Synthesis, Characterization and Antioxidant Activity of Rutin Complexes

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Abstract

Rutin, one of the excellent chelating agents, has been made to react with Zr^{4+} and MoO_4^{2-} ions to get metal complexes in methanol. The synthesis, spectral properties (UV-Vis, ^{1}H NMR, ^{13}C NMR, IR), thermal behavior and antioxidant activity of these complexes have been studied. It has been observed that rutin forms deep yellow colored complexes of mononuclear composition (through 5OH-4CO system in case of Zr^{4+}/catechol site in case of MoO_4^{2-}) having stoichiometry, 1:2 and 1:1 for Zr^{4+} and MoO_4^{2-} ions, respectively. It has been further noticed that these complexes are formed at different pHs, *i.e.* 4.0 and 8.0 pHs suitable for Zr^{4+} and MoO_4^{2-} complexes, respectively. Thermograms also confirm the results of IR and support the presence of water molecules in complexes. Relative antioxidant activity of the rutin ligand, zirconium-rutin and molybdenum-rutin complexes was determined. It was found that both metal complexes have higher antioxidant activity than rutin alone.

Keywords: Rutin; Zr(IV)-Rutin Complex; Mo(VI)-Rutin Complex; Antioxidant

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Introduction

Rutin or quercetin-3-*O*-rutinose ($C_{27}H_{30}O_{16}$) is one of the important members of flavonoid family, which is a class of chief components of plant phenolics. Many foods including vegetables and fruits as well as beverages like beer, tea, coffee and juices contain rutin. It is present in a variety of plants and found as a major component of *Sophora Japonica* flowers [1]. It acts as coloring agent, food additive and used in cosmetics [2]. In addition, rutin possesses strong therapeutic action than other flavonoid derivatives and thus mostly used as drug to cure the diseases of blood vessels [3]. Many of the metals like W, Al, Ti, V and Ni could be determined spectrophotometrically using rutin. Its structural composition is such that it does not form a range of complexes with metal cations only but can also form stable complexes with metal oxyanions. Structure of rutin contains multiple hydroxyl groups (Fig. 1), which forms complexes with metals and become the basis of rutin determination from drugs, foods and beverages [4-6]. Besides this, it can also be desirable to develop a method to enhance the antioxidant and free radical-scavenging potential of non-toxic flavonoids such as rutin without altering their basic structure. Such quality in rutin may be obtained by forming its metal complexes [7].
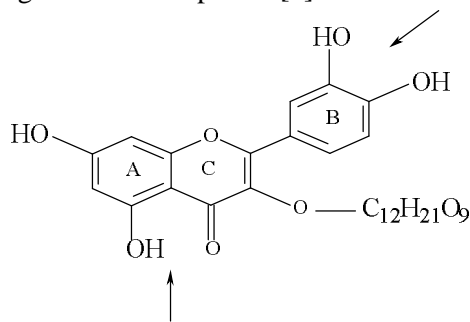

Figure 1. **Chemical Structure of Rutin.**

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The chemistry and enzymatic function of molybdenum is important in nitrogen fixation, oxygen transfer process and nitrite reduction. The knowledge of its metabolic path can be exploited to design different drugs [8]. Below (VI) oxidation state, molybdenum is characterized to form the aggregates, i.e. formation of cluster compounds. Dimeric and trimeric ionic systems are formed with the metals being connected by hydroxo or oxo bridges and coordinatively saturated by water ligands [9]. It is transported and stored as simple salt or loosely bound protein complex in body [10].

Zr^{4+} possesses higher value of charge-to-size ratio than other metal ions like Li^+, Co^{2+}, Cu^{2+}, Bi^{3+} and Sc^{3+} ions. Its compounds have tremendous coordinating ability hence, making it to behave as a strong Lewis acid and impart enormous catalytic activity. Additionally, lots of the zirconium salts are commercially available nowadays. In the last decades, interest has been increasing in the Zr^{4+} and its compounds because there is the need of very efficient and green Lewis acid catalysts in order to carry out the various organic transformations [11,12]. At the same time, Zr^{4+} complexes have remarkable biological value; their complexes also work as efficient antibacterial and antifungal agents. Recently, its complexes are reported to show antioxidant properties as well [13].

Many types of metal-flavonoid complexes are prepared and characterized in last several years. Elemental analysis, thermal analyses, conductivity, cyclic voltammetry, IR, ^1H NMR, ^{13}C NMR, UV-Vis and fluorescence spectroscopy have been used to assess relevant interactions of flavonoids and metal ions, the chelation sites, the dependence of complex structure on the metal/ligand ratio, the capability of flavonoids in binding metal ions etc [14]. Since, the complexes of metal ions of higher oxidation states, i.e. Mo^{6+} and Zr^{4+} are less available in literature, whereas those of divalent as well as trivalent metal ions are reported remarkably [15]. Therefore, the present work has been assigned to carry out the synthesis of rutin complexes of Zr^{4+} and MoO_4^{2-} ions and to assess their antioxidant activity.

