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Synthesis, Characterization, Antioxidant Studies and Scavenger Effect of Quercetin and its Copper(II) Complexes on Hydrogen Peroxide-induced Luminol Chemiluminescence

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Two copper(II) complexes of quercetin (Q) with metal:ligand stoichiometry ratios of 1:1 and 1:2 were synthesized. The synthesized complexes structures were determined by UV-Vis spectroscopy, infrared spectroscopy, thermogravimetry, elemental analyses as well as cyclic voltammetry. The spectroscopic data suggest that the chelation between Q and copper(II) can occur through a 3-hydroxy-4-carbonyl site. The antioxidant effectiveness and scavenging activity of quercetin and its complexes were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method and luminol-hydrogen peroxide chemiluminescence system. The results showed that prepared compounds have strong quenching effects on the chemiluminescence intensity of luminol in the alkaline medium and are efficient in scavenging free radicals produced in this system. Reaction system variables such as luminol, quercetin and their complexes' concentration and various pH of luminol solution were investigated. Stren-Volmer equation was also used to study the kinetics of the luminol reaction system and linear relationship was obtained between chemiluminescence (CL) intensity and the concentration of these compounds in a range of 10-100 mg L^{-1}. The Stern-Volmer quenching constant, K_q value in the presence of free quercetin is higher than the complexes, which indicates the fact that complexation with metal ions changes the chemical properties of quercetin and decreases the inhibitory ability and radical scavenging efficiency. The concentration-dependent inhibitory ability of quercetin and copper complexes is attributable to the presence of several hydroxyl groups in their structure that react with superoxide or peroxyl radicals available in luminol-H_2O_2 system. Our study strongly confirmed the potent radical scavenging property of polyphenolic compounds and their metal complexes.

Keywords: Quercetin, Copper(II) complex, Antioxidants, Luminol chemiluminescence, Stern-Volmer plot

INTRODUCTION

Flavonoids are a widely distributed group of polyphenolic compounds with over 8000 individual known compounds. They possess many biological and pharmaceutical properties such as antioxidants [1,2], free-radical scavenging ability,[8] antimicrobials,[3] anticancer [4,5], antiviral [5] and anti-inflammatory activities [6]. Fruits and vegetables along with black tea are the main dietary sources of flavonoids for humans.[9] Their antioxidant and free radical scavenging ability have been related to three main mechanisms including the direct quenching of reactive oxygen species by means of H-atom transfer or one-electron transfer [10,11] and chelating to transition metal ions [12,13]. Free radicals such as hydroxyl radical, superoxide anion radical, hydrogen peroxide and singlet oxygen, which are produced in cells and the environment, are highly reactive species that oxidize macromolecules, such as lipids, nucleic acids, and proteins leading to cell damage and human diseases [14]. The antioxidant compounds can neutralize free radicals and may play an important role in the prevention of these diseases.

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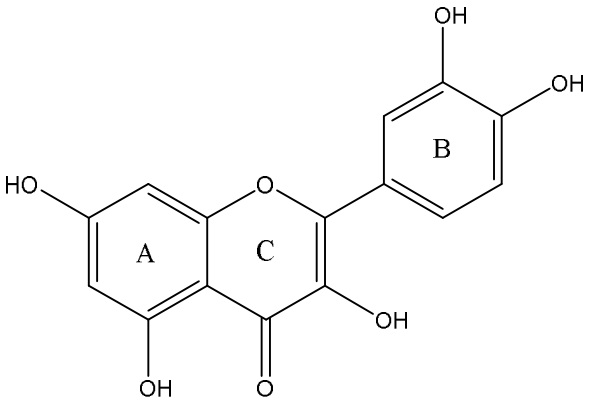


Fig. 1. Chemical structure of natural Quercetin (HQ).

Quercetin (3,3′,4′,5,7-pentahydroxyflavone) (Fig. 1) is a potential antioxidant and the main flavonoid among flavonols. It belongs to the class of organic compounds known as flavones. These are flavonoids with a structure based on the backbone of 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one). It has a wide range of biological activities that affects the presence of metal ions. Its application in the medicinal field has shown potential to improve human health due to its antioxidant activity *in vivo* [15,16]. Hydroxyl groups present in the structure of quercetin are capable of forming complexes with various metal ions. Several studies were carried out on complexation of metal cations by quercetin because of their various biological activities and their potential as therapeutic agents [17].

Quercetin possesses three possible chelating sites in competition: the 3',4'-dihydroxy group located on the B ring and the 3- or 5-hydroxy and 4-carbonyl group in the C ring (Fig. 1). The complexation of metal cations by quercetin has already been investigated for a large number of metal ions (Mg(II), Fe(III) Zn(II), Al(III), Pb(II), Ni(II), Cu(II), Co(II), Cd(II)) and different metal:ligand molar ratios were reported for them [13,18-35]. The interaction of flavonoids with metal ions may also change the antioxidant properties and also biological effects of the flavonoids because of their ability to act as a free radical acceptor. On the other hand complexation may result to less bioavalability and thus lower the toxicity of some toxic metals in the human body [27,35].

Various analytical methods can be used for the evaluation of antioxidant efficiency of phenolic compounds [36,37]. Chemiluminescence is a very useful detection method because of its simple instrumentation, selectivity, high sensitivity and low cost. It has a wide range of applications in biology, biotechnology, bioimaging and analytical technology on the basis of its CL inhibition or enhancement [38,39]. Luminol CL may also present a simple and sensitive approach for monitoring different reactive oxygen species (ROS). The oxidation of luminol by some oxidants such as hydrogen peroxide, oxygen, potassium permanganate, ferricyanide, periodate and oxygen free radicals in alkaline condition results in an intermediate species that emits light [40,41]. Many compounds were observed to enhance or quench the luminol CL. Free radical scavengers like natural phenolic compounds have been reported to decrease the luminol chemiluminescence intensity [42-50]. Antioxidants can eliminate the strong effects of oxidants thus inhibiting blue light production by interfering with oxidation of luminol. Their radical scavenging effect was shown to be concentration- and pH-dependent.

In the present work two copper(II) complexes of quercetin were synthesized and characterized by spectroscopic and analytical methods. The antioxidant activity of quercetin and its synthetic copper(II) complexes

were tested by using luminol-hydrogen peroxide system. Oxygen radicals were produced from hydrogen peroxide in alkaline medium. Addition of oxygen radical scavengers such as ascorbate, phenolics, anilines, and thiols to this system results in quenching the luminol CL signal which provides a simple and sensitive method to evaluate the antioxidant activity. The effects of concentration and pH of these compounds were also investigated. The Stern-Volmer equation was applied to explore the kinetics of the quenching process and the variation of antioxidant property of quercetin after complexation to copper(II) ion were studied.

EXPERIMANTAL

Chemicals and Reagents

All reagents and solvents used for experiments were of analytical reagent grade and used as received. Quercetin.$2H_2O$ (3,3',4',5,7-pentahydroxyflavone) were purchased from Fluka. $CuSO_4.5H_2O$ was obtained from Merck Chemical Company. A stock solution of hydrogen peroxide (30%, v/v, commercially available) was prepared by appropriate dilution of 30% solution with water. Luminol (Fluka) was dissolved in 0.01 M NaOH solution to give a 0.001 M stock standard solution and was stored in darkness at 4 ºC.

Physical Measurement

The FTIR spectroscopic studies of free and complexed quercetin were determined by using KBr pellets in the 400-4000 cm^{-1} range on an FTIR spectrometer (Tensor 27 Bruker). The UV-Vis sperctra were obtained by UNICO 4802 Double Beam UV-Vis Spectrophotometer using standard 1.00 cm quartz cells and methanol as a solvent. Elemental analyses were performed using a CHNS Elemental Analyzer model FlashEA 1112 series. Thermogravimetric analysis and differential thermal analysis of the quercetin metal complexes were carried out by use of DuPont differential scanning calorimeter model STA 504 (Bahr, Germany). pH measurements were performed using a model 710 Metrum pH meter. Electrochemical behavior of one copper complex was also studied by a Potentiostat-Galvanostat (SAMA 500, Iran).

