

This article was downloaded by: [Renmin University of China]

On: 13 October 2013, At: 10:21

Publisher: Taylor & Francis

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



## Journal of Coordination Chemistry

Publication details, including instructions for authors and subscription information:

<http://www.tandfonline.com/loi/gcoo20>

### Synthesis and evaluation of antioxidant and antibacterial properties of morin complexes

Qadeer K. Panhwar<sup>a b</sup> & Shahabuddin Memon<sup>a</sup>

<sup>a</sup> Dr M.A. Kazi Institute of Chemistry, University of Sindh, Jamshoro, Pakistan

<sup>b</sup> National Center of Excellence in Analytical Chemistry, University of Sindh, Jamshoro 76080, Pakistan

Published online: 14 Jun 2011.

To cite this article: Qadeer K. Panhwar & Shahabuddin Memon (2011) Synthesis and evaluation of antioxidant and antibacterial properties of morin complexes, Journal of Coordination Chemistry, 64:12, 2117-2129, DOI: [10.1080/00958972.2011.590192](https://doi.org/10.1080/00958972.2011.590192)

To link to this article: <http://dx.doi.org/10.1080/00958972.2011.590192>

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at <http://www.tandfonline.com/page/terms-and-conditions>

## Synthesis and evaluation of antioxidant and antibacterial properties of morin complexes

QADEER K. PANHWAR<sup>†‡</sup> and SHAHABUDDIN MEMON<sup>\*†</sup>

<sup>†</sup>Dr M.A. Kazi Institute of Chemistry, University of Sindh, Jamshoro, Pakistan

<sup>‡</sup>National Center of Excellence in Analytical Chemistry, University of Sindh,  
Jamshoro 76080, Pakistan

(Received 25 January 2011; in final form 27 April 2011)

This study examines the interaction of Mg(II) and Ca(II) with morin (2',3,4',5,7-pentahydroxyflavone) in conjunction with the appraisal of antioxidant and biological properties of the complexes. The complexes were characterized by UV-Vis spectroscopy, FT-IR, <sup>1</sup>H NMR, <sup>13</sup>C NMR, thermal, and elemental analyses. The complexes have also been studied to determine metal : ligand stoichiometry. For antioxidant studies, it may be induced that complex formation increases the scavenging ability because of the differential and selective role of metals in free radical scavenging. The bioactivity studies show that both complexes have remarkable antibacterial properties against *Micrococcus flavus* and *Staphylococcus aureus*.

**Keywords:** Synthesis; Properties; Coordination; Antimicrobial

### 1. Introduction

Flavonoids exhibit a wide spectrum of beautiful colors in flowers, fruits, and leaves [1–3]. In plants, they also work as antioxidants, antimicrobials, photoreceptors, visual attractors, and feeding repellents [4]. More than 4000 such compounds are separated and identified from plants [5]. Out of these, quercetin, morin, and rutin are best known and studied [6–8]. These compounds are potential antibacterial, anticancer, anti-inflammatory, and anti-allergic agents and their intake is very important to prevent a number of diseases, which cause free-radical-mediated damage [4, 9]. The big interest in this group of compounds is because of their biological activity; hence, many of them have been added in pharmaceuticals for therapeutic use [10–14]. They are highly useful colorimetric compounds for determining minute quantities of metals from solutions [15]. Most of the flavonoids have a common phenyl–benzopyrone skeleton called flavone; yet, they differ in hydroxyl, methoxy, and glycosyl groups [16, 17]. Free radicals damage the normal metabolic processes, ultimately causing pathological problems due to their interactions with different types of biochemicals interior and exterior to the cells. Several antioxidative compounds are known, among these the role of flavonoids is significant [18]. Many of them are less efficient, nevertheless there

\*Corresponding author. Email: shahabuddinmemon@yahoo.com

is a role played by them in removing reactive oxygen and thereby managing deadly consequences. Consequently, cost-effectiveness, healthiness, biological, and ecological significance have made it important to develop new antioxidants. Researchers try to develop/design new antioxidants with improved antioxidant power by the joint action of metal complexes and natural compounds [19, 20]. Flavonoids have ability to bind metal ions, making them outstanding reagents. Complex formation has importance in many life functions [3, 21, 22], presenting increased activity [23]. Morin (figure 1) is a phenolic compound derived from the hydroxyl substitution of flavone [24]. It is present in tea, coffee, cereal grains, fruits, vegetables, and many traditional Chinese herbal medicines [25].

Mg<sup>2+</sup> ions are found in animal cells, forming complexes with ATP and are important in enzymes like phosphohydrolases and phosphotransferases, and they catalyze various reactions concerning ATP and energy release. Mg<sup>2+</sup> is also a major constituent of chlorophyll which is present in green plants. Ca<sup>2+</sup> ions are found outside the cell in body fluids and they are also a major part of bones and teeth. They play a vital role in blood clotting, normal heart beat, and as a trigger for contraction of muscles. However, among group II metals, Mg<sup>2+</sup> and Ca<sup>2+</sup> have ability to form complexes due to their small highly charged ions, and are candidates to form complexes with oxygen donors. Mg<sup>2+</sup> reacts with different bidentate and polydentate (chelating) ligands; Ca<sup>2+</sup> also forms complexes [26, 27]. The complex formation provides the basis of complexometric titration of these metal ions with morin.

Herein, we describe the syntheses of morin complexes with Mg(II) and Ca(II) as well as the evaluation of their antioxidant and biological activities. The elemental, thermogravimetric, and spectroscopic analyses establish the proposed structure and composition of the complexes.

## 2. Experimental

### 2.1. Materials and methods

All reagents and solvents were of analytical or chemically pure grade. Morin hydrate (2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-1-benzopyran-4-one) by Sigma (St Louis, MO, USA), MgCl<sub>2</sub>·6H<sub>2</sub>O and CaCl<sub>2</sub> by Merck (Darmstadt, Germany), HPLC grade methanol by Fisher Scientific Ltd. (Leicestershire, UK), and KBr from Aldrich Chemical Co. (Taufkirchen, Germany) were purchased. All reagents were weighed with an accuracy of ±0.0001 g.

