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VIBRATIONAL SPECTROSCOPIC ANALYSIS OF KAEMPFEROL: A COMBINED EXPERIMENTAL AND THEORETICAL STUDY

D. Milenković 1, Z. Marković 2, S. Jeremić 2, D. Dimić 3 and J. Dimitrić Marković 3

1Bioengineering Research and Development Center, Prvoslava Stojanovića 6, 34000 Kragujevac, Republic of Serbia. (e-mail: deki82@kg.ac.rs)
2Department of Chemical-Technological Sciences, State University of Novi Pazar, Vuka Karadžića bb, 36300 Novi Pazar, Republic of Serbia.
3Faculty of Physical Chemistry, University of Belgrade, Studentski trg 12-16,11000 Belgrade, Republic of Serbia.

ABSTRACT
Theoretically predicted vibrational wave numbers of kaempferol were compared with available IR and Raman experimental data obtained in the 4000 to 400 cm⁻¹ range. Assignments of the experimentally obtained normal vibrational modes were done by the density functional theory using the B3LYP-D3 functional in combination with the 6-311+G(d,p) basis set implemented in the Gaussian 09 package. The optimized geometry of kaempferol, in the gas-phase was used to predict the IR and Raman spectra. The complete vibrational assignments of wave numbers were made on the basis of potential energy distribution (PED). The results of the calculations, which were applied to simulated spectra of kaempferol, showed an excellent agreement with the experimental spectra.

INTRODUCTION
Flavonoids are natural polyphenolic compounds reported to exert a wide range of positive health effects mainly arising from their antioxidant ability. Kaempferol (3,5,7-trihydroxy-2-(4-hydroxyphenyl)-4H-chromen-4-one) is a natural flavonoid that can be found mainly in broccoli, tea, kale, ginko, cabbage, endive, leek, beans, tomato, strawberries, grapes and many medical herbs used in traditional medicine [1]. It is known as a strong antioxidant which helps to prevent arteriosclerosis by inhibiting the oxidation of low density lipoprotein and the formation of platelets in the blood. By inhibiting angiogenesis in vitro and/or in vivo it may reduce the risk of developing some types of cancer, including pancreatic cancer, lung cancer, gastric cancer and ovarian cancer in post-menopausal women [1].
The present study comprises in vitro experimental (IR and Raman) and theoretical approaches in analysis of kaemepferol structure. The applied combination of the experimental vibrational spectroscopic methods and theoretical calculations could be generally applied in analyzing the structural changes of naturally occurring flavonoids under physiologically relevant conditions, providing insight into the mechanism of their bioactivity.

**EXPERIMENTAL**

**IR and Raman spectra**
The IR spectra were recorded on a Thermo Nicolet 6700 FT-IR Spectrometers with ATR and DTGS TEC detector. The Raman spectra were recorded on Thermo Scientific DXR Raman microscope. The spectra were excited with a diode pumped solid state high-brightness 532 nm laser. Both spectra were recorded in the middle IR region, 4000-400 cm⁻¹. The spectra were directly from the pure powder samples.

**DFT calculations**
The structure of most stable conformation of kaempferol (Fig.1) is defined using B3LYP-D3 [2] local density functional method in combination with 6-311+G (d, p) basis as implemented in the Gaussian 09 package [3]. In order to find the most stable rotamer of kaemepferol, all rotamers are designed. All of the conformations are obtained by combining antiperiplanar and synperiplanar arrangements of hydrogen atoms of the OH groups as well as rotation around the dihedral angle O1–C2–C1′–C2′, which defines the coplanarity of C and B rings. All geometry minima are optimized without any geometric restrictions. The structures obtained in this way were verified by normal mode analysis to be minimum on the potential energy surface. No imaginary frequencies were obtained. The calculated wavenumbers were scaled with scaling factor of 0.9873 in order to get better match between calculated and experimental wavenumber values. The vibrational modes were assigned on the basis of PED analysis using VEDA [4] software.