Preparation of the Complexes

An aqueous solution of copper(II) sulfate was added slowly under continuous stirring to an ethanolic solution of quercetin with metal-to-ligand molar ratios ranging from 1:1 to 1:2. The mixture was stirred for 4 h and was set aside for 48 h to reduce the solvent volume. Then, the reaction mixture was filtered and washed with diethyl ether to remove the uncreative part of the reagent and the product was dried in a vacuum desiccator. The olive green products of quercetin complexes were soluble in MeOH, EtOH and dimethyl sulphoxide (DMSO). Yield: 56-81%.

Antioxidant Activity of the Complexes by DPPH Method

The antioxidant activity of free quercetin and complexes were evaluated by using the stable 2,2-diphenyl-1-picrylhydrazyl radical scavenging ability ($DPPH^{\bullet}$) [51]. In this method, first standard solutions of quercetin and its complexes were prepared in methanol (25 mg L^{-1}). Then 0.1 mL of the sample was added to 2.9 mL of freshly prepared DPPH solution of 25 mg L^{-1} in ethanol, which was placed in spectrophotometer immediately and monitored for 60 min. The decrease in absorbance of DPPH at 517 nm (As) was followed every 5 min until the reaction reached the plateau. As a control the absorbance of pure DPPH solution was also measured at 517 nm (Ac). The percentage of radical scavenging ability (RSA%) of quercetin and its complexes was calculated according to following equation:

$$RSA\% = 100\ (Ac - As)/Ac$$

Chemiluminescence Measurements

The chemiluminescence measurements were performed using a Sirius Single Tube Luminometer (Berthold, Germany). The experiments were carried out in a round-bottom glass cell with 15 mm diameter at room temperature. The glass cells were filled with 50 µL of luminol (10^{-3} M, various pH in 0.1 M phosphate buffer), 50 µL of quercetin or its metal complexes (various concentrations in ethanol) and 50 µL of H_2O_2 (10^{-3} M) as the complementary reagent was injected to cause light emission. Chemiluminescence intensity was recorded as a function of time, and time

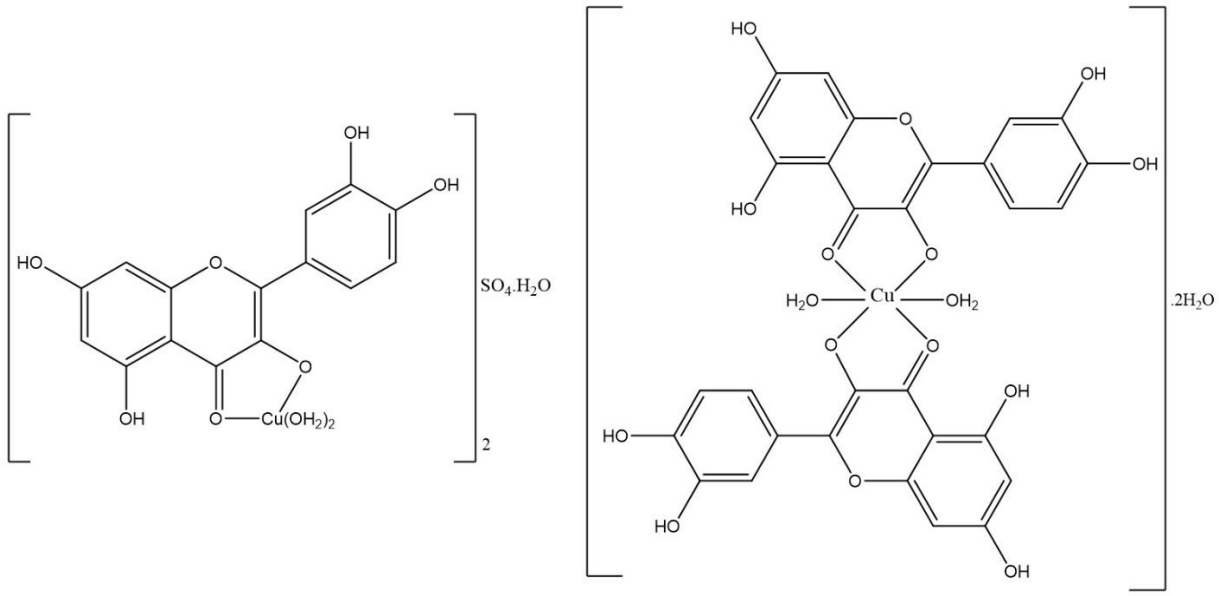


Fig. 2. Proposed chemical structure of quercetin copper(II) complexes.

Table 1. Elemental Analysis Data for Prepared Complexes

Complex	Found (Calcd.) (%)		Mol. wt.
	C	H	
$[Cu(Q)(H_2O)_2]_2SO_4.H_2O$ (1)	40.88 (39.35)	3.67 (3.08)	915
$Cu(Q)_2(H_2O)_2$ (2)	48.62 (48.82)	3.42 (3.55)	738

resolution was 5 s.

RESULTS AND DISCUSSION

Synthesis and Characterization of Complexes

The complexes were stable at room temperature. Elemental analyses of complexes were in agreement with the proposed structure of complexes (Table 1). The chemical structure of quercetin and the proposed structures of quercetin copper complexes can be observed in Fig. 2.

The electronic spectra of the complexes and the free quercetin were recorded in ethanol (Fig. 3). Two main bands were observed in UV-Vis spectrum of quercetin, which were located in ranges of 240-300 nm and 300-400 nm that were related to the conjugated system in ring A (band I, benzoyl system) and ring B (band II, cinnamoyl system), respectively [30]. These absorptions were due to π-π^* transitions in the aromatic ring of ligand. The interaction of copper(II) with quercetin results in both the formation of a new band in 428 nm and the remarkable decrease of band II. This bathochromic shift can be assigned to the extension of the conjugative system when a new ring

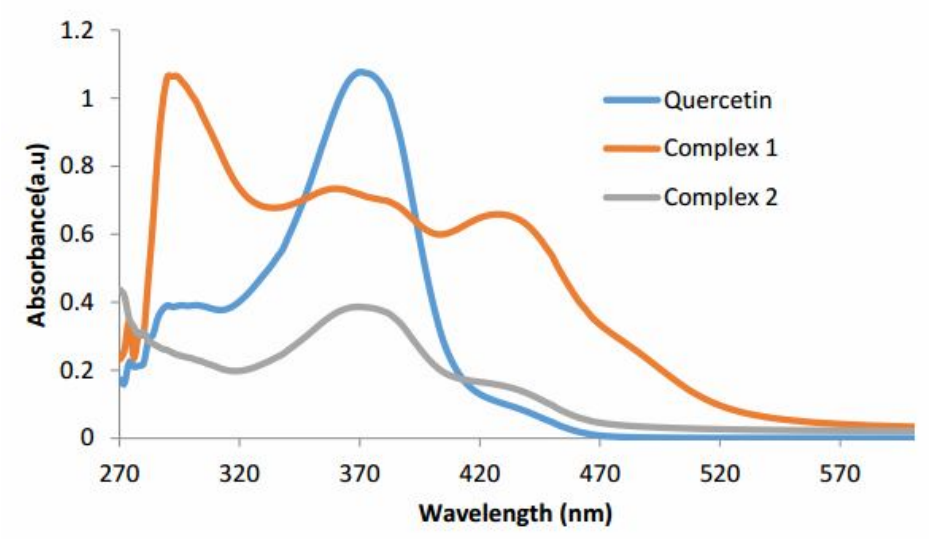


Fig. 3. The UV-Vis absorption spectra of free quercetin and complexes in methanol.

