

Spectroscopic Study on the Riboflavin - Plant Extracts Interaction

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The Riboflavin (Vitamin B₂) – plant extracts interaction was investigated by spectrophotometric methods, i.e., absorption and fluorescence emission and excitation spectra. Plant extracts were obtained by ultrasound assisted extraction. The quenching of the Riboflavin fluorescence at its interaction with the plant extracts is discussed.

Keywords: plant extract, natural extraction, riboflavin, fluorescence, antioxidant activity

Free radical induced lipid oxidation in human bodies has been related to the pathological process of many diseases, such as cardiovascular disease [1, 2], some cancerous disorders and diabetics [3]. The investigation on biological antioxidants has attracted great attention in the last decades, from both the scientific community and the general interest.

It is well known that vegetable tissues contain several substances having antioxidant properties due to the phenolic compounds (antocyanins, flavanols, flavonols, isoflavones, flavan-3-ols and hydroxycinnamic acids). Antioxidant activity of polyphenols from plants is based on their radical-scavenging capacity and on the capacity to chelate transition metals ions.

Fruits, vegetables and cereals have been explored due to their high abundance in the human diet and due to the prevention of various diseases as cancer and cardiovascular diseases [4, 5]. The plants are often used as alternative herbal treatments as astringent, antiseptic, tonic, anti-inflammation, etc. Nowadays, people are being bombarded with thousand of unhealthy products, the level of sensibility in front of diseases is very high and that is why the use of medicinal plants can represent the best solution. Riboflavin (Vitamin B₂), (fig. 1), is one of the most important hydrosoluble vitamins.

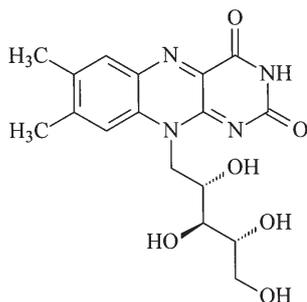


Fig. 1. The chemical structure of Riboflavin

Riboflavin deficiency can result in decreased xanthine oxidase activity and reducing blood uric acid levels [6]. In very low concentrations (μM - nM), Riboflavin is a prooxidizing agent, this action being in concordance with flavin semichinones formation, which have free radical character. In these regards, riboflavin has a powerful antioxidant potential due to its ability to regenerate glutathione via glutathione reductase. The oxidative activity of riboflavin has been previously studied by luminescence

(chemiluminescence and fluorescence) methods [7] and the effects of amino acids addition have been evidenced. Also, the effect of KI, KBr and thiourea on the oxidative activity of riboflavin has been already studied [8].

Our attention was focused on the antioxidant activity of natural plant-extracts [9-11]. The aim of this article is the study on the interaction of the riboflavin-several plant extracts, using UV-VIS absorption and fluorescence spectroscopy. The quenching effect of plant extracts on the riboflavin fluorescence leads to obtaining information on its participation in the oxidative processes.

The plant extracts that were the subject of our investigation are as follows: *Carum carvi* A. (Caraway), *Levisticum officinale* A. (Lovage), *Anethum graveolens* U. (Dill), *Brassica B.* (Mustard), *Camellia sinensis* T. (Green Tea), *Rosmarinus officinalis* R. (Rosemary), *Anthemis Nobilis* A. (Camomile), *Ocimum basilicum* L. (Sweet Basil), *Satureja Hortensis* L. (Savory), *Apium petroselinum* A. (Parsley) and *Ginkgo Biloba* G. (Ginkgo Biloba).

Experimental part

Materials. Caraway, Lovage, Mustard, Green Tea (leaves), Rosemary, Camomile, Sweet Basil, Savory and Ginkgo Biloba were purchased from "Plafar" (Romania). Dill and Parsley (*herba*) were bought from growers and dried at room temperature.

The plant extracts were obtained from dried vegetal materials by ultrasound assisted extraction. Ultrasonic irradiation was carried out using a Langford sonomatic cleaning bath operating at 33 kHz, extraction time 2 h, and 96% ethanol was used as extraction solvent, extraction time 60 min.

After the usual work up, filtration and alcohol removal using a rotary evaporator, the extracts obtained were dried in oven at 100°C for 1 hour.

Riboflavin (Vitamin B₂), 7,8-dimethyl-10-((2R,3R,4S)-2,3,4,5-tetrahydroxyethyl) benzo [g] pteridine-2,4(3H,10H)-dione, (fig. 1), one of the most important hydrosoluble vitamins, is a constituent of two enzymatic substances (flavin mononucleotide-FMN and flavin adenine dinucleotide-FAD). Riboflavin from Fluka and ethanol from Merck, with spectroscopic purity, were used. Riboflavin concentration in ethanol was 1.1×10^{-5} M, and was maintained constant in measuring solutions. 200 μL of different plant extracts in ethanol were added to riboflavin solution at a final working volume of 3000 μL .

