Biological Activities of Zn(II) and Cu(II) Complexes with Quercetin and Rutin: Antioxidant Properties and UV-Protection Capacity

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The aim of this study is to determine the changes occurred in the antioxidant capacity and in the UV-protection capacity (sun protection factor-SPF) of quercetin and rutin following the chelating with two different redox-potential cations, Zn(II) and Cu(II). The complexes were obtained using an adapted method from the literature and characterized by elemental analysis, UV-Vis and IR spectroscopy. The antioxidant capacity of the obtained complexes and the ones of the ligands (rutin and quercetin) was measured by photochemiluminometry. Sun protection factor (SPF) for both ligands and the complex compounds was determined using a spectrometric method. The SFP values in all the complexes are lower than the ones of their ligands. The antioxidant capacity is higher in the case of rutin-Zn complex, rutin-Cu(II) complex and quercetin-Cu(II) complex than of the ligands ones. These three complexes have high antioxidant activity which recommends them for further work in possible therapeutic applications identification.

Keywords: flavonoids, Zn(II) complexes, Cu(II) complexes, antioxidant activity, UV-protection capacity

Flavonoids are a broad class of low-molecular-weight secondary metabolites encompassing more than 10,000 scaffolds, and are commonly found in leaves, seeds, bark and flowers of plants. Their role in plants is to afford protection against ultraviolet radiation, pathogens and herbivore animals [1].

As their chemical structure, flavonoids are benzo-g-pyrone derivatives consisting of phenolic and pyran rings. Quercetin is a flavonoid having as substituiton pattern 3,5,7,3', 4'-OH and rutin is its 3-glycoside derivative with rutinose(α-L-Rhamnopyranosyl-(1→6))-β-D-gluco-pyranose) (fig. 1).

Most of the beneficial health effects of flavonoids are attributed to their antioxidant and chelating abilities. It is supposed that chelating complexes with divalent cations may form between the 5-OH and 4-oxo group, or between the 3'- and 4'-OH [2-4]. Zn^{2+} ions are not oxidizing agents for the phenolic structure of the flavonoids but they are able to chelate these molecules. Le Nest et al. observed two binding sites assigned, respectively to the CO-4/OH-3 on ring C with a high affinity and a second site of lower affinity on OH-3' on ring B in the case of quercetin [5, 6]. When the 3-hydroxychromone group is missing or substituted (as in case of rutin) the bonding of Zn^{2+} occurs between the CO-4 group of ring C and the OH-5 group of ring A.

Some studies showed that Cu^{2+} ions can interact with quercetin to form chelates with metal:ligand in molar ratio 1:1 [7-9] or 2:1 [7-10]. Despite the constant increases of interest concerning the changes in activity of quercetin after chelating, its reaction with Cu^{2+} ions have not been studied extensively. The recent work of

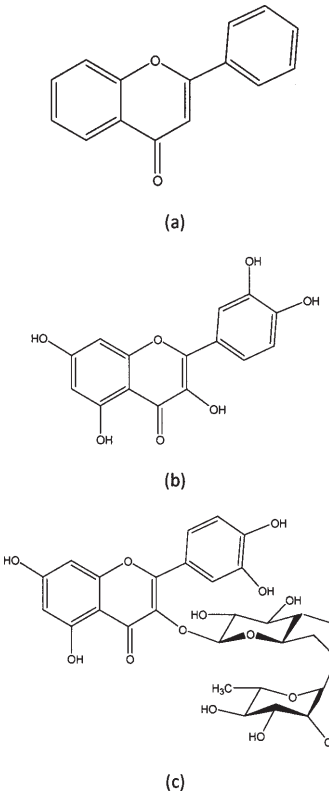

Fig. 1. Chemical structure of flavone ring (a), quercetin (b) and rutin (c)

Pekal [11] shows that the reaction of quercetin with Cu^{2+} resulted in the fast formation of 1:1 metal: ligand complex through the carbonyl oxygen and the close hydroxyl group in the A or C ring followed by the slow quercetin oxidation to the benzoquinone type products. It is

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suggested that the presence of Cu^{2+} ions promotes quercetin oxidation, probably at the hydroxyl group in B ring.

