Synthesis, Characterization and Antioxidant Activity of Cooper-Quercetin Complex and Iron-Quercetin Complex

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Complexes of cooper (II) and iron (II) with flavonoid quercetin have been synthesized. The structure of compounds has been confirmed by means of UV-Vis and FTIR spectroscopic techniques. The antioxidant activity of the flavonoid complexes has been evaluated by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. These complexes of flavonoids are much more effective free radical scavengers than the free flavonoids, an aspect which recommends them for further studies on possible therapeutic applications.

Keywords: antioxidant activity, flavonoid, quercetin, Cu(II)-quercetin complex, Fe(II)-quercetin-complex

Flavonoids, derivates of benzo-γ- pyrone, are a group of polyphenolic compounds that are extensively distributed in plant foods [1] which display a wide range of biological activities and pharmacological properties such as anti-inflammatory, antibacterial, anti-ulcer, anticancer and cardiovascular protection [2].

Most flavonoids are strong, natural antioxidant free radical scavengers as well as metal ion chelators [3]. The antioxidant action of flavonoids has been considered to be possible via two ways of action: metal chelation and radical scavenging. Radical scavenging occurs by donating hydrogen from the free hydroxyl groups on the flavonoids ring. Another possible antioxidant mechanism of flavonoids is metal chelation through phenolic OH group, which prevents metal-mediated generation of free radicals and which may shelter the potential biological target from oxidative stress [4].

Quercetin (3,3‘,4‘5,7-pentahydroxyflavone) is one of the most common flavonoids present in nature and which has attracted attention upon itself because of its biological properties [4]. Due to the fact that it is a flavonoid of flavonol type which contains five hydroxyl groups in positions 3,3‘,4‘5,7 and a carbonyl group in fourth position, quercetin easily forms complexes with a lot of metals [3]. Complexation of metal cations by quercetin has already been reported for a large number of metals (Mg (II), Fe(III), Zn (II), Al (III), Pb(II), Ni(II), Cd (II)) [4-7].

The interaction between iron ions and flavonoids is of crucial importance: flavonoids can scavenge Fe ions through charge transfer from its deprotonated hydroxyl group to form phenoxyl radicals [8]. As a result, flavonoids efficiently provide protection against oxidative damage [9].

Cooper - as transition metal ions play a vital role in the initiation of free radical processes (*via* Fenton reaction), metal chelation is considered another mechanism specific to the antioxidant activity of flavonoids [10].

In the present research, we have attempted a spectroscopic study on the coordination aspects of quercetin in the presence of Cu (II), respectively Fe(II) ions. The antioxidant activities were determined by means of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method and correlated to the oxidation potential.

The interaction of quercetin with divalent cations changes the antioxidant properties and biological effects of free flavonoids.

Due to the importance of metal chelation in the antioxidant behavior of flavonoids and our constant interest in flavonoid-metal complexes, we report here the synthesis, characterization and antioxidant activity of the Cu (II) and Fe (II) complexes with quercetin.

Experimental part

Materials

All reagents and solvents used for the experiment were of analytical reagent grade.

Quercetin · 2 H_2O dihydrate and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma Aldrich.

$CuSO_4 \cdot 5\,H_2O$; $FeSO_4 \cdot 7H_2O$ were purchased from Merck.

Synthesis of the complexes

The complexes were synthesized by means of the Bukhari modified method [10].

Synthesis of quercetin-cooper (II) complex

In a bottomed flask equipped with an electromagnetic stirrer, the solid quercetin ($C_{15}H_{10}O_7 \cdot 2H_2O$ (0.17 g, 0.01 mol) in methanol (20 mL) was added to the reaction flask; stirring until pure quercetin was completely dissolved. Consequently, $CuSO_4 \bullet 5\,H_2O$ (0.25 g, 0.02 mol) was added to the solution which was stirred at room temperature for 2 h. After stirring, the reaction mixture was filtered and the filtrate was evaporated slowly at room temperature.

The brown powder product was washed with t-butanol to remove the unreactive part of the reagent and then it was dried in a vacuum desiccator.

Synthesis of quercetin-iron (II) complex

In a bottom flask with electromagnetic stirrer, the solid quercetin ($C_{15}H_{10}O_7 \cdot 2H_2O$) (0.01 mol) in methanol (20 mL) was added in reaction flask; stirring until the pure quercetin was dissolved. Consequently, $FeSO_4 \cdot 7H_2O$ (0.0005 mol) was added into the solution, and the solution was stirred at room temperature for 2 h. After stirring, the reaction mixture was filtered and the filtrate was evaporated at room temperature.