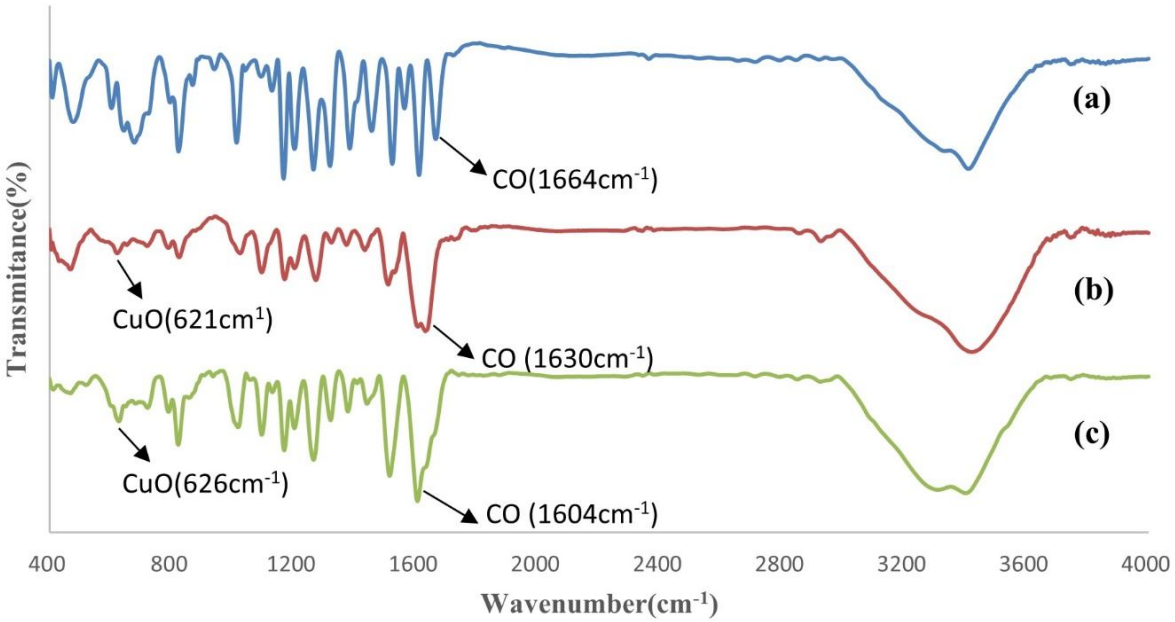


Fig. 4. FT-IR spectra of (a) free quercetin (b) complex 1 and (c) complex 2 in the frequency region of 400-4000 cm^{-1}.

is formed by complexation. The appearance of the new peak in 428 nm in spectra of complexes may arise from complexation of copper in 3-OH and 4-C=O sites. Thus, the chelating ability of quercetin is due to the presence of 3-OH and 4-C=O groups or 3',4'-dihydroxy moiety in ring B, the observed red shift can be representative of the coordinating site in the ligand. Since the hydroxyl group of 3-OH is more acidic, therefore 3-OH and oxygen of carbonyl group in

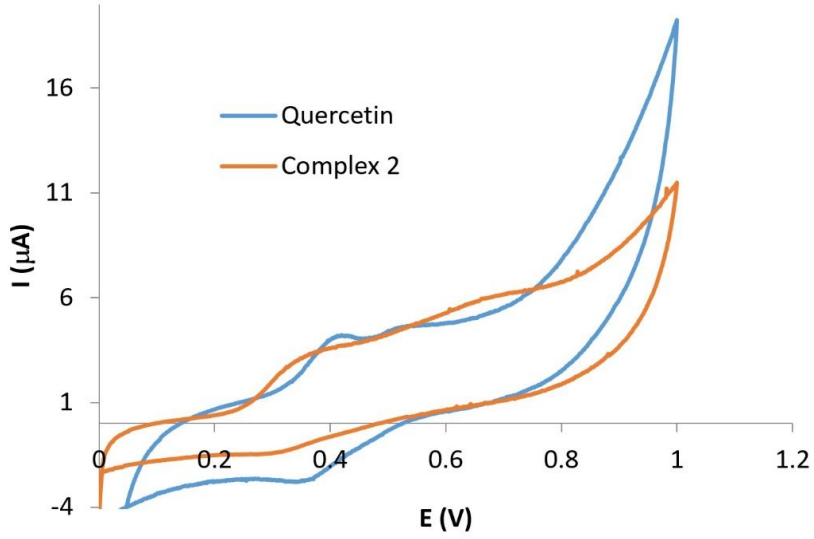


Fig. 5. Cyclic voltammograms of quercetin and copper(II) complex in 0.1 M acetate buffer solution (pH = 7), scan rate 0.025 V s^{-1}.

position 4 may be a more suitable site for complexation [52].

The FT-IR spectra of the ligand and complexes were recorded in the 400-4000 cm^{-1} region (Fig. 4). The chelating site and binding properties of the ligand were realized by comparing the IR bands of quercetin and its complexes. The C=O stretching mode of the free quercetin occurs at 1662 cm^{-1}, which were shifted to lower frequencies by interacting with copper(II) ion in the spectra of complexes. This new band is an evidence for the coordination of the carbonyl group with copper ion [30]. The C-O-H deformation mode observed at 1319 cm^{-1} in the ligand was shifted to 1370 cm^{-1}, indicating an increase in bond order which normally indicates the involvement of 3-OH or 5-OH groups of ligand in metal binding [26]. The sharp stretching vibration around 620 cm^{-1} showed M-O chelation, indicating copper(II) was chelated to quercetin ligand resulting in complex formation, whereas this stretching band was absent in the FTIR spectrum of the ligand [53]. The broad band observed at around 3400 cm^{-1} in the complexes may be assigned to the presence of water molecules, which is also supported by thermal analyses.

The cyclic voltammetry response of quercetin and its copper complex were shown in Fig. 5. All electrochemical experiments were performed in a standard three electrode cell system. The carbon paste electrode (CPE) was used as the working electrode, platinum electrode as the counter electrode and Ag/AgCl as the reference electrode. The CPE was prepared by mixing 70% graphite powder and 30% sample (quercetin and copper complex). The experiments were recorded in 0.1 M acetate buffer solutions of pH 7 at room temperature. The metal chelation resulted in changes in electrochemical behavior of the quercetin Ep (oxidation potential) and Ip (peak current). The cyclic voltammograms show two oxidation peaks indicating electrochemical processes involving two electrons. The copper complex displayed a decrease in oxidation potential (Ep = 0.376, Ip = 3.41) comparing with that of free quercetin (Ep = 0.420, Ip = 4.22). This negative shift in Ep value is assumed to arise from the ease of oxidation process caused by destabilization of the quercetin structure as a result of complexation [54].