Frequently it was observed for Cu^{2+} and Zn^{2+} complexes biologic activities which could be used in therapeutic applications [12-18].

It was observed that chelation of a divalent cation does not necessarily render the flavonoid inactive [19, 20]. Sometimes theses complexes are more effective inhibitors of metal-induced oxidation [1, 20]. The interaction of quercetin and rutin with divalent cations could change the antioxidant properties and biological effects of the free flavonoids. The clinical utility of this observation may be promising for therapy.

Our work aimed to determine the changes occurring in the antioxidant capacity and in the UV-protection capacity (sun protection factor-SPF) of quercetin and rutin following the chelating with two different redox-potential cations, at physiological *p*H value.

Experimental part

Reagents and materials

All chemicals were of the highest purity commercially available. Rutin trihydrate, quercetin dyhidrate were purchased from Fluka (Biochemica); anhydrous zinc acetate, copper chloride dihydrate and ethanol were obtained from Sigma-Aldrich.

Preparation of the complexes

The complexes were prepared by mixing stoichiometric amounts of ligand and metal ions in ethanol and heating to reflux for 3 h. A molar ratio 1:2 metal:ligand for Cu(II) and 2:1 for Zn(II) was chosen.

Cu(II)-quercetin complex (**1**), brown: a quantity of 1.510 g (0.005 mol) of quercetin was dissolved in 50 mL ethanol, under reflux. To this solution 0.427 g (0.0025 mol) of $CuCl_2 \cdot 2H_2O$ were added, dissolved in a minimal amount of ethanol and the mixture was stirred under reflux for 3 h. The precipitate formed was filtered, washed with 1:3 ethanol/water mixture and then with ether and dried under vacuum.

Analytical data (Cu %): exp. 9.36; calc. 9.60.

Zn(II)-quercetin complex (**2**), yellow-brown: to a quantity of 1.510 g (0.005 mol) of quercetin dissolved in 50 mL ethanol with heating, 1.830 g (0.01 mol) of $Zn(CH_3COO)_2$ in ethanol were added. The resulted solution was refluxed for 3 h and then cooled to room temperature, when a brown solid product separated out. It was filtered, washed and dried as above.

Analytical data (Zn %): exp. 15.2; calc. 15.25.

Cu(II)-rutin complex (**3**), yellow: colored rutin (3.05 g, 0.005 mol) and 80 mL ethanol were placed into a flask with electromagnetic stirrer and heated under reflux. After the dissolution of rutin, 0.427 g (0.0025 mol) of $CuCl_2 \cdot 2H_2O$ in ethanol were added and the reaction mixture was stirred under reflux for 3 h. The pale-yellow solid was left to stand overnight and then collected by vacuum filtration, washed 3 times with ethanol/water mixture and then with ethylic ether and dried under vacuum.

Analytical data (Cu %): exp. 4.69; calc. 4.99.

Zn(II)-rutin complex (**4**), yellow-brown, was prepared in the same way as the complex (**3**), using 3.05 g (0.005 mol) of rutin and 1.830 g (0.01 mol) of $Zn(CH_3COO)_2$ in ethanol.

Analytical data (Zn %): exp. 8.43; calc. 8.85.

Note that in the case of zinc only 1:1 complexes were obtained, although it worked in 2:1 metal:ligand molar ratio.

Chemical and spectral analysis

The metal content was determined by atomic absorption spectrometry method, using High Resolution Continuum Source Atomic Absorption Spectrometer ContrAA700 (Analytik Jena AG, Germany) with xenon short-arc lamp, a multifunctional system for flame, graphite tube and hydride/Hg cold vapour techniques, in the range 185-900 nm.

