*, *candia albicans*) were obtained from department of biology, College of Science, The University Mustansiriyah. The concentration for both morin and Cu(II)-Morin complex was 250 μM.

Instrumentation

UV-Visible spectra were obtained on a Perkin-Elmer, FT-IR spectrophotometer. Elemental analysis was carried out on a Perkin-Elmer Euro vector, Italy CHNS elemental analyzer in Al-Bayt University.

Synthesis of complex

Synthesis of morin Cu complex by addition of ethanolic solution of morin 0.0604 g, 0.2 mmol to an aqueous solution 15 mL of 0.0204 g, 0.12 mmol and was adjusted pH to 7-8 with ammonia solution. The mixture was refluxed with stirring for 12 hours, and brown precipitate formed during reflux and then allowed to cool to room temperature and filtered. The solid was washed with water and ethanol and then air-dried for 2 days.

RESULTS AND DISCUSSION

The Cu (II) complex was synthesized by aqueous solution of CuCl₂·2H₂O and the ethanolic aqueous solution of morin in 1 : 2 molar ratio (metal salt/ligand). Ligand was deprotonated by adding ammonia solution. The structure of copper (II) complex was characterized by UV-Vis, FT-IR, and elemental analysis.

UV-Vis spectrum of free ligands and their Cu complex suggested two possible metal complexing domains that can interact with metal ions: 3-or 5-hydroxy and 4-carbonyl group in the C ring (Fig. 1).

Morin has two maxima bands (Fig. 2), at 264 nm (benzoyl of ring A, band I) and the other at 358 nm (cinnamoyl of ring B, band II)¹². The maximum absorption is shifted to 226 nm and the weak band shifted to 292 nm with respect to the free morin (Fig. 2b), suggesting an interaction of the Cu (II) ion with the condensed ring of the flavonone in position 4 and 5. These results are in agreement with the results of others^{16,18-20}.

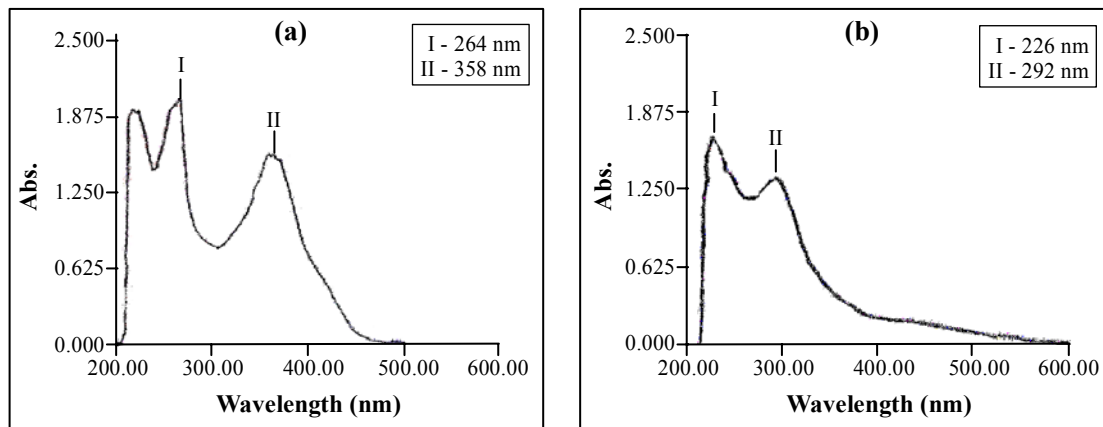


Fig. 2: UV-Vis absorption spectra of (a) free ligand morin and (b) its Cu (II) complex in ethanol

FT-IR spectrum data in KBr free ligand and its Cu complex was compared in Table 1. The absorption around 3296 cm^{-1} due to phenolic hydroxyl in the free ligand shows significantly spectral change in the Cu complex, indicating the chelate formation through hydroxyl group. The Cu complex shows a medium broad band around 3400 cm^{-1} indicating coordinated water¹². The intense absorption bands due to $\nu(\text{C}=\text{O})$ of free ligands at 1651 cm^{-1} was also shifted to lower frequency and overlapped with the broad band at 1622 cm^{-1} for the Cu (II) morin complex and was no more visible. The latter behavior can be interpreted as active evidence of the involvement of the carbonyl group in the metal binding. The $\nu(\text{C}-\text{O}-\text{C})$ at 1315 cm^{-1} shifted to 1247 cm^{-1} , thus it suggests that morin is coordinated with Cu (II) through oxygen atom of $-\text{OH}$ and $\text{C}=\text{O}$ group^{18,12}.

Table 1: FT-IR spectrum data (cm^{-1}) for (A) the free ligand morin and (B) Cu(II)–Morin complex

Compound	$\nu(\text{C}=\text{O})$	$\nu(\text{C}=\text{C})$	$\nu(\text{C}=\text{OH})$	$\nu(\text{C}=\text{H})$	$\nu(\text{C}-\text{O}-\text{C})$	$\nu(\text{Cu}=\text{O})$
Morin	1651	1608	1348	3296	1315	-
Cu (II)-Morin complex	1622	1560	1248	3439	1247	576

The most probable chelation site could be between the 5-hydroxyl and 4-oxo group since a six-membered ring can be formed compared with a less stable five-membered ring chelate, in agreement with the higher stability of the complex found in case of chelation via

5-OH^{21,22}. The dissociation pattern of individual hydroxyl groups occurs with more favourable dissociation for the 5-OH than for 3-OH group²¹.

This contention is further confirmed by the presence of (M-O) band at about 576 cm⁻¹ in the far IR frequency region (Fig. 3).