Experimental
Reagents and instrumentation

All the reagents and solvents were of analytical or chemically pure grade. Rutin and 1,1-diphenyl-2-picrylhydrazyl (DPPH•) were purchased from Sigma (St. Louis, MO, USA), zirconium nitrate and HPLC grade methanol from Fisher scientific Ltd. (Leicestershire, UK), KBr from Aldrich Chemical Co. (Taufkirchen, Germany) while sodium molybdate was purchased from Fluka (Buchs, Switzerland). All the reagents were weighed with an accuracy of ± 0.0001 g.

UV-Vis spectra were obtained by Perkins Elmer Lambda 35 UV-Vis double beam spectrophotometer. ^1H NMR spectra were recorded on a Bruker 500 MHz spectrometer. FT-IR spectra were recorded on a Thermo Scientific Nicolet iS10 FT-IR instrument. TG/DTA curves were obtained on Pyris Diamond TG/DTA (Perkin-Elmer Instrument) under nitrogen at the heating rate of 20 ℃/min. pH and conductance were measured using Inolab pH and cond 720 WTW series pH meter and conductometer.

Effect of pH

The distinctive yellow color of rutin complexes is strongly dependent on the concentration of reactants and pH of the reaction. Therefore, the complex formation has been studied over a wide pH range (2–10). For rutin, it's difficult to form complexes below 4.0 pH value because it is a weak acid hence exists in undissociated form predominately. The complex formation actually takes place within the pH range 4.0–8.0, because above 8.0 pH value there is formation of stoichiometrically different complex compounds or dissociation of already existing complex [16]. Zr^{4+} forms the complex with rutin at 4.0, while MoO_4^{2-} at 8.0 pH value [13].

Stoichiometry of the metal complexes

The equimolar solutions of rutin and salts of zirconium and molybdate were prepared in 4×10^{-4} M concentration. The stoichiometry was found using Job's method (method of continuous variation) by mixing the solutions of metals and ligands in the ratio 1:9 to 9:1. Then, the maximum

absorbance was noted at the specified wavelength to find the appropriate mole fraction value (X) [17].

Preparation of the complexes

In two separate round bottom flasks, rutin (0.664 g, 2 mmol) was added to dissolve thoroughly till 15 minutes, then added zirconium nitrate (0.215 g, 1 mmol) in one flask and sodium molybdate (0.242 g, 1 mmol) in another flask under stirring containing rutin. First, the color of rutin solutions was lemon yellow; but it immediately turned deep orange after adding metal salts. After two hours of stirring at ambient temperature, the solutions were filtered to eliminate the undissolved/unreacted part of the components used; the precipitates were collected, washed with small aliquot of diethyl ether and well dried over silica gel in a vacuum dessicator. The color of solid complexes was found as burnt orange and medium violet red formed with overall yield of 72% and 60% for Zr^{4+} and MoO_4^{2-} complexes, respectively. Elemental analysis found C, 52.40; H, 5.33; N, 1.10%. Anal. Calc. for $[Zr(C_{27}H_{29}O_{16})_2(NO_3)_2]\cdot4H_2O$: C, 52.46; H, 5.38; N, 1.13% . Similarly, elemental analysis found Na, 5.65; C, 40.26; H, 4.19%. Anal. Calc. for $Na_2[MoO_3(C_{27}H_{28}O_{16})H_2O]\cdot2H_2O$: Na, 5.71; C, 40.31; H, 4.25%, respectively.

Chemical reactions

The solution of rutin was lemon yellow but the solutions of metal salts were colorless. Upon addition of zirconium to rutin solution, the color of lemon yellow solution changed rapidly into orange yellow, while the color change with molybdate was rather slow. The main reaction of both metals with rutin completes within 1 to 2 minutes.

On complex formation, the ionic form of rutin ligand links to metal ions, therefore H^+ must be liberated to increase the acidity of solution. For this, the solution of rutin (4×10^{-4} M) was mixed with solutions of molybdate and zirconium salts (2.5×10^{-4} M). Consequently, pH of the mixture solution containing rutin and metals was found to be lower than the pH of rutin solution alone (Table 1). It indicates that the protons have been delivered in real. Though, in present case, the

reaction between molybdate and rutin shows an opposite pH effect.

The first step involves the formation of molybdenum trioxide, which links to a divalent anion of rutin through the catechol moiety that may be surrounded by ionic sphere of sodium ions. The increase in pH of mixture solution could be due to progress of reaction with the formation of molybdenic acid or the corresponding polyanions. The formation of such colorless products does not take place within maximum complex formation range or they are formed parallel to the complex formation. The chemical reaction between Zr^{4+} and rutin progresses, in which dissociated form of rutin reacts with Zr^{4+} forming a complex compound [13,18].

Table 1. **pH of rutin-molybdate and rutin-zirconium solutions.**

Solution	Na$_2$MoO$_4$, rutin	Zr(NO$_3$)$_4$, rutin	Na$_2$MoO$_4$	Zr(NO$_3$)$_4$	rutin
pH	8.49	3.84	8.75	4.5	7.84

Physical properties of the complexes

Analytical data and physical properties evidence the formation of highly colored, non-hygroscopic and thermally stable rutin complexes. Solubility test for both complexes was also carried out and found that zirconium complex is soluble in MeOH, EtOH, DMF and DMSO, slightly soluble in water but insoluble in acetone. On the other hand, molybdenum complex was more soluble in water than other solvents because it is purely ionic complex [11]. In addition, the molar conductance was determined by preparing their 10^{-3} M solutions in DMSO. Zr^{4+} and MoO_4^{2-} complexes showed 12 and 95 µS/cm conductance, respectively. Thus, zirconium formed a non-electrolytic and molybdate an electrolytic complex [19]. The analytical and spectroscopic data also show that rutin formed mononuclear type complexes. This assumption is in accord with elemental analysis, FT-IR, ^{1}H NMR and UV-Vis spectroscopy [20].

