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# Synthesis, spectral and electrochemical properties of Al(III) and Zn(II) complexes with flavonoids

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#### Abstract

The synthesis, electrochemical and spectral (UV–vis, <sup>1</sup>H NMR, IR, fluorescence) properties as well as thermal behaviors of Al(III) and Zn(II) complexes with the flavonoids quercetin  $(H_2L^1)$ , rutin  $(H_2L^2)$  and galangin  $(HL^3)$  are presented. The complexes may be formulated as  $[Al_2(L^1)(H_2O)_8]Cl_4$ ,  $[Al_3(L^2)_2(H_2O)_{12}]Cl_5$ ,  $[Al(L^3)(H_2O)_4]Cl_2$ ,  $[Zn_2(L^1)(H_2O)_4]Cl_2$ ,  $[Zn_3(L^2)_2(H_2O)_6]Cl_2$  and  $[Zn(L^3)(H_2O)_2]Cl$ . The higher fluorescence intensities of the complexes related to the free flavonoids, are attributed to the coordination of the ligands to the small, highly charged Al(III) and Zn(II) ions. The coordination effectively increases the rigidity of the ligand structure and increases the fluorescence quantum yield by reducing the probability of non-radiative energy dissipation process. Antioxidant activities of the compounds were also investigated under an electrochemical point of view. The cyclic voltammetric data show a considerable decrease of the oxidation potentials of the complexes related to that of the free flavonoids. Thus, the flavonoid–metal complexes are more effective antioxidants than the free flavonoids.

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Keywords: Flavonoids; Al(III) and Zn(II) complexes; Spectral and electrochemical properties

# 1. Introduction

Flavonoids are phenolic compounds widely distributed in plants with potential beneficial effects for human health. The basic structures (Fig. 1) consist of two aromatic rings (noted A and B) linked through three carbons that usually form an oxygenated heterocycle (C ring). In recent years, these molecules have attracted the attention of many researchers because flavonoids display a remarkable array of biological and pharmacological activities as antioxidant, antiinflammatory, antimicrobial, anticancer, cardiovascular protection, etc [1–4]. In particular, the antioxidant activity is basically a function of their abilities to act as free radical scavenger [5-7]. Furthermore, flavonoids are effective metal ions chelators [8,9]. Metal chelation is usually considered as another mechanism of the antioxidant activity of flavonoids [9,10]. The interaction of flavonoids with metal ions may change the antioxidant properties and some biological effects of the flavonoids. We have recently reported the synthesis and the antioxidant properties of complexes of flavonoids with Cu(II) and Fe(II) [11,12]. Due to the importance of metal chelation in the antioxidant behavior of flavonoids and continuing our interest in flavonoid–metal complexes, we report here the synthesis and structural investigation of the Al(III) and Zn(II) complexes with quercetin (H<sub>2</sub>L<sup>1</sup>), rutin (H<sub>2</sub>L<sup>2</sup>) and galangin (HL<sup>3</sup>). Elemental, thermogravimetric analyses, atomic absorption, molar conductance, UV–vis, IR, <sup>1</sup>H NMR, fluorescence spectroscopies and electrochemistry were used to characterize the compounds.

## 2. Experimental

#### 2.1. Materials

All reagents and solvents were analytical reagent grade. AlCl<sub>3</sub>·6H<sub>2</sub>O, ZnCl<sub>2</sub>, quercetin, rutin and galangin were purchased from Aldrich Chemical Co.

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quercetin (L1): 3, 5,7,3', 4'-OH

rutin (L<sup>2</sup>): 5, 7,3', 4'-OH and 3-*o*-rhamnosylglucoside

galangin (L<sup>3</sup>): 3, 5, 7-OH

Fig. 1. Common structures of the flavone subclass and the division of bands I and II related to the UV-vis absorption spectra.