The thermal analysis of complexes was carried out in a solid state under the flow of argon. Their TGA and DTA curves can be observed in Fig. 6. When the complex is heated, it undergoes certain physical and chemical changes

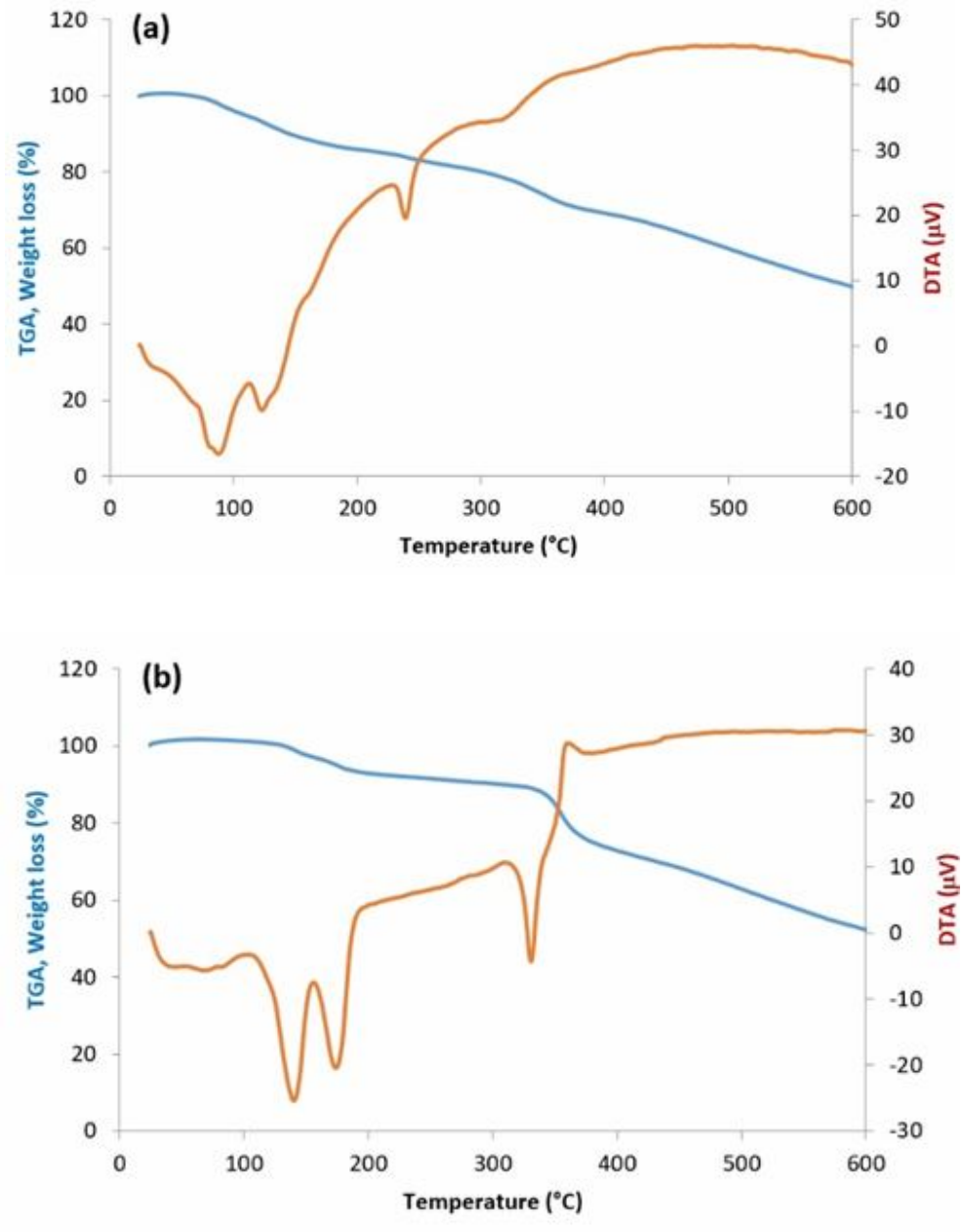


Fig. 6. TGA plots along with DTA curves for (a) complex 1 and (b) complex 2 under argon atmosphere at the heating rate of 10 °C min^{-1}.

which are resulted in absorption or liberation of heat. The chemical changes in metal complexes may include dehydration and decomposition. The data obtained indicate that complex 1 was stable up to 70 °C. In the DTA curve of this compound, an endothermic peak was observed under 100 °C (~88 °C) which, accompanied by weight loss of ~2% (calculated mass = 18 g), may be assigned to liberation of one water molecule. The second endothermic peak was

obtained within the temperature range of 100-200 °C with an estimated mass loss of 4.5% (calculated mass = 41 g) coinciding with two water molecules. The third endothermic peak at around 240 °C may be related to the melting point of the complex. This fact suggests that this complex contains one hydrated water molecule and two crystal water molecules. The thermogram of complex 2 is also presented in Fig. 6. The DSC curve shows that the complex is stable up to a temperature of 130 °C. Within the temperature range of 100-200 °C two exothermic peaks were obtained corresponding to 5% (calculated mass = 37 g) weight loss, showing the liberation of two water molecules. The DSC curve also indicated an endothermic peak around 335 °C which may be attributed to the melting point of the complex. The results suggest that this complex have two crystal water molecules. These findings were also confirmed by IR studies.

DPPH Radical Scavenging Activity

The antioxidant activity of quercetin and its complexes was measured by UV-Vis spectrophotometer using the stable DPPH. This method is based on the measurement of the ability of compounds to act as free radical scavengers or hydrogen donors. DPPH shows a strong absorption band at 517 nm due to its odd electron of the nitrogen atom and solution appears a deep violet color. When DPPH is reduced by receiving a hydrogen atom from antioxidants, the absorption vanishes as the electron pairs off [51]. The capacity of quercetin and its complexes to act as antioxidants depends on their molecular structure, especially their hydrogen donating ability. It was found that *ortho*-dihydroxy group on the B-ring (on 3', 4' position) and a hydroxyl group in the C-ring at the position 3, are the most effective radical scavengers [55]. Figure 7 shows the decrease in absorbance of DPPH ethanolic solution in the presence of quercetin and its copper(II) complexes. The reaction between quercetin and DPPH occurs in two steps: in the first step DPPH absorbance (λ_{max} = 517 nm) decays quickly and in the next step DPPH absorbance decays slowly over ~1 h to reach a constant value. The fast step refers to abstraction of most liable H-atom from the antioxidant (3'-OH, 4'-OH and 3-OH in the case of quercetin), while the slow step shows remaining activity in the oxidized products. It may be assumed that 3′-OH and 4′-OH are primarily involved in H-atom transfer reactions to DPPH and/or that each -OH group strongly facilitates H-abstraction from the other by stabilizing the corresponding radical by a combination of electronic and H-bonding effects [33]. As can be seen in Fig. 7, the antioxidant ability of Q decreased after chelation of copper(II) cation. The lower antioxidant activity of quercetin-copper complexes may be attributed to lowering the number of hydroxyl groups as a result of complexation.

The CL Quenching of Luminol by Quercetin and its Complexes

The antioxidant activity and radical scavenging ability of quercetin and its complexes are well known [31], thus here we decided to study the effect of the presence of these compounds on the CL intensity of luminol-H_2O_2 system. When quercetin and complexes were added to this system, the CL signal was decreased greatly (Fig. 8). Based on this observation, the effects of various concentrations of the compounds and different pH values of luminol solutions on the quenching behavior of these antioxidant compounds in luminol chemiluminescence were also evaluated.

Effect of Quercetin and Copper(II) Complexes Concentration on Luminol CL Reaction

First, in order to determine the optimized reaction conditions in luminol-H_2O_2 system, the effect of luminol concentration was studied from 10^{-7}-10^{-3} M. The maximum CL intensity was observed at 10^{-3} M. Thus, this concentration was selected as the optimal concentration for luminol. The effect of H_2O_2 concentration was also investigated on the CL intensity in a range of 10^{-6}-10^{-3} M at room temperature. The highest CL signal was obtained at 10^{-3} M, therefore it was chosen as the optimal H_2O_2 concentration. The influence of different concentrations of quercetin and its complexes were then recorded in the range of 10-100 mg L^{-1}. All of the investigated compounds had concentration-dependent quenching effects (Fig. 9).