Antioxidant assay

The scavenging activity on the DPPH[•] radical was analyzed using W. Brand–Williams method with little modification [21]. Almost 90

microliters of each sample solution (2 µM rutin, Zr^{4+} and MoO_4^{2-} complexes) was examined in methanol at different reaction times, *i.e.* 0, 5, 10, 15, 20 and 25 minutes. The study using $DPPH^{\bullet}$ radical (3 mL) of 0.1 mM concentration (prepared in methanol) was made at 517 nm. The absorbance of reaction was noted after each 5 minutes till the reaction has achieved the steady state and the color of solutions change from violet to yellow due to transfer of either electrons or protons (reaction (i)).

$$DPPH^{\bullet} + AH \rightarrow DPPH - H + A^{\bullet} \qquad \text{(i)}$$

The results were plotted as the percentage scavenging calculated by the expression (ii); where, A_C and A_S is the absorbance of control and sample, respectively.

$$Scavenging\ activity(\%) = \frac{(A_C - A_S)}{A_C} \times 100 \qquad \text{(ii)}$$

Results and Discussion
UV-visible study and composition of complexes

Rutin has ability to effectively chelate the metal ions *via o*-dihydroxyl and 5OH-4CO groups, but involvement of particular site in chelate formation depends more on the nature of a metal ion and some other factors such as location of hydroxyl groups and steric hindrance at ligating groups to affect the complexation [22]. The stoichiometric composition of the complexes was determined by applying Job's method and molar ratio method. Job's plots illustrate that the mole fraction values (*X*) have maxima at 0.333 and 0.5 corresponding to 1:2 and 1:1 ratios for zirconium and molybdenum complexes, respectively. Mole ratio method also confirms the same stoichiometric ratio for complexes [18].

Electronic spectroscopy further provides the important information regarding rutin complexes. From the literature, it has been noticed that the changes in the visible spectrum of rutin (350-500 nm) depend more on the nature of metal salt, complexant present and other factors. In general, the spectral changes in the 250-270 nm range are insignificant whereas, the changes in the visible range (350-500 nm) are highly significant for complexation purpose [23]. Similar to that, electronic spectra of rutin and its complexes show two clear visible peaks. The absorbance peaks in the visible region at 359, 422 and 413 nm are produced due to B ring portion (*i.e.* cinnamoyl system), whilst those visualized at lower region around 257, 275 and 271 nm are associated to A ring (*i.e.* benzoyl system) for rutin and its Zr^{4+} and MoO_4^{2-} complexes, respectively (Fig. 2) [24]. The spectra clearly demonstrate that rutin undergoes bathochromic shifts in the presence of metals due to increased conjugative effect (Table 2). These shifts take place in both the peaks (I & II) but they are more pronounced in peak I. Such behavior is identical to all kinds of subclasses of flavonoids bearing 3-hydrox-4-keto, 5-hydroxy-4-keto and/or even *o*-dihydroxyl moieties. It suggests that the formation of chelate compounds occurs through these moieties [14,25]. The spectra show the red shift of about 66 and 57 nm for Zr^{4+} and MoO_4^{2-} complexes relative to λ_{max} of rutin (356 nm), respectively. Such spectral shifts are either analogous to π–π* electronic transitions or witness the presence of charge transfer complexes (CTC) produced through the formation of coordination bonds between unshared electron pairs of oxygen atoms of the phenolic groups at rutin and the *d*-orbitals of zirconium and molybdenum ions [26].

Table 2. **Spectral shifts of band I and band II of rutin in the presence of zirconium and molybdenum ions.**

Compound	Rutin	Zr-rutin	Δλ	Mo-rutin	Δλ
Band I	356 nm	422 nm	66 nm	413 nm	57 nm
Band II	257 nm	275 nm	18 nm	271 nm	14 nm