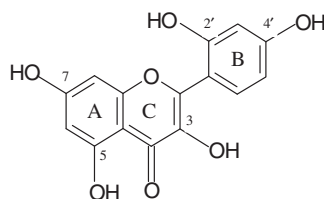


Figure 1. Molecular structure of morin.

UV-Vis spectra were obtained by a Perkin Elmer Lambda 35 UV-Vis double beam spectrophotometer using standard 1.00 cm quartz cells in methanol.  $^1\text{H}$  NMR spectra in DMSO were obtained on a Bruker AVANCE AV-400 MHz spectrometer using TMS as internal reference.  $^{13}\text{C}$  NMR spectra were recorded on a Bruker AVANCE AV-300 MHz spectrometer. IR spectra were recorded using KBr pellets from 4000 to  $400\text{ cm}^{-1}$  on a Nicolet 5700 FTIR instrument.

## 2.2. Stoichiometric composition of the complexes

For the determination of stoichiometric composition of complexes, Job's method of continuous variation was employed. In MeOH, the solutions of equimolar concentration  $4 \times 10^{-4}\text{ mol L}^{-1}$  were prepared by mixing different volumes of morin with  $\text{MgCl}_2$  and  $\text{CaCl}_2$  [28] in variable relative amounts from 1:9 to 9:1. The absorbance for both complexes was measured at 422 and 418 nm, respectively. The mole fraction of morin ( $X$ ) was found at  $XL=0.5$  (figure 2) according to the highest corresponding absorbance. From the mole fraction value it can be deduced that the complexes are formed in 1:1 ligand to metal ratio [29].

## 2.3. Synthesis of the metal complexes

Morin 0.0756 g ( $0.01\text{ mol L}^{-1}$ ) was added to a 100-mL round-bottom flask containing 25 mL of methanol. After 15 min of stirring nearly all the morin was dissolved, then 150  $\mu\text{L}$  of sodium methoxide was added to deprotonate it. Subsequently,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  (0.04066 g,  $0.01\text{ mol L}^{-1}$ ) and  $\text{CaCl}_2$  (0.0222 g,  $0.01\text{ mol L}^{-1}$ ) were added in two separate round-bottom flasks containing deprotonated morin and the color of the solution changed. The contents were refluxed for about 1.5 h and then the solution filtered to get rid of unused morin. The solvent was removed by rotary evaporator. Finally, the contents were washed with *t*-butanol, dried in a vacuum desiccator and the yield calculated as 73% and 85% for Mg and Ca complexes of morin, respectively. Elemental analysis found (%): C, 39.939; H, 4.245. Anal. Calcd for  $[\text{Mg}(\text{C}_{15}\text{H}_9\text{O}_7)(\text{H}_2\text{O})_4]\text{Cl} \cdot \text{H}_2\text{O}$  (%): C, 39.815; H, 4.258. Similarly, the elemental analysis found (%): C, 38.5899; H, 4.1, Anal. Calcd for  $[\text{Ca}(\text{C}_{15}\text{H}_9\text{O}_7)(\text{H}_2\text{O})_4]\text{Cl} \cdot \text{H}_2\text{O}$  (%): C, 38.037; H, 3.963.

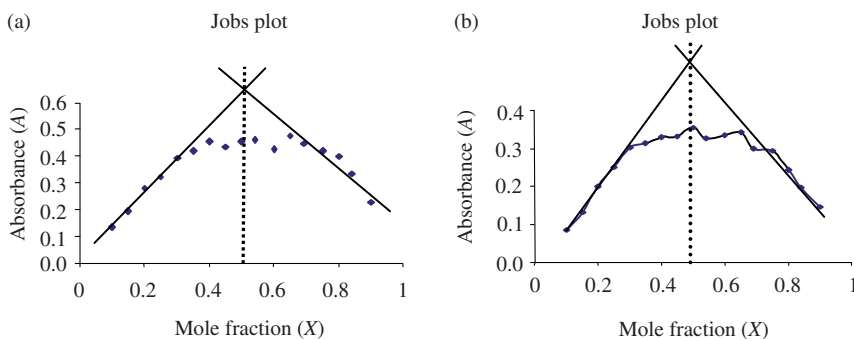


Figure 2. Jobs plot for complexes of morin with (a) Mg(II) and (b) Ca(II) by mixing equimolar solutions of  $4 \times 10^{-4}\text{ mol L}^{-1}$  each morin and Mg and Ca salts.

Table 1. Solubility of metal complexes of morin with Mg(II) and Ca(II).

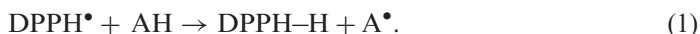
Compound	Solubility		
	Soluble	Partly soluble	Insoluble
Mg–morin complex	MeOH, EtOH, DMSO, DMF	H <sub>2</sub> O, Me <sub>2</sub> CO	CCl <sub>4</sub> , CHCl <sub>3</sub>
Ca–morin complex	MeOH, EtOH, DMSO, DMF	Me <sub>2</sub> CO, H <sub>2</sub> O	CCl <sub>4</sub> , CHCl <sub>3</sub>

#### 2.4. Solubility of the complexes

All the complexes are stable at room temperature; their solubility and other physical properties are provided in tables 1 and 2, respectively.

#### 2.5. Antioxidant activity by the 1,1-diphenyl-2-picrylhydrazyl method

Antioxidant activity was conveniently evaluated from radical scavenging of widely used stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical. The absorbance of DPPH was at 515 nm and decrease in absorbance is usually noted after the reduction of DPPH with an antioxidant to become a stable diamagnetic molecule [30, 31]. Its purple color changes to yellow, as hydrogen is abstracted (equation (1)).



