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Photophysical properties of flavonoids extracted from *Syngonanthus nitens*, the golden grass

Leonardo Schneider Berlim[a,c,*], Arandi Ginane Bezerra Jr.[b], Wallance Moreira Pazin[a], Thiago Siqueira Ramin[b], Wido Herwig Schreiner[c], Amando Siuiti Ito[a]

[a] *Departamento de Física, Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo, Av. Bandeirantes 3900, 14040-901 Ribeirão Preto, São Paulo, Brazil*
[b] *Departamento Acadêmico de Física, Universidade Tecnológica Federal do Paraná Avenida Sete de Setembro, 3165 Centro, 80230-010 Curitiba, Paraná, Brazil*
[c] *Departamento de Física, Universidade Federal do Paraná, Centro Politécnico, Jardim das Américas, 81531-980 Curitiba, Paraná, Brazil*

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ABSTRACT

Dry stems of *Syngonanthus nitens,* a plant of the *Euriocaulaceae* species, present exquisite golden shine, originated from flavonoids residing at *S. nitens* stem epidermis. We report here on the optical properties of flavonoids extracted, in different solvents, from dry stems of the plant. The extracts were analyzed by UV–Vis absorption and fluorescence emission spectroscopies, and data were obtained in solvents of different polarity and in aqueous media at different pHs. Time-resolved fluorescence experiments, made using single photon detection technique, fluorescence correlation spectroscopy and fluorescence anisotropy measurements were also employed to study flavonoid/solvent and flavonoid/flavonoid interactions. A consistent picture of the *S. nitens* flavonoid photophysical behavior and their interaction via proton transfer in aqueous suspension is obtained. The results are important to understand the photophysical process involving flavonoids in general.

1. Introduction

Handicraft products using the golden grass *Syngonanthus nitens* dry flower stem, mimic spun gold artifacts. These splendid crafts are famous and unique, being exported worldwide, representing an important income for handicraft workers in rural areas of central Brazil. However, in spite of its *sui generis* optical properties, and the importance of possible bioinspired applications which could arise from a more complete scientific understanding of *S. nitens*, still the main scientific interest regarding golden grass focuses on longtime sustainability and the sustainability of the families who explore this unique crop.

We described recently the mechanism responsible for the exquisite golden shine of dry scales [1]. The main conclusion was that flavonoids, residing at *S. nitens* stem epidermis, absorb UV, violet and blue light, mostly reflecting red-shifted light from the solar spectrum. Additionally, these flavonoids present visible light fluorescence upon UV light excitation. This red-shifted reflection, together with the smooth stem surface profile explains the golden brilliant shine.

Flavonoids are compounds of the polyphenol family with a low number of atoms, consisting of fifteen carbon atoms, arranged in a C6–C3–C6 configuration. Essentially, the structure consists of two aromatic rings, A and B, joined by a 3-carbon bridge, usually in the form of a heterocyclic ring, C. The different radicals, as well as their positions relative to the molecule, determine the biological action of the flavonoid. In fact, flavonoids are secondary plant metabolites and receive most attention due to their benefits to human health. In 1999 there were more than 6000 different flavonoids reported [2,3], and that number considerably increased since then. However, their optical properties are far less studied, being mainly found in the literature on polyphenols and anthocyanins, a class of flavonoids, which are responsible for the color of grapes, berries and carrots, for example [4].

Golden grass stem flavonoids were studied by Pacificoet al. [5] and were described as being composed by 5 flavones and 3 flavanones, identified as follows, using their original flavonoid numbering (see Fig. 1 for the structure of flavonoid 1 and Supporting Material S1 for the other flavonoids):