In the spectrophotometric titration of Zr^{4+} with rutin (Fig. 3), the original concentration of rutin and Zr^{4+} was 2 mM. In the titration procedure, the concentration of rutin was fixed and that of zirconium was varied. At first, 15 µL of rutin were analyzed and then mixed to it equal volume of Zr^{4+} with 10 equivalents. Originally, there was only rutin, but as soon as concentration of metal ions was gradually increased, the peak intensity of pure rutin started to decrease, whereas the peak due to complex formation started to increase, hence in the beginning there were more than one isobestic points (where the spectra cross each other at one common point) in the spectra (i.e. 3, for metal (M), ligand (L) as well as complex (C)) but as soon as the concentration of metal ions
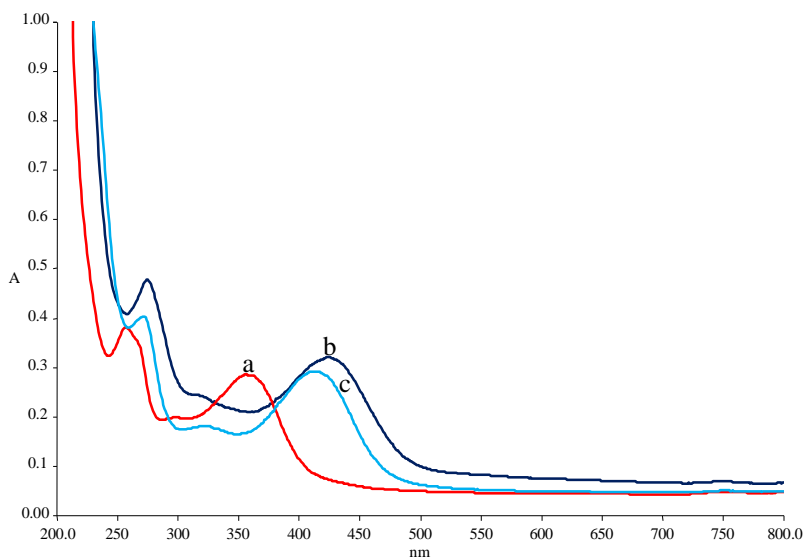
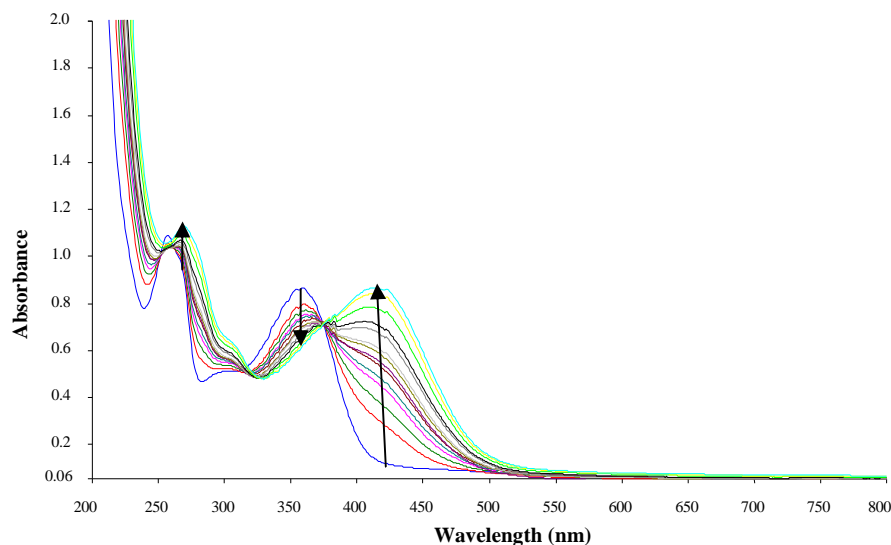*Figure 2.* **UV-Vis spectra observed for a) Rutin ligand, b) Zr-rutin and c) Mo-rutin complex.**

Figure 3. **Spectral change observed by fixing the concentration of rutin and changing the concentration of Zr^{4+}.**

reached in the excess/limiting point (because an excess of the reagent did not affect the absorbance of complex because at higher concentration of Zr^{4+}, the increase in the height of new peaks is relatively small and decrease in the original peaks is also slight) [27] the other isobestic points disappeared slowly and only one was left behind indicative of formation of only single pure complex [13]. Thus, the bands in the electronic spectra of the complexes show that the metals are coordinated to ligand molecules *in situ* and they appear either due to ligand to metal charge transfer (LMCT) or intraligand (n-π*/π-π*) transitions.

Infrared spectral study

The most important bands in the spectra of rutin and its complexes are best represented in Fig. 4. It shows that complexes exhibit some new and some shifted bands. Hence, comparing the spectra of pure rutin and its zirconium and molybdenum complexes clearly point out that the reaction products are truly the complexes of rutin and metals.

The difference of ν$_4$–ν$_1$ may be taken as an approximate measure of the covalency of metal–