#### 2.2. Instruments

Elemental analyses were performed using a CHNS-O CE Instruments model EA 1110 elemental analyzer. Aluminum and zinc were determined in a model V-85 B, Braun ICP-AES atomic emission spectrometer. Molar conductances, in MeOH, were determined in a DSS-11A Metrohm conductivity meter. <sup>1</sup>H NMR spectra, in DMSO-d<sub>6</sub>, were obtained on a RMN Bruker DR X 4009.4T spectrometer. IR spectra were recorded on a Nicolet FTIR 5ZDX spectrometer using KBr pellets. UV-vis spectra, in MeOH, were registered on a Hewlett-Packard 8453 Diode Array UV-vis spectrophotometer using standard 1.00 cm quartz cells. The  $\varepsilon$  values represent the average obtained from three different solutions. The fluorescence spectra were recorded on a CD-900 Edinburgh spectrofluorimeter using a quartz cell  $(1 \text{ cm} \times 1 \text{ cm})$ cross-section). Thermogravimetric analyses were performed by a TA Instruments SDT 2960-Simultaneous DTA-TGA-Thermal Analysis, between room temperature and 800 °C at a heating rate of  $10 \,^{\circ}$ C min<sup>-1</sup>. The cyclic voltammograms were obtained on a 273A EG&G Potentiostat/Galvanostat, using a one-compartment electrochemical cell equipped with a glassy carbon-working electrode, a platinum wire auxiliary electrode and a Ag/AgCl reference electrode, in MeOH (1  $\times 10^{-3}$  M solutions) with LiClO<sub>4</sub> as supporting electrolyte.

Table 1	
Analytical and molar conductance data for the complexes	

Electrolyses were performed at fixed applied potentials at 25  $\pm$  1 °C, using a glassy carbon plate working electrode.

#### 2.3. Preparation of the metal complexes

Job's method [13] was used to determinate, in methanol, the stoichiometric ratios for the reactions between the flavonoids and the metal ions. The solutions were prepared by mixing solutions of both components with equal molar concentrations  $(1.1 \times 10^{-4} \text{ M})$  in ratios varying from 1:9 to 9:1. The complexes were prepared by mixing, in methanol, adequate stoichiometric amounts of the flavonoids and the corresponding salts. In all preparations, pale yellow precipitates were formed immediately when the cooled mixtures were poured into H<sub>2</sub>O. The crystals were washed thrice with 1:3 EtOH/H<sub>2</sub>O mixtures and then several times with H<sub>2</sub>O and dried under vacuum.

# 3. Results and discussion

The pale yellow complexes are soluble in MeOH, EtOH and DMSO, slightly soluble in  $Me_2CO$ , but scarcely soluble in  $H_2O$  and  $CCl_4$ . Table 1 shows the analytical and molar conductance data for the complexes. The molar conductance values suggest that the chloride ions are outside the coordination sphere of the complexes. The analytical data are consistent with the stoichiometries obtained previously by Job's method.

# 3.1. IR spectra

IR spectra of the ligands and the complexes present evidence of coordination between the transition metal ions and flavonoids. The data are summarized in Table 2. IR spectra of the compounds were dominated by strong absorptions of the ligands in the carbonyl region. Specifically, in the free flavonoids these bands,  $\nu$ (C=O), appear at 1658–1682 cm<sup>-1</sup>. For all quercetin and galangin complexes, the bands were shifted to lower frequencies compared to those of free flavonoids. The ~50 cm<sup>-1</sup> displacements suggest that the coordination of quercetin and galangin involves the oxygen atoms of the carbonyl groups. The <sup>1</sup>H NMR data indicate preferential metal coordination to the deprotonated hy-

Complexes	Molecular formula	Molecular weight $(g \text{ mol}^{-1})$	Anal. found (calcd.) (%)			Molar conductance $\Lambda_{\rm M}$
			С	Н	М	$(\Omega^{-1} \operatorname{cm}^2 \operatorname{mol}^{-1})$
[Al <sub>2</sub> (L <sup>1</sup> )(H <sub>2</sub> O) <sub>8</sub> ]Cl <sub>4</sub>	Al <sub>2</sub> C <sub>15</sub> H <sub>24</sub> O <sub>15</sub> Cl <sub>4</sub>	640.12	28.35 (27.14)	3.72 (3.78)	8.49 (8.43)	547
$[Zn_2(L^1)(H_2O)_4]Cl_2$	Zn2C52H72O36Cl2	573.98	31.45 (31.39)	2.77 (2.81)	22.92 (22.78)	197
$[Al_3(L^2)_2(H_2O)_{12}]Cl_5$	Al <sub>3</sub> C <sub>52</sub> H <sub>72</sub> O <sub>44</sub> Cl <sub>5</sub>	1659.34	37.82 (37.64)	4.41 (4.37)	4.84 (4.88)	625
$[Zn_3(L^2)_2(H_2O)_6]Cl_2$	Zn3C52H60O38Cl2	1560.38	40.12 (40.03)	3.92 (3.88)	12.54 (12.58)	217
$[Al(L^3)(H_2O)_4]Cl_2$	AlC15H17O9Cl2	439.19	40.93 (41.02)	3.94 (3.91)	6.18 (6.14)	202
$[Zn(L^3)(H_2O)_2]Cl$	ZnC15H13O7Cl	406.10	44.49 (44.36)	3.24 (3.21)	16.24 (16.11)	96