1- 3′,4′,5,-tetrahydroxy-6-C-glucopyranosylflavone
3- 3′,4′,5,-trihydroxy-7-methoxy-6-C-glucopyranosylflavone
5- 3′,4′,5,-trihydroxy-7-methoxy-6-C-glucopyranosylflavanone
7- 3′,4′,5,-trihydroxy-7-methoxy-8-C-glucopyranosylflavone
8- 4′,5,-dihydroxy-3′,7-dimethoxy-6-C-glucopyranosylflavone
9- 3′,4′,5,-trihydroxy-7-methoxy-8-C-glucopyranosylflavanone
10- 4′,5,7-trihydroxy-8-C-glucopyranosylflavanone

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[*] Corresponding author at: Departamento de Física, Faculdade de Filosofia, Ciências e Letras, Universidade de São Paulo, Av. Bandeirantes 3900, 14040-901 Ribeirão Preto, São Paulo, Brazil.
E-mail address: lnrdschneider@gmail.com (L.S. Berlim).

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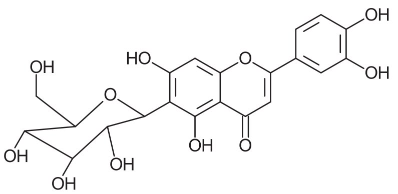

Fig. 1. - Flavonoid 1 – 3′,4′,5,-tetrahydroxy-6-C-glucopyranosylflavone. The *S. nitens* flavonoids have the hydroxy groups in position 5 of the aromatic ring A and 3′ and 4′ of the aromatic ring B. With exception of flavonoid 17, there is a glucopyranosyl radical bound in the ring A.

17- 3′,4′,5,7-tetrahydroxyflavone

More recently, Cardoso et al. [6] quantified these flavonoids using high performance liquid chromatography. Effectively, flavonoids constitute the quantitatively dominant molecules found in extracts of *S. nitens* stems. The interesting absorption and emission properties of *S. nitens* flavonoids inspired us to investigate these molecules in more detail. In order to study their optical behavior, we extracted *S. nitens* secondary metabolites by using different solvents. We employed optical absorption and fluorescence steady state and time-resolved techniques and looked at the interactions between flavonoids and between the fluorophores and solvent in the extracts. For aqueous extracts, at different pH values, the role of processes involving protonation and deprotonation of the flavonoids, and of intra and intermolecular proton transfer were evaluated. The beneficial activity of flavonoids has been recognized since long, as powerful antioxidants are effective against many diseases including cancers, tumors, allergies, and different free radical-mediated disorders [7]. From epidemiological studies, it was suggested that the consumption of foods and beverages rich in flavonoids correlates with the lower risk of certain cancers, cardiovascular diseases and oxidative stress-related diseases [8]. The increasing use of flavonoids as food additives for health purposes contributed to the growing interest in their photochemical and photophysical properties [9] and research has been devoted to evaluating the feasibility of flavonoids protecting foods and beverages against harmful photochemistry [10]. A relevant application was recently reported, where fluorescent flavonoids were employed for endoplasmic reticulum cell imaging, stressing the use of the flavonoids as template for sensor design [11]. Our hope is that the study performed here with *S. nitens* flavonoids could provide initial insight to other applications that could be expected for the use of *S. nitens* based on the properties of the flavonoids found in their composition.

2. Materials and methods

Flavonoid extraction was done by using commercial decorative items available on the market, because due to sustainability legal restrictions, we have no access to pristine stems. The flavonoids of stems of *S. nitens* were extracted for a week, at room temperature, in distilled water, p.a. methanol, isopropanol, acetone, DMSO (dimethyl sulfoxide) and propylene glycol. The crude extracts were obtained after filtration in filter paper [5,6], and used without additional procedures for isolation and purification. The solvents were acquired from Sigma-Aldrich. For the measurements in aqueous solutions of different pH, citrate-phosphate buffers with 0.1 M were prepared.

For pH measurements a mPE-210 Tecnopon pH meter was used. The UV–vis absorption spectra of the stems extract suspensions were obtained with an Ocean Optics USB2000 + spectrometer in the wavelength range 200–1000 nm. The steady-state fluorescence spectra and anisotropy measurements were obtained using a Hitachi F-7000 spectrophotometer, equipped with polarizer filters.