nitrate bonding [29]. The unidentate and bidentate nitrates show the frequency separation of 115 and 186 cm^{-1}, respectively while the combination bands (v_1+v_4) for free nitrate ion appear in the regions of 1800-1700 cm^{-1} [30,31]. The spectrum of zirconium complex shows frequencies at v_4, 1533; v_1, 1384; v_2, 1049 cm^{-1} for three non-degenerated modes of the vibrations, *i.e.* $v_a(NO_2)$, $v_s(NO_2)$ and $v(NO)$, respectively [31-33]. Hence, it is coordinated to the metal ion as unidentate ligand with C_{2v} symmetry (because the separation between two highest frequencies is below 186 cm^{-1}) [33]. The conductance data have also described the non-conducting character of the zirconium complex due to the coordinated nitrate ions [34]. Finally, the peak observed at 630 cm^{-1} is assigned to the formation of bond between metal and oxygen of rutin molecule (Zr-O).
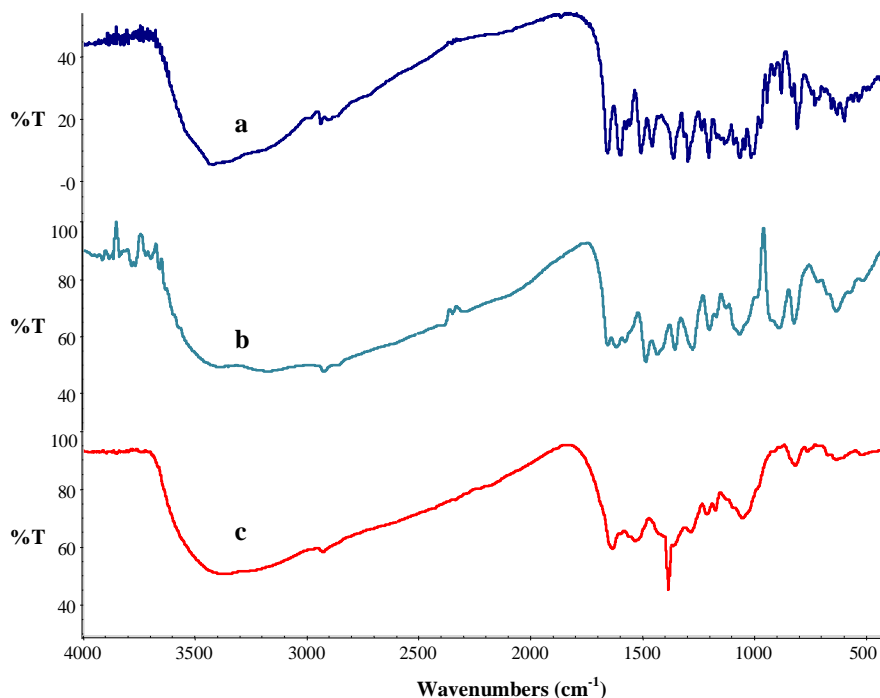

Figure 4. **IR spectra depicted for a) rutin ligand, b) Mo-rutin and c) Zr-rutin complex.**

IR spectrum of rutin molybdate complex presents the formation of two bands at 821 and 889 cm^{-1} due to MoO_2^{2+} dioxo group having symmetrical (O=Mo=O) and asymmetrical (O=Mo=O) stretching vibrations. These frequencies are not only characteristic to the presence of MoO_2^{2+} but also illustrative of its *cis*-structure [26]. This *cis* configuration is characterized as the most preferred geometry of the oxomolybdenum complexes due to maximum utilization of d_π groups [32]. The characteristic band at 1273 cm^{-1} is associated with phenolate ion formation [26]. The most well known broad peak is observed at 3000-3500 cm^{-1} along with 1613 cm^{-1} corresponding to the bending vibrations of H-O-H therefore, such band may be correlated to the presence of water molecules [11,35]. In conjunction with this, the appearance of peak at the frequency of 713 cm^{-1} due to rocking of $\rho_r(HOH)$ shows the presence of coordinated water molecules, this band is absent in the zirconium complex [36]. The presence of coordinated and crystalline water molecules may further be differentiated by the TGA studies. The peaks present at the frequency of 1362 and 1293 cm^{-1} are regarded for the C-O (phenolic) group of free rutin, which are shifted to 1355 and 1274 cm^{-1} values indicating that –OH groups have been deprotonated after coordination [37]. Lastly, the peak observed at 632 cm^{-1} is assigned to the formation of bond between molybdenum and oxygen of rutin molecule (Mo-O).

¹H NMR spectra

Table 3 lists the data for ¹H NMR signals of rutin and its zirconium and molybdenum complexes. In the case of molybdenum complex of rutin, the proper site for complexation could easily be extracted from the ¹H NMR results/studies. It shows that the rutin chelates the molybdenum *via* 3' and 4' phenolic groups; therefore, the protons at these positions have been displaced. On the other hand, the protons at 7-OH and 5-OH positions have not been displaced (Fig. 5) because they are present even after complexation; therefore, it confirms that both of these protons are not involved in complexation phenomenon.

Conversely, in the case of Zr-rutin complex the patterns are quite different because here 5-OH proton has been replaced only by the metal ion. ¹H NMR data are also strongly supported by the IR spectral results of both the complexes.

Table 3. **¹H NMR assignments tabularized for rutin ligand, Mo-rutin and Zr-rutin complex.**

