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Application of spectroscopic methods for identification (FT-IR, Raman spectroscopy) and determination (UV, EPR) of quercetin-3-O-rutinoside. Experimental and DFT based approach

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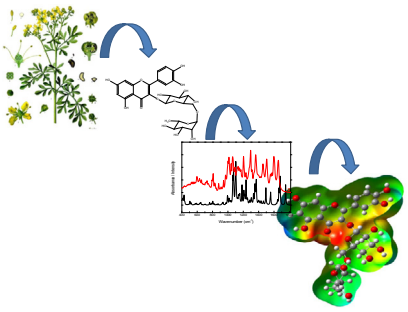
HIGHLIGHTS

- The FT-IR, Raman, EPR and UV spectra of quercetin-3-O-rutinoside were used for its identification and/or determination.
- Spectroscopic properties of the of the title compound were calculated and compared with experiment.
- The HOMO–LUMO energies and related molecular properties were evaluated.

GRAPHICAL ABSTRACT



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ABSTRACT

Vibrational (FT-IR, Raman) and electronic (UV, EPR) spectral measurements were performed for an analysis of rutin (quercetin-3-O-rutinoside) obtained from *Rutaofficinalis*. The identification of rutin was done with the use of FT-IR and Raman spectra. Those experimental spectra were determined with the support of theoretical calculations based on a DFT method with the B3LYP hybrid functional and 6-31G(d,p) basis set. The application of UV and EPR spectra was found to be a suitable analytical approach to the evaluation of changes in rutin exposed to certain physicochemical factors. Differences in absorbance observed in direct UV spectra were used to monitor changes in the concentration of rutin in degraded samples. Spectra of electron paramagnetic resonance allowed studying the process of free-radical quenching in rutin following its exposure to light. The molecular electrostatic potential (MEP) and frontier molecular orbitals (LUMO–HOMO) were also determined in order to predict structural changes and reactive sites in rutin.

Introduction

Rutin (2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyloxy]-4*H*-chromen-4-one) is a naturally occurring flavonoid in the plant *Rutaofficinalis* [1]. Structurally, rutin is aflavonolquercetin which is connected with disaccharides: rhamnose and glucose (Fig. 1) [2]. It shows antioxidant, anti-inflammatory, anticarcinogenic, antithrombotic, cytoprotective, vasoprotective and microbial activities. Due to its activity rutin is used in pharmaceutical dosage forms such as solid (e.g., tablets) and solutions (e.g., ophthalmic drops) [3–6]. Rutin is

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widely used in numerous nutraceuticals and dietary supplements due to its auxiliary, prophylactic treatment of civilization diseases, such as hypercholesterolemia, high blood pressure, hemorrhoids, phlebitis and chronic venous insufficiently [7,8].

In the case of plant substances, the achievement of biological activity depends on many factors. A purity and chemical stability are the most important parameters for efficacy of therapy based on raw substances [9]. Purity of raw materials depends on its origin. While stability of raw substance can be changed in effect of activity of affecting factors on raw substances during its storage.

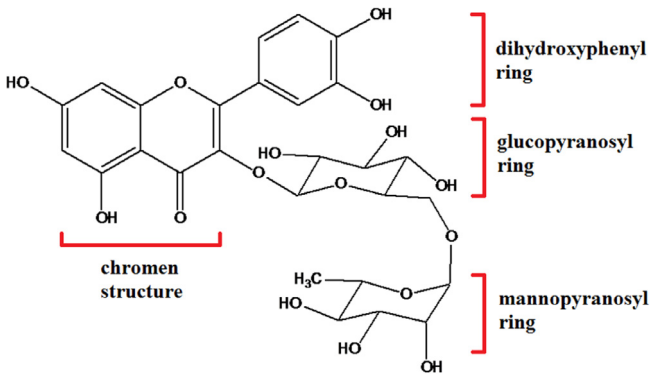
Development of analytical methods suitable for evaluation of material purity and monitoring its changes during storage. So it is important for efficacy and safety of potential phytotherapy. Up to now, for identification and determination of rutin the following techniques: gravimetry, polarography, fluorometry, ultraviolet spectrophotometry and high-performance liquid chromatography method, were reported [10–14]. A few stability studies of rutin signed the problem its susceptibility to degradation under the influence of affecting factors such as hydrogen ions, light and temperature [15–17]. To our the best knowledge, no complex procedure based on spectroscopic solvents for identification and determination of rutin, have been reported.