UV-Vis spectra were registered both in solution as well as in solid state. UV-Vis spectra in methanol were recorded on a UV/V-530 Jasco spectrophotometer (Able& Jasco Co., Tokyo, Japan) using standard 1.00 cm quartz cells and connected at a HP Photosmart 2575, printer. UV-Vis diffuse reflectance spectra were registered on a UV-Vis Jasco 650 spectrophotometer (Able& Jasco Co., Tokyo, Japan), in the range 200-900 nm.

IR-spectra were recorded on a FT-IR spectrophotometer Perkin Elmer (Massachusetts USA), with horizontal attenuated total reflectance (HATR) device with zinc selenide (ZnSe) crystal, tip Spectrum BX2.

Determination of the antioxidant capacity through photochemiluminescence method

The antioxidant capacity of the obtained complexes as well as for the ligands (rutin and quercetin) was measured using a photochemiluminometer PHOTOCHEM (Analytik Jena AG, Germany), according to the recommended protocols [21-23]. For the experiment stock solutions of each compound in methanol (0.1 g/dL) were prepared. Subsequently, aliquots of 5 mL stock solutions were used for determinations. For the samples presenting too high antioxidative capacities, which could not be reported to the calibration curve, the stock solutions were diluted 1:10 with metanol. The antioxidant capacities of the samples were quantified by comparison with the standard, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) calibration curve as equivalent units of standard substance.

Sun protection factor (SPF) determination

The absorbance measurements were done using a Jasco UV-VIS 530 spectrophotometer (Able & Jasco Co., Tokyo, Japan). For the determinations, 1 mg/mL ethanol solutions of each complex were prepared. To compare the changes induced by coordination it was determined the SPF of quercetin and rutin on 1 mg/mL solutions in ethanol. The standard solution of *p*-amino benzoic acid was prepared in ethanol, in the same way. Measurements and data analysis were done according to the methods described by Aberu Dutra and Mansur [24, 25].

Results and discussions

The results of elemental analysis are in accordance with the following formulae for the complex compounds: $[CuQu_2]$ (**1**), [ZnQu(ac)] (**2**), $[CuRu_2]$ (**3**) and [ZnRu(ac)] (**4**).

IR spectra

The interactions of the two ligands (quercetin and rutin) with the metal ions were assessed by IR spectroscopy.

The infrared spectra of both quercetin as well as rutin are complex because of the presence of three complex ring systems. On the other hand, the presence of many

Rutin	Cu(II)-rutin complex	Zn(II)-rutin complex	Assignments
1655 s	1640 s	1635 s	ν(C=O)
1610 s, 1560 m, 1500 s	1600 s, 1555 m, 1500 s	1605 s, 1560 m, 1500 s	ν(C=C)
1450 m 1240 m 1015 s 1000 s	1460 m 1245 m 1015 s 985 s	1455 m 1240 m 1015 s 980 s	O-H def. O-H def. O-H def. O-H def.
1295 s	1285 s	1280 s	ν(C-O-C)
1200 s 1170 m 1120 m	1200 s 1175 m 1110 m	1195 s 1180 s 1190 s	$\nu(C\text{-}O_{phen})$ ν(C-OH) ν(C-OH)
-	1270 s	1260 m	$\nu(C\text{-}O_{phen})$
-	-	1530 m 1340 m	$\nu_{asym}(COO)$ $\nu_{sym}(COO)$

Table 1
IR DATA FOR RUTIN, Cu(II)-RUTIN COMPLEX AMD Zn(II)-RUTIN COMPLEX (ν_{max}, cm^{-1})

Quercetin	Cu(II)-quercetin complex	Zn(II)- quercetin complex	Assignments
1658 s	1650 s	1650 s	ν(C=O)
1605 s, 1560 m, 1520 s	1600 s, 1555 m, 1510 s	1590 s, 1560 m, 1505 s	ν(C=C)
1450 m 1240 m 1010 s	1460 m 1245 m 1015 s	1455 m 1245 m 1015 s	O-H def. O-H def. O-H def.
1260 s	1255 s	1250 s	ν(C-O-C)
1200 s 1170 m 1130 m	1200 s 1175 m 1115 m	1195 s 1175 s 1120 s	$\nu(C\text{-}O_{phen})$ ν(C-OH) ν(C-OH)
-	1270 s	1260 m	$\nu(C\text{-}O_{phen})$
-	-	1535 m 1340 m	$\nu_{asym}(COO)$ $\nu_{sym}(COO)$

