ISSN-1996-918X



Pak. J. Anal. Environ. Chem. Vol. 13, No. 2 (2012) 159 - 168

Synthesis, Spectral Characterization and Antioxidant Activity of Tin(II)-Morin Complex

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Received 06 July 2012, Revised 15 December 2012, Accepted 16 December 2012

Abstract

The study focuses on the interaction between morin and Tin(II) and the resulting complex was characterized through various analytical techniques by comparing it with morin. The complexation was confirmed at first by UV-Vis study, which shows that addition of Tin(II) to morin may produce bathochromic shifts indicative of complex formation. IR spectral studies indicated that carbonyl has involved in coordination with Tin(II). Moreover, ¹H-NMR studies validated that in conjunction with carbonyl, 3-OH of morin is more appropriate to be involved in complexation by replacement of its proton. Scavenging activities of morin and its Tin(II) complex on DPPH radical showed the inhibitory rates of 65% and 49%, respectively. In addition, the reducing capacity of morin was outstanding at 0.5 and 2.0 mg/ml concentrations relative to Tin(II) complex. Overall, the study potentially shows the strong impact in order to design the anticancer drugs jointly from its cytotoxic potential and antioxidant activities, thereby selectively targeting the cancerous cells in result increasing their therapeutic index as well as extra advantages over other anticancer drugs.

Keywords: Tin; Morin; Antioxidant

Introduction

Flavonoids are largest class of natural polyphenolic compounds. The word flavonoid is derived from Latin *flavus* means yellow, but some of the flavonoids are purple, white, blue, and red and were discovered with vitamin C hence named as vitamin P by Albert Szent-Gyorgyi in 1928. Flavonoids are present in vegetables, fruits, herbs, and soya beans. Over 8,000 varieties of flavonoids have been identified so far. Their structure is characterized by a three carbon chain (C6-C3-C6) system joined together by two phenyl rings, where C3 is an aliphatic chain and two C6 groups are substituted benzene rings containing a pyran ring (Fig. 1). They are found as aglycones with hydroxyl or methoxyl substitutions or occur as Oor C-glycosides. Hence, their structures diversified by oxidation, alkylation, glycosylation patterns [1-4]. On the basis of their chemical structure, they are categorized into seven flavonols. types' viz. flavanones. flavones. isoflavones, chalcones. catechins, anthocyanidins. They show many biological properties such as antiviral, anti-allergic, antimicrobial, antiplatelet, antitumor, inflammatory, and antioxidant activities [5,6]. The antioxidant capacity of flavonoids depends upon their structural framework/molecular structure, i.e., substitution number and patterns (primarily with hydroxyl groups), chelating ability towards metal ions. Besides, they possess scavenging ability to oxygen radicals such as singlet oxygen, super oxide anion, and hydroxyl radicals. The prominent examples of strongly antioxidant flavonoids are morin, rutin, and quercetin [7,8]. Flavonoids contain such groups that undergo electron transfer reactions, i.e., 1) catechol group; 2) pyrogallol

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group; 3) 2,3-double bond conjugated with 4-oxo and 3-OH groups; and 4) some other resonance-effective substituents. In addition, there are many natural flavonoids which have strong tendency to form complexes in particular with heavy metal ions.

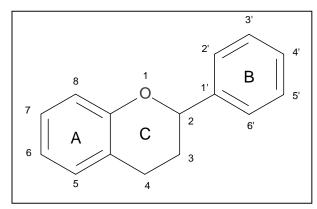


Figure 1. Basic structure of flavonoids.

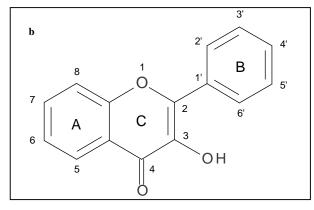


Figure 2. a) Structure of Morin (2',3,4',5,7-pentahydroxyflavone) and b) Skeleton of flavonols.