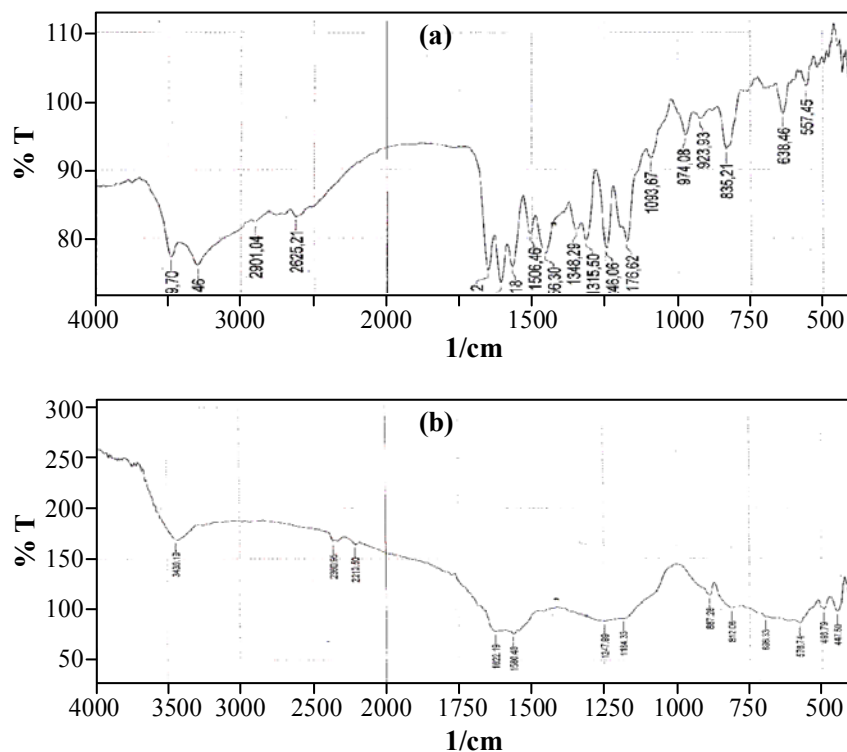


Fig. 3: FT-IR spectra for (A) the free ligand morin and (B) Cu (II)-morin complex

Elemental analysis was found to be C, 50.8; H, 3.1; N, 0.0; S, 0.0%. Calculated for [Cu(C₁₅H₉O₇)₂(H₂O)₂]: C, 51.3; H, 3.33; N, 0.0; S, 0.0%. Based on these results, the possible structure of the complex was suggested. As shown in Fig. 4, the copper (II) complex has the general formula with bidantate ligand (morin) and this complex is air stable for extended periods and soluble in methanol and scarcely soluble in water and not soluble in ether.

Antimicrobial activity

Flavonoids are phenolic compounds widely distributed in plants, which display a variety of biological activity. Many flavonoids are natural chelators and, flavonoid metal

complexes had showed significantly higher cytotoxic activity than those of the parent flavonoids, such as morin²³, chrysin²⁴, quercetin²⁵ and so forth. Besides, it is demonstrated that the coordination of copper (II) ion with bioactive ligands can actually improve the pharmaceutical activity of the drug themselves and reduce their toxicity effect²⁶.

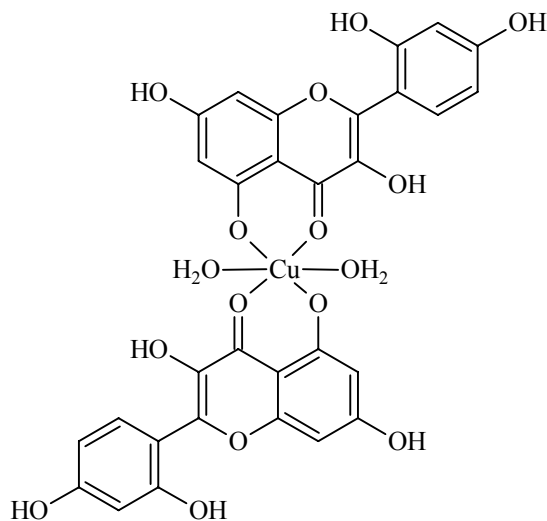


Fig. 4: The possible structure of the Cu (II)-Morin complex

The *in vitro* antimicrobial activity of morin and its complex was tested using wells-diffusion method. The susceptibility of certain strain of bacterium and fungal towards morin and its complex judged by measuring the inhibition diameter. The results reported in Table 2. Show that among the tested compounds at the concentration 250 μm , morin exhibit no inhibition of growth of studied strain, while the Cu (II)-morin complex shows higher activity for both. *S. aureus* and *Ps. aeruginosa* than for fungal *Candida albicans*. Studies of the antibacterial activity of other flavonoids and its complexes showed similar results^{10,27,28}.

Table 2: Antimicrobial activity of morin and Cu (II)-morin

Compound	Complex <i>S. aureus</i>	<i>Ps. aeruginosa</i>	<i>Candida albicans</i>
* Morin 250 μm	-	-	-
** Cu (II)-morin 250 μm	12	15	10

* No inhibition zone, ** inhibition zone (mm)

CONCLUSION

The study describes a convenient synthesis as well as the structure elucidation. From UV-Vis spectroscopic studies and FT-IR and elemental analysis for metal content of complex, it has been revealed that the complex coordinated via positions 4-carbony and 5-OH of morin. It has been noticed that in the complexation, 5-OH group shows more activity for the coordination. The Cu (II)-morin complex showed higher activity to ward microbial (bacterial, fungal) than its free ligand morin.

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