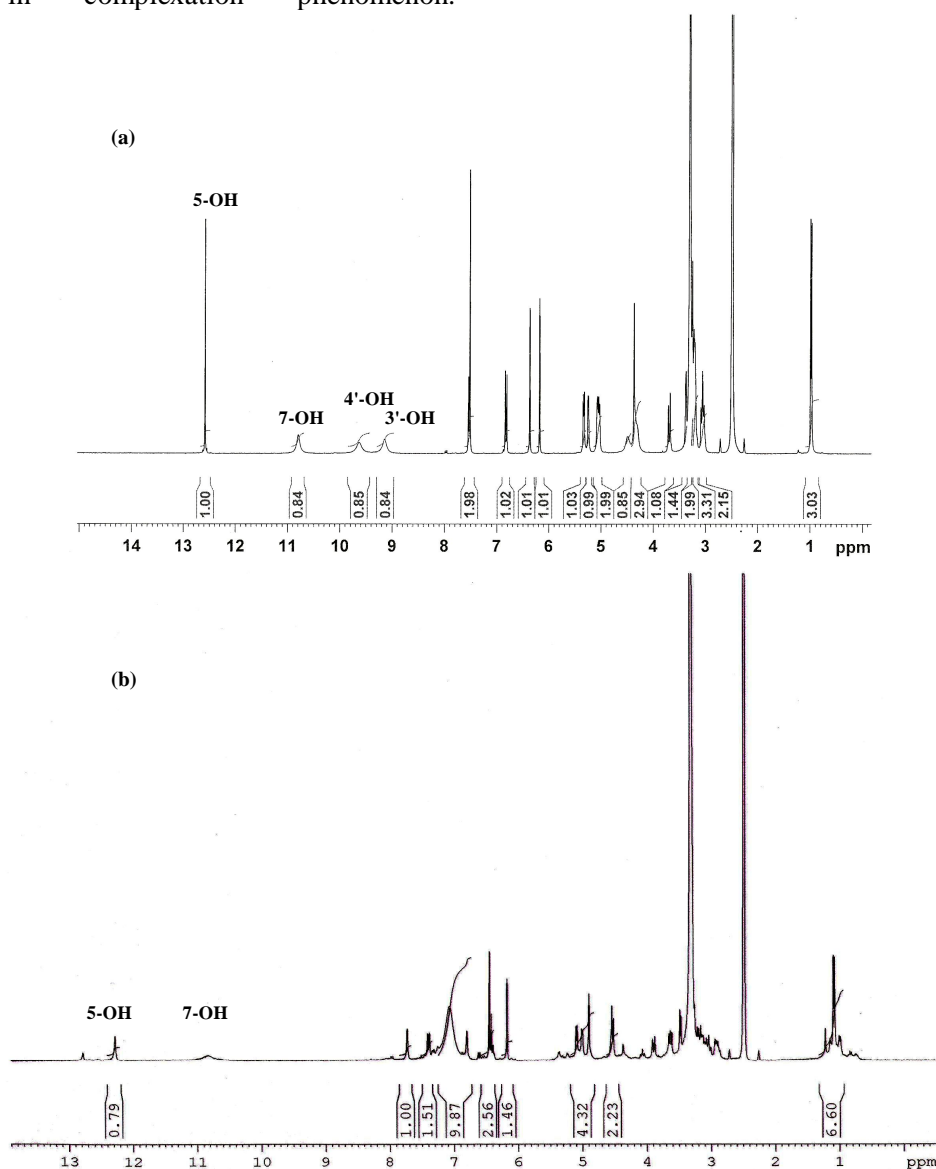| δH, Signals	rutin	Mo-rutin	Zr-rutin
1H, 5-OH	12.63	12.28	–
1H, 7-OH	10.82	10.80	10.78
1H, 4'-OH	9.69	–	9.65
1H, 3'-OH	9.28	–	9.26

Figure 5. **¹H NMR spectra illustrated for a) rutin ligand and b) Mo-rutin complex.**

^{13}C NMR spectra

The ^{13}C NMR information plays important role in support of the structural characterization of the complexes especially by showing the significant change in chemical shift values of complexes relative to ligand molecule. The chemical shift values are changed due to structural rearrangements, e.g. the –OH groups bonded to carbons undergo the complexation by deprotonation may affect the chemical shift values of carbons.

In the molybdenum complex, the most deshielded ^{13}C NMR signal corresponds to the carbonyl group (C=O), by showing the highest chemical shift value (δ 177) due to lower excitation energy of n-π* as well as the intramolecular hydrogen bonding with proton of C5-OH group. Furthermore, the chemical shift values of all the carbons are almost equal for rutin molecule and molybdenum complex except the signals at 3'-OH and 4'-OH group carbons, which may suffer the significant changes in their chemical shift values ultimately reveal the position of complex formation at the protons joined to these carbons (Table 4).

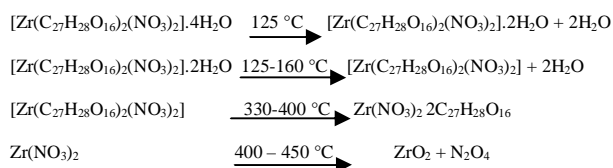*Table 4.* ^{13}C NMR assignments tabularized for rutin and Mo-rutin complex.

δC, Signals	rutin[a]	Mo-rutin complex
4C=O	178	177
5C-OH	157	157
7C-OH	164	164
3'-OH	145	147
4'-OH	149	156

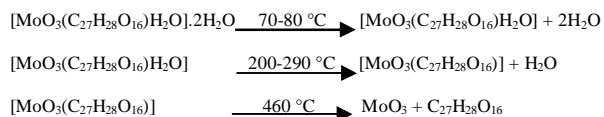**[a]Data for rutin carbons have been obtained from the literature ref. [2]**

Thermal analysis

The highly significant information can be obtained from the thermogramms of rutin complexes. Thermal patterns indicate the presence/absence of water molecules (dehydration) as well as the complete degradation of the complexes (decomposition) stepwise.