Table 2 IR data<sup>a</sup> (cm<sup>-1</sup>) for ligands and complexes

Compound	ν(C=O)	$\nu(C=C)$	ν(COC)	ν(O—H)	ν(M-O)
$\overline{H_2L^1}$	1668 s	1589 s	1260 s	3409–3144 b, m	_
$[Al_2(L^1)(H_2O)_8]Cl_4$	1590 s	1588 s	1258 s	3674–2357 b, m	612 w
$[Zn_2(L^1)(H_2O)_4]Cl_2$	1618 s	1586 s	1259 s	3633–2978 b, m	606 w
$H_2L^2$	1682 s	1590 s	1257 s	4140–2580 b, m	_
$[Al_3(L^2)_2(H_2O)_{12}]Cl_5$	1677 s	1588 s	1255 s	3725–2415 b, m	645 w
$[Zn_3(L^2)_2(H_2O)_6]Cl_2$	1680 s	1589 s	1257 s	3710–2491 b, m	628 w
HL <sup>3</sup>	1658 s	1590 s	1260 s	3607–3084 b, m	-
$[Al(L^3)(H_2O)_4]Cl_2$	1601 s	1590 s	1258 s	3706–2590 b, m	626 w
$[Zn(L^3)(H_2O)_2]Cl$	1613 s	1588 s	1258 s	3681–2395 b, m	617 w

<sup>a</sup> b: Broad; m: medium; s: strong; w: weak.

droxyl groups at 3-OH group. This fact was confirmed by the dissociation constants measured potentiometrically for both flavonoids; the pKa values for 3-OH group is  $\sim$ 8.7; the higher proton acidity at the carbon 3 position correlates well with its easy deprotonation and the enhanced metal-binding ability. For the rutin complexes, no shifts of the carbonyl bands were observed, presumably because the 3-OH group of the rutin is blocked by the sugar moiety (disaccharide glucorhamnoside). It can be noted for all complexes that: (a)  $\nu$ (C–O–C) and  $\nu_{ring}$  (C=C) frequencies are not shifted upon complexation indicating that the ring oxygen (position 1, Fig. 1) is not involved in the complexation, (b)  $\nu$ (M–O) frequencies appear in the 606–645 cm<sup>-1</sup> range, (c)  $\nu$ (O–H) frequencies appear as broad bands  $(3725-2357 \text{ cm}^{-1})$  indicating the presence of water. The presence of coordinated water is also supported by thermal analyses.

#### 3.2. UV-vis spectra

The flavonoids exhibit two major absorption bands in the ultraviolet/visible region (Fig. 1). The absorptions in the 320–385 nm range correspond to the B ring portion (cinnamoyl system, band I), and the absorptions in the 240–280 nm range correspond to the A ring portion (benzoyl system, band II) [14]. The spectra are related to the  $\pi \rightarrow \pi^*$  transitions within the aromatic ring of the ligand molecules. In comparison with flavonoids absorption spectra, those of the complexes are shifted to the long-wavelength region as shown in Table 3. Such bathochromic shift can be

Table 3					
UV-vis	data for	ligands	and	compl	exes

Compound	$\lambda_{\text{max}}$ (nm), ( $\varepsilon$ in $M^{-1}$ cm <sup>-1</sup> )			
	Band I	Band II		
$\overline{H_2L^1}$	372 (15,700)	256 (14,600)		
$[Al_2(L^1)(H_2O)_8]Cl_4$	440 (6200)	270 (7100)		
$[Zn_2(L^1)(H_2O)_4]Cl_2$	427 (9900)	265 (9800)		
$H_2L^2$	357 (18,260)	256 (22,000)		
$[Al_3(L^2)_2(H_2O)_{12}]Cl_5$	387 (74,00)	263 (10,900)		
$[Zn_3(L^2)_2(H_2O)_6]Cl_2$	409 (17,500)	272 (17,800)		
HL <sup>3</sup>	360 (16,800)	267 (24,400)		
$[Al(L^3)(H_2O)_4]Cl_2$	415 (7300)	273 (9000)		
$[Zn(L^3)(H_2O)_2]Cl$	420 (10,300)	271 (10,800)		