Morin (2',3,4',5,7-pentahydroxyflavone; a light yellowish pigment) (Fig. 2a) belongs to flavonol subclass of flavonoids (Fig. 2b). It's a bioactive

compound and found in yellow Brazil wood. Morin (indicated as **M** onwards) is also present in guava leaves, onion, apple, and other Moraceae, which are used as dietary agents and herbal medicines [9,10].

Tin is a representative metal that shows the oxidation states of 0, +2, and +4 in pure inorganic metal/allovs. tin. and organotin compounds, respectively. All of them possess different properties [11]. Metallic tin is used to line the aerosols, beverages, and food cans, but organotin compounds are used in making food packagings, plastics, pesticides, paints, plastic pipes, and pest repellents, while inorganic tin is used in toothpastes, dyes, perfumes, soaps, food additives, and pigments in ceramic and textile industries [12]. Tin is not a toxic element itself but previous studies have revealed the toxicological results of organotins. Similarly, the significant use of inorganic tin compounds also cause the acute toxicity manifested by vomiting, gastric irritation, nausea, and abdominal discomfort [11]. Tinplate is widely used in food industry and is considered its diverse industrial application. Since, it provides robust form of packaging with maximum reduction in sealed cans. It offers safe, long, and ambient shelf life with nominal or even no use of preservative [13]. Most of the foods or beverages contain very low concentration of tin usually below 10 mg/kg. United Nations' Food and Agriculture as well as World Health Organization (UN-FAO/UN-WHO) have fixed the maximum limit of 250 mg/kg tin in canned foods [11,12]. Nevertheless, there are several reports gastrointestinal perturbations and vomiting in humans consuming foods/beverages that contain tin concentrations above 200 mg/kg. As a result of tremendous use of tinplate for food and beverage packaging, there is a probability of dissolution of traces of tin in food content especially from the inside of a can body and leave a major influence on the food quality and may cause toxicological effects [13,14]. Due to risk of accumulation of large amounts of tin in foods in contact with tinplate, they must be lacquered or coated with resin. Thus, due to toxic potential of tin in humans through the contact of foods in tin coated-cans and tinfoil [11] increased interest to work with tin. Therefore, the study was focused on the possible interaction of morin present in canned foods and

beverages with toxic tin present in coating. Furthermore, the actual aim of the study was to synthesize the metal complex of morin with Tin(II) and comparison of their antioxidant activities through DPPH and ferric reducing power methods as well as cyclic voltammetric technique.

However, in literature many groups have reported the synthesis of various complexes of flavonoids and studied their antioxidant activities such as Dehghan and Khoshkam 2012, synthesized Tin(II)-quercetin complex and studied antioxidant activity relative to quercetin [15]. Afanas'ev et al. 2001, synthesized Fe- and Cu-rutin complexes and reported their antioxidant activity [16]. de Souza and De Giovani 2005, synthesized Al-quercetin, Zn-quercetin, Al-rutin, Al-galangin, Zn-galangin, and Zn-rutin complexes and studied their antioxidant activities [17]. In our previous studies we have synthesized the morin complexes of Cu(II), Mg(II), Ca(II), Zr(IV), and Mo(VI) and reported their antioxidant properties [18-20]. De Souza and De Giovani 2004, reported Cu(II), Fe(II), Al(III), and Zn(II) complexes of quercetin, rutin, galangin, and catechin and explored their antioxidant activities [21]. Chen and co-workers 2009, synthesized Cr(III) [22] and Pekal et al. 2011, Cu(II) [23] complexes of guercetin and explored their antioxidant activities. Zhou et al 2001, synthesized divalent Mn, Co, Ni, Cu, Zn, and Pb complexes of quercetin and studied their antioxidant properties relative to their respective ligand [24].