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The dark orange powder was washed with tert-butanol to remove the unreactive part of the reagent and then it was dried in a vacuum desiccator.

Chemical and spectral analysis

UV-Vis spectra in methanol were recorded on a Metertech UV-Vis spectrophotometer SP- 8001 in the range of 250-800 nm.

FTIR spectra were recorder on a Perkin Elmer FTIR spectrometer: Spectrum Two (Massachusetts, USA).

Determination of the antioxidant activity

The antioxidant activity for quercetin and the Cu(II)-quercetin and Fe(II)-quercetin complexes has been studied spectrophotometrically by the method using the stable 1,1-diphenyl-2- picrylhydrazyl (DPPH) [11].

A methanol solution (0.1 mL), containing a different concentration of quercetin (4, 8, 12, 16 and 40 μM), was added to 3.9 mL of freshly prepared (57.65 μM) DPPH in methanol.

The reduction of DPPH was followed by monitoring the decrease in absorbance at 517 nm at each 5 min for about 30 min. ($A_{t=30}$). For control purposes, the absorbance of the blank solution of DPPH (4 mL) was also determined at 517 nm. ($At_{=0}$).

Results and discussions

UV-Vis study of the complexes

The changes in the UV-Vis absorption of quercetin in the presence of Cu (II) and Fe (II) respectively, have been examined in methanol.

Quercetin, like most flavonoids, displays two main absorption bands in the ultraviolet-visible region, at 372 nm (band I) showing B ring absorption (cinnamoyl system) and 256 nm (band II). This is considered to be associated with the absorption involving the A ring (benzoyl system) [12] (fig. 1)

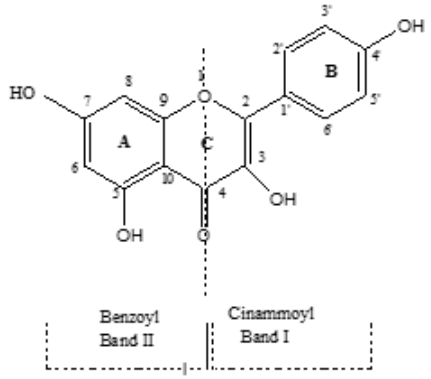

Fig. 1. Structure of quercetin and related UV-VIS absorption bands

The UV-Visible spectra, given the information on the coordination of quercetin sites: for example, the interaction of Metal (II) ions with quercetin at 2:1 metal/quercetin ratio produces a bathochromic shift in the absorbance of both bands (I and II): 275 nm and 461 nm for the Cu (II)-quercetin complex; 280 nm and 410 nm for the Fe (II)-quercetin complex. These changes support the coordination of quercetin through 4-oxo and 5-hydroxyl moieties [13].

FT-IR Spectra

The spectral data displayed the evidence of the coordination between metals and quercetin. Certain features of the spectra are discussed below. The broad bands of O-H vibration frequency located at 3432 cm^{-1}, 3240 cm^{-} indicate the existence of water in the complex and the free quercetin. The C=O stretching of free quercetin occurs at 1658 cm^{-1} and due to the interaction of quercetin with metals, the absorption band has been shifted to 1624 cm^{-1} for Cu (II)-quercetin complex and to 1651 cm^{-1} for Fe (II)-quercetin complex. The bands located at 1351 cm^{-1} for quercetin, at 1367 cm^{-1} for Cu(II)-quercetin complex and at 1312 cm^{-1} for Fe(II)-quercetin complex, were related to C-OH deformation vibrations. The bond related to the C-O-C indicates that the ring oxygen is not involved in the complexation. The appearance of a peak at 603 cm^{-1} indicates the existence of the O-Cu(II) bond in the complex and of a peak at 456 cm^{-1} indicates the existence of the O-Fe (II) bond.

The vibration at 1241 cm^{-1} for quercetin, at 1271 cm^{-1} for Cu(II)-quercetin and at 1247 cm^{-1} for Fe(II)-quercetin indicates that it is not involved in the complexation [14].

The FTIR spectra of quercetin, Cu(II)-quercetin complex and Fe(II)-quercetin complex was summarized in table 1 and the spectrum is shown in figure 2.