The DPPH-radical-scavenging activity of the samples was estimated by adding an aliquot of morin and its complexes (0.1 mL) to a solution of DPPH radical (3.9 mL) in MeOH (60  $\mu\text{mol L}^{-1}$ ). At 515 nm the drop of absorbance was examined until DPPH was completely reduced. The scavenging activity (%) was then calculated by using equation (2),

$$\text{Scavenging activity (\%)} = 100(A_c - A_s)/A_c, \quad (2)$$

where  $A_c$  and  $A_s$  are the absorbance of control and sample, respectively [32, 33].

#### 2.6. Antibacterial activity

The antibacterial actions of morin and its metal complexes were analyzed *in vitro* using different microorganisms by the well diffusion method. The bacteria used were *Micrococcus flavus* (G+ve) and *Staphylococcus aureus* (G+ve). Each culture was inoculated before pouring into the sterilized Petri dishes. Platinum wire loop was used for inoculation purpose that was first made red hot in flame and then cooled as well as ultimately used to apply bacterial strains. The medium of brain heart infusion (BHI) agar was used. Wells of 6 mm diameter were made in the agar by using a cork borer; sterilized forceps were also used as a help. The solution of morin and its metal complexes was added to the wells by using a micropipette. All the compounds were dissolved in DMSO and examined at various concentrations. For control, DMSO was employed. Finally, the plates were incubated in air at 37°C for 24 h. The antibacterial property was assessed by measuring the diameter (in mm) of the inhibition zone. Experiments were done in triplicate [34, 35].

Table 2. Physical properties of metal complexes of morin with Mg(II) and Ca(II).

Complex	Mol. wt.	Color	Yield (%)
$[\text{Mg}(\text{C}_{15}\text{H}_9\text{O}_7)(\text{H}_2\text{O})_4]\text{Cl} \cdot \text{H}_2\text{O}$	451.062	Yellowish	73
$[\text{Ca}(\text{C}_{15}\text{H}_9\text{O}_7)(\text{H}_2\text{O})_4]\text{Cl} \cdot \text{H}_2\text{O}$	466.832	Yellowish	85

### 3. Results and discussion

#### 3.1. Electronic spectra

The electronic spectra for morin and its Mg(II) and Ca(II) complexes (figure 3) were recorded in methanol from 200 to 550 nm. The spectrum of morin alone shows two absorptions. Band I at 375 nm is formed from conjugation of ring B with carbonyl group present in ring C ( $\pi$ - $\pi^*$  transitions in B ring portion of morin), while band II at 263 nm (table 3) is from conjugation in ring A with ring C containing carbonyl [36]. When methanol solution of metal ion was added it produced a bathochromic shift in band I and a new peak at 414 and 411 nm for Mg(II) and Ca(II), respectively (table 3), indicating the formation of complexes between morin and both metal ions.

From the chemical structure of morin it is obvious that it can chelate metal ions from two sites, that is, hydroxyl located at either 3- or 5-position and carbonyl group located at 4-position. The peaks present at 265 or 264 nm show no significant change. The newly formed peaks at 414 and 411 nm suggest that Mg(II) and Ca(II) form a bond with 3-OH and 4C=O present in the C ring. There are several indications that complexation involves 3-hydroxyl and 4-carbonyl: (i) 3-hydroxyl group has stronger chelating ability than 5-hydroxyl; (ii) electrons present on oxygen of 3-OH show more delocalization than 5-OH. The bathochromic shift in band I of morin takes place due to the interaction of Mg(II) and Ca(II) with 3-OH, causing the redistribution of electrons between morin and Mg(II) or Ca(II) by increasing the  $\pi$  bond system. In morin, the change in electron distribution takes place from  $n$ - $\pi^*$  to  $\pi$ - $\pi^*$  transition, which is associated with the decrease in energy during this electron transition (Supplementary material). This is also in agreement with previous work that demonstrates the involvement of 3-OH and 4C=O groups of the C ring of morin to chelate metals [37].

#### 3.2. Stability and pH study

The Mg complex of morin was dissolved in methanol and pH was 6.8. The complex was studied over a wide range of pH, starting from 2.0 to 12, which was adjusted by NaOH and/or HCl ( $0.01 \text{ mol L}^{-1}$ ) solutions. The complex does not show any change in the absorption maximum. From this, it can be supposed that metal ion does not dissociate from the flavonoids [38] (Supplementary material).

#### 3.3. Vibrational spectra

IR absorption frequencies for free ligand and its complexes with provisional assignments are presented in table 4. IR data obtained from spectra of morin and its