explained by the extension of the conjugated system with the complexation. The UV–vis spectra give significant information about the coordination sites of flavonoids, for example, the interactions of Al(III) and Zn(II) ions with quercetin at 2:1 metal:flavonoid ratio produced bathochromic shifts in the absorbances of both bands (Fig. 2). As the 3-hydroxy group has a more acidic proton, the 3-OH and 4-oxo groups are the first sites to be involved in the complexation process [15]. The 3',4'-dihydroxy groups bind a second metal ion. The 5-OH group is not involved due to lesser proton acidity and the steric hindrance caused by the first complexation.

# 3.3. <sup>1</sup>H NMR spectra

The <sup>1</sup>H NMR data for the complexes, as well as those for free ligands are listed below.

Quercetin:  $\delta$  12.50 (s, 1H, 5-OH), 10.76 (s, 1H, 7-OH), 9.60 (s, 1H, 3-OH), 9.39 (s, 1H, 4'-OH), 9.31 (s, 1H, 3'-OH), 7.68 (d,  $J_{\text{H2'/H6'}} = 1.8$  Hz, 1H, 2'-H), 7.55 (dd, 1H, 6'-H), 6.89 (d,  $J_{\text{H5'/H6'}} = 8.5$  Hz, 1H, 5'-H), 6.43 (d,  $J_{\text{H8/H6}} = 1.5$  Hz, 1H, 8-H), 6.22 (d, 1H, 6-H). [Al<sub>2</sub>(L<sup>1</sup>)(H<sub>2</sub>O)<sub>8</sub>]Cl<sub>2</sub>:  $\delta$  12.46 (s, 1H, 5-OH), 10.75 (s, 1H, 7-OH), 9.24 (s, 4'-OH), 9.20 (s, 1H, 3'-OH), 7.60 (d,  $J_{\text{H2'/H6'}} = 2.1$  Hz, 1H, 2'-H), 7.46 (dd, 1H, 6'-H), 6.80 (d,  $J_{\text{H5'/H6'}} = 8.1$  Hz, 1H, 5'-H), 6.40 (d,  $J_{\text{H8/H6}}$ 



Fig. 2. UV–vis spectra of the quercetin and metal–quercetin complexes in methanol (50  $\mu M).$ 

Compound	Dehydration temperature (°C)			Decomposition temperature (°C)		
	$\overline{T_1}$	$T_2$	Weight loss (%)	$\overline{T_3}$	$T_4$	Total weight loss (%)
$H_2L^1$	_	_	_	393	638	100.00
$[Al_2(L^1)(H_2O)_8]Cl_4$	121	208	22.65	303	732	83.90
$[Zn_2(L^1)(H_2O)_4]Cl_2$	126	197	12.63	285	655	71.46
$H_2L^2$	_	_	-	398	678	100.00
$[Al_3(L^2)_2(H_2O)_{12}]Cl_5$	156	210	13.15	347	791	90.65
$[Zn_3(L^2)_2(H_2O)_6]Cl_2$	158	215	7.01	355	828	84.24
HL <sup>3</sup>	_	_	-	265	471	100.00
$[Al(L^3)(H^2O)_4]Cl_2$	132	189	16.54	307	692	88.31
$[Zn(L^3)(H_2O)_2]Cl$	133	188	8.98	316	724	79.96

Table 4 Thermal analytical data for ligands and complexes

 $T_1-T_2$ : Temperature range corresponding to complex dehydration;  $T_3-T_4$ : temperature range corresponding to complex decomposition.

= 1.8 Hz, 1H, 8-H), 6.22 (d, 1H, 6-H).  $[Zn_2(L^1)(H_2O)_4]Cl_2$ :  $\delta$  12.47 (s, 1H, 5-OH), 10.74 (s, 1H, 7-OH), 9.24 (s, 4'-OH), 9.21 (s, 1H, 3'-OH), 7.62 (d,  $J_{H2'/H6'}$  = 2.0 Hz, 1H, 2'-H), 7.45 (dd, 1H, 6'-H), 6.82 (d,  $J_{H5'/H6'}$  = 8.3 Hz, 1H, 5'-H), 6.41 (d,  $J_{H8/H6}$  = 1.9 Hz, 1H, 8-H), 6.22 (d, 1H, 6-H).