Table 1

ASSIGNMENT OF THE MAIN FTIR BANDS OF COMPLEXES IN WAVENUMBERS (cm^{-1})

Quercetin	Cu(II)-quercetin Complex	Fe(II)-Quercetin complex	Assignments
3292	3432	3240	ν O-H
1658	1624	1651	ν C=O
1611	1605	1610	ν inel A sau B
1520	1564	1554	ν C=C
1351	1367	1312	ν C-OH, ν inel B
1241	1271	1247	ν C-O-C
596, 465	603	456	γ C-HK

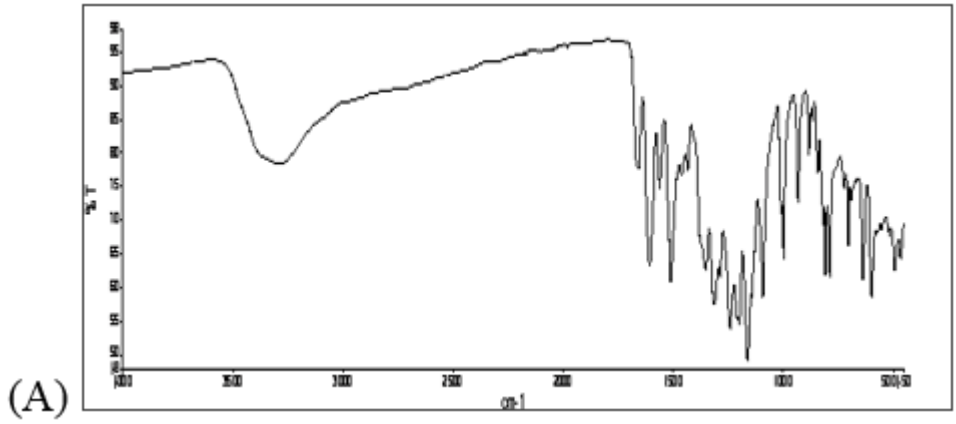

Fig. 2. The FTIR spectra of: (A)-the ligand quercetin

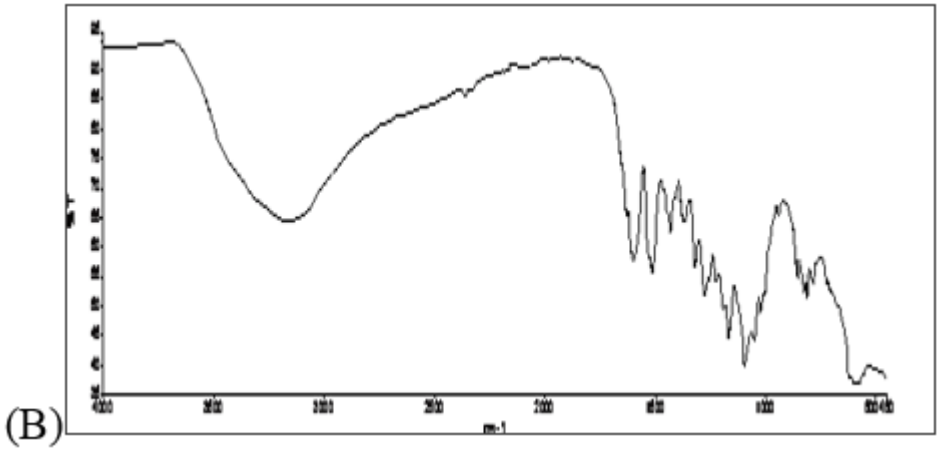

Fig. 2. The FTIR spectra of: B) Cu(II)-quercetin complex

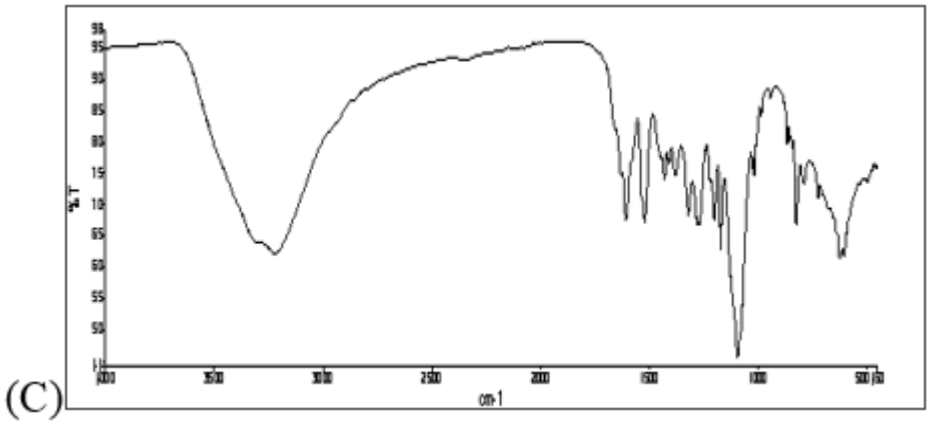

Fig. 2. The FTIR spectra of: (C)Fe(II)-quercetin complex

DPPH radical scavenging analysis

The antioxidant activity of quercetin and its complexes with Cu and Fe respectively was measured in terms of their hydrogen donating or radical scavenging ability by means of UV-Vis spectrophotometer, using the stable DPPH.