Table 2
IR DATA FOR QUERCETIN AND ITS Cu(II) AND Zn(II) COMPLEXES (ν_{max}, cm^{-1})

OH groups favours hydrogen bond network, so that some bands become broad and overlap each others.

The important frequencies exhibited by rutin and its complexes are listed in table 1 and those for quercetin and its complexes, in table 2.

The infrared spectra of the ligands show a very strong band at 1655/1658 cm^{-1}, due to the stretching vibration of the carbonyl group, ν(C=O) [26, 27]. This band shifts to lower wave numbers in the IR spectra of all the complexes, according to the coordination of the ligands through the carbonylic oxygen atom.

The strong band occurring at 1605-1610 cm^{-1} range may be attributed to ν(C=C) aromatic stretching vibrations of the rings A and B [26, 27]. Other strong or medium bands due to the C=C aromatic vibrations are located at 1500 cm^{-1} (rutin) and 1520 cm^{-1} (quercetin) and 1560 cm^{-1} for both the flavonoids [26].

A lot of bands may be assigned to the vibrations of phenolic groups, either for the O-H or C-O bonds. Thus, O-H vibrations are identified at 1450 cm^{-1} (O-H deformation, which overlaps C-H deformation), 1240 cm^{-1} (deformation) and 1015/1010 cm^{-1} (deformation) for both the ligands and 1000 cm^{-1} (deformation) for rutin [26]. The bands associated with the vibrations of C-O bonds appear at 1200 cm^{-1} (stretching), 1170 cm^{-1} and 1120 cm^{-1} (rutin)/1130 cm^{-1} (quercetin) (C-OH stretching), 1360 cm^{-1} (rutin)/1320 cm^{-1} (quercetin) (C-OH deformation) [26, 28]. The band located at 1295 cm^{-1} (rutin)/1260 cm^{-1} (quercetin) is due to ν(C-O-C) vibration frequencies [26, 29]. This last band shows a slight shift in the IR spectra of the metal complexes, indicating a little change in the electronic distribution on the ring C.

IR spectra of the complex compounds show a new band in the range of ν(C-O) phenolic vibrations, at 1260-1285 cm^{-1}, which indicates the involvement of a least one hydroxyl group in coordination. This observation, correlated with the downward shift of the band due to the carbonyl group stretching vibration, supports the conclusion that coordination takes place at 4-oxo-5-hydroxyl moieties. The conclusion is in accordance with the investigations of Ren, who found that the most likely coordination site for Fe is 3-hydroxyl-4-carbonyl group, followed by 4-carbonyl-5-hydroxyl group [30].

The IR spectra of Zn(II) complexes show two new medium absorption bands, one at 1340 cm^{-1}, which may be assigned to $\nu_{sym}(COO)$ of acetate group and another at 1530/1535 cm^{-1}, due to $\nu_{asym}(COO)$ [29]. The value of $\Delta = \nu_{asym}(COO) - \nu_{sym}(COO)$ is in the range of bidentate acetate ligand [29].

On the basis of the IR spectra we can conclude that rutin and quercetin coordinate to the metal ion through the carbonyl oxygen atom and one phenolic oxygen atom, probable of 5-hydroxyl group, as monobasic bidentate donor. Taking into account of metal:ligand molar ratio for copper(II) complexes, the metal ion will be tetra-coordinated, surrounded by two ligand molecules. In the complexes of zinc, the metal ion is also tetracoordinated, bound to one ligand molecule and a bidentate acetate ligand (fig. 2).