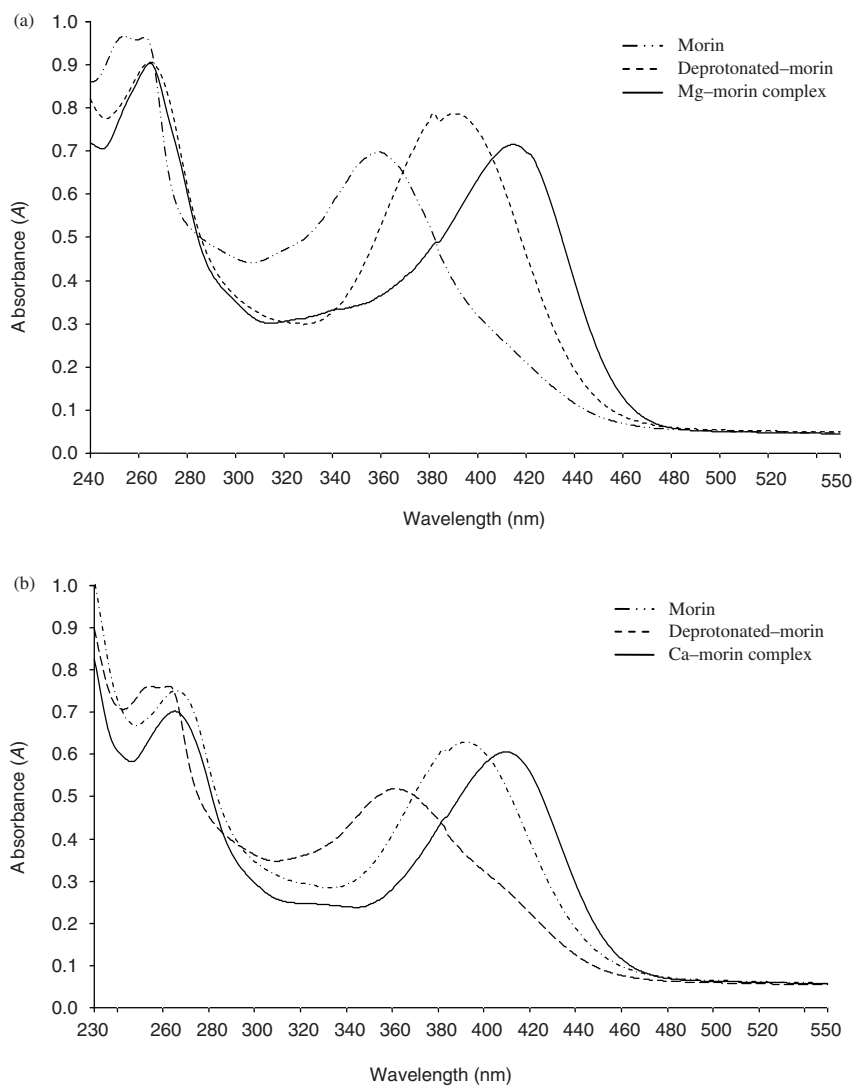


Figure 3. UV-Vis spectra of morin and its complexes with (a) Mg(II) and (b) Ca(II).

Table 3. UV-Vis data for morin and its complexes with Mg(II) and Ca(II).

Compound	$\lambda_{\max}$ (nm)	
	Band II	Band I
Morin	263.77	375.32
Mg-morin	265.60	414.88
Ca-morin	264.77	411.03

Table 4. IR data for morin and its Mg(II) and Ca(II) complexes.

Functional group	Morin	Mg(II)-morin	Ca(II)-morin
$\nu(\text{O-H})$	3381–3157b	3411	3419.7
$\nu(\text{C=O})$	1662w	1634.8	1652.9
Stretching vibration C=C in aromatic ring, ring vibrations	1613sh	–	1614.8
$\nu(\text{C=C})$	1508s	1511.4	1504.5
$\nu(\text{C=C}), \delta(\text{O-H}), \text{C-O-H}$	1460sh	1446.9	1436.6
$\nu(\text{C-OH})$	1383s	1367.8	1370.1
$\nu(\text{C-O-C})$	1310s	1321.7	1318.6
$\nu(\text{C-C})$	1258vs	1237.8	1241.9
Stretching vibrations –C–OH	1174s	–	1197.8
$\nu(\text{C-C}), \text{C-C-C}, \text{-C-OH}, \text{O-H}, \text{C-H}$ $\begin{array}{c} \parallel \\ \text{O} \end{array}$	1144m	1173.9	1171.5
Stretching vibrations –C–OH, $\rho(\text{O-H})$ in plane deformation	–	–	1094.8
(C–C) in plane deformation, deformation vibrations –C–H outside plane, related to substitution of aromatic rings of multiring compounds	969.4	975.8	980.0
(C–C) in plane, (O–H) out of plane deformation	831	800.8	834.3
(C–H) out of plane deformation	795	–	790.2
$\nu(\text{M-O})$	634.5	648.1	645.6
(C–C) out of plane deformation	564.8	–	566.2

Mg(II) and Ca(II) complexes is in accord with the structure. From significant shifts in the absorbance frequencies of metal morin complexes it may be presumed that morin is coordinated [3, 39].

- A band present in the spectra of free morin and its complexes at 3500–3000  $\text{cm}^{-1}$  corresponds to the broad band of O–H that undergoes significant variation in complexes and suggests the formation of metal–oxygen bonds [40].
- A remarkable change is observed in the region 1700–1400  $\text{cm}^{-1}$  for carbonyl group (C=O) that is diagnostic for its involvement in coordination. Upon complexation of morin with Mg(II) and Ca(II), shift in  $\nu(\text{C=O})$  is observed from 1662  $\text{cm}^{-1}$  for morin to 1634  $\text{cm}^{-1}$  with Mg(II) and 1653  $\text{cm}^{-1}$  with Ca(II). Hence, the carbonyl group participates in the bonding of metal ions and forms the chelate ring of  $>\text{C}=\text{O} \cdots \text{M}-\text{O}-$  [41].
- The characteristic  $\nu(\text{C-O-C})$  of the ligand at 1230–1383  $\text{cm}^{-1}$  are not changed significantly, suggesting that this group does not form metal–oxygen bonds [3].
- Small peaks are observed around 600  $\text{cm}^{-1}$  in both complexes, which are not observed in the spectrum of free ligand. The presence of such bands indicates metal–oxygen bonding. This is also supported by UV-Vis spectral results (table 3), where ring C coordinates with metal ions [42, 43].

### 3.4. $^1\text{H}$ NMR studies

$^1\text{H}$  NMR data show mostly an upfield shift of signals for –OH protons of morin [44] (table 5), when it forms complex with metals, because coordination increases



Table 5.  $^1\text{H}$  NMR data for morin and its Mg(II) and Ca(II) complexes.