The reaction between quercetin and DPPH occurs in two steps: (1) DPPH absorbance decays quickly (60-120 s) and (2) DPPH absorbance decays slowly in 1 h to reach a constant value [10].

Figure 3(a)- display of kinetic behavior in the presence of different concentration of quercetin (4-20μM). It is estimated that the antioxidant activity is a function of time. The kinetic behavior in the presence of quercetin and the metal-quercetin complexes is presented in figure 3(b).

The antioxidant activity of the flavonoid complexes was higher than the ligand quercetin one. This suggests that the metal ions Cu(II) or Fe(II) change significantly the chemical properties of the ligand quercetin. During the first reaction with DPPH radical as shown in figure 4 for

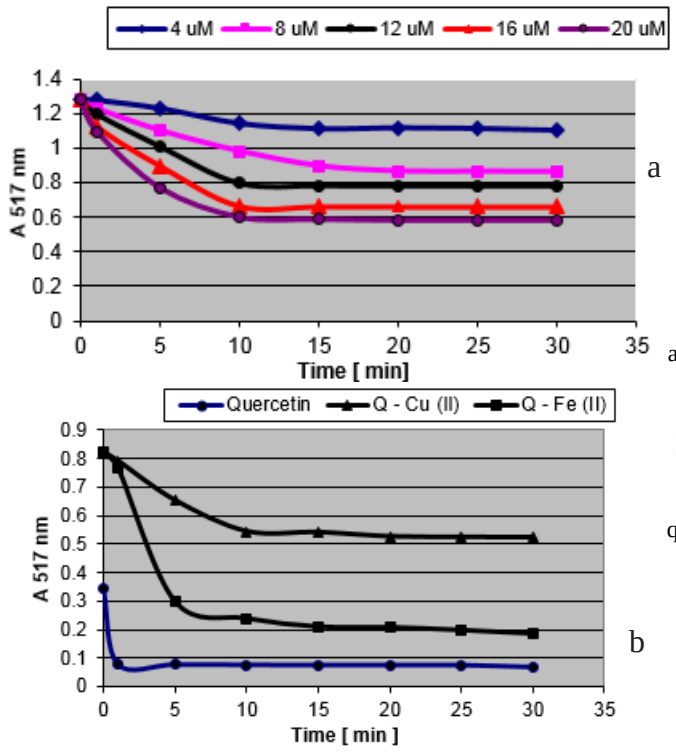

Fig. 3. Decrease in absorbance (λ 517 nm) of DPPH (methanolic solution) in the presence of different concentration of quercetin (a) and quercetin-metal (II) (b)

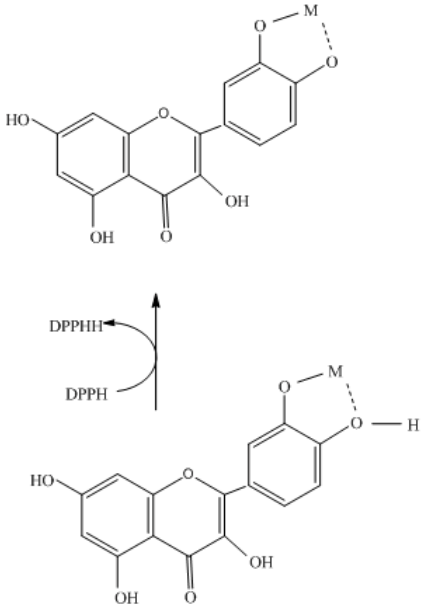

Fig. 4. Proposed metal-quercetin complex oxidation pathway by DPPH radical via a semiquinone radical intermediate

quercetin, a hydrogen atom is abstracted from the Metal (II) -quercetin complex to give a semiquinone complex stabilized by the metallic center and by the conjugation with the 3-OH group [15].

Conclusions

This study presented how a new complex of copper (II) and iron (II) with quercetin flavonoid has been prepared and characterized. The spectroscopic data show the importance of 3-OH group as coordination site. By using FTIR spectroscopy, the coordination of the carbonyl group of the ligand and of the neighboring deprotonated oxygen atom is inferred.

The antioxidant activity of flavonoid depends on the number and the position of OH group present in the flavonoid structure. The Cu(II)-quercetin complex and the Fe(II)-quercetin complex displayed higher antioxidant activity as compared to pure quercetin. This suggests that the metal ions significantly change the chemical properties of the quercetin.

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