UV-Vis spectra

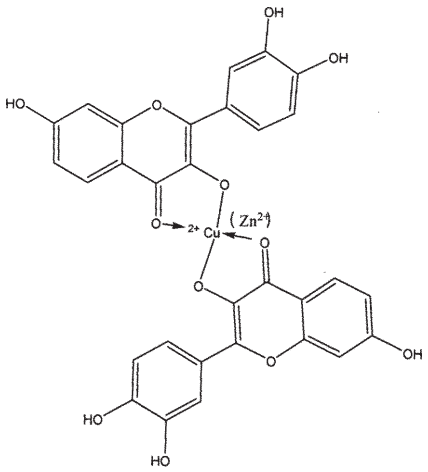

Fig. 2. Presumed structures of the Cu (Zn)- Quercetin complexes

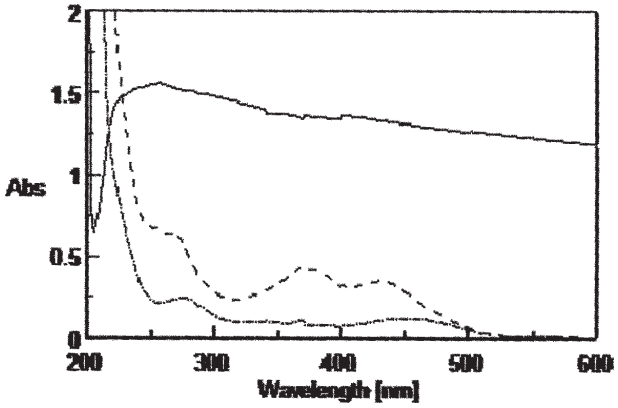

Fig. 3. UV/Vis spectra of Cu(II)-quercetin complex (-·-·-); Zn(II)-quercetin complex (—) and Quercetin (———)

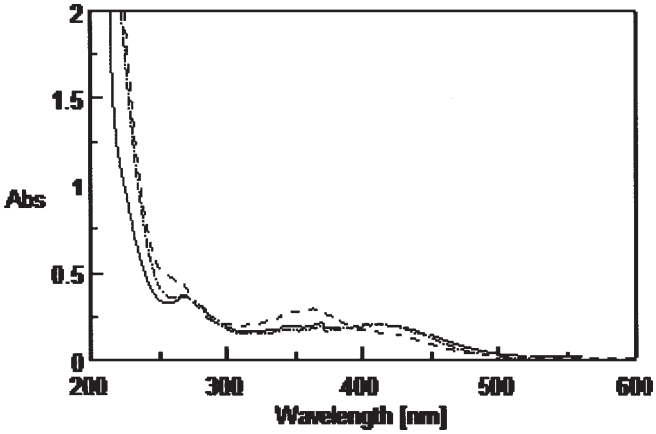

Fig. 4. UV/Vis spectra of Cu(II)-rutin complex (-·-·-); Zn(II)-rutin complex (—) and Rutin (———)

Quercetin and rutin, as well as the most flavones and flavonols, exhibits two major absorption bands in the UV-Vis region: band I, in the range 300-500 nm and band II, in the range 200-280 nm, related to the n-π* and π -π* transitions (fig. 3 and fig. 4). Most studies assign the band I to the cinnamoyl system absorption and band II, to the benzoyl system [27, 30].

In solid state, the band I appear as a strong and large absorption band, with maximum at 420 nm for quercetin and 415 nm for rutin. The UV-Vis spectra of the metal complexes show an insignificant bathochromic shift, to 430 nm (quercetin-Cu, quercetin-Zn) and 420 nm (rutin-Cu, rutin-Zn). The band II appears as two strong absorptions, at 275 nm and 235 nm, both in quercetin and in rutin spectra. A slight shift of this absorption towards higher wavelengths and a decrease of intensity are observable for all the metal complexes (290 nm for quercetin-Cu and quercetin-Zn, 285 nm for rutin-Cu, 290 nm for rutin-Zn). These changes support the coordination of the quercetin and rutin through 4-oxo-5-hydroxyl moieties [31].