In the first step, Zr-rutin complex shows the endothermic dehydration of water molecules from ambient to 125 ℃ releasing two water molecules. The second step weight loss at 125-165 ℃ also corresponds to the loss of other two water molecules. It shows that all the water molecules are of crystalline nature. After that the compound becomes stable over a little temperature range. In the third step, rutin molecule, which is actually a giant ligand first starts swelling/bulging and then undergoes the oxidative degradation in two distinctive steps around 330-400 ℃ [24]. The exothermic decomposition of aglycone part occurs before sugar moiety. The final decomposition at 400-450 ℃ belongs to the loss of nitrate ions covalently bonded to zirconium metal ion [38]. The remaining metal ion subsequently follows the different kinds of rearrangements and ultimately starts the weight gain to either form the oxides of zirconium or its other kind of products as the final residue till to a constant weight.

$$[Zr(C_{27}H_{28}O_{16})_2(NO_3)_2].4H_2O \xrightarrow{125\ °C} [Zr(C_{27}H_{28}O_{16})_2(NO_3)_2].2H_2O + 2H_2O$$

$$[Zr(C_{27}H_{28}O_{16})_2(NO_3)_2].2H_2O \xrightarrow{125-160\ °C} [Zr(C_{27}H_{28}O_{16})_2(NO_3)_2] + 2H_2O$$

$$[Zr(C_{27}H_{28}O_{16})_2(NO_3)_2] \xrightarrow{330-400\ °C} Zr(NO_3)_2\ 2C_{27}H_{28}O_{16}$$

$$Zr(NO_3)_2 \xrightarrow{400-450\ °C} ZrO_2 + N_2O_4$$

In the case of Mo-rutin complex, at first there is the endothermic loss of crystalline water molecules around 70-80 ℃ [30] and then it restores the stability and again dissociates in next step to give weight loss corresponding to the oxidative degradation of coordinated water molecules at 200-290 ℃. In third step, it shows the continuous loss regarded as the loss due to degradation of aglycone and sugar part to give complete decomposition around 460 ℃. But it also shows some weight gain either due to oxygen gain or formation of polymeric molybdenum oxides [39].

$$[MoO_3(C_{27}H_{28}O_{16})H_2O].2H_2O \xrightarrow{70-80\ °C} [MoO_3(C_{27}H_{28}O_{16})H_2O] + 2H_2O$$

$$[MoO_3(C_{27}H_{28}O_{16})H_2O] \xrightarrow{200-290\ °C} [MoO_3(C_{27}H_{28}O_{16})] + H_2O$$

$$[MoO_3(C_{27}H_{28}O_{16})] \xrightarrow{460\ °C} MoO_3 + C_{27}H_{28}O_{16}$$

Actually, there is less probability of formation of metal oxides under nitrogen

environment; hence only metal residues are more expected to form.

Structure of the complexes

Apparently, there is the marked difference between the structures of Zr^{4+} and MoO_4^{2-} complexes; however they form 1:2 and 1:1 complexes of energetically most favorable stoichiometry verified by Job's method and molar ratio method. Even if, rutin has no reactive group at 3-position nevertheless, it alternatively forms the complexes with metals by different mechanisms. The tentative structures of both the rutin complexes are illustrated in Fig. 6 [6].
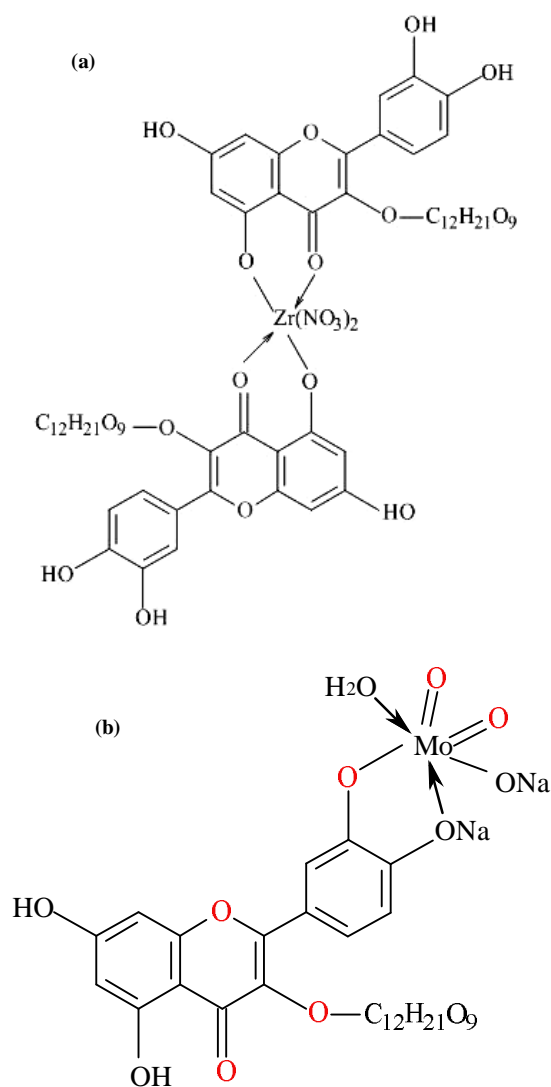
Figure 6. **Tentative structures of a) [Zr(C$_{27}$H$_{28}$O$_{16}$)$_2$(NO$_3$)$_2$]·4H$_2$O, and b) Na$_2$[MoO$_3$(C$_{27}$H$_{28}$O$_{16}$)H$_2$O]·2H$_2$O complexes.**