The study investigated experimentally and theoretically the application of spectroscopic methods for the identification (ultraviolet, UV; Fourier transform infrared spectroscopy, FT-IR; Raman spectroscopy) of rutin and the determination (UV; electron paramagnetic resonance, EPR) of changes that the compound undergoes due to degradation.

Experimental

Materials and chemicals

Rutin (purity >98%) originated from natural sours from *Rutaofficinalis* and it was supplied by Sigma–Aldrich (Poland). The impurities: quercetin and isoquercetin were obtained by Sigma–Aldrich (Poland). Hydrochloric acid, sodium hydroxide solution, phosphoric buffer and all other chemicals were obtained from P.O.Ch. (Poland). Acetonitrile of an HPLC grade was supplied by Merck KGaA (Germany) and triethylamine (99.5%) by J.T. Baker (Netherlands). High-quality pure water was prepared using an Exil SA 67120 Millipore purification system (France).



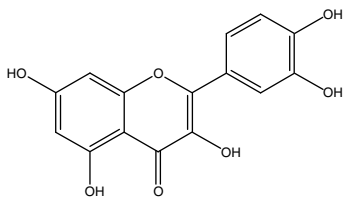


Fig. 1. Chemical structure of quercetin-3-O-rutinosideand its impurity.

Spectroscopic measurements

The vibrational infrared spectra of samples of rutin were recorded in the frequency range between 400 and 1800 cm^{-1}, at room temperature, with an FT-IR Bruker Equinox 55 spectrometer. The Raman scattering spectra were obtained with a LabRAM HR800 spectrometer (HORIBA JobinYvon) with laser excitation λ_{exc} = 633 nm (He–Ne laser). In each case the power of the laser beam at the sample was less than 1 mW to avoid sample damage. An UV–VIS Lambda 20 (Perkin Elmer) spectrophotometer equipped with 1.0 cm-in-width quarts cells and controlled by the UV WinLab software was utilized. Detection of free radicals and determination of their concentration were carried out using a Bruker ELEXSYS 500 spectrometer (X-band) at 297 K. EPR spectra were recorded as a first derivative of the absorption signal. The number of free radicals was calculated using the integration procedure described elsewhere [18].

Photodegradation stability studies of rutin in the solid state were performed using Suntest CPS$^+$ (Atlas®) with Solar ID65 filter. The cells were exposed to UV radiation (300–400 nm) in an overall illumination of $\geqslant$210 Wh/m^2 in Suntest C+.

All the calculations were made by using the Gaussian 03 package [19]. In order to interpret the experimental results of IR absorption and Raman scattering, quantum chemical calculations were performed, based on a density functional theory (DFT) method with the B3LYP hybrid functional and 6-31G(d,p) basis set.

Theoretical studies

The optimization of the molecular geometry (MG), spatial electron distribution of frontier molecular orbitals (FMOs) analysis and molecular electrostatic potential (MEP) of rutin were obtained with density functional theory calculations using Becke's three-parameter hybrid functional (B3LYP) implemented with the standard 6-31(d,p) as a basis set. The harmonic vibrational frequencies of FT-IR, Raman and UV spectra have been calculated using the same level of theory. All the calculation were made by using Gaussian 03 package and GaussView application was utilized to present the MG, FMO and MEP [19,20].

Forced studies of rutin

For the determination of concentration changes of rutin in the presence of its impurities and degradation products (quercetin and isoquercetin) as a result of factors affecting action, changes in the absorption spectra of rutin were examined. The degradation of rutin was studied in aqueous solutions: in hydrochloric acid (pH = 0.3) at 333 K up to 360 min, sodium hydroxide (pH = 13.3) at 313 K up to 15 min and in hydrogen peroxide (10%) at 353 K up to 200 min. Samples were prepared by dissolving an accurately weighed 5.0 mg of rutin in 25.0 mL of the equilibrated solution to 313 K in glass stoppered flasks. At specified times, samples of the reaction solutions (1.0 mL) were collected and instantly cooled with a mixture of ice and water, neutralized with 0.8 ml of NaOH or HCl solutions of suitable concentrations and assayed. The ionic strength of all solutions was adjusted to 0.5 mol/L with a solution of sodium chloride (4 mol/L).

While the degradation of rutin in the solid state was studies at an increased relative air humidity (RH = 76.4%) at 353 K, in dry air