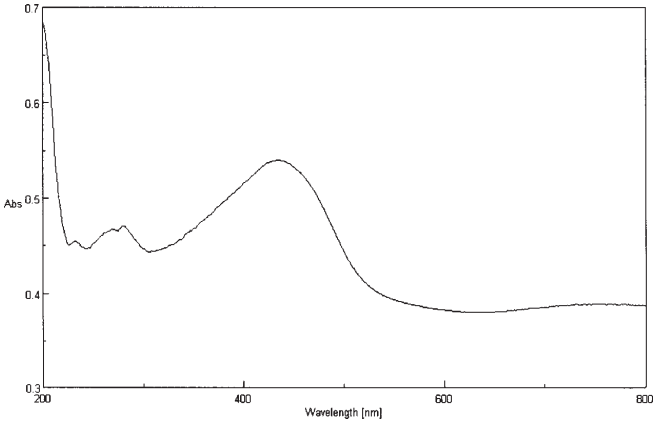

Fig. 5. UV/Vis spectrum of Cu(II)-quercetin complex in solid state

The two copper(II) complexes show also a supplementary band in the visible region, at 750 nm (quercetin-Cu) (fig. 5) and 725 nm (rutin-Cu), due to the *d-d* transitions of the Cu(II) ion [32].

UV-Vis spectra of the ligands and the metal complexes in methanol show some changes from those in solid state. Thus, both the main absorption bands are shifted to lower wavelength, as we can see from the figure 5, the difference being more important for the ligand. These changes are related to the strong interactions of the hydroxyl groups with the solvent molecules.

Antioxidant capacity

Table 3 presents the antioxidant capacities of the four synthesized complexes comparatively with the ones of the ligands.It could be observed that the antioxidant capacity is significantly higher than the ligand one in the case of rutin-Zn complex and just higher in the case of rutin-Cu(II) complex and quercetin-Cu(II) complex. The quercetin-Zn complex antioxidant capacity is ten times lower than the ligand one. The highest antioxidant activity was observed in rutin-Zn complex.

Pereira et al., describe the synthesis and the properties of a Cu (II)- naringin complex (a polyphenolic compound belonging also to the flavonoid molecular family) [31]. The antioxidant activity of this complex was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl radical) assay. The results show a significant higher antioxidant activity in complex given to the ligand (naringin), probably due to the coordination of metal in the 4 and 5 positions of the condensed ring system, which increase its capacity to stabilize unpaired electrons and scavenge free radicals.

Free radical scavenging capacity is primarily attributed to the high reactivity of hydroxyl substituent that participates in the following reaction: F-OH + R' → F-$O^{\cdot}$ + RH (free radical (R') and scavenger flavonoid molecule (F-OH).

Pekal et al. studied the antioxidant properties of a Cu (II) quercetin complex using the DPPH method and also an electrochemical method (cyclic voltammetry) [11].

Sample	Inhibition max.	TEAC/ *V*(sample) nmol echiv. Trolox/mL
Quercetin (stock solution diluted 1:10)	0.449	1.327
Rutin (stock solution diluted 1:10)	0.350	0.912
Quercetin-Cu(II) complex (stock solution diluted 1:10)	0.457	1.368
Quercetin- Zn complex	0.339	0.872
Rutin-Cu(II) complex (stock solution diluted 1:10)	0.406	1.136
Rutin-Zn complex (stock solution diluted 1:10)	0.490	1.533

Table 3
ANTIOXIDANT CAPACITIES OF THE LIGANDS AND SYNTHESIZED COMPLEXES

Sample	Concentration (mg/mL)	SPF
Quercetin	0.1	22.48
Rutin	0.1	15.88
Quercetin-Cu(II) complex	0.1	8.49
Quercetin- Zn complex	0.1	0.33
Rutin-Cu(II) complex	0.1	12.22
Rutin-Zn complex	0.1	10.39
Para-amino benzoic acid	0.02	15.05

Table 4
SUN PROTECTION FACTOR (SPF) OF THE FOUR COMPLEXES GIVEN TO THE ONES OF THE LIGANDS AND STANDARD SUBSTANCE (PARA-AMINO BENZOIC ACID)

Their studies suggest that the presence of Cu(II) ions promote quercetin oxidation. The radical scavenging activity of the Cu-quercetin complex evaluated by DPPH method and cyclic voltammetry was found to be higher than the one of the ligand.