As it can be observed the CL intensity of luminol-H_2O_2 system was decreased with the increasing concentration of

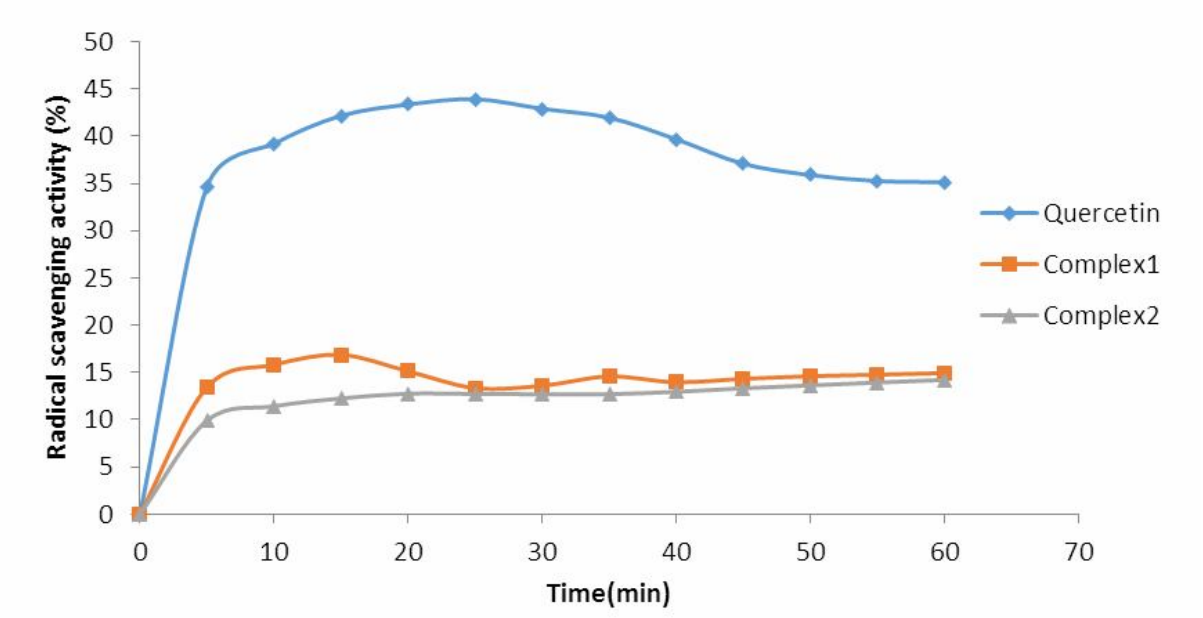


Fig. 7. Kinetic behavior of DPPH free radical scavenging activity (%) of 25 mg L^{-1} of quercetin (Q) and copper(II) complexes. The radical scavenging activity were carried out for 60 min with 5 min interval.

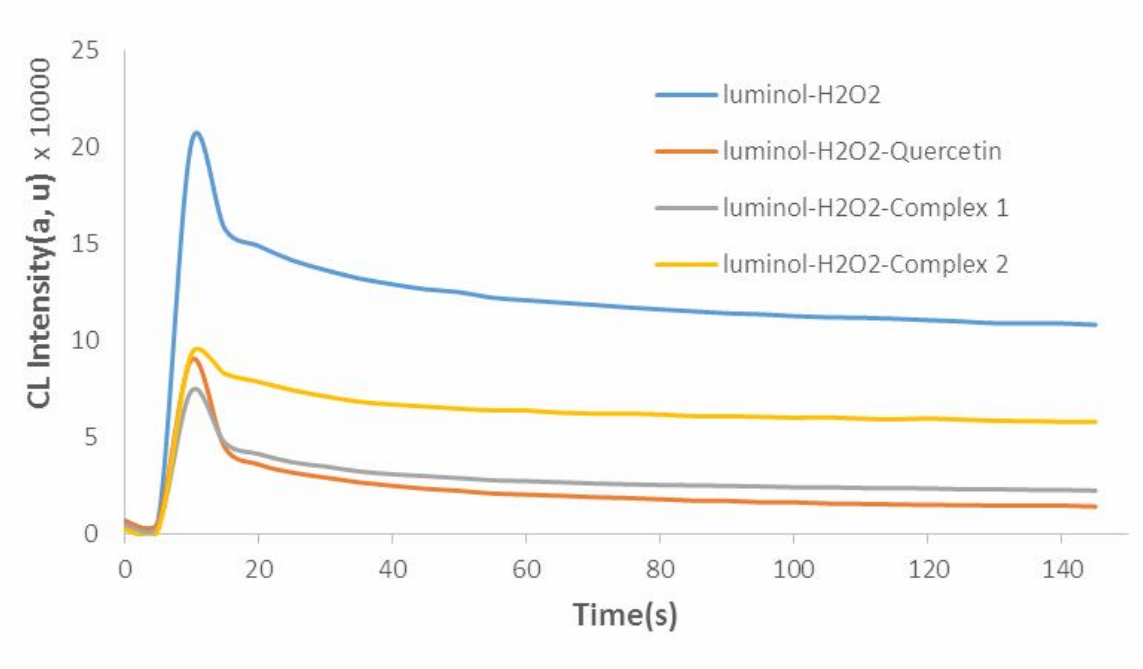


Fig. 8. Time course of the kinetic profiles of the luminal-H_2O_2 CL reaction at the presence of quercetin and complexes. 10^{-3} M luminol at pH = 12, 10^{-3} M H_2O_2, 10 mg L^{-1} quercetin and complexes.

the compounds. The strong inhibitory effect of these compounds on CL intensity of luminol may be due to radical scavenging ability of quercetin and its complexes. In general, antioxidant activity of flavonoids depends on the structure, the number and positions of the hydroxyl groups. Quercetin has a catechol structure in ring B, as well as a 2,3-double bond in conjunction with a 4-carbonyl group in ring C, allowing for delocalization of the phenoxyl radical electron to the flavonoid nucleus. The presence of hydroxyl groups in the structure of polyphenolic compounds prevented the reaction of hydroxide or superoxide radicals produced from H_2O_2 with luminol molecules by donating a hydrogen atom so the inhibitory activity of quercetin and its complexes was increased and coincidentally, the CL intensity of luminol was further decreased. They act as free radical scavengers by forming less reactive phenoxyl radicals when reacting with $OH^{\cdot}$ and $HO_2^{\cdot}$ and redistributing the unpaired electron on the aromatic core of flavonoid phenoxyl radicals leads to form a resonance structure with a much lower activity, compared to free radical and phenoxyl radicals (Scheme 1) [55]. The results represented that even a low range of these compounds can act as strong radical scavengers in luminol-hydrogen peroxide systems and have quenching effects on CL signal intensity.

To study the kinetics of quenching behavior of quercetin and complexes in the desired photoreaction system, the Stern-Volmer equation was applied. It was used to relate the reduced chemiluminescence intensity to the quencher concentration [Q]. According to the classical Stern-Volmer equation [56]:

$$I_0/I = 1 + K_q[Q]$$

where I_0 is the CL intensity in the absence of quencher; I is the CL intensity in the presence of quencher; K_q is the quenching constant and [Q] is the quencher concentration. Indeed, the Stern-Volmer equation can be used to characterize the quenching capacity of the substance. The plot of I_0/I was linear and K_q was directly proportional to the influence of substance on the photochemical reaction. The larger the K_q, the higher the inhibitory effect of the substances in CL system. The plot of I_0/I *versus* quercetin and its complexes' concentrations is presented in Fig. 10 and some of the key parameters of the plots are given in Table 2. As can be seen from the equations, the K_q value in the presence of free quercetin is higher than the complexes, which indicates the fact that complexation with metal ions changes the chemical properties of quercetin and decreases the inhibitory ability and radical scavenging efficiency. This implies that the lower hydroxyl groups in complexes as a result of deprotonation of phenolic groups, in comparison with free quercetin, may stabilize free radicals and reduce the oxidation potential and antioxidant activity of this flavonoid.