Rutin:  $\delta$  12.63 (s, 1H, 5-OH), 10.82 (s, 1H, 7-OH), 9.69 (s, 1H, 4'-OH), 9.28 (s, 1H, 3'-OH), 7.49 (d,  $J_{\text{H2'/H6'}} = 1.9$  Hz, 1H, 2'-H), 7.52 (d,  $J_{\text{H5'/H6'}} = 8.8$  Hz, 1H, 5'-H), 7.85 (dd, 1H, 6'-H), 6.41 (d,  $J_{\text{H8/H6}} = 1.5$  Hz, 1H, 8-H), 6.24 (d, 1H, 6-H). [Al<sub>3</sub>(L<sup>2</sup>)<sub>2</sub>(H<sub>2</sub>O)<sub>12</sub>]Cl<sub>2</sub>:  $\delta$  12.60 (s, 1H, 5-OH), 9.58 (s, 4'-OH), 9.23 (s, 1H, 3'-OH), 7.41 (d,  $J_{\text{H2'/H6'}} = 1.5$  Hz, 1H, 2'-H), 7.37 (d,  $J_{\text{H5'/H6'}} = 8.5$  Hz, 1H, 5'-H), 6.78 (dd, 1H, 6'-H), 6.38 (d,  $J_{\text{H8/H6}} = 1.9$  Hz, 1H, 8-H), 6.19 (d, 1H, 6-H). [Zn<sub>3</sub>(L<sup>2</sup>)<sub>2</sub>(H<sub>2</sub>O)<sub>6</sub>]Cl<sub>2</sub>:  $\delta$  12.59 (s, 1H, 5-OH), 9.56 (s, 4'-OH), 9.25 (s, 1H, 3'-OH), 7.40 (d,  $J_{\text{H2'/H6'}} = 1.5$  Hz, 1H, 2'-H), 7.36 (d,  $J_{\text{H5'/H6'}} = 8.4$  Hz, 1H, 5'-H), 6.79 (dd, 1H, 6'-H), 6.37 (d,  $J_{\text{H8/H6}} = 1.8$  Hz, 1H, 8-H), 6.20 (d, 1H, 6-H).

Galangin:  $\delta$  12.52 (s, 1H, 5-OH), 10.79 (s, 1H, 7-OH), 9.61 (s, 1H, 3-OH), 8.32 (m, 2H, 2', 6'-H), 7.64 (m, 2H, 3', 5'-H), 7.55 (m, 1H, 4'-H), 6.45 (d,  $J_{H8/H6} = 1.5$  Hz, 1H, 8-H), 6.23 (d, 1H, 6-H). [Al(L<sup>3</sup>)(H<sub>2</sub>O)<sub>4</sub>]Cl<sub>2</sub>:  $\delta$  12.49 (s, 1H, 5-OH), 10.76 (s, 1H, 7-OH), 8.29 (m, 2H, 2', 6'-H), 7.61 (m, 2H, 3', 5'-H), 7.52 (m, 1H, 4'-H),), 6.43 (d,  $J_{H8/H6} = 1.5$  Hz, 1H, 8-H), 6.24 (d, 1H, 6-H). [Zn(L<sup>3</sup>)(H<sub>2</sub>O)<sub>2</sub>]Cl:  $\delta$  12.48 (s, 1H, 5-OH), 10.75 (s, 1H, 7-OH), 8.30 (m, 2H, 2', 6'-H), 7.62 (m, 2H, 3', 5'-H), 7.50 (m, 1H, 4'-H), 6.44 (d,  $J_{H8/H6} = 1.7$  Hz, 1H, 8-H), 6.25 (d, 1H, 6-H).

The protons signal of the complexes are shifted to lower frequencies relative to the free flavonoids. This is probably due to the increase of the conjugation caused by the effect of coordination when the complex is formed, increasing the planarity of the flavonoid molecules. This is also in agreement with the considerable bathochromic shifts observed in band I of the compounds. The <sup>1</sup>H NMR spectra also allowed some conclusions about the chelation sites: quercetin and rutin are able to chelate metal ions via 3' and 4' phenolic groups; upon complexation, the metal ion displaces one hydrogen from the flavonoid [11]. The <sup>1</sup>H NMR spectra of the complexes also reveal that the resonances of the hydrogens of the 3-OH groups are not present in quercetin and galangin complexes and that the hydrogens of the 7-OH groups are not present in

the rutin complexes indicating the losses of the OH protons due to complexation.