From the above discussion, it may be extracted that the zirconium complex forms the non-electrolytic complex but conversely the molybdenum forms an electrolytic complex that is also supported by thermal study and conductance data. Furthermore, on the basis of UV and IR spectral characterization, it was deduced that the zirconium formed the complex with 5-hydroxy-4-keto system, whereas molybdenum formed the complex through catechol moiety but in both the cases rutin coordinated in bidentate fashion through the oxygen atoms of rings because they are the most effective coordination centers [26,16]. In consequence, the most probable structures have been proposed with the assistance of UV, NMR and IR spectral results as well as using literature information [26].
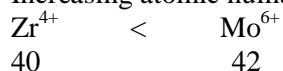
Antioxidant activity

Actually, protective role of antioxidants is displayed by three main mechanisms, 1) transfer of single electron to free radical, 2) transfer of H atom to free radical and 3) metal chelation [40-41]. Flavonoids are generally known for their outstanding antioxidant character. Such activity of natural compounds may be affected (either increased or decreased) by metal chelation [42]. But, it has been observed mostly that metal moiety increases antioxidant potential; it rarely decreases the potential of parent compound. (Fig. 7) shows the graphical representation of the relative antioxidant activity of all the three compounds. The graph has been plotted between %scavenging and time [43]. It clearly indicates the comparative results of the compounds. It shows that molybdenum and zirconium complexes show higher antioxidant potential, while rutin shows lesser activity as visualized from the plot. Zirconium complex shows almost comparable antioxidant potential to rutin molecule and may not significantly increase the antioxidant character of the parent molecule. Since, metal complexation/ chelation may significantly change the characteristic chemical properties of ligand molecule and enhance the activity overall.
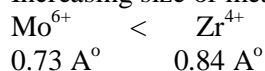
As antioxidant activity of metal complexes depends upon some factors such as size, charge, type of metal ion, coordination number and mole ratio of the complex. Since the various factors are

responsible for the increased antioxidant activity of metal complexes. Another thing is that the antioxidant activity of the ligand and its complexes also depend upon three main things; donation of protons to free radicals, donation of electrons to free radicals and increased conjugation. If the donation of proton is considered as main cause of enhanced antioxidant activity then we say that there are two types of protons present on the flavonoid structure, e.g. O-H and C-H. Here, O-H is more suspected to release the protons easily to involve in antioxidant activity, thus the proton bonded to oxygen has shorter bond length as compared to proton bonded to carbon; hence this proton can easily be broken at lower energy to donate its proton to the free radical because the other bond less easily breaks. Some data about metal ions is given blow;
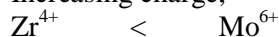
Increasing atomic number;
Zr^{4+} < Mo^{6+}
40   42

Increasing size of metal ions;
Mo^{6+} < Zr^{4+}
0.73 A^o 0.84 A^o

Increasing charge;
Zr^{4+} < Mo^{6+}

Increasing antioxidant activity of compounds;
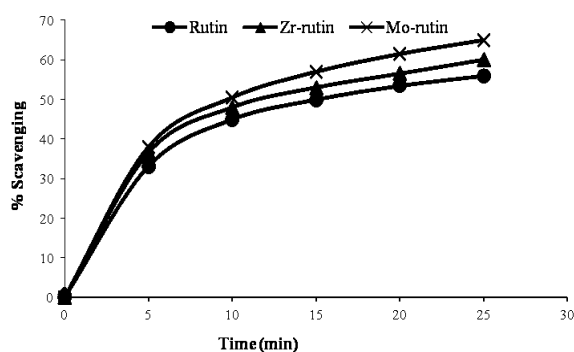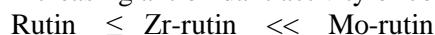Rutin   ≤   Zr-rutin   <<   Mo-rutin

Figure 7. **Antioxidant activity of rutin ligand, Zr-rutin and Mo-rutin complexes, respectively.**

Conclusion

The current study corresponds to the preparation of rutin complexes of zirconium and molybdenum metal ions. It has been concluded that zirconium and molybdenum give 1:2 and 1:1 stoichiometric composition, in addition, bathochromic shift of 66 and 57 nm, metal-oxygen bonds formed at 630 cm^{-1} (Zr-O) and 632 cm^{-1} (Mo-O) frequencies, replacement of 5-OH and 3'-OH protons, presence of water molecules, respectively, indicate strong evidences of complex formation. Their chemical formulae are found as $[Zr(C_{27}H_{29}O_{16})_2(NO_3)_2]\cdot4H_2O$ and $Na_2[MoO_3 (C_{27}H_{28}O_{16})H_2O]\cdot2H_2O$. Antioxidant activity of complexes was also studied and found that metal complexes are more antioxidants as compared to rutin alone. Out of two complexes, molybdenum complex is superior to zirconium complex.

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