A MicroTime 200 system (PicoQuant) was used to perform fluorescence correlation spectroscopy (FCS) measurements, consisting in the determination of the temporal autocorrelation of fluorescence fluctuations. The analysis of autocorrelation curves allows the determination of the diffusion coefficient of fluorophores, as described previously [12,13]. A pulsed picosecond light from a diode laser at 375 nm was reflected with a dichroic beam splitter into an inverted Olympus IX 71 microscope. Small volumes of samples placed on a cover slip were excited from the light focused by an Olympus UPlanSApo 60x water immersion objective, numerical aperture 1.2, with correction collar 0.15 Edmund Achromat (f = 175 mm) tube lens and Thorlabs 50 μm pinhole. Fluorescence emission was collected with the same objective, passing through a dichroic to remove scattered light before reaching a pinhole for confocal detection. A 405 nm long pass filter was added in the optical path to guarantee only fluorescence emission to be detected with a single avalanche photo diode (SPAD). Fluorescence intensity decay profiles were obtained in the same instrument using the time-correlated single photon counting (TCSPC) technique, and were analyzed by proprietary software of the microscope, SymPhoTime, by a non-linear least-squares formalism. The full width at half-maximum of the instrument response function was typically below 100 ps. The quality of the fit was analyzed from the reduced χ^2 values and the residuals distribution. The same software was employed to analyze the temporal correlation of intensity fluctuations in order to obtain the auto-correlation curves and diffusion coefficients.

3. Results

3.1. Solvent effects

Flavonoids were extracted from *S. nitens* using six solvents of different polarity and examined as crude extracts. Despite the high absorbance of the samples, several absorption bands are distinguishable in the spectra measured in the different solvents (Fig. 2a). In each solvent different flavonoids may be contributing to the spectrum and it is not

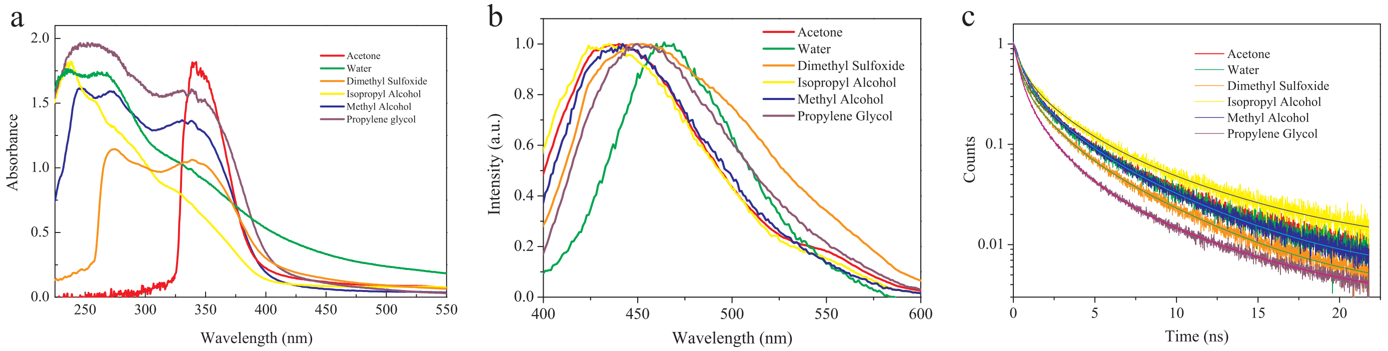

Fig. 2. a) Absorbance (left), b) fluorescence normalized to one in the maximum emission (middle) and c) lifetime measurements of *S. nitens* flavonoids in various solvents of different polarity, with excitation at 375 nm (right). The concentrations used were: water 1.8 mg/ml, methyl alcohol 1.2 mg/ml, propylene glycol 1.5 mg/ml, DMSO 3.3 mg/ml, acetone 1.5 mg/ml, isopropyl alcohol 1.3 mg/ml.