#### 3.4. Thermal analysis

The TG-DTA curves of the complexes exhibited two stages of mass loss, the mass loss with a small endothermic peak in first stage (dehydration) and the one in the second stage with an exothermic peak (decomposition). Thermal data for the dehydration and decomposition of the complexes are given in Table 4. These data show that all complexes contain coordinated water. The mass loss in the first stage indicates the number of water molecules. The complexes decompose causing ca. 80% weight loss. The final products are the metal oxides.

#### 3.5. Fluorescence spectroscopy

The fluorescence data of the ligands and complexes are listed in Table 5. Emission spectra for quercetin and quercetin complexes are shown in Fig. 3. The fluorescence data show that ligands themselves exhibit fluorescences. The emissions of the complexes are due to the fluorescences from the intraligand emission excited states. The enhanced fluorescence intensity of the complexes is attributed to the coordination of the ligand to the small, highly charged Al(III) and Zn(II)

Table 5
Fluorescence data for ligands and complexes

Compounds <sup>a</sup>	Emission wavelength (nm)	Relative fluorescence intensity
$\overline{H_2L^1}$	510	36.4
$[Al_2(L^1)(H_2O)_8]Cl_4$	478	80.1
$[Zn_2(L^1)(H_2O)_4]Cl_2$	484	60.5
$H_2L^2$	525	43.2
[Al <sub>3</sub> (L <sup>2</sup> ) <sub>2</sub> (H <sub>2</sub> O) <sub>12</sub> ]Cl <sub>5</sub>	495	74.4
$[Zn_3(L^2)_2(H_2O)_6]Cl_2$	505	55.1
HL <sup>3</sup>	535	40.3
$[Al(L^3)(H_2O)_4]Cl_2$	502	60.4
$[Zn(L^3)(H_2O)_2]Cl$	512	57.5

<sup>a</sup> Excitation wavelength: 450 nm.





Fig. 4. Cyclic voltammograms of the galangin and metal–galangin complexes (1 mM); 0.1 M LiClO<sub>4</sub>/MeOH; scan rate 0.1 V s<sup>-1</sup>.

Fig. 3. The emission spectra of the quercetin and metal–quercetin complexes in methanol (50  $\mu M$ ) at room temperature.

metal ions that effectively increases the rigidity of the ligand structure and increases the fluorescence quantum yield by reducing the probability of non-radiative energy dissipation process [16]. Another origin plausible for the enhancement of the fluorescence signal upon chelation of flavonoids with a nonparamagnetic metal (e.g., Al(III) and Zn(II)) is related to the inhibition of the excited state intramolecular proton transfer (ESPT) process between hydroxyl and





Fig. 5. Proposed structures of the complexes: (A) quercetin complexes, M = Al(III) and Zn(II), x = n = 4 for Al(III) and x = n = 2 for Zn(II); (B) rutin complexes, M = Al(III) and Zn(II), x = 4, n = 5 for Al(III) and x = n = 2 for Zn(II); and (C) galangin complexes, M = Al(III) and Zn(II), x = 4, n = 2 for Al(III) and x = 2, n = 1 for Zn(II).

 Table 6

 Electrochemical data for ligands and complexes

<i>E</i> p <sub>a</sub> vs. Ag/AgCl (mV)
631 and 934
549 and 823
573 and 853
873
693
738
856
696
715

4-oxo groups of the C ring (Fig. 1) [17,18]. It is worth to note that for some flavonoid ligands, the fluorescence intensity for Al(III) complexes is higher than for Zn(II) complexes. Moreover, the emission wavelengths of all complexes are shifted to lower wavelengths (20–33 nm) related to the free ligands, under the same excitation wavelength; this blue shift suggests that the electronic field of the metal ions decreases the energy level difference of the highest occupied molecular orbital and the lowest unoccupied orbital of the flavonoids.

#### 3.6. Electrochemical properties of the compounds

The cyclic voltammograms of the ligands and complexes show well-defined oxidation peaks and practically no reverse reduction peaks indicating EC processes involving two electrons [19]. Galangin itself presents one oxidation process at +0.856 V attributed to the 3-hydroxy group oxidation (Fig. 4). Controlled-potential electrolysis of a solution of galangin at +1.10 V shows that residual current is attained when the number of coulombs equivalent to a two-electron reaction is reached. The same behavior is observed when a controlledpotential electrolysis, at +0.75 V, is conducted in a solution of quercetin; successively, under a fixed applied potential of +1.2 V, the residual current is attained when additional number of coulombs corresponding to a two-electron reaction is reached. Nevertheless, rutin is oxidized in electrolysis by a two-electron process at +1.3 V forming quinone type compounds.