Experimental Reagents and Instrumentation

All the reagents and solvents are of analytical or chemically pure grade. Morin hydrate (2-(2, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-1-benzopyran-4-one) and DPPH* (2,2'-diphenyl-1-picrylhydrazyl) were purchased from Sigma (St. Louis, MO, USA), tin(II) chloride dihydrate, sodium di-hydrogen phosphate, and di-sodium hydrogen phosphate were purchased from Merck. HPLC grade methanol (MeOH) was obtained from Fisher scientific Ltd. (Leicestershire, UK). KBr was obtained from Aldrich Chemical Co. (Taufkirchen, Germany). Lithium perchlorate, potassium ferricyanide, and trichloroacetic acid were purchased from Fluka (Buchs, Switzerland).

Ferric chloride was obtained from Acros Organics (Geel, Belgium). All the reagents were weighed with an accuracy of $\pm 0.0001g$.

UV-Vis spectra of 4 x 10⁻⁴ M solutions of morin and its Tin(II) complex were obtained in MeOH by Perkins Elmer Lambda 35 UV-Vis double beam Spectrophotometer using standard 1.00 cm quartz cells. FT-IR spectra were recorded in the spectral range 4,000-400 cm⁻¹ on a Thermo Scientific Nicolet iS10 FT-IR instrument using KBr pellets. About 1-3 mg of sample and 100-200 mg of KBr were ground together under room temperature and higher pressures into a small disk. ¹H-NMR spectra were recorded on a Bruker-Avance-500 MHz spectrometer in DMSO using TMS as internal reference. Cyclic voltammograms were performed in MeOH on 797 VA Computrance Ω Metrohm in the potential range of -0.5 to +1.4 V.

Synthesis of complex

M (0.302 g or 0.1 mol) was dissolved in 25 ml MeOH in a two-necked round-bottomed flask (50 ml capacity) containing electromagnetic stirrer. Solution was stirred to completely dissolve the solid **M**. After 15 minutes stirring the solution became clear dark vellow. Quickly, added SnCl₂.2H₂O (0.113 g, 0.1 M) into the flask, which immediately turned the color of solution to greenish yellow, thus it shows very quick interaction between M and Tin(II). The reaction mixture was stirred at room temperature for about 2 hours. After that the solution was poured into petri dish to evaporate the solvent. Finally, scratched the petri dish and collected the coral colored compound. It was washed with 1:1 tbutanol/chloroform. The % yield of Tin(II)-Morin complex (indicated as Tin(II)-M onwards) was 66%. Elemental content found; C, 46.45; H, 2.89% and Anal. Calc. for $[Sn(C_{15}H_9O_7)_2]\cdot 2H_2O$: C, 47.59; H, 2.93%, respectively. The complex was soluble in MeOH, EtOH, DMSO, DMF, acetone, diethyl ether, partly soluble in chloroform and insoluble in H₂O, *n*-hexane, and DCM solvents.

DPPH radical scavenging activity (RSA)

Antioxidant activity was measured by monitoring the bleaching rate of DPPH (2,2'-

diphenyl-1-picrylhydrazyl radical) characteristic wavelength in the presence of sample solutions (i.e., M and Tin(II)-M complex) [25]. Since, the radical form of DPPH absorbs at 515 nm but if any antioxidative agent or radical group is added to it may decrease its absorbance. In experiment, about 0.2 mg/ml of each sample were mixed with 2 ml of DPPH (0.1 mM in MeOH) and were assessed in different time intervals, i.e., 0, 5, 10, 15, 20, 25, and 30 minutes with the difference of 5 minutes until the reaction reached the steady state. The decrease in absorbance was measured against a blank of pure MeOH. Thus, the radicalscavenging activity (RSA) of the compounds was computed as a percentage of DPPH discoloration using equation (i);

% RSA =
$$[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$$
 (i)

Where, A_S is the absorbance of sample (either **M** or Tin(II)-**M** complex) and A_{DPPH} is the absorbance of DPPH solution.