Both studies conclude that the radical scavenging activity of the Cu-flavonoid complexes is bigger than the ligand ones. Taking into account the principle of the DPPH method, one can say that Cu(II) ion lows the oxidation potential of the flavonoid structures in both cases. This means that the oxidation process of the complex is easier due to the destabilization of the flavonoid structure.

The method for antioxidant activity assessment used in this work has a system for the measurement of antioxidants based on photosensitized chemiluminescence [33, 34]. The working principle is based on the multiple acceleration of a natural reaction leading to the generation of a superoxide anion radical. This is achieved by optical excitation of a photosensitive substance and subsequent formation of the measuring radical. Subsequently, the free radicals can be detected with chemiluminogenous substances. By analyzing the antioxidant activity of our complexes using photochemilumminiscence method it was observed also a higher activity in both Zn(II) and Cu(II) complexes, except for the Zn(II)-quercetin complex which has a much lower activity given to the one of the ligand. The antioxidant activities of the synthesized complexes were not much higher comparatively to the ones of the ligands. This fact could be due to the different method for measurement given to the ones reported in the literature. These results lead to the same conclusion concerning the lack in stability of flavonoid structures subsequently to the coordination with Cu or Zn ions.

Sun protection factor (SPF)

The sun protection factor values for the four synthesized complexes given to the ligands and para-amino benzoic acid ones are presented in table 4.

It could be seen that all the synthesized complexes have the SFP values lower than their ligands. The quercetin-Zn complex has the lowest SPF value as well as the lowest antioxidant activity. SPF is defined as the UV energy required to produce a minimal erythema dose on protected skin, divided by the UV energy required producing a minimal erythema dose on unprotected skin. To be effective in preventing sunburn and other skin damage, a sunscreen product should have a wide range of absorbance between 290 and 400 nm [24]. To calculate the sun protection factor the method take into account the absorbance of compounds solutions at 290, 295,300, 305, 310, 315 and 320 nm. As it could be seen from the spectral analysis, all complexes have lower absorbance at these wavelengths given to the ones of the corresponding ligands.

Conclusions

- The IR spectra show that rutin and quercetin coordinate to the metal ions through the carboxylic oxygen atom and one phenolic oxygen atom, as monobasic bidentate donor. In the complexes of Cu(II), the metal ion will be tetracoordinated, surrounded by tow ligand molecules and in the complexes of Zn, the metal ion also tetracoordinated, bound to one ligand molecule and a bidentate acetate ligand.

- UV-Vis spectra of the metal complexes present a band to the cinnamoyl system with maximum at 430 nm for quercetin - metal complexes and 429 nm for rutin - metal complexes, and another band to the benzoyl system with maximum at 290 nm (quercetin-Cu, quercetin-Zn and rutin-Zn) and 285 nm (rutin-Cu);

-Four flavonoids-metallic complexes were synthesized using an adapted method;

-The spectral analysis (UV-Vis and IR) proves the coordination at the hydroxyl groups 4 and 5 from A and C rings for all the complexes;

- The antioxidant activity of Zn(II)-rutin, Cu(II)-rutin and Cu(II)-quercetin complexes is higher than the ones of the corresponding ligands;

- The sun protection factor (SPF) is lower in all the complexes comparatively with the ones measured for the corresponding ligands;

- Taking into account the antioxidant activities of the new compounds, further work is requested for possible therapeutic applications identification.

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