The cyclic voltammetric data (Table 6) show a considerable decrease of the oxidation potentials of the complexes related to those of the free flavonoids. The electrochemical data suggest that the coordination with metal ion makes the oxidation process easier due to the destabilization of the flavonoid structure.

Previously, we have shown that there is a relationship between the antioxidant activity and the cyclic voltammetric oxidation peak potential for flavonoids and complexes [11,12]. The lower the potential, the higher the antioxidant activity. Thus, Al(III) complexes are more effective antioxidants than the Zn(II) complexes. The postulated structural arrangements of the synthesized flavonoid complexes, based on the experimental data, are shown in Fig. 5.

#### 4. Conclusion

This work contributes to a better elucidation of the chelation chemistry of flavonoids with Al(III) and Zn(II) ions. The spectral results indicate that the chelation sites are: for quercetin 3-OH, 4-oxo and 3',4'-dihydroxy groups, for rutin 3',4'-dihydroxy and 7-OH groups, for galangin 3-OH, 4oxo groups. The results obtained have also demonstrated that the chelation influences the electrochemical properties of flavonoids. Al(III) and Zn(II) ions increase the flavonoids antioxidant activities as shown by the lower complexes oxidation potentials relative to those of free flavonoids.

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## References

- [1] E. Middleton, C. Kandaswami, Biochem. Pharmacol. 43 (1992) 1167.
- [2] J.B. Harbone, C.A. Williams, Phytochemistry 55 (2000) 481.
- [3] B.H. Havsteen, Pharmacol. Therapeut. 96 (2002) 67.
- [4] M.T.L. Ielpo, A. Basile, R. Miranda, V. Moscatiello, C. Nappo, S. Sorbo, E. Laghi, M.M. Ricciardi, L. Ricciardi, M.L. Vuotto, Fitoterapia 71 (2000) 101.
- [5] K. Ishige, D. Schubert, Y. Sagara, Free Radical Biol. Med. 30 (2001) 433.
- [6] A. Saija, M. Scalese, M. Lanza, D. Marzullo, F. Bonina, F. Castelli, Free Radical Biol. Med. 19 (1995) 481.
- [7] M.M. Silva, M.R. Santos, G. Caroco, R. Rocha, G. Justino, L. Mira, Free Radical Res. 36 (2002) 1219.
- [8] S.A.B.E. van Acker, G.P. van Balen, D.J. van der Berg, W.J.F. van der Vijgh, Biochem. Pharmacol. 56 (1998) 935.
- [9] L. Mira, M.T. Fernandez, M. Santos, R. Rocha, M.H. Florencio, K.R. Jennings, Free Radical Biol. Med. 36 (2002) 1199.
- [10] P.G. Pietta, J. Nat. Prod. 63 (2000) 1035.
- [11] R.F.V. Souza, E.M. Sussuchi, W.F. De Giovani, Synth. React. Inorg. Met-Org. Chem. 33 (2003) 1125.
- [12] R.F.V. Souza, W.F. De Giovani, Report 9 (2004) 97.
- [13] M.I. Bullatov, I.P.K Kalinkin, Practical Manual of Photometric Analysis, Chemistry, 5th ed., Leningrad, 1986.
- [14] K.R. Markham, Techniques of Flavonoids Identification, Academic Press, London, 1982.
- [15] S.V. Jovanovic, S. Steenken, M. Tosic, B. Marjanovic, M.G. Simic, J. Am. Chem. Soc. 116 (1994) 4846.
- [16] G.G. Guibault, Practical Fluorescence, Theory, Methods and Techniques, Marcel Dekker, New York, 1973.
- [17] J. Guharay, R. Chaudhuri, A. Chakrabarti, P.K. Sengupta, Spectrochim. Acta Part A 53 (1997) 457.
- [18] J. Guharay, P.K. Sengupta, Spectrochim. Acta Part A 53 (1997) 905.
- [19] A.J. Bard, L.R. Faulkner, Electrochemical Methods, Fundamentals and Applications , 2nd ed., Wiley, New York, 2001.