Ferric reducing power (FRAP)

Ferric reducing power of the **M** and Tin(II) complex was determined by means of potassium ferricyanide-ferric chloride method [26]. Various concentrations, i.e., 0.5, 1.0, 1.5, and 2.0 mg/ml of ligand molecule and its Tin(II) complex were prepared. The each sample amounting to about 0.5 ml was added to 2.5 ml 0.2 M phosphate buffer (pH 6.6) and 2.5 ml potassium ferricyanide (1%). Then, the samples were incubated for 20 minutes at 50 °C and centrifuged with speed of 3000 rpm for almost 10 minutes. Subsequently, 2.5 ml of trichloroacetic acid (10%) were added. In the last, 2.5 ml from the resulting solution were mixed with 2.5 ml distilled water and 0.5 ml FeCl₃ (0.1%). Allowed the solutions to stand for about 30 minutes and then measured their absorbance at 700 nm. For examining the relative reducing power of M and Tin(II)-M complex, the results were expressed by plotting the graph of their absorbance vs. various concentrations.

Cyclic Voltammetry (CV)

The conventional three electrode system consisting of glassy carbon, platinum wire, and

Ag/AgCl as working, auxiliary, and reference electrodes, respectively, was used to perform an experiment electrochemical at ambient temperature. The cleaning of working electrode prior to electrochemical measurement was carried out by polishing it with alumina powder on polishing cloth [27]. The solutions of 0.01 M concentrations for M and its Tin(II) complex were prepared in MeOH. To get the electrochemical results for M, its solution was prepared by mixing 1 ml of M, 5 ml of LiClO₄, and 4 ml of MeOH solvent. While the Tin(II)-M complex was prepared by mixing 1 ml M, 1 ml SnCl₂·2H₂O, 5 ml LiClO₄, and 3 ml MeOH, respectively. Total 10 ml of the solution was prepared for an analysis [28].

Results and Discussion *UV-Vis studies*

In the absorption spectrum of M, two prominent characteristic absorption peaks may be visualized with corresponding maxima at 368 and 264 nm for band I and band II, respectively. Here, band **I** is annotated for the absorption of cinnamoyl system (ring B), while band II is interpreted for benzoyl system (A ring). From the molecular structure of M, it can also be inferred that M can chelate the metal ions via two sites, i.e., 5hydroxy-4-oxo and 3-hydroxy-4-oxo systems. Besides, it possesses another potential chelating site at 3,2'-dihydroxy system, which forms sevenmembered chelate ring to complexate any metal ion [29]. When Tin(II) solution was added to methanolic solution of M, it caused the significant change in the M spectrum due to appearance of new peak at 422 nm λ_{max} (band III). It shows that the bathochromic shift of about 54 nm takes place. The spectral change can be easily observed in (Fig. 3) [30]. It confirms that complex formation takes place between \mathbf{M} and Tin(II). That change takes place band I; conversely the shift of band at 264 nm is relatively insignificant (band IV). Therefore, it supports the clue that newly appeared peak at 422 nm in Tin(II) complex of M may arise due to complexation of Tin(II) at 3OH and 4CO of M.

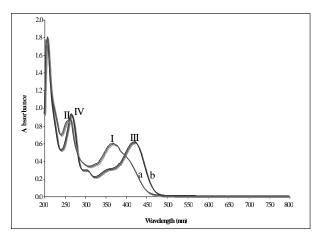
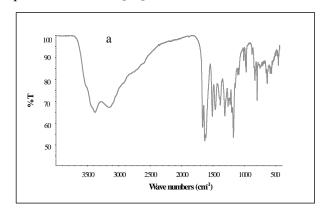


Figure 3. UV-Vis spectra of a) Morin and b) ${\rm Tin}({\rm II})$ -Morin complex.