Ligand/complex	Chemical shifts (in $\delta$ values or ppm)									
	5-OH	7-OH	3-OH	4'-OH	2'-OH	H-6'	H-5'	H-8	H-3'	H-6
Morin	12.612	10.689	9.761	9.365	9.278	7.248	6.468	6.174	6.438	6.19
Mg-morin	13.374	11.647	–	9.667	10.73	7.60	6.911	6.469	6.889	6.450
Ca-morin	12.56	10.78	–	10.789	9.725	7.949	6.928	6.467	6.889	6.475

the conjugation. Since morin is likely to undergo chelation *via* 3-OH phenol, the complex formation removes phenolic hydrogen of flavonoid. From the above information it becomes clear that upon complexation only one proton in free morin undergoes deprotonation and morin is monobasic bidentate [45, 46].

The complexes show only four signals for hydroxyl groups in spectra of the Mg(II) and Ca(II) complexes of morin; the resonance of hydrogen of 3-OH is not present, indicating loss of –OH proton due to complexation (table 5). Only this proton is replaced by metal, confirming coordination of Mg(II) or Ca(II) with oxygen of 3-OH (Supplementary material) [47, 48].

### 3.5. $^{13}\text{C}$ NMR spectra

$^{13}\text{C}$  NMR spectra (Supplementary material) for morin and its Mg(II) or Ca(II) complexes have also been discussed comparatively. From the data (table 6), the main feature of complex formation is that almost every carbon in morin is influenced but C3 and C4 show significant difference relative to the other carbons. The signals at 176 and 177 ppm (C4=O) indicate that Mg(II) or Ca(II) interacts strongly and their position is near C=O. From this it may be established that chelation takes place *via* C4=O and C3–O. Hence, the substitution of intramolecular C3–OH  $\cdots$  O=C4 hydrogen bonding for coordination results in frequency shift of C3 and C4 (4.64, 2.83, 0.28, and 1.1 ppm for Mg(II) and Ca(II), respectively), because C3 and C4 become electron deficient in the formation of chelates with Mg(II) or Ca(II). Mg(II) and Ca(II) draw electrons from C3 and C4 carbons because they offer their electrons for bonding. When metal complexes are formed, both mesomeric forms, i.e., pyronium and cinnamoyl, contribute, altering the electronic charge density (figure 4) [49]. The signals of carbons located as C2, C1', C2', and C4' show only negligible upfield shifts, because cinnamoyl has substantial contribution to stabilize the complexes.

Intramolecular hydrogen bonding plays a vital role in the stability of morin, formed by the transfer of hydrogen from 2'-hydroxyl to oxygen of 3-hydroxyl group and simultaneously to oxygen of 4-carbonyl. As soon as the complexes form, the proton of –OH group located at C3 is lost, disrupting the hydrogen bonding. No appreciable shift in the signals of C4 and C5 shows that they have intramolecular bonding C5–OH  $\cdots$  O=C4. The orientation of C7–OH is unclear due to insignificant shielding differences shown by C6 and C8. If the hydroxyl substituent is in the plane of the aromatic ring, then the shieldings of proximal carbons is marked, suggesting that C7–OH is twisted out of the plane. The relative change in the position of morin by

Table 6.  $^{13}\text{C}$  NMR (chemical shift) data for morin and its Mg(II) and Ca(II) complexes.

Position	Chemical shifts (in $\delta$ values or ppm)		
	(Morin)	(Mg–morin)	(Ca–morin)
C2	148.97	148.32	148.58
C3	136.16	140.80	138.99
C4	176.16	176.28	177.29
C5	156.74	156.70	158.63
C6	93.32	93.45	93.26
C7	163.85	163.62	163.45
C8	97.99	97.97	97.82
C9	160.68	159.78	159.22
C10	106.77	107.49	106.70
C1'	115.41	115.42	115.43
C2'	156.77	156.14	156.60
C3'	103.51	104.97	103.94
C4'	160.89	159.78	159.22
C5'	109.19	107.49	106.70
C6'	131.63	129.45	129.96

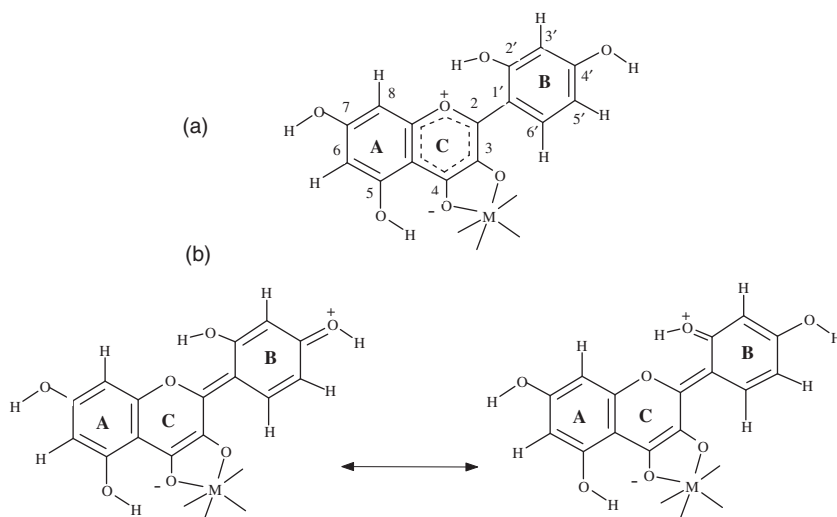


Figure 4. (a) Pyronium and (b) cinnamoyl mesomeric forms participating in the resonance of morin complexes. Only one ligand has been displayed.

rotation and rocking produce different chemical environments and creates different intramolecular bonding, resulting in considerable shielding differences [9, 38].

Spectroscopic data reveal that coordination of morin with Mg(II) or Ca(II) takes place *via* C4=O and C3–O oxygen donors [50, 51].