Effect of pH on CL Reaction

In general, luminol oxidation by oxidants often occurs in basic conditions. The CL intensity of the luminol-hydrogen peroxide system is strongly affected by the solution pH. In a basic medium, the rate of H_2O_2 decomposition and the amount of superoxide radical production increases which results in CL intensity enhancement. Since the antioxidant activity of flavonoids change in different pH values [48,57], we decided to investigate the pH-dependent behavior of quercetin and prepared complexes for CL signal quenching. Considering the highest CL intensity of luminol in the presence of 10 mg L^{-1} solutions of these compounds, luminol solutions (10^{-3} M) were prepared in a phosphate buffer at various pH values from 5 to 11, then CL plots of luminol were recorded in the presence of 10 mg L^{-1} concentration of quercetin and it's complexes. Due to the strong quenching effect of quercetin in low pH values, luminol-H_2O_2 system showed no observable CL signal so, in order to accelerate the oxidation process, hemin solution (10^{-6} M, 10 μL) was added to luminol. Hemin, is an iron containing compound derived from heme which can catalyze luminol CL reaction in the presence of hydrogen peroxide and its potential application in blood detection have been investigated [58]. As shown in Figs. 11a and b, increasing pH values causes CL enhancement. It is clear that there are several hydroxyl groups in quercetin compounds which reduce the generation of HOO radicals from hydrogen peroxide. The reduction of free radicals

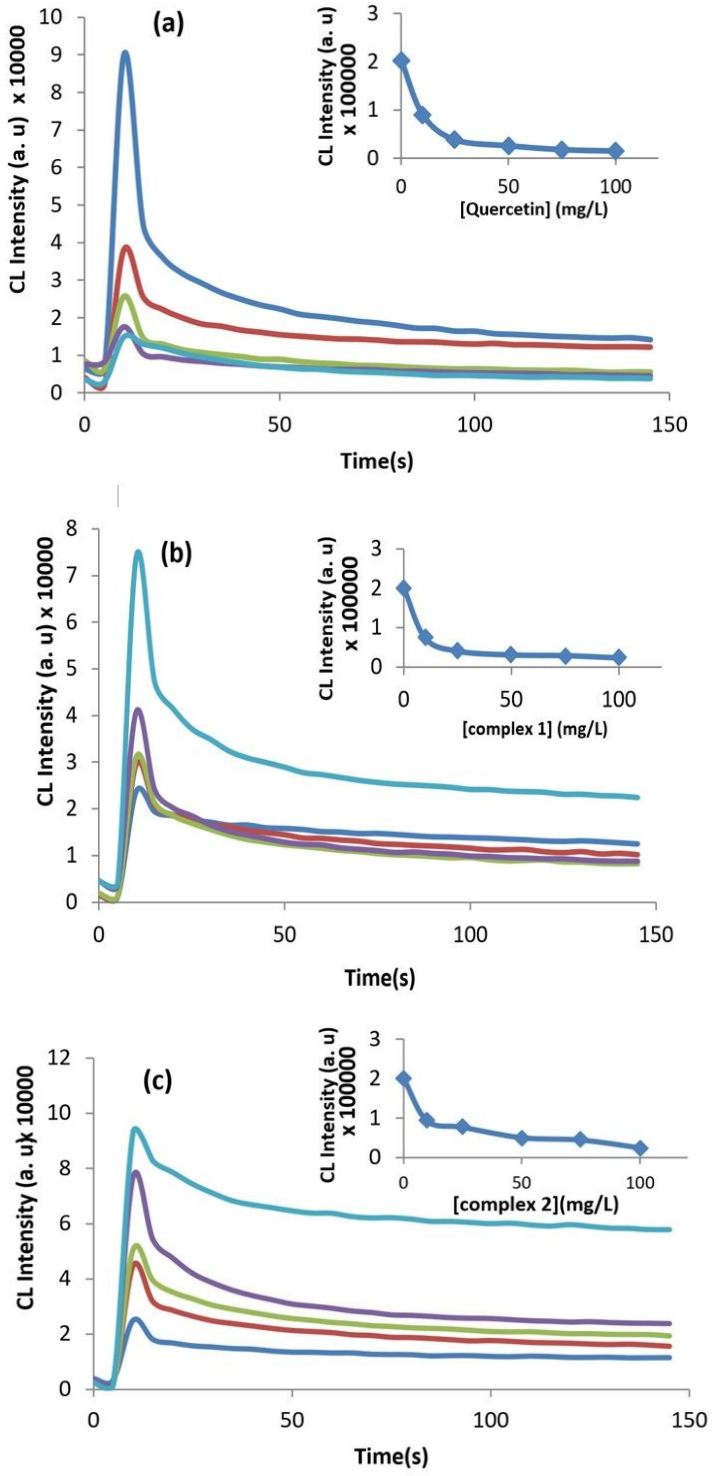
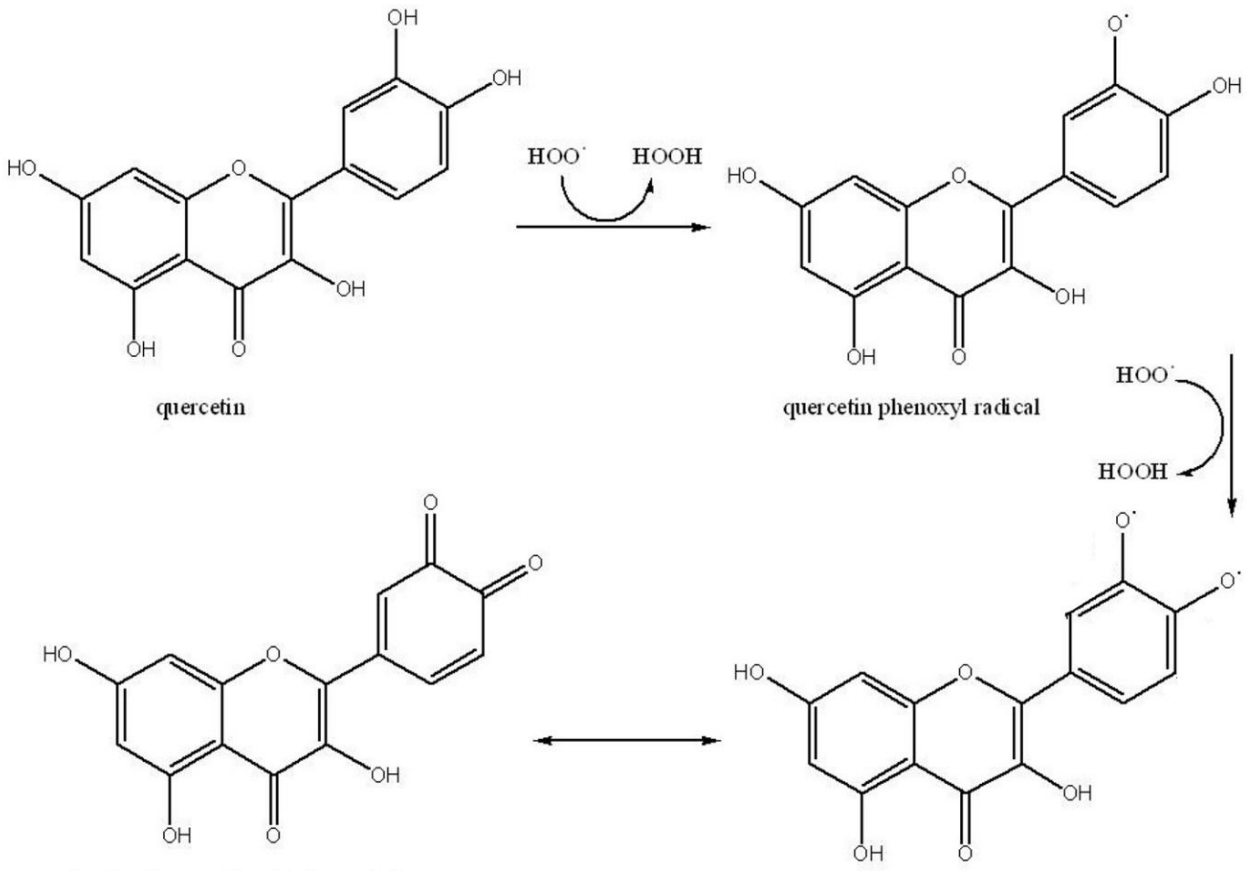


Fig. 9. Effects of Quercetin and complexes concentration on the luminol-H_2O_2 CL system. (a) luminol-H_2O_2-quercetin, (b) luminol-H_2O_2-complex 1, (c) luminol-H_2O_2-complex 2. 10^{-3} M luminol at pH = 12, 10^{-3} M H_2O_2 and varying concentrations of Q and complexes. Inset: The correlation diagram for the chemiluminescence emission with samples concentrations.