In fact, there are many proofs, which indicate that complex formation in M involves the 3-hydroxyl-4-keto moiety; (i) because 3-hdroxyl possesses more chelation power than 5-hydroxyl group; ii) also because of higher delocalization of oxvgen electrons of 3-hydroxyl than 5-hydroxyl group, thereby facilitating the π electrons delocalization. Thus, complex formation at band I caused by the interaction of 3-hydroxyl group of M with Tin(II) subsequently follows the electronic redistribution between the Tin(II) and M to form a big extended π bond system. It changes the electronic distribution in **M** from n- π^* to π - π^* transition of lower energy [31]. Thus, new ring formation in complex is caused by the increased conjugative effect with inclusion of C ring, which provides the additional molecular stabilization resulting from the formation of extended 4 bond system [32]. Hence, this information may be supportive in the sense that the chelation ability in M is more attributable to the presence of 3OH and 4CO groups in ring C. Thus, the red shift in the M spectrum is highly informative for the coordination site in ligand having multiple chelating sites, but due to more acidic nature of 3OH proton and more suitable location of 4CO, they may be the proper sites to be involved in complex formation. Since, the orientation of 2' and 4' hydroxyl groups is such that they can not bind the metal ion, whereas the 50H group has lesser proton acidity and much hindrance created by the formation of first complexation, therefore its involvement becomes less probable in complexation process [33].

IR Spectroscopy

IR spectra provide very useful information regarding the complex structure (Fig. 4). Major difference between the spectrum of ligand molecule and complex compound may provide the information of shifting of certain peaks as well as disappearance/formation of some other peaks. Some of the selected peak values have been shown in Table 1. It is observed that highly notable spectral change between the spectra of two compounds occurs at 1400-1700 cm⁻¹ region. Where, the position of v(C=O) is significantly shifted in **M** from 1662 cm⁻¹ to 1647 cm⁻¹ ($\Delta v = 15$ cm⁻¹), hence its involvement in complex formation becomes more clear. Consequently, participation in complex formation may cause the lengthening of C-O bond and decrease in force constant that result in shift of carbonyl chromophore towards even smaller value of wave number after coordination. Therefore, involvement of CO in complexation can not be ignored. Furthermore, the formation of chelate ring of >C=O···M-O- may be recognized from the peak at 1558 cm⁻¹ [32].



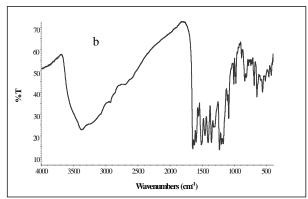


Figure 4. IR spectra of a) Morin and b) Tin(II)-Morin complex.

At the same time, appearance of new bands in the Tin(II) complex at 519 and 427 cm⁻¹ may indicate the formation of metal oxygen (M–O) bond [34], while such bands can not be observed in the ligand spectrum. On the other hand, shift in peak position of v(C–O–C) from 1310 to 1320 cm⁻¹ is quite negligible; hence it confirms that the ring oxygen is not involved in complex formation. Finally, the most prominent and broad peak at 3000-3600 cm⁻¹ reveals the presence of water molecules v(O–H) [35].

 ${\it Table \ 1.} \ Assignments \ of \ IR \ spectral \ peaks \ for \ Morin \ and \ Tin(II)-Morin \ complex$

Compound/ Complex	v(O–H)	v(C=O)	v(C-O-C)	v(C=C)	v(M-O)
Morin	3381–3157	1662	1310	1613	-
Tin(II)-Morin	3375	1647	1320	1623	427

¹H-NMR Studies

The chemical shift values for the hydroxyl protons of \mathbf{M} have been assigned as well as compared with already existing literature. Table 2 shows the chemical shift values of ${}^{1}\text{H-NMR}$ signals for the \mathbf{M} and Tin(II) complex.

M shows a sharp singlet at 12.61 ppm produced from the intramolecular hydrogen bond. This is the hydroxyl group of ring A located at C5 position, which can interact with the acceptor carbonyl group of ring B at C4 position. Similarly, one more hydrogen bond may be formed by the hydroxyl group of C2' by interacting with furan oxygen of ring C or the hydroxyl group oxygen placed at C3, but it depends more upon the orientation of ring C or water molecules [36].