### 3.6. Thermal analysis

Thermal data obtained from TG-DTA curves (Supplementary material) show dehydration and decomposition of the complexes, heated at  $20^\circ\text{C min}^{-1}$ . The results

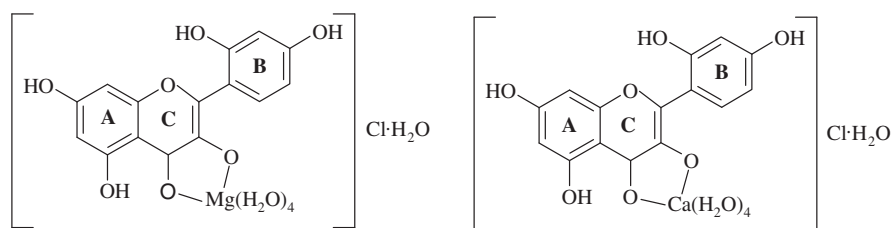


Figure 5. Proposed structures of morin-Mg(II) and morin-Ca(II) complexes.

of Mg–morin complex displayed three main stages from 40°C to 600°C. The first stage from 90°C to 110°C is associated with dehydration with mass loss of 2.5% from loss of hydration water. The next dehydration process from 140°C to 155°C with corresponding mass loss value of 2.2% is due to the coordinated water molecules. The final mass loss was the decomposition of the ring of the ligand at 380–500°C. As thermogravimetric analysis is performed with nitrogen, residues of metal oxide are not expected to form. Ca–morin complex exhibits weight loss at 60–93°C that corresponds to dehydration. Sharp weight loss at 270–500°C corresponds to continuous thermal degradation of the ligand [52]. The proposed structures of the complexes are given in figure 5.

### 3.7. Gravimetric analysis of chloride

Appropriate amounts of  $[\text{Mg}(\text{C}_{15}\text{H}_9\text{O}_7)(\text{H}_2\text{O})_4]\text{Cl}\cdot\text{H}_2\text{O}$  and  $[\text{Ca}(\text{C}_{15}\text{H}_9\text{O}_7)(\text{H}_2\text{O})_4]\text{Cl}\cdot\text{H}_2\text{O}$  were dissolved in methanol and subsequently 0.5 mL of conc.  $\text{HNO}_3$  was added. After that, the solution was heated to 70°C and 5 mL of  $\text{AgNO}_3$  was added to the warm solution with constant stirring. The precipitates were further warmed and allowed to cool in a dark place. After some time, the supernatant liquid was tested for chloride ions by adding  $\text{AgNO}_3$  with the sides of beaker in order to check the completion of precipitation. Again, the precipitates were allowed to stand in the dark for a few hours, preferably overnight. Finally, the precipitates were filtered through previously cleaned, dried, and weighed sintered glass crucible. Washing of the precipitate was done with dil.  $\text{HNO}_3$  (0.01 N) followed by distilled water. Subsequently, the precipitates were dried above 100°C, cooled in a desiccator, and finely weighed. Equation (3) shows precipitate formation.



Ignition of  $\text{AgCl}$  precipitates was performed in ashless filter paper [53]. At last, the percentage of  $\text{Cl}^{-1}$  was calculated from the amount of complexes dissolved. When the formulae of complexes were compared for chloride ions, the gravimetric determination proved that the experimental values, i.e., 7.295% and 7.138% are in good agreement with theoretical values of 7.870% and 7.604% in the proposed formulae, i.e.,  $[\text{Mg}(\text{C}_{15}\text{H}_9\text{O}_7)(\text{H}_2\text{O})_4]\text{Cl}\cdot\text{H}_2\text{O}$  and  $[\text{Ca}(\text{C}_{15}\text{H}_9\text{O}_7)(\text{H}_2\text{O})_4]\text{Cl}\cdot\text{H}_2\text{O}$ , respectively.

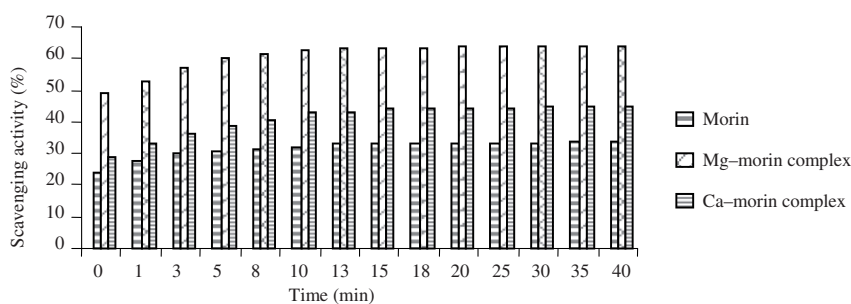


Figure 6. Scavenging activity of morin, morin-Mg(II), and morin-Ca(II) complexes to DPPH radical.

### 3.8. Metal-content determination

Metal ion concentrations were determined through complexometric titration, in which suitable volume of morin complexes was titrated by adding standard solution of  $0.1 \text{ mol L}^{-1}$  EDTA [53]. From the titrimetric results, the amount of  $\text{Mg}^{2+}$  and  $\text{Ca}^{2+}$  was 4.731% and 8.194% in the complexes, consistent with theoretically calculated values, i.e., 5.3873% and 8.5684% in  $[\text{Mg}(\text{C}_{15}\text{H}_9\text{O}_7)(\text{H}_2\text{O})_4]\text{Cl}\cdot\text{H}_2\text{O}$  and  $[\text{Ca}(\text{C}_{15}\text{H}_9\text{O}_7)(\text{H}_2\text{O})_4]\text{Cl}\cdot\text{H}_2\text{O}$ , respectively, indicating 1:1 metal:ligand composition involving deprotonation at single  $-\text{OH}$ .