Scheme 1. Proposed mechanism of free radical scavenging activity of quercetin

Table 2. Quenching Parameters of Quercetin and Complexes from Stern-Volmer Plot in Luminol-H_2O_2 CL System

	K_q (L mg^{-1})	Stern–Volmer equation ($y = I_0/I$, x = [Q])	R^2	N^a
Luminol-H_2O_2-Quercetin	0.124	$y = 1.619 + 0.124x$	0.986	5
Luminol-H_2O_2-Complex 1	0.057	$y = 2.921 + 0.057x$	0.918	5
Luminol-H_2O_2-Complex 2	0.060	$y = 1.136 + 0.060x$	0.892	5

[a]Number of points.

effects on the oxidants' potentials for oxidation of luminol molecules, resulting in the CL quenching process. In higher pH values, deprotonation of hydroxyl groups may decrease the inhibitory ability and radical scavenging capacity of these compounds, resulting in CL intensity enhancement. The results demonstrate that the antioxidant property of

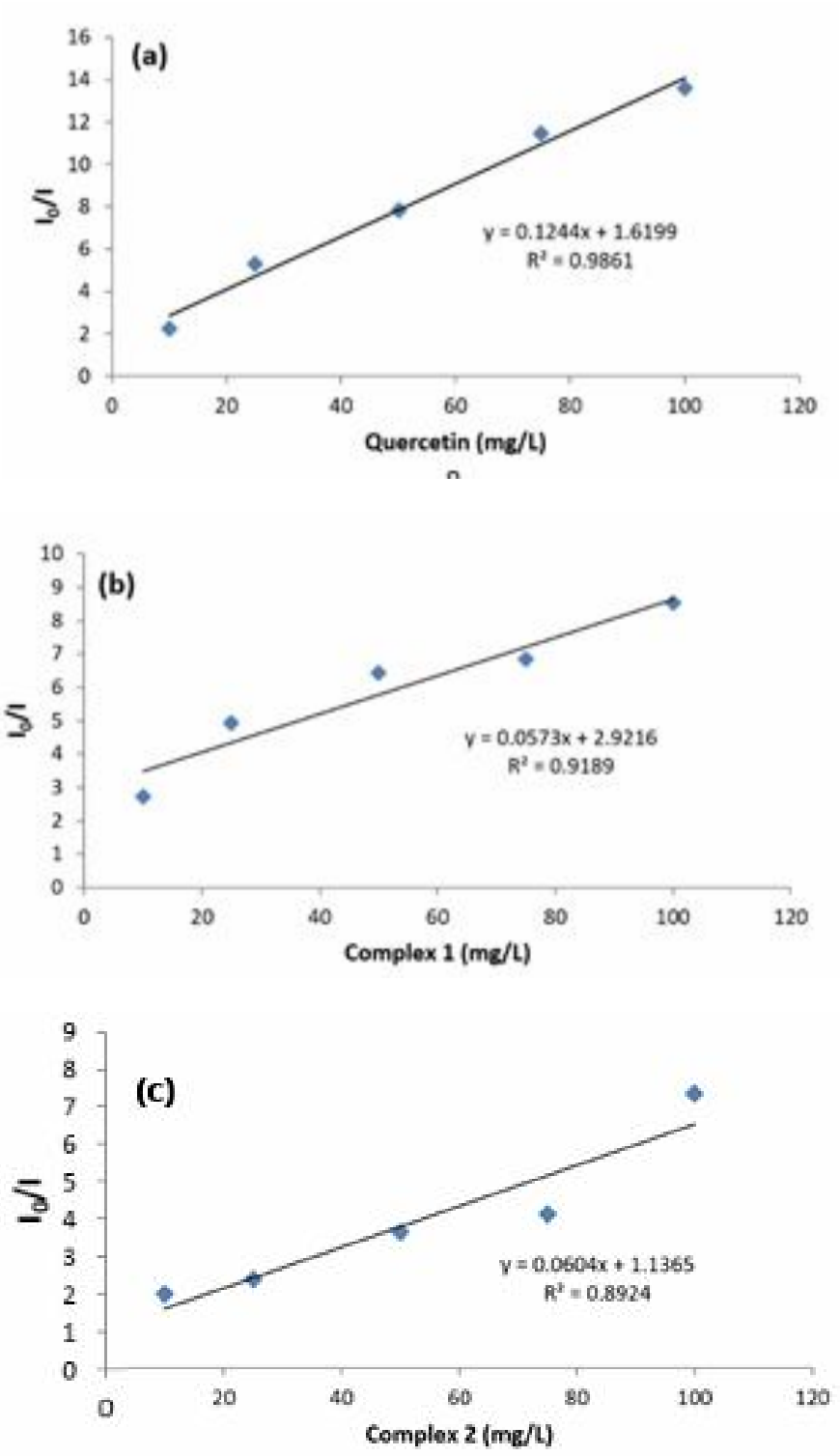


Fig. 10. Stern-Volmer plots for quenching effect of (a) quercetin (b) complex 1 and (c) complex 2 on luminol-H_2O_2 CL system.