Absorption spectra have been performed with a Perkin Elmer Lambda 35 Spectrometer.

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Compound	λ_{abs} (nm)	Abs	λ_{em} (nm)	If (a.u.)
Riboflavin (RF)	357.8; 448.4	0.14; 0.17	514	600
RF- Caraway	270.4; 337.21; 445.50	0.29; 0.13; 0.12	513	568
RF-Lovage	269.23; 326.44; 368.04; 409; 472.7; 505.84; 537.05; 67.90; 666.40	0.56; 0.35; 0.32; 0.38; 0.16; 0.13; 0.13; 0.13; 0.22	511	520
RF-Dill	269.57; 329.06; 370.19; 407.64; 468.60; 505.32; 536.91; 608.15; 665.44	0.49; 0.34; 0.43; 0.57; 0.20; 0.12; 0.11; 0.10; 0.24	513	556
RF-Mutard	269.84; 350.60; 447.57	0.27; 0.10; 0.13	514	548
RF-Green Tea	271.9; 360.9; 410.1; 472.8; 666.9	1.51; 0.30; 0.22; 0.14; 0.13	513	460
RF-Rosemary	271.8; 334.3; 446.1	0.26; 0.12; 0.06	512	501
RF-Camomille	270.7; 324.5; 443.7; 472.8	0.56; 0.35; 0.15; 0.13	512	537
RF-Sweet Basil	271.58; 331.40; 406.96; 466.78 536.05; 609.09; 666.40	0.54; 0.27; 0.26; 0.14 0.08; 0.08; 0.13	513	496
RF- Savory	272.06; 332.11; 410.94; 444.72 474.75; 666.19	0.50; 0.24; 0.20; 0.16; 0.13; 0.11	513	511
RF-Parsley	269.53; 407.81; 469.53; 665.40	0.48; 0.44; 0.20; 0.20	513	434
RF-Biloba	272.65; 333.12; 415.20; 466.18; 666.12	0.78; 0.43; 0.19; 0.16; 0.12	512	419

Table 1
ABSORPTION AND FLUORESCENCE
PROPERTIES IN ETHANOL OF THE
RIBOFLAVIN (RF) – PLANT
EXTRACTS MIXTURES; $\lambda_{ex} = 365$ nm

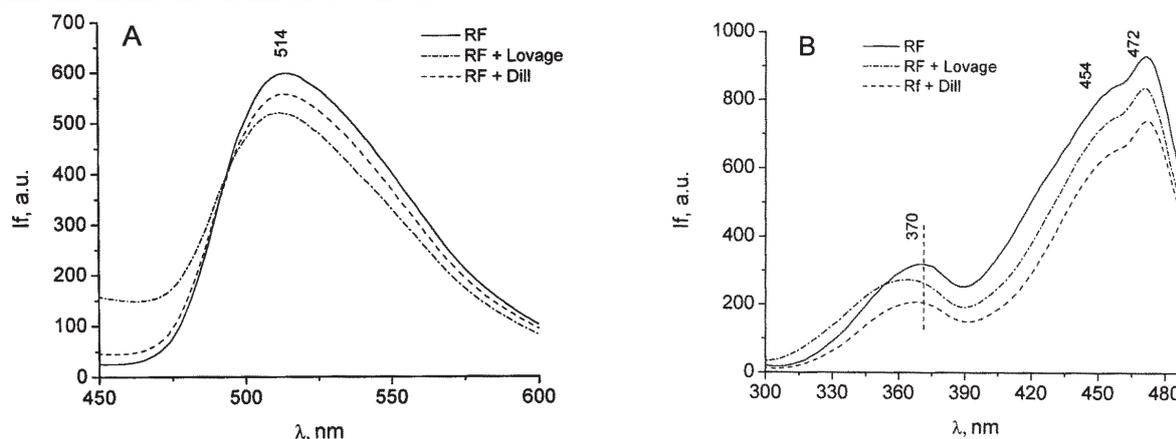


Fig. 2. The fluorescence emission (A) and excitation (B) spectra in ethanol of the Riboflavin and some Riboflavin –Plant Extracts mixture; $\lambda_{ex} = 365$ nm

The fluorescence emission spectra were recorded with Perkin Elmer 204 spectrophotometer, interfaced to a computer, permitting a pre-established reading time of the data. Usually the time range between two measurements is 550 ms. The excitation wavelength was 365 nm.

Results and discussions

The fluorescence emission and excitation spectra of Riboflavin and Riboflavin – Plant Extracts mixtures ($\lambda_{ex} = 365$ nm for emission spectra, $\lambda_{em} = 550$ nm for excitation spectra), are shown in figure 2 and the fluorescence characteristics are presented in table 1.