### 3.9. Antioxidant activity

The results of DPPH-radical-scavenging analysis can be seen in figure 6. Morin and its complexes scavenge DPPH effectively with scavenging activity of complexes greater than that of free morin, indicating that complexes are much stronger free radical scavengers and antioxidants than free morin. The substantial antioxidant action of complexes, relative to free morin, is from coordination of metals at the 3 and 4 positions of the fused ring, stabilizing unpaired electron and thus scavenging the free radicals [44, 54–56].

### 3.10. Antibacterial activity

The antibacterial activities of ligand and its complexes were carried out against *M. flavus* and *S. aureus* by using the well diffusion method. Morin is active against *S. aureus* but inactive against *M. flavus* and its complexes are even more active against both strains relative to the ligand (table 7). Metallation increases the antimicrobial activity by chelate formation [57, 58].

## 4. Conclusion

Morin forms 1:1 metal:ligand complexes with Mg(II) and/or Ca(II). UV-Vis spectra show significant bathochromic shifts that confirm the formation of complexes in the

Table 7. Antibacterial activity of morin and its metal complexes with Mg(II) and Ca(II).

Compounds	Diameter of inhibition zone of bacteria in ligand and complexes (mm)									
	<i>M. flavus</i>					<i>S. aureus</i>				
	0.1%	0.2%	0.3%	0.4%	0.5%	0.1%	0.2%	0.3%	0.4%	0.5%
Morin	–	–	–	–	–	2.5	4.3	8	8.5	8.75
Mg–morin	1	2.2	3	3.7	4.5	3	4	9	9.32	9.51
Ca–morin	1.3	2.5	3.4	4	5.1	3.3	4.5	9.4	9.7	9.9
DMSO	–ve	–ve	–ve	–ve	–ve	+ve	+ve	+ve	+ve	+ve
Kanamycin			–					25		
Vancomycin			20					–		

B ring portion and consequently verify deprotonation of the 3-OH, which is further confirmed by NMR studies. FT-IR spectroscopy shows involvement of oxygen of carbonyl in coordination, while chloride exists as a counter anion. Thermal analysis confirms the presence of coordinated as well as hydrated water molecules, also supported by FT-IR. From significant thermal degradation values of the complexes, stable bonding is formed between metals and morin. The antioxidant efficacy of the complexes is more pronounced with significant DPPH-scavenging capacity in contrast to morin from complexation occurring at the 2,3-double bond and the 3-OH group. The complexes have promising activity against *S. aureus* and *M. flavus*.

### Acknowledgments

This research work was supported by the National Centre of Excellence in Analytical Chemistry, University of Sindh, Jamshoro/Pakistan. The authors thank and gratefully acknowledge for this financial assistance and express their appreciations on the provision of necessary facilities.

### References

- [1] I.F. Cheng, B. Kevin. *Biometals*, **13**, 77 (2000).
- [2] J.B. Harborne, T.J. Mabry. *The Flavonoids: Advances in Research*, Chapman and Hall, London (1982).
- [3] E.G. Ferrer, M.V. Salinas, M.J. Correa, L. Naso, D.A. Barrio, S.B. Etcheverry, L. Lezama, T. Rojo, P.A.M. Williams. *J. Biol. Inorg. Chem.*, **11**, 791 (2006).
- [4] L. Naso, E.G. Ferrer, L. Lezama, T. Rojo, S.B. Etcheverry, P. Williams. *J. Biol. Inorg. Chem.*, **15**, 889 (2010).
- [5] V. Cody, E. Middleton, J.B. Harborne. *Plant Flavonoids in Biology and Medicine: Biochemical, Pharmacological and Structure-Activity Relationships*, Alan R. Liss, New York (1986).
- [6] J. Pusz, M. Kopacz, S. Kopacz. *Koord. Khim.*, **7**, 966 (1990).
- [7] Y.M. Song, J.W. Kang, Z.H. Wang, X.O. Lu, J.Z. Guo, L.F. Wang. *J. Inorg. Biochem.*, **91**, 470 (2002).
- [8] J. Zhou, L.F. Wang, N. Tang. *J. Inorg. Biochem.*, **83**, 41 (2001).
- [9] J. Pusz, B. Nitka, A. Zielińska, I. Wawer. *Microchem. J.*, **65**, 245 (2000).
- [10] J. Pusz, E. Woznicka, S. Wolowicz, M.H. Umbreit. *J. Therm. Anal. Calorim.*, **97**, 987 (2009).
- [11] V. Cody, E. Middleton, J.B. Harborne, A. Beretz. *Plant Flavonoids in Biology and Medicine II: Biochemical, Cellular and Medicinal Properties*, Alan R. Liss, New York (1988).