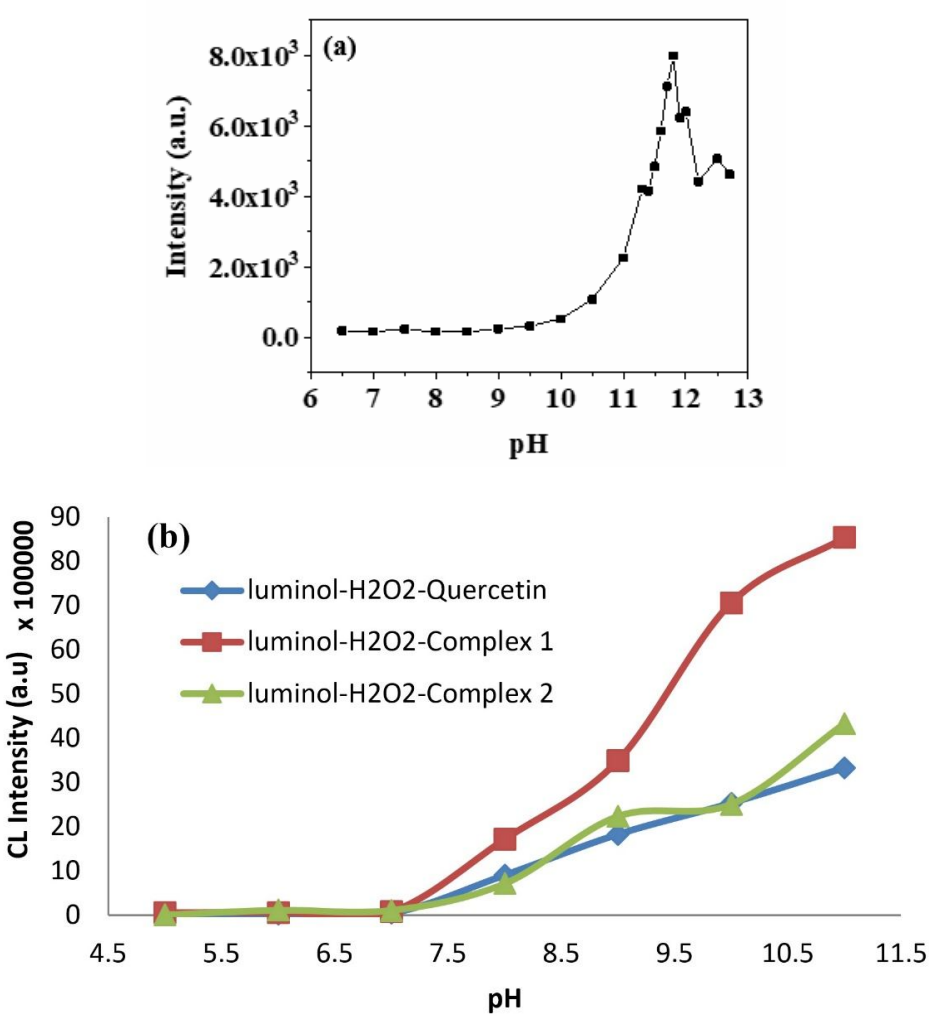


Fig. 11. Effects of pH of luminol solution on quenching behavior of quercetin and the complexes. (a) In the absence and (b) In the presence of quercetin and their complexes. Conditions: 10^{-3} M luminol, 10^{-3} M H_2O_2, 10^{-6} M hemin, 10 mg L^{-1} quercetin and complexes.

quercetin and its complexes in physiological pH values is great; therefore, they can be considered as good scavengers for free radicals. It is also suggested that the use of different buffer pH can influence selectivity and sensitivity of these types of compounds in CL measurements.

The Possible Mechanism

The antioxidant activity of quercetin and its complexes have been extensively studied [18,19,23,26-28,30,31,33,34]. It was observed that the radical scavenging ability in phenolic compounds resulted from three mechanisms: hydrogen atom transfer, electron donation and metal chelation [59]. The antioxidant behavior of quercetin and its complexes were strongly dependent on their structure, particularly their hydrogen donating free radical scavengers and this property was measured by UV-Vis spectroscopy

method using the stable DPPH. In general, flavonoids containing more hydroxyl groups have demonstrated higher antioxidant properties, which in the case of quercetin, 3'-OH, 4'-OH and 3-OH groups are the most liable H donating groups involved in free radical scavenging. The chemiluminescence method is considered a sensitive approach for studying free radicals in reactive metabolites and the mechanism of the reaction has been extensively investigated [60]. The applied luminol-H_2O_2 method is a useful tool for evaluating the oxygen radicals' scavenging ability of desired compounds. These properties of phenolic compounds can influence luminol CL signal in such a way that demonstrate their potential for neutralizing hydroxyl radicals produced in this system and is determined by their concentrations. In a basic medium, the luminol nitrogen protons are removed leaving a negative charge which moves onto the carbonyl oxygen to form an enolate structure. The hydrogen peroxide is also converted into hydroxyl and superoxide radicals in the basic solution which then attacks luminol radicals and oxidizes them into carboxylate anions and leaves 3-aminophthalate in an excited state. This process is accompanied by the loss of nitrogen molecules and the emission of a photon of light (hν). The reaction scheme could be described as follows:

$$LH_2 + OH^{\cdot\cdot} \rightarrow LH^{\cdot\cdot} + H_2O \quad (1)$$

$$LH^{\cdot\cdot} + OH^{\cdot} \rightarrow L^{\cdot\cdot} + H_2O \quad (2)$$

$$H_2O_2 + OH^{\cdot} \rightarrow HO_2^{\cdot} + H_2O \quad (3)$$

$$HO_2^{\cdot} + OH^{-} \rightarrow O_2^{\cdot\cdot} + H_2O \quad (4)$$

$$L^{\cdot\cdot} + O_2^{\cdot\cdot} \rightarrow LO_2^{2-\cdot} \quad (5)$$

$$LO_2^{2-\cdot} \rightarrow AP^{2-\cdot *} + N_2 \quad (6)$$

$$AP^{2-\cdot *} \rightarrow AP^{2-\cdot} + h\nu \quad (7)$$

where LH_2, $LH^{\cdot\cdot}$, $L^{\cdot\cdot}$, $LO_2^{2-\cdot}$, $O_2^{\cdot\cdot}$ and $AP^{2-\cdot *}$refer to luminol, luminol monoanion, luminol radical, luminol endoperoxide, superoxide radical and excited 3-aminophatalate, respectively.

In the presence of quercetin and the synthesized complexes, CL signal inhibition was observed in the luminol-H_2O_2 system and this quenching effect was concentration-dependent. These inhibitor compounds contain *ortho*-dihydroxy groups that can react with radical intermediates of luminol CL reaction like $OH^{\cdot}$, $O_2^{\cdot\cdot}$ and $L^{\cdot\cdot}$. Phenolic compounds are first oxidized to semiquinone radicals by superoxide anions and further oxidation may result in the production of quinone form [48,61]. So this competition for scavenging radicals may reduce the available radicals for luminol oxidation. This process may interfere with light emission in this system which results in quenching the luminol CL intensity. The reaction pathways could be as follows [48]:

$$IH_2 + OH^{\cdot\cdot} \rightarrow IH^{\cdot\cdot} + H_2O \quad (8)$$

$$IH^{\cdot\cdot} + OH^{\cdot} \rightarrow I^{\cdot\cdot} + H_2O \quad (9)$$

$$I^{\cdot\cdot} + O_2^{\cdot\cdot} \rightarrow IO_2^{2-\cdot} \quad (10)$$

$$IO_2^{2-\cdot} + L^{\cdot\cdot} \rightarrow LO_2^{2-\cdot} + I^{\cdot} \quad (11)$$

where IH_2 presents the inhibitor (quercetin and the complexes). According to this mechanism, compounds with strong reducing abilities could have stronger CL quenching capacities. Thus, complex formation between quercetin and copper(II) ions may diminish the inhibitory activity of quercetin due to less electron or hydrogen transfer ability in the complexed forms. So chelation of phenolic compounds with metal ions can affect the reducing ability by coordinating with OH groups and thus reducing the light quenching potential of phenolic compounds.

CONCLUSIONS

In this study, two complexes of quercetin and copper(II) were prepared in ethanolic solution. The spectroscopic measurements suggest that the interaction of quercetin and copper(II) can occur through the 3-hydroxy and 4-carbonyl ligand site. It can be attributed to the more acidic property

of 3-OH group which makes it more suitable for the coordination to metal ions. The DPPH method and luminol chemiluminescence was used to investigate the radical scavenging capacity of quercetin and its complexes. Since light emission was observed as a result of oxidation of luminol by some oxidants in alkaline solution, antioxidants inhibited this blue light by interfering with the oxidation of the luminol. Under optimal conditions, low concentration of these compounds leads to strong quenching of the observed chemiluminescence signal intensity. Decrease in the CL signal was proportional to the concentration of analytes in the range of 10-100 mg L^{-1}. It was confirmed that the *ortho*-dihydroxy groups in polyphenolic compounds were responsible for the antioxidant and free radical scavenging properties, and the competition of OH groups in tested compounds with luminol for O_2 and OH radicals led to the inhibition of CL. The results showed that quercetin, compared with the complexes, indicates more quenching effect which is probably due to more contribution of hydroxyl groups in quercetin for electron or hydrogen transfer. Chelation with metal ions may change the redox potential of metal complexes while also decreasing the hydrogen atoms available for scavenging to free radicals. Since several protonable groups are present in these compounds, changing the environmental pH can influence their scavenging ability. It was found that an increase in the pH, decreases the inhibitory activity of polyphenolic compounds, resulting in an enhancement of luminol CL intensity. The present work provided a sensitive, simple and fast method for screening the antioxidant properties of substances which act as natural and synthetic radical scavengers.

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