As it can be observed in figure 2, Riboflavin exhibits an intense fluorescence with an emission maximum at 514 nm and that interaction between Riboflavin and natural plant extracts leads to a decrease in the fluorescence intensity of the riboflavin as well as to a slight shift of its fluorescence emission maximum. An energy transfer from Riboflavin to Chlorophyll *a* takes places. It is known that

flavin fluorescence is strongly quenched when bound to protein; Weber first proposed the likelihood that quenching could be primarily due to the interaction of the flavin with nearby aromatic amino acid residues [12].

Quenching of fluorescence can arise from two different mechanisms; in the static mechanism, quenching is a result of the formation of charge-transfer complexes and it can readily be identified by no change occurred in the mean fluorescence lifetime, whereas the dynamic mode of quenching is strictly collisional in nature and results in a proportional decrease in the mean of the fluorescence lifetime [13]. According to previously reported results, flavins form strong complexes with the aromatic compounds, phenols [14, 15] and indoles, respectively [6]. In these regards and in direct comparison, figure 3 presents the fluorescence emission and excitation spectra of some plant extracts.

It can be observed that the fluorescence emission of chlorophyll *a*, the band at 680 nm, is more or less intense

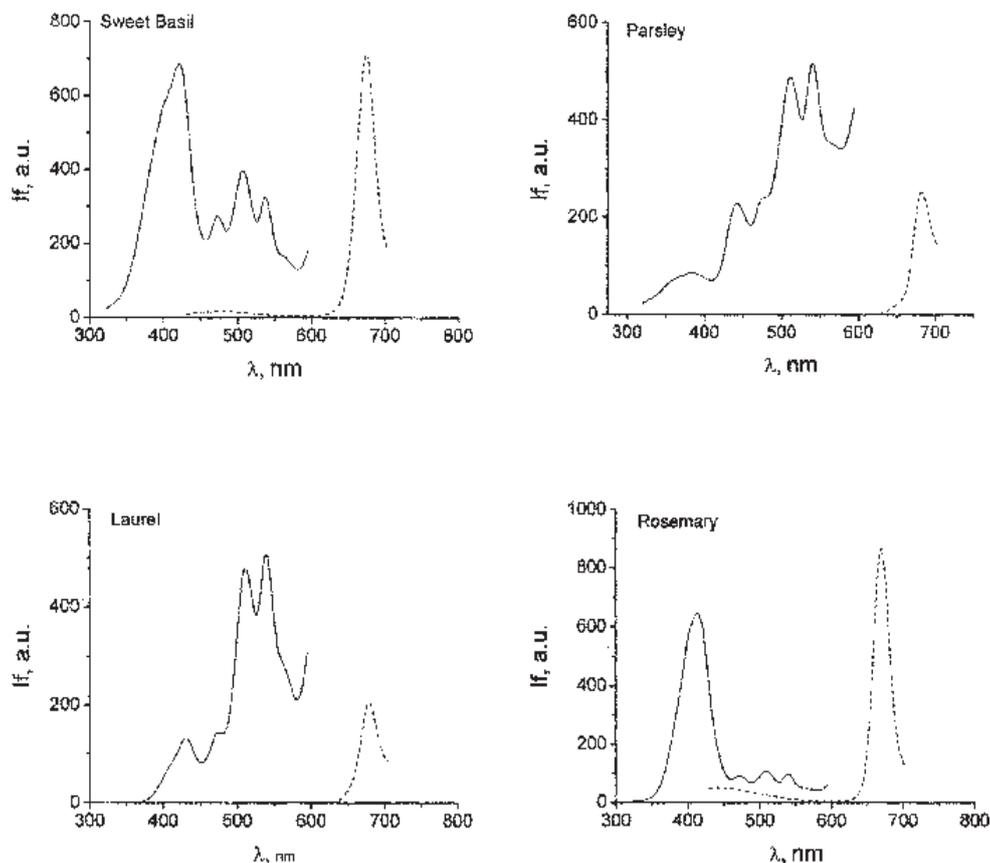


Fig. 3. The fluorescence excitation (left) and emission (right) spectra in ethanol of some Plant Extracts; $\lambda_{ex} = 400 \text{ nm}$, $\lambda_{em} = 680 \text{ nm}$

as a function of the plant extract. In the case of Parsley, a slight red-shift of the fluorescence emission is observed. Subsequently, the energy transfer from riboflavin to chlorophyll *a* may depend on the total chlorophyll *a* content found in each plant extracts. As can be observed, in the case of Sweet basil and Rosemary, another fluorescence emission band is evidenced around 460 nm. According to Lichtenthaler and Schweiger, 1998, this fluorescence emission is attributed to the blue-green fluorophore, probably ferulic acid [16].