- [12] F.I. Kanaze, E. Kokkalou, I. Niopas, M. Georgarakis, A. Stergiou, D. Bikiaris. *J. Therm. Anal. Calorim.*, **83**, 283 (2006).
- [13] J. Pusz, B. Nitka. *Microchem. J.*, **56**, 373 (1997).
- [14] Z. Jerzmanowska. *Wiad. Chem.*, **27**, 623 (1973).
- [15] M. Katyál, S. Prakash. *Talanta*, **24**, 367 (1977).
- [16] L.D. Mello, R.M.S. Pereira, A.C.H.F. Sawaya, M.N. Eberlin, L.T. Kubota. *J. Pharm. Biomed. Anal.*, **45**, 706 (2007).
- [17] M.G. Alvarez, G. Alzuet, J.L.G. Gimenez, B. Macias, J. Borrás. *Z. Anorg. Allg. Chem.*, **631**, 2181 (2005).
- [18] S.B. Etcheverry, E.G. Ferrer, L. Naso, J. Rivadeneira, V. Salinas, P.A.M. Williams. *J. Biol. Inorg. Chem.*, **13**, 435 (2008).
- [19] H.L. Wang, Z.Y. Yang, B.D. Wang. *Transition Met. Chem.*, **31**, 470 (2006).
- [20] S.F. Lo, V. Mulabagal, C.L. Chen, C.L. Kuo, H.S. Tsay. *J. Agric. Food Chem.*, **52**, 6916 (2004).
- [21] Y. Masataka, K. Murakami. *Anal. Biochem.*, **257**, 404 (1998).
- [22] J.E. Brown, H. Khodr, R.C. Hider, C.A.R. Evans. *J. Biochem.*, **330**, 1173 (1998).
- [23] J. Zhou, L. Wang, J. Wang, N. Tang. *J. Inorg. Biochem.*, **83**, 41 (2001).
- [24] M. Balcerzak, A. Kosiorek, E. Swiecicka. *J. Anal. Chem.*, **60**, 2229 (2004).
- [25] G. Zhang, J. Guo, J. Pan, X. Chen, J. Wang. *J. Mol. Struct.*, **923**, 114 (2009).
- [26] M.Z. Iqbal. *Text book of Inorganic Chemistry*, Revised and Enlarged Edn, Markazi Kutub Khana, Kabir Street, Urdu Bazar, Lahore (2001/2002).
- [27] J.D. Lee. *Concise Inorganic Chemistry*, 5th Edn, Blackwell Science Ltd., London (1996).
- [28] M.I. Sancho, S.E. Blanco, F.H. Ferretti. *J. Argent. Chem. Soc.*, **94**, 143 (2006).
- [29] D.C. Harris. *Quantitative Chemical Analysis*, 3rd Edn, W.H. Freeman and Company, New York (1991).
- [30] S.B. Bukhari, S. Memon, M.M. Tahir, M.I. Bhangar. *J. Mol. Struct.*, **892**, 39 (2008).
- [31] S.B. Bukhari, S. Memon, M.M. Tahir, M.I. Bhangar. *Spectrochim. Acta, Part A*, **71**, 1901 (2009).
- [32] R.F.V. de Souza, W.F. de Diovani. *Redox Rep.*, **9**, 97 (2004).
- [33] W. Chen, S. Sun, W. Cao, Y. Liang, J. Song. *J. Mol. Struct.*, **918**, 194 (2009).
- [34] H.D. Aliyu. *Pak. J. Sci. Ind. Res.*, **52**, 312 (2009).
- [35] E. Canpolat, M. Kaya. *Turk. J. Chem.*, **28**, 235 (2004).
- [36] G.T. Castro, S.E. Blanco. *Spectrochim. Acta, Part A*, **61**, 1985 (2005).
- [37] R.M.S. Pereira, N.E.D. Andrades, N. Paulino, A.C.H.F. Sawaya, M.N. Eberlin, M.C. Marcucci, G.M. Favero, E.M. Novak, S.P. Bydlowski. *Molecules*, **12**, 1352 (2007).
- [38] A.A. Ansari. *Main Group Chem.*, **7**, 133 (2008).
- [39] A.A. Ansari. *Main Group Chem.*, **7**, 43 (2008).
- [40] E. Woznicka, M. Kopacz, M. Umbreit, J. Klos. *J. Inorg. Biochem.*, **101**, 774 (2007).
- [41] A.A. Ansari. *J. Coord. Chem.*, **61**, 3869 (2008).
- [42] R.F.V. de Souza, E.M. Sussuchi, W.F. de Giovani. *Synth. React. Inorg. Met-Org. Nano-Met. Chem.*, **33**, 1125 (2003).
- [43] E. Yoshimura, Z.D. Liu, H. Khodr, R.C. Hider. *Japan Soc. Anal. Chem.*, **51**, 979 (2002).
- [44] S. Tachakittirungrod, F. Ikegami, S. Okonogi. *Sci. Pharm.*, **75**, 179 (2007).
- [45] T.W. Wu, K.P. Fung, L.H. Zeng, J. Wu, A. Hempel, A.A. Grey, N. Camerman. *Biochem. Pharmacol.*, **49**, 537 (1995).
- [46] R.F.V. de Souza, W.F. de Giovani. *Spectrochim. Acta, Part A*, **61**, 1985 (2005).
- [47] Q.K. Panhwar, S. Memon, M.I. Bhangar. *J. Mol. Struct.*, **967**, 47 (2010).
- [48] N. Bunyapraphatsara, S. Dechsree, C. Yoosook, A. Herunsalee, Y. Panpisutchai. *Phytomedicine*, **6**, 421 (1999/2000).
- [49] B. Ternai, K.R. Markham. *Tetrahedron*, **32**, 565 (1976).
- [50] M. Quan-zhang, L. Qi-Xun. *Acta Botanica Sinica*, **24**, 147 (1982).
- [51] M. Tan, J. Zhu, Y. Pan, Z. Chen, H. Liang, H. Liu, H. Wang. *Bioinorg. Chem. Appl.*, Article ID 347872, 9pp. (2009). doi:10.1155/2009/347872.
- [52] F. Shrififar, N. Yassa, A. Shafiee. *Iranian J. Pharm. Res.*, **2**, 235 (2003).
- [53] D.A. Skoog, D.M. West. *Analytical Chemistry: An Introduction*, 4th Edn, CBS College Publishing, New York (1986).
- [54] J. Yin, C.L. Si, M.H. Wang. *J. Appl. Biol. Chem.*, **51**, 57 (2008).
- [55] A.P.M. Bernardi, C.L. Alarcón, A. Aspee, S. Rech, G.L.V. Poser, R. Bride, E. Lissp. *J. Chil. Chem. Soc.*, **52**, 1326 (2007).
- [56] N.A. Khalaf, A.K. Shakya, A. Al-Othman, Z. El-Agbar, H. Farah. *Turk. J. Biol.*, **32**, 51 (2008).
- [57] M.V. Kaveri, R. Prabhakaran, R. Karvembu, K. Natarajan. *Spectrochim Acta, Part A*, **61**, 2915 (2005).
- [58] A. Bravo, J.R. Anaconda. *Transition Met. Chem.*, **26**, 20 (2001).