In addition, after complexation **M** shows the upfield and downfield chemical shifts in the resonances of aromatic ring protons in the Tin(II) complex spectrum. In complex spectrum, the upfield shift arises due to an increase in ring current shielding effect in the resonances of **M**. The chemical shift in hydroxyl protons of **M** located at 5OH, 7OH, and 3OH may appear at 12.61, 10.68, and 9.76 ppm, respectively, which shows change in characteristic chemical shift upon coordination with Tin(II). Thus, it seems that the

signals of 5OH and 7OH protons are present at 12.59 and 10.69 ppm with little change in their chemical shifts but 3OH proton is completely missing. Therefore, it can be presumed that metal ions most probably replace 3OH proton by undergoing coordination through oxygen atom [37].

Table 2. Assignments of $^1\text{H-NMR}$ signals for Morin and Tin(II)-Morin complex

Compound/Complex	3-ОН	5-OH	7-OH	H ₂ O
Morin	9.76	12.61	10.68	3.336
Tin(II)-Morin	-	12.59	10.69	3.315

Thus, it becomes self explanatory that after coordination the shifting of signals to higher field takes place due to more proton shielding originating from extension in conjugated system of complex [38]. It was also observed that in complex spectrum there is relative signal broadening due to different metal proton distances but the broader peaks are mostly those protons nuclei which are closer to Tin(II) [39].

Scavenging Activity on DPPH Radical

DPPH test shows that the antioxidants have inherent potential to reduce the DPPH radical from violet to yellow colored diphenylpicrylhydrazine. Therefore, in the chemical reaction antioxidants donate the hydrogen to DPPH and convert it into DPPH-H that is well illustrated in Scheme 1. Therefore, using DPPH' method the antioxidant activity of both the compounds was evaluated [40]. Actually antioxidant activity of **M** and Tin(II)-complex depends upon their structures especially their hydrogen donating ability. During experiment, it was observed that the reaction between M and its complex occurs in two main steps, where step one corresponds to the quick decrease in DPPH' absorbance at 515 nm and second step shows quite slow decrease in absorbance finally reaching the steady state. Fast step corresponds to abstraction of most labile H-atom, whereas slow step shows the oxidation degradation in the remaining product.

Scheme 1. Proposed mechanism for DPPH' scavenging activity of ${\bf Tin}({\bf II})$ -Morin complex.

However, antioxidant activity of the compounds depends more upon their molecular structures, but complexation made by the metal ions may affect the chemical properties of ligand molecules hence the resulting complexes may be of higher or lower activity [32]. Thus, coordination may affect the ability of parent antioxidants. The antioxidant activity of the compounds has been studied in time dependent manner [41], which is determined in every established period of time for both compounds [42]. Thus, the plot illustrated in (Fig. 5) shows that **M** scavenged free radical to about 65% while Tin(II) complex scavenged to about 49%, thus **M** shows better inhibitory effect compared to the complex compound [43].

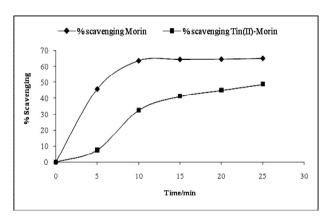


Figure 5. Antioxidant activity of Morin and Tin(II)-Morin complex against DPPH', a stable free radical.

Cyclic Voltammetry

Application of electrochemical methods for antioxidant activity investigation is a well

complementary to the previously used method such as UV–Vis spectroscopy [41]. Cyclic voltammetry characterizes the antioxidant activity compounds/complexes via redox potentials. The compounds oxidized at relatively low potential values have strong scavenging abilities. The main characteristic of all the flavonoids is that they contain one or more hydroxyl groups attached to rings. Therefore, the compounds either having more hydroxyl groups or containing more electron donating groups have higher antioxidant activities and show anodic peaks at lower potentials than those containing small number of hydrogen or electron donating groups. Although, the oxidation pathways of flavonol flavonoids have been extensively investigated, their however, mechanism is still not completely understood.