Figure 4 shows the fluorescence emission spectra of RF and some RF-Plant Extracts, at an excitation wavelength of 365 nm. It was found that in the presence of riboflavin, the fluorescence emission band of chlorophyll *a* is 10 nm blue-shifted (670 nm) by comparison to that observed in the extract plant without Riboflavin, 680 nm respectively.

The spectral regions of the absorption maxima in ethanol of the Riboflavin (RF) and RF - Plant Extracts mixture are presented in table 1. Figure 5 presents a

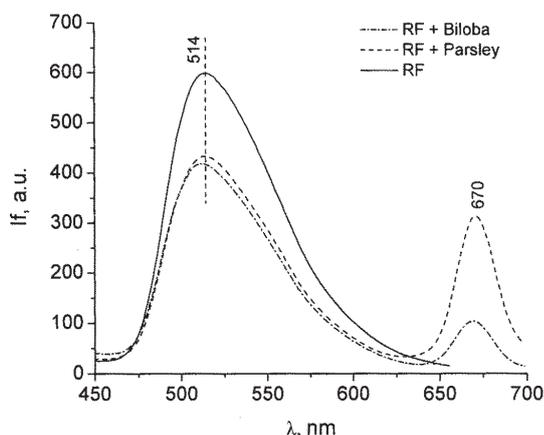


Fig. 4. The fluorescence emission spectra in ethanol of Riboflavin and some Riboflavin -Plant Extracts mixture; $\lambda_{ex} = 365 \text{ nm}$

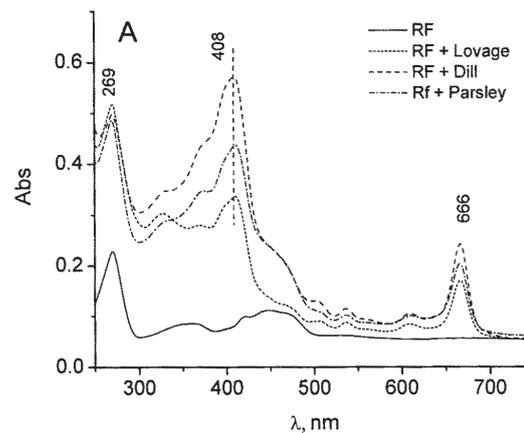


Fig. 5. Absorption spectra in ethanol of the Riboflavin (RF) and some RF - Plant Extracts mixtures

comparison of the absorption spectra of some plant extracts.

The major contribution of Chlorophyll *a* is evidenced, by the absorption band at 408 and 666 nm, respectively. Another major contribution found, may be attributed to flavones or flavonols, with the absorption band at 269 nm. According to the literature, the band II (benzoyl) from flavonoids is in the absorption range of 250-290 nm [17].

It is known that Riboflavin represents the most elementary form of the naturally occurring flavins. The electronic spectrum of riboflavin consists of four bands centered at 220, 265, 375, and 445 nm in water. The 375 nm band is particularly sensitive to solvent effects, and generally shifts to shorter wavelengths with decreasing solvent polarity, but the 445 nm band is much less affected showing only a slight bathochromic shift with decreasing solvent polarity.

The two longer wavelength transitions are π, π^* in character, the 357.8 nm band having some contribution from an intramolecular charge-transfer process leading to a polar excited state [18].

As it can be observed, in the Riboflavin- plant extracts mixtures, the modifications of the absorption spectra consist of a decrease in the height of the two absorption maxima at 357.8 and 448.4 nm, as well as and in a red edge shift of the absorption band towards longer wavelengths.

Conclusions

The compounds present into plant extracts are intense fluorescent due to the most prominent fluorescent pigment found in plant extracts that is Chlorophyll *a*.

The interaction between Riboflavin and the natural extracts that were the subject of our investigation showed some modifications of the absorption spectrum that consist of a decrease in the height of the two absorption maxima at 357.8 and 448.4 nm and in a displacement of the red edge of the absorption band towards longer wavelengths. The absorption bands correspond to the $\pi-\pi^*$ and $n-\pi^*$ transitions; the position of the absorption band evidence the influence of each compound into plant – extract as is shown in literature data [17].

In the fluorescence spectra of Riboflavin-natural extract solution, a decrease of fluorescence intensity was observed, as a result of the formation of charge-transfer complexes between Riboflavin and aromatic compounds from natural plant extracts.

Further studies are needed in order to get an accurate contribution of the Riboflavin (Vitamin B₂) in each plant extracts. Also, a study of the antioxidant capacity of these plant extracts by chemiluminescence method and the way in which the antioxidant activity of Riboflavin could be improved by these plant- extracts, needs to be taken into consideration.

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