**RESULTS AND DISCUSSION**

**Conformational analysis**
From the optimized molecular structure of the kaempferol, it is found that torsion angle, τ, defined by the C3–C2–C1′–C2′ atoms is ~180°. Thus, as expected, investigated molecule is a planar with the facilitated electron delocalization between the B and C rings.
**IR and Raman spectra analysis**

The observed and calculated infrared and Raman spectra of kaempferol and scaled band positions, wavenumbers, along with the corresponding assignments for first 66 vibrational modes (of the total 87) appearing in the 4000 to 400 cm\(^{-1}\) region, are calculated. The experimentally obtained and scaled theoretical frequencies, IR intensities, and Raman activities, PEDs and mode of description are also calculated.

![Figure 1](image1.png)

**Figure 1.** The optimized structure and numbering of kaempferol: a) HOMO and LUMO orbitals of kaempferol b)

![Figure 2](image2.png)

**Figure 2.** Experimental and theoretical IR spectrum of kaempferol

The most distinct bands in the IR spectrum of kaempferol are those in high frequency region (4000–2000 cm\(^{-1}\)) assigned to different modes of OH vibrations. This region is dominated by very massive and intense band. Taking into account the polyhydroxylated structure of kaempferol the intense band positioned at 3420 cm\(^{-1}\) could be taken as possible absorption for (O5–H5---O4) hydrogen bond. This is also the result confirmed by the calculations which predict hydrogen bonding. The high frequency region is also very characteristic to CH stretching modes (3206 cm\(^{-1}\)). In the 4000–3000 cm\(^{-1}\) region of the experimental Raman spectrum there are no bands easily assignable to O–H and C–H stretching modes.

The majority of the intense bands both in kaempferol IR and Raman spectra are to be found in the 1800-500 cm\(^{-1}\) wavenumber region (Fig. 2).
The DFT wavenumbers are adjusted by a scaling factor of 0.9873. The scaling factor is determined by means of the least squares method, on the basis of the experimental data for the IR spectrum. The low frequency region involves combination of the C=O stretching (1660 (IR) and 1612 (IR) cm\(^{-1}\); 1660 (R) and 1607 (R) cm\(^{-1}\))(modes \(v_{77}\) and \(v_{74}\)), C2=C3 stretching (1660 (IR) and 1568 (IR) cm\(^{-1}\); 1660 (R) and 1561 (R) cm\(^{-1}\))(modes \(v_{77}\) and \(v_{72}\)) and C–C stretching (1660 (IR), 1612 (IR), 1568 (IR), 1509 (IR) cm\(^{-1}\); 1660 (R), 1607 (R), 1561 (R), 1509 (R) cm\(^{-1}\))(modes \(v_{77}\), \(v_{74}\), \(v_{72}\) and \(v_{70}\)). The bands between 1500 and 1000 cm\(^{-1}\) mostly involve C–C stretching, O–C stretching and in-plane C–C–H, C–O–H, C–C–O and C–C–C bending vibrations of the rings. The most intense bands in this region belong to \(v_{65}\), \(v_{62}\), \(v_{61}\), \(v_{58}\), \(v_{57}\) and \(v_{53}\) IR modes and only to \(v_{55}\) Raman mode. Bands appearing below 1000 cm\(^{-1}\), medium to low intensity, are assigned to bending modes (C–C–O, C–C–C, C–O–H, H–C–C and C–O–C) of all three rings and also to the combination of various in plane (C–C–C–H, H–C–C–H, C–C–C–C, C–C–O–C) and out of plane (O–C–C–C and C–C–O–C) modes.

CONCLUSION
The results of the applied B3LYP-D3/6-311+G(d,p) density functional method in determination of the spectroscopic and electronic features of kaempferol point to a planar molecule, characterized by facilitated electron delocalization between the B-ring and the C-ring. Spectral assignments, done on the basis of a best-fit comparison between the experimentally obtained and theoretically calculated IR and Raman spectra, match quite well, indicating DFT calculations as a very accurate source of normal mode assignments.

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