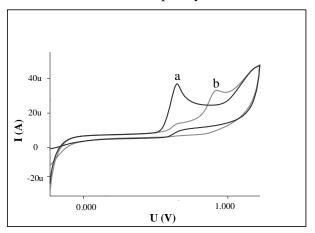


Figure 6. Cyclic voltammograms of a) Morin and b) ${\rm Tin}({\rm II})$ -Morin complex.

In the cyclic voltammograms of M ligand and Tin(II) complex, only one well defined anodic oxidation peak is observed with no reverse reduction peak (Fig. 6). Absence of cathodic peak in the reverse scan indicates that the oxidation process is followed by a chemical reaction, which rapidly removes the generated products. The oxidation of **M** leads to the formation of phenoxy radical, which can undergo the further chemical reactions like coupling, proton loss or nucleophilic attack. Hence, the voltammetric peak in M is unequivocally attributed to the oxidation of C-3 hydroxyl group. Another possible antioxidant mechanism is via metal chelation [44]. But in the case of Tin(II) complex, the peak potentials of oxidation signals are shifted towards more positive value. Where the potential value for M is +0.658 V

and for Tin(II)-complex is +0.925 V, which indicates that **M** molecule is more antioxidant than its corresponding complex. Hence, the voltammetric method can also be used for the determination of antioxidant activity, in the same way as DPPH assay because of the correlation found between oxidation potentials and anti-radical power [45].

Reducing activity

Reducing power acts as an important marker of the compounds' possible antioxidant activity. An antioxidant may act as a common reductant but a reductant may not necessarily act as an antioxidant [46]. Thus, in the FRAP (ferric reducing antioxidant power) method, the direct reduction of Fe³⁺(CN⁻)₆ to Fe²⁺(CN⁻)₆ may be used as a measure of reducing power of \mathbf{M} and its Tin(II)-complex. The mechanism of complex is shown in Scheme 2. The reducing power of compounds was determined by measuring the absorbance of Perl's Prussian blue complex formed and followed by subsequent reaction with ferric chloride (equation (ii)) to yield the ferric ferrous complex with λ_{max} at 700 nm. The method indicates that greater the absorbance, greater is the antioxidant's reducing capacity for iron from Fe³⁺ to Fe²⁺ [40].

Potassium ferricyanide + Ferric chloride

Antioxidant Potassium ferrocyanide + Ferrous chloride (ii).

Scheme 2. Ferric reducing power of Tin(II)-Morin complex.

The assay is based on the change of yellow color into various shades of green color and finally to blue based on compounds' reducing power. With increasing concentration the reducing power increases. Reducing power of **M** and Tin(II)-**M**

complex as a function of their concentration is shown in (Fig. 7) [47]. It shows that the reducing power of **M** is higher than its corresponding Tin(II) complex.

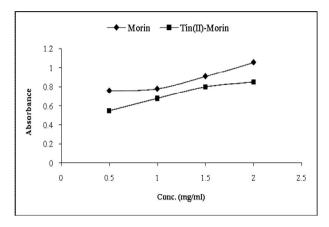


Figure 7. Ferric reducing power of Morin and its Tin(II)-Morin complex.

Conclusion

It has been concluded that Tin(II) cations form complex with morin. The resulting complex was characterized by UV-Vis, IR, and NMR techniques. From the spectral characterization, it was concluded that in Tin(II)-Morin complex the coordination of Tin(II) with morin takes place through 3-OH and 4-CO groups. From the antioxidant investigation (evaluated by DPPH' method) of ligand molecule and the complex compound, it was observed that complexation of Tin(II) with morin may reduce the antioxidant potential of ligand molecule. DPPH assay measures the ability of compounds to donate hydrogen to the radical. While the ferric reducing power measures the ability of compounds to donate electron to Fe(III). The results demonstrate the strong impact of study in designing various anticancer drugs.

Acknowledgements

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

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